A CLINICAL AND LABORATORY INVESTIGATION OF SYNTHETIC MATERIALS TO ENHANCE HEALING AND AID REGENERATIVE PROCEDURES IN PERIODONTAL SURGERY

by

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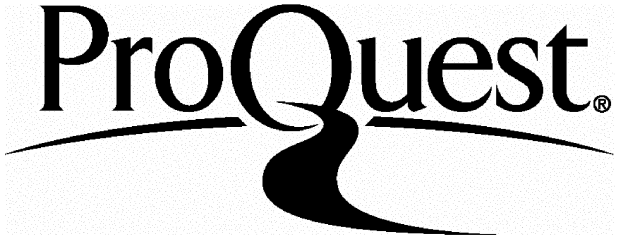
A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

University College
University of London 1992



ABSTRACT

This study investigated the use of synthetic materials to enhance healing and to encourage regeneration of periodontal tissues after surgical therapy. The study was in two parts, a clinical investigation of the use of hydroxy-apatite implant material, and a laboratory investigation of biodegradable polymer membranes being developed for use as barrier membranes in the technique known as guided tissue regeneration.

The aim of this study was to evaluate the efficacy of these materials in surgical wounds, to observe their limitations and to establish their potential to enhance regeneration of tissues.

The hydroxy-apatite material was well tolerated by the tissues, but clinically significant benefits could not be established. Radiographically significantly more infill occurred in the sites which received the implant material, with some evidence of the material being modified with time so that the density and structure became more similar to that of the approximating host bone. Histological assessment of specimens from three individuals who were not in the clinical trial demonstrated resorption and osteoid formation over a longer time period. Both the radiographic and histological investigations indicated that there was a wide variation in the tissue responses to this material.

The membrane materials were also well tolerated by the tissues, but a highly variable tissue response to them was also noted. Absorption of the biodegradable materials was irregular and unpredictable. The non-degradable Gore-Tex material was

associated with prolonged inflammation, poor wound healing and epithelial downgrowth.

It was therefore concluded that variable and unpredictable tissue responses occur in relation to these materials. The variations described were attributed to localised tissue responses, and individual site and host variations, rather than to the materials themselves. Other factors like the surface roughness, hydrophobicity or electrostatic surface changes of the materials may also be of importance.

Although in general, for the clinical study, results were found to be equal to, or marginally better than control procedures, the enhancement of tissue regeneration using the synthetic implant materials or barrier membranes employed in this study to augment periodontal surgery is not entirely predictable.

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CHAPTER 1

INTRODUCTION

1.1 THE PURPOSE OF THE STUDY

Periodontal diseases are widespread in the adult and teenage populations throughout the world. Epidemiological evidence suggests that periodontal destruction is present in more than 95.9% of the adult population in the U.K. (Jenkins & Mason, 1984). However, other studies have demonstrated a wide variation in the incidence of periodontal destruction (Lennon & Davies, 1974; Greene, J.C., 1960; Lindhe et al 1983). These discrepancies may be attributed to differences in criteria for diagnosis and assessment of the presence of disease, and variations in their application (Schluger et al 1990). Workers in different parts of the world have used a variety of clinical and radiographic criteria and indices in reporting their results. In a well controlled longitudinal study by Loe et al (1978), it was found that the progression of periodontal diseases was slow in a Norwegian population, but rapid in a Sri Lankan population of adults. The difference in progression was attributed to differences in oral hygiene status of the two populations. In the Norwegian group oral hygiene was good, whereas in the Sri Lankan group, oral hygiene was very poor. It was also assumed in this, and other studies, that the progression of periodontal diseases was by means of slow chronic deterioration with progressive destruction of periodontal

tissues leading to tooth loss. More recent research (Goodson, J.M. et al 1982, Socransky, S.S. et al 1984) has indicated that periodontal diseases are episodic with periods of exacerbation having the characteristics of acute inflammation, and quiescence, occurring simultaneously at different sites in the same individual. In addition, a number of different periodontal diseases are now recognised, and these are associated with different rates of progression (Page & Schroeder, 1982). Other workers have observed that a small minority of sites and a small minority of individuals exhibit a high susceptibility to periodontal destructive activity (Haffajee et al 1983, Lindhe et al 1983). The episodic nature and varied susceptibilities of different sites and individuals to periodontal destruction present difficulties in the clinical management of the disease. Nevertheless, once periodontal destruction has occurred, the loss of soft tissue and alveolar bone was considered to be irreversible. The present work addresses the practical problems of treatment of periodontal diseases with a view to achieving healing of bone defects and regeneration of the tissues lost as a result of the destructive process. Regeneration of new periodontal attachment has been defined by Nyman et al (1982) as the regeneration of the principal fibres of the periodontium and the insertion of such fibres into newly formed cementum on a root surface which has previously been exposed to the oral environment. True regeneration of the periodontal tissues after therapy has

not been possible using surgery and root debridement alone (Listgarten & Rosenberg, 1979; Caton & Zander, 1979). The purpose of this study was to investigate a number of synthetic materials which have been advocated as having the potential to achieve healing of bone defects and regeneration of new attachment.

The overall aim of the study was to investigate the possibility of achieving enhanced bone healing and tissue regeneration by using the one or the other of these techniques with a view to developing a suitable composite procedure incorporating both techniques.

The study has been undertaken in two parts. In the first part commercially produced synthetic hydroxy-apatite (Periograf, Sterling-Winthrop, Guildford, UK) was placed into infrabony defects in order to provide a matrix for new bone growth to encourage filling in or healing of bone defects. In the second part a number of barrier membranes were evaluated in respect of their tissue responses, for use in the technique known as "guided tissue regeneration".

The use of synthetic bone implant material in laboratory animals and to a lesser extent clinically, is well documented and a number of commercial products are available for this purpose. The investigation of these materials took the form of a clinical study. As the technique of guided tissue regeneration is relatively new, and there are comparatively few reports of biodegradable membranes having been developed and tested in vitro or vivo, the study of barrier membranes was undertaken in

animals using an extraoral site. This had the additional advantage of allowing evaluation of the healing response to the membranes without the complicating factors of saliva, contaminated root surfaces and functional disturbances which occur in the mouth.

1.2 HISTORICAL PERSPECTIVE

The arrest of progressive periodontal destruction is the immediate aim of periodontal treatment, and the restitution of the periodontal and supporting tissues lost as a result of the disease process has been the ultimate, but elusive goal. A number of workers (Hancock & Wirthkin, 1981) have claimed to have achieved regeneration of the periodontal supporting tissues, as assessed by means of clinical parameters such as periodontal probing. However, histological evaluation of the healing wounds has shown that, although resolution of inflammation occurred with the presence of a long junctional epithelial attachment in apposition to the root surfaces (Caton & Zander, 1979, Caton et al 1980), connective tissue re-attachment did not occur. There has, therefore, been some confusion in the literature in respect of which periodontal procedures actually result in regeneration, as opposed to repair, of the tissues.

In addition, confusion has arisen in the use of the words regeneration and repair, with some workers claiming regeneration of tissues when in fact only repair of damaged tissues occurred. In this study, repair and regeneration are defined according to the definition by Nyman et al (1982) as follows:-

- Repair is the replacement of tissues lost, by new tissue which is dissimilar to the original tissues. This occurs when fibrous tissue covered by a junctional epithelium develops in apposition to the root surfaces.

- Regeneration is the replacement of tissues lost, by new tissue which is identical to the original tissues. This may occur if a new periodontal ligament and new cementum with inserting Sharpey's fibres were to form after periodontal therapy.

- New attachment is the regeneration of a new periodontal ligament containing functionally orientated inserting Sharpey's fibres with new cementum and bone associated with root surfaces previously exposed as a result of destructive periodontal disease.

A number of treatments for periodontal defects have been advocated over the years. Essentially the therapeutic aims of these treatments can be divided into three broad groups:-

1) Therapies aimed at defect resection
2) Therapies aimed at tissue repair
3) Therapies aimed at tissue regeneration

Arrest of periodontal disease by resection of the inflamed tissues has been advocated in the past, Goldman (1949). The gingivectomy procedure was utilised for this purpose. In a review article, Prichard (1972) observed that this procedure could not be used in defects which extended beyond the mucogingival line. Some early attempts at achieving regeneration, by removal of coronal tissue and gingival epithelium over the defect were attempted. It was thought that this would delay the migration of the oral epithelium into the healing subgingival defects, enabling a greater time lag for regeneration of the tissues.

Consistent regeneration of the periodontal support tissues was not obtained, and the surgical techniques employed were very destructive to the supporting tissues. These limitations led to attempts to treat periodontal defects using less radical procedures.

Early resection techniques like the gingivectomy procedure were subsequently replaced by inverse bevel flap surgery, in which a flap of mucogingival tissue was raised buccally and palatally or lingually in order to gain access to the bony defects which were then resected and recontoured. When the soft tissue was repositioned apical to the original position, the procedure became known as the "apically repositioned flap procedure". This was a great improvement on the gingivectomy procedure, because healing was quicker without creating large wound areas in the superficial tissues (Tolmie et al 1988). However, the procedure still resulted in considerable recession of soft tissue and often included the resection of the bony walls of osseous defects. New attachment with new periodontal ligament was not possible due to the loss of tissue height, (Schluger, 1949)and the rapid ingress of epithelial cells into the surgical wound, developing a long junctional epithelial apposition against the root surface (Hancock & Wirthkin, 1981). Research effort was directed at overcoming these two problems.

Ellegaard et al (1974) described a gingival grafting procedure which aimed at limiting epithelial ingress into the surgical wounds. After exposing the defects by means

of inverse bevel flap surgery and thorough debridement of the root surfaces, gingival grafts were positioned over the defects prior to flap replacement. It was believed that the sloughing of epithelial cells on the graft would delay new epithelial cells from getting to the surgical site for an extended period of time. Although clinical and histological evidence of "regeneration" was observed in up to 60% of defects, complete and predictable results could not be achieved by this technique. Although "regeneration" was claimed to have been achieved by these workers, albeit unpredictably, it is questionable whether this was in fact the case, as the degree of preoperative destruction was not known. Current philosophy concerning guided tissue regeneration (see p.30) dictates that epithelial cells and gingival fibroblasts must be excluded from the wound, to prevent repair of the defects by non-functionally orientated fibrous tissue derived from the gingival tissues. As gingival fibroblasts would not have been excluded with a gingival tissue graft, true regeneration of the periodontal tissues would be uncertain. This technique had the further disadvantage that an additional surgical wound had to be created in order to obtain the gingival graft material detracting from its clinical applicability. As a result, the technique was not widely used, and no further work has been undertaken using this technique. Nevertheless, this was an important publication, because it was the precursor to the more recent techniques for achieving regeneration by means of

synthetic barrier membranes.

Other attempts to obtain predictable tissue regeneration with new periodontal attachment have not been successful. Stahl et al (1983) compared a number of surgical procedures and demonstrated histologically that regeneration of lost periodontal attachment was possible only in close proximity to viable periodontal ligament cells, irrespective of which regenerative procedure was used. Karring et al (1980) in meticulously executed animal studies, demonstrated that new attachment did not occur on root surfaces which had been exposed to the oral environment. They observed that new connective tissue attachment was dependent upon the types of cells which re-populated the surgical sites initially. Nyman et al (1980) found that gingival connective tissue did not have the ability to induce the formation of new connective tissue periodontal attachment.

From these studies it can be concluded that, using conventional periodontal surgery procedures, only minimal regeneration of new attachment is possible in teeth which have been exposed to the oral environment by destructive periodontal diseases. Irrespective of the surgical technique used, what little regeneration occurs is dependent upon the availability of periodontal ligament precursor cells which may be present at the margins of the defect.

1.3 REGENERATION OF DEFECTS BY BONE GRAFTING PROCEDURES

Mature alveolar bone is a complex structure, consisting of peripheral cortical plates and internal cancellous bone which is trabeculated. The alveolar bone is continually being remodelled in response to functional forces to which it is subjected, and it is rapidly resorbed after loss of the teeth which it supports. Bone deposition is characterised by the laying down of an organic matrix consisting of extracellular proteins like chondroitin phosphate and collagen. The uncalcified connective tissue matrix begins to form islands of calcification in the presence of high levels of chondroitin sulphate and a group of small molecular weight proteins (<50,000 Daltons) known collectively as bone morphogenic protein (Withkin, 1987). These islands of calcification enlarge to form ossicles which are invaded by capillaries. Fibroblasts differentiate into osteoblasts which begin actively to lay down bone and become incorporated in the bone as osteocytes (Schluger et al 1990). Osteoclasts derived from the haemopoetic tissues resorb bone in order to remodel it or remove excess bone as it develops. Thus, a dynamic balance is established between the bone-forming osteoblasts and the bone-eroding osteoclasts, which enables continuous remodelling of the bone throughout life. In the presence of inflammation, this dynamic balance is disturbed, resulting in osteoclastic resorption of the bone (Schluger et al 1990). If the inflammatory process is shortlived and minimal loss of the organic matrix has occurred, bone

regrowth may occur after resolution of the inflammation. If the inflammatory process continues for a protracted period, as occurs in periodontal diseases, progressive and permanent bone loss will occur, with loss of the organic bone matrix. When this occurs, repair of the area can occur only by means of the deposition of fibrous scar tissue with minimal or no bone regeneration (Hancock & Wirthkin, 1981).

It could be argued that regeneration may be encouraged if some of the lost bone could be replaced as part of the surgical treatment of infra-bony defects. Replacement of the lost bone by means of bone grafting or implant materials might encourage regeneration of a new periodontal support structure by occluding the infra-bony defect. This would facilitate the ingrowth of periodontal ligament and osteoblast precursor cells into the maturing blood clot. As a result, a large number of workers have utilised different materials to try to achieve regeneration of bone lost as a result of destructive periodontal diseases, utilising bone grafting or implanting techniques (Wirthlin, 1987). The materials utilised for this purpose have largely been heterogenous or autologous organic materials.

1.3.1 Organic bone grafting materials

An excellent review article by Wirthlin in (1987) has considered a number of autologous bone grafting materials which have been shown to have the potential to encourage tissue regeneration. Bone, dentin and cementum chips (Grant, 1967; Stallard & Hiatt, 1968) have all been shown

to activate regenerative processes by causing resorption and deposition of osteoid on the implanted fragments. These studies were interesting and some successes were achieved, but they should be viewed with caution as the conclusions were based on subjective observations and untested hypotheses. One interesting hypothesis which was developed at that time, and which has become increasingly popular, is that the materials themselves are not important. The organic matrices of these materials contained osteo-inductive proteins which can stimulate bone growth (Wirthlin, 1987). These proteins have became known as "bone morphogenic protein" (BMP)(Urist, 1965; Urist et al 1967).

These early investigations produced small bone ossicles in fibrous tissue which would not normally contain bone. As a result a bone induction principle known as BMP was proposed. These were visionary papers when they were written, but with subsequent research, more detailed knowledge has become available. More recent studies using cell culture techniques have demonstrated a large number of polypeptide growth factors which act as local regulators of cell growth, and interact with systemic growth regulators and hormones in a finely balanced interactive system (Somerman et al 1982; Canalis, 1985; Canalis et al 1988; Schluger et al 1990). Although the current work on growth regulating substances is very exciting, the complexities of the substances involved and their interactions are not yet fully understood. This work should therefore be considered

experimental at the present time and probably not be applied clinically until more is known about the functions of BMPs.

The ideal material for bone grafting sites in the oral cavity would be cancellous bone autografts derived from sites within the oral cavity, as this bone is functionally identical to the bone which has been lost through destructive periodontal diseases (Froum et al, 1975; Schluger, 1990). However, the availability of bone for grafting purposes within the oral cavity is limited, so other sources of autologous bone had to be sought. Iliac crest bone has been used as an alternative (Froum et al, 1975) but this has the disadvantages of possibly causing root resorption and ankylosis (Ellegaard et al 1976; Klinge et al 1985) and requiring a second operative procedure to procure the graft material. Allogenic bone derived from stored tissue banks has also been used in the past. The published studies using these materials are confusing, because some histologic investigations have shown hardly any tissue regeneration with new periodontal attachment (Kromer, 1962), while others have shown results equivalent to sites grafted with autologous bone material (Haggerty, 1977; Hiatt et al 1978). Some of the confusion can be attributed to the material itself, which was preserved in tissue banks and preserved in frozen form which would probably have damaged the vitality of the osteogenic cells, or the bone growth factors. Some of the studies were in animals which demonstrated less osteogenesis than others in

humans where the use of frozen preserved bone was more prevalent (Wirthlin, 1987). More recently, lyophilised bone has been advocated as a suitable bone graft material (Pearson et al 1981), but consistently successful results have not been achieved (Sanders et al 1983). Decalcified lyophilised bone has been found to have a higher osteogenic potential than uncalcified material (Mellonig, 1981), but the degree of new bone formation was still not complete. Other materials which have been used include lyophilised decalcified dentin chips (Movin et al 1982), allogenic cartilage (Schaffer, 1958) and sclera (Iacono et al 1980) but these materials have shown little success in terms of tissue regeneration. A major limitation in the use of allogenic materials has been their lack of consistently predictable results in clinical trials (Wirthlin, 1987), and difficulty of procurement and storage of the tissue. More recently, the concern of the public and the profession over the use of blood and tissue derived products which might transfer disease from donor to recipient has been a further limiting factor.

Heterogenic materials might possibly eliminate some of the disadvantages of the allogenic materials. Materials used have included sheep bone powder (Beube et al 1934), boiled cow bone powder (Beube, 1941), defatted freeze dried cow bone (Arrocha et al 1968) and demineralised bone (Sonis et al 1985). With the exception of the study by Sonis et al (1985) this work was done a long time ago and should be viewed within an historical perspective of the

controlled with test and control sites, but relied on subjective observations in a small number of cases. While some successes have been reported, these materials have been shown to have poor osteogenic potential and they suffer from the disadvantages of being tissue-derived substances with consequent difficulties in preparation, storage, and the possibility of rejection and cross-infection.

1.3.2 Synthetic bone implant materials

In view of the limitations and difficulties discussed in the previous section, non-organic, synthetic materials might provide an alternative to the organic bone grafting materials, without the associated disadvantages. There has therefore been a considerable effort to develop synthetic implant materials. Various materials have been tried, including Plaster of Paris (Radentz & Collins, 1965), pyrolytic carbon (Klinge et al 1985) and zirconium oxide (Bump et al 1975), but with only limited success. More recently hydroxy-apatite and tricalcium phosphate materials have been developed which have been advocated as potential bone implant materials. Hydroxy-apatite and tricalcium phosphate, manufactured as implant materials, have been shown to be well tolerated by the tissues and to have the ability to become directly bonded to the bone with which they are in contact (Jarcho, 1981). Jarco (1981) has suggested that these materials might be useful as bone implant substitutes for organic bone grafting material when sufficient natural material was not available. These

ceramic materials are absorbable by means of two biological pathways, a solution-mediated process which causes the implant to dissolve, and a cell-mediated process which involves phagocytosis of the particles of ceramic. The degree to which the materials are absorbed is determined by their crystalline structure and density. High density and crystalinity have been shown to result in low and limited absorption (Han et al 1984) and animal studies have indicated that biocompatibility of both hydroxy-apatite and tricalcium phosphate are good (Han et al 1984). Tricalcium phosphate is absorbed and replaced by new bone, hydroxy-apatite is non-absorbable and therefore incorporated in the tissues without absorption. Although some studies (Levin et al 1974; Mors & Kaminski, 1975) have indicated that tricalcium phosphate ceramics induce bone formation, histological investigations have demonstrated that animal subjects show minimal amounts of bone particles. These particles are found only close to the natural bone, and are associated with persistence of foreign body giant cells and very slow absorption over extended periods of time (Nery et al 1975). In these studies, surgically created defects in animal models cannot necessarily be directly compared with destructive periodontal diseases in human subjects, where long-term contamination of the root surfaces has occurred. Human studies have demonstrated good clinical and radiographic results, (Nery & Lynch, 1978) but histological examination has shown implant particles with minimal absorption, some residual inflammation, and only minimal

bone formation (Bowers et al 1986; Stahl & Froum, 1986). These studies suffer from the disadvantage that only small numbers of specimens were assessed over a short period of time. Nevertheless, taken together with the animal studies, it is not unreasonable to conclude that the absorption and induction of bone associated with these materials is minimal, with the additional disadvantage of being associated with long-term inflammatory cell infiltrate, which it has been speculated, might interfere with any potential regenerative activity; they might, however, stimulate some new bone to grow into areas which it would not otherwise occupy (Han et al 1984).

A review of the literature has shown that tricalcium phosphate bone implant materials used in the treatment of periodontal defects, result in fibrous encapsulation of the particles without bone formation at 9 months (Baldock et al 1985), and bone with osteoid formation and granulation tissue around the particles at 1 year (Bowers et al 1986). This interpretation implies that regeneration of bone occurs only after preliminary fibrotic encapsulation, over a protracted period of time. Alternatively, it may indicate that the conflicting results obtained by different workers are due to non-standardised clinical procedures. Both studies quoted above were in human subjects so the number of treated defects which could be used for evaluation was small, and not all from the same subject. The method of implant placement is not clearly defined in the papers and these factors may account for the

contradictory results in the literature.

The results of studies utilising hydroxy-apatite are also contradictory (Han et al 1984). The literature is complicated by the number of different hydroxy-apatite implant materials available commercially. These materials differ in crystalinity, physical shape, and porosity. Human studies have shown particles of hydroxy-apatite surrounded by connective tissue only (Ogilvy et al 1987) at 6 months post-operatively although by 12 months post-operatively some calcified tissue was apparent around the crystals. Other studies (Sapkos, 1986; Kenney et al 1986) have tended to confirm that some osteoid develops at about 1 year post-operatively. In surgically created defects in animals Boyne & Flemming (1982) have shown that new bone formation occurs in the more apical regions of the implanted material, while only fibrous tissue encapsulation occurs more coronally. The implant material may also be susceptible to exfoliation (West & Burnstein, 1985)

Comparisons between commercially available tricalcium phosphate and hydroxy-apatite in dogs have demonstrated that tricalcium phosphate particles were absorbed by means of giant cells and macrophages and incorporated into a new bone matrix, whereas hydroxy-apatite was encapsulated by fibrous connective tissue with some bone formation occurring around the hydroxy-apatite particles with time (Han et al 1984). These workers demonstrated that hydroxy-apatite particle absorption was by phagocytosis as evidenced by large multinucleated cells and macrophages

which contained some of the grafted material within their cytoplasm. Other workers comparing these two materials (Ellinger et al 1986) have also shown that hydroxy-apatite implant materials act mostly as filler materials with minimal or no absorption and bone deposition, whereas tricalcium phosphate facilitated bone growth by progressively being absorbed and replaced by bone-like material. Amler (1987) investigated a number of implant materials: these included Plaster of Paris and Periograf hydroxy-apatite bone implant material. He concluded that results with these synthetic materials were generally unpredictable. He could not establish whether the bone which formed as a result of osteogenesis was induced as a result of osteogenesis by the implant material, or as a result of physical irritation, by stimulation of the regenerative process in the surrounding bone.

Despite the plethora of different implant materials which have been clinically evaluated, and the different surgical procedures in both human and animal subjects, a number of tentative conclusions can be drawn. Only minimal regeneration of bone is possible with the synthetic bone implant materials. The new bone only begins to form after a protracted period of time, and may be limited to those crystals which are in close apposition to natural bone from which osteogenic cells might be derived.

Regeneration by means of surgery alone has therefore been shown to be limited only to the deepest margins of periodontal defects, and when surgery is augmented by means

of synthetic implant materials, only limited bone regrowth has been shown to occur. Organic bone grafting materials may result in more new bone formation than the synthetic implant materials, but difficulties in their procurement and preparation have precluded their use in routine clinical practice.

Thus, attempts to achieve regeneration of bone and new periodontal attachment by surgery augmented by grafting or implant techniques have given unsatisfactory and unpredictable results.

A different approach to the problem of regeneration of the tissues may therefore be necessary. After debridement of infrabony defects, bleeding into the area results in the formation of a blood clot. The clot then organises to form granulation tissue. Angiogenesis occurs, carrying nutrients and oxygen to the region. Primitive mesenchymal cells in the granulation tissue mature and differentiate in order to produce different types of tissue. The differentiation of these primordial mesenchymal cells depends on the type of cells in the immediate vicinity of the granulation tissue. Thus bone, periodontal ligament, and infrabony scar tissue will develop from osteoblast, periodontal ligament and gingival fibroblast precursor cells respectively (Nyman et al 1982, Boyko et al 1981, Isidor et al 1986). If the maturing blood clot could be isolated in such a way as to exclude unwanted cells like epithelial cells and gingival fibroblasts, it might be possible to facilitate enhanced bone and periodontal

ligament regeneration, and minimise fibrous scar tissue formation during healing. Using this argument, the technique of "guided tissue regeneration" has been proposed (Nyman et al 1982, Gottlow et al 1986), in which a barrier membrane is placed over the defect prior to flap replacement to prevent the ingress of epithelial cells and gingival fibroblasts into the healing wound.

1.4 ENHANCEMENT OF REGENERATIVE CAPACITY OF THE TISSUES USING BARRIER MEMBRANES

The cells of newly regenerated fibrous attachment are derived from periodontal ligament cells present at the margins of the defect (Karring et al 1985) and these are a prerequisite for the formation of new attachment on denuded root surfaces (Isidor et al 1986). Although it is possible for regenerated fibrous attachment to occur, Listgarten and Rosenberg (1979) have shown histologically that regeneration does not in fact routinely occur in humans due to the downgrowth of epithelial cells into the healing wound to form a long junctional epithelium.

It has been shown that new attachment by means of fibroblasts and new cementum with inserting fibres is possible, (Lindskog et al 1987) but that in the human subject the regeneration of new attachment only occurs in the apical 1-3mm of defects (Genco, 1987).

A technique in which a physical barrier membrane is introduced to prevent epithelial cells entering a surgical site has been termed "Guided Tissue Regeneration" (Gottlow et al 1986). A number of membranes have been used under soft tissue flaps for this purpose. These have included Millipore filter, Teflon, Biobrane knitted nylon membrane coated with collagen and lyophylised collagen (Nyman et al 1982; Gottlow et al 1986; Aukhill et al 1985; Magnusson et al 1985; Aukhill et al 1986, Nyman et al 1987; Galgut, 1990). If the downgrowth of epithelium could be inhibited, and the environment of the healing wound controlled

sufficiently to achieve optimal conditions for tissue regeneration, more predictable regrowth of tissue might occur. This might be possible if a protective membrane is placed over the surgical site before replacement of the soft tissue flaps. A number of studies have demonstrated both in the animal and human subjects that enhanced new connective tissue attachment and bone regeneration is possible utilising a number of different membrane materials (Nyman et al 1982, Gottlow et al 1986, Caffese et al 1988, Pontoreiro et al 1988).

1.4.1 Collagen derived membrane materials - autologous.

A variety of autologous membrane materials have been used to stabilise the blood clot to encourage the development of new periodontal attachment. A composite procedure has been described by Ellegaard et al (1974, 1976) in which mucoperiosteal flaps of tissue, derived from the palate, were grafted over surgically exposed infra-bony defects which had received autologous cancellous bone to fill in the defects. In defects which received the palatal tissue grafts, necrosis of the epithelium occurred, initially exposing the underlying fibrous tissue, which subsequently became re-epithelialised. Histological examination revealed no evidence of epithelial migration along the root surfaces up to 20 days after surgery in the sites which received palatal tissue graft, whereas the sites which were closed without receiving the palatal tissue graft demonstrated downgrowth of epithelium along the root surface of between 0.2 and 2.0 mm by 1 week

after surgery. It was concluded that it was possible to prevent epithelial migration into healing infra-bony defects after surgery by the use of a palatal soft tissue graft in addition to autologous bone grafting material, which was used to fill in the defects and encourage new bone formation. This occurred because the palatal graft acted as a barrier to ingrowth of epithelial cells into the maturing blood clot. In the immediate period after placement of the graft, sloughing of the epithelial cells on the graft itself occurred, exposing the fibrous tissue. By the time the epithelium had regrown over the grafted fibrous tissue, sufficient differentiation of the blood clot had occurred to prevent ingress of the epithelium into the region. The placement of autologous bone graft alone was not able to prevent epithelial downgrowth into the wound. This technique had the disadvantage of requiring a secondary surgical procedure to procure the graft material, which resulted in painful areas of exposed tissue at the donor sites. In addition, the amount of graft material which could be harvested was limited. Although the technique demonstrated the potential to achieve "regeneration" it could not be determined if true regeneration with new periodontal attachment had occurred, as the original degree of periodontal destruction was uncertain. Thus, for practical clinical reasons in addition to the basic premise on which the technique was based, the work on this technique ceased and alternative membrane materials have been developed; an additional

objective being to prevent the ingress of gingival connective tissue into the healing defect.

1.4.2 Collagen derived membrane materials - heterogenous

Collagen heterograft materials have long been used as biological dressings extra-orally and intra-orally. Turnbull and Stross (1983) observed that porcine skin could be used as a biologic dressing in periodontal mucogingival grafting procedures. Busschop and De Boever (1983) have used lyophylised allogenic collagen derived from processed human dura mater as an implant material to cover interdental two wall infra-bony craters after surgery. Clinically, a significant increase in probing attachment level with greater reduction in pocket depths was observed together with less gingival recession and significant bone regeneration in those sites in which the material was used. In both of these studies (Turnbull & Stross, 1983, Busschop & De Boever, 1983) histological examination showed that the implant remodelled completely and was replaced gradually by enzymic breakdown and fibroblast infiltration to form new collagen. New bone formation occurred underneath the implant, but not within it, and it behaved in the same way as a barrier membrane preventing downgrowth of epithelium and gingival fibroblasts into the healing surgical wound.

Collagen membranes, therefore, have the potential for use as barrier membranes in periodontal therapy. As the material is tissue derived, it suffers from the same

disadvantages as the tissue derived bone grafting materials discussed previously. These include difficulties in preparation and sterilisation of the material, and the possible transfer of disease from donor to recipient. In addition, published work has indicated that the use of this material clinically results in variable and unpredictable results (Galgut, 1990), without preventing the formation of a long junctional epithelial attachment (Tanner et al 1988).

1.4.3 Synthetic membrane materials - Polytetra-Fluoro-Ethylene (PTFE)

As collagen membranes have been shown to be unpredictable in achieving regeneration of the tissues, other materials have been sought for this purpose. One of the most extensively investigated materials has been expanded polytetra-fluoro-ethylene manufactured and marketed by the Gore Company, Arizona, U.S.A., under the trade name of Gore-Tex.

