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A NOVEL METHOD OF ARTICULAR CARTILAGE REPAIR

by

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Abstract

Articular cartilage repair of post-traumatic articular cartilage defects and well-defined articular cartilage pathology is challenging in clinical practice and has been the focus of investigations for many years. In the present thesis a newly developed polymer system, based on poly-ethyl-methacrylate [PEMA] polymer and tetra-hydro-furfuryl methacrylate [THFMA] monomer has been exploited for the repair of large, full-thickness articular cartilage defects, created in a weight-bearing surface in the rabbit knee joint. The method of implantation is simple and easily reproducible and can be performed in one stage with open arthrotomy or arthroscopy in clinical applications.

Intravenous administration of the monomer did not elicit significant cardiorespiratory side effects. The repair tissue in defects treated with PEMA/THFMA was compared to control defects that healed 'naturally'. Macroscopic and histological/histochemical evaluation using the newly developed Articular Cartilage Repair Scoring System, immunohistochemistry, electron microscopy, image analysis as well as biochemical analysis were used for the characterisation of the repair tissue. The results demonstrated that the PEMA/THFMA polymer enhanced significantly the quality of repair up to 1 year post-operatively. The repair tissue contained numerous chondrocytes producing large amounts of proteoglycans and collagen type II, and it was completely bonded to the adjacent normal articular cartilage in the vast majority of the specimens.

The enhancing effect of PEMA/THFMA in articular cartilage defects was also demonstrated in three age groups of rabbits at 6 weeks, thus increasing the potential clinical applications of the polymer. Furthermore, PEMA/THFMA was compared to the conventional bone cement PMMA/MMA. At 6 weeks post-implantation PEMA/THFMA produced significantly superior repair tissue, compared to PMMA/MMA, confirming the importance of the properties of the new polymer. Finally, PEMA/THFMA was exploited as a potential drug delivery system in vivo by loading human growth hormone in the polymer. It was shown that the loaded polymer repaired the defects with a proliferative type of tissue, resembling immature articular cartilage.

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CHAPTER I

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COMPOSITION OF ARTICULAR CARTILAGE

Articular cartilage varies in thickness, cell density, matrix composition, and mechanical properties within the same joint, amongst joints, and amongst species; however, in all synovial joints it consists of the same general structure and performs the same functions (Athanasiou et al 1991). On wet weight basis the water content may lie in the range of 65 to 80%, collagen in the range of 15 to 25%, and proteoglycans in the range of 3 to 10% (Maroudas, 1979). The cells contribute little to the volume of the tissue, about 1% in human articular cartilage (Buckwalter et al 1988).

Although cells and matrix are structurally separate, they are functionally very interdependent. Chondrocyte activity is necessary for the synthesis and physiological degradation of matrix components, while the matrix plays an important part in maintaining the homeostasis of the chondrocyte's environment (Mitchell and Shepard, 1989). Despite its unimpressive appearance and low level of metabolic activity, detailed study of the morphology and biology of adult articular cartilage shows that it has an elaborate, highly-ordered structure and that a variety of complex interactions between the chondrocytes and the matrix actively maintain the tissue (Buckwalter and Mankin, 1997).

CHONDROCYTES

Cartilage first forms from undifferentiated mesenchymal cells that cluster together and synthesize cartilage collagens, proteoglycans, and non-collagenous proteins. The tissue becomes recognizable as cartilage under light microscopy when an accumulation of matrix separates the cells and they assume a spherical shape (Buckwalter and Mankin, 1997). It was previously believed that chondrocytes in adult cartilage cannot undergo division; however, cell division has been demonstrated (Rothwell and Bentley, 1973, Bentley 1985). The internal structure of a chondrocyte is typical of cells active in producing and secreting proteins. Under the electron microscope they reveal prominent nucleoli, and a basophilic cytoplasm with abundant rough endoplasmic reticulum and prominent Golgi apparatus.

All of the major components of the matrix are synthesized and secreted by chondrocytes. The same cell can sythesize the precursors of collagen and the proteoglycans. The chondrocytes also secrete metabolic enzymes which play a role in the slow turn-over of the matrix components. Individual chondrocytes are surprisingly active metabolically. They have a glycolytic rate per cell similar to that of cells in vascularized tissues but the total metabolic activity of the tissue is low because of the low cell density. To produce a tissue that can provide normal function of the synovial joint, the chondocytes must first synthesize appropriate types and amounts of macromolecules and then assemble and organize them into a highly ordered framework. Maintenance of the articular surface requires turnover of the matrix macromolecules and probably alterations in the macromolecular framework of the matrix in response to the use of the joint. To accomplish these activities, chondrocytes must sense changes in the composition of the matrix as well as changes in the demands placed on the articular surface; the cells must then respond by synthesizing appropriate types and amounts of macromolecules (Buckwalter and Mankin, 1997).

During skeletal growth the chondocytes produce new tissue to expand and remodel the articular surface. The cells proliferate rapidly and synthesize large volumes of matrix. The cell density is high and the cells reach their highest level of metabolic activity (Buckwalter and Mankin, 1997). After completion of skeletal growth, most chondrocytes probably never divide and the volume of the cartilage tissue does not change substantially. The synthetic activity of the cells, however, continues suggesting ongoing internal remodeling of the macromolecular framework of the matrix. Enzymes produced by chondrocytes presumably are responsible for degradation of the matrix macromolecules, and chondrocytes probably respond to the presence of fragmented matrix molecules by increasing their synthetic activity to replace the degraded components (Buckwalter, 1995). With aging, the capacity of the cells to synthesize some types of proteoglycans and their response to stimuli, including growth factors, decrease (Guerne et al 1995). Age-related changes may limit the ability of the chondrocytes to maintain the tissue and therefore contribute to the degeneration of the articular cartilage (Martin and Buckwalter, 1996).

MATRIX

Tissue fluid

Fluid contributes 60% to 80% of the wet weight of cartilage and consists of water with disolved gases, small proteins and metabolites (Maroudas and Schneiderman, 1987). Tissue fluid also contains a high concentration of cations to balance the negatively charged proteoglycans. This high water concentration is characteristic and determines the properties of the articular cartilage. The water interacts with the structural matrix molecules [particularly the large aggregating proteoglycans] and it is loosely bound to them so to be able to maintain hydration of the cartilage and at the same time to allow exchange with fluids outside the tissue. The articular cartilage has no vascular supply and, therefore, the function of the chondrocytes depends entirely on the interaction of the tissue fluid and the matrix macromolecules. The diffusion properties of the articular cartilage do not change during skeletal maturation (Torzilli et al 1998).

Structural macromolecules

Structural macromolecules constitute about 20-40% of the wet weight of the tissue. Collagens contribute about 60% of the dry weight of cartilage; proteoglycans 25-35%, and non-collagenous proteins and glycoproteins 15-20% (Buckwalter et al 1990).

Collagens

Articular cartilage contains mutliple genetically distinct collagen types, specifically types II, VI, IX, X, and XI (Eyre et al 1992). Types II, IX, and XI form the cross-banded fibrils seen with electron microscopy. The organisation of these fibrils into a tight meshwork that extends throughout the tissue provides the tensile stiffness and strength of articular cartilage and contributes to the cohesiveness of the tissue by mechanically entrapping the large proteoglycans (Sandell, 1995).

The principle collagen is collagen type II_1 which accounts for 90% to 95% of the total cartilage collagen (Buckwalter et al 1990) and forms the primary component of the cross-banded fibrils. Collagen type II is found in tissues with high water and proteoglycans content, suggesting that this type of collagen has specific properties, to create and maintain a highly-hydrated matrix.

Type II collagen consists of 3 α (II)-chains. Each alpha chain forms a tight left-handed helix and three helico-alpha chains wrapped around each other in a right-handed helix to form a tropocollagen molecule with the configuration being made more stable by covalent and hydrogen bonds between the polypeptide molecules. The tropocollagen molecule [molecular weight 280,000-540,000] is 300nm long and 1.4nm thick. The tropocollagen molecules then aggregate into microfibrils [3.5nm thick] which, in turn, aggregate into collagen fibres. The collagen fibres are again stabilized by covalent and hydrogen bonds. The precursor molecule [procollagen] of the collagen fibres is synthesised and secreted by the chondrocytes, but fibrogenesis occurs extracellularly (Grant and Prockop, 1972, Eyre, 1980). The procollagen consists of 100 repeating amino acid triplets each with Glycine at the third position, the other two varying in type but often being the amino acids hydroxyproline and hydroxylysine – substances rarely found outside collagen.

The functions and interrelationships of the quantitatively minor collagens remain unclear but the speculation is that they contribute to the formation and stability of the type II collagen fibril meshwork (Mayne and Irwin, 1986, Apone et al 1987). Type VI collagen appears to form an important part of the matrix immediately surrounding the chondrocytes and to help chondrocytes attach to the matrix (Hagiwara et al 1993, Marcellino and McDevitt, 1995). Type IX collagen molecules bind covalently to the superficial layers of the cross-banded fibrils and project into the matrix, where they also can bind covalently to other type IX collagen molecules. Type X collagen is present only near the cells of the calcified cartilage zone suggesting that it has a role in the mineralization of the cartilage (Buckwalter and Mankin, 1997). Type XI collagen molecules bind covalently to type II collagen molecules and probably form part of the interior structure of the cross-banded fibrils. The functions of type IX and type XI collagens remain uncertain, but presumably they help to form and stabilize the collagen fibrils assembled primarily from type II collagen The projecting portions of type IX

collagen molecules may also help to bind together the collagen fibril meshwork and to connect the meshwork with proteoglycans (Bruckner et al 1988, Diab et al 1996).

Proteoglycans

Proteoglycans form the major macromolecule of the cartilage matrix and exist mostly in the form of proteoglycan aggregates (Carney and Muir, 1988). They have multiple forms, including large aggregating proteoglycans, large non-aggregating proteoglycans and small non-aggregating proteoglycans. Large aggregating proteoglycan monomers [aggrecans] fill most of the interfibrillar space of the cartilage matrix, contributing about 90% of the total cartilage matrix proteoglycan mass; large non-aggregating proteoglycans contribute 10% or less; and small non-aggregating proteoglycans contribute about 3%, although because of their small size they may present in equal or higher molar amounts (Hardingham et al 1992, Rosenberg, 1992, Poole et al 1996). Proteoglycan monomers are large hydrophilic molecules and consist of a protein core and one or more glycosaminoglycan chains [long unbranched polysaccharide chains consisting of repeating disaccharides that contain an amino sugar]. Each unit of disaccharide has at least one negatively charged carboxylate or sulfate group, so the glycosaminoglycans form long strings of negative charges that repel other negatively charged molecules and attract cations (Roughley and Lee, 1994). The resultant high osmolality accounts for the retention of water in cartilage. Glycosaminoglycans found in cartilage include hyaluronic acid, chondroitin sulfate, keratan sulfate, and dermatan sulfate. The concentrations of these molecules varies among sites within articular cartilage and also with age, injury to the cartilage, and disease (Hardingham et al 1992, Rosenberg, 1992). Most aggrecans non-covalently associate with hyaluronic acid [hyaluronan] and link proteins [small non-collagenous proteins] to form proteoglycan aggregates. The proteoglycan-hyaluronide aggregates are very large [molecular weight about 50 x 10^6]. Large aggregates may have more than 300 associated aggrecan molecules (Buckwalter et al 1994). Proteoglycan aggregates have a central backbone of hyaluronan. Link proteins stabilize the association between monomers and hyaluronic acid and appear to have a role in directing the assembly of aggregates. The formation of aggregates helps to anchor proteoglycans within the matrix, preventing their displacement during deformation of the tissue, and helps to organize and stabilize the relationship between proteoglycans and the collagen meshwork (Tang et al 1996).

Non-aggregating proteoglycans include decorin, biglycan, and fibromodulin. Decorin has one dermatan-sulfate chain, biglycan has two dermatan-sulfate chains, and fibromodulin has several keratan-sulfate chains (Roughley and Lee, 1994). The small non-aggregating proteoglycans have shorter protein cores than the aggrecan molecules and they do not fill large volume of the tissue and do not directly contribute to the mechanical behaviour of cartilage. Instead, they bind to other macromolecules and probably influence cell function. Fibronectin and albumin have also been detected in the surface of bovine and human articular cartilage (Noyori et al 1998). Decorin and fibromodulin bind with type II collagen and may have a role in organizing and stabilizing the type II collagen meshwork (Hedbom and Heinegard, 1993, Hedlund et al 1994). Biglycan is concentrated in the pericellular matrix and may interact with type VI collagen (Roughley and Lee, 1994). The small proteoglycans also can bind transforming growth factor-β and may influence the activity of this cytokine in cartilage (Hildebrand et al 1994).

Non-collagenous proteins and glycoproteins

There is a wide variety of these molecules within normal articular cartilage. They primarily consist of protein and have a few attached monosaccharides and oligosaccharides (Heinegard et al 1995). Anchorin CII, a collagen-binding chondrocyte surface protein, may help to anchor chondrocytes to the collagen fibrils of the matrix (Pfaffle et al 1990). Cartilage oligomeric protein, an acidic protein, is concentrated primarily within the territorial matrix of the chondrocyte and appears to be present only within cartilage and to have the capacity to bind chondrocytes (Hedbom at al 1992, DiCesare et al 1994). This molecule may have value as a marker of cartilage turnover and of the progression of cartilage degeneration in patients who have osteoarthrosis (Lohmander et al 1994, Sharif et al 1995). Fibronectin and tenascin, non-collagenous matrix proteins found in a variety of tissues, also have been identified within cartilage. They may have roles in matrix organization, cell-matrix interactions, and the responses of the tissue in inflammatory arthritis and osteoarthrosis (Salter, 1993, Chevalier et al 1994, Hayashi et al 1996, Savarese et al 1996).

STRUCTURE OF ARTICULAR CARTILAGE

ZONES

Human articular cartilage varies from 1mm to 7mm in thickness. Morphological and biochemical examinations demonstrated four layers or zones proceeding from the articular surface to the subchondral bone. The differences among zones reflect transition from resistance to prominent shear force at the joint surface to more compressive force deeper in the cartilage (Singh, 1994). Although the boundaries between the zones cannot be sharply defined, zonal organization has functional importance (Aydelotte et al 1992).

Zone I (superficial, tangential, gliding): This thinnest zone consists of two layers, a sheet of fine fibrils with no cells that covers the joint surface, and a deeper layer of flattened ellipsoid chondrocytes arranged with their axes parallel to the articular surface. Chondrocytes in the superficial zone synthesize matrix with high collagen and low (the lowest of all zones) proteoglycan concentration (Aydelotte et al 1992). The superficial zone has its abundant collagen fibrils arranged parallel to the articular surface to resist tensile and shear forces (Mow and Rosenwasser, 1988). Changes in this zone change the mechanical behaviour of the tissue and may contribute to the development of osteoarthrosis. The densely packed collagen fibrils also act as a barrier to the passage of large molecules such as antibodies and other proteins, thus isolating the cartilage from the immune system. Disruption of this zone may release cartilage molecules that stimulate an immune or inflammatory response (Guilak et al 1994).

Zone II (intermediate, transitional, middle): This zone has several times the volume of the superficial zone. The chondrocytes are spheroidal with more synthetic organelles. They synthesize a matrix with higher concentration of proteoglycans and lower concentration of water and collagen although the fibrils are of larger diameter (Buckwalter and Mankin, 1997). The collagen fibrils lie at oblique angles relative to the articular surface.

Zone III (deep, mediate, middle, radial): In this zone spheroidal cells are aligned in columns perpendicular to the joint surface. This zone contains the largest in diameter collagen fibrils, the highest concentration of proteoglycans, and the lowest concentration of water. The collagen fibrils lie also perpendicular (radial) to the articular surface. The

collagen fibres pass into the tidemark, a thin basophilic line seen on light microscopy sections. This is decalcified articular cartilage that roughly corresponds to the boundary between calcified and uncalcified cartilage. One study revealed a band of fine fibrils corresponding to the tidemark suggesting that it represents a well defined matrix structure (Redler et al 1975). Oegema and Thompson (1995) believe that the tidemark represents a high watermark for calcification. Zones II and III form the bulk of the cartilage thickness and provide most of the resistance to static compression loading (Singh, 1994). Zones I, II and III merge imperceptibly while zones III and IV meet at the weakly basophilic tidemark (Oegema and Thompson, 1995).

Zone IV (calcified): This zone lies adjacent to the subchondral bone. Most of the collagen fibres are arranged perpendicular to the subchondral bone. The matrix is impregnated with hydroxyapatite, bonding the cartilage to the irregular surface of the subchondral bone (Buckwalter, 1983). It has few chondrocytes which are smaller in volume and they have low level metabolic activity which may have a role in the development of osteoarthrosis (Oegema and Thompson, 1995).

REGIONS OF THE MATRIX

Variations in the matrix within zones allow the distinction of three regions (Buckwalter et al 1990):

-**the pericellular region** surrounds the cell surface and is rich in proteoglycans. It has little type II non-fibrillar collagen fibres (Hagiwara et al 1993, Marcellino and McDevitt, 1995) and it also contains the non-collagenous protein anchorin CII (Pfaffle et al 1990).

-**the territorial region** surrounds cells or clusters of cells and their pericellular matrix. Nearer to the cell, thin collagen fibrils adhere to the pericellular matrix. At a distance from the cell, the collagen fibrils intersect and form a fibrillar basket around the cells which may provide protection for the chondrocytes during loading and deformation of the cartilage.

-**the interterritorial region** makes up most of the volume of the mature articular cartilage and contains the largest diameter collagen fibrils (Buckwalter and Mankin, 1997).

THE BENNINGHOFF MODEL

Damage to the collagen fibril network is likely to be one of the earliest signs of osteoarthrosis. The local stresses and strains in the collagen fibrils, which cause the damage, cannot be determined without taking the local arcade-like collagen-fibril structure into account (Wilson et al 2004). In the classic Benninghoff model of cartilage organisation, the tangential surface fibres are presented as an extension of radial fibres, which have arched in the transitional zone thus forming arcades of collagen extending from the bone to the cartilage surface (Benninghoff, 1925). While modern microscopy indicates that these do not exist as discrete collagen bundles, the collagen orientation carries out a similar function. The more or less vertically oriented collagen fibres of the transitional zone and the radial zone below it are designed to resist the stress of load bearing (Lehman, 1973). Clark in 1985 found by scanning electron microscopy on adult rabbit articular cartilage that the fibrous framework was found to be similar to that proposed by Benninghoff (Clark, 1985). The same author in 1990 traced fibres in both animal and human articular cartilage, which originated in the radial zone into the surface where they flattened and overlapped in a common direction (Clark, 1990). This study shows that the fibrous tangential zone is continuous to the fibres of the radial zone, similar to that described in the classic Benninghoff model. The findings, however, differ to those originally reported by Benninghoff in two ways: the fibres do not interdigitate in the transitional zone but instead overlap as thin lamellae and also the fibrous lamellae on the surface are not anchored in the periosteum. De Bont et al in 1986 could not confirm by scanning electron microscopy the arcade model of Benninghoff concluding that the organization of collagen fibrils in hyaline cartilage shows a three-dimensional network of randomly oriented fibrils (De Bont et al, 1986). Hwang et al in 1992 using optical silver staining technique on 60 human cases concluded that in the normal articular cartilage, the collagen fibrils in the superficial zones were compactly arranged into layers of decussating flat ribbons mostly parallel to the artificial split lines. The fibrils showed a tendency to condense into vertical arcade columns under girded by tangential bundles in the intermediate zone. In the deep zone, the fibrils formed a random meshwork (Hwang et al, 1992). A cryotechnical processing technique applied on adult human articular cartilage showed that the tendency of fibrils to form bundles is greater in deeper zones than in more superficial ones. A duality in the orientation of fibrils and fibril bundles is observed within interterritorial matrix compartment: superimposed upon the well-characterised arcade-like structure formed by one subpopulation is another more randomly arranged one (Hunziker et al, 1997). A more recent clinical study (Seidel and Grunder, 2005) using non-invasive magnetic resonance images detects early osteoarthritic changes in joints based on the changes of the arcade model of collagen fibres in the articular cartilage.

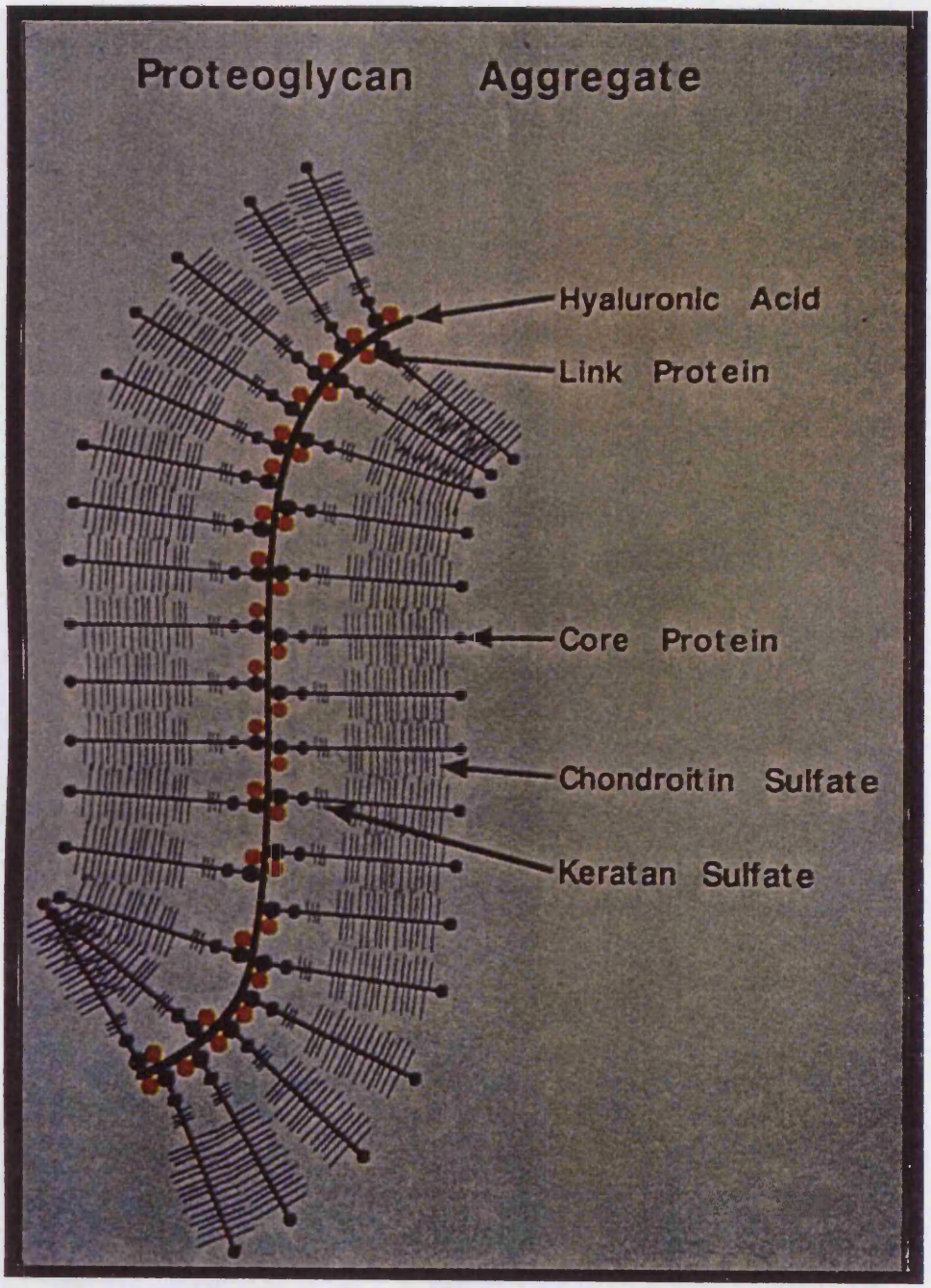

Proteoglycan Aggregate
Hyaluronic Acid
Link Protein
Core Protein
Chondroitin Sulfate
Keratan Sulfate

Fig.I.1

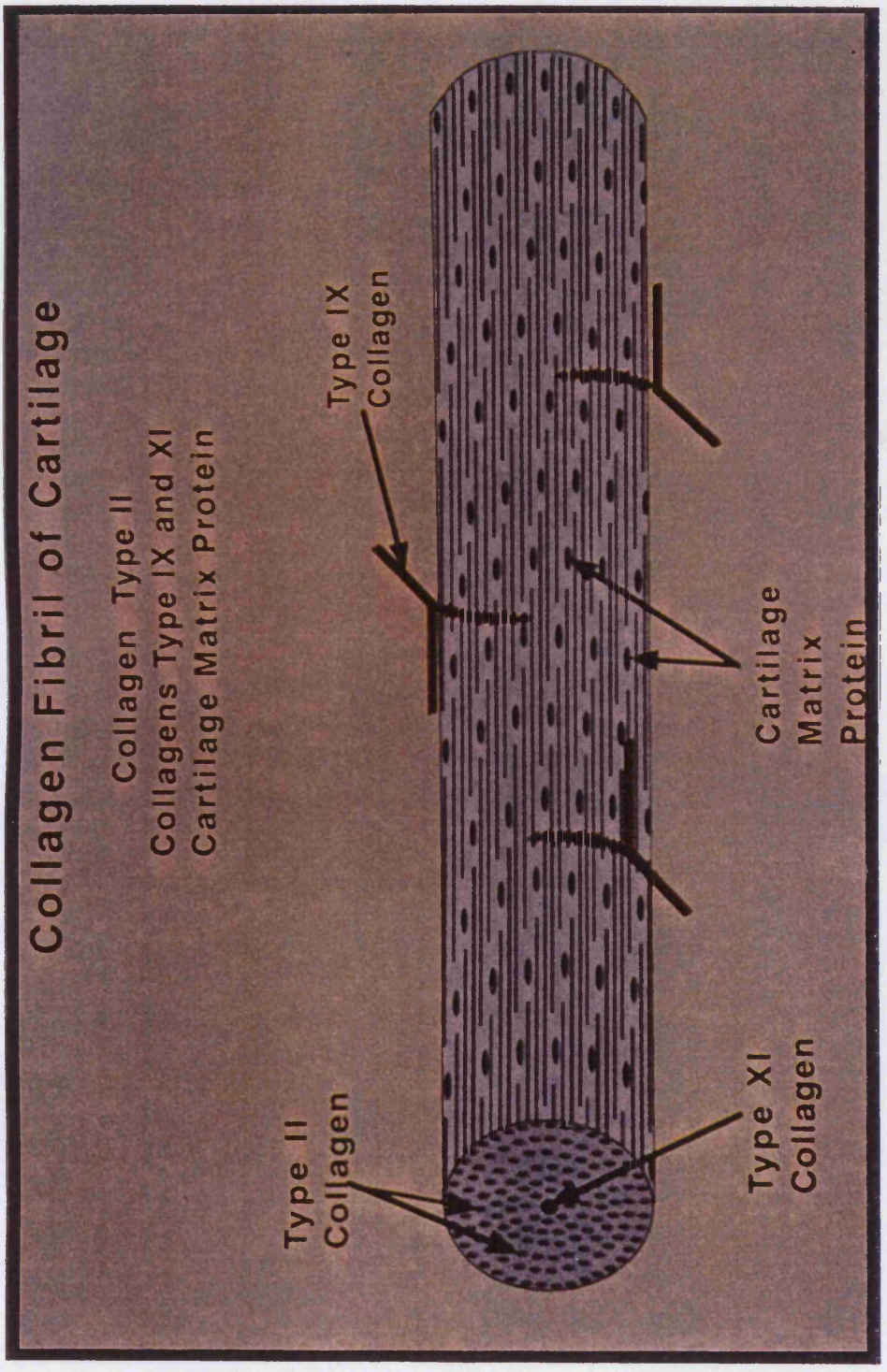

Collagen Fibril of Cartilage
Collagen Type II
Collagens Type IX and XI
Cartilage Matrix Protein
Type IX
Collagen
Cartilage
Matrix
Protein
Type II
Collagen
Type XI
Collagen

Fig.I.2

Fig.I.3.a

Histochemical appearance of normal articular cartilage:
The chondrocytes in the superficial **[s]** zone are flattened, lying parallel to the articular surface, while the chondrocytes in the deep **[d]** zones are round, lying in vertical columns. Proteoglycans are detected in all zones with higher concentration in the deeper zones [zonal differentiation]. *(Safranin-O staining, x 66)*

Fig.I.3.b

Immunohistochemical appearance of normal articular cartilage:
Collagen type II is detected in the deep zone **[cart]** and to a lesser extent the calcified zone below the tidemark **[►]**. The subchondral bone does not contain collagen type II and is stained negative. *(Silver-enhanced colloidal gold immuno-staining, x 66)*

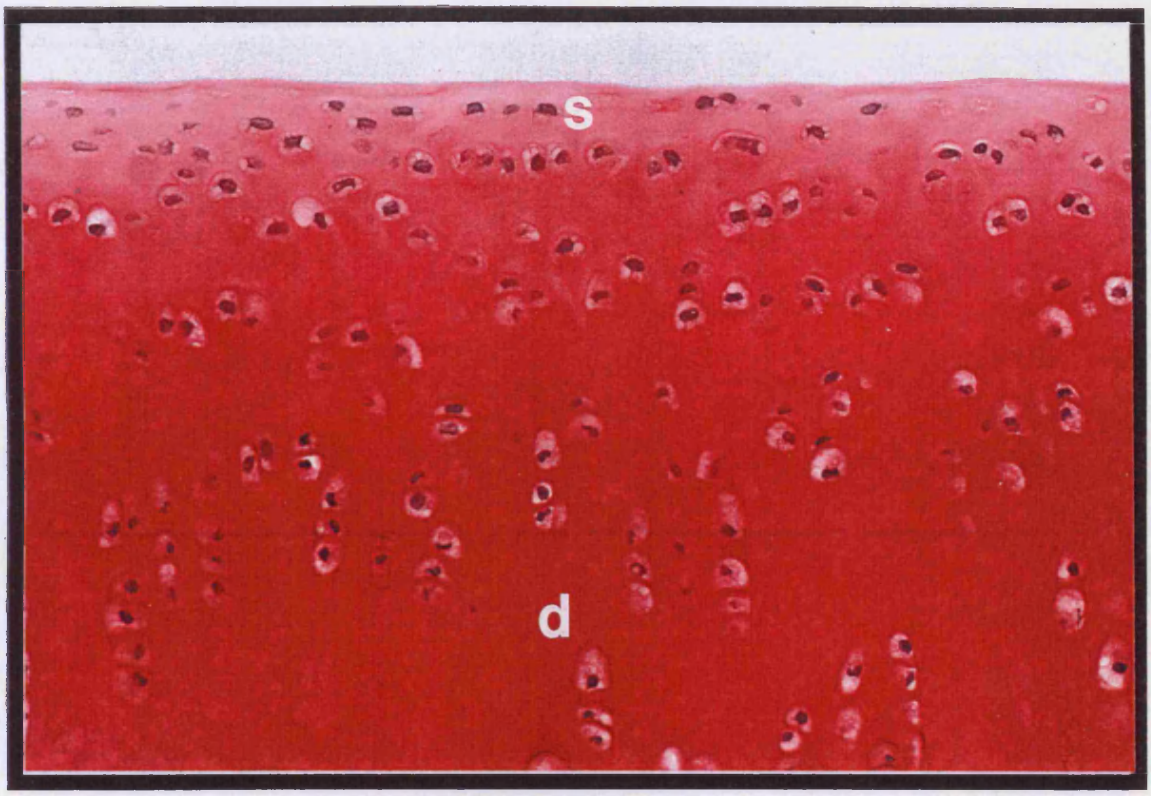


Fig.I.3.a

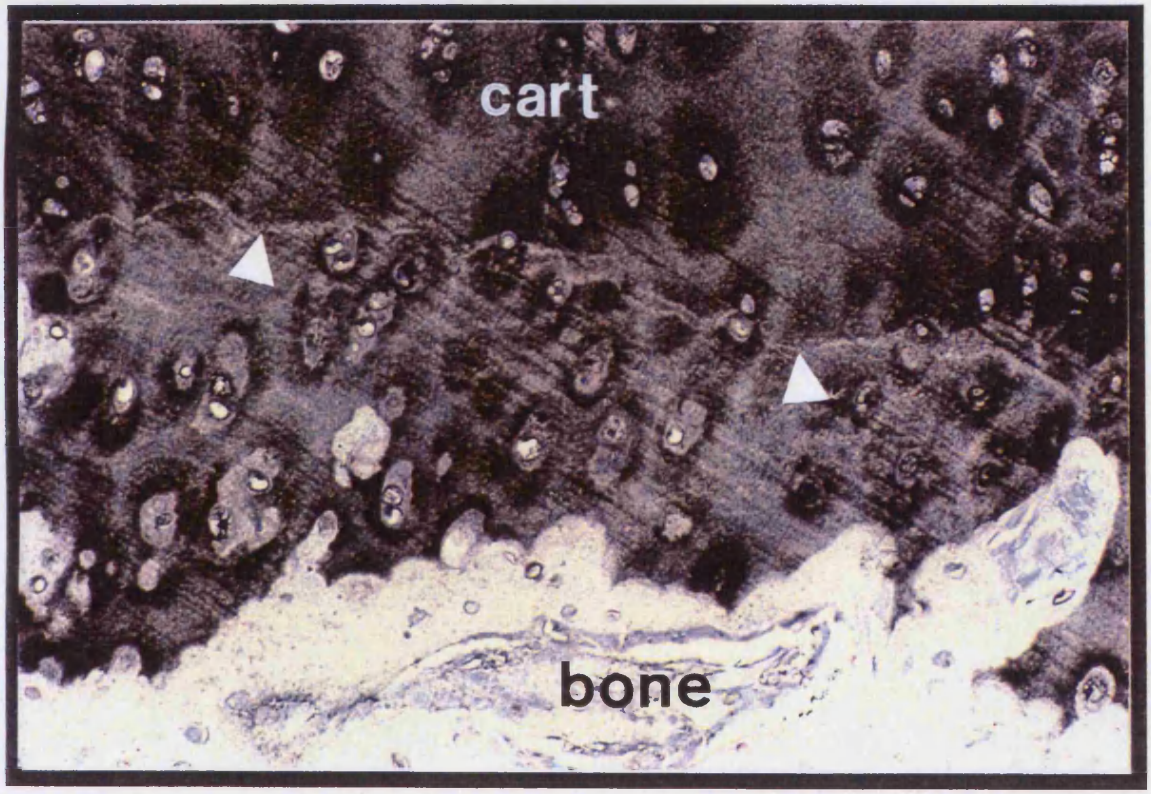
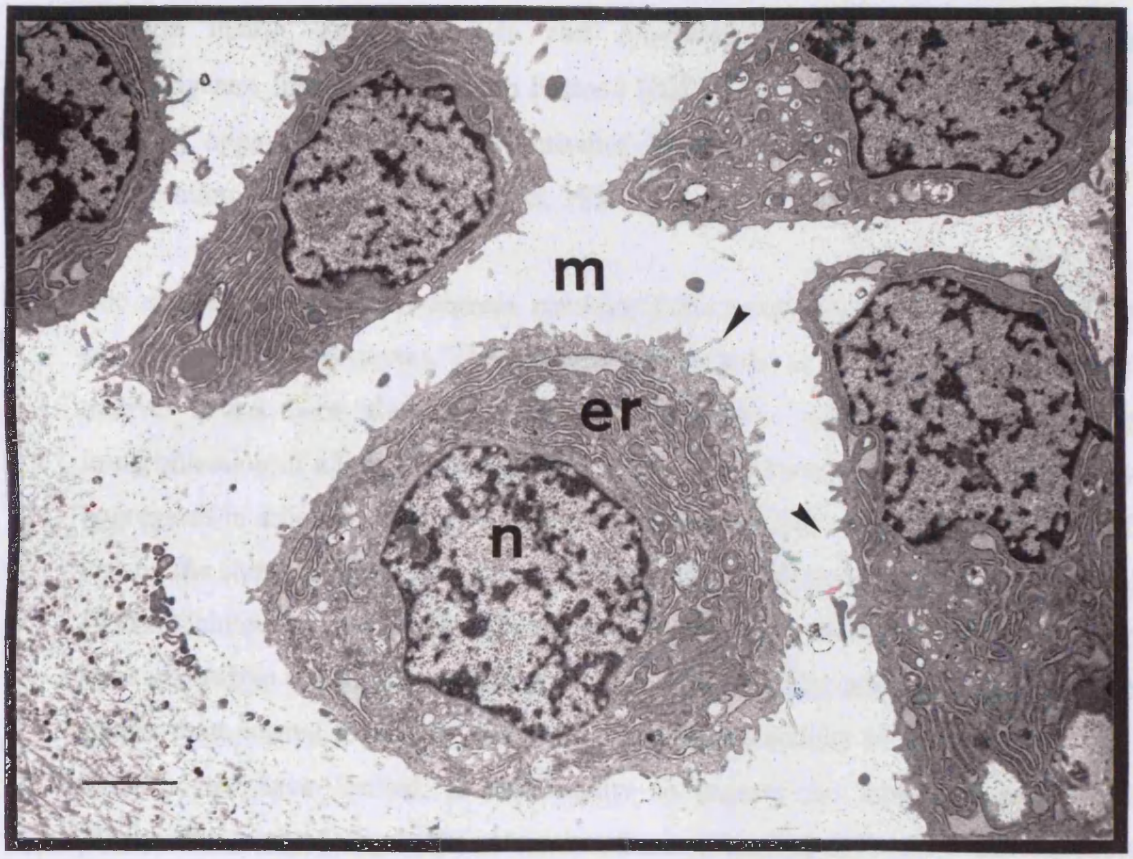


Fig.I.3.b

Fig.I.4

Ultrastructural appearance of normal articular cartilage:
Chondrocytes contain pronounced rough endoplasmic reticulum **[er]** and large nucleus **[n]**. Numerous cytoplasm processes **[►]** are extending into the extracellular matrix **[m]**.
(Uranyl acetate and Reynold's lead citrate staining, x 6477, the bar equals 2μm)

FIG.1.4



PHYSIOLOGY OF ARTICULAR CARTILAGE

CHONDROCYTE – MATRIX INTERACTIONS

Chondrocytes and matrix interact throughout life. Chondrocytes synthesize and degrade matrix macromolecules. The balance between these activities appears to be controlled by cytokines (which are synthesized and released by the cells) with catabolic and anabolic effects. Interleukin-1 [IL-1] induces the expression of matrix metalloproteases that degrade matrix macromolecules and interferes with the synthesis of matrix proteoglycans. Insulin-like Growth Factor-I [IGF-1] and Transforming Growth Factor-β [TGF-β] oppose these catabolic activities by stimulating matrix synthesis and cell proliferation (Lotz et al 1995, Trippel, 1995).

The matrix also transmits signals resulting from mechanical loading of the articular surface to the chondrocytes. The chondrocytes respond to these signals by altering the matrix. It has been shown that persistent abnormal decrease in joint loading or immobilization of a joint decreases the concentration of proteoglycans and proteoglycan aggregates in articular cartilage and alters the mechanical properties. Resumption of the use of the joints restores the composition and the mechanical properties (Buckwalter, 1995). Maintenance of the normal composition of articular cartilage requires a minimum level of loading and motion of the joint. Repetitive loading and motion of the joint at greater than normal levels may increase the synthetic activity of chondrocytes, but the chondrocytes have limited, if any, ability to expand the tissue volume in adults (Buckwalter and Mow, 1992). How mechanical loading influences the function of chondrocytes is not well known but deformation of the matrix produces mechanical, electrical and physicochemical signals that may stimulate chondrocytes (Gray, 1988, Grodzinsky, 1993, Lee and Bader, 1995)

BIOMECHANICAL PROPERTIES

Articular cartilage has two main functions: to distribute joint loads and reduce stresses, and to allow movement between opposing surfaces with the minimum of friction and

wear. Articular cartilage does not exist in complete anatomical isolation and the properties of the synovial fluid and the functions of the synovial membrane are clearly important in cartilage homeostasis, as well as the mechanical effects of the subchondral bone (Mow et al 1989).

Articular cartilage is a viscoelastic substance that is biphasic. The fluid phase is composed of water with disolved inorganic salts. The solid phase is made up of an organic solid matrix, ie collagen and proteoglycans. The water concentration differs in the various layers of cartilage, being 80% at the surface and falling to 65% in the deep zone. Seventy per cent of the water is intermolecular and is available to move when a load or pressure gradient is applied. Mature articular cartilage is also anisotropic. It has different mechanical properties depending on the direction it is loaded. This is due to the relationships of the collagen fibre arrangements, their cross-linking, and variations in the collagen-proteoglycan interactions (Mow et al 1989). The mixture of collagen fibrils and proteoglycan aggregates gives hyaline cartilage its unique physiological properties. The collagen fibrils provide elements of high tensile strength, while the proteoglycan aggregates exert an internal swelling pressure due to their hydrophilic character.

In situ, in normal cartilage, the proteoglycan aggregates are compressed to about one fifth of their potential volume and have a constant tendency to swell. They are protected from swelling to their maximum volume by their topographical entanglement within the fibrous collagen meshwork and, in turn, the collagen fibres and fibrils are put under stretch, thereby increasing their tensile properties. Thus the biomechanical properties of cartilage are dependent on this interaction between the collagen fibres and the proteoglycan aggregates (Broom, 1984). If a constant load is applied to articular cartilage, it will initially deform rapidly and then as the stress decreases, cartilage will show slow progressive increasing deformation, known as creep, until a steady state of deformation is reached, known as stress relaxation. Both creep and stress relaxation occur through interstitial fluid movement and macromolecular movement. Compression tends to cause movement of fluid, whereas shear tends to cause movement of macromolecules. Kerin et al (1998) have shown that cartilage strength increased following creep-induced water loss, and initial mechanical damage could propagate under moderate cyclic loading. The structure of the collagen of articular cartilage

exhibits a zone-specific deformation that is dependent on the magnitude and type of load (K'a'ab et al 1998).

Articular cartilage is porous and freely permeable. However, under high loads the movement of water in hindered by the effect of the frictional drag of the macromolecules. This causes a decrease in the flow and therefore stiffens the tissue, allowing greater resistance to the higher load. Tension alters the molecular structure of articular cartilage, the organization of collagen fibres, and the collagen fibre cross-links. They are, in effect, pulled apart. The tissue will become more swollen as permeability is increased and therefore fluid moves in leading to a decrease in the compressive stiffness (Mow et al 1989). The role of the stiffness of the collagen network in limiting hydration in normal cartilage and ensuring a high proteoglycan concentration in the matrix, is essential for effective load-bearing and is lost in osteoarthritis (Basser et al 1998).

The surface of cartilage is believed not to be perfectly smooth but to have structure resembling that of a golf ball and it is thought that these pits play an importnant role in lubrication (Bentley, 1987). The lubrication system of the articular cartilage is a sophisticated system and is a combination of an absorbed lubricant on the articular surface (boundary lubrication), and lubricant in a film between the articulating surfaces [fluid film lubrication]. The lubricant in boundary lubrication is a glycoprotein called lubricin. Fluid film lubrication is thought to be elastohydrodynamic. The sliding action of the articular surfaces forms a film and the pressure generated deforms the cartilage to increase the bearing contact area which, in turn, decreases the ability of the film to be dissipated. It therefore allows high load-bearing. Under load, fluid is also forced out of the cartilage adding lubricant to the system during movement. Under extreme loading conditions, such as prolonged standing after impact, surface-to-surface contact may occur, but the surfaces are still protected by the layer of lubricin (Mow et al 1989).

NUTRITION

Articular cartilage is a highly specialized tissue and relatively isolated. It has neither blood, lymphatic nor nerve supply. Nutrients and waste products are diffused through cartilage by movement of synovial fluid. The movement comes about by the changing loads and pressure gradients that occur during physiological activity. In vivo work on

human femoral heads has suggested that the pumping effect is only important for large molecules, whereas small molecules such as oxygen and glucose can disolve freely even when cyclical loading occurs (O'Hara et al 1990).

Chondrocytes derive their nutrition from nutrients in the synovial fluid. To reach the cell, these nutrients must pass through a double diffusion barrier: the synovial tissue and fluid, and the cartilage matrix. The matrix is not only restrictive with respect to the size of the materials but also with respect to their charges and to other features such as molecular configuration. The nature of this system leaves chondrocytes with a low concentration of oxygen relative to most other tissues, therefore, they depend primarily on anaerobic metabolism (Fischer et al 1995).

DEGENERATION OF ARTICULAR CARTILAGE

AETIOLOGY

Isolated defects of articular cartilage and osteochondral defects that result from trauma often leave most of the articular surface intact (Levy et al 1996). Clinical experience has shown, however, that, when left untreated, chondral injuries fail to heal and that defects that involve a major portion of the articular surface may progress to symptomatic degeneration of the joint (Maletius and Messner, 1996, Messner and Gillquist, 1996).

Infectious as well as inflammatory diseases of the joints, some anti-inflammatory drugs, joint immobilization, surgical intervention to the joints, ligamentous instability, and, even, possibly, joint irrigation can stimulate degradation of proteoglycans or suppress proteoglycan synthesis (Reimann et al 1982, Donohue et al 1983, Reagan et al 1983, Brandt and Adams, 1989, Branstein et al 1990, Gradinger et al 1995). Papain in large doses causes diffuse superficial breakdown of the surface of the cartilage via disruption of the organic matrix (Bentley, 1971). Ovalbumin immunization and subsequent intra-articular injection simulate the effects of rheumatoid arthritis (Pettipher et al 1989). Inborn errors of collagen metabolism have a direct effect on the organic matrix (Hull and Pope, 1982). Hemarthrosis and septic arthritis produce free radicals that also have destructive effects (Blake et al 1989).

AGE-RELATED CHANGES

Superficial fibrillation of articular cartilage occurs in many joints in association with increasing age and does not appear to cause symptoms or to affect the function of the joint adversely. Age-related, non-progressive, superficial fibrillation of cartilage and focal lesions of the articular surface must be distinguished from degeneration of articular cartilage occuring as part of the syndrome of osteoarthrosis (Mankin and Buckwalter, 1996).

Chondrocyte apoptosis plays a role in some aspect of maintenance, remodeling, or turnover of mature articular cartilage. The increase in apoptosis associated with aging could contribute to the greater risk of cartilage degeneration (Adams and Horton, 1998). Chondrocyte apoptosis and proteoglycan depletion are anatomically linked and may be mechanistically related (Hashimoto et al 1998).

CARTILAGE BREAKDOWN

The key to cartilage destruction is degradation of the collagen-proteoglycan matrix (Mow et al 1989). Collagen-proteoglycan matrix disruption can occur either at a mechanical level, eg by high stress from joint incongruity or ligament rupture, or at a biochemical level, eg by disordered collagen metabolism, or proteolytic enzyme degradation. Traumatic effects will initially cause localized injury, whereas at a biochemical level articular cartilage damage is likely to be diffuse.

Mechanical aspects

Wear is the removal of material from solid surfaces by mechanical action (Mow et al 1989). It can be interfacial, due to the action of the bearing surfaces, or fatigue, due to bearing deformation under load. The macroscopic effects of cartilage breakdown can cause vertical splits which may penetrate through the whole depth of the cartilage. The critical point is that once the collagen-proteoglycan matrix is disrupted, the rate of interfacial and fatigue wear will accelerate. Fissure formation and propagation is a significant element of cartilage degeneration (Chin-Purcell and Lewis, 1996). Splits and

cracks observed in diseased cartilage may be initiated, or propagated, by tensile stress (Kelly and O'Connor, 1996). A resultant swelling of the cartilage is due to distension of the proteoglycan aggregates; further damage causes stress fractures of more collagen fibrils and proteoglycan and collagen escape (Roberts et al 1986).

Biochemical aspects

There are two cells directly involved in cartilage breakdown, the synoviocyte and the chondrocyte. Early synoviocyte-initiated damage will cause diffuse superficial cartilage destruction, whereas early chondrocyte-initiated damage can be local and involve the deeper layers, as would be expected after trauma, or more diffuse, if production of abnormal collagen is involved. Once the stage of collagen-proteoglycan disruption has occured to any significant extent, then the destructive processes on the cartilage will be both mechanical and biochemical. Disintegrating cartilage fragments have a strong inflammatory effect on synovium, and areas of abnormal cartilage will be subject to adverse mechanical loading. A cycle of progressive destruction is then in place, as is typically observed in osteoarthritis (Donnell, 1994).

Both the chondrocytes and the synoviocytes produce the pro-inflammatory cytokines Tumour Necrosis Factor-α [TNF-α] and Interleukin-1 [IL-1] which in turn cause these cells to synthesize enzymes [proteinases] which degrade proteoglycans and collagens. The source of these proteinases is dependent upon the type of disease. In osteoarthritis, the chondrocytes are the most probable source, whilst in highly inflamed rheumatoid joints, synovial fibroblasts and macrophages as well as the chondrocytes play a role in tissue destruction (Cawston and Rowan, 1998).

There are four main classes of proteinases and they are named according to the chemical group involved in catalysis. The aspartate proteinase [cathepsins D] and the cysteine proteinases [cathepsins B,H,K,L,S] act at low pH and are thought to act within the lysosomal system where they degrade proteins intracellularly. Secreted serine proteinases [elastase, cathepsin G, plasmin, plasminogen activator] and metalloproteinases [collagenases, stromelysins, gelatinases, membrane-bound MMPs] act extracellularly at neutral pH (Barrett, 1997).

Cathepsins have a direct action on proteoglycan breakdown (Bentley et al 1981). Cathepsins B,H and L are present in elevated levels in antigen-induced arthritis whilst cathepsin B and cathepsin D are raised in osteoarthritic tissues (Everts et al 1996).

The metalloproteases have direct effects on the collagen-proteoglycan matrix causing it to breakdown (Pelletier and Martel-Pelletier, 1989). Matrix metalloproteinases [MMPs] can collectively degrade all the components of the extracellular matrix. They all contain a zinc atom [Zn^{2+}] at their catalytic centre and are all synthesised as inactive proenzymes which require activation before thay can degrade matrix. Activation of pro-MMPs by removal of the propeptide that maintains MMPs in inactive form is an important control point in tissue turnover (Vincenti et al 1994). Plasmin activity as well as other serine proteinases may activate pro-MMPs (Nagase and Woessner, 1993).

All connective tissues contain endogenous Tissue Inhibitors of Metalloproteinases [TIMPs 1-4] which bind tightly to active MMPs. This family of small polypeptide inhibitors plays an important role in controlling tissue breakdown by blocking MMP activity (Cawston, 1996).

TNF-α and IL-1 also stimulate the chondrocytes to produce interleukin 6 [IL-6] which may have an inhibitory effect on metalloprotease synthesis (Shinmei et al 1990). TNF-α also stimulates synoviocytes to synthesize prostaglandins, in particular prostaglandin E2 [PGE2]. This causes plasma to be extravasated into the synovium leading to synovitis, but inhibits lymphocyte proliferation. PGE2 also has an inhibitory effect on IL-1 thus reducing its effect on metalloprotease production and cartilage matrix breakdown (Bunning and Russell, 1989). TNF-α is inhibited by γ-interferon [γ-IFN] which is produced by T lymphocytes in response to IL-2. The latter is found in large quantities in rheumatoid arthritis (Pettipher et al 1989, Vignon et al 1990).

JOINT USE

Chondrocytes adapt to the unloaded condition by decreasing proteoglycan synthesis (Jurvelin et al 1989). Cyclical loading of cartilage stimulates matrix synthesis, whereas

prolonged static loading or the absence of loading and motion causes degradation of the matrix and, eventually, degeneration of the joint (Buckwalter, 1995). Moderate and possibly even strenuous regular activity does not cause degeneration in normal joints. Repetitive intense joint loading may lead to an early onset of degeneration. Participation in sports or other activities that repetitively expose joints to high levels of impact or torsional loading may increase the probability of joint degeneration (Buckwalter and Lane, 1996). Newton et al (1997) showed that a lifetime of regular weight-bearing exercise in dogs with normal joints did not cause alterations in the structure and mechanical properties of articular cartilage that might lead to degeneration. Otterness et al (1998) concluded that a sedentary lifestyle in the hamster leads to a lower proteoglycan content in the cartilage and a lower synovial fluid volume, associated with cartilage fibrillation, pitting and fissuring.

OSTEOARTHRITIS

CLINICAL SYNDROME

The degeneration, or the progressive loss of normal structure and function of articular cartilage is an integral part of the clinical syndrome of osteoarthrosis, also referred to as degenerative joint disease, degenerative osteoarthritis, osteoarthritis, and hypertrophic osteoarthritis. The diagnosis of osteoarthrosis requires the presence of symptoms and signs that may include joint pain, restriction of motion, crepitus with motion, joint effusions, and deformity. The progressive breakdown of cartilage and the manifest clinical features distinguish osteoarthritis from age-related changes. Evidence of synovitis is frequently present, but inflammation is not a major component (Buckwalter and Mankin, 1997).

EPIDEMIOLOGY

Osteoarthrosis occurs most commonly in the foot, knee, hip, spine, and hand joints. Thirty-nine million men and 115 million women is one estimate of the number of people world-wide with knee osteoarthritis alone. No country studied so far is free of the

problem, and the prevalence in China is as high as it is in Iceland, America or the Middle East (Murray and Lopez, 1996). Radiographically based studies suggest that, at the age of 70 years, the prevalence of moderate and severe osteoarthritis of the finger joints rises to 70% among women and 50% among men, that of the knees affects 30% of women and 50% of men, and that of the hips 13% of women and 10% of men. Reported pain correlates best with radiographic grade and severity at the hip, and then at the knee, with less than 40% of people with severe radiographic hand osteoarthritis reporting pain (Erhardt, 1995). Osteoarthritis is the indication for 21,000 hip and 7,000 knee arthroplasties annually in the United Kingdom (Wynne, 1998). The proportion of subjects over 50 years in the general population who report knee pain and disability of sufficient severity to justify early knee joint replacement is about 2%, twice as common in women compared with men (Tennant et al 1995).

GENETICS

Recent studies have shown significant familial clustering for hand and knee osteoarthritis (Hirsch et al 1998, Felson et al 1998). A greater association in radiological features of hand and knee osteoarthritis has also been shown amongst monozygotic twins when compared with dizygotic twins, confirming a genetic influence at these sites (Spector et al 1996).

Osteoarthritis has been linked to the COL2A1 gene on chromosome 12 encoding type II procollagen (Vikkula et al 1993). Some reports have suggested linkage to chromosome 2q. Potential candidate genes mapping to this region include fibronectin, the alpha-2 chain of collagen type V, and the interleukin-8 receptor (Wright et al 1996). Some research has also shown that candidate genes associated with osteoarthritis are the vitamin D receptor gene (Keen et al 1997), the oestrogen receptor gene (Ushiyama et al 1998) and the Insulin-like growth factor 1 gene (Meulenbelt et al 1998).

The immediate clinical relevance of these findings is that they identify family history as a major risk factor for osteoarthritis. Studies of family aggregation, however, show that there is considerable variation in the distribution of joint involvement in those at equal risk. This suggests that the action of biomechanical or other local environmental factors

on predisposed joints determine the rate of development of disease (Felson and Zhang, 1998).

AETIOLOGY

Osteoarthrosis develops most commonly in the absence of a known cause (primary or idiopathic osteoarthrosis). It can also develop as a result of joint injury, infection, or one of a variety of hereditary, developmental, metabolic, and neurological disorders (secondary osteoarthrosis). The age of onset associated with secondary osteoarthrosis depends on the underlying cause; thus, it may develop in young adults and even children as well as the elderly. In contrast, there is a strong association between the prevalence of primary osteoarthrosis and increasing age (Peyron, 1988).

PATHOGENESIS

Osteoarthritis is initiated by multiple factors. Protein-kinase C, gas mediators [superoxide anion and nitric oxide] and proteinases are all involved (Tanaka et al 1998). Nitric oxide is a highly reactive, cytotoxic free radical that has been implicated in tissue injury in a variety of diseases. Cartilage obtained from patients with osteoarthritis produces significant amounts of nitric oxide ex vivo. In vitro, nitric oxide exerts detrimental effects on chondrocyte functions, including the inhibition of collagen and proteoglycan synthesis, and enhances apoptosis (Amin and Abramson, 1998).

The primary changes consist of loss of articular cartilage, remodelling of subchondral bone, and formation of osteophytes (Schiller, 1995). It has been suggested that osteoarthritis may be secondary to stress fracture of the collagen fibrils (Kempson et al 1968). Loss of the larger aggregates appears to be one of the earliest changes associated with osteoarthrosis and immobilization of the joint. Increasing age is also associated with loss of large aggregates from articular cartilage (Buckwalter et al 1994). Early osteoarthritis is characterized by fibrillation of the superficial surface, loss of proteoglycans and collagen, and increase in water content. The basal calcified layer expands, and thickening of the subchondral bone occurs. It has been suggested that the

zone of calcified cartilage may be reactivated in osteoarthritis and may progressively calcify the unmineralized cartilage (Oegema et al 1997).

The arthritic joints become hypervascularized but the venous outflow is delayed, resulting in a rise in the intraosseous pressure. Cartilaginous debris in the synovial fluid may lead to inflammation and subsequent fibrosis of the synovial membrane and joint capsule. Osteophyte formation involves *de novo* regeneration of fibrocartilage from the tissues at the transitional zone at the margin of the joint surface. As the disease progresses, the surface irregularities become clefts, more of the articular surface becomes roughened and irregular, and the fibrillation extends deeper into the cartilage until fissures reach subchondral bone. As the cartilage fissures grow deeper, the superfical tips of the fibrillated cartilage tear, releasing free fragments into the joint space and decreasing the thickness of the cartilage. At the same time, enzymatic degradation of the matrix further decreases the volume of the cartilage. Eventually the progressive loss of articular cartilage leaves only dense and often necrotic eburnated bone (Buckwalter and Mankin, 1997).

IMAGING

Plain radiographs are the simplest and most widely use method of joint evaluation and monitoring of the disease. The Altman and the members of the American Rheumatism Association Subcommittee on Classification of Osteoarthritis system based on clinical and radiographic criteria (Altman et al 1986, Altman et al 1991) are most commonly adapted. The presence of osteophytes seems to best differentiate osteoarthritis (Menkes, 1991) although part of the osteophyte is ossific and radiopaque, but part is cartilaginous and not visible on x-ray films (Bentley, 1985, Bentley, 1987). Other methods of imaging such as nuclear scans, arthrography and computed tomography are of limited use because they are unable to detect early cartilage abnormalities (Blackburn et al 1996). Magnetic resonance imaging [MRI] has the advantages of multiplanar imaging, soft tissue contrast, and non-invasiveness. Like radiography, it can underestimate the extent of cartilage abnormality. The most sensitive technique for measuring superficial articular abnormalities is arthroscopy of the joint (Blackburn et al 1996). Wada et al (1998) reported a high correlation of medial compartment knee osteoarthrits between

radiography and arthoscopic findings and a poor correlation of lateral compartment knee osteoarthritis.

TREATMENT

Analgesics and NSAIDs

Simple analgesics and non-steroidal anti-inflammatory drugs [NSAIDs] are the most commonly used agents for the relief of the symptoms. Paracetamol is the preferred analgesic for long-term use because of its minimal side effects. Aspirin inhibits cathepsin production from chondrocyte lysosomes. A beneficial effect of aspirin on induced superficial lacerations has been shown (Ginsberg et al 1968). No effect, however, was noted with the use of aspirin in patients with chondromalacia patellae (Bentley et al 1981). NSAIDS inhibit cyclo-oxygenase production which converts arachidonic acid into prostaglandins. This therefore reduces both the pain and swelling of synovial inflammation. Unfortunately, it also suppresses the inhibitory effect of prostaglandin on IL1 and so it accelerates cartilage breakdown (Pettipher et al 1989, Vignon et al 1990). Corticosteroids and some NSAIDs have also been found to decrease glycosaminoglycan synthesis (Dingle, 1996).

Chondroprotective agents

Glucosamine-sulphate given exogenously has been shown to have stimulatory effects on chondrocytes and synoviocytes in vivo (Jimenez and Dodge, 1996, Helio et al 1996). High grade chondroitin-sulphate has been shown to have clinical and anti-catabolic effects, including the competitive inhibition of metalloproteinases and down-regulation of interleukin-1 (Verbruggen et al 1998, Busci and Poor, 1998). Because glucosamine and chondroitin-sulphate have different but complementary mechanisms of action, using these two compounds together produces a synergistic response in articular cartilage (Hungerford, 1998).

Bracing

The use of braces specifically designed for osteoarthritis sufferers can be effective in taking the load off the affected compartment of the joint. During gait, the knee joint is subjected to a varus moment, which shifts the joint load to the medial compartment. Higher loads intensify pain and may contribute to degenerative changes. Valgus bracing applies valgus correction to the knee and reduces medial compartment load (Horlick and Loomer, 1993).

Pulsed electromagnetic fields

Pulsed Electromagnetic Fields (PEMF) have been used widely to treat non-healing fractures. In cartilage, PEMF produce an electromagnetic phenomenon which stimulates the synthesis of the matrix components from the chondrocyte (Trock, 1994). No useful clinical affect has been demonstrated as yet.

Intra-articular steroids

The use of intra-articular steroids is strongly contested in the literature. Chondroprotection by corticosteroids has been indicated (Williams and Brandt, 1985, Pelletier et al 1987). Others have argued against their use in humans because of direct and side effects (Salter et al 1964, Behrens et al 1975). Low-dose long-acting steroids intra-articularly seem to overcome the adverse reactions. They are thought to act directly on the chondrocyte and inhibit metalloprotease production (Pelletier and Martel-Pelletier, 1989, Pelletier et al 1995). Papachristou et al (1997), however, showed that intra-articular injections of hydrocortisone alters the shape of articular cartilage chondrocytes, producing abnormal changes in the cytoplasm and nucleus, leading to cell degeneration.

Viscosupplementation

Treatment with viscosupplementation consists of intra-articular injections of elastoviscous solutions of hyaluronan to augment the elastoviscosity of the synovial fluid and the matrix of the cartilage. The injected elastoviscous solution, however, has only a short residence time in the joint, therefore the injection should be repeated (Scale et al 1994, Lussier et al 1996). Randomised controlled clinical trials are required to validate the efficacy of this method of treatment.

Surgical treatments

These include arthroscopic techniques of debridement and bone marrow stimulation, corrective osteotomies and resurfacing of the diseased cartilage in the early stages of osteoarthritis. These methods are described in the section on the Repair of Articular Cartilage later on in this chapter. The end-stage treatment of an osteoarthritic joint remains its replacement with artificial joint components either partially or totally.

Gene therapy

The future prospect of identifying disease susceptibility genes that explain a significant part of the genetic component of osteoarthritis will make possible primary prevention of the disease (Evans and Robbins, 1995). The identification of disease genes also leads to identifying pathogenetic pathways, finding targets for new drug therapies. Gene therapy directed at replacing defective gene products will become the future approach to treatment of osteoarthritis, known as disease modification.

With regard to the repair of the damaged articular cartilage, gene therapy and work with growth factors and cytokines and genetically modified chondrocytes all offer the promise of regenerating cartilage (Bandara et al 1992, Pelletier et al 1997, Doherty et al 1998). The possible uses of gene therapy range from the simple delivery of an inhibitor of a cartilage-destructive enzyme, to the very complex production of a range of factors that reproduce fetal development signals (Gerich et al 1996, Gerich et al 1997).

INJURIES OF ARTICULAR CARTILAGE

INJURY TYPES

Acute injuries to articular cartilage can be caused by blunt trauma, penetration, friction abrasion, or sharp concentration of weight-bearing forces. Acute chondral lesions of the knee have been divided into two groups: osteochondral fractures and chondral fractures / separations without bone involvement (Urrea and Silliman, 1995).

Osteochondral fractures

Osteochondral fractures in the knee are generally restricted to the medial femoral condyle, lateral femoral condyle, and patella. Rarely do lesions occur on the tibial articular surface. Osteochondral fractures are caused by impaction or avulsion and can be produced by direct blow, by twisting shearing forces on a nearly fully extended knee, and by subluxation or dislocation of the patella (Johnson-Nurse and Dandy, 1985). Because of the mechanism of injury, these lesions are often associated with soft tissue injuries including anterior cruciate ligament tears, meniscal tears, and parapatellar ligament tears (Terry and Flandry, 1988, Butler and Andrews, 1988).

Osteochondral fractures tend to occur mainly in adolescents (Bradley and Dandy, 1989). In this younger age group, the tidemark has not yet developed. The hyaline articular cartilage has firm attachment to subchondral bone through directly connecting collagen fibres. The shearing forces are transmitted deep to the osteochondral junction, and the fracture line passes through the relatively weaker subchondral cancellous bone (Bauer and Jackson, 1988).

Chondral fractures / separations

These were first described by O'Donoghue in 1966. Usually there is a history of blunt trauma, twisting, shearing weight-bearing type of injury, impaction or direct blow on a flexed or extended knee (Terry and Flandry, 1988).

Chondral fracture tend to occur in skeletally mature individuals. The fracture line occurs at the tidemark (Bradley and Dandy, 1989). The tidemark represents a weak zone between calcified and uncalcified tissue marking the junction of two biomechanically dissimilar materials. This junction provides a cleavage plane where shear stress can produce a purely cartilaginous fracture leaving the subchondral plate undisturbed.

DIAGNOSIS OF ARTICULAR CARTILAGE DEFECTS

Clinical

Patients with osteochondral fractures will experience sudden pain aggravated by weight-bearing. Usually there is swelling. The symptoms often mimic torn meniscus, the knee may lack full extension and may be locked. The involved femoral condyle may be tender to palpation. Patellar stability assessment and tenderness of the medial patellar retinaculum fibres may indicate lateral patella dislocation (Johnson-Nurse and Dandy, 1985). Hemarthrosis with fat globules seen on aspiration is of significant importance (Sanberg and Balkfors, 1986, Butler and Andrews, 1988).

In chondral fractures / separations pain and swelling are common. Isolated chondral fractures may mimic torn menisci with mechanical symptoms of locking, catching and giving way and joint line tenderness (Terry and Flandry, 1988). Hemarthrosis is rare because the subchondral plate is not penetrated. A bloody effusion means that an associated injury has been sustained (De Haven, 1980, Butler and Andrews, 1988).

Although the cartilage itself has no nerve ends, the subchondral bone is often compromised, resulting in pain. Partial-thickness defects allow more pressure to the subchondral bone when the weight load passes through the defect area. This is more

pronounced during athletic activities when increased stress is applied to the joint surfaces. In these cases the pain is intermittent and often relieved by rest. Full-thickness defects, however, where the subchondral bone is directly affected, present with more or less continuous pain. Differential diagnosis from a meniscal injury is often necessary. During clinical examination, a knee with a damaged meniscus is tender at the joint line level. A knee with an articular cartilage defect in the femoral condyle is tender above the joint line level. Often there is a false positive McMurray's test. Anterior knee pain with positive patello-femoral grinding test are also common when the defect lies on the articular surface of the patella.

