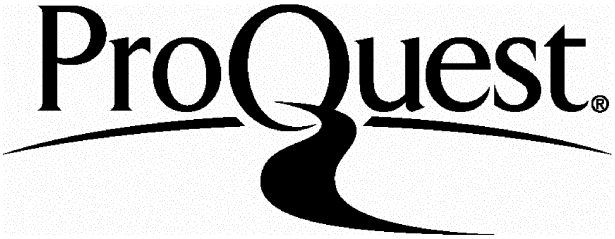
CELLULAR AND MOLECULAR ASPECTS OF ARTICULAR CARTILAGE RESURFACING.

by

Bena Hemmen.

Submitted in fulfilment of the requirements for a Doctorate of Philosophy in the Faculty of Science at the University of London.
June 1992.

University Department of Orthopædic Surgery,
Institute of Orthopædics,
University College and Middlesex School of Medicine.
Brockley Hill,
Stanmore, Middlesex.



Abstract.

The aim of this project is to find alternative ways of repairing articular cartilage defects in rabbits by implanting carbon fibre, collagen gel or hydrogel PC 97 plugs with associated chondrocytes. The results were assessed using histology, electron microscopy, biomechanical testing and immunocytochemistry using antibodies against collagen types I and II, chondroitins -4- and -6- sulphates and keratan sulphate. The antibodies were used as qualitative markers of extracellular matrix composition.

Isolated chondrocytes, cultured in either a carbon fibre, collagen gel or hydrogel PC 97 plug, were transplanted into full-thickness defects of articular cartilage in the tibiae of mature rabbits. Grafts were examined 3, 6 and 12 months post-implantation using the techniques outlined above.

The carbon fibre plugs with associated chondrocytes showed a cartilaginous matrix with incorporation of the carbon fibres at 3 weeks *in vitro* culture and 3 months post-implantation; after 6 and 12 months cartilage-like tissue was shown in all layers except the surface. After implantation of carbon fibre plugs without chondrocytes the repair tissue was fibro-cartilaginous. The stiffness of the carbon fibre implants at 6 and 12 months post-implantation showed values in the same range as normal rabbit articular cartilage of similar age (native cartilage).

The collagen gel plugs with associated chondrocytes showed that after 3 weeks *in vitro* culture most of the chondrocyte-like cells were present at the surface of the gel, with more fibroblast-like cells within the gel. At 3, 6 and 12 months post-implantation, a variety of repair responses were observed, ranging from repair tissue resembing articular cartilage to fibrous-like graft tissue. Fibrocartilaginous repair tissue generated in the control joints was sparse, with little evidence of chondrogenesis. The stiffness of the collagen gel plugs was lower than in native cartilage.

The hydrogel PC 97 plugs plus associated chondrocytes at 3 weeks *in vitro* culture were surrounded by a cartilage-like matrix. At 3 months post-implantation some areas of the matrix showed pericellular metachromatic staining, and the plugs were well incorporated into the bone, but not into the adjacent cartilage. The control plugs showed the presence of a growth response encapsulating the hydrogel PC 97. The hydrogel PC 97 plugs had a higher stiffness than in native cartilage.

Contents.

Acknowledgements.

I would like to acknowledge the support and advice of Prof. G.Bentley, Professor of Orthopædic Surgery, supervisor, who laid the facilities of his department at my disposal and performed most of the transplants described in this thesis. Also, I am indebted to Prof. Charles W. Archer, Prof. of Anatomy, supervisor, for giving me the chance to deepen my theoretical and practical knowledge of articular cartilage biology and for his help throughout my time in Stanmore, especially in the final phase. Miss Lucia Caravia and Dr. John Fisher, Dept of Mechanical Engineering, University of Leeds, who performed the indentation tests in this study and were pleasant and supportive collaborators (despite receiving wet parcels). Also, I wish to thank Miss Helen Fitton, Dr. Phil Corkhill and Prof. Brian Tighe, Dept. of Chemical Engineering and Applied Chemistry, Aston University, Birmingham, for generously preparing the hydrogel PC 97 and its predecessors, thereby considerably increasing the profitability of British Rail on the line between Watford and Birmingham.

Space does not permit me to mention all those in the Institute who have helped me over the years, but personal thanks go to Mr. Mike Kayser, 'homo universalis', who advised and taught me on histological and electron microscopical processing and re-photographed all the plates in this thesis; Dr. Gordon Blunn and Miss Mary Wait, Dept. of Bio Medical Engineering (BME), who performed the scanning electron microscopy; Mr. Hugh Crawford (BME), whose expert computer advise was extremely valuable as was that of Mr. Paul Unwin (BME); Mr. Gary Batchelor, Mr. Paul de Silva and assistants for husbrandry and technical assistance; Dr. Peter Smith and Mrs. Sandra Bollard of the Medical Library at Stanmore for their help and advice; the members of the Medical Photography Department for photographing the knee joints post-implantation; the Department of Morbid Anatomy for their help with decalcifying specimens and the vacuum embedding of hydrogel PC 97 plugs (Mr. John Collins) and the expert advise from Dr. J. Pringle on the interpretation of the histological sections of synovial and capsular tissues.

Most antibodies used in this study were kindly supplied by Dr. Mike T. Bayliss, Kennedy Institute of Rheumatology, London. For the statistical advice I acknowledge the help of Drs. R. Brooks and S. Pitt, Dept. of Statistics, University College London. Thanks to the many secretaries in the Professorial department and the administrative staff of Admissions/Medical Records of the Royal National Orthopaedic Hospital trust who informed me about admissions of patients.

Financial support for this project was provided by the Science and Engineering Research Council. I would like to thank Surgicraft Ltd, Redditch, for their financial support in the final months of this study and especially my thanks to Dr. C. Gee and Dr. T. O'Brien.

I thank Josette, Marjan and Margaret for their friendship during the last three years. Finally, I would like to thank my partner, Paul, for his continous support and encouragement, preventing too much 'double dutch' from entering this thesis.

to my parents, my sister, with love.

List of Tables.

List of Figures.

Appendix 2.

INTRODUCTION

1.1. Structure and properties of articular cartilage.

Cartilage is the major skeletal material of all embryonic vertebrates and of chondrichthyan fishes throughout life. The wide distribution of cartilage across many phyla suggests that it is an ancient feature of multicellular animals. It is also found in parts of the skeleton of adult vertebrates such as the tip of the nose, the outer ear, the ventral area of the rib cage and the articulating joints, where flexibility and toughness are more important than rigidity and at sites of skeletal growth and healing. Its most familiar and in many ways its most unusual property is the complete lack of blood supply.

The tissue consists of extracellular matrix and chondrocytes with a high matrix to cell volume ratio. The matrix consists of collagen, proteoglycans, non-collagenous proteins and water. The proper balance between synthesis and degradation of proteoglycans, collagen and other matrix constituents is essential to normal cartilage function. Articular cartilage is subjected to a wide range of mechanical loading forces. In humans contact pressures as high as 3-18 MPa have been estimated in the femoropatellar and hip joints (Huberti and Hayes, 1984; Hodge *et al.*, 1986; Afoke *et al.*, 1987). The ability of cartilage to withstand the resulting compressive, tensile and sheer strains depends critically on the structure, composition and integrity of its extracellular matrix (Buckwalter *et al.*, 1988b). Whilst collagen fibrils are strong in tension, proteoglycans can resist compression, owing to their bulk compressive stiffness and to electrostatic repulsive interactions between (a) individual glycosaminoglycan chains and (b) glycosaminoglycan chains and water (Kempson, 1980; Maroudas, 1980; Grodzinsky, 1983; Mow and Rosenwasser, 1988). Tissue stiffness is also affected to a significant extent by the frictional interactions between interstitial fluid and the extracellular matrix.

1.1.1. Collagen.

Collagen is the most abundant protein in mammals, constituting a quarter of the total (Stryer, 1975). At present, 14 different collagen types are known. Collagen types I, II and III are the main type of collagens found in connective tissue and collagen types II, V, VI, IX, X and XI are found in articular cartilage. The collagen molecule is characterised by a triple-stranded helical structure of polypeptide chains (α-chains) each of which is about 1000 amino acids long. The three strands form a right-handed superhelix. The whole molecule is about 300 nm long and 1.5 nm in diameter. An α-chain is composed of a series of triplet Gly-X-Y sequences in which X and Y can be any amino acid, but is often proline (Grassmann *et al.*, 1965).

The three-dimensional architecture of articular cartilage has been described by various models. Benninghoff (1925) described collagen fibres arising in the subchondral bone, passing towards the surface radially to become tangentially oriented at the surface and then returning to the bone to form the other half of an arcade. Further research showed that the orientation in the radiate zone was radial to the subchondral bone, the orientation

in the surface zone was tangential, but that the orientation in the intermediate zone was for the most part random (McCall, 1969; Mulholland, 1974; Redler, 1974; Minns and Steven, 1977; Speer and Dahners, 1979a). Clark *et al.*, (1991) and Jeffery *et al.*, (1991) described a so-called 'leaf'-model. The collagen in the intermediate and superficial zones is arranged in a series of closely packed layers of 'leaves'. The leaves themselves are composed of a fine meshwork of fibres, and adjacent leaves merge or are closely linked by bridging fibrils. The leaves that are formed in the intermediate zone then arch over to form the horizontally orientated leaves of the superficial zone. In the surface layer there is no distinct fibrillar structure as there is in the deeper layers.

Collagen type I.

Collagen type I is the most abundant collagen type in nature and it is found in many types of connective tissues such as skin, tendon, bone and fibrocartilage, but not in articular cartilage. It consists of two $\alpha 1(1)$ chains and one $\alpha 2(1)$ chain.

Collagen type II.

Collagen type II is the major collagen in cartilage, consisting of 3 $\alpha 1(II)$-chains. It is also present in the vitreous humour of the eye (Swann *et al.*, 1972), in the nucleus pulposus and annulus fibrosus of the intervertebral disc (Eyre and Muir, 1974). It can be found in small quantities in developing chicken embryos at the epithelio-mesenchymal interfaces during morphogenesis of the cartilaginous neurocranium as well as in other noncartilaginous tissues (Thorogood and Hinchcliffe, 1975; Kosher and Solursh, 1989). It is synthesized as a procollagen monomer, assembled into molecular trimers and processed extracellularly to remove NH_2- and COOH-terminal extension propeptides. The procollagen monomer contains a non-interrupted Gly-X-Y protein domain of about 1,014 amino acids, flanked by the globular propeptides. The COOH-terminal domain of about 275 amino acids is connected to the main triple helix by a short telopeptide, which is thought to be involved in the formation of the triple-helical molecule. The NH_2-terminal propeptide shows structural and sequence diversity, but generally consists of a short globular domain followed by a cysteine-rich domain, a Gly-X-Y domain of 40-60 residues, and a short connecting telopeptide (Sandell and Boyd, 1990). It has been suggested that the propeptides, particularly the NH_2-propeptide, are involved in feedback regulation of collagen synthesis and in the formation of fibrils in the extracellular matrix (Wu, 1986). Type II procollagen can be expressed in two forms by differential splicing of the primary gene transcript: the two mRNAs either include (type IIA) or exclude (type IIB) an exon (exon 2) encoding the major portion of the amino-propeptide (Ryan and Sandell, 1990). Each type of procollagen mRNA has a distinct tissue distribution during chondrogenesis, with type IIB expressed in chondrocytes in cartilaginous tissue, and type IIA expressed in cells surrounding cartilage in the prechondrogenic area. Also, the morphology of the cells expressing the two collagen

types is distinct with the morphology of the cells expressing type IIA being fibroblast-like, whilst the cells expressing type IIB are large, round and typically chondrocyte-like (Sandell *et al.*, 1991).

Collagen type III.

Collagen type III is formed by three α1(III)-chains. It has a high content of hydroxyproline and a low content of hydroxylysine and bound carbohydrate compared to other types of collagen. The primary structure of the procollagen chains is similar to that described for collagen types I and II, which reflects the strong homology between the collagens type I, II and III (Hofmann *et al.*, 1980). Collagen type III accompanies collagen type I, in different ratios, in all tissues except bone (Gay and Miller, 1978; Sandberg *et al.*, 1989a). It has also been observed in developing (pre) articular cartilage (Sandberg *et al.*, 1989b), but not in non-diseased mature articular cartilage. A considerable fraction of type III collagen is present as the partially processed monomer in the form of pNcollagen III (Fessler *et al.*, 1981; Timpl and Glanville, 1981). Romanic *et al.*, (1991) suggested that pNcollagen III can regulate the diameter of collagen I fibrils by coating the surface of the fibrils, thereby allowing tip growth but not lateral growth of the fibrils. Collagen type III appears to be important in the healing process of skin (Barnes *et al.*, 1976), tendons (Williams *et al,* 1984) and bone fractures (Page *et al.*, 1986).

Collagen type VI.

Collagen type VI represents 1-2% of the total collagen in (steer) cartilage (Eyre *et al.*, 1987a). It is a short triple-helical collagen molecule with a very large globular domain at each end (Keene *et al.*, 1988). The collagen type VI monomers are assembled into well-defined oligomers which are the building blocks of microfibrils found both in tissues and cell cultures. It is found throughout the matrix of cartilage (Keene *et al.*, 1988) and/or directly surrounding the chondrocyte (Ayad *et al.*, 1984; Poole *et al.*, 1988; Keene *et al.*, 1988). It has been suggested that it has a role in mediating fibril-fibril or fibril-proteoglycan interactions (Eyre *et al.*, 1987a).

Collagen type IX.

Collagen type IX is a nonfibrillar collagen that comprises 5-20% of the total collagen in cartilage. It comprises three distinct polypeptide chains marked α1(IX), α2(IX) and α3(IX) encoded by separate genes (Ninomiya and Olsen, 1984; van der Rest *et al.*, 1985). The molecule contains three collagenous domains (COL1- COL3) and four noncollagenous domains (NC1-NC4). One of the polypeptide subunits α2(IX) contains a glycosaminoglycan side chain (Vaughan *et al.*, 1985; Bruckner *et al.*, 1985). In the cartilage matrix, type IX collagen appears to be covalently cross-linked to the surface of type II collagen fibrils (van der Rest and Mayne, 1988). Mayne and Irwin (1986) using immunocytochemical techniques described binding for type IX collagen in embryonic

and adult chick sternum cartilage, possibly in co-distribution with collagen type II (Irwin *et al.*, 1985). This suggests that type IX collagen is distributed throughout the cartilage extracellular matrix, but is not present in other connective tissues or skeletal muscle. Kulyk *et al.*, (1991) found that type IX collagen gene expression appears to be restricted to differentiating chondrogenic cells.

Collagen type X.

Many studies have shown that type X collagen is predominantly synthesized by hypertrophic chondrocytes during the process of endochondral ossification (Schmid and Linsenmayer, 1985; Poole and Pidoux, 1989; Kirsch and von der Mark, 1990). In the foetal and adolescent skeleton, the molecule is a transient intermediate in the cartilage which will be replaced by bone. In the adult, this collagen type persists in the zone of calcified cartilage which separates the hyaline cartilage from the subchondral bone. Grant *et al.*, (1987) demonstrated the synthesis of collagen type X at sites of endochondral ossification in pathological circumstances, namely in the cartilaginous callus which is formed during bone healing. Others demonstrated its widespread occurrence in osteoarthritic cartilage (Walker *et al.*, 1991, Kirsch *et al.*, 1991b).

Type X collagen is a short-chained collagen comprised of three apparently identical polypeptides, the α1(X)-chains. Type X collagen has been isolated from rabbit epiphyseal cartilage by Remington *et al.*, (1983) and Sussman *et al.*, (1984). Rabbit, human and chicken type X collagen are very similar in that in all three the intermolecular disulphide bond is absent. In contrast, bovine and sheep type X collagen do appear to form disulphide-stabilized aggregates (Gibson *et al.*, 1991). The human type X collagen is composed of α-chains of 59 kDa, which is composed of a helical region of 45 KDa with non-helical extensions. The molecular size is similar to the chick type X collagen, but is smaller than the bovine or sheep type X collagen (Grant *et al,* 1985; Gibson *et al.*, 1991). Schmid and co-authors suggested that collagen type X may possibly facilitate the removal of the hypertrophic cartilage, after finding that type X collagen is more rapidly degraded than type II collagen by vertebrate collagenase (Schmid *et al.*, 1986). The second possible function of type X collagen may be in the formation of an extracellular matrix susceptible to calcification. More recent work demonstrated the link between collagen synthesis and cartilage mineralization by *in vivo* studies with rachitic chickens (Kwan *et al.*, 1989), and *in vitro* studies using hypertrophic chondrocytes (Thomas *et al.*, 1990) and foetal human chondrocytes (Kirsch and von der Mark, 1991a). Using rotary shadowing Kwan *et al.*, (1991) have shown that *in vitro,* chicken type X collagen forms large aggregates based on a regular hexagonal lattice. This network may be part of the modification process of the extracellular matrix to prepare it for calcification. Using cDNA cloning of the rabbit α1(VIII)-collagen chain a high degree of similarity has been found between the primary structures of type VIII, a product of endothelial cells and type X collagens (Yamaguchi *et al.*, 1989). It could be postulated that the collagen X

framework may influence the migration of invading endothelial cells during angiogenesis within the cartilage matrix (Kwan *et al.*, 1991).

Collagen type XI.

Type XI collagen ($1\alpha 2\alpha 3\alpha$) forms, with collagen type V, a closely related sub-class of the class 1 group of fibril-forming collagens (Eyre *et al.*, 1987b). It co-localises with type II collagen, and in a ratio in mature cartilage of collagen types XI:II = 1:30 (Eyre and Wu, 1987b). Type XI collagen molecules and fibrils have a specific and firm binding to sulphated glycosaminoglycans (Smith jr.*et al.*, 1985), which could possibly allow them to mediate physical interaction between collagen fibrils and proteoglycans in cartilage.

1.1.2. **Proteoglycans.**

Cartilage proteoglycans exist mostly in the form of proteoglycan aggregates formed by noncovalent association of proteoglycan monomers, hyaluronan and link proteins (reviewed by Heinegard *et al.*, 1986 and Carney and Muir, 1988). Many proteoglycan monomers are bound to a single central hyaluronan filament via the hyaluronan-binding region of proteoglycan monomer core protein (Hardingham and Muir, 1974; Hascall and Heinegard, 1974; Rosenberg *et al.*, 1975). The link proteins appear to bind simultaneously to hyaluronan and to the hyaluronan-binding region of proteoglycan monomer core protein, and stabilize the proteoglycan aggregate against dissociation (Baker and Caterson, 1979; Hardingham, 1979). The proteoglycan monomers in mature articular cartilage consist of the glycosaminoglycans, chondroitin sulphate and keratan sulphate, the chain being covalently bound to a protein core (Roughley and White, 1980; Garg and Swann, 1981; Bayliss *et al.*, 1983). The protein core consists of three different major domains, namely

(1) the chondroitin-sulphate-rich region (Heinegard and Hascall, 1974, Hascall and Kimura, 1982) which is the portion of the protein core that extends peripherally in the proteoglycan aggregate. It contains most of the chondroitin sulphate chains and about 30% of the keratan sulphate chains. Its length is variable.

(2) the hyaluronan-binding region, which is the opposite end of the protein core consisting of a portion of the polypeptide chain folded into a globular structure. It is essentially devoid of glycosaminoglycan chains and contains about 7 disulphide-bridges, which are the binding site of the proteoglycan monomer for hyaluronan (Heinegard and Hascall, 1974; Hascall and Kimura, 1982). Ratcliffe *et al.*, (1984) using electron microscopic immunolocalization and radioimmunoassay showed that hyaluronan-binding region concentration was low in the surface layer (20-23 μm from joint surface), but increased in the middle zone (80-280 μm from joint surface) and deep layers (280-480 μm) of pig articular cartilage. The concentration of hyaluronan-binding region in the deep layer was less than in the middle zone. In all layers, pericellular staining was more

intense than intercellular staining. Between the chondroitin-sulphate-rich region and the hyaluronan-binding region is located a third domain called

(3) the keratan sulphate-rich region (Heinegard and Hascall, 1974; Heinegard and Axelsson, 1977), which consists almost entirely of keratan sulphate, but also a small proportion of the chondroitin sulphate chains. Apart from the three major domains, there are two other globular domains within the core protein, called G2 and G3 (Wiedemann *et al,* 1984). Lord *et al.*, (1984) suggested that these regions were involved in the binding of cartilage matrix protein in the extracellular matrix by examining the binding of cartilage matrix protein, a glycoprotein of 148kDa, to proteoglycans. The distribution of proteoglycans throughout the articular cartilage depth varies with increasing distance from the joint surface (Stockwell and Scott, 1967b; Maroudas *et al.*, 1969; Franzen *et al.*, 1981; Bayliss *et al.*, 1983). Firstly, the surface 200 μm layer contained proteoglycans with less chondroitin sulphate and were smaller compared with the deeper layers of the cartilage, and almost all molecules formed aggregates with hyaluronan. Secondly, in the layer about 200 μm to 1040 μm from the joint surface, proteoglycans contained relatively less chondroitin sulphate, whilst the keratan sulphate content was relatively constant compared to the remaining layers of the cartilage, and 90% of the molecules were able to form aggregates with hyaluronic acid. Thirdly, the deepest layer near the calcification zone contained large proteoglycans, which predominantly contained non-aggregating molecules.

Chondroitin-sulphate.

Chondroitin-sulphate is composed of a repeating disaccharide sequence (glucuronic acid linked [β–(1-3)] to N-acetyl galactosamine residues) which can be differently sulphated on the 4-, or 6- positions of the galactosamine, or can be non-sulphated (Caterson *et al.*, 1990). Each disaccharide unit is joined [β-(1-4)] to the next.

In immature articular cartilage, more chondroitin-sulphate and longer chondroitin-sulphate chains are found in comparison with mature cartilage (Simunek and Muir, 1972a, b; Sweet *et al.*, 1979; Roughley and White, 1980; Garg and Swann, 1981; Pal *et al.*, 1981). Mourao (1988) described in immature human femoral cartilage a decrease in the relative concentration of chondroitin-6-sulphate with increasing distance from the articular surface, whilst the relative concentration of chondroitin-4-sulphate increased. Consequently, in the articular surface layer, there was a higher proportion of chondroitin-6-sulphate. In mature cartilage, the amount of chondroitin sulphate compared to keratan sulphate content decreased in comparison with immature cartilage (Kuhn and Leppelman; 1958; Venn, 1978). In contrast, a shift from the 4-sulphated to the 6-sulphated form was described in mature bovine articular cartilage (Garg and Swann, 1981). It has been suggested that chondroitin-4-sulphate might be one of the components of the matrix associated with the calcification process in humans (Michelacci *et al.*,

1979). With ageing, the type and quantity of chondroitin sulphate present in mature cartilage showed little variation (Elliot and Gardner, 1970).

Keratan sulphate.

Keratan sulphate was first discovered in 1953 in the bovine cornea (Meyer *et al.*, 1953) and was classified into a corneal type (KS I) and a skeletal type (KS II). Only the latter will be discussed here.

Keratan sulphate is composed of a repeating disaccharide sequence of galactosamine linked [β-(1-4)] to N-acetyl glucosamine residues. Each disaccharide being joined [β-(1-3)] to the next. Keratan sulphate is linked by glycosidic bonds with serine and threonine residues in the protein core (Anderson *et al.*, 1964; Seno *et al.*, 1965; Bray *et al.*, 1967). In the following pages these items will be discussed:

The presence of keratan sulphate in fetal, immature and mature cartilage, the influence of movement on keratan sulphate and the presence of keratan sulphate in different vertebrate species.

The presence of keratan sulphate in fetal, immature and mature cartilage.

The keratan sulphate present in foetal cartilage is formed by short O-linked oligosaccharides, which consist mainly of the keratan sulphate linkage region (Thonar and Sweet, 1981; Hascall and Kimura, 1982) plus perhaps a few keratan sulphate disaccharide repeating units. In immature cartilage during postnatal development the keratan sulphate content increases. This could be because either longer keratan sulphates are formed on some of the O-linked oligosaccharides (Garg and Swann, 1981), because the number of keratan sulphate chains is increased (Sweet *et al.*, 1979; Thonar and Sweet, 1981), or because a second keratan sulphate-rich proteoglycan monomer with a residual relatively short protein core - which has lost a significant proportion of the chondroitin sulphate-rich portion - appears during postnatal development (Bayliss and Venn, 1980), and which may be fibromodulin (Hardingham and Fosang, 1992). The latter is a low molecular weight leucine-rich proteoglycan, and it has been suggested that it is involved in extracellular matrix organization due to its binding to collagen types I and II *in vitro*. The addition of the keratan sulphate chains effectively increases the mass of carbohydrate and the number of anionic sites within the same hydrodynamic domain for a proteoglycan molecule. One consequence of this would be to increase the internal stiffness of the proteoglycan in such a way that it undergoes less deformation per unit compressive load (Maroudas *et al.*, 1986).

Within mature human articular cartilage, keratan sulphate concentration increases with distance from the joint surface, showing an abrupt rise in the deep cartilage, while chondroitin sulphate reaches peak concentration in the middle zone of the cartilage (Stockwell and Scott, 1965, 1967b; Maroudas *et al.*, 1969; Hjertquist and Lemperg, 1972; Venn, 1978). Stockwell and Scott (1965, 1967b) postulated that the difference in

localization of keratan sulphate and chondroitin sulphate could be explained by the fact that keratan sulphate concentration increases with distance from the source of nutrition, the synovial fluid. During 'ageing' the ratio keratan sulphate:chondroitin sulphate continues to rise slowly, but whilst some authors found a slight increase in keratan sulphate using full depth samples per dry weight of tissue (Hjertquist and Lemperg, 1972), others (Greiling and Stuhlsatz, 1969) have shown a small decrease. In elderly cartilage, positive staining for high molecular weight keratan sulphate is found in the superficial layer, up to a few microns from the articular surface (Stockwell, 1975).

The influence of movement on keratan sulphate.

Keratan sulphate is found in higher concentrations in weight bearing than in non-weight bearing areas of the joint (review by Stockwell, 1987). Experimentally, additional loading appears to promote keratan sulphate content in adult animal cartilage (Kostenszky and Olah, 1972; Tammi *et al.*, 1983), while additional exercise favours increased content of chondroitin sulphate, possibly due to an increase in biosynthesis of chondroitin sulphate, cell division and early overhydration of the matrix (Caterson and Lowther, 1978; Donahue *et al.*, 1983). In contrast, in immature cartilage both additional weight bearing and exercise increase the keratan sulphate/chondroitin sulphate ratio (Kiviranta, 1987).

The presence of keratan sulphate in different vertebrate species.

Keratan sulphate shows inter-species variation. Venn and Mason (1985) described the absence of keratan sulphate in mouse and rat costal cartilage using biochemical and immunological techniques. Stockwell (1989) described that in cat femoral condyle articular cartilage, keratan sulphate-staining is present using the Alcian blue-critical electrolyte concentration technique (Scott and Dorling, 1965), whilst in cat metatarsal articular cartilage very limited staining is found. Interestingly, there is a considerable difference in thickness of cat femoral condyle articular cartilage (300 μm) compared to articular cartilage of cat metarsal bone, which is only 80 μm thick. Zanetti *et al.*, (1985) using immunocytochemical techniques found that most cells isolated from the surface and deep layers of pig articular cartilage synthesized keratan sulphate during monolayer culture *in vitro*. In contrast, Archer *et al.*, (1990) found using human articular chondrocytes a reduction of percentage keratan sulphate-positive chondrocytes from both surface and deep layers cultured *in vitro* in monolayer for 21 days. When suspension cell culture conditions were applied, the percentage keratan sulphate-positive chondrocytes from both surface and deep layers increased, and at 21 days most cells showed immuno-positive staining for keratan sulphate. Zanetti *et al.*, (1985) postulated that there is an intrinsic heterogeneity within the cell population which is reflected in the amount of keratan sulphate produced. Also, heterogeneity with regard to keratan sulphate synthesis is observed between cells within the same zone and even within the same cell group

(Kincaid *et al.*, 1972) in dog and in human cartilage using histochemical techniques (Stockwell, 1989). According to the latter author, this kind of heterogeneity was limited to a fairly narrow zone, which did not show similar heterogeneity in relation to chondroitin-sulphate.

Other proteoglycans.

In addition to the major proteoglycans mentioned previously, there are large non-aggregating proteoglycans, small proteoglycans (PG-S), heparan sulphate proteoglycan and low buoyant density chondroitin sulphate proteoglycan with few chains (Heinegard *et al.*, 1988). The large non-aggregating proteoglycans, constituting some 1-2% of the tissue wet weight, have different core proteins and large glycosaminglycan side chains of either chondroitin sulphate (skin, metaphysis, chick skeletal muscle) or dermatan sulphate, depending on the tissue of origin (Heinegard *et al.*, 1985; Poole 1986). It is not yet clear if these proteoglycans represent a distinct molecular entity. Decorin (PG II), biglycan and fibromodulin are low molecular weight leucine-rich proteoglycans, which contain a central leucine-rich segment consisting of 10-14 repeating sequences. Decorin is found in most connective tissues. Decorin and biglycan contain, respectively, one or two chondroitin sulphate or dermatan sulphate chains close to the NH_2 terminus of the protein, whilst in fibromodulin keratan sulphate chains are N-linked to the leucine-rich repeat region within the core protein. *In vivo* and *in vitro* studies have suggested that decorin has a primary function in the organization of the extracellular matrix (Hardingham and Fosang, 1992) since, firstly, the decorin core protein interacts with fibronectin (Schmidt *et al.*, 1987) and, secondly, decorin binds to collagen types I and II. More recently, it has been suggested that decorin is involved in the control of cell proliferation (Yamaguchi and Ruoslahti, 1988). Biglycan, although structurally very similar to decorin, does not bind to collagen and is found at the cell surface and in the pericellular environments. Its function is not known (Hardingham and Fosang, 1992). Also, the low buoyant density chondroitin sulphate proteoglycan with few chondroitin sulphate chains have no known function (Mathews, 1965; Poole, 1986; Dziewiatkowski, 1987; Buckwalter and Rosenberg, 1988a; Ruoslahti, 1988). Heparan sulphate proteoglycan is composed of a repeating disaccharide sequence of either glucuronic acid linked [β-(1-4)] or iduronic acid linked [α-(1-4)] to N-acetyl-glucosamine. It is only found at cell surfaces including chondrocytes and may be involved in receptor function.

1.1.3. Chondrocytes.

The chondrocytes contribute relatively little (about 10%) to the total volume of articular cartilage. Their main importance lies in the production of matrix components. The extracellular matrix, which surrounds the chondrocytes, influences the chondrocyte function. The form and function of the chondrocytes changes with age, spatial localization and shows interspecies variation.

Immature chondrocytes proliferate rapidly and synthesize large volumes of matrix. In contrast, mature chondrocytes slow down their synthetic rate and seldom divide under normal circumstances. 'Ageing' cartilage shows a progressive decrease of cell density, especially in the superficial layer (Stockwell, 1967a; Stockwell and Meachim, 1979b). The cell density in articular cartilage decreases with increasing distance from the articular surface. In thin cartilage this is almost inversely proportional. In thicker cartilage this is true as well, but after a certain distance from the surface the cell density becomes more or less constant. The thickness of the cartilage is related to the body size, the degree of incongruity of the joint surfaces, mechanical forces and extracellular factors regulating chondrocyte metabolism, but it is not species-dependant (Stockwell, 1979a).

The morphology of adult chondrocytes changes considerably with locality in the cartilage. Articular cartilage of rabbits can be divided into four histological zones through the tissue depth from the surface, of which zones I-III are non-mineralized and IV is the calcifled cartilage zone (Schenk *et al.*, 1986). In zone I, the cells are relatively small and flat and oriented with the long axis paralled to the surface. This zone has the highest cell density. In zone II the cells are larger and rounder and are randomly distributed in the matrix. In zone III, the cells are even larger than in zone II and are arranged in columns with axes perpendicular to the surface. In zone IV mineralisation of the matrix occurs.

As discussed in chapter 1.1.2., the distribution of glycosaminoglycans throughout articular cartilage is heterogeneous. The results from Zanetti *et al.*, (1985) and Archer *et al.*, (1990) might suggest that at least two distinct populations of cells are present in articular cartilage with regard to morphology and synthetic activity. In agreement with this are the results of Aydelottte *et al.*, (1988), who cultured mature and immature bovine articular chondrocytes within agarose gels and also found differences in morphology and matrix production up to 21 days. A high percentage of the surface calf chondrocytes were flattened, irregularly shaped or spindle-shaped, whilst the chondrocytes from the deeper layers were predominantly rounded. In both mature and immature bovine chondrocytes cultured in agarose gels an alcian blue-positive staining matrix was found surrounding a low percentage of the surface chondrocytes, but most of the deeper layer cells were surrounded by an alcian blue-positive staining matrix.

Chondrocyte ultrastructure has been extensively described in humans by Roy and Meachim (1968), Weiss *et al.*, (1968), Stockwell (1979a) and Schenk *et al.*, (1986) and in rabbits by Davies *et al.*, (1962), Stockwell (1979a) and Paukkonen and Helminen (1987). Ultrastructural studies of adult rat articular cartilage found two types of chondrocytes, either glycogen-rich or those with abundant granular ER. Using autoradiography ($^{35}SO_4$ and ^{3}H-proline) it was found that the glycogen-rich cells mainly synthesised proteoglycans, whilst the other cells produced mainly collagen, although in both cell types some proteoglycans were produced (Mazhuga and Cherkasov, 1974). In contrast, Aydelotte *et al.*, (1988) described two cell types in bovine articular cartilage after 9 and 12 days *in vitro* culture with distinct electron microscopical characteristics.

They found that the superficial cells possessed many electron-lucent vacuoles and numerous small vesicles associated with the cell membrane, whilst the deep cells showed a more extensive rough endoplasmatic reticulum and accumulation of glycogen and lipid. Archer *et al.*, (1990) described that freshly isolated human articular chondrocytes of the surface layers often had abundant fine cell processes and significant amounts of rough endoplasmatic reticulum and lysosomes, whilst freshly isolated deep zone chondrocytes had few cell processes and fewer lysosomes, but relatively more Golgi vesicles.

1.1.4. Repair of articular cartilage.

Intrinsic repair.

One of the features of mature cartilage is the lack of cellular proliferation (Mankin, 1962a,b, 1963; Dustmann *et al.*, 1974a). Previously, it was thought that adult chondrocytes were incapable of dividing (Mankin 1963). In contrast, Green (1971) showed that isolated chondrocytes in culture can regain proliferative potential. This has been confirmed by Mohr and Wild (1977), Benya and Schaffer (1982) and Sokoloff (1985). In some pathological conditions such as osteoarthritis (Rothwell and Bentley, 1973; Dustmann *et al.*, 1974b) chondrocytes initiate division forming distinctive clusters. Hirotani and Ito (1975) described mitosis of chondrocytes in aseptic necrosis of the femoral head. Bentley (1985) demonstrated cellular proliferation in human chondromalacic cartilage. Experimentally damaged cartilage also shows chondrocyte division (Johnell and Telhag, 1977, 1978; Havdrup and Telhag, 1978; Kunz *et al.*, 1979; Lee, 1991 and Scully *et al.*, 1991).

The repair of a cartilage defect appears to depend on the defect depth. Superficial defects, that is, those which do not penetrate the calcified zone of cartilage, do not heal (Buckwalter *et al.*, 1988c). Within one day of an operation to create a defect in articular cartilage, a 50-100μm zone of cartilage neighbouring the defect undergoes cellular necrosis (Mankin, 1962b; Calandruccio and Gilmer, 1962; DePalma *et al.*, 1966). After 2-3 weeks, some chondrocytes near the site of the lesion proliferate, form small cell clusters and synthesize new matrix which remains pericellular. The marginal superficial cartilage forms a lip or sloping shoulder at the side of the crater-like defect, due to matrix flow (Calandruccio and Gilmer, 1962; DePalma *et al.*, 1966; Ghadially *et al.*, 1971). The synthesis of matrix by chondrocytes near the site of the lesion and the formation of a sloping shoulder at the side of the crater-like defect takes place in both partial, and, full thickness defects. Consequently, the defect is not repaired and it rarely progresses. The type of response to superficial penetrating trauma of the cartilage can be found in lacerations perpendicular or tangential to the articular surface (Ghadially *et al.*, 1977).

Hunziker and Rosenberg (1992) compared superficial articular cartilage defects in the patellar groove and the medial femoral condyle of rabbits with and without enzymatic removal of the superficial (1 μm thick) layer of proteoglycan molecules. In the former group after 1 month, the amount of repair tissue increased to about 55% of the defect

surface area instead of 30% of the defect surface area in the rabbits not treated with enzymatic removal, but this additional effect disappeared after 3 and 6 months. The authors explained their findings in terms of the chondrocytes underlying the defect surface reconstructing the matrix surface, and this apparantly inhibits further cell adhesion.

Extrinsic repair.

Full-thickness defects, caused by injuries that extend through the cartilage into the subchondral bone, elicit an inflammatory response. The repair response is generated by bone marrow stromal cells as demonstrated by De Palma *et al.*, (1966), Meachim and Roberts (1971) and Mitchell and Sheppard (1976) in animals, and by Meachim and Osborne (1970) in humans. One experiment involving full-thickness defects in rabbits will be described in more detail. Furukawa *et al.*, (1980) created defects in rabbit patellar grooves with a diameter of 3 mm, which extended into subchondral bone. After three to four weeks post-trauma, the collagen type II content of the repair tissue was less than 40% of the total amount of collagens type I and II. After eight weeks, the collagen type I content in the repair tissue was still more than 40% of the total amount of collagens type I and II, although histologically (staining with Safranin-O) the tissue was hyaline cartilage-like in appearance. In the period from 8 to 24 weeks post-trauma, the repair tissue contained 25-33% collagen type I, the proteoglycans decreased in concentration and the tissue underwent fibrillation and degeneration. After 52 weeks post-trauma, about 20% of the repair tissue was collagen type I, whilst about 80% was collagen type II. This showed that the repair tissue was not comparable to the normal articular cartilage with regard to structure and chemical composition.

The process of formation of repair tissue could be explained thus - the bone marrow stromal cells (e.g. mesenchymal osteo- and chondro-precursor cells) proliferate and undergo differentiation into fibroblast-like and chondrocyte-like cells. There are several factors which are important in the differentiation of post-foetal mesenchymal cells into chondrocytes (as reviewed by Urist, 1983). The three most important factors are, firstly, competence, which is a state of unexpressed readiness to synthesize cartilage-like extracellular matrix, secondly, the presence of an inductive agent (to enhance cell-cell communication and reciprocal interactions) and, thirdly, other extrinsic factors like pO_2 (Caplan and Koutroupas, 1973), endocrine and/or paracrine factors, nutrients and essential minerals. A brief summary of the effects of growth factors on mesenchymal or cartilaginous tissue is provided. Basic fibroblastic growth factor (bFGF) stimulates DNA-synthesis in immature bovine chondrocytes (Scully *et al.*, 1991). Transforming growth factor-β (TGF-β) is suggested to have a regulatory function in proteoglycan synthesis by chondrocytes (Morales and Hascall, 1989) as have the insulin-like growth factors (IGFs) (Daughady *et al.*, 1972; Vetter *et al.*, 1985; McQuillan *et al.*, 1986; Luyten *et al.*, 1988). The IGFs are also progression factors for cell division. The roles of

growth factors can be characterised as competence or progression factors. The names are derived from the two phases necessary for a quiescent mammalian cell to enter before DNA-synthesis can be induced (Pledger *et al.*, 1977). Competence factors include fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF)(Pledger *et al.*, 1978) and they are necessary before a progression factor, such as IGF-1, epidermal growth factor (EGF) or insulin (Pledger *et al.*, 1978; Osborn *et al.*, 1989), can become active. Whilst growth factors have a profound effect on chondrocyte division and synthesis, the addition of growth factors (hGH, IGF-1, TGF-ß, bFGF and EGF) into superficial rabbit articular cartilage defects did not stimulate healing of the defect (Hunziker and Rosenberg, 1992).

Apart from the repair response of the bone marrow stromal cells, Stockwell (1979a) suggested that new tissue might also reach the (partial and full-thickness) defect from the synovium at the margin of the articular cartilage area or by 'seeding' from the synovial fluid.

In conclusion, the repair tissue which is formed in full-depth articular cartilage defects appears structurally, biochemically and biomechanically (Whipple *et al.*, 1985) of inferior quality compared to normal articular cartilage and eventually leads to osteoarthritis. Consequently, without treatment articular cartilage defects caused by trauma, osteochondritis dissecans, tumour or infection will not, and if caused by chondromalacia patellae may not, heal adequately, will predispose the affected joints to pain and dysfunction, and eventually to further degenerative changes with ensuing reduction in the quality of life. There is a need for treatment of articular cartilage defects in such a way that the quality and quantity of the repair tissue parallels that of normal articular cartilage. Because rabbits and humans show a similar type of response to articular cartilage damage the rabbit is a useful model for studies in fundamental mechanisms of cartilage repair.

1.2. Allografting of articular cartilage defects.

1.2.1. Historical background.

Because of the limited self-repair capacity of articular cartilage, various treatments have been sought to replace the articular surface. Although the use of artificial joint replacements has been a success, especially in hip and knee joints, there is the problem of young patients who, having once had an artificial joint implanted, are unfortunately faced with the inevitable one or more revision operations due to wear and loosening. Also, patients with high functional demands are faced with (early) prosthetic failure. An alternative is to replace the cartilage, either with or without the underlying bone. In diseases affecting the cartilage such as chondromalacia patellae and osteochondritis dissecans, where limited degeneration occurs, we do not at present have enough knowledge to treat them definitively. In these cases, an operation which minimises whole joint trauma would be preferred. Again, a resurfacing operation of the cartilage is a possibility.

A resurfacing operation can be performed using joint allografts, which means replacing either the whole joint (total joint allograft) or one articulating bone (half joint allograft). The first total and half joint (massive) operations were performed on humans by Lexer (1908) and in rabbits by Judet (1908). Further work was carried out in humans by Wrede (1909), Dalla Vedova (1911), Ducuing (1912), Impallomeni (1911) and Minoura (1914). When techniques for bone preservation such as storage at temperatures below 0° C were developed, the possibility of osteochondral allografting attracted the attention of surgeons like Ottolenghi (1966, 1972), Parish (1966, 1973, who used block allografts, in which only a part of the articulating end of a bone was transplanted) and Volkov (1970, 1976). All used frozen grafts, which were stored at temperatures between -20° C to -30° C. They emphasized the importance of technical factors such as precise fit and stable internal fixation and Volkov (1970) also described the influence of the immune response on the success of the implant. In agreement with those findings are the results of many other authors (Pap and Krompecher, 1961; Brooks *et al.*, 1963; Heiple*et al.*, 1963; Chesterman and Smith, 1968; Porter and Lance, 1974; Rodrigo *et al.*, 1978; Malinin *et al.*, 1985; Prolo and Rodrigo, 1985; Schachar and McGann, 1986).

With regard to the immune response it has been shown that chondrocytes are immunogenic, but in articular cartilage the immune response is prevented by the presence of an intact extracellular matrix (Bacsich and Wyburn, 1947; Langer and Gross, 1974). In contrast, bone appears to be immunogenic (Bonfiglio *et al.*, 1955). Therefore, implantation of any graft containing bone will be rejected. This possibility might be reduced by careful selection of the graft in such a way that its histocompatability antigens match those of the recipient (although Burwell *et al.*, 1985, contradicted this) or by the use of specific physical or chemical treatments e.g.freezing the graft. The latter only slightly decreases the immunogenicity of the bone (Langer *et al.*, 1975 and 1978), but it

was also found that after freezing the viability of chondrocytes within the cartilage prior to implantation was about 50% using trypan blue dye exclusion assay (Tomford and Mankin, 1983; Henry *et al.*, 1985; Tomford *et al.*, 1985). Gross and co-workers emphasized the importance of viable cartilage being transplanted and they began clinical application by implanting fresh shell allografts in which only a small area of about 5 mm of osteochondral tissue was grafted (Pap and Krompecher, 1961; Gross *et al.*, 1975 and 1976; Locht *et al,* 1984; McDermott *et al.*, 1985; Czitrom *et al.*, 1986 and Zukor *et al.*, 1989a).

Earlier, Chesterman and Smith (1968) successfully isolated chondrocytes in rabbits, which were subsequently frozen (-79^{o} C) and thawed with or without dimethyl sulphoxide prior to implantation into cancellous bone of the wing of the rabbit ilium or into an articular cartilage defect in mature and immature rabbits (see below). They found that isolated chondrocytes survived freezing in the presence of dimethyl sulphoxide and at 6 weeks post-implantation, the cancellous bone was filled with cartilage-like repair tissue, whilst in the articular cartilage defect fibro-cartilaginous repair tissue was shown. In contrast to Gross and co-workers, Mankin and co-workers believed that freezing of osteo-articular grafts decreased antigenicity, which offered the possibility of storing and banking tissues. They went on to develop further techniques for storing cartilage at low temperature. Their major indication for implantation of osteo-articular allografts after storage is reconstruction following excision of musculoskeletal tumours (Mankin *et al.*, 1987; Wood *et al.*, 1989; Power *et al.*, 1991). Although 70% or more of the patients showed good or excellent results functionally, a problem with this type of allograft was the limited revascularization of the bone (Mankin *et al.*, 1983). Other groups used vascularized grafts as a living supplement to the large osteoarticular allo- and autografts in animals (Slome and Reeves, 1966; Judet and Padovani, 1968; Goldberg and Lance, 1972; Stewart *et al.*, 1983) and in humans (Buncke jr *et al.*, 1967; Cobbett, 1969). The latter described improved speed of incorporation of the graft and healing of the defect. Stewart *et al.*, (1983) found that a significant number of these grafts survived if an immunosuppressive drug (cyclosporin A) was used.

1.2.2. Types of allograft.

Isolated and cultured articular chondrocytes.

In 1965 Smith isolated mature articular chondrocytes and three years later Chesterman and Smith published the results of implantation of isolated immature rabbit chondrocytes into full-thickness defects of rabbit humeral articular cartilage. After 12 weeks post-implantation, the grafts showed mainly fibrous tissue with some areas of cartilaginous tissue. Also in 1965, Moskalewski and Kawiak isolated rabbit immature nasal septum chondrocytes and implanted them into muscles of rabbits. They found that nasal cartilage-like tissue was formed up to 42 days after implantation and suggested that the presence of an interstitial substance might protect transplants. Bentley and Greer (1971)

used isolated immature rabbit articular chondrocytes, which were implanted into a drill hole (3 mm wide and 3 mm deep) in the articular surface of the lateral tibial plateau of mature rabbits. After 8 weeks, they found that the grafts were not incorporated into the defect and inflammatory cells and fibrous tissue were present around them. Instead of using isolated chondrocytes, Green (1977) described the results of implantation of cultured rabbit immature articular chondrocytes on decalcified bone into full-thickness defects of rabbit femoral articular cartilage at 10 days and 6 weeks post-implantation and showed good repair in approximately 25% of the grafts after 6 weeks. Aston (1980) implanted cultured immature rabbit articular chondrocytes into full-thickness defects (3 mm wide and 3 mm deep) in lateral tibial plateau of adult rabbits and found after 12 weeks partial and complete repair with cartilage-like tissue in 25% and 50% of cases, respectively. After 52 weeks, 25% of the defects showed partial and 25% complete filling with cartilaginous tissue. Since in each group there were only 2-4 rabbits, the results need to be interpreted with some caution.

Isolated and cultured epiphyseal chondrocytes.

Laurence and Smith (1968) were the first to implant epiphyseal plate chondrocytes into fractures of rabbit radii. These fractures appeared to unite faster than corresponding control fractures. Bentley and Greer (1971) implanted immature rabbit epiphyseal chondrocytes into defects as described above, which at 8 weeks post-implantation showed a histologically definable cartilage-like repair tissue well incorporated into the defect. They concluded that isolated epiphyseal chondrocytes were superior to those of articular origin for repair of cartilage defects, although the possibility of bone marrow stromal cells and other host tissue invading the defect could not be excluded. Bentley *et al.*, (1978) extended this work and used chondrocytes both partially and completely separated from their matrix, and after 8 weeks the chondrocytes partially separated from their matrix were more successful in filling the defects compared to completely separated chondrocytes. As described above for articular chondrocytes, Aston (1980) implanted cultured epiphyseal chondrocytes into articular cartilage defects. After 12 weeks, complete filling with cartilage-like tissue was found in 67% of the defects and partial filling in none of them; but after 52 weeks 25% of the grafts showed partial filling and none complete filling with cartilaginous tissue. These results suggest breakdown of the original completely filled grafts.

Itay *et al.*, (1987) implanted chicken embryonic epiphyseal chondrocytes cultured in a fibrinogenic gel into full-thickness defects in the tibial condyles of rooster articular cartilage. The gel consisted mainly of cartilaginous extracellular matrix, fibrinogen, trypsin-inhibitor and thrombin. After 6 months (and after 18 months - Itay *et al.*, 1988) the defect was filled with histologically definable cartilage-like tissue, whilst in the control groups (either implanted fibrinogenic gel only or an empty drill hole defect without implant) the defect was filled with fibrous repair tissue. The authors suggest that

the fibrinogenic gel, firstly, fixates the chondrocytes in the defect sites and, secondly, provides an extracellular environment which stimulates chondrogenesis and inhibites the formation of fibrous tissue. However, it is known that chicken articular cartilage has a fibrous component and is, therefore, not comparable to mammalian cartilage.

Chondral and osteochondral allografts.

Chesterman and Smith (1965) implanted chondral allografts (grafts consisting of intact immature rabbit articular cartilage) into cancellous bone of the wing of the ilium of mature rabbits. At 12 weeks post-implantation some of the grafts seemed reduced in size by ingrowth of osteoid tissue or bone marrow cells, whilst in other areas, strips of Alcian blue staining cartilage resembling epiphyseal cartilage was present.

McKibbin (1971) continued research along this line by implanting immature sheep articular cartilage in full-thickness defects in the femoral condyle of immature and mature sheep. After 10 and 14 months, respectively, the graft tissue histologically resembled normal articular cartilage, but the results were more successful in the mature group. McKibbin suggested that failure of some grafts, especially in the immature sheep, was caused by displacement of the graft. This displacement was mainly below the level of the surrounding cartilage and could be explained by the immature animals' lack of a supporting subchondral bone. Implantation of intact immature rabbit articular cartilage into full-thickness defects of mature rabbits was found to be more successful compared to implantation of cultured immature chondrocytes (Aston and Bentley, 1986). The authors suggested this might possibly be due to the culture methods used for immature chondrocytes.

The use of osteochondral allografts in animals and humans is well documented (see above). Because of the relatively small number of patients treated with allograft transplantation, lack of uniform criteria with regard to assessment of the graft tissue, and the lack of long term (20 years) follow-up studies there are at present no ways of comparing the different results. Therefore, it might be more informative to look at the failures rather than the successes. Kandel *et al.*, (1985) reported on the radiological, histological and electron micros-copical examination of failed osteoarticular shell allografts in the tibial plateaux of humans. The implant consisted of necrotic bone and bone marrow, often in close proximity to areas of metachromatic-staining tissue. Therefore, the authors suggested that survival of articular cartilage was not dependent on bone remodelling. In the graft tissue areas of cartilage-like tissue were surrrounded by areas of necrotic cartilage. In 40% of the grafts pannus was shown growing over the resorbing articular cartilage surface. Yablon *et al.*, (1977) suggested that synovial pannus might be one of the main factors in graft failure. The articular cartilage of the donor implant showed a variety of histological and ultra-structural changes suggesting cartilage degeneration. Kandell and co-workers did not find any immune-response directed against bone, bone marrow or cartilage and as possible reasons for graft failure they

suggested (1) an incomplete repair of the cartilaginous surface by fibrocartilage-like tissue and (2) mechanical factors. With regard to the latter they suggested (a) an imbalance between the speed of graft resorption and the ingrowth of host bone could have caused either stress fractures (which they found in about half of the grafts) or (b) overloading or improper axial loading. The major influence of mechanical factors on the success of the allograft is stated by many authors (DePalma *et al.*, 1963; Rodrigo *et al.*, 1978; Tanaka *et al.*, 1980; Zukor *et al.*, 1989a). In their article, Kandell and co-workers concluded that viable cartilage was present as late as seven years post-implantation. Unfortunately, $^{35}SO_4$- or ^{3}H-cytidine-incorporation studies were not done to assess the function and the viability of the chondrocytes. Oakeshott *et al.*, (1988) described the graft tissue in patients with failed allografts using histology and electron microscopy. In 67% of the patients they found cartilaginous tissue at 9.5 years post-implantation. Failure of grafts was associated with poorly sized grafts, grafts of less than 1 cm in thickness and grafts where internal fixation was not used.

Perichondral allografts.

In 1853 Paget wrote that perichondrium was essential in the healing of wounds of cartilage. Since then many have tried to use perichondrium to repair articular cartilage defects, although perichondrium can not be found in articular cartilage *in vivo*. Engkvist *et al.*, (1979) used organ cultures of ear and rib perichondrium from immature rabbits and after 1 week *in vitro* chondrogenesis was found, whilst *in vivo* studies showed that rabbit rib perichondrium has a chondrogenic potential which appeared to be greater than that of rabbit ear perichondrium. Further research of Engkvist and co-workers confirmed this finding by implanting perichondrial grafts, dissected from both rib and ear, in full-thickness articular cartilage defects in mature and immature rabbits with a follow-up period of 13 and 17 weeks (Engkvist and Ohlsen, 1979; Engkvist and Wilander, 1979). Because histological techniques could not definitively determine the precise nature of the repair tissue, Amiel *et al.*, (1988) grafted defects of the femoral condyle with rib perichondrial grafts and the repair tissue was assessed using histological and biochemical techniques. At 12 months post-implantation, the defects were filled with cartilage-like tissue, with 82% collagen type II compared to total collagen types I and II, and an increased amount of glycosaminoglycans (expressed as mgs hexosamine per gm dry tissue) compared to native perichondrium, but a reduced amount compared to non-diseased articular cartilage (80% of the value for 'healthy' articular cartilage). Although the biochemical assessment increased the state of knowledge about quantitative composition of articular cartilage little was known about its mechanical properties. Kwan *et al.*, (1989) described implantation of perichondrium into full-thickness articular cartilage defects in the femoral condyles of rabbits. The biomechanical properties of the graft tissue using a dynamic shear tester showed that the complex shear modulus for the neocartilage approached the values for normal articular cartilage at 1 year post-

implantation. They did not find any significant difference in complex shear modulus at 1 year post-implantation with or without passive motion once every 45 sec for eight hours daily during a two week period post-implantation. They also described the following criteria for acceptance of graft tissue: confluence with the surrounding cartilage, basilar attachment of the neocartilage, peripheral attachment of the neocartilage points, the quality of the neocartilage with respect to Safranin-O uptake, cellular appearance and height and orientation of the neocartilage column. Their failure rate was 35%, which was caused mainly by detachment of the graft from the defect.

Periosteal allografts.

Nakahara *et al.*, (1991) suggested that periosteal-derived cells of young chickens contain mesenchymal cells, which possess the potential to undergo terminal differentiation into chondrogenic (and osteogenic) phenotypes, depending on the local environment or on positional cues. The implantation of free periosteal grafts both with (Rubak *et al.*, 1982a, b; O'Driscoll *et al.*, 1986) and without additional joint motion (Rubak, 1982) in full-thickness defects in femoral condylar articular cartilage of 6 months-old rabbits have shown that periosteum has the potential to facilitate the repair of relatively large defects. Naito and Hirotani (1991) implanted free periosteal grafts in half-thickness articular defects in immature rabbits, and found after 8 weeks repair of the defects with histologically definable cartilaginous matrix, but only after fixation with Kirschner wires had been implemented.

A question not previously addressed is 'does the repair tissue originate from the donor graft only?' Zarnett and Salter (1989) described transplantion of free periosteal grafts into full-thickness defects, whilst using karyotyping of graft cells to determine the origin of the graft tissue. At three weeks post-implantation, the hyaline cartilage-like graft tissue was derived from the periosteal graft only in 33% of the rabbits and from periosteal graft and subchondral tissues in 67% of the rabbits. Moran *et al.*, (1991) determined the origin of repair tissue after implantation of free allografts of periosteum into full-thickness defects in femoral condyle cartilage of mature rabbits using RLA-DQ alpha restriction fragment length polymorphism analysis. At 6 weeks post-implantation, the cartilage-like repair tissue in 25% of the rabbits came from an unknown cell in the recipient animal, whilst in the rest of the animals the repair tissue came from the transplanted periosteum.

Demineralized bone grafts.

The differences in repair of articular cartilage defects caused by superficial and deep penetrating injuries (see 1.1.4.) has convinced some authors that the bone matrix plays an important role in the repair of cartilage defects (Urist, 1965; Reddi, 1983, 1985). By placing demineralized allogeneic bone matrix within a diffusion chamber which in turn is placed orthotopically or heterotopically, local mesenchymal cells from the marrow cavity differentiate into chondrocyte-like cells and osteoblast-like cells (Urist *et al.*, 1967,1969;

Reddi and Huggins, 1972; Urist, 1983; Reddi *et al.*, 1987; Nogami *et al.*, 1989). Dahlberg and Kreicbergs (1991) implanted demineralized allogeneic bone matrix in full-depth articular cartilage defects. They found that cartilage-like tissue was formed of a highly variable quality, which does not restore surface integrity. It has been suggested that this property of the demineralized bone matrix is due to the presence of bone morphogenetic protein (Iwata *et al.*, 1991). Remarkably, the size of the defect filled with demineralized bone matrix did not have any influence on the outcome.

1.2.3. Use of biomaterials.

Several materials have been utilized as a supporting graft tissue such as nylon (Kuhns, 1964), collagen sponge (Speer *et al.*, 1979b), silicon (Engkvist and Ekenstam, 1982) and coralline hydroxyapatite coated with polylactides (Jamshidi *et al.*, 1988).

Kuhns (1964) described that 3 years or more post-operative wear had adversely affected the nylon membrane rendering it unsuitable as an implant material.

Holmes *et al.*, (1975) and Speer *et al.*, (1979b) described the use of a collagen type I sponge (isolated from bovine skin), which was cross-linked with hexamethylene di-isocyanate for repair of osteochondral defects in rabbits. It has continuous channels with a pore size of 50-120 μm. After 12 weeks the sponge had resorbed and it was replaced by bone and unspecified cartilage-like tissue. Ulreich *et al.*, (1987) used the collagen sponge to culture *in vitro* rabbit articular chondrocytes up to 14 days and they described that this maintained chondrocyte phenotype and produced a proteoglycan-rich matrix using histological techniques and $^{35}SO_4$-incorporation studies. ^{3}H-thymidine-incorporation studies for cell proliferation showed labelling in chondrocytes in low density culture areas.

The idea of combining a biomaterial with articular chondrocytes as an articular replacement is very appealing, and in the following pages the use of three materials in this way will be enlarged upon.

1.2.4. Combination of allografts and biomaterials.

Carbon fibre.

The structure of carbon fibre is dominated by hexagonal rings of carbon atoms, linked edge to edge to form layers and it is chemically inert. The use of carbon fibre was initially suggested on account of its biological and biomechanical properties (Benson 1971), although its possible application as an implant material was recognised in 1967 as a technological spin-off from the NASA-funded aerospace programme. There are three forms of carbon used clinically: pyrolitic (low-temperature-isotropic), glassy or vitreous carbon and vapor-deposited carbon (Haubold *et al.*, 1981). The pyrolitic form will be discussed only. In table 1.1. some mechanical properties of pure LTI carbon are listed. It is a very strong, stiff and light material. Bokros (1977) suggested that carbon does not biochemically inhibit tissue growth and that any device that depends on tissue ingrowth

to perform its function could possibly be improved by surfacing with carbon. In the past, carbon fibre has been utilized and is still used in various clinical applications (McKibbin, 1983,1984) like the reconstruction of parallel fibred tissues such as ligaments and tendons in animals (Jenkins *et al.*, 1977; Forster *et al.*, 1978; Jenkins 1978) and in humans (Jenkins and McKibbin, 1980; McKibbin, 1990).

Minns and Flynn (1978) introduced carbon fibre for the repair of articular cartilage and bone. Their work showed stimulation of the amount of collagen fibres within bone and the formation of a new surface over defects of articular cartilage mainly consisting of fibrous tissue. Minns *et al.*, (1982) implanted carbon fibre into full-depth defects of articular cartilage in rabbits with instability-induced knee osteoarthritis. The cells of the subchondral plate, and presumably the marrow cavity, were stimulated to produce repair tissue. Thus, the carbon fibre acted as a scaffold for the invading cells, which synthesized a matrix, predominantly fibrocartilaginous in appearance. This repair tissue was not comparable biologically or biomechanically to the original cartilage tissue, although the significance of this is unclear.

Implantation of artificial materials into the joint has the disadvantage of eliciting immune responses. Several authors have shown that carbon fibre is neither toxic, nor carcinogenic (Tayton *et al.*, 1982) but could result in a limited inflammatory response (Helbing *et al.*, 1977) depending on the way the carbon fibre is processed.

The long term effects of carbon on the synovium are described by many authors (Forster *et al.*, 1978; Dandy *et al.*, 1982; Rushton *et al.*, 1983; Rushton and Rae, 1984; Claes and Neugebauer, 1985; Parsons *et al.*, 1985; King and Bulstrode, 1985 and Thomas, 1986). After implantation of a carbon fibre cruciate ligament graft, an atypical foreign body reaction surrounding the neo-tendon was described by most authors, except Rushton *et al.*, (1983) who described synovial inflammation with a mild foreign body-giant cell reaction. Past experience (Charnley, 1969) has shown that a material in bulk form may elicit only a mild biological reaction, whilst the response to particulate matter may be severe. Injections of carbon particles into knee joints of mice and dogs showed (1) absence of carbon fibre and no histologically detectable changes in articular cartilage, (2) presence of carbon in synovium up to 52 weeks post-injection, (3) presence of slight synovial hypertrophy up to 52 weeks post-injection, (4) after 4 weeks post-injection a foreign body response was found, localized in the surface layers of synovium, which gradually decreased till 8 weeks post-injection and did not change further up to 52 weeks. In those studies a single injection was given but, of course, under clinical conditions a slow but continuous release of particulate matter is present.

Minns *et al* ., (1987) have used carbon fibre clinically in 145 patients with (grade II) osteoarthritis of the knee or osteochondritis dissecans with a 5 year follow up-period. The repair tissue appears fibrous or fibrocartilaginous, which is in agreement with previous studies carried out on rabbits. A clinical study from Bentley *et al.*, (1990) in 96 patients with osteochondritis dissecans, chondromalacia patellae and osteoarthritis (grade

I or II) showed good results with regard to improvement of knee function, pain relief and patient'satisfaction' at 3 years post-implantation of either carbon fibre mesh or rods.

Collagen gel.

The use of collagen gels was introduced by Ehrmann and Gey (1956). Collagen gels consist of a hydrated collagen lattice. It contains about 0.1% (w/w) collagen in the form of native bundles with a diameter from 500-5000 Å which possess a 640 Å periodicity (Elsdale and Bard, 1972). Collagen gel can be used to provide a 2- or 3- dimensional network or to overlay cells already attached to a substratum (Elsdale and Bard, 1972). With regard to the latter, Schor (1980) showed that chick embryo sternal chondrocytes plated on the surface of the collagen gel and cultured for 10-14 days, did not infiltrate the collagen gel matrix, thus demonstrating the lack of mobility of chondrocytes. Chondrocytes cultured within a collagen gel are capable of maintaining the characteristics of differentiated chondrocytes (Yasui *et al.*, 1982; Kimura *et al.*, 1984). Gibson *et al.*, (1982) cultured chick embryo sternal chondrocytes within a collagen gel and described the following observations:

(1) Chondrocytes cultured within the collagen gel matrix undergo a change to a fibroblast-like morphology after 1-2 days *in vitro*, but after 5-6 days in culture they adopted a rounded cell shape, which was retained for up to 3 weeks. Also, cells cultured within a collagen gel organise themselves into an irregular chain resembling 'growth' cartilage. (2) During the first week the (collagen) gel volume decreased by 50% and, thereafter, the reduction was only marginal, which could be explained by the presence of new extracellular matrix in the gel. This observation has been confirmed by other authors (Storm and Michalopoulos, 1982; Allen *et al.*, 1984). Storm and Michalopoulos (1982) indicated that if the cells cover 60% or more of the gel surface, the gel will retract from the periphery. Harris *et al.*, (1981) described that cells exert tractional forces on the extracellular matrix, which may account for this shrinkage. Cuprak and Lever (1974) showed that contraction of the gel is not caused by breakdown of collagen, whilst addition of cytochalasin prevented contraction of the collagen gel (Bell *et al.*, 1979). The latter experiment adds weight to the argument that the shrinkage was initiated mechanically by the chondrocytes since cytochalasin depolymerizes the actin cytoskeleton. (3) Chondrocytes grown in a collagen gel synthesized more collagen precursor polypeptides than α–chain, suggesting slower conversion of procollagen to collagen. (4) Chondrocytes grown in collagen gels produced after 2-3 weeks collagen type II and rarely collagen type I, whilst chondrocytes grown in monolayer produced collagen type I and some collagen type II. (5) Chondrocytes grown in a collagen gel produced after 1 week *in vitro* culture a low molecular weight collagenous protein, which is also produced in plastic cultures, but in far lower quantities. Its molecular weight by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) analysis was calculated at 59,000 Daltons and its triple helical structure is more stable than similar-

sized fragments of normal type II collagen. This molecule was later classified as type X collagen. (6) Chondrocytes grown in a collagen gel deposited about 60% of *de novo* synthesized collagen as insoluble matrix, whilst in chondrocytes grown on plastic dishes this was only 10-20%. Collagen gels have the advantage that processing can be done using standard histological embedding and sectioning techniques. Collagen gels can be fixed in formol-saline or ethyl alcohol (Storm and Michalopoulos, 1982).

Wakitani *et al.*, (1989) suspended immature articular rabbit chondrocytes within a collagen gel, which after stabilizing for 10 minutes (37° C) was implanted into full-thickness defects (4 mm diameter, 4 mm deep) in the articular cartilage of femoral patellar grooves of mature rabbits. After 24 weeks the repair tissue was histologically similar to the surrounding cartilage. Using ^{14}C proline labelling followed by SDS-PAGE analysis, they showed that at 2 and 26 weeks post-implantation mainly collagen type II was being synthesised. As control groups, a collagen gel without added cells or an empty drill hole were used. The two control results were similar with regard to histology and showed fibrous repair tissue at 24 weeks post-implantation. Using the same biochemical techniques as described above, in both control groups predominantly type I collagen was being synthesised.

Hydrogels.

Hydrogels are water-swollen polymer networks, of either natural or synthetic origin. Unsatured (vinyl) monomers that contain functional groups capable of interacting with water undergo polymerization to form hydrogel polymers (Corkhill *et al.*, 1990). More than 100 vinyl monomers are available, which makes the production of over 1,000 copolymers possible. Hydrogels are characterised by the introduction of three or more monomers in the polymer, which can provide specific properties (Corkhill *et al.*, 1991). More detailed information about the state of knowledge of the chemical aspects of hydrogels, hydrogel testing, production and fabrication is given by Peppas (1986, 1987a, b), Corkhill *et al.*, (1990) and Stoy and Kliment, (1990).

Folkman and Moscona (1978) introduced the use of the non-adhesive polymer poly-2-hydroxyethyl methacrylate (poly-HEMA) to coat tissue culture dishes for *in vitro* culture of fibroblasts and epithelial cells. They described that, with thinner coatings of poly-HEMA the cells spread as well as they did on tissue culture plastic, whilst at higher poly-HEMA thicknesses cell attachment and spreading was prevented. Archer *et al.*, (1982) used poly-HEMA as a semi-adhesive substratum to study *in vitro* the relationship of cell shape in the differentiation of chick embryo mesenchyme cells into chondroblasts.

Several factors play an important role in cell adhesiveness of the hydrogel: (a) surface chemistry, (b) surface charge, (c) water structuring, (d) surface rigidity and (e) rugosity. Below some examples will be given for (a) and (b).

(a) Some authors report that the concentration of ethyl methacrylate polymers is related to the proliferation rate (Lentz *et al.*, 1985; Lydon *et al.*, 1985).

(b) Tanzawa *et al.*, (1980) examined the growth of neonatal human preputial cells in serum-free medium on:

i. negatively charged heparinized (vinyl chloride/ethylene vinyl acetate){R} (methoxy-poly (ethylene glycol)-mono-methacrylate){S} and quaternized N-N-dimethyl aminoethyl methacrylate {D}(RSD);

ii. nonionic, neutral poly (n-vinyl pyrrolidone-methyl- methacrylate) copolymer;

iii. positively charged RSD;

iv. hydrophobic poly methyl methacrylate.

They found that the degree of spreading of the cells adhering to the substrate increased in the order that the polymers were listed above. Also, that cell adhesion decreased with increasing water content.

Other factors involved in the adhesion of cells to hydrogels were (i), using mouse lung fibroblasts, cell spreading was eliminated when the polar component of the free energy was less than 5 mNm^{-1}, but when this was more than 15 mNm^{-1} significant spreading was observed (Van der Valk *et al.*, 1983), and (ii) attachment and spreading onto a planar substrate involves the dynamic reorganization of three filamentous structures: microtubules, microfilaments and intermediate filaments (Ireland *et al.*, 1987).

Wichterle and Lim (1960) were the first to suggest the use of lightly crosslinked polymers of 2-hydroxyethyl methacrylate (HEMA) for biomedical use on account of its stability to varying conditions of pH, temperature and tonicity. If articular cartilage is regarded as a natural composite hydrogel of collagen, proteoglycans with their many hydrophilic groups, and a high percentage of water, then the main difference from a mechanical-chemical engineering point of view is the anisotropy due to (1) the increasing concentration of proteoglycans with increasing distance from the joint surface, (2) the decreasing water content with increasing distance from the joint surface and (3) the change in collagen architecture in different layers of cartilage. This explains the wide variety in values for the tensile strength and tensile (Youngs) modulus for articular cartilage.

Kon and Visser (1981) implanted a polyHEMA sponge with an average pore size of either less than 50 μm or, less than 100 μm into full-thickness defects (3 mm diameter, 2 mm depth) in lateral tibial plateau of immature and mature rabbits. At 12 weeks post-implantation, all sponges with pore size up to 100 μm could not withstand weightbearing and the articular surfaces were destroyed, whilst the sponges with up to 50 μm pore size remained intact and a histologically definable fibrous/fibro-cartilaginous tissue was formed covering the hydrogel. A foreign body-giant cell response was not observed.

Bray and Merrill (1973) suggested the use of crystallized polyvinyl alcohol as a synthetic cartilage. Crystallisation was achieved by heating to 90° C and this, together with the crosslinking, decreased the water content and increased the tensile modulus to the range of normal articular cartilage. Cationic molecules were inserted onto the PVA backbone during the radiation crosslinking of PVA-H_2O solutions to absorb hyaluronan (which is

the main lubricant of synovial fluid), ensuring low-friction lubrication in the same range as normal joint structures. Peppas (1977) used a similar polyvinyl alcohol, but by using short-term-high-temperature annealing could achieve values of mechanical strength closer to those of articular cartilage. Oka *et al.,* (1990) improved the polyvinyl alcohol hydrogel further by modifying the annealing process. To ensure firm attachment to the bone, an implant composed of PVA-hydrogel and a porous titanium fibre mesh was made. The authors emphasize the importance of early stable attachment of these porous materials to the bone for a successful result. The hydrogel plugs were implanted into full-thickness defects (4mm diameter, 4mm depth) in the patellar groove of the distal femur of mature rabbits. At 52 weeks post-implantation, using histological techniques Noguchi *et al.,* (1990) showed that the structure of articular cartilage was maintained in the graft tissue, but was also present in the control groups (ultra-high molecular-weight polyethylene (UHMWPE) and sham-operated animals), but that only in the PVA-hydrogel implant was Safranin-O-staining seen almost in comparable levels to normal articular cartilage, whilst in the two control groups the staining was less. At 2 weeks post-implantation synovial tissue showed a mild inflammatory and proliferative response, which stabilised by 8 weeks. In the sham-operated control group only slight inflammation was detected.

1.3. Indications for allografting in articular cartilage defects in humans

1.3.1. Chondromalacia patellae.

Etiology/pathogenesis.

Chondromalacia patellae was first described by Aleman in 1928 as the degeneration of the articular cartilage of the patella. Most authors define the term 'chondromalacia' as soft cartilage (Bentley, 1970; Goodfellow *et al.,* 1976; Abernathy *et al.,* 1978; Radin, 1985; Fulkerson and Hungerford, 1990). In recent years an increasing number of authors have rejected the term chondromalacia patellae and instead used terms such as patello-femoral pain (Goodfellow *et al.,* 1976) or patellar pain (Arnoldi, 1991) to describe pathological processes in the articular cartilage of the patella. In addition, Fulkerson and Shea (1990) stated that the cause of anterior knee pain is strain on the peripatellar retinaculum, brought about by abnormal patellar alignment and damage to the patellar articular cartilage. In contrast, Bentley suggested that chondromalacia patellae is the only common clinical condition of articular cartilage which presents with symptoms before gross breakdown has occured (Bentley, 1985). Because the term 'chondromalacia patellae' is still commonly used in literature to describe pathological processes in the articular cartilage of the patella, this term is also used in this section.

The basic pathologic lesion in chondromalacia patellae is a disorder of the middle and deep layers of the cartilage which involves the surface layer only late in its development (Goodfellow *et al.,* 1976). The latter authors described this lesion as 'basal degeneration'.

This defect is predominantly found in

Symptoms.

Chondromalacia patellae has no specific signs or symptoms and can be asymptomatic, but most patients experience a dull aching discomfort, localized at the anterior part of the knee, at the medial or lateral joint line or at the anterior tibia. The pain is provoked by sitting in one position for a long time, squatting or ascending and descending stairs (Ficat, 1970; Goodfellow *et al.,* 1976; Sisk, 1992). Sometimes during the latter movements the pain is associated with a sudden giving way. When extension against resistance is tested, this will reproduce the pain as well as compression of the patella against the sulcus femoris. In addition, the medial part of the patellar articular surface is tender on palpation. In the previously described group of inactive teenage females, the symptoms are frequently worse after sitting for a long period with the knees flexed and are relieved by walking around for a few minutes. In the group of highly active males there was aggrevation by activity, especially involving stresses on the knee joint in flexion and twisting.

Retropatellar crepitation is variably felt or heard. The range of motion is normal and wasting of quadriceps muscles may be present. Swelling of the joint may be present (Sisk, 1992), although rarely found in other studies (Goodfellow *et al.,* 1976; Arnoldi, 1991).

Clinical examination will usually reveal a subluxation, instability syndrome, a tilt/compression syndrome or a combination of these (Fulkerson and Shea, 1990). Diagnostic radiology is also used and the following measurements can be done: values of sulcus angle as an index of subluxation of the patella (Brattstrom, 1964; Merchant *et al.,* 1974; Aglietti and Ceruli, 1979; Bentley and Dowd, 1984), congruence angle as an index of subluxation (Merchant *et al.,* 1984), lateral patello-femoral angle as an index of tilt (Laurin *et al.,* 1978) and patellar tendon-patella ratio to measure the position of the patella (Insall and Salvati, 1971).

Computerized tomography is capable of producing a precise image of the patello-femoral relationships and can show normal alignment, excessive lateral tilt and subluxation of the patella (Schutzer *et al.,* 1986). Also the use of Magnetic Resonance Imaging has been described (Shellock *et al.,* 1988).

Treatment.

In general, arthroscopy is used to finalize the diagnosis. With the arthroscope it is possible to assess the presence and extent of a lesion of the articular cartilage, and by careful probing of the patella with a blunt hook the degree of softness of the articular surface can also be assessed (Bentley and Dowd, 1984). The treatment of chondromalacia patellae is mostly directed to the underlying causes. Firstly, conservative measures such as non-steroidal anti-inflammatory drugs and isometric quadriceps exercises are suggested. Fulkerson and Shea (1990) suggested an intensive rehabilitation

program. It is important that the patient is reassured that chondromalacia patellae is not a serious condition. Most authors (DeHaven *et al.*, 1979; Insall *et al.*, 1980) believe that the majority of the patients with chondromalacia patellae do not need operative treatment. Nevertheless, many operative techniques have been described and can be divided into (Sisk, 1992):

(1) treatment directed at malalignment and other abnormalities of the extensor mechanism and the patella-femoral joint, and:

(2) treatment of the diseased cartilage.

Examples of (1) are lateral retinacular release, corrections of malalignment of the extensor mechanism, whilst examples of (2) are patellar shaving (open or arthroscopic), local excision of defects with drilling of the subchondral bone (Insall *et al.*, 1967; Ficat *et al.*, 1979), mechanical decompression of the patellofemoral joint by anterior elevation of the tibial tuberosity (Maquet procedure)(Maquet, 1963) and patellectomy (West, 1962).