Polytetra-fluoro-ethylene (PTFE) has been shown to be highly biocompatible. In a study using blocks of PTFE placed transcutaneously through the skin on the dorsum of pigs, Winter (1974) has produced a stable ring of epithelium without any downgrowth of cells into the underlying tissue with a collagenous matrix passing through the pores to incorporate the membrane. Recent research into specially designed PTFE membranes have demonstrated the potential for encouraging coronal migration of periodontal ligament cells (Nyman et al 1982, Aukhill et al

1985, Magnusson et al 1985 and Gottlow et al 1986). Membranes of PTFE have been especially produced by Gore Associates for the purpose of guided tissue regeneration in periodontal defects. The material has been expanded to increase porosity, and has been manufactured with a collar to improve adaptation and inhibition of epithelial downgrowth at the tooth/gingiva interface. Becker et al (1987), using this PTFE membrane (Gore-tex (R)) placed sub-gingivally, have demonstrated new bone, cementum and periodontal fibre attachment occurring in periodontal defects of poor regenerative capacity. A study by Pontoriero et al (1988) has also shown that regeneration was possible in molars with furcation involvement.

A number of studies into the tissue responses of a variety of membranes and Millipore filters has shown that epithelial cells migrate down alongside the membrane surface, and may reach a mean depth of 4.6 mm by the 15th day (Aukhill et al 1983, Gottlow et al 1984, Magnusson et al 1985). Evidence of bacterial infection and persistent inflammatory reaction around all of the membranes, with the exception of porous PTFE membrane, was noted.

There are, however, a number of disadvantages of PTFE when used clinically. These include:-

1) the difficulty of placement,

2) time consuming technique

3) the necessity for a secondary procedure to remove the material after a number of weeks in situ.

The latter disadvantage could be overcome if a

suitable biodegradable alternative to the material was found.

1.4.4 Synthetic membrane materials - biodegradable materials

Holland et al (1986) have reviewed the biodegradable materials used in medicine. Biodegradable polymers have been used extensively in drug release systems. Poly-alpha-esters, like polylactic acid and polyglycolic acid, polyhydroxybutyrate, polydioxanones, polylactones, polyester hydrogels and polyanhydride compounds are the most suitable as they have polymeric biodegradable matrices (Holland et al 1986). They degrade to nontoxic products, so are suitable for subcutaneous implantation.

"Biodegradable" is defined as the biological breakdown of polymeric material by enzymic, hydrolytic or bacteriological action.

"Bio-erosion" is often used synonymously with biodegradation, but implies the conversion of a material from a water-insoluble to water soluble material usually by physical or chemical means.

The biodegradable materials used in medicine include (Holland et al 1986):-

1. Polyglycolic acid (PGA)(Dexon, Cyanamid Co) is the most widely used biodegradable polymer. Erosion occurs as a 2-stage process:

   1. Water diffusion into amorphous regions of the polymer leads to hydrolysis (± 21 days).

2. Crystalline hydrolysis occurs where the crystals are now exposed (± 49 days).

PGA is the most hydrophilic of the polyesters currently in use, but it is too crystalline for drug release.

2. Polylactic Acid (PLA) occurs in 3 forms. D (-), L (+) and D/L (Racemic) as determined by their optical characteristics. D and L forms are highly crystalline, so their degradation is slow. D/L form is amorphous, so degradation is better. The degradation and elimination of PLA is similar to PGA. PLA is extensively used in absorbable sutures.

3. Copolymers of PLA/PGA: Confusion in the literature is due to the multiplicity of copolymer forms and combinations. 25/75% PLA/PGA copolymers are amorphous, whereas if rich in either of the constituents they become much more resistent to hydrolytic attack. Vicryl sutures are made from a copolymer containing 92% PGA (also called Polyglactin), and this material has been used clinically for many years.

4. Polydioxanones and polyoxalates have been utilised and they display similar degradation characteristics to PGA with the amorphous regions preferentially eroded, but the degradation products are removed as products in the urine rather than respiration (as in PLA and PGA). Their degradation may be by different mechanisms.

Detailed information on these materials is not available.

5. Lactone/Caprolactone group of polymers have also been used. Their degradation is similar to PLA and PGA. When copolymers are made with other lactones, or PGA/PLA, quicker degradation occurs, but generally degradation tends to be prolonged with these materials.
6. Hydroxybutyrates are manufactured by ICI as Biopol (copolymer with 30% polyhydroxyvalerate). The in-vivo rate of degradation is slow, probably by a combination of hydrolysis and enzymic activity.
7. Polyester hydrogels: Very little information is available on these materials, in clinical usage.
8. Other esters are available but very little is known of their characteristics as they have not been fully investigated.

The most labile of these materials are the PLA, PGA or PLA/PGA copolymers. Degradation is governed by the molecular weight, molecular weight distribution and polymer morphology of the constituents (Holland et al 1986).

Biodegradable materials have not been used extensively in tissue regeneration procedures. Magnusson et al (1988) have shown in dogs that a mean value of 46% connective tissue regeneration was possible using polylactic acid membranes produced by solution casting on glass to a

thickness of 70 microns. As only 25% connective tissue regeneration was observed in the sites which received Millipore filter, the PLA membrane demonstrated superior regenerative capacity and this type of membrane may therefore offer advantages for use in the guided tissue regeneration technique in periodontal treatment. As the membrane is completely absorbed, it has the additional advantage not requiring a secondary procedure to remove it after placement. Regeneration of new cementum with inserting connective tissue fibres and bone were observed in this study.

The guided tissue regeneration technique has been shown to have the potential to enhance the regeneration of tissues after periodontal treatment (Nyman et al 1982, Aukhill et al 1983, Gottlow et al 1984). Results have shown that only partial regeneration occurs, and that this may be variable (Gottlow et al 1986). The limitations of the guided tissue technique may include (Ben-Yehuda et al 1989):

1. Impaired nutrition to the surgical flap, as a result of the membrane interposed over the bone resulting in damage or necrosis of the overlying soft tissue.
2. The protruding section of the membrane, or the custom made collar of the commercially produced Gore-Tex membrane, may become contaminated by saliva and oral debris and may present oral hygiene maintenance problems during

the healing phase, resulting in inflammation rather than regeneration of the tissue.

3. The non-degradable membranes require a secondary surgical procedure to remove them, and they remain in situ for a prolonged period of time (4-6 weeks). Both of these factors may result in additional loss of attachment due to recession which limits the amount of regeneration which can take place.

Other factors relating to healing of wounds in general may contribute to the observed variation in regenerative responses to clinical treatment (Buser et al 1990). These include traumatic surgical technique in placement, contamination of the membrane during handling prior to placement of the material, and variations in wound healing characteristics due to host factors.

It therefore seems that the guided tissue regenerative technique offers considerable potential for achieving new periodontal attachment and, less certainly, bone growth after surgery. However, there may be a number of limiting factors which prevent consistent and predictable regeneration of the tissues with this technique. In addition, it may be that many of the problems with regeneration materials only become evident some time after placement. The research papers that are available report only short term results, usually in very few defects (Gottlow et al 1986, Becker et al 1987, Pontoriero et al 1988, Lekovic et al 1989). As no long term studies are

available with carefully analysed comparisons between test and control sites, the technique may not prove to be predictable at achieving regeneration, and more importantly, preventing recurrent breakdown of the tissues in the longer term.

1.5 CONCLUSION

It is concluded that synthetic materials have been shown to have the potential to enhance the regenerative capacity of the tissues during healing. The use of hydroxy-apatite based materials to fill in periodontal defects in which excessive bone loss has occurred has been shown to be effective. The guided tissue regeneration technique has been shown to inhibit epithelial downgrowth post-operatively although problems remain in respect of the clinical application of this technique. Biodegradable materials used for this technique may overcome some of the difficulties, and have been shown to offer advantages when used for this purpose.

As the results using the synthetic bone implanting materials do not achieve predictable regeneration, and the guided tissue regeneration technique has been shown to give variable results, particularly with regard to bone healing, it is possible that a combination technique utilising both of these procedures may enhance the results and overcome some of the difficulties occurring with each individual technique.

1.6 AIM OF THIS STUDY

The aim of this study was to evaluate the efficacy of synthetic hydroxy-apatite bone implant material and a range of biodegradable membranes, which, inhibiting the ingress of gingival epithelium and gingival fibroblast cells into healing surgical wounds, might have the potential to enhance selective healing and regeneration of tissues lost as a result of destructive periodontal diseases.

1.7 HYPOTHESIS

In the light of previous studies it was decided to test a number of hypotheses in this study:-

1. Commercially available hydroxy-apatite bone implant material (Periograf (R)) has the potential to enhance tissue healing and reduce penetrability of the tissue to probing by initiating the deposition of new osteoid or other hard tissue in periodontal defects.
2. A number of biodegradable membranes are biocompatible with epithelial and epidermal fibrous tissue, and may be suitable for transcutaneous placement in techniques like guided tissue regeneration.
3. Biodegradable membranes in different physical dimensions and with different physical characteristics may affect the healing responses of the tissues.
4. The tissue responses to the membranes varies longitudinally throughout the healing period and the changes which take place in relation to the materials tested may affect the outcome of treatment using these materials.

1.8 OBJECTIVES

It was anticipated that the clinical trial utilizing commercially produced hydroxy-apatite (Periograf) would determine the efficacy of this material in human subjects in a long term, carefully monitored trial. The trial would also demonstrate the limitations of this material in respect of its ability to induce repair and/or regeneration of tissues lost as a result of inflammatory periodontal disease.

In view of the indications that regenerative procedures using these implant materials were not consistently good, it was decided to investigate the possibility of developing a biodegradable membrane which could be placed over the implanted synthetic material incorporating the principles of guided tissue regeneration techniques. Future aspirations were that a suitable biodegradable membrane for this purpose might be developed as a result of the second part of the study.

CHAPTER 2

HYDROXYAPATITE: THE CLINICAL TRIAL

2.1 INTRODUCTION

The review of the literature has shown limited and unpredictable results using bone substitute implant materials (Chapter 1.3.2). Clinical trials using both tricalcium phosphate (Snyder et al 1984) and hydroxyapatite (Yukna et al 1985, Yukna et al 1989) have shown that this group of materials has the potential to enhance infill and encourage some new hard tissue formation in infrabony defects in human subjects. These studies are limited by the lack of control sites in the former study, and because assessment was carried out by means of re-entry procedures in the latter, which may have affected the results and conclusions drawn from this work

2.2 AIM

The aim of this investigation was to evaluate the clinical results of treatment of periodontal infrabony defects by means of synthetic bone implant material, as compared to treatment of similar defects without the use of the bone implant material. The other clinical studies (Yukna et al 1985, 1989) had evaluated only bony infil over a short period of time and by means of re-entry procedures alone. It was therefore decided that in this study the efficacy of this material would be observed by means of a number of clinical variables over a period of four years after placement. This would have the advantage of comparison of defects which received the implant material

with those that did not, in a carefully monitored long term clinical trial.

2.3 MATERIALS AND METHODS

2.3.1. Study design

The study was designed to evaluate the performance of the implant material clinically over an extended period of time. As a result, clinical assessment variables were chosen for monitoring the response to treatment. These included:-

Plaque
Gingival bleeding
Pocket depth measurement
Soft tissue recession
Loss or gain of clinical attachment
Tooth mobility
Tooth vitality

All of these variables were assessed pre-operatively, 1 month post-operatively and 6 monthly thereafter for the duration of the study. In addition, at the time of surgery, measurements were taken of the actual defects and the height of the bony walls of the defects.

As clinical assessment criteria could not determine the response of the alveolar bone to the implant material and it was felt that re-entry procedures could not be justified ethically, particularly if successful responses to the procedures occurred, it was decided to use a standardised radiographic technique to observe the changes which took place in the healing defects with time. Moskow and Lubarr (1983) have suggested that radiographic evaluation of healing for synthetic implant material placed into infrabony defects may be a suitable non-invasive method for assessing their status longitudinally. This method could therefore serve as a useful additional

procedure in the assessment of long-term healing of sites which received the implant material. Thus in addition to the clinical variables described before, it was decided to evaluate radiographically the healing of sites which were implanted with hydroxy-apatite ceramic implant material over the period of 4 years. The technique employed for this study incorporated a Rinn paralleling system modified to incorporate an aluminium step wedge and a removable inter-occlusal platform for the purpose of obtaining occlusal registrations of the teeth. This was designed to ensure consistency of the radiographic images throughout the study. The radiographs were subjected to densitometric analysis as described by Verrier et al (1989) to see if significant differences between test and control sites could be found.

Although successful treatment was anticipated for the experimental and control teeth used in the study, a number of very poor prognosis teeth were also included. It was anticipated that some of these teeth would be lost with time, and that tissue specimens might be collected for histological assessment of the cellular responses to the implanted material.

2.3.2 Subjects

Ten individuals were selected for periodontal surgery after completing an initial phase of treatment which included oral hygiene instruction, superficial scaling, deep scaling and root planing. Criteria for selection to the study included the presence of multiple pockets with

bone loss after completion of the initial phase of treatment, and a consistent ability to maintain high levels of oral cleanliness, during the maintenance phase of six months prior to entry into the study. Informed consent also was required before admission to the trial.

The group consisted of 7 females and 3 males with a mean age of 46 (range 26-58). All but 1 of the individuals were Caucasian, the other one being Negroid.

A medical history was taken to eliminate the presence of systemic conditions such as diabetes, pregnancy, rapidly progressing infective states (other than periodontal disease), diseases involving bone (osteoporosis, hyperparathyroidism, osseous metastases associated with malignancy, Paget's disease, sarcoidosis), cardiovascular or respiratory disease, blood dyscrasias or long-term steroid therapy. In addition, blood samples were taken pre-operatively, 6 months and 12 months post-operatively to ensure normal values were maintained for red blood cells, white blood cells, platelets and blood chemistry (liver and kidney function, and blood calcium and phosphate levels).

For admission to the study each patient was required to have at least two infra-bony defects of similar radiological appearance to be used for test and control sites. Where a greater number of lesions were present, these were also used in the study.

2.3.3 Assessment criteria

The clinical variables of recession, and pocket depths, were assessed pre-operatively and at six-month

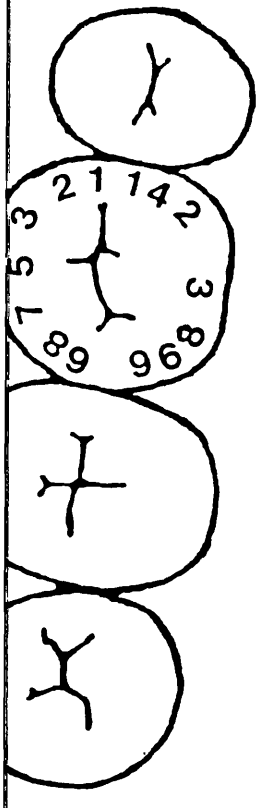
intervals post-operatively for a period of four years using a Hu-Friedy periodontal probe with a probe tip diameter of 0.62 mm. Plaque and gingival bleeding scores were assessed at the same time periods using the O'Leary Plaque Control Record (1972) to ensure that oral cleanliness was maintained and to remotivate the subjects to maintain their oral hygiene regimen when necessary. At each of the above time intervals the measurements were taken from the cemento-enamel junctions to the base of the defects along the vertical axes of the teeth. Measurements were made at multiple sites for each bone defect defined as:-

1. At the interproximal surfaces with the probe resting against the contact points.
2. At the buccal and linguo-palatal line angles of the tooth surfaces associated with the defects.
3. At the points of maximum convexity of the roots.
4. At the entrance to the furcations.

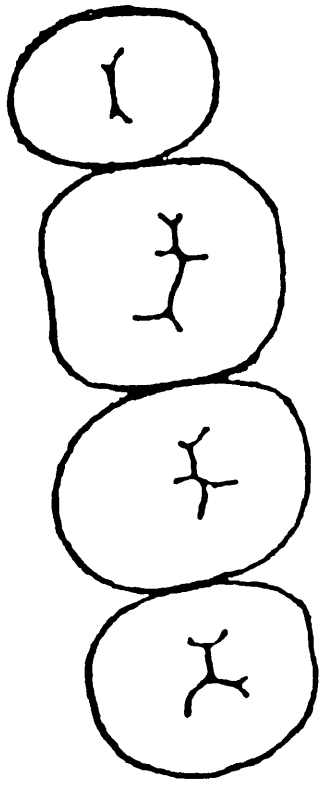
Single rooted teeth could thus be measured at up to five sites on both vestibular and oral aspects, whereas molar teeth could be measured at seven sites on both aspects according to the number of sites involved. The distribution of these sites is illustrated in diagram 2:1.

This use of multiple assessment points around each tooth resulted in up to 14 measurements per tooth. These points of measurement were approximately 1-2mm from each other, so that although care was taken to ensure that the periodontal probe was placed in the same place at each

Fig. 2.1 TO ILLUSTRATE THE SITE NUMBERING SYSTEM FOR INFRA-BONY DEFECTS



1 = Mesial interproximal
2 = Mid-vestibular/mid-oral (of mesial root)
3 = Mid-vestibular/mid-oral
4 = Mesial furcation (palatal)
5 = Vestibular or lingual furcation
6 = Distal furcation (palatal)
7 = Mid-vestibular/mid-oral (of distal root)
8 = Disto-vestibular/disto-oral (of distal root)
9 = Distal interproximal



assessment, minor variations in position would be averaged out by the adjacent measurement points when the defects were statistically analysed. This use of a multiplicity of adjacent sites avoided the necessity for rigid standardisation of sites by use of stents which had been found to inhibit access to sites in an initial pilot study, particularly when these were deep, or in the posterior regions of the mouth.

2.3.4 Statistical analysis

The defects were randomly assigned to test and control defects utilising computer generated randomisation tables. Each site was then further subdivided into those which were initially shallow (< 3mm), moderate (3-5 mm) or deep (>6mm). The mean values for each pocket were taken as the units for the statistical analysis as described by Blomqvist (1985).

Although every attempt was made to achieve equal numbers of test and control defects, clinically this was not always possible. As a result there were different numbers of test and control sites in some of the patients. This occurred because some adjacent defects, which were treated during the procedure, were included in the study and also because some defects were so extensive that the teeth had to be extracted at the time of surgery.

2.3.5 Radiographic assessment

In addition to the clinical investigations, periapical radiographs were taken of all sites pre-operatively, one week post-operatively and 6-monthly thereafter until the

end of the study using a parallel long cone X-ray technique.

The radiographs of 9 of the 10 subjects described previously were utilised in this part of the study. The 10th individual was excluded because the radiographic series was not of sufficient quality to be analysed densitometrically.

The radiographic procedures were carried out using a standardized technique. A commercially available film holder and tube aligning system were modified to enable a removable film-locating impression to be incorporated in the occlusal block, together with an aluminum step wedge, as described by Verrier et al (1989). A locating impression was used to improve the reproducibility of the alignment of the film and X-ray tube to the teeth. The individual impressions were utilised for reducing variations in film and tooth alignment.

The serial radiographs of the sites at which bone defects had been treated were examined to ensure that the required anatomical landmarks could be discerned; those series that were suitable were subjected to densitometric analysis. A total of 15 test and 12 control series of radiographs were assessed. These series will be referred to subsequently as the experimental sites. As a result of having to reject three radiographic series, there were, by chance, fewer control series than test series.

The radiographs were assessed densitometrically using a system based on a microcomputer as described by Verrier

et al (1989). The system comprised a solid state camera which was used to obtain a video image from a given radiograph placed on an illuminated light box. The resultant image was digitized and stored in a microcomputer. The image of the aluminum step wedge, which was incorporated in the film holder, was used to achieve a constant density reference, and to enable compensation to be made by computer analysis for variations in radiographic technique. Discrepancies in technique for exposing and developing of films in serial radiographs of the same site could be corrected as a result.

The level of the interproximal bone was measured for the various sites by means of a specially developed algorithm. The digitized radiograph was viewed on the screen and the cursor was used to mark and record reference points. For each interproximal region to be assessed a quadrilateral "zone of interest" was marked out, bounded by the two cemento-enamel junctions and by two points marked in the periodontal ligament spaces. The level of these points was not critical, being ascribed to a level about 2 mm apical to the base of the bone defects. A calculation was then made by the computer program of the gray-level histogram of the enclosed region. From this calculation, the appropriate threshold gray level to delineate the height of interproximal bone was determined. The interproximal bone level was then determined by means of three measurements for each interspace, the first being taken along the line from the point midway between the

cemento-enamel junctions to the midpoint between the periodontal ligament markers. Two further measurements were taken at the equidistant, tertiary points along the above two reference lines. For each interproximal site the mean of these three values was used to represent the level of the hard tissue at that site.

The values obtained for each radiograph in each series were then subtracted from the values on the initial radiograph to give a value representing the changes which had taken place in the hard tissues with time.

2.3.6 Reproducibility of the Clinical Investigator

All the measurements throughout the study were carried out by the same examiner (PNG). Reproducibility was determined prior to the study for the various measurement criteria by assessing 6 patients who were receiving treatment in the periodontal department at UCH Dental Hospital. Measurements were taken on two separate occasions with an interval of at least 60 minutes between the examinations. The percentage reproducibility for measurement by site was 92% for pocket measurement, 88% for recession of the gingival margins, and 89.5% for the level of tissue attachment.

2.3.7 The pilot investigation

A pilot investigation was undertaken prior to the main study, as a number of uncertainties in respect of the assessment of the clinical data could not be resolved. The two main areas of concern related to reproducibility of sites of measurement in a long-term study, and whether a

standard periodontal probe, or a pressure controlled periodontal probe should be used in the study.

In the case of reproducibility of sites in the study, a small pilot study was undertaken utilizing 6 hygienists in the Periodontal Department. Impressions were taken and stents were manufactured. Reproducibility utilizing the stents, and the multiple site measuring system described previously was investigated. Measurements of crevicular depths were taken using a Willis periodontal probe on two occasions on different days. Points of measurement on the occlusal stents were demarcated by grooves, or by embedding orthodontic tubes into the stents with cold cure acrylic.

Site readings were similar with and without utilizing the stents and reproducibility was in excess of 90% utilizing both methods of site demarcation. The stents, however, were found to impede site measurement, particularly in posterior interdental regions, and in furcations. In addition, the pressure controlled probe gave inaccurate readings due to resistence to probing occurring in the grooves and orthodontic tubes. It was therefore decided to use the multiple site system for derivation of the data in preference to the stents.

The decision as to whether or not a pressure controlled probe or a standard manual probe should be used could not be resolved in the pilot study, as measurements were taken in sites with minimal crevicular depths, in individuals with excellent oral hygiene. With this in mind, it was decided to take all pocket depth readings with

both a manual probe (Hu-Friedy Willis type probe with a point diameter of 0.62mm) and an electrically operated pressure regulating probe (Vine Valley Research Probe Model 200, fitted with an identical probe tip to the manual probe with the probing force set at 0.25 Newtons) as described by Galgut and Waite (1990). It was decided to evaluate the results of using both of these probes prior to analysis of the data for the study itself, by comparing the data for each probe with measurements taken at surgery to determine which probe gave a better approximation of the actual defects measured at surgery.

2.3.8 The surgical procedure

Utilising randomisation tables the test sites which were to receive the implant material and the control sites which received no implant material were determined. The surgical sites were then anaesthetised with Lignocaine local anaesthetic containing 1:80,000 parts of adrenaline, and the patient requested to wash his mouth with chlorhexidine mouthwash for 1 minute.

The defects were exposed utilising inverse bevel incisions designed to preserve as much of the soft tissue as possible, particularly in the interdental regions. After surgical exposure, inflamed soft tissue, granulation tissue and reparative fibrous tissue was removed. The root surfaces were then meticulously scaled and root planed, and flushed with sterile saline.

Synthetic hydroxy-apatite bone implant material (Periograf (R), Winthrop, Guildford, U.K.) was mixed with

sterile saline in accordance with the manufacturers instructions and introduced into the test sites only, on the round end of a Cumine scaler. The implant material was then lightly compressed and contoured into the defects.

The soft tissue flaps were then replaced and care was taken to seal over as much of the defect area as possible to minimise contamination of the sites. The flaps were sutured using interrupted sutures. The patients were instructed to maintain their oral hygiene regimens, but to supplement them with chlorhexidine mouthwash twice a day during the healing period. The sutures were removed one week postoperatively.

2.3.9 Histological specimens

The specimens for histological evaluation were obtained from three individuals with severe periodontal breakdown and bone loss who had been treated with hydroxy-apatite implant material. Samples were obtained from teeth which were scheduled for extraction some time after placement of the implant material. Reasons for extraction included failure of the procedure with recurrent periodontal pocketing and abscess formation, or because it was decided that the tooth in question had a prognosis post-operatively which was too poor to be included in an overall restorative treatment plan. Removal of the specimens was by means of an inverse bevel flap, created so as to retain a cuff of soft tissue around the affected tooth, and some of the natural bone surrounding the implanted site. The material was removed and placed in

10% formal saline solution until it could be processed for histological examination. The specimens were then decalcified and mounted in paraffin wax. The samples were then cut longitudinally along the middle of the tooth to include the apex of the tooth and the graft material. A section was taken for processing, and a further sample was taken, approximately 1 mm into each half of the specimen. The samples were then stained with haematoxaline and eosin stain and examined by means of a light microscope.

2.4 RESULTS

2.4.1 Results - pilot investigation

Tables 2a and 2b give the mean values for pocket depth and attachment levels for mild, moderate, and severe pockets before treatment and 26 weeks after surgical treatment. It can be seen from these tables that over the 26-week period a reduction in pocket depths and a gain in attachment occurred in all of the groups irrespective of whether this was assessed by means of the manual probe, or the electronic pressure probe. In all cases mean pocket depths and loss of attachment measurements using the electronic pressure probe were less than those using the manual probe.

Statistical analysis indicated that the differences between the measurements taken with the two instruments were significant at baseline, especially in the case of the moderate and severe groups. At 26 weeks after surgery the differences between the measurement techniques were much smaller, significance only being found between pocket depths in the severe group and loss of attachment in the moderate group ($P>0.05$).

The mean values of pocket depth measurements and attachment levels using manual and pressure probes immediately before periodontal surgery are shown in Table 2c. These measurements are related to corresponding values at the same sites taken at open flap surgery. The results for the manual probe were found to be higher than those for the pressure probe. It can also be seen that they were closer to the values for the bone levels derived at

TABLE 2.a **MEAN (± SD) VALUES FOR POCKET DEPTHS DERIVED USING THE MANUAL AND PRESSURE PROBES FOR MILD, MODERATE AND SEVERE POCKETS AT BASELINE AND 26 WEEKS AFTER SURGERY**

WEEKS NO	CLASSIFICATION	MANUAL PROBE	PRESSURE PROBE
Week 0 (baseline)	Mild	1.57 ± 0.58	1.14 ± 0.51*
	Moderate	3.89 ± 0.38	2.16 ± 0.48**
	Severe	7.13 ± 0.72	4.30 ± 1.19**
26 weeks	Mild	1.16 ± 0.47	0.84 ± 0.34
	Moderate	1.97 ± 0.58	1.36 ± 0.30
	Severe	2.81 ± 0.95	1.91 ± 0.73*
Significance levels (manual *v.* pressure probe): *P<0.05; **P<0.001.			

TABLE 2.b MEAN (± SD) VALUES FOR LOSS OF ATTACHMENT USING THE MANUAL AND PRESSURE PROBES FOR MILD, MODERATE AND SEVERE POCKETS AT BASELINE AND 26 WEEKS AFTER SURGERY

WEEKS NO	CLASSIFICATION	MANUAL PROBE	PRESSURE PROBE
Week 0 (baseline)	Mild	1.90 ± 0.72	1.43 ± 0.57
	Moderate (3.5-5.0 mm)	4.57 ± 0.70	2.89 ± 0.37***
	Severe	7.86 ± 1.32	5.11 ± 1.62**
26 weeks			
	Mild	1.98 ± 0.81	1.71 ± 0.78
	Moderate	3.42 ± 0.57	2.79 ± 0.51*
	Severe	4.91 ± 1.09	4.01 ± 1.02
Significance levels (manual *v.* pressure probe): *P<0.05; **P<0.01; ***P<0.001.			

TABLE 2.c **MEAN (± SD) VALUES OF POCKET DEPTHS AND LEVELS OF ATTACHMENT BEFORE SURGERY, RELATED TO MEAN (± SD) BONE LEVEL DERIVED AT THE TIME OF SURGERY**

	POCKET DEPTH	ATTACHMENT LEVEL	BONE LEVEL
Manual	4.19 ± 0.59	5.15 ± 0.91	5.63 ± 2.54
Pressure	3.05 ± 0.71	3.57 ± 0.85	

periodontal surgery.

In addition, a number of problems with the pressure probe occurred. Due to the bulk of the handpiece head difficulty in placement of the instrument accurately and difficulty in reading pocket depths occurred. Difficulty in obtaining true pocket depth readings were found, particularly posteriorly and in deep defects due to loss of tactile sensation and fouling of the instrument by the oral tissues and root anatomy. This resulted in premature attainment of the preset pressure for probing. Because the manual probe gave readings closer to those of the actual defect depths, and as it was easier and more effective to use, and as reproducibility with this investigator had been shown to be high, it was decided to analyse only the data derived from readings taken with the manual probe.

2.4.2 Results - clinical variables at the various time intervals

In this study a total of 10 individuals received treatment, (7 females and 3 males) with a mean age of 42.5 and a range of 33-59 years at the point of entry into the study. Table 2d shows the defect and site distribution which was analysed from the data derived from the group. From this table it can be seen that the test and control defects were evenly matched (58 test defects and 59 control defects). However, due to differences in defect morphology, the actual site distributions between test and control defects varied to some extent. There were more control sites in the shallow and moderate categories (152 and 241

TABLE 2.d TO ILLUSTRATE THE NUMBERS OF TEST AND CONTROL DEFECTS AND SITES AT THE BEGINNING OF THE STUDY

	TEST	CONTROL
Defects	58	59
Shallow Sites	146	152
Moderate Sites	216	241
Deep Sites	140	133

control compared with 146 and 216 test sites), but fewer control sites (140 test and 133 control sites) in the deep category. Thus, although every effort had been made to obtain matched pairs of defects for test and control sites, it can be seen from this table that differences in the site distribution were present at baseline.