Imaging

Various imaging methods have been applied for the assessment of articular cartilage. These include standard radiography, arthrography, computerized tomography, CT arthrography, ultrasonography, and magnetic resonance imaging (Hodler and Resnick, 1996). Plain radiographs of the injured knee may not show the loose or undisplaced cartilaginous fragment. It is important to obtain multiple views including antero-posterior, lateral, right and left obliques, tunnel, and patellar views (Mathewson and Dandy, 1978). Castriota-Scanderbeg et al (1996) described that sonographic measurement of articular cartilage is precise enough to be used in clinical practice. MRI is a promising tool of investigation for the articular cartilage. Its modalities are still imprecise and debated, but, because of its non-invasiveness, it is destined to be preferred over arthro-CT (Drapé et al 1998). Specialized magnetic resonance imaging techniques such as fast-spin-echo MR imaging (Potter et al 1998), magnetic resonance chondro-crassometry (Eckstein et al 1996), three-dimensional computerized representations (Marshall et al 1995, Lavid et al 1996, Tebben et al 1997) as well as magnetic resonance microscopy (Goodwin et al 1998, Cova et al 1998) provide more accurate assessment of lesions of articular cartilage.

Arthroscopy / Classification systems

Arthroscopy is the best procedure to establish the diagnosis and also to determine the extent and depth of lesion. (De Haven, 1980, Hopkinson et al 1985, Dzioba, 1988). This is reflected in the development of a number of **classification systems** that define the lesions arthroscopically:

Outerbridge, 1961	**grade I:** softening and swelling **grade II:** partial thickness, early fissuring **grade III:** full thickness, fissuring to subchondral bone **grade IV:** subchondral bone exposed
Insall et al 1976	**grade I:** softening and swelling **grade II:** deep fissures to subchondral bone **grade III:** fibrillation – 'crab meat' **grade IV:** thinning, course granular appearance, exposure of subchondral bone
Bentley and Dowd, 1984	**grade I:** fibrillation or fissuring, <0.5cm **grade II:** fibrillation or fissuring, 0.5 – 1cm **grade III:** fibrillation or fissuring, 1- 2cm **grade IV:** fibrillation or fissuring, >2cm
Bauer and Jackson, 1988	Acute traumatic **type I:** linear crack **type II:** stellar fracture **type III:** flap **type IV:** crater Degenerative **type V:** fibrillation **type VI:** degrading
Dzioba, 1988	**age of lesion:** acute, subacute, chronic **diameter:** small (<1cm), medium (1-3cm), large (>3cm) **depth:** superficial, partial, complete **cartilage defect localization:** weightbearing zone, femur, condyle, submeniscal, tibial, intercondylar region, non-weight-bearing zone
Nojes and Stabler, 1989	**grade I:** cartilage surface intact grade IA: softening, <10mm grade IB: softening (deformation), >15mm **grade II:** cracks, fissuring, fibrillation, fragmentation grade IIA: <1/2 thickness grade IIB: >1/2 thickness **grade III:** bone exposed grade IIIA: bone surface intact grade IIIB: bone surface excavation

A number of systems have been described in the recent years which take into account more variables (Koshino and Machida, 1993, Lewandrowski et al 1996) and also statistical analysis as in the SFA [Société Francaise d'Arthroscopie] Score (Dougados and Ayral, 1994).

REPAIR OF ARTICULAR CARTILAGE

THE 'NATURAL' REPAIR

The type of cartilage injury, including its depth and size and whether it is acute or chronic, distinctly affects the healing process (Suh et al 1995). Acute injuries to the articular cartilage have been biologically distinguished into two types: the injuries with loss of matrix macromolecules without mechanical damage to cells or the collagen fibril meshwork and the lesions with mechanical disruption of cells and matrix (Buckwalter et al 1988).

In partial-thickness injury, because cartilage is avascular, it is up to the chondrocytes to mount the entire healing process. Cells near the defect are able to increase their synthesis of matrix elements. Chondrocyte multiplication and cell proliferation which can form abnormal multicellular clusters of chondrocytes was shown by Meachim in 1963. The increased metabolic activity has been also shown through autoradiographic studies using tritiated thimidine (Rothwell and Bentley, 1973) and also during ultrastructural studies (Ghadially, 1977). A non-osseous cartilage formation has also been described deriving from juxta-articular or synovial tissue that applies to the defects at the joint margin (Stockwell and Meachim 1979). The dense extracellular matrix, however, keeps the newly synthesized macromolecules from reaching the defect area and the healing of the defect cannot be completed (Buckwalter et al 1988). Partial-thickness injuries healing depends on the size of the lesion, its location within the joint and the age of the individual. Generally, however, partial-thickness injuries do not heal, although they seldom progress (Suh et al 1995).

Full-thickness injuries affect the vascularized and innervated subchondral bone and therefore activate the inflammatory response. Reparative mesenchymal cells originate from the undifferentiated bone marrow cells. Within a few weeks, a good proportion of the mesenchymal cells in the chondral portion of the defect, differentiate into chondrocyte-like cells and secrete proteoglycans and collagen type II. In the bony portion of the defect, endochondral and intramembranous bone formation reconstructs the bone defect. By 6 to 8 weeks, some of the repair tissue exhibits an hyaline cartilage appearance, while the remainder resembles fibrocartilaginous or fibrous tissue containing

predominantly collagen type I (Stockwell, 1987, Shapiro et al 1993). By 1 year, however, the majority of the chondrocyte-like cells transform to fibroblasts and there is depletion of matrix proteoglycans. Fibrillation, fissuring and thinning of the chondral tissue follow. This fibrocartilage usually degenerates in a relatively short time (Furukawa et al 1980).

Full-thickness injuries' healing depends on the size, the location and possibly the age of the individual. Smaller defects heal more consistently and effectively. In younger patients the chondrocyte response to stimuli is more effective (Buckwalter et al 1988). Osteochondral defects in weight-bearing areas that articulate with another cartilage surface are less likely to heal than those that do not articulate (Stover et al 1987).

REGULATION OF CARTILAGE BREAKDOWN AND REPAIR

Breakdown and repair of articular cartilage can be regulated mechanically (Caterson and Lowther, 1978, Radin et al 1984, Helminen et al 1992) and biologically by growth factors (Morales and Hascall, 1989) and other biologial factors (Cawston and Rowan, 1998).

Mechanical load

Immobilization or reduced loading of a joint results in a decrease in proteoglycan synthesis, whereas moderate exercise leads to an increase in proteoglycan synthesis (Caterson and Lowther, 1978) and thickening of the cartilage matrix (Helminen et al 1992). Severe mechanical loading causes thinning of the matrix and leads to degenerative changes (Radin et al 1984). *In vitro* cartilage explant studies showed that dynamic compression forces at frequencies of 0.01 to 1.0 Hz stimulate the synthesis of matrix macromolecules (Palmoski and Brandt, 1984, Sah et al 1989, Hall et al 1991), but dynamic compression forces of large magnitude appear to accelerate the loss of matrix macromolecules and destroy the matrix (Sah et al 1991). It seems that early controlled loading and motion can promote healing (Buckwalter, 1996). Torzilli and Grigiene in

1998 presented results that indicated that proteoglycan release from the cartilage matrix is inhibited by continuous cyclic mechanical loading.

Growth factors

Biological regulation has been shown to take place through polypeptides known as growth factors such as the Transforming Growth Factor-β [TGF-β], the Insulin-like Growth Factor-1 [IGF-1] and the basic Fibroblast Growth Factor [bFGF] (Morales and Hascall, 1989). The source of growth factors varies; they may be produced remote from the cartilage and diffuse in, they may be sunthesized by the chondrocytes or they may be released from the matrix as it is broken down (Cawston and Rowan, 1998).

TGF-β stimulates the synthesis rate of matrix macromolecules such as decorin and biglycan (Morales and Roberts, 1988). TGF-β also promotes the repair of damaged cartilage (Joyce et al 1990). TGF-β upregulates plasminogen activator inhibitor and TIMP production whilst down-regulating production of MMP-1; however, recent evidence suggests that it can also up-regulate MMP-13 in normal cartilage (Moldovan et al 1997). IGF-1 has the capacity to stimulate chondrocyte proliferation and the synthesis of aggrecan and hyaluronic acid (Guenther et al 1982, McQuillan et al 1986, Schalkwijk et al 1989). IGF-1 also can decrease the cartilage degradation by anatagonizing Interleukin-1 [IL-1] action (Tyler, 1989). bFGF is a potent mitogenic agent for chondrocytes and can stimulate synthesis of cartilaginous matrix (Kato and Gospodarowicz, 1985).

Other biological factors

Other factors may also have protective effects under certain circumstances. IL-4, IL-13 and IL-10 oppose the effects of pro-inflammatory agents by blocking proteinase secretion, increasing inhibitor production and synthesis of new matrix in many cells. Both IL-4 and IL-13 can prevent IL-1-stimulated matrix resorption. Platelet-derived growth factor (PDGF) has a mitogenic effect on chondrocytes and also stimulates collagenase production in synovial fibroblasts (Cawston and Rowan, 1998).

METHODS OF ARTICULAR CARTILAGE REPAIR

Methods for articular cartilage repair have become popular in clinical practice. In the past, defects of the articular cartilage used to remain untreated causing severe pain and disability to the patients. The older patient used to proceed earlier to corrective osteotomy [high tibial or distal femoral] to reduce the weight stress on the area of cartilage repair (Tippet, 1991) or to total joint replacement. The young and active patient used to have to refrain from sporting activities as these would aggravate the symptoms. The clinical application of methods for the resurfacing of chondral defects has been brightening the prognosis of the repair of the articular cartilage. The early identification of the symptoms is essential for the future management.

Arthroscopy of the joint is still indispensable in order to identify the grade, the size and the location of the defect. This arthroscopic information in conjunction with the patient's expectations and compliance with rehabilitation, will dictate the choice of the appropriate method of treatment. A number of treatments have been explored. The goal of articular cartilage repair is to restore the integrity of the joint surface and to provide full range of pain-free motion, preventing further tissue deterioration.

Arthroscopy and debridement

Arthroscopic lavage and debridement clears the joint of loose articular debris and inflammatory mediators generated by the synovial lining of damaged joints. It may offer temporary relief of pain, but no prospect of long-term cure (Jackson, 1991).

Bone marrow stimulation

In marrow-stimulation techniques [arthroscopic abrasion, subchondral drilling, microfracture] the subchondral bone is penetrated to reach a zone of vascularization, stimulating the formation of a fibrin clot containing stem cells. This clot then differentiates and remodels, resulting, however, in fibrocartilage repair tissue (Johnson, 1991, Rodrigo et al 1994). If the surface is protected from excessive loading,

undifferentiated mesenchymal cells migrate into the clot, proliferate, and differentiate into cells with the morphological features of chondrocytes (Shapiro et al 1993).

Ficat et al (1979) reported good or excellent results in 79% of their patients (85 patients in total) following spongialization (excision of damaged cartilage along with underlying subchondral bone). Johnson (1986) examined joint surfaces after arthroscopic abrasion and found that in some individuals this procedure resulted in the formation of a fibrocartilaginous articular surface that varied in composition. Baumgaertner et al (1990) reported less successful results following arthroscopic abrasion in 44 patients.

The formation of a new articular surface after penetration of the subchondral bone or after abrasion does not necessarily relieve pain. The lack of beneficial clinical outcome may be attributable to the inability of the newly formed tissue to replicate the properties of articular cartilage. It does not have the structure, the composition, the mechanical properties and the durability of articular cartilage. Therefore, even though this tissue covers the subchondral bone, it may fail to distribute loads across the articular surface in a way that avoids pain with loading and additional degeneration of the joint (Mitchell and Shepard, 1976, Buckwalter and Lohmander, 1994). A clinical study by Akizuki et al (1997) comparing the results of high tibial osteotomy alone with osteotomy and abrasion arthroplasty showed no difference in the clinical outcome at 2 to 9 years. Favorable results were reported also by Blevins et al (1998) following microfracture treatment of 178 athletes at mean follow-up 3.7 years.

Continuous passive motion [CPM]

Salter et al (1980) found no beneficial effect of continuous passive motion on the repair of cartilage injuries although in defects that penetrated the subchondral bone, continuous passive motion accelerated repair. O' Driscoll et al (1984, 1986) showed evidence of improving cartilage repair with periosteal grafts when CPM is used. The forced circulation of interstitial fluid during cyclic compression might benefit cartilage metabolism by promoting the transport of various macromolecules, such as growth factors, cytokines, and other enzymes necessary for cartilage metabolism (O'Hara et al

1990). Rodrigo et al (1994) also reported improvement of full-thickness chondral defects in the human knee after debridement and microfracture using CPM.

Corrective osteotomy

An osteotomy is planned in order to decrease loads on the most severely damaged regions of the joint surface, to bring regions of the joint surface that have remaining articular cartilage into opposition with regions that lack articular cartilage, or to correct malalignment that may contribute to symptoms and dysfunction of the joint. Clinical experience has shown that osteotomies of the hip and the knee can decrease symptoms and stimulate the formation of a new articular surface. Osteotomy leads to a decrease in the radiographic signs of joint degeneration, with the improvement including the resolution of subchondral cysts or radiolucent lines, decreased density of subchondral bone, and increased radiographic joint space (Buckwalter and Lohmander, 1994). Long term follow-up of patients who were managed with osteotomy for osteoarthrosis of the hip and the knee have shown, however, that the clinical results deteriorate with time (Insall et al, 1984, Reigstad and Gronmark, 1984, Matthews et al 1988, Berman et al 1991).

Periosteal and perichondrial grafts [Soft tissue arthroplasty]

Studies have shown that perichondrial and periosteal grafts placed in articular cartilage defects can produce new cartilage (Engkvist and Johansson, 1980, Rubak 1982, Rubak et al 1982, Homminga et al 1990, Billings et al 1990). O'Driscoll and Salter (1984) showed that neochondrogenesis in free periosteal autografts was facilitated with continuous passive motion. Synovium-fat-periosteum autograft taken from the medial side of the femoral condyle and sutured into the defect of the knee of mature sheep showed failure of the graft two years later (Rothwell, 1990). Perichondrial graft taken from the cartilaginous covering of a rib can be placed in a joint where it develops into hyaline cartilage (Kon, 1981, Woo et al 1987, Coutts et al 1992). The graft size is limited by the rib size, and several ribs often must be used to harvest sufficient perichondrium to graft

large joints. These techniques are also limited by the tendency towards ossification of the repair tissue (Ritsila, 1994).

A study in sheep, where osteochondral lesions were filled with rib perichondrial grafts secured by either collagen sponges or fibrin glue, showed sufficient filling of the defects and ingrowth to the surrounding cartilage up to 16 weeks post-transplantation (Bruns et al 1997). Another study on 88 patients with up to 52 months mean follow-up reported 91% good results (Bouwmeester et al 1997). However, an 1-year study in rabbits using allogenic perichondrium cell polylactic acid, although it showed grossly successful repairs it also revealed inconsistent subchondral bone formation suggesting that these repairs may not represent structurally normal articular cartilage (Chu et al 1997).

Bone and Meniscal grafts

Bone grafting with callus or cortical bone performed in rats showed hyaline-like repair tissue, although hypocellular and fibrillated (Göransson et al 1995). Frozen meniscal allografts transplanted in rabbits showed good cellular response and matrix synthesis in 24 weeks (Sumen et al 1995).

Osteochondral grafts [Transplantation of articular cartilage]

Osteochondral autografts

This method introduces a new cell population that participates in the repair of articular cartilage. The small number of donor sites limits this method to selected regions of cartilage defect. Clinical studies have shown that this technique can restore the articular surface (Campanacci et al 1985, Yamashita et al 1985, Outerbridge et al 1995, Bobic, 1996). The use of osteochondral plugs derived from the intercondylar notch or femoral trochlea has demonstrated satisfactory repair of cartilage defects with good clinical outcome. This is a technique which also allows the transplantation of multiple grafts (mosaicplasty) and therefore the cover of large defects (Outerbridge et al 1995, Bobic, 1996, Hangody et al 1998).

Osteochondral allografts

Clinical experience with fresh and frozen osteochondral allografts has shown that they can heal to the host tissue and restore articular surface (Bayne et al 1985, Aston and Bentley, 1986, Convery et al 1991, Beaver et al 1992, Garett, 1994, McDermott et al 1985, Mahomed et al 1992, Meyers et al 1989). Gross et al (1992) used fresh allografts for the repair of post-traumatic osteoarticular defects and reported 75% successful results in five years, 64% at ten years and 63% at fourteen years. Frozen allografts are more available and can be prepared in any size. They permit elective reconstruction and allow time for more extensive testing for viral and bacterial infections (Flynn et al 1994). Frozen storage of osteochondral allografts appears to maintain the viability of chondrocytes (Schachar et al 1989) and not to alter the viscoelastic properties of the tissue (Kiefer et al 1989). Larger grafts seem to have higher rate of failure than smaller ones (Lord et al 1988). Stevenson et al (1991) showed that frozen grafts' structure deteriorated and proteoglycan concentration was lower in comparison to fresh grafts. Flynn et al (1994), however, reported results with frozen osteochondral allografts that compared favourably with those using fresh allografts. Toolan et al (1998) reported improved healing up to 12 weeks post-implantation of a chimeric xenograft.

Electrical and sonic stimulation

Enhanced healing has been demonstrated with the use of implanted electrodes in animal models (Lippiello et al 1990). Huang et al (1997) suggested that ultrasound enhances cartilage repair in early stages of degenerative disease and has the effect of arresting further deterioration in the later stages.

Laser Stimulation

Schultz et al (1985) found that superficial cartilage defects exposed to low-dose laser energy demonstrated a reparative process superior to that found in untreated injuries and in injuries treated with higher doses of laser energy. Carbon dioxide experimental laser surgery showed fibrous repair in deep lesions at early assessment and in superficial lesions at later 6 to 12 months assessment (Vangsness et al 1995). The effects of holmium: yttrium-aluminium-garnet [Ho:YAG] laser energy on cartilage have been studied showing no difference between treated and control lesions in horses (Pullin et al 1996). Chondral damage and cartilage slough secondary to Ho:YAG laser following knee arthroscopy has been reported by Thal et al (1996). Reduction of cell viability and the expression of proteoglycans in the repaired articular cartilage and damage in the architecture and histology of the subchondral bone have also been reported (Lane et al 1997).

Chondrogenesis stimulated with Bone Matrix Protein, Growth Hormone and Growth Factors.

Syftestad and Caplan (1986) showed enhanced experimental chondrogenesis following stimulation with bone matrix protein. Growth hormone enhances osteogenesis and chondrogenesis in cells in culture; some of these effects are mediated by Insulin-like Growth Factor-I [IGF-I] which also affects bone matrix formation and cell replication (Hock et al 1988, Slootweg et al 1988, Kassem et al 1993).

Local treatment of chondral or osteochondral defects with growth factors has the potential to stimulate the restoration of the damaged articular surface. Hunziker and Rosenberg (1994, 1996) treated partial-thickness cartilage defects with transforming growth factor-β [TGF-β] and showed that it can stimulate cartilage repair. The responsiveness of cells to growth factors may decline with age (Pfellschifter et al 1993, Martin and Buckwalter, 1996). For the younger patient with isolated chondral or osteochondral defects growth factor based treatment appears promising.

Cell transplantation

A number of experimental studies has shown that transplantation of chondrocytes can promote the restoration of articular cartilage (Bentley and Greer, 1971, Bentley et al 1978, Itay et al 1988, Wakitani et al 1989, Robinson et al 1990, Kamade and Yoshimao, 1991, Brittberg et al 1994, Noguchi et al 1994, Brittberg et al 1996, Shortcroff et al 1996). Brittberg et al (1994) described the use of transplants of autologous chondrocytes for the treatment of localized cartilage defects of the femoral condyle or the patella in twenty three patients. Autologous chondrocyte implantation involved harvesting of chondrocytes from the upper medial femoral condyle of the affected knee. The population of the chondrocytes was cultured in vitro for 5 weeks and was expanded ten-fold. During a second procedure, the defect was covered with a periosteal patch from the proximal tibia, and the cultured chondrocytes were injected beneath the patch. The resulting cartilage was of the hyaline type in twelve of the twenty-three patients and the method showed good or excellent clinical results in sixteen patients.

Some studies have also shown that mesenchymal cells aspirated from bone can produce cartilaginous tissue in goats (Butnariu-Ephrat et al 1996) and that these mesenchymal cells can also repair large osteochondral defects in rabbits (Wakitani et al 1994).

Synthetic materials

A number of synthetic biological and non-biological materials has been developed in the recent years for the repair of articular cartilage defects. Most of these have been trialed as carrier matrices for the delivery and stabilization of growth factors or chondrocytes in the defects (Peppas and Langer, 1994, Parsons, 1985). An artificial matrix, however, may allow, and in some instances stimulate ingrowth of host cells, matrix formation, and binding of new cells and matrix to host tissue (Paletta et al 1992).

Fibrin has been used to implant and allow timed release of a growth factor (Hunziker and Rosenberg, 1994). Fibrin has also been used as an adhesive (Kaplonyi et al 1988) and as chondrocyte carrier (Hendrickson et al 1994). Collagen matrices have been trialed on their own (Speer et al 1979) or as scaffolds for chondrocytes (Sams and Nixon, 1995,

Frenkel et al 1997). Silastic sheet spacers showed no beneficial results (Engkvist and af Ekenstam, 1982). An aromatic porous polyurethane material has also been used with the formation, however, of fibrocartilage as a result (Klompmaker et al 1992). Corkhill et al (1990, 1993) studied in vitro hygrogel gels. Bioactive glasses, hydroxyapatite and hydroxyapatite-glass have also been tried (Suominen et al 1996).

Carbon fibre patches have been used in rabbits (Minns et al 1982, Minns and Muckle, 1989) and then in clinical practice to fill large osteochondral defects. The patch is used as a support to the fibrocartilaginous matrix. Seventy five per cent excellent or good results were reported three years later (Muckle and Minns, 1990). Encouraging results were also reported by Brittberg et al (1994) in 36 patients and by Bentley et al (1998) in 78 patients followed for 6 years. Hemmen et al (1991) also used carbon fibre plugs loaded with chondrocytes. However, Meister et al (1998) in 27 patients with patellar defects, reported discouraging results with low patient satisfaction and with carbon-fibre debris and histiocytic giant-cell reaction in the synovium.

The resorbable polymers polyglycolic acid and polylactic acid have been tried as chondrocyte delivery systems (Freed et al 1993) and in cartilage tissue engeneering attempts (Sittinger et al 1996, Cao et al 1998, Vacanti and Upton, 1994, Grande et al 1997). Although promising in their applications, sterilization and tissue inflammation remain problematic with these biomaterials (Athanasiou et al 1996). No cartilage was found formed in defects which received polyglycolic acid polymer alone without carrying cells (Vacanti et al 1994).

New biomaterials for resurfacing articular cartilage defects are constantly under continuing investigations. Of these, the new poly-ethyl-methacrylate/tetra-hydro-furfuryl methacrylate polymer [PEMA/THFMA] has been experimentally tested in preliminary studies up to 6 weeks post-implantation with promising results (Reissis et al 1994a, Reissis et al 1994b, Reissis et al 1995). This thesis will present results on the PEMA/THFMA polymer's *in vivo* toxicity and comprehensive evaluation of the repair tissue following PEMA/THFMA implantation in articular cartilage defects in rabbits up to 1 year. The efficacy of PEMA/THFMA to repair articular cartilage defects will also be trialed in different rabbit age groups and in comparison to conventional bone cement. Finally, the new polymer will be tested as a drug delivery system for growth hormone.

CHAPTER II

THE NEW POLY-ETHYL-METHACRYLATE / TETRA-HYDRO-FURFURYL-METHACRYLATE *[PEMA/THFMA]* POLYMER

- **Introduction to PEMA/THFMA**
- **In vivo toxicity test of THFMA**

OBJECTIVE

MATERIALS

- 2 young, 2 adult, 2 old rabbits
- THFMA vs MMA monomer

METHOD

- Intravenous administration of monomer concentrations
- Evaluation of heart rate, arterial blood pressure, O_2 saturation

RESULTS

DISCUSSION

INTRODUCTION TO PEMA/THFMA

PEMA/THFMA is an heterocyclic methacrylate polymer system that had originally been developed for use as hearing aid and temporary crown and bridge materials in dentistry. It is based on poly-ethyl-methacrylate (containing 0.6% benzoyl peroxide) powder polymer and tetra-hydro-furfuryl-methacrylate (containing 2.5% v/v N,N,-dimethyl-p-toluidine) liquid monomer.

The conventional poly-methyl-methacrylate/methyl-methacrylate [PMMA/MMA] system that is used as bone cement in orthopaedic surgery, for denture bases, for artificial teeth and orthodontic appliances and for moulds for hearing aid devices, has a number of disadvantages, mainly high exothermic reaction and high polymerisation shrinkage. Various other methacrylate esters have been studied to overcome these problems. Low shrinkage is obtained with high molar volumes, i.e. big molecules. Above C_6, however, alkyl-methacrylates are rubbers. Hence, cyclic methacrylates must be used. The heterocyclic monomer tetra-hydro-furfuryl-methacrylate [THFMA] emerged as a suitable candidate. THFMA is a 5 membered ring with a hetero atom. It is a colourless, transparent, odourless liquid insoluble in water.

The new PEMA/THFMA system, compared to existing poly-methyl-methacrylate [PMMA] based materials, exhibitis much lower shrinkage (Bhusate and Braden, 1985, Patel et al 1987), lower exotherm during polymerisation (Patel and Braden, 1991ii), excellent biological properties (Pearson et al 1986), reasonable mechanical properties with good flexural strengths and exhibited ductile fracture (Patel and Braden, 1991i) and hydrophilicity (Patel and Braden, 1991iii).

The extent of the exothermic reaction during polymerization is of great clinical importance. Tetra-hydro-furfuryl-methacrylate gives lower exotherm with poly-ethyl-methacrylate than with conventional poly-methyl-methacrylate systems. The PEMA/THFMA system polymerizes at room temperature. N,N-dimethyl-p-toluidine is satisfactorily used as activating amine (Patel and Braden, 1991ii).

Tetra-hydro-furfuryl-methacrylate is potentially useful because it does not have the unpleasant odour of n-butyl-methacrylate and has been shown to be relatively non-

irritant in dental use (Pearson et al 1986). PEMA/THFMA is biocompatible in bone and cartilage although not biodegradable (Patel et al 1994). This is probably the reason why it elicits no inflammatory or foreign body response in the host tissues.

An interesting and important feature of this polymer is that it exhibits high water absorption -up to 34%- in vitro (Patel and Braden, 1991iii), not reaching equilibrium for several months. In vivo this polymer should absorb tissue fluids that contain growth factors and hence gain similar biological properties to those of the host tissue. This may create an environment which encourages cartilage overgrowth. The high water content of this polymer is a charcteristic property of normal hyaline articular cartilage. The water uptake process is protracted. Hydration of cartilage is essential because chondrocytes rely upon diffusion for the supply of nutrients. The material swells due to water uptake after polymerization and a tight bond occurs between the polymer and the bone, providing a mechanically stable implant (Downes et al 1994). The high water uptake of the PEMA/THFMA polymer system is also the key to its success as a drug release polymer (Patel et al 1994).

Early results of the new polymer's effect on cartilage repair have been published with regard to macroscopic, histological, immonohistochemical, and ultrastructural characteristics of the repair tissue (Reissis et al 1994a, 1994b, Reissis et al 1995). These results showed that PEMA/THFMA is biocompatible and it encourages the formation of hyaline-like cartilage tissue in full-thickness articular cartilage defects. The present thesis provides equally encouraging long-term results.

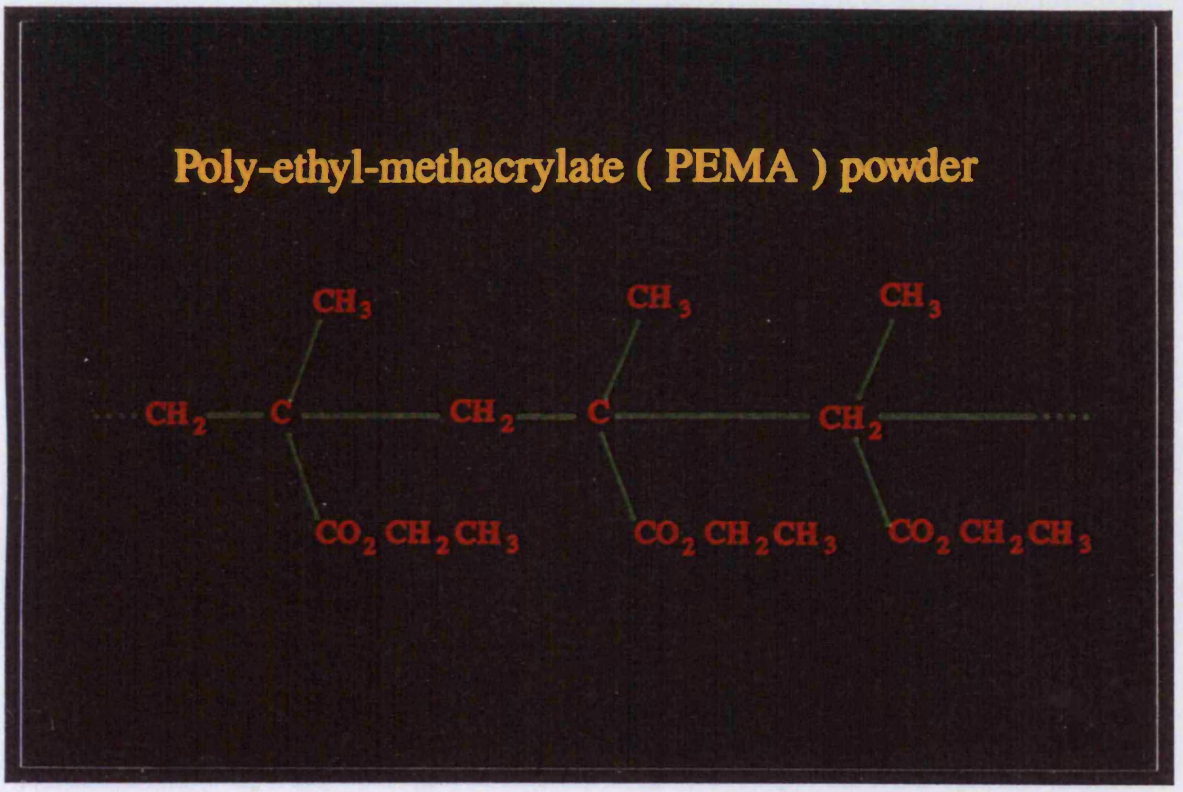


Fig.II.A.1

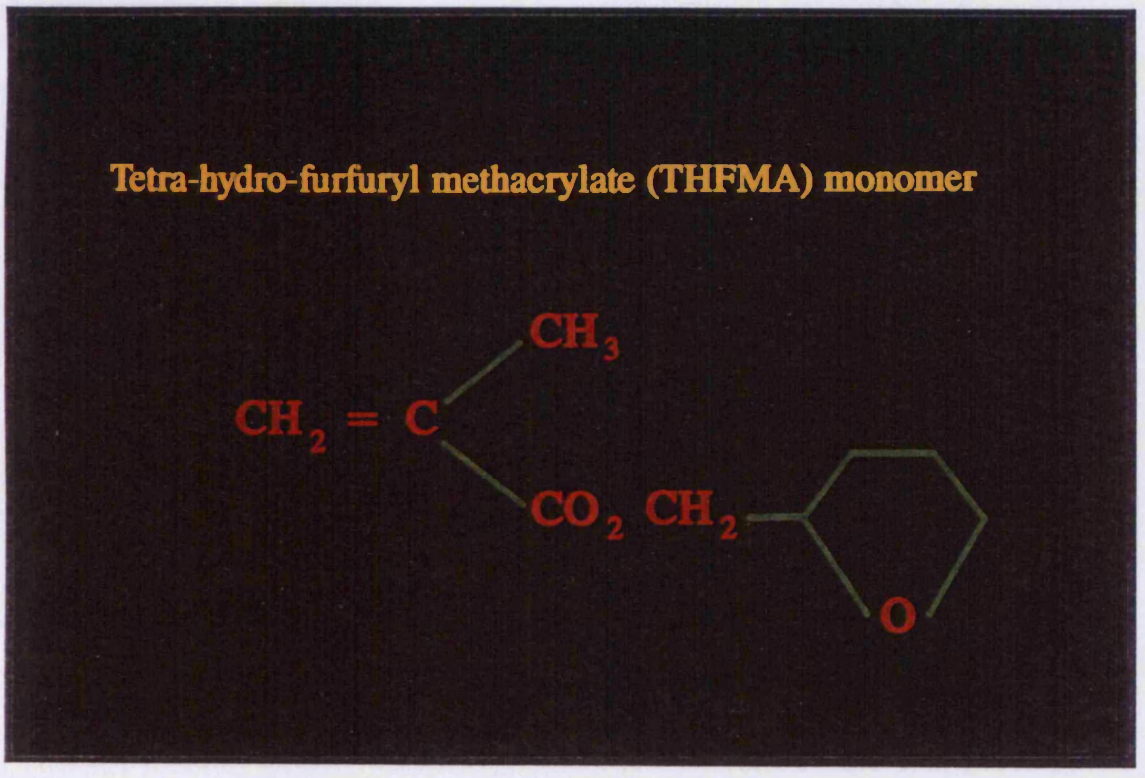


Fig.II.A.2

IN VIVO TOXICITY TEST OF THFMA

OBJECTIVE

This study tests the in vivo toxicity of the new monomer tetra-hydro-furfuryl-methacrylate [THFMA] compared to methyl-methacrylate monomer [MMA]. The monomers were administered intravenously in rabbits of three different age groups.

MATERIALS

Two young [6 months old], two adult [12 months old] and two old [18 months old] female Sandy-Lop rabbits were used. The young rabbits weighed 4.859 kg and 4.880 kg, the adult 5.100 kg and 5.120 kg, and the old rabbits 5.150 kg and 5.180 kg. One rabbit in each age group was injected with THFMA monomer and the other with MMA monomer.

METHOD

The rabbits were anaesthetised with oxygen 1litre/min, nitrous oxide 1litre/min and halothane 0.5-1.5%, breathing spontaneously via a mask throughout the course of the experiment. The injections were performed intravenously via a 23G butterfly in a marginal ear vein. In the THFMA group, 1 ml bolus injection of 60/40 alcohol/saline was followed by injection of 1 ml boluses of 2% [4mg/kg], 4% [8mg/kg], and 10% [20mg/kg] THFMA diluted in 60/40 alcohol/saline. In the MMA group, 1 ml bolus injection of 60/40 alcohol/saline was followed by injection of 1 ml boluses of 2% [4mg/kg], 4% [8mg/kg] and 10% [20mg/kg] MMA also diluted in 60/40 alcohol/saline. The injections were given every 10 minutes and measurements of heart rate, arterial blood pressure [systolic and diastolic] and O_2 saturation [using a pulse oximeter] were recorded every 1 minute using a standard AS3 anaesthesia monitor [Dàtex, Finland].

RESULTS

The results of the monitoring of the vital signs of heart rate, blood pressure and oxygen saturation are demonstrated in tables II.1, II.2, II.3 and II.4 and graphically for both monomers.

Table II.1:
In vivo toxicity assay.
Heart rate monitoring following intravenous injections of THFMA vs MMA monomer

	Heart rate THFMA					Heart rate MMA			
	Young	Adult	Old	THFMA mean		Young	Adult	Old	MMA mean
Obs 1	180	168	153	167	*Baseline*	168	172	153	164
Obs 2	174	154	160	163		176	176	152	168
Obs 3	186	153	156	165		174	163	152	163
Obs 4	184	151	158	164		188	156	160	168
Obs 5	182	158	156	165		193	168	152	171
Obs 6	194	156	148	166		196	163	160	173
Obs 7	185	163	162	170		182	162	162	169
Obs 8	180	168	160	169		189	158	151	166
Obs 9	190	165	158	171		184	159	156	166
Obs 10	184	162	154	167	*60/40 alcohol*	182	168	152	167
Obs 11	182	165	152	166		182	153	150	162
Obs 12	186	168	156	170		186	162	151	166
Obs 13	183	172	152	169		192	158	156	169
Obs 14	189	168	140	166		193	166	152	170
Obs 15	198	164	138	167		201	163	153	172
Obs 16	201	160	146	169		186	162	156	168
Obs 17	190	152	148	163		201	171	162	178
Obs 18	186	160	146	164		192	173	153	173
Obs 19	182	163	152	166		193	168	160	174
Obs 20	186	171	156	171	*2% monomer*	190	169	158	172
Obs 21	180	152	143	158		180	160	146	162
Obs 22	176	156	149	160		178	173	142	164
Obs 23	172	158	158	163		180	174	160	171
Obs 24	179	159	162	167		186	169	154	170
Obs 25	184	158	151	164		196	173	156	175
Obs 26	189	168	143	167		192	182	158	177
Obs 27	185	162	148	165		198	181	160	180
Obs 28	178	153	148	160		206	168	153	176
Obs 29	178	163	146	162		202	173	156	177
Obs 30	183	160	152	165	*4% monomer*	193	182	150	175
Obs 31	165	148	133	149		180	186	133	166
Obs 32	163	143	138	148		178	182	138	166
Obs 33	172	148	133	151		180	176	146	167
Obs 34	178	156	136	157		163	159	152	158
Obs 35	183	159	148	163		184	168	162	171
Obs 36	186	163	146	165		193	173	160	175
Obs 37	184	166	151	167		194	172	168	178
Obs 38	192	162	156	170		195	156	172	174
Obs 39	204	163	152	173		198	162	160	173
Obs 40	189	165	148	167	*10% monomer*	201	182	152	178
Obs 41	157	138	126	140		186	153	143	161
Obs 42	162	133	123	139		183	158	140	160
Obs 43	159	139	128	142		192	168	146	169
Obs 44	168	146	124	146		196	163	150	170
Obs 45	178	158	141	159		190	172	150	171
Obs 46	191	162	148	167		192	176	152	173
Obs 47	186	168	140	165		186	173	162	174
Obs 48	185	162	152	166		182	168	150	167
Obs 49	182	160	158	167		201	190	148	180
Obs 50	183	159	162	168		186	186	152	175

Table II.2:
In vivo toxicity assay.
Systolic blood pressure monitoring following intravenous injections of THFMA vs MMA monomer

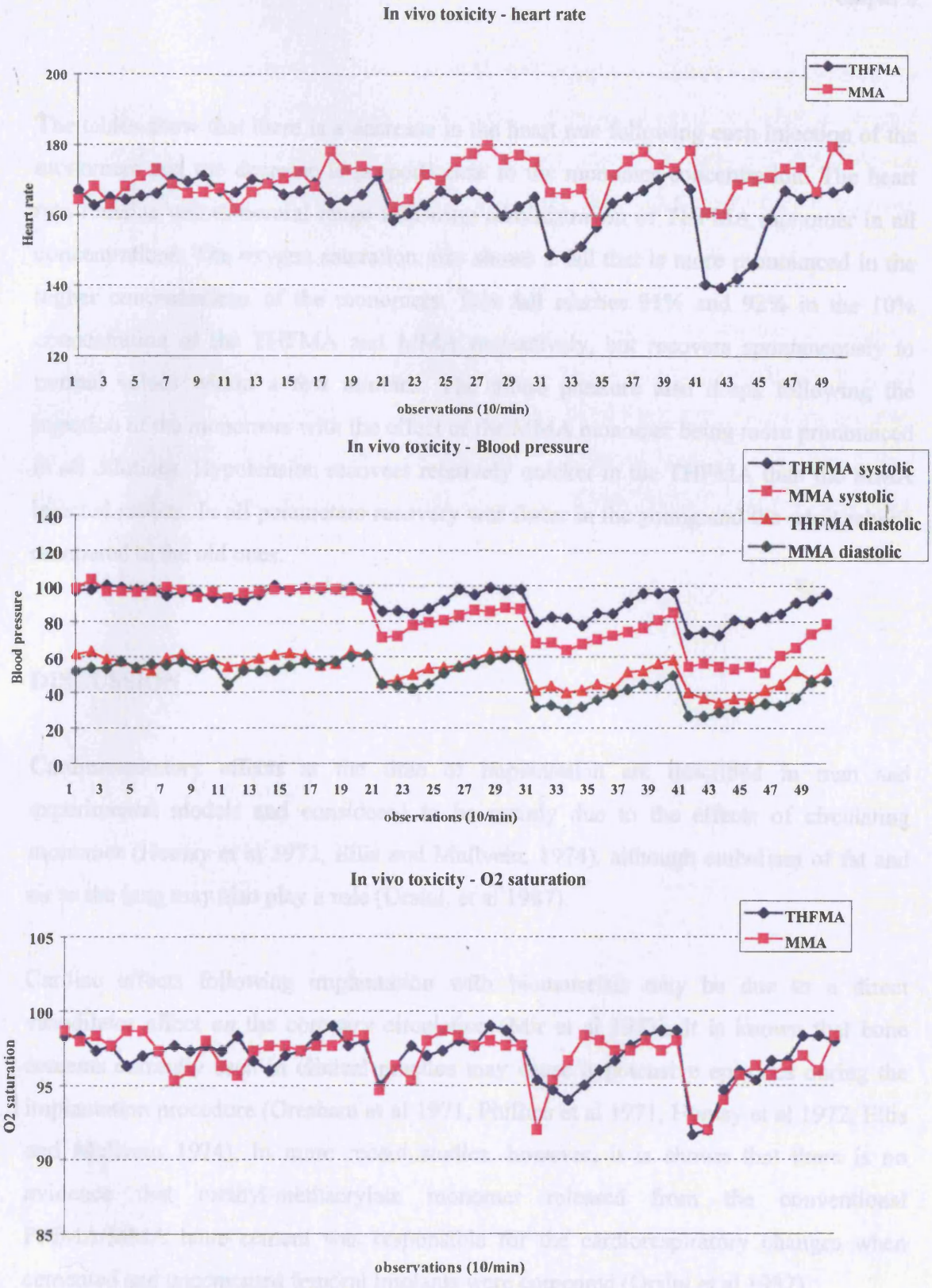
	BP Young	Systolic Adult	THFMA Old	THFMA mean		BP Young	Systolic Adult	MMA Old	MMA mean
Obs 1	96	96	102	97	*Baseline*	96	98	102	99
Obs 2	96	92	106	98		99	106	106	104
Obs 3	98	99	104	100		86	102	103	97
Obs 4	98	100	100	99		89	104	98	97
Obs 5	94	102	98	98		92	102	96	97
Obs 6	93	108	96	99		96	96	99	97
Obs 7	90	93	100	94		98	98	102	99
Obs 8	92	96	102	97		98	93	102	98
Obs 9	92	98	98	96		90	92	99	94
Obs 10	94	96	98	96	*0% monomer*	96	94	102	96
Obs 11	88	92	99	93		92	89	98	93
Obs 12	86	90	100	92		96	92	99	96
Obs 13	89	96	100	95		92	96	102	97
Obs 14	96	98	106	100		96	96	102	98
Obs 15	94	94	104	97		99	92	102	98
Obs 16	96	98	102	99		101	93	100	98
Obs 17	94	94	106	98		102	94	100	99
Obs 18	96	96	102	98		98	96	100	98
Obs 19	93	98	106	99		96	98	98	97
Obs 20	96	94	100	96	*2% monomer*	89	89	98	92
Obs 21	85	88	84	86		72	68	73	71
Obs 22	86	86	86	86		76	63	76	72
Obs 23	83	88	82	84		78	82	73	78
Obs 24	89	83	89	87		76	86	76	79
Obs 25	86	89	100	92		79	85	78	81
Obs 26	92	96	106	98		86	91	73	83
Obs 27	93	98	94	95		84	92	83	86
Obs 28	98	101	98	99		83	86	88	86
Obs 29	95	106	92	98		89	88	86	88
Obs 30	96	102	96	98	*4% monomer*	86	92	83	87
Obs 31	82	80	76	79		71	72	60	68
Obs 32	86	83	78	82		68	78	58	68
Obs 33	83	86	76	82		69	68	54	64
Obs 34	85	74	74	78		73	69	60	67
Obs 35	86	82	86	85		78	70	62	70
Obs 36	82	89	82	84		72	76	68	72
Obs 37	89	94	89	91		81	78	63	74
Obs 38	94	98	96	96		86	76	67	76
Obs 39	96	93	99	96		91	82	69	81
Obs 40	93	96	102	97	*10% monomer*	90	89	76	85
Obs 41	81	73	64	73		62	53	48	54
Obs 42	76	78	68	74		68	58	43	56
Obs 43	74	74	69	72		63	52	48	54
Obs 44	78	79	85	81		66	50	43	53
Obs 45	76	76	86	79		69	51	43	54
Obs 46	82	84	81	82		63	50	40	51
Obs 47	86	89	78	84		72	61	49	61
Obs 48	91	92	89	91		76	68	52	65
Obs 49	96	81	99	92		78	73	68	73
Obs 50	93	91	103	96		86	81	69	79

Table II.3:
In vivo toxicity assay.
Diastolic blood pressure monitoring following intravenous injections of THFMA vs MMA monomer

	BP Young	Diastolic Adult	THFMA Old	THFMA mean		BP Young	Diastolic Adult	MMA Old	MMA mean
Obs 1	68	63	52	61	*Baseline*	48	46	52	52
Obs 2	72	65	52	63		56	49	56	54
Obs 3	68	52	56	59		54	53	53	53
Obs 4	62	53	58	58		62	56	54	57
Obs 5	52	56	53	54		54	62	46	54
Obs 6	50	53	54	52		59	61	48	56
Obs 7	63	61	54	59		62	58	46	55
Obs 8	64	62	58	61		68	60	43	57
Obs 9	59	51	59	56		64	49	48	54
Obs 10	61	51	60	58	*60/40 alcohol*	63	56	49	56
Obs 11	58	51	54	54		30	52	50	44
Obs 12	61	50	56	56		53	56	48	52
Obs 13	62	56	59	59		52	58	46	52
Obs 14	60	62	61	61		54	60	43	52
Obs 15	62	62	62	62		56	62	46	55
Obs 16	59	63	60	61		58	66	46	57
Obs 17	56	51	58	55		60	64	43	56
Obs 18	59	56	53	56		62	62	48	57
Obs 19	68	61	61	63		68	60	52	60
Obs 20	68	63	61	61	*2% monomer*	66	64	53	61
Obs 21	58	38	40	45		46	46	42	45
Obs 22	56	42	43	47		43	48	40	44
Obs 23	62	40	48	50		43	43	40	42
Obs 24	68	46	46	53		48	48	38	45
Obs 25	62	49	51	54		52	52	48	51
Obs 26	59	53	52	55		58	56	48	54
Obs 27	59	62	53	58		64	58	46	56
Obs 28	63	68	56	62		63	60	52	58
Obs 29	72	59	58	63		66	62	50	59
Obs 30	71	62	56	63	*4% monomer*	60	68	48	59
Obs 31	52	33	38	41		38	33	24	32
Obs 32	56	38	36	43		36	38	24	33
Obs 33	50	31	38	40		32	36	22	30
Obs 34	48	32	43	41		38	33	24	32
Obs 35	61	31	42	45		39	40	28	36
Obs 36	62	30	34	42		39	48	30	39
Obs 37	62	46	46	51		46	43	36	42
Obs 38	59	48	49	52		48	49	36	44
Obs 39	61	49	58	56		46	52	32	43
Obs 40	68	53	53	58	*10% monomer*	51	56	41	49
Obs 41	60	31	28	40		30	28	22	27
Obs 42	54	30	26	37		31	26	22	26
Obs 43	43	29	30	34		38	28	20	29
Obs 44	46	27	36	36		36	30	22	29
Obs 45	49	31	28	36		34	40	20	31
Obs 46	52	36	36	41		41	42	18	34
Obs 47	46	39	48	44		38	42	18	33
Obs 48	56	48	53	52		42	48	20	37
Obs 49	59	39	43	47		64	46	28	46
Obs 50	64	52	42	53		62	46	31	46

Table II.4:
In vivo toxicity assay.
Oxygen saturation monitoring following intravenous injections of THFMA vs MMA monomer

	O2 Saturation THFMA					O2 Saturation MMA			
	Young	Adult	Old	THFMA mean		Young	Adult	Old	MMA mean
Obs 1	99	98	98	98	*baseline*	99	99	98	99
Obs 2	98	98	99	98		98	98	98	98
Obs 3	97	96	99	97		96	100	99	98
Obs 4	96	99	98	98		96	99	98	98
Obs 5	94	99	96	96		98	99	99	99
Obs 6	96	99	96	97		99	98	99	99
Obs 7	98	99	95	97		98	96	98	97
Obs 8	99	98	96	98		94	96	96	95
Obs 9	99	98	95	97		96	94	98	96
Obs 10	99	98	96	98	*60/40 alcohol*	98	98	98	98
Obs 11	99	98	95	97		99	96	94	96
Obs 12	98	99	98	98		99	94	94	96
Obs 13	99	96	95	97		96	98	98	97
Obs 14	94	98	96	96		96	99	98	98
Obs 15	98	96	97	97		96	99	98	98
Obs 16	98	98	96	97		96	98	98	97
Obs 17	99	98	98	98		98	96	99	98
Obs 18	99	99	99	99		96	98	99	98
Obs 19	98	99	96	98		98	99	98	98
Obs 20	99	98	97	98	*2% monomer*	99	99	97	98
Obs 21	94	97	94	95		96	93	95	95
Obs 22	96	98	95	96		98	96	95	96
Obs 23	99	98	96	98		99	93	94	95
Obs 24	99	98	94	97		99	99	96	98
Obs 25	98	98	96	97		99	99	98	99
Obs 26	99	99	96	98		98	99	98	98
Obs 27	98	98	97	98		96	98	99	98
Obs 28	99	99	99	99		98	98	98	98
Obs 29	99	99	98	99		96	98	99	98
Obs 30	99	98	96	98	*4% monomer*	96	98	99	98
Obs 31	96	98	92	95		93	90	93	92
Obs 32	94	96	94	95		96	94	96	95
Obs 33	94	94	94	94		96	98	96	97
Obs 34	93	98	94	95		98	99	98	98
Obs 35	96	98	94	96		99	99	96	98
Obs 36	96	98	96	97		99	99	94	97
Obs 37	99	96	98	98		98	98	96	97
Obs 38	99	98	98	98		98	98	98	98
Obs 39	99	99	98	99		96	98	98	97
Obs 40	99	99	98	99	*10% monomer*	98	99	97	98
Obs 41	90	93	92	92		92	93	93	93
Obs 42	92	92	92	92		92	92	92	92
Obs 43	91	99	94	95		96	93	93	94
Obs 44	98	96	94	96		99	94	94	96
Obs 45	98	92	96	95		99	98	92	96
Obs 46	98	96	96	97		99	98	90	96
Obs 47	96	98	96	97		98	93	98	96
Obs 48	98	99	98	98		96	98	97	97
Obs 49	99	98	98	98		94	99	95	96
Obs 50	98	99	97	98		98	99	98	98



Graphic presentation of vital sings *[mean values]* following intravenous administration of THFMA vs MMA monomers:

observations 1-10:baseline, observations 11-20: 60/40 alcohol/saline, observations 21-30: monomer 2%, observations 31-40: monomer 4%, observations 41-50: monomer 10%.

The tables show that there is a decrease in the heart rate following each injection of the monomers and the decrease is proportionate to the monomer concentration. The heart rate remains within normal range following administration of THFMA monomer in all concentrations. The oxygen saturation also shows a fall that is more pronounced in the higher concentrations of the monomers. This fall reaches 91% and 92% in the 10% concentration of the THFMA and MMA respectively, but recovers spontaneously to normal values within a few minutes. The blood pressure also drops following the injection of the monomers with the effect of the MMA monomer being more pronounced in all dilutions. Hypotension recovers relatively quicker in the THFMA than the MMA injected rabbits. In all parameters recovery was faster in the young and the adult rabbits compared to the old ones.

DISCUSSION

Cardiorespiratory effects at the time of implantation are described in man and experimental models and considered to be mainly due to the effects of circulating monomer (Homsy et al 1972, Ellis and Mullvein, 1974), although embolism of fat and air to the lung may also play a role (Orsini, et al 1987).

Cardiac effects following implantation with biomaterials may be due to a direct vasodilator effect on the coronary circulation (Mir et al 1973). It is known that bone cements currently used in clinical practice may cause hypotensive episodes during the implantation procedure (Gresham et al 1971, Phillips et al 1971, Homsy et al 1972, Ellis and Mullvein 1974). In more recent studies, however, it is shown that there is no evidence that methyl-methacrylate monomer released from the conventional PMMA/MMA bone cement was responsible for the cardiorespiratory changes when cemented and uncemented femoral implants were compared (Orsini et al 1987).

Revell et al (1992) when testing the toxicity of MMA and n-butyl-methacrylate monomers, reported dose-related hypotensive response when injected intravenously in rabbits. In our study intravenous injection of the THFMA and MMA monomers also

caused reduction in heart rate, blood pressure and oxygen saturation which seems to be dose-related. These effects on the vital sings, however, showed recovery to normal values within a very short time. THFMA had the less dramatic effects in all dilutions and thus it appears to be the safer of the two in its effects on the cardiovascular / respiratory system.

Homsy et al (1972) reported blood levels of MMA following hip arthroplasty at 2 mg/kg. This is a level considerably below that administered to the rabbits in our study. It seems that THFMA is no more toxic in rabbits than MMA in all three age groups studied and, since MMA levels in clinical practice produce no apparent toxic effects, it is highly unlikely that THFMA monomer released from the PEMA/THFMA polymer will have any toxic side effects in humans.

CHAPTER III

PEMA/THFMA POLYMER IN THE REPAIR OF FULL-THICKNESS ARTICULAR CARTILAGE DEFECTS

A. Scoring System for the assessment of articular cartilage repair

B. Macroscopic and Histolocical/Histochemical assessment of repair tissue

C. Immunohistochemical assessment of repair tissue

D. Electron Microscopy of repair tissue

E. Image Analysis of repair tissue

CHAPTER III.A

SCORING SYSTEM FOR THE ASSESSMENT OF ARTICULAR CARTILAGE REPAIR

A scoring system for the assessment of the repair tissue in articular cartilage defects should be simple to use and should take into consideration characteristics of the articular cartilage that have been proven to be of clinical significance. O'Driscoll et al (1988) have developed a grading scale for the histochemical and histological assessment of the repair tissue in full-thickness articular cartilage defects. The scale included no macroscopic criteria i.e. evaluation of the total area of the defect resurfaced, which is of paramount clinical importance. The grading scale applied was different for each criterion depending on the number of sub-groups noted rather than the consistency of the particular parameter throughout the repair or its clinical significance. In 1992, Pineda et al developed a semiquantitative scale for histological grading of the natural healing process of defects drilled into articular cartilage. Their scale composed of four paramenters: percent filling of the defect, reconstitution of the osteochondral junction, matrix staining and cell morphology. This grading scale is also based on histological only criteria up to 4 months of observations and, as the authors addressed, it may not necessarily correspond to clinical pathology.

The scoring system that has been developed for this study is numerical, based on seven macroscopic and histological/histochemical criteria. These can be assessed during experimental studies for evaluation and comparison between treatments and also in clinical applications in humans by using arthroscopy and simple histology. When applied in humans, this scoring system may also be complemented with clinical criteria that will monitor the patient's progress.

The seven criteria of the scoring system include:

1. **Area**

   The total area of the defect that has been resurfaced is of paramount importance. Complete resurfacing of the defect allows more physiological use of the joint and inevitably reduces the amount of inflammation and pain. The larger the percentage of

the defect resurfaced, the larger the area score of the repair, keeping in mind that wider lesions can also cause abrasion of the articular cartilage that articulates with the defect.

2. Level

The ideal repair comprises repair tissue that grows at the same level as the adjacent normal articular cartilage. Minimal depression/elevation do not appear to cause significant functional disability, although gross depression of the repair tissue leads to formation of intra-articular 'steps' that can cause secondary damage to the opposing articular cartilage and to the normal articular cartilage adjacent to the defect.

3. Surface

The smooth and glistering surface of the repair tissue allows frictionless movement to occur in the joint and causes no damage to the opposing articular cartilage. Moreover, the more irregular the repair tissue surface is, particularly when disrupted by fissures, the less resilient the tissue can be to mechanical loading.

4. Cells

Predominance of chondrocytes in the repair tissue is of paramount importance, although a percentage of fibroblast-like cells has been noted in all methods of clinical and experimental repair of articular cartilage reported to date. However, the presence of other cells, such as fat cells and osteoblasts can compromise the biological and mechanical properties of the repair tissue and can lead to early degenerative changes.

5. Proteoglycans [PGs]

The amount of proteoglycans in the repair tissue characterises the total amount of water that can be retained in the tissue, thus reflecting the ability of the repair tissue to sustain compression loading. Low concentration of proteoglycans can lead to earlier degeneration of the repair tissue in the long-term.

6. **Structure**

Hyaline-like appearance of the repair tissue, comprising of high concentration of proteoglycans in the deeper layers of the repair tissue, combined with even distribution of chondrocytes throughout the whole of the repair tissue, promises more physiological function of the repair tissue in the joint. Reversely, grossly disorganized matrix and the presence of clusters of chondrocytes will score lower in the repair tissue.

7. **Bonding**

Complete bonding of the repair tissue to the adjacent normal articular cartilage creates a stable foundation that helps in the even distribution of the mechanical loading on the repair tissue, thus protecting the repair tissue and the adjacent to the defect normal articular cartilage from early degeneration. On the contrary, minimal or incomplete bonding results in abnormal micro-movement between the repair tissue and the adjacent normal articular cartilage with subsequent degeneration in both areas.

In a pilot study conducted prior to formulating this scoring system, macroscopic and histological/histochemical assessments were performed on sections from different sites of the repair tissue. It was noted that the level of resurfacing, the surface appearance of the repair tissue and its structure as well as the amount of proteoglycans in the newly formed tissue can vary significantly from one site to another in the same repair tissue. A solution could have been to assess separately numerous sections in the same defect taken from different sites and to calculate mean values. This method, however, would have introduced new variables, the most important being the different diameter of the defect in different sites and would have complicated the scoring system. Thus, it was decided to score all criteria from sections taken from the centre of the defect where the diameter of the repair is at its maximum length, and to score these four criteria [level, surface, PGs and structure] with lower maximum points [10 points].

By contrast, the total area of the defect resurfaced, the quality of the bonding of the repair tissue to the adjacent normal articular cartilage and the predominant type of cells in the repair tissue scored similarly throughout the whole of the repair tissue and

therefore it was hypothesised that the score in the centre of the repair represented more accurately the mean score in the whole repair. Thus, it was decided to score these three criteria [area, cells and bonding] with higher maximum points [20 points].

The total maximum points that the repair tissue can score are 100 and the lowest score is 0. The repair tissue can be poor [total score 0-45], good [total score 50-80] or excellent [85-100]. The articular cartilage repair scoring system has as follows:

Articular Cartilage Repair Scoring System.

AREA	**Score**
100% resurfacing	20
>50% resurfacing	10
<50% resurfacing	0
LEVEL	**Score**
Normal cartilage level	10
Minimal depression / elevation	5
Gross depression	0
SURFACE	**Score**
Smooth, glistering	10
Irregular, opaque	5
Disrupted by fissures	0
CELLS	**Score**
Chondrocytes	20
Fibroblasts	10
Other	0
PGs	**Score**
Normal	10
Moderate	5
Low	0
STRUCTURE	**Score**
Hyaline-like tissue	10
Moderately disorganised	5
Grossly disorganised	0
BONDING	**Score**
Complete	20
Incomplete	10
Minimal	0
ARTICULAR CARTILAGE REPAIR	**TOTAL SCORE**
Excellent	85 - 100
Good	50 - 80
Poor	0 - 45

CHAPTER III.B

MACROSCOPIC AND HISTOLOGICAL/HISTOCHEMICAL ASSESSMENT OF THE REPAIR TISSUE FOLLOWING IMPLANTATION OF PEMA/THFMA POLYMER IN FULL-THICKNESS ARTICULAR CARTILAGE DEFECTS

OBJECTIVE

MATERIALS

- 120 adult rabbits
- PEMA vs CONTROL

METHOD OF IMPLANTATION

METHODS OF ASSESSMENT

- Macroscopic evaluation at 6 weeks, 3 months, 6 months and 1 year
- Histological/Histochemical evaluation at 6 weeks, 3 months, 6 months and 1 year

RESULTS

DISCUSSION

OBJECTIVE

This study aims to assess the chondrogenic potential of PEMA/THFMA polymer in large full-thickness articular cartilage defects in the weight-bearing area of the knee joint.

MATERIALS

One hundred and twenty female Sandy-Lop adult rabbits (10-12 months of age and 3.6-4.7 [mean 4.1] kg of weight) were used for the experiment. Both knees were operated on in each animal, the one knee receiving the new biomaterial [PEMA] and the contralateral knee receiving no biomaterial and allowed to repair 'naturally' [CONTROL].

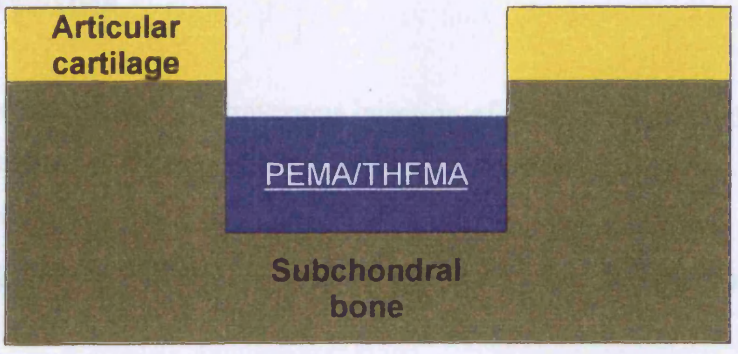
METHOD OF IMPLANTATION

Anaesthesia was induced by intravenous injection of Methohexitone Sodium (Brietal, 10mg/kg) and maintained by 2% Halothane and a 3:2 mixture of Nitrous Oxide and Oxygen, through a fixed face mask. The knee joint was exposed through a 2cm straight midline skin incision and a medial parapatellar approach. The osteochondral defect was created in the femoral trochlea, 10mm proximal to the level of insertion of the anterior cruciate ligament into the distal femur. The femoral trochlea articulates directly with the patella throughout the whole range of movement of the knee joint, and is a heavily loaded area of articular cartilage with significant shear forces applied by the articulating patella. A circular articular cartilage and subchondral bone defect was created by hand, using a 4.5mm drill bit. The depth of drilling was carefully controlled to be 4mm in the biomaterial group and 2mm in the control group. All osteochondral debris from the drill holes were thoroughly washed with normal saline prior to polymer implantation.

Preparation of the polymer system was made at room temperature using a sterile spatula (Patel and Braden, 1991ii) by mixing by hand 1gr PEMA (powder containing 8% Barium Sulphate) with 0.6ml THFMA (liquid containing 2.5% V/V N,N - dimethyl - p - toluidine) for two minutes. The resulting paste was then sucked into a 1ml sterile syringe and 0.1ml of this was injected into the created osteochondral defect of one knee

[PEMA group]. This polymer volume forms a 2mm deep plug and therefore, subsequently, a 2mm deep by 4.5mm wide osteochondral defect was left to be biologically repaired in both the biomaterial and the control groups.

Polymerisation occurred in situ. In most cases bleeding from the defects was minimal, not interfering with accurate positioning of the polymer plugs and setting of the polymer. During polymerisation, using the flat end of a 4.5mm wide cylindrical metallic rod, constant pressure was applied at the top surface of the plugs, thus carefully controlling the level of implantation into the subchondral bone, 2mm below the level of the adjacent articular cartilage in order to allow full-thickness repair tissue to form on top. At this part of the joint the thickness of the articular cartilage is 1.4mm (range 1.3mm – 1.6mm). The positioning of the polymer plug is illustrated in the scheme below:



Bleeding was carefully controlled and wounds were closed using absorbable sutures. The operated limbs were not immobilised. The rabbits were group-housed in floor-pens (Batchelor, 1991). Full range of movement and full weight bearing were allowed immediately post-operatively allowing maximum loading on the defect area.

METHODS OF ASSESSMENT

Macroscopic and Histological/Histochemical assessment was done at 6 weeks, 3 months, 6 months and 1 year post-implantation. Thirty rabbits were assigned in each observational period. General clinical observations were made and the assessment of the repair tissue in the PEMA/THFMA biomaterial group and the CONTROL group was based on the seven criteria of the Articular Cartilage Repair Scoring System. Three examiners assessed all specimens independently and blindly. The score given to each criterion for each specimen represents the score given by at least two of the three independent examiners. In 86% of the specimens the score given was the same by all three examiners, and in 14% the score was given the same by two examiners. During this study, none of the specimens was given a different score by each one of the three examiners.

Macroscopic evaluation

The rabbits were sacrificed by intravenous injection of pentobarbitone sodium (Euthatal 600mg). The presence or absence of knee swelling and/or contractures was recorded. The knee joints were exposed and the presence of excessive synovial fluid, intra-articular adhesions or degenerative changes was also noted. Samples of the adjacent to the defect synovium were placed in 10% neutral buffered formalin and sent for histology, to investigate an inflammatory or foreign body reaction to the biomaterial [biocompatibility]. The total resurfaced area of the defect [AREA criterion in the Scoring System], the repair tissue level in relation to the adjacent articular cartilage [LEVEL criterion] and the repair surface appearance [SURFACE criterion] were assessed.

Histological and histochemical evaluation

Distal femora, including the resurfaced defect area, were fixed in 4% paraformaldehyde and 0.3% glutaraldehyde in 0.1M sodium cacodylate for seven days at 4^0C. Excessive bone of the distal femur was removed and specimens were decalcified in neutral EDTA (14% solution in distilled H_2O) at 4^0C, until mineral (calcium) could no longer be detected radiographically.

Specimens were washed in 0.1M sodium cacodylate for two days and dehydrated through a graded series of ethanols (70%, 90%, 100%) and xylene for 24 hours at room temperature, with three changes of fresh xylene. Specimens were then impregnated into wax over 24 hours at 60^0C, with three changes of fresh wax, before being embedded into wax blocks and stored at room temperature. Sagittal sections, 5 μm thick, cut from the central part of the defect and including the whole of the repair tissue and part of the adjacent to the defect normal articular cartilage, were stained with haematoxylin/eosin and safranin-O stains.

The haematoxylin/eosin and safranin-O stained sections were assessed by means of the predominant type of cell in the repair tissue [CELLS criterion in the Scoring System], the concentration of extracellular proteoglycans [PGs criterion], the structural characteristics of the repair tissue in terms of cell/matrix organisation [STRUCTURE criterion] and the quality of bonding between the repair tissue and the adjacent normal articular cartilage [BONDING criterion].