In the literature there is no consensus about the appropriate treatment(s). Fulkerson (Fulkerson and Hungerford, 1990) suggested the treatment shown in table 1.4., and the same authors also extensively described all operations.

1.3.2. Osteochondritis dissecans.

Etiology/pathogenesis.

Osteochondritis dissecans is believed to be the result of trauma (Paget 1870; Fairbank 1933; Aichroth, 1971) or local ischaemia (Rieger, 1920; Rosenberg, 1964; O'Donoghue, 1966; Carlson *et al.*, 1991). Ischaemia of the subchondral bone will lead to insufficient support of the overlying cartilage. Although this should not affect the nutrition of the cartilage, which occurs via the synovial fluid, it renders the cartilage more easily damaged by loading (Radin and Rose; 1986). In other cases, the cartilage becomes separated from its base and forms a loose body in the joint. Hereditary and familial factors are also important in the etiology of osteochondritis dissecans (Bernstein; 1925; Stougaard, 1961). Roberts and Hughes (1950) described cases in which dwarfs and patients with endocrinological disorders were affected. Nambu *et al.*, (1988) found using finite element analysis that the mechanical strain in the femoral condyles yielded by knee flexion plays an important role in the initiation and progression of osteochondritis dissecans. More than 85% of the lesions in the knee joint are located in the lateral aspect of the medial condyle of the femur near the intercondylar fossa, but other joints can be involved (Hay, 1950; Roberts and Hughes, 1950; Zinman and Reiss, 1982). The incidental peak of the first onset of osteochondritis dissecans appears between the age of ten and twenty years. The male:female incidence of osteochondritis dissecans is about 3:1.

Some authors report that osteochondritis dissecans healed well in children and does not progress to osteoarthritis (Linden, 1977; Hughston *et al.*, 1984).

Symptoms.

The lesions may cause symptoms, but they can be asymptomatic especially in the elbow and ankle. Aichroth (1971) found that in his study 60% of the patients had taken part in first class sports or athletics. Wilson (1967) described a diagnostic test for osteochondritis dissecans, whilst other authors, such as Aichroth (1971), found this test to be positive only in 1% of patients. Additionally, there is usually an association with mechanical abnormalities of the knee like genu varum/valgum or recurvatum, ligament laxity and patellar dislocation or subluxation.

Treatment.

Crawfurd *et al.,* (1990) advised treatment of stable osteochondritis dissecans localised in the medial femoral condyle, because it does not heal spontaneously (31 knees, 7.5 years follow-up period) regardless of size of lesion, age of onset or the sex of the patient. The lesions elsewhere in the knee joint have a tendency to heal spontaneously. In contrast, Twyman *et al.,* (1991) reported on 22 knees in 18 patients with a follow-up of 33.6 years (range 26-54) and found that about one-third of the knees had radiographically moderate or severe osteoarthritis with a preference for large defects or defects localized in the lateral femoral condyle. Unfortunately, they only examined about one-third of the original number of patients and the patients received different treatments.

Because of lack of exact knowledge of the natural history of this disease, and the different views on pathogenesis, the treatment shows enormous variation (Green and Banks, 1953; Smillie, 1957; Guhl, 1982; Hughston *et al.,* 1984; Bradley and Dandy, 1989). Aichroths (1971) treatment advice can be found in Table 1.5.

Project aim.

Against the above background of data, the aim of the project was to test three materials experimentally (one already used clinically) as reparative agents in articular cartilage defects, and to assess their suitability as vehicles of cultured cartilage as a means of augmenting the repair process.

Tensile Strength (MPa)*	**40**
Stiffness (GPa)*	**21-26**
Toughness (Nm/m^3)*	**35**
Surface Free Energy ($ergs/cm^2$)+	**50**
Wear factor K (m^2/kg)^	**2.84×10^{-14}**
Friction coefficient μ ^	**0.06**

***values from Haubauld *et al.*, (1981)**
+values from Bokros (1977)
^values from Dumbleton *et al.*, (1974)

Table 1.1. Mechanical properties of pure LTI-carbon. Toughness is the ability to absorb energy in impacts.

Grade 1: Articular cartilage softening (closed chondromalacia) only.
Grade 2: Fibrillation of less than 1.3 cm ($^1/_2$ inch) in diameter.
Grade 3: Fibrillation of more than 1.3 cm ($^1/_2$ inch) in diameter.
Grade 4: Erosion of articular cartilage down to subchondral bone.

Table 1.2. The Outerbridge classification of chondromalacia patellae (Outerbridge, 1961).

1. **Lesions grade 1-2**: arthroscopy and patellar tendon transfer.
2. **Lesions grade 3-4**: patellar tendon transfer, except in elderly patients. In the latter group, patellectomy is preferred as well as for those patients who fail in the more conservative operations.

Table 1.3. The treatment of chondromalacia patellae according to Bentley and Dowd (1984).

1. **Lesions grade 1-2** : arthroscopic debridement of grade 2 lesion.
2. **Lesions grade 3-4**: arthroscopic debridement and possible tibial tubercle anteriorization.
3. **Lesions grade 1-2 with patellar subluxation**: lateral release
4. **Lesions grade 3-4 with patellar subluxation**: lateral release and possible anteromedial tibial tubercle transfer.
5. **Lesions grade 1-2 with patellar tilt with or without subluxation**: lateral retinacular release.
6. **Lesions grade 3-4 with patellar tilt with or without subluxation**: lateral retinacular release, careful debridement, and possible anteromedial tibial tubercle transfer.

Table 1.4. Treatment of chondromalacia patellae according to Fulkerson (Fulkerson and Hungerford,1990).

***If symptoms were not severe and fragments remained in situ:** conservative measures.

***If the fragment was loose,** it must be removed. The site from which the fragment came must be explored carefully and the crater must be cleared. If the cartilage was intact the lesion might be drilled.

***If the fragment was separated, but remained in the crater,** simple excision and trimming of the edges of the defect would be advisable.

Table 1.5. Treatment of osteochondritis dissecans (Aichroth, 1971).

MATERIALS AND METHODS

2.1. Pre- and peri-implantation procedures.

2.1.1. Preparation of plugs prior to implantation.

In these experiments, three different type of plugs, each consisting of cultured articular cartilage chondrocytes and a resurfacing material, were used with a follow up period of 3, 6 and 12 months respectively.

The three materials were carbon fibre, collagen gel and hydrogel.

i. Carbon fibre. The carbon fibre plugs were provided ready-made and sterile by Surgicraft Ltd (Medicarb, Redditch, England, radius=3.5 mm). They were made using modified textile techniques. Carbon fibres were fabricated from organic fibres like rayon and polyacrylonitrile (PAN) The precursor fibres were treated under temperatures in excess of 1000° C, in an inert atmosphere of nitrogen. Under these conditions, constituent elements, such as hydrogen, oxygen and nitrogen, become volatile and are expelled from the polymer chains, leaving a solid interlocked residue of pure carbon. The pyrolysed fibres (from PAN precursor) were typically 6-9 μm in diameter, and possessed a relatively smooth surface texture.

ii. Collagen gel. The collagen gel was prepared by a modification of the method described by Elsdale and Bard (1972). First, 40 mg. collagen type I (Sigma type III collagen, C-3511, from calf skin, St. Louis, USA) was dissolved in 20 mls acetic acid 0.5M at 4° C. This was followed by dialysis of the resulting solution against 4 litres of distilled water, eventually adding 4 mls of 10x F10-medium (Flow Labs, Paisley, Scotland). The solution was changed at least twice in 24 hours, and was in total dialyzed for a minimum period of 48 hours. Afterwards the solution was placed in a high speed refrigerated centrifuge at 17,000 rpm for 24 hours. The supernatant, containing the dissolved collagen, was stored at 4° C.

The gel was prepared (quantities for 3 dishes) by mixing 3 mls collagen solution, 0.2 ml 10x F10 medium substituted with 1.6 μl 11.2M $NaHCO_3$ (Gibco, Paisley, Scotland) and 0.25ml NaOH (0.5M).

One ml of collagen solution was inoculated into 35 mm Petri-dishes which were then transferred to a CO_2-incubator at 37 °C. The gelling took place in about 60 minutes.

iii. Hydrogel. The hydrogel PC 97 was developed and provided by Drs. B. Tighe, P. Corkhill and H. Fitton (Dept. of Chemical Engineering and Applied Chemistry, Aston University, Birmingham). PC 97 consisted of

80% [N-vinyl-pyrrolidone (56%)
[Methyl methacrylate (29%)
[Cellulose acetate butyrate (15%)
20% [Dextrin particles with a diameter of less than, or equal to 38 μm.

The basic principles of preparation of this type of hydrogel are described by Corkhill *et al.*, (1990). Cellulose acetate butyrate was dissolved in a mixture of N-vinyl-pyrrolidone and methyl methacrylate. Dextrin was then dispersed in this solution. N-vinyl-pyrrolidone and methyl methacrylate polymerized around the cellulose. When

polymerization took place, semi-interpenetrating networks were formed. The cellulose improved mechanical properties like strength and stiffness. The hydrogel PC 97 was swollen in hot methanol and distilled water, then individual plugs with a diameter of 3 mm were punched out of the hydrogel sheet and autoclaved in dilute HCl (pH 1.8-2.0). The autoclaving hydrolyses the dextrin and the pores appear. Following sterilization it was washed thoroughly with sterile deionized water for at least 7 days, and stored in sterile phosphate buffered saline (PBS) at 4° C prior to use.

2.1.2. Isolation and culture of chondrocytes.

Articular cartilage slices were aseptically dissected from distal femora, proximal tibiae and patellae of immature Sandy half-lop rabbits. The cartilage preparations were washed in phosphate-buffered-saline (PBS) and diced finely. Chondrocytes were isolated by sequential enzymatic digestion with Hepes-buffered Ham's F12 medium (pH 7.2)(Flow Labs, Paisley, Scotland) supplemented with pronase (70,000.u./ml)(BDH, Poole, England), 10% foetal calf serum (FCS, Gibco), 2% Hepes buffer (Gibco) and 1% antibiotic/antimycotic (100 u./ml penicillin, 100 µg/ml Streptomycin and 0.25 µg Amphotericin/ml medium) (Gibco, Paisley, Scotland) for 1 hour at 37° C on a roller. The supernatant was discarded and the cartilage was further digested in Hepes-buffered Ham's F12 medium supplemented with collagenase (Clostridiopeptidase A from Clostridium histolyticum; Sigma, type 1A) (300 i.u./ml), 10% FCS (Gibco), 2% Hepes buffer (Gibco) and 1% antibiotic/antimycotic for 1-2 hours, depending on the rate of digestion at 37° C on the roller. The trypan blue dye exclusion test for cell viability showed that 99% of the cells were viable immediately after isolation.

Depending on the resurfacing material used, one of the following procedures was followed:

a. The cells were inoculated into the carbon fibre plug at high density (2 x 10^7 cells/ml; 1 aliquot of 30 µl or 6 x10^5 cells per 3.5mm plug; 1 plug per 35mm dish)(Becton Dickinson). This was carried out by placing 3 consecutive aliquots of 10 µl within each carbon fibre plug. A small amount of medium was placed around the edge of the dish to prevent the carbon fibre plug plus associated 30 µl medium from drying out. The dishes were kept at 37° C, 5% CO_2 for two hours, after which they were carefully flooded with medium and then returned to the incubator.

b. The cells were inoculated into the collagen gel at high density (2x10^7 cells/ml; 1 aliquot of 30 µl per 35 mm dish). This was carried out by placing 3 consecutive aliquots of 10 µl in the centre of the collagen gel. Subsequently, the procedure was similarly to that described in 'a' above.

c. The cells were inoculated onto the hydrogel PC 97 plug (radius=3 mm) at high density (2x10^7 cells/ml; 1 aliquot of 30 µl per plug; 1 plug per 35 mm dish).

In all three culture types the cells were fed every second day with 1 ml Ham's F12 medium supplemented with 10% (v/v) FCS, 1.4 mM L-glutamine (Gibco), 150 µg/ml

ascorbic acid (Gibco), 1% antibiotic/ antimycotic and 1% Nystatin (Gibco) and, if necessary, 0.75% (v/v) Gentamycin (Gibco). This medium was used in all culture experiments, except when otherwise stated. The chondrocytes were cultured for 21 days.

All media and digest solutions were made up and sterilized using a 0.22 μm membrane filter unit (Millipore, Millipore Ltd, Watford, England). Sterilized media and digest solutions were stored in 20 ml universal vials at -20° C. Phosphate buffered saline (PBS, Oxoid, Unipath Ltd, Basingstoke, England) was sterilized at 10 lbs/in^2 pressure for 20 minutes and stored at 4° C.

All dissecting instruments were dry/heat sterilized (180° C for 2 hours) before use.

2.1.3. Transplantation of plugs and associated chondrocytes.

Seventy, one year-old, skeletally mature, Sandy Lop rabbits weighing 4 kg (range 3.7-4.4 kg) were used. A full-thickness defect (3 mm in diameter, 3 mm in depth) in the articular cartilage and subchondral bone of the lateral tibial plateau of the right knee was made using a hand drill. Chondrocyte plugs prepared as described above were transplanted into the defect. Care was taken that the filled defect was level with the surrounding area. The left knee joint was used as a control and similar defects were filled with either carbon fibre plugs or hydrogel PC 97 plugs without chondrocytes. With regard to the collagen gel control a different procedure was followed. During the preparation of the collagen gel by cutting it the right size before implantation the collagen gels became very distensible and it was not possible to fixate the gel into the drill hole sufficiently.

Elsdale and Bard (1972) also noticed that collagen gel could not be cut with a knife and explained this due to collapse of the collagen lattice and loss of water caused by cutting the gel. Due to the transparancy of the gel it was also very difficult to see if the gel had been transplanted into the defect or not.

Wakitani *et al.*, (1989) suspended immature articular rabbit chondrocytes in collagen-medium mixture and a full-thickness defect (4 mm diameter, 4 mm deep) was drilled in the articular cartilage and subchondral bone and the chondrocyte-gel mixture was added. They used as control groups collagen gel only or empty drill hole. The results of both control groups were similar with regard to histology and ^{14}C proline labelling followed by SDS-PAGE. Therefore, in the left knee joint the drill hole was left empty as a control.

2.2. Morphological studies.

2.2.1. Outline of procedures.

Cultures were examined and photographed routinely using an Olympus inverted microscope model IMT under phase contrast optics.

After 3 weeks *in vitro* culture and 3, 6 and 12 months post-implantation, specimens were fixed for histological, immuno-histological and electron microscopical studies. The hydrogel PC 97 was developed late in the project and, consequently, specimens with hydrogel PC 97 have been analysed only at 3 months post-implantation. The graft tissue after 3, 6 and 12 months post-implantation was divided transversely into articular surface and deep regions and fixed for immunohistological studies. Biomechanical testing took place within 24 hours post-mortem and the joints were subsequently fixed in formal saline for histological processing. The graft tissues for biomechanical testing were transported on ice to the University of Leeds as part of whole knee joints in tissues soaked in PBS.

2.2.2. Histological assessment of graft tissue.

Two different protocols were followed depending on the type of tissue used, either graft tissue only or the superior (of the corpus tibiae) part of the tibia inclusive graft tissue.

Graft tissue samples were fixed in 10% formal saline and dehydrated at 4° C in 50%, 70% and 90% alcohol (30 mins each), three changes of 100% alcohol (30 mins each) and two changes of xylene (30 mins each) and allowed to reach room temperature before embedding in paraffin wax (melting point 56° C). The embedded tissue was stored at 4° C. Serial 5 μm sections were cut on a rotary microtome, mounted on glass slides and stored at 4° C. The sections were dewaxed in xylene, rehydrated through a graded series of alcohols to water and stained with Coles hematoxylin and Safranin-O for assessment of glycosaminoglycan distribution within the tissue (Rosenberg, 1971) or with 1% toluidine blue in 1% borax for metachromasia (Bancroft and Stevens, 1990).

Except for histological assesment of the graft tissue the surrounding synovial and capsular tissues were examined histologically for presence of graft material and the reaction of the synovial and capsular tissues on that material. The protocol as described above was used.

The superior (of the corpus tibiae) part of the tibia which had been used for biomechanical testing and (as described in chapter 2.1.3.) subsequently fixed, was then decalcified in 1M tetra-sodium EDTA (pH 7)(Nervanaid B 30 Soln, Ubi chem, Southampton, England). The tissue was dehydrated in 90% alcohol for 8 hours, 100% alcohol for 8-10 hours (x4), two changes of chloroform for 8-10 hours and allowed to reach room temperature before embedding in paraffin wax (melting point 56° C). The wax embedding took place under vacuum conditions. The embedded tissue was stored at 4° C. Serial 5 μm sections were cut on a sledge microtome, mounted on glass slides

and stored at 4° C. The sections were dewaxed in xylene, rehydrated through a graded series of alcohols to water and stained with Coles hematoxylin and Safranin-O or toluidine blue as described previously. The slides were coated with egg albumen (Culling, 1974). This was made by mixing equal parts of glycerin, distilled water and egg albumen. The solution was filtered through coarse filter paper. To prevent mould a crystal of thymol is added.

2.2.3. Scanning electron microscopical assessment of graft tissue.

Collagen gel, hydrogel PC 97 or carbon fibre plugs cultured for three weeks with associated chondrocytes were fixed in 2.0 % glutaraldehyde in 0.1M sodium cacodylate buffered to pH 7.2. The specimens were washed twice in cacodylate buffer (pH 7.2) at 4° C for 4 hours and dehydrated at 4° C in a graded series of alcohols (30, 50, 75, 85, 95 and 100 %) (each step for 1 hour). The specimens were transferred through a graded mixture of acetone and alcohol (1:3, 1:1, 3:1 v/v) through to 100% acetone for 2-4 hours each at 4° C before being dried in a critical point drying apparatus (Polaron) using liquid N_2. The specimens were mounted on to aluminium stubs (Jeol), sputter coated with a 20 nm layer of gold (Polaron E 500 sputter coater) and examined using a Jeol JSM 35C scanning electron microscope.

Because the hydrogel PC 97 plugs partially dissolved in acetone a different protocol was devised. The hydrogel PC 97 plugs were fixed and dehydrated as described for collagen gel and carbon fibre plugs. They were stored in 100% alcohol and then freeze fractured. This was achieved by rapidly freezing the specimens in a slush of Freon 12 for approximately 1 min before freeze drying (personal communication Dr. G. Blunn). The specimens were sputter coated with gold as described for carbon fibre or collagen gel plugs. Finally, the specimens were examined and photographed using a Jeol JSM-35C scanning electron microscope.

The surrounding synovial and capsular tissues were examined histologically for the presence of graft material and the reaction of the synovial and capsular tissues on that material. Because the synovial and capsular tissue-reaction of hydrogel PC 97 plug could not be done completely without using the scanning electron microscope, the protocol for hydrogel PC 97 plug processing as described above was used.

2.2.4. Transmission electron microscopical assessment of graft tissue.

The tissue was fixed overnight in 2.0% glutaraldehyde (TAAB Labs, Reading, England) made up in 0.1M sodium cacodylate buffer (BDH, Poole, England). After washing in

0.1 M sodium cacodylate (x3) for 5 mins each, the tissue was post-fixed for 60 mins with 1% osmium tetroxide (TAAB Labs) in 0.1M sodium cacodylate. The post-fixed tissue was then dehydrated in a graded series of ethanols and embedded as follows:

70% alcohol	5 mins.	x1.
1% uranyl acetate in 70% alcohol	20 mins.	x1.
90% alcohol	5 mins.	x2.
96% alcohol	5 mins.	x2.
100% alcohol plus sodium sulphate	10 mins.	x3.
100% alcohol:Spurrs resin (1:1)	60 mins.	x1.
Spurrs resin	overnight.	
embedding and polymerisation of resin	16 hours at 60° C.	

The use of Spurr's resin (Agar Scientific, Stansted, England) was described by Spurr (1969).

Semi-thin, 1 μm sections were cut using a diamond knife on a L.K.B. ultramicrotome, stained with 1% toluidine blue in 1% borax for approximately 1 min (Bancroft and Stevens, 1990), examined and photographed on a Zeiss photomicroscope III. Thin sections (80-100 nm) were then cut and mounted on copper grids (Gilder, G 200 HS grids, TAAB Labs). The sections were stained for 10 mins with 2% aqueous uranyl acetate, washed three times in distilled water 15 secs each, post-stained with lead citrate solution (Reynolds, 1963) for 10 mins, washed (x3) for 10 secs each in distilled water and, finally, viewed on a Philips CM12 transmission electron microscope using an accelerating voltage of 80 kV.

2.2.5. Immunocytochemical assessment of graft tissue.

Tissue samples were fixed at 4° C in 95% ethanol, dehydrated in three changes of absolute ethanol (30 mins each) followed by three changes of xylene (30 mins each) and allowed to reach room temperature before embedding in paraffin wax (melting point 56° C). The embedded tissue was stored at 4° C until sectioned. Serial 5 μm sections were cut on a rotary microtome, mounted on glass slides and stored at 4° C.

Sections were dewaxed in xylene, rehydrated through a series of alcohols and rinsed in three changes of PBS (0.02M)(15 mins each). The sections were pretreated with chondroitinase ABC (Sigma)(0.25 i.u./ml PBS) at 37° C for 15 mins, washed three times with PBS (5 mins each) and then incubated with the primary antibody for 45 mins.

The primary antibodies used were:

1. GAR I, an affinity purified polyclonal antibody recognising an epitope on collagen type I, a kind gift from Dr.D. Ashhurst, Dept. Anatomy, St. Georges Hospital Medical School, London. It was used at a concentration of 1: 50 in PBS. From July 1991 a monoclonal antibody (ascitic fluid) recognising an epitope on collagen type I was used (Chemicon International, London, United Kingdom). It was used at a concentration of 1: 50 in PBS.

2. CIICI, a monoclonal antibody recognising an epitope on collagen type II (Developmental Studies Hybridoma Bank, Iowa, USA). This was supplied as a hybridoma culture supernatant and used at a concentration of 1:1 in PBS.
3. 2B6, a monoclonal antibody recognising an epitope on chondroitin -4- sulphate.
It was used at a concentration of 1:100 in PBS (ascitic fluid)(Caterson, 1985).
4. 3B3, a monoclonal antibody recognising an epitope on chondroitin -6- sulphate.
It was used at a concentration of 1:100 in PBS (ascitic fluid)(Caterson, 1985).
5. MZ 15, a monoclonal antibody recognising an epitope on keratan sulphate (hybridoma culture supernatant)(Zanetti *et al.*, 1985). It was used at a concentration of 1:1 in PBS.
(Antibodies 3., 4. and 5. were all kind gifts from Dr. M.T. Bayliss, Kennedy Institute of Rheumatology, London.).
After 3 washes in PBS of 5 mins each, the secondary antibody (either fluorescein isothiocyanate conjugated rabbit anti-goat IgG after primary antibody 1, or fluorescein isothiocyanate conjugated rabbit anti-mouse IgG after primary antibodies 2 to 5, both diluted 1:40 in PBS)(Dako, Denmark) was applied in subdued lighting and kept for 45 mins at room temperature in the dark. The sections were then washed in PBS (3x 5 mins each) and mounted in glycerol/water (9:1) containing 1, 4-diazobicyclo [2.2.2.] octane (25 mg/ml) (Sigma) to retard photo-bleaching (Johnson *et al* ., 1982).
Control sections for all antibodies were processed substituting the primary antibody for PBS or in myeloma medium from a non-IgG secreting cell line (Developmental Studies Hybridoma Bank, Iowa, USA)(diluted 1:20 in PBS). In these cases little or no fluorescence was observed. The sections were examined on a Zeiss Photomicroscope III under epifluorescent illumination. More extensive controls were not utilized since all primary antibodies were well characterised and extensive controls had been carried out previously in this laboratory.
Limited chondroitinase pre-treatment is essential for localising chondroitins -4- and -6- sulphate since the enzyme pre-treatment generates the epitopes recognised by the primary antibodies within the glycosaminoglycan chain (Caterson *et al.*, 1990). In addition, chondroitinase treatment will partially remove chondroitin-sulphate and so enhance staining by making keratan sulphate more accessible to the primary antibody, MZ 15 (Oike *et al.*, 1980; Smith and Watt 1985). Similarly, chondroitinase pre-treatment also makes collagen type II epitopes more accessible to the primary antibody CIICI.

2.2.6. Silver enhancement immunocytochemical assessment of graft tissue.

Tissue samples were fixed at 4^{o} C in 95% ethanol, dehydrated in three changes of absolute ethanol (30 mins each), followed by three changes of xylene (30 mins each) and allowed to reach room temperature before embedding in paraffin wax (melting point

56° C). The embedded tissue was stored at 4° C until sectioned. Serial 5 μm sections were cut on a rotary microtome, mounted on glass slides and stored at 4° C.

For silver amplification, a kit from Amersham International, United Kingdom, was purchased and the procedure was carried out as per the makers' instructions. Briefly, sections were de-waxed in xylene and rehydrated through a series of alcohols and rinsed in three changes of TBS (100mM Tris, 150 mM NaCl at pH 7.2)(5 mins). The sections, except the ones that will be treated with the primary antibody 7D4, were pretreated with chondroitinase ABC (Sigma)(0.1 i.u./ml TBS) at 37° C for 30 mins, washed two times with TBS (5 mins each) and once with distilled water for 2 minutes. The sections were dehydrated through a series of alcohols and treated with 2.5% H_2O_2 in methanol for 30 mins to block endogenous peroxidase activity in the tissue. The sections were rehydrated down a graded series of alcohols and rinsed in two changes of TBS (5 mins) before application of normal lamb serum (20% in TBS)(blocking agent) for 30 mins and then incubated with the primary antibody for 45 mins at room temperature or overnight at 4° C.

The primary antibodies used were:

1. MC 7, a monoclonal antibody (ascitic fluid) recognising an epitope within the triple helical domain of collagen type X (Kwan *et al.*, 1989). This antibody was diluted 1:100 in TBS. This antibody was a kind gift from Dr. A. Kwan, Department of Biochemistry and Molecular Biology, University of Manchester, Manchester.
2. 7D4, a monoclonal antibody recognising over-sulphated regions on chondroitin sulphate chains. This antibody was applied without chondroitinase pre-treatment and used at a dilution of 1:100 in TBS.
3. MZ 15, a monoclonal antibody recognising an epitope on keratan sulphate. This antibody was used in dilution 1:1 in TBS. The antibodies 2 and 3 were kind gifts from Dr. M. Bayliss, Kennedy Institute of Rheumatology, London.). The titrations for all the antibodies were carried out previously in this laboratory.

After 3 washes in TBS of 5 mins each, peroxidase anti-mouse IgG (332 peroxidase units/ml, Sigma)(diluted 1:50 in TBS) was applied for 30 mins at room temperature. The sections were then washed in TBS (three times, 5 mins each) and incubated with 3,3'-diaminobenzidine (DAB) -solution (15 μl fresh 30% H_2O_2 in 50 mls of diluted DAB(1:50 in TBS) (Sigma) for 2-3 minutes, while carefully watching that the DAB-deposition did not exceed a minimal (sub-visual) level. Only a very pale yellow deposition was required and when this was achieved the reaction was stopped by submersion in water. The sections were washed in TBS three times for 5 mins each and then washed in deionized double-distilled water three times for 3 minutes each wash .

Non-specific metal binding sites (agyrophilic control) were blocked by treating the sections twice with acetic acid (1%) for 1 min, and afterwards $CuSO_4$ (10mM aq) was applied for 10 mins. After two washes in distilled water of 1 mins each, the tissue was treated with 3% H_2O_2 in 1% sodium acetate trihydrate, for 20 mins. The sections then

were treated with 1% sodium acetate (aq) twice for 1 min each and afterwards washed with deionized double-distilled water three times for 5 minutes each.
For the silver amplification the sections were treated with reagent 'A' for 6 mins and washed three times with deionized double-distilled water for 5 mins each. Reagent 'B' was applied for 6 mins and the sections were washed again three times with deionized double-distilled water for 5 mins each. The sections then were incubated with the silver amplification mixture (reagents 'C', 'D' and 'F') for 8 mins, whilst monitoring the silver deposition with a microscope under low illumination. After briefly washing the sections with deionized double-distilled water the sections were treated with reagent 'F' for 2 mins, and afterwards were washed three times with deionized double-distilled water for 5 mins each, dehydrated and mounted.
Control sections for all antibodies were processed substituting the primary antibody with myeloma medium from a non-IgG secreting cell line for monoclonal antibodies or PBS for polyclonal antibodies. In these cases little or no silver deposition was found. The sections were examined and photographed on a Zeiss photomicroscope III.

2.3. Biomechanical assesment of the graft tissue.

The graft tissue at 6 months and 1 year post-implantation was sent still within the knee joint and within 24 hours post mortem, to Miss L. Caravia and Dr. J. Fisher, Dept. of Mechanical Engineering, University of Leeds, Leeds.
The knee joints were dissected on arrival and photographs taken to record the macroscopic appearance of the graft tissue. Indentation tests were carried out on the graft tissue plugs in the tibial plateaux. An indenter load of 2.58N was used with a spherical indenter of 3 mm radius. The plug thickness was measured using a needling device at the same load. The velocity of the indenter and needler was approximately 0.5 mm/s (Fig. 2.1).
Using these results the elastic modulus of the material can be calculated according to Hayes' formula (Hayes *et al.*, 1972). Four calibration materials of different moduli were also tested. The materials were tested before and after the rabbit knee indentation tests. These materials consisted of layers of 1 mm thickness or less mounted on a rigid base. The testing was carried out in the same manner as the rabbit knee specimens. The materials used were:

a. Tecoflex (polyurethane) Shore hardness 93A
b. Tecoflex (polyurethane) Shore hardness 80A
c. Silicone rubber RTV3112
d. Silicone rubber RTV11

The modulus (MPa) was 20, 8, 6 and 2, respectively. After testing, the graft tissue was fixed in formol saline and processed for histology (see paragraph 2.1.2.).

Fig. 2.1. Photograph of apparatus used for performing indentation tests. The measurements made by the indentation machine (I) which feeds the signals through an analogue/digital converter connected to a microcomputer. Indentation tests were carried out on the graft tissue plugs in the tibial plateaux (S). An indenter load of 2.58N was used with a spherical indenter of 3 mm radius. The plug thickness was measured using a needling device at the same load. The velocity of the indenter and needler is approximately 0.5 mm/s (courtesy of Ms L. Caravia, Dept. of Mechanical Engineering, University of Leeds, Leeds).

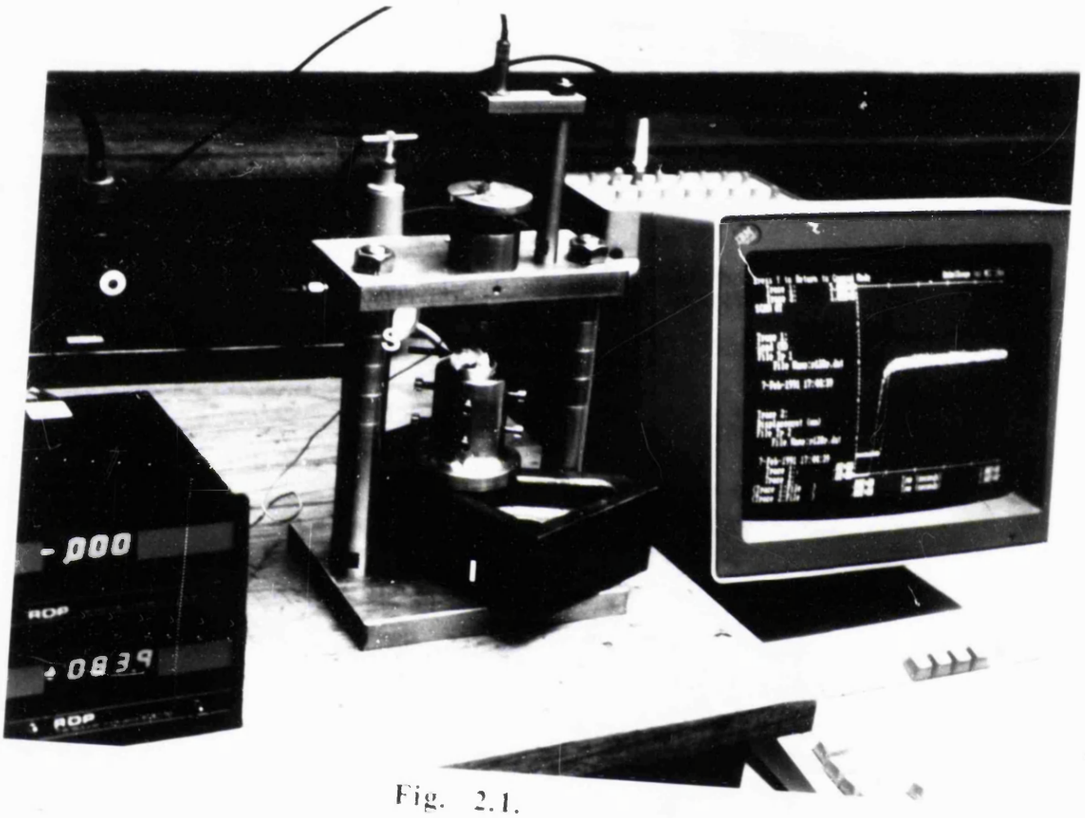


Fig. 2.1.

RESULTS

3.1. *In vitro* culture of chondrocytes within a carbon fibre meshwork, on a collagen gel, and on a hydrogel PC 97.

3.1.1. *In vitro* culture of chondrocytes within carbon fibre meshwork.

Photomicrographs of isolated rabbit articular chondrocytes cultured *in vitro* within a carbon fibre meshwork for 7 and 21 days are shown in Fig. 3.1. a.-e. The carbon fibre pads (Medicarb) were unravelled in such a way that the number of fibres per cm^3 decreased, facilitating the injection of isolated chondrocytes. Cells implanted into carbon fibre plugs adopted two types of morphology which remained throughout the culture period; in areas of high cell density on days 7 and 21 the cells were typically polygonal or rounded (Fig. 3.1. a + c.), whilst on the same days in regions of low cell density the chondrocytes tended to be fibroblastic in appearance. This was also evident with cells elongated along, and in contact with, the carbon fibres (Fig 3.1. b.+ d.). For comparative purposes, the histology of the various cultures is described later in this section. Fig. 3.1.e. shows the unravelled carbon fibre plug only, which formed a loose meshwork.

3.1.2. *In vitro* culture of chondrocytes on a collagen gel.

Isolated rabbit articular chondrocytes cultured *in vitro* on a collagen gel for 7 and 21 days are shown in Fig. 3.1. f.-i. After 7 days *in vitro* culture the cells in high cell density areas retained their rounded-polygonal shape (Fig. 3.1.f.), whilst cells in low cell density areas adopted a flattened appearance (Fig. 3.1.g.). After 21 days of *in vitro* culture the rounded cells were surrounded by large amounts of refractile extracellular matrix, making resolution of detail difficult (Fig. 3.1.h + i.). Again, for comparative purposes, the histology of the cultures is shown later in this section.

3.1.3. *In vitro* culture of chondrocytes on a hydrogel PC 97.

Isolated rabbit articular chondrocytes cultured *in vitro* on a pad of hydrogel PC 97 for 7 and 21 days are shown in Fig. 3.1. k.-l. After 7 days of culture the chondrocytes had a preference to grow either on top of or under the hydrogel PC 97, forming an envelope-like sheet around it. On only one occasion did chondrocytes grow within the hydrogel PC 97. One problem encountered was that some of the hydrogels PC 97 with associated chondrocytes did not adhere to the bottom of the Petri-dish and became a free-floating plug. As shown on Fig. 3.1.k. some cells growing on top of the gel had lost their rounded-polygonal shape; however, due to the semi-opaque nature of the hydrogel PC 97 it was difficult to see chondrocytes on top, within or beneath the hydrogel PC 97 with the inverted microscope. After 21 days culture two morphologies were evident: some cells had an elongated, flattened appearance, while other chondrocytes retained a polygonal-rounded shape. Most of the cells were found at the margins of the hydrogel PC 97, although the cells were probably present all around the gel. But this could not be

seen using phase contrast microscopy, as explained previously (Fig. 3.1.l.). Histological aspects of the cultures are detailed later in this section.

Fig. 3.1.a. Rabbit chondrocytes cultured *in vitro* within carbon fibre plug for 7 days in area of high cell density (x 250). The cells adopt a polygonal-rounded morphology.

Fig. 3.1.b. Rabbit chondrocytes cultured *in vitro* within carbon fibre plug for 7 days in area of low cell density (x 550). The arrows show a cell elongated along the carbon fibre.

Fig. 3.1.c. Rabbit chondrocytes cultured *in vitro* within carbon fibre plug for 3 weeks in area of high cell density (x 250). The arrows show a rounded-polygonal chondrocyte.

Fig. 3.1.d. Rabbit chondrocytes cultured *in vitro* within carbon fibre plug for 3 weeks in area of low cell density (x 250). The cells (see arrow) have a tendency to become fibroblast-like.

Fig. 3.1.e. Unravelled carbon fibre plug, which forms loose meshwork (x 125).

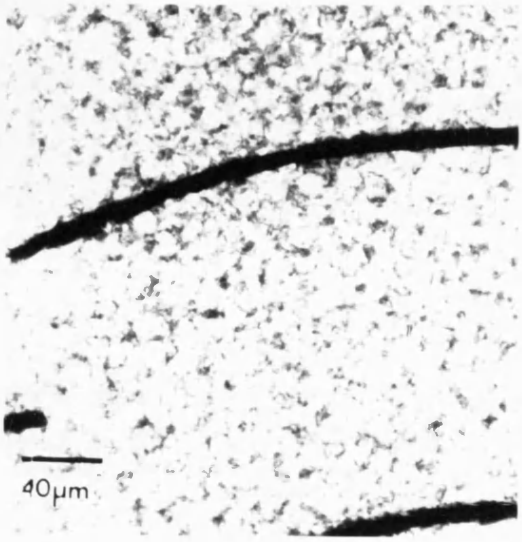


Fig. 3.1.a.

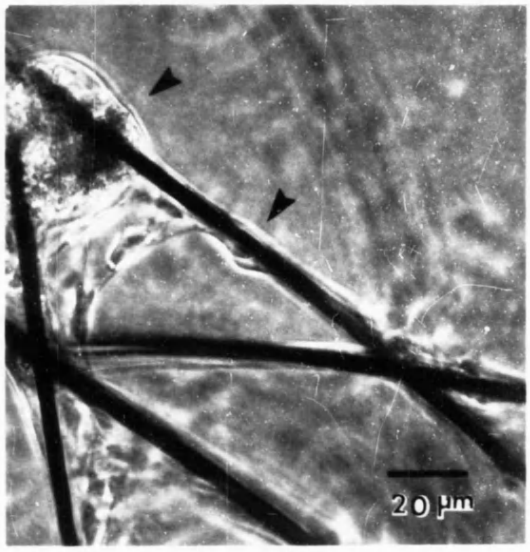


Fig. 3.1.b.

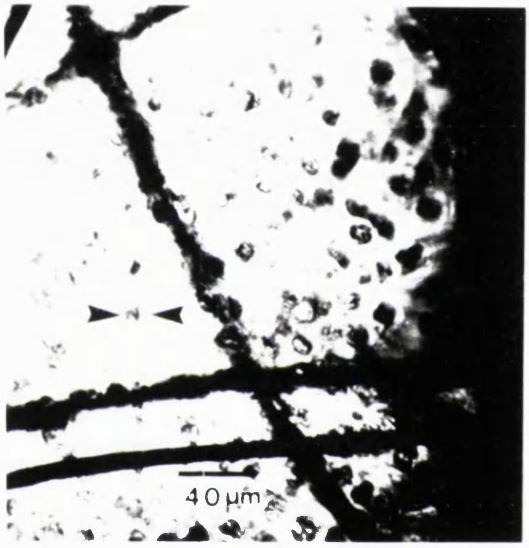


Fig. 3.1.c.

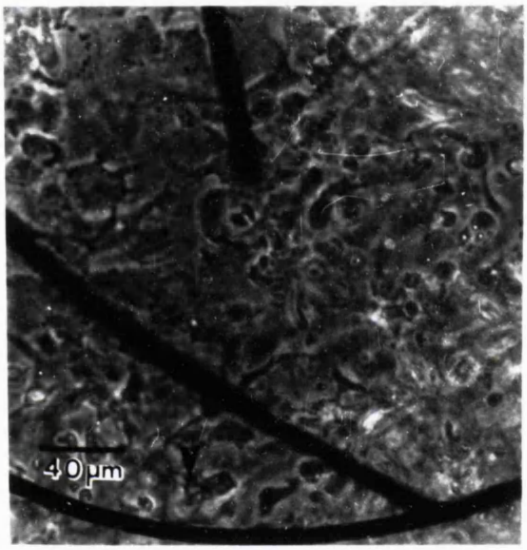


Fig. 3.1.d.

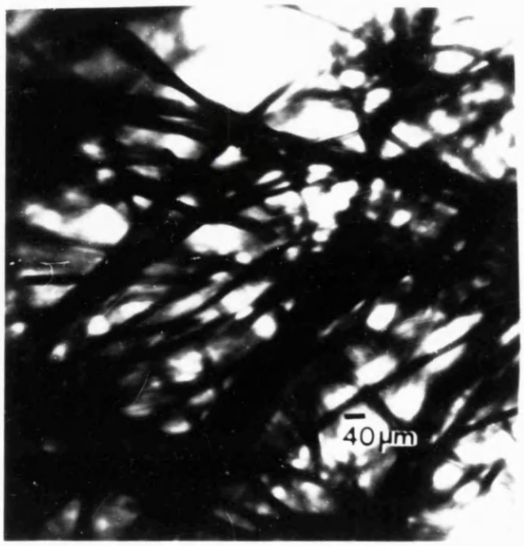


Fig. 3.1.e.

Fig. 3.1.f. Rabbit chondrocytes cultured *in vitro* within a collagen gel for 7 days in area of high cell density (x 250). The chondrocytes retain their rounded-polygonal morphology.

Fig. 3.1.g. Rabbit chondrocytes cultured *in vitro* within a collagen gel for 7 days in area of low cell density (x 250). The cells adopt a flattened appearance.

Fig. 3.1.h. Rabbit chondrocytes cultured *in vitro* within a collagen gel for 3 weeks in area of high cell density (x 250). As after 7 days *in vitro* culture the cells are characterized by their rounded-polygonal morphology. The cells are surrounded by large amounts of refractile matrix.

Fig. 3.1.i. Rabbit chondrocytes cultured *in vitro* within a collagen gel for 3 weeks in area of low cell density (x 250).

Fig. 3.1.k. Isolated chondrocytes cultured *in vitro* on a hydrogel PC 97 plug for 7 days (x 200). (HG=hydrogel PC 97).

Fig. 3.1.l. Isolated chondrocytes cultured *in vitro* on a hydrogel PC 97 plug for 21 days (x 200). (HG=hydrogel PC 97).

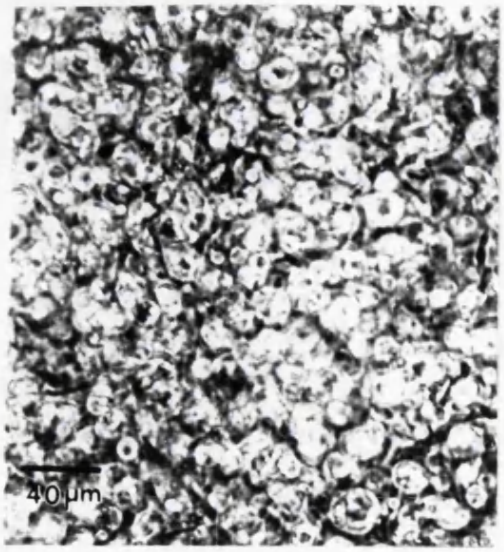


Fig. 3.1.f.

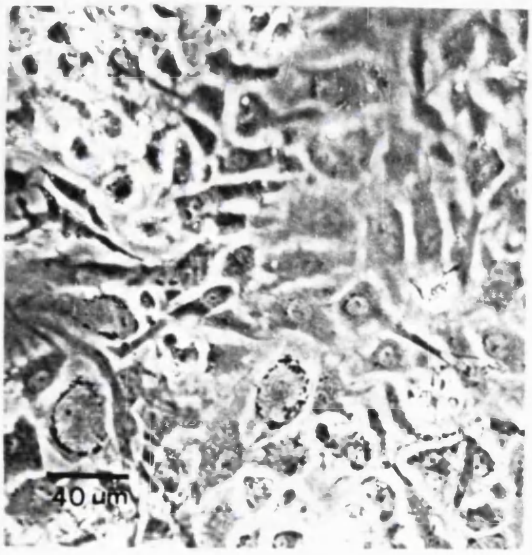


Fig. 3.1.g.

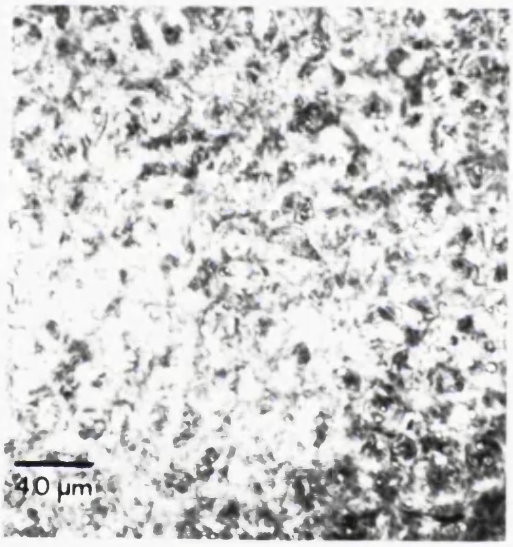


Fig. 3.1.h.

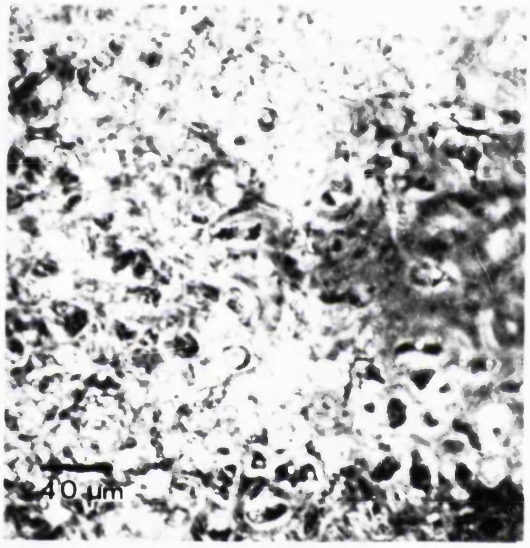


Fig. 3.1.i.

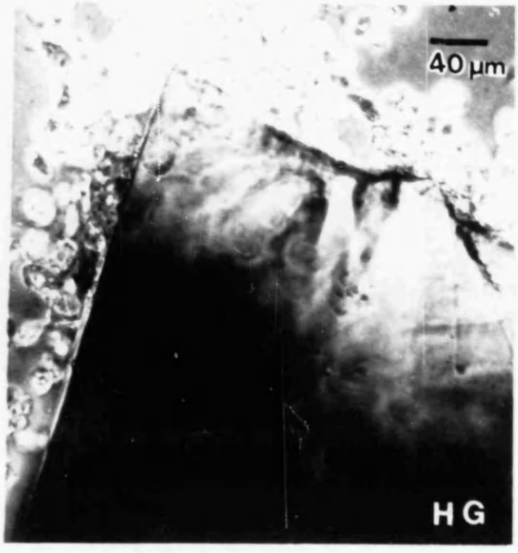


Fig. 3.1.k.

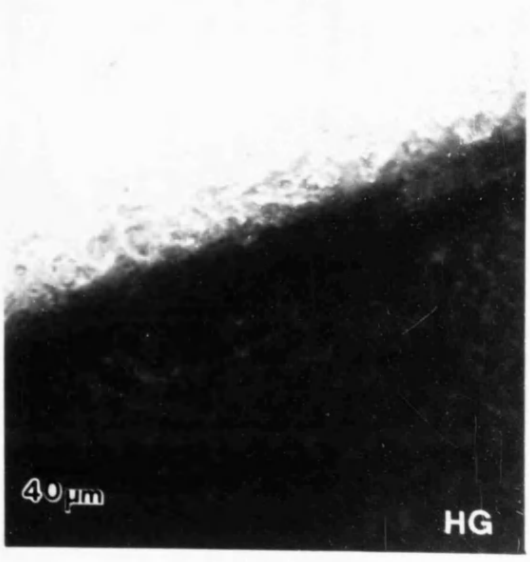


Fig. 3.1.l.

3.2. Morphological studies.

3.2.1. Macroscopic assessment of the grafts.

The acceptance/rejection rates of the different graft tissue plugs are shown in tables 3.1-3.7. In table 3.8 both grafts (e.g. carbon fibre plugs, either with or without chondrocytes, 6 months post-implantation) were compared. The criterion for acceptance of the graft tissue was defined as the presence of graft tissue in the drill hole filling at least one-third of it macroscopically. If there was less tissue present, or if the graft was not attached to the defect and, in effect, only 'lying' in the drill hole, the graft was considered to be rejected.

Except for the following two categories, all the groups are represented by ten animals: (a) 3 months post-implantation of carbon fibre plug with and without cells. One rabbit had a collagen gel plug with cells implanted in the right knee and a carbon fibre plug without chondrocytes in the left knee. This was not consistent with the rest of the study and, therefore, this animal was not included.

(b) 3 months post-implantation collagen gel with and without cells. The graft tissue of one rabbit was unfortunately lost during processsing.

At 3, 6 and 12 months post-implantation of the carbon fibre plugs.

At 3 months post-implantation.

The acceptance/rejection rates of the carbon fibre plugs at 3 months post-implantation are shown in table 3.1. At 3 months post-implantation of the carbon fibre plug with chondrocytes, 6 (67%) grafts were accepted. The tissue in the accepted grafts was level with the surrounding cartilage and the edges of the defect were not visible. A moderately thickened synovium was observed. With regard to the other 3 knee joints, one joint became infected and the other two rabbits died (data not shown).

The control grafts (carbon fibre plugs without chondrocytes) after 3 months were covered with a layer of white tissue. A similar 6 (67%) of the grafts were accepted, which were level with the surrounding cartilage. Again, moderate thickened synovial tissue was present (data not shown).

At 6 months post-implantation.

The acceptance/rejection rates of the carbon fibre plugs at 6 months post-implantation are shown in table 3.2. At 6 months post-implantation, the carbon fibre plug with chondrocytes 8 (80%) of the grafts were accepted. Of these grafts five were level with the surrounding cartilage and the edges of the defect were not visible (Fig. 3.2.1.a). In three cases, the surface was depressed below the surrounding cartilage (Fig. 3.2.1.b.+c.). With regard to the two rejected grafts, in one rabbit the graft had detached from the surrounding tissue and the defect was filled with fibrous repair tissue (Fig. 3.2.1.d.), whilst in the other rabbit the plug was found in the synovial and capsular tissues anterior to the joint, with partial attachment to the lateral meniscus (Fig. 3.2.1.e.).

The control grafts after 6 months were accepted in 8 knees, but in half of these the grafts were not level with the surrounding cartilage (Fig. 3.2.1.f.). Of the two rejected grafts, one plug was detached from the drill hole and could be found in the synovial and capsular tissues anterior of the joint (Fig. 3.2.1.g.). The other rejected graft was caused by the drill hole being made too anteriorly, which led the plug to fuse with the meniscus (Fig. 3.2.1.h.). In most joints, especially in the rejected control carbon fibre plug ones, a thickened synovium was observed.

At 12 months post-implantation

The acceptance/rejection rates of the carbon fibre plugs at 12 months post-implantation are shown in table 3.3. At 12 months post-implantation, the carbon fibre plugs plus chondrocytes were accepted in six (60%) rabbits. The grafts were well incorporated and the surface of the grafts were smooth (Fig. 3.2.1.i.). The degree of synovial thickening was markedly less than in knee joints examined at 3 and 6 months post-implantation .

The implants of the control knee joints at 12 months post-implantation were accepted in seven (70%) rabbits, with most of the grafts level with the surrounding cartilage (Fig. 3.2.1.k.). The surfaces of the grafts were mostly smooth and the edges of the defects were not visible. The one rabbit in whom both grafts were rejected had multiple capsulated swellings in connection with the left knee joint and a hydrops of the right knee joint.

At 3, 6 and 12 months post-implantation of the collagen gel plugs.

At 3 months post-implantation.

The acceptance/rejection rates of the collagen gel plugs at 3 months post-implantation are shown in table 3.4. At 3 months post-implantation, collagen gel plugs and associated chondrocytes were accepted in 5 (56%) joints and the grafts were level with the surrounding cartilage. The edges of the defects were not visible, which made it difficult to see the graft (Fig. 3.2.1.l.+m.).

The control grafts after 3 months showed 6 (67%) accepted grafts. These grafts were similar in appearance to those described above, except in 1 case which displayed a small crater-like defect (Fig. 3.2.1.n.). There were no rejected control grafts. No synovial changes were observed in any of the groups.

At 6 months post-implantation.

The acceptance/rejection rates of the collagen gel plugs at 6 months post-implantation are shown in table 3.5. At 6 months post-implantation, it was difficult to detect the grafts in the eight (80%) accepted cases (Figures 3.2.1.o.and 3.2.1.p.).

The 8 (80%) accepted control grafts at 6 months post-implantation were either below the level (40%)(Fig. 3.2.1.q.+r.) or slightly proud of the host cartilage surface (40%). Synovial changes were not observed in any of the groups.

At 12 months post-implantation.

The acceptance/rejection rates of the collagen gel plugs at 12 months post-implantation are shown in table 3.6. At 12 months post-implantation, 4 (40%) grafts were accepted. The collagen gel plugs with associated chondrocytes were well incorporated and the edges of the defect were most difficult to locate (Fig. 3.2.1.s.). One graft of collagen gel plus chondrocytes was rejected.

The 5 (50%) control grafts were easier to detect because all 5 (50%) accepted grafts were below the level of the surrounding cartilage (Fig. 3.2.1.t.). None of the control grafts were rejected. No changes in synovial tissue were observed in any of the groups.

At 3 months post-implantation of the hydrogel PC 97 plug.

The acceptance/rejection rates of the hydrogel PC 97 plugs at 3 months post-implantation are shown in table 3.7. At 3 months post-implantation, hydrogel PC 97 plugs plus chondrocytes were accepted in 9 (90%) joints. In the majority of the joints the grafts were level with the surrounding cartilage and well incorporated (Fig. 3.2.1.u.). The hydrogel PC 97 plug was relatively easy to detect because of it's white colour and the absence of new tissue covering the hydrogel at the joint surface. In some joints, the graft appeared only attached to one side of the defect. However, in 5 joints degenerative changes were present in the surrounding cartilage. In the hydrogel PC 97 plugs without chondrocyte co-culture 6 (60%) grafts were accepted. The grafts were all level with the surrounding cartilage (Fig. 3.2.1.v.), but half of these did not seem to be well incorporated into the defects (Fig. 3.2.1.w.). A thickened synovium was observed in all joints.

		CARBON FIBRE PLUG WITH CHONDROCYTES			
CARBON FIBRE PLUG	3 months post-implant-ation	accepted	rejected	missing	total
WITH-	accepted	5	0	1	6
OUT	rejected	0	0	0	0
CHONDRO	missing	1	0	2	3
-CYTES	total	6	0	3	9

Table 3.1. Acceptance/rejection rates of carbon fibre plugs and control plugs at 3 months post-implantation. For each rabbit the right (carbon fibre graft with cells) and left knee (carbon fibre graft without cells) were compared. In 5 rabbits the plugs were accepted in both knees and in 1 rabbit the graft was accepted in the right knee (carbon fibre graft with cells), but the graft in the left knee was missing. In another rabbit the graft in the right knee was missing, but the graft in the left knee (carbon fibre graft without cells) was accepted and in two rabbits the plugs in both knees were missing (animal died or got infected).

		CARBON FIBRE PLUG WITH CHONDROCYTES			
CARBON FIBRE PLUG	6 months post-implant-ation	accepted	rejected	missing	total
WITH-	accepted	7	1	0	8
OUT	rejected	1	1	0	2
CHONDRO	missing	0	0	0	0
-CYTES	total	8	2	0	10

Table 3.2. Acceptance/rejection rates of carbon fibre plugs and control plugs at 6 months post-implantation.

		CARBON FIBRE PLUG WITH CHONDROCYTES			
CARBON FIBRE PLUG	12 months post-implant-ation	accepted	rejected	missing	total
WITH-	accepted	6	1	0	7
OUT	rejected	0	1	0	1
CHONDRO	missing	0	0	2	2
-CYTES	total	6	2	2	10

Table 3.3. Acceptance/rejection rates of carbon fibre plugs and control plugs at 12 months post-implantation.

		COLLAGEN GEL PLUG WITH CHONDROCYTES			
COLLAGEN GEL PLUG	3 months post-implant-ation	accepted	rejected	missing	total
WITH-OUT CHONDRO-CYTES	accepted	4	0	1	5
	rejected	1	0	0	1
	missing	1	0	2	3
	total	6	0	3	9

Table 3.4. Acceptance/rejection rates of collagen gel plugs and control plugs at 3 months post-implantation.

		COLLAGEN GEL PLUG WITH CHONDROCYTES			
COLLAGEN GEL PLUG	6 months post-implant-ation	accepted	rejected	missing	total
WITH-OUT CHONDRO-CYTES	accepted	7	1	0	8
	rejected	1	0	0	1
	missing	0	0	1	1
	total	8	1	1	10

Table 3.5. Acceptance/rejection rates of collagen gel plugs and control plugs at 6 months post-implantation.

		COLLAGEN GEL PLUG WITH CHONDROCYTES			
COLLAGEN GEL PLUG	12 months post-implant-ation	accepted	rejected	missing	total
WITH-OUT CHONDRO-CYTES	accepted	4	1	0	5
	rejected	0	0	0	1
	missing	0	0	5	5
	total	4	1	5	10

Table 3.6. Acceptance/rejection rates of collagen gel plugs and control plugs at 12 months post-implantation.

		HYDRO GEL PC 97 PLUG WITH CHONDROCYTES			
HYDRO GEL PLUG	3 months post-implant-ation	accepted	rejected	missing	total
WITH-OUT CHONDRO-CYTES	accepted	6	0	0	6
	rejected	2	0	0	2
	missing	1	0	2	3
	total	9	0	2	11

Table 3.7. The acceptance/rejection rates of hydrogel PC 97 plugs and control plugs at 3 months post-implantation.

CARBON FIBRE PLUGS			COLLAGEN GEL PLUGS			HYDRO GEL PC97 PLUGS
months follow up time			months follow up time			months follow up time
3	6	12	3	6	12	3
5/5	7/10	6/8	4/5	7/9	4/5	6/8

Table 3.8. The observed proportions that both plugs were accepted. Any missing or dead animals were excluded.

Fig. 3.2.1.a. Photograph of a carbon fibre plug with chondrocytes at 6 months post-implantation (x 4). Most of the grafts were level with the surrounding cartilage and the edges of the defect were not visible. The arrow points to the defect.

Fig. 3.2.1.b. Photograph of a carbon fibre plug with chondrocytes at 6 months post-implantation (x 4). This showed one of the three accepted grafts with the surface depressed below the level of the surrounding cartilage. The arrow points to the defect (t = tibial plateau).

Fig. 3.2.1.c. Photograph of a carbon fibre plug with chondrocytes at 6 months post-implantation (x 2.5). This showed a graft which was accepted, but the surface was depressed below the surrounding cartilage. The arrow points to the defect.

Fig. 3.2.1.d. Photograph of a carbon fibre plug with chondrocytes at 6 months post-implantation (x 2.5). This showed one rejected graft, where the graft had detached from the surrounding tissue and the defect was filled with repair tissue. The arrow points to the defect (f = femoral condyle).

Fig. 3.2.1.e. Photograph of a carbon fibre plug with chondrocytes at 6 months post-implantation (x 3). This showed another rejected graft, where the plug was found in the synovial and capsular tissues anterior to the joint (see three small arrows) with partial attachment to the lateral meniscus. The two big arrows point to the defect.

Fig. 3.2.1.f. Photograph of a carbon fibre plug without chondrocytes at 6 months post-implantation (x 4). This showed an accepted graft which was not level with the surrounding cartilage. The arrow points to the defect.

Fig. 3.2.1.g. Photograph of a carbon fibre plug without chondrocytes at 6 months post-implantation (x 3). This showed a rejected graft which was detached from the drill hole and could be found in the synovial and capsular tissues anterior of the joint (see two arrows).

Fig. 3.2.1.h. Photograph of a carbon fibre plug without chondrocytes at 6 months post-implantation (x 3). This showed another rejected graft caused by a technical mistake i.e. the drill hole (see one arrow) was made too anteriorly. This led the plug to fuse with the meniscus (see two double arrows).

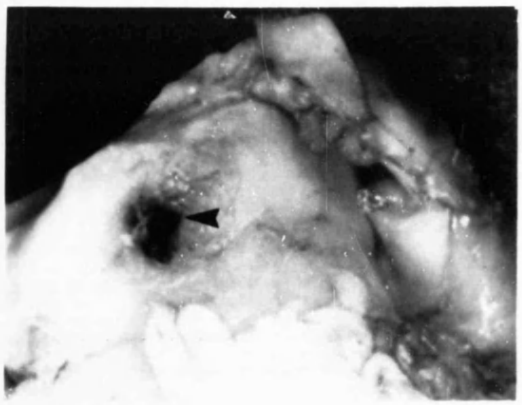


Fig. 3.2.1.a.

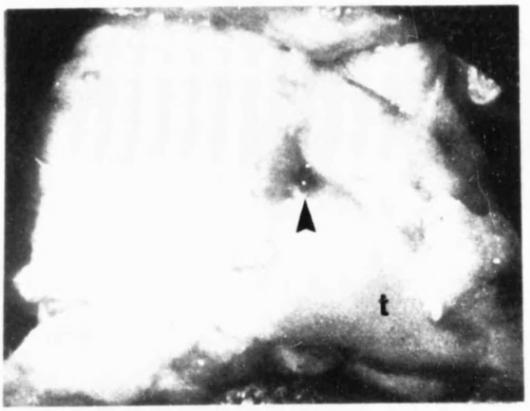


Fig. 3.2.1.b.

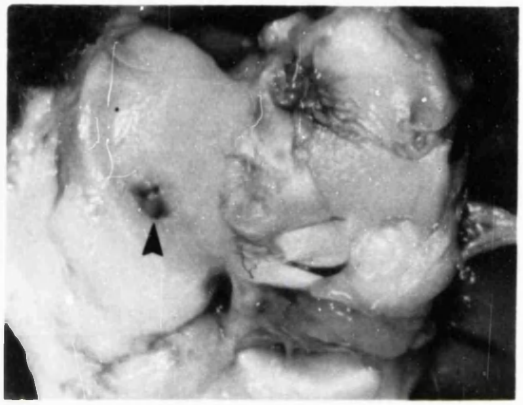


Fig. 3.2.1.c.

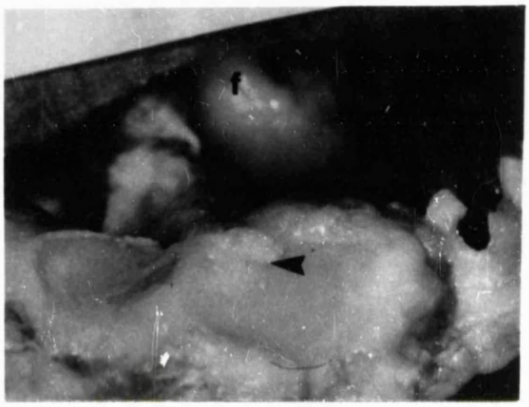


Fig. 3.2.1.d.

Fig. 3.2.1.e.

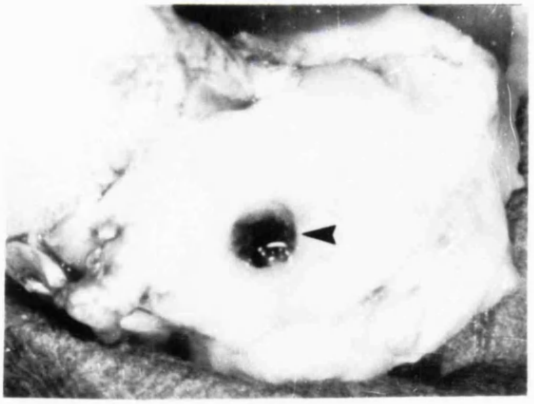


Fig. 3.2.1.f.

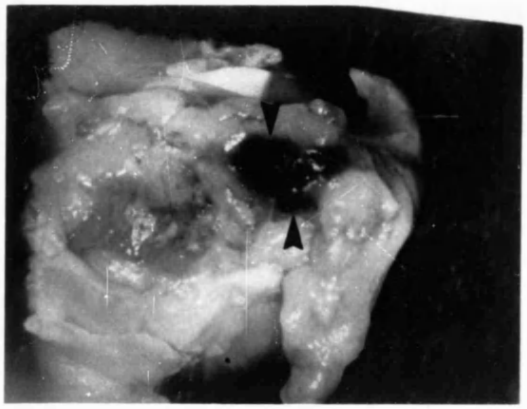


Fig. 3.2.1.g.

Fig. 3.2.1.h.

Fig. 3.2.1.i. Photograph of a carbon fibre plug with chondrocytes at 12 months post-implantation (x 3). This showed an accepted graft, which was well incorporated and the surface of the graft were smooth. The arrow points to the defect.

Fig. 3.2.1.k. Photograph of a carbon fibre plug without chondrocytes at 12 months post-implantation (x 2). This showed one of the accepted grafts which were level with the surrounding cartilage. The surface was mostly smooth and the edges of the defect were not visible. The arrow points to the defect (f = femoral condyle, t = tibial plateau).

Fig. 3.2.1.l. Photograph of a collagen gel plug and associated chondrocytes at 3 months post-implantation (x 4). The accepted graft was level with the surrounding cartilage and the edges of the defect were not visible. The arrow points to where the defect originally was made.

Fig. 3.2.1.m. Photograph of a collagen gel plug and associated chondrocytes at 3 months post-implantation (x 4). The accepted graft was difficult to detect, because it was level with the surrounding cartilage and the edges of the defect were not visible.

Fig. 3.2.1.n. Photograph of a collagen gel plug control at 3 months post-implantation (x 4). This showed an accepted graft and the edges of the defects were not visible, but there was a little crater-like defect left (see arrows).

Fig. 3.2.1.o. Photograph of a collagen gel plug and associated chondrocytes at 6 months post-implantation (x 2). It was difficult to detect the accepted graft in the lateral tibial plateau. The arrow points to the defect (f = femoral condyle, t = tibial plateau).

Fig. 3.2.1.p. Photograph of a collagen gel plug and associated chondrocytes at 6 months post-implantation (x 4). The accepted graft was well incorporated into the surrounding cartilage and there was no crater-like depression left.

Fig. 3.2.1.q. Photograph of a collagen gel plug control at 6 months post-implantation (x 3.5). This showed an accepted graft which was below the level of the surrounding cartilage. The arrows point to the defect.

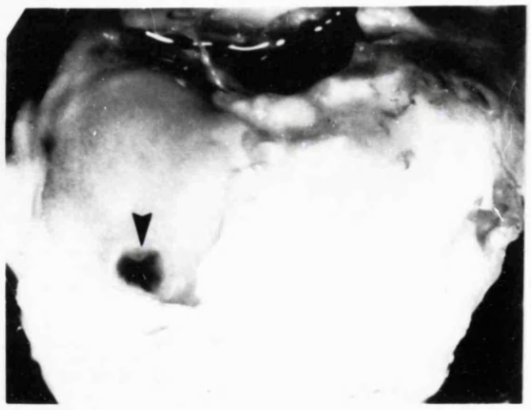


Fig. 3.2.1.i.

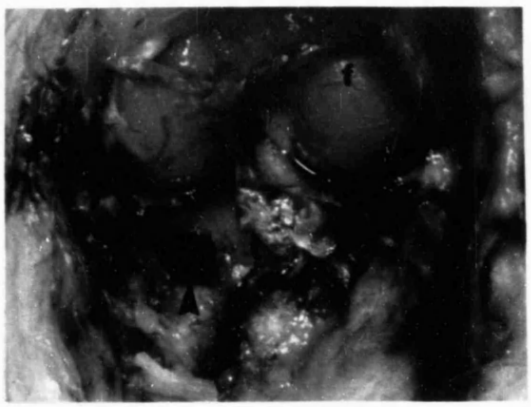


Fig. 3.2.1.k.

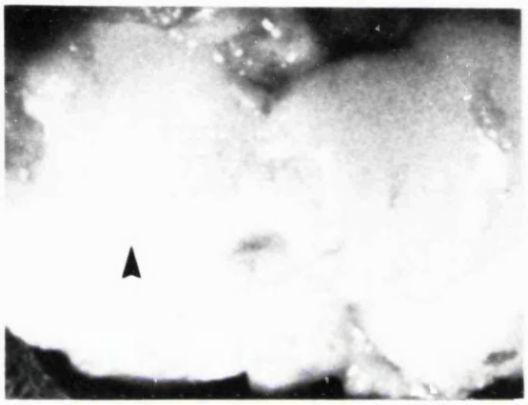


Fig. 3.2.1.l.

Fig. 3.2.1.m.

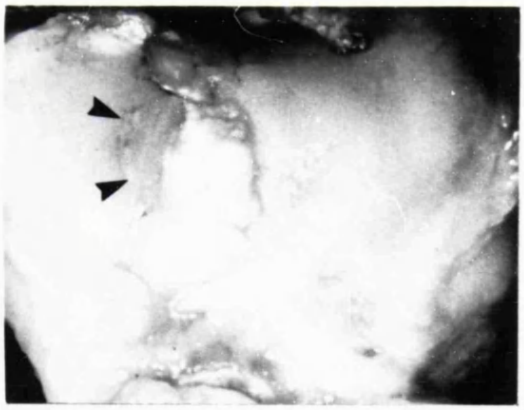


Fig. 3.2.1.n.

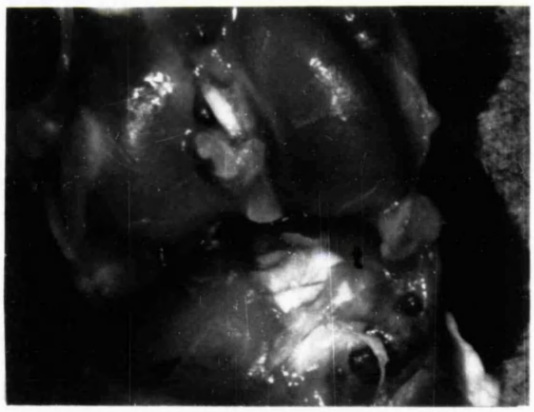


Fig. 3.2.1.o.

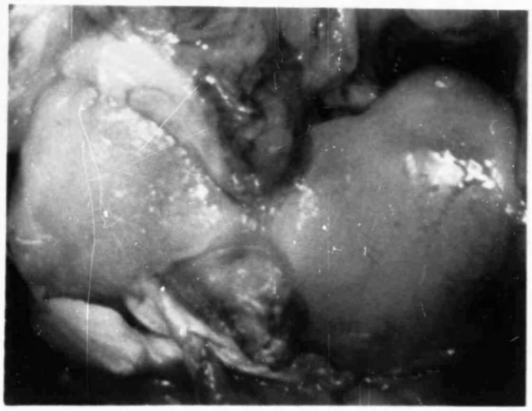


Fig. 3.2.1.p.

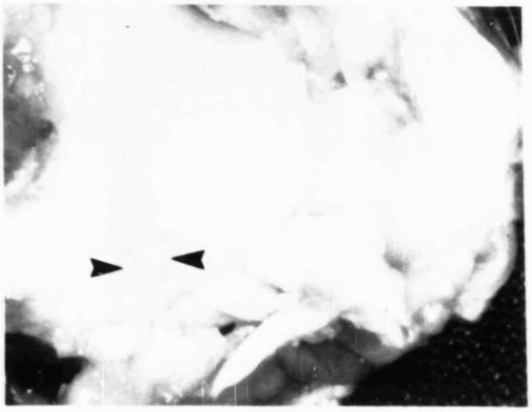


Fig. 3.2.1.q.

Fig. 3.2.1.r. Photograph of a collagen gel plug control at 6 months post-implantation (x 3). One of the accepted grafts was relatively easy to detect because it was below the level of the surface of the surrounding cartilage. The arrow points to the defect.

Fig. 3.2.1.s. Photograph of a collagen gel plug and associated chondrocytes at 12 months post-implantation (x 4). The collagen gel plugs with associated chondrocytes were well incorporated and the edges of the defect were most difficult to locate (f = femoral condyle, t = tibial plateau, l = lig. cruciata).

Fig. 3.2.1.t. Photograph of a collagen gel plug control at 12 months post-implantation (x 4). The control graft was more easy to detect because it was below the level of the surface of the surrounding cartilage. The arrows point to the defect (f = femoral condyle, t = tibial plateau).

Fig. 3.2.1.u. Photograph of a hydrogel PC 97 plug and associated chondrocytes at 3 months post-implantation (x 3). This showed one of the accepted grafts which was level with the surface of the surrounding cartilage and well incorporated into the former defect. The hydrogel PC 97 plug was relatively easy to detect because of its white colour and the absence of any tissue layer being formed covering the hydrogel at the joint surface. The arrows point to the defect.

Fig. 3.2.1.v. Photograph of a hydrogel PC 97 plug without chondrocytes at 3 months post-implantation (x 2.5). This showed one of the accepted grafts. The graft was level with the surface of the surrounding cartilage. The arrows point to the defect (t = tibial plateau).

Fig. 3.2.1.w. Photograph of a hydrogel PC 97 plug without chondrocytes at 3 months post-implantation (x 3). This showed one of the grafts which did not seem to be well incorporated into the defect. The arrow points to the defect (t = tibial plateau).

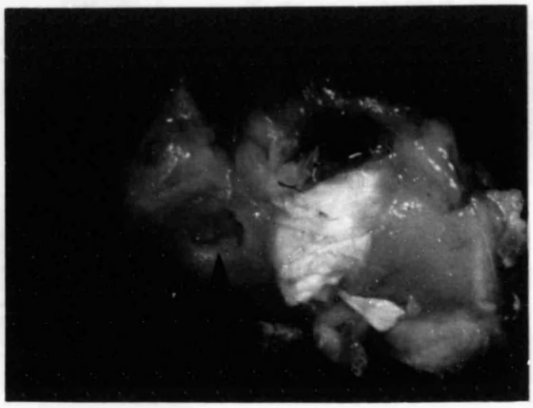


Fig. 3.2.1.r.

Fig. 3.2.1.s.

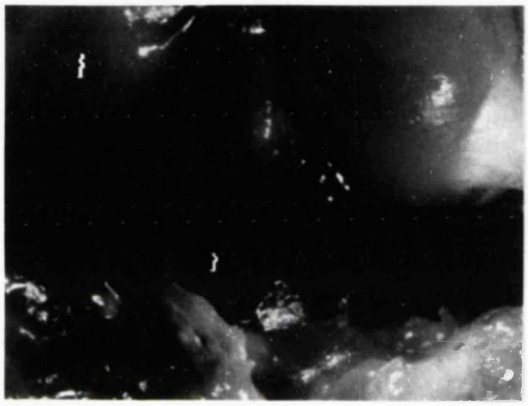


Fig. 3.2.2.1.t.

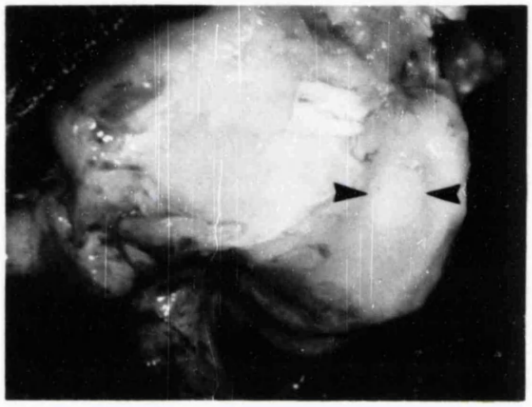


Fig. 3.2.1.u.

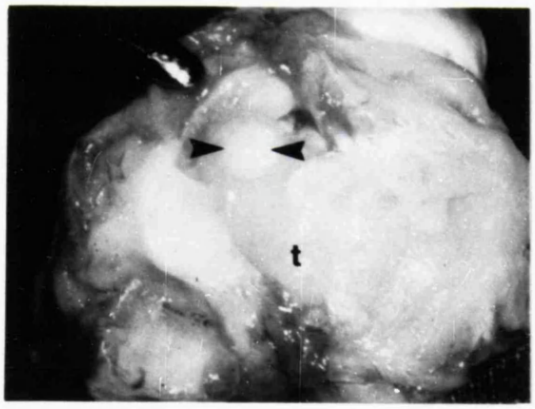


Fig. 3.2.1.v.