Table 2e shows the means and standard deviations in the clinical variable of recession at each time period during the study for the test and control sites in shallow, moderate and deep subgroups, From this table it can be seen that recession occurred in all of the subgroups. At baseline, the values for recession ranged from 0.45mm to 0.7mm and the test defects had more recession than the control defects in all of the categories. By 6 months post-operatively, there was an increase in recession with a range of 1.03mm in the shallow (control) sites to 2.18mm in the deep (test) sites. There was a tendency for recession to increase with time, with a range from 1.5mm (shallow control) to 2.44 (deep test) 4 years postoperatively. None of the differences between the test and control defects were statistically significant, although more recession occurred in the test sites initially than the control sites. With time, more recession occurred in the control sites so that by the end of the study the differences between the test and control sites were less marked. This was particularly noticeable in the deep defects where test sites showed 2.18mm of recession by 6 months post-operatively, increasing to

TABLE 2.e SHOWING THE MEANS AND STANDARD DEVIATIONS FOR RECESSION FOR TEN PATIENTS AT THE VARIOUS TIME INTERVALS FOR INITIAL SUBCATEGORIES IN TEST AND CONTROL SITES

POCKETS	SHALLOW INITIAL < 3 MM		MODERATE INITIAL 3-6 MM		DEEP INITIAL > 6 MM	
TIME INTERVAL	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATION)	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATIONI)	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATION)
BASELINE	0.45 (0.27)	0.22 (0.20)	0.50 (0.55)	0.46 (0.54)	0.70 (0.71)	0.35 (0.55)
SIX MONTHS	1.18 (0.57)	1.03 (0.45)	1.54 (0.86)	1.23 (0.59)	2.18 (1.13)	1.63 (1.05)
ONE YEAR	1.24 (0.64)	0.98 (0.63)	1.35 (0.67)	1.22 (0.69)	2.04 (0.89)	1.70 (1.21)
TWO YEARS	1.30 (0.59)	1.13 (0.45)	1.49 (0.67)	1.26 (0.50)	2.22 (1.19)	1.63 (1.43)
THREE YEARS	1.46 (0.52)	1.36 (0.50)	1.66 (0.64)	1.49 (0.74)	2.40 (1.21)	1.90 (1.36)
FOUR YEARS	1.69 (0.50)	1.50 (0.72)	1.90 (0.60)	1.67 (0.78)	2.44 (0.76)	2.31 (1.36)

2.44mm, while the control sites increased from 1.63mm to 2.31mm over the same time period.

Table 2f shows the means and standard deviations for pocket depths in both the test and control groups. It can be seen that pocket depths decreased as a result of surgery. This decrease progressively continued in both test and control sites with time, in all of the subgroups. Minimal differences in pocket depths between test and control sites existed at baseline. Only minor reductions in pocket depths occurred in the shallow sites, but the test sites decreased from 1.76mm to 1.42mm while the control sites decreased from 1.77mm to only 1.58mm. Thus in the sites with shallow pockets initially, test sites showed greater pocket depth reduction than control sites. This was more evident in the moderate and deeper pockets where test sites were shallower than control sites by 4 years post-operatively. Although by the end of the study the test sites were 0.41mm shallower than the control sites in the moderate category, and 0.51mm shallower in the deep category, these differences were not statistically significant.

Table 2g describes the changes which took place in respect to attachment levels. This variable was derived by the summation of the degree of recession and the pocket depths at each site. Comparison of the results for this variable show that attachment loss occurred in the shallow defects progressively throughout the time period of study. There was, however a progressive gain in attachment levels

TABLE 2.f SHOWING THE MEANS AND STANDARD DEVIATIONS FOR POCKETS FOR TEN PATIENTS AT THE VARIOUS TIME INTERVALS FOR INITIAL SUBCATEGORIES IN TEST AND CONTROL SITES

POCKETS	SHALLOW INITIAL < 3 MM		MODERATE INITIAL 3-6 MM		DEEP INITIAL > 6 MM	
TIME INTERVAL	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATION)	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATIONI)	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATION)
BASELINE	1.76 (0.16)	1.77 (0.17)	3.80 (0.30)	3.69 (0.32)	7.40 (0.91)	7.10 (0.61)
SIX MONTHS	1.59 (0.25)	1.44 (0.41)	2.09 (0.76)	1.99 (0.41)	2.98 (0.71)	3.02 (0.81)
ONE YEAR	1.64 (0.30)	1.56 (0.36)	2.12 (0.53)	2.15 (0.31)	3.07 (0.69)	3.37 (0.81)
TWO YEARS	1.43 (0.33)	1.33 (0.32)	1.92 (0.55)	1.95 (0.56)	2.69 (1.10)	3.03 (1.18)
THREE YEARS	1.32 (0.50)	1.40 (0.51)	1.34 (0.60)	1.38 (0.51)	2.50 (0.88)	2.89 (0.97)
FOUR YEARS	1.42 (0.40)	1.58 (0.75)	1.78 (0.36)	2.19 (0.76)	2.39 (0.70)	2.90 (1.12)

TABLE 2.g SHOWING THE MEANS AND STANDARD DEVIATIONS FOR ATTACHMENT LEVELS FOR TEN PATIENTS AT THE VARIOUS TIME INTERVALS FOR INITIAL SUBCATEGORIES IN TEST AND CONTROL SITES

POCKETS	SHALLOW INITIAL < 3 MM		MODERATE INITIAL 3-6 MM		DEEP INITIAL > 6 MM	
TIME INTERVAL	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATION)	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATIONI)	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATION)
BASELINE	2.21 (0.31)	1.99 (0.22)	4.31 (0.72)	4.15 (0.56)	8.10 (1.09)	7.45 (0.99)
SIX MONTHS	2.77 (0.70)	2.46 (0.70)	3.62 (1.06)	3.22 (0.42)	5.16 (0.96)	4.65 (1.29)
ONE YEAR	2.89 (0.63)	2.54 (0.90)	3.47 (0.59)	3.36 (0.79)	5.10 (0.96)	5.07 (1.45)
TWO YEARS	2.73 (0.56)	2.46 (0.65)	3.40 (0.78)	3.21 (0.79)	4.91 (1.21)	4.67 (1.78)
THREE YEARS	2.98 (0.65)	2.76 (0.84)	3.50 (0.56)	3.37 (0.92)	4.90 (0.89)	4.79 (1.48)
FOUR YEARS	3.11 (0.56)	3.08 (1.35)	3.69 (0.53)	3.86 (1.32)	4.83 (1.07)	5.21 (1.80)

in the moderate and deep defects, which was observed throughout the duration of the study, particularly for the test sites. It can be seen that initially, loss of attachment values ranged from 1.99mm (shallow control) to 8.10mm (deep test) sites. At the end of the study the shallow sites had lost further attachment in both the test and control groups, while the deep sites had gained attachment to a value of 4.83mm in the test sites, and 5.21 in the control sites. In the shallowest sites at the final assessment, the test sites had slightly lower values for levels of attachment than the control sites (test 3.11mm, control 3.08mm), while in the moderate and deep sites the test sites showed greater gain in attachment compared to the control sites. Although comparing initial and final values, moderate sites had 0.17mm and deep sites had 0.38mm more attachment gain in the test sites than the control sites, this was not statistically significant.

2.4.2.1 Changes from baseline

The magnitude of the differences between recession in test and control sites described previously are less evident when the change occurring relative to baseline is observed for each subgroup, as shown in Table 2h. Increased recession ranging 0.73 to 1.48 occurred in the first 6 months after surgery. This increased over the period of study to approximately 1.6mm (range 1.21-1.95) by the end of the study, being more marked with the deeper defects. The change from baseline to final assessment between the control and the test sites was 0.21mm in the deep defects

TABLE 2.h SHOWING THE MEANS AND STANDARD DEVIATIONS FOR CHANGE IN RECESSION FROM BASELINE FOR TEN PATIENTS AT THE VARIOUS TIME INTERVALS FOR INITIAL SUBCATEGORIES IN TEST AND CONTROL SITES

POCKETS	SHALLOW INITIAL < 3 MM		MODERATE INITIAL 3-6 MM		DEEP INITIAL > 6 MM	
TIME INTERVAL	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATION)	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATIONI)	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATION)
SIX MONTHS	0.73 (0.55)	0.80 (0.34)	1.03 (0.56)	0.77 (0.30)	1.48 (0.68)	1.28 (0.72)
ONE YEAR	0.80 (0.57)	0.75 (0.52)	0.84 (0.44)	0.76 (0.27)	1.33 (0.39)	1.35 (0.87)
TWO YEARS	0.86 (0.48)	0.91 (0.41)	0.98 (0.39)	0.80 (0.24)	1.51 (0.69)	1.28 (1.16)
THREE YEARS	1.01 (0.39)	1.14 (0.50)	1.15 (0.34)	1.03 (0.40)	1.69 (0.07)	1.55 (1.04)
FOUR YEARS	1.24 (0.51)	1.28 (0.65)	1.40 (0.39)	1.21 (0.54)	1.74 (0.59)	1.95 (1.03)

(control 1.95mm, test 1.74mm).

Table 2i shows greater change in pocket depths in all categories for the test subjects than the control defects. The values at the final assessment were 5.0mm and 4.20mm, there being 0.8mm more change in the deep test defects by the end of the study. This difference was only significant ($p < 0.05$) at the four-year post-treatment assessment period. Less significant change at the final assessment could be observed in the moderate sites (test 2.02mm, control 1.50mm) which amounted to a difference of 0.5mm between test and control sites. There was only a difference of 0.16mm (test 0.34mm, control 0.18mm) in the shallow sites.

In Table 2j, which shows changes in attachment levels, a similar pattern can be seen. The test sites show greater change than the control sites. Thus greater gain in attachment occurred in sites which received the implant material. Although pocket depth results (Table 2i) showed significantly greater reductions in the test sites for the deep defects at the final assessment, this difference was not significant for the assessment of change in attachment levels, due to an ameliorating effect from the reduction in recession in these test sites. However, for the deep defects, the results approached statistical significance ($p < 0.058$).

TABLE 2.I SHOWING THE MEANS AND STANDARD DEVIATIONS FOR CHANGE IN POCKET DEPTHS FROM BASELINE FOR TEN PATIENTS AT THE VARIOUS TIME INTERVALS FOR INITIAL SUBCATEGORIES IN TEST AND CONTROL SITES

POCKETS	SHALLOW INITIAL < 3 MM		MODERATE INITIAL 3-6 MM		DEEP INITIAL > 6 MM	
TIME INTERVAL	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATION)	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATIONI)	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATION)
SIX MONTHS	0.17 (0.24)	0.33 (0.39)	1.72 (0.60)	1.71 (0.50)	4.42 (1.28)	4.08 (0.88)
ONE YEAR	0.12 (0.26)	0.20 (0.39)	1.68 (0.44)	1.55 (0.48)	4.33 (1.01)	3.73 (0.70)
TWO YEARS	0.33 (0.32)	0.44 (0.34)	1.89 (0.38)	1.74 (0.56)	4.70 (1.28)	4.07 (1.22)
THREE YEARS	0.24 (0.57)	0.37 (0.58)	1.96 (0.67)	1.81 (0.58)	4.90 (0.92)	4.21 (1.06)
FOUR YEARS	0.34 (0.42)	0.18 (0.83)	2.02 (0.56)	1.50 (0.71)	5.00* (0.81)	4.20 (0.83)

Analysis of differences between procedures:

*Statistically significant difference ($P<0.05$)
**Approaching significance ($P<0.058$)

TABLE 2.j SHOWING THE MEANS AND STANDARD DEVIATIONS FOR CHANGE IN ATTACHMENT FROM BASELINE FOR TEN PATIENTS AT THE VARIOUS TIME INTERVALS FOR INITIAL SUBCATEGORIES IN TEST AND CONTROL SITES

POCKETS	SHALLOW INITIAL < 3 MM		MODERATE INITIAL 3-6 MM		DEEP INITIAL > 6 MM	
TIME INTERVAL	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATION)	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATIONI)	TEST MEAN (ST. DEVIATION)	CONTROL MEAN (ST. DEVIATION)
SIX MONTHS	-0.56 (0.69)	-0.48 (0.65)	0.68 (0.62)	0.93 (0.55)	2.94 (1.60)	2.80 (1.17)
ONE YEAR	-0.68 (0.59)	-0.55 (0.88)	0.84 (0.52)	0.79 (0.59)	3.00 (1.12)	2.38 (0.88)
TWO YEARS	-0.52 (0.61)	-0.47 (0.59)	0.90 (0.53)	0.94 (0.51)	3.19 (1.33)	2.79 (1.43)
THREE YEARS	-0.77 (0.64)	-0.78 (0.80)	0.81 (0.60)	0.78 (0.63)	3.21 (0.82)	2.66 (1.05)
FOUR YEARS	-0.90 (0.66)	-1.09 (1.35)	0.62 (0.58)	0.29 (0.90)	3.27(*) (1.16)	2.24 (0.96)

Analysis of differences between procedures:

* Statistically significant difference (P<0.05)
(*)Approaching significance (P<0.058)

2.4.3 Results - radiographic analysis

Table 2k shows the results for the test sites of the analysis of the change from baseline in the height of the interproximal hard tissue, measured in pixel units from the cemento-enamel junctions (CEJ). At the various sites and time intervals relative to baseline measurements, there were 35 analyses showing gain in hard tissue and only 10 showing a loss.

Table 2l shows the changes from baseline of the interproximal bone height for the control sites. These results indicate that 16 of the analyses show a gain in height, whereas 18 show a loss.

The compiled data in Table 2m show that at all the time intervals compared to baseline there was a significant mean gain in pixel units of hard tissue height on the implanted sites, whereas there was a slight mean loss of bone height on the control sites. Statistical analysis was carried out by using all of the data in an unpaired Student's "t" test, and also by restricting the data to matched test and control sites, using a paired test. Both methods of statistical analysis indicated that the test sites showed statistically significant gains in hard tissue height compared with the control sites.

2.4.4 Results - histological specimens

The results of histological assessment of the specimens derived from teeth with implanted defects which had been extracted are presented below.

TABLE 2.k **CHANGE FROM BASELINE IN LEVELS OF INTERPROXIMAL BONE MEASURED IN PIXEL UNITS FROM THE CEJ FOR THE NINE PATIENTS (TEST SITES) (17 PIXEL UNITS ARE APROXIMATELY EQUIVALENT TO 1mm)**

PATIENT	6 MONTHS	1 YEAR	2 YEARS
1	4.17	7.60	6.50
	8.87	9.90	16.20
2	22.10	21.67	23.23
	52.37	5.00	-3.00
3	10.40	4.87	2.33
	14.97	16.63	17.10
4	8.80	12.03	14.03
	1.50	13.33	14.67
	42.03	41.23	39.93
5	9.00	1.67	3.33
6	9.50	-0.90	1.17
7	25.60	16.03	25.00
8	-0.27	-0.67	-8.37
	0.40	-3.30	-1.60
9	-15.23	-11.43	-18.17

(Negative values indicate further loss of hard tissue)

TABLE 2.I **CHANGE FROM BASELINE IN LEVELS OF INTERPROXIMAL BONE MEASURED IN PIXEL UNITS FROM THE CEJ FOR THE NINE PATIENTS (CONTROL SITES) (17 PIXEL UNITS ARE APROXIMATELY EQUIVALENT TO 1mm)**

PATIENT	6 MONTHS	1 YEAR	2 YEARS
1	4.33	3.20	0.17
2	-2.67	-2.67	-1.67
3	6.37 -12.30	5.37 -20.10	0.00 -21.13
4	2.63 2.03	7.13 12.87	6.33 18.20
5	-6.23	-4.40	-0.10
6	-5.43	0.63	-4.13
7	-5.67	-6.77	-3.63
8	1.50	-1.13	0.47
9	-3.73	-2.70	-5.30

(Negative values indicate further loss of hard tissue)

TABLE 2.m **MEAN VALUES AND STANDARD DEVIATIONS FOR CHANGES FROM BASELINE IN LEVEL OF INTERPROXIMAL HARD TISSUE, MEASURED IN PIXEL UNITS FROM THE CEJ FOR TEST AND CONTROL SITES, WITH STATISTICAL ANALYSIS (17 PIXEL UNITS ARE APPROXIMATELY EQUIVALENT TO 1 MM)**

TIME	TEST		CONTROL		STUDENT'S t		STUDENT'S t (PAIRED)	
	Mean	SD	Mean	SD	t	P	t	P
6 months	12.95	17.01	-2.24	5.10	2.84	0.0007	2.92	0.016
1 Year	8.98	12.54	-0.96	8.21	2.26	0.021	3.07	0.014
2 Years	8.82	14.71	-0.46	8.95	1.83	0.061	3.16	0.012

Patient One

The histological section from the right upper premolar region in the first patient (Fig.2.2a) shows a mature collagen matrix in which can be seen a number of clear angular spaces, which prior to decalcification would have been occupied by the implanted hydroxy-apatite particles. There is minimal evidence of inflammatory response, indicating that the particles were relatively inert.

Examination at higher magnification (Fig.2.2b) shows a field that includes portions of the periphery of four particles and, centrally, a complete particle. Around the particle, on the lower right corner, there is no evidence of either absorption or deposition of bone. The two particles in the upper left corner of the illustration have a number of cells bordering their periphery, but these seem not to have resulted in any obvious changes to the particles. On the periphery of the central particle, towards the left side, there is a large multinucleated giant cell, with similar morphology to an osteoblast. The margin of the particle in juxtaposition with the multinucleated giant cell is less clearly defined than in other regions, which may be indicative of absorption of the crystal in association with this cell. Other cells with single nuclei, which are suggestive of spindle-shaped fibroblasts, or osteoblast precursor cells, can be seen along the upper border of the particle.

Patient Two

The tissue sample was obtained from this individual 40

weeks after placement. The tooth had developed a periapical abscess, and therefore required extraction.

Figure (2.3a) shows a section viewed at low power with an aggregate of particles embedded in a mature connective tissue matrix in the lower part of the section. On the upper left-hand side of the illustration there is an acute inflammatory cellular infiltrate which was probably related to the apical abscess. A higher magnification of the interface between the inflammatory infiltrate and the particles (Fig.2.3b) shows that the particles have retained an intact outline even in the presence of extraneous inflammation. There is no evidence of the inflammatory infiltrate affecting the tissue surrounding the implanted particles, even those in closest proximity to the site of inflammation. A medium-power view of particles in another field on the same slide (Fig.2.3c) shows a diffuse loss of the borders of some of the particles, indicating a degree of absorption, and the laying down of an amorphous eosinophilic material which seems to contain large numbers of pale cells. Higher magnification of one of these regions (Fig.2.3d) confirms that cells are present in this eosinophilic material, which is suggestive of early osteoid formation. Considerable variation in healing response can be seen at different locations in the implanted area, with some particles merely acting as inert space fillers whilst those nearby show absorption with or without deposition of osteoid-like material.

Patient Three

In this individual, synthetic implant material was placed in deep infrabony defects in teeth which were hypermobile. The extraction was carried out 80 weeks after the placement of the implant due to continued hypermobility. Figure (2.4a) shows implant particles imbedded in a mature fibrous matrix. To the left of the photomicrograph there is evidence of inflammatory infiltrate. This probably represents a response to plaque bacteria, rather than being directly related to the implant material as indicated by the clearly demarcated border of fibrous tissue adjacent to the particles closest to the inflammatory infiltrate. Differences can be seen in the responses to the implant. On the left-hand side of the photomicrograph, there is minimal change in the particles in spite of the presence of inflammation. Particles on the right side show signs of irregularity and loss of definition of the outline form. A high-power view of these particles (Fig.2.4b) shows a particle on the right of the slide which has a clearly delineated outline, whereas the majority of the other particles show evidence of absorption with lacunae evident on most of the surfaces of the crystals. This illustration shows clearly that the cells associated with the absorption of the material are multinucleated giant cells suggestive of osteoclasts. In some of these cells vacuoles are seen in the cytoplasm, again consistent with osteoclastosis.

A very variable host response to the implant is seen

Fig. 2.2 (a) Lower-power view 22 weeks after surgery showing decalcified particles of hydroxyapatite surrounded by a mature collagen matrix. (Mag: x6.3)

(b) Medium-power view showing multiple cells with single nuclei surrounding the particles. Near the centre of the field there is a multinuclear cell. (Mag: x16)
(F=fibrous tissue, HA=hydroxyapatite, GC=giant cell).

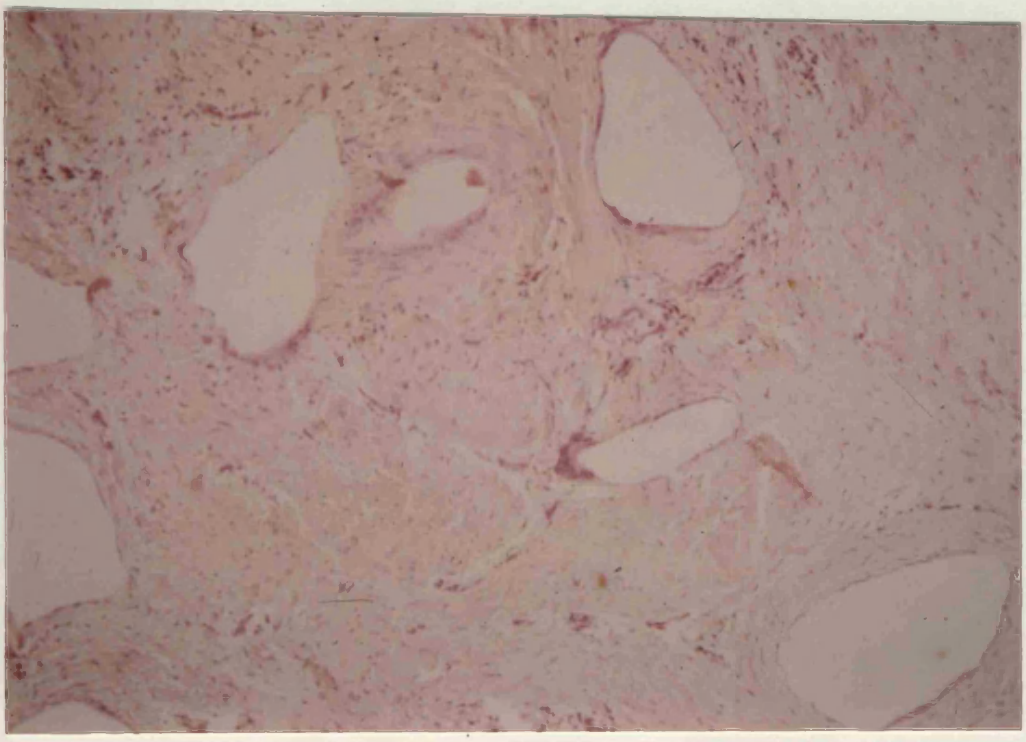


Fig. 2.2.a

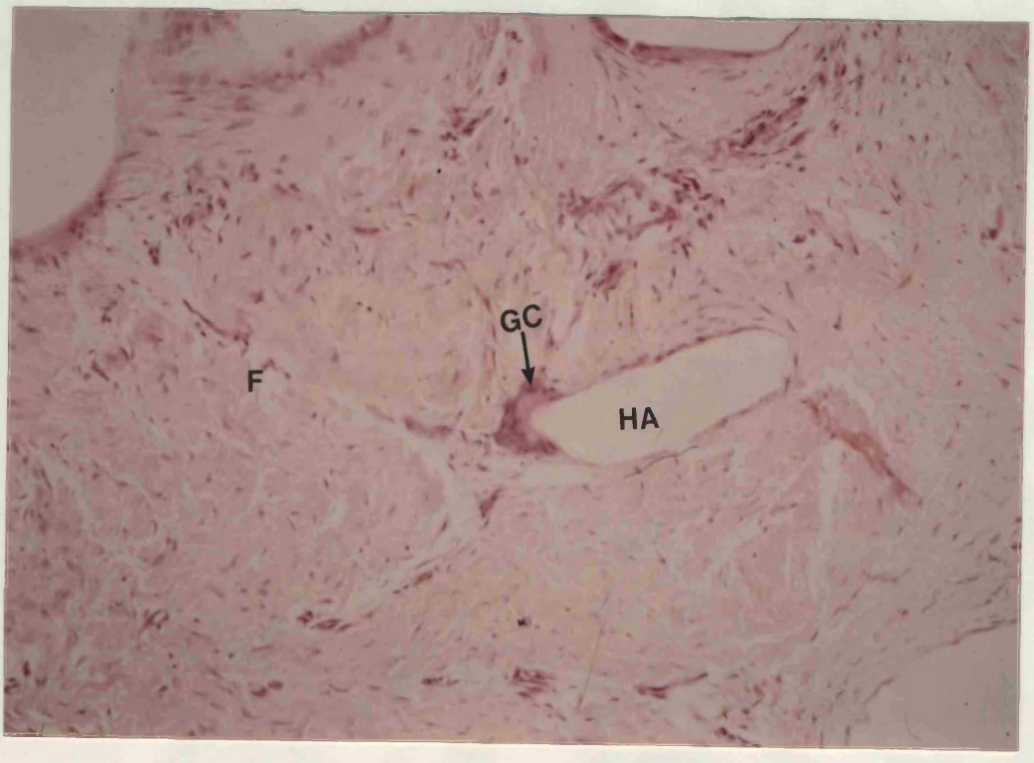


Fig. 2.2.b

Fig. 2.3 (a) Low-power view 40 weeks after surgery showing aggregate of particles in a mature collagen matrix with areas of inflammatory exudate. (Mag: x6.3)

(b) Medium-power view of particle boundary in proximity to a zone of inflammation; the outline of the particle appears intact. (Mag: x16) (F=fibrous tissue, HA=Hydroxy-apatite, IE=inflammatory exudate).

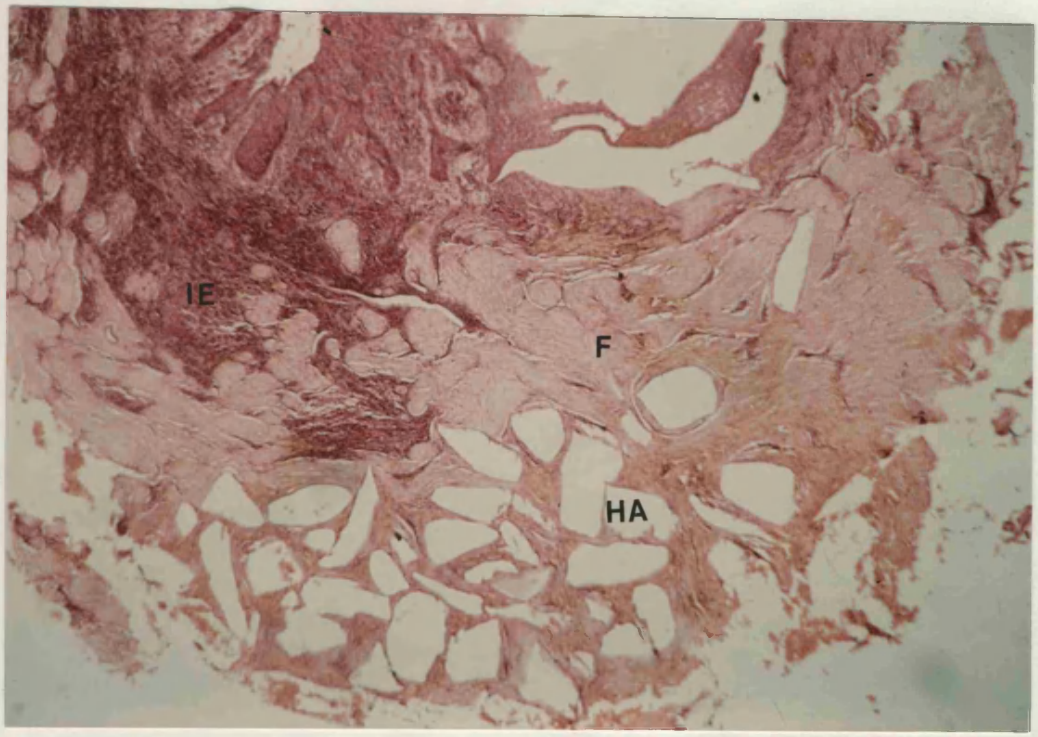


Fig. 2.3.a

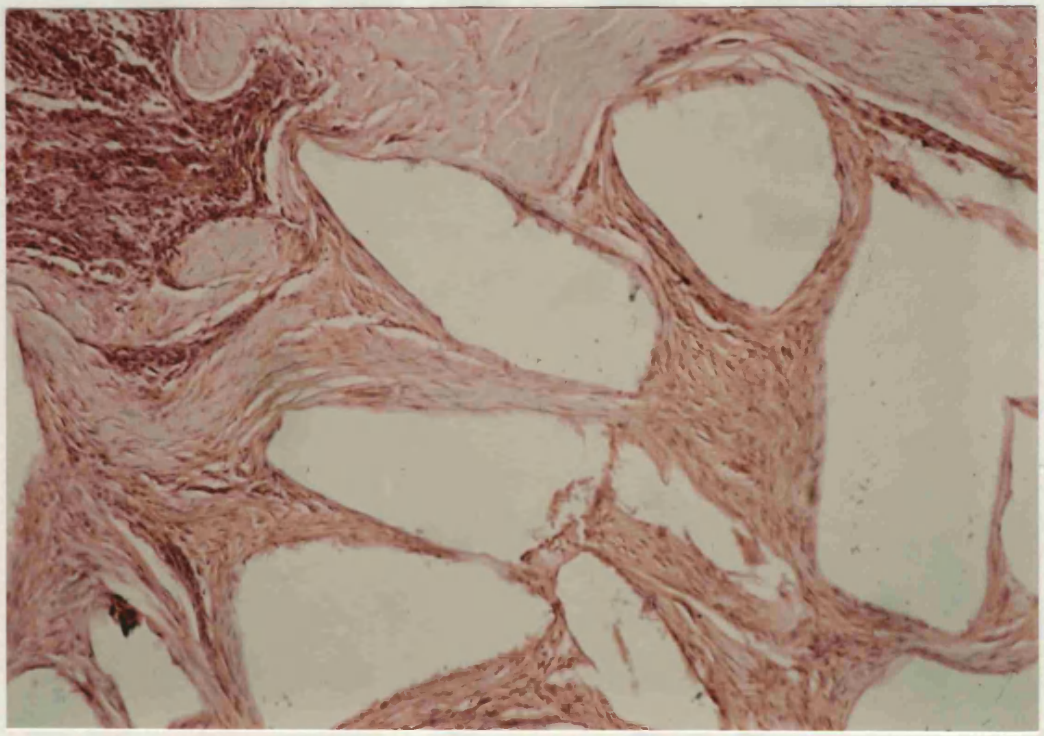


Fig. 2.3.b

Fig. 2.3 (cont.) (c) Medium-power view of an area where there is diffuse loss of the outline of some particles with replacement by pale eosinophilic cells. (Mag: x16)

(d) High-power view of the eosinophilic cells and pale coloured nuclei, perhaps representing early osteoid formation. (Mag: x40) (F=fibrous tissue, HA=hydroxyapatite, E=eosinophilic material).