Sagittal sections, 1 μm thick were cut with a diamond knife from 16 randomly selected specimens [2 from the PEMA group and 2 from the control group at 6 weeks, 3 months, 6 months and 1 year] and were stained with 1% toluidine blue in 1% borax (Robinson and Gray, 1990). These sections were later prepared for electron microscopy according to the method described in Chapter III.D.

RESULTS

Clinical observations

- **Deaths and excluded animals**

The rabbits lived happily in the open pens during the experimental period. Five rabbits died unexpectedly and two other rabbits were culled as a result of continuing otitis media that was impossible to be controlled on antibiotics. The incidents happened at 5 weeks [3 rabbits, 2 died and I was culled, finally assigned in the 6 week experimental period], at 7 months [1 rabbit died, assigned in the 6 month experimental period] and at 11 months [3

rabbits, 2 died and 1 was culled, assigned at the 1 year experimental period]. All seven rabbits were replaced in their original groups.

- **Contractures**

Twenty seven knees were detected with fixed flexion deformity [21 (17.5%) in the control group and 6 (5%) in the biomaterial group]. The deformities consisted of loss of extension in the knee joint although flexion was complete. The fixed flexion deformity ranged between 25 and 50 degrees in the control group and between 30 and 40 degrees in the biomaterial group.

- **Intra-articular adhesions**

Twenty-eight (23.3%) knees in the control group and 7 (5.8%) knees in the biomaterial group were found to have intra-articular adhesions. Twelve of these knees from the control group were also found to have fixed flexion deformity while none of the knees with intra-articular adhesions demonstrated fixed flexion deformity in the biomaterial group. All intra-articular adhesions were primarily in the anterior compartment of the knee although additional adhesions in the medial compartment were noted in 7 knees in the control group.

- **Effusion**

Four (3.3%) knees in the control group and three (2.5%) in the biomaterial group were noted to have significantly increased amount of synovial fluid which was cultured and was found sterile in all cases.

- **Osteoarthritic changes**

Moderate/advanced osteoarthritic changes were noted in 2 (3.3%) of the knees of the PEMA group at 6 months and in 7 (11.6%) of the knees in the 1-year specimens (5 in the control group and 2 in the PEMA group). The changes consisted primarily of destruction of the articular cartilage in the femoral condyles (predominantly the medial condyle) and formation of peripheral osteophytes.

- **Non-specific inflammation of the synovium and foreign body reaction**

Mild inflammatory response of the synovium was noted in 26 (21.6%) of the control knees and in 11 (9.1%) of the knees in the biomaterial group. The changes in the biomaterial group were noted exclusively in the 6-week and 3-month specimens (9 and 2 knees respectively). In the control group the inflammatory response was more common in the 6-month and 1-year specimens (7 and 10 knees respectively) with less reaction noted at 6 weeks (4 knees) and 3 months (4 knees). There was no evidence of infection or foreign body reaction in the synovial membrane samples. There was prominent vilous formation in the synovial tissue with chondroid metaplasia of the stroma with occasional incorporated calcified bony particles, some of which were surrounded by multinucleate giant cells, in 19 of the control knees. Mild vilous formation without chondroid metaplasia of the stroma was noted in 3 of the knees of the biomaterial group.

Macroscopic and Histological/Histochemical results

All results are demonstrated in Tables III.B.1, III.B.2, III.B.3, III.B.4, and III.B.5 followed by statistical analysis and Figures III.B.1 – III.B.28.

At all observational periods the PEMA group scored higher than the control group in all seven criteria. At 6 weeks, the highest mean scores in the PEMA group were for the surface (9 out of 10), for the cells (18 out of 20) and for the proteoglycans (9.5 out of 10). The total mean scores were 80.83 in the PEMA and 42.83 in the control groups. At 3 months, the highest mean scores in the PEMA group were for the cells (15.33 out of 20), for the bonding (15.33 out of 20) and for the proteoglycans (8.67 out of 10). The total mean scores in the PEMA group was 73.50 and in the control group 50.00. At 6 months, the highest mean scores were for the cells (15.33 out of 20), for the proteoglycans (8.5 out of 10), for the level (8 out of 10) and for the area resurfaced (15 out of 20). The total mean scores in the PEMA group was 73.33 and in the control group 50.33. Finally at 1 year, the higher mean scores were for the area (16 out of 20), for the cells (15 out of 20) and for the level (8.17 out of 10). The total mean scores were 69.33 in the PEMA and 46.00 in the control groups.

Table III.B.1:

Macroscopic and Histological/Histochemical assessment per criterion
Scoring at 6 weeks. Rabbits *1-30*
PEMA vs CONTROL

PEMA	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*
Area	20	20	10	20	20	10	0	20	10	20	20	20	20	20	10	20	0	0	20	20	10	20	10	20	10	20	0	20	20	20
Level	10	5	10	10	10	0	0	10	10	10	10	5	10	10	10	10	0	5	10	10	10	10	10	10	10	5	0	10	10	10
Surface	10	5	10	10	10	10	5	10	10	10	10	10	10	10	10	10	0	5	10	10	10	10	10	10	10	10	5	10	10	10
Cells	20	20	20	20	20	20	10	20	20	20	20	20	20	20	20	20	10	10	10	20	20	20	20	20	20	20	0	20	20	20
PGs	10	10	10	10	10	10	5	10	10	10	10	10	10	10	10	10	5	10	10	10	10	10	10	10	10	10	5	10	10	10
Structure	10	5	5	5	10	5	0	5	5	5	5	10	5	10	10	10	0	0	5	5	10	10	5	5	5	0	0	0	10	5
Bonding	20	20	20	20	10	20	10	20	20	20	20	10	10	20	20	10	0	10	20	20	10	20	20	20	10	20	0	20	20	20
TOTAL SCORE	**100**	**85**	**85**	**95**	**90**	**75**	**30**	**95**	**85**	**95**	**95**	**85**	**85**	**100**	**90**	**90**	**15**	**40**	**85**	**95**	**80**	**100**	**85**	**95**	**75**	**85**	**10**	**90**	**10**	**95**

CONTROL	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*
Area	10	0	10	10	10	20	10	10	20	0	0	0	10	20	10	10	10	10	10	20	20	10	0	10	0	0	0	10	20	0
Level	5	0	5	5	5	10	5	10	10	0	5	0	10	5	5	5	5	10	10	10	5	0	0	0	10	10	0	5	5	0
Surface	0	5	5	5	10	10	10	5	0	0	5	0	10	5	5	5	5	5	5	10	5	0	5	5	10	10	5	5	5	0
Cells	10	10	10	10	10	20	10	10	20	10	0	0	10	10	10	10	0	10	10	20	10	0	10	10	0	0	10	20	10	10
PGs	5	0	5	10	5	10	10	5	0	5	0	0	10	10	5	5	0	10	5	5	10	0	10	5	0	5	10	10	0	5
Structure	0	0	0	0	0	5	0	0	0	5	0	0	0	0	5	5	5	0	0	0	0	0	0	5	0	0	0	5	5	5
Bonding	10	10	0	0	10	10	0	10	10	10	0	0	10	10	20	10	10	0	0	20	20	10	0	10	0	0	0	10	10	10
TOTAL SCORE	**40**	**25**	**35**	**40**	**50**	**85**	**45**	**50**	**60**	**30**	**10**	**0**	**60**	**60**	**60**	**50**	**35**	**45**	**40**	**85**	**70**	**20**	**25**	**45**	**20**	**25**	**25**	**65**	**55**	**30**

	PEMA MEANS	*CONTROL MEANS*	*PEMA SD*	*CONTROL SD*
Area	15.00	9.00	7.31	7.12
Level	8.00	5.17	3.62	3.82
Surface	9.00	5.17	2.42	3.34
Cells	18.00	9.33	4.84	5.83
PGs	9.50	5.33	1.53	3.92
Structure	5.33	1.50	3.46	2.33
Bonding	16.00	7.33	6.21	6.40
TOTAL SCORE	**80.83**	**42.83**	**24.21**	**20.33**

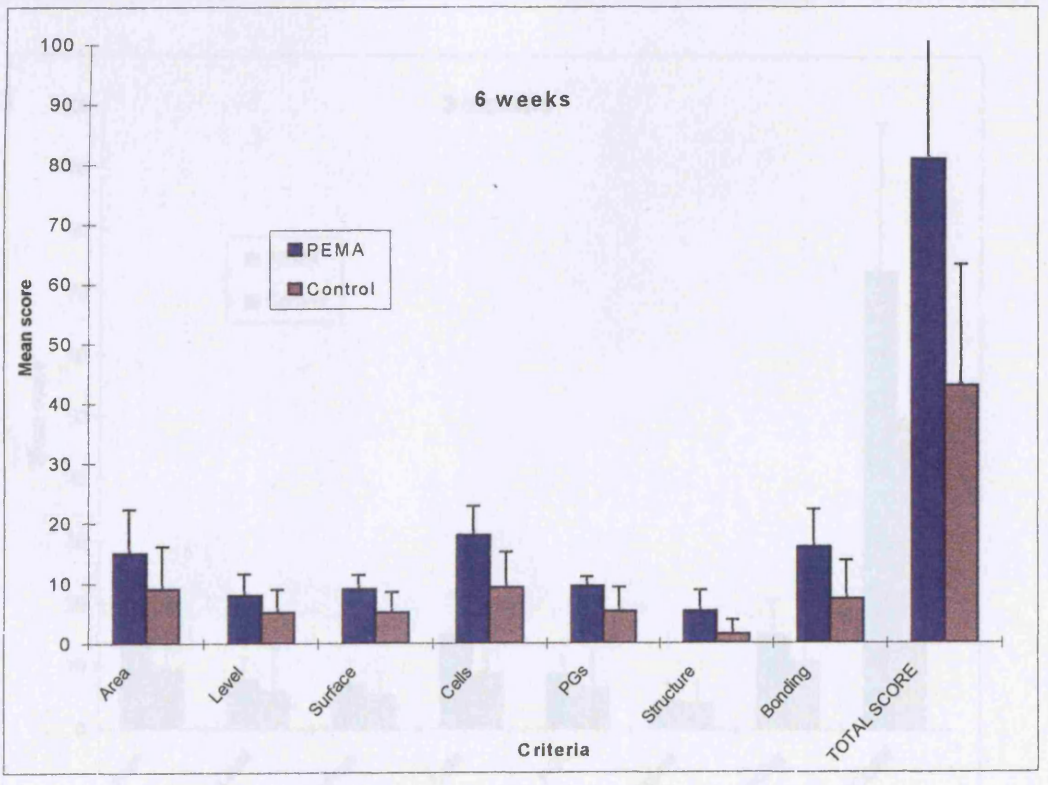


Table III.B.2:

Macroscopic and Histological/Histochemical assessment per criterion
Scoring at 3 months. Rabbits *31 – 60*
PEMA vs CONTROL

PEMA	*31*	*32*	*33*	*34*	*35*	*36*	*37*	*38*	*39*	*40*	*41*	*42*	*43*	*44*	*45*	*46*	*47*	*48*	*49*	*50*	*51*	*52*	*53*	*54*	*55*	*56*	*57*	*58*	*59*	*60*
Area	20	10	20	20	20	20	10	20	0	20	20	20	10	20	0	0	20	20	10	20	20	10	0	20	0	20	20	20	20	10
Level	10	10	10	10	5	10	0	10	0	10	10	10	5	10	0	5	10	10	10	10	5	10	0	10	10	10	5	10	5	10
Surface	10	10	10	5	10	10	5	5	0	10	10	10	10	5	0	0	10	10	10	10	5	5	0	10	10	10	10	5	5	10
Cells	20	20	20	20	20	20	10	10	10	20	10	20	20	10	10	0	10	20	20	20	10	10	10	20	20	20	10	20	10	20
PGs	10	10	10	5	10	10	10	10	0	10	10	10	10	10	5	0	10	10	10	5	10	5	10	10	10	10	10	10	10	10
Structure	5	5	5	0	5	5	5	5	0	5	5	0	5	5	0	0	0	5	5	5	10	5	5	10	5	10	10	5	5	0
Bonding	20	20	20	20	10	20	20	20	10	20	20	20	10	10	10	0	10	10	20	20	20	10	10	20	20	20	10	10	20	10
TOTAL SCORE	**95**	**85**	**95**	**80**	**80**	**95**	**60**	**80**	**20**	**95**	**85**	**90**	**70**	**70**	**25**	**5**	**70**	**85**	**85**	**90**	**80**	**55**	**35**	**100**	**75**	**100**	**75**	**80**	**75**	**70**

CONTROL	*31*	*32*	*33*	*34*	*35*	*36*	*37*	*38*	*39*	*40*	*41*	*42*	*43*	*44*	*45*	*46*	*47*	*48*	*49*	*50*	*51*	*52*	*53*	*54*	*55*	*56*	*57*	*58*	*59*	*60*
Area	10	10	10	10	0	10	10	10	10	0	0	0	10	20	20	10	20	0	0	10	10	10	20	20	10	0	10	20	10	0
Level	10	5	5	5	0	5	10	10	5	0	5	0	10	5	10	5	10	0	5	10	5	10	5	10	10	0	0	5	10	5
Surface	5	5	10	0	5	5	10	5	0	5	5	0	10	5	10	5	10	5	0	10	5	10	5	10	0	5	0	5	10	5
Cells	20	10	10	0	10	10	10	0	10	10	20	20	10	10	20	10	0	0	10	10	10	10	10	10	0	10	0	10	10	0
PGs	5	5	5	10	10	5	10	0	5	10	10	10	10	5	5	5	5	0	10	5	10	5	5	10	5	0	10	5	10	10
Structure	0	0	5	5	0	5	0	5	5	0	5	5	0	0	0	5	5	5	10	10	5	5	5	0	5	5	5	10	10	5
Bonding	10	10	20	10	0	10	10	20	10	20	10	0	10	10	20	10	10	10	20	10	10	10	20	20	0	0	10	10	10	10
TOTAL SCORE	**60**	**45**	**65**	**40**	**25**	**50**	**60**	**50**	**45**	**45**	**55**	**35**	**60**	**55**	**85**	**50**	**60**	**20**	**10**	**65**	**55**	**60**	**70**	**80**	**30**	**20**	**35**	**65**	**70**	**35**

	PEMA MEANS	*CONTROL MEANS*	*PEMA SD*	*CONTROL SD*
Area	14.67	9.33	7.76	6.91
Level	7.67	5.83	3.65	6.91
Surface	7.33	5.50	3.65	3.73
Cells	15.33	9.00	5.71	3.56
PGs	8.67	6.67	2.92	6.07
Structure	4.50	4.17	3.04	3.30
Bonding	15.33	11.00	5.71	3.24
TOTAL SCORE	**73.50**	**50.00**	**23.86**	**18.05**

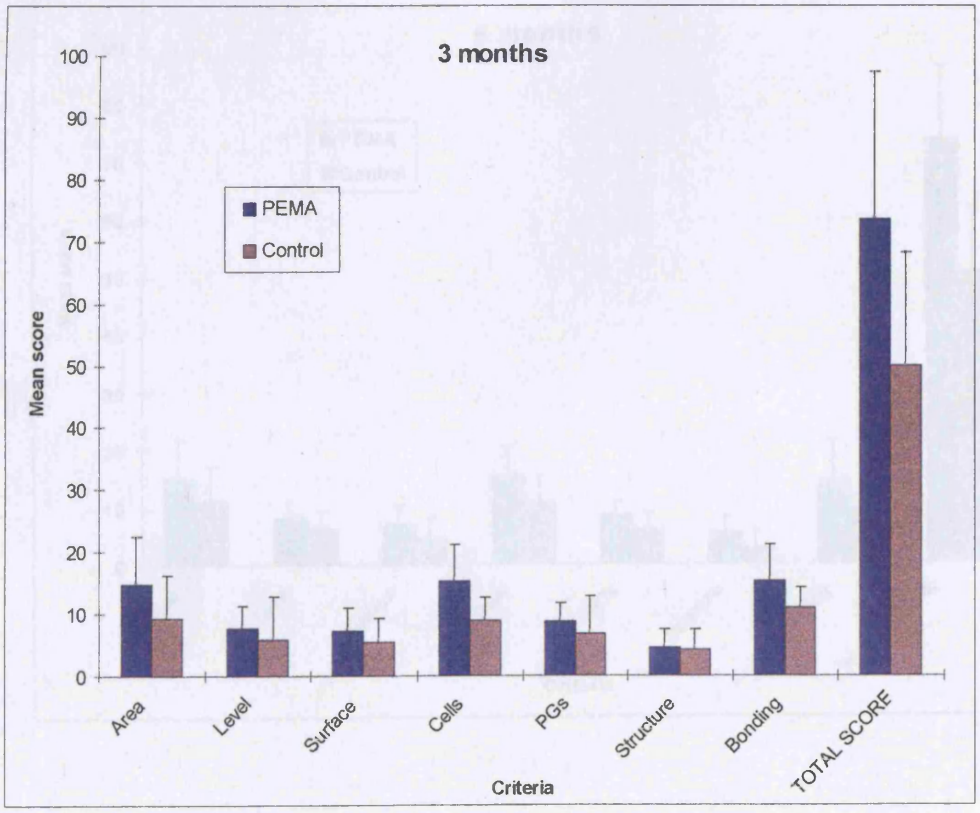


Table III.B.3:

Macroscopic and Histological/Histochemical assessment per criterion
Scoring at 6 months. Rabbits *61 – 90*
PEMA vs CONTROL

PEMA	*61*	*62*	*63*	*64*	*65*	*66*	*67*	*68*	*69*	*70*	*71*	*72*	*73*	*74*	*75*	*76*	*77*	*78*	*79*	*80*	*81*	*82*	*83*	*84*	*85*	*86*	*87*	*88*	*89*	*90*
Area	20	10	10	20	20	20	20	10	20	10	0	20	20	20	20	10	20	0	20	10	20	10	20	20	20	10	0	20	20	10
Level	10	5	0	10	10	5	10	10	10	10	5	10	5	10	10	10	5	0	10	10	5	10	10	10	5	10	10	10	10	5
Surface	10	10	5	10	5	5	10	5	10	10	10	5	10	10	5	5	5	0	10	10	5	10	10	5	5	0	10	0	10	5
Cells	20	20	10	20	20	20	10	20	10	10	10	20	10	20	10	20	10	20	10	10	20	10	10	10	20	20	20	20	10	20
PGs	10	10	10	5	10	10	10	10	10	5	5	10	5	10	5	10	5	10	10	10	10	10	10	5	10	5	10	10	10	5
Structure	5	5	5	0	5	10	5	10	5	5	5	5	5	5	5	5	5	5	0	10	5	10	5	10	5	5	5	0	0	5
Bonding	20	20	10	10	20	10	20	10	10	20	20	20	20	20	10	0	20	10	0	20	10	20	10	20	10	20	20	20	10	0
TOTAL SCORE	**95**	**80**	**50**	**75**	**90**	**80**	**85**	**75**	**75**	**70**	**55**	**90**	**75**	**95**	**65**	**60**	**70**	**45**	**60**	**80**	**75**	**80**	**75**	**80**	**75**	**70**	**75**	**80**	**70**	**50**

CONTROL	*61*	*62*	*63*	*64*	*65*	*66*	*67*	*68*	*69*	*70*	*71*	*72*	*73*	*74*	*75*	*76*	*77*	*78*	*79*	*80*	*81*	*82*	*83*	*84*	*85*	*86*	*87*	*88*	*89*	*90*
Area	10	20	10	0	10	10	10	0	10	20	20	10	10	20	10	20	0	0	10	10	10	20	10	10	10	10	10	10	10	20
Level	10	5	0	5	10	5	10	5	10	5	0	0	10	5	10	10	5	5	10	10	5	5	10	5	5	5	5	5	5	5
Surface	10	5	5	5	5	5	5	10	10	5	5	0	5	10	5	0	5	0	0	5	0	5	10	5	10	5	0	0	0	5
Cells	20	10	10	10	10	20	10	10	10	10	20	0	10	10	10	10	10	20	10	10	10	10	10	0	10	10	10	10	10	10
PGs	10	5	5	10	10	10	5	5	5	5	0	5	0	5	0	5	5	5	10	10	10	5	5	5	5	10	5	5	5	5
Structure	0	0	5	5	0	0	0	5	0	0	5	0	5	10	5	5	5	5	10	0	0	5	5	5	0	0	0	0	0	0
Bonding	20	10	20	10	10	10	0	0	10	10	10	0	10	0	10	0	10	10	10	10	10	10	10	10	10	10	10	10	20	10
TOTAL SCORE	**80**	**55**	**55**	**45**	**55**	**60**	**40**	**35**	**55**	**55**	**60**	**15**	**50**	**60**	**50**	**50**	**40**	**45**	**60**	**55**	**45**	**60**	**60**	**40**	**50**	**50**	**40**	**40**	**50**	**55**

	PEMA MEANS	*CONTROL MEANS*	*PEMA SD*	*CONTROL SD*
Area	15.00	11.00	6.82	6.07
Level	8.00	6.17	3.11	3.13
Surface	7.00	4.67	3.37	3.46
Cells	15.33	10.67	5.07	4.50
PGs	8.50	5.83	2.33	2.96
Structure	5.17	2.67	2.78	3.14
Bonding	14.33	9.33	6.79	5.21
TOTAL SCORE	**73.33**	**50.33**	**12.62**	**11.29**

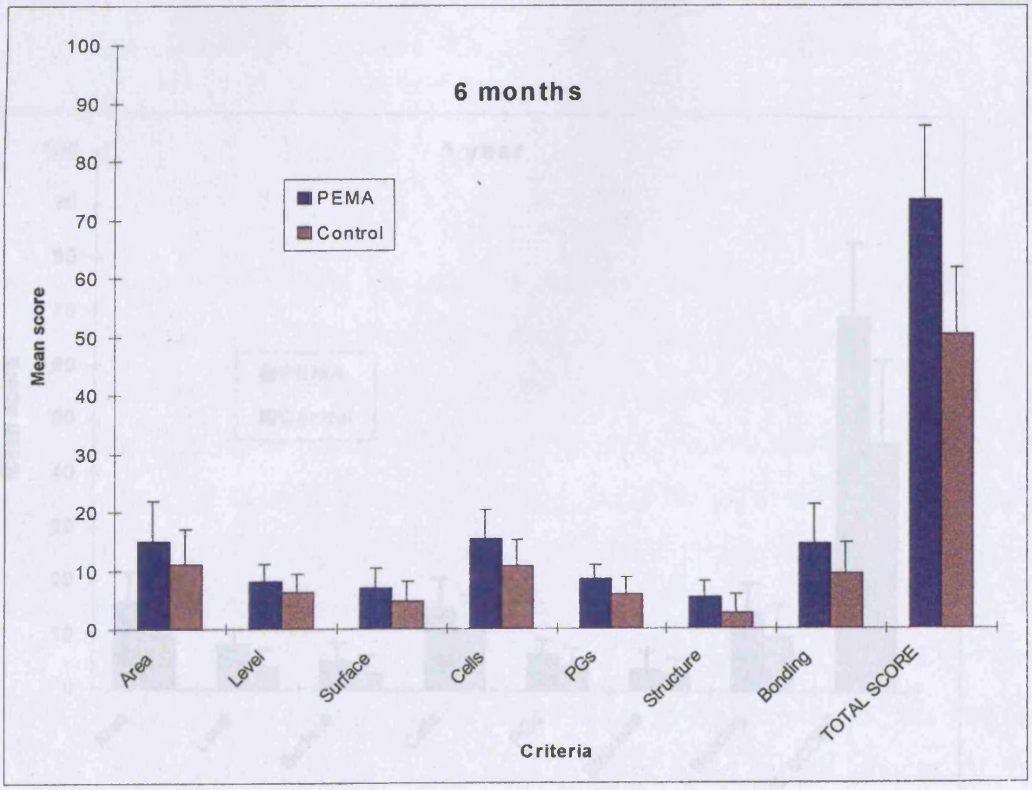


Table III.B.4:

Macroscopic and Histological/Histochemical assessment per criterion
Scoring at 1 year. Rabbits *91 – 120*
PEMA vs CONTROL

PEMA	*91*	*92*	*93*	*94*	*95*	*96*	*97*	*98*	*99*	*100*	*101*	*102*	*103*	*104*	*105*	*106*	*107*	*108*	*109*	*110*	*111*	*112*	*113*	*114*	*115*	*116*	*117*	*118*	*119*	*120*
Area	20	20	10	20	10	20	10	20	10	20	20	20	20	20	10	20	0	20	20	20	20	10	20	10	20	10	20	10	20	10
Level	10	10	5	10	10	10	10	10	10	10	10	10	5	5	10	10	5	10	0	10	10	5	10	10	5	10	10	10	5	0
Surface	0	5	5	5	10	5	10	5	0	0	5	5	5	10	0	10	10	10	5	5	5	5	5	0	0	10	0	10	5	5
Cells	20	10	20	20	20	20	20	20	10	10	10	10	20	20	0	10	20	20	10	20	20	20	10	10	10	10	20	10	20	10
PGs	10	10	5	5	10	10	10	10	10	5	5	5	5	10	5	5	5	5	5	5	10	10	10	5	0	5	5	5	5	5
Structure	5	5	0	0	5	5	0	0	5	0	5	0	0	5	10	5	5	5	10	10	5	5	5	5	0	0	0	5	5	10
Bonding	20	10	20	20	20	20	10	10	10	10	20	20	20	10	20	10	20	20	10	20	20	10	10	10	0	10	10	10	20	10
TOTAL SCORE	**85**	**70**	**65**	**80**	**85**	**90**	**70**	**75**	**55**	**55**	**75**	**70**	**75**	**80**	**55**	**70**	**65**	**90**	**60**	**90**	**90**	**65**	**70**	**50**	**35**	**55**	**65**	**60**	**80**	**50**

CONTROL	*91*	*92*	*93*	*94*	*95*	*96*	*97*	*98*	*99*	*100*	*101*	*102*	*103*	*104*	*105*	*106*	*107*	*108*	*109*	*110*	*111*	*112*	*113*	*114*	*115*	*116*	*117*	*118*	*119*	*120*
Area	20	10	10	20	10	20	0	20	10	20	0	0	10	20	10	0	0	0	0	0	10	20	10	10	20	20	20	0	0	10
Level	10	10	5	5	5	5	0	5	5	5	5	0	0	5	5	5	5	5	5	5	0	5	0	10	10	5	0	5	0	0
Surface	0	0	5	0	0	0	5	0	0	0	5	10	0	0	5	5	5	5	0	0	5	5	0	10	5	10	5	5	0	0
Cells	10	10	0	10	10	20	10	20	20	10	10	10	20	0	20	10	10	20	10	10	20	10	10	20	10	10	10	10	0	0
PGs	5	5	5	0	0	5	5	5	5	0	0	5	0	0	10	10	5	10	5	5	5	5	5	0	10	5	5	5	0	10
Structure	0	0	5	5	0	5	5	0	0	0	5	5	5	0	10	5	0	0	0	0	5	0	0	0	0	10	5	5	5	5
Bonding	10	10	10	20	20	20	20	10	10	20	10	10	0	0	10	10	0	0	10	10	10	10	10	10	10	10	10	10	0	10
TOTAL SCORE	**55**	**45**	**40**	**60**	**45**	**75**	**45**	**60**	**50**	**55**	**35**	**40**	**35**	**25**	**70**	**45**	**25**	**40**	**30**	**30**	**55**	**55**	**35**	**60**	**65**	**70**	**55**	**40**	**5**	**35**

	PEMA MEANS	*CONTROL MEANS*	*PEMA SD*	*CONTROL SD*
Area	16.00	10.00	5.63	8.30
Level	8.17	4.33	3.07	3.14
Surface	5.17	3.00	3.59	3.37
Cells	15.00	11.33	5.72	6.29
PGs	6.67	4.50	2.73	3.31
Structure	4.00	2.83	3.32	3.13
Bonding	14.33	10.00	5.68	5.87
TOTAL SCORE	**69.33**	**46.00**	**13.94**	**15.61**

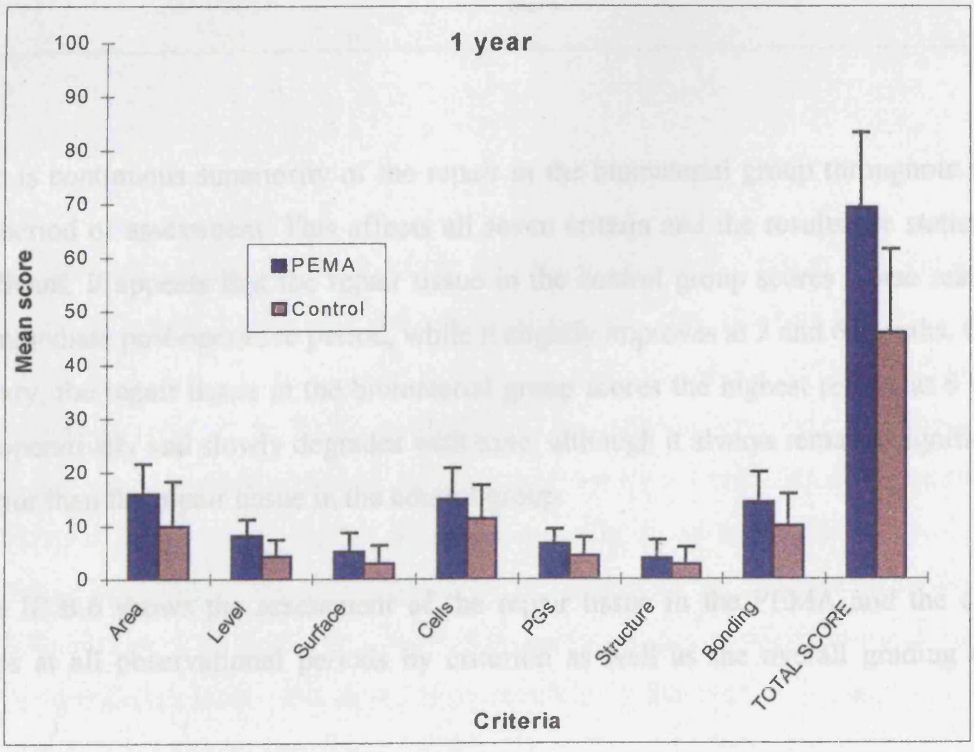
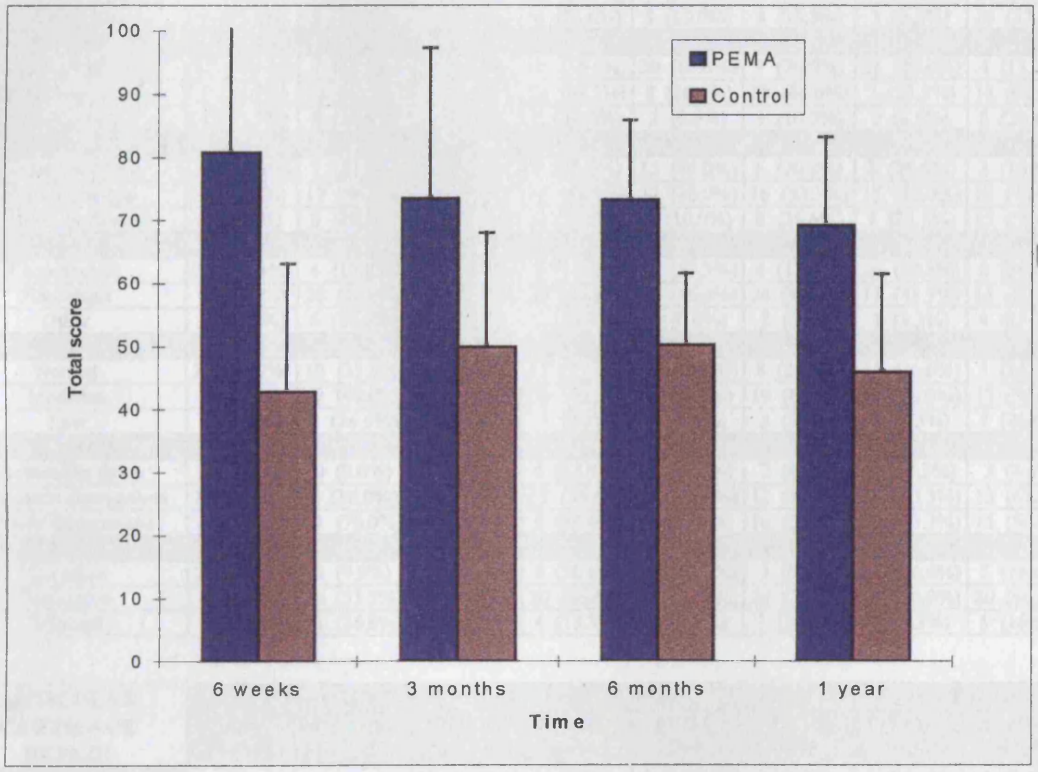


Table III.B.5:
Macroscopic and Histological/Histochemical assessment.
TOTAL SCORES at 6 weeks, 3 months, 6 months and 1 year. Rabbits *1-120*
PEMA vs CONTROL.

	PEMA MEANS	*CONTROL MEANS*	*PEMA SD*	*CONTROL SD*
6 weeks	80.83	42.83	24.21	20.33
3 months	73.50	50.00	23.86	18.05
6 months	73.33	50.33	12.62	11.29
1 year	69.33	46.00	13.94	15.61



There is continuous superiority of the repair in the biomaterial group throughout the 1-year period of assessment. This affects all seven criteria and the results are statistically significant. It appears that the repair tissue in the control group scores worse results in the immediate post-operative period, while it slightly improves at 3 and 6 months. On the contrary, the repair tissue in the biomaterial group scores the highest results at 6 weeks post-operatively and slowly degrades with time, although it always remains significantly superior than the repair tissue in the control group.

Table III.B.6 shows the assessment of the repair tissue in the PEMA and the control groups at all observational periods by criterion as well as the overall grading of the repair.

Table III.B.6:

Macroscopic and Histological/Histochemical assessment of repair tissue by criterion and the overall repair at 6 weeks, 3 months, 6 months and 1 year
PEMA vs CONTROL

CRITERION	6 weeks		3 months		6 months		1 year	
	PEMA n=30	CONTROL n=30	PEMA n=30	CONTROL n=30	PEMA n=30	CONTROL n=30	PEMA n=30	CONTROL n=30
AREA								
100% resurfacing	19 (63.3%)	6 (20.0%)	19 (63.3%)	6 (20.0%)	18 (60.0%)	7 (23.3%)	19 (63.3%)	10 (33.3%)
>50% resurfacing	7 (23.3%)	15 (50.0%)	6 (20.0%)	16 (53.3%)	9 (30.0%)	19 (63.3%)	10 (33.3%)	10 (33.3%)
<50% resurfacing	4 (13.3%)	9 (30.0%)	5 (16.6%)	8 (26.6%)	3 (10.0%)	4 (13.3%)	1 (3.3%)	10 (33.3%)
LEVEL								
Normal cartilage level	22 (73.3%)	9 (30.0%)	20 (66.6%)	11 (36.6%)	20 (66.6%)	9 (30.0%)	21 (70.0%)	4 (13.3%)
Minimal depression	4 (13.3%)	13 (43.3%)	6 (20.0%)	13 (43.3%)	8 (26.6%)	18 (60.0%)	7 (23.3%)	18 (60.0%)
Gross depression	4 (13.3%)	8 (26.6%)	4 (13.3%)	6 (20.0%)	2 (6.6%)	3 (10.0%)	2 (6.6%)	8 (26.6%)
SURFACE								
Smooth, glistering	25 (83.3%)	7 (23.3%)	18 (60.0%)	9 (30.0%)	15 (50.0%)	6 (20.0%)	8 (26.6%)	3 (10.0%)
Irregular, opaque	4 (13.3%)	17 (56.6%)	8 (26.6%)	15 (50.0%)	12 (40.0%)	16 (53.3%)	15 (50.0%)	12 (40.0%)
Disrupted by fissures	1 (3.3%)	6 (20.0%)	4 (13.3%)	6 (20.0%)	3 (10.0%)	8 (26.6%)	7 (23.3%)	15 (50.0%)
CELLS								
Chondrocytes	25 (83.3%)	4 (13.3%)	17 (56.6%)	4 (13.3%)	16 (53.3%)	4 (13.3%)	16 (53.3%)	8 (26.6%)
Fibroblasts	4 (13.3%)	20 (66.6%)	12 (40.0%)	19 (63.3%)	14 (46.6%)	24 (80.0%)	13 (43.3%)	18 (60.0%)
Other	1 (33.3%)	6 (20.0%)	1 (3.3%)	7 (23.3%)	0 (0.0%)	2 (6.6%)	1 (3.3%)	4 (13.3%)
PGs								
Normal	27 (90.0%)	10 (33.3%)	24 (80.0%)	13 (43.3%)	21 (70.0%)	8 (26.6%)	11 (36.6%)	5 (16.6%)
Moderate	3 (9.9%)	12 (40.0%)	4 (13.3%)	14 (46.6%)	9 (30.0%)	19 (63.3%)	18 (60.0%)	17 (56.6%)
Low	0 (0.0%)	8 (26.6%)	2 (6.6%)	3 (9.9%)	0 (0.0%)	3 (9.9%)	1 (3.3%)	8 (26.6%)
STRUCTURE								
Hyaline-like tissue	8 (26.6%)	0 (0.0%)	4 (13.3%)	4 (13.3%)	5 (16.6%)	2 (6.6%)	4 (13.3%)	2 (6.6%)
Moderately disorganised	16 (53.3%)	9 (30.0%)	19 (63.3%)	17 (56.6%)	21 (70.0%)	12 (40.0%)	16 (53.3%)	13 (43.3%)
Grossly disorganised	6 (20.0%)	21 (70.0%)	7 (23.3%)	9 (30.0%)	4 (13.3%)	16 (53.3%)	10 (33.3%)	15 (50.0%)
BONDING								
Complete	20 (66.6%)	3 (9.9%)	17 (56.6%)	6 (20.0%)	16 (53.3%)	3 (9.9%)	14 (46.6%)	5 (16.6%)
Incomplete	8 (26.6%)	16 (53.3%)	12 (40.0%)	20 (66.6%)	11 (36.6%)	22 (73.3%)	15 (50.0%)	20 (66.6%)
Minimal	2 (6.6%)	11 (36.6%)	1 (3.3%)	4 (13.3%)	3 (9.9%)	5 (16.6%)	1 (3.3%)	5 (16.6%)

ARTICULAR CARTILAGE REPAIR	6 weeks		3 months		6 months		1 year	
	PEMA n=30	CONTROL n=30	PEMA n=30	CONTROL n=30	PEMA n=30	CONTROL n=30	PEMA n=30	CONTROL n=30
EXCELLENT	22 (73.3%)	2 (6.6%)	12 (40.0%)	1 (3.3%)	5 (16.6%)	0 (0.0%)	6 (20.0%)	0 (0.0%)
GOOD	4 (13.3%)	10 (33.3%)	14 (46.6%)	18 (60.0%)	24 (80.0%)	20 (66.6%)	23 (76.6%)	13 (43.3%)
POOR	4 (13.3%)	18 (60.0%)	4 (13.3%)	11 (36.6%)	1 (3.3%)	10 (33.3%)	1 (3.3%)	17 (56.6%)

Table III.B.6 shows that the **area** of the defects in the PEMA group was completely resurfaced by 6 weeks in 63.3% and remained so over time showing 63.3% complete cover also at 1 year. In the control group the majority of the defects (80%) were incompletely resurfaced. In 73.3% in the PEMA group the defects were resurfaced up to the **level** of the surrounding normal articular caritlage by 6 weeks and they remained so in 70% up to 1 year. In the control group the majority of the defects (70% by 6 weeks) were found depressed. This phenomenon in the control group deteriorates further with time (86.7% depressed repairs at 1 year). The **surface** appearance of the repair tissue in both groups deteriorated with time, more severely in the control group (50% of the repairs were disrupted by fissures at 1 year).

Table III.B.6 also shows that there was striking **chondrocyte** predominance in the repair tissue in the biomaterial group (83.3% by 6 weeks) compared to the fibroblastic predominance (66.6% by 6 weeks) in the control group. The repair tissue in the biomaterial group presented high concentration of **proteoglycans** (90% by 6 weeks), much higher than that in the control group (33.3% at 6 weeks). There was, however, in both groups a gradual decrease in the proteoglycan concentration over time.

In both groups the **structure** of the cells and the matrix was inferior compared to normal articular cartilage. In the biomaterial group the cells of the repair tissue were numerous, oriented parallel to the surface of the repair in the superficial layer and more vertical in the deeper layers, without the characteristic formation of columns. Collagen fibres were scattered and disorganised particularly in the long-term specimens (86.6% in 1 year). In the control group, however, there was complete disorganisation of the cellular/matrix components in both the superficial and the deep layers of the repair tissue, particularly in the short-term specimens (100% at 6 weeks and 93.3% at 1 year).

There was significantly superior **bonding** of the repair tissue to the surrounding the defect normal articular cartilage in the biomaterial group at all times post-operatively (66.6% complete bonding at 6 weeks and 46.6% at 1 year). The majority of the control group specimens, however, showed incomplete/minimal bonding of the fibrous tissue to the adjacent articular cartilage (90.1% at 6 weeks and 83.4% at 1 year). The overall complete bonding of the repair tissue to the surrounding the defect normal articular cartilage in the PEMA group was 55.8% throughout the 1-year period.

Statistical analysis of the results:

The most important aspect of the trial design is the pairing of the PEMA and CONTROL knees within animals because this automatically removes the inter-animal variability. The measurements are ordinal, rather than scalar, and thus the ***McNemar's test*** was used. This test demonstrates the possible asymmetry in the results that would indicate that the score was dependent on whether the articular cartilage defect was resurfaced with PEMA or not, or that it could be produced at random. This test produces a p-value to quantify this effect.

For each animal, there are seven pairs of scores corresponding to different criteria of each knee's repair. The number of rows/columns (20) is too large and hence McNemar's test would have insufficient power. Instead, we assume the 20 point ordinal score produced is an approximation to a continuous scale. This allows the use of ***Wilcoxon's rank sum test***. This counts how many of the PEMA scores are 'greater', 'equal' or 'less than' the corresponding control score. An imbalance between the 'greater' and 'less than' counts indicates that PEMA/control has an effect on the overall score. Wilcoxon's test also produces a p-value to quantify this.

To surmount multiple testing errors and the inability to detect trends in the 'size' of the treatment's effect, a more complex statistical approach is taken, namely ***polychotomous logistic regression***. Firstly, the pairing is ignored and the knees are assumed to be individual units, independent from each other. Secondly, the knees' total score is trichotomised into three categories, excellent, good and poor, and Table III.B.6 for the tissue repair is used where the numbers are counts of knees. From this we fit a model where the probability of falling into a particular repair category is a function of the time and treatment. This allows the detection of any trends or interaction and improves the statistical power. The exact details are:

$\Pr(\text{repair} = i \mid \text{treatment, time}) = \theta_i$, i = poor, good, excellent;
treatment = PEMA, Control;
time = 6 weeks, 3 months, 6 months, 1 year

where,
$\log(\theta_i) = \alpha_i + \beta_{treatment\ i} + \beta_{time\ i}$

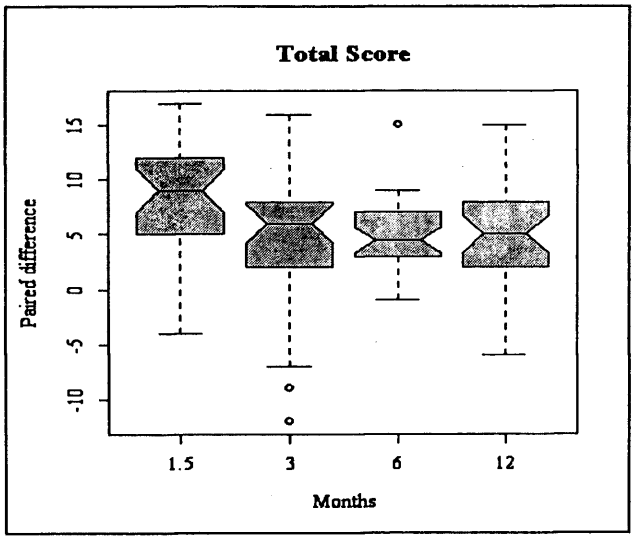
The data determines the choice of α, β, and we assess the goodness of fit using an ***analysis of deviance*** table which uses the difference between predicted and observed values to test if the covariates should be included in the model. The values of the coefficients, α, β, tell us the size and direction of any trends.

P-values for McNemar's and Wilcoxon's test

SCORES	6 weeks	3 months	6 months	12 months	Using combined time groups
Area	0.019	0.011	0.149	0.022	1.5e-6
Level	0.031	0.163	0.039	0.001	7.4e-8
Surface	6.4e-4	0.162	0.055	0.080	8.7e-7
Cells	1.1e-4	0.011	0.010	0.133	5.1e-10
PGs	0.002	0.006	0.005	0.041	1.4e-8
Structure	0.002	0.749	0.014	0.506	2.6e-4
Bonding	6.7e-4	0.059	0.015	0.079	1.4e-8
Total score *(Wilcoxon's)*	1.5e-5	0.002	7.8e-6	7.3e-5	2.7e-15

The higher mean scores of the repair tissue in the PEMA group in all time intervals show the superior quality of repair in the biomaterial group. This affects all criteria and total scores and the results are statistically significant.

It is therefore manifested that the PEMA group is significantly better than the control group. The effect of time, however, is not very clear. All the 6 week scores are significant while 4 scores at 3 months are non-significant at the 5% level. At 6 months, 2 scores are non-significant and at 1 year 4 scores are non-significant at the 5% level of significance. It is difficult to tell from these tests if there is a trend over time. Using the box-plot below, however, it is shown that there is a trend over time and the logistic regression confirms this:



Analysis of deviance for the overall repair is as follows:

Variable	d.f.	Deviance	residual d.f.	residual deviance	p-value
Treatment	2	79.577	12	50.909	.004
Time	6	42.014	6	8.895	.040

The analysis of deviance tells us that both treatment with PEMA and time have a significant effect on the repair. The next question is to show *how* these factors affect the repair.

To interpret the size of the effect of any covariates, we use odds ratios. To systematically present the predicted odds of a repair outcome given the type of treatment and time we present the odds of all events relative to some arbitrary reference event i.e. P(event of interest)/P(reference event). The reference event is an arbitrary bench-mark used for ease of interpretation. In this analysis the reference event used is Excellent repair having had treatment with PEMA and being measured at 6 months. If we wish to compare two events neither of which are the reference event, we use the relationship:

$$\frac{P(A)}{P(C)} = \frac{P(A)}{P(reference)} \times \frac{P(reference)}{P(C)} = \frac{P(A)}{P(reference)} \Big/ \frac{P(C)}{P(reference)} = \frac{odds(A)}{odds(C)}$$

In fact we present the logarithm of the odds, which converts multiplication into addition and division into subtraction. With this we can calculate any odds ratio we desire. The first row tells us that:

log (Pr(Good | PEMA, 6 weeks) / Pr(Excellent | PEMA, 6 weeks)) = -1.3932 ,

which, since it is negative, tells us that with the conditioning factors, PEMA & 6 weeks, having good repair is less likely than having excellent repair. The statistical results are presented in the table below:

Conditioning Factors	Repair	Log odds coefficient	S.D.	p-value
Intercept	good	-1.3932	0.4212	0.000942
	poor	-2.2495	0.5136	1.19e-05
Control	good	2.9237	0.6632	1.04e-05
	poor	4.6189	0.7097	7.61e-11
Time 3 months	good	1.6246	0.5375	0.002507
	poor	0.5312	0.6313	0.400098
Time 6 months	good	2.9803	0.6344	2.63e-06
	poor	1.3226	0.7363	0.072456
Time 1 year	good	2.6688	0.6100	1.21e-05
	poor	1.7690	0.6874	0.010068

The values of the coefficients indicate that, overall, the PEMA enhanced repairs are significantly superior to controls and that as time progresses the quality of the repair tissue decreases marginally.

Fig.III.B.1

Macroscopic appearance of the defect of the articular cartilage in the femoral trochlea [ft] at operation:
The → indicates the periphery of the defect.

Fig.III.B.2

Macroscopic appearance of the defect in the articular cartilage of the femoral trochlea [ft] following the implantation of the polymer [p] at operation:
The polymer plug is recessed below the surrounding normal articular cartilage, at the level of the subchondral bone. The → indicates the periphery of the defect.

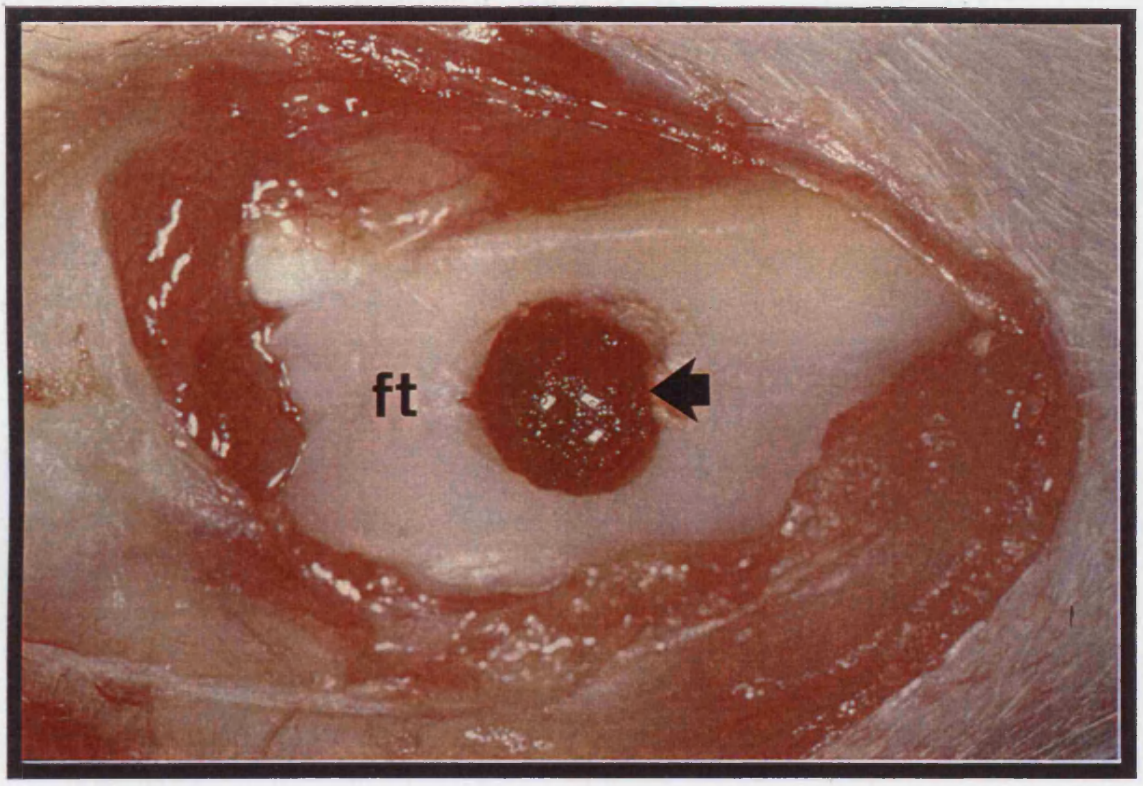


Fig.III.B.1

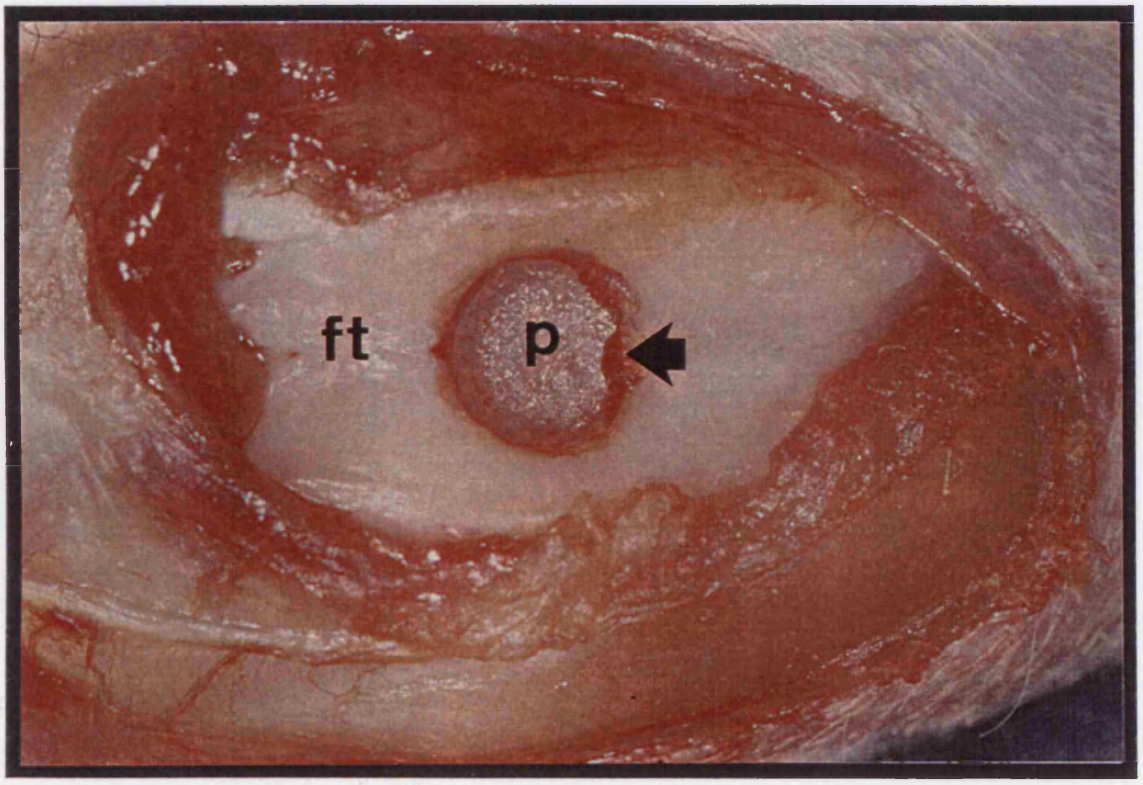


Fig.III.B.2

Fig.III.B.3

Histological appearance of a sample of synovium of the knee joint in the polymer group at 6 weeks:

There is no apparent inflammatory or foreign body reaction around the polymer particles [→]. *(Haematoxylin/Eosin staining, x 66)*

Fig.III.B.4

Histological appearance of a sample of synovium of the knee joint in the polymer group at 1 year:

A polymer particle can be noted [▶] with no apparent inflammatory or foreign body reaction around it. *(Haematoxylin/Eosin staining, x 66)*

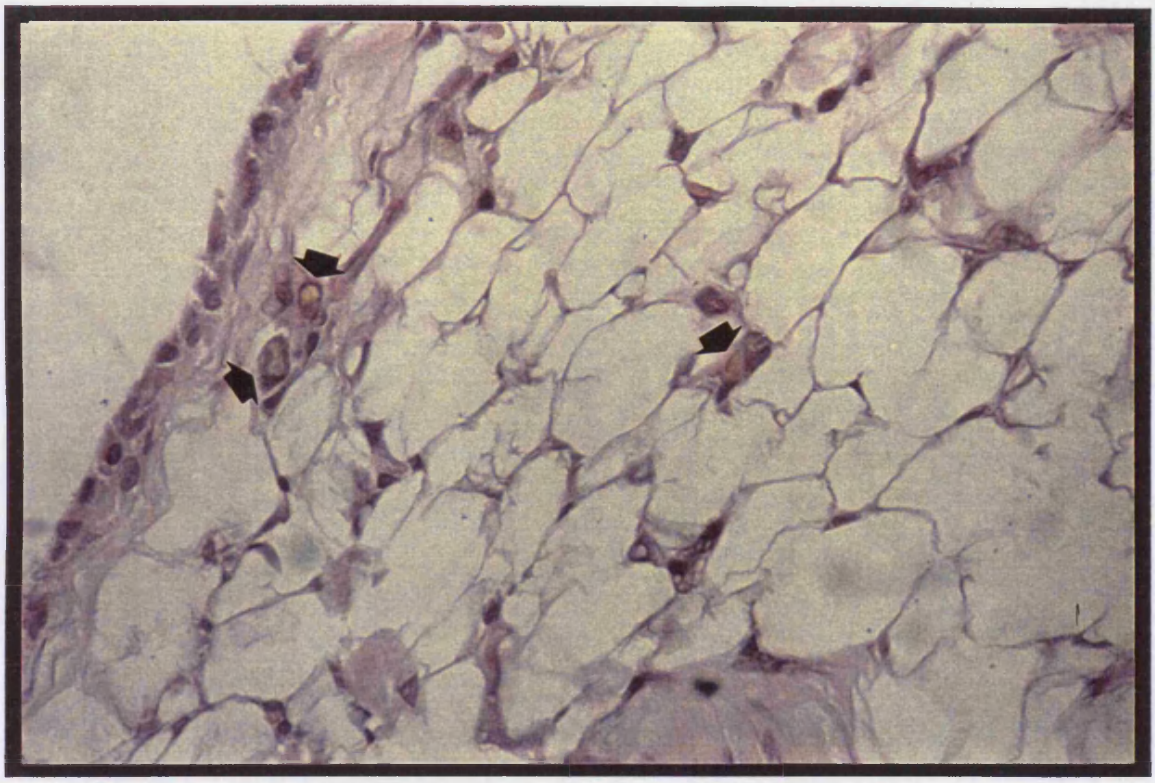


Fig.III.B.3

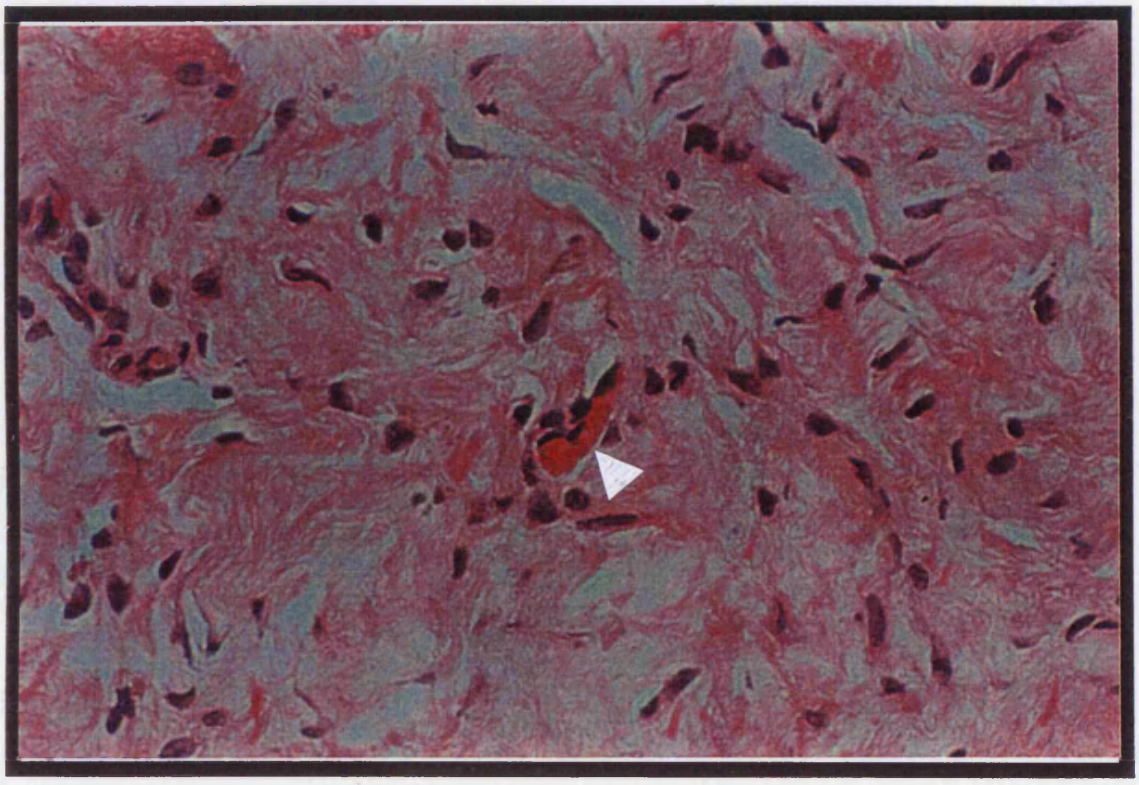


Fig.III.B.4

Fig.III.B.5

Macroscopic appearance of the repair tissue in the polymer group at 6 weeks:
The defect in the femoral trochlea **[ft]** has been fully resurfaced and the repair tissue **[rt]** is smooth and glistering, fully bonded to the surrounding the defect normal articular cartilage. The ▶ indicates the periphery of the defect.