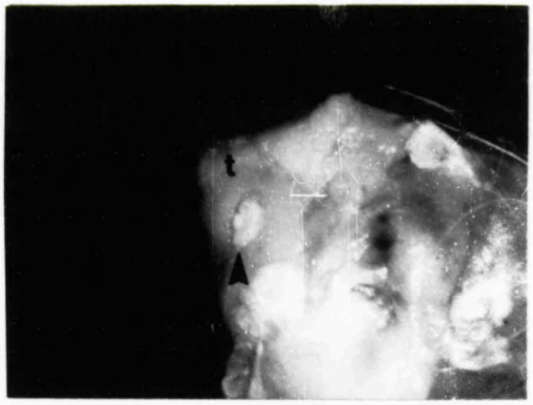


Fig. 3.2.1.w.

3.2.2. Histological assessment of the grafts.

Histological assessment of carbon fibre plugs with associated chondrocytes.

Photomicrographs of sections of graft tissue of isolated rabbit articular chondrocytes cultured *in vitro* within a carbon fibre meshwork after 3 weeks culture, and at 3, 6 and 12 months post-implantation are shown in Fig. 3.2.2.1.a.-l.

Tissue samples at 3 weeks showed chondrocytes and carbon fibres within an intensely metachromatic-staining matrix, which suggested active accumulation of proteoglycans (Fig. 3.2.2.1.a.).

At 3 months post-implantation.

At 3 months post-implantation, the graft tissue consisted of carbon fibres which again were totally incorporated into the cartilage-like tissue (Fig. 3.2.2.1.b.). In contrast, the host tissue within the graft region of the control knee joint without chondrocytes consisted of two histological types of tissues; fibrous-like tissue with incorporation of carbon fibres, and some areas of metachromatic-staining tissue in which carbon fibres were also embedded (Fig. 3.2.2.1.c.).

At 6 months post-implantation.

Due to the limited number of specimens available, only 1 or 2 specimens for 6 and 12 months follow-up periods after implantation of carbon fibre and collagen gel plugs, and for 3 months follow-up period after implantation of hydrogel PC 97, could be examined histologically together with the surrounding cartilage and bone. Consequently, the mid-region of the tibiae were cut using a band-saw, decalcified, and serially-sectioned.

Low-power examination of decalcified sections of the whole graft region showed that the surface of the articular cartilage of the experimental knee was irregular and fibrillations were present. The surface of the graft was seen to be continuous with the surrounding cartilage, apart from one sectioning artefact through the cartilage and bone. The carbon fibre plug consisted of fibro-cartilaginous repair tissue and was surrounded by bone-like tissue. A layer consisting of superficial fibrocartilaginous tissue and deep bone-like tissue had grown over the carbon fibre plug. In addition, the thickness of the subchondral bone was reduced in some areas of the section. Whilst the graft-cartilage interface could not be found, the graft-bone interface showed highly cellular fibrous tissue (Fig. 3.2.2.1.d.). Part of the carbon fibre plug was found in the extra-articular structures. High-power examination of the graft tissue at 6 months post-implantation of the carbon fibre plugs with associated chondrocytes showed many carbon fibres, which were surrounded by mainly metachromatically-staining matrix, whilst areas of fibrous tissue were found closer to the joint surface (Fig. 3.2.2.1.e.).

The host tissue within the graft region of the control knee joint at 6 months post-implantation showed an irregular surface with fibrillations. The defect was filled with fibrous/fibro-cartilaginous graft tissue, but no carbon fibre was detected. The host repair tissue was well incorporated into the bone, but vascular elements were seen in close proximity to the deep layers. The interface between cartilage and repair tissue was

marked by clefts and there was no continuity between these two tissues. The adjacent cartilage showed signs of breakdown (Fig. 3.2.2.1.f.). The carbon fibres were found in bone within the proximity of the original defect as well as in bone in areas distant to the original defect and in other articular structures such as synovium. High-power examination of the host tissue within the graft region of the control knee joint at 6 months post-implantation showed mainly fibrous-like tissue in areas devoid of carbon fibres, whilst some areas of cartilage-like staining tissue were also found surrounding the carbon fibres (Fig. 3.2.2.1.g.).

At 12 months post-implantation.

Low-power examination of decalcified sections of the entire graft region at 12 months post-implantation showed that the surface of the graft tissue was irregular. Carbon fibres were extremely difficult to cut and being unsupported in the wax block, these pulled out during sectioning, which might explain the absence of carbon fibres in the section. The graft tissue was cartilaginous and a histologically distinct interface between the graft tissue and the surrounding cartilage and bone was not found (Fig. 3.2.2.1.h.). High-power examination of the graft tissue at 12 months post-implantation showed metachromasia of the matrix in the middle and deep layers, whilst the surface layer did not show metachromatic staining. Around the carbon fibres no metachromatic staining was found. In addition, the spatial organization of the chondrocytes seen in normal mature articular cartilage was not evident (Fig. 3.2.2.1.i.).

The host tissue within the graft region of the control knee joint after 12 months showed an intact surface layer, but the middle and deep host tissue within the graft region layers were fragmented. The fragmentation of the host tissue within the graft region could again be explained by the difficulty in sectioning carbon fibre, which resulted in damage to the surrounding tissue. The fragmentation also made it difficult to find the interface between the host tissue within the graft region and the neighbouring cartilage and bone. In the deep layer of the tissue which had formed within the graft region, carbon fibres were surrounded by a fibrocartilaginous tissue in which chondrocyte clusters were embedded (Fig. 3.2.2.1.k and l.). The subchondral plate formed within the graft region was depressed compared to the subchondral plate of the surrounding cartilage.

The reaction to carbon fibre of the surrounding synovial and capsular tissues.

In addition to the histological assessment of the graft tissue, the surrounding synovial and capsular tissues were examined histologically for presence of graft material and the reaction of the synovial and capsular tissues to that material. Photomicrographs of sections of carbon fibre in synovial and capsular tissues surrounding the rabbit knee joint at 6 and 12 months post-implantation are shown in Fig. 3.2.2.1.m.-n. At 6 and 12 months post-implantation, foreign body giant cells and histiocytes were found around the carbon fibre (Fig. 3.2.2.1.m.). Granulomas, however, were not evident. The synovium showed evidence of hyperplasia (Fig. 3.2.2.1.n.).

Histological assessment of collagen gel plugs with associated chondrocytes.

Photomicrographs of sections of graft tissue of isolated rabbit articular chondrocytes cultured *in vitro* on a collagen gel after 3 weeks culture and at 3, 6 and 12 months post-implantation are shown in Fig. 3.2.2.2.a.-l. After 3 weeks *in vitro* culture most chondrocyte-like cells were present at the surface of the gel, with more fibroblast-like cells within the gel (Fig. 3.2.2.2.a).

At 3 months post-implantation.

At 3 months post-implantation, the graft tissue showed the presence of cells with differing morphologies: rounded-polygonal cells, both in surface and deep layers, elongated cells mainly in the deep layer and cells in clusters located mainly in the surface layer. Metachromatic-staining of the extracellular matrix increased with increasing distance from the articular surface. In the deep layer, pericellular metachromatic-staining was also observed (Fig. 3.2.2.2.b.).

The host tissue within the graft region of the control knee joint at 3 months post-implantation showed a metachromatic staining in the deep layer of the host tissue within the graft region with a diversity of cell configurations as described above, whilst in the surface layers metachromatic staining was not observed (Fig. 3.2.2.2.c.).

At 6 months post-implantation.

Low-power examination of decalcified sections of the whole graft region showed that the surface of the graft was similar to the surrounding cartilage; the repair tissue appeared cartilaginous and there was no histologically distinct interface seen between graft tissue and neighbouring cartilage or bone at 6 months post-implantation (Fig. 3.2.2.2.d.). High-power examination of the graft tissue at 6 months post-implantation showed metachromatic staining of the matrix in the deeper layers of the graft tissue, whilst in the superficial layers less staining was evident (Fig. 3.2.2.2.e.).

Low-power examination of decalcified sections of the whole graft region in the the control knee at 6 months post-implantation showed a fibrillated surface, which was continuous with the surface of the surrounding cartilage. The spatial distribution of the chondrocytes as seen in normal non-diseased cartilage was not present, but rather the cells were distributed at random and at an apparantly reduced cell density throughout the host tissue within the graft region. The host tissue within the graft region was well incorporated into the surrounding bone and cartilage, and no histologically distinct interface was seen (Fig. 3.2.2.2.f.). High-power examination of the host tissue within the graft region of the control knee joint at 6 months post-implantation showed metachromatic staining of the matrix of the deep layers in an irregular pattern, whilst the surface layers were negative. As was described at 3 months post-implantation, the presence of cell clusters was found within the surface layers (Fig. 3.2.2.2.g.).

At 12 months post-implantation.

Low-power examination of decalcified sections of the whole graft region at 12 months post-implantation, graft tissue could only be detected by the difference in spatial

distribution of the chondrocytes. The surface of the graft tissue was continuous with the surrounding cartilage, and an interface between the graft and host tissue was not detected (Fig. 3.2.2.2.h.). At 12 months post-implantation high-power examination of the graft tissue showed that in the extracellular matrix of the graft tissue, areas of pericellular metachromatic-staining as well as areas of fibrous-like tissue were found (Fig. 3.2.2.2.i.).
Low-power examination of decalcified sections of the whole graft region in the control knee after 12 months showed that the repair tissue varied greatly in cell density and that a thickened subchondral plate was found. The host tissue within the graft region was fibro-cartilaginous (Fig. 3.2.2.2.k.). High-power examination of the host tissue within the graft region of the control knee joint at 12 months post-implantation showed metachromatic-staining of the matrix in the deep layers, whilst the matrix of the surface and middle layers did not stain metachromatically (Fig. 3.2.2.2.l.).

Because previous work (Wakitani *et al.*, 1989) has shown that the collagen (type I) gel used did not elicit an immune rejection response in rabbits, the synovial and capsular tissues were not examined histologically.

Histological assessment of hydrogel PC 97 plugs with associated chondrocytes.
Photomicrographs of sections of graft tissue of isolated rabbit articular chondrocytes in a hydrogel PC 97 after 3 weeks *in vitro* culture and at 3 months post-implantation are shown in Fig. 3.2.2.3.a.-e.
After 3 weeks in culture a metachromatic-staining matrix was produced covering the hydrogel PC 97 (Fig. 3.2.2.3.a.). Due to difficulties in embedding the hydrogel PC 97 it was not always possible to preserve the continuity of the tissue.
At 3 months post-implantation.
During the processing of the hydrogel PC 97 graft and surrounding joint tissues a problem arose, which will be discussed briefly. The wax embedding procedure as described in ch. 2.2.2. was used. Unfortunately, the graft tissue showed a tendency to come out of the original drill hole during sectioning, presumably because of inadequate tissue infiltration. However, using vacuum wax embedding techniques (ch. 2.2.2.) better results were obtained. Stoy and Kliment (1990) have stated that modification of the standard histological methods was sometimes necessary, as hydrogels dehydrated with the tissue and, when embedded in soft embedding media, might shatter during cutting and even damage the surrounding tissue.
Although vacuum wax embedding was used, at 3 months post-implantation low-power examination of decalcified sections of graft tissue showed detachment of the majority of the plugs. It was assumed that this detachment occured during graft tissue processing as remains of the hydrogel PC 97 plugs with associated chondrocytes were still present. In addition, the grafts were clearly visible and *in situ* during macroscopic examination. This

made it difficult to interprete the results. These plugs were not incorporated into the surrounding cartilage, but bone showed integration in the pores of the gel. In the surrounding cartilage, chondrocyte clones were present near the margins of the original drill hole (Fig. 3.2.2.3.b.). At 3 months post-implantation, high power examination of the graft tissue showed areas of fragmentation and some areas of the matrix showed pericellular metachromatic staining (Fig. 3.2.2.3.c.).

Low-power examination of decalcified sections of the whole graft region in the the control knee at 3 months post-implantation showed that the host tissue within the graft region appeared to be split in two parts, a superior and an inferior part. The superior part was only attached to one side of the surrounding cartilage, which might have been an artefact caused by processing. In the superior part, two layers of cartilage could be observed, namely, a surface layer with elongated cells, and in the deeper layers chondrocytes in a random distribution present both as single cells and cell clusters. The distal part was well incorporated into the bone (Fig. 3.2.2.3.d.).

High-power examination of the host tissue within the graft region in the control knee after 3 months showed that the hydrogel PC 97 was surrounded by a weak metachromatic-staining matrix with elongated cells and a few localized areas of cells with a rounded-polygonal shape. The latter ones showed pericellular metachromatic staining (Fig. 3.2.2.3.e.).

Reaction to hydrogel PC 97 of synovial and capsular tissue.

As well as histological assessment of the graft tissue the surrounding synovial and capsular tissues were examined for presence of graft material and the reaction of the synovial and capsular tissues on that material.

Photomicrographs of sections of hydrogel PC 97 in synovial and capsular tissues surrounding the rabbit knee joint at 3 months post-implantation are shown in Fig. 3.2.2.3.f.-h. In the synovial and capsular tissues, a greyish-purple material was seen surrounded by histiocytes and foreign body giant cells. Also, proliferating fibrous tissue was observed (Fig. 3.2.2.3.f.). The synovium showed a mild synovi(ali)tis and hyperplasia and hypertrophy of the surface layer (Fig. 3.2.2.3.g.). In some specimens the subsurface contained a greyish-purple material present 'in puddles', stimulating a foreign body cell reaction (Fig. 3.2.2.3.h.) and, in addition, a perivascular inflammatory infiltrate was seen. This suggested that the hydrogel PC 97 was either degrading, or that whilst cutting the plug (before *in vitro* culture or during transplantation to adapt the plug size to the drill hole size) some fragments were released into the joint. Subsequently, either part of the degrading material or the fragments were being encapsulated by macrophages in the synovial and capsular tissues. It was unlikely that the hydrogel PC 97 was degrading, since the constituent components are known to have a long half-life time under physiological conditions. Thus, further examination of the greyish-purple material with the scanning electron microscope was used to determine if this material

consisted of fragments of hydrogel PC 97 or, possibly, another material (see below). The scanning electron microscope was used, because (1) the physical structure of hydrogel PC 97 was orginally studied by its developers using scanning electron microscopy, and later for the present study and (2) it was a reliable and relatively non-time consuming method (see below).

Fig. 3.2.2.1.a. Photograph of tissue sample at 3 weeks (toluidine blue, x 300). This showed chondrocytes and carbon fibres within an intensely metachromatic-staining matrix, which suggested active accumulation of proteoglycans.

Fig. 3.2.2.1.b. Photograph of the carbon fibre graft plus associated chondrocytes at 3 months post-implantation (toluidine blue, x 175). The graft tissue consisted of carbon fibres which were totally incorporated into the metachromatic-staining tissue (S = surface layer of the repair tissue, D = deep layer of the repair tissue).

Fig. 3.2.2.1.c. Photograph of the carbon fibre graft without chondrocytes at 3 months post-implantation (toluidine blue, x 100). The host tissue within the graft region of control knee joint consisted of two histological types of tissues: fibrous-like tissue with incorporation of carbon fibres, and some areas of metachromatic-staining cartilaginous-like tissue in which carbon fibres were embedded (S = surface layer of the graft tissue; D = deep layer of the graft tissue).

Fig. 3.2.2.1.d. Photograph of low-power examination of decalcified section of the carbon fibre graft with chondrocytes at 6 months post-implantation (haematoxylin-eosin, x 50). The surface (S) of the graft was seen to be continuous with the surrounding cartilage, apart from one sectioning artefact through cartilage and bone. A layer consisting of a superficial fibrocartilaginous tissue and deep bone-like tissue had grown over the carbon fibre plug. The carbon fibre plug consisted of fibro-cartilaginous repair tissue and was surrounded by bone-like tissue. The graft-bone interface showed highly cellular fibrous tissue.

Fig. 3.2.2.1.e. Photograph of high-power examination of the carbon fibre graft with chondrocytes at 6 months post-implantation (toluidine blue, x 175). The graft tissue showed many carbon fibres, which were surrounded by mainly metachromatic-staining matrix, whilst areas of fibrous tissue were found closer to the joint surface (S = surface layer of the graft tissue, D = deep layer of the graft tissue).

Fig. 3.2.2.1.a.

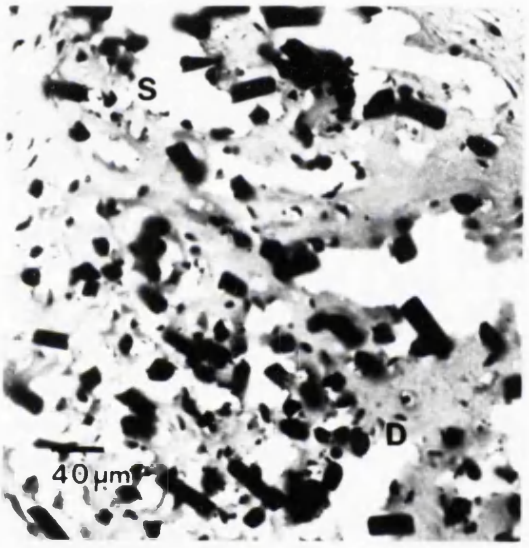


Fig. 3.2.2.1.b.

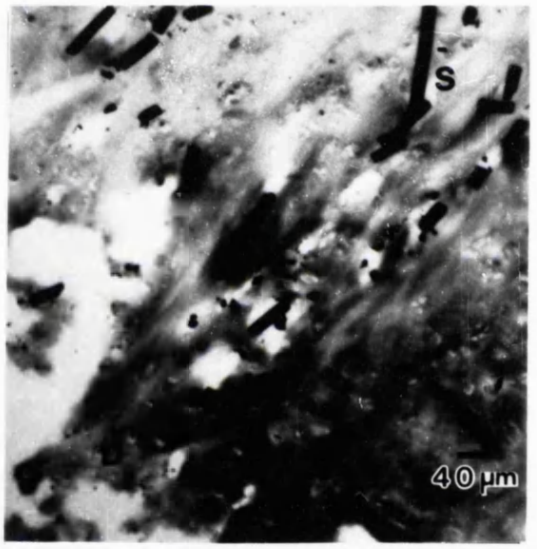


Fig. 3.2.2.1.c.

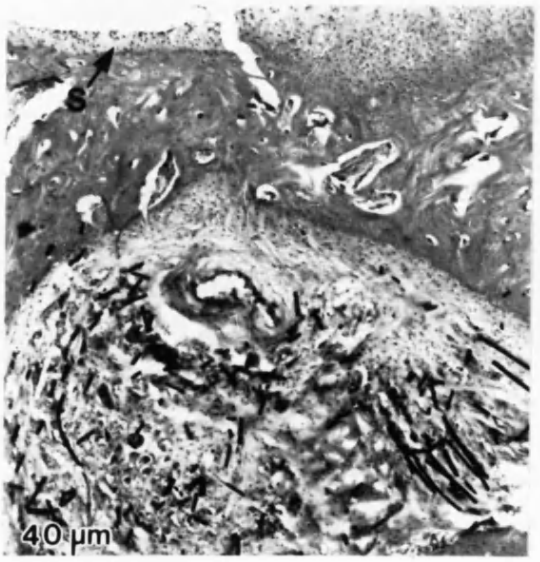


Fig. 3.2.2.1.d.

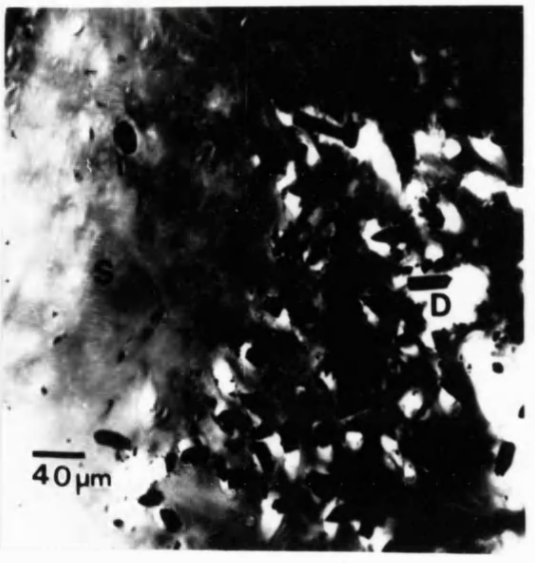


Fig. 3.2.2.1.e.

Fig. 3.2.2.1.f. Photograph of low-power examination of decalcified section of the whole graft region at 6 months post-implantation of the carbon fibre graft without chondrocytes (haematoxylin-eosin, x 50). The host tissue within the graft region showed an irregular surface (S) with fibrillations. The defect was filled with fibrous/fibrocartilaginous repair tissue. The repair tissue was well incorporated into the bone, but vascular elements were seen in close proximity of the deep layers of the graft tissue.

Fig. 3.2.2.1.g. Photograph of high-power examination of the carbon fibre plug without chondrocytes at 6 months post-implantation (toluidine blue, x 100). The host tissue within the graft region showed mainly fibrous-like tissue without the presence of carbon fibres, whilst some areas of cartilage-like staining were found surrounding the carbon fibres (S = surface layer of the tissue, D = deep layer of the tissue).

Fig. 3.2.2.1.h. Photograph of low-power examination of decalcified section of the carbon fibre graft with chondrocytes at 12 months post-implantation (haematoxylin-eosin, x 50). The surface (S) of the graft tissue was irregular and the graft tissue was partly fragmented. The carbon fibres were absent in the section. A histologically distinct interface between the graft tissue and the surrounding cartilage and bone was not found.

Fig. 3.2.2.1.i. Photograph of high-power examination of the carbon fibre graft with chondrocytes at 12 months post-implantation (toluidine blue, x 350). The graft tissue showed metachromasia of the matrix in the middle and deep layers, but not in the surface layer. The spatial localization of the cells as seen in normal mature articular cartilage was lost (S = surface layer of the graft tissue, D = deep layer of the graft tissue).

Fig. 3.2.2.1.k. Photograph of low-power examination of decalcified section of the whole graft region at 12 months post-implantation of the carbon fibre graft without chondrocytes (haematoxylin-eosin, x 50). The host tissue within the graft region showed an intact surface layer (S), but the middle and deep repair tissue layers were fragmented. The carbon fibre plug was found in the deep layers of the repair tissue and subchondral bone.

Fig. 3.2.2.1.l. Photograph of high-power examination of the carbon fibre graft without chondrocytes at 12 months post-implantation (toluidine blue, x 350).The host tissue within the graft region showed cartilage-like staining in some areas and around the carbon fibres, whilst in other areas no metachromasia of matrix or surrounding the carbon fibres was found (S = surface layer of the repair tissue, D = deep layer of the repair tissue).

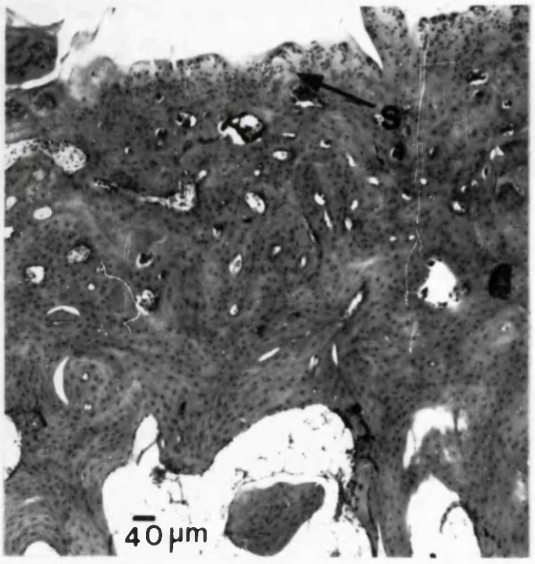


Fig. 3.2.2.1.f.

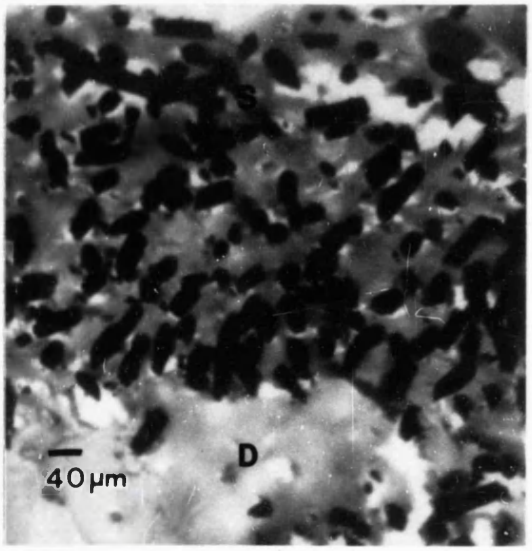


Fig. 3.2.2.1.g.

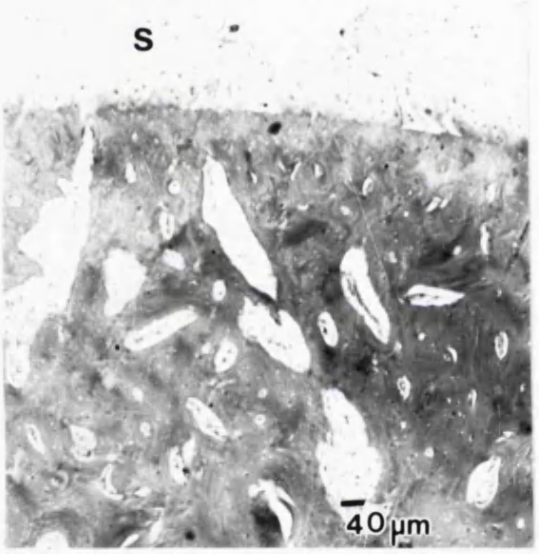


Fig. 3.2.2.1.h.

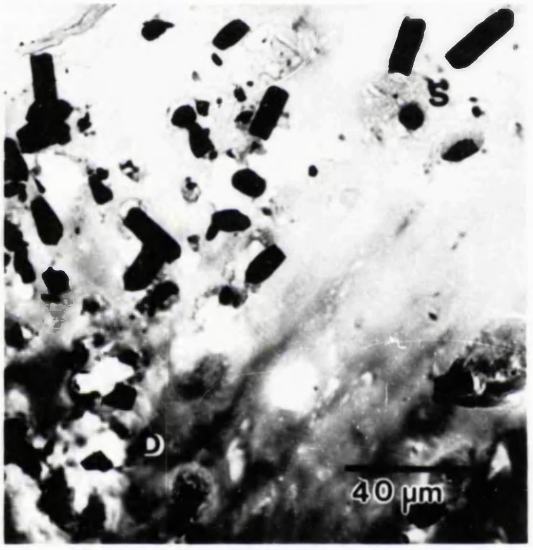


Fig. 3.2.2.1.i.

Fig. 3.2.2.1.k.

Fig. 3.2.2.1.l.

Fig. 3.2.2.1.m. Foreign body giant cells (F) and histiocytes (see arrow) surrounding carbon fibre (CF) in capsular tissue (haematoxylin-eosin, x 400).

Fig. 3.2.2.1.n. Thickened synovium with foreign body giant cells (F), histiocytes (see arrow) and carbon fibres (CF) (haematoxylin-eosin, x 400).

Fig. 3.2.2.2.a. Photograph of the collagen gel graft tissue sample at 3 weeks *in vitro* culture showed that most chondrocyte-like cells (CH) were present at the surface of the gel (toluidine blue, x 175). Throughout the gel more elongated cells were seen (F = fibrocyte). The pericellular environment of some of the chondrocyte-like cells were staining metachromatically.

Fig. 3.2.2.2.b. Photograph of the collagen gel plug with associated chondrocytes at 3 months post-implantation (toluidine blue, x 175). The graft tissue showed the presence of cells with differing appearances, rounded-polygonal cells, both in surface (S) and deep (D) layers, elongated cells, mainly in the deep layer and cells in clones, mainly in the surface layer. With increasing distance from the surface the metachromasia of the extracellular matrix increased and in the deep layer pericellular metachromatic-staining was found.

Fig. 3.2.2.2.c. Photograph of the collagen gel control plug at 3 months post-implantation (toluidine blue, x 175). The host tissue within the graft region showed a metachromatic-staining matrix in the deep layer (D) of the host tissue with a diversity of cell morphologies as described for Fig. 3.2.2.2.b. (S = surface layer of the repair tissue).

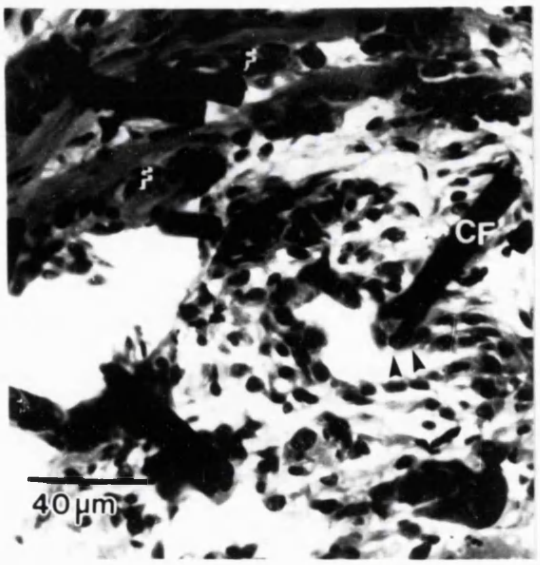


Fig. 3.2.2.1.m.

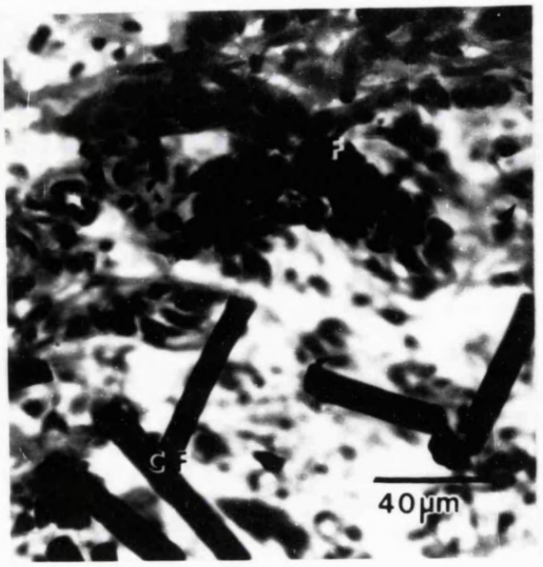


Fig. 3.2.2.1.n.

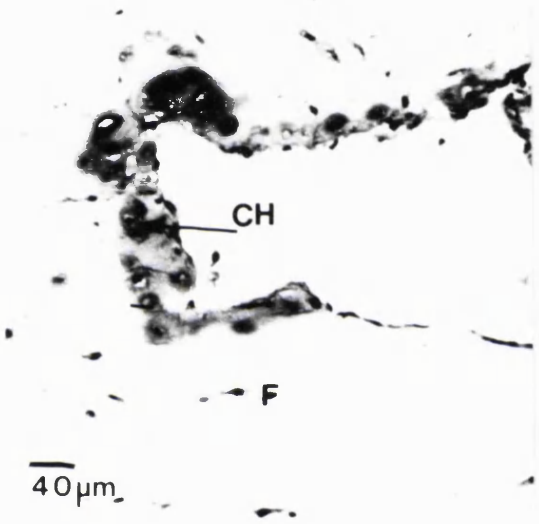


Fig. 3.2.2.2.a.

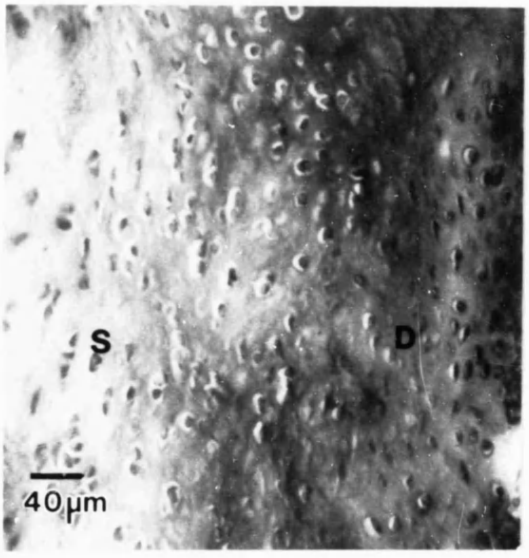


Fig. 3.2.2.2.b.

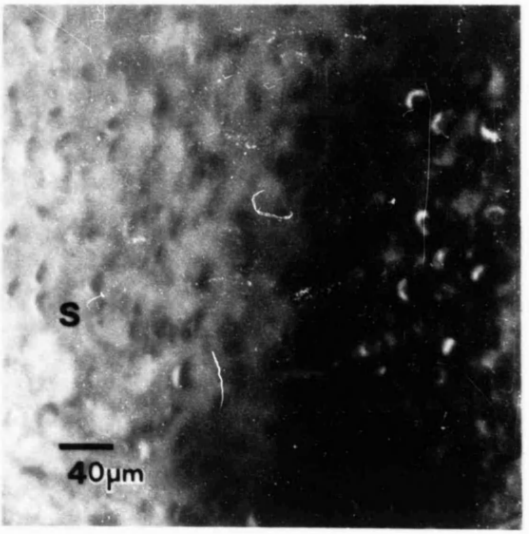


Fig. 3.2.2.2.c.

Fig. 3.2.2.2.d. Photograph of low-power examination of decalcified section of the whole graft region at 6 months post-implantation of the collagen gel plug with associated chondrocytes (haematoxylin-eosin, x 50). The surface (S) of the graft was similar to the surrounding cartilage and there was no histologically distinct interface detected between the graft tissue and neighbouring cartilage or bone.

Fig. 3.2.2.2.e. Photograph of high-power examination of the collagen gel plug with associated chondrocytes at 6 months post-implantation (toluidine blue, x 175). The graft tissue showed metachromatic staining of the matrix in the deeper layers (D) of the graft tissue, whilst in the superficial layer (S) less staining was found.

Fig. 3.2.2.2.f. Photograph of low-power examination of decalcified sections of the whole graft region at 6 months post-implantation (haematoxylin-eosin, x 50). The host tissue showed a fibrillated surface (S), which was continuous with the surface of the surrounding cartilage. The spatial distribution of the chondrocytes as seen in normal non-diseased cartilage was not present, but rather the cells were distributed at random and at an apparantly reduced cell density throughout the host tissue. The latter was well incorporated into the surrounding bone and cartilage, and no histologically distinct interface was seen.

Fig. 3.2.2.2.g. Photograph of high-power examination of the host tissue within the graft region at 6 months post-implantation (toluidine blue, x 175). The host tissue showed metachromatic staining of the matrix of the deep layer (D) in an irregular pattern, whilst the surface (S) layers were negative. Cell clusters were found within the surface layers.

Fig. 3.2.2.2.h. Photograph of low-power examination of decalcified sections of the whole graft region at 12 months post-implantation of the collagen gel graft with associated chondrocytes (haematoxylin-eosin, x 50). The surface (S) of the graft tissue was continuous with the surrounding cartilage. A histologically distinct interface between the graft tissue and the surrounding cartilage and bone was not observed.

Fig. 3.2.2.2.i. Photograph of high-power examination of the graft tissue at 12 months post-implantation of the collagen gel graft with associated chondrocytes (toluidine blue, x 175). In the extracellular matrix of the graft tissue, areas of cartilaginous tissue as well as areas of fibrous-like tissue were found. It was not possible to point out which was surface and deep layers of the graft tissue. Chondrocytes (CH) were shown in groups or single as well as fibrocytes (F).

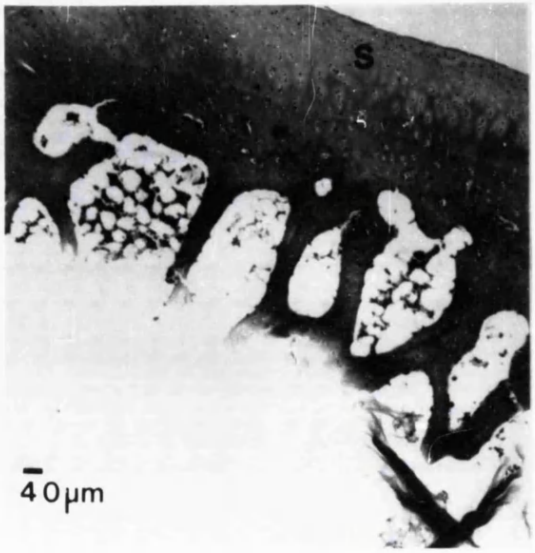


Fig. 3.2.2.2.d.

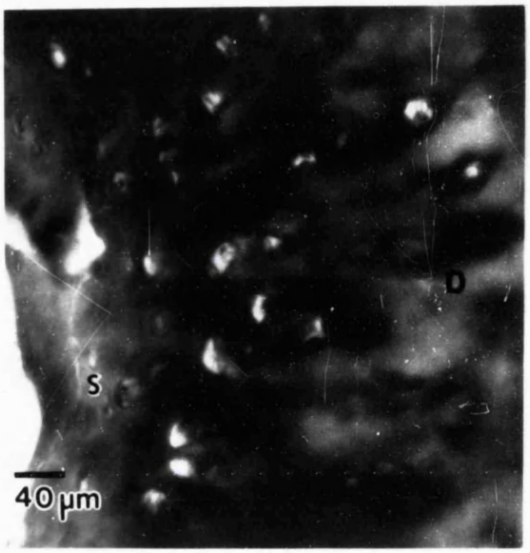


Fig. 3.2.2.2.e.

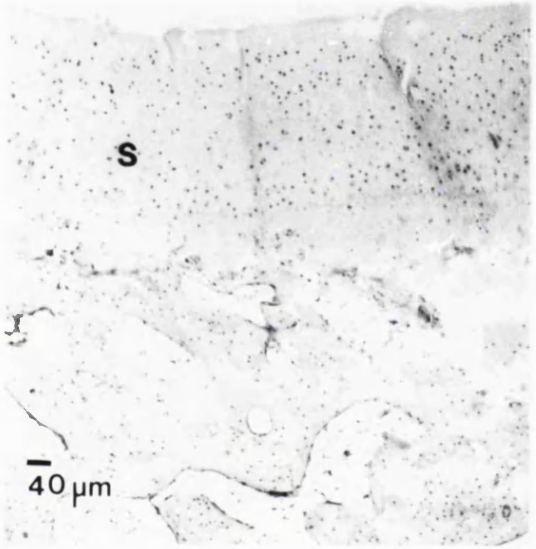


Fig. 3.2.2.2.f.

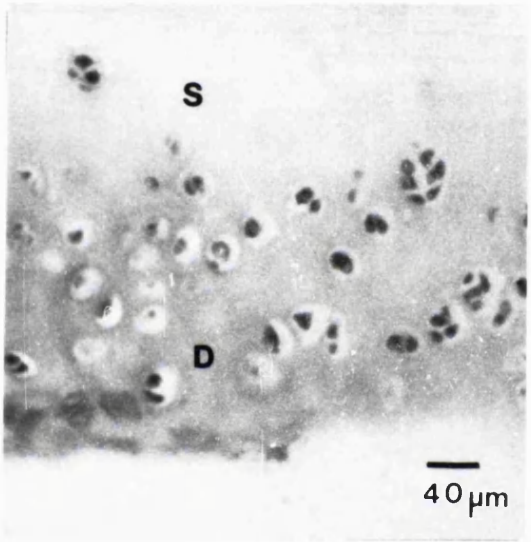


Fig. 3.2.2.2.g.

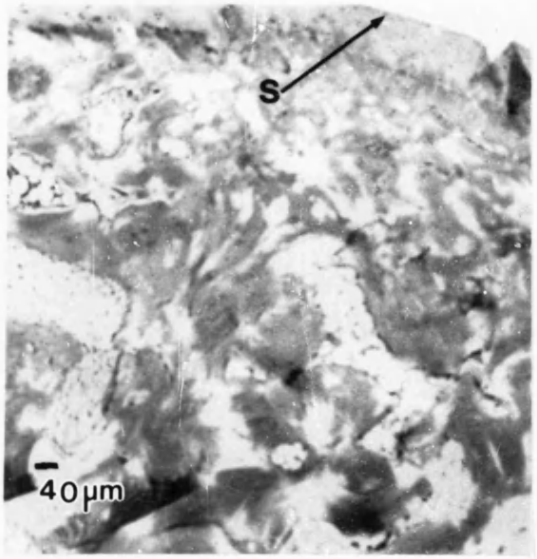


Fig. 3.2.2.2.h.

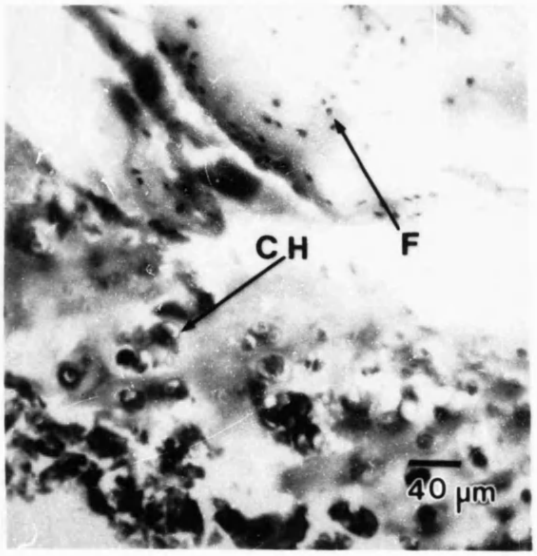


Fig. 3.2.2.2.i.

Fig. 3.2.2.2.k. Photograph of low-power examination of decalcified section of the whole graft region at 12 months post-implantation (haematoxylin-eosin, x 50). The repair tissue varied greatly in cell density and a thickened subchondral plate was found. The host tissue within the graft region was fibro-cartilaginous (S = surface layer of the host tissue)

Fig. 3.2.2.2.l. Photograph of high-power examination of the host tissue within the graft region at 12 months post-implantation (toluidine blue, x 100). The host tissue showed metachromatic-staining of the matrix of the deep layers (D), whilst the matrix of the surface (S) and middle layers did not stain metachromatically.

Fig. 3.2.2.3.a. Photograph of the hydrogel PC 97 graft tissue samples at 3 weeks *in vitro* culture (toluidine blue, x 175). After 3 weeks in culture a metachromatic-staining matrix (M) was produced covering the hydrogel PC 97 (HG).

Fig. 3.2.2.3.b. Photograph of low-power examination of decalcified section of the hydrogel PC 97 plug with associated chondrocytes at 3 months post-implantation (haematoxylin-eosin, x 100). Detachment of the majority of the plugs was observed. These plugs were not incorporated into the surrounding cartilage, but bone showed integration in the pores of the gel. In the surrounding cartilage chondrocyte clones were present near the margins of the original drill hole (S = surface layer of the graft tissue).

Fig. 3.2.2.3.c. Photograph of high power examination of the hydrogel PC 97 plug with associated chondrocytes at 3 months post-implantation (toluidine blue, x 175). The graft tissue showed areas of fragmentation and some areas of the matrix showed pericellular metachromatic staining (S = surface layer of the graft tissue; D = deep layer of the graft tissue).

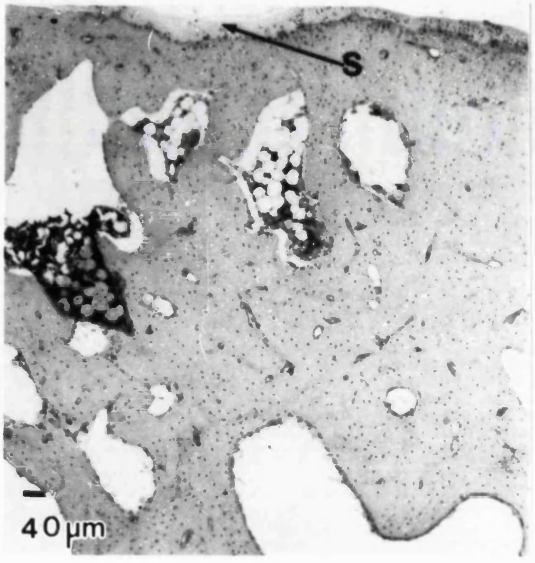


Fig. 3.2.2.2.k.

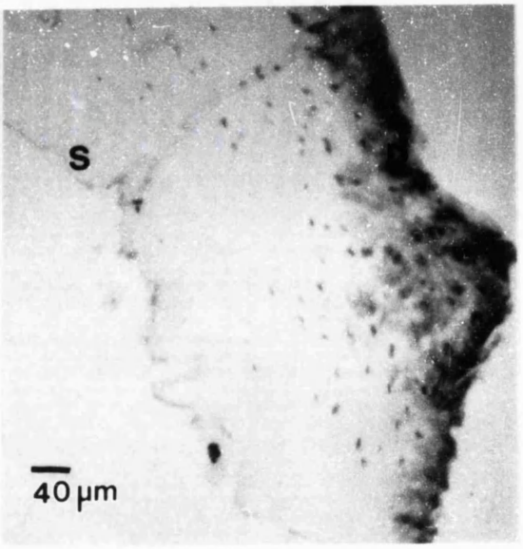


Fig. 3.2.2.2.l.

40 µm

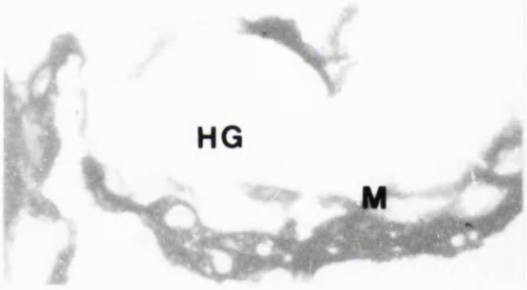


Fig. 3.2.2.3.a.

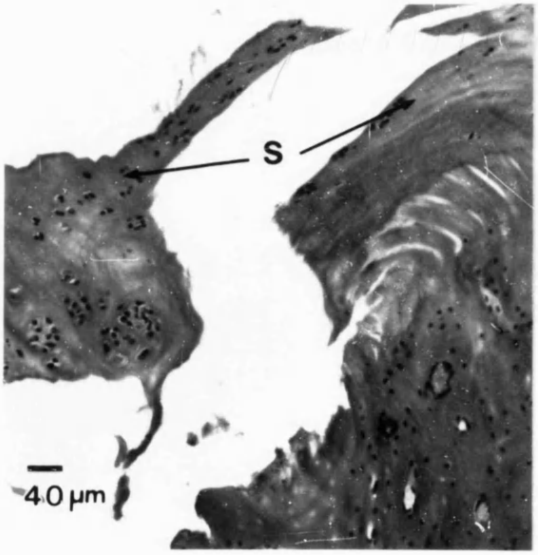


Fig. 3.2.2.3.b.

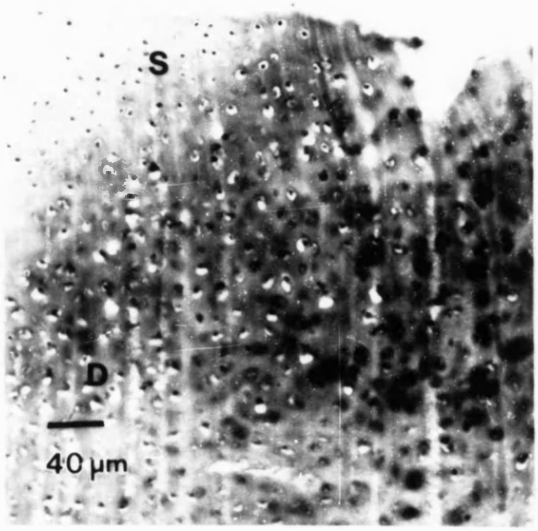


Fig. 3.2.2.3.c.

Fig. 3.2.2.3.d. Photograph of low-power examination of decalcified section of the whole graft region at 3 months post-implantation of the hydrogel PC 97 graft tissue without associated chondrocytes (haematoxylin-eosin, x 100). The host tissue appeared to be split in two parts, a superior and an inferior part. The superior part was only attached to one side to the surrounding cartilage. In the superior part, two layers of cartilage could be observed, namely, a surface layer with elongated cells, and in the deeper layers chondrocytes in a random distribution present both as single cells and cell clusters. The distal part was well incorporated into the bone (S = surface layer of the graft tissue).

Fig. 3.2.2.3.e. Photograph of high-power examination of the host tissue within the graft region at 3 months post-implantation of the hydrogel PC 97 plug without associated chondrocytes (toluidine blue, x 200).The hydrogel PC 97 (HG) was surrounded by weak metachromatic-staining matrix with elongated cells and occasionally discrete areas of rounded-polygonal shaped cells, which showed pericellular metachromatic-staining.

Fig. 3.2.2.3.f. Capsular tissue with hydrogel PC 97 (HG) and presence of foreign body giant cells (F) and histiocytes (see arrow) (haematoxylin-eosin, x 150).

Fig. 3.2.2.3.g. Synovial tissue with parts of the hydrogel PC 97 (HG) (haematoxylin-eosin, x 75). The villous-like structure of the thickened synovium was shown.

Fig. 3.2.2.3.h. Detail of Fig. 3.2.2.3.g. showed synovium and part of hydrogel PC 97 (haematoxylin-eosin, x 175). The villous-like surface layer of the synovium showed hyperplasia and hypertrophy with plump cells.

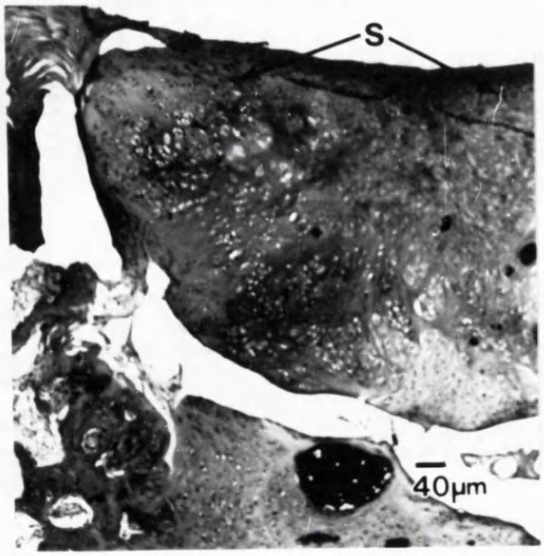


Fig. 3.2.2.3.d.

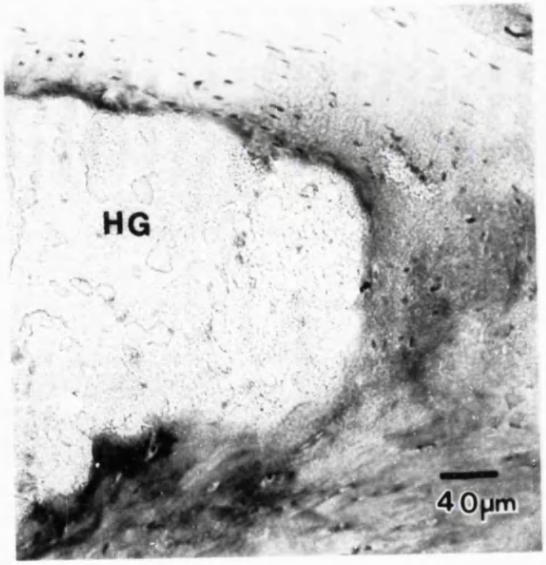


Fig. 3.2.2.3.e.

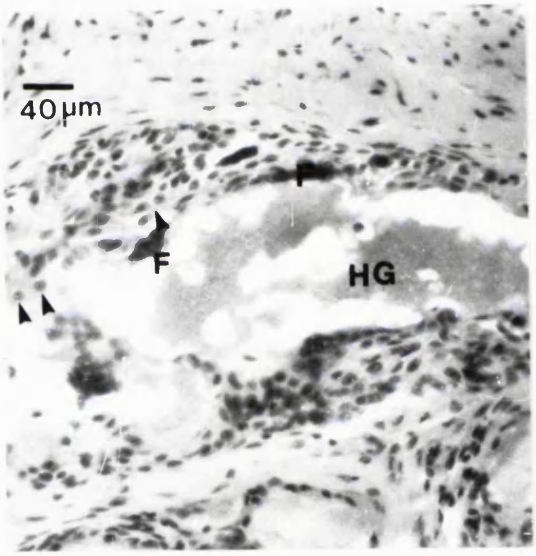


Fig. 3.2.2.3.f.

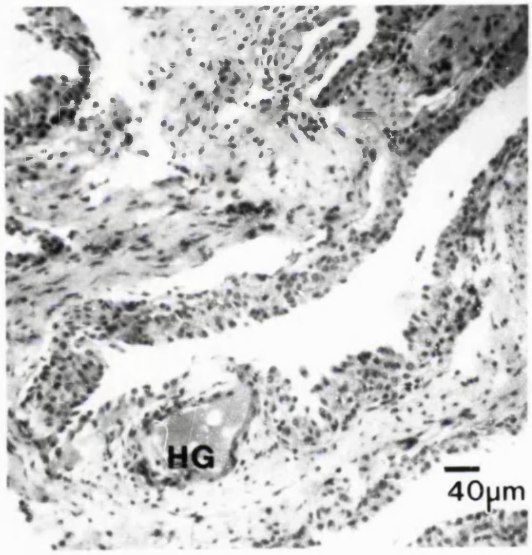


Fig. 3.2.2.3.g.

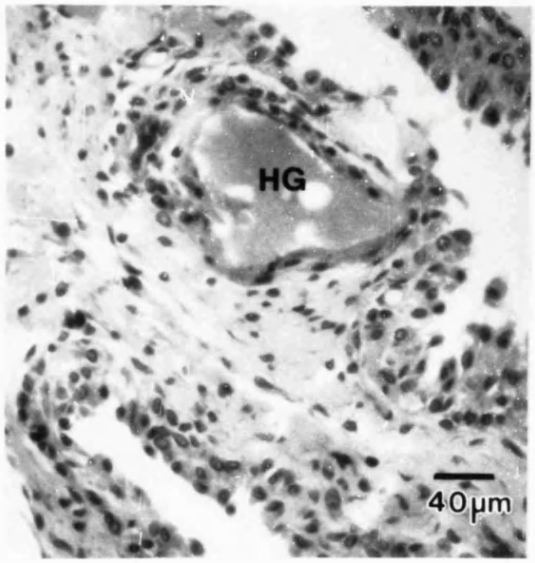


Fig. 3.2.2.3.h.

3.2.3. Scanning and transmission electron microscopical assessment of the grafts.

Electron microscopical assessment of carbon fibre plugs with associated chondrocytes.
Scanning electron micrographs of sections of graft tissue of isolated rabbit articular chondrocytes cultured *in vitro* within a carbon fibre meshwork after 3 weeks culture are shown in Fig. 3.2.3.a.-d. The chondrocytes within the carbon fibre plug produced matrix which was laid down against and between the carbon fibres (Fig 3.2.3.a.+b.). The fine fibrillar nature of the synthesised matrix is shown in Fig. 3.2.3.c. Note how chondrocytes attach to the individual carbon fibres (Fig. 3.2.3.d.).

Electron microscopical assessment of collagen gel plugs with associated chondrocytes.
Transmission electron micrographs of sections of graft tissue of isolated rabbit articular chondrocytes on a collagen gel plug after 7 days *in vitro* culture are shown in Fig. 3.2.3.e.-h. The collagen gel cultures were examined after 7 days *in vitro* and not after 21 days since most comparable literature on cells cultured on or within a collagen gel gives results during the first week *in vitro*. Similar to the results described by Gibson *et al.*, (1982) chondrocytes cultured within a collagen gel maintain a rounded-polygonal configuration, in addition to some elongated cells. Ultrastructurally, the cells show features of active synthesis, for example, abundant, distended rough endoplasmatic reticulum, mitochondria and Golgi vesicles (Fig. 3.2.3.e.-h.).

Electron microscopy assessment of hydrogel PC 97 plugs with associated chondrocytes.
Scanning electron micrographs of sections of graft tissue of isolated rabbit articular chondrocytes within a hydrogel plug after 21 days *in vitro* culture are shown in Fig. 3.2.3.i.-l. The structure of the hydrogel PC 97 is shown in detail in Fig. 3.2.3.i. The pore size varied from 20 μm to 38 μm and some cellular ingrowth into these pores was observed, although the chondrocytes at the margin did not migrate very far into the hydrogel PC 97 (Fig. 3.2.3.k.+l.).
As described above the surrounding synovial and capsular tissues were examined histologically for presence of graft material and the reaction of the synovial and capsular tissues to hydrogel PC 97. Further examination using scanning electron microscopy was carried out.
In order to confirm my interpretation of the wax sections (3.2.2.3. above), two sections were processed in tandem for wax and scanning electron microscopical examination, respectively. One section was stained with haematoxylin-eosin, whilst the other was dehydrated up to 70% alcohol and air-dried. Subsequently, the air-dried section was processed as described for the hydrogel PC 97 plug in chapter 2.2.3. The two tissue sections were compared and the foreign material was found (Fig. 3.2.3.m.-o.). Because of the similarities in physical structure and pore size of the foreign material in the

synovial and capsular tissues (Fig. 3.2.3.p.) and of the hydrogel PC 97 (Fig. 3.2.3.i.), it was concluded that the foreign material was hydrogel PC 97.

Fig. 3.2.3.a. Scanning electron micrograph of the interaction between chondrocyte and carbon fibre after 3 weeks *in vitro* culture (x 150). The chondrocytes within the carbon fibre plug (CF) produced matrix which was laid down against the carbon fibres.

Fig. 3.2.3.b. Scanning electron micrograph of the interaction between chondrocyte and carbon fibre after 3 weeks *in vitro* culture (x 240). The chondrocytes within the carbon fibre plug (CF) produced matrix (M) which was laid down between the carbon fibres.

Fig. 3.2.3.c. Scanning electron micrograph of the interaction between chondrocyte and carbon fibre after 3 weeks *in vitro* culture (x 2 500). The fine fibrillar nature of the produced matrix (M) is shown (CF = carbon fibre).

Fig. 3.2.3.d. Scanning electron micrograph showing attachment of the chondrocyte to the individual carbon fibre (CF) (x 2 500).

Fig. 3.2.3.e. Transmission electron micrograph of chondrocytes cultured on a collagen gel after 7 days *in vitro* culture (x 4 000). Three chondrocytes are shown with little matrix.

Fig. 3.2.3.f. Transmission electron micrograph of chondrocytes cultured on a collagen gel after 7 days *in vitro* culture (x 10 000). More detailed view of the chondrocytes showing some organelles: mitochondria (M), lipid drops (D) and vacuoles (V). (N = nucleus).

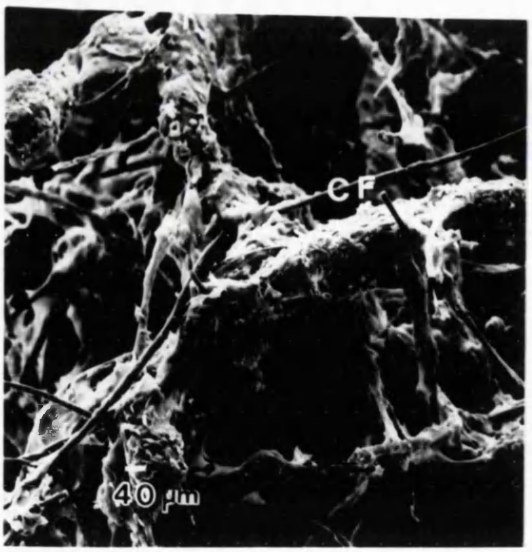


Fig. 3.2.3.a.

Fig. 3.2.3.b.

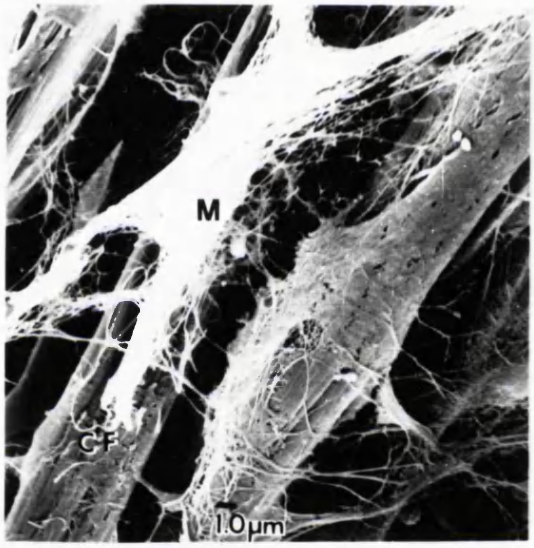


Fig. 3.2.3.c.

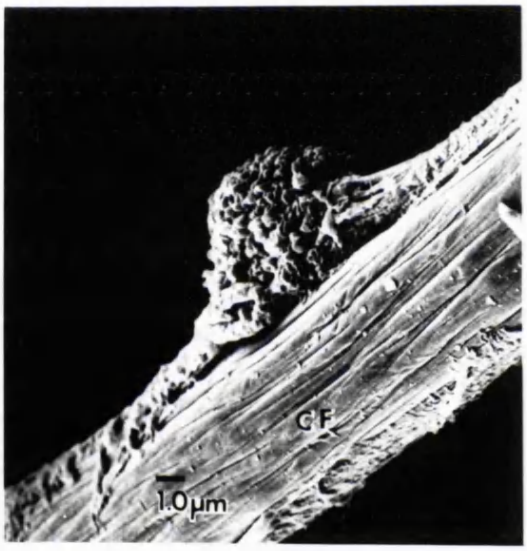


Fig. 3.2.3.d.

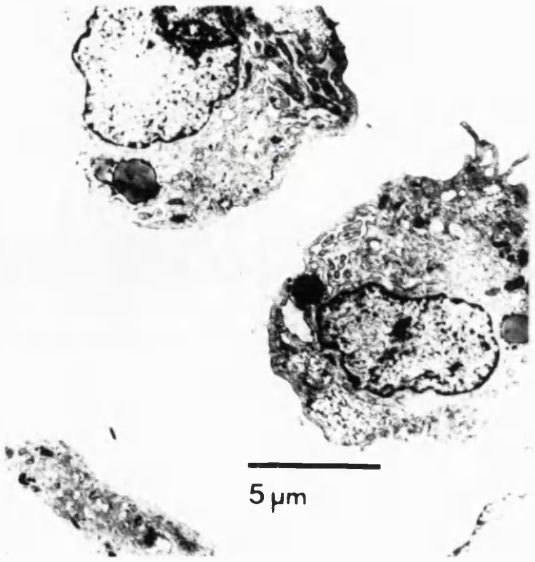


Fig. 3.2.3.e.

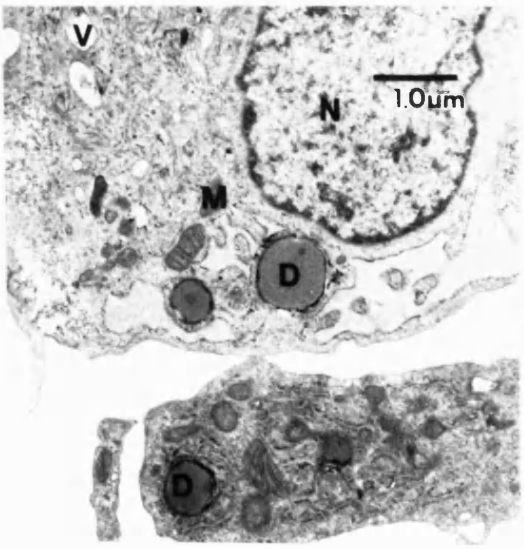


Fig. 3.2.3.f.

Fig. 3.2.3.g. Transmission electron micrograph of chondrocytes cultured on a collagen gel after 7 days *in vitro* culture (x 37 500). Ultrastructure of the cells showed the presence of endoplasmatic reticulum (E). (N = nucleus).

Fig. 3.2.3.h. Transmission electron micrograph of chondrocytes cultured on a collagen gel after 7 days *in vitro* culture (x 18 000). Ultrastructure of the cells showed widened cisternae of endoplasmatic reticulum (E) and mitochondria (M). (N = nucleus).

Fig. 3.2.3.i. Scanning electron micrograph of section of a hydrogel PC 97 plug prior to use (x 2 500). The structure of the hydrogel PC 97 (HG) can be seen in more detail. The pore size varies from 20 μm to 38 μm (D = dextrin).

Fig. 3.2.3.k. Scanning electron micrograph of section of graft tissue of isolated rabbit articular chondrocytes within a hydrogel plug after 21 days *in vitro* culture (x 550). This showed some ingrowth of the chondrocytes into the pores of the hydrogel (S = surface of the graft tissue).

Fig 3.2.3.l. Scanning electron micrograph of section of graft tissue of isolated rabbit articular chondrocytes within a hydrogel plug after 21 days *in vitro* culture (x 500). This gave more detailed view of the surface layers (S) of the graft tissue showing the presence of matrix at the margins of the hydrogel PC 97 plug.

Fig. 3.2.3.m. Photograph of wax-embedded section of synovial and capsular tissues at 3 months after implantation of hydrogel PC 97 plug (haematoxylin-eosin, x 150). A foreign material (hydrogel PC 97) was found.

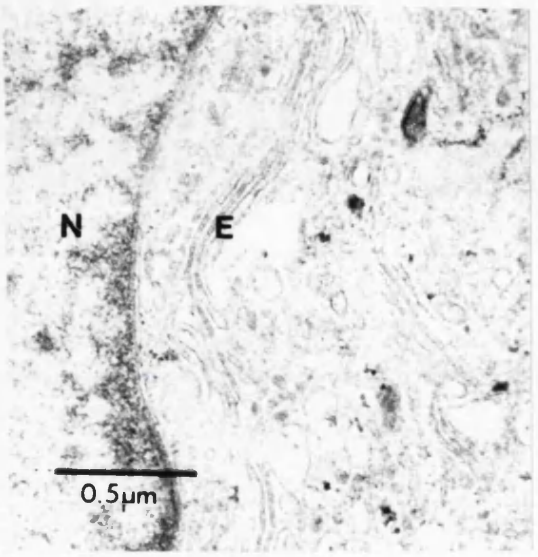


Fig. 3.2.3.g.

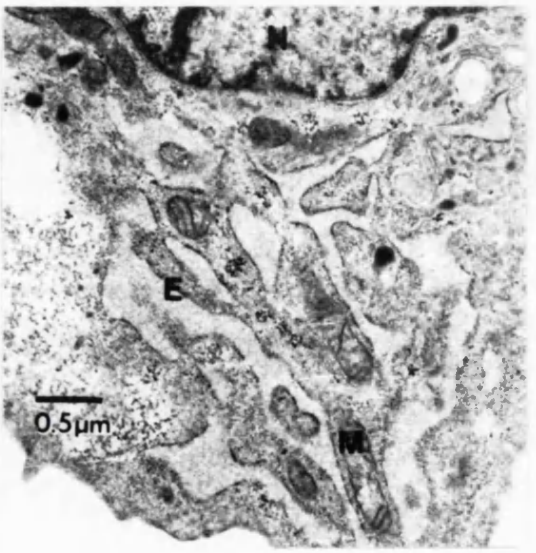


Fig. 3.2.3.h.

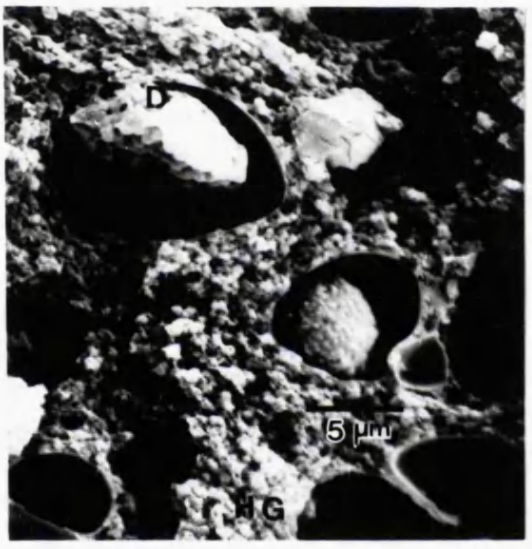


Fig. 3.2.3.i.

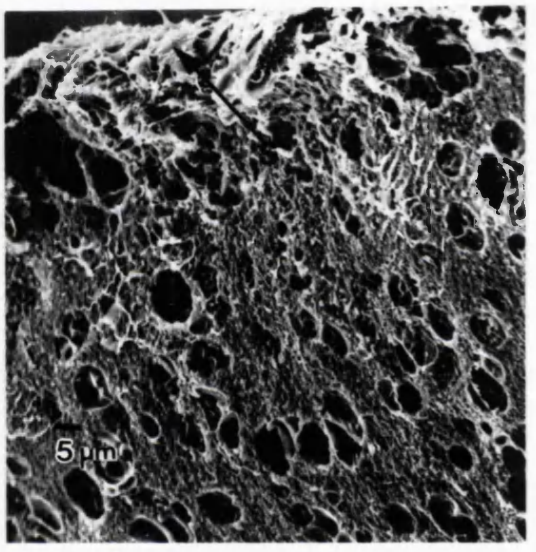


Fig. 3.2.3.k.

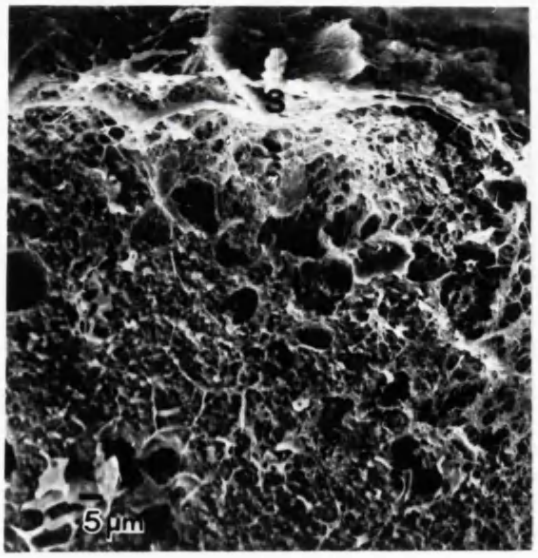


Fig. 3.2.3.l.

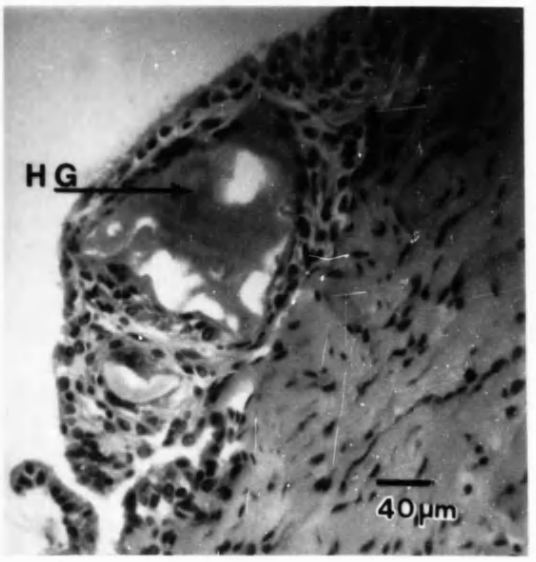


Fig. 3.2.3.m.

Fig. 3.2.3.n. Scanning electron micrograph of wax-embedded section of synovial and capsular tissues at 3 months after implantation of hydrogel PC 97 plug (x 125). This section was similar to the one shown in Fig. 3.2.3.m., but it was processed for SEM and hydrogel PC 97 (see arrows) was found.

Fig. 3.2.3.o. Scanning electron micrograph of wax-embedded section of synovial and capsular tissues at 3 months after implantation of hydrogel PC 97 plug (x 1 000). It showed the porous structure of the hydrogel PC 97 present in the synovial and capsular tissues.

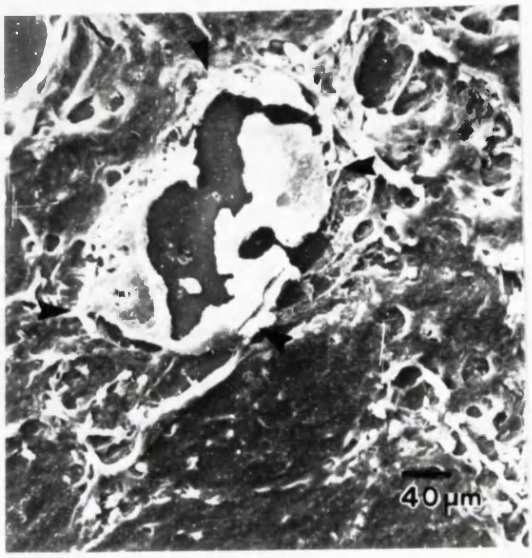


Fig. 3.2.3.n.

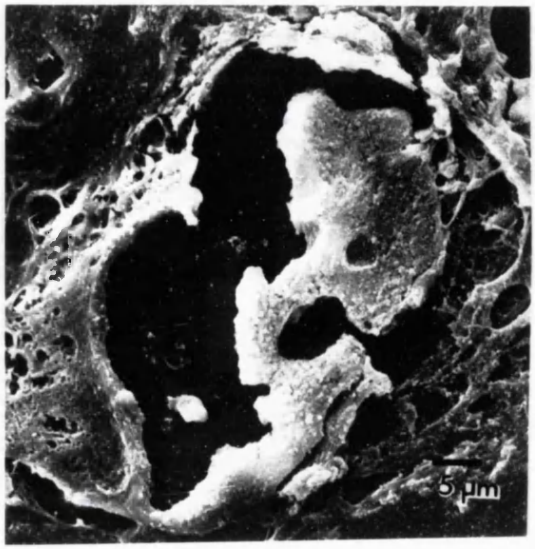


Fig. 3.2.3.o.

3.2.4. Immunocytochemical assessment of the grafts.

Immunocytochemical assessment of carbon fibre plugs with associated chondrocytes.
Phase contrast micrographs and fluorescence micrographs of isolated rabbit articular chondrocytes cultured *in vitro* within a carbon fibre meshwork after 3 weeks culture are shown in Fig. 3.2.4.1. 01-12.
The tissue at 3 weeks *in vitro* culture showed the presence of collagen type II (Fig. 3.2.4.1.01 + 02) and chondroitins -4- and -6- sulphates (Fig. 3.2.4.1.03-06). Collagen type I-positive staining was not detected (Fig. 3.2.4.1.07 + 08). Keratan sulphate-staining was found pericellularly and around some carbon fibres (Fig. 3.2.4.1.09 + 10). Control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.1.11 + 12).

Phase contrast micrographs and fluorescence micrographs of graft tissue at 3 months post-implantation are shown in Fig. 3.2.4.1. 13-24.
At 3 months post-implantation staining for collagen type II was found in the deep graft tissue directly around the carbon fibres and in localised areas of tissue, and this was in contrast to the superficial graft tissue where it was present throughout the matrix (Fig. 3.2.4.1.13 + 14). Immuno-positive staining for chondroitin -4- sulphate was shown mainly pericellularly in the superficial and deep regions of the graft tissue (Fig. 3.2.4.1.15 + 16). A difference in staining pattern was found in the distribution of chondroitin -6- sulphate - in the superficial graft tissue it was found throughout the matrix (Fig. 3.2.4.1.17 + 18), whilst in the deep graft tissue it was located pericellularly. Staining for collagen type I was found in a few localised areas in the superficial and deep graft tissue (Fig. 3.2.4.1.19 + 20). Immuno-positive staining for keratan sulphate was detected intercellularly in the superficial (Fig. 3.2.4.1.21 + 22) and pericellularly in the deep region. Control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining
(Fig. 3.2.4.1.23 + 24).

Phase contrast micrographs and fluorescence micrographs of host tissue within the graft region of the control knee joint at 3 months post-implantation are shown in
Fig. 3.2.4.1. 25-36.
Only in a few circumscribed areas of the host tisssue within the graft region of the control knee showed positive staining for collagen type II, chondroitins -4- and -6-sulphates and keratan sulphate found. Collagen type II-staining was found pericellularly (Fig. 3.2.4.1.25+26). Similarly, immuno-positive staining for chondroitins -4- and -6-sulphates was also located pericellularly (Fig. 3.2.4.1.27-30). The host tissue within the graft region of the control knee joint at 3 months post-implantation showed in the superficial region immuno-positive staining for collagen type I throughout the matrix

(Fig. 3.2.4.1.31+32). Keratan sulphate-staining was found mainly around the carbon fibres (Fig. 3.2.4.1.33+34). In the deep region of the host tissue within the graft region of the control knee joint, a similar pattern of distribution as in the superficial region was detected, but there were even less areas of immuno-positive staining for collagen type II, chondroitins -4- and -6- sulphates and keratan sulphate. As in the superficial host tissue within the graft region, staining for collagen type II, chondroitins -4- and -6- sulphates and keratan sulphate was present pericellularly in discrete areas. Immuno-positive staining for collagen type I was distributed widely through the matrix. Control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.1.35+36).