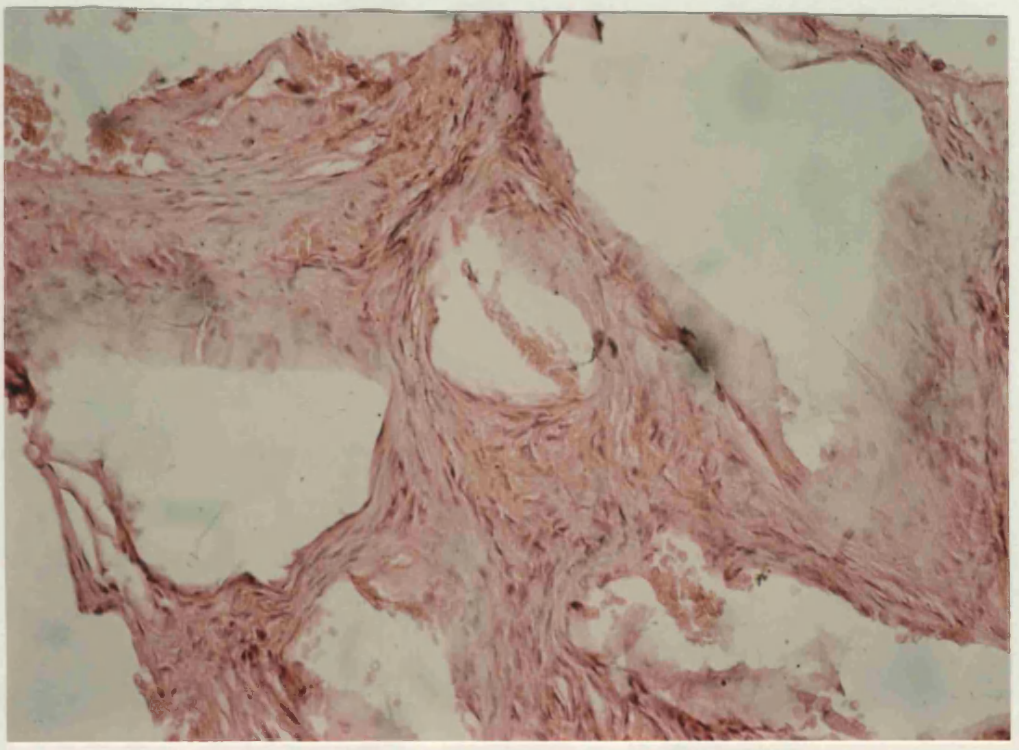


Fig. 2.3.c

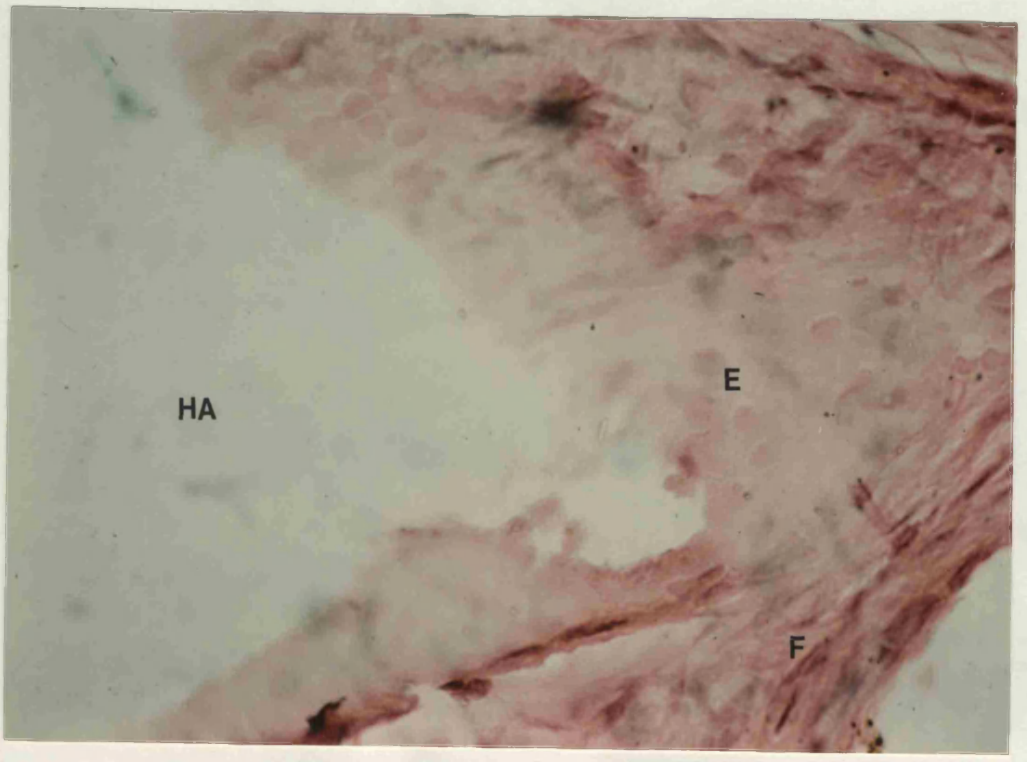


Fig. 2.3.d

Fig. 2.4

(a) Medium-power view of particles in fibrous tissue matrix 80 weeks after surgery. On the lower right of the section the outline of the particles is irregular due to degradation of the particles. These particles do not demonstrate uniform degradation characteristics. (Mag: x16)
(HA=hydroxyapatite, F=fibrous tissue, IE=inflammatory exudate)

(b) High-power view showing absorption lacunae around a number of particles. These lacunae contain mononuclear cells, and multinucleate giant cells in different sites. Some evidence of vacuolation in some of the multinuclear giant cells can be seen. (Mag: x40)
(HA=hydroxyapatite, A=absorption lacuna, GC=giant cell).

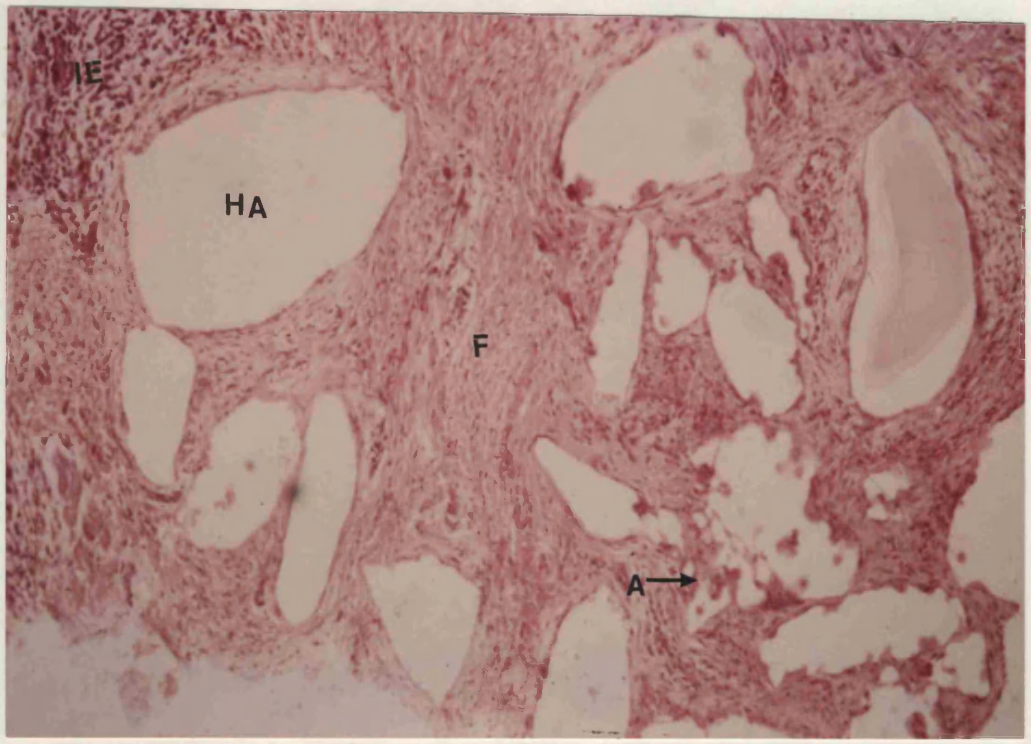


Fig. 2.4.a

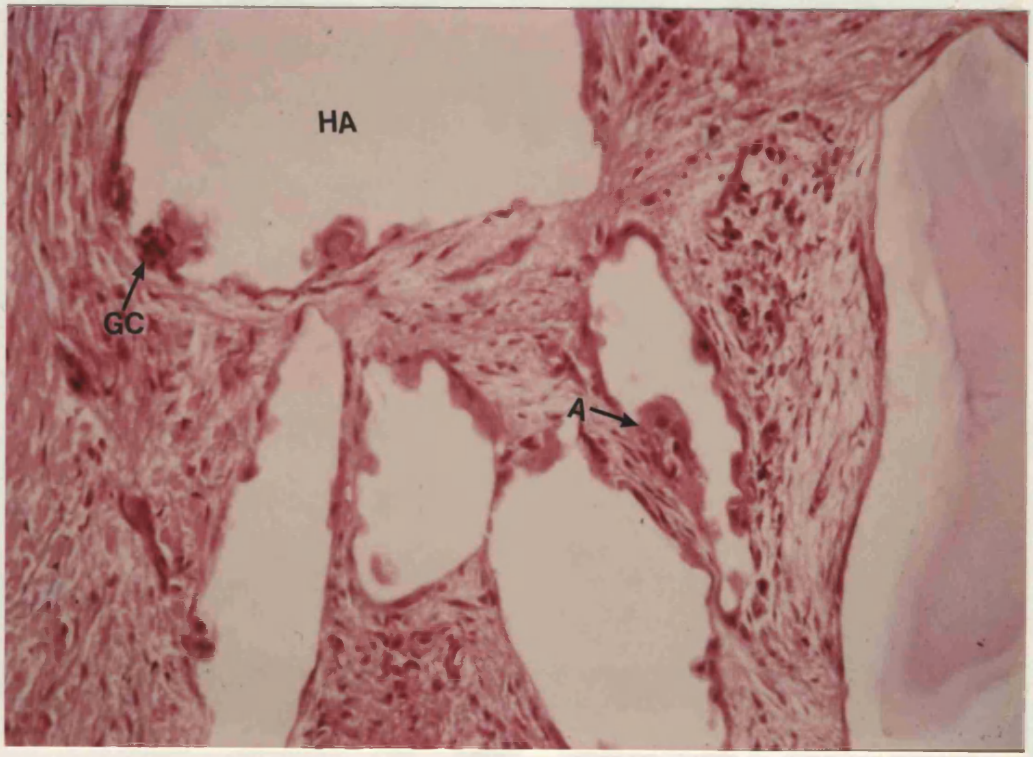


Fig. 2.4.b

Fig. 2.4 (cont.) (c) High-power view at a different site to show eosinophilic material laid down at the periphery of some particles while adjacent particles show only fibrous encapsulation. (Mag: x40)
(HA=hydroxyapatite, E=eosinophilic material, B=bone).

(d) Particles at another site also showing osteoid material. (HA=hydroxyapatite, O=osteoid). (Mag: x16)

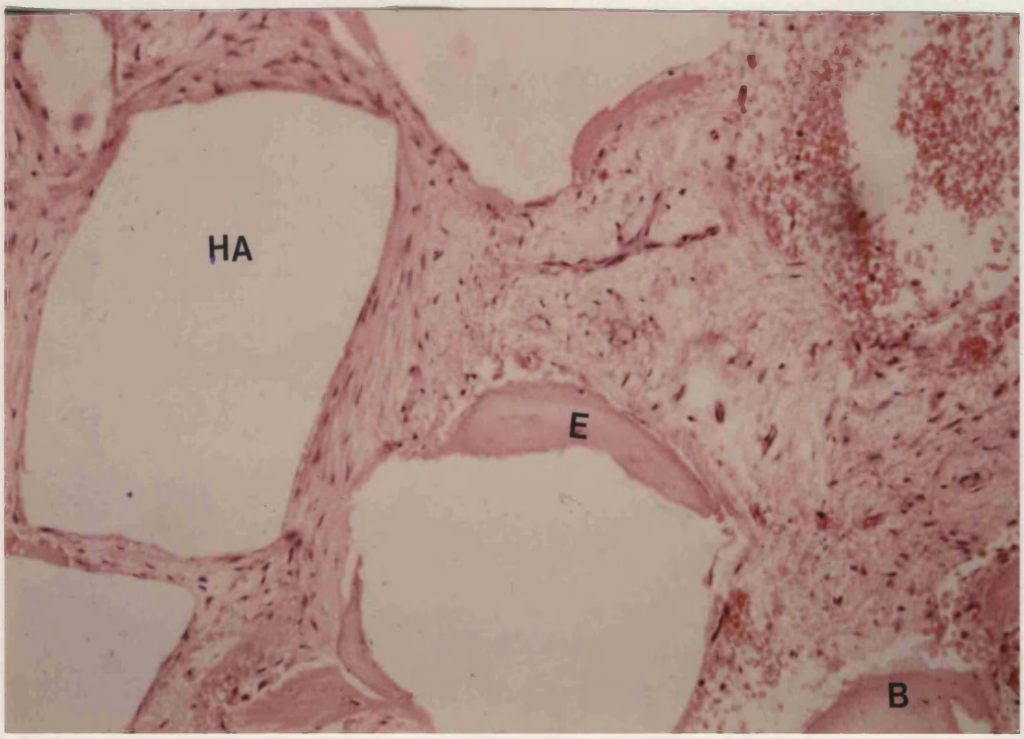


Fig. 2.4.c

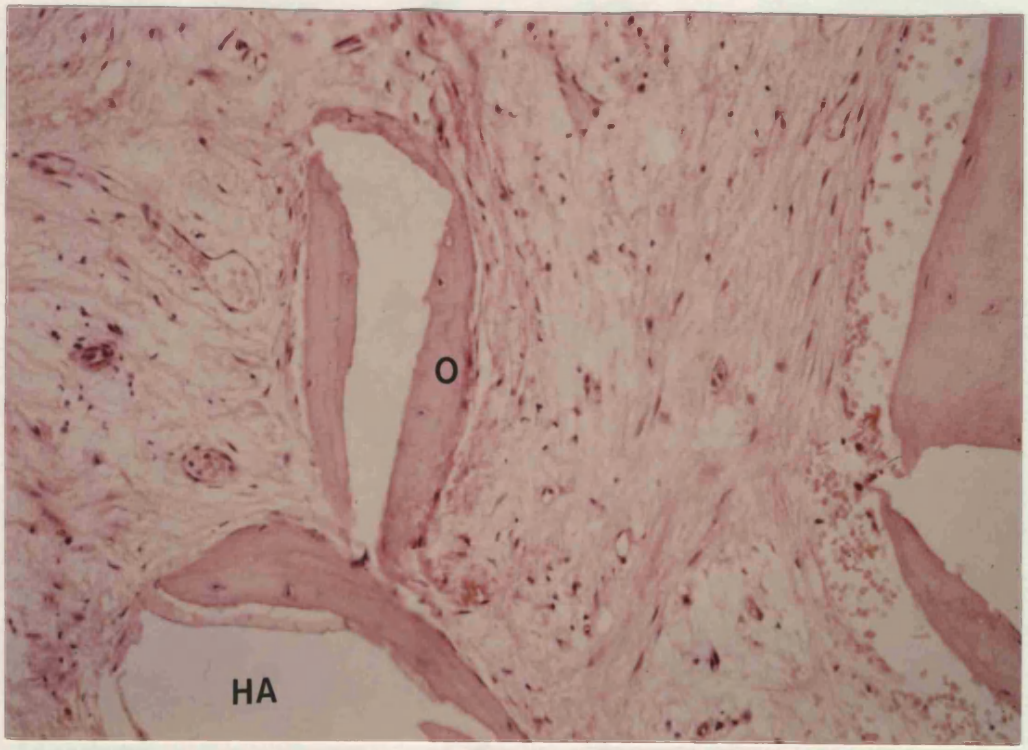


Fig. 2.4.d

Fig. 2.4 (cont.) (e) High-power view to show incremental lines similar to those in woven bone. (Mag: x40)
(HA=hydroxyapatite, O=osteoid, F=fibrous tissue).

(f) A particle at another site showing abundant bone-like material around most of the periphery, but with evidence of osteoclastic absorption and lacunae in the lower part of the picture. (Mag: x40)
(HA=hydroxyapatite, O=osteoid, A=absorption lacunae).

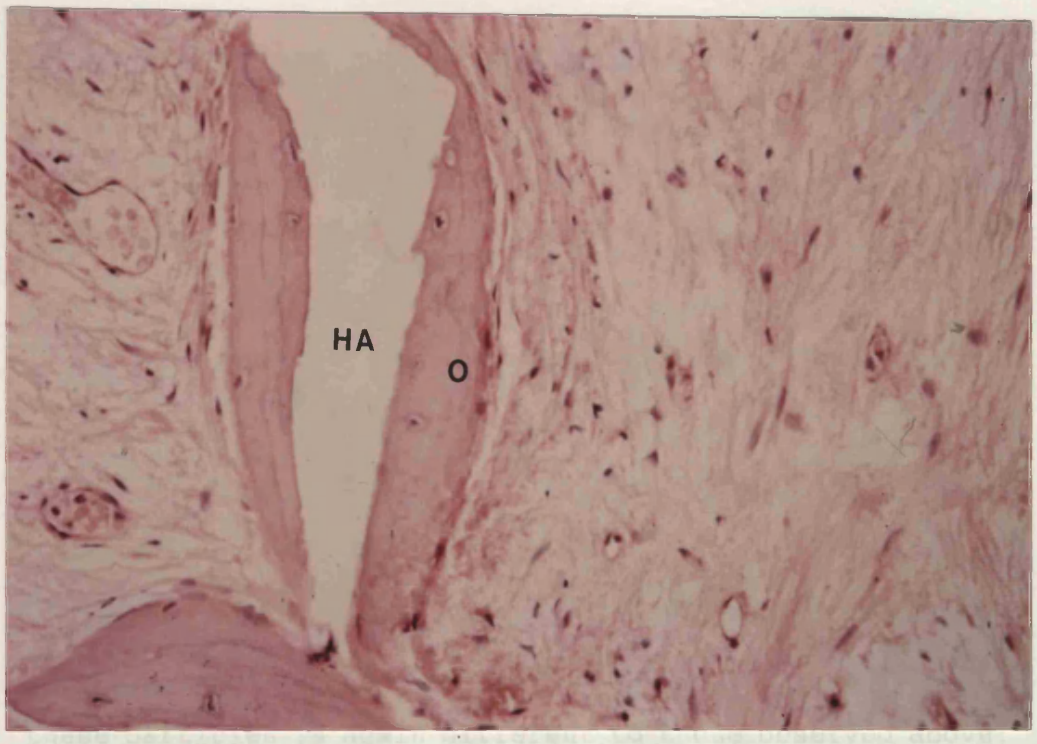


Fig. 2.4.e

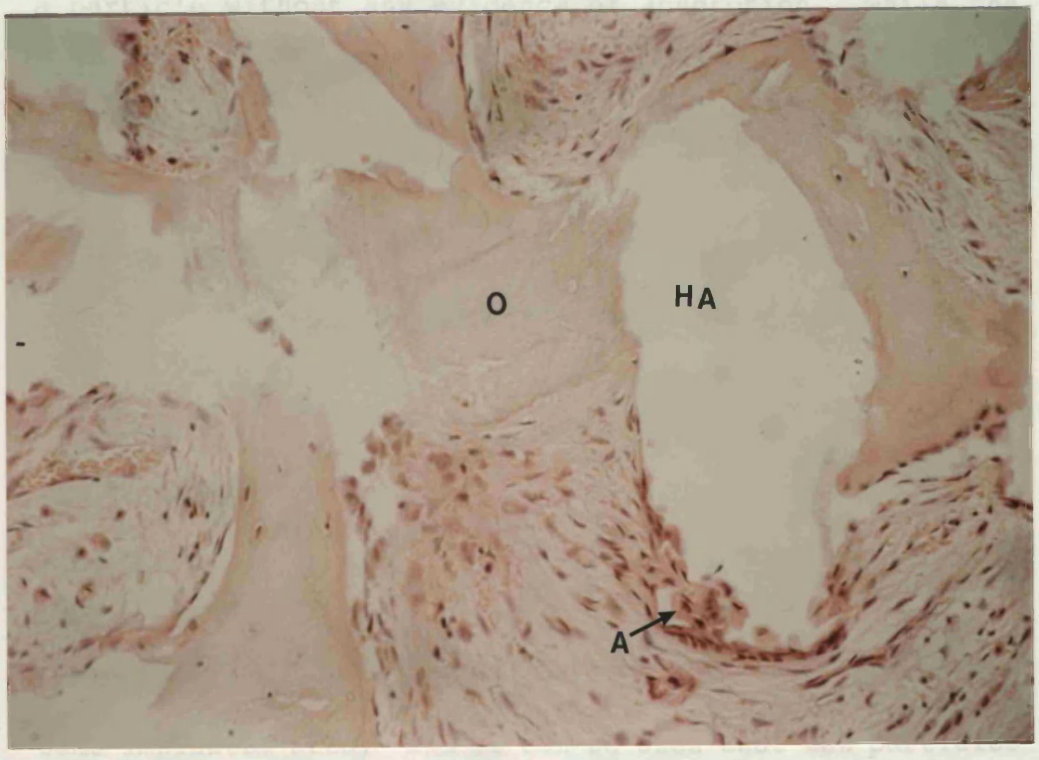


Fig. 2.4.f

in (Fig.2.4a). In the upper right region a particle is acting as an inert filler, whereas below and to the left the particles are being subjected to degradation around their periphery. One crystal is being rapidly degraded at multiple foci by multinucleated giant cells and in some areas small amounts of eosinophilic material are present. Thus not only is the type of healing response different between adjacent particles, but the rate of activity at adjacent sites may also vary.

Figure (2.4c) demonstrates particles from another region of the same implant site. The host reaction to these particles is again different to those observed above: Centrally, on the lower margin of the illustration, eosinophilic material is being laid down on the surface of a particle without any evidence of absorption, while the particles immediately adjacent to it do not seem to be initiating any soft tissue response and are merely acting as inert space fillers.

Eosinophilic material has been observed being laid down on or replacing hydroxy-apatite particles. This material has some of the characteristics of bone, with evidence of osteocytes being present in lacunae. Particles in close proximity to the host bone seem to initiate a variety of responses in the surrounding tissue, osteoid material being laid down at some sites and minimal effect at others. Figure (2.4d) is from a different site in the same implanted area, and it can be seen that the particles are being replaced or covered by osteoid-like material.

A higher-power magnification of this region (Fig.2.4e) shows that this tissue has the charateristics of woven bone with osteocytes in lacunae and with incremental lines. At another site on the same specimen (Fig.2.4f) an implant particle can be seen which is all but surrounded with mature bone-like material, but in the lower part of the picture there is evidence of osteoclastic absorption by multinucleated giant cells similar to those observed previously. Therefore, at different sites on the same particle of hydroxy-apatite implant material, a variety of host responses are possible, with osteoid being laid down at one site and osteoclastic absorption occurring at another.

2.5 DISCUSSION OF THE RESULTS

2.5.1 Discussion of results - the clinical study

The clinical trial utilising hydroxy-apatite synthetic material was undertaken to evaluate the efficiency of the material when used in periodontal defects associated with extensive tissue destruction and bone loss. The results have been presented in three parts namely, clinical results, radiographic results and histological findings. A discussion of these results follows.

A number of options were considered for statistical analysis. These included the parametric Student's "t" tests, and non-parametric tests such as the chi-square, ranking Mann-Whitney, and Kruskall-Wallis tests. Analysis of variance was considered but rejected as it was considered that the data would not represent a straight line progression with time, which is an essential prerequisite for this type of analysis of data. As the data to be analysed was parametric, paired Student's "t" tests were considered most appropriate to compare test and control sites, in order to establish whether or not significant differences could be discerned at each time interval throughout the study. However due to the uneven distribution of test and control sites in the database, this was not possible, and unpaired Student's "t" tests were conducted.

The statistical analysis of the clinical variables has shown that slightly better although not significant pocket reductions and attachment level gains can be expected in

defects which receive hydroxy-apatite implant material than those which do not. More recession tended to occur in the sites which received the implant material. These differences were not significant except in the deepest defects four years after treatment. At this assessment period it was seen that continuous change had occurred in the test sites while the control sites had remained stable. This may indicate continuing long-term healing of the test sites, or possibly recurrent disease in the control sites which is reflected in some of the pockets recurring. Clinically some sites demonstrated recurrent peridontal disease with increasing pocket depths while others remained stable, irrespective of whether the sites had received the implant material or not. The significant difference in pocket depth change with time may be reflecting a tendency for the sites which received the bone implant material to inhibit the progression of periodontal disease. This could be attributed to the formation of new hard tissues in the deeper regions of the defects in association with the implanted hydroxy-apatite.

It could be argued that the clinical variables used in this study were not ideal assessment criteria as they evaluate soft tissue changes only, without reference to changes in the underlying bone or bone implant material. Such changes could only be evaluated using surgical re-entry procedures, probing through soft tissue to bone, or radiographic procedures. Yukna (1981, 1985) and his group have investigated the same bone implant material

(Periograf) in both human and animal subjects. He adopted a different approach to assessing the efficacy of this material, in that he applied a secondary re-entry procedure to the sites and assessed the amount of infil which had occurred. The results of his studies are summarised in Table 2n. The study was in two parts. The first part was at 6 months post-operatively (Rabalais et al 1981) and the second part at 12 months post-operatively (Yukna et al 1985). It can clearly be seen from this table that although over half of the sites showed greater than 50% infil when treatment included hydroxy-apatite implant material (Periograf) a significant percentage showed poor infil (45%). Conversely, 31% of sites spontaneously filled in without implant material.

The Yukna studies (1981,1985) are particularly relevant to this investigation because the current work was designed with reference to the work in progress at that time by Yukna and his group. The protocol used for the Yukna study was made available by the manufacturers of Periograf so as to try to achieve some degree of compatibility between the two studies. A number of important differences between the two studies exist. Re-entry procedures for assessment of the implant material were not undertaken in the present study because it was felt that, in addition to the ethical consideration of performing surgery on healed sites, each surgical procedure would result in alterations in the baseline soft tissue variables due to remodelling and healing of the tissues

TABLE 2.n TO SUMMARISE THE OVERALL PERCENTAGE DEFECT INFILL OBTAINED IN THE STUDIES BY YUKNA RESEARCH GROUP

		> 50% INFILL	< 50% INFILL
DEBRIDEMENT + DURAPATITE	6 Months	54%	46%
	12 Months	55%	45%
DEBRIDEMENT ONLY	6 Months	32%	68%
	12 Months	31%	69%

6 Months: Rabalais, Yukna + Mayer (1981)
12 Months: Yukna, Harrison, Caudill, Evans, Mayer + Miller (1985)

after surgical exposure. Also, the Yukna protocol specifically excluded defects deeper than 7mm, and teeth with furcation exposures. In this study, these poor prognosis teeth were included as it was felt that it was in these extensive infrabony defects that the implant material might be most beneficial. Notwithstanding these differences, the Yukna study is complementary to this investigation in that it highlights the fact that infill or regeneration of infra-bony defects is not predictable subsequent to the placement of a bone implant material into the defects. Considerable variation in the amount of infil of sites is seen to occur. This would in part account for the lack of statistical significance in the results of the present study, some control sites filling spontaneously without bone implant material, with others failing to fill even with the bone implant. This effect would mask attempts to derive significance from the clinical variables assessed in this study. In a five year follow-up of the Yukna study, (Yukna et al 1989) it was found attachment levels in 86% of the sites which received bone graft were stable or improved as opposed to only 62% of the sites which received surgery without any implant material. The failure rate, in terms of recurrent loss of attachment was 3 times greater in the sites which were treated by surgery alone, than those which received the implant material. Only 2% of sites treated with synthetic bone implant material had deeper pockets than pre-operatively, whereas 20% of the sites treated with surgery alone had recurrent

pockets that were deeper than at baseline. Thus, the conclusion drawn is that clinically the sites which received the bone implant material showed better long-term stability and resistance to recurrent periodontal breakdown than those sites which were treated by means of surgery without any implant material. The results of the Yukna studies (1981,1985,1989) reinforce the observations of this study, in that it has been shown that the implanted sites demonstrated better results than the non-implanted sites, with the differences becoming more obvious with time. In the Yukna studies, only six patients were investigated, and the results of such a small number of subjects could be questioned. However, the Yukna study was the only previous well controlled long-term study that has been published. It was carefully designed, controlled and monitored, and the results may be considered to give an accurate record of efficacy of bone implant materials used clinically in human subjects.

By contrast, in this study, only soft tissue variables were assessed. Pocket depth changes and attachment level changes assessed by clinical variables and bone infil as assessed by means of re-entry procedures do not necessarily correlate. Thus, although the Yukna studies demonstrated better infill of the defects which received the implant material, this study showed that at some sites recurrent periodontal disease occurred in both test and control sites post-operatively, although less so in the test sites. In the long term, both studies demonstrated better resistance

to recurrent periodontal breakdown at the implanted sites. Thus, although this study was limited to only 10 individuals, and the Yukna study was limited to only 6 individuals, and the assessment criteria in both studies were not totally compatible, when observed in conjunction with one another, they can be seen to be largely complementary.

2.5.2 Discussion of results - the radiographic study

Radiographic assessment techniques have been extensively used in periodontal research, but the results have had to be interpreted with caution because of a number of inherent problems. Errors are liable to result from discrepancies in radiographic techniques caused by variations in exposure, and development of the films, non-standardised alignment of the radiographic films and the X-ray source throughout the series, and the fact that visual methods of estimating radiographic changes on radiographs are highly subjective. These difficulties were overcome in this study by utilising an adapted radiographic parralelling system which incorporated an impression of the occlusal surfaces of the teeth being investigated, an aluminium stepwise wedge which would allow for densitometric correction of different film intensities, and semi-automated measurement techniques utilising computer technology.

Nevertheless, clinical assessment of the series of radiographs indicated that there was evidence of some discrepancies in exposure geometry, such as changes in the

degree of interproximal overlap or changes in position of the teeth on the film. Alternatively, minor changes in the position of individual teeth in the arches, as a result of minor tooth movement after healing, may have been one of the possible causes of the changes in radiographic geometry with time. The exclusion of some sites from the study was necessitated, for example, by the overlap of the images of the cemento-enamel junctions of neighbouring teeth. Accurate film placement and tube alignment is still under human control and the initial placement, recorded by the impression, relies on the skill and judgement of the operator.

A gain in the levels of the hard tissue was seen to be significantly greater in those sites which were treated with hydroxy-apatite than those that were not and this gain was statistically significant compared with the results for the control sites. This finding supports the clinical and surgical re-entry data reported by the Yukna group which have shown that 12 months after surgery there had been an average filling in of the osseous defects of between 1 mm for the shallower sites and 2.1 mm for the deeper sites implanted with hydroxy-apatite (Yukna et al 1985). The densitometric analysis system was not designed to differentiate between bone levels and hydroxy-apatite implant material interproximally.

A major disadvantage of the densitometric analysis is that it is limited to a quantitative evaluation of the radiographs without any reference to qualitative

variations. The assessment of subtle variations in the radiolucency of the tissues is not possible using current densitometric techniques, but these subtle variations form the basis of clinical evaluation in periodontology. The disadvantage of clinical evaluation of radiographs is that it is subjective and therefore open to different interpretations. Nevertheless, the range variation in crestal bone density evident on the radiographs throughout the study may warrant some comment.