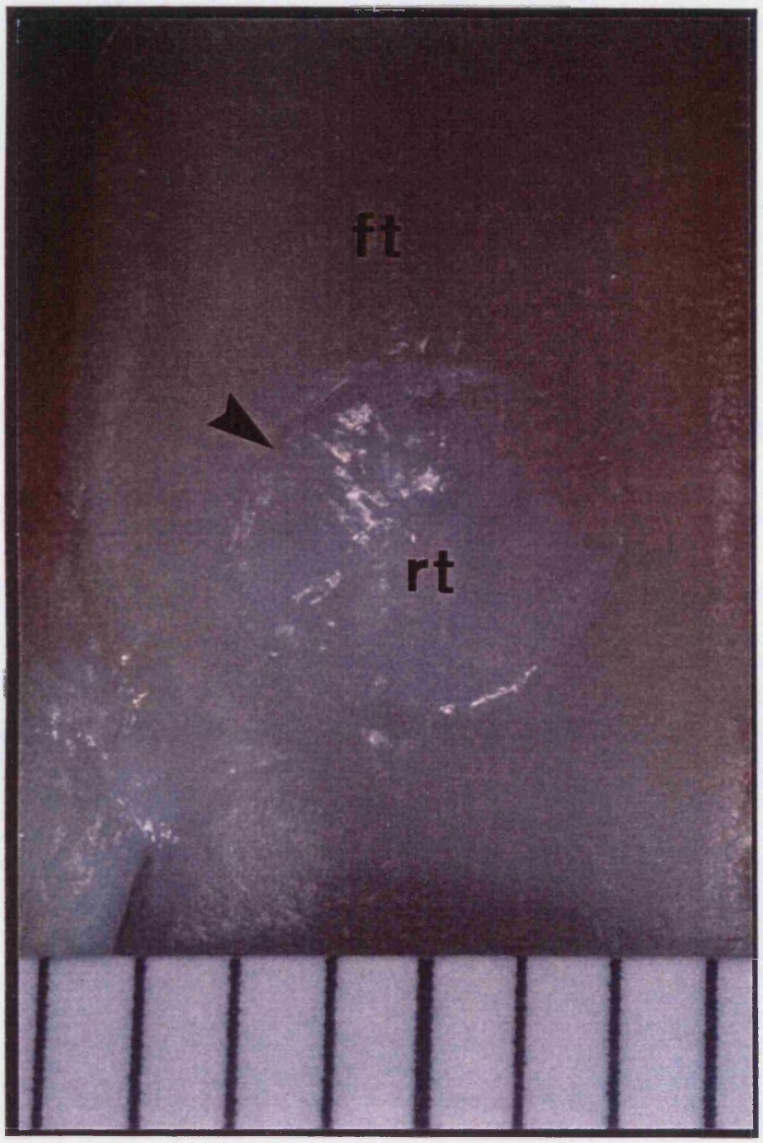


Fig.III.B.5

Fig.III.B.6

Histological appearance of the repair tissue in the polymer group at 6 weeks:
Complete integration of the repair tissue **[rt]** with the surrounding the defect normal articular cartilage **[c]**. The polymer **[p]** has been dissolved through the tissue process **[b**=subchondral bone**]**. The → indicates the smooth transition between the normal articular cartilage and the repair tissue. *(Haematoxylin/Eosin staining, x 13.2)*

Fig.III.B.7

Histological appearance in the centre of the repair tissue in the polymer group at 6 weeks:
Numerous chondrocytes can be noted both in the supreficial **[s]** as well as the deeper layers in the repair tissue. The chondrocytes in the superficial layer are smaller in size than those in the deeper layers. *(Haematoxylin/Eosin staining, x 66)*

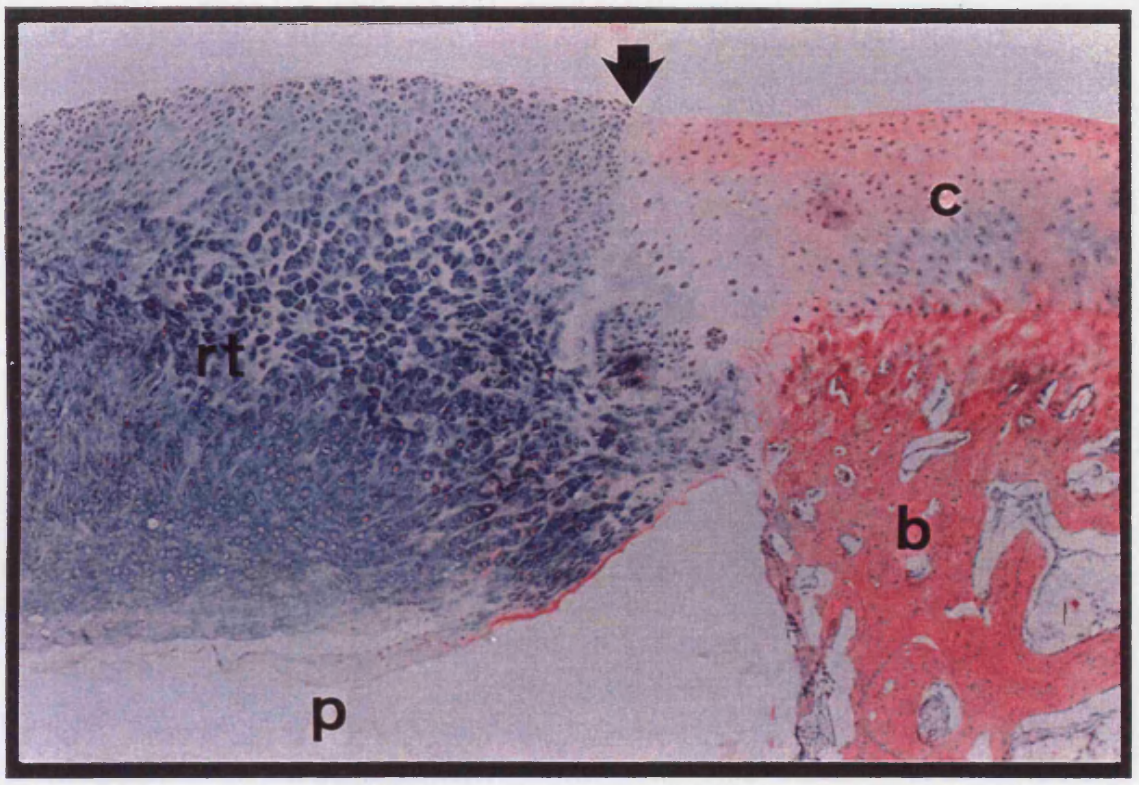


Fig.III.B.6

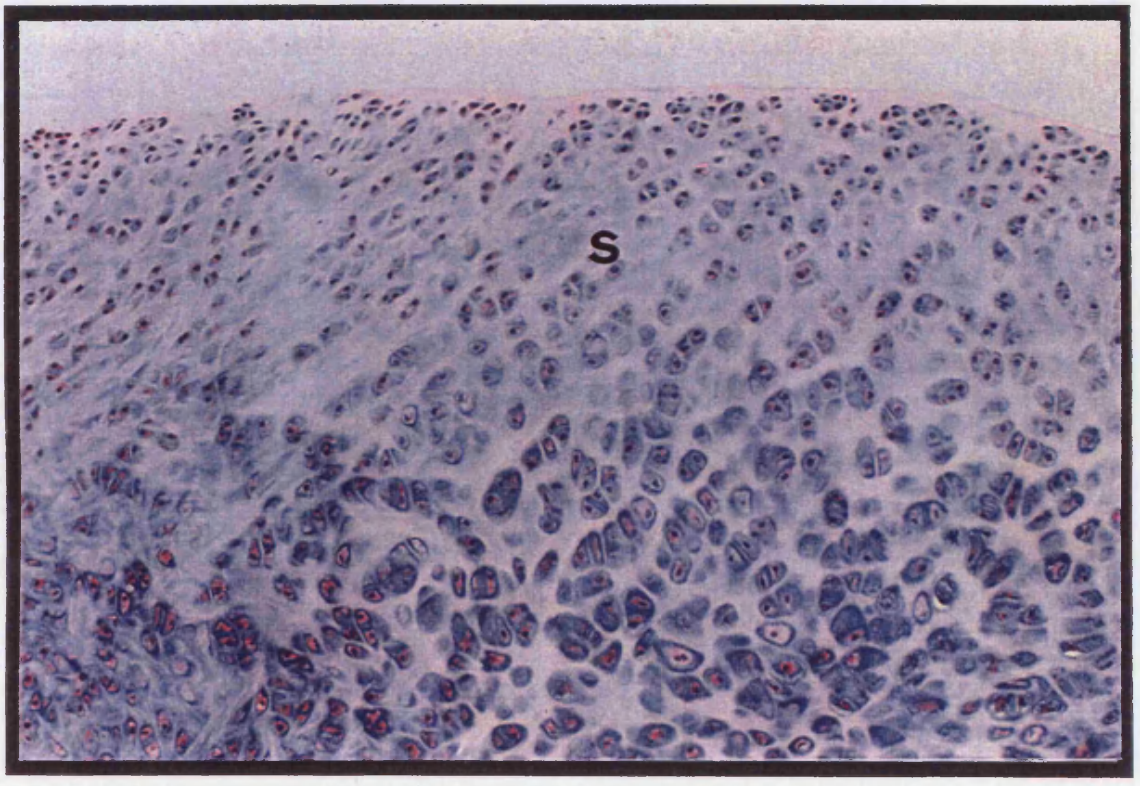


Fig.III.B.7

Fig.III.B.8

Histochemical appearance of the repair tissue in the polymer group at 6 weeks:

Abundant concentration of proteoglycans can be noted in the repair tissue **[rt]** similar to the concentration in the surrounding the defect normal articular cartilage **[c]**. The subchondral bone **[b]** does not contain proteoglycans and stained negative. The polymer **[p]** has been dissolved through the tissue process. *(Safranin-O staining, x 13.2)*

Fig.III.B.9

Histological appearance of the junction between the repair tissue and the normal articular cartilage in the polymer group at 6 weeks:

The repair tissue **[rt]** is fully integrated with the surrounding the defect normal articular cartilage **[c]**. *(1μm thick section, Toluidine blue staining, x 132)*

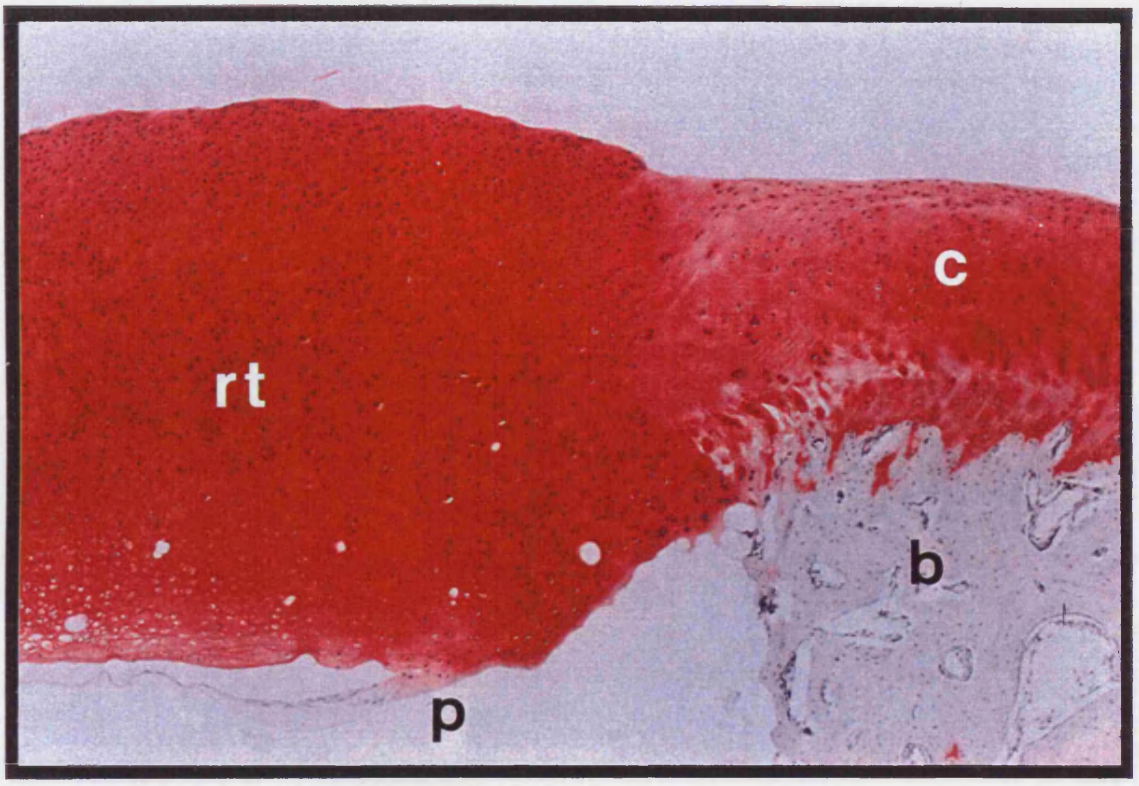


Fig.III.B.8

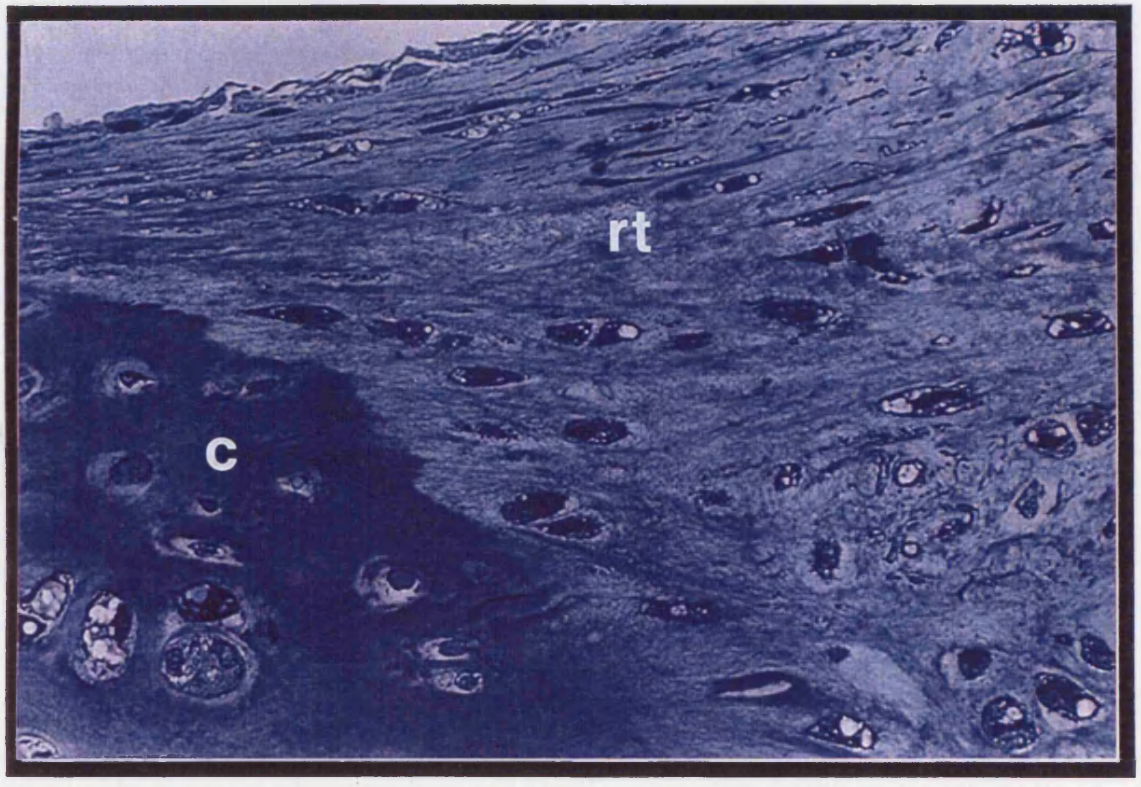


Fig.III.B.9

Fig.III.B.10

Macroscopic appearance of the repair tissue in the control group at 6 weeks:
The defect in the femoral trochlea **[ft]** has been partially resurfaced with repair tissue **[rt]**. The **star** indicates the unresurfaced area of the defect.

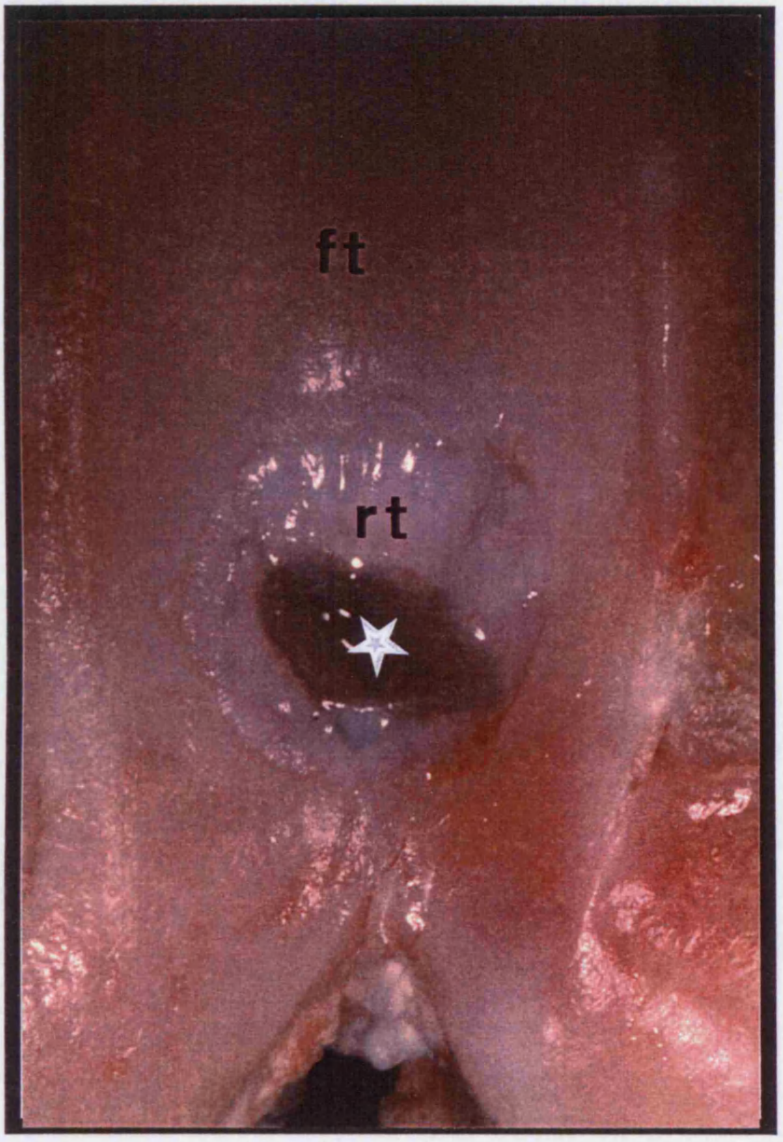


Fig.III.B.10

Fig.III.B.11

Histological appearance of the repair tissue in the control group at 6 weeks:
The repair has been covered with fibroblastic tissue with irregular superficial layer **[s]**. The → indicate large lipid droplets. *(Haematoxylin/Eosin staining, x 66)*

Fig.III.B.12

Histological appearance of the tissue in the unresurfaced area of the defect in the control group at 6 weeks:
Accumulation of debris and undifferentiated mesenchymal stem cells can be noted. **[s**=superficial layer] *(Haematoxylin/Eosin staining, x 13.2)*

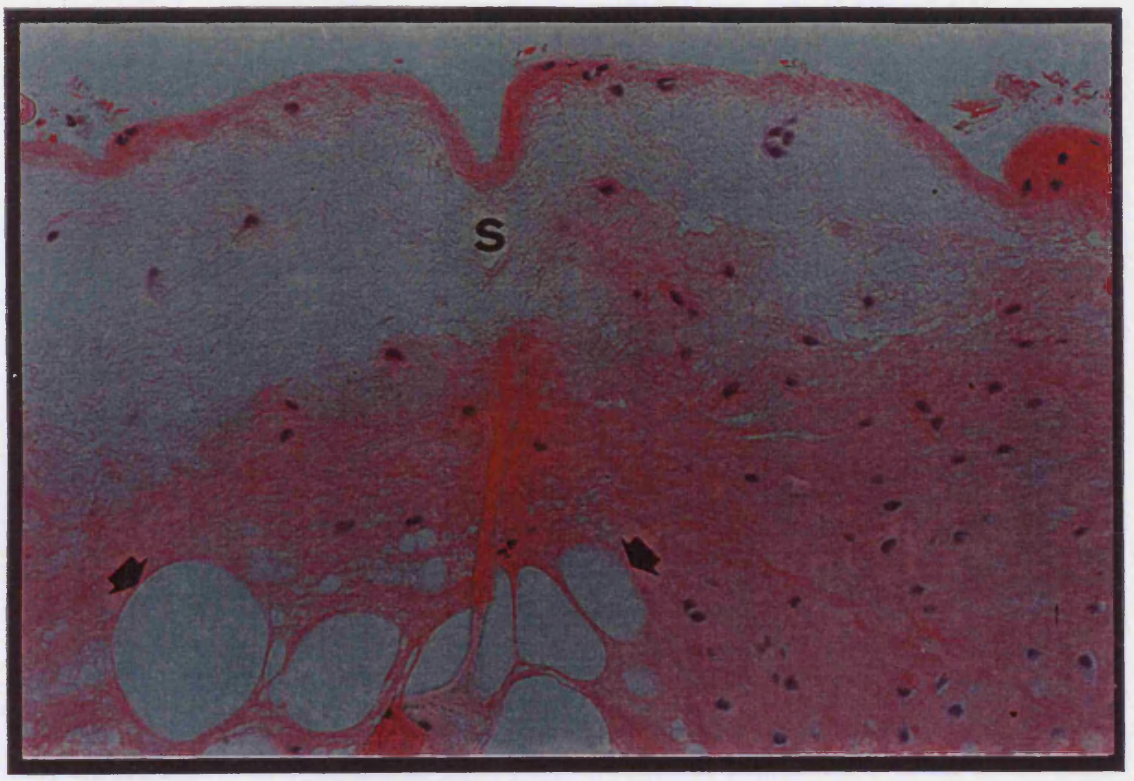


Fig.III.B.11

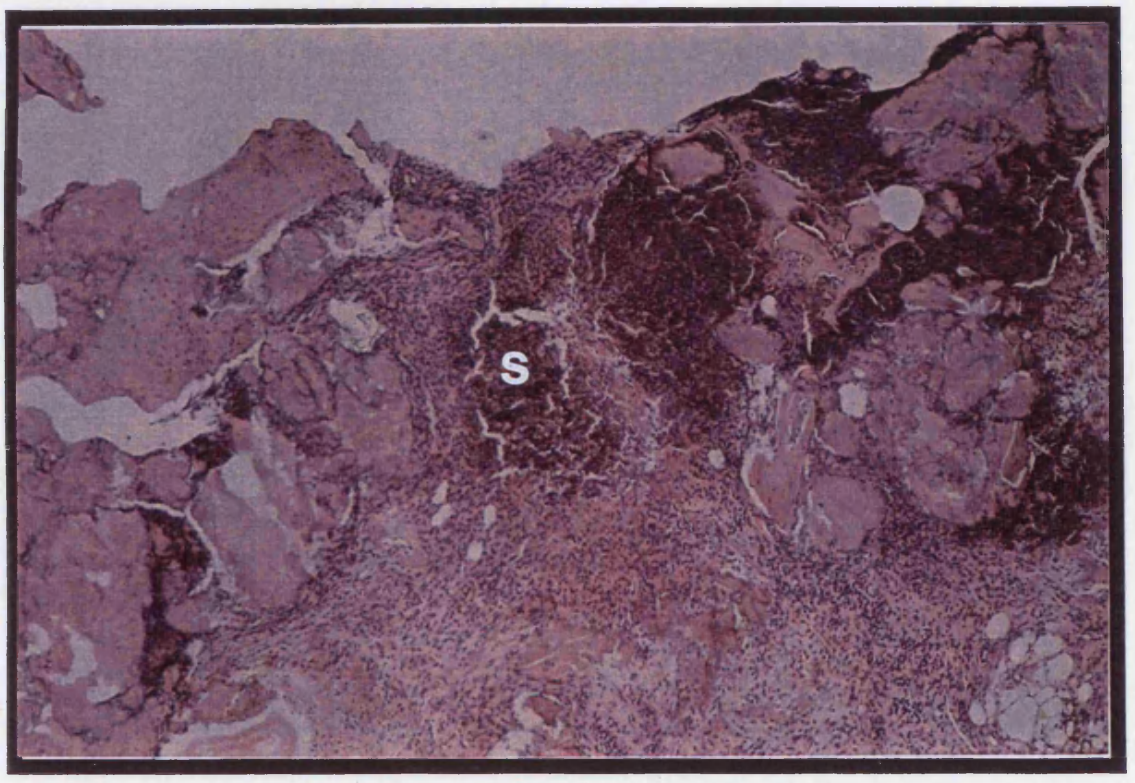


Fig.III.B.12

Fig.III.B.13

Histochemical appearance of the repair tissue of the polymer group at 3 months:
The repair tissue **[rt]** and the normal articular cartilage **[c]** contain large amounts of proteoglycans. The subchondral bone **[b]** contains no proteoglycans and stained negative. The → indicates the smooth transition between the repair tissue and the normal articular cartilage, **[p**=polymer**]**. *(Safranin-O staining, x 13.2)*

Fig.III.B.14

Histological appearance of the repair tissue in the polymer group at 3 months:
Numerous cells with chondrocytic phenotype can be noted in both the superficial **[s]** layer and the deeper **[d]** layers. The cells in the superficial layer appear to be more flattened, lying parallel to the surface, while the cells in the deep layers are round. *(1μm thick section, Toluidine blue staining, x 132)*

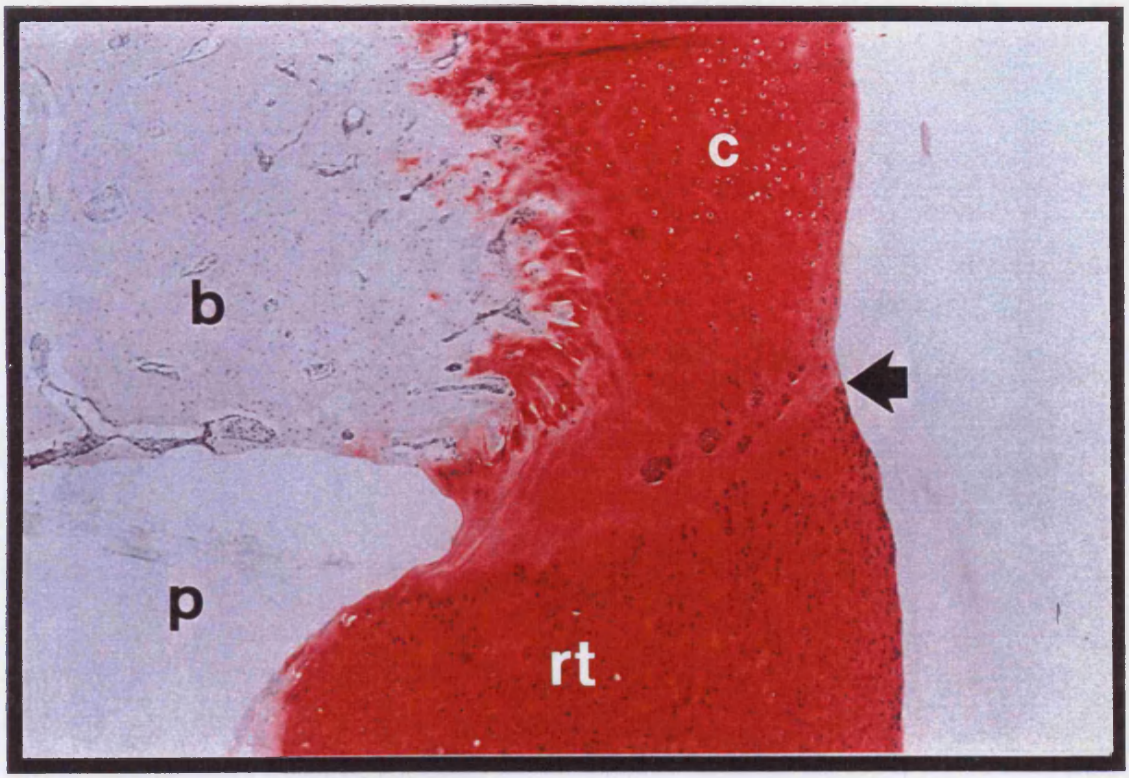


Fig.III.B.13

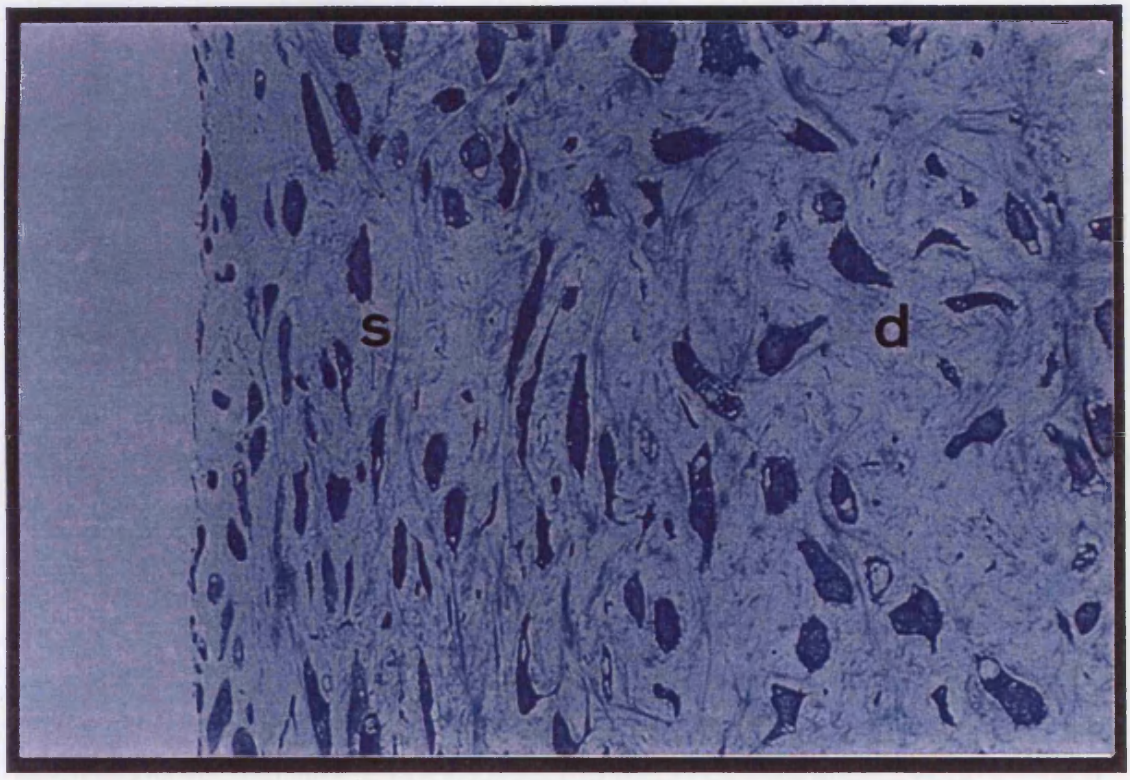


Fig.III.B.14

Fig.III.B.15

Histological appearance of the repair tissue in the control group at 3 months:
The repair tissue **[rt]** is disorganised and comprises primarily of cells with fibroblastic phenotype. The articular cartilage **[c]** surrounding the defects has also been replaced by fibroblastic tissue **[b**=subchondral bone**]** *(Haematoxylin/Eosin staining, x 13.2)*

Fig.III.B.16

Histological appearance in the centre of the repair tissue in the control group at 3 months:
Higher magnification in the centre of the repair tissue shown in Fig.II.B.15, illustrating the disorganised appearance of the fibroblastic repair. *(Haematoxylin/Eosin staining, x 33)*

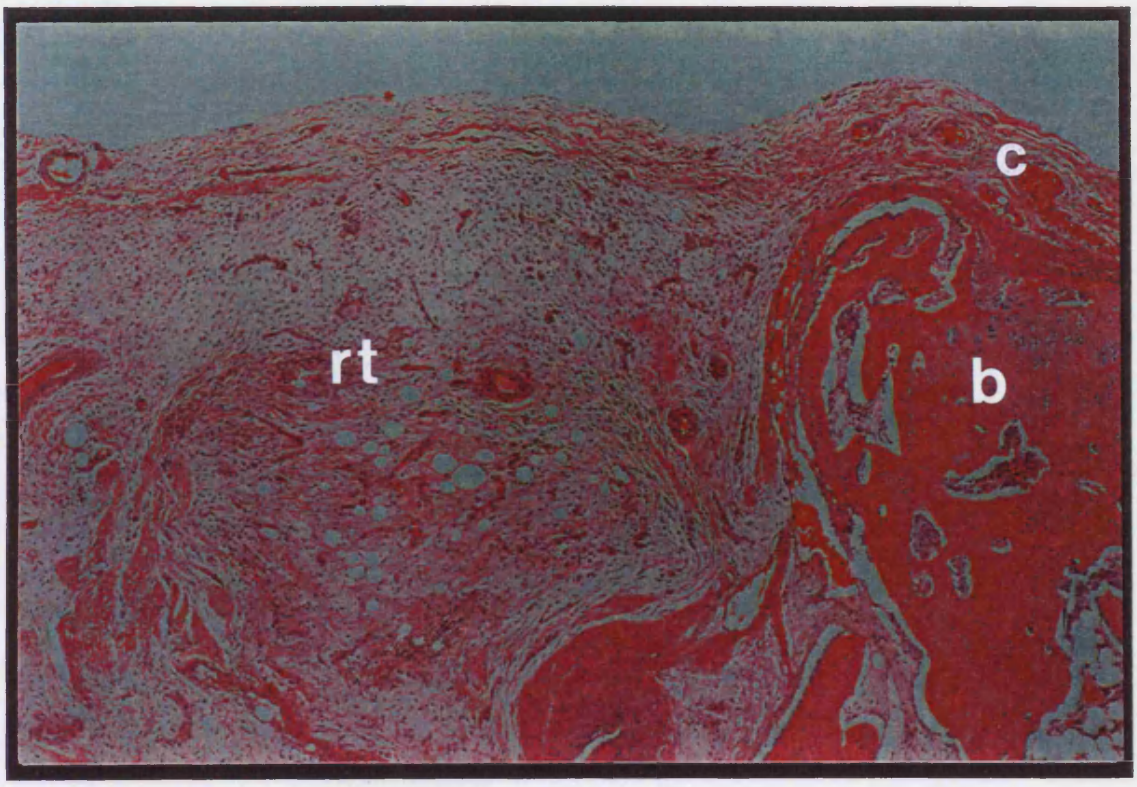


Fig.III.B.15

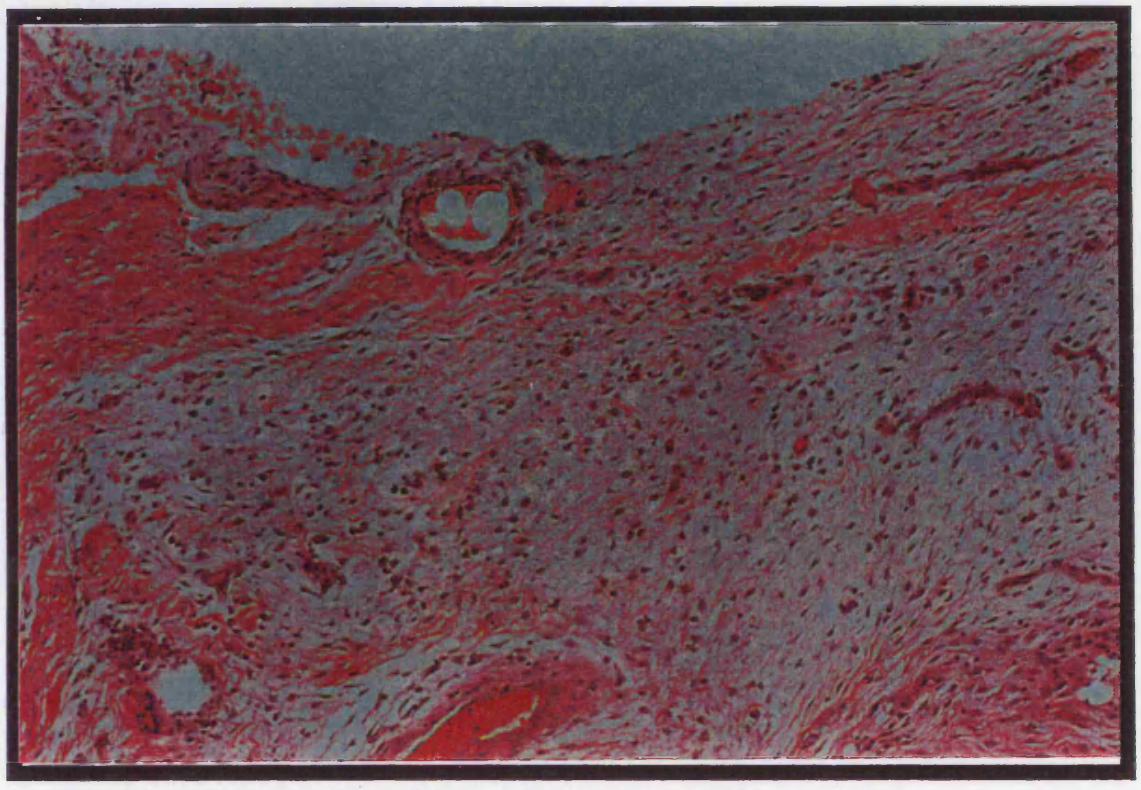


Fig.III.B.16

Fig.III.B.17

Macroscopic appearance of the repair tissue in the polymer group at 6 months:
The defect in the femoral trochlea **[ft]** has been fully resurfaced with smooth and glistering repair tissue fully contoured to the shape of the femoral trochlea. The ▶ indicates the periphery of the defect.

Fig.III.B.18

Macroscopic appearance of the posterior aspect of the femoral condyle of the specimen shown in Fig.III.B.17:
Early osteoarthritic changes can be noted with destruction of the articular cartilage **[c]** and eburnation of the subchondral bone **[b]**, suggesting that the polymer enhances articular cartilage repair despite the presence of osteoarthritic changes elsewhere in the joint. The ▶ indicates the periphery of the articular cartilage destruction.

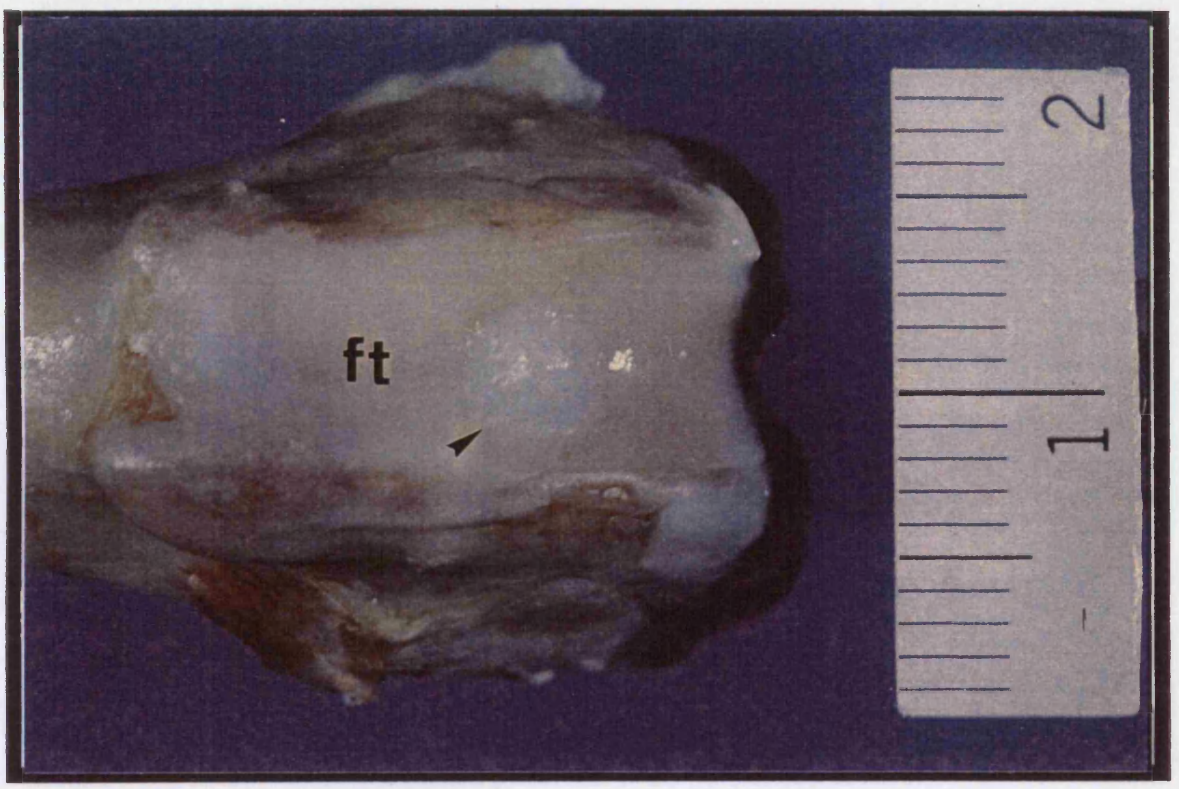


Fig.III.B.17

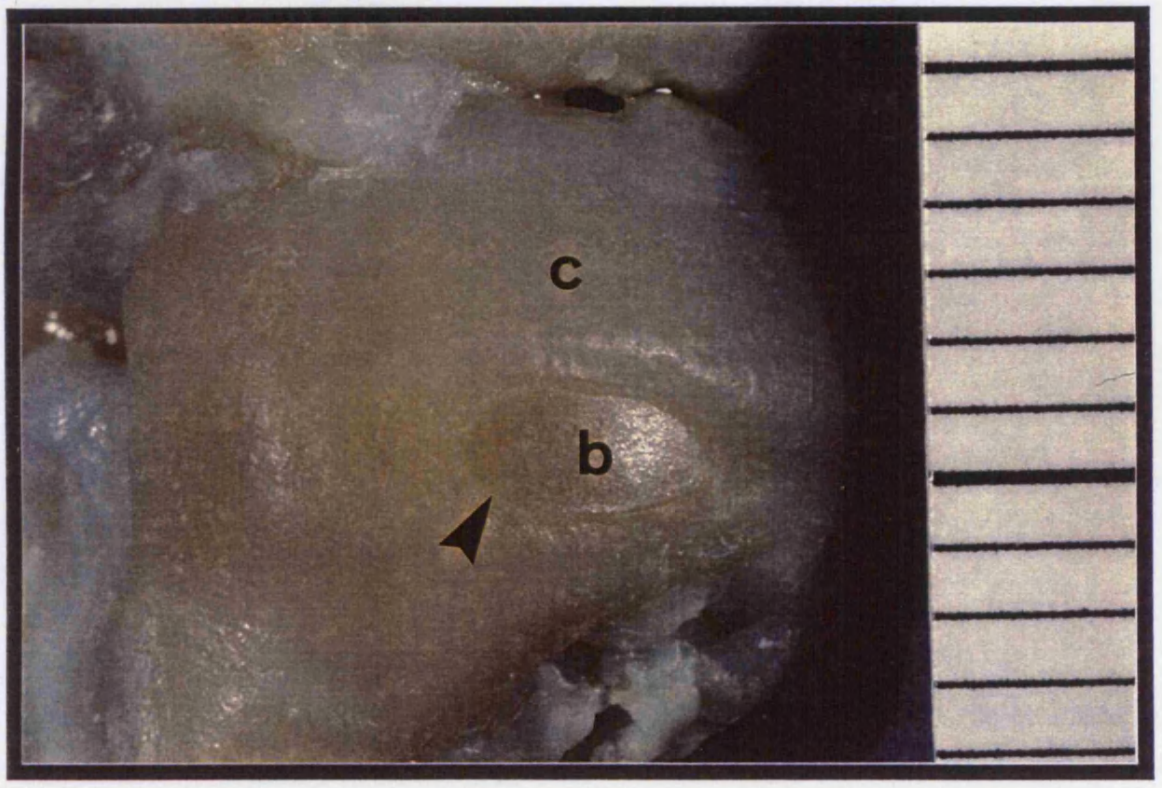


Fig.III.B.18

Fig.III.B.19

Histological appearance of the repair tissue in the polymer group at 6 months:
The repair tissue **[rt]** is fully integrated with the surrounding the defect normal articular cartilage **[c]**. The → indicates the transition from the repair tissue to the normal articular cartilage. [**b**=subchondral bone, **p**=space occupied by the polymer that has been dissolved through the tissue process]. *(Haematoxylin/Eosin staining, x 13.2)*

Fig.III.B.20

Histological appearance of the repair tissue in the polymer group at 6 months:
The picture focuses on the junction between the repair tissue and the normal articular cartilage on the opposite edge of the defect shown in Fig.III.B.19. The repair tissue **[rt]** is fully bonded to the normal articular cartilage **[c]** and it comprises of cells with chondrocytic phenotype. The → indicates the transition from the repair tissue to the normal articular cartilage. [**b**=subchondral bone]. *(Haematoxylin/Eosin staining, x 33)*

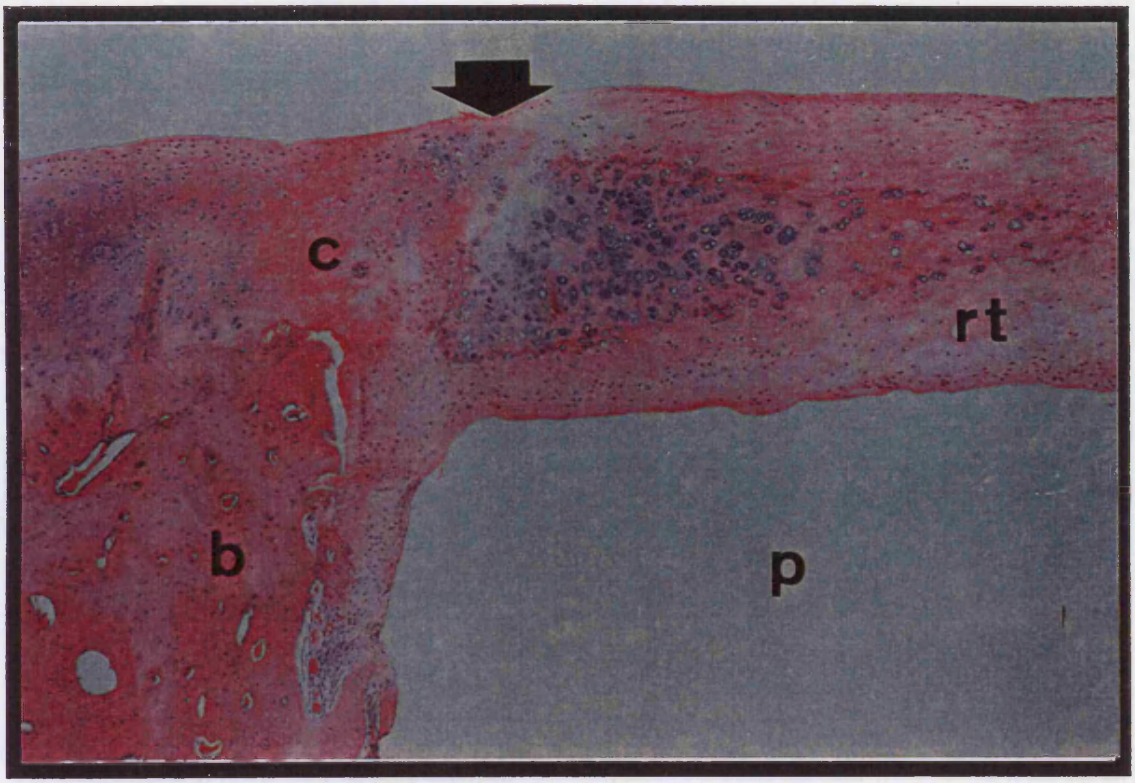


Fig.III.B.19

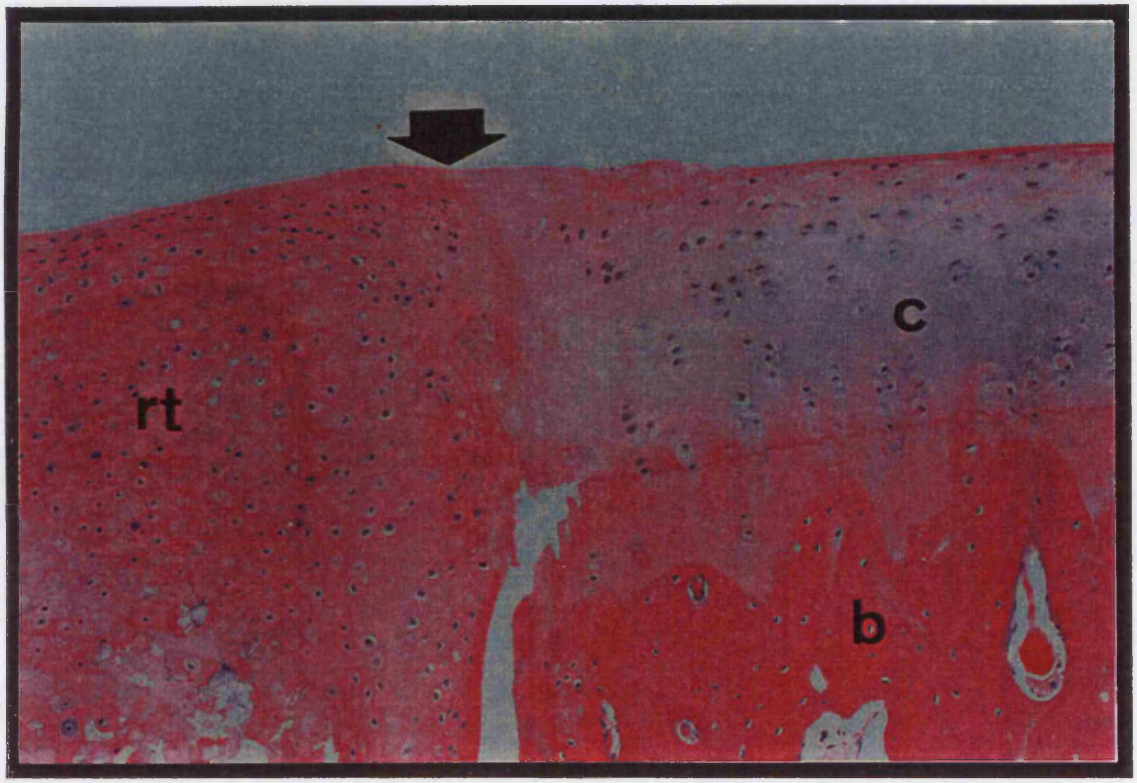


Fig.III.B.20

Fig.III.B.21

Histological appearance of the repair tissue in the control group at 6 months:
The repair tissue **[rt]** comprises predominantly of fibroblasts arranged in a multidirectional pattern. The normal articular cartilage **[c]** shows signs of degeneration with deep erosions. The → indicates a deep erosion, **[b**=subchondral bone**]** *(Haematoxylin/Eosin staining, x 33)*

Fig.HI.B.22

Histological appearance of the repair tissue in the control group at 6 months:
The repair tissue is primarily fibroblastic with irregular surface and deep fissures **[f]**. Chondrocyte islands **[c]** are scattered in the repair tissue. The ▶ indicate the deep fissure, splitting the repair tissue. *(Haematoxylin/Eosin staining, x 33)*

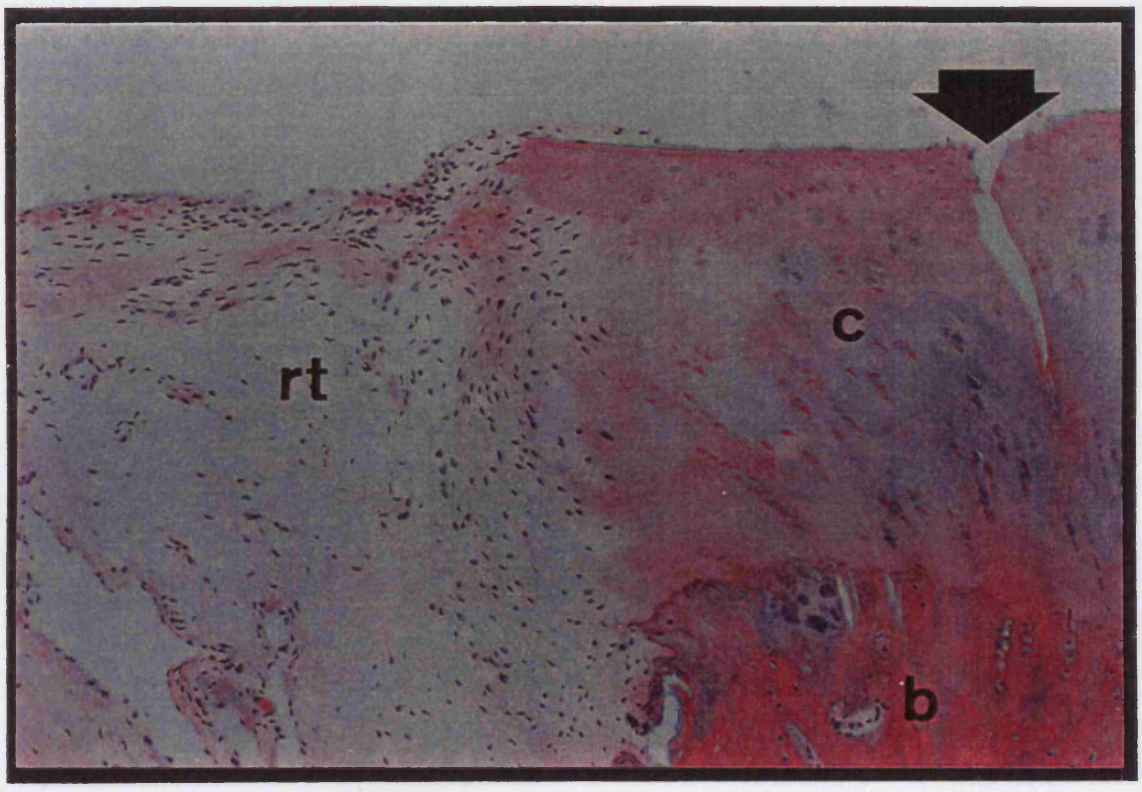


Fig.III.B.21

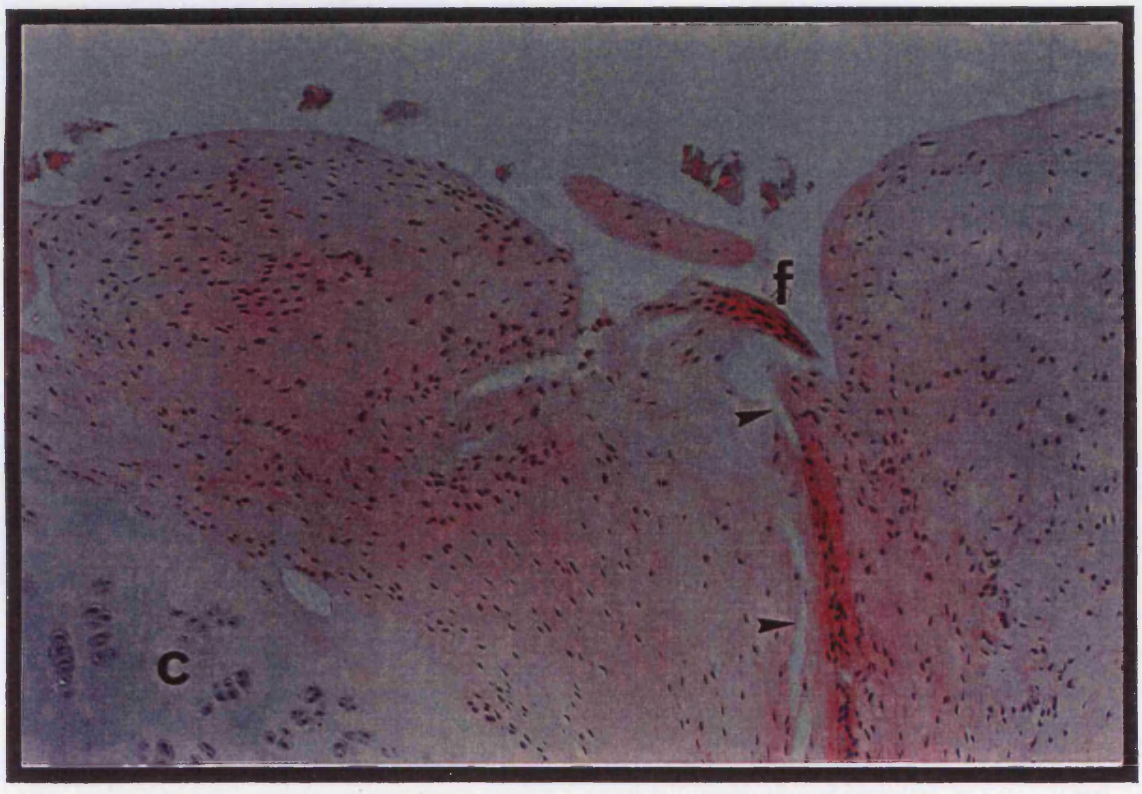


Fig.III.B.22

Fig.III.B.23

Macroscopic appearance of the repair tissue in the polymer group at 1 year:
The defect in the femoral trochlea **[ft]** has been fully resurfaced with smooth and glistering repair tissue, fully integrated with the surrounding normal articular cartilage. The small ► indicates the periphery of the defect, the large → indicates an osteophyte in the femoral condyle.

Fig.III.B.24

Macroscopic appearance of the posterior aspect of the femoral condyle of the specimen shown in Fig.III.B.23:
The articular cartilage **[c]** is disrupted by advanced degenerative changes with a large area of subchondral bone **[b]** being exposed, indicating that the polymer can enhance articular cartilage repair despite the presence of degenerative changes elsewhere in the joint. The ► indicates the periphery of the degeneration of the articular cartilage.

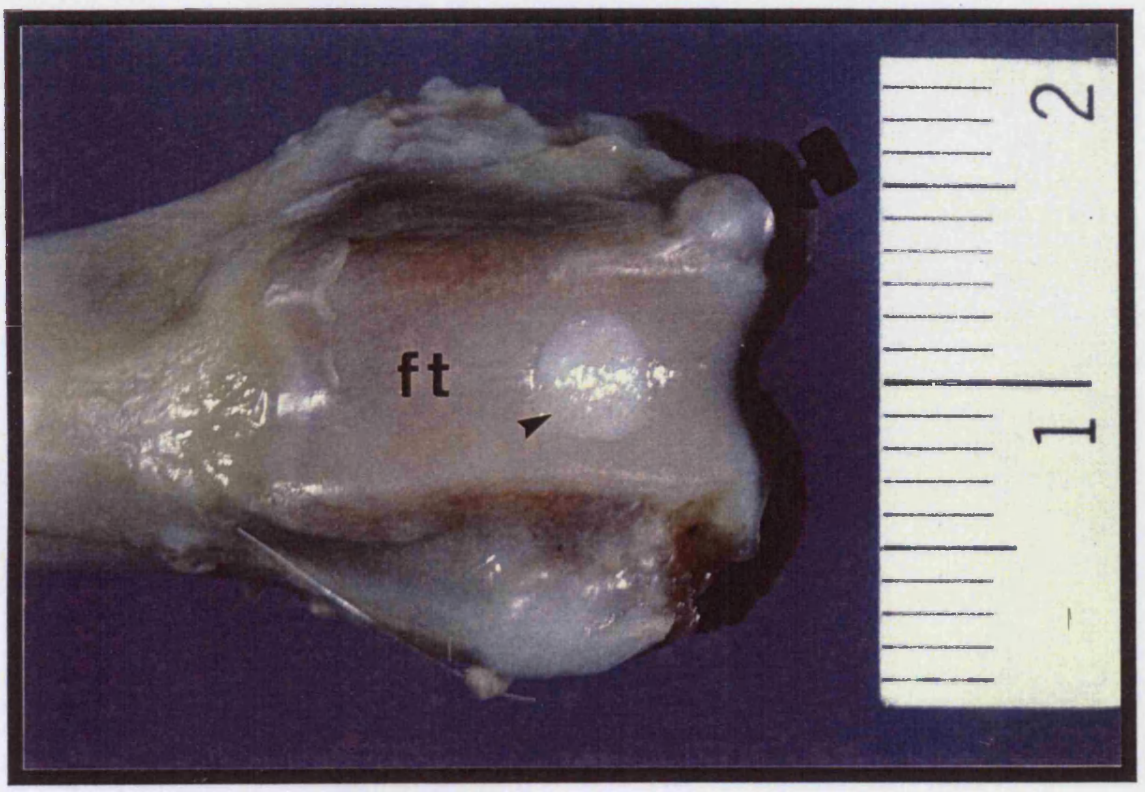


Fig.III.B.23

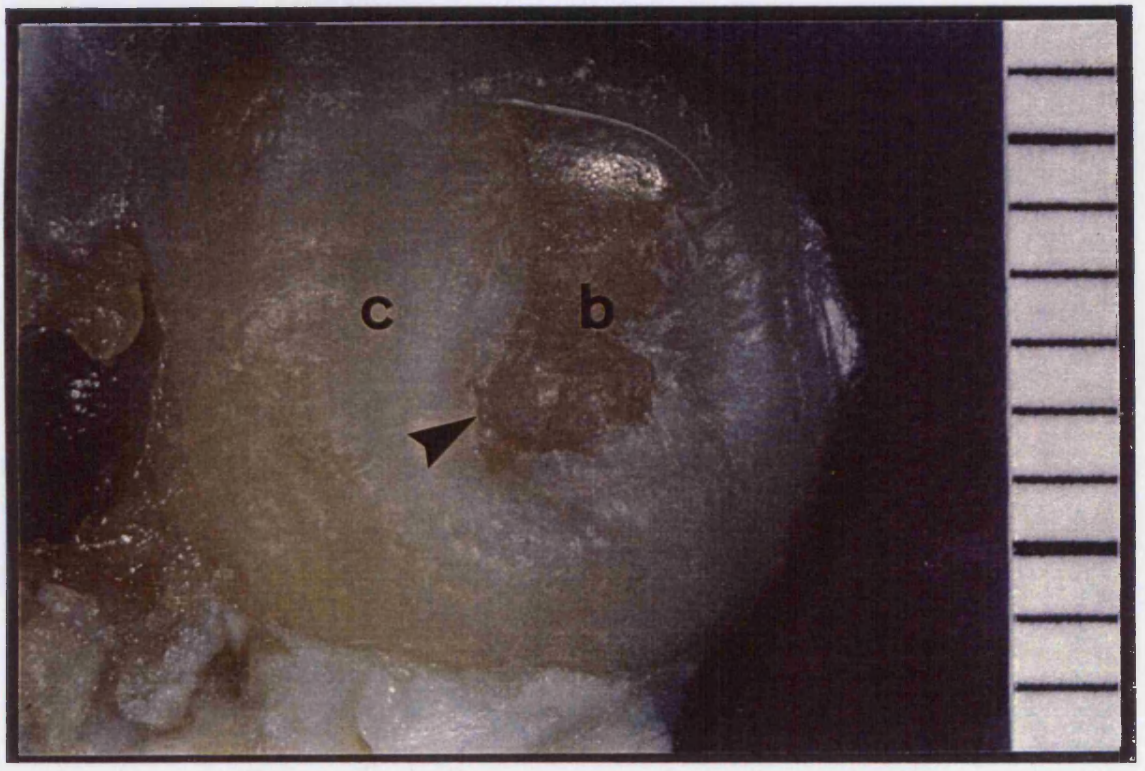


Fig.III.B.24

Fig.III.B.25

Histological appearance of the repair tissue in the polymer group at 1 year:
The repair tissue **[rt]** is fully integrated with the surrounding normal articular cartilage **[c]** and comprises of cells with chondrocytic phenotype. The → indicates the transition from the repair tissue to the normal articular cartilage, **[b**=subchondral bone] *(Haematoxylin/Eosin staining, x 33)*

Fig.III.B.26

Histochemical appearance of the repair tissue in the polymer group at 1 year:
The repair tissue **[rt]** and the normal articular cartilage **[c]** contain abundant amounts of proteoglycans. The subchondral bone **[b]** contains no proteoglycans and stained negative. The → indicates the transition from the repair tissue to the normal articular cartilage, **[p**=polymer] *(Safranin-O staining, x 33)*

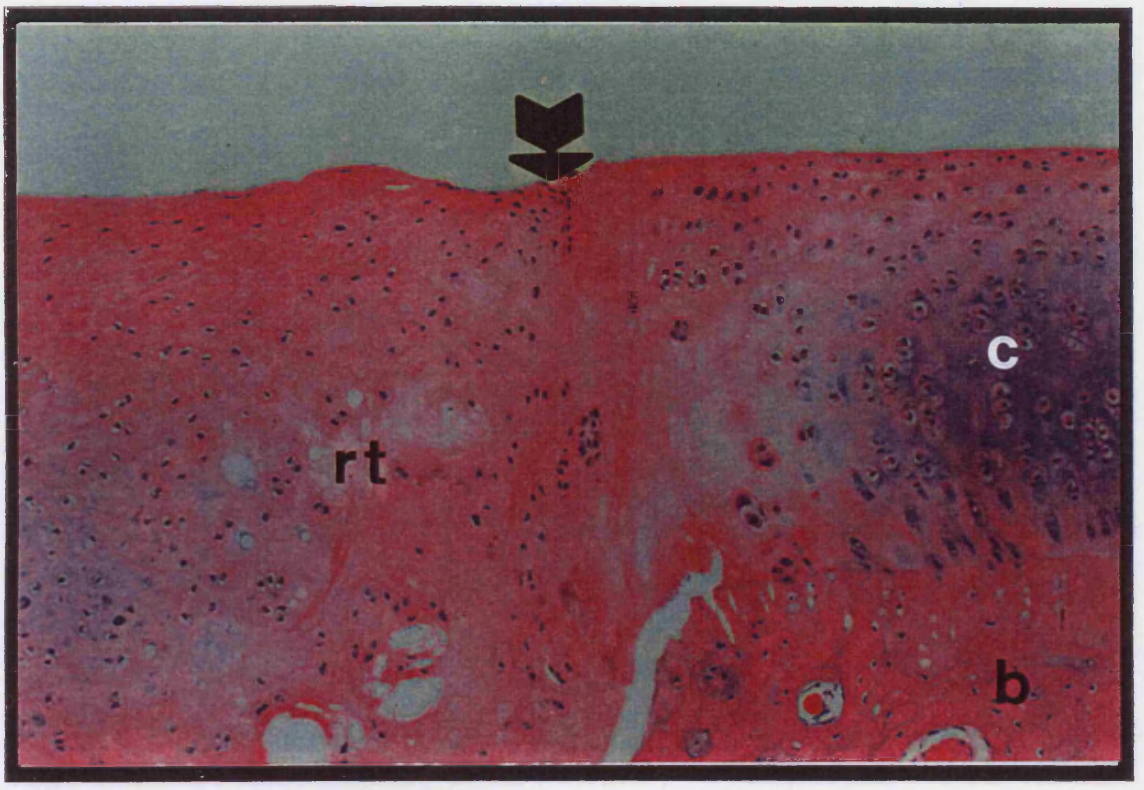


Fig.III.B.25

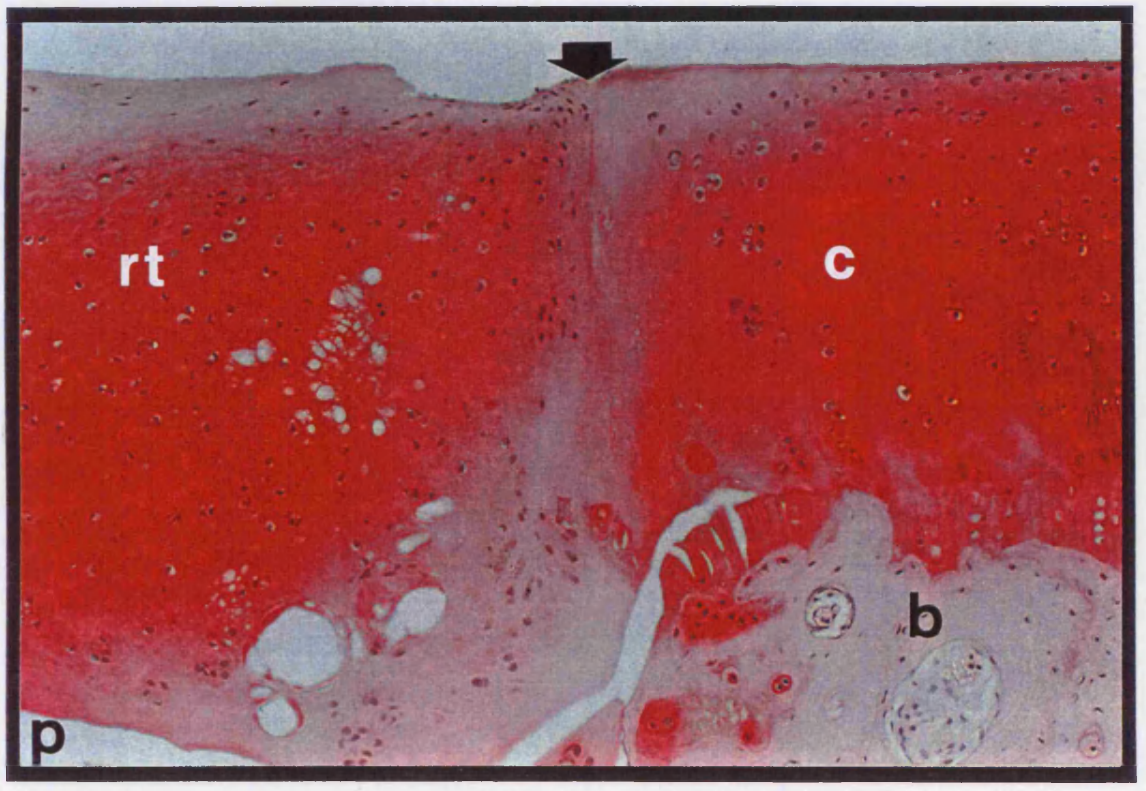


Fig.III.B.26

Fig.III.B.27

Histological appearance of the repair tissue in the control group at 1 year:
The repair tissue **[rt]** is fibrocartilaginous, not integrated with the surrounding the defect articular cartilage **[c]**. The latter shows signs of degeneration with acellular areas and destruction of the superficial layer. **[b**=subchondral bone**]** *(Haematoxylin/Eosin staining, x 33)*

Fig.III.B.28

Histochemical appearance of the repair tissue in the control group at 1 year:
The repair tissue **[rt]** contains very small amounts of proteoglycans, mainly distributed around the scattered chondrocyte aggregations. Proteoglycan concentration is also abnormal at the edge of the normal articular cartilage **[c]** due to tissue degeneration. The subchondral bone **[b]** contains no proteoglycans and stained negative. *(Safranin-O staining, x 33)*

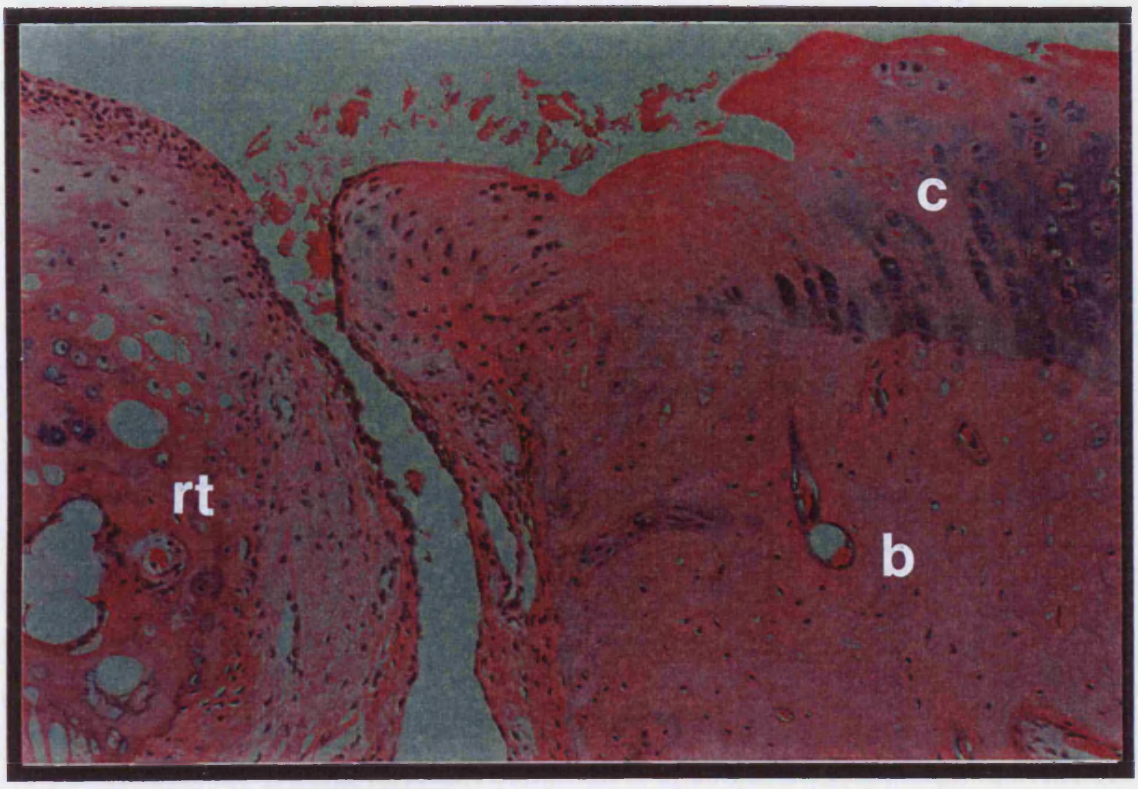


Fig.III.B.27

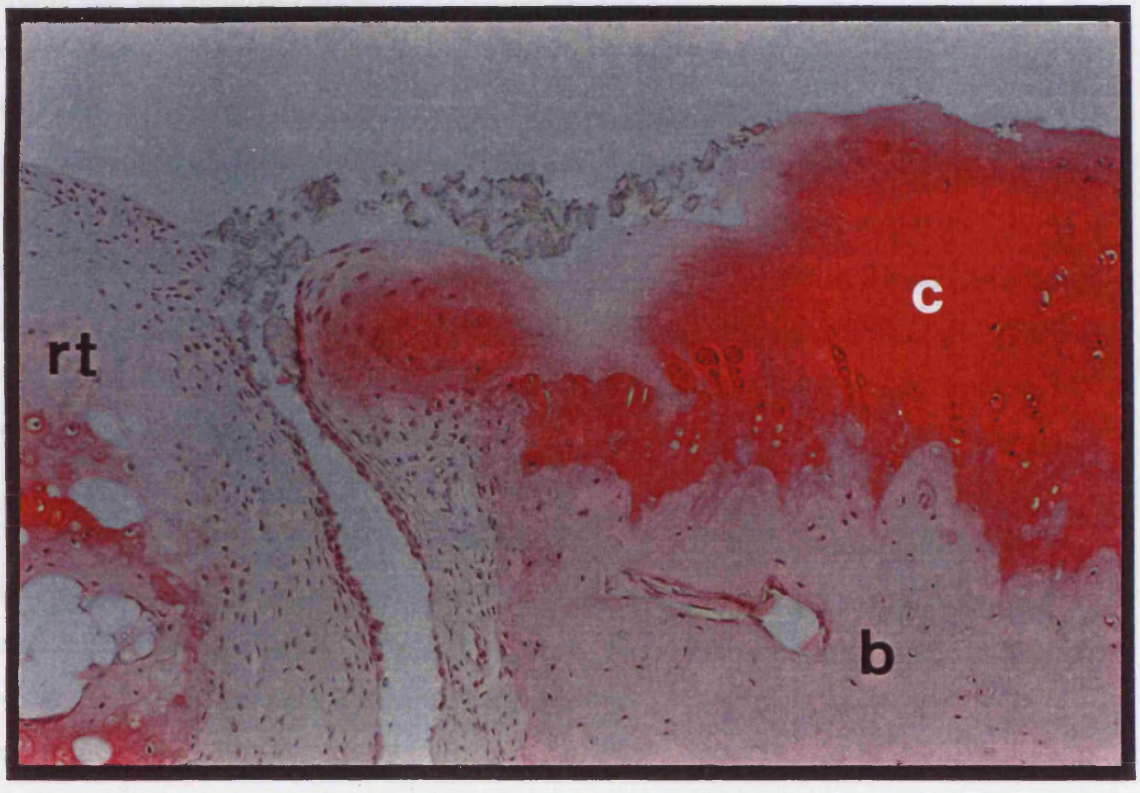


Fig.III.B.28

DISCUSSION

The present in vivo experimental study utilised the new hydrophilic polymer system PEMA/THFMA as a potential biomaterial to enhance the biological resurfacing of large, full-thickness defects, in a weight-bearing area of the articular cartilage. The material has been found to have excellent biological properties in the oral mucosa (Pearson et al 1986). In the present study, no foreign body reaction was noted in neither the synovium nor the repair tissue in any of the specimens. Foreign body responses are common following implantation of biomaterials in orthopaedic surgery. Such reactions have been shown to exist following implantation of poly-propylene-fumarate based materials (Peter et al 1998), of polyglycolide, polylactide or glycolide-lactide based copolymers (Bostman, 1998) and also following implantation of hydroxyapatite coated materials (Morscher et al 1998).