Phase contrast micrographs and fluorescence micrographs of graft tissue at 6 months post-implantation are shown in Fig. 3.2.4.1. 37-48.
At 6 months post-implantation collagen type II-staining was found around the carbon fibres in some areas in the surface layers (Fig. 3.2.4.1.37+38), but in the deep layers positive staining was seen around the carbon fibres and intercellularly. Staining for chondroitin -4- sulphate was found around some carbon fibres and pericellularly in some areas of the surface layers (Fig. 3.2.4.1.39+40). Chondroitin-6-sulphate-positive staining was found in a few areas of the surface layers (Fig. 3.2.4.1.41+42), but not in the deep layers. Collagen type I-staining was occasionally observed in discrete areas of the graft tissue (Fig. 3.2.4.1.43+44). Immuno-positive staining for keratan sulphate in the superficial graft tissue was shown peri- and inter-cellularly (Fig. 3.2.4.1.45+46). In contrast, immuno-positive staining for keratan sulphate was not detected in the deep zone. Control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.1.47+48).

Phase contrast micrographs and fluorescence micrographs of host tissue within the graft region of the control knee joint at 6 months post-implantation are shown in
Fig. 3.2.4.1. 49-60.
At 6 months post-implantation collagen type II-staining was only found around some carbon fibres in both surface and deep layers (Fig. 3.2.4.1.49+50). Staining for chondroitin -4- sulphate in the superficial control graft layers was found pericellularly and around carbon fibres (Fig. 3.2.4.1.51+52). Staining for chondroitin -6- sulphate in the superficial control graft layers was found intercellularly and around carbon fibres
(Fig. 3.2.4.1.53+54). Collagen type I-staining was shown around the carbon fibres in the surface layers (Fig. 3.2.4.1.55+56), but was not present in deep layers. Immunopositive staining for keratan sulphate of the superficial and deep host tissue within the graft region of the control knee joint was shown pericellularly (Fig. 3.2.4.1.57+58). Control sections substituting the primary antibody for PBS or myeloma

medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.1.59+60).

Phase contrast micrographs and fluorescence micrographs of graft tissue at 12 months post-implantation are shown in Fig. 3.2.4.1. 61-72.
At 1 year post-implantation collagen II-positive staining was only found pericellularly in a few areas in surface and deep layers (Fig. 3.2.4.1.61+62). Staining for chondroitin -4-sulphate in the surface layers was seen either pericellular (on most sections, carbon fibres were lost during processing)(Fig. 3.2.4.1.63+64) or it was undetected, whilst in the deep layers staining was shown pericellularly and around the carbon fibres. Chondroitin-6-sulphate-positive staining was found pericellularly in the surface layers of some, but not all, grafts (Fig. 3.2.4.1.65+66). However, in the deep layers it was found around the carbon fibres and pericellularly in about half of the sections. Collagen type I-positive staining was found only in a few localised areas of surface and deep layers (Fig. 3.2.4.1.67+68). Immuno-positive staining for keratan sulphate of the superficial graft tissue was detected mainly pericellularly, and was also found inter-cellularly (Fig. 3.2.4.1.69+70), but was not detected in the deep zone tissue. Control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.1.71+72).

Phase contrast micrographs and fluorescence micrographs of host tissue within the graft region of the control knee joint at 12 months post-implantation are shown in
Fig. 3.2.4.1. 73-84.
At 1 year post-implantation immuno-positive staining for collagen type II was not seen in either layer of the graft (Fig. 3.2.4.1.73+74). Weak staining for chondroitin -4- sulphate pericellularly and/or intercellularly in the surface and deep layers was detected
(Fig. 3.2.4.1.75+76). Chondroitin-6-sulphate-positive staining was found superficially around the carbon fibres (Fig. 3.2.4.1.77+78), but in the deep layers it was not detected. Collagen type I-positive staining was found throughout the tissue of the surface and deep layers (Fig. 3.2.4.1.79+80). Immuno-positive staining for keratan sulphate of the superficial and deep layers of the host tissue within the graft region of the control knee joint was located intercellularly (Fig. 3.2.4.1.81+82). Control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.1.83 +84).
The above data for carbon fibre plugs are summarized in Tables 3.9 and 3.10.

CARBON FIBRE PLUG WITH CELLS	3 WEEKS	3 MONTHS	6 MONTHS	1 2 MONTHS
COLLAGEN I I	++	++	++ (S) around the carbon fibres (D) around the carbon fibres and intercellular	+
CHONDROI-TIN-4-SULPHATE	++	++ pericellular	++ around carbon fibres and pericellular	-/++ (S) pericellular ++ (D) pericellular and around carbon fibres
CHONDROI-TIN-6-SULPHATE	++	++ (S) throughout the tissue (D) pericellular	+(S) - (D)	-/++ (S) pericellular ++ (D) pericellular and around carbon fibres
COLLAGEN I	-	+	+	+
KERATAN SULPHATE	++ pericellularly and around carbon fibres	++ (S) intercellular (D) pericellular	++ (S) peri- and intercellular - (D)	++ (S) peri-cellular - (D)

INDEX

(S) surface layer of the plug.
(D) deep layer of the plug.
- no staining.
+ occasional positive staining in discrete areas of the repair tissue.
++ positive staining throughout the repair tissue.
-/++ on some sections positive staining, on others no staining.

Table 3.9. Immunocytochemistry of carbon fibre plugs with chondrocytes at 3 weeks, 3, 6 and 12 months post-implantation. The used antibodies recognise epitope(s) on collagen types I and II, chondroitins -4- and -6- sulphates or keratan sulphate.

CARBON FIBRE PLUG WITHOUT CELLS	3 MONTHS	6 MONTHS	1 2 MONTHS
COLLAGEN I I	+	++ around carbon fibres	-
CHONDROI-TIN-4-SULPHATE	+	++ pericellular and around carbon fibres	++/- weak peri- and inter-cellularly
CHONDROI-TIN-6-SULPHATE	+	++ intercellular and around carbon fibres	++ (S) around carbon fibres - (D)
COLLAGEN I	++ throughout the tissue	++ (S) around carbon fibres - (D)	++ throughout the tissue
KERATAN SULPHATE	+	++ pericellular	+

INDEX

(S) surface layer of the plug.
(D) deep layer of the plug.
- no staining.
+ occasional positive staining in discrete areas of the repair tissue.
++ positive staining throughout the repair tissue.
-/++ on some sections positive staining, on others no staining.

Table 3.10. Immunocytochemistry of carbon fibre plugs without chondrocytes at 3, 6 and 12 months post-implantation. The used antibodies recognise epitope(s) on collagen types I and II, chondroitins -4- and -6- sulphates and keratan sulphate.

Immunocytochemical assessment of collagen gel plugs with associated chondrocytes.

Phase contrast micrographs and fluorescence micrographs of isolated rabbit articular chondrocytes cultured *in vitro* on a collagen gel after 3 weeks culture are shown in Fig. 3.2.4.2. 01-12.

After 3 weeks culture, immuno-positive staining for collagen type II was found pericellularly (Fig. 3.2.4.2.01+02). Staining for chondroitin -4- sulphate was found intercellularly in some areas of the tissue (Fig. 3.2.4.2.03+04), whilst chondroitin -6-sulphate-positive staining was only present in a few areas of the tissue (Fig. 3.2.4.2.05+06). Immuno-positive staining for collagen I was found throughout the tissue (Fig. 3.4.2.07+08). Keratan sulphate staining was located throughout the tissue (Fig. 3.4.2.09+10). Control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.2.11+12).

Phase contrast micrographs and fluorescence micrographs of graft tissue at 3 months post-implantation are shown in Fig. 3.2.4.2. 13-24.

At 3 months post-implantation, weak intercellular staining for collagen type II was found in the surface layer (Fig. 3.2.4.2.13+14), but was not present in the deep layers. Chondroitin-4-sulphate-positive staining was not found in the deep layers of the graft, but was detected in the superficial layers (Fig. 3.2.4.2.15+ 16). Staining for chondroitin -6- sulphate was found in surface and deep layers both inter- and peri-cellularly (Fig. 3.2.4.2.17+18). Collagen type I-positive staining was detected in the superficial and deep layers of the graft tissue, but was discretely located (Fig. 3.2.4.2.19+20). Immuno-positive staining for keratan sulphate was seen in the superficial layers intercellularly (Fig. 3.2.4.2.21+22), but was not found in the deep layers. Control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.2.23+24).

Phase contrast micrographs and fluorescence micrographs of host tissue within the graft region of the control knee joint at 3 months post-implantation are shown in Fig. 3.2.4.2. 25-36.

At 3 months post-implantation, collagen type II-staining was occasionally observed in discrete areas in the surface and deep layers of the host tissue within the graft region of the control knee joint (Fig. 3.2.4.2.25+26). Chondroitin-4-sulphate-positive staining was not detected in the deep layers, but was observed in the superficial layers intercellularly in most areas (Fig. 3.2.4.2.27-28). Staining for chondroitin -6- sulphate was not found in surface layers (Fig. 3.2.4.2.29-30), but in the deep layers was located pericellularly. Staining for collagen type I in the surface and deep layers was located intercellularly (Fig. 3.2.4.2.31+32). Immuno-positive staining for keratan sulphate was not detected in either surface or deep layers (Fig. 3.2.4.2.33+34). Control sections

substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.2.35+36).

Phase contrast micrographs and fluorescence micrographs of graft tissue at 6 months post-implantation are shown in Fig. 3.2.4.2. 37-48.
At 6 months post-implantation immuno-positive staining for collagen type II was not detected in either surface or deep layers (Fig. 3.2.4.2.37+38). Chondroitin-4-sulphate-positive staining was found pericellularly in some areas of the deep layers, but was not present in others. Similarly, in the centre of the surface layers of the graft tissue, pericellular immuno-positive staining was observed, but it was not detected in other areas (Fig. 3.2.4.2.39+40). Chondroitin-6-sulphate immuno-positivity was largely pericellular in both tissue layers, but was also located intercellularly in the superficial graft tissue (Fig. 3.2.4.2.41+42). Collagen type I-positive staining was not detected in surface or deep layers (Fig.3.2.4.2.43+44). Immuno-positive staining for keratan sulphate was only seen pericellularly in the surface layers (Fig. 3.2.4.2.45+46) and also intercellularly in a few areas of the deep graft tissue. Control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.2.47+48).

Phase contrast micrographs and fluorescence micrographs of host tissue within the graft region of the control knee joint at 6 months post-implantation are shown in Fig. 3.2.4.2. 49-60.
At 6 months post-implantation, collagen type II-staining was not found in the surface layers (Fig.3.2.4.2.49+50), but was present both peri- and intercellularly in a few areas of the deep layers. Chondroitin-4-sulphate-positive staining was found pericellularly in both the surface and deep layers (Fig. 3.2.4.2.51+52). Weak staining for chondroitin -6-sulphate was also found in surface and deep layers pericellularly (Fig. 3.2.4.2.53+54). The superficial and deep layers of the host tissue within the graft region were negative for collagen type I-immuno-reactivity (Fig.3.2.4.2.55+56). Immuno-positive staining for keratan sulphate was not detected (Fig.3.2.4.2.57+58). Control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.2.59+60).

Phase contrast micrographs and fluorescence micrographs of graft tissue at 12 months post-implantation are shown in Fig. 3.2.4.2. 61-72.
At 1 year post-implantation collagen II-positive staining was observed occasionally intercellularly in discrete areas of the surface graft tissue (Fig. 3.2.4.2.61+62). In the deep layers, collagen type II-staining was not detected. Chondroitin-4-sulphate positive staining was found pericellularly in both the surface and deep layers of the graft (Fig. 3.2.4.2.63+64). Immuno-positivity for chondroitin -6- sulphate was located

pericellularly in surface and deep layers (Fig. 3.2.4.2.65+66). The superficial graft tissue was positive in a few circumscribed areas for collagen type I (Fig. 3.2.4.2.67+68), whilst the deep graft tissue showed weak intercellular staining with collagen type I. Immuno-positive staining for keratan sulphate was not found in superficial layers (Fig. 3.2.4.2.69+70), but was present in the deep layers intercellularly. Control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.2.71+72).

Phase contrast micrographs and fluorescence micrographs of host tissue within the graft region of the control knee joint at 12 months post-implantation are shown in Fig. 3.2.4.2. 73-84.

At 1 year post-implantation, all layers of the host tissue within the graft region were negative for collagen type II (Fig. 3.2.4.2.73+74). Chondroitin-4-sulphate-positive staining was found pericellularly in a few areas only (Fig. 3.2.4.2.75+76). However, immuno-positivity for chondroitin -6- sulphate was found intercellularly in superficial and deep layers (Fig. 3.2.4.2.77+78). Immuno-positive staining for collagen type I was not detected in either layers (Fig. 3.2.4.2.79+80), whilst staining for keratan sulphate was located only in the superficial layers of the tissue (Fig. 3.2.4.2.81+82). Control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.2.83+84).

The above data for collagen gel grafts are summarized in Tables 3.11 and 3.12.

COLLAGEN GEL PLUG WITH CELLS	3 WEEKS	3 MONTHS	6 MONTHS	1 2 MONTHS
COLLAGEN I I	++ pericellular	++ (S) weak inter-cellular - (D)	-	+ (S) - (D)
CHONDROI-TIN-4-SULPHATE	++ intercellular	++ (S) pericellular - (D)	+	++ pericellular
CHONDROI-TIN-6-SULPHATE	+	++ peri- and intercellular	++ pericellular	++ pericellular
COLLAGEN I	++ intercellular	+	-	+
KERATAN SULPHATE	++ pericellular	++,(S) inter-cellular -, (D)	++ (S) pericellular + (D) intercellular	- (S) ++ (D) intercellular

INDEX

(S) surface layer of the plug.
(D) deep layer of the plug.
- no staining.
+ occasional positive staining in discrete areas of the repair tissue.
++ positive staining throughout the repair tissue.
-/++ on some sections positive staining, on others no staining.

Table 3.11. Immunocytochemistry of collagen gel plugs with chondrocytes at 3 weeks, 3, 6 and 12 months post-implantation. The used antibodies recognise epitope(s) on collagen types I and II, chondroitins -4- and -6- sulphates and keratan sulphate.

COLLAGEN GEL CONTROL PLUG	3 MONTHS	6 MONTHS	12 MONTHS
COLLAGEN II	+	- (S) + (D)	-
CHONDROITIN-4-SULPHATE	++ (S) intercellular - (D)	++ pericellular	+
CHONDROITIN-6-SULPHATE	- (S) ++ (D)	++ weak pericellular	++ intercellular
COLLAGEN I	++ intercellular	-	-
KERATAN SULPHATE	-	-	++ (S) intercellular - (D)

INDEX

(S) surface layer of the plug.
(D) deep layer of the plug.
- no staining.
+ occasional positive staining in discrete areas of the repair tissue.
++ positive staining throughout the repair tissue.
-/++ on some sections positive staining, on others no staining.

Table 3.12. Immunocytochemistry of collagen gel control plugs at 3, 6 and 12 months post-implantation. The used antibodies recognise epitope(s) on collagen types I and II, chondroitins -4- and -6- sulphates and keratan sulphate.

Immunocytochemical assessment of hydrogel PC 97 plugs with associated chondrocytes.

Because it was not the intention to culture chondrocytes within the hydrogel PC 97, but only at the margins, the results of immunocytochemistry of the hydrogel PC 97 plug after 3 weeks *in vitro* culture would not give any additional information and was not carried out for that reason.

Phase contrast micrographs and fluorescence micrographs of graft tissue at 3 months post-implantation are shown in Fig. 3.2.4.3. 01-12.

At 3 months post-implantation immuno-positive staining for collagen type II was seen in some areas of the surface layers of the graft tissue intercellularly (Fig. 3.2.4.3.01+02), but no staining was seen in the deep layers. Immuno-positive staining for chondroitin -4- sulphate and chondroitin -6- sulphate was not detected in either surface nor deep layers of the graft (Fig. 3.2.4.3.03-06). Similarly, collagen type I positive staining was not detected in superficial nor deep layers (Fig. 3.2.4.3.07+08). Immuno-positive staining for keratan sulphate was, however, found in some areas of the surface (Fig. 3.2.4.3.09+10), but not in the deep layers of the graft tissue. Control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (Fig. 3.2.4.3.11+12).

The above data for hydrogel PC 97 grafts are summarized in Table 3.13.

The host tissue within the graft region at 3 months post-implantation showed in the superficial and deep regions of the graft tissue no staining for any of the antibodies used.

For comparative purposes the phase contrast micrographs and fluorescence micrographs of sections of normal tibial articular cartilage of 24 months old Sandy Half Lop rabbits are shown in Fig. 3.2.4.3. 13-20. This showed that collagen type II-staining was found pericellularly and weak immuno-positivity was also observed in the interterritorial matrix (Fig. 3.2.4.3.13+14). Immuno-positivity for chondroitins -4- and -6- sulphates was detected pericellularly (Fig. 3.2.4.3.15+18). Keratan sulphate positive staining was not found in the surface layer of the cartilage, but was present intercellularly in the deeper layers (Fig. 3.2.4.3.19-20). Collagen type I-staining was not detected (data not shown) and control sections substituting the primary antibody for PBS or myeloma medium from a non-IgG secreting cell line showed no immuno-positive staining (data not shown).

HYDROGEL PLUG WITH CELLS	3 MONTHS
COLLAGEN II	++ (S) intercellular - (D)
CHONDROITIN-4-SULPHATE	-
CHONDROITIN-6-SULPHATE	-
COLLAGEN I	-
KERATAN SULPHATE	++ (S) intercellular - (D)

INDEX

(S) surface layer of the plug.
(D) deep layer of the plug.
- no staining.
+ occasional positive staining in discrete areas of the repair tissue.
++ positive staining throughout the repair tissue.
-/++ on some sections positive staining, on others no staining.

Table 3.13. Immunocytochemistry of hydrogel PC 97 plugs with chondrocytes at 3 months post-implantation. The used antibodies recognise epitope(s) on collagen types I and II, chondroitins -4- and -6- sulphates and keratan sulphate.

Silver enhancement immunocytochemical assessment of the graft tissue.

Using Silverenhancement immunocytochemistry, carbon fibre grafts plus chondrocytes were examined for the presence of collagen type X, keratan sulphate and 7D4 -epitope, 1 year post-implantation. Photomicrographs of sections of graft tissue at 12 months post-implantation are shown in Fig. 3.2.4.4. 01.-08.

Type X collagen was present in calcifying zone of surrounding cartilage outside the defect (Fig 3.2.4.4.02.), but not within the graft tissue (Fig 3.2.4.4.01.).

Keratan sulphate immuno-positivity was detected pericellularly, particularly in the surface graft tissue (Fig. 3.2.4.4.03.). The surrounding cartilage showed peri- and intercellularly staining in surface and deeper layers (Fig. 3.2.4.4.0.04).

7D4 immuno-positivity was detected in both the surface and calcifying zone of the graft tissue and was similarly distributed in the surrounding host cartilage (Fig. 3.2.4.4.05+06). In all the control sections staining was not detected (Fig. 3.2.4.4.07.-08.).

Fig. 3.2.4.1.01. Phase contrast micrograph of graft tissue at 3 weeks pre-implantation .

Fig. 3.2.4.1.02. Fluorescence micrograph of graft tissue at 3 weeks pre-implantation. Collagen type II-staining was found throughout the tissue.

Fig. 3.2.4.1.03. Phase contrast micrograph of graft tissue at 3 weeks pre-implantation.

Fig. 3.2.4.1.04. Fluorescence micrograph of graft tissue at 3 weeks pre-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown throughout the tissue.

Fig. 3.2.4.1.05. Phase contrast micrograph of graft tissue at 3 weeks pre-implantation.

Fig. 3.2.4.1.06. Fluorescence micrograph of graft tissue at 3 weeks pre-implantation. Immuno-positive staining for chondroitin -6- sulphate was found throughout the tissue.

Fig. 3.2.4.1.07. Phase contrast micrograph of graft tissue at 3 weeks pre-implantation.

Fig. 3.2.4.1.08. Fluorescence micrograph of graft tissue at 3 weeks pre-implantation. Collagen type I-staining was not detected.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

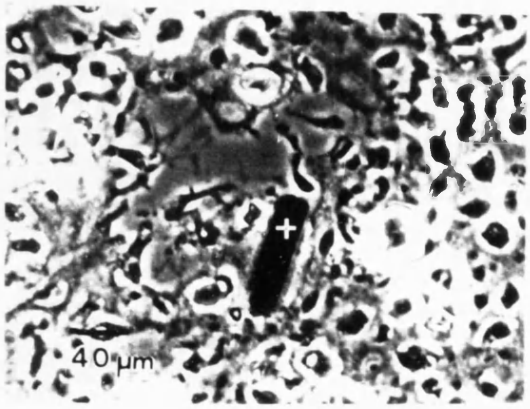


Fig. 3.2.4.1.01

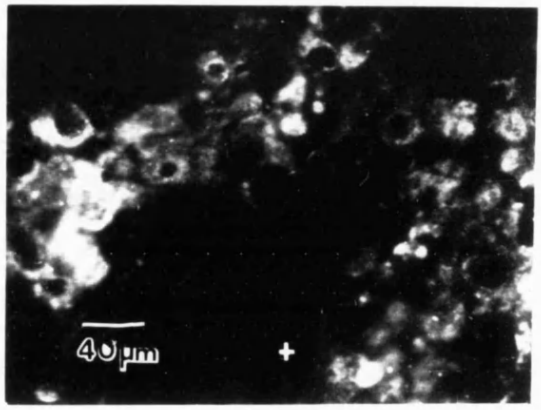


Fig. 3.2.4.1.02

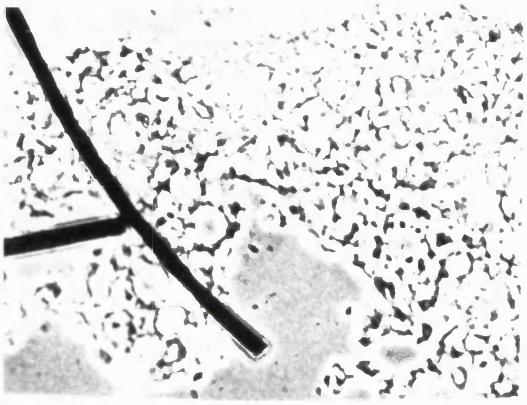


Fig. 3.2.4.1.03

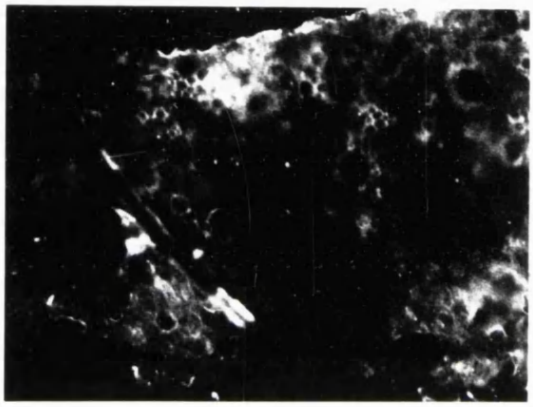


Fig. 3.2.4.1.04

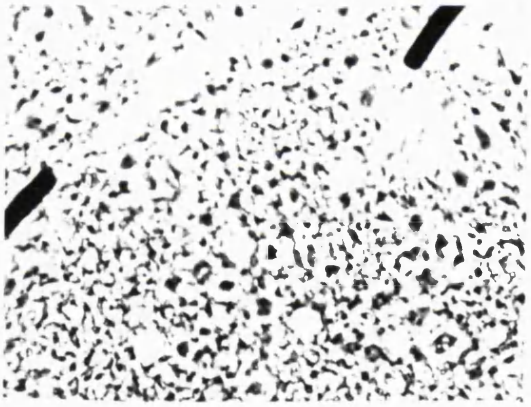


Fig. 3.2.4.1.05

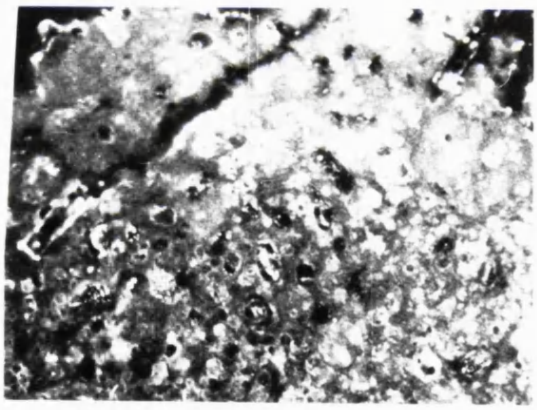


Fig. 3.2.4.1.06

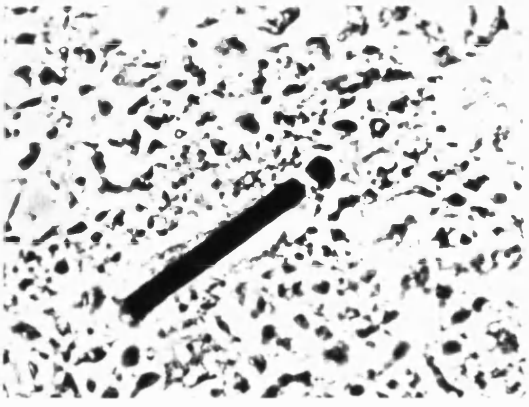


Fig. 3.2.4.1.07

Fig. 3.2.4.1.08

Fig. 3.2.4.1.09. Phase contrast micrograph of graft tissue at 3 weeks pre-implantation.
Fig. 3.2.4.1.10. Fluorescence micrograph of graft tissue at 3 weeks pre-implantation. Keratan sulphate-staining was found pericellularly and around some fibres.

Fig. 3.2.4.1.11. Phase contrast micrograph of graft tissue at 3 weeks pre-implantation.
Fig. 3.2.4.1.12. Fluorescence micrograph of graft tissue at 3 weeks pre-implantation. The control only showed background staining.

Fig. 3.2.4.1.13. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation .
Fig. 3.2.4.1.14. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. Collagen type II-staining was found throughout the matrix.

Fig. 3.2.4.1.15. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.
Fig. 3.2.4.1.16. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown mainly pericellularly.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

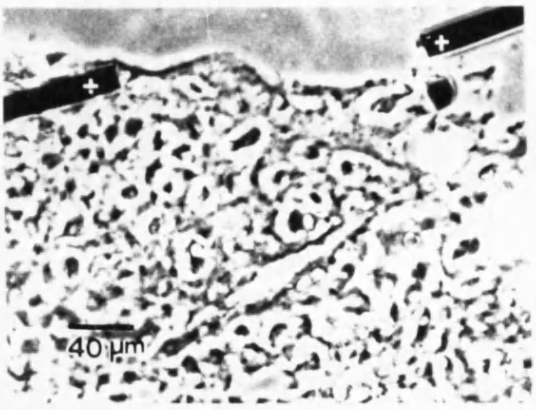


Fig. 3.2.4.1.09.

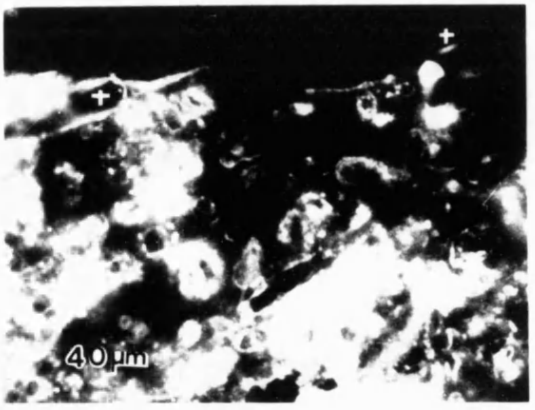


Fig. 3.2.4.1.10.

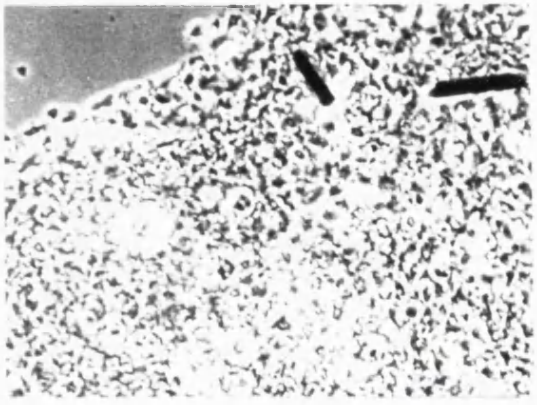


Fig. 3.2.4.1.11.

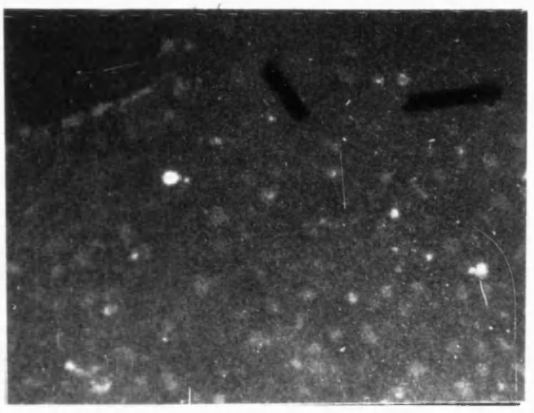


Fig. 3.2.4.1.12.

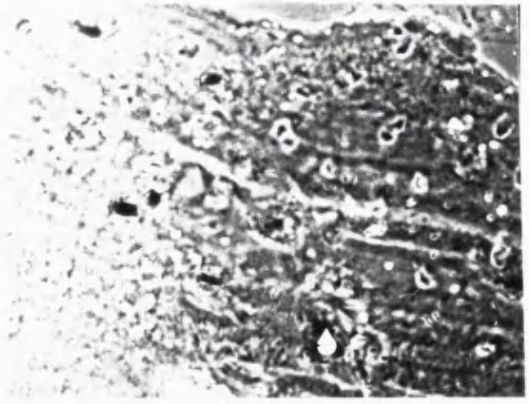


Fig. 3.2.4.1.13.

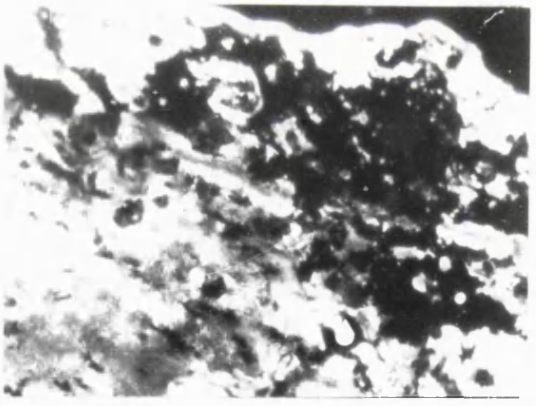


Fig. 3.2.4.1.14.

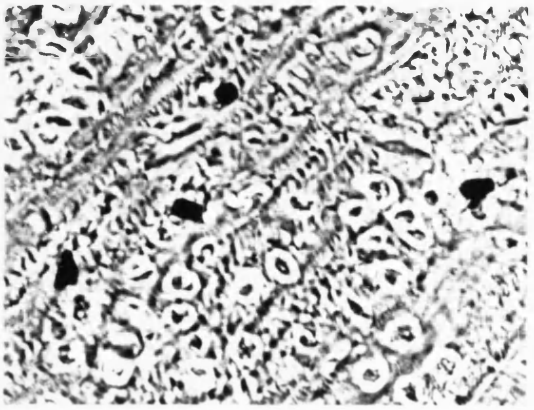


Fig. 3.2.4.1.15.

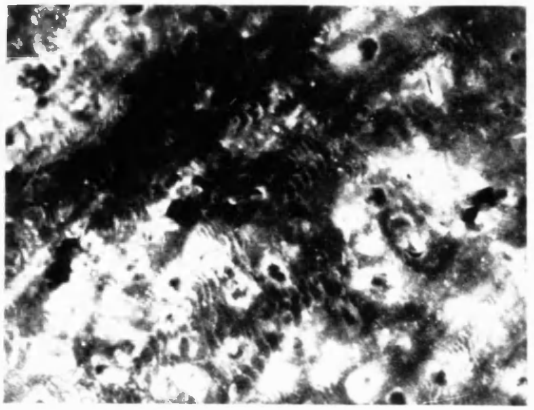


Fig. 3.2.4.1.16.

Fig. 3.2.4.1.17. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.
Fig. 3.2.4.1.18. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. Immuno-positive staining for chondroitin -6- sulphate was found throughout the matrix.

Fig. 3.2.4.1.19. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.
Fig. 3.2.4.1.20. Fluorescence micrograph of superficial graft tissue after 3 months post-implantation. Collagen type I-staining was shown occasionally in discrete areas of the graft tissue.

Fig. 3.2.4.1.21. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.
Fig. 3.2.4.1.22. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. Keratan sulphate-positive staining was found mainly intercellularly.

Fig. 3.2.4.1.23. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.
Fig. 3.2.4.1.24. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. The control section only showed background staining.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

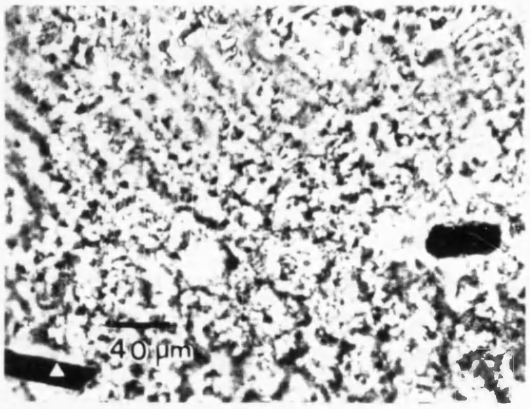


Fig. 3.2.4.1.17.

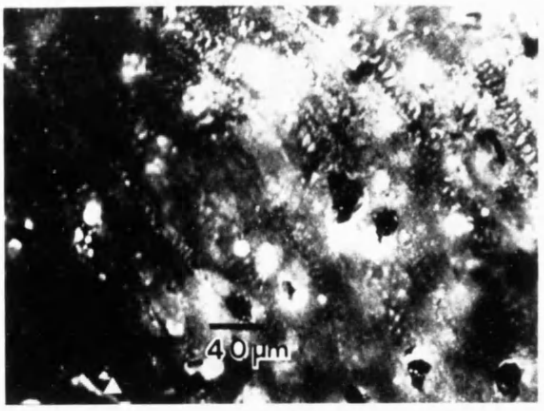


Fig. 3.2.4.1.18.

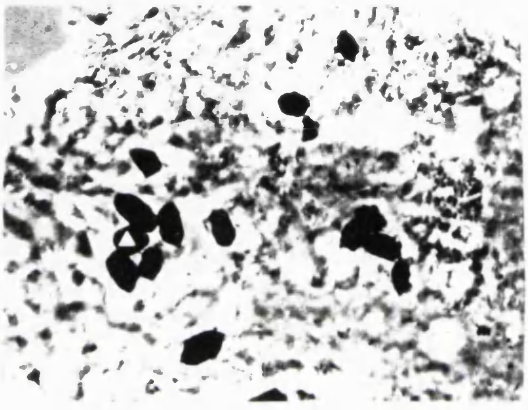


Fig. 3.2.4.1.19.

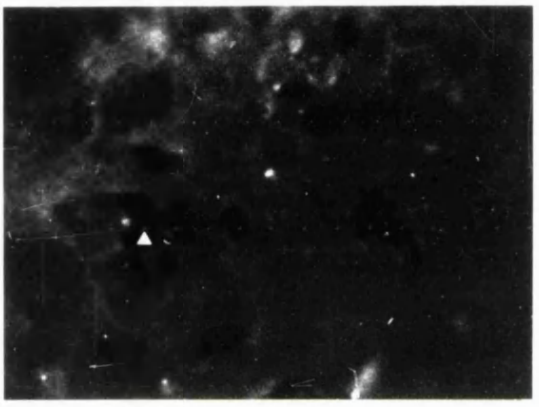


Fig. 3.2.4.1.20.

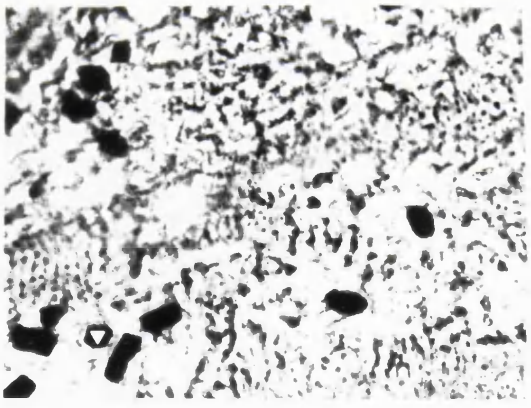


Fig. 3.2.4.1.21.

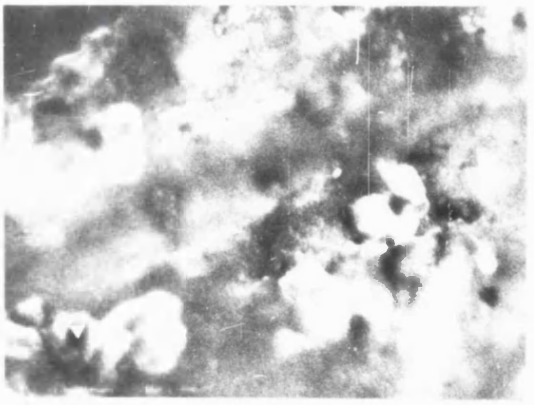


Fig. 3.2.4.1.22.

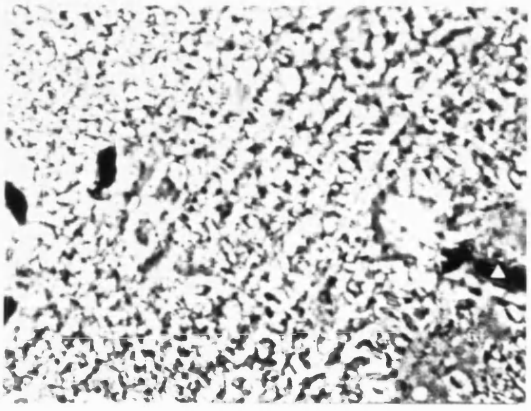


Fig. 3.2.4.1.23.

Fig. 3.2.4.1.24.

Fig. 3.2.4.1.25. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation .
Fig. 3.2.4.1.26. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation. Collagen type II-staining was found only occasionally in discrete areas of the repair tissue.

Fig. 3.2.4.1.27. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation.
Fig. 3.2.4.1.28. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown only in discrete areas of the tissue.

Fig. 3.2.4.1.29. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation.
Fig. 3.2.4.1.30. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation. Immuno-positive staining for chondroitin -6- sulphate was found only occasionally in discrete areas of the tissue.

Fig. 3.2.4.1.31. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation.
Fig. 3.2.4.1.32. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation. Collagen type I-staining was shown throughout the matrix.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

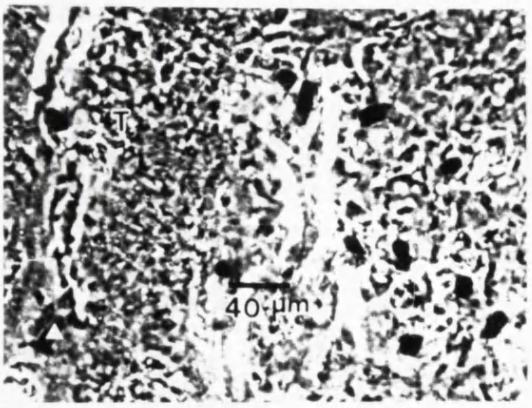


Fig. 3.2.4.1.25.

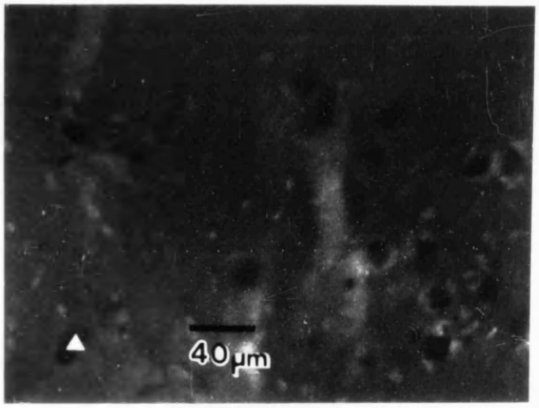


Fig. 3.2.4.1.26.

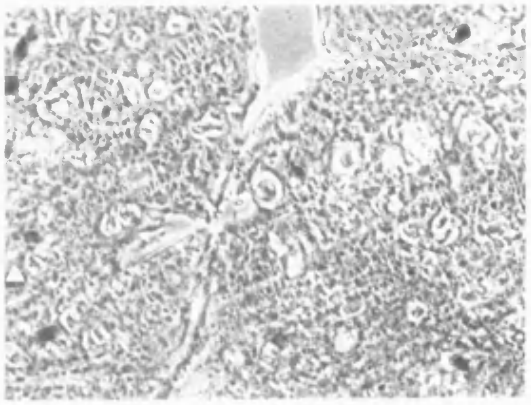


Fig. 3.2.4.1.27.

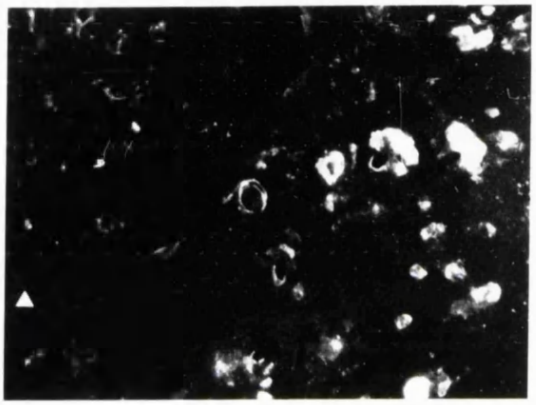


Fig. 3.2.4.1.28.

Fig. 3.2.4.1.29.

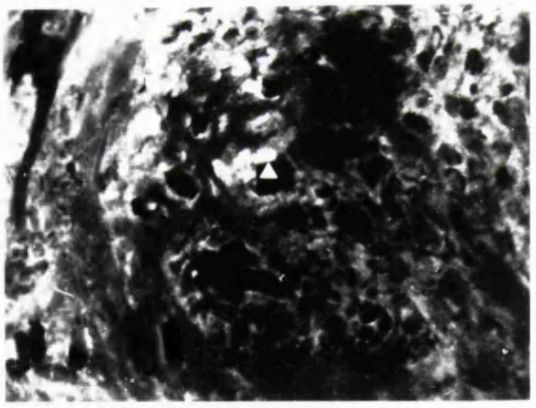


Fig. 3.2.4.1.30.

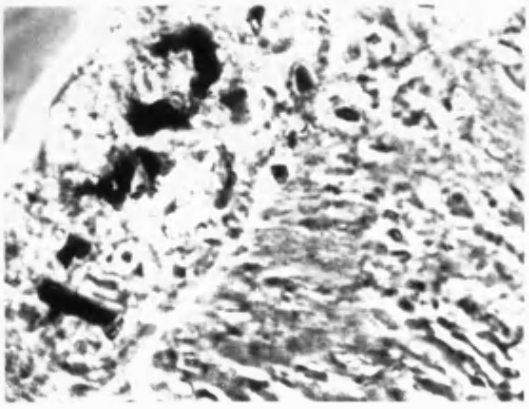


Fig. 3.2.4.1.31.

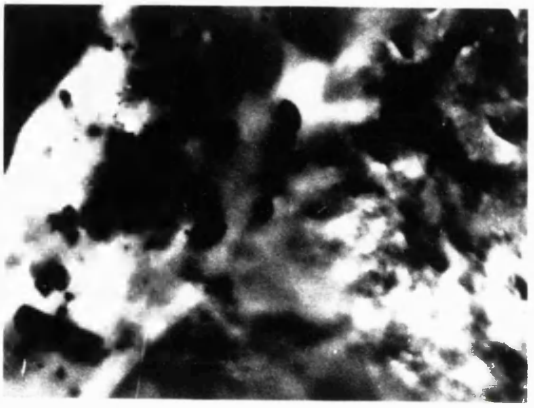


Fig. 3.2.4.1.32.

Fig. 3.2.4.1.33. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation.
Fig. 3.2.4.1.34. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation. Immuno-positive staining for keratan sulphate was found occasionally in discrete areas of the tissue.

Fig. 3.2.4.1.35. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation.
Fig. 3.2.4.1.36. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation. The control only showed background staining.

Fig. 3.2.4.1.37. Phase contrast micrograph of superficial graft tissue at 6 months post-implantation .
Fig. 3.2.4.1.38. Fluorescence micrograph of superficial graft tissue at 6 months post-implantation. Collagen type II-staining was found in some areas around the carbon fibres.

Fig. 3.2.4.1.39. Phase contrast micrograph of superficial graft tissue at 6 months post-implantation.
Fig. 3.2.4.1.40. Fluorescence micrograph of superficial graft tissue at 6 months post-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown around some carbon fibres and pericellularly in some areas of the graft tissue.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

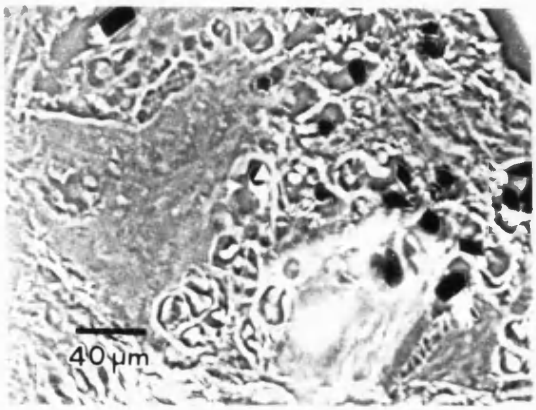


Fig. 3.2.4.1.33.

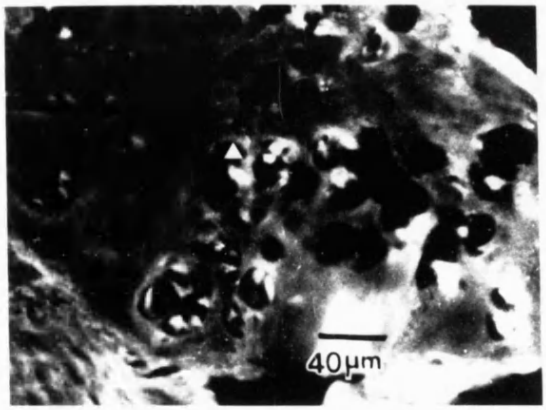


Fig. 3.2.4.1.34.

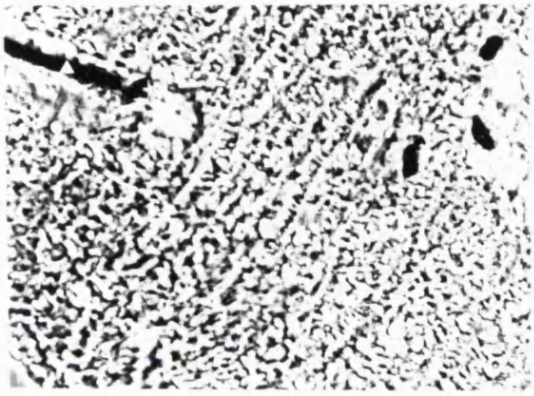


Fig. 3.2.4.1.35.

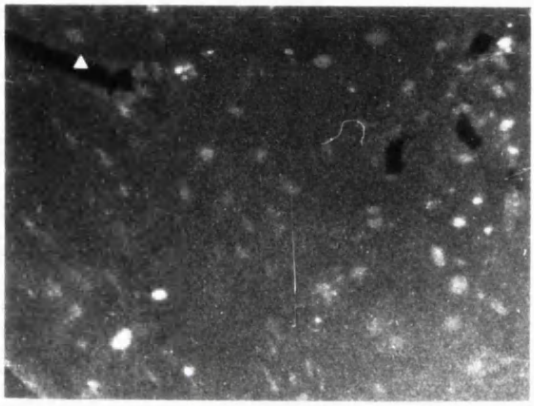


Fig. 3.2.4.1.36.

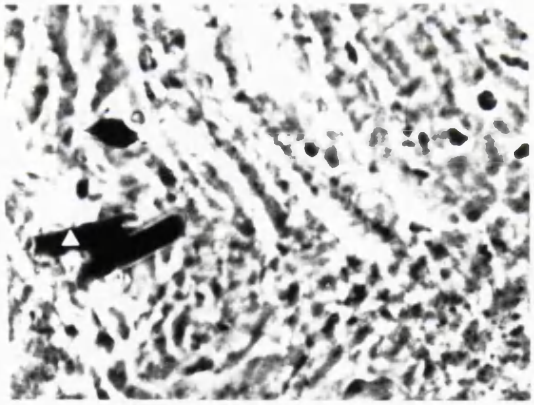


Fig. 3.2.4.1.37.

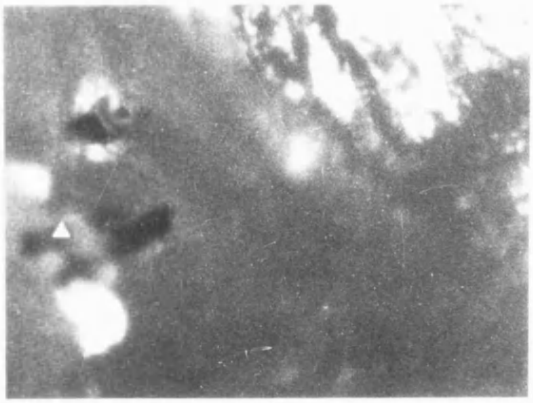


Fig. 3.2.4.1.38.

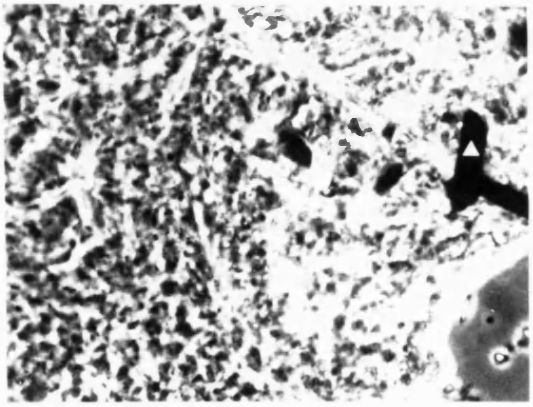


Fig. 3.2.4.1.39.

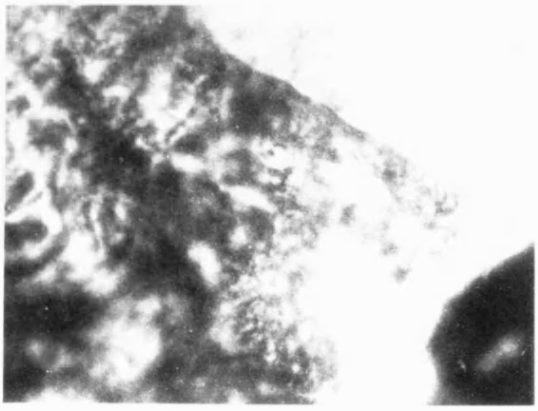


Fig. 3.2.4.1.40.

Fig. 3.2.4.1.41. Phase contrast micrograph of superficial graft tissue at 6 months post-implantation.
Fig. 3.2.4.1.42. Fluorescence micrograph of superficial graft tissue at 6 months post-implantation. Immuno-positive staining for chondroitin -6- sulphate was found occasionally in discrete areas.

Fig. 3.2.4.1.43. Phase contrast micrograph of superficial graft tissue at 6 months post-implantation.
Fig. 3.2.4.1.44. Fluorescence micrograph of superficial graft tissue after 6 months post- implantation. Collagen type I-staining was shown occasionally in discrete areas.

Fig. 3.2.4.1.45. Phase contrast micrograph of superficial graft tissue at 6 months post-implantation.
Fig. 3.2.4.1.46. Fluorescence micrograph of superficial graft tissue at 6 months post-implantation. Keratan sulphate-positive staining was found peri- and intercellularly.

Fig. 3.2.4.1.47. Phase contrast micrograph of superficial graft tissue at 6 months post-implantation.
Fig. 3.2.4.1.48. Fluorescence micrograph of superficial graft tissue at 6 months post-implantation. Only background staining was found

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

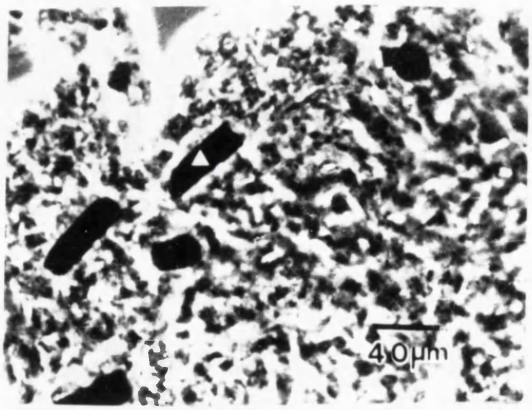


Fig. 3.2.4.1.41.

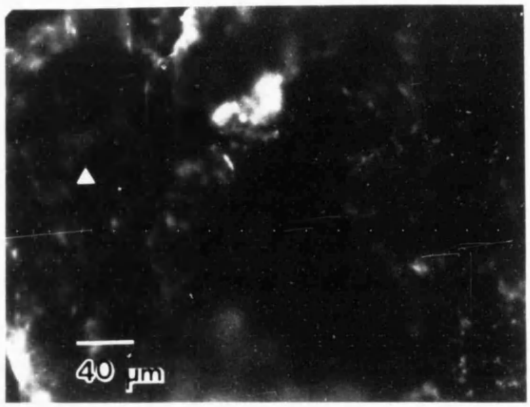


Fig. 3.2.4.1.42.

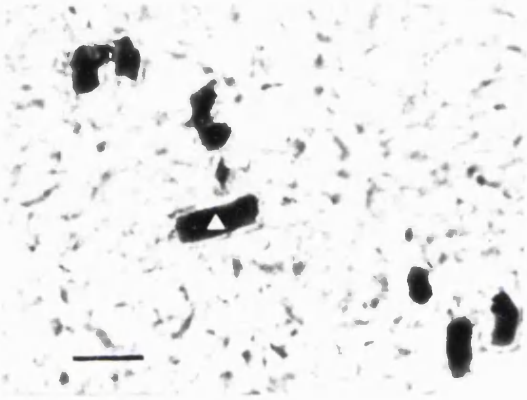


Fig. 3.2.4.1.43.

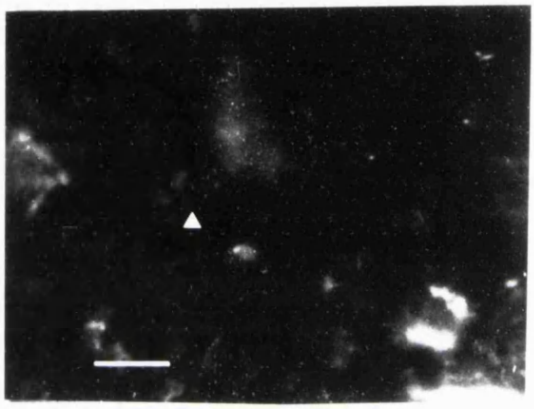


Fig. 3.2.4.1.44.

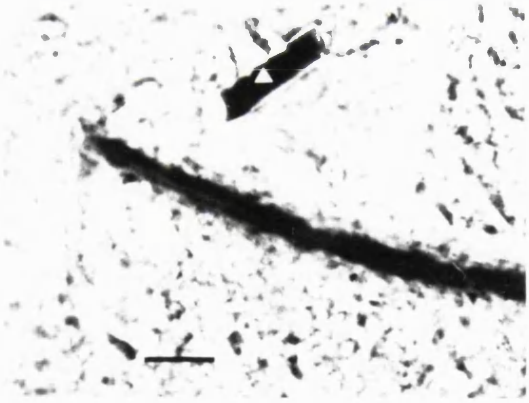


Fig. 3.2.4.1.45.

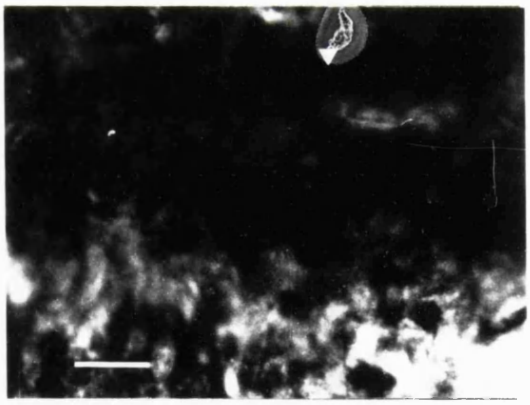


Fig. 3.2.4.1.46.

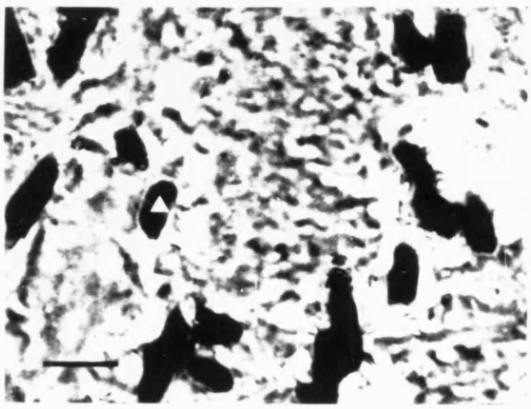


Fig. 3.2.4.1.47.

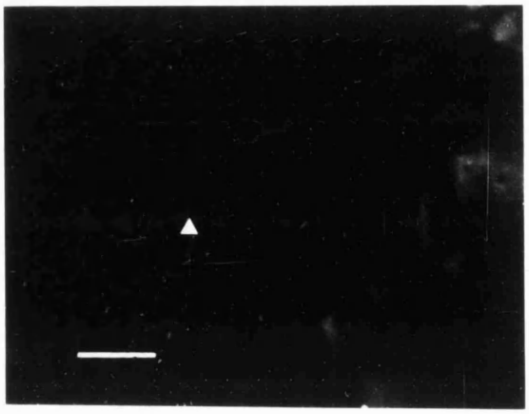


Fig. 3.2.4.1.48.

Fig. 3.2.4.1.49. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation .
Fig. 3.2.4.1.50. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation. Immuno-positive staining for collagen type II was only found around some carbon fibres.

Fig. 3.2.4.1.51. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation.
Fig. 3.2.4.1.52. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown pericellularly and around the carbon fibres.

Fig. 3.2.4.1.53. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation.
Fig. 3.2.4.1.54. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation. Immuno-positive staining for chondroitin -6- sulphate was found intercellularly and around the carbon fibres.

Fig. 3.2.4.1.55. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation.
Fig. 3.2.4.1.56. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation. Collagen type I-staining was shown around the carbon fibres.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

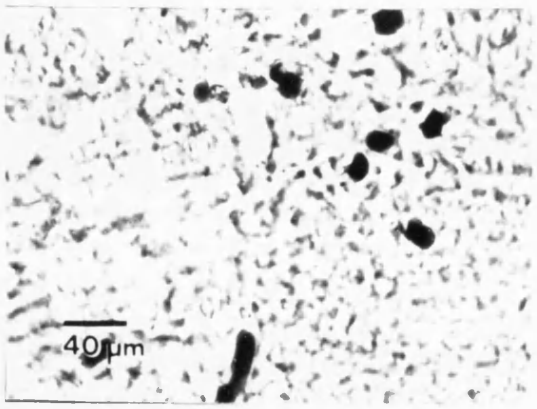


Fig. 3.2.4.1.49.

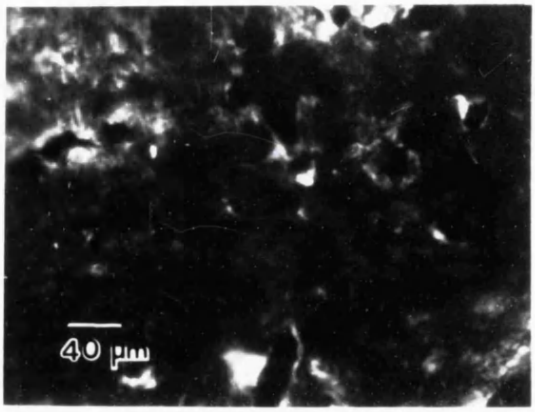


Fig. 3.2.4.1.50.

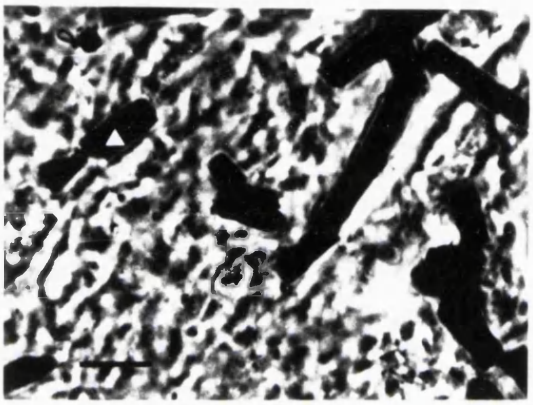


Fig. 3.2.4.1.51.

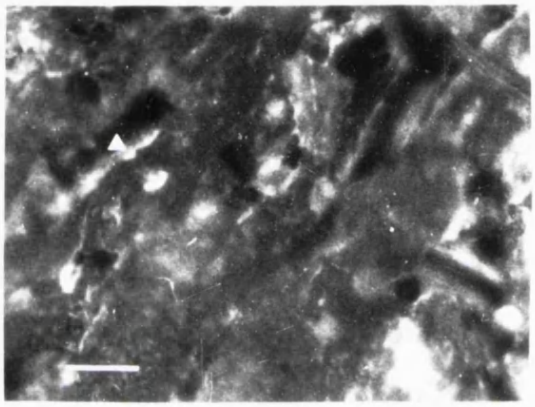


Fig. 3.2.4.1.52.

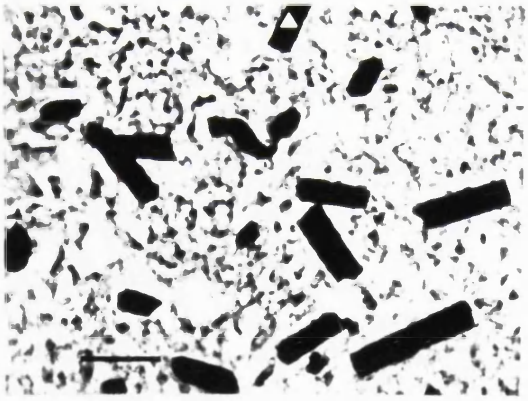


Fig. 3.2.4.1.53.

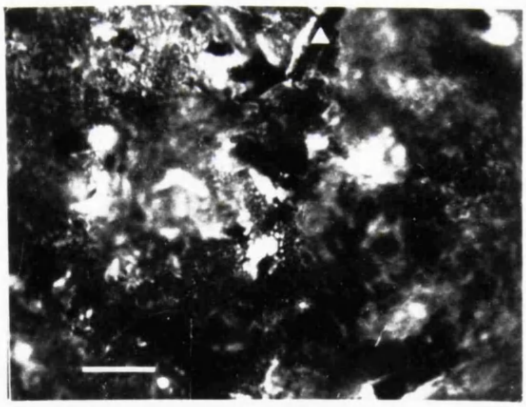


Fig. 3.2.4.1.54.

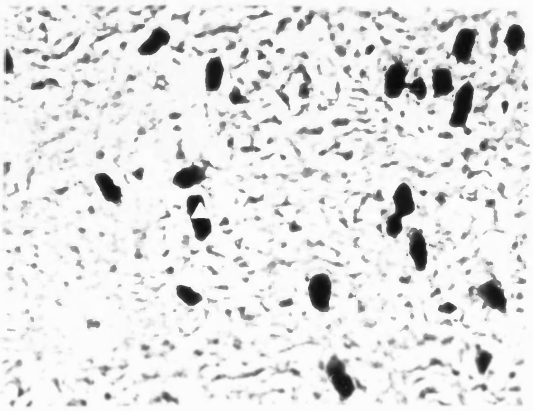


Fig. 3.2.4.1.55.

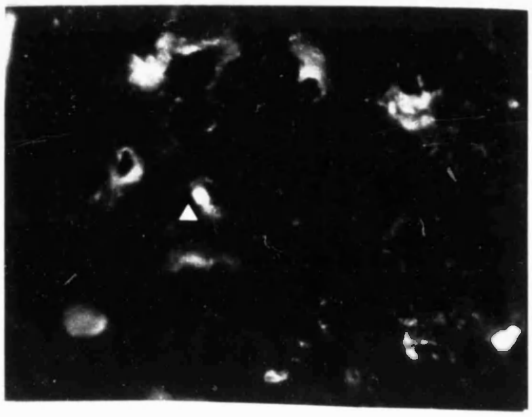


Fig. 3.2.4.1.56.

Fig. 3.2.4.1.57. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation.
Fig. 3.2.4.1.58. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation. Immuno-positive staining for keratan sulphate was found pericellularly.

Fig. 3.2.4.1.59. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation .
Fig. 3.2.4.1.60. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation. Only background staining was found.

Fig. 3.2.4.1.61. Phase contrast micrograph of superficial graft tissue at 12 months post-implantation .
Fig. 3.2.4.1.62. Fluorescence micrograph of superficial graft tissue at 12 months post-implantation. Collagen type II-staining was found occasionally pericellularly in discrete areas of the graft tissue.

Fig. 3.2.4.1.63. Phase contrast micrograph of superficial graft tissue at 12 months post-implantation.
Fig. 3.2.4.1.64. Fluorescence micrograph of superficial graft tissue at 12 months post-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown pericellularly.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

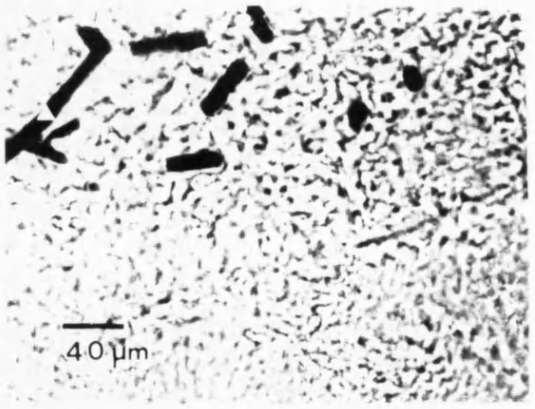


Fig. 3.2.4.1.57.

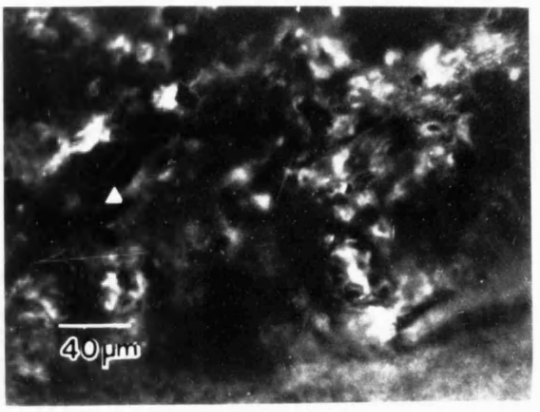


Fig. 3.2.4.1.58.

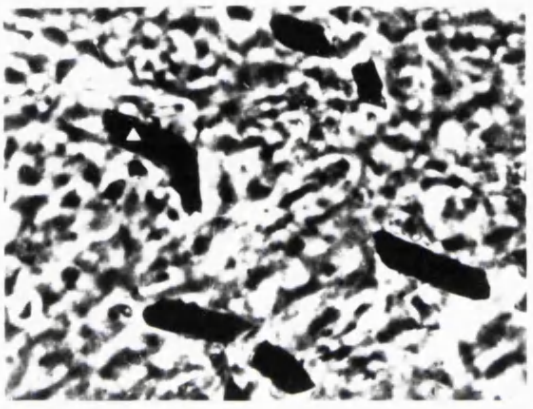


Fig. 3.2.4.1.59.

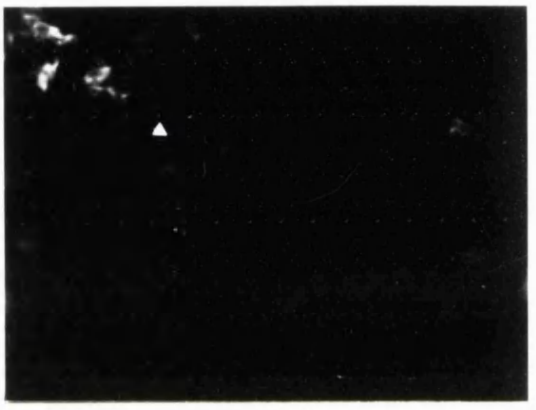


Fig. 3.2.4.1.60.

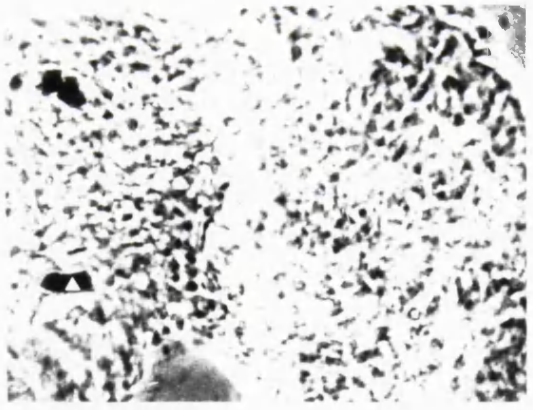


Fig. 3.2.4.1.61.

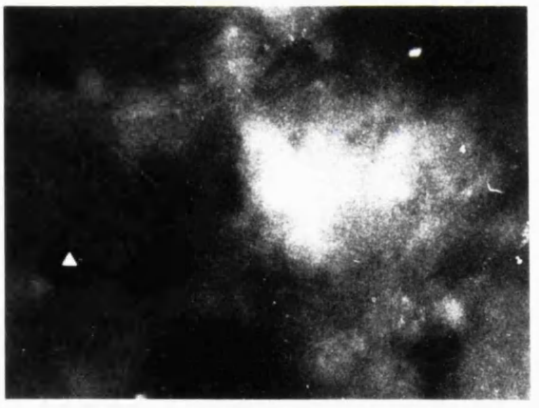


Fig. 3.2.4.1.62.

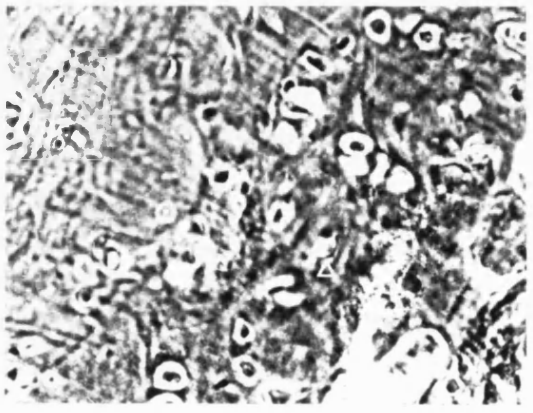


Fig. 3.2.4.1.63.

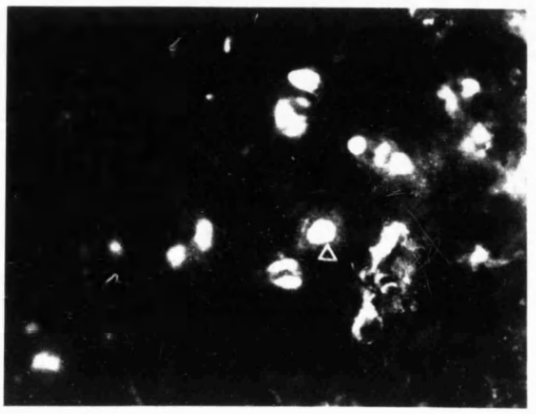


Fig. 3.2.4.1.64.

Fig. 3.2.4.1.65. Phase contrast micrograph of superficial graft tissue at 12 months post-implantation.
Fig. 3.2.4.1.66. Fluorescence micrograph of superficial graft tissue at 12 months post-implantation. Immuno-positive staining for chondroitin -6- sulphate was found occasionally pericellularly in some areas of the graft tissue.

Fig. 3.2.4.1.67. Phase contrast micrograph of superficial graft tissue at 12 months post-implantation.
Fig. 3.2.4.1.68. Fluorescence micrograph of superficial graft tissue after 12 months post-implantation. Collagen type I-staining was shown in occasionally in discrete areas of the graft tissue.

Fig. 3.2.4.1.69. Phase contrast micrograph of superficial graft tissue at 12 months post-implantation.
Fig. 3.2.4.1.70. Fluorescence micrograph of superficial graft tissue at 12 months post-implantation. Keratan sulphate-positive staining was found mainly pericellularly.

Fig. 3.2.4.1.71. Phase contrast micrograph of superficial graft tissue at 12 months post-implantation.
Fig. 3.2.4.1.72. Fluorescence micrograph of superficial graft tissue at 12 months post-implantation. The control section only showed background staining.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

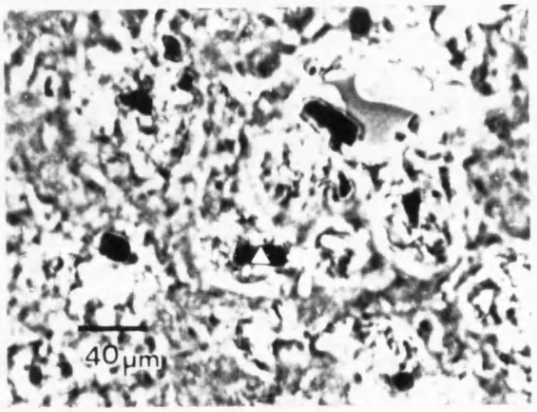


Fig. 3.2.4.1.65.

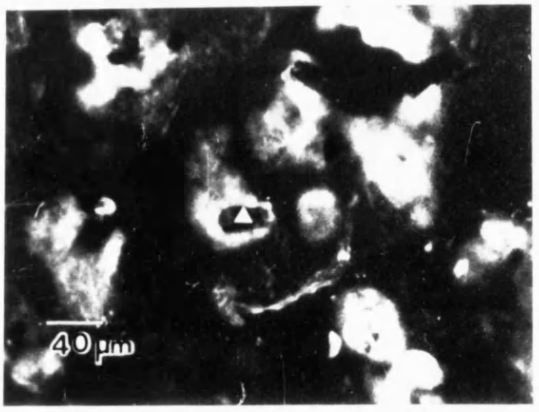


Fig. 3.2.4.1.66.

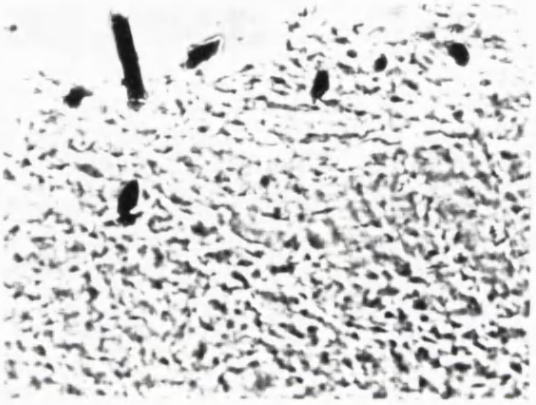


Fig. 3.2.4.1.67.

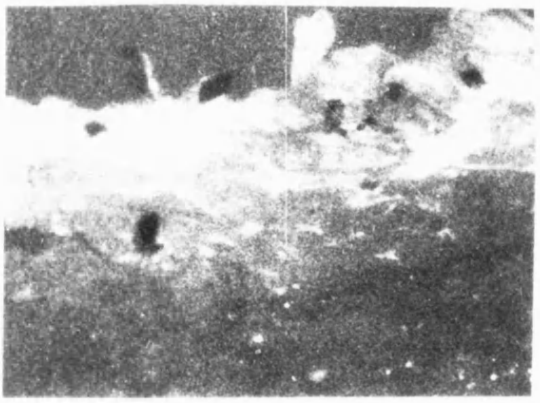


Fig. 3.2.4.1.68.

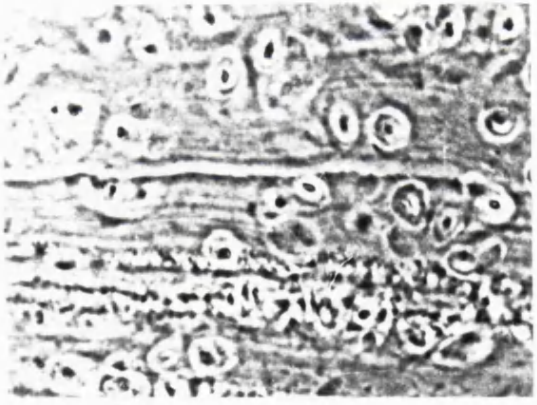


Fig. 3.2.4.1.69.