Two patients have been described in a paper relating to this work (Galgut, 1990), in which a number of variations in radiographic appearance have been observed with time. In this paper, visual examination of the radiographs was carried out.

It was shown that changes in the appearance of the implant material can in fact occur over a protracted period of time, and that these changes are not necessarily complete 3 years after treatment. These changes, which mimic new bone formation with replacement of the implant material are inconsistent. In one individual wide ranges in response to the material were observed in different sites. These variations included exfoliation of the material, retention of the material which remained unchanged with time, retention of the material with changes in its appearance reminiscent of bone formation, and areas of degeneration all occurring concurrently in different regions of the mouth of this subject. In the second individual progressive absorption of the implant material

occurred. The implant material was replaced by trabeculated bone. Thus, a wide variety of radiographic responses have been observed.

The conclusions drawn from this paper should be interpreted with caution. Only two individuals are described in the paper, and the conclusions were drawn from visual interpretation of the radiographs. The resultant subjective interpretation of the radiographs relating to only two individuals in the study may be open to misinterpretation. However, observations described in this paper could be seen in the other individuals in the study. It has been noted that the histological sections also demonstrated a wide variation in cellular response to the implant material. Similar differences seen on the histological speciments may account for the differences noted radiographically.

The radiographic assessment of the individuals in the study therefore confirms the observations clinically, histologically and densitometrically. The clinical variables have shown that the implanted sites had better long-term healing than the non-implanted sites which was not significant except towards the end of the study and in the deepest defects. The radiographic assessment has shown a significant difference between implanted and non-implanted sites. Thus, although recurrent soft tissue disease may have occurred as assessed by the clinical variables, the underlying infrabony defects had significantly better infil in the implanted sites. This

would explain the lack of significance obtained from the clinical assessment data, in that recurrent periodontal diseases in these individuals may have been restricted primarily to the soft tissues. Recurrent periodontal pocketing due to gingival hyperplasia and increased probe penetration into the junctional epithelium could have occurred at both test and control sites; and thus any fill-in of defects resulting from the implant would be masked by the disease process affecting the soft tissues. Nevetheless, the clinical variables do indicate that the implanted sites heal better and are affected less by recurrent soft tissue disease over a longer period of time.

The radiographic study has a number of disadvantages. These include the fact that the densitometric analysis could only be carried out over the first two years of the study, and problems of assessment of radiographic changes in bone height occurred. Although the standardisation technique minimised the chances of differences occurring between serial radiographs due to placement or processing, clearly some changes in alignment did occur and some experimental regions had to be excluded from the study as a result. In addition, radiographs present two dimensional representations of three dimensional infrabony defects. Thus, what might appear to be defect infil on the radiographs may in fact only represent partial infil of one aspect of the defect. However, because the significant differences described in this chapter are in agreement with the re-entry data described by Yukna and his group (1985)

the conclusions drawn from this investigation are likely to be correct.

The radiographic response to the material has been shown to be variable when viewed visually. This is consistent with the variations in the histological responses to the material. Thus, a number of different cellular interactions are possible with the hydroxy-apatite implant material, and these different responses would explain the differences in the radiographic appearance of the material and the changes which take place with time.

2.5.3 Discussion of results - histological study

It has been shown in the review of the literature for this thesis that tricalcium phosphate and hydroxy-apatite degrade by different means, but previous workers have used different methods and models to assess the degradation of these materials. Comparisons are therefore difficult. Nevertheless, a variety of healing responses have been demonstrated by several workers using these materials.

Hydroxy-apatite has been shown to form some new bone which may be time dependent (Boyne & Flemming,1982). Workers like Moscow & Lubarr (1983) have shown no osteoid formation 8 weeks after placement of the graft material, while other workers (Sapkos, 1986; Ogilvy et al 1987; Barney et al 1986) have shown osteoid formation at up to 12 months after placement of the material.

Gameles et al (1986) have observed that histologically not all specimens show evidence of bone formation one year after placement implying either a different healing

response between subjects, or that bone formation may progress at different rates or in some cases not at all. In this histological investigation, some crystals have been associated with osteoid and bone formation while others within the same site have been merely encased in fibrous tissue. As has been pointed out in the literature review, the published histological studies are confusing as variations in results have been obtained by different study designs and different materials. Variations in the host tissue healing response have been described, some within the same defect (Ogilvy et al 1987), and others with time (Sapkos, 1986; Ogilvy et al 1987; Barney et al 1986), while other studies (Amler, 1987) have simply concluded that healing is unpredictable. In this investigation different rates of healing and different types of healing have been observed in various individuals, and in adjacent regions of the same defect. Indeed, different types of healing have even been observed on different surfaces of a single particle. It is not known what factors determine either the type or the rate of healing which takes place in the presence of these particles. As with the Amler study (1987) it cannot be determined if healing is by induction, increased regenerative mechanisms or irritation by the material which may determine the type of healing which is observed.

Ogilvy et al (1987) have described bone resorption by osteoclasts with vacuolation of the cytoplasm, and absorption of the particles. Absorption is clearly

observable on several particles described in the histological sections, but also within the same specimen, osteoid seems to be laid down on particles without any apparent particle absorption taking place. The factors which determine whether absorption or osteoid deposition occur in relation to hydroxy-apatite implant material are unknown, but as both processes can continue simultaneously in adjacent sites, it would imply that local factors either pertaining to the host healing response or surface characteristics of the particles are operative. The fact that different types of healing were observed on different surfaces of a particle may indicate that surface characteristics of the particle might be the initiating factors if the surface characteristics of the particles are not uniform. Whether this is the case is unknown, but degradation and bone deposition occurring simultaneously are a normal feature of the bone remodelling process with incremental lines of resorption and bone deposition seen in normal bone. These observations may therefore be demonstrating a normal physiological process which occurs naturally in bone.

Even in the presence of extensive inflammatory infiltrate the particles remain inert and do not enhance or contribute to the inflammatory response in any way. The inflammatory exudate clearly bypasses the particles, and it might therefore be inferred that the concept of irritation resorption (Amler et al 1987) or foreign body reactions to the particles with the production of foreign body giant

cells is probably not the case. This argument is strengthened, when observing the relatively early specimens taken from individual 1 which show only fibrous encapsulation with a total absence of any cells associated with inflammatory reactions. As this observation is in agreement with all of the studies published using this material, it would seem that what is being observed is a process whereby healing initially occurs by means of fibrous encapsulation of the implant material, and that the osteoconductive effect of the material results in a process not unlike bone remodeling with osteoclastic resorption and osteoblastic bone deposition occurring concurrently. However, inferences drawn from this histological investigation should be viewed with extreme caution because only three specimens were evaluated. Nevertheless, the specimens are from human material which is difficult to obtain and therefore only small numbers of samples are usually available. Taken in conjunction with other published work, a consistent picture emerges, which implies that the conclusions drawn from this histological investigation are valid. In addition, it should be borne in mind that histological results have limitations in that they present a picture at a single point of time, and no longitudinal assessment of the site is possible. In addition, the histological specimens are usually derived from sites of recurrent disease in the human subject, which is not necessarily indicative of the type of healing which may be expected within a normal physiologically healed

defect.

It is therefore impossible to make definitive statements based on the limited material available from only three human specimens which were obtained as a result of less than ideal clinical conditions requiring extraction of the teeth concerned. However, human histological material is extremely rare, and if the material is considered in conjunction with the extensive animal research which has been undertaken, some tentative conclusions can be drawn:-

1) Healing takes place by means of fibrous encapsulation initially, and induction of bone formation occurs only after severals months have elapsed.

2) When bone formation is initiated, it is associated with concurrent resorption and bone deposition, not unlike the normal physiological process of bone remodelling.

3) When bone formation does occur, it does not progress at the same rate uniformly throughout a site, or at the same rate in different regions of the same specimen. In some areas bone formation may not occur at all.

4) The particles are inert, even in the presence of inflammatory infiltrate from concurrent infection. This implies that the changes in the particles with time are probably as a result of normal osteoclastic and osteoblastic activity rather than foreign body reactions to the material itself.

5) Not withstanding these observations, the

placement of these materials clinically results in healing patterns which are unpredictable due to inadequate material retention in the site, and excessive recession of the overlaying gingival soft tissue after surgery. This results in variable gain in clinical attachment levels and variations in the formation of new bone in the sites which receive the implanted material.

2.6 INTERPRETATION OF RESULTS

It therefore would seem that the ability of these materials to facilitate healing is variable and probably only possible in the deepest infra-bony defects. This would explain the unpredictable results cited in other studies, and the lack of significant differences between sites receiving the implant material and those which did not in this clinical study.

The histological investigation has highlighted the inconsistency of the tissue response to the implanted granules of implanted material, and the variations in cellular responses within individual sites. This may go some way to explaining the variability in results obtained by means of the clinical assessment variables. The longitudinal assessment of the changes occurring in the infrabony defects using standardised radiographs, taken at regular intervals throughout the study, concur with the observations of the histological specimens, in that the tissue responses to the implanted material were shown to be variable, even in different sites within the same individual.

Consistently significant clinical differences could not be established because of variable soft tissue responses, which included excellent healing at some sites, and recurrent periodontal disease at others during the time period under investigation. Thus, a wide variation in the healing responses to this material may occur.

2.7 OVERALL CONCLUSIONS

It is concluded that, although hydroxy-apatite is a useful infil material which results in better healing and long-term stability when placed in deep infrabony defects, a wide variation in the healing responses to this material may occur. These variations may be attributable to recurrent periodontal destruction, or ingress of epithelium into healing defects, precluding regeneration of a new periodontal ligament. Improved results might be possible if the soft tissue epithelium and gingival fibroblasts could be excluded from the healing wound. This would facilitate maturation of the granulation tissue and the implanted material within the defects. This might be achieved by using a barrier membrane interposed between the defect and the overlaying soft tissue flap prior to closure of the surgical wounds.

CHAPTER 3

THE MEMBRANE STUDY

3.1 INTRODUCTION

A review of the literature (Chapter 1) relating to regenerative techniques in periodontal treatment has shown that the response to both synthetic and non-synthetic implant materials has been limited and variable. The histological and radiographic investigations of human tissue described in Chapter 2 have shown that variations in the cellular interactions with the particles of synthetic implant materials occur within a single site and this is in agreement with other published work using animal models.

The guided tissue regeneration technique described in Chapter 1 has also been shown to result in limited and variable results (Gottlow et al 1986, Magnusson, 1990). A number of different membrane materials have been studied, in different animal models, and comparisons between studies are therefore difficult. Most of the published work relates to the use of non-degradable membranes for this purpose. It was therefore decided to develop a membrane for use in the guided tissue regeneration technique which would be biodegradable and suitable for use in human subjects with a view to devising a composite technique incorporating implantation with hydroxy-apatate material and guided tissue regeneration.

Little is known of the patterns of healing and the histological responses to the placement of membranes in tissue. In view of the variation in clinical results

reported with different membranes, it was decided to investigate the tissue responses to a variety of membranes and to compare these tissue responses with those already known to occur with the synthetic bone implanting materials. It would be highly desirable if those membranes could be studied in relation to periodontal defects in human subjects using the guided tissue regeneration technique, but, for practical and ethical reasons, this experimental procedure was considered to be premature. An animal model was therefore used. As the study of tissue healing intra-orally is complicated by the presence of saliva, food, dental plaque, functional forces and root surface characteristics, it was decided to use an extra-oral site where these complicating factors do not exist. In view of the number of different materials under investigation the dorsal surface of the rat was selected as the most appropriate model for this investigation.

3.2 AIM OF THE STUDY

The aim of this study was threefold:-

(1) To evaluate longitudinally the healing responses to a number of membranes and to examine the progression of the healing process associated with the transcutaneous materials.

(2) To establish if any differences in the healing response could be attributed to the characteristics of the materials under investigation.

(3) To ascertain the differences in tissue response between the degradable and non-degradable materials under investigation.

3.3 STUDY DESIGN

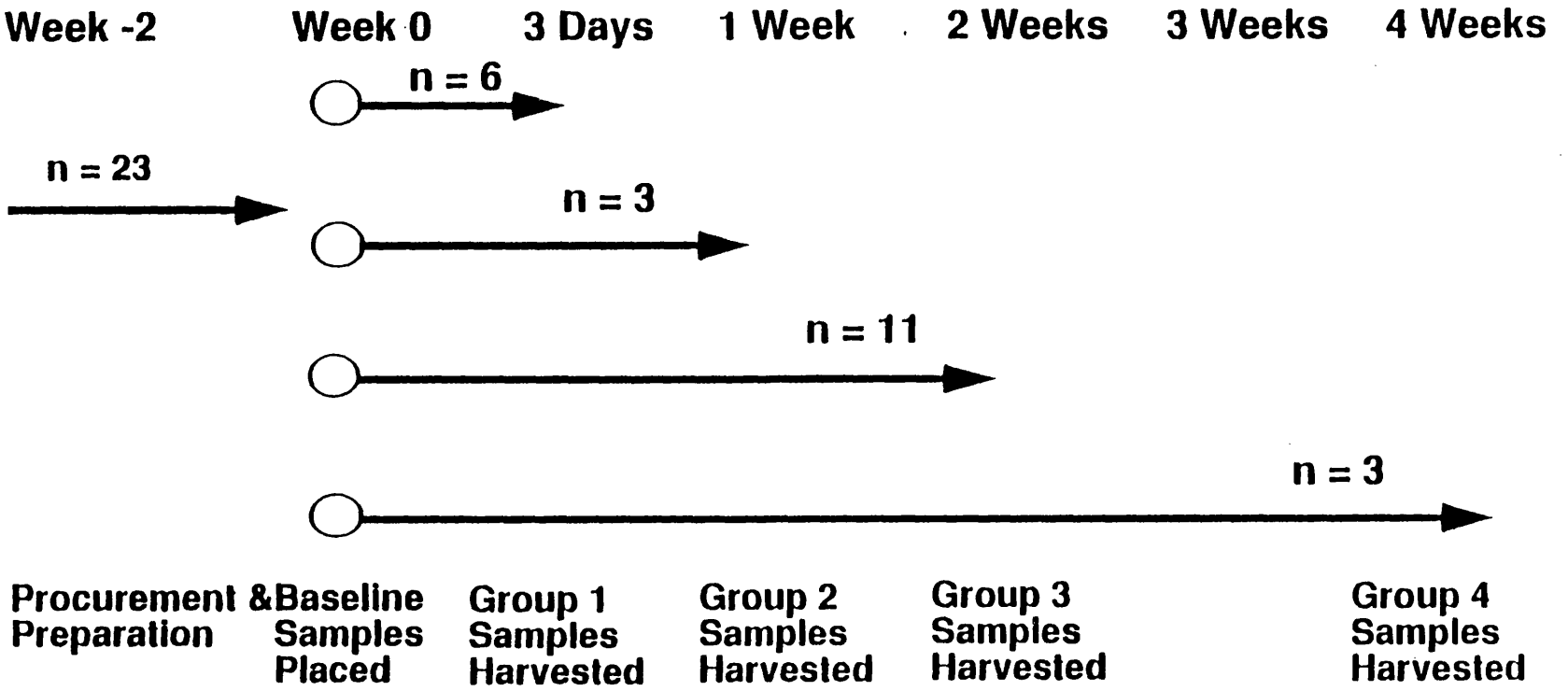
The study took place over a period of four weeks, four groups of rats were used in accordance with the protocol shown in Table 3.a.

3.4 MATERIALS AND METHODS

Twenty-three adult male Wistar rats weighing between 200-300 gms each were obtained and placed in separate cages. Adjustable plastic collars were placed on the animals for a period of 10 days prior to the start of the study and retained during the study so as to prevent the animals from scratching the surgical sites after placement of the materials.

The animals were anaesthetized with Hypnoral dispensed at the rate of 0.05ml/100gms weight (fentanyl citrate 0.315 mg/ml and fluanisone 10mg/ml). Approximately 0.35ml was

TABLE 3.a STUDY DESIGN FOR THE MEMBRANE STUDY IN RATS



therefore administered to each animal to achieve anaesthesia. Once general anaesthesia had been achieved, the fur on the dorsum of each animal was shaved to expose the skin. The skin surface was sterilised with 0.5% chorhexidine in alcohol solution, and transcutaneous incisions were made 1 cm on either side of the spinal vertebrae, parallel to the long axis of each animal in order to receive the test materials as shown in Fig.3.1. The materials were placed transcutaneously and the wounds were sutured with 4/0 braided silk suture material to form crossover mattress sutures over the test materials. The materials were prepared in a T-shape as shown in Fig.3.2 and placed in the wounds so that the horizontal section of the membrane protruded externally and prevented the vertical section from being dislodged from the wound during healing. The crossover mattress sutures were placed over the protruding section so as to hold it in tight apposition to the skin surface stabilising the membrane in situ.

The animals were divided into groups consisting of 3 animals each for the 1 week and 4 week investigations, 6 animals for the 3 day investigation, and a group of 11 animals for the 2 week investigation as illustrated in the study design (Table 3.a). More animals were assigned to the three day and two week groups as a number of additional materials were included for evaluation at these stages.

Prior to each surgical procedure all of the samples under investigation were randomly assigned to one of the eight incisions on each animal allowing for a control site

Fig. 3.1 TO ILLUSTRATE THE SURGICAL PLACEMENT OF THE MEMBRANES

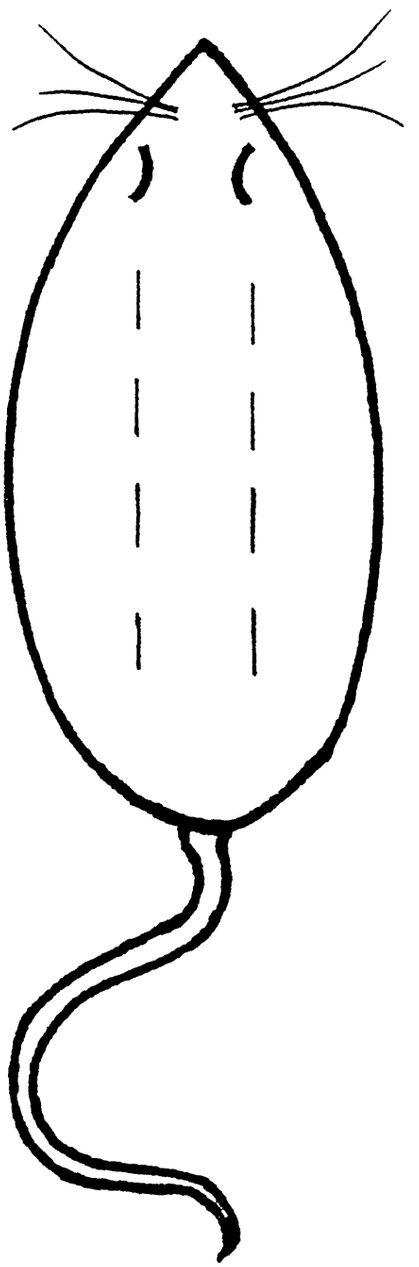
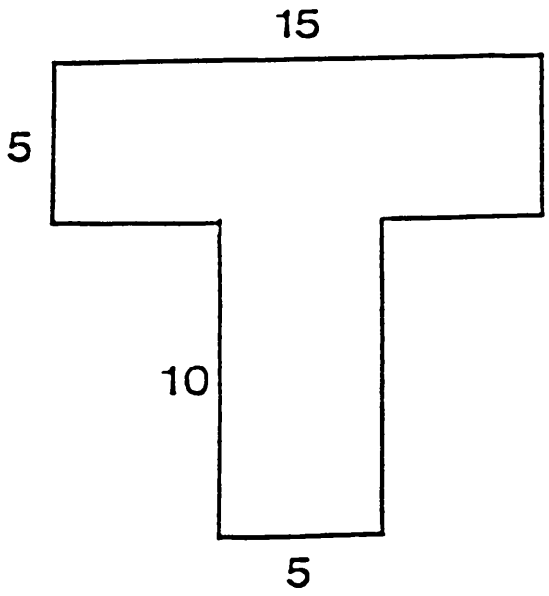


Fig. 3.2 DIMENSIONS OF IMPLANT SPECIMENS IN MILLIMETRES



which received no membrane on each animal. This site was designated the "sham" site in each case.

The materials were left *in situ* for the designated time period at which time the animals were placed in a CO_2 chamber. After sacrifice the dorsal skin was dissected free, and cut into individual pieces containing the membranes to enable samples to be taken for histological evaluation as shown in Figure 3.3. The medio-posterior corner of each specimen was cut off so as to retain the orientation of the specimen during preparation. The specimens were placed in 10% formalin solution, and subsequently embedded in wax blocks. Serial sections were cut from each block, ignoring the first 10 cuts. Subsequently, every 10th cut was mounted for staining with haematoxylin and eosin tissue stain. In addition, specimens of the 3 day, 1 week and 4 week periods were stained with Van Gieson's stain for collagen to highlight the maturation of collagen within the healing wounds.

A number of materials were evaluated in the study and these included:

PTFE Millipore filter (Millipore, Harrow, UK)

Gore-Tex Periodontal Membrane (Gore Assocs. Arizona, USA)

Polylactic acid membrane (PLA)

Polyhydroxy butyrate/valerate copolymer P(HB-HV)

Dexon Polyglycolic acid copolymer membrane (Cyanamid, UK)

Zenoderm Bovine collagen membrane (Ethicon, Edin.UK)

Fig. 3.3 TO ILLUSTRATE THE CUT SPECIMENS PRIOR TO PROCESSING FOR HISTOLOGICAL INVESTIGATION

The biodegradable polymer membranes were produced by the biomaterials department of Queen Mary College London in 3 separate thicknesses, viz: 0.02 mm, 0.04 mm and 0.07 mm by dissolving the polymers in a chloroform solvent solution and casting the resulting solution onto freshly cleaved, anatomically clean mica. Controlled evaporation was achieved by placing the specimens in a vacuum chamber maintained at room temperature (22°C). The resulting films were then cut into T-shaped pieces illustrated in diagram 3.2. Each piece was packaged and sealed and sterilised prior to surgery. Additionally, a number of other biodegradable materials were assessed only at the two week period to see if the tissue reactions differed markedly between different biodegradable materials. These materials included three samples each of collagen membrane, commercially available polyglycolic acid copolymer membrane, polyhydroxy butyrate/valerate copolymer membrane and polyactic acid membrane with altered surface characteristics to modify the surface free energy of the material.

Prior to the start of the study, the materials were characterised in the laboratory (Galgut et al 1991) to establish the surface hydrophobicity/hydrophilicity of each material as this might affect the cell adhesion and growth properties on the materials. The membranes were prepared and characterised at the biomaterials department (Queen Mary College) prior to the animal study, to ensure that the specifications of size, thickness, purity and sterility

were attained.

3.5 RESULTS

3.5.1 Characterisation of test materials

The contact angles of each material were determined by the research assistant designated to this project (Dr. R. Pitrola), and the surface free energy of each material was calculated by measurement of the angles formed by a number of hydrophobic and hydrophilic liquids placed on the surface of each sample. The results of this calculation are presented in Table 3b which shows the total surface free energy, and also the dispersion and polar components of the total energy. From this table it can be seen that the total surface free energy, as well as the polar and dispersion components were higher for the biodegradable polymers than for the non-biodegradable PTFE materials. This indicates that the biodegradable polymers are more hydrophilic than the PTFE materials. The polar component of surface free energy is directly related to the hydrophobicity/hydrophilicity of the material under investigation. It can be seen that the polar component is highest for PLA, less with P(HB-HV) and very low with the PTFE materials. Molecular weight made no difference to the surface free energey of the substance being evaluated. Thus, it would be anticipated that the best cellular adhesion would occur with the most hydrophilic material, which was PLA, and the poorest cell adhesion would be associated with the most hydrophobic materials which were PTFE. Cell adhesion might be less good with the P(HB-HV)

TABLE 3.b SURFACE FREE ENERGIES OF THE VARIOUS POLYMERS (FOR SMOOTH SURFACES)

	SURFACE FREE ENERGY (mJm^{-2})		
POLYMER	DISPERSION COMPONENT	POLAR COMPONENT	TOTAL
PLA[a]	32.5 ± 2.0	5.0 ± 0.4	37.5 1.8
PLA[b]	33.7 ± 1.7	5.2 ± 0.3	38.9 1.4
expanded PTFE	18.1 ± 1.0	0.4 ± 0.2	18.5 0.8
PTFE[c]	18.6	0.5	19.1
P(HB-HV)	36.9 ± 1.5	3.4 ± 0.7	40.3 1.0

[a]Poly (L-lactic acid) of molecular weight 50,000 g mol^{-1}.
[b]Poly (L-lactic acid) of molecular weight 200,000 g mol^{-1}.
[c]Derived from the literature (Owens & Wendt 1969).

polymer than the PLA polymers as the polar component of the surface free energy of this material was less than that of PLA.

3.5.2 Surgical results

In this investigation the wounds healed with the formation of a small blood clot, and with no obvious signs of infection. Most sites healed with close approximation of the wound margins; in a small proportion of sites there was a loss of the sutures, or separation of the wound margins or both. No pattern of loss of sutures, or wound opening could be seen. With time, wound closure occurred in all of the samples as assessed visually, but this occurred at different rates. In some samples, the wounds were virtually healed by 1 week after placement, while in other samples, wounds were still open with a bloodclot at this stage. By four weeks post-operatively most, although not all, of the wounds had virtually healed. This made accurate determination of the surgical sites difficult. In addition, the fur had re-grown, and the India Ink marked sites had in many cases faded. Thus, the harvesting of specimens at the 4-week period was less easy than in the early stages of the study.

At each time period during the study, some animals demonstrated faster healing than others, some lost more of their sutures while others lost hardly any, and in some, wounds tended to open more readily than others. Thus, variations between animals were observed in terms of the

post-operative sequelae of the placement of the membranes.

3.5.3 Histological results

The results of the histological evaluation are illustrated in Tables 3c-3m. Each table lists the responses to each material under investigation for the different time periods evaluated.

3.5.3.1 Loss of samples from the surgical sites

Table 3c records the number of samples lost at each time period. From this table it would seem that no clear trend emerges between the various materials. Initially more Gore-Tex expanded PTFE membranes (36%) were lost, than biodegradable PLA (7%, 23%). At the end of the study, more Gore-Tex samples were <u>in situ</u> (80%) than PTFE filter (33%), which may indicate a different host response between the two types of PTFE. The large percentage of PLA membranes lost (60% of each sample) at the end of the study by comparison to the Gore-Tex membranes was probably partially due to absorption of the material rather than loss from the sites. Nevertheless, the great variation between results for the two thicknesses of the material in the intervening intervals should be noted.

3.5.3.2 Tissue response

Table 3d records the tissue response to the materials at each time period in terms of epithelial downgrowth into the wound which might indicate attempts to exfoliate the material, or fibrous condensation which might indicate incorporation of the material into the tissues.

Initially 44% of control (sham) sites demonstrated

TABLE 3.c TO ILLUSTRATE THE PERCENTAGE SAMPLES LOST FROM SURGICAL SITES FOR EACH MATERIAL AT EACH TIME INTERVAL DURING THE STUDY

MATERIAL	3 DAYS	1 WEEK	2 WEEKS	4 WEEKS
SHAM	-	-	-	-
GORE	36% (4/11)	60% (3/5)	67% (4/6)	20% (1/5)
FILTER	-	0% (0/3)	80% (4/5)	67% (2/3)
PLA 02	7% (2/12)	0% (0/3)	20% (3/15)	60% (3/5)
PLA 04	23% (3/13)	0% (0/3)	66% (4/6)	60% (2/3)

(Figures in brackets indicate actual numbers of samples from which the percentages were derived).

TABLE 3.d TO ILLUSTRATE THE PERCENTAGE OF SAMPLES DEMONSTRATING EPITHELIAL DOWNGROWTH OR FIBROUS CONDENSATION FOR EACH MATERIAL AT EACH TIME INTERVAL DURING THE STUDY

MATERIAL	EPITHELIAL DOWNGROWTH				FIBROUS CONDENSATION			
	3 DAYS	1 WEEK	2 WEEKS	4 WEEKS	3 DAYS	1 WEEK	2 WEEKS	4 WEEKS
SHAM	44% (4/9)	25% (1/4)	0% (0/2)	33% (1/3)	0% (0/9)	0% (0/4)	0% (0/8)	0% (0/3)
GORE	9% (1/11)	20% (1/5)	50% (3/6)	80% (4/5)	91% (10/11)	20% (1/5)	50% (3/6)	40% (2/5)
FILTER	-	67% (2/3)	40% (2/5)	33% (1/3)	-	33% (1/3)	20% (1/5)	33% (1/3)
PLA 02	87% (8/12)	67% (2/3)	7% (1/15)	20% (1/5)	75% (9/12)	100% (4/4)	7% (1/15)	20% (1/5)
PLA 04	62% (8/13)	50% (2/4)	0% (0/6)	0% (0/5)	69% (9/13)	100% (4/4)	17% (1/6)	40% (2/5)

(Figures in brackets indicate actual numbers of samples from which the percentages were derived).

epithelial invagination of the wound which varied at each time period, reducing to 33% by the end of the study. These observations show great variation at each time interval.

The PTFE membranes demonstrated different tissue responses. The percentage of Gore-Tex membranes which demonstrated epithelial downgrowth was small initially (9%), but increased to 80% by the end of the study. The Millipore filter, however, showed a reducing percentage of samples with epithelial downgrowth throughout the study, and a consistently reduced tendency for fibrous tissue deposition.

The two different thicknesses of PLA showed similar responses in that the majority of samples showed epithelial downgrowth and fibrous tissue deposition, which reduced with time.

The reduction in epithelial downgrowth with time was probably due to absorption and/or exfoliation of the biodegradable membrane in the superficial part of the wound with subsequent healing, which could not occur with the Gore-Tex material. The Gore-Tex material, by contrast, showed increasing numbers of sites with epithelial downgrowth, possibly indicating a tendency for long-term exfoliation by epithelial downgrowth.

Once again the great variation in responses to materials at each time interval should be noted.

3.5.3.3 Cellular response

Table 3e shows the type of cellular responses which were observed at each time period subdivided into monocytic

and polymorphic responses.

It can be seen that the control sites demonstrated a high incidence of both monocytes and polymorphic nucleocytes initially which progessively reduced with time, to a complete absence by the end of the study.

In response to both the absorbable polymers and the non-absorbable PTFE membranes, a high monocytic response occurred initially which remained high, albeit with some reduction throughout the study. The high residual monocytic response to the degradable materials can be attributed to continuing absorption of the material with time. The Millipore filter demonstrated no polymorphic response throughout the study, while the Gore-Tex material had a marked polymorphic infiltrate (73%) initially which was still evident at the end of the study (40%). This may indicate better tolerance of the Millipore material by the tissues. The Gore-Tex PTFE material is not absorbable so that the high levels of monocytes and polymorphic nucleocytes remaining at the end of the study is possibly indicative of irritation of the tissues by the membrane itself.