Providing that PEMA/THFMA maintains high water uptake *in vivo*, similar to its hydrophilic property *in vitro* (Patel and Braden, 1991iii), the material may absorb tissue fluids and the dissolved proteins, including growth factors, from the synovial fluid, the bone matrix (Syftestad and Caplan, 1986) and the bone marrow, thus creating an artificial environment that encourages cartilage overgrowth. High water concentration is characteristic and determines the properties of articular cartilage. The water interacts with the structural matrix molecules [particularly the large aggregating proteoglycans] and it is loosely bound to them so to be able to maintain hydration of the cartilage and at the same time to allow exchange with fluids outside the tissue (Torzilli et al 1998a). It seems that the hydrophilicity of the PEMA/THFMA polymer is a major advantage in its role in the regeneration of hyaline articular cartilage.

The resurfaced defects in the biomaterial group in this study were in their majority levelled (mean 69.1% throughout the 1 year of assessments) with the adjacent normal articular cartilage, whereas the control defects were resurfaced with tissue depressed below the level of the surrounding articular cartilage (mean 20.8% gross depression in 1 year). Formation of 'steps' in the articular cartilage surface creates areas of excessive mechanical stress in the adjacent to the repair normal articular cartilage and also in the opposing the repair articulating surface, that can lead to early degeneration and damage (Minas and Nehrer, 1997).

In this study, the polymer was positioned into the subchondral bone, with the surface level recessed just below the level of the adjacent normal articular cartilage. The subchondral marrow cells have been shown to be able to potentially differentiate to chondrocytes (Sporn et al 1986, Johnstone et al 1998). Bone marrow also contains a multipotential stromal cell known as colony-forming unit-fibroblastic [CFU-F] which is capable of differentiating into chondrocytes, osteoblasts, fibroblasts and adipocytes (Beresford, 1989, Caplan, 1991, Wakitani et al 1994) and, therefore, exposure of the subchondral bone could be a biological advantage in articular cartilage repair.

Meachim (1972) as well as Salter et al (1980) reported on the significance of the repaired subchondral plate in the subsequent healing of the overlying articular cartilage. Mechanical loading has been shown to influence the metabolism of undifferentiated mesenchymal cells with regions under compressive loads becoming cartilaginous (Sah et al 1989, Lee and Bader, 1995). When the biomaterial is placed at the level of the subchondral bone, it instantly restores the structural integrity of the subchondral plate, allowing at early stages the much more physiological mechanical loading onto the repair tissue and the adjacent articular cartilage and this, may enhance formation and maturation of the articular cartilage. It is also possible that the blood supply of the subchondral bone plays an essential role in the nutrition of the newly formed articular cartilage above, since it has been shown that ischaemia of the underlying bone results in degenerative changes in the articular cartilage (Graf et al 1992, Hodler et al 1992, Simank et al 1992).

In our study we observed deterioration in the surface appearance in both the PEMA enhanced and the control repairs with time, the surface irregularities being worse in the control group. This might be a sign of progressive degradation of the repair tissue in both groups or it could reflect the inferior biological or mechanical properties of the repair tissue that become apparent with time. The predominance of fibroblasts in the repair tissue in the control defects explains the gradual increase of fissure formation and breakdown of the repair tissue in the control group, since fibrous tissue is mechanically less resilient than articular cartilage (Shapiro et al 1993). Although the proteoglycan concentration is much higher in the biomaterial group compared to the control, in both groups the proteoglycan concentration decreases with time. This decrease is more

apparent in the PEMA group at 1 year (36.6% of repairs have high proteoglycan concentration compared to 90% at 6 weeks) and may reflect a gradual return to 'normal' production of proteoglycans from the chondrocytes that were overproducing in the initial stages following the implantation of the biomaterial. Reduction of proteoglycan formation was mainly noted in the supreficial layers at 1 year, but not in the deeper layers of the repair tissue in the biomaterial group. Hunziker and Kafinger (1998) showed that removal of proteoglycans from the surface of defects in articular cartilage transiently enhanced coverage by repair cells migrating in the defect.

Bonding of the repair tissue to the surrounding normal articular cartilage was significantly superior in the PEMA group showing complete bonding in the majority of repaired defects. This may be explained by the chemical/physical/mechanical resemblance between the normal articular cartilage and the repair tissue in the PEMA group or may reflect the properties of the PEMA/THFMA polymer. Because the polymer swells slightly during polymerisation (Patel and Braden, 1991), it forms an excellent bond with the surrounding tissues, thus providing a stable foundation for the growth of the repair tissue over it. In the control specimens in the present study, severe trauma was noted at the edges of the surrounding the defect 'normal' articular cartilage, possibly caused by uneven mechanical loading. This could also be one of the etiologic factors, responsible for the significantly inferior bonding of the repair tissue to the adjacent articular cartilage, found in the control group. Shapiro et al (1993) and Newman (1998) also noted that the collagen fibrils in 'naturally' healing defects were not well integrated with those of the surrounding cartilage. They suggested that this leads to vertical shear forces, precipitating micromotion and early failure.

In general, the PEMA group showed significantly better results than the control group. Particularly in the early stages the repair is excellent in the majority of the defects, although gradual deterioration is seen with time. The rate of deterioration of the repair tissue in the biomaterial group is low and can be safely speculated that the repair tissue will continue to function satisfactorily after the first year. On the other hand, the rate of improvement of the repair tissue in the control group is very low and it does not appear that the repair tissue in this group will score as high as the biomaterial group at a later observational period.

In conclusion, PEMA/THFMA is a biocompatible material, which enhanced articular cartilage repair and could, therefore, be used for the repair of full-thickness articular cartilage defects. In this study, the use of the biomaterial resulted in marked improvement of the repair tissue, both macroscopically and histologically/histochemically throughout the one year of assessment. The method of implantation is easily reproducible and technically simple. Compared to other to date methods for articular cartilage repair, it requires no harvesting of osteochondral material and it does not involve complicated cell culture prior to implantation. In clinical practice, this method could be performed arthroscopically or by an open procedure in patients with localised articular cartilage defects.

CHAPTER III.C

IMMUNOHISTOCHEMICAL ASSESSMENT OF THE REPAIR TISSUE FOLLOWING IMPLANTATION OF PEMA/THFMA POLYMER IN FULL-THICKNESS ARTICULAR CARTILAGE DEFECTS

OBJECTIVE

MATERIALS

- 80 adult rabbits (specimens randomly taken from those used in Chapter III.B)
- PEMA vs CONTROL

METHOD OF IMMUNOHISTOCHEMICAL EVALUATION

- Silver-enhanced colloidal gold immunostaining
- Collagen type II, keratan-sulfate, chondroitin 4-sulfate and chondroitin 6-sulfate
- At 6 weeks, 3 months, 6 months and 1 year

RESULTS

DISCUSSION

OBJECTIVE

The aim of the immunohistochemical study is to detect the presence of hyaline articular cartilage components in the repair tissue in the biomaterial group, including collagen type II, keratan-sulphate, chondroitin 4-sulphate and chondroitin 6-sulphate.

MATERIALS

The specimens used for the immunohistochemical study of the repair tissue were selected randomly from the rabbits used in the macroscopic and histological/histochemical assessment in Chapter III.B. Specimens were taken from both PEMA and CONTROL groups - 10 rabbits from each observational period (6 weeks, 3 months, 6 months and 1 year). In total 80 repair tissue specimens were assessed, 40 in the PEMA group and 40 in the CONTROL group.

METHOD OF IMMUNOHISTOCHEMICAL EVALUATION

The silver-enhanced colloidal gold immunostaining method was adopted (Hacker et al 1985). The proprietary kit for the immunogold-silver staining was provided by Bioclin (Cardiff, Wales). Four selected monoclonal antibodies were applied individually: the anti-type II collagen antibody (CIICI, Developmental Studies Hybridoma Bank, Iowa, USA, dilution 1:1), the anti-keratan-sulphate (5D4, ICN Biomedicals, Bucks, UK, dilution 1:500), the anti-chondroitin 4-sulphate antibody (2-B-6 ICN Biomedicals, Bucks, UK, dilution 1:100) and the anti-chondroitin 6-sulphate antibody (3-B-3, ICN Biomedicals, Bucks, UK, dilution 1:100).

Five μm thick sections taken from the centre of the repair tissue, were dewaxed in xylene and rehydrated through graded series of ethanols (100%, 90%, 70%, 50%). The epitopes were exposed by incubating the sections with hyaluronidase (10 IU/ml, Sigma, Poole, UK) and chondroitinase ABC (0.25 IU/ml, Sigma, Poole, UK) for 2 hours at 37°C. Incubation with the primary antibody was made overnight at 4°C in a dark, humidified atmosphere.

The antibodies were diluted individually in phosphate buffered saline with 0.6% bovine serum albumin (Sigma, Poole, UK). Sections were incubated with the colloidal gold conjugated anti-mouse Ig secondary antibody (BioClin, Cardiff, Wales, dilution 1:50) for 2 hours at room temperature in a dark, humidified atmosphere, followed by fixation in 1% glutaraldehyde. The colloidal gold was visualised by use of a physical silver development solution [enhancement] and the sections were counter-stained with Mayer's haematoxylin.

RESULTS

Table II.C.1 demonstrates analysis of the immunolocalisation of the cartilage components in the repair tissue in both the biomaterial and the control groups.

Table III.C.1:
Immunohistochemical assessment of repair tissue
PEMA vs CONTROL

ANTIGEN	6 weeks		3 months		6 months		1 year	
	PEMA n=10	CONTROL n=10	PEMA n=10	CONTROL n=10	PEMA n=10	CONTROL n=10	PEMA n=10	CONTROL n=10
Collagen type II								
>50% of matrix	5 (50%)	0 (0%)	5 (50%)	0 (0%)	3 (30%)	2 (20%)	3 (30%)	2 (20%)
<50% of matrix	4 (40%)	2 (20%)	5 (50%)	4 (40%)	7 (70%)	2 (20%)	5 (50%)	3 (30%)
no detection	1 (10%)	8 (80%)	0 (0%)	6 (60%)	0 (0%)	6 (60%)	2 (20%)	5 (50%)
Keratan Sulphate								
>50% of matrix	6 (60%)	0 (0%)	4 (40%)	1 (10%)	6 (60%)	1 (10%)	5 (50%)	2 (20%)
<50% of matrix	4 (40%)	0 (0%)	6 (60%)	7 (70%)	4 (40%)	4 (40%)	4 (40%)	4 (40%)
no detection	0 (0%)	10 (100%)	0 (0%)	2 (20%)	0 (0%)	5 (50%)	1 (10%)	4 (40%)
Chondroitin Sulphate								
>50% of matrix	6 (60%)	0 (0%)	6 (60%)	2 (20%)	6 (60%)	2 (20%)	7 (70%)	2 (20%)
<50% of matrix	3 (30%)	2 (20%)	4 (40%)	6 (60%)	4 (40%)	2 (20%)	2 (20%)	0 (0%)
no detection	1 (10%)	8 (80%)	0 (0%)	2 (20%)	0 (0%)	6 (60%)	1 (10%)	8 (80%)
Chondroitin 6-Sulphate								
>50% of matrix	7 (70%)	0 (0%)	8 (80%)	2 (20%)	5 (50%)	2 (20%)	8 (80%)	1 (10%)
<50% of matrix	3 (30%)	4 (40%)	2 (20%)	6 (60%)	3 (30%)	2 (20%)	2 (20%)	4 (40%)
no detection	0 (0%)	6 (60%)	0 (0%)	2 (20%)	2 (20%)	6 (60%)	0 (0%)	5 (50%)

Fifty per cent of the specimens in the biomaterial group showed normal distribution of collagen type II throughout the matrix of the repair tissue at 6 weeks and 3 months post-operatively. For the same observational periods all control specimens showed limited only distribution of collagen type II, arranged mainly around chondrocyte clusters. By 1

year, collagen II was detected in 80% of the specimens (30% showing normal distribution).

Glycosaminoglycans were detected in the extracellular matrix in over 80% of the specimens in all observational periods in the PEMA group, compared to less than 20% in the control group.

Statistical analysis of the results:

For the antigen detection, the data did not have paired values. All that was recorded was the row and column sums with the scores as >50%, <50% and no detection for the PEMA and the CONTROL knees at 6 weeks, 3 months, 6 months and 1 year. To analyse such data, ***Fisher's exact test*** was used, which tests for independence between the row distribution and column distribution.

The table below shows P-values from Fisher's exact test for PEMA vs CONTROL stratified by time of inspection:

Antigen	6 weeks	3 months	6 months	1 year	Using combined time groups
Collagen type II	.004	.002	.011	.523	3e-7
Keratan-sulphate	1e-5	.164	.012	.289	6e-8
Chondroitin 4-sulphate	.001	.097	.021	.009	4e-8
Chondroitin 6-sulphate	.001	.033	.210	.004	6e-8

The values indicate that the biomaterial enhances significantly the production of collagen type II and glycosaminoglycans in the repair tissue in all observational periods, compared to the control specimens. PEMA repair is significantly superior to control up to 1 year post-implantation, although the enhancement effect decreases as the time of observation increases.

Fig.III.C.1

Immunohistochemical appearance of the repair tissue in the polymer group at 6 weeks:

The repair tissue **[rt]** contains collagen type II like the normal articular cartilage **[c]**. The subchondral bone **[b]** does not contain collagen type II and stained negative. The → indicates the transition from the repair tissue to the normal articular cartilage, **[p**=polymer**]** *(Silver-enhanced colloidal gold immuno-staining, x 13.2)*

Fig.III.C.2

Immunohistochemical appearance of the repair tissue in the polymer group at 3 months:

Large amounts of keratan-sulfate can be detected in the deep **[d]** layers in the repair tissue, with smaller amounts also noted in the superficial **[s]** layer. *(Silver-enhanced colloidal gold immuno-staining, x 66)*

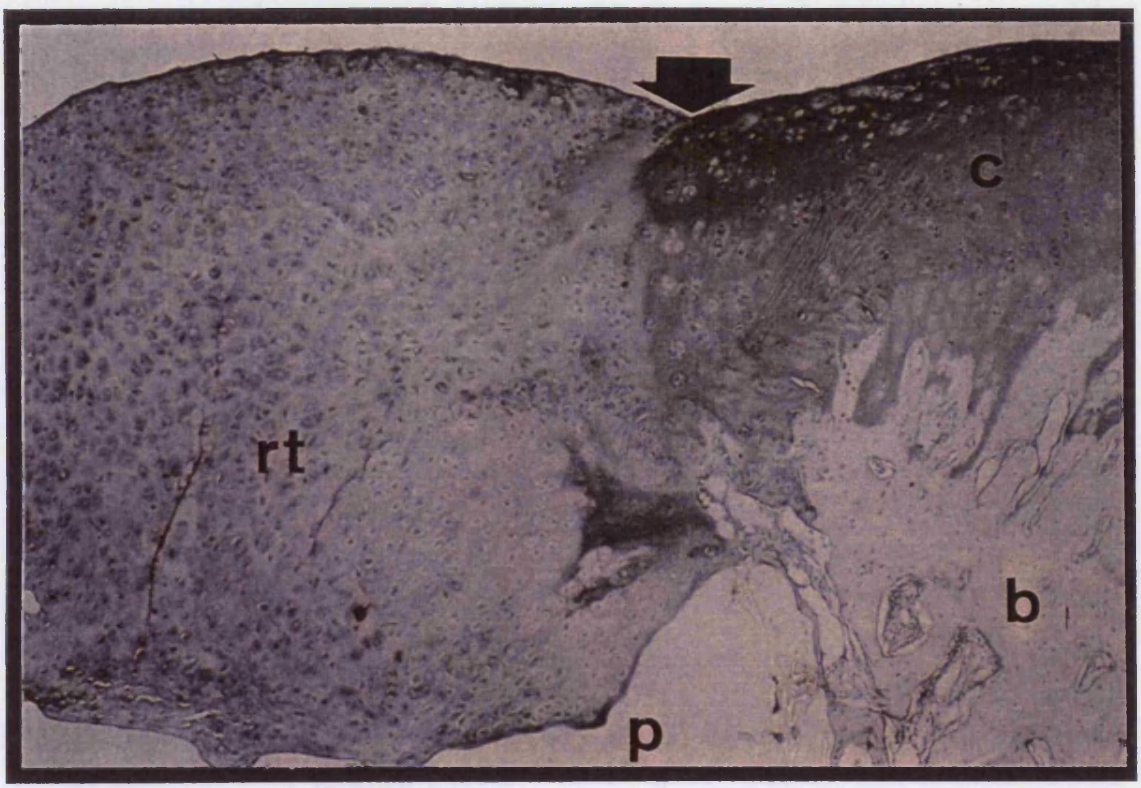


Fig.III.C.1

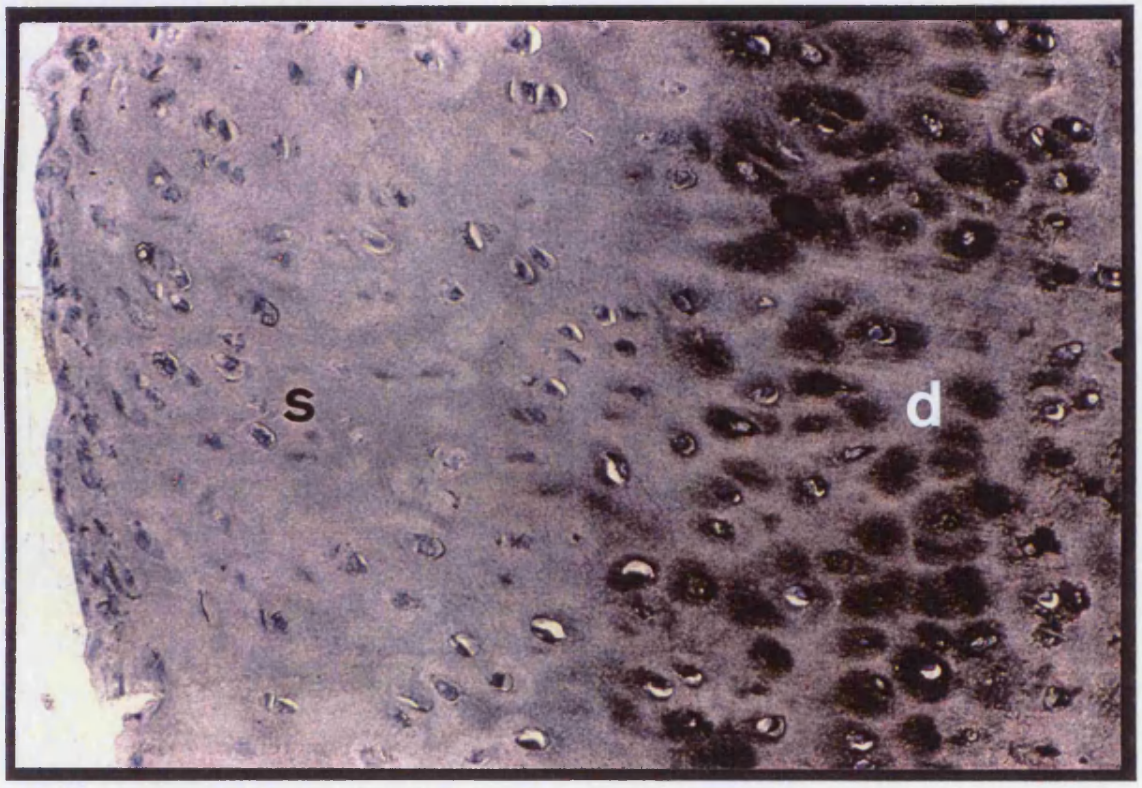


Fig.III.C.2

Fig.III.C.3

Immunohistochemical appearance of the repair tissue in the polymer group at 6 months:

The repair tissue contains collagen type II evenly distributed throughout the matrix with larger concentration in the superficial **[s]** layer. *(Silver-enhanced colloidal gold immuno-staining, x 66)*

Fig.III.C.4

Immunohistochemical appearance of the repair tissue in the polymer group at 1 year:

Chondroitin 4-sulphate can be detected in all layers of the repair tissue, with higher concentrations in the deep **[d]** layers than in the superficial **[s]** layer. In this particular specimen subchondral bone **[b]** has been formed that contains no chondroitin 4-sulphate and stained negative. *(Silver-enhanced colloidal gold immuno-staining, x 66)*

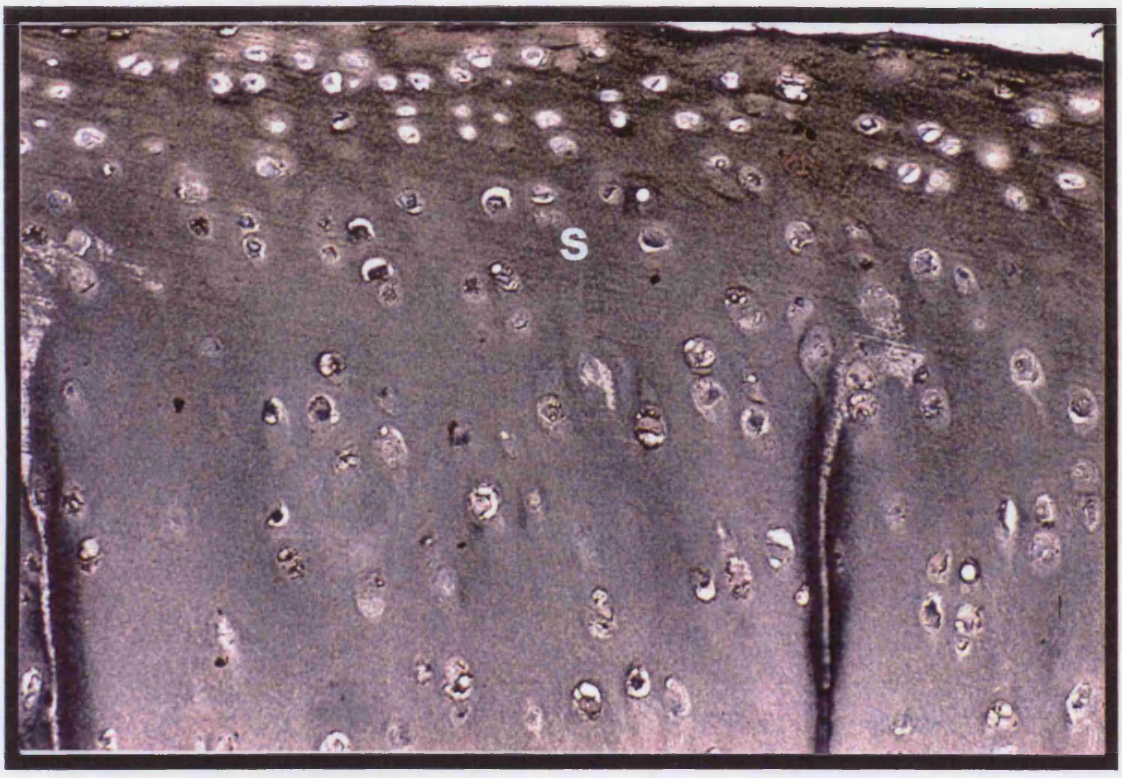


Fig.III.C.3

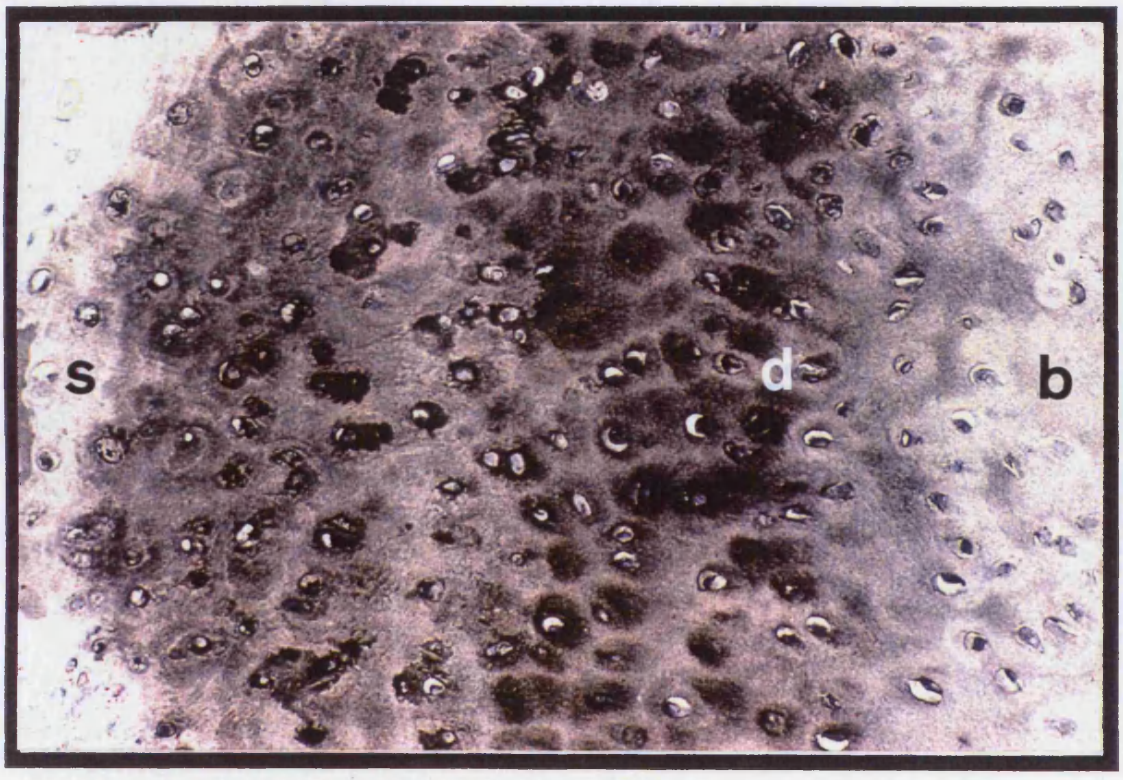


Fig.III.C.4

Fig.III.C.5

Typical immunohistochemical appearance of the repair tissue in the control group:
This specimen is of the 6-month group and shows complete absence of collagen type II in the repair tissue **[rt]**. Collagen type II is detected in the articular cartilage **[c]** surrounding the defect, but the subchondral bone **[b]** contains no collagen type II and stained negative. *(Silver-enhanced colloidal gold immuno-staining, x 33)*

Fig.III.C.6

Immunohistochemical appearance of the normal articular cartilage surrounding the defect in the specimen shown in Fig.III.D.5:
Small concentration of collagen type II around the cells with minimal detection in the extracellular matrix, suggesting degeneration of the normal articular cartilage adjacent to the defect in the control group. *(Silver-enhanced colloidal gold immuno-staining, x 132)*

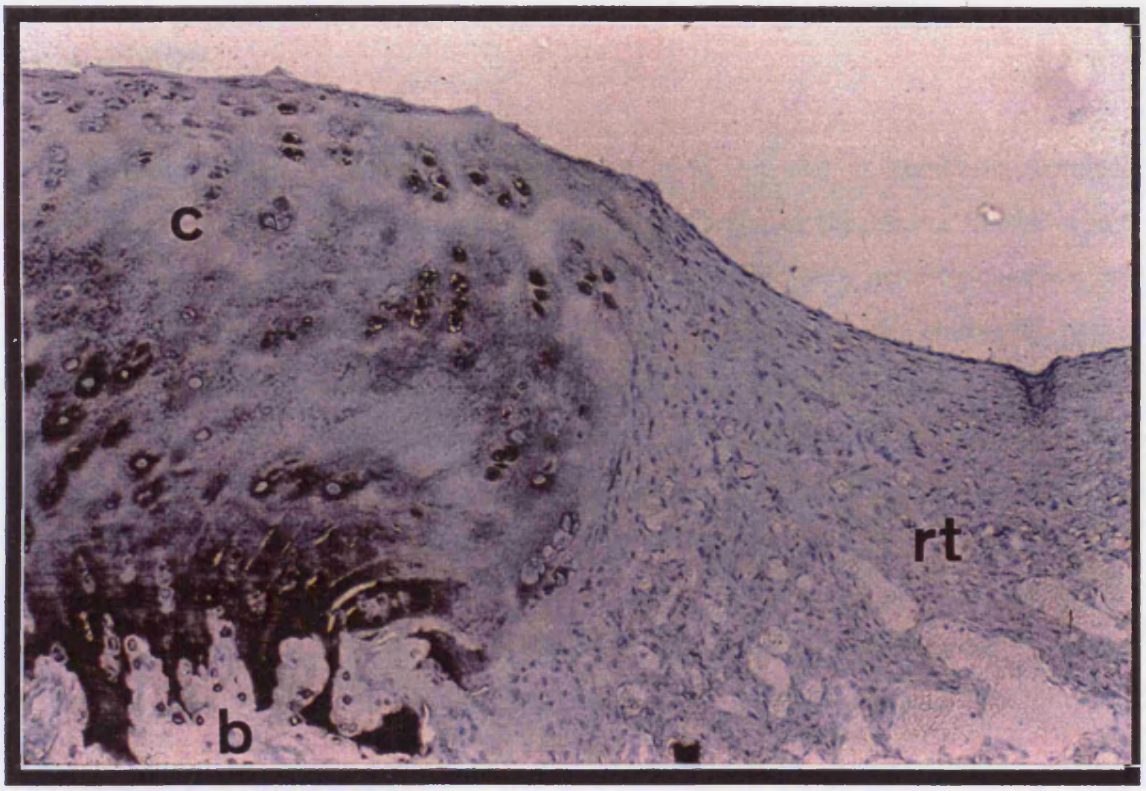


Fig.III.C.5

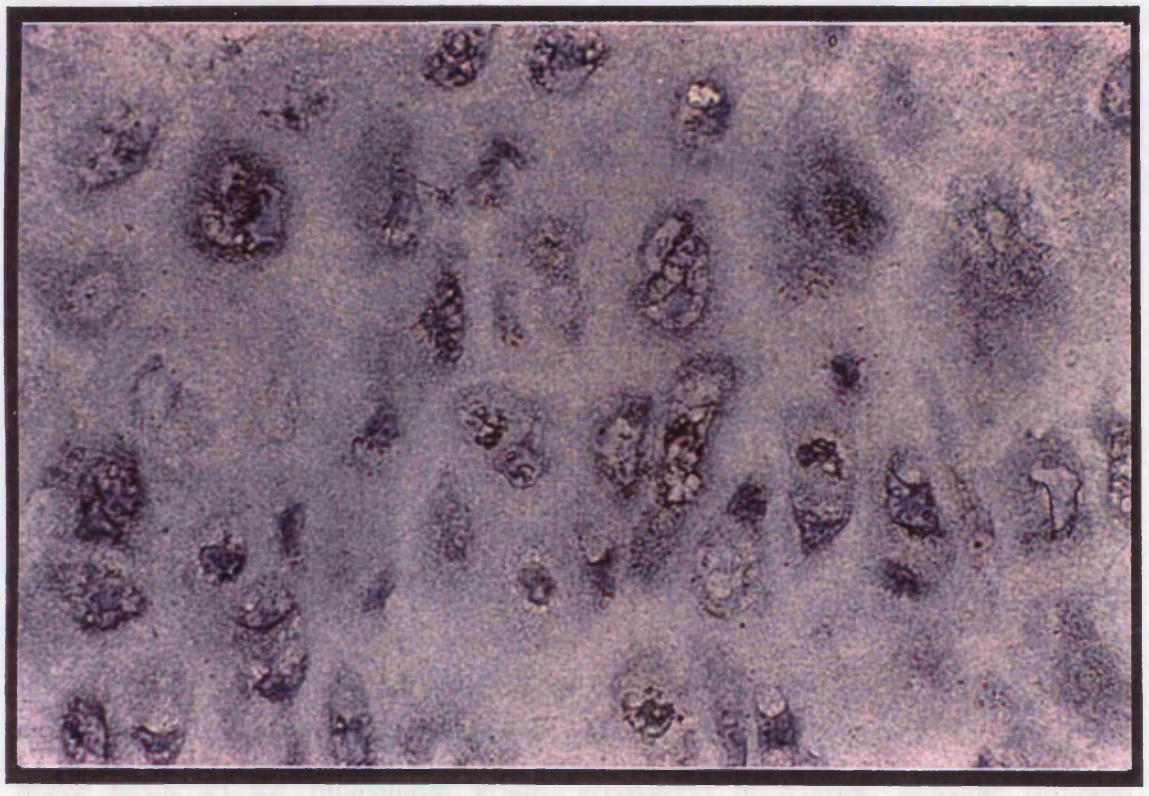


Fig.III.C.6

DISCUSSION

The silver-enhanced colloidal gold technique used for the immunohistochemical detection of collagen type II and the glycosaminoglycans in the matrix of the repair tissue, is a very sensitive method and allows the use of routine histological counter-staining, such as Haematoxylin and Eosin, which helps in the study of tissue morphology. The method also allows use of low magnification, that helps in the comparative analysis between groups (Hacker et al 1985). In the present study the use of low magnification also helped in the comparative assessment of the repair tissue with the adjacent normal articular cartilage.

Detection of collagen type II and glycosaminoglycans was constantly high in the PEMA/THFMA group at all time intervals studied. In the control group detection was very low at 6 weeks and 3 months and although it improved at 6 months and 1 year, it always remained significantly lower than the biomaterial group. Collagen type II is found in tissues with high water and proteoglycan content, suggesting that this type of collagen has specific properties, to create and maintain a highly hydrated matrix (Buckwalter et al 1997). The glycosaminoglycans, which are side chains of the proteoglycan monomer, form long strings of negative charges that repel other negatively charged molecules and attract cations (Roughley and Lee, 1994). The resultant high osmolarity accounts for the retention of water in cartilage. Glycosaminoglycans also repel each other tending to keep the molecules in distended state (Buckwalter et al 1988).

The hydrophilicity of the new polymer must be of great importance as the specific to hyaline cartilage collagen type II and glycosaminoglycans maintain high water concentrations. Nutrition of the chondrocytes, which occurs by diffusion, may therefore be facilitated and the distension of the newly formed articular cartilage may also be advantageous by improving its bonding with the surrounding normal tissues.

In this immunohistochemical study, four principal matrix components collagen type II, keratan-sulphate, chondroitin 4-sulphate and chondroitin 6-sulphate, were detected in the repair tissue in the biomaterial group. Production of hyaline articular cartilage components in the repair tissue treated with PEMA/THFMA was significantly higher at the early stages post-implantation. It remained higher than the control group up to 1 year.

CHAPTER III.D

ELECTRON MICROSCOPY OF THE REPAIR TISSUE FOLLOWING IMPLANTATION OF PEMA/THFMA POLYMER IN FULL-THICKNESS ARTICULAR CARTILAGE DEFECTS

OBJECTIVE

MATERIALS

- 16 adult rabbits (specimens randomly taken from those used in Chapter III.B)
- PEMA vs CONTROL

METHOD

- Transmission electron microscopy
- At 6 weeks, 3 months, 6 months and 1 year

RESULTS

DISCUSSION

OBJECTIVE

The electron microscopy study aims to demonstrate the ultrastructural characteristics of the chondrocytes and the extracellular matrix in articular cartilage repair tissue treated with PEMA/THFMA, in an attempt to provide information on the potential mode of action of the polymer in terms of chondrocyte metabolism and matrix organisation.

MATERIALS

Sixteen specimens [2 from the PEMA and 2 from the control group at 6 weeks, 3 months, 6 months and 1 year] were randomly taken from those used in the macroscopic and histological/histochemical assessment in Chapter III.B and were prepared for transmission electron microscopy.

METHOD OF TRANSMISSION ELECTRON MICROSCOPY

The specimens were cut with a blade into small pieces 3 x 3 mm each, and were fixed in 2% glutaraldehyde in 0.1M sodium cacodylate buffer at pH 7.2 for 24 hours at 4^{o}C followed by secondary fixation in an aqueous solution of 1% osmium tetroxide and 1.5% potassium ferrocyanide (de Bruijn, 1973, Dvorak et al, 1972, Farnum and Wilsman, 1983) for one hour. The samples were washed in the same buffer and dehydrated through a graded series of ethanols (70%, 90%, 100%). Residual polymer was carefully removed prior to impregnation with a 1:1 ethanol/Spurrs' resin mixture (Agar Scientific, Stansted, UK) for six hours including two hours vacuum impregnation at 150 mbar. The 1:1 mixture of ethanol/Spurrs' resin underwent four changes over twelve hours each, of the Spurrs' resin alternating every six hours with vacuum infiltration. The specimens were embedded in 8mm flat polythene capsules and cured at 70^{o}C for 18 hours.

One μm thick sections were cut with a diamond knife for light microscopy and stained with 1% Toluidine Blue in 1% Borax (Robinson and Gray, 1990). The resin was removed prior to staining with Sodium Methoxide and 90 nm thick sections were cut on

an L.K.B. Ultratome III. The sections were stained with 2% Uranyl Acetate (10 minutes) and Reynold's Lead Citrate (10 minutes) and were picked up on 3 mm diameter copper grids. The electron microscope used was Phillips CM 12 fitted with EDAX PV 9800 X-ray Microanalysis system.

Unstained sections were used for the energy dispersive microanalysis. They were probed at 80 KV with a specimen tilt of 20^{o}, giving a final take-off angle of 40^{o}, and counted for 200 live seconds.

RESULTS

Figures III.D.1 to III.D.6 show the ultrastructural characteristics of the repair tissue and the control groups.

The PEMA/THFMA biomaterial used in this study appeared to have enhanced chondrocyte migration / formation in both the superficial and the deep zones of the repair tissue in all observational periods. In the superficial layer, chondrocytes were flat in appearance, orientated parallel to the articular surface and containing pronounced rough endoplasmic reticulum and mitochondria. Electron-dense granules were found in the matrix of the repair tissue. By contrast, cells in the superficial zone of the repair tissue in the control specimens appeared to be less productive and no granules were noted around these cells or in the matrix. The latter was characteristically depleted of fibres, particularly immediately adjacent to cells.

In the deep layers of the repair tissue in the biomaterial group the chondrocytes were round and large with numerous electron-dense molecules forming a 'halo' around the cells and into the adjacent matrix in all studied periods. Abundant rough endoplasmic reticulum, numerous secretory vesicles, mitochondria and large Golgi apparatei were noted in the cytoplasm of these cells. Multiple cytoplasm processes emerging from the cell periphery were also seen. Energy dispersive microanalysis detected Sulphur in these molecules up to 1 year post-implantation, suggesting the presence of proteoglycans. In the control group no pericellular granules were seen at any observation period and the matrix showed large calibre fibrils consistent with collagen type I.

Another ultrastructural characteristic observed in both groups, more common in the polymer group, was the formation of chondrocyte clusters. They were found mainly in the deep layers of the repair tissue enclosed in thin pericellular layer of small calibre fibres. Many short processes were seen emerging from the cells within the clusters. These were significantly more numerous in the surfaces of the chondrocytes looking at the centre of the cluster rather than those looking at its periphery.

The matrix of the neocartilage in the biomaterial group appeared to have a well-organised arrangement of predominantly small calibre collagen fibrils, consistent with collagen type II. This collagen mesh appeared to be more pronounced around the chondrocytes in the 1 year specimens. However, compared with normal articular cartilage, the matrix in the repair tissue appeared to be significanlty depleted of fibres, although it outnumbered hyaline articular cartilage in cells. In the control specimens the newly formed tissue revealed a disorganised arrangement of multidirectional large calibre collagen fibrils and few only cells with chondrocyte phenotype that appeared to be flattened in all layers. Chondrocyte degeneration with ghost cells, empty lacunae and excessive intracellular deposition of lipids were frequently noted in the control specimens at all observational periods. Mild chondrocyte degeneration was also noted at 1 year in the repair tissue in the PEMA/THFMA group.

Fig.III.D.1

Ultrastructural characteristics of the repair tissue in the polymer group at 6 weeks: Large, highly productive, round chondrocytes **[c]** with multiple cytoplasmic processes. Electron dense granules are noted at the periphery of the cells and in the extracellular matrix **[m]**. *(Uranyl acetate and Reynold's lead citrate staining, x 17000, the bar equals 1μm)*

Fig.III.D.2

Energy dispersive micro-analysis of the repair tissue in the polymer group at 6 weeks:

The electron-dense granules seen in Fig.III.D.1 were probed in unstained, 90 nm thick sections. Molecular sulphur [SK_a peak] was detected suggesting the presence of proteoglycans in these granules. The osmium [OsL_a peak] is due to fixation of the specimen with osmium, while the copper [CuK_a peak] molecule derives from the copper grid used for the mounting of the specimen.

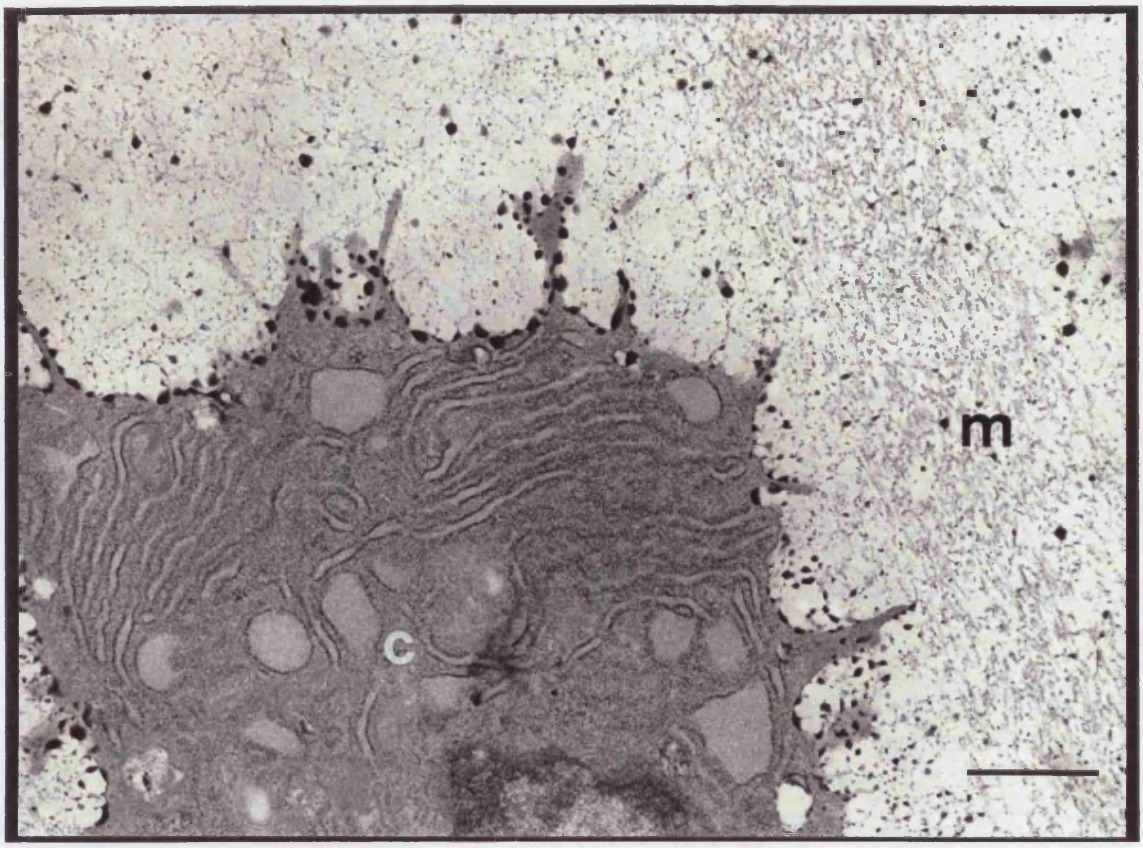


Fig.III.D.1

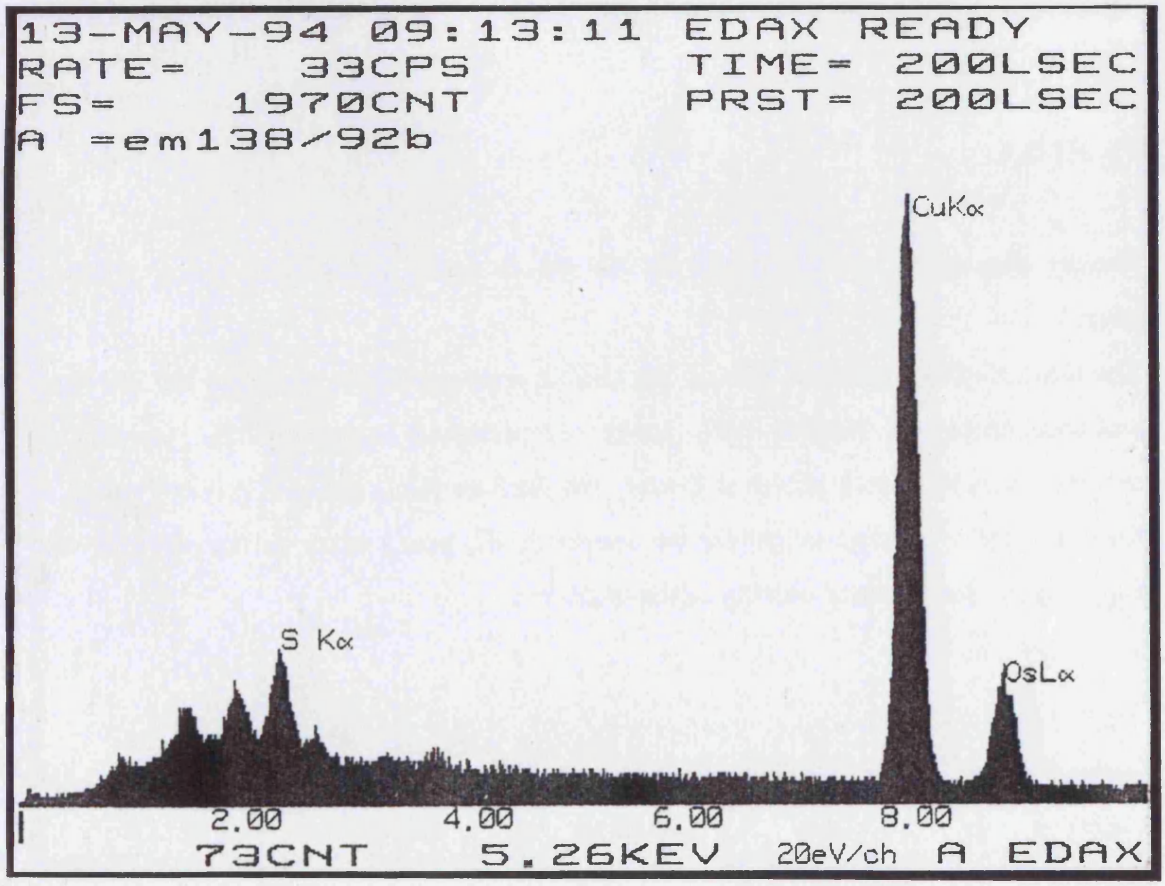


Fig.III.D.2

Fig.III.D.3

Ultrastructural characteristics of the repair tissue in the polymer group at 1 year: Highly productive chondrocytes with multiple cytoplasm processes and numerous electron-dense granules at the periphery of the cells and in the adjacent extracellular matrix. The collagen fibres in the matrix **[m]** are forming characteristic 'lacunae' around each chondrocyte. *(Uranyl acetate and Reynold's lead citrate staining, x 5100, the bar equals 3μm)*

Fig.III.D.4

Energy dispersive micro-analysis of the repair tissue in the polymer group at 1 year:

The electron-dense granules seen in Fig.III.D.3 were probed in unstained, 90 nm thick sections. Molecular sulphur [SK_a peak] was detected suggesting the presence of proteoglycans in these granules at 1 year. The osmium [OsL_a peak] is due to fixation of the specimen with osmium, while the copper [CuK_a peak] molecule derives from the copper grid used for the mounting of the specimen.

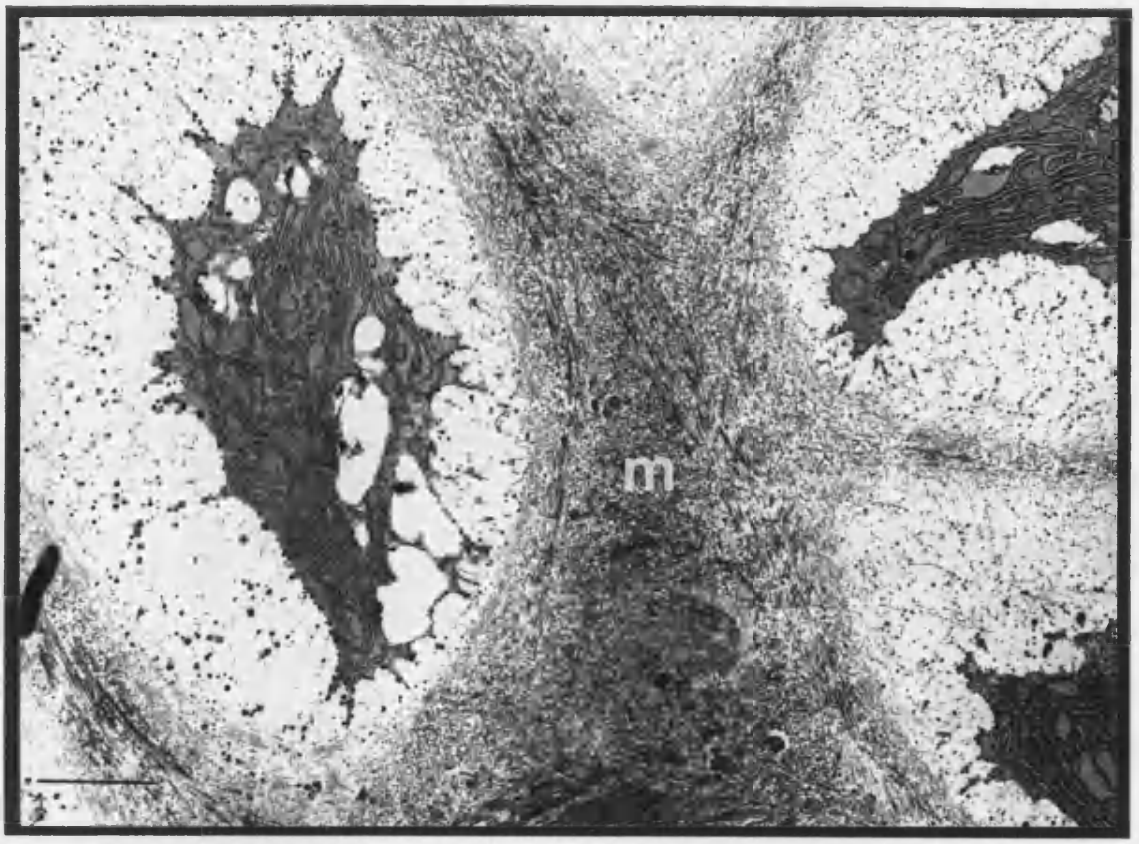


Fig.III.D.3

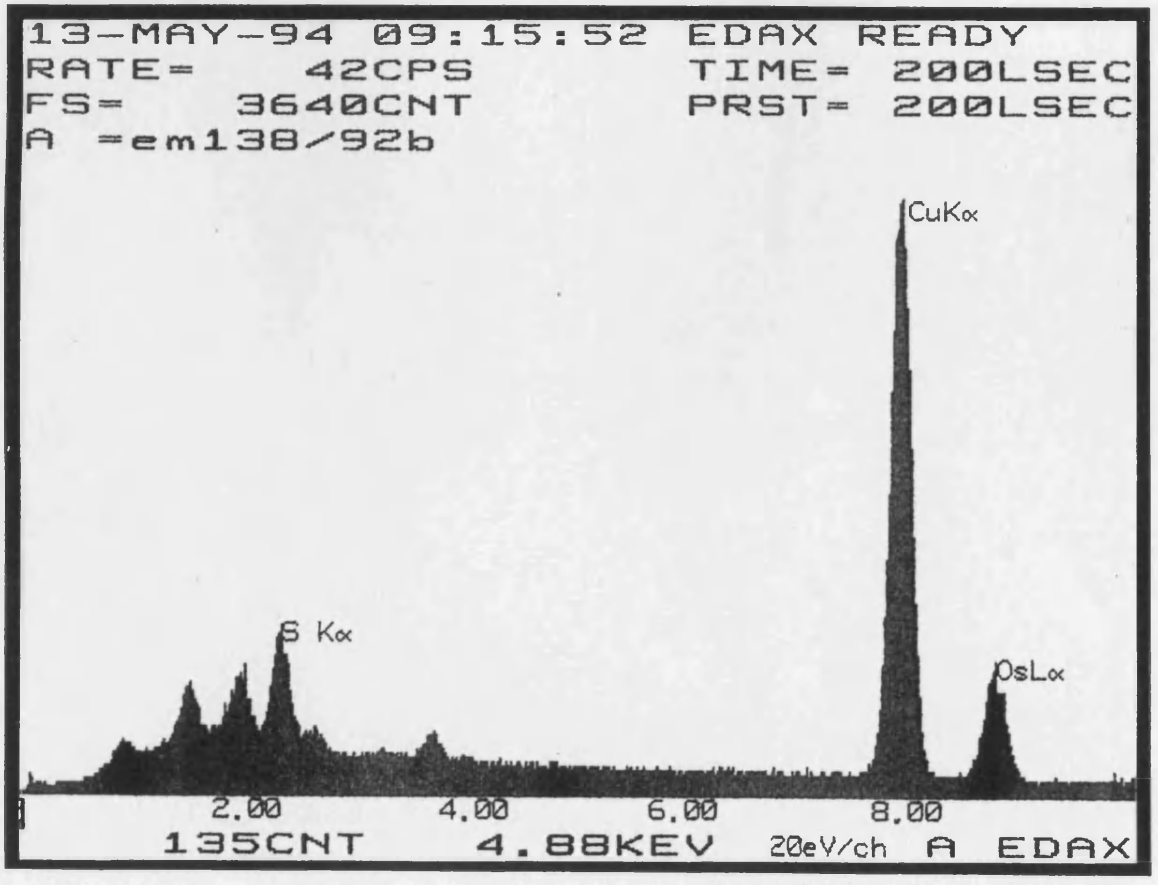


Fig.III.D.4

Fig.III.D.5

Ultrastructural characteristics of the repair tissue in the control group at 6 weeks: The chondrocytes are surrounded by loose extracellular matrix **[m]** without the characteristic electron-dense granules seen in the polymer group. *(Uranyl acetate and Reynold's lead citrate staining, x 5100, the bar equals 3μm)*

Fig.III.D.6

Ultrastructural characteristics of the repair tissue in the control group at 1 year: The extracellular matrix **[m]** is dense with large-calibre collagen fibrils arranged in a multidirectional, disorganised pattern around the scattered chondrocytes **[c]**. *(Uranyl acetate and Reynold's lead citrate staining, x 5100, the bar equals 3μm)*

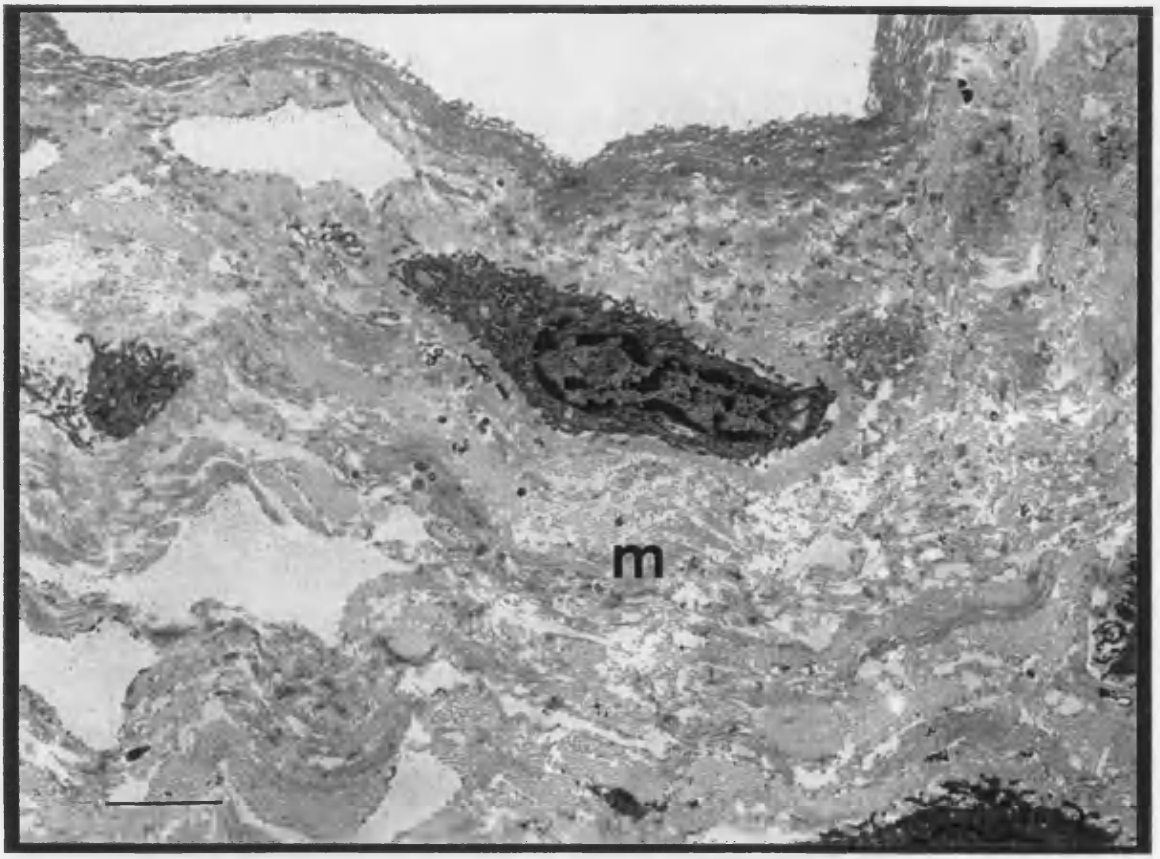


Fig.III.D.5

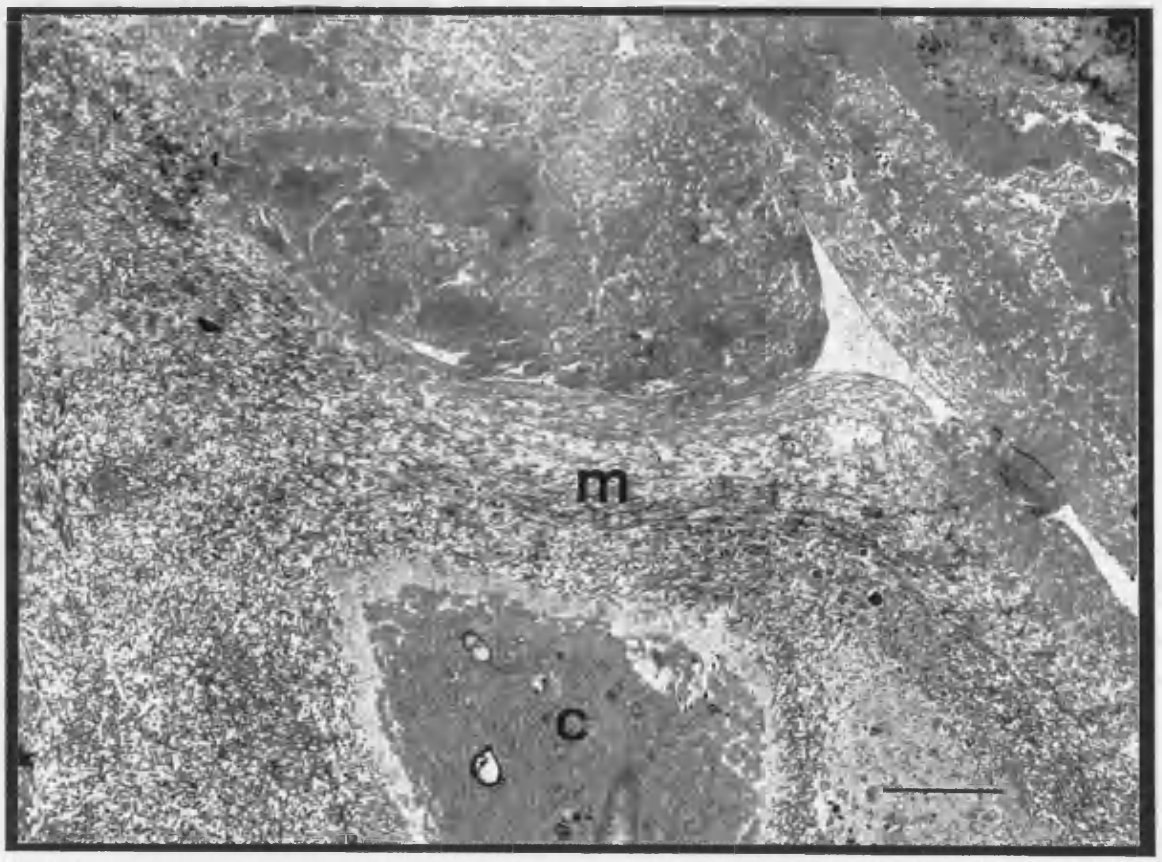


Fig.III.D.6

DISCUSSION

The ultrastructure of the repair tissue in the biomaterial group showed large number of highly productive chondrocytes with multiple electron-dense granules around the cells and in the extracellular matrix. These granules contain molecular Sulphur and we speculate that they represent proteoglycan molecules secreted by the cells.

It is well known that the proteoglycan content of cartilage matrix is correlated with compressive strength (Buckwalter, 1983, Carlson et al 1986) and that loss of this component leads to increased mechanical stress and subsequent dysfunction of chondrocytes (Reimann et al 1982). Moreover, the capability of the articular cartilage to repair is related to the stress placed upon it (Carlsson et al 1986) and excessive loading can be harmful. If therefore PEMA/THFMA enhances proteoglycan production by the chondrocytes, it can provide a strong mechanical shelter, which prevents cell dysfunction and death, thus improving the formation as well as the longevity of the repair tissue. It can also be hypothesised that the grossly disorganised multiple orientation of the collagen fibrils that has been recorded in the control specimens, is the result of excessive abnormal mechanical loading applied during joint movement on the repair tissue.