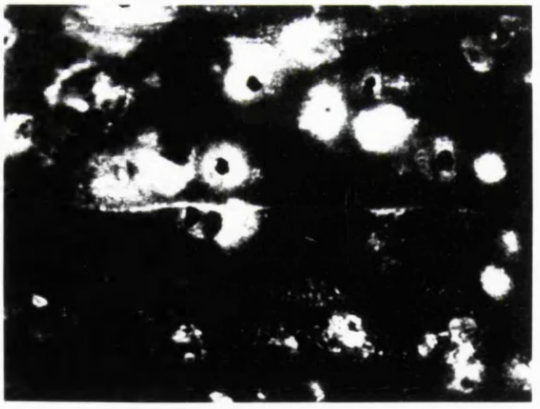


Fig. 3.2.4.1.70.

Fig. 3.2.4.1.71.

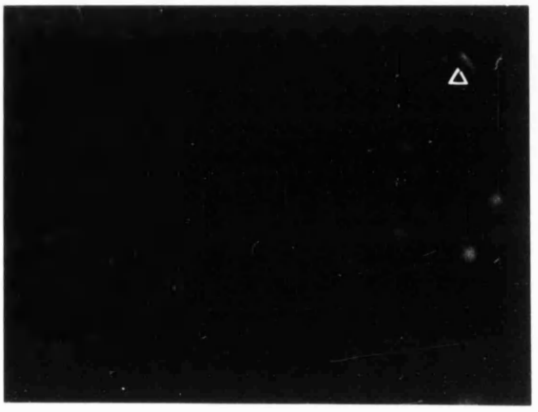


Fig. 3.2.4.1.72.

Fig. 3.2.4.1.73. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation .
Fig. 3.2.4.1.74. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation. Collagen type II-staining was not found.

Fig. 3.2.4.1.75. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation.
Fig. 3.2.4.1.76. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown pericellularly. On other micrographs staining was observed inter- and peri-cellularly.

Fig. 3.2.4.1.77. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation.
Fig. 3.2.4.1.78. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation. Immuno-positive staining for chondroitin -6- sulphate was found around the carbon fibres.

Fig. 3.2.4.1.79. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation.
Fig. 3.2.4.1.80. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation. Collagen type I-staining was shown throughout the tissue.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

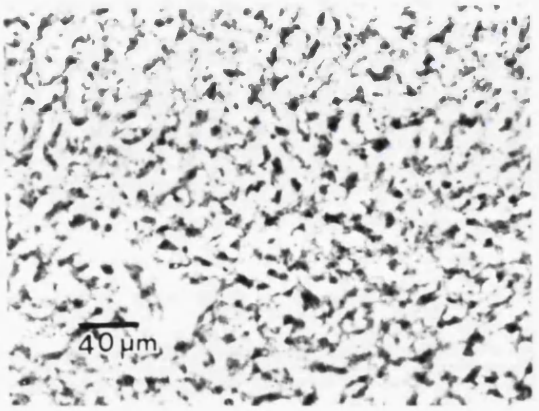


Fig. 3.2.4.1.73.

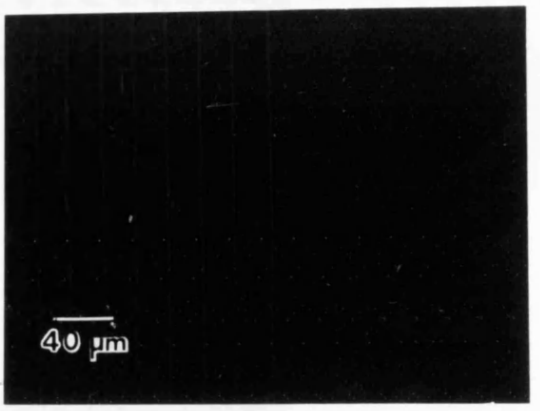


Fig. 3.2.4.1.74.

Fig. 3.2.4.1.75.

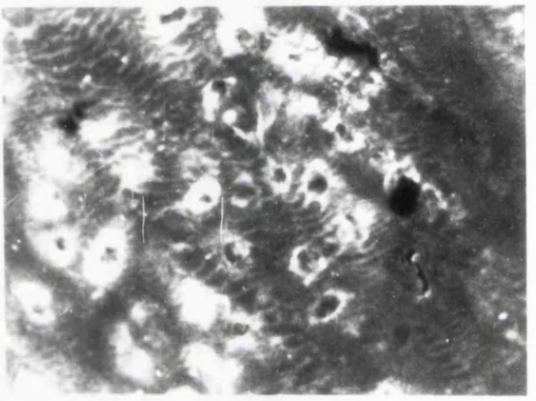


Fig. 3.2.4.1.76.

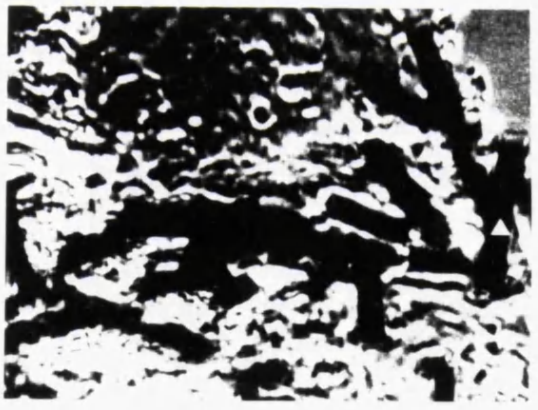


Fig. 3.2.4.1.77.

Fig. 3.2.4.1.78.

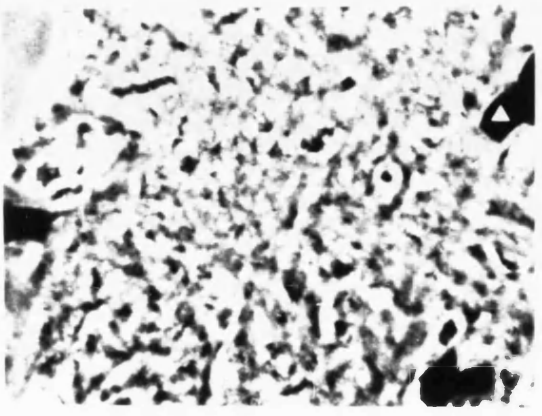


Fig. 3.2.4.1.79.

Fig. 3.2.4.1.80.

Fig. 3.2.4.1.81. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation.
Fig. 3.2.4.1.82. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation. Immuno-positive staining for keratan sulphate was found intercellularly.

Fig. 3.2.4.1.83. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation.
Fig. 3.2.4.1.84. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation. The control only showed background staining.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

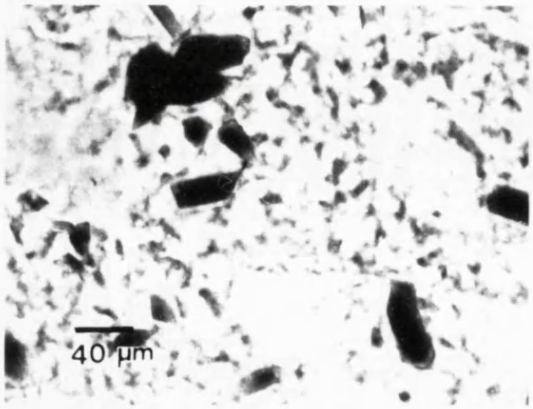


Fig. 3.2.4.1.81.

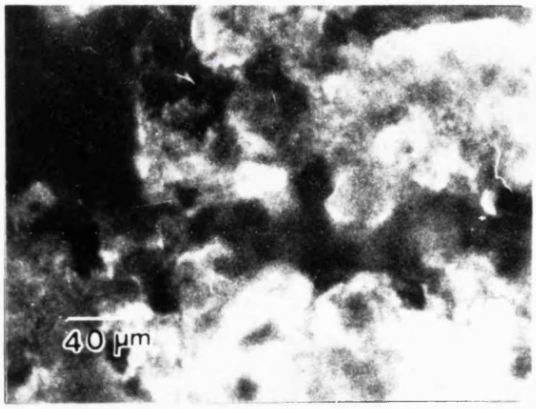


Fig. 3.2.4.1.82.

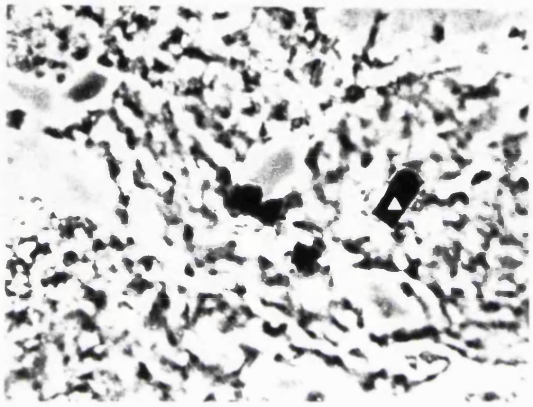


Fig. 3.2.4.1.83.

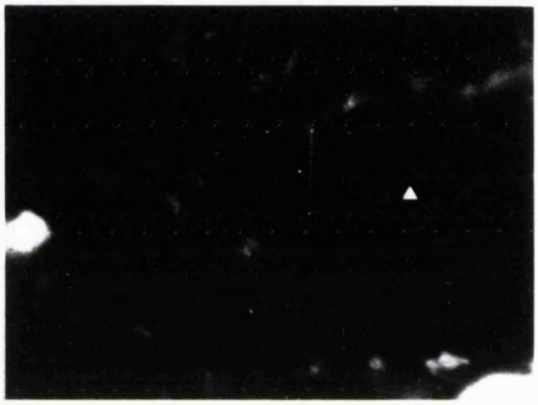


Fig. 3.2.4.1.84.

Fig. 3.2.4.2.01. Phase contrast micrograph of graft tissue at 3 weeks pre-implantation .

Fig. 3.2.4.2.02. Fluorescence micrograph of graft tissue at 3 weeks pre-implantation. Collagen type II-staining was found pericellularly.

Fig. 3.2.4.2.03. Phase contrast micrograph of graft tissue at 3 weeks pre-implantation.

Fig. 3.2.4.2.04. Fluorescence micrograph of graft tissue at 3 weeks pre-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown intercellularly in some areas of the graft tissue.

Fig. 3.2.4.2.05. Phase contrast micrograph of graft tissue at 3 weeks pre-implantation.

Fig. 3.2.4.2.06. Fluorescence micrograph of graft tissue at 3 weeks pre-implantation. Immuno-positive staining for chondroitin -6- sulphate was found occasionally in discrete areas of the graft tissue.

Fig. 3.2.4.2.07. Phase contrast micrograph of graft tissue at 3 weeks pre-implantation.

Fig. 3.2.4.2.08. Fluorescence micrograph of graft tissue at 3 weeks pre-implantation. Collagen type I-staining was found throughout the tissue.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

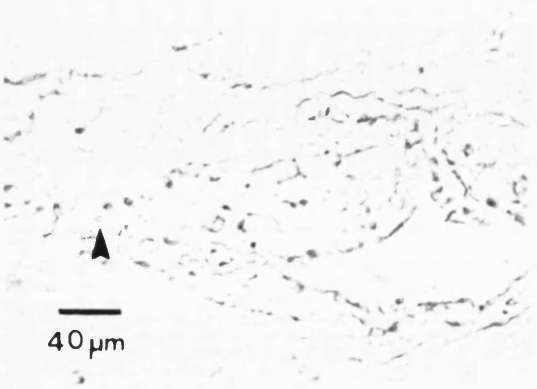


Fig. 3.2.4.2.01.

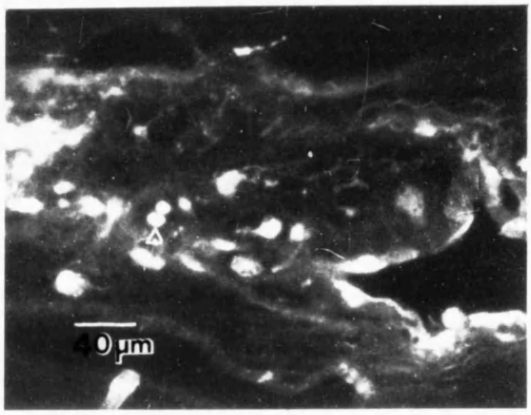


Fig. 3.2.4.2.02.

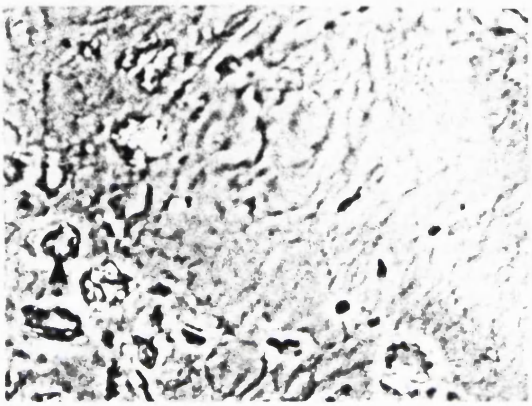


Fig. 3.2.4.2.03.

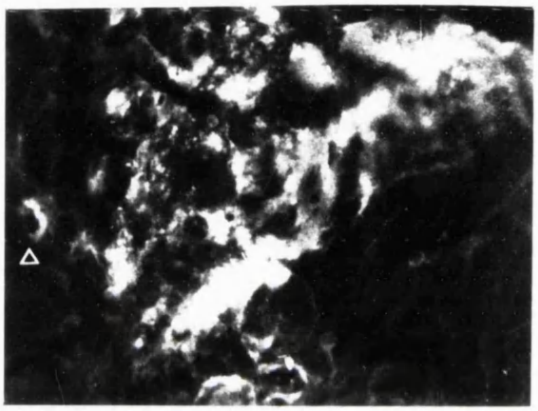


Fig. 3.2.4.2.04.

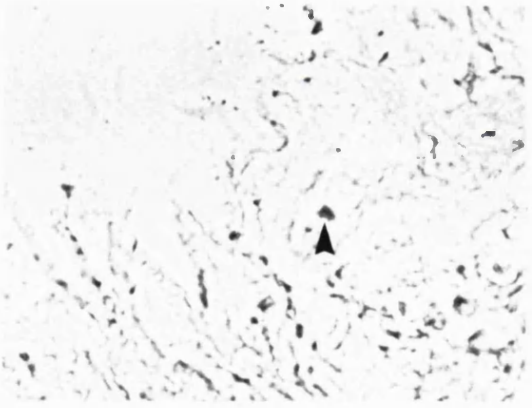


Fig. 3.2.4.2.05.

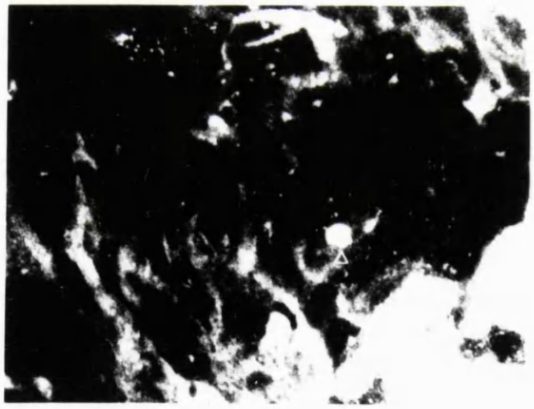


Fig. 3.2.4.2.06.

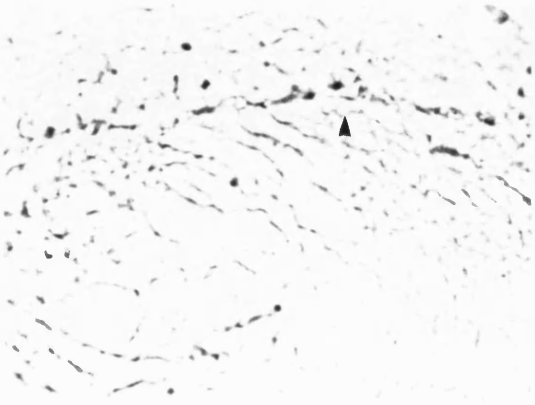


Fig. 3.2.4.2.07.

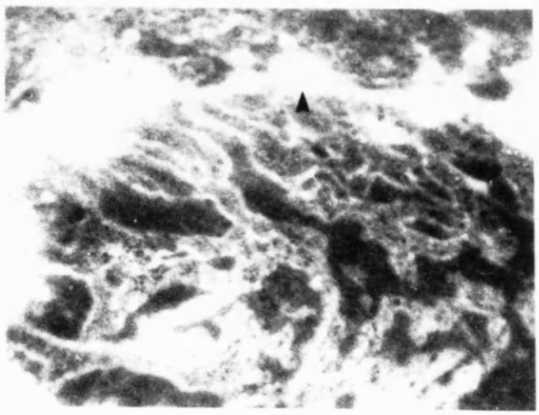


Fig. 3.2.4.2.08.

Fig. 3.2.4.2.09. Phase contrast micrograph of graft tissue at 3 weeks pre-implantation.
Fig. 3.2.4.2.10. Fluorescence micrograph of graft tissue at 3 weeks pre-implantation. Keratan sulphate-staining was found throughout the graft tissue.

Fig. 3.2.4.2.11. Phase contrast micrograph of graft tissue at 3 weeks pre-implantation.
Fig. 3.2.4.2.12. Fluorescence micrograph of graft tissue at 3 weeks pre-implantation. The control only showed background staining.

Fig. 3.2.4.2.13. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation .
Fig. 3.2.4.2.14. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. Weak collagen type II-staining was found intercellularly.

Fig. 3.2.4.2.15. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.
Fig. 3.2.4.2.16. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown pericellularly.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

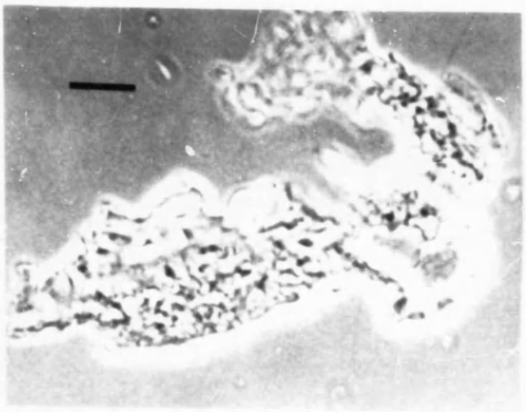


Fig. 3.2.4.2.09.

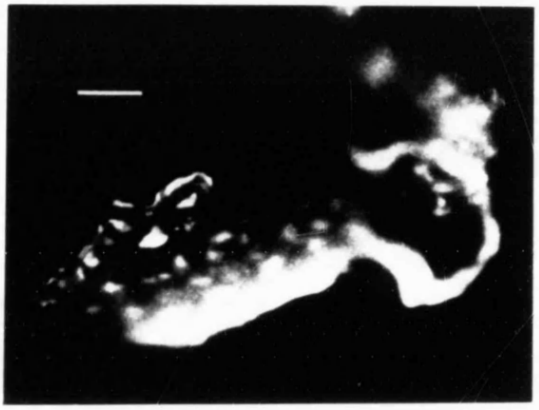


Fig. 3.2.4.2.10.

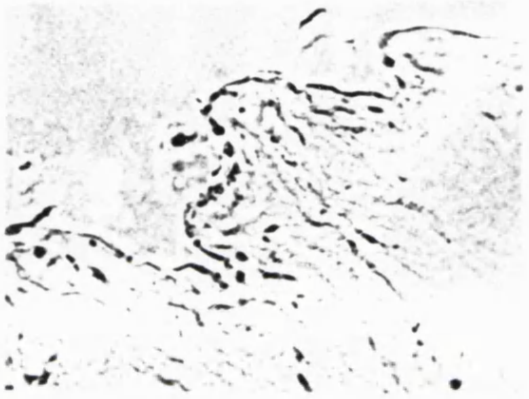


Fig. 3.2.4.2.11.

Fig. 3.2.4.2.12.

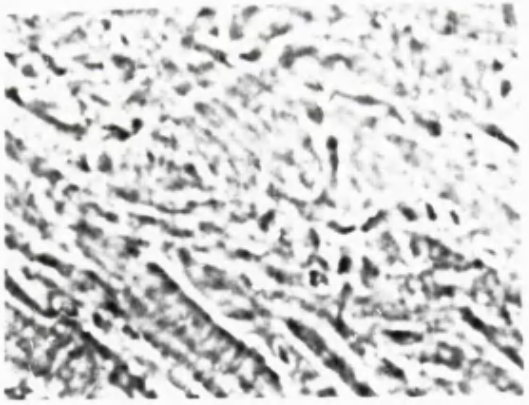


Fig. 3.2.4.2.13.

Fig. 3.2.4.2.14.

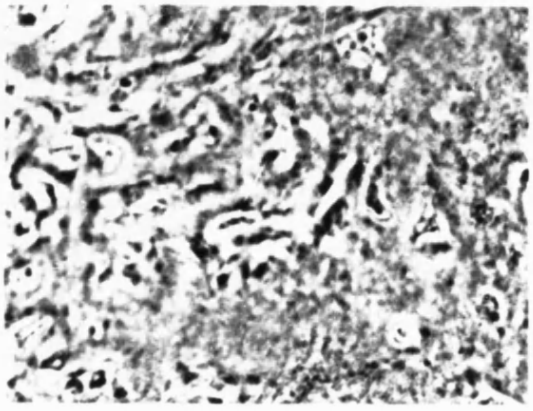


Fig. 3.2.4.2.15.

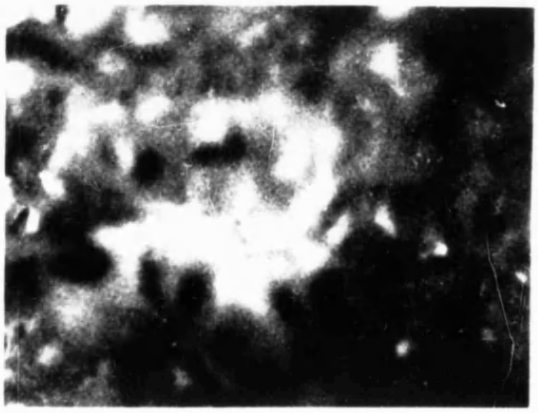


Fig. 3.2.4.2.16.

Fig. 3.2.4.2.17. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.
Fig. 3.2.4.2.18. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. Immuno-positive staining for chondroitin -6- sulphate was found inter- and pericellularly.

Fig. 3.2.4.2.19. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.
Fig. 3.2.4.2.20. Fluorescence micrograph of superficial graft tissue after 3 months post-implantation. Collagen type I-staining was shown occasionally in discrete areas.

Fig. 3.2.4.2.21. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.
Fig. 3.2.4.2.22. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. Keratan sulphate-positive staining was found mainly intercellularly.

Fig. 3.2.4.2.23. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.
Fig. 3.2.4.2.24. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. The control section only showed background staining.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

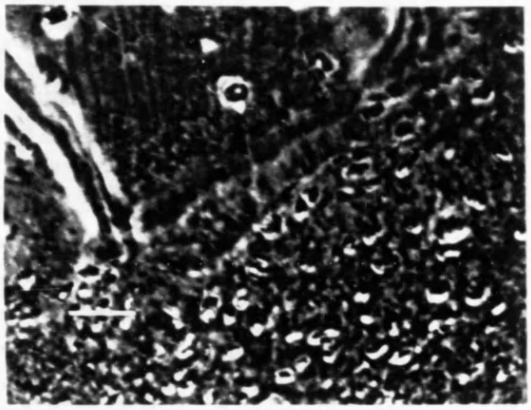


Fig. 3.2.4.2.17.

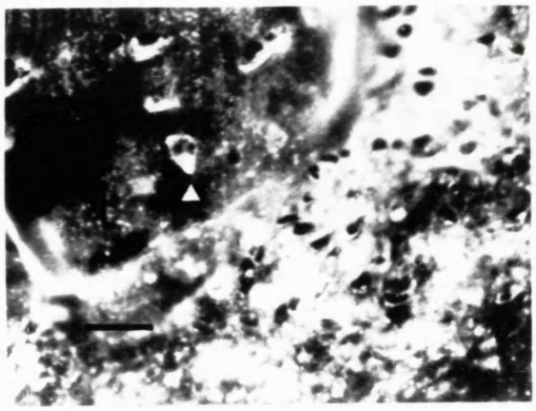


Fig. 3.2.4.2.18.

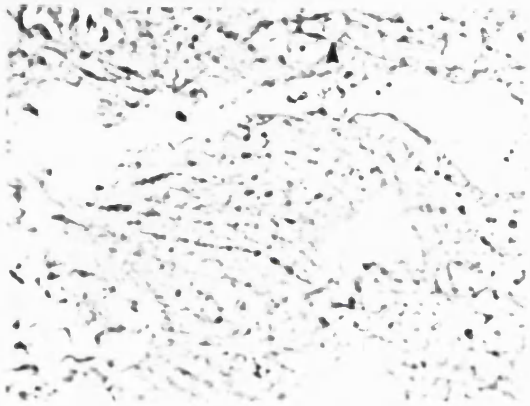


Fig. 3.2.4.2.19.

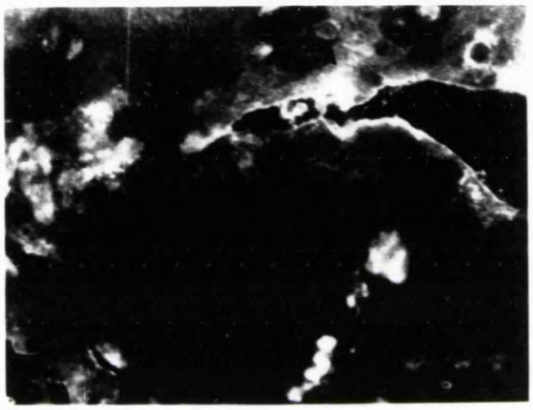


Fig. 3.2.4.2.20.

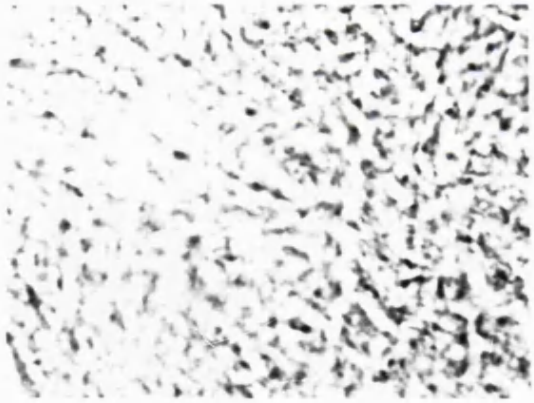


Fig. 3.2.4.2.21.

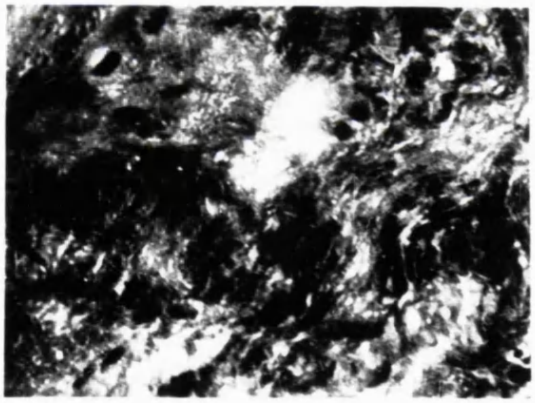


Fig. 3.2.4.2.22.

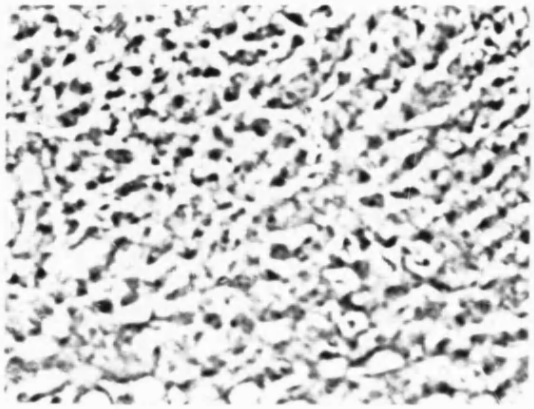


Fig. 3.2.4.2.23.

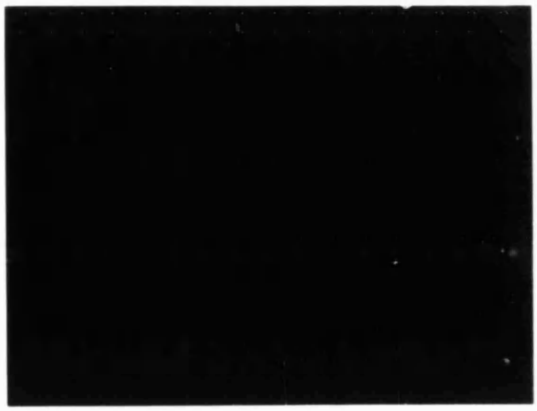


Fig. 3.2.4.2.24.

Fig. 3.2.4.2.25. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation .
Fig. 3.2.4.2.26. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation. Collagen type II-staining was found occasionally in discrete areas of the graft tissue.

Fig. 3.2.4.2.27. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation.
Fig. 3.2.4.2.28. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown intercellularly in most areas of the tissue.

Fig. 3.2.4.2.29. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation.
Fig. 3.2.4.2.30. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation. Immuno-positive staining for chondroitin -6- sulphate was not found.

Fig. 3.2.4.2.31. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation.
Fig. 3.2.4.2.32. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation. Collagen type I-staining was shown intercellularly.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

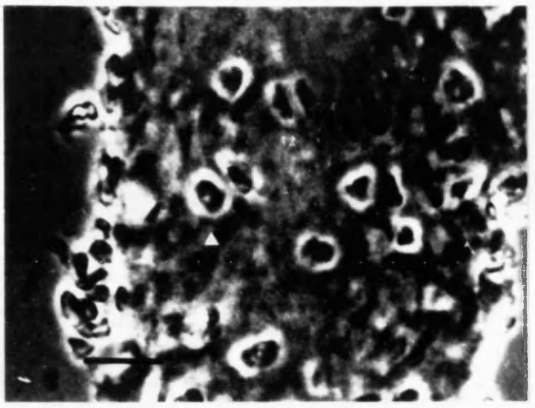


Fig. 3.2.4.2.25.

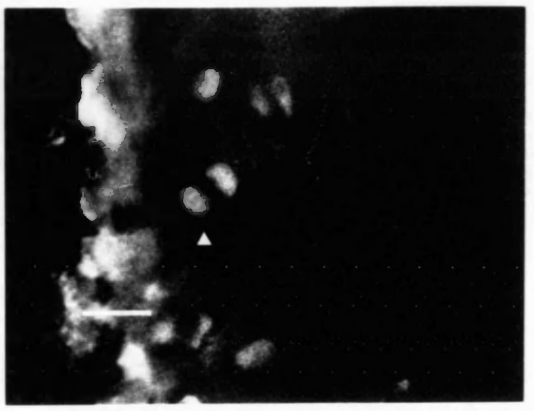


Fig. 3.2.4.2.26.

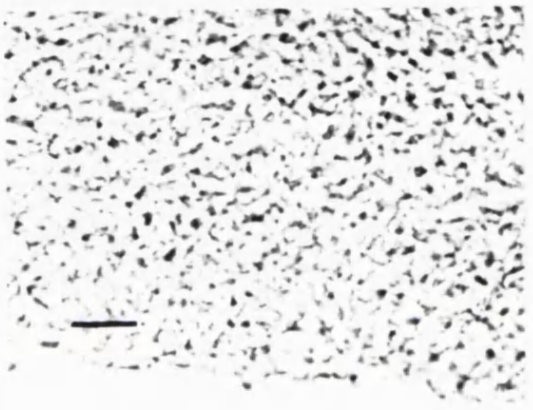


Fig. 3.2.4.2.27.

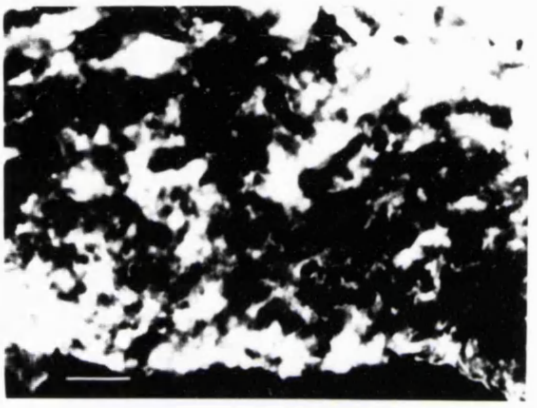


Fig. 3.2.4.2.28.

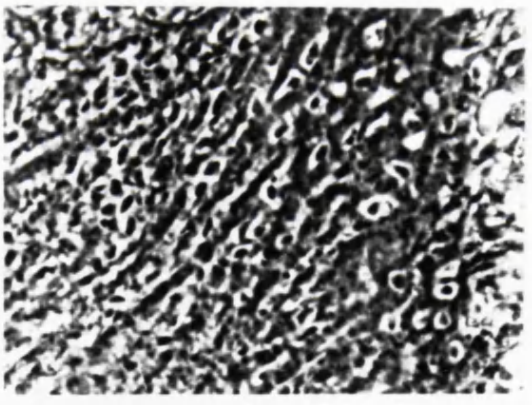


Fig. 3.2.4.2.29.

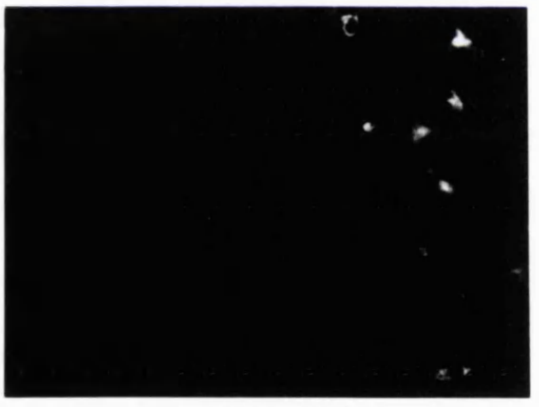


Fig. 3.2.4.2.30.

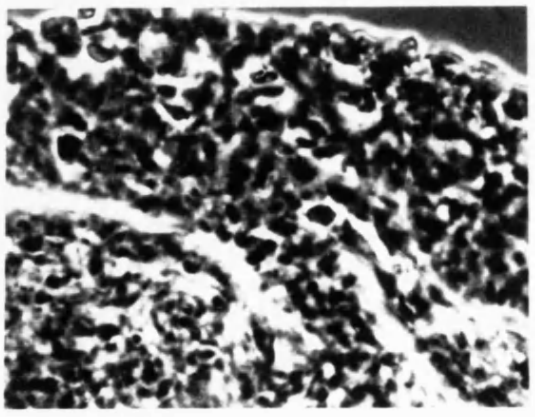


Fig. 3.2.4.2.31.

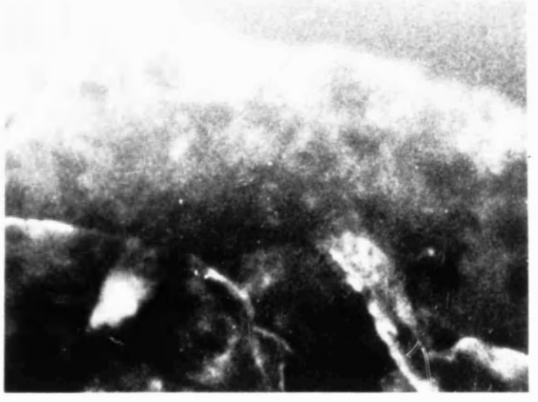


Fig. 3.2.4.2.32.

Fig. 3.2.4.2.33. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation.
Fig. 3.2.4.2.34. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation. Immuno-positive staining for keratan sulphate was not found.

Fig. 3.2.4.2.35. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation.
Fig. 3.2.4.2.36. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 3 months post-implantation. The control only showed background staining.

Fig. 3.2.4.2.37. Phase contrast micrograph of superficial graft tissue at 6 months post-implantation .
Fig. 3.2.4.2.38. Fluorescence micrograph of superficial graft tissue at 6 months post-implantation. Collagen type II-staining was not found.

Fig. 3.2.4.2.39. Phase contrast micrograph of superficial graft tissue at 6 months post-implantation.
Fig. 3.2.4.2.40. Fluorescence micrograph of superficial graft tissue at 6 months post-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown pericellularly in some areas of the graft tissue.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

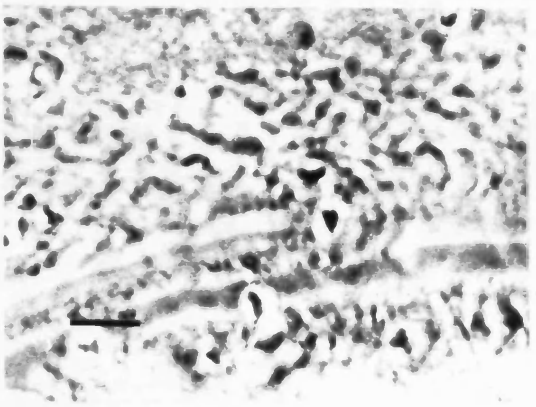


Fig. 3.2.4.2.33.

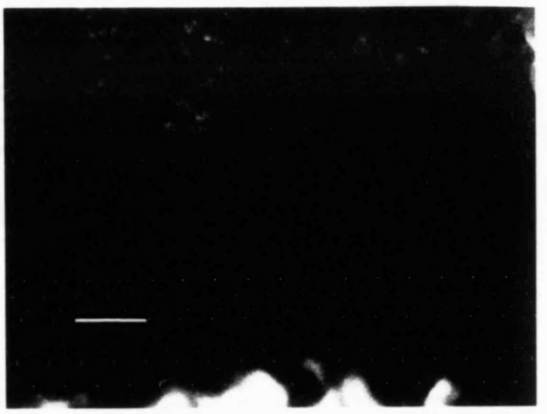


Fig. 3.2.4.2.34.

Fig. 3.2.4.2.35.

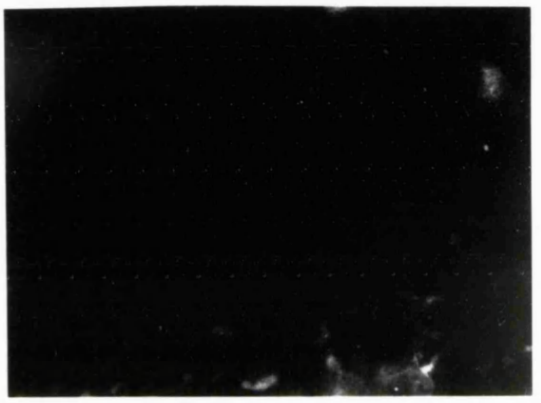


Fig. 3.2.4.2.36.

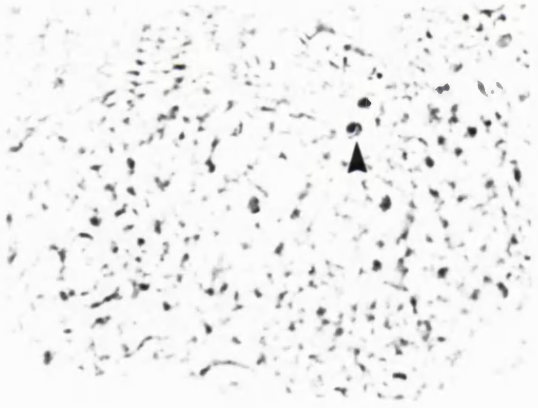


Fig. 3.2.4.2.37.

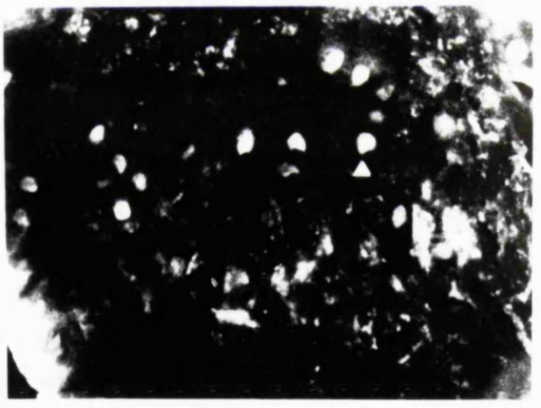


Fig. 3.2.4.2.38.

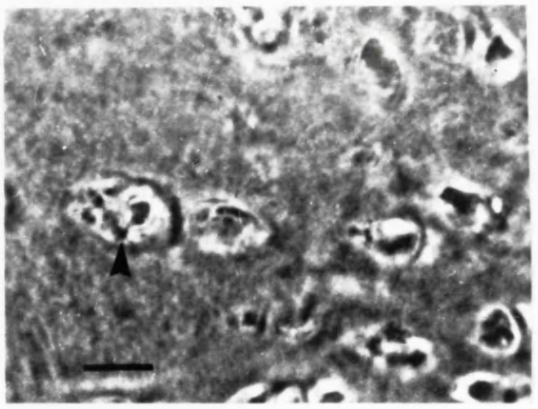


Fig. 3.2.4.2.39.

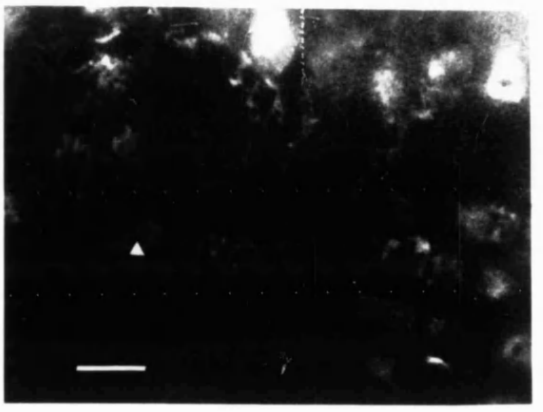


Fig. 3.2.4.2.40.

Fig. 3.2.4.2.41. Phase contrast micrograph of superficial graft tissue at 6 months post-implantation.
Fig. 3.2.4.2.42. Fluorescence micrograph of superficial graft tissue at 6 months post-implantation. Immuno-positive staining for chondroitin -6- sulphate was found pericellularly.

Fig. 3.2.4.2.43. Phase contrast micrograph of superficial graft tissue at 6 months post-implantation.
Fig. 3.2.4.2.44. Fluorescence micrograph of superficial graft tissue after 6 months post-implantation. Collagen type I-staining was not observed.

Fig. 3.2.4.2.45. Phase contrast micrograph of superficial graft tissue at 6 months post-implantation.
Fig. 3.2.4.2.46. Fluorescence micrograph of superficial graft tissue at 6 months post-implantation. Keratan sulphate-positive staining was found pericellularly.

Fig. 3.2.4.2.47. Phase contrast micrograph of superficial graft tissue at 6 months post-implantation.
Fig. 3.2.4.2.48. Fluorescence micrograph of superficial graft tissue at 6 months post-implantation. Only background staining was found.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

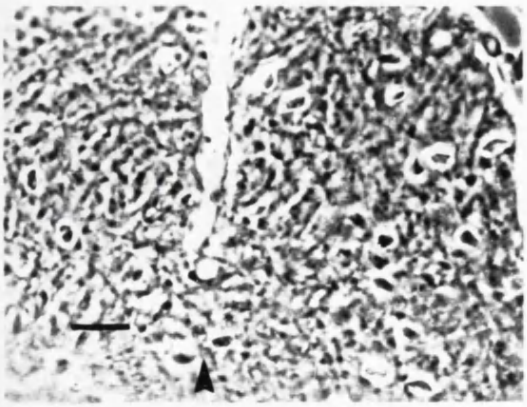


Fig. 3.2.4.2.41.

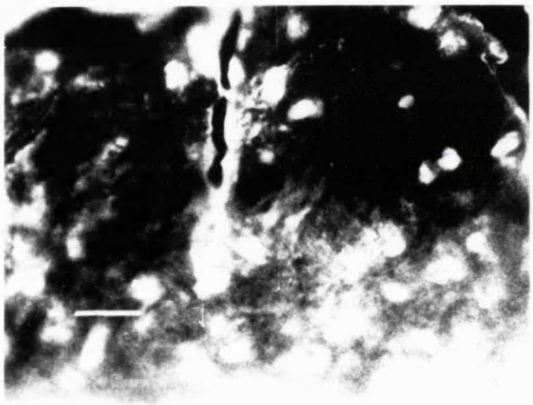


Fig. 3.2.4.2.42.

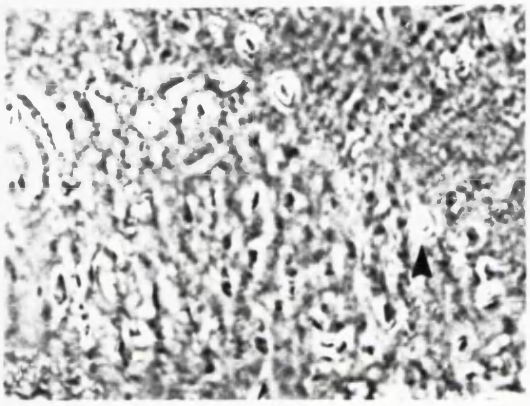


Fig. 3.2.4.2.43.

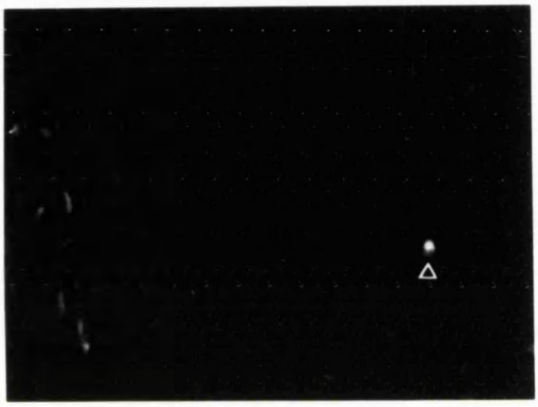


Fig. 3.2.4.2.44.

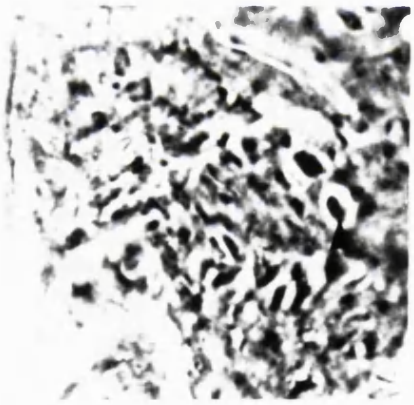


Fig. 3.2.4.2.45.

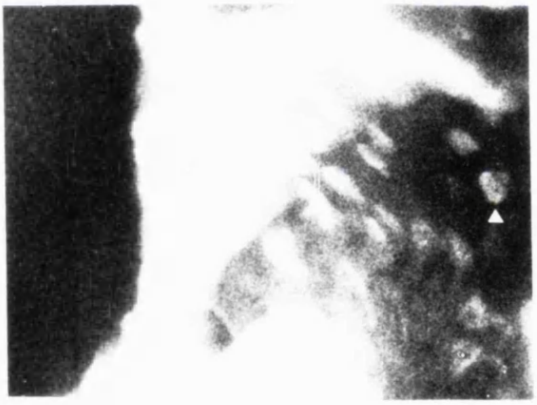


Fig. 3.2.4.2.46.

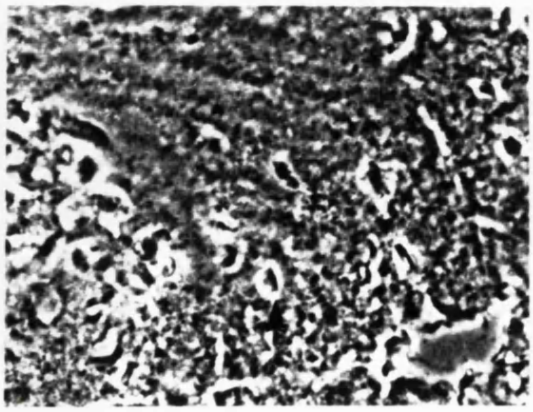


Fig. 3.2.4.2.47.

Fig. 3.2.4.2.48.

Fig. 3.2.4.2.49. Phase contrast micrograph of superficial host tissue within the graft region of the control knee after 6 months post-implantation.
Fig. 3.2.4.2.50. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation. Immuno-positive staining for collagen type II was not found.

Fig. 3.2.4.2.51. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation.
Fig. 3.2.4.2.52. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown pericellularly.

Fig. 3.2.4.2.53. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation.
Fig. 3.2.4.2.54. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation. Immuno-positive staining for chondroitin -6- sulphate was found pericellularly.

Fig. 3.2.4.2.55. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation.
Fig. 3.2.4.2.56. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation. Collagen type I-staining was not shown.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

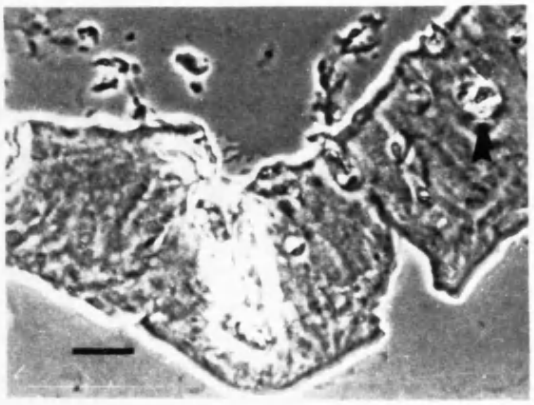


Fig. 3.2.4.2.49.

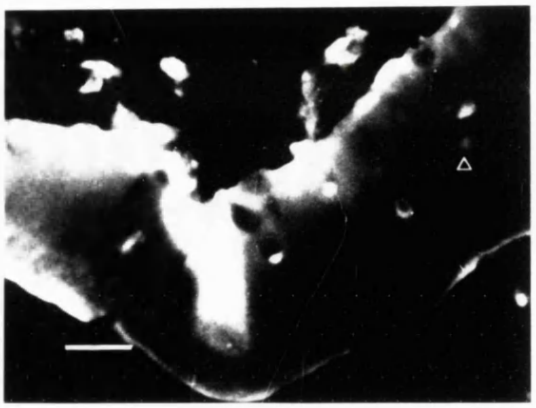


Fig. 3.2.4.2.50.

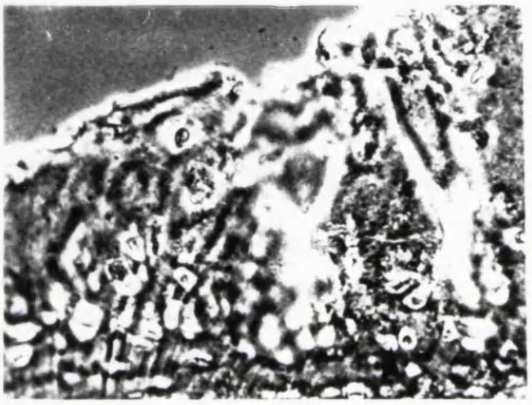


Fig. 3.2.4.2.51.

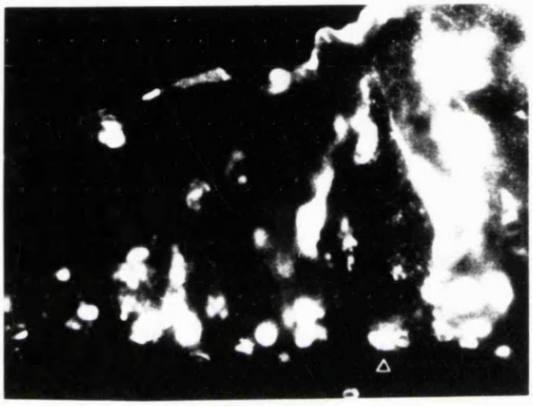


Fig. 3.2.4.2.52.

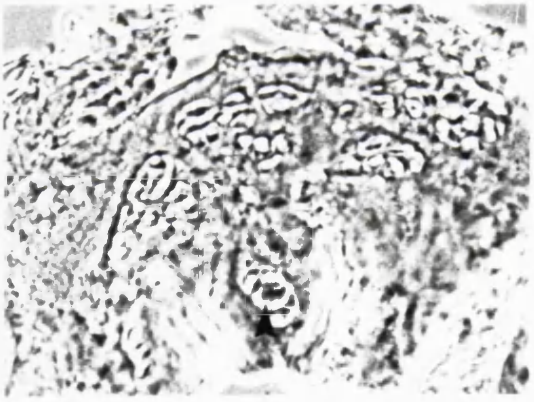


Fig. 3.2.4.2.53.

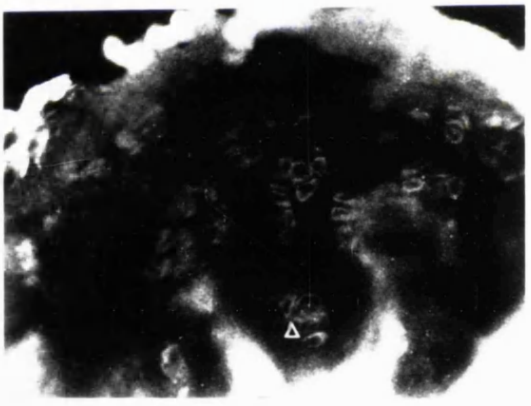


Fig. 3.2.4.2.54.

Fig. 3.2.4.2.55.

Fig. 3.2.4.2.56.

Fig. 3.2.4.2.57. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation.
Fig. 3.2.4.2.58. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation. Immuno-positive staining for keratan sulphate was not found.

Fig. 3.2.4.2.59. Phase contrast micrograph of superficial host tissue within the graft region of the control knee after 6 months post-implantation .
Fig. 3.2.4.2.60. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 6 months post-implantation. Only background staining was found.

Fig. 3.2.4.2.61. Phase contrast micrograph of superficial graft tissue at 12 months post-implantation .
Fig. 3.2.4.2.62. Fluorescence micrograph of superficial graft tissue at 12 months post-implantation. Collagen type II-staining was found occasionally intercellularly in discrete areas of the graft tissue.

Fig. 3.2.4.2.63. Phase contrast micrograph of superficial graft tissue at 12 months post-implantation.
Fig. 3.2.4.2.64. Fluorescence micrograph of superficial graft tissue at 12 months post-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown pericellularly.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

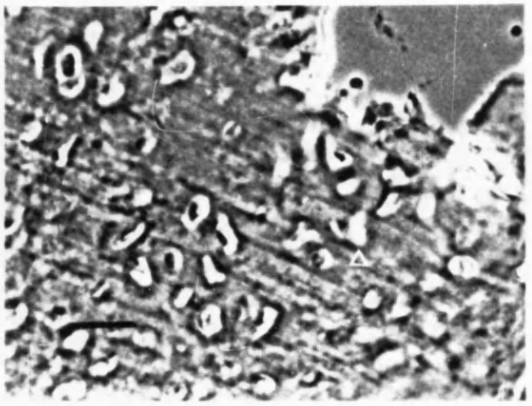


Fig. 3.2.4.2.57.

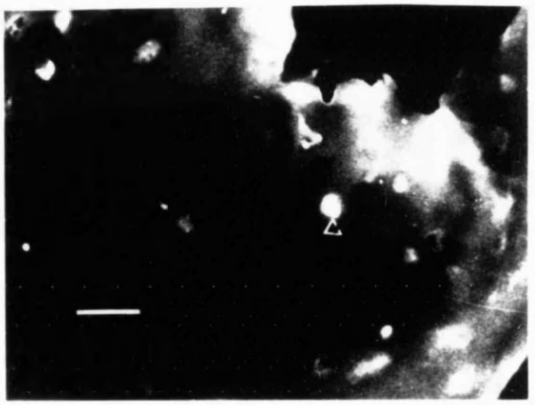


Fig. 3.2.4.2.58.

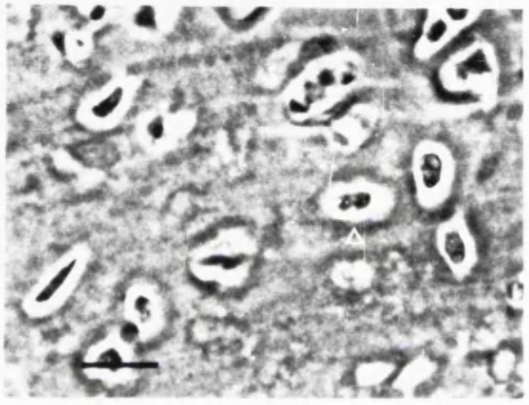


Fig. 3.2.4.2.59.

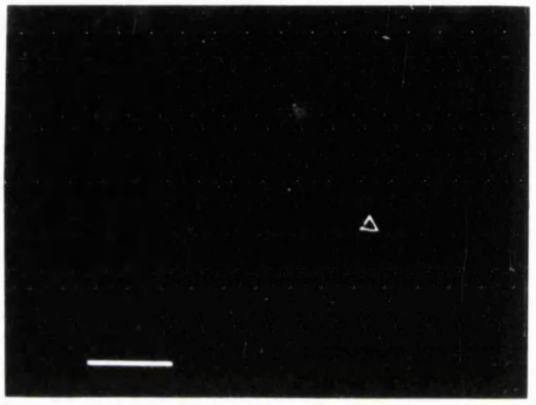


Fig. 3.2.4.2.60.

Fig. 3.2.4.2.61.

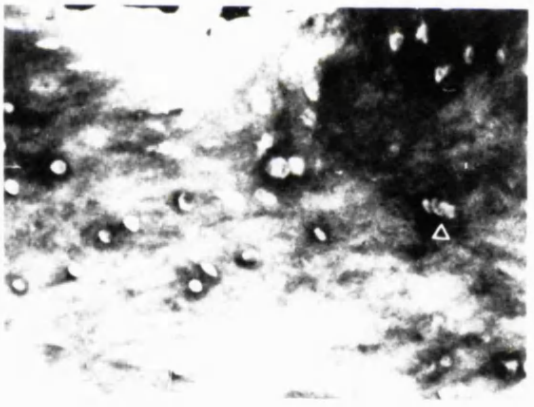


Fig. 3.2.4.2.62.

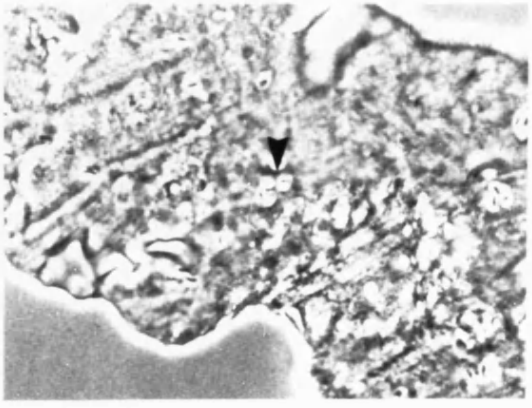


Fig. 3.2.4.2.63.

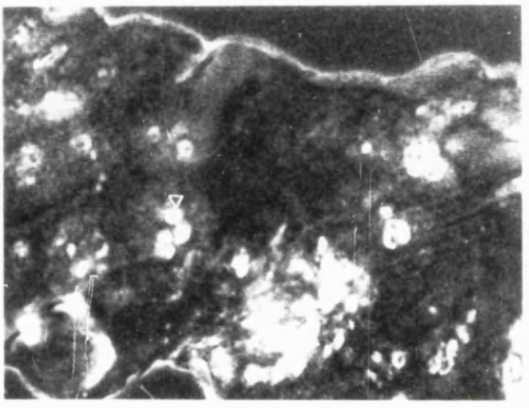


Fig. 3.2.4.2.64.

Fig. 3.2.4.2.65. Phase contrast micrograph of superficial graft tissue at 12 months post-implantation.
Fig. 3.2.4.2.66. Fluorescence micrograph of superficial graft tissue at 12 months post-implantation. Immuno-positive staining for chondroitin -6- sulphate was found pericellularly.

Fig. 3.2.4.2.67. Phase contrast micrograph of superficial graft tissue at 12 months post-implantation.
Fig. 3.2.4.2.68. Fluorescence micrograph of superficial graft tissue after 12 months post-implantation. Collagen type I-staining was shown occasionally in discrete areas of the graft tissue.

Fig. 3.2.4.2.69. Phase contrast micrograph of superficial graft tissue at 12 months post-implantation.
Fig. 3.2.4.2.70. Fluorescence micrograph of superficial graft tissue at 12 months post-implantation. Keratan sulphate-positive staining was not found.

Fig. 3.2.4.2.71. Phase contrast micrograph of superficial graft tissue at 12 months post-implantation.
Fig. 3.2.4.2.72. Fluorescence micrograph of superficial graft tissue at 12 months post-implantation. The control section only showed background staining.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

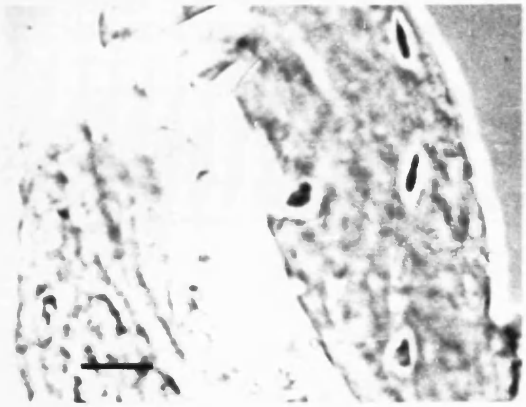


Fig. 3.2.4.2.65.

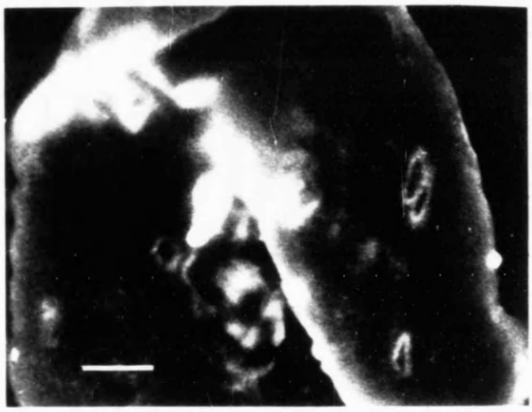


Fig. 3.2.4.2.66.

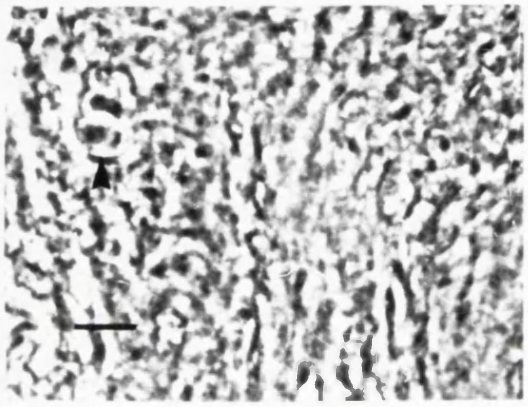


Fig. 3.2.4.2.67.

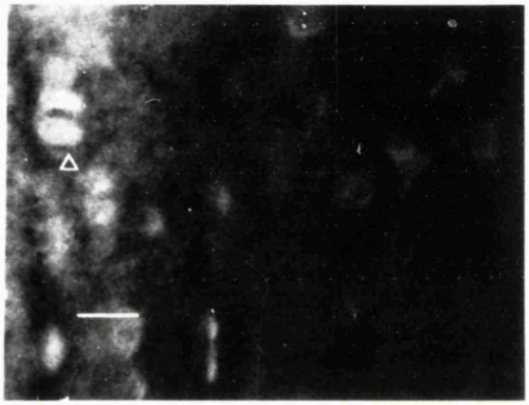


Fig. 3.2.4.2.68.

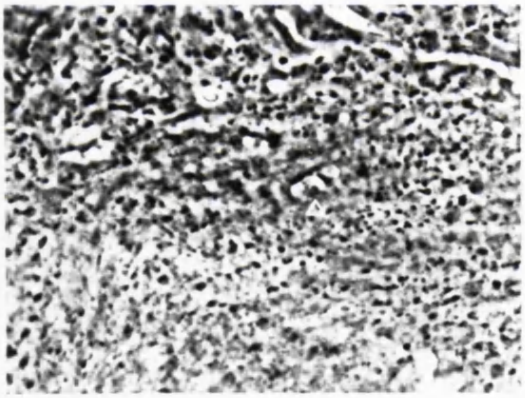


Fig. 3.2.4.2.69.

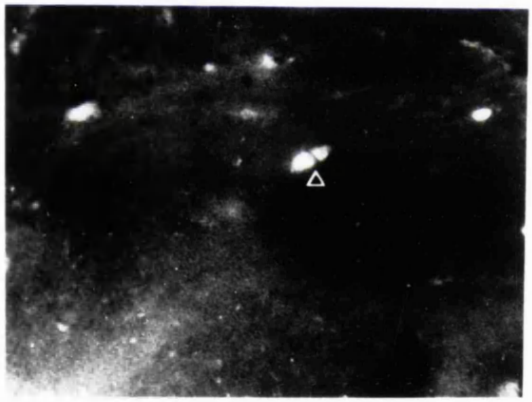


Fig. 3.2.4.2.70.

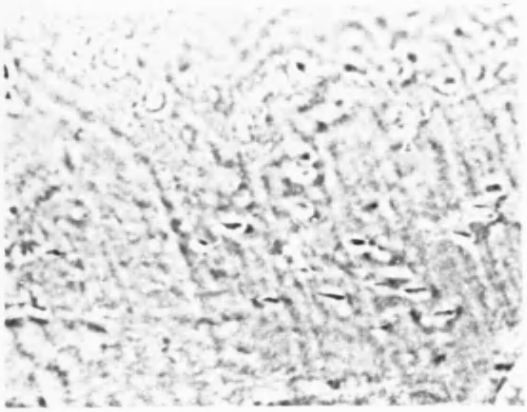


Fig. 3.2.4.2.71.

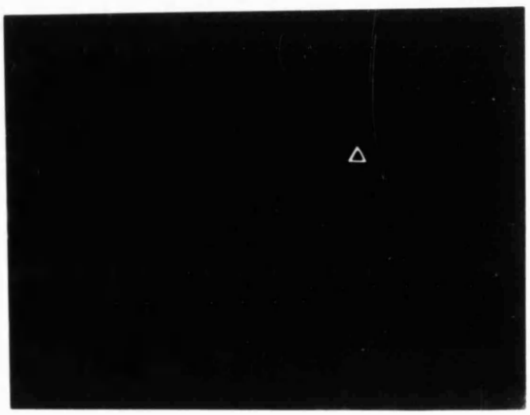


Fig. 3.2.4.2.72.

Fig. 3.2.4.2.73. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation .
Fig. 3.2.4.2.74. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation. Collagen type II-staining was not found.

Fig. 3.2.4.2.75. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation.
Fig. 3.2.4.2.76. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation. Immuno-positive staining for chondroitin -4- sulphate was shown occasionaly pericellularly in discrete areas.

Fig. 3.2.4.2.77. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation.
Fig. 3.2.4.2.78. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation. Immuno-positive staining for chondroitin -6- sulphate was found intercellularly.

Fig. 3.2.4.2.79. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation.
Fig. 3.2.4.2.80. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation. Collagen type I-staining was not detected.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

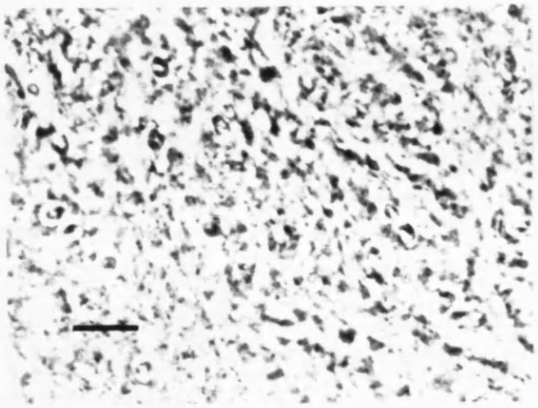


Fig. 3.2.4.2.73.

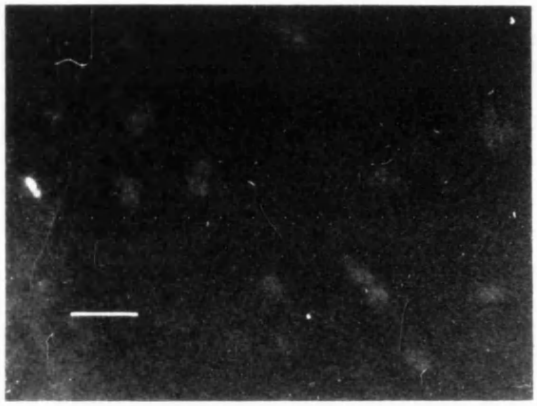


Fig. 3.2.4.2.74.

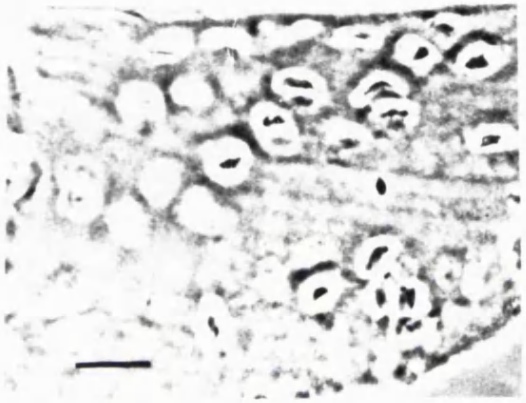


Fig. 3.2.4.2.75.

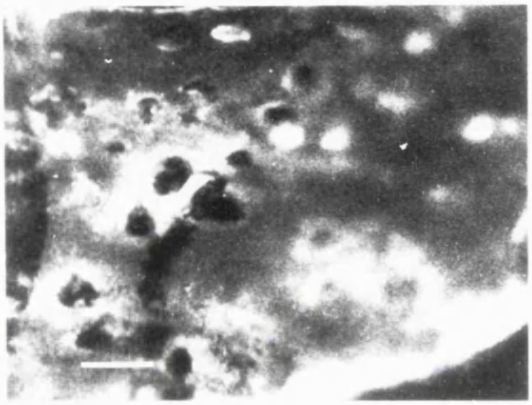


Fig. 3.2.4.2.76.

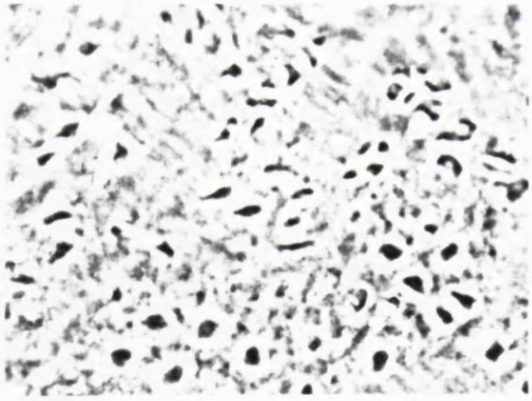


Fig. 3.2.4.2.77.

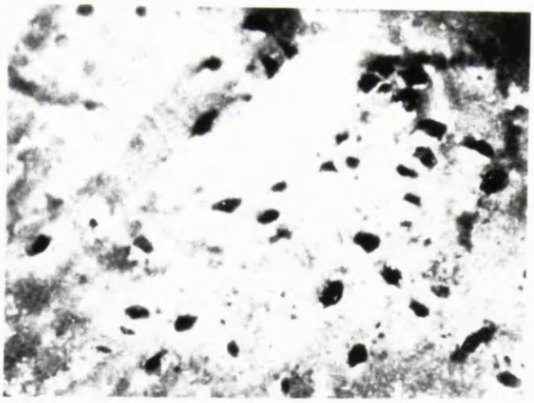


Fig. 3.2.4.2.78.

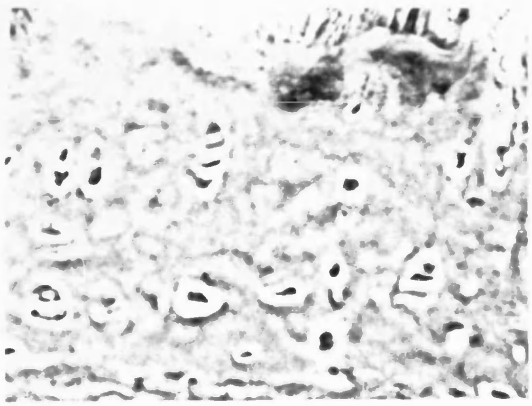


Fig. 3.2.4.2.79.

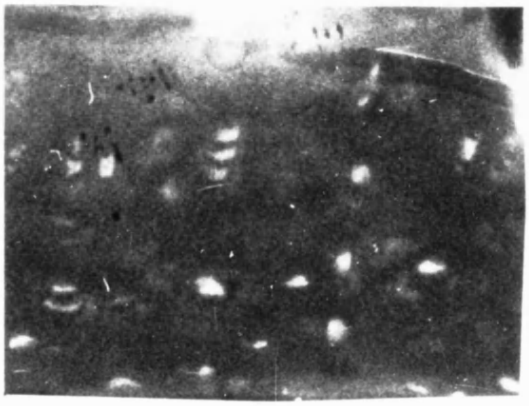


Fig. 3.2.4.2.80.

Fig. 3.2.4.2.81. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation.
Fig. 3.2.4.2.82. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation. Immuno-positive staining for keratan sulphate was found intercellularly.

Fig. 3.2.4.2.83. Phase contrast micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation.
Fig. 3.2.4.2.84. Fluorescence micrograph of superficial host tissue within the graft region of the control knee at 12 months post-implantation. The control only showed background staining.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

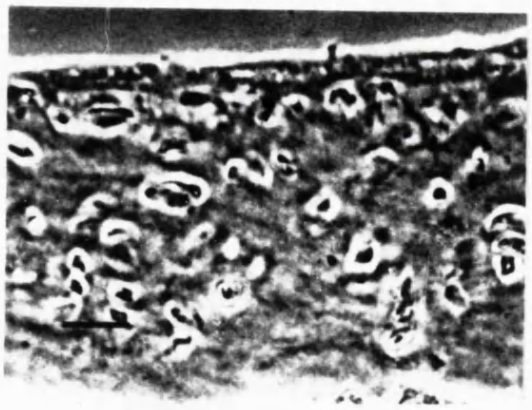


Fig. 3.2.4.2.81.

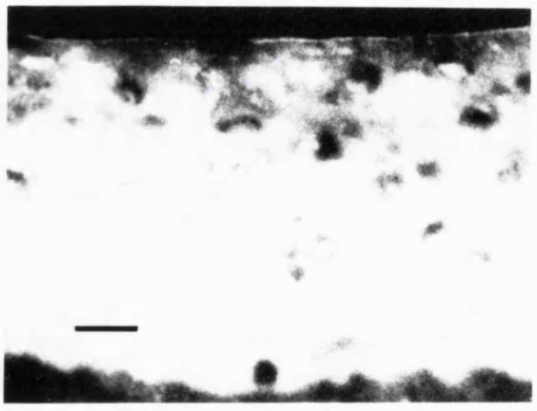


Fig. 3.2.4.2.82.

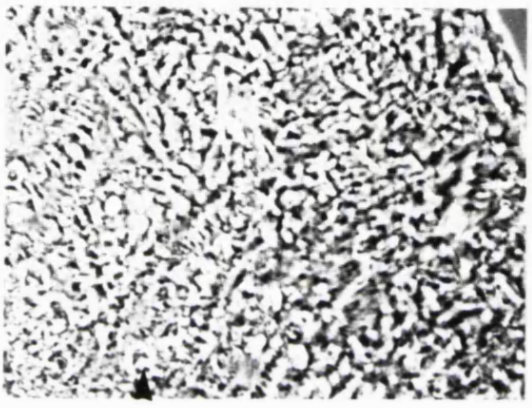


Fig. 3.2.4.2.83.

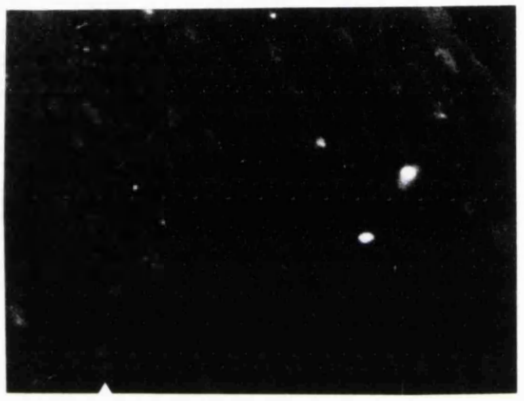


Fig. 3.2.4.2.84.

Fig. 3.2.4.3.01. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation .

Fig. 3.2.4.3.02. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. Collagen type II-staining was found in some areas of the graft tissue intercellularly.

Fig. 3.2.4.3.03. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.

Fig. 3.2.4.3.04. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. Immuno-positive staining for chondroitin -4- sulphate was not shown.

Fig. 3.2.4.3.05. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.

Fig. 3.2.4.3.06. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. Immuno-positive staining for chondroitin -6- sulphate was not found.

Fig. 3.2.4.3.07. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.