The polymorph response was expected in so far as the majority of sites had polymorphic cells present initially which reduced with time, except in the case of Gore-Tex PTFE. The polymorphic cells remained present in a large percentage of Gore-Tex samples throughout the study period. This would indicate prolonged acute inflammation in these sites, possibly due to irritation from the material itself,

TABLE 3.e TO ILLUSTRATE THE TYPE OF CELLULAR RESPONSES ASSOCIATED WITH EACH MATERIAL AT EACH TIME INTERVAL DURING THE STUDY

MATERIAL	MONOCYTES				POLYMORPHONUCLEOCYTES			
	3 DAYS	1 WEEK	2 WEEKS	4 WEEKS	3 DAYS	1 WEEK	2 WEEKS	4 WEEKS
SHAM	89% (8/9)	50% (2/4)	-	0% (0/3)	67% (6/9)	50% (2/4)	-	0% (0/3)
GORE	82% (9/11)	100% (5/5)	50% (3/6)	60% (3/5)	73% (8/11)	20% (1/5)	50% (3/6)	40% (2/5)
FILTER	-	100% (3/3)	40% (2/5)	33% (1/3)	-	0% (0/3)	20% (1/5)	0% (0/3)
PLA 02	92% (11/12)	100% (3/3)	53% (8/15)	60% (3/5)	58% (7/12)	100% (3/3)	20% (3/15)	0% (0/5)
PLA 04	77% (10/13)	100% (3/3)	33% (2/6)	80% (4/5)	54% (7/13)	67% (2/3)	17% (1/6)	0% (0/5)

(Figures in brackets indicate actual numbers of samples from which the percentages were derived).

TABLE 3.f TO DEMONSTRATE THE ABSORPTION CHARACTERISTICS OF THE PLA POLYMER WITH TIME

MATERIAL	NON ABSORPTION				MIN ABSORPTION				50% ABSORPTION				TOTAL ABSORPTION			
	3 DAY	1 WK	2 WKS	4 WKS	3 DAY	1 WK	2 WKS	4 WKS	3 DAY	1 WK	2 WKS	4 WKS	3 DAY	1 WK	2 WKS	4 WKS
PLA 02	8% (0/3)	0% (0/3)	0% (0/15)	0% (0/5)	33% (4/12)	0% (0/3)	53% (0/3)	0% (0/5)	33% (8/15)	100% (3/3)	46% (7/15)	20% (1/5)	0% (0/12)	0% (0/3)	0% (0/15)	80% (4/5)
PLA 04	7% (1/13)	0% (0/3)	0% (0/6)	0% (0/5)	15% (2/13)	0% (0/3)	50% (3/6)	0% (0/5)	15% (2/13)	100% (3/3)	33% (2/6)	60% (3/5)	23% (3/13)	0% (0/3)	0% (0/6)	40% (2/5)

(Figures in brackets indicate actual numbers of samples from which the percentages were derived).

or else a tendency for secondary infection to occur in the surgical wounds. As this was not a characteristic of the Millipore PTFE filter, it is assumed that the polymorphic response was due to the physical characteristics of the Gore-Tex material itself, rather than to the non-absorbability, or surface free energy of the material.

3.5.3.4 Absorption characteristics

In Table 3f the absorption characteristics of the PLA polymer was evaluated.

It is apparent from this table, that many of the samples were undergoing absorption as early as 3 days after placement with less than 10% of samples showing no absorption at this time. Absorption of up to 50% of the body of the material occurred in all of the samples evaluated at 1 week with many samples only reaching the 50% absorption mark by the second week period. 80% of PLA 02 samples and 40% of PLA 04 samples had been fully absorbed by the four week post-operative period.

Careful examination of the table shows that while all of the samples were 50% absorbed by the 1 week period, 20% of PLA 02 and 60% of PLA 04 samples were not fully absorbed by the end of the study. This difference between the two materials in the latter part of the study could be explained by differences in the material. As both of the materials were identical, except in their thicknesses, this is unlikely. It is more likely that this disparity is due to a chance error as a result of small numbers of samples, or alternatively due to the variation in healing responses

occurring in different sites observed before.

3.5.3.5 Other materials

As part of this study a number of other materials were evaluated at the two week period. These materials included PLA 0.07 mm thick and PLA 0.04mm thick with its surface modified to reduce the surface free energy. Neither of these materials demonstrated markedly different healing characteristics from the PLA in the main study, except in so far as the PLA 0.07 tended to be retained in situ without absorption for a longer period than the thinner material. Other copolymers included polhydroxy butyrate/valerate and polyglycolic acid/polylactic acid (available commercially as Dexon Cyanamid UK) were also investigated. These polymers demonstrated healing responses which were indistinguishable from the PLA membranes. An organic biodegradable membrane made from bovine collagen (Xenoderm, Ethicon, Edinburgh, UK) was included for preliminary investigation but handling characteristics of the material and an excessive tissue response precluded its further use.

Thus, although a number of other materials were also investigated in this study, the lack of advantageous responses compared to polylactic acid membranes, handling characteristics or poor tissue tolerance resulted in their elimination from the main study without further investigation or evaluation.

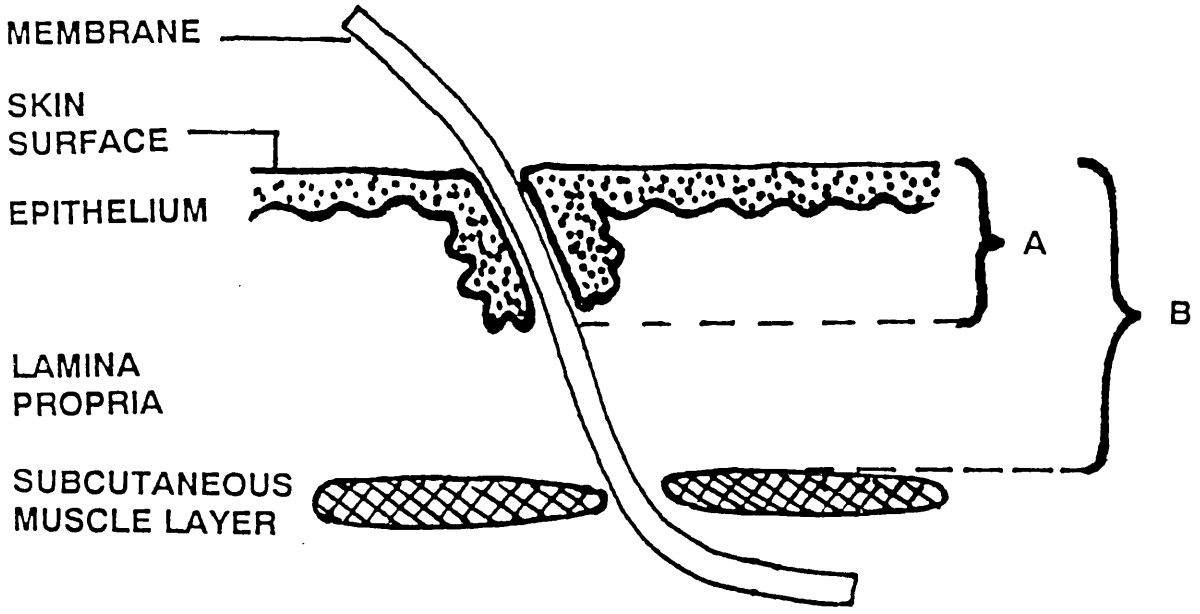
3.5.3.6 Variations between samples

A constant feature of the analysis has been a degree

of variation which was observed between samples. A further, more detailed investigation of epithelial invagination, exfoliation/loss of membrane samples, presence or absence of fluid exudate at the wound surface, and tissue maturation was undertaken to see if any discernable differences were observable between the different membrane materials at each time interval during the study.

In Table 3g it can be seen that distances of epithelial migration for each material at each time period were assessed. This assessment was achieved by counting the number of squares in a graticule placed in the microscope eyepiece and applying the formula A/B x 100 = %, where A represented epithelial downgrowth, and B represented the full thickness of the surgical wound as far as the subepithelial muscular layer (see Fig. 3.4). At 3 days the greatest ingress of epithelium into the wounds was seen with the Gor-Tex material (79%). By comparison, there was slightly more than 50% epithelial migration in the two PLA absorbable materials. By 1 week after placement the amount of ingrowth of epithelium had decreased in the Gore-Tex while the PLA samples remained unchanged. The samples of Gore-Tex 4 weeks after surgery also showed relatively less epithelial downgrowth. As the Millipore PTFE was not showing the same reduction in epithelial downgrowth at the 1-week period, it is possible that the marked reduction seen with the Gore-Tex material was in fact a chance finding due to the small number of samples, rather than a

Fig. 3.4 TO ILLUSTRATE THE METHOD OF CALCULATION OF EPITHELIAL DOWNGROWTH



A= EPITHELIAL DOWNGROWTH
B=TOTAL DEPTH OF EPITHELIUM TO SUBCUTANEOUS MUSCLE LAYER

TABLE 3.g TO SHOW THE DISTANCE OF EPITHELIAL DOWNGROWTH INTO THE WOUND AS A PERCENTAGE OF THE TOTAL THICKNESS OF THE TISSUE ABOVE THE SUBCUTANEOUS MUSCLE LAYER

MATERIAL	EPITHELIAL INVAGINATION			
	3 DAYS	1 WEEK	2 WEEKS	4 WEEKS
SHAM	56% n=8 (0-86)	25.3% n=2 (13-38)	44% n=2 (-)	 n=1 (-)
GORE	79% n=8 (22-93)	28.1% n=3 (20-33)	48.2% n=16 (5-100)	53.8% n=3 (39-67)
FILTER		61.4% n=3 (42-83)		50% n=1 (-)
PLA 02	58% n=11 (18-86)	59.5% n=3 (43-67)		18.8% n=2 (10-33)
PLA 04	65% n=10 (40-100)	64.9% n=3 (40-95)	49.8% n=12 (30-72)	41.7% n=1 (-)

(n may be more than the sample number if different effects on both sides of the membrane gave 2 readings.)

(Figures in brackets indicate the range.)

real difference in responses. This interpretation would tend to be supported by the fact that epithelial downgrowth increased again by the 4-week period to a level not dissimilar to that of the PTFE filter. Alternatively this pattern does tend to follow that of the sham sites which might indicate that this pattern of epithelial downgrowth reducing between three days and 1 week after placement, and then increasing with time, is a true reflection of what occurs. However, there are even fewer samples in the sham sites which were amenable to this analysis, so that these observations must be interpreted with caution.

As noted previously, a wide range in variation between samples was observed throughout the study, in the sham operated sites as well as all of the sites which received the membranes. This emphasizes that the preponderance of variations observed previously were due to site or subject variations which include different rates of healing, or factors like wound infection or trauma, which may cause delayed healing, rather than the nature of the materials placed in the wound. Therefore it can be concluded that the majority of the epithelial downgrowth occurs within 3 days of placement of the material, and this reduces with time. A greater percentage of downgrowth occurs with the Gore-Tex material initially, than with the biodegradable materials, or the sham operated sites. The biodegradable materials show similar amounts of downgrowth to the sham operated sites which received no membrane, possibly indicating that no interference with the wound healing

characteristics in relation to the epithelial tissue response occurs with these materials.

Table 3h describes the percentage of samples which lost the membrane sample (due to exfoliation or absorption) for each time period in the study. It can be seen that about 50% of the membranes were absent by 3 days after placement. By one week, all of the absorbable materials were showing absence of some or all of the material. When these materials were lost, the histological features were typical of those described earlier in this chapter with retained fragments in the deeper regions of the tissues and superficial exfoliation or absorption. Half of the Gore-Tex samples were lost, while none of the filter PTFE samples were absent, again highlighting the differences in the tissue responses to these two forms of PTFE. By 2 weeks post-operatively, 67% of the Gore-Tex samples and 80% of the Filter PTFE samples had been lost, demonstrating the progressive loss of these non-degradable membranes. The biodegradable polymers show that 50% of the membranes were totally lost from the sites by 2 weeks. This is in contrast with the one-week results which demonstrated loss of all the membranes. This disparity in results cannot be interpreted due to the small number of samples available for assessment, but possible explanations may include chance observations, varying rates of healing between different animals, or variations in the harvesting and processing of the specimens. By four weeks after placement, loss of some samples of all of the materials had

TABLE 3.h **TO COMPARE THE NUMBER OF SITES WITH LOSS OF MEMBRANE FOR EACH MATERIAL AT EACH TIME PERIOD DURING THE STUDY, CALCULATED AS A PERCENTAGE OF THE TOTAL NUMBER OF SITES BEING ASSESSED**

MATERIAL	TIME PERIOD			
	3 DAYS	1 WEEK	2 WEEKS	4 WEEKS
SHAM				
GORE	45.5% (5/11)	50% (3/6)	66.7% (4/6)	100% (6/6)
FILTER	-	0% (0/0)	80% (4/5)	100% (3/3)
PLA 02	54.5% (6/11)	100% 4/4	20% (3/5)	100% (6/6)
PLA 04	50% (6/12)	100% 3/3%	50% (3/6)	100% (6/6)

occurred in all cases. Thus it can be seen that the majority of the absorption of the biodegradable materials takes place in the early stages (within 3 days of placement) while the non-absorbable materials take longer to be removed from the wounds (by whatever means - exfoliation, fragmentation, or absorption).

Another factor which may play a part in the differences observed in the epithelial migration noted on each side of the membranes is the angulation of the samples to the surface of the wound. Due to the flexibility of the materials, and the angulation of the tissue on the dorsum of the animals, only very few samples were placed so as to transept the surgical sites vertically. Thus the epithelium abutted the materials at an acute angle (towards the midline) and an obtuse angle (laterally) as shown in Figure 3.5 and this may have affected the migration characteristics of the membrane. Table 3i shows the results of epithelial downgrowth assessments on the sides of the membranes at acute and obtuse angles to the epithelial surfaces. In this table, no consideration was given to the time periods at which the measurements were taken, because by this stage in the analysis it was clear that epithelial migration was not a function of time, but rather a manifestation of different migration rates in different animals or sites in the same animal. The greatest amount of epithelial migration occurred in those few samples which were placed at 90° to the surface of the wound, with less migration associated with those at an

Fig. 3.5 TO DEMONSTRATE THE METHOD OF DETERMINING THE ANGULATION OF ENTRY OF THE MATERIAL IN RESPECT OF THE SURGICAL WOUND

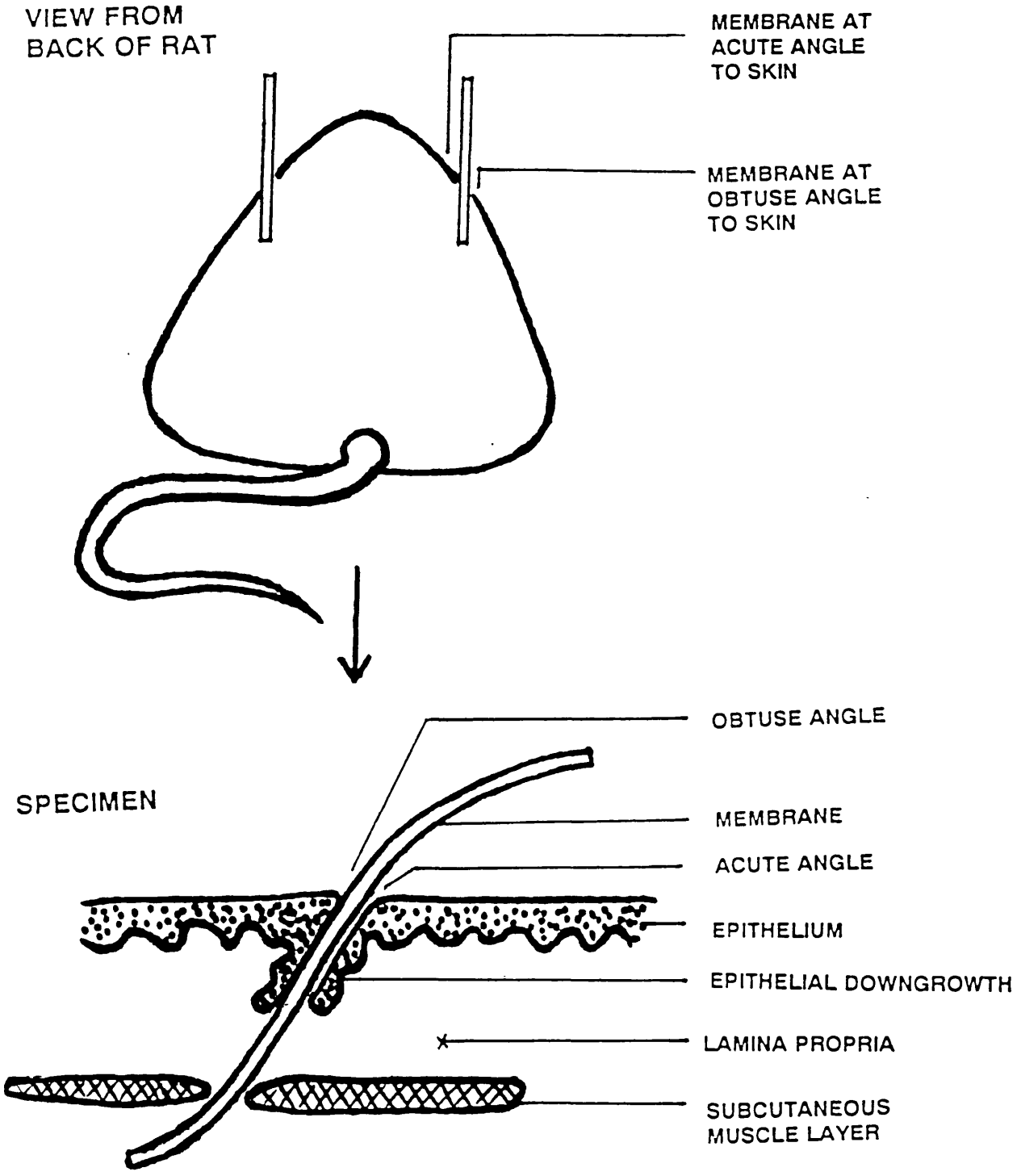


TABLE 3.I **TO DETERMINE IF ANGULATION OF THE MEMBRANES TO THE WOUND SURFACE RESULTED IN DIFFERENCES IN EPITHELIAL DOWNGROWTH**

MATERIAL	ACUTE ANGLE	OBTUSE ANGLE	90° ANGLE
SHAM	80.4% (n = 3)	74.7% (n = 3)	-
GORE	72.2% (n = 10)	74.1% (n = 11)	94.6% (n = 1)
PLA 02	65.9% (n = 9)	70.4% n = 11)	91.0% (n = 2)
PLA 04	54.1% (n = 8)	78.3% (n = 10)	54.8% (n = 2)
FILTER	74.6% (n = 3)	97.3% (n = 3)	-
OVERALL MEAN **SD** **n**	64.9 16.26 33	72.6 19.38 38	80.1 22.01 5

obtuse angle and the least migration with those at an acute angle. The relatively large standard deviation associated with these mean values emphasizes the wide variation in actual downgrowth which was observed when individual materials were assessed. Although all of the materials showed less downgrowth of epithelium on the acute angled surfaces, a greater percentage of downgrowth occurred with both types of PTFE non-degradable membranes than with the PLA degradable membranes. A high percentage of epithelial migration is also observed in the sham control sites which received no membranes. This indicates that in a surgical wound, where the epithelial margins are not in close apposition, extensive epithelial migration down the cut surfaces of the wound occurs until wound closure is achieved, after which infil of the underlying lamina propria occurs as a secondary healing process. Within this context, it should be noted that similar epithelial migration occurred with both of the non-absorbable PTFE samples and noticeably less migration occurred with the unmodified PLA, especially when the membrane was at an acute angle to the wound surface.

The biodegradable membranes were produced by the solution casting method. Slight variations in the surfaces of the membrane might be anticipated in respect of the side which was on the mica casting surface, and the side that was exposed to air during casting. As these surface effects might account for some of the variation observed previously due to different cell adhesion characteristics

on different surfaces, it was decided to investigate whether or not the air side of the membranes showed greater epithelial downgrowth than the mica side. The sections were observed under a low power, in a light microscope which was fitted with a measuring graticule. The results of this investigation are presented in Table 3j which shows the percentage of epithelial downgrowth for each material at each time period throughout the study, subdivided into the upper (air side) and the lower (mica side) surfaces.

Initially the sham (control) incisions showed more downgrowth on the "upper" surface than the "lower" surface after which the situation was reversed, the differences between the two surfaces being 12.5% and 17.6% respectively. As no membranes were present, this could be interpreted as approximating the normal range of variation which might be expected in a healing wound.

All of the membranes showed differences between the upper and lower surfaces which were initially opposite to those of the sham sites. The membrane sites showed less downgrowth on the upper surface (air side) than the lower surfaces (mica side), but the range of difference was 5.2% (Gore-Tex), 12% (PLAO2) and 9.5% (PLAO4) respectively which is lower than the range of differences between the two wound margins of the sham sites, and therefore not likely to be significant. By 1 week after placement, the Gore-Tex membrane was showing more downgrowth on the upper side than the lower side (15.6%) although these differences were again reversed at two weeks (11.2%) and at 4 weeks (5.3%).

TABLE 3.j TO ESTABLISH IF DIFFERENT DEGREES OF INVAGINATION OF EPITHILIAL CELLS WERE OBSERVABLE ON EITHER SIDE OF THE MEMBRANE

MATERIAL	UPPER SURFACES (AIR SIDE)				LOWER SURFACE (MICA SIDE)			
	3 DAYS	1 WEEK	2 WEEKS	4 WEEKS	3 DAYS	1 WEEK	2 WEEKS	4 WEEKS
SHAM	86.4% (n = 14)	42.9% (n = 8)	50% (n = 2) *	None - Full Healing	73.9% (n = 14)	60.5% (n = 8)	36.4% (n = 2) *	None - Full Healing
GORE	75.3% (n = 23)	58.1% (n = 8)	42.2% (n = 16)	51.4% (n = 8)	80.5% (n = 23)	42.5% (n = 8)	53.4% (n = 16)	46.1% (n = 8)
FILTER		76.3% (n = 5)		76.7% (n = 2) *		93.2% (n = 5)		100% (n = 2) *
PLA 02	77.1% (n = 22)	57.8% (n = 8)		37.5% (n = 2) *	89.1% (n = 22)	80.2% (n = 8)		44% (n = 2) *
PLA 04	62.8% (n = 22)	58.1% (n = 6)	50.9% (n = 12)	33.3% (n = 2) *	72.3% (n = 22)	72.3% (n = 6)	49.4% (n = 12)	20% (n = 2) *
PLA 04 MOD	66.4% (n = 7)	67.7% (n = 7)		None - Full Healing	65.5% (n = 7)	67.4% (n = 7)		None - Full Healing

* Indicates that only 1 sample was evaluated. Full Healing had taken place in the rest of the samples.

These differences are also well within the threshold levels of the sham sites described above, and therefore probably do not represent anything of significance. This is not unexpected, as the Gore-Tex woven mesh would be equally compatible with the tissues on both sides due to its woven meshlike structure.

The biodegradable PLA.02 shows a difference between the upper and lower surfaces (12%) 3 days after surgery, which is markedly increased by one week after surgery (22.4%). This is clearly above the threshold of variation in the sham sites, and therefore may be indicative of greater ingrowth of epithelium on the lower (i.e. mica) sides of the membranes than the upper (i.e. air) sides. By 4 weeks these differences are minimal, but wound healing had taken place by this stage in the majority of samples so this observation is probably not of relevance. A similar result is seen with PLA 0.04, with a difference between sides noticeable at 3 days (9.5%) increasing by 1 week (to 14%) and then reducing to negligible differences by 2 and 4 weeks after placement. Although these differences are within the thresholds of the sham sites, they could be considered to be not significant. However taken in conjunction with the PLA 02 membranes which are made from exactly the same material, the overall trend towards greater downgrowth of epithelium on the mica (lower) side of the membranes would tend to be confirmed.

The Millipore PTFE demonstrated a typical cuff-like configuration which is illustrated in photographs 3.16 and

3.18 at each time period. Only one sample could be analysed this way, so that no conclusions can be drawn from the fact that the upper surface showed less downgrowth than the lower surface at both time periods investigated. It is not known whether one side is in fact different to the other as this information is not available from the manufacturers of this material. It is probable that this variation merely reflects the normal range of difference between the "upper" and "lower" surfaces, as described previously in respect of the sham operated sites.

The trend indicating differences between the surfaces of the biodegradable membrane had been anticipated. As a result, a small number of samples of PLA 0.04 had been prepared so as to chemically modify the upper surface of the membranes to increase its oxidising properties.

The modified form of PLA 04 showed virtually no difference between the two sides at 3 days or 1 week after placement. Thus the chemical modification of the surface of the PLA membrane may have reduced the degree of downgrowth of epithelium to an equivalent degree to that of the air side of the membranes. Although there appears to be a tendency for less epithelial migration to occur on the air side of the PLA membrane than on the non-air side, which is not apparent when the surface is chemically modified, these effects might be attributed to other factors rather than or in addition to the surface characteristics of the materials investigated. Such factors might include the site of placement of the

material, wound infection, or the positioning of the membrane in the surgical wound.

Table 3k illustrates the presence of exudate in the superficial part of the surgical wounds. It can be seen that exudate was present in most of the samples at the 3-day period, with a greater incidence with the thinner PLA 02, and the Gore-Tex PTFE material. By 1 week post-operatively the amount of exudate was increasing in all of the biodegradable materials but reducing in the Gore-Tex material. By four weeks post-operatively there was minimal exudate for most of the materials, except for Gore-Tex where 50% of samples demonstrated continued exudation containing polymorphs. This observation may be very important clinically where the Gore-Tex material is placed for periods of 4 to 6 weeks before removal. It should be noted that none of the PTFE filter samples showed any exudation at either the 1 week or 4 week periods emphasizing how well this membranous material is tolerated by the tissues. This observation again highlights the difference between healing response to the two forms of PTFE. The continuing presence of exudate and polymorphic infiltrate reflect the more irritant nature of the Gore-Tex material by comparison to all of the other materials investigated in this study.

Early loss of material was evident in a large number of sites and this was of some concern. Exfoliation of materials placed transcutaneously is not an uncommon occurrence in animal studies of this nature, due to

TABLE 3.k TO DEMONSTRATE THE PERCENTAGE OF SAMPLES WITH WOUND EXUDATION FOR EACH MATERIAL, AT EACH TIME PERIOD UNDER INVESTIGATION

MATERIAL	TIME PERIOD		
	3 DAYS	1 WEEK	4 WEEKS
SHAM	8/9 88.9%	2/3 66/7%	0/3 0%
GORE	9/9 100%	2/6 33%	3/6 50%
FILTER		0/3 0%	0/3 0%
PLA 02	10/12 83%	4/4 100%	0/6 0%
PLA 04	7/12 58%	2/3 66.7%	0/6 0%

physical removal by the animals in rubbing or scratching themselves, or each other. Care was taken to minimise this effect by placing each animal in a separate cage, and placing a collar around their necks which limited their ability to interfere with the surgical sites. This probably reduced the incidence of membrane loss, but it was noted that in those animals in which two samples of the same material were placed, some sites seemed to be associated with greater loss of whatever material than others. It was decided to investigate this phenomenon by dividing the placement regions into four quadrants thus:

UL	UR
LL	LR

Those sites which had two samples in each animal and in which one of the two sites had demonstrated exfoliation were then tabulated as shown in Table 31.

From this table it can be seen that the exfoliated samples were almost exclusively associated with the upper left quadrant and were predominantly associated with the non-degradable PTFE materials. The site distribution exclusively to the upper left region is interesting, but as only a very few samples could be evaluated, interpretation of this finding should be met with caution. What is of greater interest, is the complete lack of any PLA absorbable membranes in this table. This would imply that the absence of these membranes might be due to early absorption of the PLA material with healing, rather than loss of the material due to exfoliation or physical

TABLE 3.I TO DETERMINE IF THE POSITION OF THE SURGICAL WOUND COULD BE RELATED TO EXFOLIATION OF THE MATERIAL

	3 DAYS	1 WEEK	2 WEEKS	4 WEEKS	3 DAYS	1 WEEK	2 WEEKS	4 WEEKS
SHAM GORE PLA 02 PLA 04 FILTER P(HB-HV) TOTAL	(1)	(1)(1)	(1)	1				
SHAM GORE PLA 02 PLA 04 PLA 04 MED FILTER P(HB-HV) TOTAL					1	11	1	(1)

Numbers in brackets indicate sites at which exfoliation had occurred

dislodgement.

3.5.3.7 Wound maturation characteristics

The observed variations in tissue response could possibly also be related to the maturation characteristics of the healing tissues. With this in mind the specimens were stained with Von Gieson's stain for collagen and assessed for differences in maturation of collagen between different materials.

In Table 3m, the observations of sections which were stained with van Gieson's stain are shown. This stain differentially stains mature collagen red and immature collagen pale yellow. It can be seen from this table that the Gore-Tex material was associated with both mature and immature collagen initially, then immature collagen at 1 week and 4 weeks after placement, indicating poor long-term wound maturation and healing. This feature is also present in respect of the PTFE filter, and this would tend to indicate that factors relating to the nature of the materials themselves like surface electronegativity, hydrophobicity, or low surface energy may play a part in the maturation of the fibrous tissue with which they were associated, in addition to the surface roughness characteristics of the Gore-Tex PTFE discussed previously.

The biodegradable PLA materials had more immature collagen in their proximity at 3 days and 1 week after placement. By 4 weeks after placement, more samples had mature collagen in the healing wound, but immature collagen was still present. When this is compared to the sham

TABLE 3.m **TO ILLUSTRATE THE NUMBER OF SAMPLES WHICH HAD MATURE AND/OR IMMATURE COLLAGEN WHEN STAINED WITH VAN GIESON'S STAIN FOR COLLAGEN AT 3 DAYS, 1 WEEK AND FOUR WEEKS AFTER PLACEMENT OF THE TEST MATERIALS**

	3 DAYS		1 WEEK		4 WEEKS	
MATERIAL	MATURE COLLAGEN ONLY	IMMATURE COLLAGEN	MATURE COLLAGEN ONLY	IMMATURE COLLAGEN	MATURE COLLAGEN ONLY	IMMATURE COLLAGEN
SHAM	1	2	1	2	2	0
GORE	3	3	1	4	1	4
FILTER			0	3	0	1
PLA 02	0	4	0	4	2	2
PLA 04	1	4	0	3	3	2
PLA 04 MOD	0	2	0	4		

(control) sites it can be seen that initially more immature collagen was present which had completely disappeared by the 4th week. This demonstrates that a degree of delay in the maturation of tissues during wound healing occurs even with the biodegradable materials albeit that this is less marked than with the non-degradable materials. A possible explanation of this is that some delay in maturation of the tissues is inevitable to allow for absorption and removal of the biodegrading substances before full healing can be achieved. As this cannot occur with the non-degradable materials, the features of immature tissue remain, unless exfoliation can occur.