It has been postulated that insulin-like growth factors can be crucial for the homeostasis of the extracellular matrix of articular cartilage in vivo (McQuillan et al 1986) and therefore it is possible that the hydrophilic PEMA/THFMA attracts/adsorbs growth factors, which effectively enhance the overall repair process.

The chondron concept was introduced by Benninghoff in 1925 to describe the functional and metabolic unit of the chondrocyte and its pericellular micro-environment in hyaline cartilage (Poole et al 1990). Ultrastructural studies have shown that the chondron in adult articular cartilage consists of a chondrocyte and its pericellular matrix, both enclosed within an impacted fibrillar capsule (Poole et al 1987), similar to that observed in the 1 year specimens in the biomaterial group in this study. Chondrons exist in the middle and deep layers of normal adult articular cartilage and their pericellular matrix is rich in proteoglycans (Bayliss et al 1983) bound with strong cross-links to the collagen fibril meshwork (Poole et al 1990). The latter consists, among other collagens, of

collagen types II, VI and IX (Poole et al 1988) of which type VI collagen provides an adhesive mechanism for substratum attachment (Linsenmayer et al 1986). It is also believed that collagen type VI is exclusive to the capsule of the chondron and does not form a general component of the territorial and inter-territorial matrices. As far as the function role of the chondron is concerned, Poole also postulates that it acts hydrodynamically to protect the chondrocyte during compressive loading. In this study formation of chondrons was observed predominantly in the biomaterial group.

Transmission electron microscopy showed that PEMA/THFMA enhanced chondrocyte formation and matrix collagen organisation in the repair tissue. Energy dispersive microanalysis helped in the characterisation and chemical analysis of the multiple electron dense granules seen exclusively in the biomaterial specimens at all observational periods up to 1 year post-implantation. We postulate that the mode of action of PEMA/THFMA is by formation of chondrons, distributed through the whole of the repair tissue, which produce large amounts of proteoglycans. The integrity of these chondrons provides further mechanical protection to the chondrocytes against excessive/abnormal loading and therefore, particularly in the proteoglycan-depleted matrix, they improve the tissue characteristics of the repair tissue.

CHAPTER III.E

IMAGE ANALYSIS OF THE REPAIR TISSUE FOLLOWING IMPLANTATION OF PEMA/THFMA POLYMER IN FULL-THICKNESS ARTICULAR CARTILAGE DEFECTS

OBJECTIVE

MATERIALS

- 40 adult rabbits (specimens randomly taken from those used in Chapter III.B)
- PEMA vs CONTROL

METHOD

- Cell count using Image Analysis from periphery and centre of repair and from adjacent normal articular cartilage
- At 6 weeks, 3 months, 6 months and 1 year

RESULTS

DISCUSSION

OBJECTIVE

This study aims to assess quantitatively histological characteristics [number of cells] of the repair tissue in the PEMA/THFMA group, compared to the control group and to normal articular cartilage, using a computerised technique.

MATERIALS AND METHOD

The image analysis system used in this study comprised of an Olympus microscope, connected to a SONY video camera and connected to an Apple Macintosh Quadra computer with a Neotec image grabber programme and an Optilab (Groftec, Paris, France) image analysis programme.

Five specimens in each observational period [6 weeks, 3 months, 6 months and 1 year] from both the biomaterial and the control group were randomly taken from those used in the macroscopic and histological/histochemical assessment in Chapter III.B. In all specimens the number of cells in the repair tissue was calculated in the centre of the defect and also at the periphery. Evaluation of the normal articular cartilage was performed at the closest to the defect normal articular cartilage that was fully supported by subchondral bone.

Sections 5 μm thick stained with Safranin-O were used and selected areas were calibrated. For each section a number of images were taken from the edge and from the centre of the repair tissue and also from the adjacent normal articular cartilage. Twenty-four bit colour images were obtained at 44 [2.2 x 20] magnification. Images were then thresholded to select a biniary image of only the chondrocyte lacunae. The images were then further processed using filters, which enhanced the lacunae. The number of lacunae was then determined within the image.

The Image Analysis system an example of the computerised images are shown in Fig.III.E.1 and III.E.2.

Fig.III.E.1:

The Image Analysis system:

The microscope is connected to a video camera and to a computer with an image grabber and an image analysis programme

Fig.III.E.2:

Image analysis picture following the filter process that selected only the chondrocyte lacunae:

<u>Centre / repair tissue</u>
Intermediate Particles Detection Report

- Lower Gray: 1
- Upper Gray: 1
- Minimum detectable: 8.54372e-06
- Maximum detectable: 2.79961e+00
- Detected particles: 74
- Touching border: 2
- Minimum area: 9.39810e-06
- Maximum area: 3.33632e-04
- Mean area: 8.73769e-05
- Standard deviation: 7.18380e-05
- Sum area: 6.46589e-03
- Total area: 1.31232e-01
- Percentage: 4.92708e+00

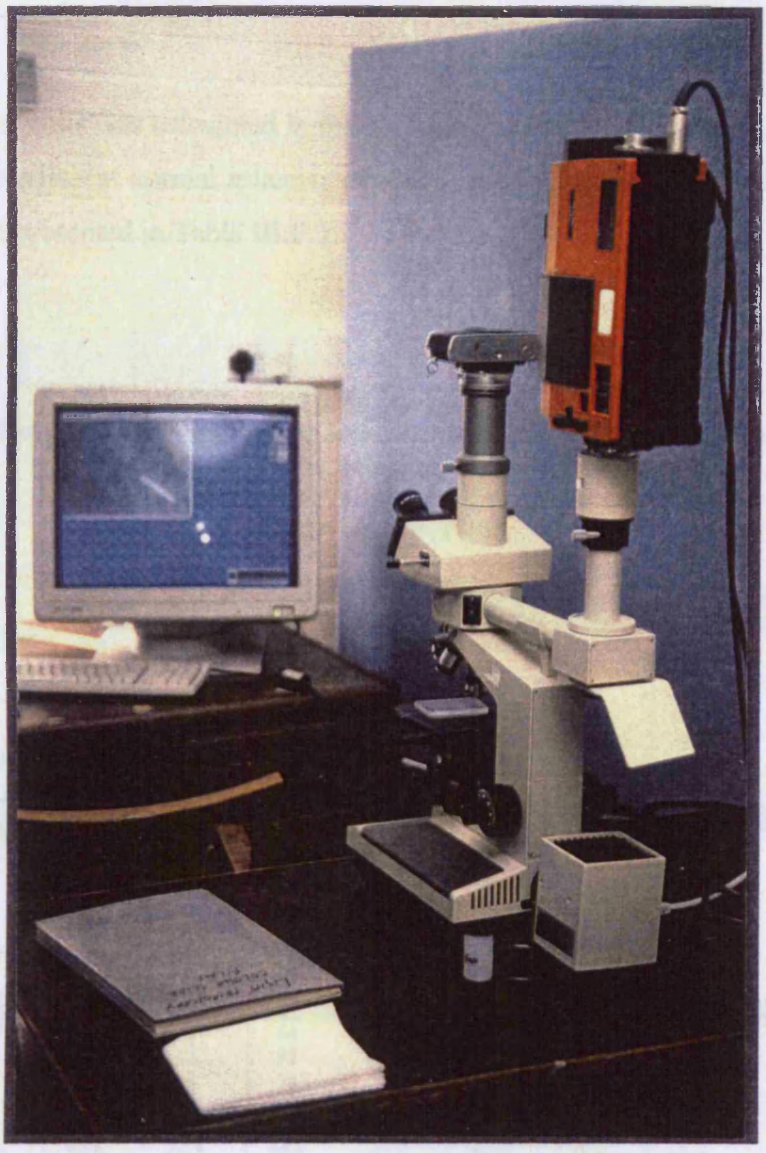


Fig.III.E.1

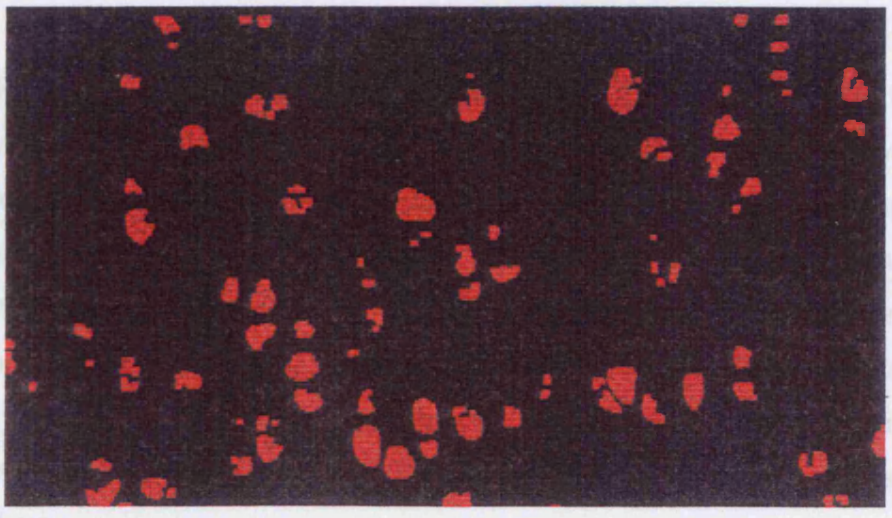


Fig.III.E.2

RESULTS

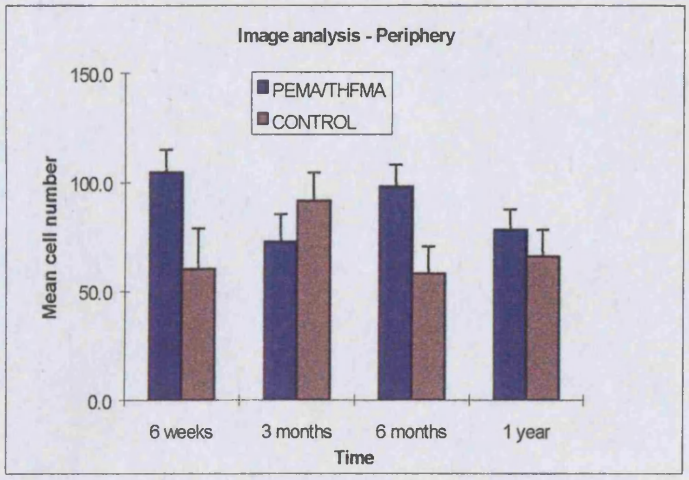
The number of cells was calculated in the periphery and the centre of the repair tissue as well as in the adjacent normal articular cartilage, and the mean values and the standard deviations are presented in Table III.E.1.

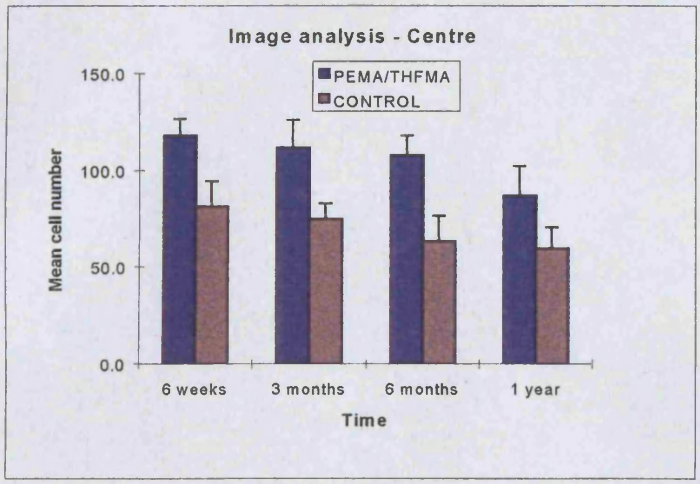
Table III.E.1:
Number of cells in the periphery and the centre of the repair tissue and the adjacent to the defect normal articular cartilage Image Analysis at 6 weeks, 3 months, 6 months and 1 year PEMA vs CONTROL

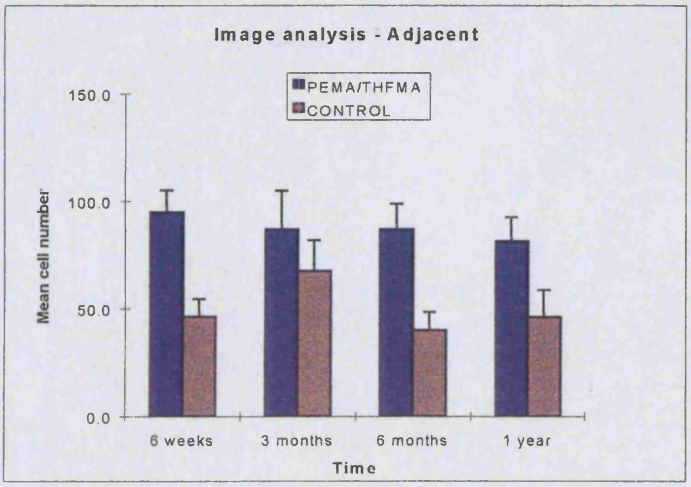
	6 weeks		3 months		6 months		1 year	
	PEMA	CONTROL	PEMA	CONTROL	PEMA	CONTROL	PEMA	CONTROL
PERIPHERY	108	86	79	93	106	48	68	74
	92	47	91	78	94	72	76	79
	99	38	71	99	111	43	84	52
	106	62	60	108	87	67	72	54
	119	68	62	79	92	61	91	71
MEAN	**104.8**	**60.2**	**72.6**	**91.4**	**98.0**	**58.2**	**78.2**	**66.0**
SD	**10.1**	**18.7**	**12.8**	**12.9**	**10.1**	**12.4**	**9.3**	**12.2**
CENTRE	114	68	98	69	108	73	98	45
	132	79	121	78	124	79	76	68
	118	101	131	64	101	54	105	71
	109	86	100	81	98	63	68	51
	116	71	109	82	108	47	87	62
MEAN	**117.8**	**81.0**	**111.8**	**74.8**	**107.8**	**63.2**	**86.8**	**59.4**
SD	**8.6**	**13.2**	**14.1**	**7.9**	**10.1**	**13.2**	**15.2**	**11.1**
ADJACENT	78	35	64	78	93	31	82	48
	93	48	93	86	78	38	89	27
	104	56	74	52	104	46	64	43
	101	51	96	58	85	34	78	61
	98	41	108	64	74	51	93	51
MEAN	**94.8**	**46.2**	**87.0**	**67.6**	**86.8**	**40.0**	**81.2**	**46.0**
SD	**10.2**	**8.3**	**17.7**	**14.1**	**12.0**	**8.3**	**11.3**	**12.5**

Table III.E.1 shows that PEMA/THFMA increased the cellularity of the repair tissue in all observational periods by an average of 43.3 cells, compared to the control group. The repair tissue contained more cells in the centre than in the periphery in both groups. The number of cells, however, in the centre of the defect in the polymer group was significantly higher than that in the control group. The cellularity in the normal articular cartilage adjacent to the defect was significantly lower in the control group. This hypo-cellularity in the adjacent to the defect normal articular cartilage was more apparent in the 6 month and 1 year specimens. Graphic presentation and statistical analysis of the results follows.

Graphic presentation of number of cells in the periphery and the centre of the repair tissue and the adjacent normal articular cartilage at 6 weeks, 3 months, 6 months and 1 year

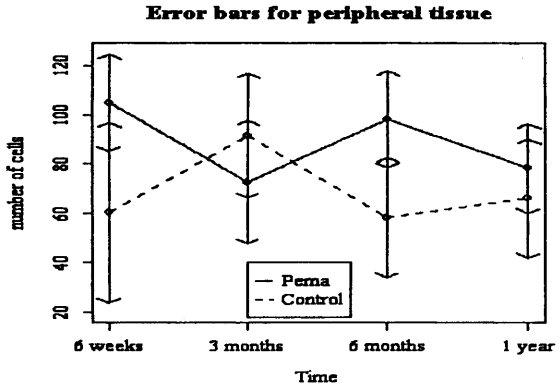


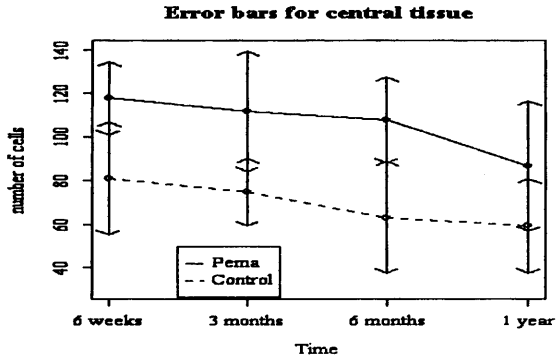


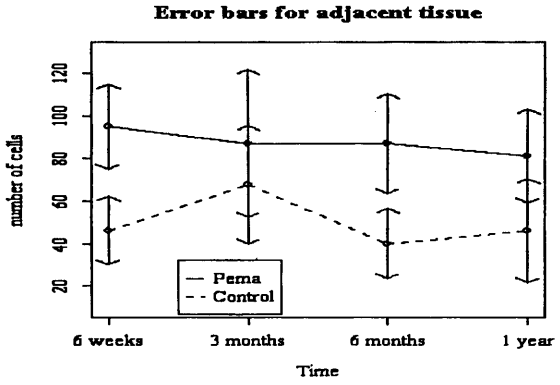


Statistical analysis of the results:

As a precursor to any detailed analysis, it is useful to examine the data visually. This is done in this study by producing ***error bars*** for the means of each of the cells, in separate plots for each of the tissue regions.







These plots give the means and 95% confidence intervals for each set of five measurements. They suggest that:

- PEMA/THFMA produces higher cell counts than control.
- The cell counts decrease over time.
- An increasing order of cell count by tissue type is: adjacent, peripheral, centre.

The most serious threat to these assertions is the measurements made at 3 months at the peripheral tissue. One option is to simply ignore these particular measurements and assume they are simply due to randomness. With this assumption, the trends are quantified using a ***3-way ANOVA***. The stepwise selection procedure settled on a model, which included the principal effects and one interaction term between treatment and time.

	Df	Sum Sq	Mean Sq	F value	p-value
treatment	1	34995	34995	224.7419	< 2.2e-16
time	3	4474	1491	9.5773	1.286e-05
tissue	2	7370	3685	23.6648	3.850e-09
treatment - time	3	2072	691	4.4348	0.005712
Residuals	100	15571	156		

The p-value column tells us that treatment, time and tissue all have a significant effect on the cell count. The significance of the treatment-time interaction suggests that treatment and time effect is not simply additive.

To estimate the *size* of the effects we need to decide on a reference level for each of the factors. This reference level is an arbitrary benchmark which is used to see how much the other levels deviate from. The reference levels are shown below:

Factor	Reference level
Treatment	Pema
Time	6 weeks
Tissue	Peripheral

Comparisons:

	Estimate	Std. Error	p-value
Intercept	106.900	3.720	< 2e-16
treatment = control	-43.333	4.556	1.11e-15
time = 3 months	-5.850	5.178	0.261319
time = 6 months	-8.267	4.556	0.072636
time = 1year	-23.733	4.556	1.02e-06
tissue = middle	7.912	3.120	0.012748
tissue = adjacent	-11.213	3.120	0.000507
control . 3 months	15.133	7.204	0.038195
control . 6 months	-0.400	6.444	0.950627
control . 1 year	18.400	6.444	0.005227

The intercept tells us the model's predicted value for the reference level. This is 106.9 cells with a standard error of 3.720 cells. The other rows make comparisons with this. The 'treatment = control' row says that the average control group is 43.3 cells less than the PEMA group.

DISCUSSION

Histological analysis of articular cartilage and of the repair tissue has been traditionally performed qualitatively. While new techniques and grading systems have attempted to improve the quantitative nature of histological assessment, the advent of computer-based analysis systems has enabled development of more quantitative methodologies for cartilage repair (Hacker et al 1997). In this study, in an attempt to quantify certain characteristics of the repair tissue in articular cartilage defects, the image analysis system has been employed. It is a versatile technique that has found applications in many scientific disciplines. Advantages include the high reproducibility of the system as well as the high accuracy (Hunt et al 1993).

This study focuses on the quantitative analysis of the number of cells in the repair tissue and in the surrounding the defect normal articular cartilage in defects treated with PEMA/THFMA, compared to control specimens where no biomaterial has been implanted. In previous studies it has been shown that there was a five-fold difference between manual and automatic counts (Hunt et al 1993). It was found that, when manually assessed, the cell count in tissues was up to 87% higher due to the fact that cell debris and non-cellular material were included in the count. The advantage of the image analysis system is that these objects can be safely deleted because the system recognises only stained cells in a particular frame.

Many parameters were initially analysed in this study including number of cells, size of cells and total area covered by cells, the final comparison between the normal and the repaired articular cartilage is based on the number of cells alone. This is because the size of cells was found to vary enormously in the same section, thus presenting difficulty in the statistical analysis of the values. The number of cells in the repair tissue, however, was more constant and easily comparable with the number of cells in the adjacent to the defect normal articular cartilage. Finally, the total area of the repair tissue occupied by cells was also difficult to be statistically correlated since it depends on the highly variable size of cells.

The centre of the repair tissue was found to have more cells per measured area than the periphery. Both values were higher compared to the number of cells in the adjacent

normal articular cartilage. It appears that the number of cells in the centre of the repair tissue is higher at 6 weeks and 3 months as compared to the 6 month and 1 year specimens. This probably illustrates the attraction of mesenchymal cells by the biomaterial in the first weeks of implantation that results in the differentiation of more cells to chondrocytes. Similarly, in the centre of the repair tissue there are more cells compared to the periphery and this most probably reflects the higher concentration or rather the more accumulative action of the biomaterial in the centre of the defect.

The number of cells in the repair tissue remains higher than the adjacent normal articular cartilage by 1 year in the biomaterial group, with no apparent degeneration in the adjacent articular cartilage. This continuing hypercellularity of the repair tissue suggests that PEMA/THFMA may initially attract the undifferentiated mesenchymal cells while later on it creates the necessary micro-environment / extracellular matrix for the support / function of the formed chondrocytes.

It is concluded that the image analysis system used in this study for the quantitative evaluation of the number of cells in the repair tissue was found easy to use, although the existing great variations in other tissue characteristics among different areas/sections of the repair tissue present a difficulty in their statistical correlation.

CHAPTER IV

BIOCHEMICAL ANALYSIS OF THE REPAIR TISSUE FOLLOWING IMPLANTATION OF PEMA/THFMA POLYMER IN FULL-THICKNESS ARTICULAR CARTILAGE DEFECTS

OBJECTIVE

MATERIALS

- 15 adult rabbits
- PEMA vs CONTROL, PEMA vs SHAM (no defect), CONTROL vs SHAM

METHOD

- Quantitative assessment of water, DNA, glycosaminoglycans and hydroxyproline
- At 6 weeks

RESULTS

DISCUSSION

OBJECTIVE

The aim of this study is to quantitatively assess the composition of the repair tissue in the biomaterial group, compared to the control group and also to normal articular cartilage.

MATERIALS AND METHODS

Fifteen female adult Sandy-Lop rabbits were used in this study. Both knees were operated on in all rabbits, 10 knees receiving the new biomaterial PEMA/THFMA [PEMA group], ten knees receiving no biomaterial and allowing natural repair [CONTROL group], and ten knees receiving 'sham' operations with no cartilage defect [SHAM group]. The pattern of the knee operations [right knee/left knee] in all 15 rabbits had as follows:

Rabbit 1: **PEMA / CONTROL**	*Rabbit 6:* **SHAM / PEMA**	*Rabbit 11:* **SHAM / CONTROL**
Rabbit 2: **SHAM / CONTROL**	*Rabbit 7:* **PEMA / CONTROL**	*Rabbit 12:* **SHAM / PEMA**
Rabbit 3: **SHAM / PEMA**	*Rabbit 8:* **SHAM / CONTROL**	*Rabbit 13:* **PEMA / CONTROL**
Rabbit 4: **PEMA / CONTROL**	*Rabbit 9:* **SHAM / PEMA**	*Rabbit 14:* **SHAM / CONTROL**
Rabbit 5: **SHAM / CONTROL**	*Rabbit 10:* **PEMA / CONTROL**	*Rabbit 15:* **SHAM / PEMA**

The knees in the PEMA and the control groups were operated using the same approach and method of implantation as described in Chapter III.B. In the ten knees that received 'sham' operations, an arthrotomy was performed but no defect was created in the articular cartilage, the joint was washed with normal saline and the wound was closed using absorbable sutures. No immobilisation was applied to any of the operated knees in all groups and the rabbits were group-housed in floor-pens.

All rabbits were sacrificed at 6 weeks after the operation by intravenous injection of Pentobarbitone sodium (Euthatal 600mg). Full-thickness cartilage slices from the repair tissue in the knees of the PEMA and control groups, and from anatomically similar sites of the normal articular cartilage in the femoral trochlea in the knees who received 'sham' operations, were taken with a 4.5 mm biopsy punch. The cartilage plugs were trimmed of underlying bone and they were weighed and dried at 60°C until a constant weight was obtained in three consecutive measurements. One ml papain digest buffer (cysteine hydrochloride 10mM, EDTA 10mM in phosphate buffered saline, buffered to pH 6.0 with

NaOH) containing 5µl of papain (Sigma P3125) was added to the dried cartilage slices, activated at 60°C and allowed to digest overnight.

Quantitative biochemical analysis included water content, DNA, glycosaminoglycan and hydroxyproline contents. All samples were assayed blindly.

Water content:

The percentage water was calculated from the formula:

$$\frac{\text{wet weight - dry weight}}{\text{wet weight}} \times 100$$

DNA content:

The DNA content of the papain digest samples was measured using the fluorimetric assay of Kapuscinski and Skoszylas (1977). Duplicate samples (200µl) of papain digest of DNA solutions, containing 0-15.0 µg /ml in 0.01M NaCl, were diluted with 300µl 0.01M NaCl and 400µl 0.01M Tris-HCl buffer, pH 7.0. One hundred µl of a solution containing 1µg/ml fluorimetric dye 4',6-diamidino-2-phenylindole-2HCl (DAPI) was then added to each sample and mixed. Fluorescence was measured at 460nm on a Perkin-Elmer Fluorimeter.

Glycosaminoglycan content:

The glycosaminoglycan (GAG) concentration was measured in papain digests of the cartilage by a modification of the 1,9-dimethylmethylene blue (DMB) dye binding assay described by Farndale et al (1982). Forty µl of the digest was added in duplicate to a 96 well plate. Two hundred and fifty µl of the DMB dye reagent (containing 16mg DMB, 0.5% ethanol, 0.2% sodium formate and 0.2% formic acid in a litre, stored in a dark bottle at room temperature) were added. Standards containing 5-50µg/ml shark chondroitin-4-SO4 were treated in the same way. The metachromatic shift in absorption at 600-535 nm, when the dye is complexed with sulphated GAG, was measured on a Bio-Rad Plate reader and the results were calculated using Microplate Manager© on an Apple Mac computer.

Hydroxyproline:

Hydroxyproline was measured in acid hydrolysed papain digests by a modification of the method of Bergman and Loxley (1970). After overnight hydrolysis in 0.5 ml concentrated HCl at 100°C, 1.0 ml of an oxidant solution (containing sodium acetate trihydrate 57 g, trisodium citrate 37.5 g, citric acid 5.5 g and 385 ml propan-2-ol made up to 1 litre with distilled water) was added. The pH was adjusted to 5-6 with saturated lithium hydroxide and the volume of each sample was made up to 5.0 ml with distilled water and stored at 4°C until assay. One hundred µl of test sample of standard solution containing 0-20.0 µg/ml hydroxyproline in propan-2-ol were added in duplicate to a 96 well plate. An equal volume of propan-2-ol was added to each well followed by 10 ml of freshly prepared chloramine T (7 g per 100 ml distilled water), the plate was sealed and mixed for 5 min on a plate mixer. One hundred µl of freshly prepared Ehrlich's [DMAB] reagent, (containing p-dimethylamino-benzaldehyde (17.6 g) and 44.3 ml of perchloric acid (SG 1.54) made up to 100 ml with propan-2-ol immediately before use), was added to each well, the plate was sealed, mixed for 5 minutes at room temperature and heated to 60°C for 30 minutes in an oven. After cooling, the absorbance was read at 540 nm on a Bio-Rad Plate reader and the results were calculated using Microplate Manager© on an Apple Mac computer.

RESULTS

In one of the PEMA group knees [left knee, rabbit no 6], dislocated patella and severe inflammation/adhesions in the joint were noted at termination. This knee was excluded from the study. The overall results of the biochemical analysis are presented in Table IV.1.

Table IV.1:

Biochemical analysis of the repair tissue in the PEMA/THFMA group compared to control specimens and to normal articular cartilage at 6 weeks

group	wet weight	dry weight	% water	DNA mg/g	GAG mg/g	oh-pro mg/g
R2: SHAM	0.0131	0.0071	45.80	18.85	0.64	35.41
R3: SHAM	0.0152	0.0084	44.74	35.69	0.96	45.72
R5: SHAM	0.0181	0.0084	53.59	8.90	0.80	30.67
R6: SHAM	0.0174	0.0087	50.00	25.21	0.60	32.56
R8: SHAM	0.0053	0.0024	54.72	31.51	1.18	49.46
R9: SHAM	0.0141	0.0068	51.77	9.61	0.78	37.49
R11: SHAM	0.0098	0.0038	61.22	8.24	1.06	88.50
R12: SHAM	0.0188	0.0098	47.87	22.71	0.60	31.97
R14: SHAM	0.0133	0.006	54.89	13.84	0.71	45.59
R15: SHAM	0.0128	0.0061	52.34	34.47	0.90	43.82
			516.94	*209.03*	*8.23*	*441.19*
SHAM mean			**51.694**	**20.903**	**0.823**	**44.119**
SHAM SD			**4.884**	**10.662**	**0.199**	**16.947**
R1: PEMA	0.0625	0.0341	45.44	37.73	1.20	43.90
R3: PEMA	0.046	0.0216	53.04	40.59	1.58	64.80
R4: PEMA	0.0652	0.0348	46.63	36.33	1.13	40.12
R7: PEMA	0.0299	0.0141	52.84	43.02	1.89	69.16
R9: PEMA	0.0132	0.0048	63.64	50.27	1.34	54.51
R10: PEMA	0.0341	0.0187	45.16	37.26	1.40	38.33
R12: PEMA	0.0617	0.0348	43.60	36.97	1.09	41.24
R13: PEMA	0.0247	0.011	55.47	45.60	2.05	54.07
R15: PEMA	0.0028	0.002	28.57	39.56	1.80	65.99
			434.39	*367.33*	*13.48*	*472.12*
PEMA mean			**48.266**	**40.814**	**1.498**	**52.458**
PEMA SD			**9.76**	**4.688**	**0.350**	**12.104**
R1: CONTROL	0.0396	0.0168	57.58	7.62	0.24	11.56
R2: CONTROL	0.0464	0.0261	43.75	3.51	0.18	10.65
R4: CONTROL	0.0374	0.0204	45.45	2.64	0.16	6.73
R5: CONTROL	0.038	0.0212	44.21	2.21	0.17	9.40
R7: CONTROL	0.038	0.021	44.74	3.07	0.15	7.00
R8: CONTROL	0.042	0.0257	38.81	5.74	0.17	9.51
R10: CONTROL	0.0403	0.0287	28.78	3.81	0.17	8.11
R11: CONTROL	0.0426	0.0318	25.35	1.43	0.14	4.48
R13: CONTROL	0.0407	0.0205	49.63	3.10	0.18	8.68
R14: CONTROL	0.0417	0.0248	40.53	3.27	0.16	7.75
			418.83	*36.4*	*1.72*	*83.87*
CONTROL mean			**41.883**	**3.64**	**0.172**	**8.387**
CONTROL SD			**9.384**	**1.794**	**0.027**	**12.692**

Statistical analysis of the results:

The biochemical analyses were all calculated using the dry weight of tissue and statistical comparison was made using the un-paired ***student "t" test***:

Water

	SHAM	*PEMA*
SHAM	-	-
PEMA	non-sign.	-
CONTROL	p≤.005	non-sign

GAG

	SHAM	*PEMA*
SHAM	-	-
PEMA	p≤.0005	-
CONTROL	p≤.0005	p≤.0005

DNA

	SHAM	*PEMA*
SHAM	-	-
PEMA	P≤.0005	-
CONTROL	p≤.0005	p≤.0005

Hydroxyproline

	SHAM	*PEMA*
SHAM	-	-
PEMA	non-sign	-
CONTROL	p≤.005	p≤.005

Water Content

There was no significant difference between the percentage of water in normal articular cartilage [sham] and the repair tissue in the PEMA group. However, there was a significant lowering (approximately 20%) in the water content of the repair tissue in the control group.

DNA

The DNA content of the repair tissue in the PEMA group was significantly higher than the DNA content in normal articular cartilage. There was a high degree of consistency within the group demonstrated by the small (≤9.0%) standard deviation. The increase in cellularity was not due to the effect of operation itself, as in the control group it was significantly reduced.

GAG

There was a statistically significant increase in the GAG concentration in the repair tissue in the PEMA group and decrease in the control group when compared to normal articular cartilage.

Hydroxyproline

There is no significant difference in the small elevation in the hydroxyproline content of the PEMA group compared to normal articular cartilage, although the reduction of hydroxyproline in the control group was significant when compared to normal articular cartilage.

DISCUSSION

Biochemical assessment is essential in the evaluation of the repair tissue in articular cartilage defects. Biochemical analysis has also been used extensively for the assessment of articular cartilage following cryopreservation of osteoarticular allografts (Schachar et al 1992), following joint disuse and joint instability (Muller et al 1994), and for the changes affecting the opposing to the defect articular surface (Richardson and Clark, 1990). Non-invasive techniques such as magnetic resonance imaging of the articular cartilage appear to correlate with the collagen content of the articular cartilage (Fragonas et al 1998) and it is a possibility that other techniques will be developed in the future to enable the non-invasive analysis of the articular cartilage composition.

In this study, the repair tissue in the PEMA group showed high concentration of water and hydroxyproline, similar to that in the normal articular cartilage in the 'sham' group. The high water concentration is characteristic and determines the properties of hyaline articular cartilage (Maroudas and Schneiderman, 1987). Water interacts with the matrix structural macromolecules maintaining hydration of the cartilage tissue and allowing exchange with fluids outside the tissue. Water also contributes to joint lubrication and to nutrition of the chondrocytes through diffusion (Newman, 1998). Hydroxyproline is a characteristic amino acid of the polypeptide chain of collagen, rarely found in other tissues (Grant and Prockop, 1972, Eyre, 1980). The total collagen content of articular cartilage specimens can be determined on the basis of the hydroxyproline content (Vachon et al 1990).

This study showed that the DNA content of the repair tissue in the PEMA group was significantly higher than that in the normal articular cartilage. The DNA concentration reflects the cellularity of the tissue (Kim et al 1988), thus confirming that the repair tissue in the PEMA group is significantly more cellular than the normal articular cartilage. This increased cellularity of the repair tissue in the biomaterial group has already been reported in the histological/histochemical assessment [Chapter III.B] and in the image analysis assessment [Chapter III.E] of the repair tissue in all observational periods up to 1 year post-implantation.

Glycosaminoglycans form part of the proteoglycan molecule (Roughley and Lee, 1994). Their concentrations vary with injury and disease of cartilage (Hardingham et al 1992, Rosenberg, 1992). The significant increase in the amount of glycosaminoglycans detected in the repair tissue in the biomaterial group reflects either the increased amount of proteoglycans synthesized by each chondrocyte or it results from the larger number of chondrocytes in the repair tissue, although each cell produces normal amounts of proteoglycans. The overall increased amounts of proteoglycans make the repair tissue able to sustain higher compression loading without tissue degeneration and failure.

In the control group, by contrast, all studied components of the repair tissue were found to be significantly reduced confirming the presence of a less cellular type of repair with less proteoglycans in the extracellular matrix, changes similar to those in osteoarthritis. Guilak et al (1994) reported 37% decrease in the proteoglycan content and 36% decrease in the collagen content in canine osteoarthritic articular cartilage.

It is concluded that the repair tissue in full-thickness articular cartilage defects treated with PEMA/THFMA is biochemically similar to hyaline articular cartilage, although it demonstrates hypercellularity and overproduction of proteoglycans at 6 weeks. Control specimens present biochemical composition similar to that noted in degenerative arthritis.

CHAPTER V

COMPARATIVE STUDY OF ARTICULAR CARTILAGE REPAIR USING PEMA/THFMA POLYMER IN THREE AGE GROUPS

OBJECTIVE

MATERIALS

- 30 adult rabbits (from the study in Chapter III.B) compared with 10 young and 10 old rabbits
- PEMA vs CONTROL
- YOUNG vs ADULT vs OLD

METHOD

- Macroscopic, Histological/Histochemical and Immunohistochemical assessment
- At 6 weeks

RESULTS

DISCUSSION

OBJECTIVE

The aim of this study is to compare the effect of the PEMA/THFMA polymer in the repair of articular cartilage defects in rabbits of three different age groups, i.e. young, adult and old. The clinical applications of the method in articular cartilage pathology would increase dramatically should the biomaterial appear effective in all ages.

MATERIALS AND METHODS

For the microscopic and histological/histochemical assessment, we compared the results of the 30 adult rabbits assessed in the study in Chapter III.B at 6 weeks [ADULT AGE GROUP, rabbits 1-30] with 10 young rabbits 3-5 months old weighing 3.1 (2.9-3.3) kg [YOUNG AGE GROUP, rabbits 121-130], and with 10 old rabbits 32-36 months old and weighing 4.9 (4.7-5.7) kg [OLD AGE GROUP, rabbits 131-140]. Female Sandy-Lop rabbits, group-housed in floor-pens were used in all groups.

For the immunohistochemical correlation involved the 10 specimens of the adult rabbits assessed at 6 weeks in the study in Chapter III.C, the 10 specimens in the young group and the 10 specimens in the old age group.

The young and the old animals underwent the same operation as the adult ones, with implantation of PEMA/THFMA in one knee and the other knee receiving no biomaterial and used as control. Animals were sacrificed at 6 weeks post-operatively by intravenous injection of pentobarbitone sodium (Euthatal, 600 mg). The specimens were assessed by Macroscopic and Histological/Histochemical Evaluation using the Articular Cartilage Repair Scoring System (described in Chapter III.A), and Immunohistochemical Evaluation using the silver-enhanced colloidal gold staining (described in Chapter III.C).

RESULTS

Mild inflammatory reaction was noted in 21.6% of the control knees and in 9.1% of the knees in the biomaterial group among the adult rabbits [Chapter III.B]. Mild

inflammatory response was also noted in the synovium in 6 (30%) knees in the young and old rabbits in the control group (1 in the young and 5 in the old group) and in 4 (20%) knees in the biomaterial group (1 in the young and 3 in the old group). No infection or foreign body reaction was noted in any of the specimens. One knee in the biomaterial group [OLD AGE GROUP] and 5 knees in the control group [1 in YOUNG AGE GROUP and 4 in OLD AGE GROUP] developed joint effusion/heamarthrosis immediately post-operatively; at termination no haemarthrosis was noted and synovial fluid was cultured and showed no bacterial growth.

Osteoarthritic changes were noted in 6 (30%) of the knees in the old group (4 in the biomaterial and 2 in the control group). These changes consisted of severe destruction of the articular cartilage in the femoral condyles and the tibial plateaux with eburnated bone and peripheral osteophyte formation. Degenerative changes were not noted in the young rabbits in this study.

The macroscopic and histological/histochemical results are presented in Tables V.1, V.2 and V.3 accompanied by graphic presentations of PEMA vs Control in each age group and of the PEMA effect compared in all age groups. Table V.4 analyses the results per criterion of assessment. Results are followed by their statistical analysis.

Table V.1:

Macroscopic and Histological/Histochemical assessment per criterion
Scoring at 6 weeks. Rabbits *121-130*
***PEMA vs CONTROL.* Young age group**

PEMA	*121*	*122*	*123*	*124*	*125*	*126*	*127*	*128*	*129*	*130*
Area	20	20	20	20	20	10	20	20	20	20
Level	10	10	10	10	5	10	10	5	10	10
Surface	10	10	10	5	10	10	10	10	5	5
Cells	20	20	10	20	20	20	20	20	20	10
PGs	10	5	5	10	10	10	10	5	10	10
Structure	5	10	5	10	10	5	5	10	10	5
Bonding	20	10	10	20	20	20	20	20	10	20
TOTAL SCORE	**95**	**85**	**70**	**95**	**95**	**85**	**95**	**90**	**85**	**80**

CONTROL	*121*	*122*	*123*	*124*	*125*	*126*	*127*	*128*	*129*	*130*
Area	10	10	20	20	10	0	0	0	10	0
Level	0	5	5	10	5	5	5	10	5	5
Surface	10	10	10	5	5	5	5	5	10	5
Cells	10	20	10	10	10	10	20	20	10	10
PGs	10	10	10	5	5	10	10	5	5	5
Structure	10	5	5	5	0	5	0	5	5	5
Bonding	20	10	10	10	0	0	0	10	10	20
TOTAL SCORE	**70**	**70**	**70**	**65**	**35**	**35**	**40**	**55**	**55**	**50**

	PEMA MEANS	*CONTROL MEANS*	*PEMA SD*	*CONTROL SD*
Area	19.00	8.00	3.16	7.89
Level	9.00	5.50	2.11	2.84
Surface	8.50	7.00	2.42	2.58
Cells	18.00	13.00	4.22	4.83
PGs	8.50	7.50	2.42	2.64
Structure	7.50	4.50	2.64	2.84
Bonding	17.00	9.00	4.83	7.38
TOTAL SCORE	**87.50**	**54.50**	**8.25**	**14.23**

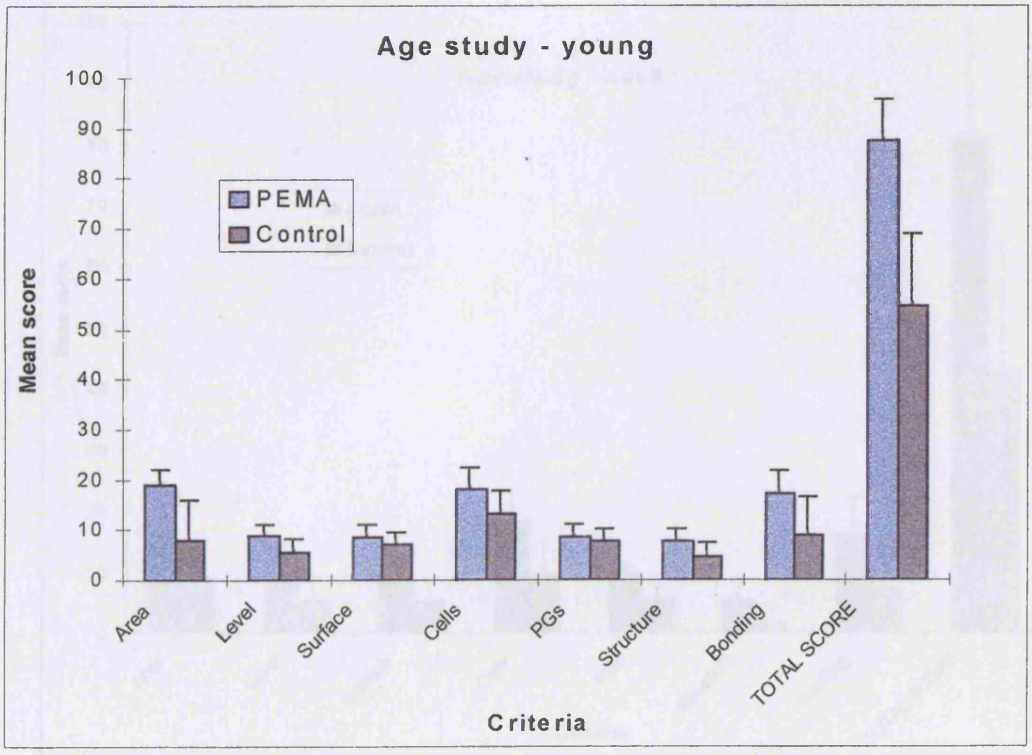


Table V.2:

Macroscopic and Histological/Histochemical assessment per criterion
Scoring at 6 weeks. Rabbits *1-30*
***PEMA vs CONTROL.* Adult age group**

PEMA	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*
Area	20	20	10	20	20	10	0	20	10	20	20	20	20	20	10	20	0	0	20	20	10	20	10	20	10	20	0	20	20	20
Level	10	5	10	10	10	0	0	10	10	10	10	5	10	10	10	10	0	5	10	10	10	10	10	10	10	5	0	10	10	10
Surface	10	5	10	10	10	10	5	10	10	10	10	10	10	10	10	10	0	5	10	10	10	10	10	10	10	10	5	10	10	10
Cells	20	20	20	20	20	20	10	20	20	20	20	20	20	20	20	20	10	10	10	20	20	20	20	20	20	20	0	20	20	20
PGs	10	10	10	10	10	10	5	10	10	10	10	10	10	10	10	10	5	10	10	10	10	10	10	10	10	10	5	10	10	10
Structure	10	5	5	5	10	5	0	5	5	5	5	10	5	10	10	10	0	0	5	5	10	10	5	5	5	0	0	0	10	5
Bonding	20	20	20	20	10	20	10	20	20	20	20	10	10	20	20	10	0	10	20	20	10	20	20	20	10	20	0	20	20	20
TOTAL SCORE	**100**	**85**	**85**	**95**	**90**	**75**	**30**	**95**	**85**	**95**	**95**	**85**	**85**	**100**	**90**	**90**	**15**	**40**	**85**	**95**	**80**	**100**	**85**	**95**	**75**	**85**	**10**	**90**	**10**	**95**

CONTROL	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*
Area	10	0	10	10	10	20	10	10	20	0	0	0	10	20	10	10	10	10	10	20	20	10	0	10	0	0	0	10	20	0
Level	5	0	5	5	5	10	5	10	10	0	5	0	10	5	5	5	5	10	10	10	5	0	0	0	10	10	0	5	5	0
Surface	0	5	5	5	10	10	10	5	0	0	5	0	10	5	5	5	5	5	5	10	5	0	5	5	10	10	5	5	5	0
Cells	10	10	10	10	10	20	10	10	20	10	0	0	10	10	10	10	0	10	10	20	10	0	10	10	0	0	10	20	10	10
PGs	5	0	5	10	5	10	10	5	0	5	0	0	10	10	5	5	0	10	5	5	10	0	10	5	0	5	10	10	0	5
Structure	0	0	0	0	0	5	0	0	0	5	0	0	0	0	5	5	5	0	0	0	0	0	0	5	0	0	0	0	5	5
Bonding	10	10	0	0	10	10	0	10	10	10	0	0	10	10	20	10	10	0	0	20	20	10	0	10	0	0	0	10	10	10
TOTAL SCORE	**40**	**25**	**35**	**40**	**50**	**85**	**45**	**50**	**60**	**30**	**10**	**0**	**60**	**60**	**60**	**50**	**35**	**45**	**40**	**85**	**70**	**20**	**25**	**45**	**20**	**25**	**25**	**65**	**55**	**30**

	PEMA MEANS	*CONTROL MEANS*	*PEMA SD*	*CONTROL SD*
Area	15.00	9.00	7.31	7.12
Level	8.00	5.17	3.62	3.82
Surface	9.00	5.17	2.42	3.34
Cells	18.00	9.33	4.84	5.83
PGs	9.50	5.33	1.53	3.92
Structure	5.33	1.50	3.46	2.33
Bonding	16.00	7.33	6.21	6.40
TOTAL SCORE	**80.83**	**42.83**	**24.21**	**20.33**

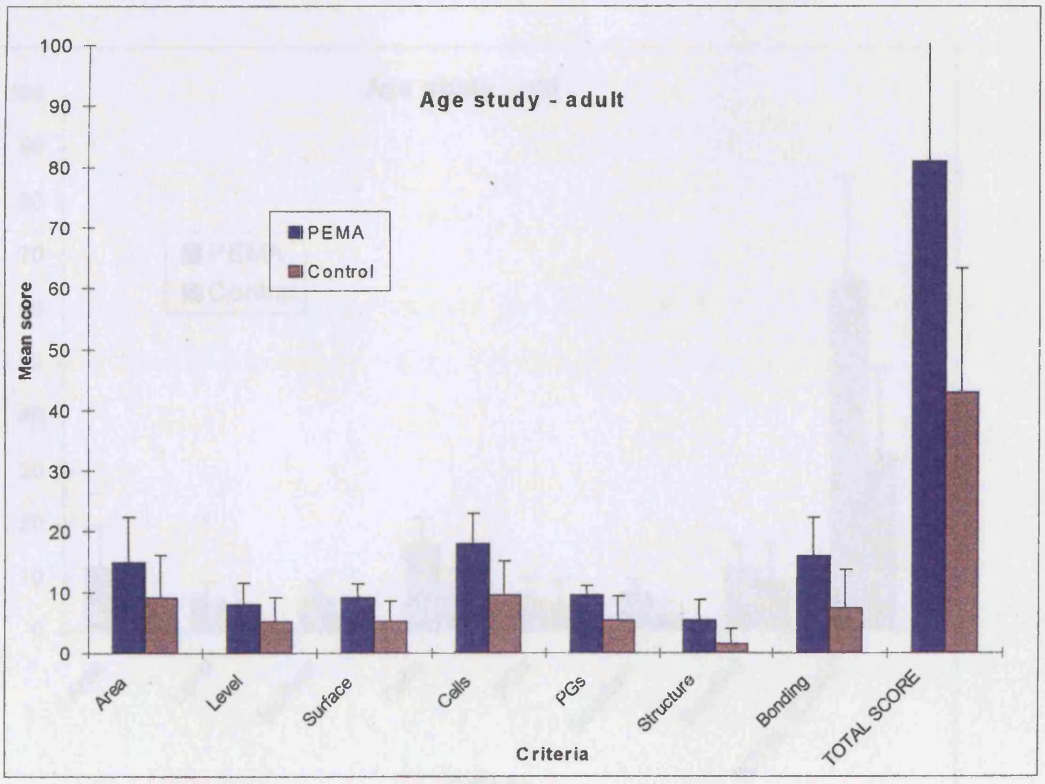


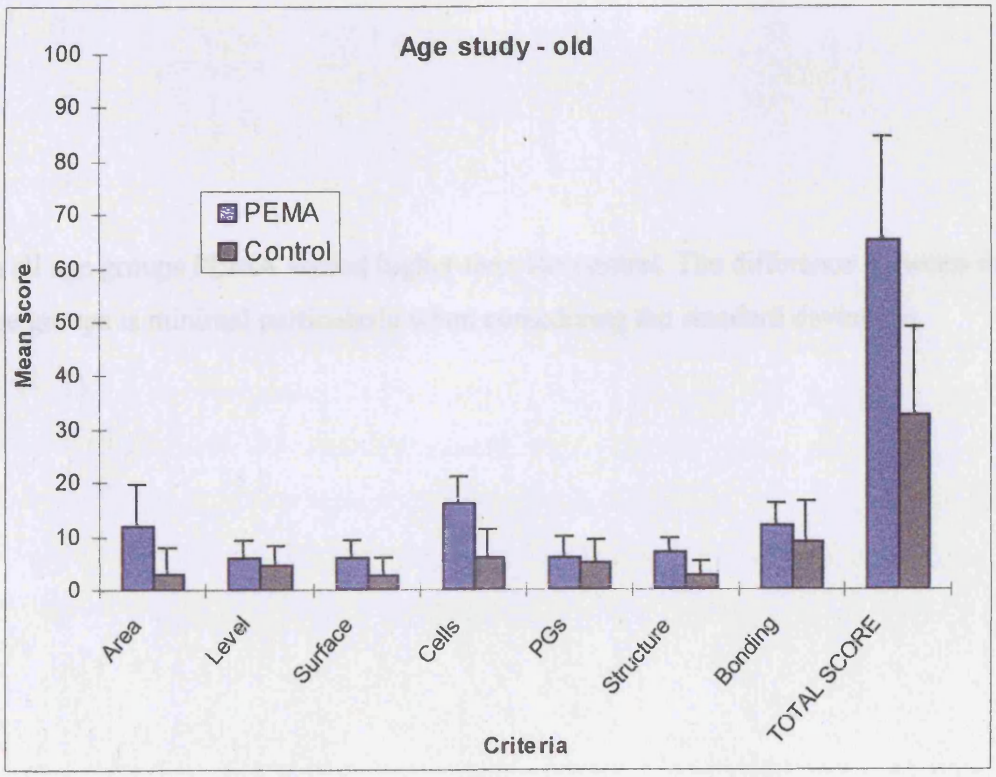
Table V.3:

Macroscopic and Histological/Histochemical assessment per criterion
Scoring at 6 weeks. Rabbits *131-140*
***PEMA vs CONTROL.* Old age group**

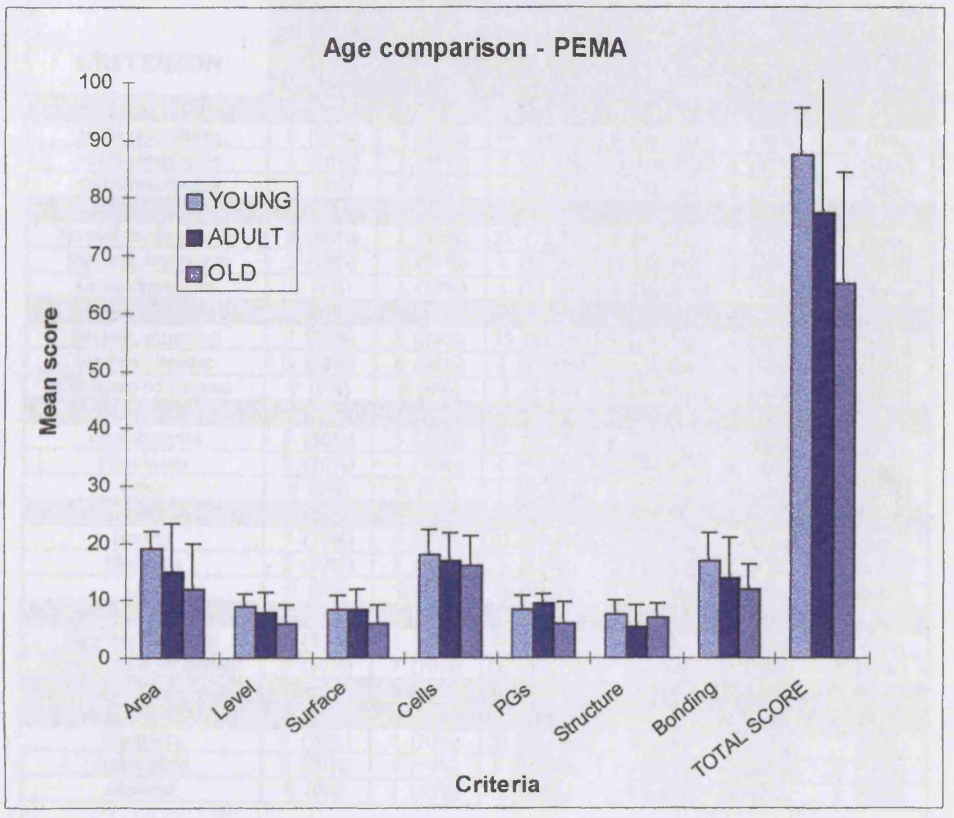
PEMA	*131*	*132*	*133*	*134*	*135*	*136*	*137*	*138*	*139*	*140*
Area	20	10	20	10	20	0	20	10	0	10
Level	10	5	5	5	10	5	10	5	0	5
Surface	5	5	5	10	10	5	10	5	0	5
Cells	20	10	10	20	10	20	20	20	10	20
PGs	10	10	5	5	5	0	5	10	0	10
Structure	10	10	5	5	5	10	5	10	5	5
Bonding	20	10	10	10	10	10	20	10	10	10
TOTAL SCORE	**95**	**60**	**60**	**65**	**70**	**50**	**90**	**70**	**25**	**65**

CONTROL	*131*	*132*	*133*	*134*	*135*	*136*	*137*	*138*	*139*	*140*
Area	10	0	10	0	0	10	0	0	0	0
Level	10	5	0	5	0	5	0	5	10	5
Surface	5	0	0	0	5	10	5	0	0	0
Cells	10	0	10	10	0	0	10	10	10	0
PGs	10	5	5	0	0	5	10	10	5	0
Structure	5	5	5	0	0	5	0	0	5	0
Bonding	20	10	10	20	10	10	0	0	0	10
TOTAL SCORE	**70**	**25**	**40**	**35**	**15**	**45**	**25**	**25**	**30**	**15**

	PEMA MEANS	*CONTROL MEANS*	*PEMA SD*	*CONTROL SD*
Area	12.00	3.00	7.89	4.83
Level	6.00	4.50	3.16	3.69
Surface	6.00	2.50	3.16	3.54
Cells	16.00	6.00	5.16	5.16
PGs	6.00	5.00	3.94	4.08
Structure	7.00	2.50	2.58	2.64
Bonding	12.00	9.00	4.22	7.38
TOTAL SCORE	**65.00**	**32.50**	**19.58**	**16.37**



Macroscopic and histological/histochemical assessment of the repair tissue at 6 weeks
Graphic presentation of the effect of PEMA/THFMA in three age groups



In all age groups PEMA scored higher than the control. The difference between the three age groups is minimal particularly when considering the standard deviations.

Table V.4:

Macroscopic and Histological/Histochemical assessment of repair tissue by criterion and the overall repair at 6 weeks
***PEMA vs CONTROL*. Three age groups**

CRITERION	YOUNG GROUP		ADULT GROUP		OLD GROUP	
	PEMA n=10	CONTROL n=10	PEMA n=30	CONTROL n=30	PEMA n=10	CONTROL n=10
AREA						
100% resurfacing	9 (90%)	2 (20%)	19 (63.3%)	6 (20.0%)	4 (40%)	0 (0%)
>50% resurfacing	1 (10%)	4 (40%)	7 (23.3%)	15 (50.0%)	4 (40%)	3 (30%)
<50% resurfacing	0 (0%)	4 (40%)	4 (13.3%)	9 (30.0%)	2 (20%)	7 (70%)
LEVEL						
Normal cartilage level	8 (80%)	2 (20%)	22 (73.3%)	9 (30.0%)	3 (30%)	2 (20%)
Minimal depression	2 (20%)	7 (70%)	4 (13.3%)	13 (43.3%)	6 (60%)	5 (50%)
Gross depression	0 (0%)	1 (10%)	4 (13.3%)	8 (26.6%)	1 (10%)	3 (30%)
SURFACE						
Smooth, glistering	7 (70%)	4 (40%)	25 (83.3%)	7 (23.3%)	3 (30%)	1 (10%)
Irregular, opaque	3 (30%)	6 (60%)	4 (13.3%)	17 (56.6%)	6 (60%)	3 (30%)
Disrupted by fissures	0 (0%)	0 (0%)	1 (3.3%)	6 (20.0%)	1 (10%)	6 (60%)
CELLS						
Chondrocytes	8 (80%)	3 (30%)	25 (83.3%)	4 (13.3%)	6 (60%)	0 (0%)
Fibroblasts	2 (20%)	7 (70%)	4 (13.3%)	20 (66.6%)	4 (40%)	6 (60%)
Other	0 (0%)	0 (0%)	1 (33.3%)	6 (20.0%)	0 (0%)	4 (40%)
PGs						
Normal	7 (70%)	5 (50%)	27 (90.0%)	10 (33.3%)	4 (40%)	3 (30%)
Moderate	3 (30%)	5 (50%)	3 (9.9%)	12 (40.0%)	4 (40%)	4 (40%)
Low	0 (0%)	0 (0%)	0 (0.0%)	8 (26.6%)	2 (20%)	3 (30%)
STRUCTURE						
Hyaline-like tissue	5 (50%)	1 (10%)	8 (26.6%)	0 (0.0%)	4 (40%)	0 (0%)
Moderately disorganised	5 (50%)	7 (70%)	16 (53.3%)	9 (30.0%)	6 (60%)	5 (50%)
Grossly disorganised	0 (0%)	2 (20%)	6 (20.0%)	21 (70.0%)	0 (0%)	5 (50%)
BONDING						
Complete	7 (70%)	2 (20%)	20 (66.6%)	3 (9.9%)	2 (20%)	2 (20%)
Incomplete	3 (30%)	5 (50%)	8 (26.6%)	16 (53.3%)	8 (80%)	5 (50%)
Minimal	0 (0%)	3 (30%)	2 (6.6%)	11 (36.6%)	0 (0%)	3 (30%)

ARTICULAR CARTILAGE REPAIR	YOUNG GROUP		ADULT GROUP		OLD GROUP	
	PEMA n=10	CONTROL n=10	PEMA n=30	CONTROL n=30	PEMA n=10	CONTROL n=10
EXCELLENT	8 (80%)	0 (0%)	22 (73.3%)	2 (6.6%)	2 (20%)	0 (0%)
GOOD	2 (20%)	7 (70%)	4 (13.3%)	10 (33.3%)	7 (70%)	1 (10%)
POOR	0 (0%)	3 (30%)	4 (13.3%)	18 (60.0%)	1 (10%)	9 (90%)

In all age groups PEMA was superior to control. Excellent overall repair was noted in 80% of the PEMA young group, in 73.3% of the PEMA adult group and in 20% of the PEMA old group specimens. In the control group 2 (6.6%) knees from the adult rabbits had excellent result. No excellent results were noted in the control young or in the control old groups. There were no poor results in the PEMA young group, compared to 30% in the controls. In the old age group 10% of the PEMA specimens showed poor repair compared to 90% in the controls.

Statistical analysis of macroscopic and histological/histochemical results:

The macroscopic and histological/histochemical results were assessed with ***McNemar's*** test. The ***Wilcoxon*** test evaluated the total scores. However, the number of rabbits used for the young and old groups was 10, rather than 30, so the statistical power is decreased. This should be born in mind when interpreting the p-values, as an insignificant p-value can be interpreted as either there is no effect or that the test has insufficient power to detect an effect; there is no way to distinguish between these two interpretations.

Score	Young	Adult	Old
Area	.046	0.019	.122
Level	.206	0.031	.721
Surface	.371	6.4e-4	.149
Cells	.074	1.1e-4	1
PGs	.683	0.002	.801
Structure	.284	0.002	.564
Bonding	.172	6.7e-4	.262
Total Score	.008	1.5e-5	.009

The adult column is a replicate of the 6 week column in the main trial [Chapter III.B]. It can be concluded from these tests that PEMA/THFMA has an effect also on young and on old animals. To determine if there is a trend in age, ***logistic regression*** is used again in a similar vein to its usage in the study in Chapter III.B, except 'time of measurement' is replaced here with 'age group'. The reference event is excellent repair with PEMA/THFMA treatment in the young age group.

Pr(score = i | treatment, age group) = θ_i, i = poor, good, excellent

$\log(\theta_i) = \alpha_i + \beta_{treatment,\ i} + \beta_{age,\ i}$

Analysis of deviance:

Variable	d.f.	Deviance	residual d.f.	residual deviance	p-value
Treatment	2	52.543	8	25.074	.011
Age	4	17.261	4	7.813	.231

The above indicates that the effect of age is non-significant. The presentation of the coefficients is unnecessary if it is decided that age does not have an effect, because the only remaining covariate is treatment, whose effect size is estimated more accurately in the study in Chapter III.B.

Table V.5 demonstrates analysis of the immunolocalisation of the cartilage components in the repair tissue in both the biomaterial and the control groups in all three age groups.

Table V.5:
Immunohistochemical assessment of repair tissue at 6 weeks.
***PEMA vs CONTROL.* Three age groups**

ANTIGEN	YOUNG GROUP		ADULT GROUP		OLD GROUP	
	PEMA n=10	CONTROL n=10	PEMA n=10	CONTROL n=10	PEMA n=10	CONTROL n=10
Collagen type II						
>50% of matrix	7 (70%)	1 (10%)	5 (50%)	0 (0%)	5 (50%)	0 (0%)
<50% of matrix	3 (30%)	4 (40%)	4 (40%)	2 (20%)	3 (30%)	0 (0%)
no detection	0 (0%)	5 (50%)	1 (10%)	8 (80%)	2 (20%)	10 (100%)
Keratan-Sulphate						
>50% of matrix	8 (80%)	2 (20%)	6 (60%)	0 (0%)	7 (70%)	0 (0%)
<50% of matrix	2 (20%)	6 (60%)	4 (40%)	0 (0%)	3 (30%)	4 (40%)
no detection	0 (0%)	2 (20%)	0 (0%)	10 (100%)	0 (0%)	6 (60%)
Chondroitin 4-Sulphate						
>50% of matrix	8 (80%)	1 (10%)	6 (60%)	0 (0%)	6 (60%)	1 (10%)
<50% of matrix	2 (20%)	5 (50%)	3 (30%)	2 (20%)	3 (30%)	2 (20%)
no detection	0 (0%)	4 (40%)	1 (10%)	8 (80%)	1 (10%)	7 (70%)
Chondroitin 6-Sulphate						
>50% of matrix	9 (90%)	2 (20%)	7 (70%)	0 (0%)	5 (50%)	2 (20%)
<50% of matrix	1 (10%)	8 (80%)	3 (30%)	4 (40%)	5 (50%)	2 (20%)
no detection	0 (0%)	0 (0%)	0 (0%)	6 (60%)	0 (0%)	6 (60%)

Collagen type II is seen evenly distributed in the extracellular matrix of the repair tissue in 70% of the PEMA repairs in the young group and 50% of the PEMA repairs in the adult and the old groups. In the control group only one repair contained collagen type II distributed in over half of the matrix. This repair occurred in a young rabbit while no repair tissue from the other age groups contained significant amounts of collagen type II.

In the control group in general, collagen type II was detected in the deepest layers in the repair tissue, while the superficial layers contained minimal or no collagen type II. This explains the early degeneration of the superficial layer in the repair tissue in the control group observed as early as 6 weeks.

With regard to detection of glycosaminoglycans in the matrix of the repair tissue, over 60% of all specimens in the PEMA group were found to contain glycosaminoglycans distributed throughout the whole of the matrix. This trend continued in all age groups.

Statistical analysis of immunohistochemical results:

The immunohistochemical results were assessed with ***Fisher's exact test*** and the p-values are presented below:

Antigen	Young	Adult	Old
Collagen type II	.007	.004	7e-4
Keratin-Sulphate	.033	1e-5	.001
Chondroitin 4-sulphate	.005	.001	.018
Chondroitin 6-sulphate	.005	.001	.016

This analysis indicates that PEMA produces repair tissue that contains significantly larger amounts of hyaline cartilage components, compared to the control group.

PEMA/THFMA appears to be equally effective in all ages and the effect of age on the quality of the repair tissue is not statistically significant.