Fig. 3.2.4.3.08. Fluorescence micrograph of superficial graft tissue after 3 months post-implantation. Collagen type I-staining was not shown.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

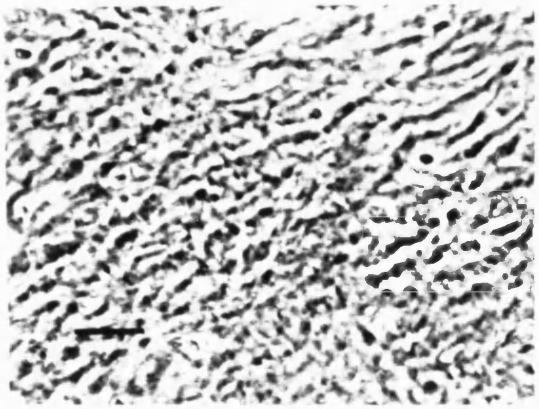


Fig. 3.2.4.3.01.

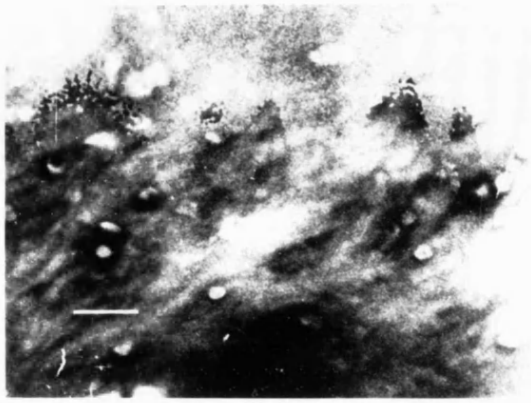


Fig. 3.2.4.3.02.

Fig. 3.2.4.3.03.

Fig. 3.2.4.3.04.

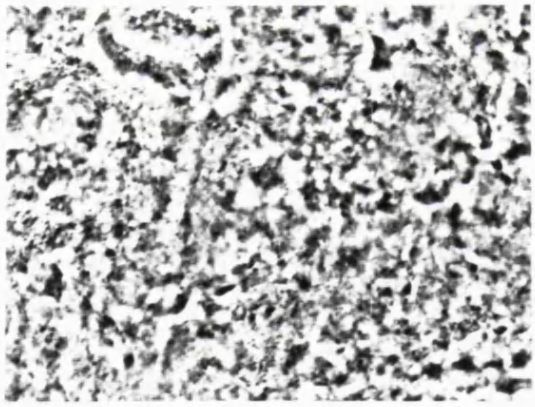


Fig. 3.2.4.3.05.

Fig. 3.2.4.3.06.

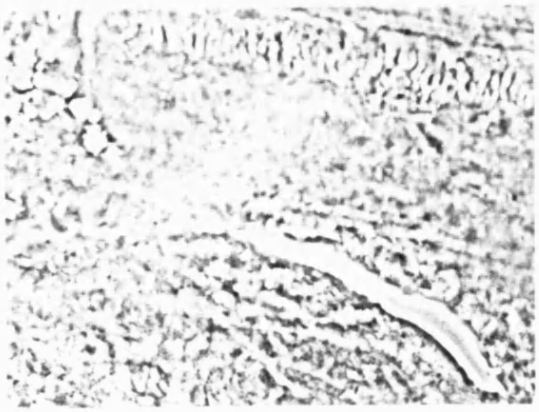


Fig. 3.2.4.3.07.

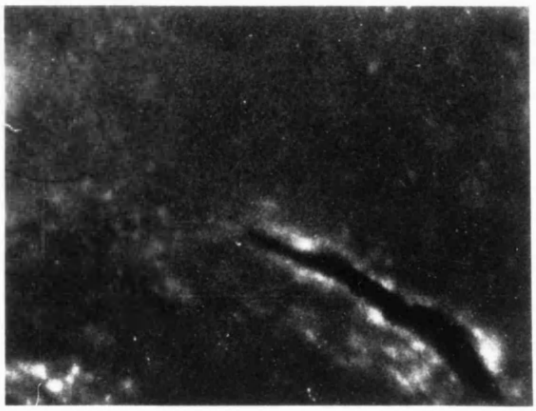


Fig. 3.2.4.3.08.

Fig. 3.2.4.3.09. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.
Fig. 3.2.4.3.10. Fluorescence micrograph of superficial graft tissue at 3 monthspost-implantation. Keratan sulphate-positive staining was found in some areas of the graft tissue.

Fig. 3.2.4.3.11. Phase contrast micrograph of superficial graft tissue at 3 months post-implantation.
Fig. 3.2.4.3.12. Fluorescence micrograph of superficial graft tissue at 3 months post-implantation. The control section only showed background staining.

Fig. 3.2.4.3.13. Phase contrast micrograph of tibial articular cartilage of 24 months old Sandy Half Lop rabbit.
Fig. 3.2.4.3.14. Fluorescence micrograph of tibial articular cartilage of 24 months old Sandy Half Lop rabbit. Collagen type II-staining was found pericellularly and weak intercellularly.

Fig. 3.2.4.3.15. Phase contrast micrograph of tibial articular cartilage of 24 months old Sandy Half Lop rabbit.
Fig. 3.2.4.3.16. Fluorescence micrograph of tibial articular cartilage of 24 months old Sandy Half Lop rabbit. Immuno-positive staining for chondroitin -4- sulphate was shown pericellularly.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

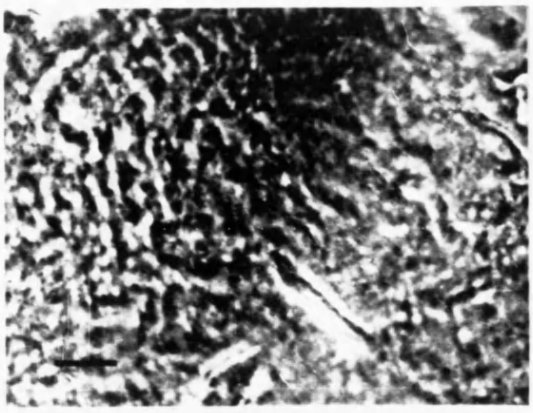


Fig. 3.2.4.3.09.

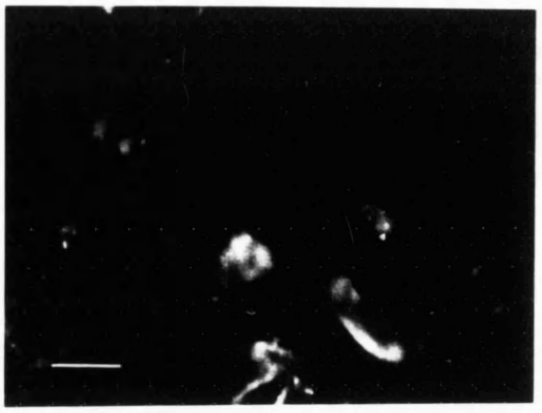


Fig. 3.2.4.3.10.

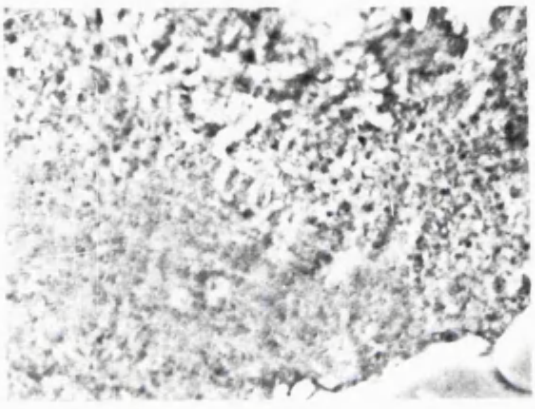


Fig. 3.2.4.3.11.

Fig. 3.2.4.3.12.

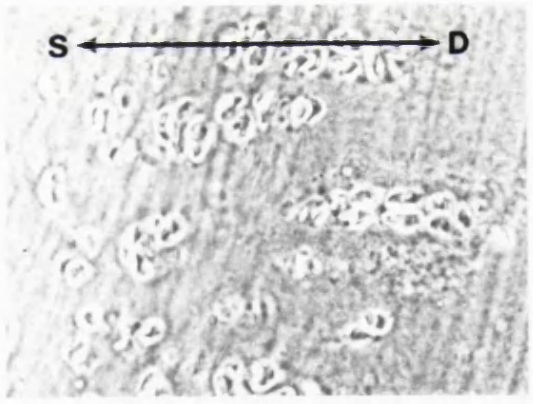


Fig. 3.2.4.3.13.

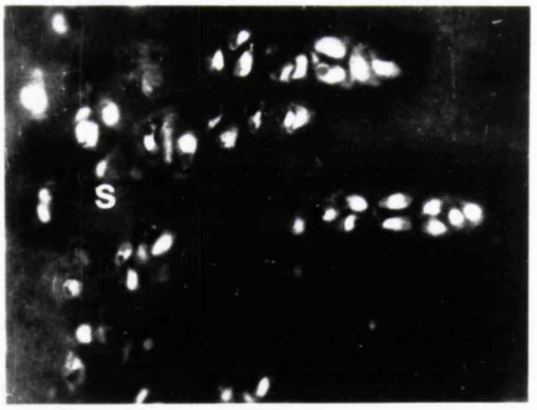


Fig. 3.2.4.3.14.

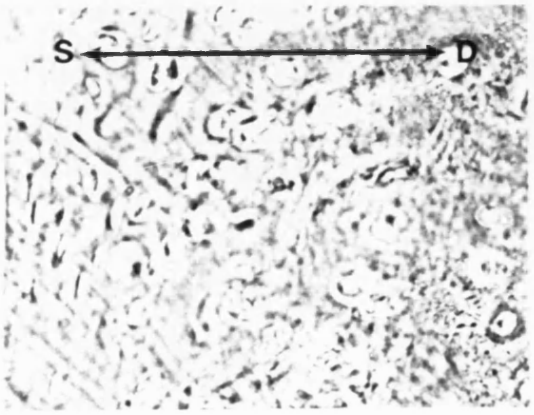


Fig. 3.2.4.3.15.

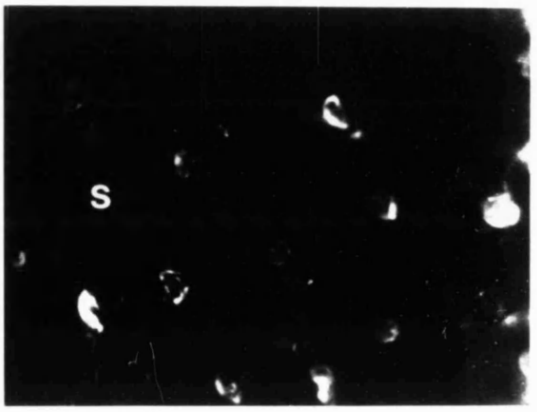


Fig. 3.2.4.3.16.

Fig. 3.2.4.3.17. Phase contrast micrograph of tibial articular cartilage of 24 months old Sandy Half Lop rabbit.
Fig. 3.2.4.2.18. Fluorescence micrograph of tibial articular cartilage of 24 months old Sandy Half Lop rabbit. Immuno-positive staining for chondroitin -6- sulphate was found pericellularly.

Fig. 3.2.4.3.19. Phase contrast micrograph of tibial articular cartilage of 24 months old Sandy Half Lop rabbit.
Fig. 3.2.4.3.20. Fluorescence micrograph of tibial articular cartilage of 24 months old Sandy Half Lop rabbit. Keratan sulphate-positive staining was not found in surface layers, but was present intercellularly in deep layers.

Note: magnification x 200, which is shown with scale bar (40 μm) on first phase contrast micrograph and first fluorescence micrograph of each page. When x 260 magnification is used, a scale bar (40 μm) can be found on the phase contrast micrograph and fluorescence micrograph concerned. Identical areas of the phase contrast micrographs and fluorescence micrographs were given markers to make comparison easier.

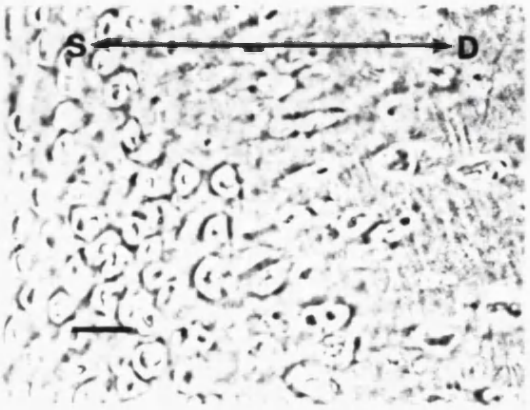


Fig. 3.2.4.3.17.

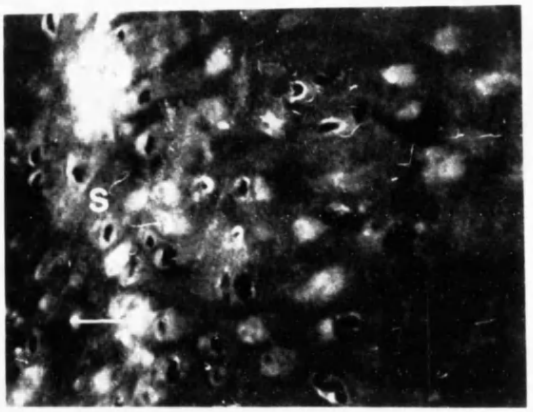


Fig. 3.2.4.3.18.

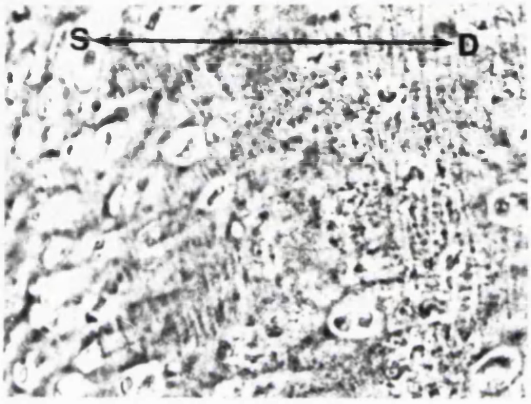


Fig. 3.2.4.3.19.

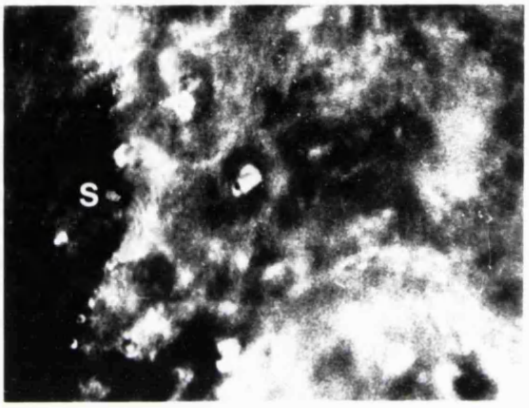


Fig. 3.2.4.3.20.

Fig. 3.2.4.4.01. Photomicrograph of section of graft tissue at 12 months post-implantation of carbon fibre plug with chondrocytes applying Silver enhancement immunocytochemical techniques (SEIT). Immuno-positive staining for collagen type X was not found within the graft tissue.

Fig. 3.2.4.4.02. Photomicrograph of section of the neighbouring cartilage of the graft region at 12 months post-implantation of carbon fibre plug with chondrocytes applying SEIT. Immuno-positive staining for collagen type X was found in the calcifying zone of the surrounding cartilage.

Fig. 3.2.4.4.03. Photomicrograph of section of graft tissue at 12 months post-implantation of carbon fibre plug with chondrocytes applying SEIT. Immuno-positive staining for keratan sulphate was found pericellularly in the graft tissue, especially in the surface layers of the graft tissue.

Fig. 3.2.4.4.04. Photomicrograph of section of the neighbouring cartilage of the graft region at 12 months post-implantation of carbon fibre plug with chondrocytes applying SEIT. Immuno-positive staining for keratan sulphate was observed in the surface and deep layers, both, pericellularly, and, intercellularly.

Fig. 3.2.4.4.05. Photomicrograph of section of graft tissue at 12 months post-implantation of carbon fibre plug with chondrocytes applying SEIT. Immuno-positive staining for 7D4 (without chondroitinase pre-digestion) was detected in surface layer and in the calcifying zone of the graft tissue.

Fig. 3.2.4.4.06. Photomicrograph of section of the neighbouring cartilage of the graft region at 12 months post-implantation of carbon fibre plug with chondrocytes applying SEIT. Immuno-positive staining for 7D4 (without chondroitinase pre-digestion) was observed in both, the superficial layers, and, the calcifying zone, of the graft tissue.

Fig. 3.2.4.4.07. Photomicrograph of section of graft tissue at 12 months post-implantation of carbon fibre plug with chondrocytes applying SEIT. Control section showed minimal staining.

Fig. 3.2.4.4.08. Photomicrograph of section of the neighbouring cartilage of the graft region at 12 months post-implantation of carbon fibre plug with chondrocytes applying SEIT. Control section showed minimal staining.

Note: magnification x 200, which is shown with scale bar (10 μm) on first two photomicrographs of this page.

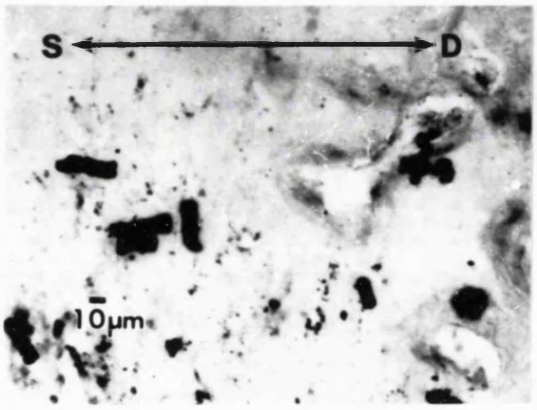


Fig. 3.2.4.4.01.

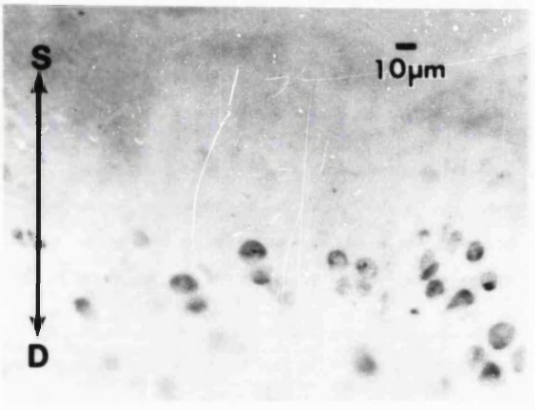


Fig. 3.2.4.4.02.

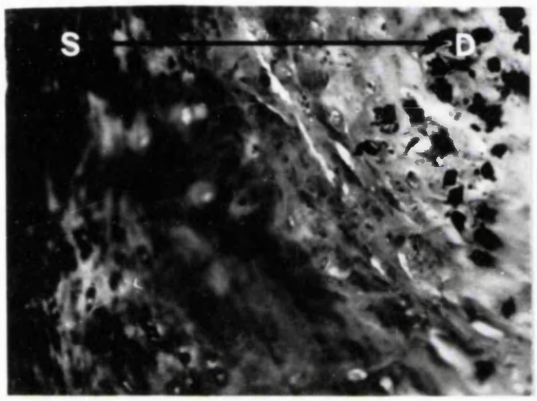


Fig. 3.2.4.4.03.

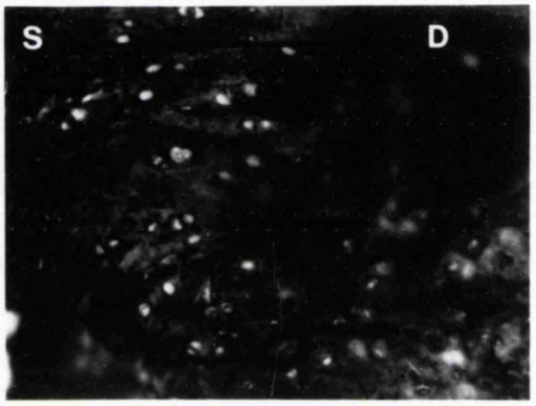


Fig. 3.2.4.4.04.

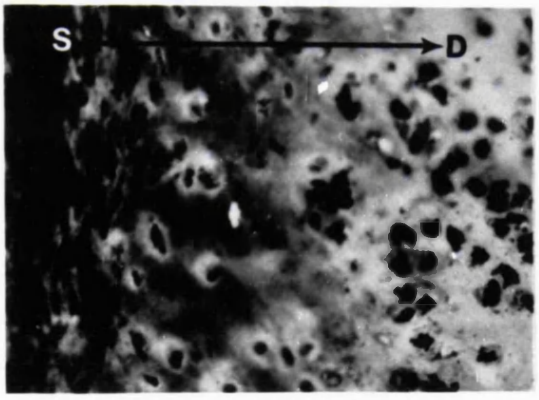


Fig. 3.2.4.4.05.

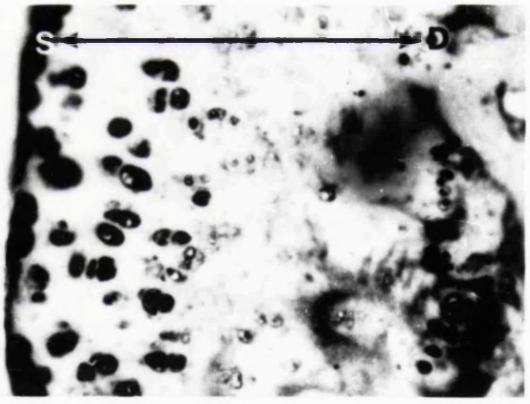


Fig. 3.2.4.4.06.

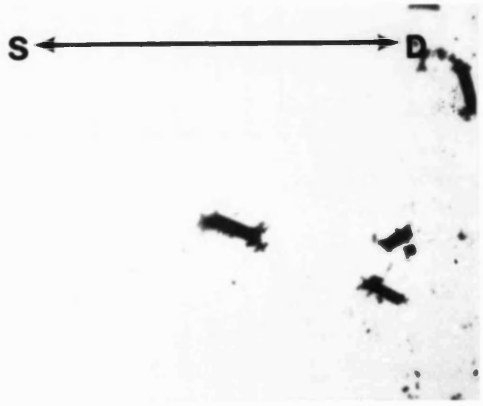


Fig. 3.2.4.4.07.

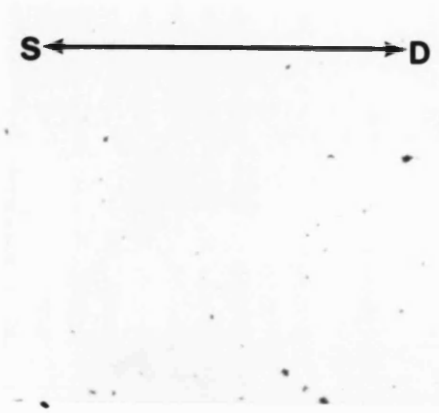


Fig. 3.2.4.4.08.

3.3. Biomechanical assesment of the grafts.

The results of the elastic modulus of the grafts are listed in tables 3.14-3.21. Because of the variation found in values for the plug thickness measurements were carried out to determine the effect of layer thickness on the Youngs modulus (Fig. 3.6.).

3.3.1. Biomechanical testing of carbon fibre plugs and collagen gel plugs with chondrocytes at 6 months post-implantation.

Tables 3.14, 3.16 and 3.19. give the results at 6 months post-implantation.

Due to the limited number of specimens available, these results need to be interpreted with some caution. The carbon fibre grafts without associated chondrocytes showed values for Youngs modulus in the range of 0.67-0.91MPa (after 1 sec) after 6 months, whilst the carbon fibre grafts with chondrocytes showed values for Youngs modulus of 1.45MPa or 1.74MPa (after 1 sec). The measured graft thickness varied in the group of carbon fibre without cells between 0.51mm-1.00mm, whilst in the group of carbon fibre graft with cells these values showed less variation (0.71mm and 0.84mm). The a/R (a=geometric modulus, R=radius) ratio showed little variation in both groups.

The collagen gel plugs with chondrocytes (graft thickness: 0.23mm and 0.34mm) showed at 6 months post-implantation values of Youngs modulus of 0.28MPa and 0.15MPa (after 1 sec). The a/R ratio of the collagen gel plugs with chondrocytes (0.37 and 0.5) showed more variation than both the carbon fibre plugs with and without cells after 6 months. To place the above data in context, the cartilage of lateral tibial plateau of right and left knee joints of 18 months old Sandy Half Lop rabbits, which were of similar age to the rabbits analysed above, was tested (Figs. 3.3.-4.). The specimen was placed in the indentator in such a way that the area of the lateral tibial plateau where the drill hole was made in the experimental rabbits, was tested. Youngs modulus analyses showed values between 0.69-1.75MPa (after 1 sec), whilst the graft thickness varied between 0.17-0.50mm. The a/R ration varied between 0.26-0.40 (after 1 sec).

3.3.2. Biomechanical testing of carbon fibre plugs and collagen gel plugs with chondrocytes at 12 months post-implantation.

Tables 3.15, 3.17 and 3.20 list the results at 12 months post-implantation.

The carbon fibre plugs with and without cells showed at 12 months post-implantation, the Youngs modulus was 0.79MPa and 1.29MPa, and the graft thickness was 0.45mm and 0.90mm, respectively. The a/R ratio (a=geometric modulus, R=radius) was 0.37 and 0.41, respectively.

The collagen gel plugs only after 12 months showed variation in the Youngs modulus; 0.75MPa and 1.84MPa (after 1 sec), respectively. In the graft, little variation was found in the thickness (0.29mm and 0.36mm) as was the case for the a/R ratio: 0.34 and 0.30.

The collagen gel plugs with cells after 12 months showed wide variation in the Youngs modulus; 0.67MPa and 5.86MPa (after 1 sec) and a wide variation was also detected in

the graft thickness 0.15mm and 0.44mm. The a/R ratio showed little variation: 0.27 and 0.24.

To compare these results with non-operated articular cartilage, normal articular cartilage of similar age was tested as described above. The Youngs modulus (after 1 sec) was 1.72MPa or 1.11MPa and the tissue thickness was 0.45mm and 0.44mm. The a/R ratio was 0.32 and 0.35. Table 3.21. and Figs. 3.3.-4. compare the values for Youngs modulus of carbon fibre plugs and collagen gel grafts after 6 and 12 months with those of normal rabbit articular cartilage of the same age. It can be seen that the values for the mean elastic modulus of carbon fibre plugs at 6 and 12 months were in the same range, whilst the values for the mean elastic modulus of collagen gel plugs at 6 and 12 months were rather dissimilar. Also, the mean value for the elastic modulus of the collagen gel plugs at 12 months was, firstly, about twice the median value and, secondly, showed a wide range of values. The mean value for the elastic modulus of the carbon fibre plugs at 6 months was in the same range as the mean value for normal rabbit articular cartilage, whilst it was higher than the value for the elastic modulus of the collagen gel plugs at the same time period. At 12 months post-implantation, similar conclusions for the carbon fibre plugs and the normal rabbit articular cartilage could be drawn, whilst the mean value for the elastic modulus of the collagen gel plugs was higher than those of the carbon fibre plugs, but as stated previously the range of values of the elastic modulus was wide. Because of the small sample size, statistical analyses were not carried out.

3.3.3. Biomechanical testing of hydrogel PC 97 plugs with chondrocytes at 3 months post-implantation.

Table 3.18. showed the results at 3 months post-implantation for the hydrogel PC 97 plugs with and without chondrocytes. At 3 months post-implantation the hydrogel PC 97 plugs without chondrocytes showed wide variation in the Youngs modulus (after 1 sec) 1.56-11.63MPa. Also, in the values for the graft thickness a wide variation was also found 0.45-1.36mm, whilst the a/R (a=geometric modulus, R=radius) ratio varied little (0.22-0.36). The hydrogel PC 97 plugs with cells also showed a variation in Youngs modulus (after 1 sec): 1.34-9.85MPa, whilst the graft thickness varied between 1.27-1.54mm. The a/R ratio was in the range 0.24 - 0.42. Table 3.21. and Fig. 3.5. show a comparison between the values for the elastic modulus of the hydrogel PC 97 plugs at 3 months post-implantation with the values of normal rabbit articular cartilage at 6 and 12 months. The hydrogel PC 97 plugs were stiffer, meaning it had a higher mean value for the elastic modulus; but it appeared that the standard deviation and, and to a lesser extent the standard error, were high. Because of the small sample size and different time intervals used, again, statistical analyses were not carried out.

NO. RABBIT + RIGHT/LEFT KNEE USED	THICKNESS OF GRAFT (mm)	INDENTATION DEPTH (mm)	MODULUS (MPa)	a/R RATIO
carbon fibre 126L	1.00	0.34(1s) 0.36(2s)	0.91 0.80	0.46 0.48
127L	0.51	0.27(1s) 0.29(2s)	0.67 0.58	0.41 0.43
128L	0.64	0.29(1s) 0.31(2s)	0.79 0.66	0.43 0.45
+chondrocytes 127R	0.71	0.24(1s) 0.25(2s)	1.45 1.28	0.39 0.40
193R	0.84	0.21(1s) 0.23(2s	1.74 1.62	0.38 0.42

Table 3.14. Elastic modulus of carbon fibre grafts with and without associated chondrocytes at 6 months post-implantation.

NO. RABBIT + RIGHT/LEFT KNEE USED	THICKNESS OF GRAFT (mm)	INDENTATION DEPTH (mm)	MODULUS (MPa)	a/R RATIO
carbon fibre 159L	0.90	0.27(1s) 0.30(2s)	1.29 1.06	0.41 0.43
+chondrocytes 159R	0.45	0.23(1s) 0.27(2s	0.79 0.56	0.37 0.38

Table 3.15. Elastic modulus of carbon fibre grafts with and without associated chondrocytes at 12 months post-implantation.

NO. RABBIT + RIGHT/LEFT	THICKNESS OF GRAFT (mm)	INDENTATION DEPTH (mm)	MODULUS (MPa)	a/R RATIO
collagen gel +chondrocytes				
126R	0.23	0.21(1s) 0.23(2s)	0.28 0.21	0.37 0.39
196R	0.34	0.41(1s) 0.45(2s)	0.15 0.12	0.50 0.53

Table 3.16. Elastic modulus of collagen gel grafts with associated chondrocytes at 6 months post-implantation.

NO. RABBIT + RIGHT/LEFT KNEE USED	THICKNESS OF GRAFT (mm)	INDENTATION DEPTH (mm)	MODULUS (MPa)	a/R RATIO
collagen gel				
112L	0.29	0.19(1s)	0.75	0.34
113L	0.36	0.13(1s) 0.17(2s)	1.84 1.17	0.30 0.33
+chondrocytes				
112R	0.15	0.09(1s) 0.11(2s)	0.67 0.72	0.27 0.27
113R	0.44	0.08(1s) 0.09(2s)	5.86 4.72	0.24 0.25

Table 3.17. Elastic modulus of collagen gel grafts with associated chondrocytes and control grafts at 12 months post-implantation.

NO. RABBIT + RIGHT/LEFT KNEE USED	THICKNESS OF GRAFT (mm)	INDENTATION DEPTH (mm)	MODULUS (MPa)	a/R RATIO
hydrogel				
286L	1.36	0.13(1s) 0.16(1.5s)	5.82 3.95	0.28 0.31
287L	1.16	0.08(1s)	11.63	0.22
290L	0.72	0.21(1s) 0.24(1.5s)	1.56 1.23	0.36 0.38
291L	0.45	0.15(1s) 0.16(1.5s)	1.83 1.71	0.30 0.31
292L	1.33	0.14(1s)	4.74	0.30
+chondrocytes				
287R	1.27	0.09(1s) 0.11(1.5s)	9.85 7.52	0.24 0.26
291R	1.25	0.29(1s) 0.29(1.5s)	1.34 1.32	0.42 0.42
292R	1.54	0.25(1s) 0.29(1.5s)	2.00 1.53	0.37 0.42

Table 3.18. Elastic modulus of hydrogel PC 97 grafts with and without associated chondrocytes at 3 months post-implantation.

NO. RABBIT + RIGHT/LEFT KNEE USED	THICKNESS OF GRAFT (mm)	INDENTATION DEPTH (mm)	MODULUS (MPa)	a/R RATIO
cartilage 156R	0.50	0.25(1s) 0.26(2s)	0.79 0.69	0.40 0.41
control R	0.23	0.10(1s) 0.10(2s)	1.75 1.74	0.26 0.26
control L	0.17	0.16(1s) 0.17(2s)	0.69 0.64	0.26 0.32

Table 3.19. Elastic modulus of normal tibial articular cartilage of 18 months old Sandy Half Lop rabbits.

NO. RABBIT+ RIGHT/LEFT KNEE USED	THICKNESS OF GRAFT	INDENTATION DEPTH (mm)	MODULUS (MPa)	a/R RATIO
cartilage 199R	0.45	0.16(1s) 0.18(2s)	1.72 1.26	0.32 0.34
199L	0.44	0.19(1s) 0.21(2s)	1.11 0.90	0.35 0.37

Table 3.20. Elastic modulus of normal tibial articular cartilage of 24 months old Sandy Half Lop rabbits.

nature of implant	carbon fibre plugs with and without cells		collagen gel plugs with cells and control plugs.		hydrogel plugs with and w/o cells	rabbit articular cartilage	
follow up time	6 months	12 months	6 months	12 months	3 months	6 months	12 months
elastic modulus median	0.91	1.04	0.22	1.30	3.37	0.79	1.42
mean (MPa)	1.11	1.04	0.22	2.28	4.84	1.08	1.41
S.D.(MPa)	0.46	0.35	0.09	2.45	4.01	0.58	0.43
S.E. (MPa)	0. 21	0.25	0.07	1.22	1.41	0.34	0.31

INDEX

S.D. standard deviation
S.E. standard error

Table 3.21. Comparison of values for Youngs modulus for carbon fibre plugs and collagen gel plugs at 6 and 12 months, hydrogel PC 97 plugs at 3 months compared to normal rabbit articular cartilage of 18 and 24 months old rabbits. Individual data can be found in Tables 3.14-3.19.

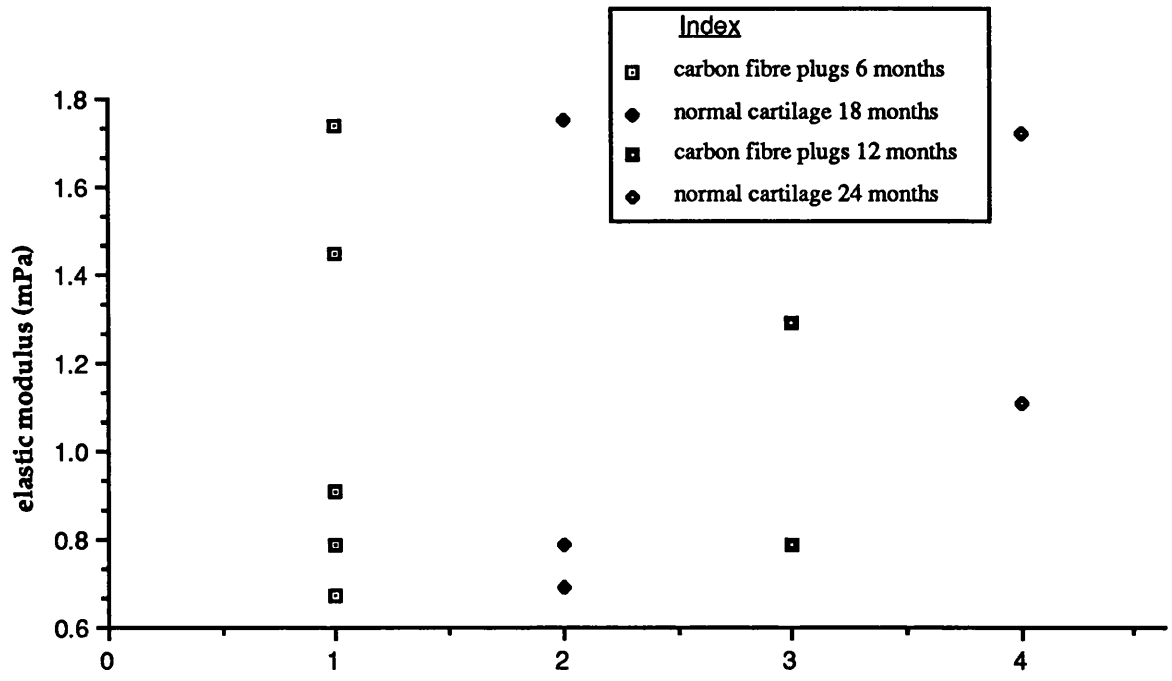


Fig. 3.3. The elastic moduli of carbon fibre plugs at 6 and 12 months post-implantation compared to normal (non-diseased) articular cartilage of rabbits of similar age as experimental rabbits.

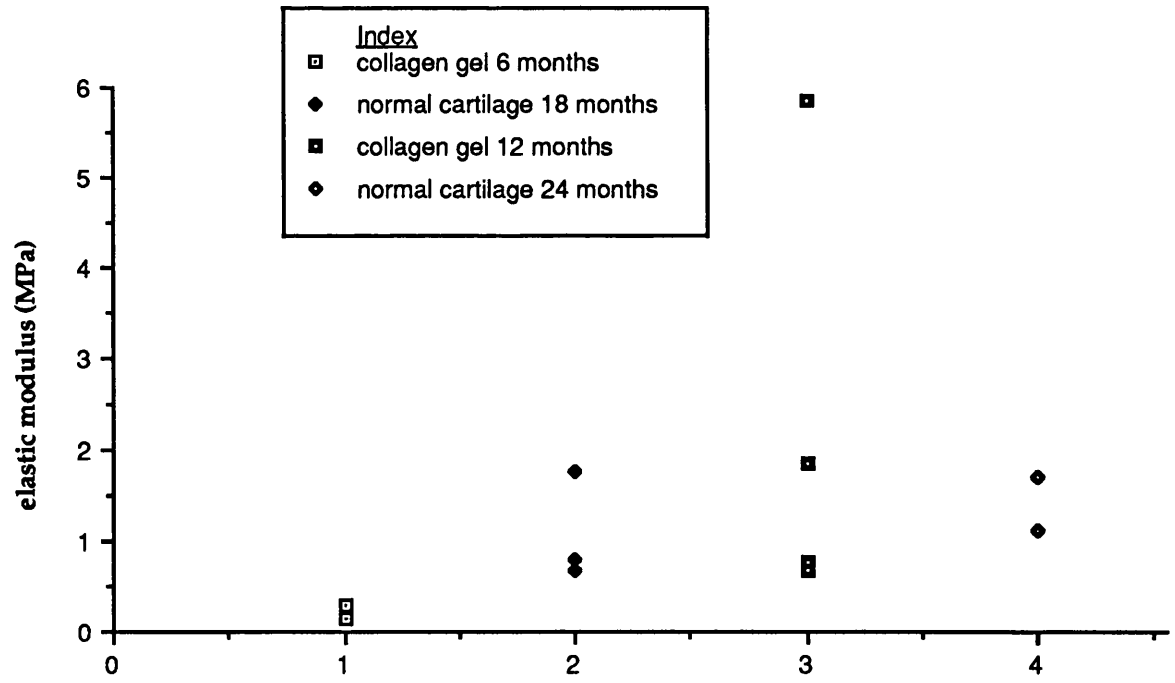


Fig. 3.4. The elastic moduli of collagen gel plugs at 6 and 12 months post-implantation compared to normal (non-diseased) articular cartilage of 18 and 24 months old rabbits.

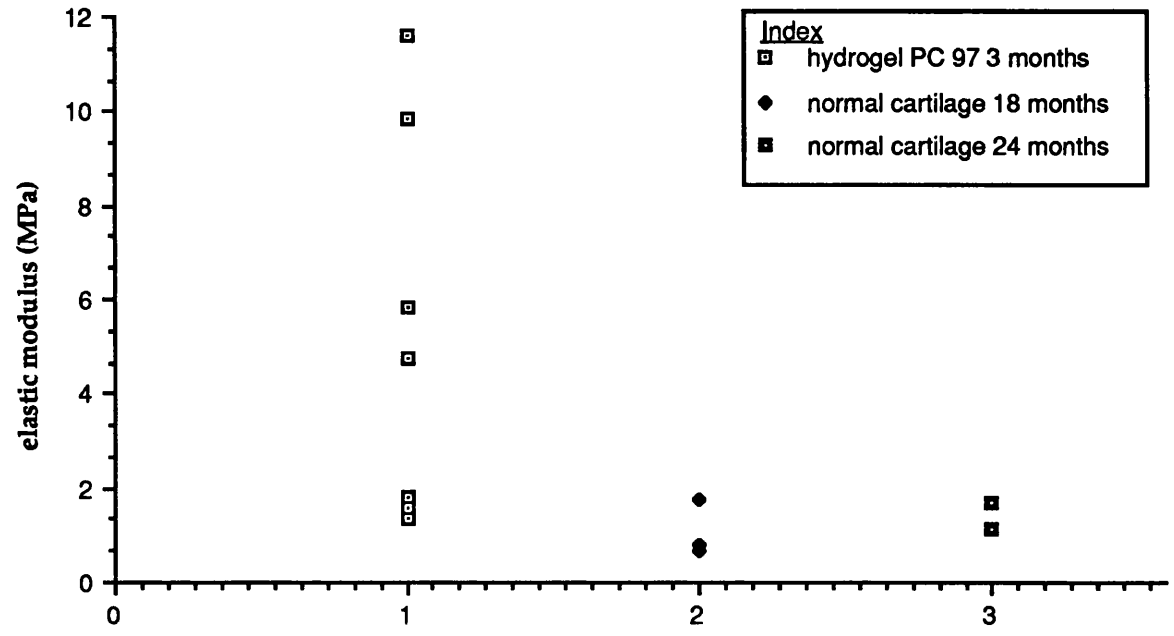


Fig. 3.5. The elastic moduli of hydrogel PC 97 plugs at 3 months post-implantation compared to normal (non-diseased) articular cartilage of 18 and 24 months old rabbits.

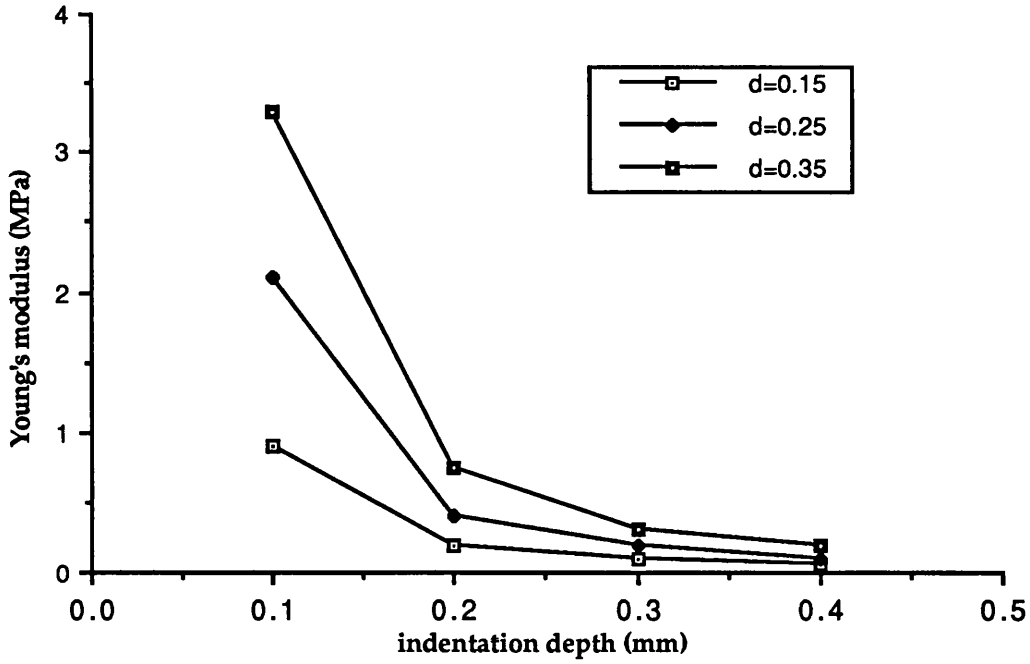


Fig. 3.6. Effect of layer thickness (d) on the Youngs modulus.

In table 3.22 the results of the water content, tensile strength, initial modulus, elongation to break and surface free energy for hydrogel PC 97 before and after autoclaving are given. It can be concluded that autoclaving of the hydrogel PC 97 did not significantly alter its mechanical properties with regard to the mechanical properties shown in table 3.22.

	before autoclaving	after autoclaving
EWC(%)	**54.9**	**57.6**
Tensile Strength (MPa)	**3.3+-0.3**	**3.4+-0.1**
Initial Modulus (MPa)*	**21.0+-5**	**22.0+-3**
Elongation to Break	**65+-11**	**64+-8**
Surface Free Energy(mN/m)	**67.9**	**68.0**
-dispersive component	**26.7**	**24.4**
-polar component	**41.2**	**43.6**

Tabel 3.22. Mechanical data for the hydrogel PC 97.

DISCUSSION

The aim of the study was to investigate the cellular and molecular aspects of repair tissue in full-thickness rabbit articular cartilage defects after implantation of three different materials which had been cultured with isolated articular chondrocytes.
Immature rabbit chondrocytes were isolated from articular cartilage of femoral condyle, tibial plateau and patella by sequential digestion with pronase and collagenase. The cells were injected at high density into an unravelled carbon fibre plug, into a type I collagen gel, or inoculated onto a hydrogel PC 97 at high density and then cultured for 3 weeks. The carbon fibre, collagen gel and hydrogel PC 97 plugs with associated chondrocytes were implanted into full-thickness defects of articular cartilage in one year old Sandy Half Lop rabbits. After 3 weeks *in vitro* culture, and at 3 months post-implantation, the graft tissue was examined using histology, electron microscopy, immunocytochemistry and biomechanical testing (hydrogel PC 97 only). At 6 and 12 months post-implantation of the carbon fibre and collagen gel plugs with associated chondrocytes, the grafts were examined using histology, immunolocalization and biomechanical testing. Controls comprised either the carbon fibre or hydrogel PC 97 plugs without chondrocytes, or the drill hole defect was left empty (as a control for the collagen gel), because the collagen gel plugs without cells could not be fixed in the defect sufficiently during implantation.

In this chapter, firstly, the results (presented in chapter 3) are discussed and compared to other studies. Secondly, the three implant materials are compared and contrasted and, thirdly, a conclusion with suggestions for future work is given.

Carbon fibre plugs.

The morphology assumed by chondrocytes during *in vitro* culture within the carbon fibre plug was found to depend on the cell density at inoculation. In areas of high cell density the cells retained their rounded-polygonal shape, whilst in low density culture areas the chondrocytes became fibroblast-like in appearance. It has been described that embryonic chick chondrocytes in high density culture maintained their differentiated state, whilst when inoculated at lower densities, nodules of aggregates of chondrocytes surrounded by elongated fibroblast-like cells were present (Oakes *et al.*, 1977). Chondrocyte phenotype is partly dependent on cell shape (Archer *et al.*, 1982; Benya and Shaffer, 1982). Carbon fibres don't appear to actively alter cell shape and thus the overall chondrocyte phenotype.

The acceptance rate of the carbon fibre plugs plus chondrocytes after 3, 6 and 12 months was 67%, 80% and 60%, and the acceptance rate of carbon fibre plugs without cells was 67%, 80% and 70% respectively. Using the same rabbit system, Aston (1980) showed that at 3 and 12 months post-implantation of cultured immature rabbit articular chondrocytes, partial and complete filling of the defect with histologically definable

cartilage-like tissue was achieved in 50% and 67% of cases respectively. The data of the present study showed that most carbon fibre plugs with or without chondrocytes remained in the defect, and hence supported the idea that carbon fibre could act as a scaffolding material for the cells, not only cells applied extrinsically, but also for mesenchymal cells migrating from the marrow.

Using histological techniques, the carbon fibre graft with associated chondrocytes showed a cartilaginous matrix with incorporation of the carbon fibres after 3 weeks *in vitro* and at 3 months post-implantation. The graft after 6 and 12 months also showed a cartilage-like tissue in all layers except the surface. In contrast, histology of the carbon fibre plug without chondrocytes at 3 months post-implantation, showed a fibrous-like matrix with a few localized areas of cartilaginous tissue. At 6 and 12 months post-implantation, the repair tissue was a combination of fibrous-like and cartilaginous tissue. These results are in agreement with the studies carried out by Minns *et al.*, (1982), but contrast with some of the findings described by Furukawa *et al.*, (1980). The latter study will be discussed in more detail below.

When comparing the types of repair tissue formed after implantation of carbon fibre with and without chondrocytes, it appears that the graft tissue with associated chondrocytes is able to maintain a cartilage-like consistency up to three months. Therefore, it has to be concluded that the presence of a carbon fibre meshwork supports the chondrogenic phenotype up to 3 months post-implantation. With time, however, the surface of the repair tissue becomes fibrous. This might be caused, firstly, by the shift from a rounded to a flattened morphology of the cultured cells or, secondly, by invasion of fibroblasts from elsewhere. The loss of the differentiated state of the cultured chondrocytes has been attributed to the local micro-environmental conditions (Gospodarowicz *et al.*, 1980). Thirdly, other authors (as referred to by Radin *et al.*, 1979) suggested the influence of mechanical factors. Too high a stress in the initial healing period probably increases the tensile force in the joint surface to such an extent that the surface layer of the repair tissue is damaged. Other authors, such as Furukawa *et al.*, (1980) and Koide *et al.*, (1979) also described changes in the surface layer of the repair tissue with time. Furukawa and co-workers (1980) created 3 mm (diameter) defects in rabbit patellar grooves, which extended into the subchondral bone. They found after eight weeks that histologically (staining with Safranin-O), the repair tissue was hyaline cartilage-like in appearance, and that this lasted up to 24 weeks. However, after 24 weeks the repair tissue underwent fibrillation and degeneration. A similar study by Koide and co-workers (1979) showed that after drilling two 3 mm (diameter) holes through the distal femoral articular cartilage of mature and immature rabbits, histologically definable cartilage-like tissue was generated up to 8 weeks, but, thereafter, degeneration of the surface layer of the host tissue within the graft region occurred.

At 6 and 12 months post-implantation of the carbon fibre plugs without chondrocytes, chondrogenesis was increasingly evident compared to the situation at 3 months post-implantation. *In vitro* studies have shown that mesenchymal cells will differentiate into chondrocytes when they are grown at high density (Levitt and Dorfman, 1972). The presence of the carbon fibre meshwork could be acting such that, *in vivo,* it keeps the mesenchymal cells in close contact. Also, it may be that the carbon fibre meshwork influences the differentiation of mesenchymal cells directly or indirectly. Another explanation might be the influence of mechanical factors. Radin *et al.*, (1979) described mechanical factors influencing the nature of the repair tissue in articular cartilage defects. It was suggested that when mesenchymal cells were subjected experimentally to equal pressure from all directions, fibro-cartilage-like tissue was formed. If the joint involved was moved continuously, synovial fluid was produced and stresses were formed in and over the articular surfaces. If these surfaces were relatively congruent, and the stresses not excessive, the pressurized synovial fluid transmitted hydrostatic pressure. This tended to promote differentiation to cartilage-like tissue. However, rigorous experimental testing of these ideas remains to be carried out.

The repair tissue formed after the implantation of cultured chondrocytes with (the present study) or without carbon fibre [as described by Aston (1980)] showed a tendency to consist of a fibrous surface layer and cartilaginous deeper layers. In the present study, this appeared after 6 months, whilst in the study of Aston, it was apparent after 3 months. There were several differences between the studies which might explain these results. (i) Aston cultured chondrocytes *in vitro* for four weeks in monolayer (5×10^5 cells per 30mm dish) followed by a two week period on a organ culture grid. In the present study, the chondrocytes were cultured *in vitro* as high density cultures (6×10^5 cells per 3.5mm plug; 1 plug per 35mm dish) for 3 weeks. Previously, it has been shown that cells cultured in monolayer have a tendency to spread and become flattened, thereby modulating away from the differentiated phenotype (Benya *et al.*, 1978). In contrast, chondrocytes cultured in suspension culture maintain their phenotype, thus re-inforcing the relationship between cell shape and chondrogenesis.

Nevertheless, changes in the extracellular matrix, as seen in osteoarthritis, alter the material properties of cartilage in such a way as to increase the compliance and decrease the stiffness of cartilage as described by Vignon *et al.*, (1984), whilst other authors (Altman *et al.*, 1984), observed an increase in stiffness. Both an increase and a decrease of stiffness will reduce the capacity of cartilage to function as a load bearing material for the joint. It might be assumed that changes in the extracellular matrix, caused by processes other than osteoarthritis such as *in vitro* culture, will also influence the function of cartilage as a joint bearing material.

(ii). The Youngs modulus of cultured articular chondrocyte grafts was lower (not published data) than the elastic modulus of carbon fibre plugs with chondrocytes, which

was in the same range as normal rabbit articular cartilage. It might be that the chondrocyte graft was not able to withstand the forces at the joint surface, whilst the carbon fibre plugs with associated chondrocytes were capable of resisting those forces for a longer period of time.

(iii). The presence of a carbon fibre meshwork may play an important role. Perhaps the carbon fibre functions as a fixation device for the chondrocytes in the defect, where as if the carbon fibres were absent, the chondrocytes would lose contact with the surrounding cartilage and bone more easily. This would make them more vulnerable to dislodging forces working at the joint surface.

(iv). The rabbits at the animal house of the Institute of Orthopaedics were mobilised in pens, whilst the rabbits in the study of Aston and colleagues were kept in cages. It has been suggested that normal cage activity produces a fibrous/fibrocartilaginous repair tissue in articular cartilage defects, whilst continuous passive motion produces cartilage-like repair tissue in the short term (Salter *et al.*, 1975). Although housing rabbits in pens is not equivalent to continuous passive motion, it increases the activity of the rabbit and avoids disuse phenomena.

Although at present there are no comparable studies using articular chondrocytes cultured within a carbon fibre plug, Itay *et al.*, (1987) implanted cultured chicken embryonal epiphyseal chondrocytes embedded in a fibrin gel into full-thickness defects in rooster condylar articular cartilage. The gel was described as a biologically resorbable immobilization vehicle (BRIV). At 2 months post-implantation, the repair tissue was found to be highly cellular and the matrix stained intensely with Alcian blue. In the subchondral zone, the newly formed cartilage was surrounded by increased vascular formation. After 6 months the repair tissue showed histological staining of the repair tissue comparable to normal chicken articular cartilage, but the deeper layers of the graft tissue were invaded by vascular elements and hypertrophic chondrocytes. There is a difference in structure between rooster and mammalian articular cartilage, because chicken articular surface cartilage has a fibrous component in contrast to mammalian cartilage. The carbon fibre meshwork could function in a similar way, fixing the chondrocytes in the defect.

In the present study, it was found that when the regenerated subchondral plate in the repair tissue was level with the surrounding subchondral plate the articular cartilage surface was also restored. McKibbin (1971) implanted intact articular cartilage grafts of immature sheep into full-thickness articular defects in the femoral condyle of immature and mature sheep. He suggested that failure of some grafts after 10-14 months, especially in the immature sheep, was caused by displacement of the graft, in most cases below the level of the surrounding cartilage. The author explained this because of the immature animals' lack of subchondral bone. Radin *et al.*, (1972) stressed the importance of the subchondral bone in maintaining the integrity of the articular cartilage.

In agreement with the observations of the present study were the results of O'Driscoll *et al.*, (1988). They showed that in cartilaginous repair tissue formed after implantation of periosteal grafts into full-thickness articular cartilage defects, the level of the subchondral plate appeared to be normal compared to the neighbouring cartilage, whilst when the graft tissue was mainly fibrous the level of the subchondral plate was elevated. Wakitani *et al,* (1989) also emphasized the importance of reconstituting the subchondral bone for achieving a biomechanically adequate tissue. Therefore, the restoration of the subchondral bone might be essential for successful articular cartilage repair.
When comparing 12 months with 3 months post-implantation, the carbon fibre plug was found at an increased distance from the joint surface, allowing repair tissue to grow over the carbon fibres. Minns *et al.*, (1982) also described that repair tissue was found between the joint surface and the carbon fibre plug and that bone-like tissue grew into the carbon fibre plug. The latter could be important in reconstitution of the subchondral plate at the level of the surrounding subchondral bone.

In the synovial and capsular tissues surrounding the rabbit knee joint a histiocytic reaction was observed around the carbon fibre after implantation of carbon fibre plugs without associated chondrocytes. This conforms with the observations of many authors, mainly using carbon fibre as (part of) an arteficial ligament (Forster *et al.*, 1978; Dandy *et al.*, 1982; Rushton *et al.*, 1983; Rushton and Rae, 1984; Claes and Neugebauer, 1985; Parsons *et al.*, 1985; King and Bulstrode, 1985; Thomas 1986). The described observation of synovium-hypertrophy and the mild foreign body-giant cell reaction in the present study was also shown by Rushton *et al.*, (1983). It has been suggested that the way in which the carbon fibre is processed is important in relation to the type of foreign body reaction elicited (Helbing *et al.*, 1977). Thomas (1986) implanted anterior cruciate ligament replacements made of carbon fibres in rabbits and noticed that the operated knee joints after 3 months showed a capsular and synovial tissue reaction, which was reduced after 12 months. The present study also showed this phenomenon after implantation of.carbon fibre plugs with and without cells. A possible reason why the tissue response was less after 12 months compared to 3 months might be that the carbon fibre meshwork was damaged by the forces working on the implant, leading to breakage of the carbon fibre. Subsequently, the carbon fibres were lost in the synovial fluid, followed by uptake in the capsular and synovial tissues. After 6 months, the defect (present study) or the ligament (Thomas, 1986) was found to be covered by tissue, and it has been suggested that this could prevent further release of particles of carbon fibre into the joint space.

Using immunocytochemistry, the carbon fibre graft plugs with and without associated chondrocytes, were compared. The former showed after 3 weeks *in vitro* culture, and at 3 months post-implantation the presence of graft tissue with a cartilage-like composition

due to the presence of chondrocytes which synthesised collagen type II, chondroitins -4- and -6- sulphates and keratan sulphate, but not collagen type I, whilst the latter showed mainly fibrous tissue with occasional areas of cartilage-like staining. This supports the conclusion drawn from the histological appearance of the repair tissue that the presence of a carbon fibre meshwork supports the chondrogenic phenotype up to 3 months post-implantation. At 6 months post-implantation, the differences between the two types of plug were less pronounced. In the case of carbon fibre plugs without chondrocytes, positive staining for collagen type I was found throughout the tissue, whilst in the carbon fibre plugs with cells collagen type I was present in discrete areas. This interpretation supports the histological data. At 12 months post-implantation of the carbon fibre plugs with chondrocytes, collagen types I and II were only seen occasionally in discrete areas of the graft tissue. In contrast, collagen type II positive staining was not found in the repair tissue formed after implantation of cell-free carbon fibre plugs. This seems not to support the histological findings, but suggests that after implantation of carbon fibre plugs with and without chondrocytes, the repair tissue eventually becomes fibro-cartilaginous. Low-power examination of decalcified sections of the whole graft region at 12 months post-implantation of carbon fibre plugs with cells showed that the surface of the graft tissue was irregular. Also, fibrillations of the surface of the articular cartilage were present, and this might suggest that the repair tissue underwent degenerative changes (Gay *et al.*, 1976), but more data are necessary to substantiate this. For example, Von der Mark *et al.*, (1991) examined samples of osteoarthritic human articular cartilage, and found that inter-territorial collagen type II gradually disappeared focally, whilst intracellular collagen type I and III appeared, although collagen type I was never observed in the interterritorial matrix. Therefore, for further studies it would be necessary to apply antibodies which recognise one or more epitopes on collagen type III. If collagen type III immuno-positive staining could be shown, this would demonstrate that the presence of a reparative process as seen in the osteoarthritic cartilage (study of Von der Mark *et al.*, 1991) was also found in the repair tissue at 12 months post-implantation of carbon fibre plugs with cells (present study). In addition, it has to be taken into account that histological examination and immunocytochemical analysis were not performed on the same specimen. Dahlberg and Kreicbergs (1991) described variation between specimens with the same implant and same follow-up period using only one method of assessment, namely, histology. It is clear, therefore, that the variable nature of the repair response requires large sample sizes.

Furukawa *et al.*, (1980) described the repair of 3 mm (diameter) full-thickness defects created by drilling holes in rabbit patellar grooves. They found a relative increase in the percentage of collagen II compared to total collagen types I and II with time. It is not possible to compare Furukawa's study and the repair tissue formed after implantation of cell-free carbon fibre plugs, primarily because the results of the immunofluorescence

study as described above are qualitative and the study of Furukawa *et al.*, (1980) is quantitative. Nevertheless, there is a tendency for collagen type II in both studies to increase in amount with time or to increase in distribution throughout the graft tissue up to 6 months. Amiel *et al.*, (1988) described that after implantation of perichondrium into full-thickness defects of rabbit articular cartilage the collagen type II content (as percentage of total collagen) at 3, 6 and 12 months was 62%, 79% and 82%, respectively. The collagen type I content for the same time periods was 38%, 21% and 18%, respectively.

In the studies of Furukawa and co-workers and Amiel and co-workers, it was noticed that there was, quantitatively, an increase in the collagen type II content after implantation of cells, whilst the graft tissue after implantation of carbon fibre plugs with chondrocytes as described in the present study showed a qualitative decrease. Furakawa and co-workers and Amiel and co-workers used two entirely different systems, which, in addition, show differences with the system used in the present study. Also, both research groups analysed the repair tissue only for collagen types I and II. It was shown recently that mammalian articular cartilage contained at least six different types of collagen [types II, V, VI, IX, X and XI)(Eyre *et al.*, 1987a; Van der Rest and Mayne, 1988; Ronziere *et al.*, 1990)]. Therefore, other collagens have to be considered when examing the nature of repair tissue in articular cartilage defects.

Furukawa *et al.*, (1980) described that the proteoglycan content (hexosamine content as % dry weight) in the repair tissue decreased with time and at 24 weeks post-implantation was significantly reduced compared to the non-drilled control knee joint. This was in contrast with the histological analysis after 24 weeks in the same study. Amiel *et al.*, (1988) also found that the glycosaminoglycan content (mg hexosamine per g dry tissue) was lower in the graft tissue than in normal articular cartilage. In contrast, O'Driscoll *et al.*, (1986) found after implantation of periosteal grafts that the glycosaminoglycan content was in the same range as normal articular cartilage at 4 weeks post-implantation but only if the animals were treated with continuous passive motion. O'Driscoll and co-workers did not describe the results of glycosaminoglycan content after 1 year, but determined the total collagen content and collagen type II content using biochemical techniques (O'Driscoll *et al.*, 1988). In the present study, the presence of chondroitins -4- and -6- sulphates and keratan sulphate was found in most sections of the repair tissue after implantation of carbon fibre plugs with and without cells up to 12 months. Quantitative data combined with immunocytochemical data using comparable systems are necessary to clarify whether the results of the present study are in agreement with the studies referred to above.

Using silver enhancement immunocytochemistry, carbon fibre grafts plus chondrocytes were examined for the presence of 7D4 -epitope, collagen type X and keratan sulphate at

1 year post-implantation. In this study, 7D4-epitope was present in the surface of the graft tissue and also in the calcifying zone. The 7D4-epitope indicates oversulphated regions within chondroitin sulphate chains (Caterson *et al.*, 1990), and expression of this epitope was probably an indication of altered sulphation during chondroitin sulphate synthesis. It has been detected in experimentally-induced osteoarthritis and *in vitro* culture of explants of bovine articular cartilage (Hardingham *et al.*, 1989; Caterson *et al.*, 1989,1990; Lee, 1991), but no definitive correlation with any joint pathology has yet been found (Slater *et al.*, 1992). In the cartilage neighbouring the graft, 7D4-epitope was found in both the surface layer and calcifying zone. Further examination of age-matched non-operated articular cartilage needs to be carried out.

In addition to 7D4, oversulphated regions within the chondroitin sulphate chains could be carried out using monoclonal antibody 3B3 in the absence of chondroitinase pre-digestion (Caterson *et al.*, 1991). Slater *et al.*, (1992) suggested that the expression of 3B3-epitope in osteoarthritic and rheumatoid arthritic cartilage might indicate that this epitope was expressed on chondroitin sulphate chains by chondrocytes attempting to repair and/or remodel the cartilage extracellular matrix in these diseases. Immuno-positive staining for 3B3 (without chondroitinase pre-treatment) was found pericellularly around chondrocytes cultured within a carbon fibre plug for 3 weeks (data not shown). The data suggest that expression of the 3B3-epitope is a reaction of the chondrocytes to a changed external environment. It might be interesting to examine if the expression of 3B3-epitope is dependent on specific stimuli in the chondrocytes' environment and if, after implantation of the graft tissue, the expression of the epitope in the repair tissue undergoes changes with time.

Collagen type X was present in the calcifying zone of normal articular cartilage and that surrounding the defect, but it was not found within the graft tissue. Its absence might possibly suggest that the graft tissue did not calcify, and, therefore, did not integrate with the bone

At 6 and 12 months post-implantation, the elastic moduli of carbon fibre plugs with and without cells was tested in compression using an indentation machine. The measured values were in about the same range for both time periods, and comparable to the range of values for Youngs modulus of normal articular cartilage of a similar age (Fig. 3.3.). Nevertheless, few measurements were done per implant and, therefore, the results can only give an indication of the stiffness.

Some difficulties arose when carrying out the measurements in three situations which made the measurements unreliable. Firstly, in the case of a plug placed very anteriorly on the tibial plateau, secondly, when some of the implant material became detached from its insertion site and, thirdly, when the drill hole was too close to the insertion of the cruciate ligaments. Possible reasons might be that false indentation depths were

measured when the free movement of the indenter into the implant was impeded; the anterior lateral tibial plateau is very curved compared to the more posterior areas, and this could explain the difficulty in measuring the indentation depth in defects placed too anteriorly. In the case of incomplete filling of the drill hole the indenter could be impeded by the sides of the remaining 'crater'. In rabbit number 193L(left knee) an almost empty defect was found, which made it impossible to measure accurately the indentation depth and this tissue was excluded from the study. If the drill hole was too close to the ligament insertion it was not possible to determine the indentation depth precisely, subsequently, resulting in a wrong calculation of the elastic modulus using Hayes' formula.

Woo *et al.*, (1987) examined the complex shear modulus of perichondrial allografts implanted into full-thickness defects in the rabbit medial femoral condyle at 26 weeks post-implantation. The authors found that with time the complex shear modulus increased and after 1 year approached the value of normal articular cartilage (Kwan *et al.*, 1989). The relationship between the elastic modulus and the shear modulus is given by the following equation (Gere and Timoshinko, 1984):

$$g = \frac{e}{2(1+\upsilon)}$$

(g= shear modulus, e= Youngs or elastic modulus, υ= Poisson's ratio) This shows that the elastic modulus is related linearly to the shear modulus. An increase in shear modulus would give rise to an increase in elastic modulus presuming that the Poisson's ratio remained constant. In the present study, we did not find an increase in value for the elastic modulus after implantation of carbon fibre plugs with or without cells with time, although the number of specimens tested was very small. It would be interesting to repeat the measurements in more animals as well as at more frequent intervals during a one year follow-up period and at 2 years post-implantation.

Collagen gel plugs.

After 1-2 days *in vitro* culture of chick embryo chondrocytes within a collagen gel, Gibson *et al.*, (1982) observed a change from a chondrocyte-like morphology to a fibroblast-like morphology during the first 5-6 days, after which the fibroblast-like cells adopted a more rounded-polygonal shape. The authors suggested that the fibroblast-like cells exerted tractional forces on the collagen gel similar to those exerted by skin fibroblasts on gels (Bell *et al.*, 1979). The results in the present study suggest that a similar phenomenon is operating, since the gels also contracted by 5 or 6 days in culture.

This resulted in retraction of the collagen gel from the sides of the tissue culture dish, thereby losing about half of its volume (Gibson *et al.*, 1982). The retraction-phenomenon has been described by several authors (Bell *et al.*, 1979; Gibson *et al.*, 1982; Storm and Michaolopoulos, 1982; Allen *et al.*, 1984). Bell *et al.*, (1979) cultured fibroblasts within a collagen gel and described that the rate of retraction of the collagen gel depended on the protein content of the gel, the number of cells present within the gel or the presence of cytochalasin B. A possible explanation why Gibson and co-workers found the gel to retract earlier, in contrast to the present study, might be that in the present study spot cultures were used and the cells were not dispersed throughout the gel as described by Gibson *et al.*, (1982).
The handling of the collagen gel cultures needed to be done very carefully as the collagen gel could lose its stability because of mechanical disturbances. Elsdale and Bard (1972) reported this previously. They also noticed that collagen gel could not be cut with a knife because this caused collapse of the collagen lattice and loss of water. Our experience confirmed this property. During the preparation of the collagen gel by cutting it to the right size before implantation (described in ch. 2.1.1.) the collagen gels became distensible and it was not possible to fix the gel into the drill hole securely.

The acceptance rate of the collagen gel plugs with associated chondrocytes was at 3, 6 and 12 months post-implantation 67%, 80% and 40%, respectively. The relatively low percentage of accepted grafts after 1 year could be explained as follows: 5 rabbits in this group could not be used due to infection or death. If these 5 rabbits were not included, the results would have been 80%. After 3 months, 4 rabbits became infected or died and if they were discarded the acceptance rate was 100%.
The acceptance rate of the collagen gel plug controls was at 3, 6 and 12 months post-implantation 56%, 80% and 50%, respectively. Again, if the animals which died or became infected were not taken into account the percentages would be more favourable; 100%, 80% and 100%.

The collagen gel plugs with associated chondrocytes were assessed histologically. The chondrocytes were mainly found on the collagen gel after 7 and 21 days *in vitro* culture. This observation resembled that of Schor (1980) using chick embryonal sternal chondrocytes grown on a collagen gel. At 3 months and 6 months post-implantation, the matrix of the surface layer of the graft tissue was fibrous, whilst the matrix of the deeper layers was cartilage-like. Both layers were incorporated well into the defect. When these results were compared with the repair tissue formed in a control drill hole (consisting of fibrous surface layers and a cartilage-like deep layer) it appeared that the collagen gel plug with chondrocytes was capable of maintaining a cartilaginous repair tissue, except for the surface layer. Possible explanations for the presence of this fibrous component in

the surface layers were discussed in the section concerning histological assessment of the graft tissue after implantation of carbon fibre plugs. The use of collagen gels with chondrocytes as an implant material to repair articular cartilage defects has been described by others, such as Wakitani *et al.*, (1989). After implanting a suspension of collagen gel and chondrocytes into full-thickness defects (4 mm diameter, 4 mm deep) in the articular cartilage of femoral patellar grooves of mature rabbits, Wakatani and co-workers found, histologically, the presence of a cartilage-like matrix up to 24 weeks post-implantation. There are several differences between this and the present study. Wakitani and co-authors used as controls a collagen gel plug without cells and a sham operated knee joint. At 24 weeks post-implantation the repair tissue in both controls was predominantly fibrous, although weak metachromatic staining was found. It was an unexpected finding that sham operated knee joints had weak metachromatic staining compared with the metachromatic staining of the experimental knee joints at 24 weeks post-implantation. Another difference with the present study is that Wakitani did not culture the chondrocytes *in vitro* within or on a collagen gel, but instead added the mixture of freeze-thawed chondrocytes and collagen gel directly into the articular cartilage defect. Therefore, extracellular matrix produced during culture which surrounded the chondrocytes was not, or in only limited amounts, present at the time of implantation. The presence of the extracellular matrix gives cartilage *in vivo* its specific physico-chemical and mechanical properties. It might be suggested that chondrocytes implanted in the absence of an extracellular matrix will function less well than those implanted with extracellular matrix.

Other authors (Furukawa *et al.*, 1980; Minns *et al.*, 1982; Aston and Bentley, 1986; Itay *et al.*, 1987) have described contrasting results of the histological appearance of the repair tissue in control drill hole defects with time. Furukawa *et al.*, (1980) described that repair tissue became more cartilage-like with time and they also observed that one in ten drill holes retained a deep plug of cartilage which was often overgrown by fibrocartilaginous tissue, but which in bone failed to differentiate. Those plugs were similar to ones described in the present study, but the latter were well incorporated into the bone. There are experimental differences between the study of Furukawa and co-workers and the present study, which might account for the different results. Firstly, defects in Furakawa's study were made in the (non-weight bearing) patellar groove of the femur and not in the (weight bearing) lateral tibial plateau. These dissimilar anatomical areas differ with regard to the mechanical properties as is expressed in the ratio of their elastic moduli (E= elastic modulus; the patellar surface of the femur = the area of the femur which faces the patella)(Dr. Jin, University of Leeds, personal communication).

$$\frac{E_{\text{tibia covered with menisci}}}{E_{\text{patellar surface of the femur}}} = 1.4$$

and the described areas might be subjected to different forces. Secondly, the rabbits were permitted full cage activity (Furukawa and co-workers) or mobilized in pens (present study). Thirdly, Furukawa *et al.* used both rabbits of 6-7 months (immature) and 10-12 months (mature), whilst in the present study 12 months old (mature) rabbits were used.
Bentley *et al.*, (1978) implanted isolated rabbit epiphyseal chondrocytes into full-thickness articular cartilage defects of mature and immature rabbits, and as controls, full-thickness defects were drilled without injecting chondrocytes. Using histological techniques the authors described after 8 weeks for the controls no significant difference in the results with the skeletally mature compared to the immature rabbits. Koide *et al.*, (1979) also described the repair capacity of articular cartilage after drilling holes in the distal femoral cartilage of 6 and 12 months old rabbits, and reported no differences with regard to the histological appearance of the repair tissue in mature and immature rabbbits at 8 weeks post-trauma. This would suggest that the age of the recipient animal does not significantly influence the type of repair tissue formed in drilled articular cartilage defects.
Apart from Furukawa *et al.*, (1980), Itay *et al.*, (1987) also described using histological techniques their findings about the nature of the repair tissue formed after drilling full-thickness defects in articular cartilage. Itay and co-workers created drill hole defects in the tibial condyles of rooster articular cartilage.without implantation of cells or gel (controls). The repair tissue up to 6 months appeared fibrous-like. As discussed previously, Itay and co-workers used chickens as experimental animals, whilst rabbits were employed in the present study. In contrast, others (Minns *et al.*, 1982; Aston and Bentley, 1986) found that in the empty drill holes in articular cartilage and bone (controls) after 26 and 52 weeks histologically definable fibrocartilaginous tissue was overlying the newly formed subchondral bone. The results from the present study support these findings.

Using immunocytochemical analysis, the collagen gel plugs with associated chondrocytes were compared with the control drill hole defects. At 3 months post-implantation at least two major differences between the repair tissue formed by the collagen gel plus associated chondrocytes and the tissue in the control drill hole defect were evident. First, collagen type I was found occassionally in localized areas of the graft tissue after implantation of collagen gel plug with cells, whilst in the control defect collagen type I-staining was found throughout the repair tissue. Second, the presence of collagen type II was found throughout the graft tissue after implantation of the collagen

gel plus cells, whilst in the control defect it was detected occasionally in discrete areas. At 6 months post-implantation there was no significant collagen type I found in the repair tissue formed in the experimental and control knees. Furthermore, staining for collagen type II was not found in the collagen gel plugs with cells, but was found occasionally in discrete areas of the repair tissue of the cell-free collagen gel plugs. This distribution of collagen types changed at 12 months post-implantation, because collagen type II staining was present occasionally in discrete areas the graft tissue formed after implantation of collagen gel plugs with cells, whilst immuno-positive staining for collagen type II was not found in the repair tissue in the control drill hole. The results of the immunocytochemical assessment of the collagen gel plugs with chondrocytes up to 1 year post-implantation contrast with the macroscopical appearance (Fig. 3.2.1. l.+m., 3.2.1. o.+p. and 3.2.1.s.) which showed grafts so well incorporated that they were very difficult to find in the tibial plateau. Other authors have described similar contrasting results (Aston and Bentley, 1986; Dahlberg and Kreicbergs, 1991).

The absence of collagen type II at 6 months post-implantation may be explained by the presence of fibrous graft tissue. This could also explain the differences from the results of Wakitani *et al.*, (1989) with regard to the presence of collagen type II. They found using ^{14}C proline labelling followed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis of cyanogen bromide-generated peptides (SDS PAGE) at 26 weeks post-implantation, that most of the newly-synthesised collagens were type II. At 12 months post-implantation of collagen gel plugs with chondrocytes collagen type II-positive staining was present and, added to the presence of chondroitins -4- and -6- sulphates and keratan sulphate indicates that after 12 months, chondrogenesis is occurring.

The absence of collagen type I at 6 and 12 months post-implantation was unexpected. If collagen type II alone was present, this would give the impression of similarity to normal articular cartilage. However, after 6 months, immuno-positive staining for collagen type II was also absent. Page *et al.*, (1986) described the process of fracture healing in rabbits and they used the same collagen type I antibody as was applied in the present study. Page and co-workers reported that collagen type I-positive staining could not be detected in frozen and wax-embedded sections of the periosteum, although collagen type I is reported to be one of the main components. The authors could not find an explanation, but the fact that collagen type I was not detected does not necessarily mean that it is absent. It might be that the epitope on the collagen type I, which is recognised by the antibody, is masked. This masking problem has been described for collagen type V (Linsenmayer *et al.*, 1983), but since collagen type I positive staining was found in other defects, its absence could not be purely a fixation artefact.