3.5.4 Photographic results

A number of different responses to the materials under investigation have been described. The photographs which follow illustrate the typical appearance of the materials under investigation when stained sections are viewed using light microscopy, over a period of four weeks after placement.

3.5.4.1 Photographs 3.1-3.30 show the typical healing observed in the sham, Gore-Tex PTFE, Filter PTFE and PLA sites respectively for each time interval in the study. It can be seen from these illustrations that the healing characteristics of each material were different.

The Gore-Tex PTFE material showed a characteristically greater cellular infiltrate which was present throughout the study period. The cellular infiltrate was characteristically of a mixed monocytic and polymorphic

nature. The fibrillar nature of this material as opposed to the Millipore Filter PTFE, which was smooth surfaced and associated with a very mild cellular infiltrate, may account for the differences in tissue responses observed. In some sections, there was evidence of invagination of the fibrils of Gore-Tex by monocytes, with evidence of cleavage of the mesh fibrils which could lead to exfoliation of the superficial portion of the membrane (Photograph 3.14). The PTFE Millipore filter (Photographs 3.16-3.19) was tolerated as a highly biocompatible transcutaneous implant with a very mild, diffuse infiltrate. Some epithelial downgrowth occurred which was arrested to form an "attachment" apparatus against the material reminiscent of the epithelial attachment apparatus in the gingival crevice. It should also be noted that the tissue in apposition to this material was consistently detached from the material itself. Although this observation might be attributed to artefactual defects during preparation, the consistency of this finding may be due to the hydrophobicity of this material which reduces cell adhesion to it.

The biodegradable polymers showed a totally different tissue reaction. The material illustrated in photographs 3.20-3.30 demonstrates the absorption of the material with time. The absorption of the material was highly variable and was not directly related to time in situ, as some sites had virtually eliminated the material by 3 days after placement, while in others there was residual material present 4 weeks after placement. Absorption of the

material also varied in different parts of the wound. Epithelial downgrowth occurred down the sides of the membrane material. Rapid absorption of the fragments in the superficial parts of the lamina propria enabled epithelial contact to occur with exfoliation of the transcutaneous fragment of the material and healing by collagen deposition in the lamina propria. The deeper fragments of the material in the region of the subcutaneous muscle layers were encapsulated in fibrous tissue, and more slowly absorbed. The absorption of the material was highly variable with some fragments being absorbed while others were degrading and yet others being eroded in the same field of view (Photograph 3.27). In this photograph monocytes are present in some areas and giant cells in others. The monocytes seemed to be associated with superficial erosion of the fragments (as on the left side of photograph) while giant cells were associated with erosion of the material (as on the right side of the photograph).

A number of fragments demonstrated transverse hairline bands in some areas (Photograph 3.28). These fragments were examined under polarised light to see if they were due to crystalline configurations within the material or lines of stress which had developed due to distortion during placement of the material. Polarised light showed some bi-refringence (Photograph 3.29) but this was not specifically related to the observed lines, or the degradation of the fragments, or any of the bent (and

therefore stressed) fragments.

Photographs 3.24-3.26 show a sample in serial section with fragments undergoing absorption. It can be seen that uneven degradation occurs throughout the depth of the material as absorption of fragments varies between the serial sections. In addition, each photograph shows different absorption characteristics, with dissolution of the material occurring in some fragments, and phagocytic erosion occurring in others. Concurrently some fragments or parts of the fragments showed no signs of absorption.

Fig. 3.6 Low-power view of the sham operated wound at 3 days which received no membrane. The photograph shows a section of skin with the epithelium towards the top, and the lamina propria below. The surgical site can be seen where the tissue has been severed centrally. Minimal wound exudate is visible as amorphous material in and around the wound opening. (Mag: x 6.3)

Fig. 3.7 Sham operated site at 1 week. Epithelialisation of the wound has occurred, and immature connective tissue is present in the lamina propria, with a mild inflammatory infiltrate around the wound site, and some exudate still present on the surface. (Mag: x 6.3)

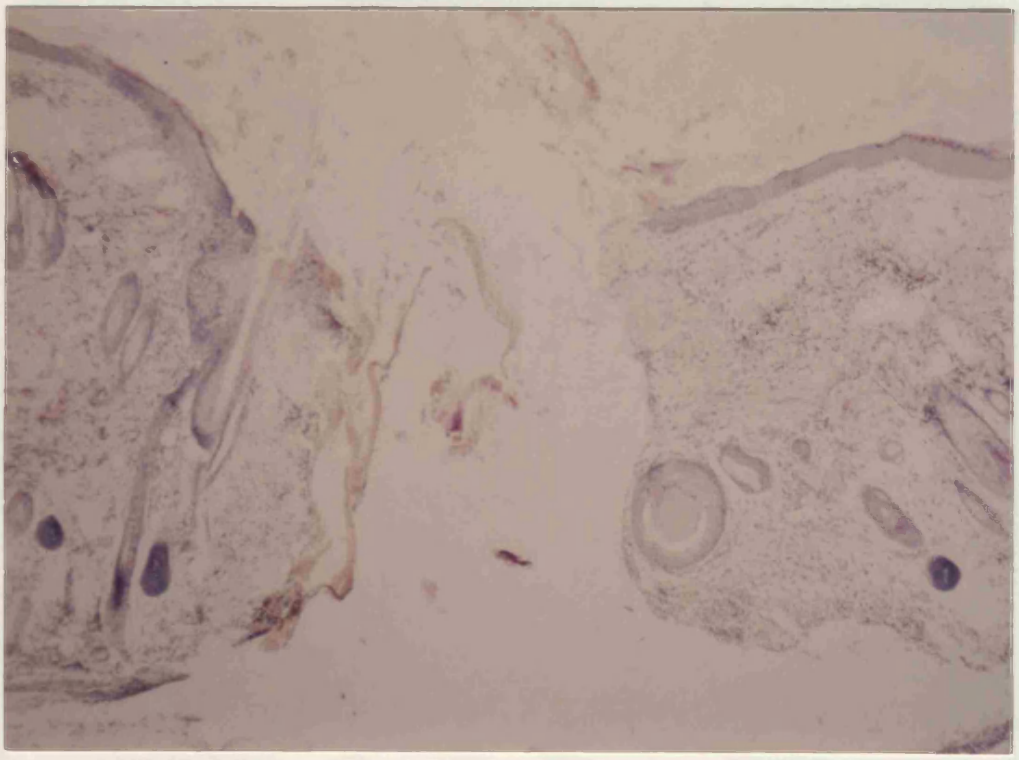


Fig. 3.6

Fig. 3.7

Fig. 3.8 Sham operated site at 2 weeks showing almost complete healing of the wound. Inflammatory cells reducing but still noticeable as a band of mild cellular infiltrate adjacent to the healing surgical wound. The epithelial layer is virtually normal, and immature connective tissue is still present in the wound site. (Mag: x 6.3)

Fig. 3.9 4 weeks post-operatively the sham site has fully healed with normal epithelium and mature connective tissue at the wound site. (Mag: x 6.3)

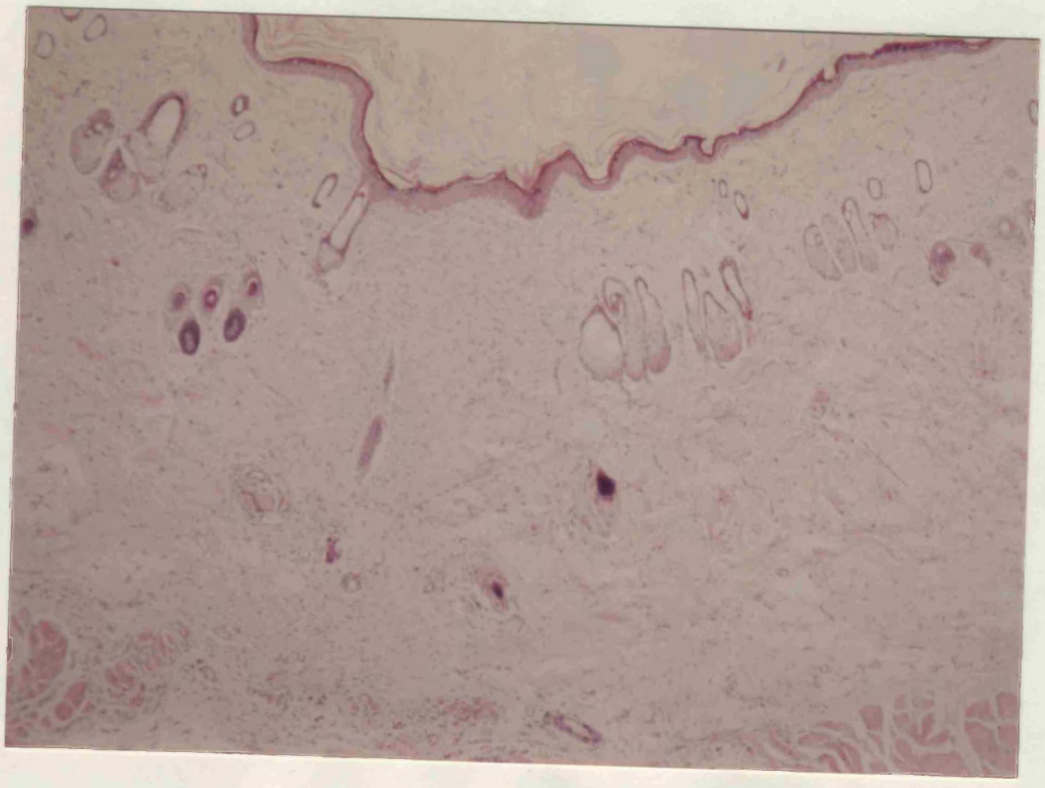


Fig. 3.8

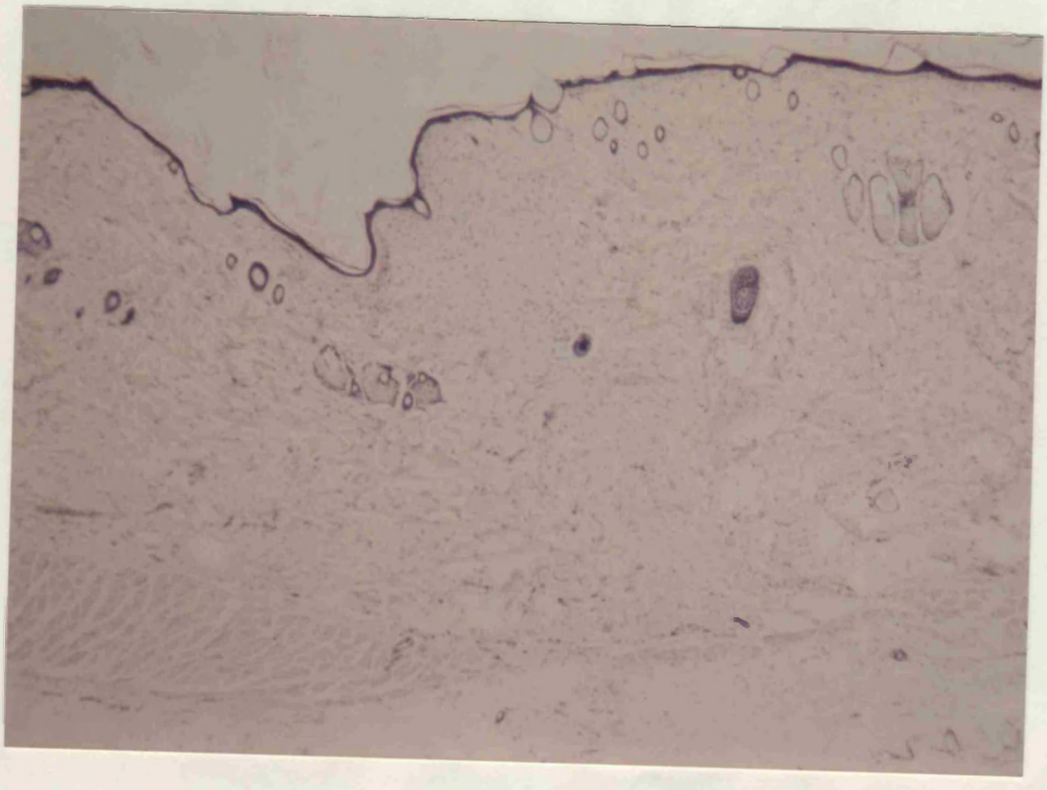


Fig. 3.9

Fig. 3.10 This photograph demonstrates the typical characteristics of the Gore-Tex membrane, 3 days after placement. Epithelial downgrowth is evident along the surfaces of the membrane, and a mild exudate containing some inflammatory cells is present. An inflammatory cell infiltrate is present in the deeper regions of the wound, which is more marked than in the sham operated site illustrated previously (Fig. 3.6). (Mag: x 6.3)

Fig. 3.11 In this photograph Gore-Tex can be seen one week post-operatively. A marked monocytic inflammatory response is still present in the sub-epithelial connective tissue, epithelial downgrowth into the wound is observable. Wound exudate is visible as amorphous material around the superficial exposed part of the Gore-Tex membrane.
(Mag: x 6.3)

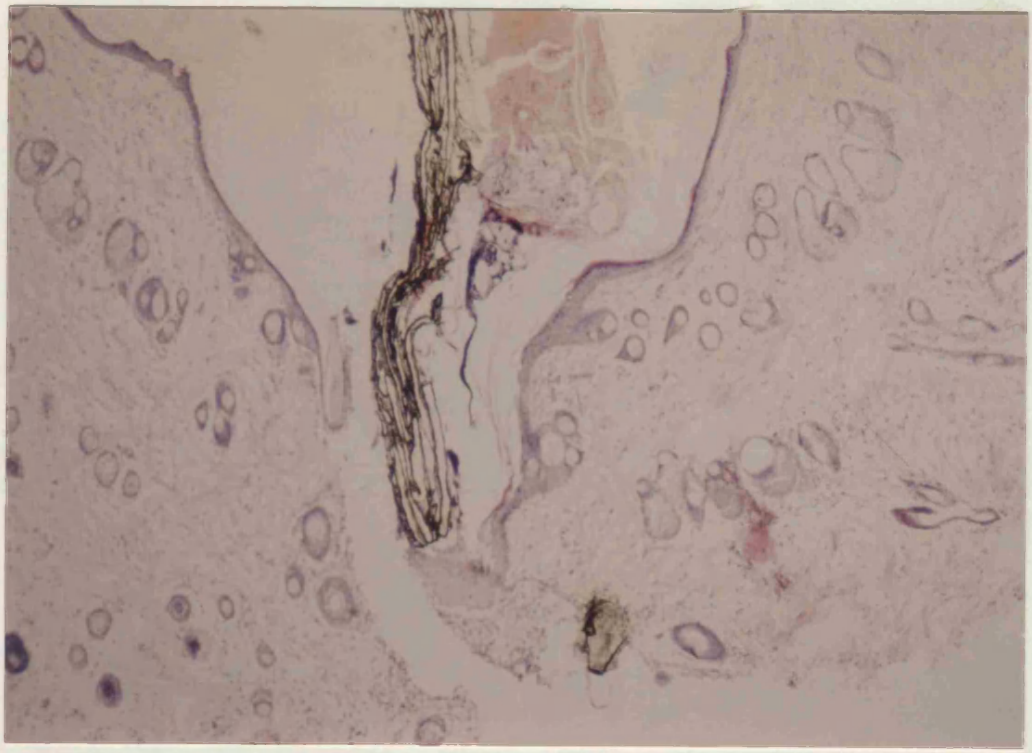


Fig. 3.10

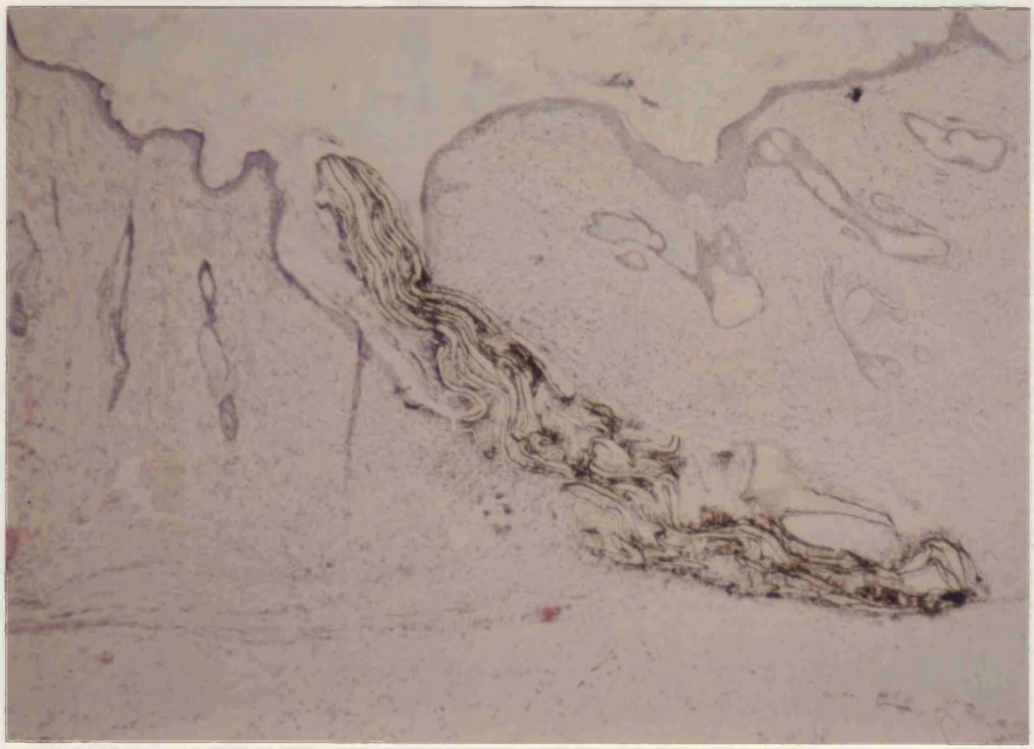


Fig. 3.11

Fig. 3.12 Shows the Gore-Tex membrane at 2 weeks after placement. The characteristics are similar to those observed at 1 week period. Epithelial downgrowth, and exudation from the wound surface is present. The fibrillar mesh-like structure of this material is evident. One fibre has become detached from the main body of the material on the right side. (Mag: x 6.3)

Fig. 3.13 Medium-power view of the section described in Fig. 3.12 showing the fibril which is detached from the main body of the membrane associated with more marked monocytic activity. On the opposite side of the membrane the cellular infiltrate appears to be "streaming" in towards the membrane surface with distortion of the membrane as a result. Monocytes are also evident in between the fibrils, and in the wound exudate superficial to the epithelium, is infiltrated with monocytes. (Mag: x 16)

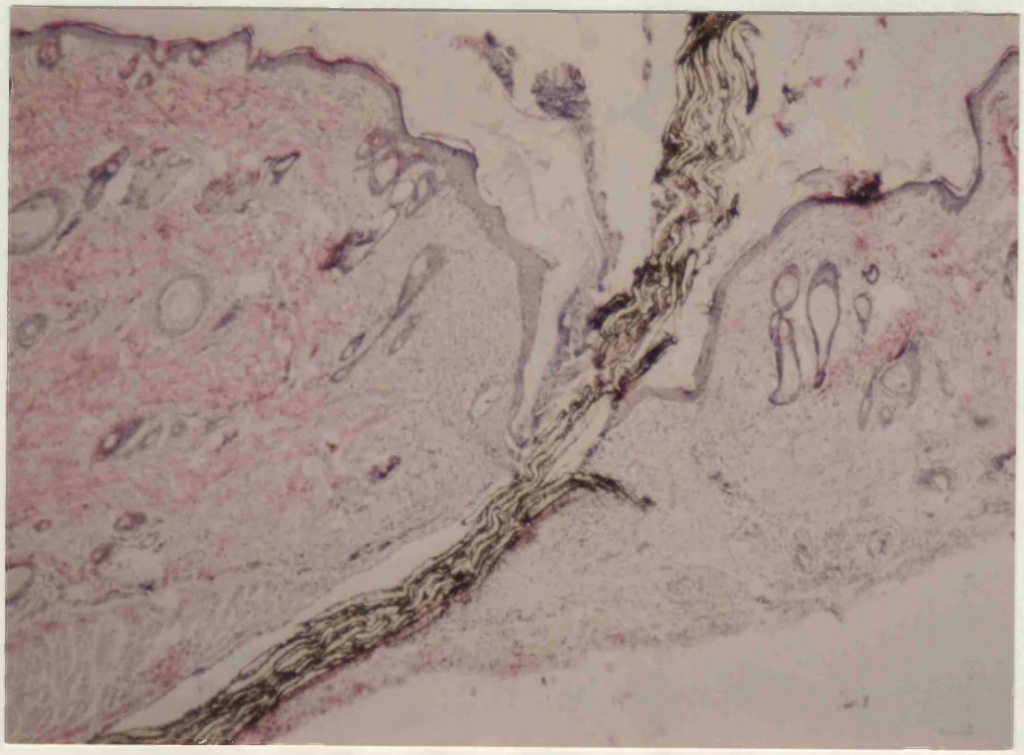


Fig. 3.12

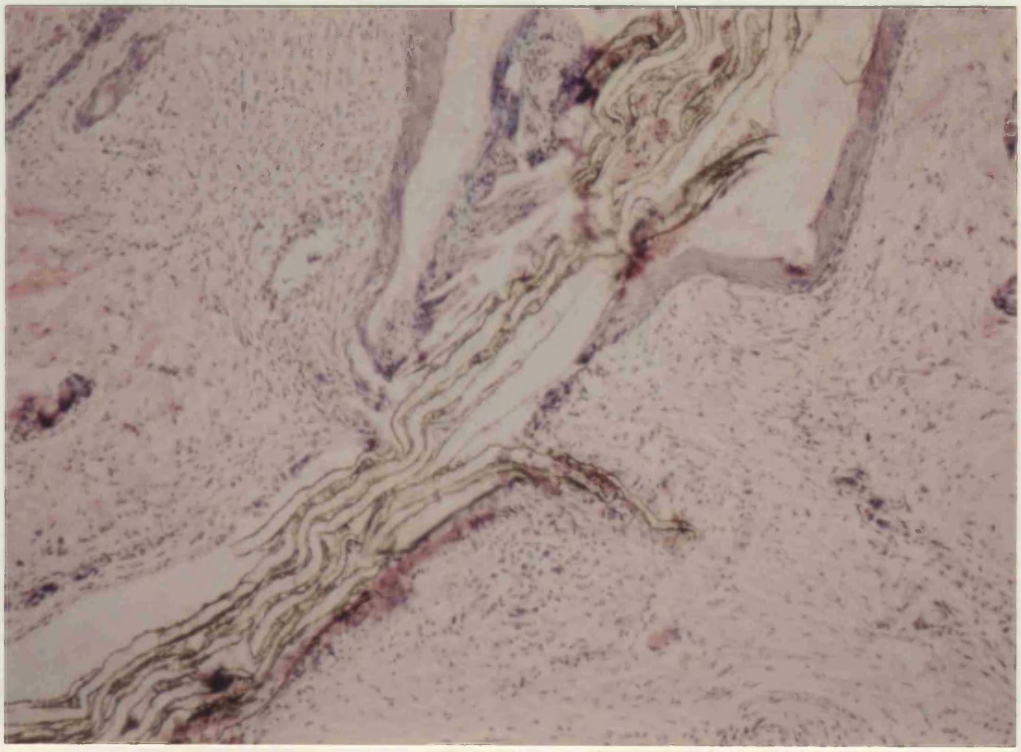


Fig. 3.13

Fig. 3.14 Gore-Tex at 4 weeks after placement shows persistence of inflammation and exudation with epithelial downgrowth and poor maturation of the wound. A tendency for fragmentation of the membrane can be observed. The wound exudate is now heavily infiltrated with cells, with breakdown of the epithelial apposition to the material on the right hand side of the membrane. The cellular infiltrate in the tissue and in the superficial exudate are characteristic of the acute infection at the wound site. (Mag: x 6.3)

Fig. 3.15 In some cases, the Gore-Tex seemed to have been separated with exfoliation of the superficial portion, and encapsulation of the deeper portion in the lamina propria. This photograph illustrates the superficial fragment. By this stage, the superficial portion was often associated with a marked acute inflammatory response with polymorphonucleocytes in addition to monocytes invaginating the fibrils and the production of an inflammatory cell containing exudate. (Mag: x 6.3)

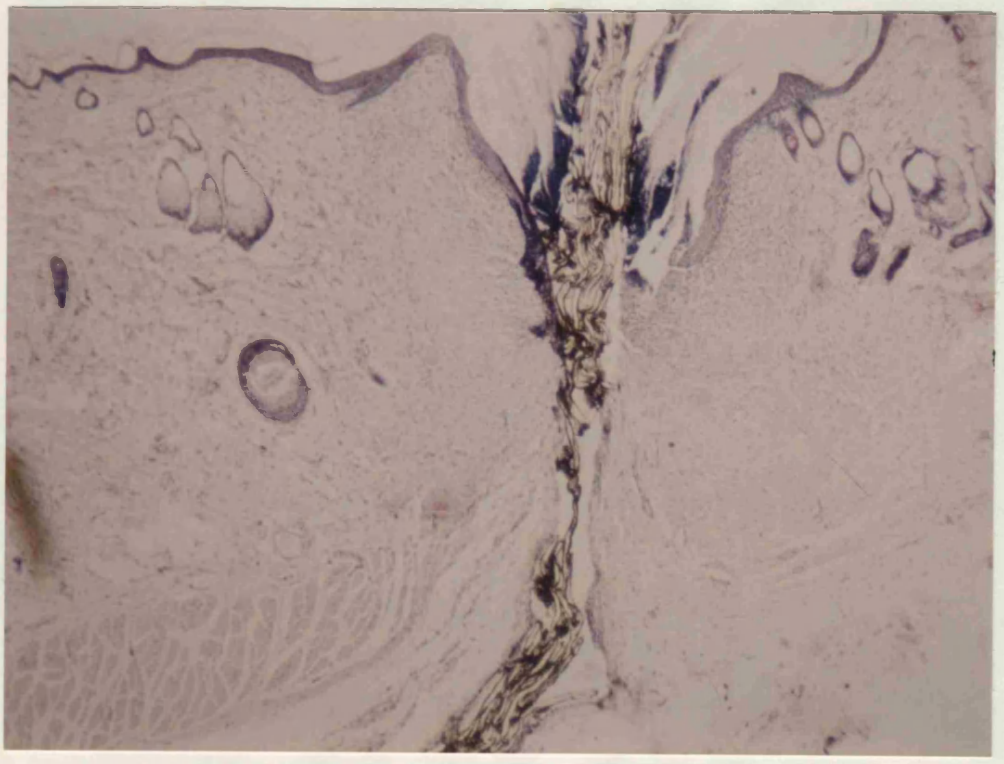


Fig. 3.14

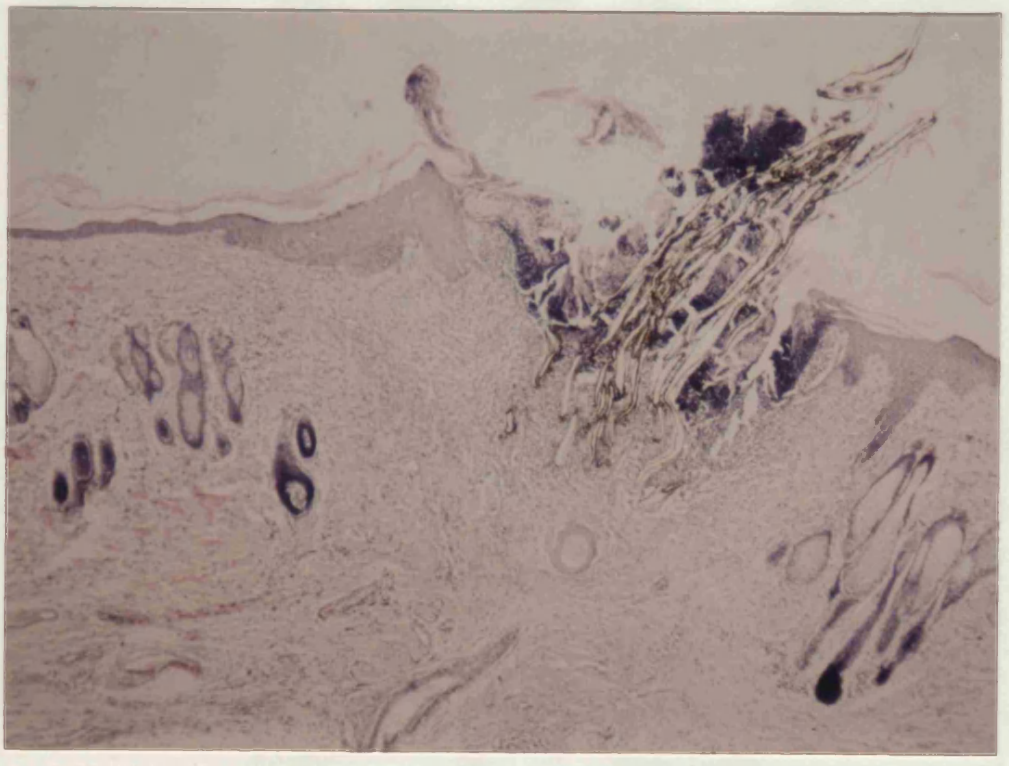


Fig. 3.15

Fig. 3.16 PTFE filter one week post-operatively has a completely different histological response to the Gore-Tex PTFE. A mild diffuse predominantly monocytic cellular infiltrate is present, with epithelial downgrowth which seems to have been arrested. Thickening in the deeper portion of the epithelium and apposition to the material by the epithelial cells is reminiscent of the anatomical features of the gingival crevice epithelial attachment. (Mag: x 6.3)

Fig. 3.17 A medium-power view of the section described in Fig. 3.17 demonstrating the diffuse monocytic cellular infiltrate and the limited epithelial downgrowth into the wound. The epithelial apposition to the membrane has anatomical features not unlike those of the epithelial attachment of the gingival epithelium to the teeth. (Mag: x 16)