Fig.V.1

Histological appearance of the repair tissue in the polymer group in a young rabbit: The repair tissue **[rt]** comprises of numerous cells with chondrocytic phenotype, evenly distributed within the extracellular matrix. The arrow indicates the smooth transition from the normal articular cartilage to the repair tissue. **[p**=polymer, **b**=subchondral bone, **c**= normal articular cartilage] *(1μm thick section, Toluidine blue staining, x 13.2)*

Fig.V.2

Histological appearance of the repair tissue in the polymer group in a young rabbit: Higher magnification of the specimen shown in Fig.V.1 focusing on the transitional area **[→]** between the repair tissue **[rt]** and the surrounding the defect normal articular cartilage **[c]**. *(1μm thick section, Toluidine blue staining, x 33)*

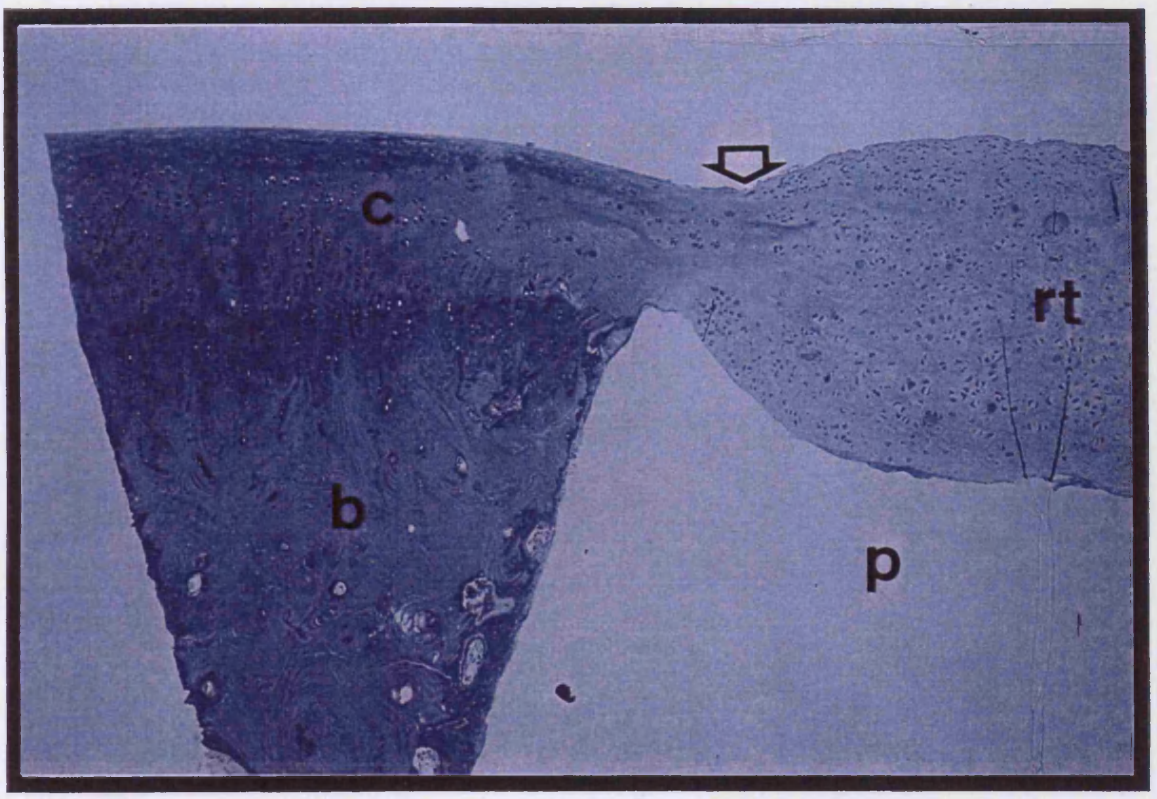


Fig.V.1

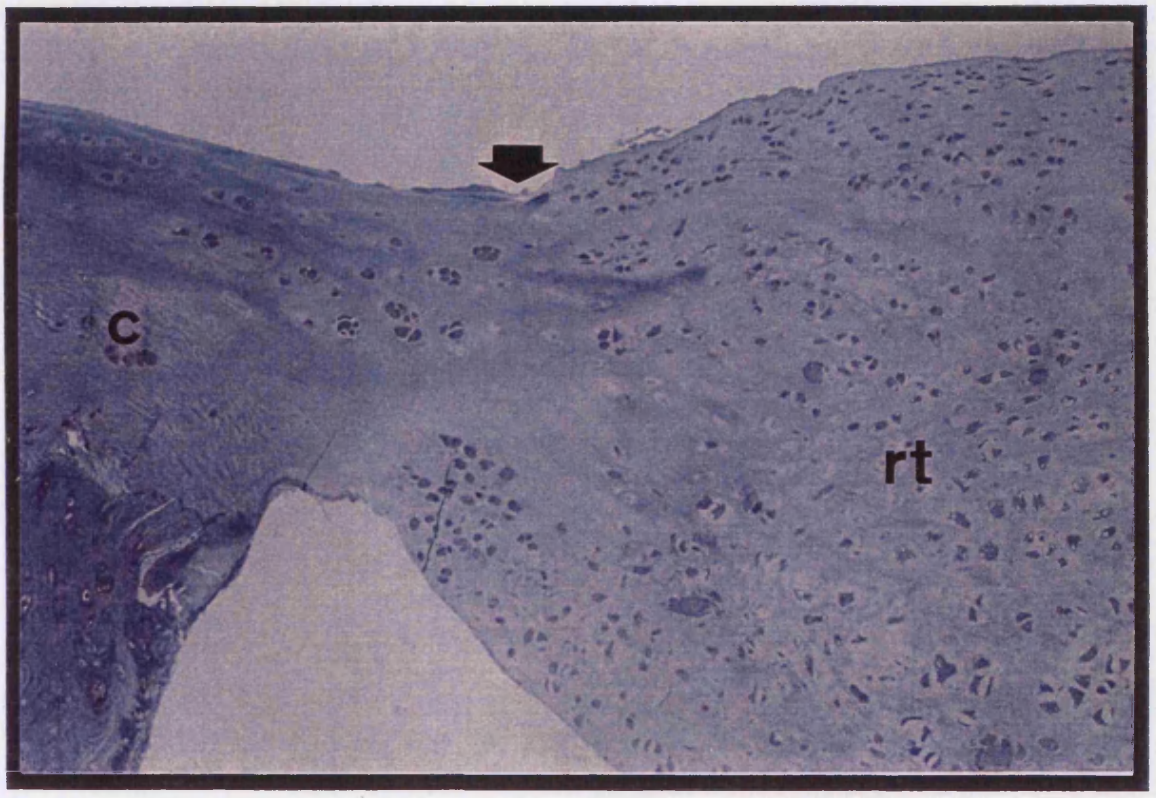


Fig.V.2

Fig.V.3

Histological appearance of the repair tissue in the polymer group in a young rabbit: The repair tissue comprises of numerous cells with chondrocytic phenotype; multiple mitoses of the cells are noted in this age group. *(Haematoxylin/Eosin staining, x 132)*

Fig.V.4

Histological appearance of the repair tissue in the polymer group in a young rabbit: Complete integration of the repair tissue **[rt]** with normal articular cartilage **[c]**. The cells in the edge of the repair tissue outnumber those in the edge of the normal articular cartilage. *(1μm thick section, Toluidine blue staining, x 132)*

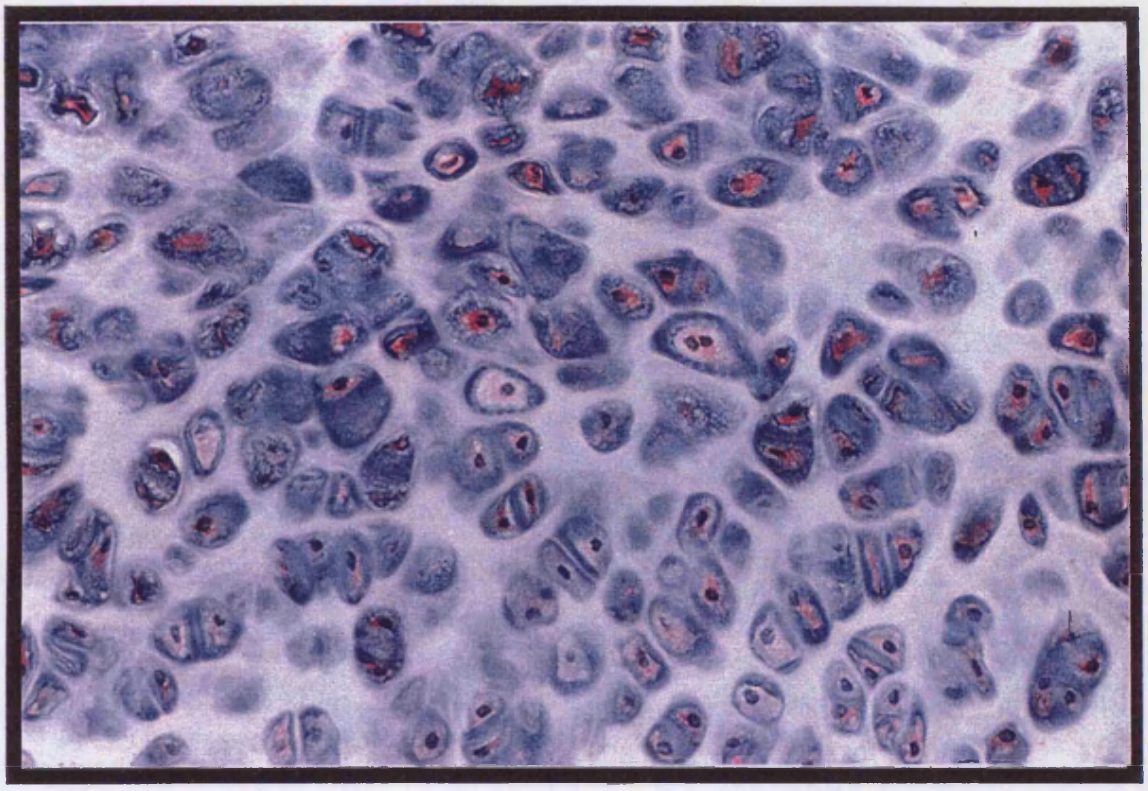


Fig.V.3

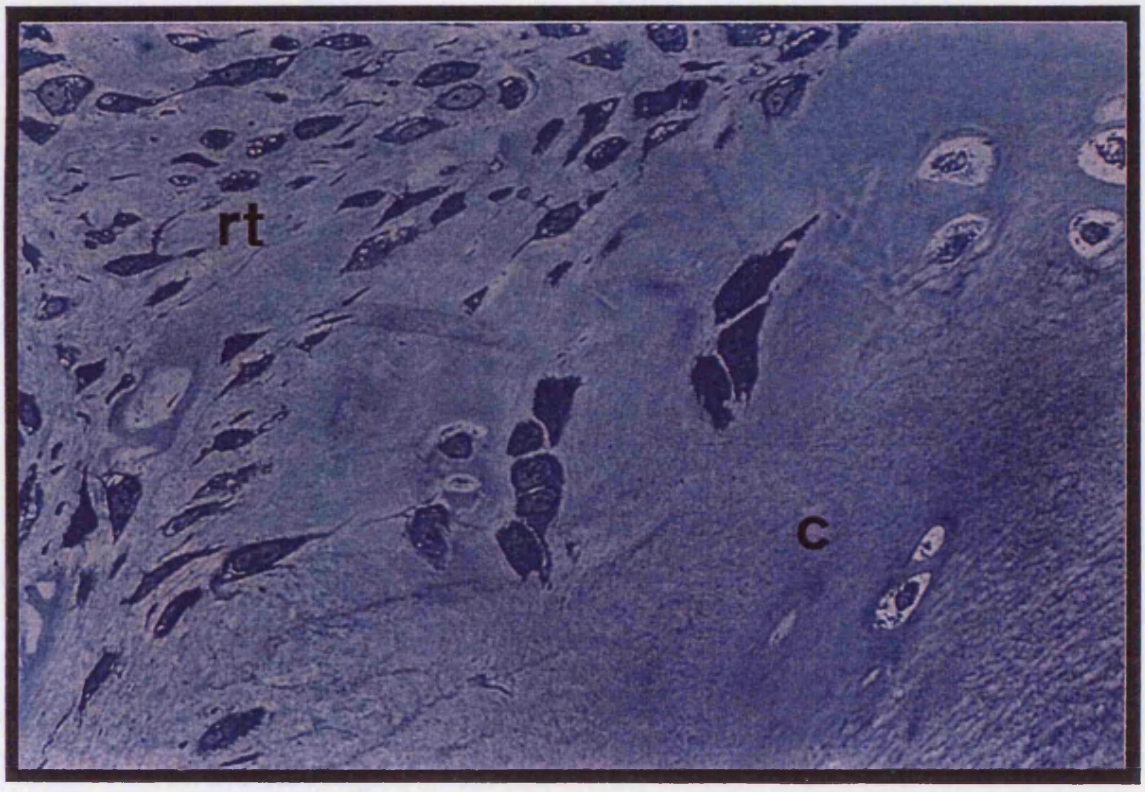


Fig.V.4

Fig.V.5

Histochemical appearance of the repair tissue in the polymer group in a young rabbit:

The extracellular matrix of the repair tissue shows zonal differentiation between the deep **[d]** layers containing higher concentration of proteoglycans and the superficial **[s]** layer showing lower concentration of proteoglycans and smaller cells lying parallel to the articular surface. *(Safranin-O staining, x 66)*

Fig.V.6

Immunohistochemical appearance of the repair tissue in the polymer group in a young rabbit:

The repair tissue contains collagen type II evenly distributed throughout the matrix, with higher concentration in the superficial **[s]** layer and lower concentration in the deep **[d]** layers. *(Silver-enhanced colloidal gold immuno-staining, x 66)*

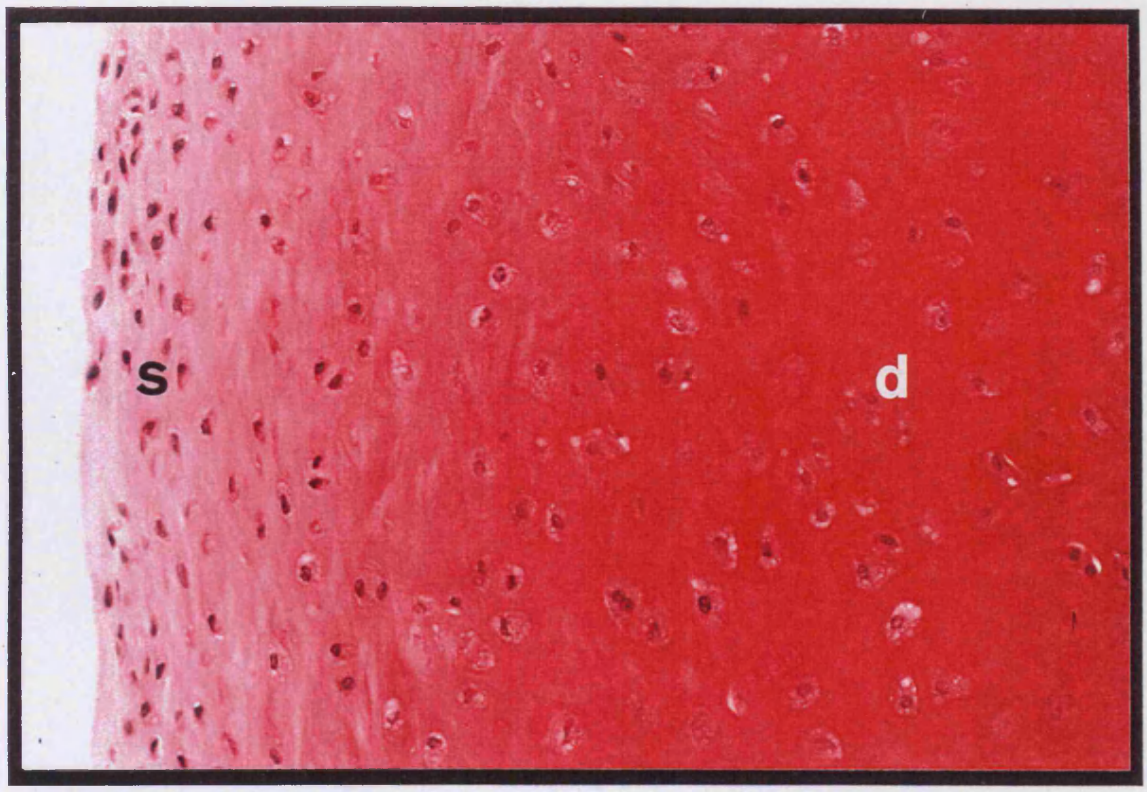


Fig.V.5

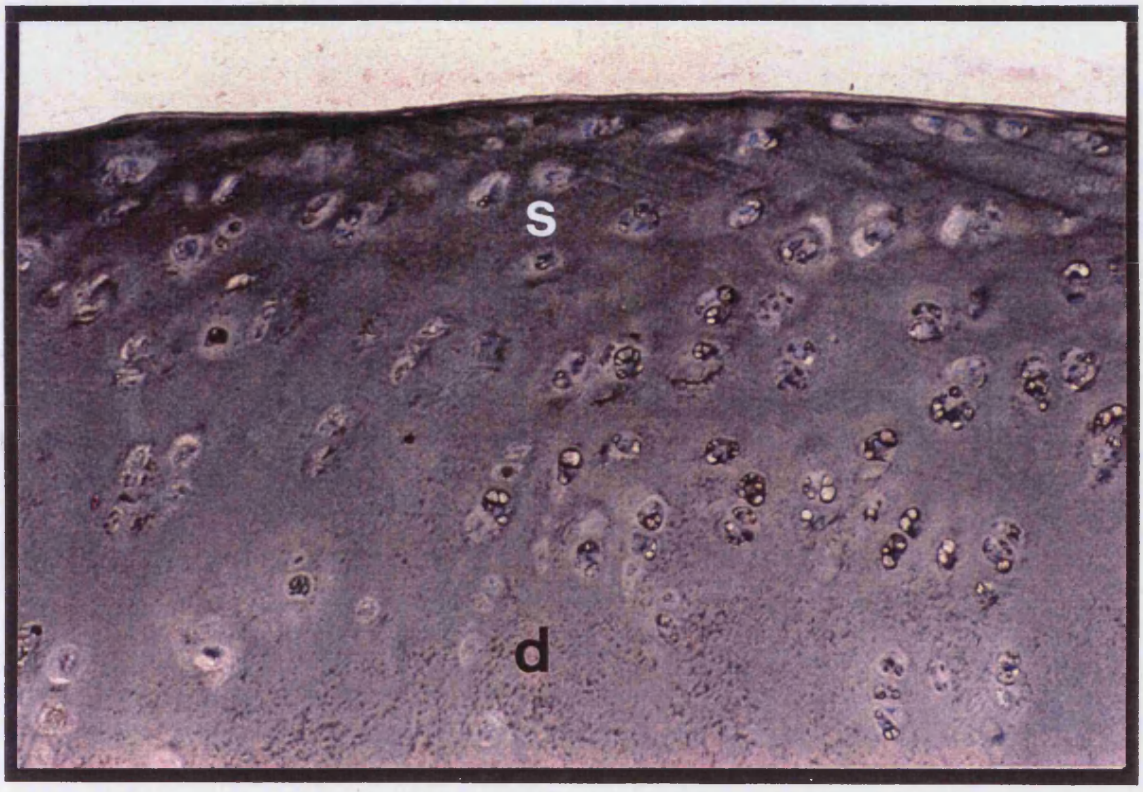


Fig.V.6

Fig.V.7

Histological appearance of the repair tissue in the polymer group in an adult rabbit:

The repair tissue **[rt]** contains numerous cells with chondrocytic phenotype evenly distributed throughout the matrix with large numbers of cells in the middle layers. The → indicates the transition from the repair tissue to the normal articular cartilage **[c]**. **[b=** subchondral bone, **p=** polymer] *(Haematoxylin/Eosin staining, x 13.2)*

Fig.V.8

Histochemical appearance of the repair tissue in the polymer group in an adult rabbit:

The repair tissue **[rt]** contains high concentration of proteoglycans, similar to the concentration in the normal articular cartilage **[c]**. The subchondral bone has no proteoglycans and stained negative. **[p=**polymer] *(Safranin-O staining, x 13.2)*

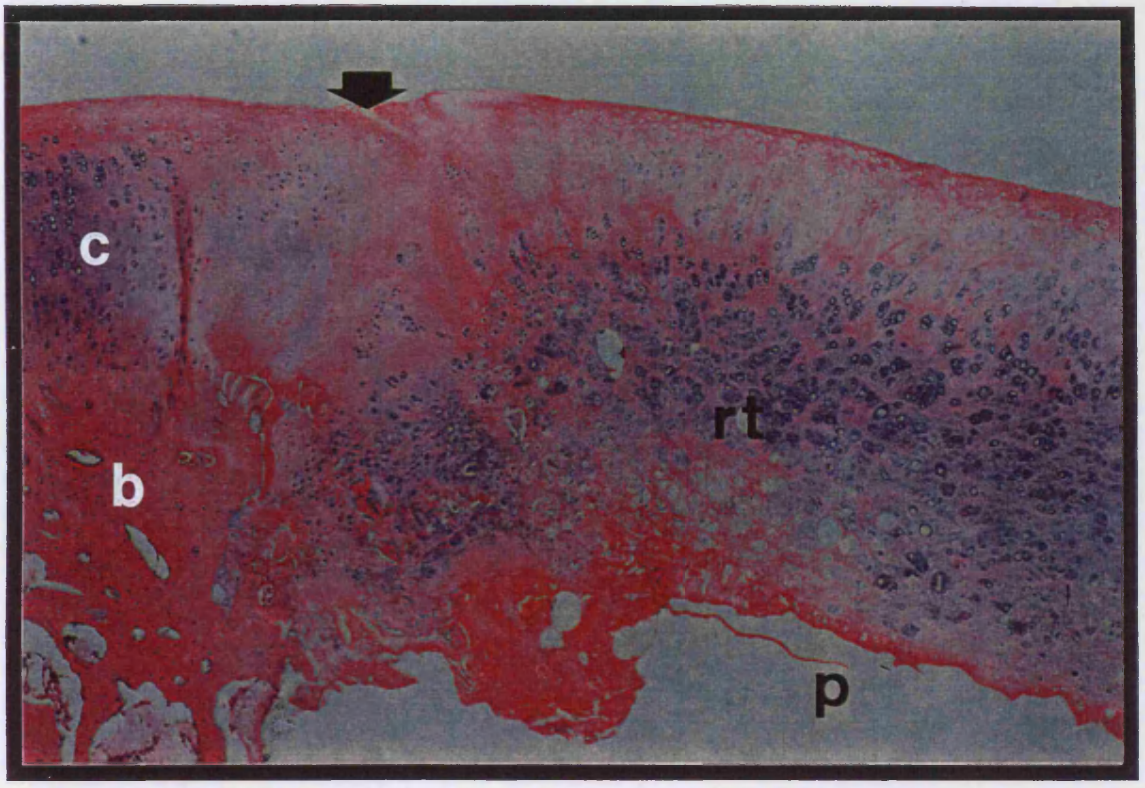


Fig.V.7

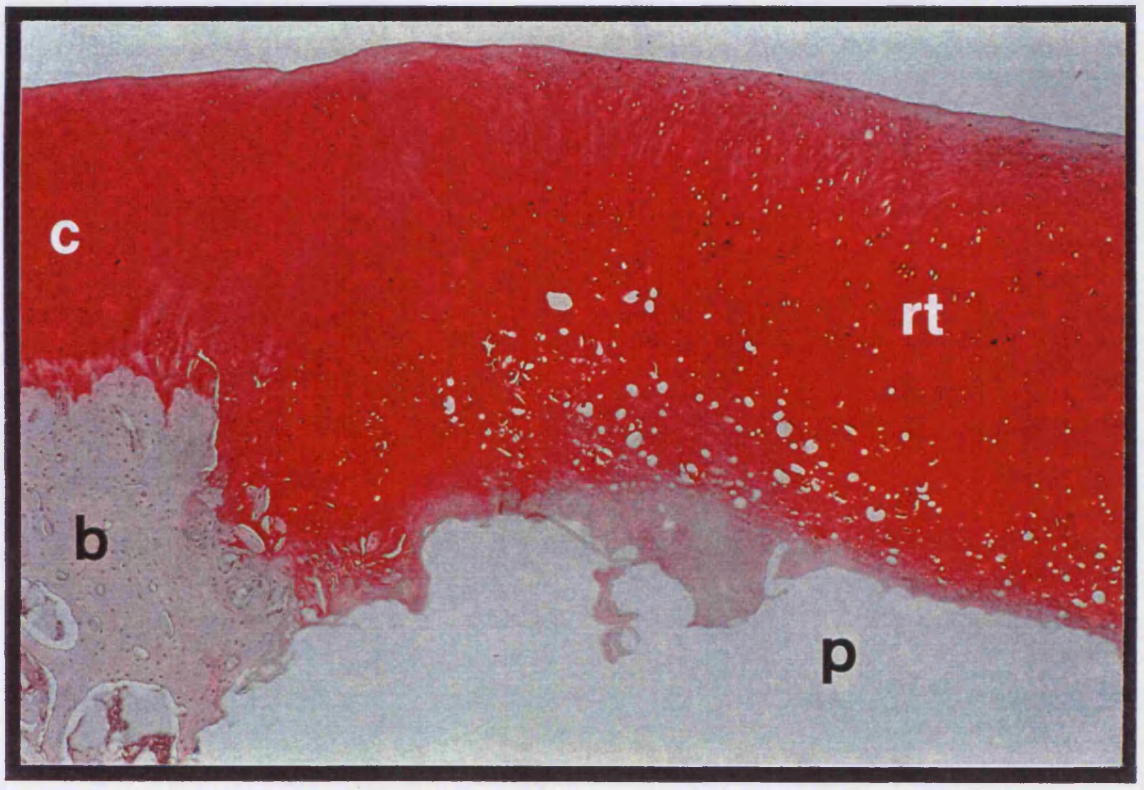


Fig.V.8

Fig.V.9

Macroscopic appearance of the repair tissue in the polymer group in an old rabbit:
Complete resurfacing of the defect in the femoral trochlea **[ft]**. The repair tissue is fully integrated with the surrounding the defect normal articular cartilage **[►]** and the transitional area is smooth, except for a small region which is irregular **[→]**.

Fig.V.10

Macroscopic appearance of the posterior aspect of the femoral condyle in the specimen shown in Fig V.9:
Severe osteoarthritic changes with complete destruction of the articular cartilage **[c]** and exposure of the subchondral bone **[b]**. The ► indicate the remaining parts of the articular cartilage that show signs of advanced degeneration.

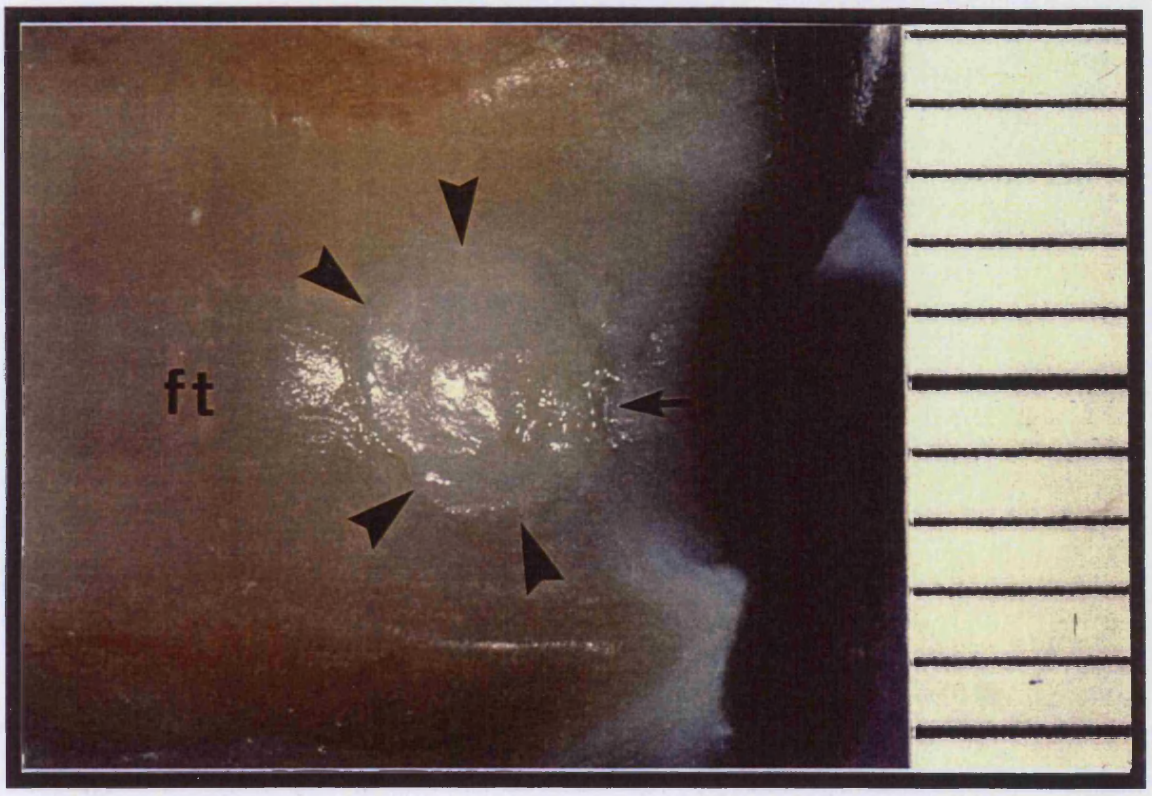


Fig.V.9

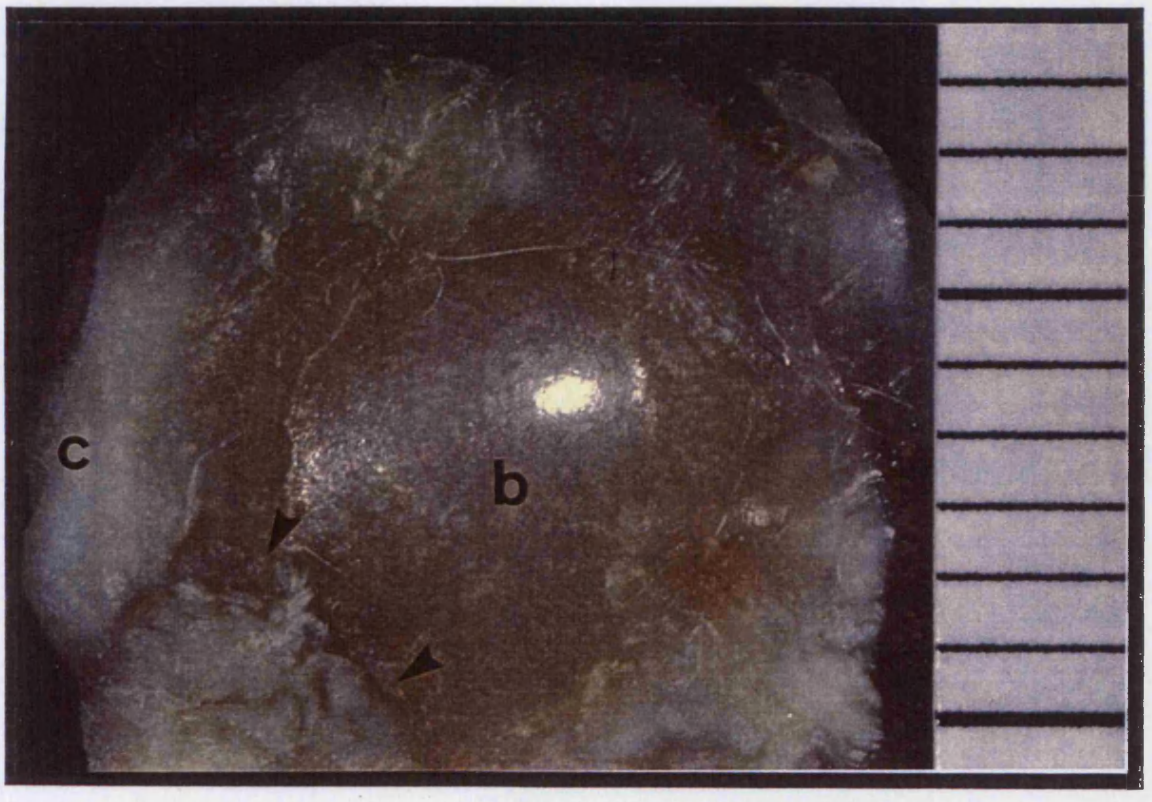


Fig.V.10

Fig.V.11

Histological appearance of the repair tissue in the polymer group in an old rabbit:
The repair tissue **[rt]** is fully integrated with the surrounding the defect normal articular cartilage **[c]**. The ▶ indicates the transition from the repair tissue to the adjacent articular cartilage. **[b**=subchondral bone, **p**=polymer] *(Haematoxylin/Eosin staining, x 13.2)*

Fig.V.12

Histochemical appearance of the repair tissue in the polymer group in an old rabbit:
The repair tissue **[rt]** contains high concentration of proteoglycans, similar to that of the normal articular cartilage **[c]**. The subchondral bone contains no proteoglycans and stained negative. **[p**=polymer] *(Safranin-O staining, x 13.2)*

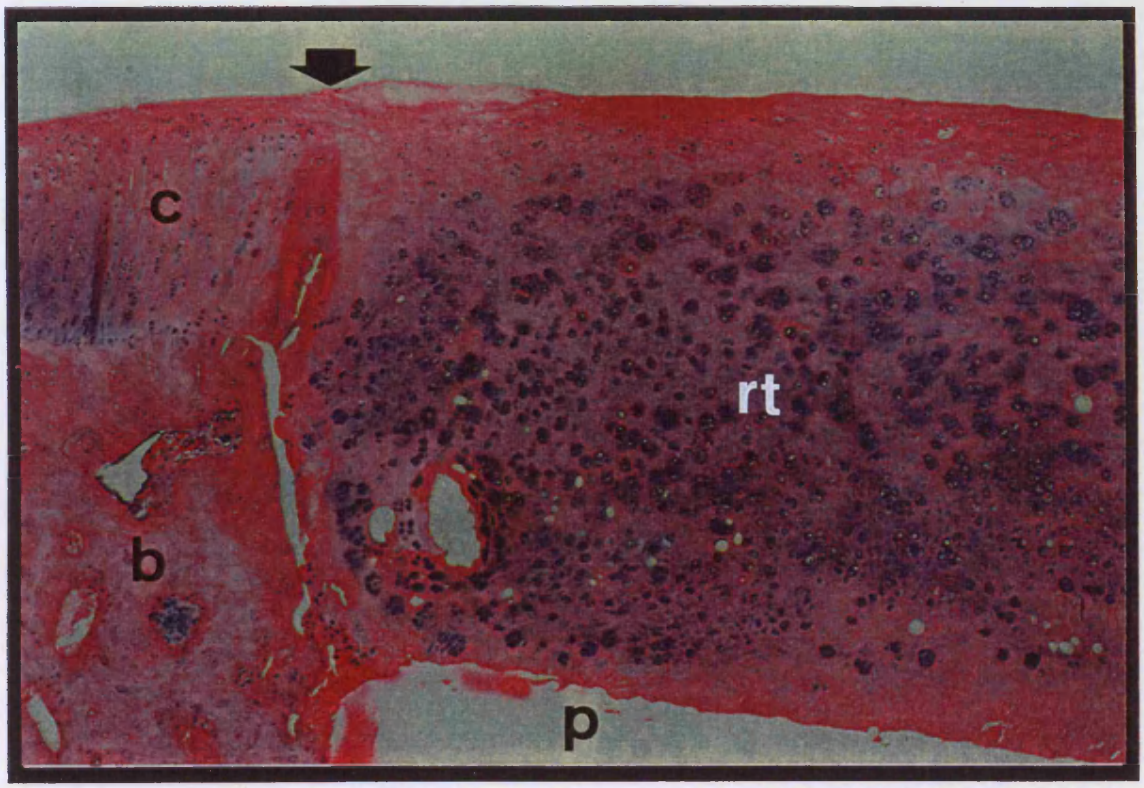


Fig.V.11

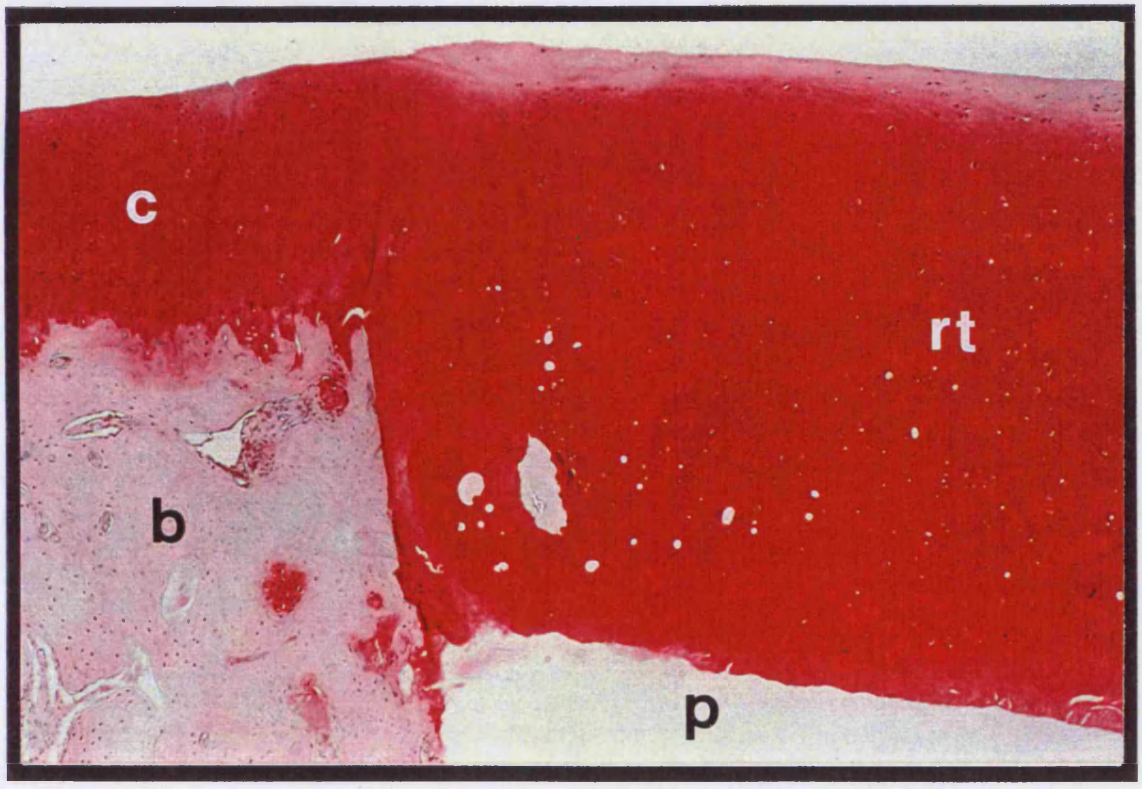


Fig.V.12

Fig.V.13

Histological appearance of the repair tissue in the polymer group in an old rabbit:
Complete bonding of the repair tissue **[rt]** to the surrounding the defect normal articular cartilage **[c]**. The cells in the repair tissue outnumber those in the normal articular cartilage, a finding similar to that in the young and adult age groups. *(1 μm thick section, Toluidine blue staining, x 132)*

Fig.V.14

Immunohistochemical appearance of the repair tissue in the polymer group in an old rabbit:
The repair tissue **[rt]** contains collage type II like the normal articular cartilage **[c]**. The subchondral bone **[b]** contains no collagen type II and is stained negative. *(Silver-enhanced colloidal gold immuno-staining, x 33)*

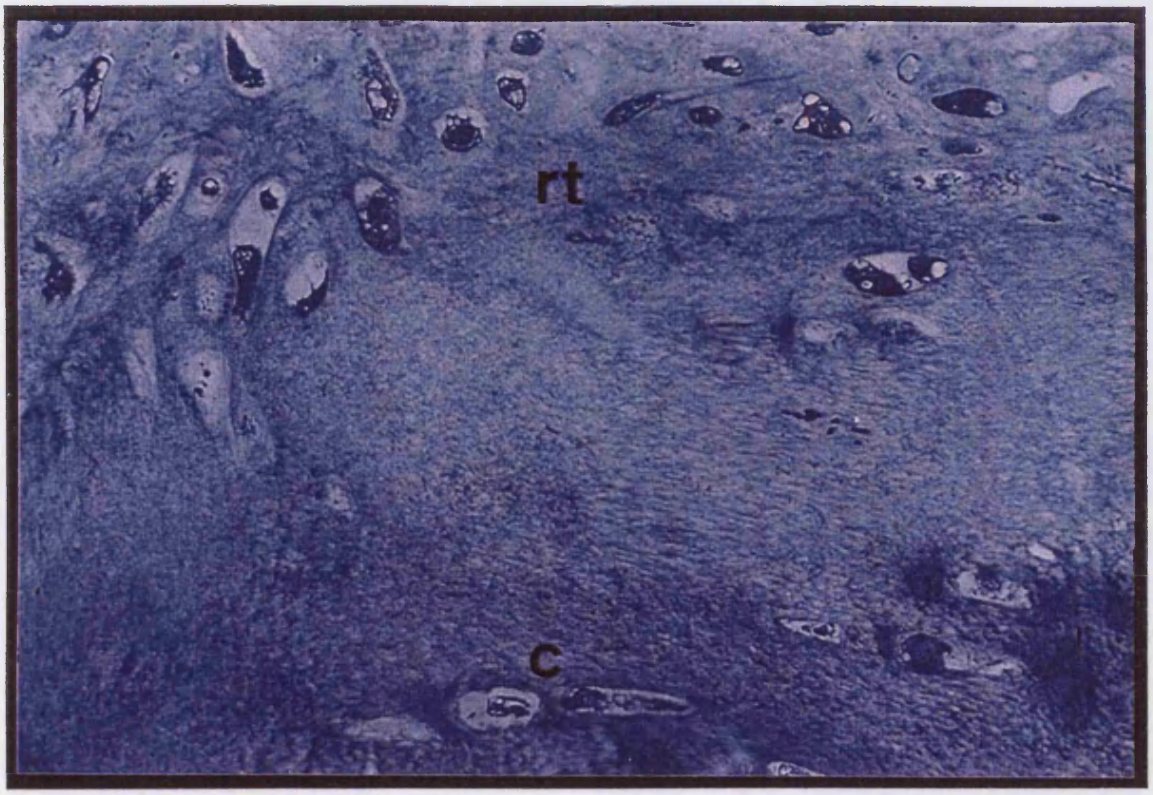


Fig.V.13

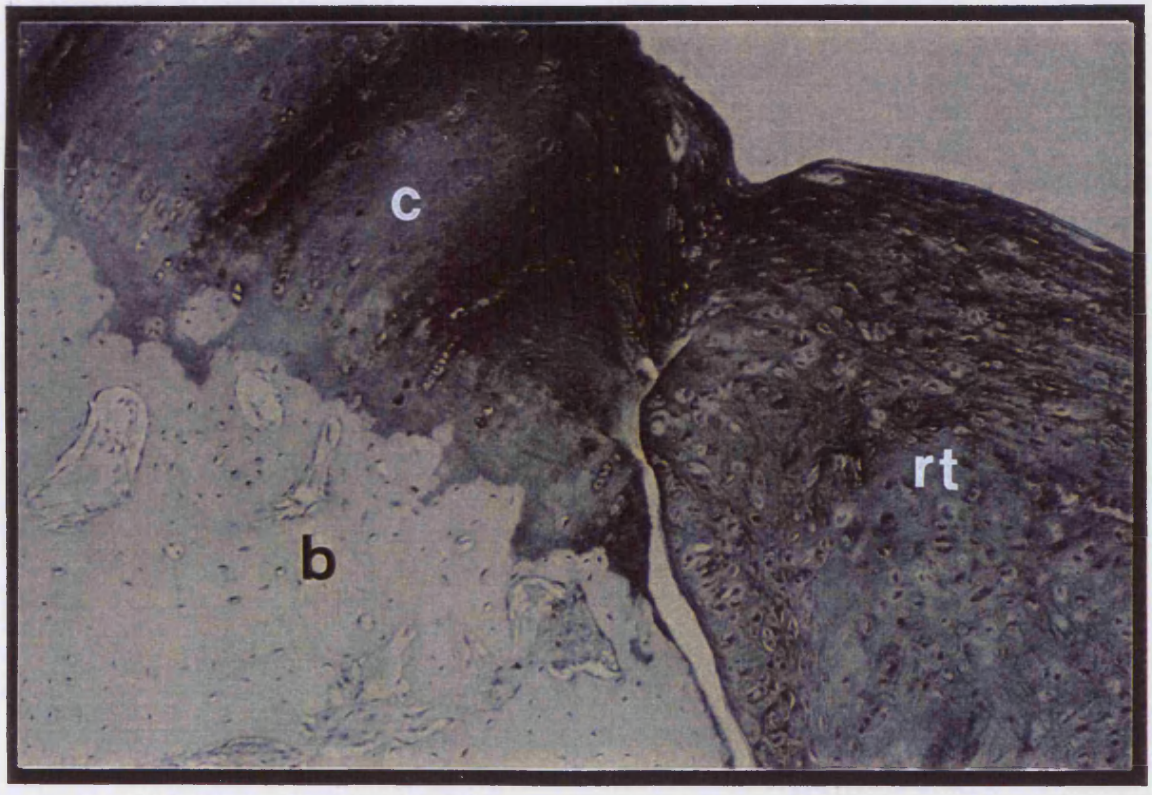


Fig.V.14

Fig.V.15

Histological appearance of the repair tissue in the control group in a young rabbit: The repair tissue **[rt]** is fibrocartilaginous and contains pieces of subchondral bone **[*]**. The adjacent to the defect normal articular cartilage **[c]** shows signs of degeneration with fibrillation and empty cell lacunae. The ► indicate the incomplete bonding of the repair tissue, **[b**=subchondral bone**]** *(Haematoxylin/Eosin staining, x 33)*

Fig.V.16

Histological appearance of the repair tissue in the control group in an old rabbit: The repair tissue **[rt]** is fibroblastic and there is significant degeneration and fibrillation **[►]** in the superficial layer of the normal articular cartilage **[c]** adjacent to the defect. **[b**=subchondral bone**]** *(Haematoxylin/Eosin staining, x 33)*

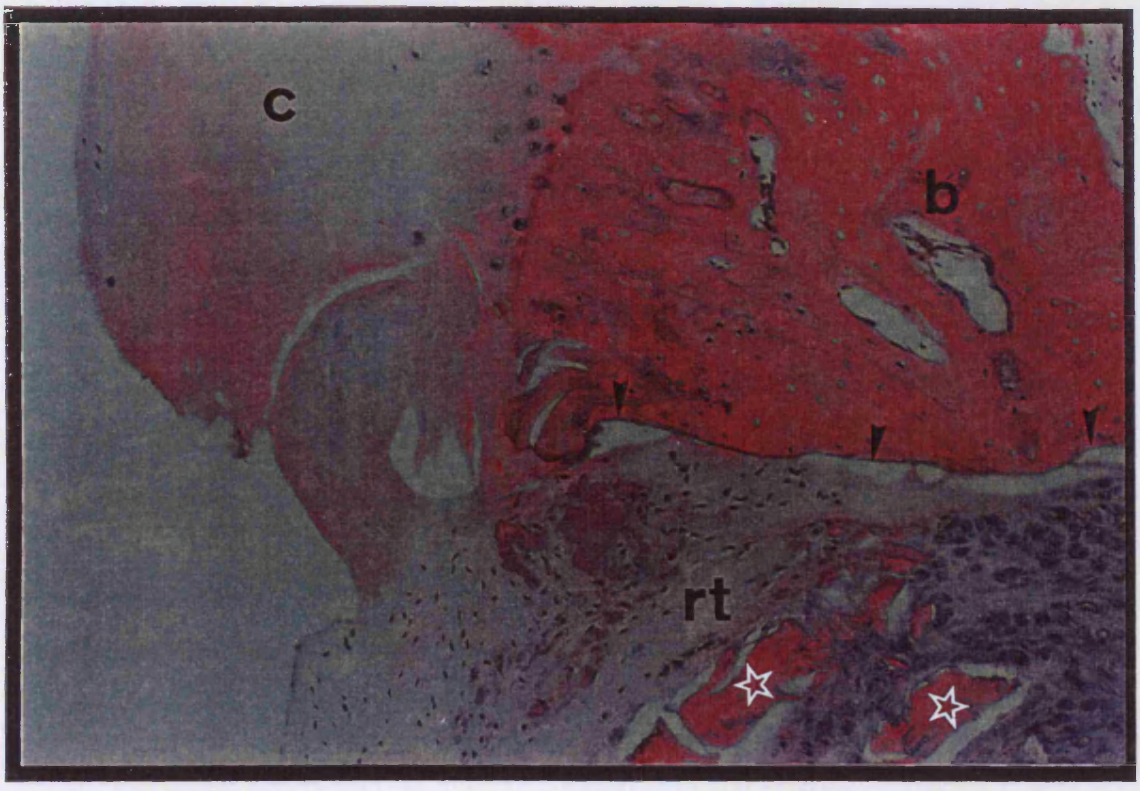


Fig.V.15

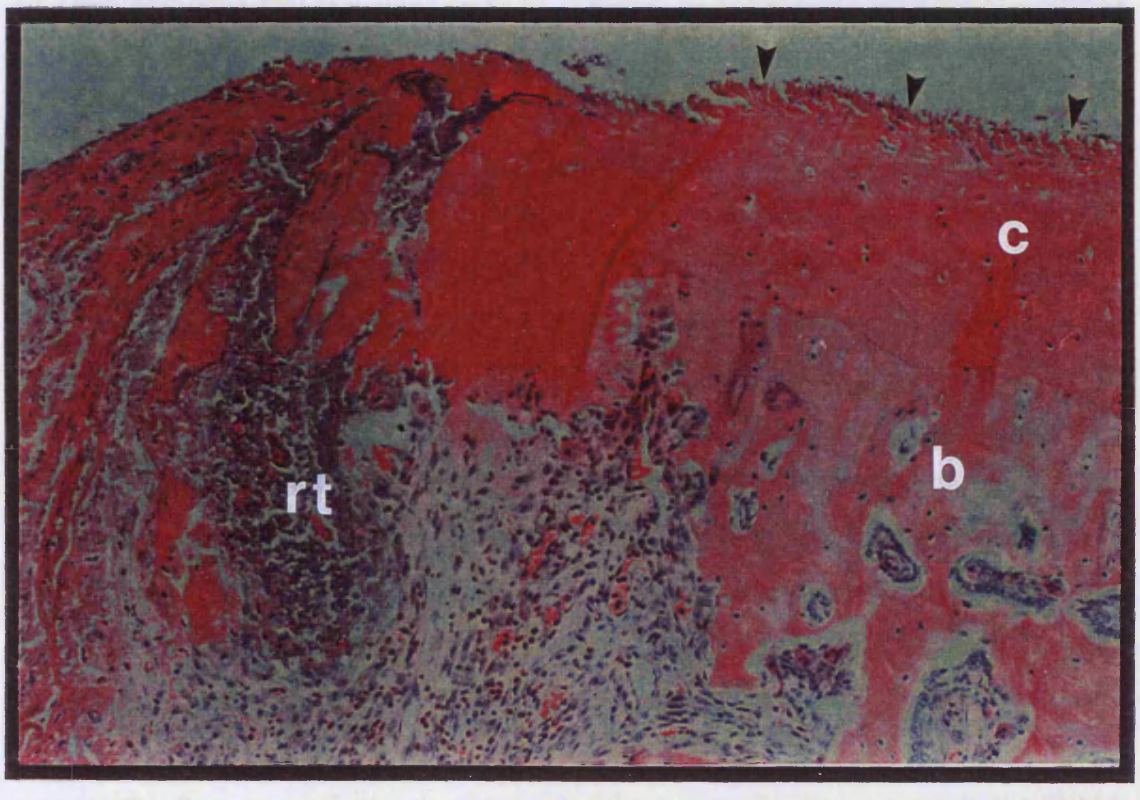


Fig.V.16

DISCUSSION

The structure, composition and metabolism of the articular cartilage are known to change with age (Buckwalter et al 1985, Webber et al 1987, Stanescu et al 1988, Flannery et al 1990, Arner, 1994). There are many macroscopic changes as well as microscopic alterations in the cartilage cellularity and extracellular matrix structure, which are age-related. The present study used the new PEMA/THFMA polymer system for articular cartilage repair in three different age groups of rabbits and showed enhancement of the biological repair in all ages.

Vignon et al (1976) studied the femoral head in humans and found an age-related decrease in cell numbers that was more pronounced in the superficial as compared to the deeper zones of cartilage. It was also apparent that there is a close correlation between reduced cellularity and increased frequency of fibrillation in the superficial zone of the articular cartilage. Chondrocyte growth factor responsiveness shows qualitative changes during development and, after skeletal maturity, there is a profound decline in the levels of DNA synthesis and cell replication in response to the known chondrocyte growth factors (Guerne et al 1995). Our results showed predominance of chondrocytes in the repair tissue in the PEMA group at 6 weeks in all ages without a statistically significant change with age.

Hollander et al (1995) examined articular cartilage explants from human knees using immunohistochemistry. They specifically studied denaturation [loss of triple helix] of collagen type II and found that in ageing the first damage to collagen type II occurs in the superficial and upper middle zones extending to the lower middle and deep zones with increasing degeneration. They also noted that the initial damage of the collagen fibrils always occurs around chondorcytes, thus implicating them in the denaturation of collagen type II. In our study in the control group, immunohistochemistry detected collagen type II in the deeper layers of the repair tissue only, correlating with the early degeneration of the superficial layer of the repair tissue observed in this group from as early as 6 weeks. Only one repair tissue specimen (10%) in the young age control group contained collagen type II in more than half of the matrix. On the contrary, in the PEMA group, 70% of the young specimens, 50% of the adult specimens and 50% of the old age specimens contained abundant collagen type II evenly distributed throughout the

extracellular matrix of the repair tissue. Collagen type II provides mechanical protection of the repair tissue against tensile stresses and shear forces and thus it can be speculated that the early degenerative changes seen in the repair tissue in the control group in all ages could reflect the mechanical disadvantage of the depleted of collagen type II repair tissue in this group.

Bank et al (1998) showed that the age-related accumulation of non-enzymatic glycation affects biomechanical properties of human articular cartilage. They indicated extensive tissue remodelling at young age, with slow turnover of collagen after maturity. They also showed that maturation starts in the second decade of life in the upper half of the tissue, and occurs last in the tissue close to bone. Madsen et al (1983) studied articular chondrocytes in rabbits of different age groups and found that ageing chondrocytes produce qualitatively and quantitatively different matrix components. Levels of elastin, collagen and proteoglycan decreased to 50% or even lower levels in 60-month-old rabbits as compared to 1-week-old animals. The decreased ability of mature cartilage to replace lost proteoglycans rapidly (Arner, 1994) would increase the susceptibility of cartilage to degeneration. These findings would also suggest a delay in the repair of articular cartilage defects in old age, and thus we can speculate that the repair tissue in the PEMA group in the old rabbits will continue improving after the 6-week observation period in our study. Some degree of improvement is also anticipated in the control knees in the old rabbits, although, taken into account the results in the young and adult rabbits, this improvement is expected to be minimal.

The articular cartilage of older individuals has xanthous appearance as opposed to the white colour of young cartilage (Sokoloff, 1983). This may be due to increased intracellular lipofuscin and to non-enzymatic advanced glycosylation of the extracellular matrix constituents (Kirstein et al 1990). The expression of extracellular matrices seems to be closely related to aging and degenerative changes (Ishibashi et al 1996). In our study, no significant colour changes were noted in the normal articular cartilage and the repair tissue in the three age groups.

Proteoglycans in ageing cartilage are smaller in size (Hardingham and Bayliss, 1990), while the total glycosaminoglycan content in human articular cartilage may not significantly change with ageing, although there is a decrease in the relative amount in

chondroitin-sulphate and an increase in keratan-sulphate content (Roughley and White, 1980). The changes in chondroitin-sulphate are thought to account for the decreased water content in aged cartilage. Ageing chondrocytes appear to assemble link protein-stabilized aggregates more slowly (Plaas et al 1988). Chondrocytes also synthesize qualitatively different proteoglycans with age. Proteoglycans produced by cells from older animals showed decreased size and aggregation and an increase in the amount of chondroitin 6-sulphate (Madsen et al 1983). This change does not appear to occur in the repair tissue itself. In our studies using the silver-enhanced colloidal gold immunostaining, it was shown that the repair tissue in the old age rabbits, at least for the first 6 weeks, contains smaller amounts of chondroitin 6-sulphate in both the PEMA and the control groups.

The cartilage reduces its thickness in weight-bearing and also in non-weight-bearing joints with age (Meachim, 1971) and it shows fibrillations and softening of the superficial layers as a result of loss of proteoglycans and discontinuity in the collagen network (Meachim, 1972). Wachtel et al (1995) using x-ray scattering techniques found a statistically significant tendency towards less dense collagen packing in human articular cartilage as a result of ageing.

The mechanical properties of cartilage, including tensile fracture stress and tensile stiffness also change adversely with increasing age (Kempson, 1981, Kempson, 1991), rendering the cartilage susceptible to mechanical failure. Luder in 1998, following a microscopic study on human mandibular condyles from adolescence to old age, believes that both maturational and later age changes seem to depend markedly on articular load bearing. The rabbits in our study were fully weight bearing immediately post-operatively and they were group-housed in floor pens that allowed more significantly more physiological mobility and weight bearing of the operated knees.

Osteoarthritic changes were noted in 4 (20%) of the knees treated with PEMA/THFMA in the old group of rabbits. The changes were severe, showing complete destruction of the articular cartilage in the posterior aspect of the femoral condyles with eburnated subchondral bone and osteophyte formation. In all these 4 knees, the articular cartilage defects in the femoral trochlea were fully resurfaced by 6 weeks [3 good, 1 excellent repair]. It is therefore possible to assume that PEMA/THFMA enhances articular

cartilage regeneration and repair, even in the presence of osteoarthritic changes in the treated joint.

In Chapter III.B it was shown that the repair tissue following implantation of PEMA/THFMA in articular cartilage defects in adult rabbits maintains satisfactory structure and composition up to 1 year post-operatively. It is not yet determined how the newly formed repair tissue will behave in the long term in the young and old age groups. However, it appears from this study that the biomaterial is equally effective in all three age groups. Thus, the use of PEMA/THFMA polymer in clinical practice could potentially challenge the traditional methods of treatment of articular cartilage defects in patients of all ages.

CHAPTER VI

COMPARATIVE STUDY OF ARTICULAR CARTILAGE REPAIR USING PEMA/THFMA POLYMER AND POLY-METHYL-METHACRYLATE POLYMER [PMMA/MMA]

OBJECTIVE

MATERIALS

- 10 adult rabbits
- PEMA vs PMMA

METHOD

- Macroscopic, Histological/Histochemical and Immunohistochemical assessment
- At 6 weeks

RESULTS

DISCUSSION

OBJECTIVE

Full-thickness articular cartilage defects were treated either with PEMA/THFMA or with the conventional bone cement PMMA/MMA. Both polymers were implanted using the same technique. The aim of this study is to clarify, among others, whether the method of implantation matters mostly, or whether the chemistry of the polymer is critical.

MATERIALS AND METHODS

This study used 10 female adult Sandy-Lop rabbits. Both knees were operated on in each rabbit, one knee received PEMA/THFMA [PEMA] and the other knee PMMA/MMA [PMMA]. The method of implantation and preparation of the PEMA/THFMA polymer was the same as described in Chapter III.B. For the preparation of the PMMA/MMA system, 1 gr PMMA powder (containing 10% Barium Sulphate) was mixed with 0.5 ml MMA liquid (containing 2.5% V/V N,N-dimethyl-p-toluidine).

Animals were sacrificed at 6 weeks after operation with intravenous injection of Pentobarbitone Sodium (Euthatal 600 mg). Specimens were evaluated using the Macroscopic and Histological/Histochemical Assessment of the Articular Cartilage Repair Scoring System [Chapter III.A], and also using Immunohistochemistry involving the 10 control specimens of the adult rabbits assessed at 6 weeks with the silver-enhanced colloidal gold immunostaining [Chapter III.C]. Furthermore, the results in the PMMA group were also compared with the results in the control group at 6 weeks from the study in Chapter III.B, in order to assess whether or not implantation of conventional bone cement in articular cartilage defects is preferable to 'natural' healing of the defect.

RESULTS

No infection was recorded in neither group. There was mild inflammatory reaction of the synovium in 2 (20%) knees in the PEMA group and 5 (50%) knees in the PMMA group of which three knees also showed significant foreign body reaction of the synovium to particles of bone cement. The macroscopic and histological/histochemical results are presented in Tables VI.1, VI.2 and VI.3 followed by statistical analysis.