In contrast to the present study, Amiel *et al.*, (1988) implanted perichondrium into full-thickness defects of rabbit articular cartilage, and found by determining collagen concentration on the basis of hydroxyproline content and HPLC (high performance

liquid chromatography) that the collagen type II content increased with time, and that at 52 weeks about 80% of collagen present was type II, whilst the collagen type I content decreased with time. It has been suggested that collagen type III might be present in the repair tissue, and it would be interesting to apply immunocytochemical techniques using a collagen type III-antibody. In addition, the presence of collagen type III has been suggested to be essential to most healing processes (Page *et al.*, 1986).
The glycosaminoglycan content (mg hexosamino per g dry tissue) in the graft tissue after implantation of perichondrium was shown to be lower than in normal articular cartilage Amiel *et al.*, (1988). Quantitative data of the present study are necessary to compare both studies. O'Driscoll *et al.*, (1988) implanted periosteal grafts in full-thickness, 5mm wide and 10mm deep, defects in patellar groove articular cartilage of rabbits. After 1 year, the percentage collagen type II (of total collagen) was 76% using electrophoresis of cyanogen bromide-generated peptides. When the animals were additionally mobilized directly after surgery using continuous passive motion (CPM) for 2 weeks this percentage increased to 84%. They also showed the presence of chondrocyte clusters in 72% of the rabbits, but additional mobilization using CPM for a 2 week period directly after surgery decreased this to 33%.
Chondrocyte clusters are a characteristic of osteoarthritis (Rothwell and Bentley, 1973; Dustmann *et al.*, 1974b; Gay *et al.*, 1976), chondromalacia patellae (Bentley 1985) and experimentally induced cartilage damage (Johnell and Telhag, 1977, 1978; Havdrup and Telhag, 1978; Kunz *et al.*, 1979; Lee, 1991; Scully *et al.*, 1991). In the present study chondrocyte clones were found in graft tissue with carbon fibre plugs and collagen gel plugs at 3, 6 and 12 months post-implantation and hydrogel plugs at 3 months post-implantation and also in the neighbouring host-cartilage (Fig. 3.2.2.1.b.-l., 3.2.2.2.b.-l., 3.2.2.3.b.-e.). The clones could be a reparative action of the chondrocytes in order to restore the damaged surface or a response to the altered local micro-environment (Meachim and Collins, 1962).

Using an indentation machine the elastic moduli of the collagen gel plugs plus associated chondrocytes were determined at 6 and 12 months post-implantation. Fibro-cartilaginous repair tissue formed after 6 and 12 months implantation of the collagen gel plugs with associated chondrocytes had a mean (average value of measurements) elastic modulus higher, and a median (middle value of measurements) value lower, than the elastic modulus of normal articular cartilage. This contrast in values is caused by the presence of two values for collagen gel plug plus cells at 12 months post-implantation, where one measured value was 8 times higher than the other value. Both values were found to have an indentation depth less than 0.10 mm. Fig. 3.6. showed the effect of indentation depth measurement on the modulus values and it demonstrates that small measurement errors in the thickness measurement of the plug give a large error in the elastic modulus value.

When the two values mentioned above were omitted, the mean elastic modulus for all collagen gel plugs was 0.95 MPa. This value was lower than the Youngs modulus of normal rabbit articular cartilage of a similar age.
Part of the volume of the collagen gel plug was taken by the collagen gel itself, which is an easily distensible material. Although native collagen has a high tensile strength, the chemical treatment necessary for isolation leads to a material with poor mechanical properties (Gilbert and Lyman, 1987). This could partly explain the low value of the elastic modulus for collagen gel plugs with cells.
After 12 months the Youngs modulus of collagen gel plugs with chondrocytes were 0.67MPa and 5.86MPa (after 1 sec), whilst the values for Youngs modulus of the collagen gel control after 12 months were 0.75MPa and 1.84MPa (after 1 sec). Because there are in each category only 2 values, which are rather dissimilar, discussion of the results is rather difficult. When comparing the elastic modulus after 6 and 12 months it appeared that this value showed at least a two-fold increase from 6 to 12 months.
As discussed in the paragraph on biomechanical testing of carbon fibre plugs with and without chondrocytes, there is a linear relationship between the shear modulus and elastic modulus. Woo *et al.*, (1987) described an increase with time in the shear modulus after implantation of perichondrial allografts, which is consistent with the results presented here.

Hydrogel PC 97 plugs.

Isolated chondrocytes were cultured *in vitro* on a hydrogel PC 97 plug for 21 days. Most of the cells had an elongated, flattened appearance, although a few chondrocytes retained a polygonal-rounded shape. Van der Valk *et al.*, (1983) found that mouse lung fibroblasts *in vitro* spread on polymer films when the polar component of the surface free energy exceeded 15 mNm-1. The polar component of the surface free energy for hydrogel PC 97, after autoclaving, was 43.6 mNm-1. Therefore, it appears that chondrocytes *in vitro* spread on a hydrogel with a polar component of the surface free energy of about 45 mNm^{-1}, but more extensive studies of hydrogel PC 97 with a variety of surface free energies are necessary. Only then can it be concluded that one of the factors influencing spreading of chondrocytes on hydrogels *in vitro* is a value for the polar component of the surface free energy exceeding 15 mNm^{-1}, since cell spreading is governed by many factors in addition to the surface free energy.

Using histological techniques the hydrogel PC 97 plugs with and without associated chondrocytes were examined. At 3 months post-implantation the plugs were not incorporated into the adjacent cartilage. Possible explanations might be, firstly, that autoclaving reduces the volume of the plug by about 5%, although there was a time dependent recovery post-autoclaving, with equilibrium reached after 48 hours. The

hydrogel PC 97 plugs without cells were stored at 4° C prior to implantation and autoclaved 1 day before the operation and would not have reached equilibrium, whilst the hydrogel PC 97 plugs with cellls were autoclaved 22 days prior to the implantation and, subsequently, used for *in vitro* culture for 21 days and would have recovered from the volume reducing effect of autoclaving. Secondly, detachment could be due to processing, because (i) organic solvents such as alcohol, chloroform and xylene cause hydrogels to swell. Using cryosectioning instead of wax-embedding techniques would possibly give better results, and (ii) at 3 months post-implantation the surface and deep layer of hydrogel PC 97 grafts were divided with a scalpel which may have mechanically dislodged the hydrogel. Many authors described problems with fixation of the graft tissue after implantation into an articular cartilage defect (Pap and Krompecher, 1961; McKibbin, 1971; Naito and Hirotani, 1991). Keller *et al.*, (1985) compared fibrin glue with Kirschner wires in the treatment of fixation of osteochondral fractures in the mongrel femoral condyle. The tendency of displacement was less in the fibrin glue treated group. Nevertheless, there are contrasting views on the tendency of fibrin glue to stimulate bone ingrowth (Zilch and Wolff, 1987; Schlag and Redl, 1988). Other authors described the clinical use of fibrin glue associated with an external fixation device (Homminga 1989), chondronectin (Ulreich *et al.*, 1985) or internal fixation devices. It might be useful to compare the different fixation methods when implanting hydrogel PC 97 plugs into defects in order to improve integration.

Of course, the presence of a cartilage-like matrix with chondrocytes might also be important for the improved acceptance rate of the PC 97 plug plus chondrocytes compared to the hydrogel PC 97 plug only. However, a study performed by Kon and Visser (1981), who implanted a poly-HEMA sponge (average pore size less than 50 μm) without cells into full-thickness defects in lateral tibial plateau of rabbits, described the prescence of a cartilaginous repair tissue using histological techniques. Also, Noguchi *et al.*, (1990) showed that at 52 weeks post-implantation of PVA-hydrogel combined with a porous titanium fibre mesh without cells into a full-thickness defect, histologically definable cartilage-like tissue was present.

Reaction of synovial and capsular tissue to hydrogel PC 97.

In the synovial and capsular tissues a foreign body giant cell-reaction was seen. Granulomas were also present. The synovium showed a mild synovi(ali)tis and hyperplasia of the surface layer. These results contrast with the findings of Kon and Visser (1981), who reported the lack of a foreign body giant cell response. Noguchi *et al.*, (1990) found a mild inflammatory response and slight proliferation of synovium after 2 weeks, which settled down after 8 weeks. The different findings can be explained because the polymers examined in the present study and in the studies of Kon and Visser and Noguchi and co-workers are entirely dissimilar. The reaction in the present study can

be explained if when cutting the hydrogel PC 97 plugs to size (3mm diameter-3mm depth) using a corkbore, debris was formed, which was subsequently released into the joint space and became phagocytosed in the capsular and synovial tissues. This can be prevented in further experiments by using pre-cut hydrogels.

At 3 months post-implantation of hydrogel PC 97 plug with cells, immuno-positive staining for collagen type II and keratan sulphate was seen in some areas of the surface layers of the graft tissue. However, in the case of keratan sulphate this was only shown in one graft, whilst in the others no staining was found. Histologically, cartilage-like tissue was found, but when applying immunocytochemical techniques other matrix components such as chondroitins -4- and -6- sulphate were not found in detectable quantities. This may be explained by the technique used, because during the lenghty procedure of immunolocalization some of the sections detached from the slide. Also, the hydrogel PC 97 was not adherent to the surrounding tissues in some sections, which could have reduced the quantity of repair tissue available for immunocytochemistry. In addition, it is possible that epitopes for chondroitins -4- and -6- sulphate and collagen type I were masked, due to processing of the hydrogel PC 97.

Biomechanical testing to calculate the elastic modulus of the hydrogel PC 97 plugs with and without chondrocytes after 3 months showed values in the range of 1.34-9.85MPa and 1.56-11.63MPa, respectively. Normal articular cartilage specimens were not tested, but presuming that the elastic modulus after 3 and 6 months does not differ significantly, the values of 6 months articular cartilage from the collagen gel and carbon fibre experiments can be used (Fig. 3.5; Table 3.19). The median value (middle value of measurements) of the elastic modulus, tested in compression, of hydrogel PC 97 with and without chondrocytes was higher than in normal articular cartilage. This might be expected to mean that when a shearing force is applied across both the hydrogel PC 97 plug and the articular cartilage, the cartilage would distort further, putting a strain on the interface between the two, and may lead to separation. This might explain to some extent why most of the hydrogels PC 97 were not well incorporated into the surrounding cartilage.

The three resurfacing materials used: carbon fibre, collagen (I) gel and hydrogel PC 97 were employed towards one objective using different strategies: the carbon fibre and collagen gel merely provided a vehicle to implant chondrocytes, whilst the hydrogel PC 97 was to form a permanent and integral part of the articular cartilage, with the chondrocytes incorporated in the margins to improve attachment of the graft tissue.

The major function of adult articular cartilage is to provide a suitable covering material for the articular ends of bones at synovial joints (Meachim and Stockwell, 1979). It is able to fulfill this function, because of its specific mechanical properties such as a low elastic modulus compared to bone. Changes in the elastic modulus could influence the ability of cartilage to withstand normal physiological stresses (Kempson, 1979). Therefore, an articular cartilage resurfacing material should possess an elastic modulus more or less in the same range as the native cartilage. This becomes more critical if only a part of the articular surface is replaced, as it would tend to minimize a tendency of the graft (tissue) to shear away from the native cartilage. In this context, it is also important that the graft tissue shares some of the tendency of the native cartilage to lose fluid under pressure.

Biomechanical testing to determine the Youngs (elastic) modulus showed that the mean value for carbon fibre repair tissue at 6 and 12 months post-implantation was in the same range as normal rabbit articular cartilage, the mean and median value for collagen gel repair tissue at 6 and 12 months post-implantation were higher or lower, respectively, than rabbit articular cartilage, whilst the mean and median values for the hydrogel PC 97 repair tissue at 3 months post-implantation were higher than in normal rabbit articular cartilage. It has to be taken into account that the mean value of the elastic modulus of hydrogel PC 97 plugs showed a high standard deviation, thus indicating a wide range of values. Also, the values of the elastic moduli of hydrogel plugs PC 97 did not follow a 'normal' distribution (Fig. 3.5).

A surprising finding was that the Youngs modulus of carbon fibre plugs with and without chondrocytes was in the same range as that of normal rabbit articular cartilage. Histological examination showed a difference in structure between normal articular cartilage and carbon fibre plugs with and without associated chondrocytes. The carbon fibre plug with associated chondrocytes showed after 6 and 12 months cartilage-like tissue in all layers, except the surface, whilst the carbon fibre plugs without cells at 6 and 12 months post-implantation consisted of fibro-cartilage-like tissue throughout. This would suggest that the presence of cartilaginous or fibro-cartilaginous tissue and/or the presence of carbon fibre significantly influenced the elastic modulus of the repair tissue. As can be seen in table 1.1. the tensile strength of carbon fibre is 40 MPa., but the exact elastic modulus of the carbon fibre plug as used in the present study is not known. More detailed examination of the extracellular matrix components of these tissues is necessary before it is possible to compare them. The collagen content as well as the alignment of the collagen fibres determine significantly the value of the elastic modulus (Kempson, 1979), but the amount of glycosaminoglycan present in the tissue is also important in determining the ability of articular cartilage to withstand mechanical deformation (Caterson and Lowther, 1978). A loss of proteoglycans from the cartilage and an increased tissue hydration can significantly reduce the compressive stiffness of cartilage

(Maroudas, 1980; Armstrong and Mow, 1982), whilst loosening or failure of the collagen network, associated with fibrillation, decreases tensile stiffness (Akizuki *et al.*, 1986). Apart from articular cartilage, fibro-cartilage-like and fibrous-like tissue was also found in the present study. Fibrocartilage is stronger in tension than in compression or under shear forces (Coletti *et al.*, 1972), whilst this is even more so in the case of fibrous-like tissue. At 3 months post-implantation of hydrogel PC 97 plugs with or without chondrocytes the Youngs modulus was almost 5 times as high as the normal rabbit articular cartilage (of 18 and 24 months old Sandy Lop rabbits. Although only few values were measured there was no obvious difference between the hydrogel PC 97 plugs with and without cells, which suggests that the presence of a fibrocartilaginous or cartilage-like tissue does not alter the elastic modulus to a large extent. Unfortunately, there is not much research carried out yet into the biomechanical properties of materials with associated cells.

In conclusion, this study has shown that it is possible to culture *in vitro* chondrocytes for 21 days within or on carbon fibre, collagen gel and hydrogel while maintaining the chondrogenic phenotype and cartilage-like matrix. More detailed studies of the matrix components synthesized (e.g. collagens type III, X) would be of interest, particularly of the organisational level of the newly synthesized matrix.

After implantation of the carbon fibre plug, it was shown that cartilage-like repair tissue was produced up to 3 months, whilst after 12 months the cartilage-like repair tissue was confined to a smaller area of the graft. The remaining area of the graft tissue did not stain for collagen type I, which does suggest that other collagen types such as collagen type III might be present. This will require further immunocytochemical study and biochemical analysis. An advantage of applying biochemical techniques is that quantitative data can be obtained which would complement the qualitative data (histology and immunocytochemistry). The synovial and capsular tissues showed a histiocytic reaction and a fibrous response to the carbon fibres. These might be reduced or even prevented if a technique was developed to, for example, coat the carbon fibre with a resin or to create a carbon fibre-like sponge, but using a biodegradable material in which chondrocytes were cultured *in vitro* pre-implantation. This sponge could function as a scaffold until sufficient matrix is produced, allowing the newly formed cartilage to function adequately. Unfortunately, degradation of materials in general influences the overall biocompatability phenomena (Williams, 1992), which must be considered when developing such materials.

After implantation of the collagen gel plug, it was found that cartilage-like tissue was formed up to 3 months, but after 6 and 12 months fibro-cartilaginous tissue was

observed. A problem to be addressed is the fixation of the graft tissue. Although the acceptance rate in this study was never below 70% it might be interesting to examine the effect of a fixation device. The Youngs modulus of collagen gel was lower than that of normal rabbit articular cartilage. Although the elastic modulus is only one of the parameters determined, the collagen gel needs to gain more stiffness, for example, by combining it with a hydrogel of an appropriate stiffness.

After implantation of the hydrogel PC 97 plug, the results were described up to 3 months post-implantation. It would be worth increasing the follow-up period to 2 years because very little is known about the longterm results of hydrogel PC 97 implantation in animals. The repair tissue was not well incorporated into the neighbouring cartilage. This might partly be due to processing, but improved fixation is probably required as well as more compatible mechanical properties. The synovial and capsular tissues showed a foreign body giant cell reaction with granulomas, which was probably caused by the presence of debris due to the technique used to prepare the gels prior to implantation. This technique has now been revised. The hydrogel PC 97 plugs are pre-cut to size (3 mm by 3 mm) and autoclaved by its developers at Aston University, Birmingham, and, subsequently, sent to the Institute of Orthopaedics, Stanmore. The elastic modulus of the implant was much higher than that of cartilage, and may be changed by altering either, the percentage or the type of compounds used in the hydrogel, or both. Another possibility would be to develop a hydrogel which allowed cell ingrowth throughout the gel, for example, by creating interconnecting pores and utilizing various growth factors to promote cell ingrowth and matrix synthesis.

Finally, similar studies in larger animals need to be carried out, because of (a) technical reasons such as comparing the implantation of a plug in either weight bearing or non-weight bearing surface area and also, the size of the defect can be controlled more precisely and (b) to predict results in human joints, which are different from rabbit knee joints in most respects. Although the initial response of cartilage to injury is probably similar in different species, the long-term results of cartilage repair probably differ significantly. Compared with human cartilage, the rabbit knee cartilage is thin, usually less than 1 mm thick and the molecular composition and organization of rabbit cartilage is significantly different to human cartilage, as are the mechanical properties. Nevertheless, most of the information concerning the responses of articular cartilage to penetrating injury is based on experimental studies of rabbit knee joints.

Final conclusions.

In the present study the repair of articular cartilage defects in rabbits was examined using implants of articular chondrocytes cultured *in vitro* within or on three different materials: carbon fibre, type I collagen gel and hydrogel PC 97. This is one of the first studies to report on the immuno-cytochemical/histological as well as on the biomechanical aspects of articular cartilage resurfacing using implants consisting of chondrocytes and a biomaterial.

1. It is possible to culture chondrocytes *in vitro* for 21 days within a carbon fibre plug whilst maintaining the chondrogenic phenotype and cartilaginous matrix. In addition, this study has shown that the graft tissue, consisting of carbon fibre with associated chondrocytes is able to maintain a cartilage-like consistency up to 3 months post-implantation. However, at 6 and 12 months post-implantation the surface of the graft tissue becomes fibroblast-like and the consistency of the graft can no longer be described as cartilage-like. With regard to the biomechanical properties it was found that the stiffness of the grafts with carbon fibre and chondrocytes was in the same range as normal rabbit articular cartilage of comparable age. Therefore, carbon fibre plugs loaded with articular chondrocytes can be used as a means of repair for small cartilage defects, but only in the short-term.

2. When implanting cell-free carbon fibre plugs it appears that at 3 months post-implantation they produce a fibrous matrix, whilst at 6 and 12 months post-implantation chondrogenesis becomes more and more evident. This confirms the idea that carbon fibre could function *in vivo* as a scaffolding material for mesenchymal cells migrating from the marrow and keep them close together, which could make differentiation of these cells into chondrocytes possible. So far, the immunohistochemical studies have not supported other evidence that carbon fibre plugs without chondrocytes after implantation can be compared to and used as cartilage plugs, although one of the biomechanical properties (elastic modulus) is in the same range as that of normal cartilage.

3. In the present study, chondrocytes were cultured for 21 days within a type I collagen gel and immuno-cytochemical analysis confirmed the synthesis of a cartilage-like matrix. Up to 6 months post-implantation the collagen gel plug with cells was capable of maintaining a cartilage-like repair tissue in all layers, except the articular surface. Clearly, more work is needed in stabilizing the chondrocyte phenotype in the articular surface and producing mechanical properties compatible with those of the native cartilage. Only then can collagen gel plugs be of possible clinical use.

4. It was shown that chondrocytes cultured *in vitro* on a hydrogel PC 97 plug for 21 days formed a cartilage-like matrix covering the hydrogel. At 3 months post-implantation only some areas of the matrix showed the presence of cartilaginous tissue, whilst unfortunately the graft tissue was not well incorporated into the surrounding cartilage. Technical problems of implantation as well as excessive values for the stiffness of the implant could be responsible for this failure. If those two factors were to be corrected, the hydrogel might be a very promising implant for cartilage resurfacing.

5. The reconstitution of the subchondral bone in the repair tissue may be important in the success of the implant. In this study, it was found that when the regenerated subchondral plate in the repair tissue was level with the surrounding subchondral plate the articular surface was restored.

6. The biomechanical properties of the implant could be essential in preventing failure of the implant. In this study it was shown that when an articular cartilage resurfacing material possessed an elastic modulus in the same range as the native cartilage, the incorporation of the implant into the surrounding cartilage *in vivo* was improved.

7. Immunocytochemical techniques proved invaluable in elucidating the distribution of matrix components. Given the heterogeneous distribution that emerged, it is recommended that these techniques be used in follow-up studies as such details do not emerge using conventional histology.

APPENDICES

APPENDIX 1.

Instrumentation.

The procedures were carried out under the aegis of The Animals (Scientific Procedures) Act 1986. This protective legislation involves a licensed designated establishment, both personal and project licences and ensures the 'minimisation of pain, suffering and stress on animals used in scientific research'.

The experimental rabbits, female Sandy half-lops, were looked after in accordance with the Home Office (50 Queen Anne's Gate, London SW1H 9AT) rules. The rabbits used had an average age of 12.9 months (range 12 - 17), except for the rabbits used for implantation of the hydrogel PC 97 due to delay in the preparation of hydrogel PC 97. They had an average age of 18.3 months (range 18-19). All rabbits were skeletally mature at the beginning of the experiments and an average weight of 4 kg (range: 3.7-4.4 kg).They were bred in-house at the Institute of Orthopaedics. Rabbits were housed in groups of either 6 or 9 on floor pens, with a floor area of $3.5m^2$ or $4.5m^2$, respectively (Fig. APP. 1.). All rabbits used were housed in this way starting from the age of 6-24 weeks. The pens were cleaned out once per week using barley straw and woodchips as bedding. They were fed a commercial pellet diet, *ad libitum* initially, then restricted to approximately 180 g per day. Water was available *ad libitum* from bottles and hay was given as fodder on a daily basis.

Rabbits housed in this way are physically active, thus avoiding skeletal disuse phenomena such as osteoporosis. Female rabbits housed in groups on floor pens have an increased behavioural repertoire compared with individual caged rabbits, which is beneficial to the animal and also prevents abnormal and stereotypic behaviours being performed. Whilst housing in pens is desirable and represents an advance in 'environmental enrichment' certain drawbacks are inevitable. For exemple, since a social order is established, animals at the low end of the 'pecking-order' can be injured during bouts of aggression.

<u>Pre-, peri- and post-operative procedures:</u>

Pre-operatively the animals were removed from a pen and boxed individually. Monohexitone ('Brietal')(3 ml) was injected in the marginal ear vein followed by inhalation anaesthesia - halothane, N_2O and O_2 through a face mask. Anaesthesia was maintained at 1.5% halothane, N_2O and O_2 (ratio N_2O:O_2 3:2; total flow rate 2 l/min). When the level of anaesthesia was satisfactory the operative procedure was performed. Each operation usually took about 20 minutes. In one session 5-6 rabbits could be operated. After the operation the rabbits were returned to their box unconscious with the lid open allowing regular observation until consciousness returned.

All rabbits were returned to the pen after consciousness was regained. This 'recovery' method has proved very successful and no difficulties with re-introduction into the pens have occurred.
Animals were culled with an i.v. injection of sodium pentobarbitone ('Sagatal' or 'Euthalal') after 3, 6 or 12 months post-operation.

Rabbits excluded from this study

As mentioned in tables 3.1-3.7 the category 'missing carbon fibre, collagen gel or hydrogel PC 97 plugs with cells' or 'missing carbon fibre, collagen gel or hydrogel PC 97 plugs only' consisted of animals which suffered from infection of the limb(s) or animals which died. In total 17 animals were found in this category. The graft tissue of those rabbits was not used in the study.
The causes of infection or death of the rabbits were:

gastro-intestinal symptoms followed by sudden death	:3
infection of limbs	:3(S.aureus and S. epidermidis)
	:1(S.aureus)
	:1(S. aureus, coryneforme/coliforme types)
	:1(moderate growth of 2 types of coliform
	:1(unknown)
unknown	:7

Fig. APP. 1. Rabbits mobilised in pens with cardboard boxes. The latter is part of the environment enrichment program for rabbits at the Institute of Orthopaedics, Stanmore, United Kingdom.

Fig. APP. 1.

Fig. APP. 2. Schematic representation of the experimental set up of the project.

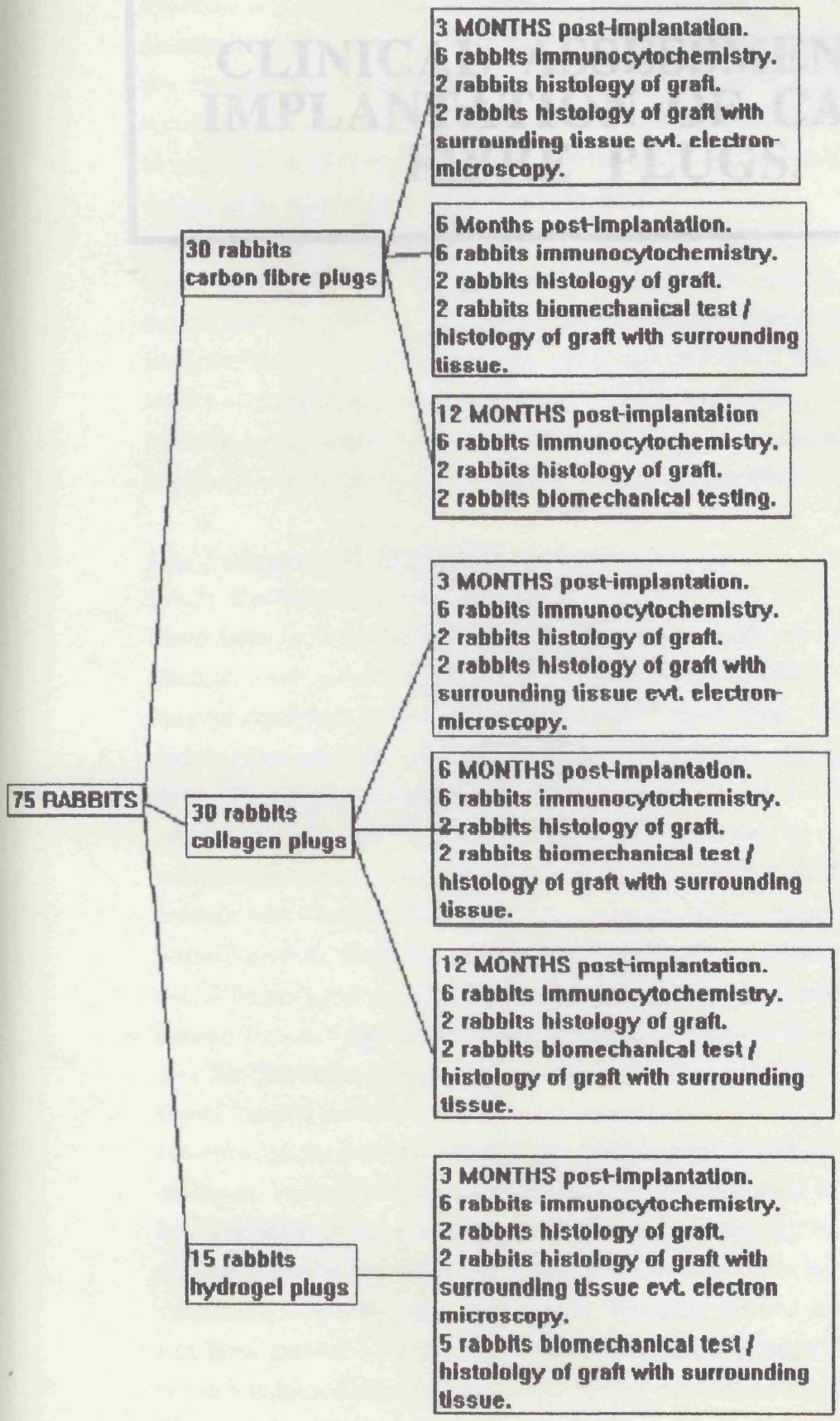

75 RABBITS
30 rabbits carbon fibre plugs
3 MONTHS post-implantation. 6 rabbits immunocytochemistry. 2 rabbits histology of graft. 2 rabbits histology of graft with surrounding tissue evt. electron-microscopy.
6 Months post-implantation. 6 rabbits immunocytochemistry. 2 rabbits histology of graft. 2 rabbits biomechanical test / histology of graft with surrounding tissue.
12 MONTHS post-implantation 6 rabbits immunocytochemistry. 2 rabbits histology of graft. 2 rabbits biomechanical testing.
30 rabbits collagen plugs
3 MONTHS post-implantation. 6 rabbits immunocytochemistry. 2 rabbits histology of graft. 2 rabbits histology of graft with surrounding tissue evt. electron-microscopy.
6 MONTHS post-implantation. 6 rabbits immunocytochemistry. 2 rabbits histology of graft. 2 rabbits biomechanical test / histology of graft with surrounding tissue.
12 MONTHS post-implantation. 6 rabbits immunocytochemistry. 2 rabbits histology of graft. 2 rabbits biomechanical test / histology of graft with surrounding tissue.
15 rabbits hydrogel plugs
3 MONTHS post-implantation. 6 rabbits immunocytochemistry. 2 rabbits histology of graft. 2 rabbits histology of graft with surrounding tissue evt. electron microscopy. 5 rabbits biomechanical test / histology of graft with surrounding tissue.

Fig. APP. 2.

APPENDIX 2.

CLINICAL ASSESSMENT OF IMPLANTATION OF CARBON FIBRE PLUGS.

5.1. Introduction.

In the University Department of Orthopaedic Surgery at the Institute of Orthopaedics, a selection of patients with symptomatic chondromalacia patellae or osteochondritis dissecans had been treated with cartilage allografts. Although the results were promising, this program had to be stopped when the possible transmission of the HIV-virus and hepatitis B-virus could not be excluded. Carbon fibre implantation had been described by Minns *et al.*, (1982) and seemed to offer the possibility of restoring articular cartilage defects in humans without using human tissue.

The intention of this small clinical study was to get an impression of other factors which might be important in the treatment of patients with chondromalacia patellae or osteochondritis dissecans, apart from the ones described in chapters 1-3. The present study was part of a prospective double blind randomized trial where patients were treated with or without carbon fibre plugs, but only the patients with carbon fibre implants were included in the present study. Also, there was the possibility of identifying the group(s) of patients who might benefit most from this specific treatment.

5.2. Patients and methods.

5.2.1. Patient selection

Since January 1989 thirty-three patients with intractable anterior knee pain due to a discrete lesion, either in the articular cartilage of the patella (Outerbridge grade II) or femoral condyles, confirmed by arthroscopic examination, were treated with woven carbon fibre implants provided by 'Surgicraft Ltd'. Twenty-six patients (79%) with carbon fibre implants were retrospectively re-arthroscoped and reviewed clinically at an average of 14 months (Standard Deviation (S.D.) 4)(range 10 - 27). Surgical technique and clinical management were under the direction of Prof. G. Bentley. There were 16 patients with chondromalacia patellae, 6 men and 10 women whose average age was 29 years (S.D. 6.4)(range 21 - 44). Affected knees were as follows: 12 right and 4 left. In the 10 patients with osteochondritis dissecans, there were 6 men and 4 women, with 10 knees affected, 4 right and 6 left, and with an average age of 28 years (S.D. 4.8)(range 20 - 36). The defect was localised on the medial femoral condyle in 8 patients and on the lateral femoral condyle in 2 patients. There was no statistically significant difference in age between the patients with chondromalacia patellae and those with osteochondritis dissecans. Patients with chondromalacia had failed treatment by conservative measures and continued to have retro-patellar pain, reduced activity level and patello-femoral crepitus. Thus, in this series, the indication for operation was persistence of troublesome symptoms, especially retro-patellar pain. When the patients were first seen, symptoms had been present for a mean of 95 months (S.D. 68)(range 18 - 240). Patients with osteochondritis dissecans had complained of pain for an average of 95 months (S.D. 72)(range 24 - 240).

5.2.2. Surgical procedure.

The medial parapatellar approach was used through a medial arthrotomy incision. The patella was everted to demonstrate and assess the pathological changes of cartilage and then the area of diseased cartilage was excised to its full thickness. The area of the defect was carefully cleared of all diseased cartilage down to subchondral bone and a dental burr was used to remove all vestiges of cartilage. A portion of carbon fibre implant was then cut slightly larger than the defect. The graft was thoroughly washed in normal saline to remove loose particles of carbon fibre. Subsequently, the implant was pressed into position and, after the tourniquet was released, a small amount of blood was impregnated into the patch in order to induce clot formation. The joint was then irrigated with normal saline and taken through a range of motion to insure placement and stability of the implant. Post-operatively, the patient was placed in a cylinder cast with instructions for elevation in the first 24 hours, after which immediate straight leg raising was encouraged. After 2 weeks of immobilization the plaster was removed, and isometric quadriceps strengthening and range of motion exercises were begun.

5.2.3. Method of assessment.

The objective assessment of results in chondromalacia patellae and osteochondritis dissecans is not precise, since the main cause for surgery is persistence of troublesome subjective symptoms. Recently, many authors have tried to assess the treatment of anterior knee pain in general, and in chondromalacia patellae and osteochondritis dissecans in particular, using knee pain scoring systems e.g. Clinical Assessment of Chondromalacia Patellae (Bentley, 1970), Criteria for Evaluating Patellofemoral Dysfunction (Ficat *et al.*, 1979), Scoring system as described by Hughston *et al.*, (1984), Clinical Rating Scale (Worrell 1986), Knee Disability Assessment of the Hospital of Special Surgery by Ranawat (Homminga 1989), Rating Scale for Knee Function as described by Larson (Brittberg and Peterson, 1990), Gillespie's scoring system (Kampmann and Thomsen, 1991). To avoid adding another scoring system to the existing ones, and because the character of this study was purely exploratory, the predominant data were listed with appropriate statistical tests. In our series, a special questionnaire was developed, based on the Royal National Orthopaedic Hospital questionnaire for knee pain assessment, which was divided into 9 categories:

1. Pain score
2. Pain analysis
3. Principal symptoms
4. Medications
5. Past medical and orthopaedic surgical history
6. Orthopaedic examination
7. Radiology
8. Pre-operative investigations

9. Arthroscopy

The questionnaire was used pre- and post-operatively. Also, a section about the operation itself was added, but this will not be discussed here.

5.3. Results.

1. Pain score.

Pain was reduced significantly post-surgery ($p<0.001$, Wilcoxon signed rank). Pre-operatively, 50% of the patients had tolerable pain permitting limited activity, 15% had pain preventing all activity and 4% had severe spontaneous pain. In total 2/3 of the patients had severe pain (Table 5.1). Post-operatively, 31% of the patients had no pain, 4% slight or intermittent pain and 23 % had pain after some activity. Severe pain was felt by 31% of the patients (Fig. 5.2). In figures 5.3-5.6 the pre- and post-operative pain score for patients with chondromalacia patellae and osteochondritis dissecans is shown. The reduction in pain in the group of chondromalacia patellae patients as well as osteochondritis dissecans patients was statistically significant ($p<0.013$ and $p<0.018$, respectively, Wilcoxon signed rank).

Only 15% of the patients did not suffer from night pain before surgery, whilst after surgery this was increased to 38%.

Squatting (80% of the patients), kneeling (70% of the patients) and running (58% of the patients) were the factors aggravating pain pre-operatively, whilst after the operation running (50% of the patients) was the only significant factor which aggravated pain. Other factors, such as walking up and down stairs were aggravating the pain in less than half of the patients pre- and post-operatively.

Pre-operatively, 12% of the patients could walk less than one mile, 19% of the patients could walk less than half a mile and 8% of the patients could walk less than 100 yards. Nevertheless, 23% had unlimited walking ability. After the operation this number increased to 58%.

Before and after surgery 62% of the patients were not using any walking aid. Pre-operatively, 1 patient (4%) used 1 stick, whilst after the operation 2 sticks were used. Before the operation 2 patients (8%) used 2 crutches before surgery, whilst after the operation one of those patients did not need any walking aid, but the other patient still used 2 crutches. One patient (4%) used 2 crutches after the operation instead of none before the operation. This result needs to be interpreted with caution, because the number of 'unknown' answers was 27%.

With regard to pre-operative work status almost half (46%) of the patients were secretarial or clerical, 23% light manual, 15% domestic and 8% heavy manual. Before the operation 62% of the patients were still working in the same job, but almost half of those with considerable difficulties and 19% of all patients had to change jobs. After the operation 77% of the patients were still working in the same job and only 12% with

considerable difficulties. Also, 4% of the patients still had to change their job after the operation. Pre- and post-operatively, 14% of the patients were unable to work.
The family history of these patients revealed osteoarthritis in 19% of the patients and rheumatoid arthritis and osteoarthritis in 1 patient. Strikingly, 46% of the patients did not know for certain if there was a family history of the above mentioned diseases in their family.

2. Pain analysis.

The site of most pain in the patients with chondromalacia patellae (75% of the patients) was in the anterior region of the knee joint over the patella, whilst in the patients with osteochondritis dissecans the site of pain was more varied in localization. The latter complained about pain at the medial femoral condyle, the lateral femoral condyle and the anterior region of the knee joint in similar numbers. Post-operatively, the patients with chondromalacia patellae indicated the most painful site as being the anterior compartment of the knee joint, whilst most osteochondritis dissecans patients were pain-free.
Symptoms re-occurred after the operation at an average of 7 months (S.D. 6.5)(range 0-18) prior to post-operative assesssment and there was a statistically significant difference between both groups of patients ($p<0.05$, paired t-Test). Whilst the patients suffering from chondromalacia patellae had symptoms re-occurring on average 5 months (S.D. 6.5) after surgery, in the osteochondritis dissecans patients this was 10 months (S.D. 5.1)

3. Principal symptoms.

'Giving way' of the knee joint was found in 65% of the patients before the operation (Fig. 5.7), 63% of the chondromalacia patellae patients and 70% of the osteochondritis dissecans patients, whilst after the operation only 20% complained about this (Fig. 5.8), 25% of the chondromalacia patellae patients and 10% of the osteochondritis dissecans patients.
Locking of the knee joint was seen in 50% of the patients pre-operatively, but only in 15% after the operation (Figs. 5.9 + 5.10). The improvement was more striking in the chondromalacia patients (pre-op. 50% versus post-op. 12.5%) compared to the osteochondritis group (pre-op. 50% versus post-op. 20%).
Swelling of the knee joint was found in 62% of the patients, 50% of the chondromalacia patellae patients and 80% of the osteochondritis dissecans patients, whilst post-operatively only 42% of the joints were swollen, 50% of the chondromalacia patellae and 30% of the osteochondritis dissecans (Figs. 5.11 + 5.12).
Of all patients, 54% were not able to play any sports at all, while 19% could not play contact sports. After the operation 23% were not able to play any sports, whilst 19% could play unlimited sports, that means they were not restricted in their choice of sports, compared to none before the operation.

4. Medications.

Before surgery, 43% of the patients were using either analgesics (33%), non-steroidal anti-inflammatory drugs (NSAIDs)(5%) or oral steroids (5%). One patient was using antibiotics. Fifty-two percent of the patients did not use any medication. Around 25% of the chondromalacia patellae patients were using analgesics/NSAIDs as were about 40% of the osteochondritis dissecans patients, whilst after surgery only 6% of the chondromalacia patellae patients used analgesics and about 20% of the osteochondritis dissecans patients were using painkillers. In total, 86% of all patients were not using any medication after the operation. From the 14% of the patients using medication after surgery, all but one patient were using analgesics already before surgery.

5. Past medical and orthopaedic surgical history.

In 45% of the patients there were no important diseases as listed in the questionnaire and 64% of the patients had no new medical problems after the operation. Most patients (93%) did not have any previous hip surgery. In contrast, only 1 patient (4%) had never had any knee surgery. Previous operations carried out were: medial or lateral meniscectomy (19% of patients), patella tendon repair (4% of patients), lateral ligament release (15% of the patients; these were 3 chondromalacia patellae patients and 1 osteochondritis patient), arthroscopy and wash out (92% of the patients) and other operations (23% of the patients).

An ankle operation was carried out on one patient with chondromalacia patellae previously, but no lumbar spine operations had been performed on any of the patients, although 12% (all patients with chondromalacia patellae) suffered from back pain.

6. Orthopaedic examination.

With regard to pre-operative mobility of the affected knee joint 8% of the patients could flex between 90-95°, 8% of the patients had 110-115° flexion, 8% of the patients had 120-125° flexion, 15% of the patients had 130-135° flexion and 58% of the patients had a flexion more or equal to 135° (Fig. 5.13). The mean pre-operative flexion was 131° (S.D. 15.9)(range 90-150°), whilst post-operatively this increased slightly to 133° (S.D. 13)(range 110-150°). With regard to post-operative flexion of the affected knee joint 12% of the patients had 110-115° flexion, 8% of the patients could flex between 115-120°, 8% of the patients had 120-125° flexion and 4% of the patients had 130-135° flexion and 68% of the patients had a flexion more or equal to 135° (Fig. 5.14). There was no significant difference between pre- and post-operative flexion ($1<p<0.375$, T-test).

With regard to extension of the affected knee joint 96% and 92%, respectively, of the patients could extend to 0°, pre-and post-operatively, and one patient had 5° extension lag pre-and post-operatively (Figs. 5.15 + 5.16).

One patient had -10° extension pre-operatively which decreased to -5° after the operation, whilst another patient lost extension after the operation (pre-op: -5° and post-op: 15°). The mean extension was -.4° (S.D. 2.5) pre-operatively and 6° (S.D. 3.4) postoperatively. There was no significant difference between pre- and post-operative extension ($1<p<0.375$, T-test).

Pre- and post-operatively the maximum valgus deformity was recorded in only 10 patients (38% of total) so the results need to be interpreted with caution. The pre-operative valgus deformity was 0° (35%) and 5°(3.8%), compared to 0° (38%) post-operatively.

Pre- and post-operatively the maximum varus deformity was recorded in only 9 patients (35% of total) so, again, the results need to be interpreted with caution. The varus deformity was, pre-operatively, 0° (31%) and 5° (3.8%), whilst all patients had 0° maximum varus deformity post-operatively.

Lachman's test was positive in 3 patients (12%) pre-operatively - one with chondromalacia patellae and 2 with osteochondritis dissecans.

Wasting of Mm. quadriceps femoris was measured 6 cm above the patella. In the affected knee the circumference of the thigh was on average 1.9 cm (S.D. 1.9)(range 0 - 8) less than at the same position on the non-affected side.

Effusion of the knee joint was found in 54% of the knee joints; 32% of the patients with chondromalacia patellae and 90% of the patients with osteochondritis dissecans.

Crepitus was found in 81% of the patients, including 75% of those with chondromalacia patellae and 90% of those with osteochondritis dissecans.

Ankle pain was found in only one patient.

7. Radiology.

The bone density was found to be normal in 65% of the patients, including 56% of the patients with chondromalacia patellae and 80% of those with osteochondritis dissecans, whilst the bone density was reduced in 12% of all patients - 13% of the patients with chondromalacia patellae and in 10% of those with osteochondritis dissecans. No data was available for 23% of all patients

Loose bodies were found in 4 patients, all suffering from osteochondritis dissecans, and soft tissue calcification in 3 patients - two with osteochondritis dissecans and 1 patient with chondromalacia patellae. The patella was never subluxed.

8. Pre-operative investigations.

Haemoglobin values were as follows. Pre-operatively for women 13.5 g/100ml (S.D. 0.9)(range 12.0 - 15.9) and for men 14.9 g/100ml (S.D. 0.8)(13.5 - 16.3), whilst from 6 patients the haemoglobin value was not known.

9. Arthroscopy.

Follow-up arthroscopy after 14 months revealed intact carbon fibre implants in all knees. The implants were fully covered with material which was described macroscopically as fibrous/fibrocartilaginous, and was proven to be stable with arthroscopic probing. However, there was speckling of the joint with fragments of carbon fibre in all knees. There was a minimal synovial reaction to the carbon fibre fragments.

On some questions too many patients had replied "unknown" and, therefore, those questions will not be discussed here. It was difficult, for example, to get conclusive answers to the questions about the frequency of the pain, how long the pain lasts, or on the character of the pain.

There were 5 patients who required manipulation under anaesthetic for residual stiffness, and one patient developed a sympathetic dystrophy. There were no wound infections and there were no problems with skin breakdown.

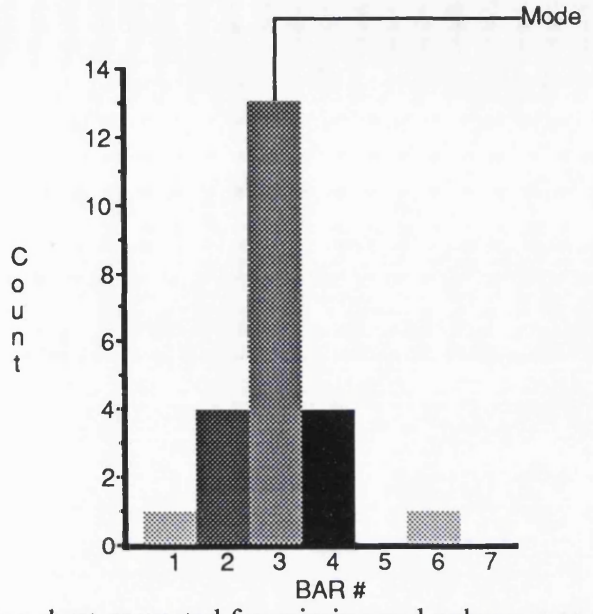
5.4. Discussion.

In the present study 35 patients suffering from osteochondritis dissecans and chondromalacia patellae were treated with implantation of carbon fibre plugs. After follow-up of on average 14 months, 26 patients were assessed. The important symptoms (pain, night pain, the phenomenons of 'giving way', 'locking' or 'swelling' of the knee joint, walking distance and playing unlimited sports) were significantly reduced post-operatively compared to pre-operatively. The mobility (flexion/extension) did not change significantly after the operation.

Minns *et al.*, (1987) implanted carbon fibre pads or rods in 145 knees with grade II articular cartilage defects, mostly in the patellae. There were 79 males and 59 females, with a mean age of 39 years (range 16-64). The results were excellent in most patients, and they could return to normal activity early. The present study did not confirm this, although it was shown that more patients could work in their job without difficulties than before the operation. There were also differences between the two studies: in the present study the male: female ratio was 6:7 with a lower mean age of 28 years (range 20 - 44) at the time of operation. A similar study by Muckle *et al.*, (1990) described 47 patients, average age 30 years (range 18-72) who had been followed up 3 years post-operatively. The authors described in 77% of the patients an excellent or good clinical response. Unfortunately, the criteria for excellent or good were not well defined in their article.
Brittberg and Peterson (1990) described the implantation of carbon fibre pads in 30 patients (male:female= 3:2), mean age 43 years (range 27 - 53) with localized gonarthrosis. They found good-to-excellent results in 83% of the patients using the Larsen rating and visual analogue scales. They described that pain relief was remarkable, but that the effect of the operation on functional recovery was less. The present study confirmed this. Also, they found that one-third of the patients developed pain after 8-12 weeks, but this disappeared gradually with time. In contrast, the present study showed that pain re-occured at an average of 7 months prior to post-operative assessment. Since the average follow-up period was 14 months this means that, on average, the pain re-occured 7 months after the operation. Possible explanations for the differences found between the study of Brittberg and Peterson and the present study might be the different mean age, male:female ratio and indication for surgery.
Zukor and co-authors (1989b) described their experience with osteochondral allografts for reconstruction of articular cartilage defects. They suggested that anatomical site, allograft size, stability of the graft and limb alignment were important factors in successful implantation. With regard to the former, the anatomical site (patella versus femoral condyle) did not show any statistical significant difference in the present study with regard to reduced post-operative pain score ($p< 0.46$, Wilcoxon signed rank). The other factors were not examined in the present study.

Rushton *et al.*, (1983) described problems with skin breakdown due to fragments of the carbon fibres deposited subcutaneously. The present study did not confirm this.
In conclusion, our prelimenary investigations suggest that woven carbon fibre pads may have a role in the treatment of articular cartilage defects such as osteochondritis dissecans and chondromalacia patellae in which conservative treatment has failed, with the result that the patient becomes disabled in work and daily activity due to persistent anterior knee pain and related symptoms. In future, further studies with longer follow up time, control groups and larger patients numbers will be necessary.

Fig. 5.1. Assessment of pain pre-operatively in all patients.

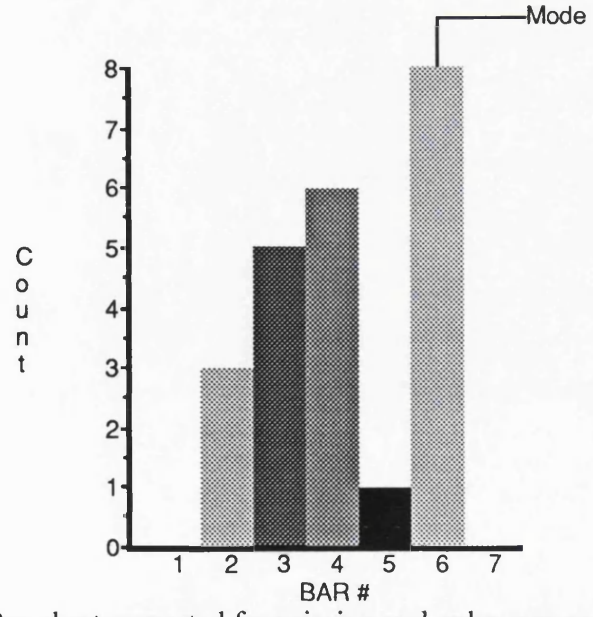


Bar chart corrected for missing and unknown answers.

INDEX

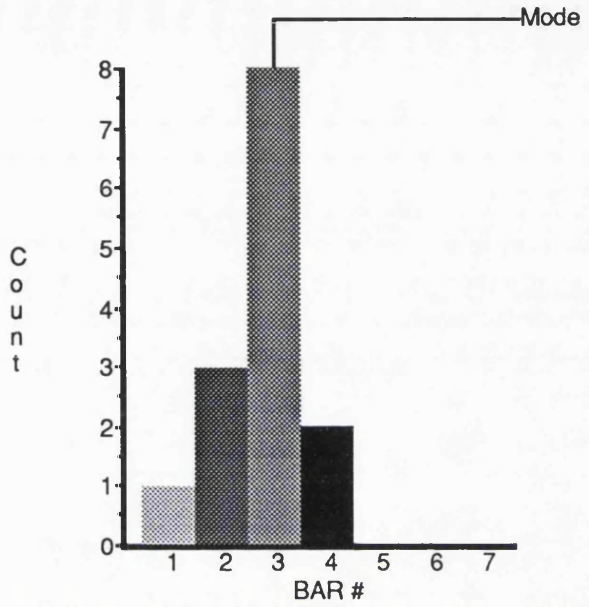
1= severe spontaneous pain
2= pain severe on attempting to walk, prevents all activity
3= tolerable pain permitting limited activity
4= pain only after some activity, goes quickly with rest
5= slight or intermittend pain
6= no pain

Fig. 5.2. Assessment of pain post-operatively in all patients.



Bar chart corrected for missing and unknown answers.

Fig. 5.3. Assessment of pain pre-operatively in patients with chondromalacia patellae.

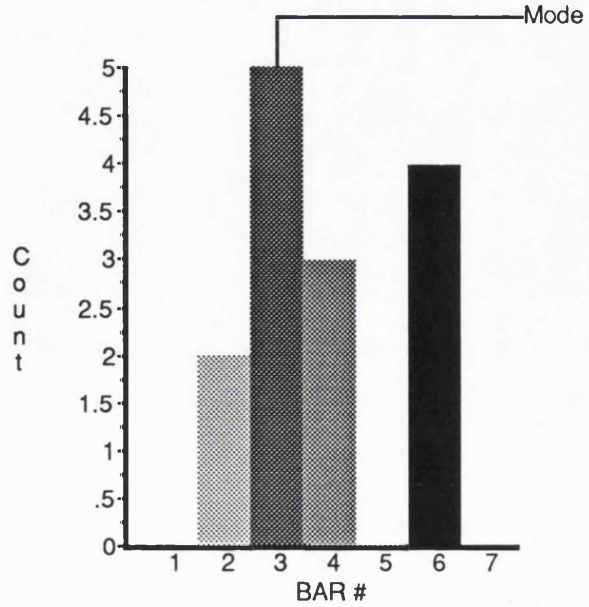


Bar chart corrected for missing and unknown answers.

INDEX

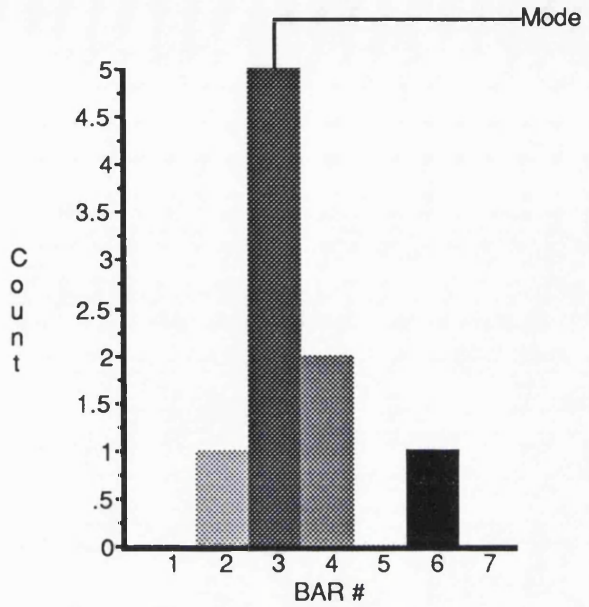
1= severe spontaneous pain
2= pain severe on attempting to walk, prevents all activity
3= tolerable pain permitting limited activity
4= pain only after some activity, goes quickly with rest
5= slight or intermittend pain
6= no pain

Fig. 5.4. Assessment of pain post-operatively in patients with chondromalacia patellae.



Bar chart corrected for missing and unknown answers.

Fig. 5.5. Assessment of pain pre-operatively in patients with osteochondritis dissecans.

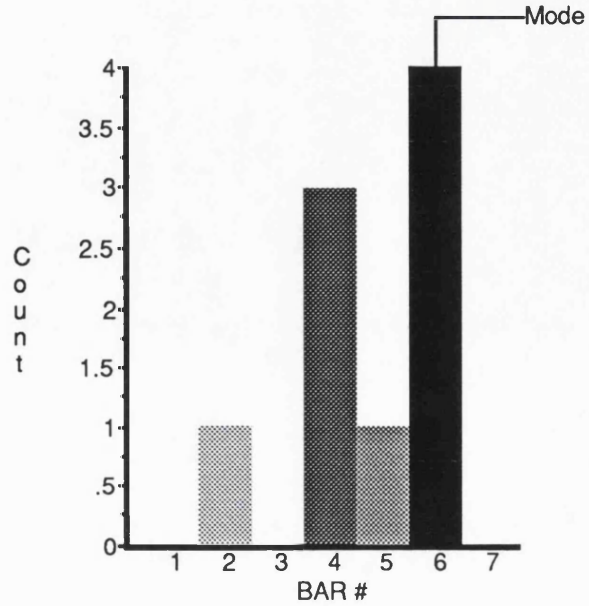


Bar chart corrected for missing and unknown answers.

INDEX

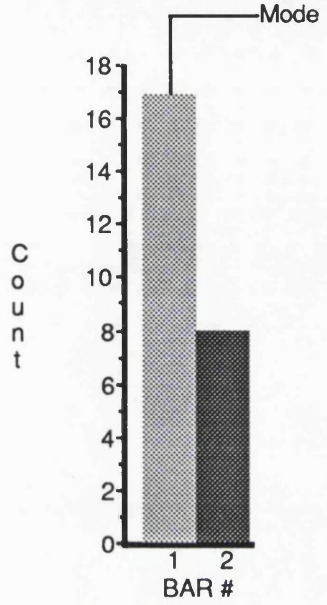
1= severe spontaneous pain
2= pain severe on attempting to walk, prevents all activity
3= tolerable pain permitting limited activity
4= pain only after some activity, goes quickly with rest
5= slight or intermittend pain
6= no pain

Fig. 5.6. Assessment of pain post-operatively in patients with osteochondritis dissecans.



Bar chart corrected for missing and unknown answers.

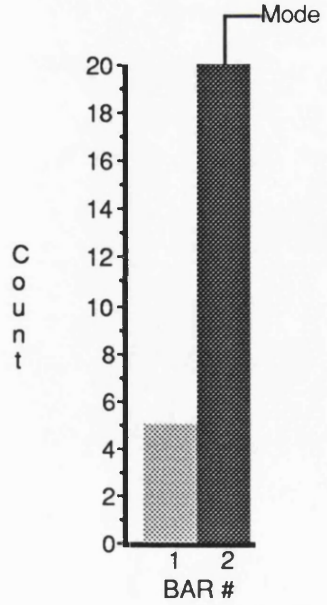
Fig. 5.7. The presence of giving way of affected knee joints in all patients pre-operatively.



Bar chart corrected for missing and unknown answers.

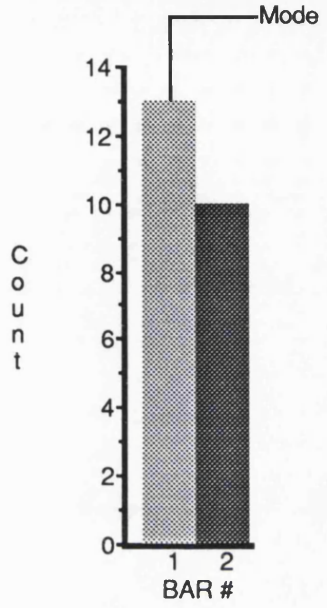
INDEX	
bar 1	yes
bar 2	no

Fig. 5.8. The presence of giving way of affected knee joints in all patients post-operatively.



Bar chart corrected for missing and unknown answers.

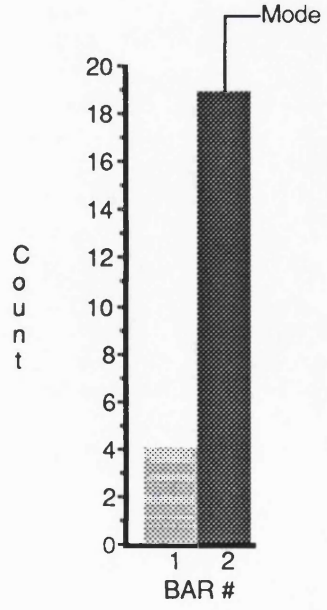
Fig. 5.9. The presence of 'locking' of the affected knee joints in all patients pre-operatively.



Bar chart corrected for missing and unknown answers.

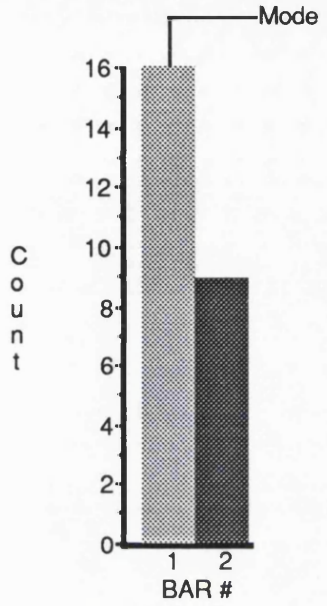
INDEX	
bar 1	yes
bar 2	no

Fig. 5.10. The presence of 'locking' of affected knee joint in all patients post-operatively.



Bar chart corrected for missing and unknown answers.

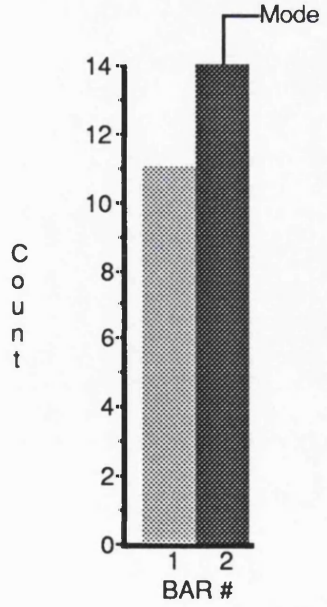
Fig. 5.11. The presence of swelling of affected knee joints in all patients pre-operatively.



Bar chart corrected for missing and unknown answers.

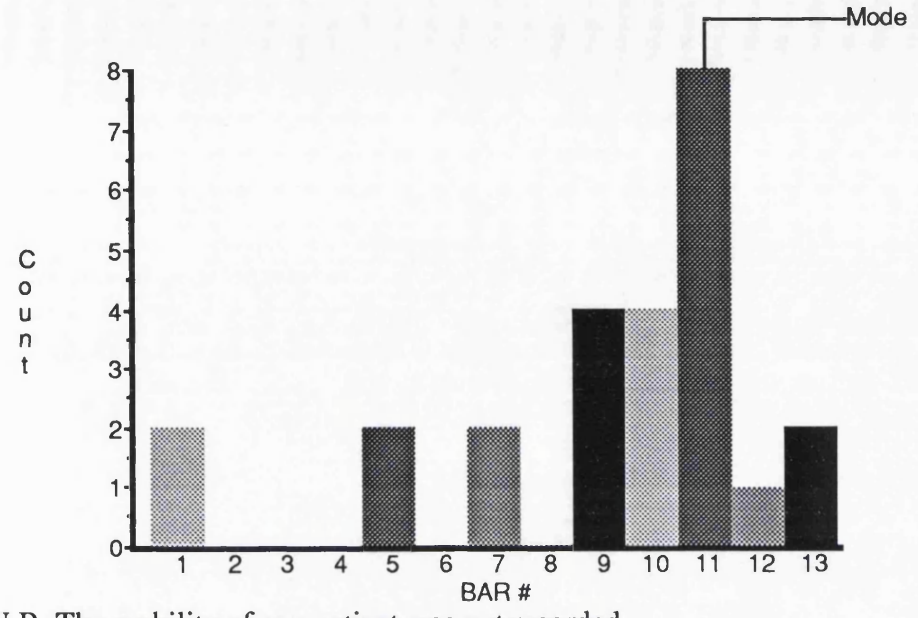
INDEX	
bar 1	yes
bar 2	no

Fig. 5.12. The presence of swelling of affected knee joints in all patients post-operatively.



Bar chart corrected for missing and unknown answers.

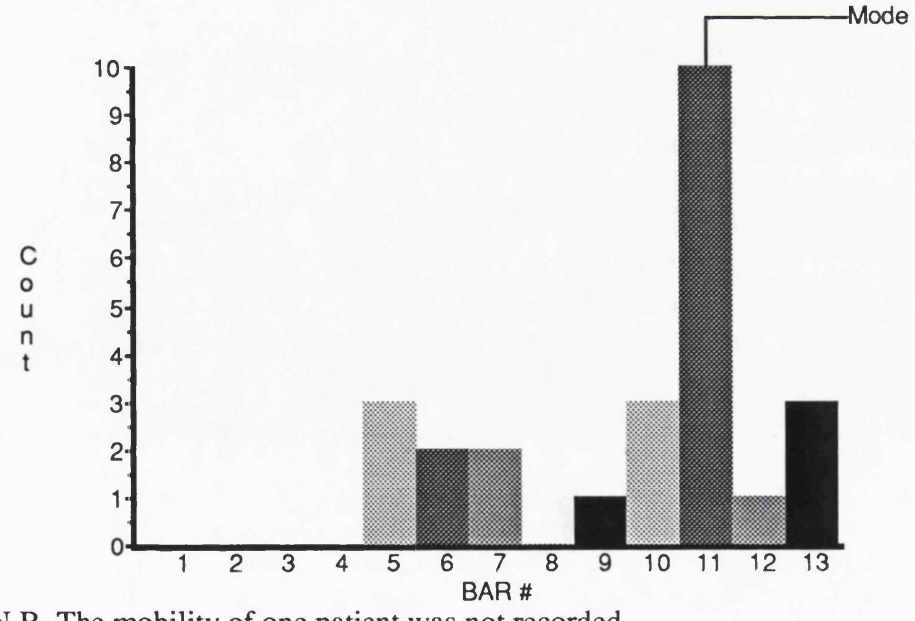
Fig. 5.13. Mobility of affected knee joints in all patients pre-operatively- flexion.



N.B. The mobility of one patient was not recorded.

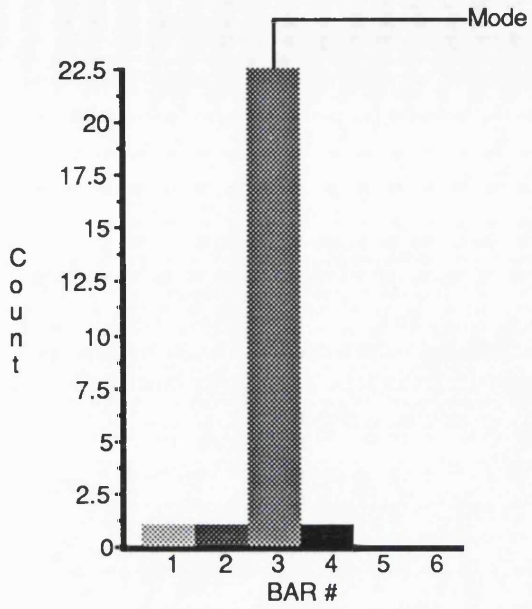
INDEX			
bar 1 90^{o}-95^{o}	bar 4 105^{o}-110^{o}	bar 7 120^{o}-125^{o}	bar 10 135^{o}-140^{o}
bar 2 95^{o}-100^{o}	bar 5 110^{o}-115^{o}	bar 8 125^{o}-130^{o}	bar 11 140^{o}-145^{o}
bar 3 100^{o}-105^{o}	bar 6 115^{o}-120^{o}	bar 9 130^{o}-135^{o}	bar 12 145^{o}-150^{o}
			bar 13 150^{o}-155^{o}

Fig. 5.14. Mobility of affected knee joints in all patients post-operatively- flexion.



N.B. The mobility of one patient was not recorded.

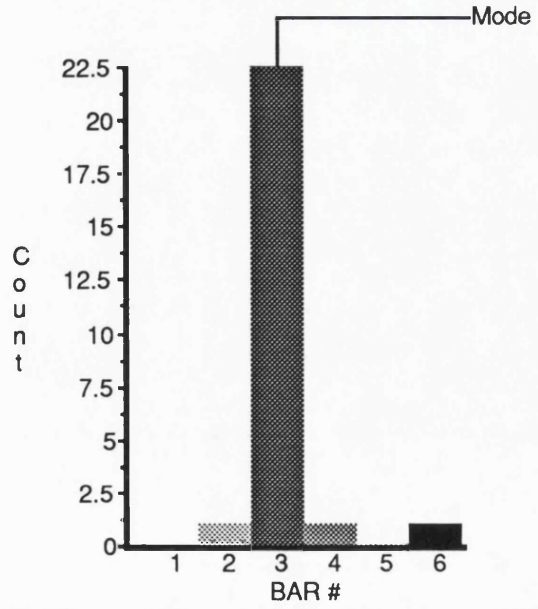
Fig. 5.15. Mobility of affected knee joints in all patients pre-operatively - extension.



N.B. The mobility of one patient was not recorded.

INDEX		
bar 1 -10^{o} - -5^{o}	bar 3. 0^{o} - 5^{o}	bar 5. 10^{o}-15^{o}
bar 2. -5^{o} - 0^{o}	bar 4. 5^{o} - 10^{o}	bar 6. 15^{o}- 20^{o}

Fig. 5.16. Mobility of the affected knee joints in all patients post-operatively - extension.



N.B. The mobility of one patient was not recorded.

REFERENCES

Abernathy, PJ, Townsend, P, Rose, R, Radin, EL. (1978). Is Chondromalacia Patella a Separate Clinical Entity? *J. Bone Jt..Surg.[Br]* **60B**, 205-210.

Afoke, NYP, Byers, P, Hutton, WC. (1987). Contact pressures in the human hip joint. *J. Bone Jt. Surg.* **69B**, 536-541.

Aglietti, P, Cerulli, GL. (1979). Chondromalacia and recurrent subluxation of the patella. *Ital. J. Orthop. Traumatol.* **5**, 187.

Aichroth, PM. (1971). Osteochondritis dissecans of the knee: a clinical survey. *J. Bone Jt. Surg. [Br]* **53B**, 440-447.

Akizuki, S, Mow, VC, Muller, F, Pita, JC, Howell, DS, Manicourt, DH. (1986). The tensile properties of human knee joint cartilage: I. Influence of ionic conditions, weight bearing, and fibrillation on the tensile modulus. *J. Orthop. Res.* **4**, 379-392.

Allen, TD, Schor, SL, Schor, AM. (1984). An ultrastructural review of collagen gels, a model system for cell-matrix, cell-basement membrane and cell-cell interactions. *Scanning Electron Micr.* **1**, 375-390.

Altman, RD, Tenenbaum, J, Latta, L, Riskin, W, Blanco, LN, Howell, DS. (1984). Biomechanical and biochemical properties of dog cartilage in experimentally induced osteoarthritis. *Ann. Rheum. Dis.* **43**, 83-90.

Amiel, D, Coutts RD, Harwood FL, Ishizue KK, Kleiner JB. (1988). The chondrogenesis of rib perichondrial grafts for repair of full thickness articular cartilage defects in a rabbit model: a one year post-operative assesment. *Conn. Tissue. Res.* **18**, 27-39.

Anderson, B, Seno, N, Sampson, P, Riley, JG, Hoffman, P, Meyer, K. (1964). Threonine and serine linkages in mucopolysaccharides and glycoproteins. *J. Biol. Chem.* **239**, 2 716 - 2 717.

Archer, CW, Rooney, P, Wolpert, L. (1982). Cell shape and cartilage differentiation of early chick limb bud cells in culture. *Cell differentiation* **11**, 245-251.

Archer, CW, McDowell, J, Bayliss, MT, Stephens, MD, Bentley, G. (1990). Phenotypic modulation in sub-populations of human articular chondrocytes in vitro. *J. Cell. Sci.* **97**, 361-371

Armstrong, CG, Mow, VC., Wilson, P., Straub, LR., (1982). Biomechanics of normal and osteoarthrotic articular cartilage. In: "Clinical trends in Orthopaedics". Eds. Thieme-Stratton, Inc., New York, 189-197.

Aston, JE. (1980). Biological repair of articular surfaces - an experimental study. Liverpool (Ph.D. thesis).

Aston, JE, Bentley, G. (1986). Repair of articular surfaces by allografts of articular cartilage and growth-plate cartilage. *J. Bone Jt. Surg. [Br]* **68**, 29-35.

Ayad, SH, Evans, J, Weiss, B, Holt, L. (1984). Type VI collagen but not type V collagen is present in cartilage. *Coll. Rel. Res.* **4**, 165-168.

Aydelotte, MB, Greenhill, RR, Kuettner, KE. (1988). Differences between sub-populations of cultured bovine articular chondrocytes. II. proteoglycan metabolism. *Conn. Tiss. Res.* **18**, 223-234.

Aydelotte, MB, Schmid, TM, Greenhill, RR, Luchene, BL, Schumacher, BL, Kuettner, KE. (1991). Synthesis of collagen by cultured bovine chondrocytes derived from different depths of articular cartilage. *Trans. Orthop. Res. Soc.* USA. **37**, 26.

Bacsich, P, Wyburn, GM. (1947). The significance of the mucoprotein content on the survival of homografts of cartilage and cornea. *Proc. R. Soc. Edinb.* **62**, 321-327.

Baker, JR, Caterson, B. (1979). The Isolation and Characterization of the Link Proteins from Proteoglycan Aggregates of Bovine Nasal Cartilage. *J. Biol. Chem.* **254**, 2 387 - 2 393.

Bancroft, JD, Stevens, A. (1990). In: "Theory and Practice of Histological Techniques". Churchill Livingstone, 548

Barnes, ML, Morton, CF, Bennett, RC, Bailey, AJ, Sims, TJ. (1976). Presence of type III collagen in guinea-pig dermal scar. *Biochem. J.* **157**, 263-266.

Bayliss, MT, Venn, MF. (1980). Chemistry of articular cartilage. In: "Studies in Joint Diseases I". Eds. Maroudas, A, Holborow, EJ, 2-58. Pitman Medical, Tunbridge Wells.

Bayliss, MT, Venn, MF, Maroudas, A, Ali, SY. (1983). Structure of proteoglycans from different layers of human articular cartilage. *Biochem. J.* **209**, 387-400.

Bell, E, Ivarsson, B, Merill, C. (1979). Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. *Proc. Natl. Acad. Sci. USA* **76**, 1 274-1 278.

Benninghoff, A. (1925). Form und Bau der Gelenkknorpel in ihren Beziehungen zur Funktion. II. Der Aufbau des Gelenkknorpels in seinen Beziehungen zur Funktion. *Z. Zellforsch U Mikr Anat (Berlin)* **2**, 783-862.

Benson, J. (1971). Elemental Carbon as a biomaterial. *J. Biomed. Mater. Res. Symposium* **2**, 41-47.

Bentley, G. (1970). Chondromalacia Patellae. *J. Bone Jt. Surg.* **52A**, 221-232.

Bentley, G, Greer III, RB. (1971). Homotransplantation of isolated epiphyseal and articular cartilage chondrocytes into joint surfaces of rabbits. *Nature* **230**, 385-388.

Bentley, G, Smith, AU, Mukerjee, R. (1978). Isolated epiphyseal chondrocyte allografts into joint surfaces. *Ann. Rheum. Dis.* **37**, 449-458.

Bentley, G, Dowd, G. (1984). Current Concepts of Etiology and Treatment of Chondromalacia Patellae. *Clin. Orthop.* **189**, 209-228.

Bentley, G. (1985). Articular cartilage changes in chondromalacia patellae. *J. Bone Jt. Surg. [Br]* **67B**, 769-774.

Bentley, G. (1989). The relationship between chondromalacia patellae and osteoarthritis of the knee. Presented at British Orthopaedic Association (BOA) Autumn Meeting.

Bentley, G, Pongor, P, Betts, J, Muckle, DS. (1990). Surface replacement of the knee with woven carbon.Presented at British Orthopaedic Association Autumn Meeting.

Benya, PD, Padilla, S, Nimni, ME. (1978). Independent regulation of collagen types of chondrocytes during the loss of differentiated function in culture. *Cell* **15**, 1 313 - 1 321.

Benya, PD, Shaffer, JD. (1982). Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. *Cell* **30**, 215-224.

Bernstein, MA. (1925). Osteochondritis dissecans. *J. Bone Jt. Surg.* **7**, 319-329.

Bjorkstrom, S, Goldie, IF. (1982). Hardness of the subchondral bone of the patella in the normal state, in chondromalacia, and in osteoarthrosis. *Acta Orthop. Scand.* **53**, 451-462.

Bokros, JC. (1977). Carbon biomedical devices. *Carbon* **15**, 355-371.

Bonfiglio, M, Jeter, WS, Smith, C. (1955). The immune concept: Its relation to bone transplantation. *Ann. N. Y. Acad. Sci.* **59**, 417.

Bradley, J, Dandy, DJ. (1989). Results of drilling osteochondritis dissecans before skeletal maturity. *J. Bone Jt. Surg. [Br]* **71B**, 642-4.

Brattstrom, H. (1964). Shape of the intercondylar groove normally and in recurrent dislocation of the patella. A clinical and X-ray-anatomical investigation. *Acta Orthop. Scand. (Suppl)* **68**, 135.

Bray, BA, Lieberman, R, Meyer, K. (1967). Structure of human skeletal keratosulfate: the linkage region. *J. Biol. Chem.* **242**, 3 373 - 3 380.

Bray, JC, Merrill, EW. (1973). Poly(vinyl alcohol) Hydrogels for Synthetic Articular Cartilage Material. *J. Biomed. Mater. Res.* **7**, 431-443.

Brittberg, M, Peterson, L. (1990). Biological articular cartilage resurfacing in osteoarthritic knee with carbon fibre. *Acta Orthop. Scand. (Suppl.)* **61**, 237.

Brooks, DB, Heiple, KG, Herndon, CH, Powell, AE. (1963). Immunological factors in homogeneous bone transplantation. *J. Bone Jt. Surg. [Am]* **45A**, 1 617-1 626.

Bruckner, P, Vaughan,L, Winterhalter, KH. (1985). Type IX collagen from sternal cartilage of chicken embryo contains covalently bound glycosaminoglycans. *Proc. Natl. Acad. Sci. USA* **82**, 2 608 - 2 612.

Buckwalter, JA, Rosenberg, LC. (1988a). Electron microscopic studies of cartilage proteoglycans. *Electron Microsc. Rev.* **1**, 87-112.

Buckwalter, JA, Hunziker, E, Rosenberg, L, Coutts, R, Adams, M, Eyre, D. (1988b). Articular cartilage: composition and structure. In:"Injury and repair of the

musculoskeletal soft tissue". Eds. Woo, SL-Y., Buckwalter, JA., 405-425. American Academy of Orthopaedic Surgeons, Park Ridge.

Buckwalter, JA, Rosenberg, L, Coutts, R, Hunziker, E, Reddi, AH, Mow, V (1988c). Articular cartilage: Injury and Repair. In: "Injury and Repair of the Musculoskeletal Soft Tissues". Eds. Woo, SL-Y., Buckwalter, JA., 465-482. American Academy of Orthopaedic Surgeons, Park Ridge.

Buncke jr, HJ, Daniller, AI, Schulz, WP, Chase, RA. (1967). The fate of autogenous whole joints transplanted by microvascular anastomoses. *Plast. Reconstr. Surg.* **39**, 333.

Burwell, RG, Friedlaender, GE, Mankin, HJ. (1985). Current Perspectives and Future Directions: The 1983 Invitational Conference on Osteochondral Allografts. *Clin. Orthop.* **197**, 141-157.

Calandruccio, RA, Gilmer, WS. (1962). Proliferation, regeneration and repair of articular cartilage of immature animals. *J. Bone Jt. Surg. [Am]* **44A**, 431-55.

Caplan, AI, Koutroupas, S. (1973). The control of muscle and cartilage development in the chick limb: the role of differential vascularization. *J. Embryol. Exp. Morphol.* **29**, 571-583.

Carlson, CS, Meuten, DJ, Richardson, DC. (1991). Ischaemic necrosis of cartilage in spontaneous and experimental lesions of osteochondrosis. *J. Orthop. Res.* **9**, 317-329.

Carney, SL, Muir, H. (1988). The structure and function of cartilage proteoglycans. *Physiological Rev* **68**, 858-910.

Caterson, B, Lowther, DA. (1978). Changes in the metabolism of the proteoglycans from sheep articular cartilage in response to mechanical forces. *Biochim. Biophys. Acta* **540**, 412-22.

Caterson, B, Christner, JE, Baker, JR, Couchman, JR. (1985). Production and characterization of monoclonal antibodies directed against connective tissue proteoglycans. *Fedn. Proc. Fedn. Am. Socs. exp. Biol.* **44**, 386-393.

Caterson, B, Mahmoodian, F, Sorrell, JM, Bayliss, MT, Hardingham, TE, Muir, H. (1989). Monoclonal antibodies that recognise novel chondroitin sulfate structures that are

specifically expressed during development and in disease. 35th Trans Orthop Res Soc USA, **14**, 12.

Caterson, B, Mahmoodian F, Sorrell JM, Hardingham TE, Bayliss MT, Carney ST, Ratcliffe A, Muir H. (1990). Modulation of native chondroitin sulphate structure in tissue development and in disease. *J. Cell.Sci.* **97**, 411-417.

Caterson, B, Blankenship-Paris, T, Chandrasekhar, S, Slater, R. (1991). Biochemical characterization of guinea pig cartilage proteoglycans with the onset of 'spontaneous' osteoarthritis. 37th Trans. Orthop. Res. Soc. USA, **16**, 251.

Charnley, J. (1969). Total prosthetic replacement of the hip. In: "Reconstructive Surgery and Traumatology", 9. Phiebig, New York.

Chesterman, P, Smith, AU. (1968). Homotransplantation of articular cartilage and isolated chondrocytes: An experimental study in rabbits. *J. Bone Jt. Surg. [Br]* **50B**, 184-197.

Claes, L, Neugebauer, R. (1985). *In vivo* and *in vitro* investigation of the long-term behavior and fatigue strength of carbon fibre ligament replacement. *Clin. Orthop.* **196**, 99-111.

Clark, JM. (1991). Variation of collagen fiber alignment in a joint surface: A scanning electron microscope study of the tibial plateau in dog, rabbit, and man. *J. Orthop. Res.* **9**, 246-257.

Cobbett, JR. (1969). Free digital transfer. *J. Bone Jt. Surg. [Br]* **51**, 677.

Coletti, JM, Akeson, WH, Woo, SL-Y. (1972). A comparison of the physical behaviour of the normal articular cartilage and the arthroplasty surface. *J Bone Jt Surg[Am]* **54A**, 147-160.

Corkhill, PH, Hamilton, CJ, Tighe, BJ. (1990). The design of hydrogels for medical applications . In: "Critical Reviews in Biocompatibility", 363-436.

Corkhill, PH, Trevett, AS, Tighe, BJ. (1991). The potential of hydrogels as synthetic articular cartilage. *Proc Inst. Mech. Eng. Part. H Engineering in Medicine* in press.

Crawfurd, EJP, Emery, RJH, Aichroth, PM. (1990). Stable osteochondritis dissecans - does the lesion unite? *J. Bone Jt. Surg.* **72B**, 320.

Culling, CFA. (1974). Handbook of Histopathological and Histochemical Techniques, 141-386. Butterworths, London.

Cuprak, LJ, Lever, WF. (1974). Growth characteristics of a hamster cell line on collagen gel. *Proc. Soc. Exp. Biol. Med.* **146**, 309-315.

Czitrom, AA, Langer, F, McKee, N, Gross, AE. (1986). Bone and cartilage allotransplantation: A review of 14 years of research and clinical studies. *Clin. Orthop.* **208**, 141-145.

Dahlberg, L, Kreicbergs, A. (1991). Demineralized allogeneic bone matrix for cartilage repair. *J. Orthop. Res.* **9**, 11-19.

Dalla Vedova, R. (1911). Di alcune ricerche sperimentali sul trapianto libero osteo-articolare. *Policlini Sezione Chirurg.* **18**, 529-545.

Dandy, DJ, Flanagan, JP, Steenmeyer, V. (1982). Arthroscopy and the management of the ruptured anterior cruciate ligament. *Clin. Orthop.* **167**, 43-49.

Daughady, WH, Hall, K, Raben, MS, Salmon, WD, Vanden Brande, JL, van Wyk, JJ. (1972). Somatomedin-proposed designation for sulphation factor. *Nature* **235**, 107.

Davies, D, Barnett, CH, Cochrane, W, Palfrey, AJ. (1962). Electron microscopy of articular cartilage in the young adult rabbit. *Ann. Rheum. Dis.*. **21**, 11-22.

DePalma, AF, Tsultas, TT, Mauler, GG. (1963). Viability of osteochondral grafts as determined by uptake of 35S. *J. Bone Jt. Surg. [Am]* **45A**, 1 565-1 578.

DePalma, AF, McKeever, CD, Subin, DK. (1966). Process of repair of articular cartilage demonstrated by histology and autoradiography with tritiated thymidine. *Clin. Orthop.* **48**, 229-42.

Donohue, JM, Buss, D, Oegema, TR, Thompson, RC. (1983). The effects of indirect blunt trauma on adult canine articular cartilage. *J. Bone Jt. Surg. [Am]* **65A**, 948-57.

Ducuing, J. (1912). Les greffes articulaires totales chez l'homme. *Rev. Chir. (Paris)* **46**, 238-279 and 452-498.

Dumbleton, JH, Shen, C, Miller, EH. (1974). A study of the wear of some materials in connection with the total hip replacement. Annual Meeting Orthop. Res. Soc. USA.

Dustmann, HO, Puhl, W and Krempien, B. (1974a). Die Zellteilung im Gelenkknorpel. *Arch. Orthop. Unfall-Chir.* **79**, 171-182.

Dustmann, HO, Puhl, W, Krempien, B. (1974b). Das Phaenomen der Cluster im Arthroseknorpel. *Arch. Orthop. Unfall-Chir.* **79**, 321-333.

Dziewiatkowski, DD. (1987). Binding of Calcium by Proteoglycans In Vitro. *Calcif. Tissue Int.* **40**, 265-269.

Ehrmann, RL, Gey, GO. (1956). The growth of cells on a transparent gel of reconstituted rat-tail collagen. *J. Nat. Cancer Inst.* **16**, 1 375-1 390.

Elliott, RJ, Gardner, DL. (1979). Changes with age in the glycosaminoglycans of human articular cartilage. *Ann. Rheum. Dis.* **38**, 371-377.

Elsdale, TR, Bard, JBL. (1972). Collagen substrata for studies on cell behaviour. *J. Cell. Biol.* **54**, 626-637.

Engkvist, O, Ohlsen, L. (1979). Reconstruction of articular cartilage with free autologous perichondral grafts. *Scand. J. Plast. Reconstr. Surg.* **13**, 269-74.

Engkvist, O, Skoog, V, Pastacaldi, P, Yormuk, E, Juhlin, R. (1979). The cartilaginous potential of the perichondrium in rabbit ear and rib. *Scand. J. Plast. Reconstr. Surg.* **13**, 275-80.

Engkvist, O, Wilander E. (1979). Formation of cartilage from rib perichondrium grafted to an articular defect in the femur condyle of the rabbit. *Scand. J. Reconstr. Surg.* **13**, 371-376.

Engkvist, O, Ekenstam, F. (1982). Joint cartilage formation induced by silastic sheet spacer. *Scand. J. Plast . Reconstr. Surg.* **16**, 191-194.

Eyre, DR, Muir, H. (1974). Collagen polymorphism: two molecular species in pig intervertebral disc. *FEBS Lett.* **42**, 192-196.

Eyre, DR, Wu, JJ, Apone, S. (1987a). A growing family of collagens in articular cartilage: Identification of 5 Genetically Distinct Types. *J. Rheum.* **14**, 25-27.

Eyre, DR, Wu, JJ., (1987b). Type XI or 1a2a3a. In: "Structure and Function of Collagen Types". Eds. Mayne, R., Burgeson, RE., 261-281. Academic Press, Orlando.

Fairbank, HAT. (1933). Osteo-chondritis Dissecans. *Brit. J. Surg.* **21**, 67.

Fessler, LI., Timpl, R, Fessler, JH. (1981). Assembly and Processing of Procollagen Type III in Chick Embryo Blood Vessels. *J. Biol. Chem.* **256**, 2 531-2 537.

Ficat, R. (1977). Excessive lateral pressure syndrome. In: "Disorders of the Patella-femoral Joint ". Eds. Ficat, R., Hungerford, DS., 127. Masson, Paris.

Ficat, R, Ficat, C, Gedeon, P, Toussaint, JB. (1979). Spongialization: A New Treatment for Diseased Patellae. *Clin. Orthop.* **144**, 74-83.

Folkman, J, Moscona, A. (1978). Role of cell shape in growth control. *Nature* **273**, 345-349.

Forster, IW, Ralis, ZA, McKibbin, B, Jenkins, DHR. (1978). Biological reaction to carbon fiber implants: the formation and structure of a carbon-induced "neotendon". *Clin. Orthop.* **131**, 299-307.

Franzen, A, Inerot, S, Hejderup, SO, Heinegard, D. (1981). Variations in the composition of bovine hip articular cartilage with distance from the articular surface. *Biochem. J.* **195**, 535-543.

Freeman, MAR. (1974). Collagen fatigue failure as a cause of osteoarthrosis. In: "Normal and Osteoarthrotic Articular Cartilage". Eds. Ali, SY, Elves, MW, Leaback, DH., 173. The Institute of Orthopaedics, London.

Fulkerson, JP, Edwards, C, Chrisman, OD. (1987). Articular cartilage. In: "The Scientific Basis of Orthopaedics". Eds. Albright, J, Brand, R. Appleton and Lange, East Norwalk.

Fulkerson, JP, Hungerford, DS. (1990). In: "Disorders of the Patellofemoral Joint", 176-225. Williams and Wilkins, Baltimore.

Furukawa, T, Eyre, DR, Koide, S, Glimcher, MJ. (1980). Biochemical studies on repair cartilage resurfacing experimental defects in the rabbit knee. *J. Bone Jt. Surg. [Am]* **62A**, 79-89.

Garg, HG, Swann, DA. (1981). Age-related changes in the chemical composition of bovine articular cartilage. The structure of high-density proteoglycans. *Biochem. J.* **193**, 459-468.

Gay, S, Mueller, PK, Lemmen, C, Remberger, K, Matzen, K, Kuehn, K. (1976). Immunohistological study on collagen in cartilage-bone metamorphosis and degenerative osteoarthrosis. *Klin. Wschr.* **54**, 969-976.

Gay, S, Miller, EJ. (1978). In: "Collagen in the physiology and pathology of connective tissue", 1-110. Gustav Fisher Verlag, Stuttgart.

Gere, J, Timoshinko, FP. (1984). Mechanics of materials.

Ghadially, FN, Fuller, JA, Kirkaldy-Willis, WH. (1971). Ultrastructure of full-thickness defects in articular cartilage. *Archs. Path.* **92**, 356-69.

Ghadially, FN, Thomas, I, Oryschak, AF, Young, NK. (1977). Long-term results of superficial defects in articular cartilage: A scanning electron-microscope study. *J. Pathol.* **121**, 213-217.

Gibson, GJ, Schor, SL, Grant, ME. (1982). Effects of Matrix Molecules on Chondrocyte Gene Expression: Synthesis of a Low Molecular Weight Collagen Species by Cells cultured within Collagen Gels. *J Cell Biol* **93**, 767-774.

Gibson, GJ, Francki, KT, Hopwood, JJ, Foster, BK. (1991). Human and sheep growth-plate cartilage type X collagen synthesis and the influence of tissue storage. *Biochem. J.* **277**, 513-520.

Gilbert, DL, Lyman, DJ. (1987). *In vitro* and *in vivo* characterization of synthetic polymer/biopolymer composites. *J. Biomed. Mat. Res.* **21**, 643-655.

Glowacki, J, Trepman, E, Folkman,J. (1983). Cell shape and phenotypic expression in chondrocytes. *Proc. Soc. Exp. Biol. Med.* **172**, 93-98.

Goldberg, VM, Lance, EM. (1972). Revascularization and accretion in transplantation. Quantitative studies of the role of the allograft barrier. *J. Bone Jt. Surg. [Am]* **54A**, 807-816.

Goodfellow, JW, Hungerford, DS, Woods, C. (1976). Patello-Femoral Mechanics and Pathology: II. Chondro-Malacia Patellae. *J. Bone Jt. Surg.[Br]* **58B**, 291.

Gospodarowicz, D, Delgado, D, Vlodavsky, I. (1980). Permissive effect of the extracellular matrix on cell proliferation in vitro. *Proc. Natl. Acad. Sci. USA* **77**, 4094-4098.

Grant, WT, Sussman, MD, Balian, G. (1985). A Disulfide-bonded Short Chain Collagen Synthesized by Degenerative And Calcifying Zones of Bovine Growth Plate Cartilage. *J. Biol. Chem.* **260**, 3 798-3 803.

Grant, WT, Wang, GJ, Balian, G. (1987). Type X collagen synthesis during enchondral ossification in fracture repair. *J. Biol. Chem.* **262**, 9 844-9 849.

Grassmann, W, Engel, J, Hannig, K, Hoermann, H, Kuehn, K, Nordwig, A . (1965). In: "Kollagen", 195-314. Springer Verlag, Vienna.

Green, W. (1971). Behavior of articular chondrocytes in cell culture. *Clin. Orthop.* **75**, 248-260.

Green, WT, Banks, HH. (1953). Osteochondritis dissecans in children. *J. Bone Jt. Surg. [Am]* **35A**, 26-47.

Green jr, WT (1977). Articular cartilage repair. Behavior of rabbit chondrocytes during tissue culture and subsequent allografting. *Clin. Orthop.* **124**, 237-250.

Greiling, H, Stuhlsatz, HW. (1969). Glykosaminoglykan-peptide aus dem humanen Kniegelenk-knorpel. Hoppe-Seyler's Z. Physiol. Chem. **350**, 449-456.

Grodzinsky, AJ. (1983). Electromechanical and physicochemical properties of connective tissue. *CRC Crit Rev Bioeng* **9**, 133-199.

Gross, AE, Silverstein, EA, Falk, J, Falk, R, Langer, F. (1975). The allotransplantation of partial joint in the treatment of osteoarthritis of the knee. *Clin. Orthop.* **108**, 7-14.

Gross, AE, Langer, F, Houpt, J, Pritzker, K, Friedlaender,G. (1976). Allotransplantation of partial joints in the treatment of osteoarthritis of the knee. *Transplant Proc* **VIII**, 129-132.

Guhl, JF. (1982). Arthroscopic treatment of osteochondritis dissecans. *Clin. Orthop.* **167**, 65-74.

Hardingham, TE, Muir, H. (1974). Hyaluronic acid in cartilage and proteoglycan aggregation. *Biochem. J.* **139**, 565-581.

Hardingham, TE. (1979). The role of link-protein in the structure of cartilage proteoglycan aggregates. *Biochem. J.* **177**, 237-247.

Hardingham, TE, Caterson, B, Bayliss, MT, Carney, SL, Ratcliffe, A, Muir, H. (1989). Appearance of novel chondroitin sulphate structures in the articular cartilage from experimental canine osteoarthritic joints. 35th Trans. Orthop. Res. Soc., USA, **14**, 505.

Hardingham, TE, Fosang, AJ. (1992). Proteoglycans: many forms and many functions. *FASEB J.* **6**, 861-870.

Harris, AK, Stopak, D, Wild, P. (1981). Fibroblast traction as a mechanism for collagen morphogenesis. *Nature* **290**, 249-251.

Hascall, VC, Heinegard, D. (1974). Aggregation of Cartilage Proteoglycans. II. Oligosaccharide competitors of the proteoglycan-hyaluronic acid interaction. *J. Biol. Chem.* **249**, 4 242 - 4 249.

Hascall, VC, Kimura, JH. (1982). Proteoglycans: Isolation and Characterization. In: "Methods in Enzymology". Eds. Cunningham, L., Frederiksen, DW, 769-800. Academic Press, New York.

Haubold, AD, Shim, HS, Bokros, JC. (1981). Carbon in medical devices. In: "Biocompatibility of clinical implant materials". Eds. Williams, D. CRC Press, Boca Rotan.

Havdrup, T, Telhag, H. (1978). Scattered mitosis in adult joint cartilage after partial chondrectomy. *Acta Orthop. Scand.* **49**, 424-429.

Hay, BM. (1950). Two cases of osteochondritis dissecans affecting several joints. *J. Bone Jt. Surg.* **32B**, 361-367.

Hayes, WC, Keer, LM., Herrmann, G, Mockros, LFA. (1972). Mathematical analysis for indentation tests of articular cartilage. *J. Biomech.* **5**, 541-551.

Heinegard, D, Hascall, VC. (1974). Aggregation of Cartilage Proteoglycans. III. Characteristics of the proteins isolated from trypsin digests of aggregates. *J. Biol. Chem.* **249**, 4 250-4 256.

Heinegard, D, Axelsson, I. (1977). Distribution of Keratan Sulfate in Cartilage Proteoglycans. *J. Biol. Chem.* **252**, 1 971-1 979.

Heinegard, D, Bjorne-Persson, A, Coster, L, Franzen, A, Gardell, S, Malmstrom, A, Paulsson, M, Sandfalk, R, Vogel, KV. (1985). The core proteins of large and small intersitial proteoglycans from various connective tissues form distinct subgroups. *Biochem. J.* **230**, 181-194.

Heinegard, D, Franzen, A, Hedbom, E, Sommarin, Y., CIBA, (1986). Common structures of the core proteins of interstitial proteoglycans. In: "CIBA Symp", 204-214.

Heinegard, D, Antonsson, P, Hedbom, E, Larsson, T, Oldberg, A, Sommarin, Y, Wendel, M. (1988). Noncollagenous Matrix Constituents of Cartilage. *Pathol. Immunopathol. Res.* **7**, 27-31.

Heiple, KG, Chase, SW, Herndon, CH. (1963). A Comparative Study of the Healing Process Following Different Types of Bone Transplantation. *J. Bone Jt. Surg. [Am]* **45**, 1 593-1 616.

Helbing, G, Wolter, D, Neugebauer, R, Coldewey, J. (1977). The reaction of tissue to friction particles of LTI carbon and carbon reinforced carbon in the knee joint of the rat. Transactions of the 3rd Annual Meeting for Biomaterials, **1**, 151.

Henry jr, WB, Schachar, NS, Wadsworth, PL, Castronovo, FP, Mankin, HJ. (1985). Feline model for the study of frozen osteoarticular hemijoint transplantation: Qualitative and quantitative assesment of bone healing. *Am. J. Vet. Res.* **46**, 1 714-1 720.

Hirotani, H, Ito, T. (1975). Chondrocyte mitosis in the articular cartilage of femoral heads with various diseases. *Acta Orthop. Scand.* **46**, 979-986.

Hjertquist, S-O, Lemperg, R. (1972). Identification and concentration of the glycosaminoglycans of human articular cartilage in relation to age and osteoarthritis. *Calcif. Tissue Res.* **10**, 223-237.

Hodge, WA, Fijan, RS, Carlson, KL, Burgess, RG, Harris, WH, Mann, RW. (1986). Contact pressures in the human hip joint measured *in vivo*. *Proc. Natl. Acad. Sci. USA* **83**, 2 879-2 883.

Hofmann, H, Fietzek, PP, Kuehn, K. (1980). Comparative analysis of the sequences of the three collagen chains alfa1(I), alfa2 and alfa1(III). Functional and genetic aspects. *J. Mol. Biol.* **141**, 293-314.

Holmes, M, Volz, RG, Chvapil, M. (1975). Collagen sponge as a matrix for articular cartilage regeneration. *Surgical Forum* **XXVI**, 511-513.

Homminga, GN. (1989). Perichondrial arthroplasty of the knee. Maastricht (PhD thesis).

Homminga, GN, Bulstra, SK, Kuyer, R, van der Linden, AJ. (1991). Repair of sheep articular cartilage defects with a rabbit costal perichondrial graft. *Acta Orthop. Scand.* **62**, 415-418.

Huberti, HH, Hayes,WC. (1984). Patellofemoral contact pressures: the influence of Q-angle and tendofemoral contact. *J. Bone Jt. Surg[Am]* **66A**, 715-724.

Hughston, JC, Hergenroeder, PT, Courtenay, BG. (1984). Osteochondritis dissecans of the femoral condyles. *J. Bone Jt. Surg. [Am]* **66A**, 1340-8.

Hunziker, EB, Rosenberg, LC. (1992). Biological basis for repair of superficial articular cartilage lesions. 38th Trans. Orthop. Res. Soc. USA., 17, 231.

Impallomeni, G. (1911). Sul trapianto della aricolozioni. *Archivio di Ortopedia* **28**, 342.

Insall, JN. (1967). Intra-articular surgery for degenerative arthritis of the knee. A report of the work of the Late K.H. Pridie. *J. Bone Jt. Surg. [Br]* **49B**, 211-228.

Insall, JN, Salvati, E. (1971). Patella position in the normal knee joint. *Radiology* **101**, 101.

Insall, JN, Tria, AJ, Aglietti, P. (1980). Resurfacing of the Patella. *J. Bone Jt. Surg. [Am]* **62A**, 933-936.

Ireland, GW. (1987). Effects of patterned surfaces of adhesive islands on the shape, cytoskeleton, adhesion and behaviour of swiss mouse 3T3 fibroblasts. *J. Cell. Sci. (Suppl)* **8**, 19-33.

Irwin, MH, Silvers, SH, Mayne, R. (1985). Monoclonal antibody against chicken type IX collagen: preparation, characterization and recognition of the intact form of type IX collagen secreted by chondrocytes. *J. Cell. Biol.* **101**, 814-823.

Itay, S, Abramovici, A, Nevo, Z. (1987). Use of cultured embryonal chick epiphyseal chondrocytes as grafts for defects in chick articular cartilage. *Clin. Orthop.* **220**, 284-303.

Itay, S, Abramovici, A, Yosipovitch, Z, Nevo, Z. (1988). Correction of defects in articular cartilage by implants of cultured embryonic chondrocytes. 34th Trans.Orthop. Res. Soc. USA., **13**, 112.

Iwata, H, Ono, S, Sato, K, Kawamara, M, Sato, T. (1991). Cartilage formation on monolayer cell culture regulated by bone matrix protein containing bone morphogenetic protein (BMP). Combined Meeting of the Orthopaedic Research Societies of USA, Japan and Canada, 50.

Jamshidi, K, Shimizu, T, Usui, Y, Eberhart, RC, Mooney, V. (1988). Resorbable structured porous materials in the healing process of hard tissue defects. *Transactions Am. Soc. Artif. Intern. Organs* **34**, 755-760.

Jeffery, AK, Blunn, GW, Archer, CW, Bentley, G. (1991). Three-dimensional collagen architecture in bovine articular cartilage. *J. Bone Jt. Surg. [Br]* **73B**, 795-801.

Jenkins, DHR, Forster, IW, McKibbin, B, Ralis, ZA. (1977). Induction of tendon and ligament formation by carbon implants. *J. Bone Jt. Surg. [Br]* **59B**, 53-7.

Jenkins, DHR. (1978). The repair of cruciate ligaments with flexible carbon fibre. A longer term study of the induction of new ligaments and of the fate of the implanted carbon. *J. Bone Jt. Surg. [Br]* **60B**, 520-522.

Jenkins, DHR, McKibbin, B. (1980). The role of flexible carbon-fibre implants as tendon and ligament substitutes in clinical practice. A preliminary report. *J. Bone Jt. Surg. [Br]* **62**, 497-499.

Johnell, O, Telhag, H. (1977). The effect of osteotomy and cartilage damage on mitotic activity. *Acta Orthop. Scand.* **48**, 263-265.

Johnson, GD, Davidson RS, McNamee KC, Russell G, Goodwin D, Halbarow EJ. (1982). Fading of immunofluorescence during microscopy: A study of the phenomenon and its remedy. *J Immunol Methods* **55**, 231-242.

Judet, H. (1908). La greffe des tissue articulaires. Recherches de Chirurgie Experimentale. Eds. Libraire, J. Bailliere et Fils., Paris.

Judet, H, Padovani, JP. (1968). Transplantation d'articulation complete avec retablissement circulatoire immediat par anastomoses arterielles et veineuses. *Rheum Acad Chir* **94**, 520.

Kampmann, J, Thomsen, PB. (1991). Biological repair of osteochondral defects with carbon rods. *Acta Orthop. Scand. (Suppl)* **62**, 246.

Kandel, RA, Gross, AE, Ganel, A, McDermott, AGP, Langer, G, Pritzker, KPH. (1985). Histopathology of failed osteoarticular shell allografts. *Clin. Orthop.* **197**, 103-110.

Karlson, S. (1940). Chondromalacia patellae. *Acta Chir. Scand.* **83**, 347-381.

Keene, DR, Engvall, E, Glanville, RW. (1988). Ultrastructure of type VI collagen in human skin and cartilage suggests an anchoring function for this filamentous network. *J. Cell. Biol.* **107**, 1 995-2 006.

Keller, J, Andreassen, TT, Joyce, F, Knudsen, VE, Joergensen, PH, Lucht, U. (1985). Fixation of osteochondral fractures. Fibrin sealant tested in dogs. *Acta Orthop. Scand.* **56**, 323-6.

Kempson, GE. (1979). Mechanical properties of articular cartilage. In: "Adult articular cartilage". Eds. Freeman, MAR., 333-414. Pitman Medical, Tunbridge Wells.

Kempson, GE. (1980). The mechanical properties of articular cartilage. In: "The joints and synovial fluid". Eds. Sokoloff, L., 140-176. Academic Press, New York.

Kimura, T, Yasui, N, Ohsawa, S, Ono, K. (1984). Chondrocytes embedded in collagen gels maintain cartilage phenotype during long-term cultures. *Clin. Orthop.* **186**, 231-239.

Kincaid, SA, Van Sickle, DC, Wilsman, NJ. (1972). Histochemical evidence of a functional heterogeneity of the chondrocytes of adult canine articular cartilage. *Histochem. J.* **4**, 237-243.

King, JB, Bulstrode, C. (1985). Polylactate-coated carbon fibre in extra-articular reconstruction of the unstable knee. *Clin. Orthop.* **196**, 139-142.

Kirsch, T, von der Mark, K. (1990). Isolation of bovine type X collagen and immunolocalization in growth-plate cartilage. *Biochem. J.* **265**, 453-459.

Kirsch, T, von der Mark, K. (1991a). Type X Collagen synthesis and matrix calcification in cultures of differentiating fetal human chondrocytes (abstract). Fifth International Conference on Cell-mediated calcification and matrix vesicles. Hilton Head, USA.

Kirsch, T, Weseloh, G, von der Mark, K. (1991b). Increased synthesis of type X collagen in osteoarthrotic cartilage - a sign of premature chondrocyte hypertrophy? (abstract). Deutsche Rheuma Liga Meeting, 10a.

Kiviranta, I, Jurvelin, J, Tammi, M, Saamanen, AM, Helminen, HJ. (1987). Weight-bearing controls of glycosaminoglycan concentration and articular cartilage thickness in the knee joints of young Beagle dogs. *Arthritis Rheum.* **30**, 801-809.

Koide, S, Shapiro, F, Glimcher, MJ. (1979). Nature and source of repair tissue in articular cartilage defects. 25th Trans. Orthop. Res. Soc. USA., **4**, 160.

Kon, M, de Visser, AC. (1981). A poly(HEMA) sponge for restoration of articular cartilage defects. *J. Plastic and Reconstr. Surg.* **67**, 289-293.

Kosher, RA, Solursh, M. (1989). Widespread distribution of type II collagen during embryonal chick development. *Dev. Biol.* **131**, 558-566.

Kostenszky, KS, Olah, EH. (1972). Effect of increased functional demand on the glucosaminoglycans (mucopolysaccharide) content of the articular cartilage. *Acta Biol. Hung.* **23**, 75-82.

Kuhn, R, Leppelmann, HJ. (1958). Galakttosamin und Glukosamin im Knorpel und Abhangigkeit vom Lebensalter. *Justus Liebigs Annin. Chem.* **611**, 254-458.

Kuhns, JG. (1964). Nylon membrane arthroplasty of the knee in chronic arthritis. *J. Bone Jt. Surg. [Am]* **46A**, 448-449.

Kulyk, WM, Coelho, CND, Kosher, RA. (1991). Type IX collagen gene expression during limb cartilage differentiation. *Matrix* **11**, 282-288.

Kunz, VJ, Wellmitz, G, Paul, U, Huhrmann, I. (1979). Histoautoradiographische Untersuchungen zur Chondrozyten-proliferation bei der Ausheilung experimenteller Gelenkknorpeldefekte. *Zbl. Allg. Pathol. U. Pathol. Anat.* **123**, 539-549.

Kwan, APL, Dickson, IR, Freemont, AJ, Grant, ME. (1989). Comparative studies of type X collagen expression in normal and rachitic chicken epiphyseal cartilage. *J. Cell. Biol.* **109**, 1 849- 1 856.

Kwan, APL, Cummings, CE, Chapman, JA, Grant, ME. (1991). Macromolecular Organization of Chicken Type X Collagen *In Vitro*. *J. Cell. Biol.* **114**, 597-604.

Kwan, MK, Coutts, RD, Woo, SL-Y, Field, FP. (1989). Morphological and biomechanical evaluations of neocartilage from the repair of full-thickness articular cartilage defects using rib perichondrium autografts: a long-term study. *J Biomechanics* **22**, 921-930.

Langer, F, Gross, AE. (1974). Immunogenicity of allograft articular cartilage. *J. Bone Jt. Surg. [Am]* **56A**, 297.

Langer, F, Czitrom, AA, Pritzker, KP, Gross, AE. (1975). The immunogenicity of fresh and frozen allogenic bone. *J. Bone Jt. Surg. [Am]* **57A**, 216-220.

Langer, F, Gross, AE, West, M, Urovitz, EP. (1978). The immunogenicity of allograft knee joint transplants. *Clin. Orthop.* **132**, 155-162.

Laurence, M, Smith, A. (1968). Effects on union of fractures of homografting isolated chondrocytes. *J. Bone Jt. Surg. [Am]* **50A**, 1073.

Laurin, CA, Levesque, HP, Dussault, R, Labelle, H, Peides, JP. (1978). The abnormal lateral patello-femoral angle. *J. Bone Jt. Surg. [Am]* **60A**, 55.

Lee, DA. (1991). The effect of matrix depletion on articular cartilage. London. (PhD thesis).

Lentz, AJ, Horbett, TA, Hsu, L, Ratner, BD. (1985). Rat peritoneal macrophage adhesion to hydroxyethyl methacrylate-ethyl methacrylate copolymers and hydroxystyrene-styrene copolymers. *J. Biomed. Mater. Res.* **19**, 1101-1115.

Levitt, D, Dorfman, A. (1972). The irreversible inhibition of differentiation of the limb bud mesenchyme by bromodeoxyuridine. *Proc. Natl. Acad. Sci. USA* **69**, 1 253-1 257.

Lexer, E. (1908). Substitution of the whole or half joints from freshly amputated extremities by free plastic operation. *Surg. Gynaecol. Obstet.* **6**, 601-607.

Linden, B. (1977). Osteochondritis dissecans of the femoral condyles: a long-term follow-up study. *J. Bone Jt. Surg. [Am]* **59A**, 769-76.

Linsenmayer, TF, Fitch, JM, Schmid, TM, Zak, NB, Gibney, E, Sanderson, RD, Mayne, R. (1983). Monoclonal antibodies against chicken type V collagen: production, specificity, and use for immunocytochemical localization in embryonic cornea and other organs. *J. Cell. Biol.* **96**, 124-132.

Locht, RC, Gross, AE, Langer, F. (1984). Late Osteochondral Allograft Resurfacing for Tibial Plateau Fractures. *J. Bone Jt. Surg. [Am]* **66A**, 328-335.

Lord, ND, Baker, JR, Christner, JE. (1984). Interaction of proteoglycan with cartilage matrix protein (abstract). *Federation Proc.* **43**, 1 626.

Luyten, FP, Hascall, VC, Nissley, SP, Morales, TI, Reddi, AH. (1988). Insulin-like growth factors maintain steady-state metabolism of proteoglycans in bovine articular cartilage explants. *Arch. Biochem. Biophys.* **267**, 416-425.

Lydon, MJ, Minett, TW, Tighe, BJ. (1985). Cellular interactions with synthetic polymer surfaces in culture. *Biomaterials* **6**, 396-401.

McCall, JB. (1969). Scanning electron microscopy of articular cartilage. *J. Bone Jt. Surg. [Br]* **51B**, 566.

McDermott, AGP, Langer, F, Pritzker, KPH, Gross, AE. (1985). Fresh small fragment osteochondral allografts. *Clin. Orthop.* **197**, 96-102.

McKibbin, B. (1971). Immature joint cartilage and the homograft reaction. *J. Bone Jt. Surg. [Br]* **53B**, 123-135.

McKibbin, B. (1983). New materials in orthopaedics: carbon fibre. In: "Recent advances in Orthopaedics", 179-203. Churchill Livingstone, Edinborough.

McKibbin, B. (1984). Editorial - Carbon fibre as a surgical implant. *J. Bone Jt. Surg. [Br]* **66B**, 157-8.

McKibbin, B. (1990). letter to Editor. *J. Bone Jt. Surg. [Am]* **72A**, 1 430-1 431.

McQuillan, DJ, Handley, CJ, Campbell, MA, Bolis, S, Milway, VE, Herington, AC. (1986). Stimulation of proteoglycan synthesis by serum and insulin-like growth factor 1 in cultured bovine articular cartilage. *Biochem. J.* **240**, 423-430.

Malanin, TI, Wagner, JL, Pitta, JC, Lo, H. (1985). Hypothermic storage and cryopreservation of cartilage. *Clin. Orthop.* **197**, 15-26.

Mankin, HJ. (1962a). Localisation of tritiated thymidine in articular cartilage of rabbits.I.Growth in immature cartilage. *J.Bone.J.Surg* **44A**, 682-688.

Mankin, HJ. (1962b). Localisation of tritiated thymidine in articular cartilage of rabbits.II. Repair in immature cartilage. *J.Bone J. Surg.* **45A**, 689-698.

Mankin, HJ. (1963). Localisation of tritiated thymidine in the articular cartilage of rabbits. III. mature articular cartilage. *J. Bone Jt. Surg. [Am]* **45A**, 529-540.

Mankin, HJ, Doppelt, S, Tomford, WW. (1983). Clinical experience with allograft implantation. *Clin. Orthop.* **174**, 69-86.

Mankin, HJ, Gebhardt, MC, Tomford, WW. (1987). The use of frozen cadaveric allografts in the management of patients with bone tumors of the extremities. *Orthop. Clin. North Am.* **18**, 275-289.

Maroudas, A, Muir, H, Wingham, J. (1969). The correlation of fixed negative charge with glycosaminoglycan content of human articular cartilage. *Biochim. Biophys. Acta* **177**, 492-500.

Maroudas, A. (1980). Physical chemistry of articular cartilage and the intervertebral disc. In: "The joints and synovial fluid". Eds. Sokoloff, L., 239-291. Academic Press, New York.

Maroudas, A, Mizrahi, J, Katz, EP, Wachtel, EJ, Soudry, M. (1986). Physicochemical properties and functional behavior of normal and osteoarthritic human cartilage. In: "Articular Cartilage Biochemistry". Eds. Kuettner, K., Schleyerbach, R, Hascall, V., 311-327. Raven Press, New York.

Mathews, MB. (1965). The Interaction of Collagen and Acid Mucopolysaccharides. A model for connective tissue. *Biochem. J.* **96**, 710-716.

Mayne, R, Irwin, MH. (1986). Collagen types in cartilage. In: "Articular Cartilage Biochemistry". Eds. Kuettner, K., Schleyerbach, R, Hascall, V., 23-38. Raven Press, New York.

Mazhuga, PM, Cherkasov, VV. (1974). Adaptive distribution of specific biosyntheses in a homogeneous population af the articular cartilage chondrocytes. *Z. mikrosk.-anat. Forsch.* **88**, 364-374.

Meachim, G, Collins, DH. (1962). Cell counts of normal and osteoarthritic articular cartilage in relation to the uptake of sulphate (35S) in vitro. *Ann. Rheum. Dis.*. **21**, 45.

Meachim, G, Osborne, GV. (1970). Repair at the femoral articular surface in osteo-arthritis of the hip. *J. Path.* **102**, 1-8.

Meachim, G, Roberts, C. (1971). Repair of the joint surface from subarticular tissues in the rabbit knee. *J. Anat.* **109**, 317-327.

Meachim, G, Stockwell, RA. (1979). The matrix. In: "Adult articular cartilage". Eds. Freeman, MAR., 1-68. Pitman Medical, Tunbridge Wells.

Merchant, AC, Mercer, RL, Jacobsen, RH, Cool, CR. (1974). Roentgenographic analysis of patello-femoral congruence. *J. Bone Jt. Surg. [Am]* **56A**, 1391.

Meyer, K, Linker, A, Davidson, EA, Weissman, B. (1953). The mucopolysaccharides of bovine cornea. *J. Biol. Chem.* 205, 611-616.

Michelacci, Y., Mourao, PAS, Laredo, J, Dietrich, CP. (1979). Chondroitin sulfates and proteoglycans from normal and arthrosic human cartilage. *Connect. Tiss. Res.* **7**, 29-36.

Minns, RJ, Steven, FS. (1977). The collagen fibril organization in human articular cartilage. *J. Anat.* **123**, 437-457.

Minns, RJ, Flynn, M. (1978). Intra-articular implants of filamentous carbon fibre in the experimental animal. *J. Bioeng.* **2**, 279-286.

Minns, RJ, Muckle, DS, Donkin, JE. (1982). The repair of osteochondral defects in osteoarthritic rabbit knees by the use of carbon fibre. *Biomaterials* **3**, 81-86.

Minns, RJ, Denton, MJ, Dunstone, GH, Sunter, JP. (1982). An experimental study of the use of a carbon fibre patch as a hernia prosthesis material. *Biomaterials* **3**, 199-203.

Minns, RJ, Betts, JA, Muckle, DS, Frank, PL, Walker, DI, Strover, A, Hardinge, K., (1987). Carbon fibre arthroplasty of the knee: preliminary clinical experience in a new concept of biological resurfacing. In: "Recent developments in orthopaedic surgery". Eds. Noble, J., Galasko, CSB, 282-289. Manchester University Press, Manchester.

Minoura, M. (1914). Studien ueber Gelenktransplantation (inkl Intermediaerknorpel) in Weichteile. *Frankfurter Zeitschrift fuer Pathologie* **15**, 397-458.

Mitchell, N, Shepard, N. (1976). The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. *J. Bone Jt. Surg.[Am]* **58A**, 230-233.

Mohr, W, Wild, A. (1977). The proliferation of chondrocytes and pannus in adjuvant arthritis. *Virchows Arch. B. Cell. Path.* **25**, 1-16.

Morales, TI, Hascall, VC. (1989). Factors involved in the regulation of proteoglycan metabolism in articular cartilage. *Arthr. Rheum.* **32**, 1197-1201.

Moran, ME, Wunder, JS, Dosch, HM, Salter, RB. (1991). RLA-DQ alpha Restriction fragment length polymorphism analysis to determine tissue of origin of the neocartilage produced in allograft rabbit periosteum joint resurfacing (abstract). Combined Meeting of the Orthopaedic Research Societies of USA, Japan and Canada.

Moskalewski, S, Kawiak, J. (1965). Cartilage formation after homotransplantation of isolated chondrocytes. *Transplantation* **3**, 737-747.

Mourao, PS. (1988). Distribution of chondroitin-4-sulfate and chondroitin-6-sulfate in human articular and growth cartilage. *Arthr. Rheum.* **31**, 1 028-1 033.

Mow, VC, Rosenwasser, M. (1988). Articular Cartilage: Biomechanics. In: "Injury and repair of the musculoskeletal soft tissue". Eds. Woo, S.L-Y., Buckwalter, JA, 427-463. American Academy of Orthopaedic Surgeons, Park Ridge.

Muckle, DS, Minns, RJ. (1990). Biological response to woven carbon fibre pads in the knee. A clinical and experimental study. *J. Bone Jt. Surg. [Br]* **71B**, 60-2.

Mulholland, R. (1974). Lateral hydraulic permeability and morphology of articular cartilage. In: "Normal and osteoarthritis articular cartilage". Eds. Ali, SY, Elves, MW, Leaback, DH, 85-101. Institute of Orthopadics, London.

Naito, K, Hirotani, H. (1991). The chondrogenic potential of free autogenous periosteal grafts for biological resurfacing of half-thickness defects in joint surfaces (abstract). Combined Meeting of the Orthopaedic Research Societies of USA, Japan and Canada, 49.

Nakahara, H, Dennis, JE, Bruder, SP, Haynesworth, SE, Lennon, DP, Caplan, AI. (1991). *In Vitro* Differentiation of Bone and Hypertrophic Cartilage from Periostal-Derived Cells. *Exp. Cell. Res.* **195**, 492-503.

Nambu, T, Schneider, W, Bandi, W, Perren, SM, Mueller, ME. (1988). Contribution of mechanical strain to the pathogenesis of osteochondritis dissecans in the knee. 34th Trans. Orthop. Res. Soc. USA, **14**, 239.

Ninomiya, Y, Olsen, BR. (1984). Synthesis and characterization of cDNA encoding a cartilage-specific short collagen. *Proc. Natl.Acad. Sci. USA.* **81**, 3 014-3 018.

Nogami, H, Ono, Y, Oohira, A. (1989). Bioassay of chondrocyte differentiation by bone morphogenetic protein. *Clin. Orthop.* **258**, 295-299.

Noguchi, T, Oka, M, Kumar, P Kotoura, Y, Hyon, SH, Ikada, Y, Yamamuro, T. (1990). Development of an artificial articular cartilage: evaluation of biocompatibility. In: "Advances in Biomaterials". Eds. Heimke, G, Soltesz, U, Lee, AJC, 351-354. Elsevier, Amsterdam.

O'Donoghue, DH. (1966). Chondral and osteochondral fractures. *J. Trauma.* **6**, 469-81.

O'Driscoll, SW, Keeley, FW, Salter, RB. (1986). The chondrogenic potential of free autogenous periosteal grafts for biological resurfacing of major full-thickness defects in joint surfaces under the influence of continous passive motion. *J. Bone Jt. Surg. [Am]* **68A**, 1017-1035.

O'Driscoll, SW, Salter, RB, Keeley, FW. (1988). Durability of regenerated articular cartilage produced by free autogenous periosteal grafts in major full-thickness defects in joint surfaces under the influence of continuous passive motion: A follow-up report at one year. *J. Bone Jt. Surg. [Am]* **70A**, 595-606.

Oakes, BW, Handley, CJ, Lisner, F, Lowther, DA. (1977). An ultrastructural and biochemical study of high density primary cultures of embryonic chick chondrocytes. *J. Embryol. exp. Morph.* **38**, 239-263.

Oakeshott, R., Farine, I, Pritzker, KPH, Langer, F, Gross, AE. (1988). A clinical and histological analysis of failed fresh osteochondral allograft. *Clin. Orthop.* **233**, 283-294.

Oettinger, H., Pacifici, M. (1990). Type X Collagen Gen Expression is Transiently Up-regulated by Retinoic Acid Treatment in Chick Chondrocyte Cultures. *Experimental Cell Research* **191**, 292-298.

Oike Y, Kimata K, Shinomura T, Nakazawa K, Suzuki S. (1980). Structural analysis of chick embryo cartilage proteoglycan by selective degradation with chondroitin lyases (chondroitinases) and endo-b-D-galactosidase (keratanase). *Biochem. J.* **191**, 193-207.

Oka, M, Noguchi, T, Kumar, P, Ikeuchi, K, Yamamuro, T, Hyon, SH, Ikada, Y. (1990). Development of an artificial articular cartilage. *Clin. Materials* **6**, 361-381.

Osborn, KD, Trippel, SB, Mankin, HJ. (1989). Growth factor stimulation of adult articular cartilage. *J. Orthop. Res.* **7**, 35-42.

Ottolenghi, CE. (1966). Massive osteoarticular bone grafts. *J. Bone Jt. Surg.* **48B**, 646-659.

Ottolenghi, CE. (1972). Massive osteo and osteo articular bone grafts: techniques and results in 62 cases. *Clin. Orthop.* **87**, 156-164.

Outerbridge, R. (1961). The Etiology of Chondromalacia Patellae. *J. Bone Jt. Surg.* **43B**, 752-757.

Page, M, Hogg, J, Ashhurst, DE. (1986). The effects of mechanical stability on the macromolecules of the connective tissue matrices produced during fracture healing. I. The collagens. *Histochem. J.* **18**, 251-256.

Paget, J. (1853). Healing on injuries in various tissues. *Lect. Surg. Pathol.* 262.

Paget, J. (1870). On the Production of Some of the Loose Bodies in Joints. *Saint Bartholomew's Hospital Reports.***6**, 1.

Pal, S., Tang, L, Choi, H, Habermann, E, Rosenberg, L, Roughley, P, Poole, AR. (1981). Structural changes during development in bovine fetal epiphyseal cartilage. *Coll. Relat. Res.* **1**, 151-176.

Pap, K, Krompecher, S. (1961). Arthroplasty of the knee: experimental and clinical experiences. *J. Bone Jt. Surg. [Am]* **43A**, 523-537.

Parrish, FF. (1966). Treatment of bone tumours by total excision and replacement with massive autologous and heterologous grafts. *J. Bone Jt. Surg. [Am]* **48A**, 968-990.

Parrish, FF. (1973). Allograft replacement of all or part of the end of a long bone following excision of a tumour. *J. Bone Jt. Surg. [Am]* **55A**, 1-22.

Parsons, JR, Bhayani, S, Alexander, H, Weiss, AB. (1985). Carbon fibre debris within the synovial joint. *Clin. Orthop.* **196**, 69-76.

Paukkonen, K, Helminen, HJ. (1987). Rough endoplasmic reticulum and fine intracytoplasmic filaments in articular cartilage chondrocytes of young rabbits; a

stereological morphometric study using transmission electron microscopy. *J. Anat.* **152,** 47.

Peppas, NA. (1977). Hydrogels for synthetic articular cartilage applications. *Soc. of Plastic Engineers Tech. Papers (NATEC)*, 62-63.

Peppas, NA. (1986). Hydrogels in Medicine and Pharmacy, 1 Fundamentals. CRC Press, Boca Raton

Peppas, NA, (1987a). Hydrogels in Medicine and Pharmacy, 2 Polymers. CRC Press, Boca Raton.

Peppas, NA. (1987b). Hydrogels in Medicine and Pharmacy, 3 Properties and Applications. CRC Press, Boca Raton.

Pledger, WJ, Stiles, CD, Antoniades, HN, Scher, CD. (1977). Induction of DNA synthesis in BALB/c 3T3 cells by serum components: Reevaluation of the commitment process. *Proc. Natl. Acad. Sci. USA* **71**, 4 481 - 4 485.

Pledger, WJ, Stiles, CD, Antoniades, HN, Scher, CD. (1978). An ordered sequence of events is required before BALB/c-3T3 cells become committed to DNA synthesis. *Proc. Natl. Acad. Sci. USA* **75**, 2 839-2 843.

Poole, AR. (1986). Review Article. Proteoglycans in health and disease: structures and functions. *Biochem. J.* **236**, 1-14.

Poole, AR, Pidoux, I. (1989). Immunoelectron microscopic studies of type X collagen in endochondral ossification. *J. Cell. Biol.* **109**, 2 547 - 2 554.

Poole, CA, Ayad, S, Schofield, JR. (1988). Chondrons from articular cartilage: I. Immunolocalization of type VI collagen in the pericellular capsule of isolated canine tibial chondrons. *J. Cell. Sci.* **90**, 635-643.

Porter, BB, Lance, EM. (1974). Limb and joint transplantation: A review of research and clinical experience. *Clin. Orthop.* **104**, 249-274.

Power, RA, Wood, DJ, Tomford, WW, Mankin, HJ. (1991). Revision osteoarticular allograft transplantation in weight-bearing joints. *J. Bone Jt. Surg. [Br]* **73B**, 595-599.

Prolo, DJ, Rodrigo, JJ. (1985). Contemporary bone graft physiology and surgery. *Clin. Orthop.* **200**, 322-342.

Radin, EL, Rose, RM, Paul, IP. (1972). Role of mechanical factors in pathogenesis of primary osteoarthritis. *Lancet* **i**, 519-522.

Radin, EL, Simon, SR, Rose, RM, Paul, IL. (1979). Practical Biomechanics for the orthopaedic surgeon, 112-124. John Wiley & Sons,
New York.

Radin, EL. (1985). Anterior Knee Pain: the Need for a Specific Diagnosis. *Orthop Rev* **14**, 128-134.

Radin, EL, Rose, RM. (1986). Role of subchondral bone in the initiation and progression of cartilage damage. *Clin. Orthop.* **213**, 34-40.

Ratcliffe, A, Fryer, PR, Hardingham, TE. (1984). The distribution of aggregating proteoglycans in articular cartilage. *J. Histochem. Cytochem.* **32**, 193-201.

Reddi, AH, Huggins, C. (1972). Biochemical sequences in the transformation of normal fibroblasts in adolescent rats. *Proc. Natl. Acad. Sci. USA* **69**, 1 601-1 605.

Reddi, AH. (1983). Extracellular bone matrix dependent local induction of cartilage and bone. *J. Rheumatol. (suppl 11)* **10** , 67-69.

Reddi, AH. (1985). Role of subchondral bone matrix factors in the repair of articular cartilage. In: "Degenerative Joints". Eds. Verbruggen, G, Veys, EM, 271-274. Excerpta Medica, Amsterdam.

Reddi, AH, Wientroub, S, Muthukunaran, N. (1987). Biological principles of bone induction. *Orthop. Clin. North Am.* **18**, 207-212.

Redler, I. (1974). A scanning electron microscopic study of human normal and osteoarthritic articular cartilage. *Clin. Orthop.* **103**, 262-8.

Remington, MC, Bashey, RI, Brighton, CT, Jimenez, SA. (1983). Biosynthesis of a Low Weight Collagen by Rabbit Growth Plate Cartilage Organ Culture. *Coll. Rel. Res.* **3**, 271-278.

Reynolds, ES. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell. Biol.* **17**, 208-212.

Rieger, H. (1920). Zur Pathogenese von Gelenkmausen. *M.M.W.* **67**, 719.

Roberts, N, Hughes, R. (1950). Osteochondritis Dissecans of the Elbow Joint. A clinical study. *J. Bone Jt. Surg. [Br]* **32B**, 348-360.

Rodrigo, JJ, Block, N, Thompson, EC. (1978). Joint Transplantation. *Vet. Clin. North Am.* **8**, 523-538.

Rodrigo, JJ, Sakovich, L, Travis, C, Smith, G. (1978). Osteocartilaginous allograft as compared with autografts in the treatment of knee joint osteocartilaginous defects in dogs. *Clin. Orthop.* **134**, 342-349.

Romanic, AM, Adachi, E, Kadler, KE, Hojima, Y, Prockop, DJ. (1991). Copolymerization of pNcollagen III and Collagen I. *J. Biol. Chem.* **266**, 12 703 - 12 709.

Ronziere, MC, Ricard-Blum, S, Tiollier, J, Hartmann, DJ, Garrone, R, Herbage, D. (1990). Comparative analysis of collagens solubilized from human foetal, and normal and osteoarthritic adult articular cartilage, with emphasis on type VI collagen. *Biochim. Biophys. Acta.* **1038**, 222-230.

Rosenberg, L. (1971). Chemical basis for biological use of safranin-O in the study of articular cartilage. *J. Bone Jt. Surg.* **53A**, 69-82.

Rosenberg, L, Hellmann, W, Kleinschmidt, AK. (1975). Electron Microscopic Studies of Proteoglycan Aggregates from Bovine Articular Cartilage. *J. Biol. Chem.* **250**, 1 877 - 1 883.

Rosenberg, NJ. (1964). Osteochondral fractures of the lateral femoral condyle. *J. Bone Jt. Surg.* **46A**, 1013.

Rothwell, AG, Bentley, G. (1973). Chondrocyte multiplication in osteoarthritic articular cartilage. *J Bone Joint Surg* **55B**, 588-594.

Roughley, PJ, White, RJ. (1980). Age-related Changes in the Structure of the Proteoglycan Subunits from Human Articular Cartilage. *J. Biol. Chem.* **255**, 217-224.

Roy, S, Meachim, G. (1968). Chondrocyte ultrastructure in adult human articular cartilage. *Ann. Rheum. Dis.* **27**, 544-557.

Rubak, JM. (1982). Reconstruction of articular cartilage defects with free periosteal grafts. *Acta Orthop. Scand.* **53**, 175-180.

Rubak, JM, Poussa, M, Ritsila, V. (1982a). Chondrogenesis in repair of articular cartilage defects by free periosteal grafts in rabbits. *Acta Orthop. Scand.* **53**, 181-186.

Rubak, JM, Poussa, M, Ritsila. (1982b). Effects of joint motion on the repair of articular cartilage with free periosteal grafts. *Acta Orthop. Scand.* **53**, 187-191.

Ruoslahti, E. (1988). Structure and Biology of Proteoglycans. *Ann. Rev. Cell. Biol.* **4**, 229-55.

Rushton, N, Dandy, DJ, Naylor, CPE. (1983). Arthroscopic and histological findings after replacement of the anterior cruciate ligament with carbon fibre. *J. Bone Jt. Surg. [Br]* **65**, 308-309.

Rushton, N, Rae, T. (1984). The intra-articular response to particulate carbon fibre reinforced high density polyethylene and its constituents: an experimental study in mice. *Biomaterials* **5**, 352-356.

Ryan, MC, Sandell, LJ. (1990). Differential expression of a cysteine-rich domain in the amino-terminal propeptide of type II (cartilage) procollagen by alternative splicing of mRNA. *J. Biol. Chem.* **265**, 10 334- 10 339.

Salter, RB, Simmonds, DF, Malcolm, BW, Rumble, EJ, MacMichael, D. (1975). The effects of continuous passive motion on the healing of articular cartilage defects: an experimental investigation in rabbits. *J. Bone Jt. Surg. [Am]* **57A**, 750-751.

Sandberg, M, Tamminen, M, Hirvonen, Vuorio, E, Pihlajaniemi, T. (1989a). Expression of mRNAs coding for the alfa-1-chain of type XIII collagen in human fetal tissues: comparison with expression of mRNAs for collagen types I, II and III. *J. Cell. Biol.* **109**, 1 371 - 1 379.

Sandberg, M, Maekelae, JK, Multimaeki, P, Vuorio, T, Vuorio, E. (1989b). Construction of a human pro-alfa-1-(III) collagen cDNA clone and localization of type III collagen expression in human fetal tissues. *Matrix* **9**, 82-91.

Sandell, LJ, Boyd, CD (1990). Conserved and divergent sequence and functional elements within collagen genes. In: "Extracellular Matrix Genes" Eds. Sandell, LJ, Boyd, CD, 1-56. Academic Press, New York.

Sandell, LJ, Morris, N, Robbins, JR, Goldring, MB. (1991). Alternatively spliced Type II Procollagen mRNAs Define Distinct Populations of Cells during Vertebral Development: Differential Expression of the Amino-Propeptide. *J. Cell. Biol.* **114**, 1307-1319.

Schachar, NS, McGann, LE. (1986). Investigations of low-temperature storage of articular cartilage for transplantation. *Clin. Orthop.* **208**, 146-150.

Schenk, RK, Eggli, PS, Hunziker, EB. (1986). Articular Cartilage Morphology. In: "Articular Cartilage Biochemistry". Eds. Kuettner, K, Schleyerbach, R, Hascall, VC, 3-22. Raven Press, New York.

Schlag, G, Redl, H. (1988). Fibrin sealant in orthopaedic surgery. *Clin. Orthop.* **227**, 269-285.

Schmid, TM, Linsenmayer, TF. (1985). Immunohistochemical localization of short chain cartilage collagen (type X) in avian tissues. *J. Cell. Biol.* **100**, 598-605.

Schmid, TM, Mayne, R, Jeffrey, JJ, Linsenmayer, TF. (1986). Type X Collagen Contains Two Cleavage Sites for a Vertebrate Collagenase. *J. Biol. Chem.* **261**, 4 184-4 189.

Schmidt, G, Robenek, H, Harrach, H, Gloessl, J, Nolte, V, Hoermann, H, Richter, H, Kresse, H. (1987). Interaction of small dermatan sulfate proteoglycan from fibroblasts with fibronectin. *J. Cell. Biol.* **104**, 1 683 - 1 691.

Schor, SL. (1980). Cell proliferation and migration on collagen substrata in vitro. *J. Cell. Sci.* **41**, 159-175.

Scott, JE, Dorling, J. (1965). Differential staining of acid glycosaminoglycans (mucopolysaccharides) by Alcian blue in salt solutions. *Histochemie* **5**, 221-233.

Scully, SP, DeLong, PA, Hascall, VC, Bolander, ME. (1991). Modulation of articular chondrocyte mitogenesis by the extracellular collagen matrix. Combined Meeting of the Orthopaedic Research Societies of USA, Japan and Canada, 52.

Seno, N, Meyer, K, Anderson, B, Hoffman, P. (1965). Variations in keratosulfates. *J. Biol. Chem.* **240**, 1 005-1 010.

Simunek, Z, Muir, H. (1972a). Changes in the protein-polysaccharides of pig articular cartilage during prenatal life, development and old age. *Biochem. J.* **126**, 515-523.

Simunek, Z, Muir, H. (1972b). Proteglycans of the knee-joint cartilage of young normal and lame pigs. *J. Biol. Chem.* **130**, 181-187.

Slater Jr, RR, Caterson, B, Lachiewicz, P, Bayliss, MT. (1992). The occurrence of 3B3(-) and 7D4 epitopes as markers of arthritis in humans.38th Trans.Orthop. Res. Soc. USA, **17**, 277.

Slome, D, Reeves, B. (1966). Experimental homotransplantation of the knee joint. *Lancet* **2**, 205.

Smillie, IS. (1957). Treatment of osteochondritis dissecans. *J. Bone Jt. Surg. [Br]* **39B**, 248-60.

Smith, AU. (1965). Survival of frozen chondrocytes isolated from cartilage of adult mammals. *Nature* **205**, 782-4.

Smith JC, Watt FM. (1985). Biochemical specificity of Xenopus notochord. *Differentiation* **29**, 109-115.

Smith Jr, GN, Williams, JM, Brandt, KD. (1985). Interaction of proteoglycans with the pericellular (1a2a3a) collagens of cartilage. *J. Biol. Chem.* **260**, 10 761 - 10 767.

Sokoloff, L. (1985). Factors influencing cell division in cartilage. In: "Degenerative joints" Eds. G. Verbruggen, Veys, EM, 47-53. Elsevier Science Publishers, Amsterdam.

Speer, DP, Dahners, L. (1979a). The collagenous architecture of articular cartilage: correlation of scanning electron microscopy and polarized light microscopy observations. *Clin. Orthop.* **139**, 267-75.

Speer, DP, Chvapil, M, Volz, RG, Holmes, MD. (1979b). Enhancement of healing in osteochondral defects by collagen sponge implants. *Clin. Orthop.* **144**, 326-335.

Spurr, A. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**, 31-43.

Stewart, J, Kiuchi, T, Langer, F, Halloran, P, Gross, AE. (1983). The effect of immunosuppression on vascularized bone grafts. *Orthop. Trans.* **6**, 492.

Stockwell, RA, Scott, JE. (1965). Observations on the acid glycosaminoglycan content of the matrix of ageing cartilage. *Ann. Rheum. Dis.* **24**, 341-350.

Stockwell, RA. (1967a). The cell density of human articular cartilage and costal cartilage. *J. Anat.* **101**, 753-763.

Stockwell, RA, Scott, JE. (1967b). Distribution of acid glycosaminoglycans in human articular cartilage. *Nature* **215**, 1 376-1 378.

Stockwell, RA. (1975). Stain distribution with Alcian blue in articular cartilage. *Ann. Rheum. Dis. (Suppl 2)* **34** , 17-18.

Stockwell, RA. (1979a). Biology of cartilage cells, 330. Cambridge University Press, Cambridge.

Stockwell, RA., Meachim, G. (1979b). The chondrocytes. In: "Adult articular cartilage". Eds. Freeman, MAR, 69-144. Pitman Medical, Tunbridge Wells.

Stockwell, RA. (1989). Articular cartilage keratan sulphate: maturation, ageing, biomechanical and scale effects. In: "Keratan sulphate: Chemistry, Biology, Chemical Pathology". Eds. Greiling, H, Scott, JE, 135-147. The Biochemical Society, London.

Storm, SC, Michalopoulos, G. (1982). Collagen as a substrate for cell growth and differentiation. In: "Methods in Enzymology". Eds. Cunningham, LW, Frederiksen, DW, 544-555. Academic Press, New York.

Stougaard, J. (1961). The Hereditary Factor in Osteochondritis Dissecans. *J. Bone Jt. Surg. [Br]* **43B**, 256.

Stoy, VA, Kliment, CK. (1990). Hydrogels: Specialty Plastics for Biomedical, Pharmaceutical and Industrial Applications, 438. Technomic Publishing AG., Basel.

Stryer, L. (1975). Biochemistry, 185-204. W H Freeman and Co, San Francisco.

Sussman, MD, Ogle, RC, Balian, G. (1984). Biosynthesis and processing of collagens in different cartilaginous tissues. *J. Orthop. Res.* **2**, 134-142.

Swann, DA, Constable, IJ, Harper, E. (1972). Vitreous structure. III. Composition of bovine vitreous collagen. *Invest. Ophthalmol.* **11**, 735-738.

Sweet, MBE, Thonar, EJ-MA, Marsh, J. (1979). Age-related changes in proteoglycan structure. *Arch. Biochem. Biophys.* **198**, 439-448.

Tammi, M, Saamanen, AM, Jauhiainen, A, Malminen, O, Kiviranta, I, Helminen, H. (1983). Proteoglycan alterations in rabbit knee articular cartilage following physical exercise and immobilization. *Connect. Tissue Res.* **11**, 45-55.

Tanaka, H, Inoue, M, Suzuki, M, Nojima, M. (1980). A study on experimental homocartilage transplantation. *Arch. Orthop. Traumat. Surg.* **96**, 165-169.

Tanzawa, H, Nagaoka, S, Suzuki, J, Kobayashi, S, Masabuchi, Y, Kikuchi, T. (1980). Cell adhesion and growth on the surface of synthetic hydrogels. In: "Biomedical Polymers (Polymeric materials and pharmaceuticals for biomedical use). Eds. Goldberg, EP, Nakajima, A, 189-211. Academic Press, New York.

Tayton, K., Phillips, G, Ralis, ZA. (1982). Longterm effects of carbon fibre on soft tissues. *J Bone Joint Surg [Br]* **64B**,

Thomas, MT, Boot-Handford, RP, Grant, ME. (1990). Modulation of type X collagen gene expression by calcium-b-glycerophosphate and levamisole: implications for a possible role for type X collagen in enchondral bone formation. *J. Cell. Sci.* **95**, 639-648.

Thomas, NP. (1986). A comparative analysis of synthetic anterior cruciate ligaments. Presentation for Sir Herbert Seddon Prize and Medal, Stanmore.

Thonar, EJ-MA, Sweet, MBE. (1981). Maturation-related changes in proteoglycans of fetal articular cartilage. *Arch. Biochem. Biophys.* **208**, 535-547.

Thorogood, PV, Hinchliffe, JR. (1975). An analysis of the condensation process during chondrogenesis in the embryonic chick hind limbs. *J. Embryol. exp. Morph.* **33**, 581-600.

Timpl, R, Glanville, RW. (1981). The Aminopropeptide of Collagen. *Clin. Orthop.* **158**, 224-242.

Tomford, WW, Mankin HJ. (1983). Investigational approaches to articular cartilage preservation. *Clin. Orthop.* **174**, 22-27.

Tomford, WW, Duff, GP, Mankin, HJ. (1985). Experimental freeze-preservation of chondrocytes. *Clin. Orthop.* **197**, 11-14.

Twyman, RS, Desai, K, Aichroth, PM. (1991). Osteochondritis dissecans of the knee. *J. Bone Jt. Surg. [Br]* **73B**, 461-4.

Ulreich, JB, Paicius, RM, Chvapil, M, Speer, DP. (1985). Chondronectin treated collagen sponge enhances osteochondral defect resurfacing. *J. Cell. Biol.* **101**, 96a.

Ulreich, JB, Holubec, H, Chvapil, M, Speer, DP. (1987). Collagen sponge - a three dimensional matrix for in vitro chondrocyte culture. In: "Development and Diseases of cartilage and bone matrix". Eds. Sen, A, Thornhill, T, 107-113. Alan R. Liss, Lake Tahoe.

Urist, MR. (1965). Bone: formation by auto-induction. *Science* **150**, 893-899.

Urist, MR, Silverman, BF, Buring, K, Dubac, FL, Rosenberg, JM. (1967). The bone induction principle. *Clin. Orthop.* **53**, 243-283.

Urist, MR, Hay, H, Dubac, F, Buring, K. (1969). Osteogenetic competence. *Clin. Orthop.* **64**, 194-219.

Urist, MR. (1983). The origin of cartilage: Investigations in quest of chondrogenic DNA. In: "Cartilage. Development, Differentiation, and Growth". Eds. Hall, BK, 1-85. Academic Press, New York.

van der Rest, M, Mayne, R, Ninomiya, Y, Seidah, NG, Chretien, M, Olsen, BR. (1985). The structure of collagen type IX. *J. Biol. Chem.* **260**, 220-225.

van der Rest, M, Mayne, R. (1988). Type IX collagen proteoglycan from cartilage is covalently cross-linked to type II collagen. *J. Biol. Chem.* **263**, 1 615-1 618.

van der Valk, P, van Pelt, AWJ, Busscher, HJ, de Jong, HP, Wildevuur, CRH, Arends, J. (1983). Interaction of fibroblasts and polymer surfaces: relationship between surface free energy and fibroblast spreading. *J. Biomed. Mater. Res.* **17**, 807-817.

Vaughan, L, Winterhalter, KH, Bruckner, P. (1985). Proteoglycan Lt from chicken embryo sternum identified as type IX collagen. *J. Biol. Chem.* **260**, 4 758-4 763.

Venn, G, Mason, RM. (1985). Absence of keratan sulphate from skeletal tissues of mouse and rat. *Biochem. J.* **228**, 443-450.

Venn, MF. (1978). Variation of chemical components with age in human femoral head cartilage. *Ann. Rheum. Dis.* **37**, 168-174.

Vetter, U, Helbing, G, Heit, W, Pirsig, W, Sterzig, K, Heinze, E. (1985). Clonal proliferation and cell density of chondrocytes isolated from human fetal epiphyseal, human adult articular and nasal septum cartilage. Influence of hormones and growth factors. *Growth* **49**, 229-245.

Vignon, E, Hartman, JD, Vignon, E, Moyen, B, Arlot, M, Ville, G. (1984). Cartilage destruction in experimentally induced osteoarthritis. *J. Rheumatol.* **11**, 202-207.

Volkov, MV. (1970). Allotransplantation of joints. *J. Bone Jt. Surg. [Br]* **52-B**, 49.

Volkov, MV, Imamaliev, AS. (1976). Use of allogenous articular bone implants as substitutes for autotransplants in adult patients. *Clin. Orthop.* **114**, 192-202.

von der Mark, K, Nerlich, A, Weseloh, G, Stoess, H, Mueller, PK, von der Mark, H, Swoboda, B. (1991). Immunohistochemical identification of collagen types I, II, III, VI and IX in osteoarthrotic articular cartilage (abstract). Deutsche Rheuma Liga Meeting, 10.

Wakitani, S, Kimura, T, Hirooka, A, Ochi, T, Yoneda, M, Yasui, N, Owaki, H, Ono, K. (1989). Repair of rabbit articular surfaces with allograft chondrocytes embedded in collagen gel. *J Bone Joint Surg [Br]* **71B**, 74-80.

Walker, G, Fischer, M, Thompson Jr, RC, Oegema Jr, TR. (1991). The expression of type X collagen in osteoarthritis. 37th Trans. Orthop. Res. Soc.USA, **16**, 340.

Weiss, C, Rosenberg, L, Helfet, AJ. (1968). An ultrastructural study of normal young adult human articular cartilage. *J. Bone Jt. Surg. [Am]* **50A**, 663-674.

Whipple, RR, Gibbs, MC, Lai, WM, Mow, VC, Mak, AF, Wirth, CR. (1985). Biphasic properties of repaired cartilage at the articular surface. *Trans. Orthop. Res. Soc. USA* **10**, 340.

Wichterle, O, Lim, D. (1960). Hydrophilic gels for biological use. *Nature* **185**, 117.

Wiedemann, H, Paulsson, M, Timpl, R, Engel, J, Heinegard, D. (1984). Domain structure of cartilage proteoglycans revealed by rotary shadowing of intact and fragmented molecules. *Biochem. J.* **224**, 331-333.

Williams, DF. (1992). Mechanisms of biodegradation of implantable polymers. *Clin. Mat.* **10**, 9-12.

Williams, LF, McCullagh, KG, Silver, IA. (1984). The distribution of Types I and III collagen and fibronectin in the healing equine tendon. *Conn. Tiss. Res.* **12**, 211-227.

Wilson, JN. (1967). A Diagnostic Sign in Osteochondritis Dissecans of the Knee. *J. Bone Jt. Surg. [Am]* **49A**, 477.

Woo, SL-Y., Kwan, MK, Lee, TQ, Field, FP, Kleiner, JB, Coutts, RD. (1987). Perichondrial autograft for articular cartilage. Shear modulus of neocartilage studied in rabbits. *Acta Orthop. Scand.* **58**, 510-515.

Wood, DJ, Awbrey, BJ, Tomford, WW, Mankin, HJ. (1989). Functional results of osteoarticular allograft reconstruction of the knee (abstract). British Orthopaedic Association Autumn Meeting, 66.

Worrell, RV. (1986). Resurfacing of the patella in young patients. *Orthop. Clin. of North Am.* **17**, 303-309.

Wrede, L. (1909). Experimente zur Frage der Gelenktransplantation. *Verhandlungen der Deutschen Gesellschaft fuer Chirurgie* **1909**, 238.

Wu, CH, Donovan, CB, Wu, GY. (1986). Evidence for pretranslotional regulation of collagen synthesis by procollagen propeptides. *J. Biol. Chem.* **261**, 10 482-10 484.

Yablon, IG, Brandt, KD, Delellis, Covall, D. (1977). The destruction of joint homografts - an experimental study. *Arthr. Rheum.* **20**, 1 526-1 537.

Yamaguchi, N, Benya, PD, van der Rest, M, Ninomiya, Y. (1989). The cloning and sequencing of a1(VIII) collagen cDNAs demonstrate that type VIII collagen is a short chain collagen and contains triple-helical domains similar to those of type X collagen. *J. Biol. Chem.* **264**, 16 022 - 16 029.

Yamaguchi, Y, Ruoslahti, E. (1988). Expression of human proteoglycan in chinese hamster ovary cells inhibits cell proliferation. *Nature (London)* **336**, 244-246.

Yasui, N, Osawa, S, Ochi, T, Nakashima, H, Ono, K. (1982). Primary culture of chondrocytes embedded in collagen gels. *Exp. Cell. Biol.* **50**, 92-100.

Zanetti, M, Ratcliffe, A, Watt, FM. (1985). Two subpopulations of differentiated chondrocytes identified with a monoclonal antibody to keratan sulphate. *J. Cell. Biol.* **101**, 53-59.

Zarnett, R, Salter, RB. (1989). Periosteal Neochondrogenesis for Biologically Resurfacing Joints: Its cellular origin. *C.J.S.* **32**, 171-174.

Zilch, H, Wolff, R. (1987). Fibrinekleber und Knochenneubildung. *Z. Orthop.* **125**, 214-218.

Zinman, C, Reiss, ND. (1982). Osteochondritis dissecans of the talus: Use of the high resolution computed tomography scanner. *Acta Orthop. Scand.* **53**, 697-700.

Zirn, JR, Schurman, DJ, Lane Smith, R. (1984). Keratan sulphate content and articular cartilage maturation during postnatal rabbit growth. *Biochem. J.* **2**, 143-150.

Zukor, DJ, Paitich, B, Oakeshott, RD, Brooks, PJ, Mc Auley, JP, Rudan, JF, Langer, F, Gross, AE. (1989a). Reconstruction of Post-Traumatic Articular Surface Defects Using Fresh Small-Fragment Osteochondral Allografts. In: "Bone Transplantation". Eds. Aebi, M, Regazzoni, P, 293-305. Springer-Verlag, Berlin.

Zukor, DJ, Oakeshott, RD, Gross, AE. (1989b). Osteochondral Allograft Reconstruction of the Knee. Part 2: Experience with succesful and failed fresh osteochondral allografts. *Am. J. Knee Surg.* **2**, 182-191.

References added:

Arnoldi, CC. (1991) Patellar pain. *Acta Orthop. Scand. (Suppl.)* **62**, 1-29.

Darracott, J, Vernon-Roberts, B. (1971). The bony changes in chondromalacia patellae. *Rheum. and Phys. Med.* **11**, 175-179.

DeHaven, KE, Dolan, WA, Mayer, PJ,. (1979). Chondromalacia patellae in Athletes. Clinical Presentation and Conservative Management. *Am. J. Sports Med.* **7**, 5-11.

Fulkerson, JP, Shea, KP. (1990). Current Concepts Review. Disorders of Patellofemoral Alignment. *J. Bone Jt. Surg.[Am]* **72A**, 1 424-1 429.

Jackson, RW. (1976). AAOS Instructional Course Lecture 25:36.

Maquet, F. (1963). Un traitement biomechanique de l'arthrose femoro-patellaire. *Rev. Rhum. et Osteoartic.* **30**, 779-783.

Schutzer, SF, Ramsby, GR, Fulkerson, JP. (1986). Computed Tomographic Classification of Patellofemoral Pain Patients. *Orthop. Clin. North America.* **17**, 235-248.

Shellock, FG, Mink, JH, Fix, JH. (1988). Patellofemoral joint: Kinematic MR Imaging to Assess Tracking Abnormalities. *Radiology.* **168**, 551-553.

Sisk, DL. (1992). Chondromalacia of Patella. In: "Campbell's Operative Orthopaedics".

Ed. Crenshaw, AH, 1707-12. Mosby Publishers, St. Louis.

Stougard, J. (1975). Chondromalacia of the patella - incidence, macroscopical and radiological findings at autopsy. *Acta Orthop. Scand.* **46**, 809-822.

West, FE. (1962). End Results of Patellectomy. *J Bone Jt. Surg. [Am]* **44A**, 1 089-1 108.