Table VI.1:

Macroscopic and Histological/Histochemical assessment per criterion
Scoring at 6 weeks. Rabbits *141-150*
PEMA vs PMMA

PEMA	*R141*	*R142*	*R143*	*R144*	*R145*	*R146*	*R147*	*R148*	*R149*	*R150*
Area	20	20	10	20	20	20	10	20	10	20
Level	10	10	10	5	10	10	5	5	10	10
Surface	10	10	10	10	10	10	10	5	10	10
Cells	20	10	20	20	20	10	20	10	20	20
PGs	10	10	10	10	5	10	10	5	5	10
Structure	10	10	5	5	5	5	10	5	0	5
Bonding	20	10	20	20	20	20	10	20	10	20
TOTAL SCORE	**100**	**80**	**85**	**90**	**90**	**85**	**75**	**70**	**65**	**95**

PMMA	*R141*	*R142*	*R143*	*R144*	*R145*	*R146*	*R147*	*R148*	*R149*	*R150*
Area	10	10	0	10	10	20	10	0	0	0
Level	5	0	0	5	0	5	0	10	0	5
Surface	10	5	0	5	5	5	0	5	10	10
Cells	10	20	10	10	0	20	0	10	0	10
PGs	10	5	5	5	0	5	5	5	0	10
Structure	10	10	5	5	0	0	5	0	5	10
Bonding	10	20	10	10	0	10	20	10	0	10
TOTAL SCORE	**65**	**70**	**30**	**50**	**15**	**65**	**40**	**40**	**15**	**55**

	PEMA MEANS	*PMMA MEANS*	*PEMA SD*	*PMMA SD*
Area	17.00	7.00	4.83	6.75
Level	8.50	3.00	2.42	3.50
Surface	9.50	5.50	1.58	3.69
Cells	17.00	9.00	4.83	7.38
PGs	8.50	5.00	2.42	3.33
Structure	6.00	5.00	3.16	4.08
Bonding	17.00	10.00	4.83	6.67
TOTAL SCORE	**83.50**	**44.50**	**11.07**	**20.06**

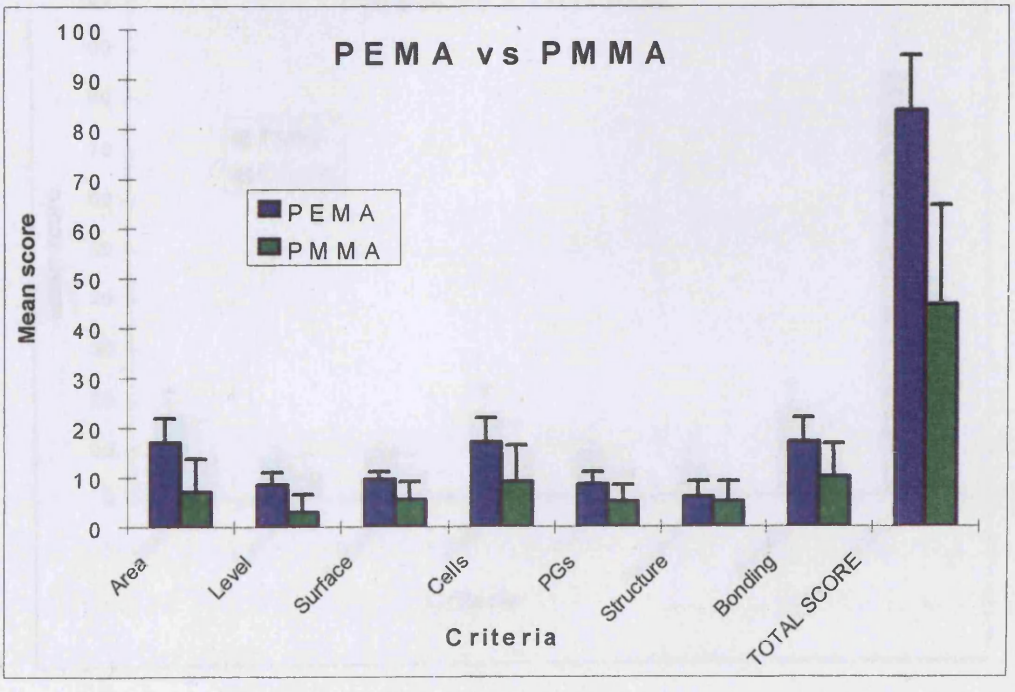


Table VI.2:

Macroscopic and Histological/Histochemical assessment per criterion
Scoring at 6 weeks. Rabbits *141-150 and 1-30*
PMMA vs CONTROL

PMMA	*R141*	*R142*	*R143*	*R144*	*R145*	*R146*	*R147*	*R148*	*R149*	*R150*
Area	10	10	0	10	10	20	10	0	0	0
Level	5	0	0	5	0	5	0	10	0	5
Surface	10	5	0	5	5	5	0	5	10	10
Cells	10	20	10	10	0	20	0	10	0	10
PGs	10	5	5	5	0	5	5	5	0	10
Structure	10	10	5	5	0	0	5	0	5	10
Bonding	10	20	10	10	0	10	20	10	0	10
TOTAL SCORE	**65**	**70**	**30**	**50**	**15**	**65**	**40**	**40**	**15**	**55**

CONTROL	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*
Area	10	0	10	10	10	20	10	10	20	0	0	0	10	20	10	10	10	10	10	20	20	10	0	10	0	0	0	10	20	0
Level	5	0	5	5	5	10	5	10	10	0	5	0	10	5	5	5	5	10	10	10	5	0	0	0	10	10	0	5	5	0
Surface	0	5	5	5	10	10	10	5	0	0	5	0	10	5	5	5	5	5	5	10	5	0	5	5	10	10	5	5	5	0
Cells	10	10	10	10	10	20	10	10	20	10	0	0	10	10	10	10	0	10	10	20	10	0	10	10	0	0	10	20	10	10
PGs	5	0	5	10	5	10	10	5	0	5	0	0	10	10	5	5	0	10	5	5	10	0	10	5	0	5	10	10	0	5
Structure	0	0	0	0	0	5	0	0	0	5	0	0	0	0	5	5	5	0	0	0	0	0	0	5	0	0	0	5	5	5
Bonding	10	10	0	0	10	10	0	10	10	10	0	0	10	10	20	10	10	0	0	20	20	10	0	10	0	0	0	10	10	10
TOTAL SCORE	**40**	**25**	**35**	**40**	**50**	**85**	**45**	**50**	**60**	**30**	**10**	**0**	**60**	**60**	**60**	**50**	**35**	**45**	**40**	**85**	**70**	**20**	**25**	**45**	**20**	**25**	**25**	**65**	**55**	**30**

	PMMA MEANS	*CONTROL MEANS*	*PMMA SD*	*CONTROL SD*
Area	7.00	9.00	6.75	7.12
Level	3.00	5.17	3.50	3.82
Surface	5.50	5.17	3.69	3.34
Cells	9.00	9.33	7.38	5.83
PGs	5.00	5.33	3.33	3.92
Structure	5.00	1.50	4.08	2.33
Bonding	10.00	7.33	6.67	6.40
TOTAL SCORE	**44.50**	**42.83**	**20.06**	**20.33**

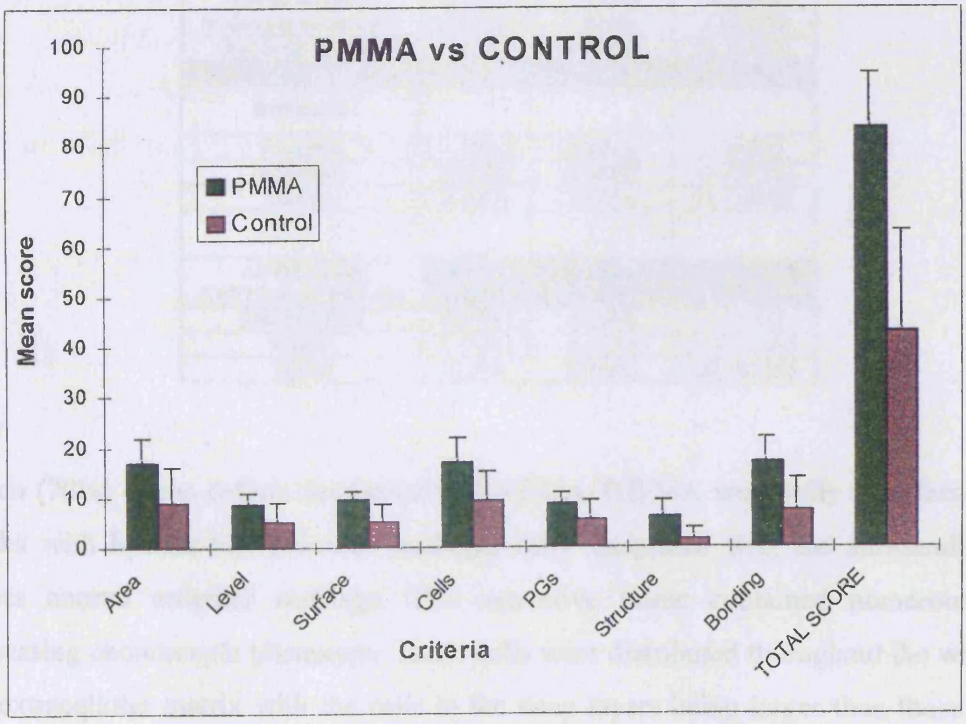


Table VI.3:

Macroscopic and Histological/Histochemical assessment of repair tissue by criterion and the overall repair at 6 weeks

PEMA vs PMMA vs CONTROL

	6 weeks		
AREA	**PEMA n=10**	**PMMA n=10**	**CONTROL n=30**
100% resurfacing	7 (70%)	1 (10%)	6 (20.0%)
>50% resurfacing	3 (30%)	5 (50%)	15 (50.0%)
<50% resurfacing	0 (0%)	4 (40%)	9 (30.0%)
LEVEL			
Normal cartilage level	7 (70%)	1 (10%)	9 (30.0%)
Minimal depression	3 (30%)	4 (40%)	13 (43.3%)
Gross depression	0 (0%)	5 (50%)	8 (26.6%)
SURFACE			
Smooth, glistering	9 (90%)	3 (30%)	7 (23.3%)
Irregular, opaque	1 (10%)	5 (50%)	17 (56.6%)
Disrupted by fissures	0 (0%)	2 (20%)	6 (20.0%)
CELLS			
Chondrocytes	7 (70%)	2 (20%)	4 (13.3%)
Fibroblasts	3 (30%)	5 (50%)	20 (66.6%)
Other	0 (0%)	3 (30%)	6 (20.0%)
PGs			
Normal	7 (70%)	2 (20%)	10 (33.3%)
Moderate	3 (30%)	6 (60%)	12 (40.0%)
Low	0 (0%)	2 (20%)	8 (26.6%)
STRUCTURE			
Hyaline-like tissue	3 (30%)	3 (30%)	0 (0.0%)
Moderately disorganised	6 (60%)	4 (40%)	9 (30.0%)
Grossly disorganised	1 (10%)	3 (30%)	21 (70.0%)
BONDING			
Complete	7 (70%)	2 (20%)	3 (9.9%)
Incomplete	3 (30%)	6 (60%)	16 (53.3%)
Minimal	0 (0%)	2 (20%)	11 (36.6%)
ARTICULAR CARTILAGE REPAIR	**PEMA n=10**	**PMMA n=10**	**CONTROL n=30**
EXCELLENT	6 (60%)	0 (0%)	2 (6.6%)
GOOD	4 (40%)	5 (50%)	10 (33.3%)
POOR	0 (0%)	5 (50%)	18 (60.0%)

Seven (70%) of the defects implanted with PEMA/THFMA were fully resurfaced by 6 weeks with hyaline-like articular cartilage, fully integrated with the surrounding the defect normal articular cartilage. The reparative tissue contained numerous cells expressing chondrocyte phenotype. These cells were distributed throughout the whole of the extracellular matrix with the cells in the deep layers being larger than those in the

superficial layer. All specimens that were stained with safranin-O showed normal or near to normal concentration of proteoglycans in the repair tissue compared to the adjacent normal articular cartilage. Moreover, a 'zonal' differentiation with lighter staining of the superficial layer compared to the deep layers, characterisitc of mature articular cartilage, was noted in all specimens in the PEMA group.

Of the defects implanted with PMMA/MMA, all were repaired with fibrocartilaginous type of tissue, incompletely bonded to the surrounding normal articular cartilage. In all but 2 specimens, the tissue of the periphery of the repair presented with a characteristic acellular edge and degenerative changes, worse in the superficial layer. Histochemistry showed proteoglycans in the deep layer of the repair tissue only and no 'zonal' differentiation.

Statistical analysis of macroscopic and histological/histochemical results:

PEMA vs PMMA

The scores of the seven criteria were tested using the ***Mc Nemar's*** test and the total score was assessed using the ***Wilcoxon*** test. However, the number of animals used for each group was 10, rather than 30, so the statistical power is decreased. This should be born in mind when interpreting the p-values, as an insignificant p-value can be interpreted as either there is no effect or that the test has insufficient power to detect an effect; there is no way to distinguish between these two interpretations.

Score	P-value
Area	.046
Level	.112
Surface	.046
Cells	.300
PGs	.072
Structure	.801
Bonding	.262
Total Score	.006

The analysis indicates that PEMA is significantly superior to PMMA, with the area and surface appearance being mostly influenced.

PMMA vs Control

In this analysis the control group is taken from the main trial and the PMMA group from the PEMA vs PMMA experiment, hence no pairing exists. Therefore ***Fisher's*** test is used and the p-values are shown below:

Score	p-value
Area	.890
Level	.296
Surface	.889
Cells	.659
PGs	.644
Structure	.007
Bonding	.596
Total score	.730

The only significant difference is in the structure score, where the PMMA group scores higher than the control group. Notably, the total score is non-significant, confirming that the PMMA bone cement when implanted in articular cartilage defects is not superior to the 'natural' healing of the control knees.

Table VI.4 demonstrates analysis of the immunolocalisation of the cartilage components in the repair tissue in the PEMA, the PMMA as well as the control groups at 6 weeks.

Table VI.4:
Immunohistochemical assessment of repair tissue at 6 weeks
PEMA vs PMMA vs CONTROL

	6 weeks		
Collagen type II	**PEMA n=10**	**PMMA n=10**	**CONTROL n=10**
>50% of matrix	6 (60%)	0 (0%)	0 (0%)
<50% of matrix	3 (30%)	0 (0%)	2 (20%)
no detection	1 (10%)	10 (100%)	8 (80%)
Keratan-Sulphate			
>50% of matrix	7 (70%)	1 (10%)	0 (0%)
<50% of matrix	3 (60%)	2 (20%)	0 (0%)
no detection	0 (0%)	7 (70%)	10 (100%)
Chondroitin 4-Sulphate			
>50% of matrix	7 (70%)	2 (20%)	0 (0%)
<50% of matrix	2 (20%)	3 (30%)	2 (20%)
no detection	1 (10%)	5 (50%)	8 (80%)
Chondroitin 6-Sulphate			
>50% of matrix	8 (80%)	0 (0%)	0 (0%)
<50% of matrix	2 (20%)	3 (30%)	4 (40%)
no detection	0 (0%)	7 (70%)	6 (60%)

In the PEMA group, immunohistochemistry detected cartilage components, including collagen type II, keratan sulphate, and chondroitin 4- and 6-sulphate, evenly distributed throughout the matrix of the reparative tissue in over 60% of the defects. In the PMMA group no collagen type II was detected in any of the specimens. In over 50% of the specimens in the same group, the repair tissue contained no glycosaminoglycans. When glycosaminoglycan aggregations were present, they were localised exclusively pericellularly and predominantly in the deep layers of the repair tissue.

Statistical analysis of immunohistochemical results:

The ***Fisher's*** exact test was used for the statistical correlation and the p-values are presented below:

Antigen	p-value
Collagen type II	1e-4
Keratin- sulphate	.002
Chondroitin 4-sulphate	.068
Chondroitin 6-sulphate	1e-4

This analysis indicates that PMMA is significantly inferior to PEMA, but it is indistinguishable from the control.

Fig.VI.1

Histochemical appearance of the repair tissue in the poly-methyl-methacrylate group:

The repair tissue **[rt]** is fibroblastic containing low concentration of proteoglycans, compared to the concentration of proteoglycans in the normal articular cartilage **[c]**. The subchondral bone **[b]** contains no proteoglycans and stained negative. The **[*]** indicates the poly-methyl-methacrylate plug. *(Safranin-O staining, x 13.2)*

Fig.VI.2

Histological appearance of the repair tissue in the poly-methyl-methacrylate group:

The repair tissue **[rt]** comprises predominantly of cells with fibroblastic phenotype. [*=poly-methyl-methacrylate plug] *(Haematoxylin/Eosin staining, x 33)*

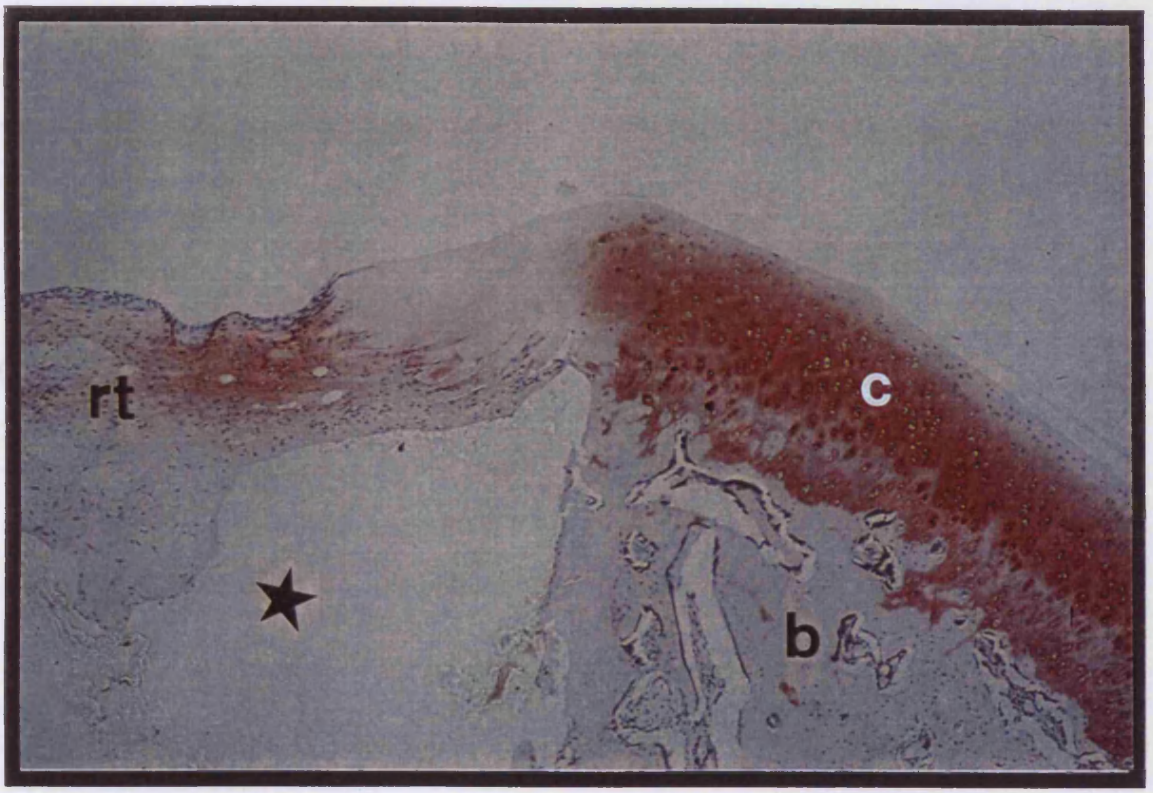


Fig.VI.1

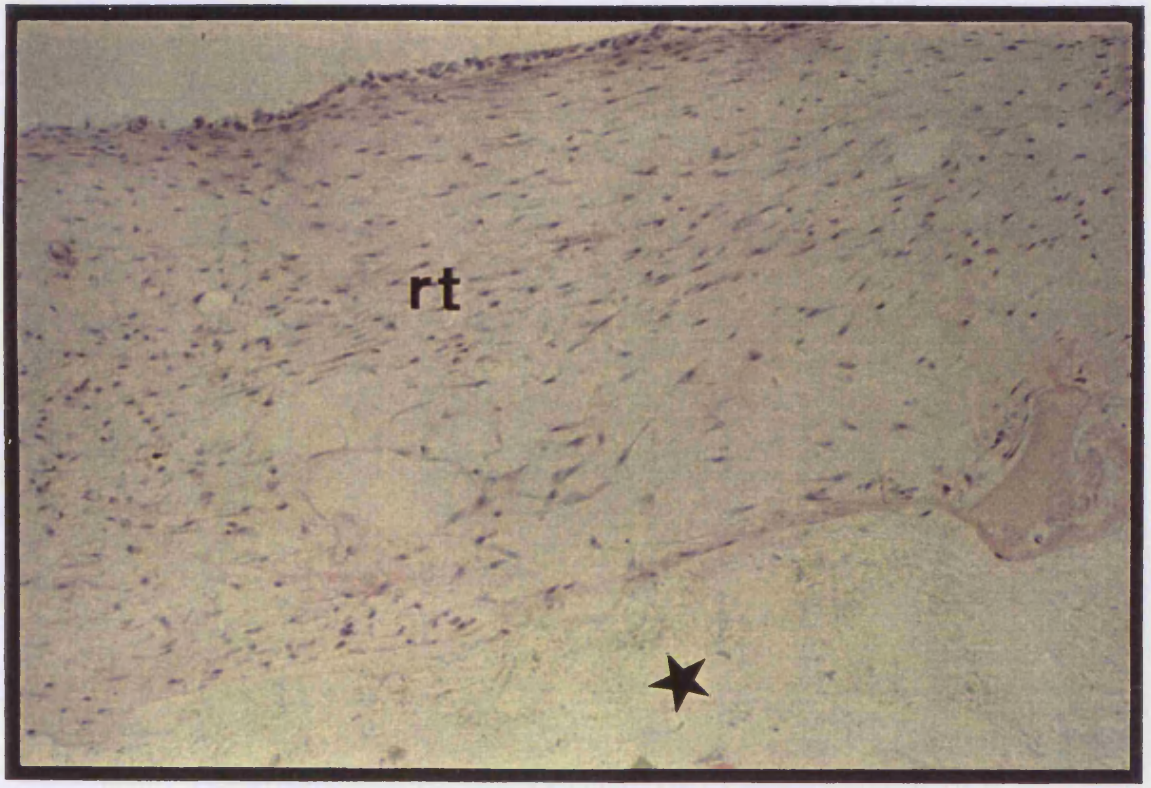


Fig.VI.2

Fig.VI.3

Histochemical appearance of the repair tissue in the polymer group:
The repair tissue **[rt]** contains high concentration of proteoglycans, similar to that noted in the normal articular cartilage **[c]**. The subchondral bone **[b]** contains no proteoglycans and stained negative. The → indicates the transition from the repair tissue to the normal articular cartilage **[p**=polymer] *(Safranin-O staining, x 33)*

Fig.VI.4

Immunohistochemical appearance of the repair tissue in the polymer group:
The repair tissue contains keratan-sulphate distributed around the chondrocytes as well as in the extracellular matrix. *(Silver-enhanced colloidal gold immuno-staining, x 132)*

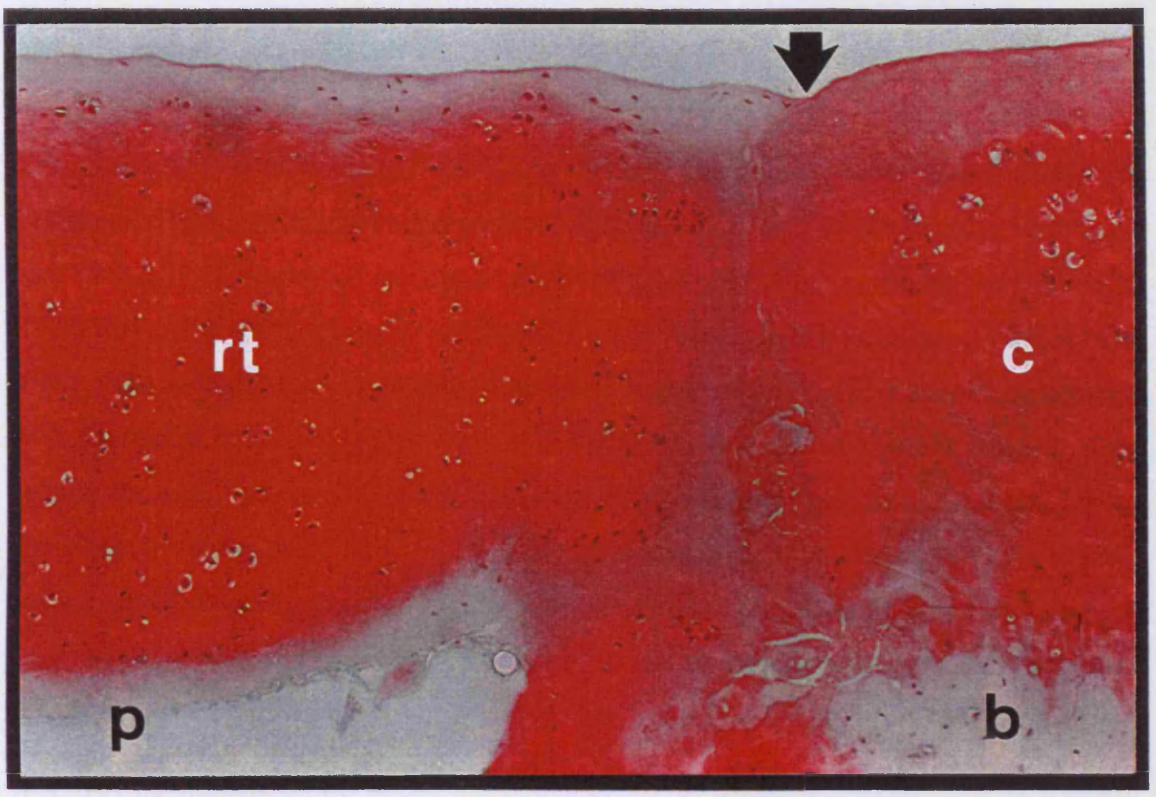


Fig.VI.3

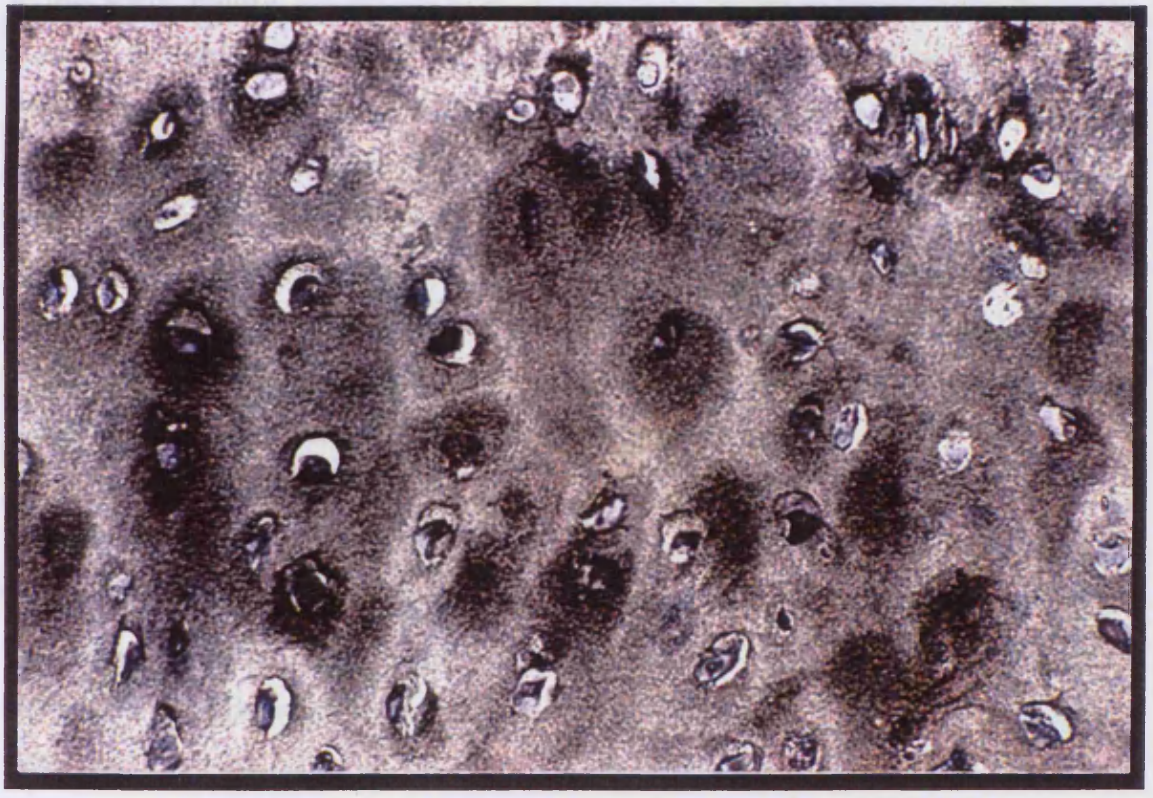


Fig.VI.4

DISCUSSION

The poly-methyl-methacrylate [PMMA] bone cement has been widely used in orthopaedic surgery mainly to fix prosthetic implants to bone. PMMA has also been used as a drug carrier system, delivering a variety of agents including antibiotics (Marks et al 1976, Elson et al 1977, Downes and Maughan, 1989) and growth hormone (Downes et al 1990). The incorporation, however, of peptide hormones in PMMA should be viewed with caution, because the heat, produced in the exothermic polymerisation reaction, may lead to the denaturation of proteins (Downes, 1991). Recently (Corry and Moran, 1998) the PMMA acrylic bone cement has also been assessed as a possible local delivery vehicle for the application of non-steroidal anti-inflammatory drugs. PMMA provides good bonding with the bone and therefore it could be successfully used to restore the continuity of the subchondral bone in articular cartilage defects by providing a stable foundation for the growth of the articular cartilage on its top. Poly-methyl-methacrylate has been well tolerated in a vast number of patients treated worldwide.

In this study, PMMA did not improve the quality of the repair tissue in full-thickness articular cartilage defects at 6 weeks, compared to the 'natural' healing of defects without implantation of a biomaterial. It is possible that the high exothermic reaction released during polymerisation of the bone cement, damages the mesenchymal cells that potentially would migrate to the defect area and differentiate to chondrocytes. It has been shown (Patel and Braden, 1991) that heterocyclic methacrylates, like the poly-ethyl-methacrylate [PEMA], gave lower exothermic reactions than the conventional methyl-methacrylate-based systems and this can probably provide an explanation as to the better results obtained by the PEMA/THFMA system in articular cartilage repair.

Contrary to the shrinkage of the PMMA/MMA system, the PEMA/THFMA system exhibits high water uptake in vitro (Patel and Braden, 1991). If the material maintains high water uptake in vivo, it may absorb tissue fluids and the dissolved proteins, including growth factors from the synovial fluid, the bone matrix (Syftestad and Caplan, 1986) and the bone marrow, thus creating an environment which could encourage cartilage overgrowth.

Vale et al (1997) assessed the interaction of PMMA on cultured human fibroblasts and found that it induced significant reduction in cell viability by liberating free radicals. Also Zambonin et al (1998) studied PMMA on human osteoblast populations and found that it inhibited cell proliferation and collagen synthesis, whereas it stimulated osteocalcin and IL-6 production, thus encouraging osteolysis. In our study there was no evidence of osteolysis in the bone adjacent to PMMA, probably because the observational period of 6 weeks was too short for the development of the osteolytic effect.

It appears from this study that the conventional bone cement PMMA/MMA does not enhance articular cartilage regeneration and repair, compared to 'natural' healing of the defects without biomaterial. Thus, it can be safely concluded that the significant enhancement of the repair tissue following implantation of PEMA/THFMA in full-thickness articular cartilage defects is related to the properties of this biomaterial.

CHAPTER VII

COMPARATIVE STUDY OF ARTICULAR CARTILAGE REPAIR USING PEMA/THFMA POLYMER AND PEMA/THFMA LOADED WITH GROWTH HORMONE

OBJECTIVE

MATERIALS

- 10 adult rabbits
- PEMA vs PEMA + GROWTH HORMONE

METHOD

- Macroscopic, Histological/Histochemical and Immunohistochemical assessment
- At 6 weeks

RESULTS

DISCUSSION

OBJECTIVE

The purpose of this study is to investigate the PEMA/THFMA polymer as a drug delivery system for articular cartilage repair. Growth hormone has been selected, although growth factors, antibiotics, etc could also be incorporated in the biomaterial.

MATERIALS AND METHODS

Ten adult female Sandy-Lop rabbits were used. Both knees were operated on in each rabbit, the one knee received PEMA/THFMA [PEMA group] and the other knee received PEMA/THFMA with Growth Hormone [PEMA+GH group]. For the preparation of the GH-loaded polymer, 12IU of lyophilized Growth Hormone (Novo Nordisk A/S) was added to the PEMA powder and thoroughly mixed prior to the addition of the monomer liquid. Further preparation of the polymer and implantation in the articular cartilage defects were performed as described in Chapter III.B.

The animals were sacrificed at 6 weeks by intravenous injection of Pentobarbitone Sodium (Euthatal 600 mg) and the specimens underwent Macroscopic and Histological/Histochemical Assessment [Chapter III.B] using the Articular Cartilage Repair Scoring System [Chapter III.A], as well as Immunohistochemical Evaluation using the silver-enhanced colloidal gold immunostaining [Chapter III.C].

RESULTS

One knee (10%) in the polymer group and two knees (20%) in the growth hormone group showed mild inflammatory reaction of the synovium.

Neither infection nor foreign body reaction was recorded in the operated knees. The macroscopic and histological/histochemical results are presented in Tables VII.1 and VII.2 followed by statistical analysis.

Table VII.1:

Macroscopic and Histological/Histochemical assessment per criterion Scoring at 6 weeks. Rabbits *151-160*
PEMA vs PEMA+GH

PEMA	*R151*	*R152*	*R153*	*R154*	*R155*	*R156*	*R157*	*R158*	*R159*	*R160*
Area	20	20	10	20	20	10	20	20	20	10
Level	10	10	10	5	10	5	10	10	10	10
Surface	10	10	10	10	10	5	10	10	10	5
Cells	20	20	20	20	10	20	20	20	10	20
PGs	10	10	10	10	10	5	5	10	5	10
Structure	10	5	5	5	10	10	10	10	5	0
Bonding	20	20	10	20	20	20	10	10	20	0
TOTAL SCORE	**100**	**95**	**75**	**90**	**90**	**75**	**85**	**90**	**80**	**55**

PEMA+GH	*R151*	*R152*	*R153*	*R154*	*R155*	*R156*	*R157*	*R158*	*R159*	*R160*
Area	20	20	20	20	20	10	20	20	10	20
Level	10	10	10	10	10	10	10	10	10	5
Surface	10	10	10	10	10	10	10	10	10	5
Cells	20	20	20	20	10	20	20	10	20	10
PGs	10	10	10	10	10	10	10	10	10	5
Structure	10	10	10	10	10	10	10	5	10	5
Bonding	20	20	20	20	20	20	10	20	10	20
TOTAL SCORE	**100**	**100**	**100**	**100**	**90**	**90**	**90**	**85**	**80**	**70**

	PEMA MEANS	*PEMA+GH MEANS*	*PEMA SD*	*PEMA+GH SD*
Area	17.00	18.00	4.83	4.22
Level	9.00	9.50	2.11	1.58
Surface	9.00	9.50	2.11	1.58
Cells	18.00	17.00	4.22	4.83
PGs	8.50	9.50	2.42	1.58
Structure	7.00	9.00	3.50	2.11
Bonding	15.00	18.00	7.07	4.22
TOTAL SCORE	**83.50**	**90.50**	**12.92**	**10.12**

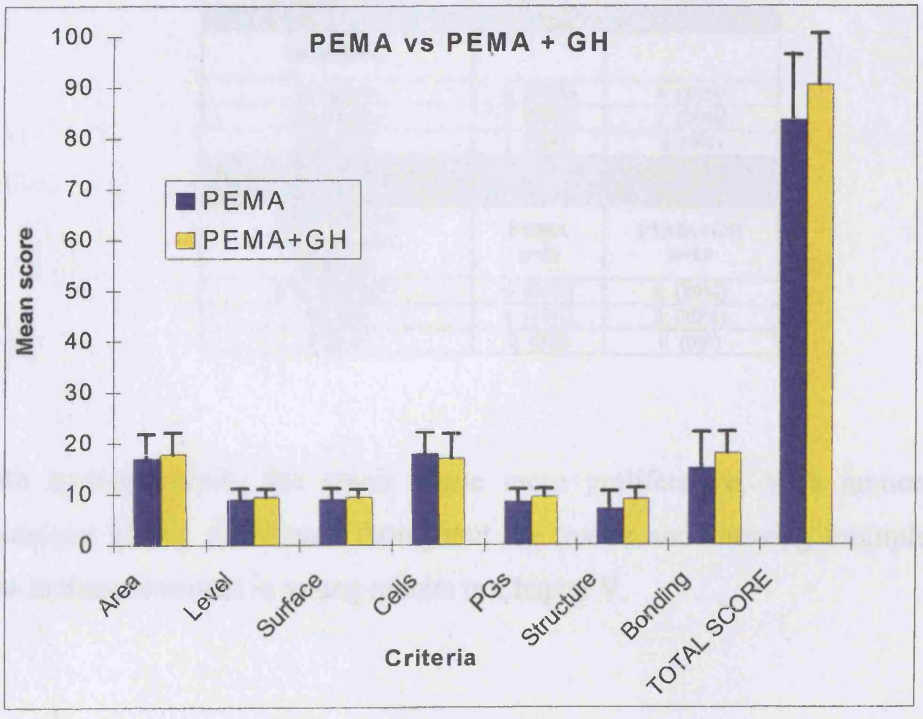


Table VII.2:

Macroscopic and Histological/Histochemical assessment of repair tissue per criterion and the overall repair at 6 weeks

PEMA vs PEMA+GH

	6 weeks	
AREA	**PEMA n=10**	**PEMA+GH n=10**
100% resurfacing	7 (70%)	8 (80%)
>50% resurfacing	3 (30%)	2 (20%)
<50% resurfacing	0 (0%)	0 (0%)
LEVEL		
Normal cartilage level	8 (80%)	9 (90%)
Minimal depression	2 (20%)	1 (10%)
Gross depression	0 (0%)	0 (0%)
SURFACE		
Smooth, glistering	8 (80%)	9 (90%)
Irregular, opaque	2 (20%)	1 (10%)
Disrupted by fissures	0 (0%)	0 (0%)
CELLS		
Chondrocytes	8 (80%)	7 (70%)
Fibroblasts	2 (20%)	3 (30%)
Other	0 (0%)	0 (0%)
PGs		
Normal	7 (70%)	9 (90%)
Moderate	3 (30%)	1 (10%)
Low	0 (0%)	0 (0%)
STRUCTURE		
Hyaline-like tissue	5 (50%)	8 (80%)
Moderately disorganised	4 (40%)	2 (20%)
Grossly disorganised	1 (10%)	0 (0%)
BONDING		
Complete	6 (60%)	8 (80%)
Incomplete	3 (30%)	2 (20%)
Minimal	1 (10%)	0 (0%)
ARTICULAR CARTILAGE REPAIR	**PEMA n=10**	**PEMA+GH n=10**
EXCELLENT	6 (60%)	8 (80%)
GOOD	4 (40%)	2 (20%)
POOR	0 (0%)	0 (0%)

Growth hormone made the repair tissue more proliferative, with numerous large chondrocytes evenly distributed throughout the matrix and showing multiple mitoses, similar to those observed in young rabbits in Chapter V.

The arrangement of the collagen fibrils in the matrix also improved and consequently the structure of the repair tissue scored higher (80% hyaline-like structure) compared to 50% in the unloaded PEMA group. With regard to the other criteria, however, significant tissue improvement was not apparent in the growth hormone group.

Statistical analysis of macroscopic and histological/histochemical results:

All scores for the macroscopic and histological/histochemical evaluation of the PEMA vs PEMA+GH repair were tested using the ***Mc Nemar's*** test and the total score was assessed using the ***Wilcoxon*** test. However, the number of rabbits used was 10, rather than 30, so the statistical power is decreased. This should be born in mind when interpreting the p-values, as an insignificant p-value can be interpreted as either there is no effect or that the test has insufficient power to detect an effect; there is no way to distinguish between these two interpretations.

Score	P-value
Area	.564
Level	.564
Surface	.317
Cells	.564
PGs	.317
Structure	.180
Bonding	.564
Total Score	.021

The overall score gives some weight to the possibility that growth hormone improves the characteristics of the repair tissue, although this is not significant.

Table VII.3 demonstrates analysis of the immunolocalisation of the cartilage components in the repair tissue in the PEMA and the PEMA + GH groups at 6 weeks.

Table VII.3:
Immunohistochemical assessment of repair tissue at 6 weeks
PEMA vs PEMA+GH

	6 weeks	
Collagen type II	**PEMA n=10**	**PEMA+GH n=10**
>50% of matrix	5 (50%)	8 (80%)
<50% of matrix	3 (30%)	2 (20%)
no detection	2 (20%)	0 (0%)
Keratan-Sulphate		
>50% of matrix	7 (70%)	8 (80%)
<50% of matrix	3 (30%)	2 (20%)
no detection	0 (0%)	0 (0%)
Chondroitin 4-Sulphate		
>50% of matrix	7 (70%)	6 (60%)
<50% of matrix	1 (10%)	4 (40%)
no detection	2 (20%)	0 (0%)
Chondroitin 6-Sulphate		
>50% of matrix	7 (70%)	7 (70%)
<50% of matrix	2 (20%)	3 (30%)
no detection	1 (10%)	0 (0%)

Immunohistochemistry showed the presence of collagen type II in all specimens in the PEMA loaded with growth hormone group. In 80% of these specimens the collagen type II was found to be evenly distributed throughout the whole of the matrix of the repair tissue (compared to 50% in the unloaded PEMA group). Formation and distribution of glycosaminoglycans in the repair tissue, however, did not improve further by incorporating growth hormone in the polymer.

Statistical analysis of the immunohistochemical results:

The immunohistochemical results were assessed with ***Fisher's exact test.***

Antigen	P-value
Collagen type II	.628
Keratin- sulphate	1
Chondroitin 4-sulphate	.164
Chondroitin 6-sulphate	1

This analysis confirms that the addition of growth hormone to PEMA/THFMA results in statistically not significant increase or decrease in the production of antigens in the repair tissue.

Fig.VII.1

Histological appearance of the repair tissue in the growth hormone group:
The repair tissue **[rt]** contains numerous cells with chondrocytic phenotype, evenly distributed throughout the matrix and is fully integrated with the surrounding the defect normal articular cartilage **[c]**. The → indicates the transition from the repair tissue to the normal articular cartilage. **[b**=subchondral bone, **p**=polymer**]** *(Haematoxylin/Eosin staining, x 13.2)*

Fig.VII.2

Histochemical appearance of the repair tissue in the growth hormone group:
The repair tissue **[rt]** contains high concentration of proteoglycans, similar to that in the normal articular cartilage **[c]**. The subchondral bone **[b]** does not contain proteoglycans and stained negative. The ► indicates the transition from the repair tissue to the normal articular cartilage, **[p**=polymer**]** *(Safranin-O staining, x 13.2)*

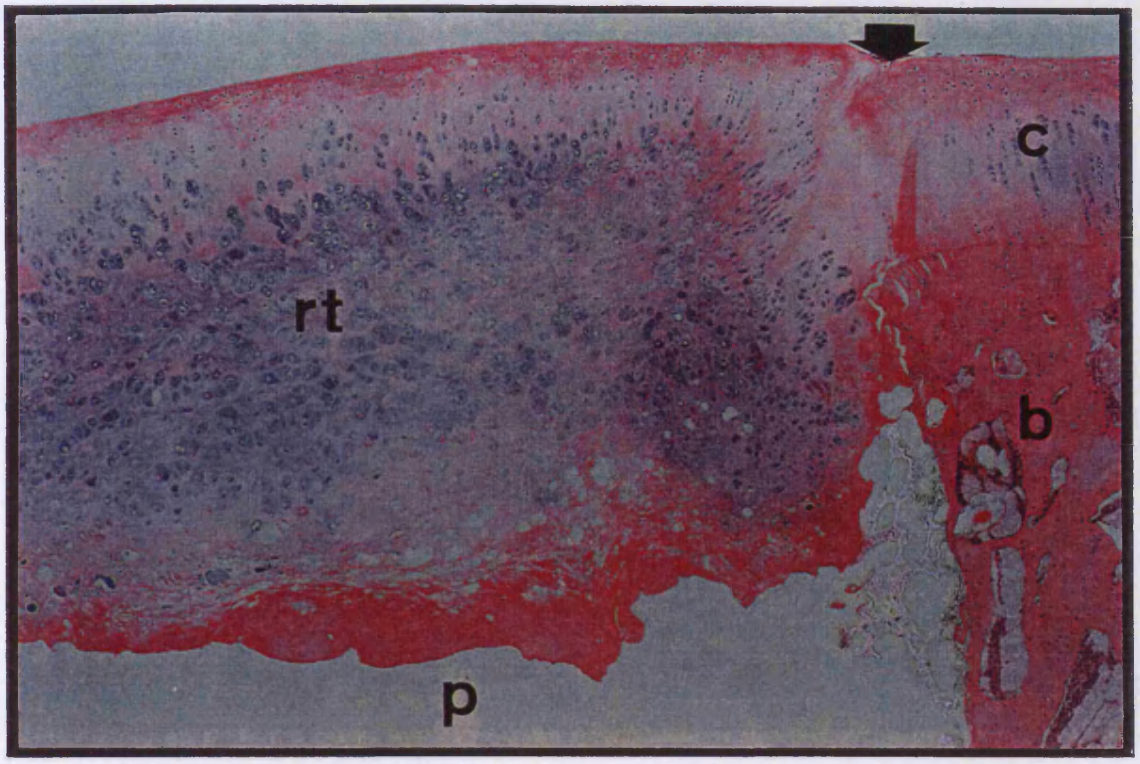


Fig.VII.1

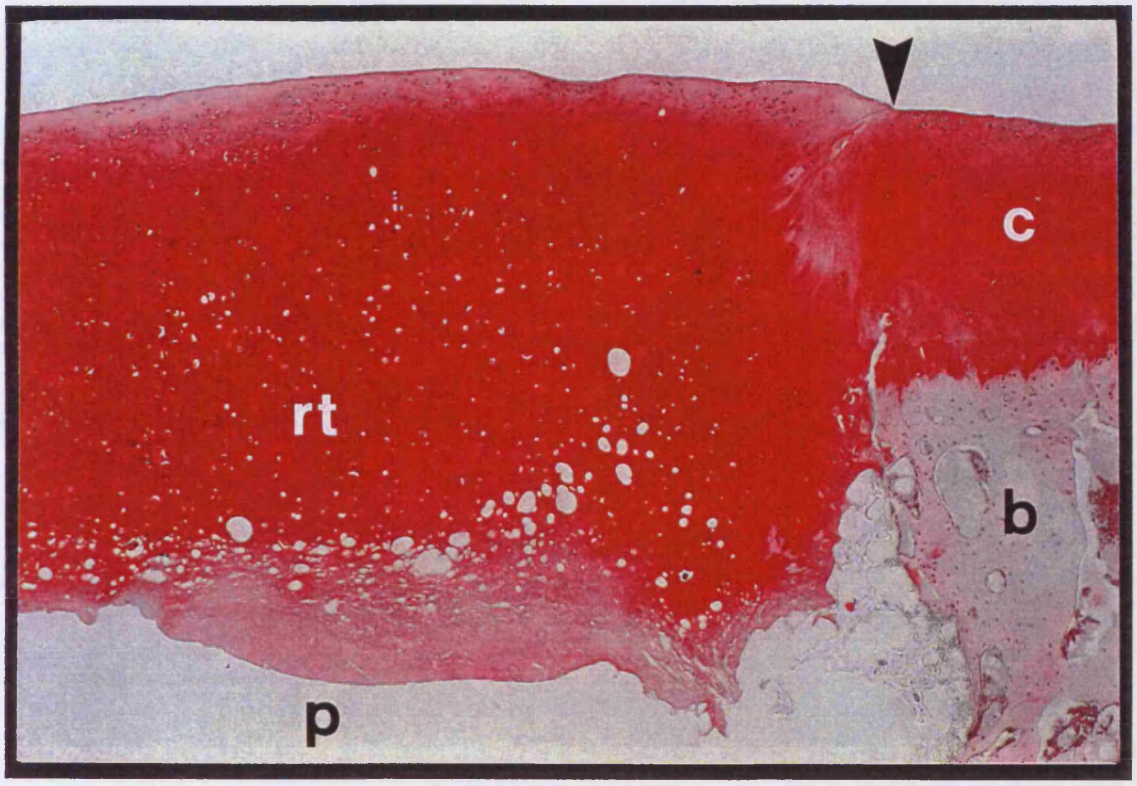


Fig.VII.2

Fig.VII.3

Histological appearance of the repair tissue in the growth hormone group:

The repair tissue contains numerous chondrocytes showing mitoses, similar to those observed in young rabbits in the experiment in Chapter V. *(Haematoxylin/Eosin staining, x 132)*

Fig.VII.4

Immunohistochemical appearance of the repair tissue in the growth hormone group:

The repair tissue contains collagen type II in large amounts in both the superficial **[s]** and the deeper layers. **[m**=middle layers] *(Silver-enhanced colloidal gold immuno-staining, x 66)*

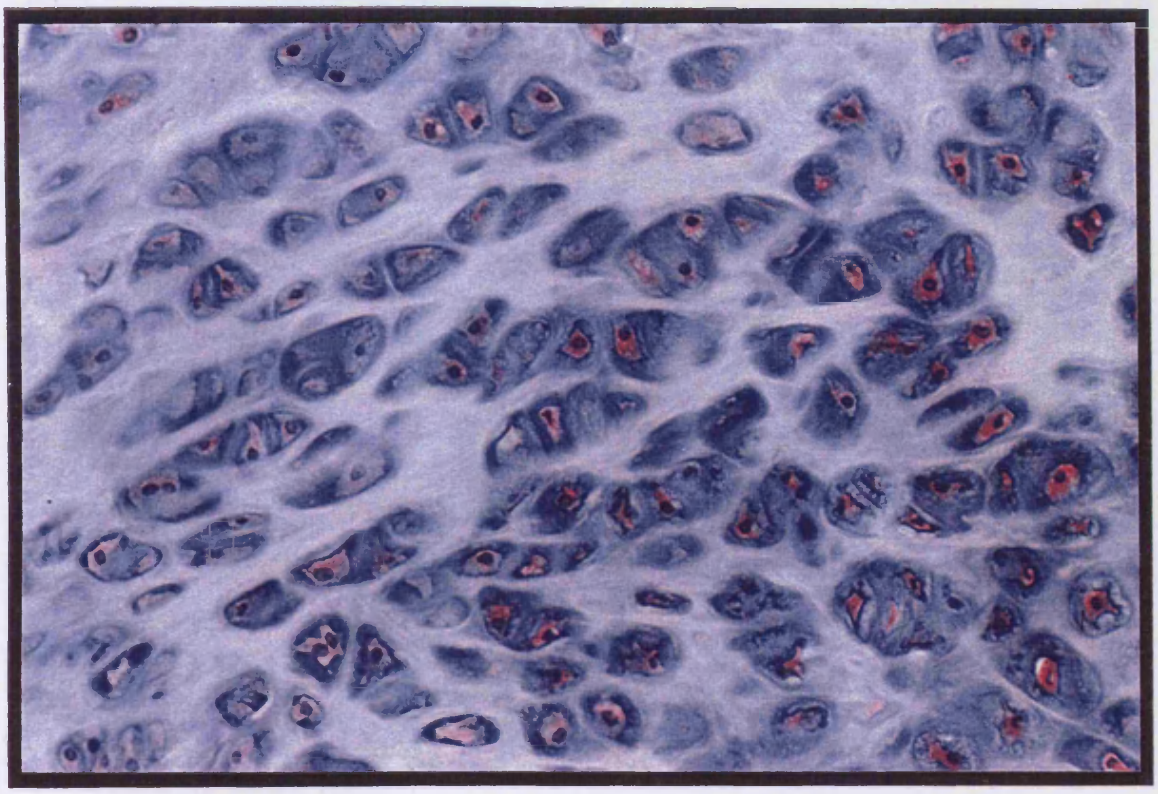


Fig.VII.3

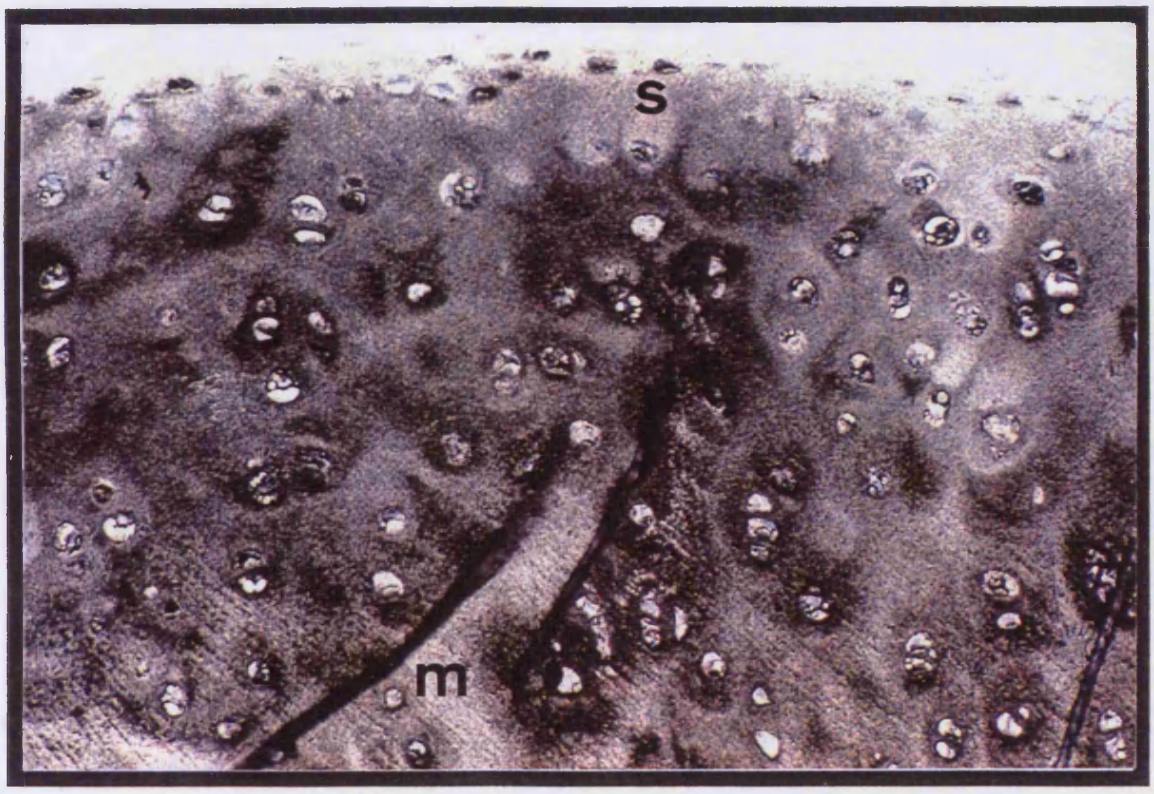


Fig.VII.4

Fig.VII.5

Histological appearance of the repair tissue in the polymer group:
The repair tissue **[rt]** is fully integrated with the surrounding the defect normal articular cartilage **[c]**. The repair tissue in the polymer group appears to be less 'mature' than that observed in the growth hormone group. **[p**=polymer, **b**=subchondral bone] *(Haematoxylin/Eosin staining, x 13.2)*

Fig.VII.6

Immunohistochemical appearance of the repair tissue in the polymer group:
The repair tissue contains chondroitin 6-sulphate throughout the matrix, with higher concentration in the deep **[d]** layers than in the superficial **[s]** layer. *(Silver-enhanced colloidal gold immuno-staining, x 66)*

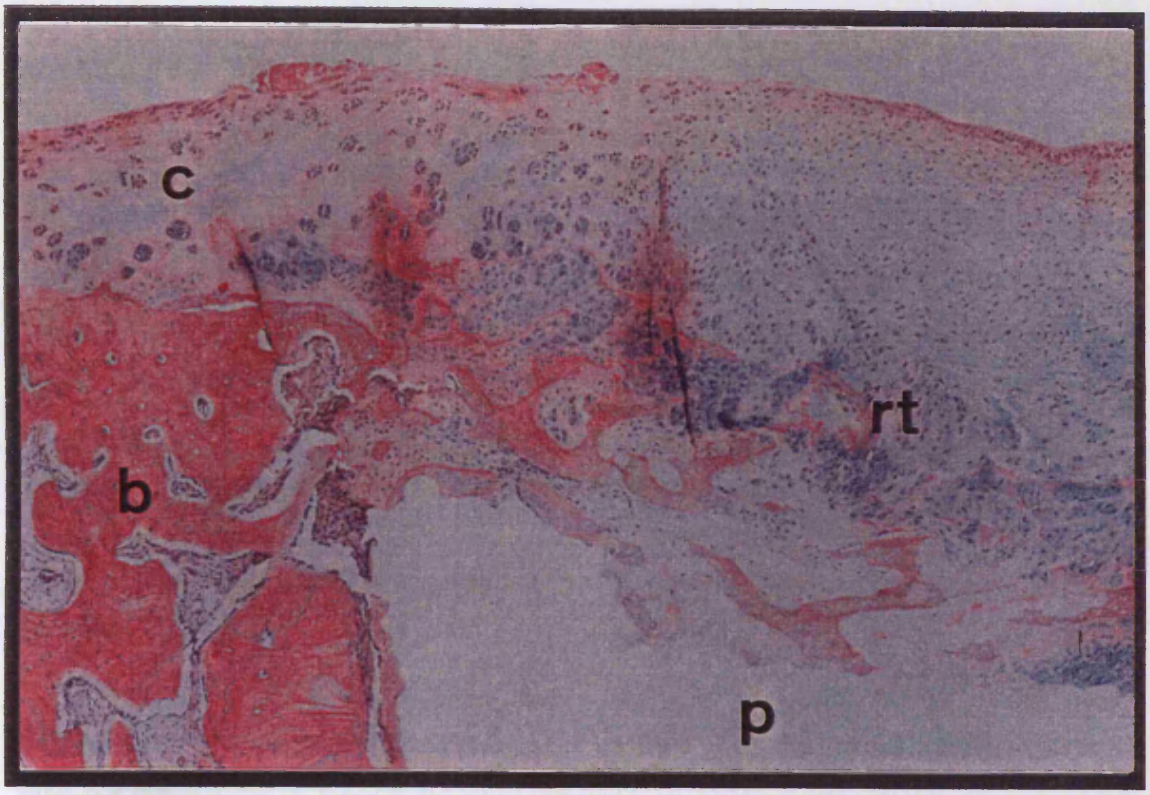


Fig.VII.5

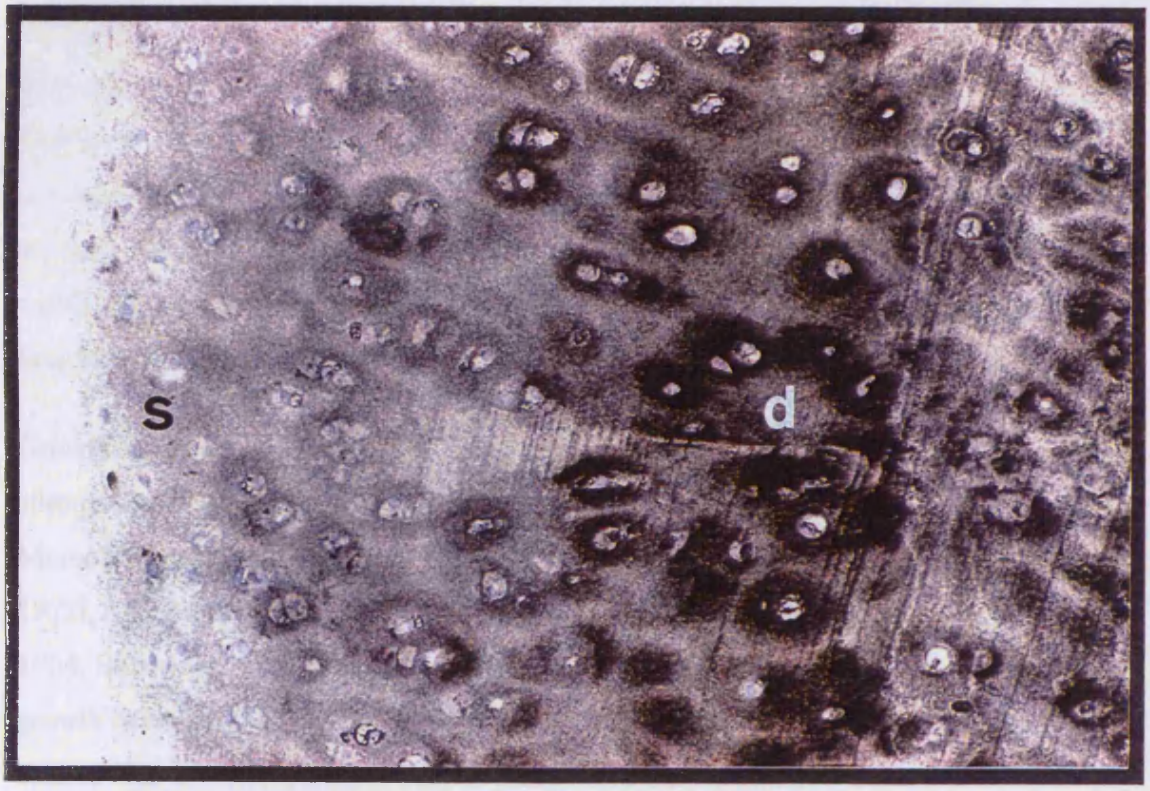


Fig.VII.6

DISCUSSION

The PEMA/THFMA polymer system polymerises at room temperature, which makes it suitable for the release of water-soluble drugs / growth factors. In this study the polymer has been exploited as a drug delivery system for the recombinant growth hormone, to achieve high local concentrations of the hormone at the site of the articular cartilage defect. The human growth hormone was preferred to be used as it has been found to be more stable than the growth hormone of other species (Purcell et al 1989, Brems et al 1990, Pikal and Dellerman, 1991, Pikal et al 1992).

It has been shown (Di Silvio et al 1994) that PEMA/THFMA releases growth hormone in vitro. The release is rapid during the first 24 hours, followed by a slower but continuous release up to nine days. The release appears to be dose-dependent and different methods of mixing have a significant effect on the amount of growth hormone released; fast mixing increases the porosity of the polymer with subsequent increase in growth hormone release. The exact mechanism of drug release from polymers remains unclear; diffusion of drugs from the polymer matrix appears to play a part (Graham, 1978, Heller et al 1978, Cardinal et al 1981, Langer et al 1981) and also release of the drug via pores or channels (Gale et al 1980, Brook and van Noort, 1985) has been suggested. A possible explanation of the mode of drug release from the PEMA/THFMA polymer (Di Silvio et al 1994) appears to be that the drug becomes 'encapsulated' by the polymer particles, and the hydrophilic nature of this polymer enables water to enter within the particles and form 'water clusters'. This in turn results in an increase in the drug released out of the polymer.

Growth hormone acts primarily by increasing serum IGF-1 (Sledge, 1973) by direct stimulation of the liver production (Salmon, 1957, Schimpff et al 1977, Wu et al 1974). Moreover, growth hormone may also exert effect on cartilage matrix (Meier and Solursh, 1972i, Meier and Solursh, 1972ii, Meier and Solursh, 1973) and cartilage growth (Regal, 1964, Silberberg et al 1964). It has also been suggested that high local concentrations of growth hormone may increase IGF-1 receptors or IGF-1 release (Nilsson et al 1986, Han et al 1987).

Stimulation of glycosaminoglycan production by growth hormone was a finding in this study, although results did not reach statistical significance compared to the unloaded

PEMA group. These findings correlate also with findings by Lane Smith et al (1989). With regard to the keratan-sulphate in particular, the fact that the levels increase with age (Simunek and Muir, 1972, Bayliss and Ali, 1978, Inerot et al 1978, Roughley and White, 1980, Zirn et al 1984) and also with chondrocyte specialisation (Zanetti et al 1985) suggests that growth hormone may contribute to early maturation of chondrocytes in the repair tissue, although it is no mitogenic to rabbit articular chondrocytes (Jones et al 1986). The precise mechanism by which growth hormone initiates IGF-1 action is unclear, but it has been hypothesized that it can lead to increased local production of IGF-1 (Watanabe et al 1985). It can therefore be speculated that, if the PEMA/THFMA polymer system releases growth hormone in vivo, the high local concentration of the hormone contributes to proliferation and maturation of the chondrocytes and further improvement of the structure of the repair tissue. Both these characteristics were indeed noted in the reparative tissue in the growth hormone group in this study.

Growth Hormone and Insulin-like Growth Factor-I [IGF-I] have been implicated in the modulation of osteoblast and chondrocyte function (Ernst and Froesch, 1988, Nilsson et al 1990ii). IGF-I produced in response to growth hormone stimulates long bone elongation by its effect on epiphyseal cartilage, and it is synthesized by a variety of cells, including bone and cartilage, so it may act as a local regulator of cell proliferation (Hock et al 1988, Linkhart and Keffer, 1991). It has also been shown that growth hormone enhances chondrogenesis and osteogenesis in cells in culture, with some of the effects being mediated by IGF-I (Scheven et al 1991) and also in vivo when incorporated in implanted biomaterials (Downes et al 1991). This study also showed significant proliferation of the chondrocytes in the growth hormone group with cell division resmbling that seen in the growing immature articular cartilage.

The PEMA/THFMA polymer system exhibits high water absorption that continues for long time and this property of the material appears to be promising in the long term release of the growth hormone in vivo. Patel and Braden (1991) showed that the water uptake amounted to 34% in two years without the system equilibrating. They also showed evidence of water 'clustering' within the polymer that can further increase the amount of the drug released from the polymer system. It can thus be speculated that the repair tissue in the growth hormone group, although it showed non-significant improvement at 6 weeks in this study, it will further improve in long term trials.

GENERAL DISCUSSION

Adult articular cartilage possesses neither blood supply nor lymphatic drainage and neural elements. Thus, the tissue remains isolated and, after they are surrounded by their extra-cellular matrix, the articular chondrocytes are sheltered even from immunological recognition. As a result, articular cartilage is ineffective in responding to injury, unless the underlined subchondral bone is penetrated and cells recruited from the marrow elements initiate a repair response. The extent to which the newly formed repair tissue resembles articular cartilage depends on the age, the species of the host and the size and location of the defect. However, complete restoration of the hyaline articular cartilage and the subchondral bone to a normal status is rarely seen and, to date, no treatment has been shown to guarantee such a repair in articular cartilage defects.

Synthetic polymer systems are easy to hanlde, cheap to produce and, particularly the conventional poly-methyl- methacrylate has been in clinical use for many years. The newly developed polymer system, based on poly-ethyl-methacrylate polymer and tetra-hydro-furfuryl methacrylate monomer, has been exploited in this study for the repair of large, full-thickness articular cartilage defects created in a weight-bearing surface of the knee joint in the rabbit model. The polymer enhanced articular cartilage repair significantly with 73.3% of the repairs being excellent by 6 weeks, compared to 6.6% in the control group where no biomaterial was used. These results are similar or even superior to those achieved by other methods of articular cartilage repair [O'Driscoll and Salter, 1986, Görandson et al, 1995, Brittberg et al, 1996, Hunziker et al, 1996, Chew et al, 1997 and Peter et al, 1998]. Moreover, it appears that using the PEMA/THFMA biomaterial, there is a consistency in the bonding of the repair tissue to the surrounding normal articular cartilage, that is complete in 66.6% at 6 weeks and remains so in 46.6% at 1 year.

The effect of age appears to be significant in this study, although PEMA was superior to control groups at all ages. Excellent overall repair was noted in 80% of the PEMA young group, in 73.3% of PEMA adult group and in 20% of the PEMA old group specimens. A large number of specimens in the old group showed osteoarthritic changes present in the treated joints, which created a challenge in using PEMA polymer for the repair of articular cartilage defects in early osteoarthritis. This study has not included defects created in areas affected by osteoarthritis and thus we cannot extrapolate any conclusions on the success of the method in treating osteoarthritic defects.

The PEMA polymer appears to be significantly more effective than the conventional bone cement [poly-methyl-methacrylate polymer system] in the repair of articular cartilage defects in the rabbit model. The high exothermic reaction produced during polymerisation of the conventional bone cement and its limited ability to attract water may have an adverse effect on articular cartilage repair. Furthermore, incorporation of growth hormone in the PEMA polymer system showed no significant improvement in the quality of the repair tissue at 6 weeks. The result can be associated with inadequate quantity of the growth hormone, loaded in the polymer system, or instead, can be due to the limited period of observation. Long-term studies of articular cartilage repair with PEMA loaded with variable doses of growth hormone will clarify whether the repair tissue is dose- or indeed time-dependent.

In-vitro analysis of the behavior of PEMA polymer system has been limited in this study simply due to a concurrent in vitro evaluation of the polymer from other co-workers in our Institute. Sawtell et al [1995], performed extensive studies confirming that the PEMA system can support chondrocytes in vitro allowing cells to remain rounded, which is important in order to maintain chondrocyte phenotype [Watt and Dudhia, 1988].

It is advisable that further studies are performed in the future to clarify the origin of the cells when the PEMA polymer is used for the repair of articular cartilage defects. Although it is possible that the origin of the cells is the same as in the control group, most probably originating from the undifferentiated cells [Shapiro et al, 1993], it appears that the PEMA polymer provides a micro-environment suitable for the differentiation of those cells to chondrocytes at the early stages of repair. Moreover, there are good reasons for studying joint development in relation to degenerative diseases and repair of the articular cartilage, since a feature of these diseases and the repair response can be manifested in renewed cell division and upregulated matrix synthesis, which resemble aspects of development [Archer et al, 1994]. The PEMA system can be used loaded with bone-morphogenetic proteins and growth and differentiation factors that have been found to play a fundamental role during skeletogenesis and repair [Francis et al, 1999].

It appears that the centuries-old supposition that the articular cartilage is incapable of healing has been reconsidered and over the last ten to fifteen years many methods have shown satisfactory results in repairing full-thickness defects in weight-bearing surfaces. Better understanding of morphogenesis will enable the future generation of researchers to reproduce fetal developmental signals for the prevention of genetic disorders affecting the articular cartilage, while local gene supplementation and transfer will improve the management of injuries, particularly when used as vehicles in the targeted delivery of growth factors.

GENERAL COMMENTS

CONCLUSIONS

- PEMA/THFMA is a biocompatible material that does not induce foreign body reaction when used intra-articularly.

- PEMA/THFMA can enhance repair in large, full-thickness articular cartilage defects in a weight-bearing area in the knee joint of the rabbit. Up to 1 year post-implantation the quality of the repair tissue is superior to that of the control defects that healed 'naturally' without treatment.

- PEMA/THFMA can be effective in young, adult and old rabbits. Up to 6 weeks post-implantation the quality of the repair tissue in articular cartilage defects is superior in the polymer group compared to the control group in all age groups.

- PEMA/THFMA can enhance articular cartilage repair in the presence of advanced osteoarthritic changes in the same joint.

- PEMA/THFMA can be used as a drug delivery system in vivo. The use of recombinant growth hormone incorporated in the polymer, however, did not improve significantly the quality of the repair tissue in articular cartilage defects, compared to the unloaded PEMA/THFMA up to 6 weeks post-implantation.

- The properties of the PEMA/THFMA system are of paramount importance, although the implantation technique, where the polymer is recessed into the subchondral bone, may also play an important role in the repair of articular cartilage defects.

- The method of implantation of PEMA/THFMA in articular cartilage defects that was applied in our studies is simple and easily reproducible. It does not require in vitro culture of cells and in clinical practice it can be performed in one stage by open or arthroscopic procedure.

- The Articular Cartilage Repair Scoring System that was developed for the macroscopic and histological/histochemical assessment of the repair tissue, helps in the comparative analysis between different treatments and in the evaluation and monitoring of the repair tissue in experimental studies and in clinical applications.

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