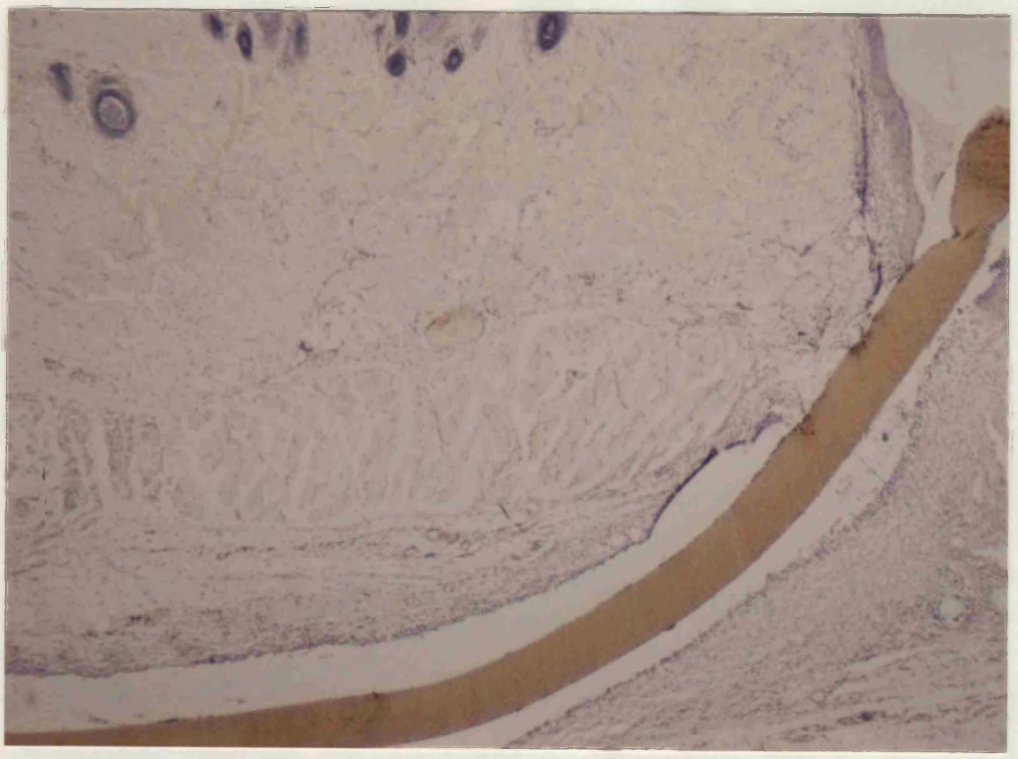


Fig. 3.16

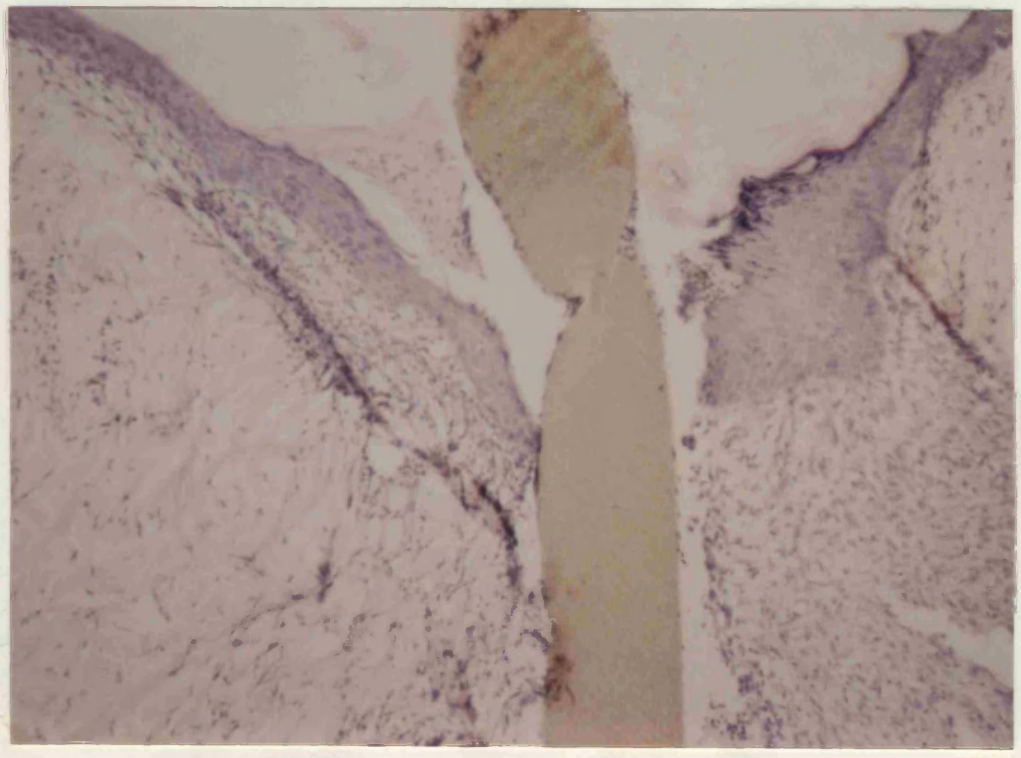


Fig. 3.17

Fig. 3.18 Two weeks after placement the membrane shows remarkable consistency in its mild tissue response demonstrating how well the material is tolerated by the tissues. Some inflammatory cells can be seen in the superficial exudate. The mild inflammatory cell infiltrate is still present adjacent to the membrane, but it is less diffuse than before. The epithelial tissue has maintained the level of minimal downgrowth with formation of an anatomical seal with similar features to those observed in the gingival cervice. (Mag: x 6.3)

Fig. 3.19 Four weeks after placement the Filter PTFE shows mild tissue response described before, is markedly reduced and occupies only a very narrow band adjacent to the membrane surfaces. The epithelial downgrowth is stable and there is no sign of any superficial inflammatory exudate. It is interesting to note how on all of the sections of this material, the tissue has separated from the membrane on preparation indicating cellular apposition to this smooth surface rather than adhesion. This may be as a result of the hydrophobic nature of this material, which tends to reduce cell adhesion. (Mag: x 6.3)

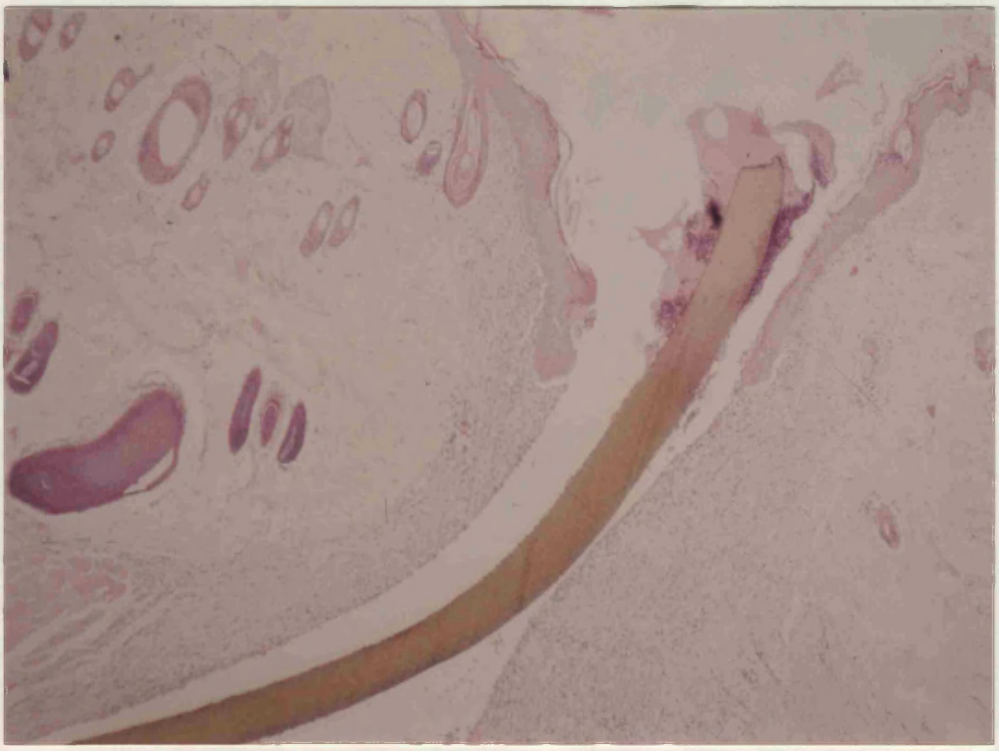


Fig. 3.18

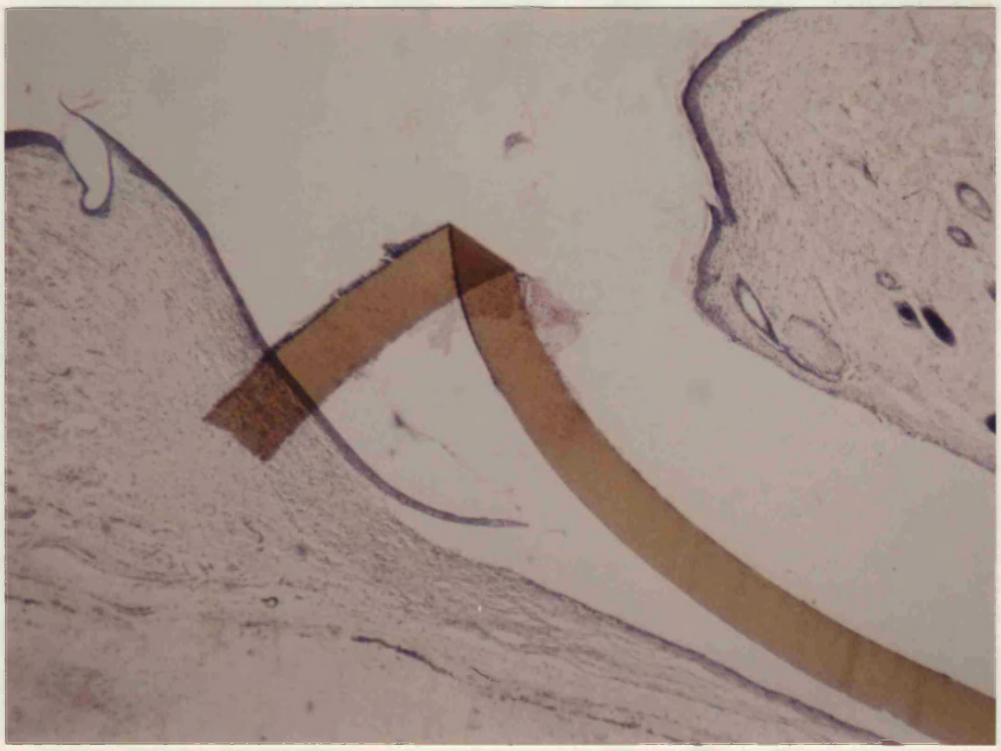


Fig. 3.19

Fig. 3.20 This photograph shows PLA biodegradable membrane 3 days after placement. The appearance of the tissue response is different to that observed with Gore-Tex, or Millipore PTFE, in that epithelial thickening has occurred, and marked superficial exudation is present. In the underlying connective tissue hardly any inflammatory cell infiltrate is observable, but some fragmentation of the membrane is visible. (Mag: x 6.3)

Fig. 3.21 One week post-operatively the membrane cavity can be seen with extensive epithelial downgrowth along the one surface of the membrane and minimal downgrowth along the other. A mild monocytic infiltrate is observable, and an acellular wound exudate is still present. (Mag: x 6.3)

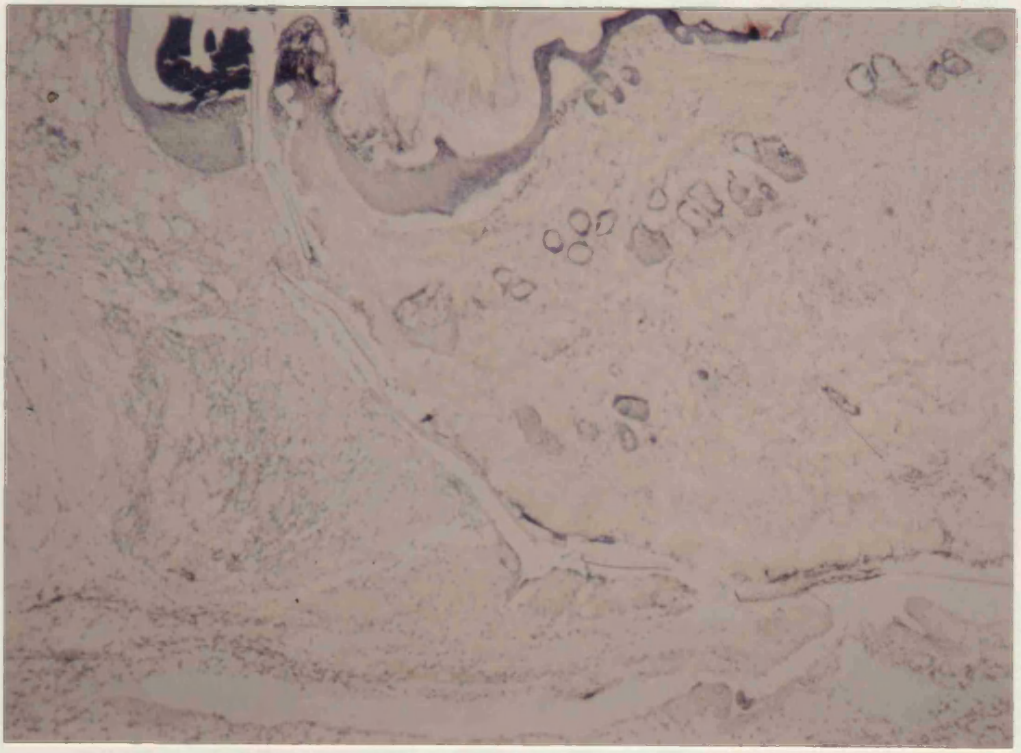


Fig. 3.20

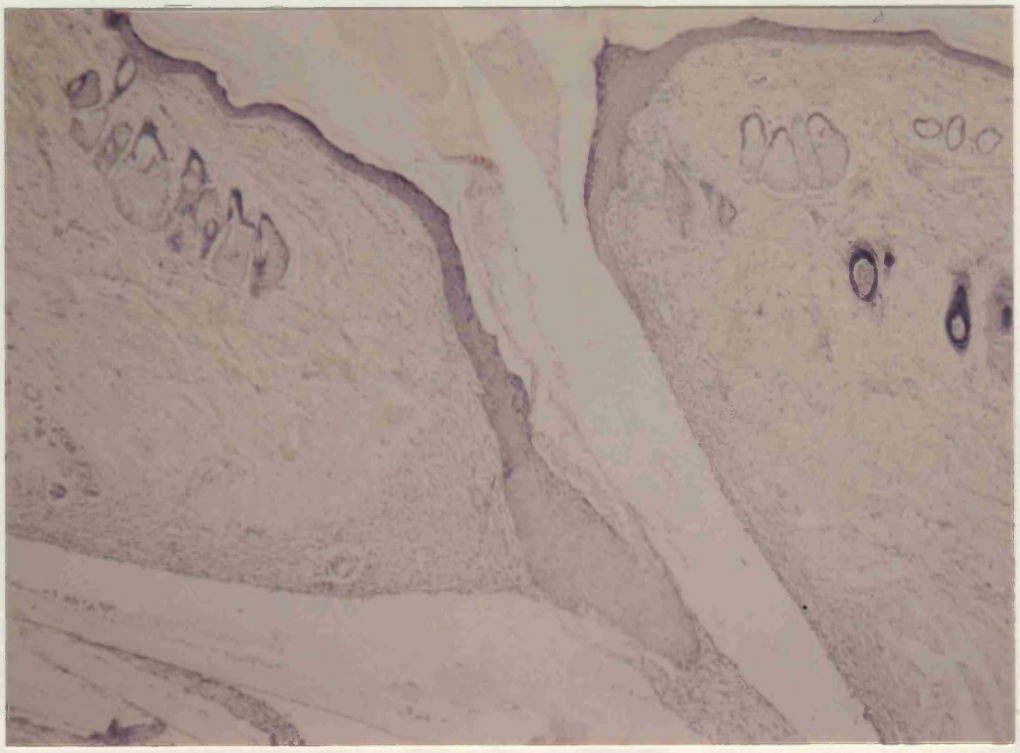


Fig. 3.21

Fig. 3.22 By 2 weeks post-operatively PLA was showing marked signs of absorption/ fragmentation. A great deal of variation in the degree of absorption was noted in different sections. It was apparent that the membrane was absorbed quickly in the superficial lamina propria, while in the deeper portions, especially in the subcutaneous muscle layers, fibrous encapsulation occurred. In this photograph, superficial exfoliation of a fragment is occurring, with absorption of the membrane in the lamina propria and encapsulation with fibrous tissue occurring in the residual fragment retained in the deeper part of the wound. No evidence of absorption of this fragment is seen in this section. (Mag: x 6.3)

Fig. 3.23 A medium-power view of the superficial fragment demonstrates the formation of an attachment apparatus similar to that which was observed with the PTFE Filter, and reminiscent of a gingival crevice type of epithelial attachment. The mild monocytic infiltrate can clearly be seen below the membrane fragment, where material has been absorbed. Spindle shaped fibroblasts are clearly visible in this region, indicative of early connective tissue repair. (Mag: x 16)

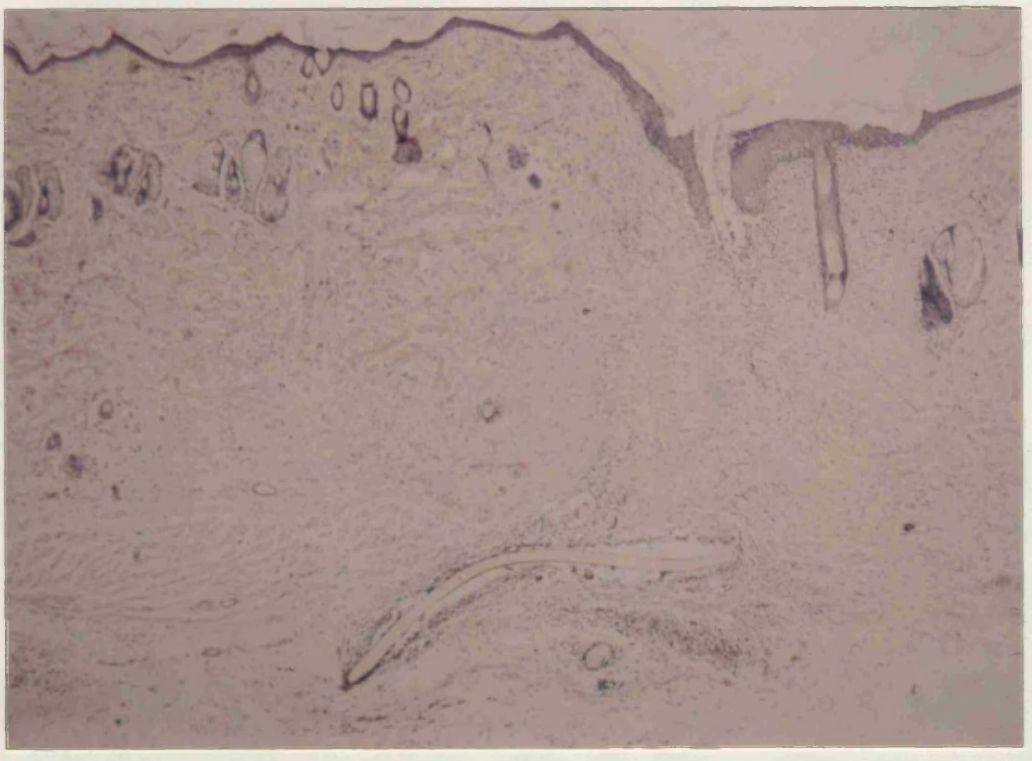


Fig. 3.22

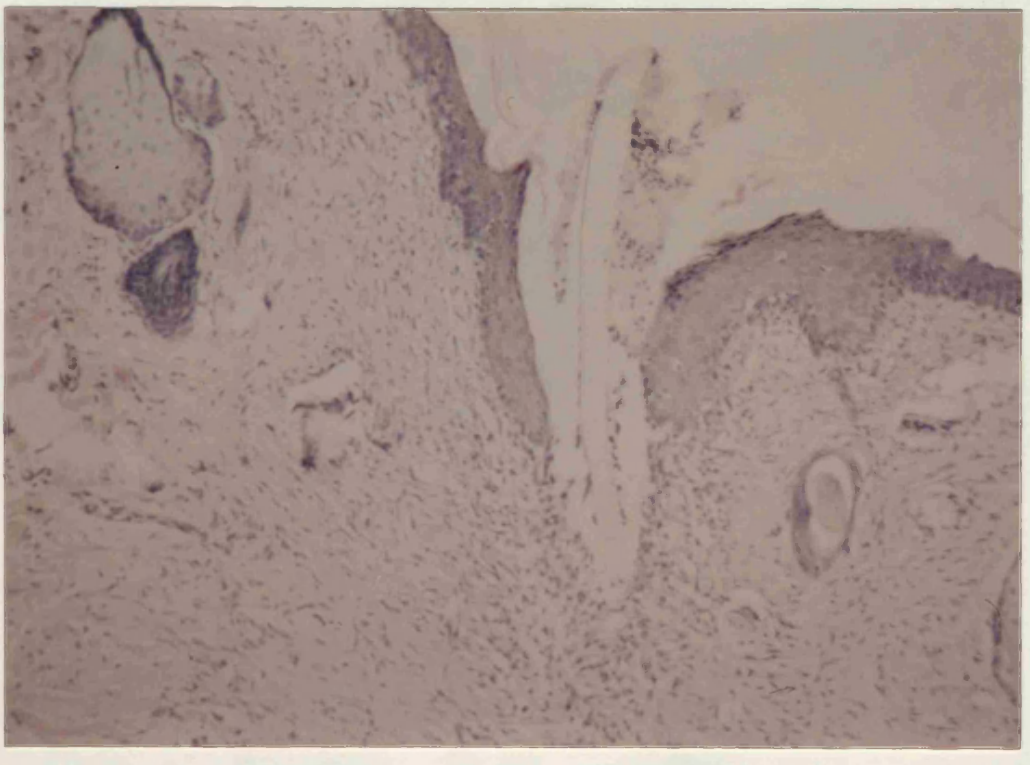


Fig. 3.23

Fig. 3.24 Shows the same field as described in Fig. 3.22, IO sections further into the specimen. It can be seen that the fragment of the material in the deeper part of the wound is being unevenly absorbed. Absorption is taking place in the central part of the fragment only. A mild cellular infiltrate is visible in the region of absorption and towards the left site of the fragment, which is less marked on the right side of the fragment. (Mag: x 6.3)

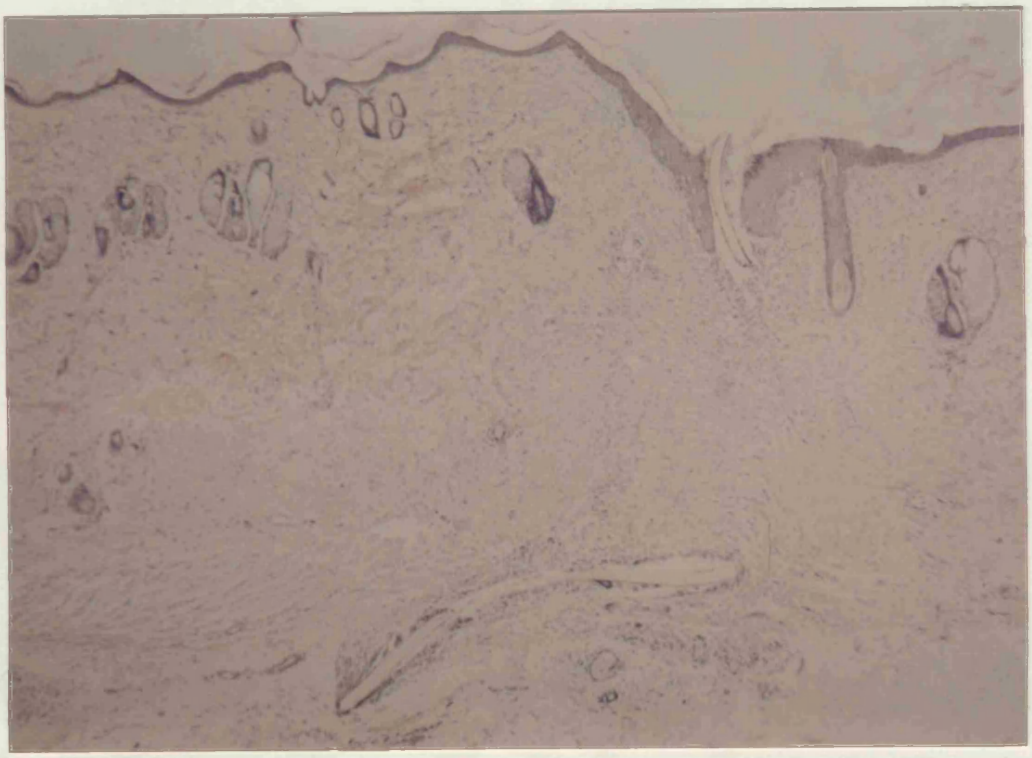


Fig. 3.24

Fig. 3.25 IO sections along the same series shows further uneven absorption of the fragment. The central part is being actively absorbed or eroded, while the ends of the fragments seem to be unaffected. A more marked cellular infiltrate is observable in the central portion than before. (Mag: x 6.3)

Fig. 3.26 IO sections along the same series shows almost complete absorption of the fragment with only small residual pieces remaining. The central part of the fragment demonstrates reduced cellularity by comparison to the previous section with some evidence of early fibrous tissue formation. (Mag: x 6.3)

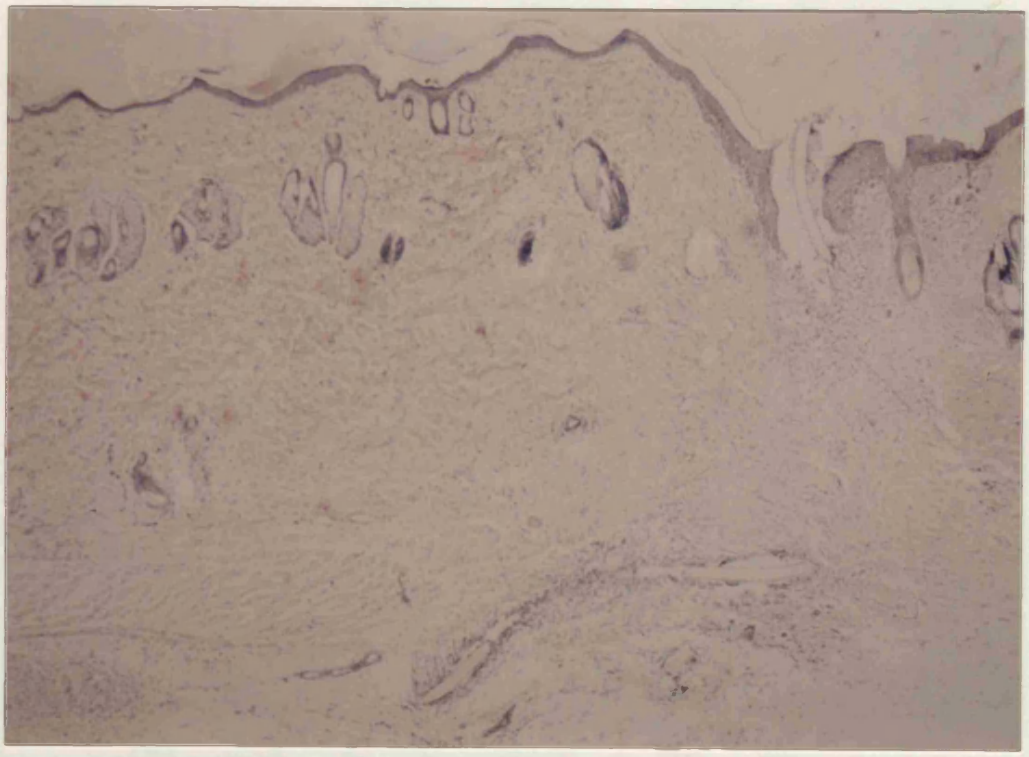


Fig. 3.25

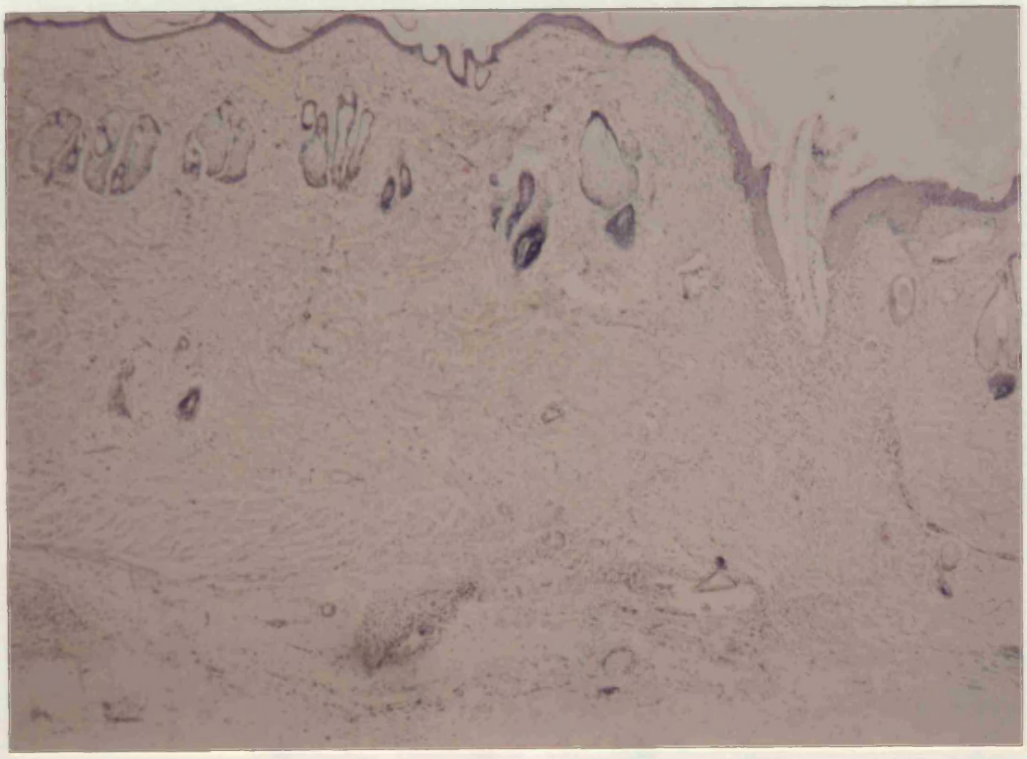


Fig. 3.26

Fig. 3.27 A high-power view of a different two week section of PLA fragments highlights the variations in absorption characteristics of the material. The uppermost fragment is swollen and distorted possibly due to absorption of water due to the hydrophilicity of the material. The fragment below and to the left has superficial erosion lacunae while the one on the right has thinned out at one end and swollen at the other. The lower left fragment is virtually unaltered. On the adjacent fragment, immediately to the right of it, two giant cells are clearly visible on the upper surface. This fragment has been almost totally absorbed. The giant cells are vacuolated indicating phagocytic activity. A characteristic mild monocytic infiltrate surrounds all of the fragments. Note the transverse lines across the fragments (see Figs. 3.28-3.29). (Mag: x 40)

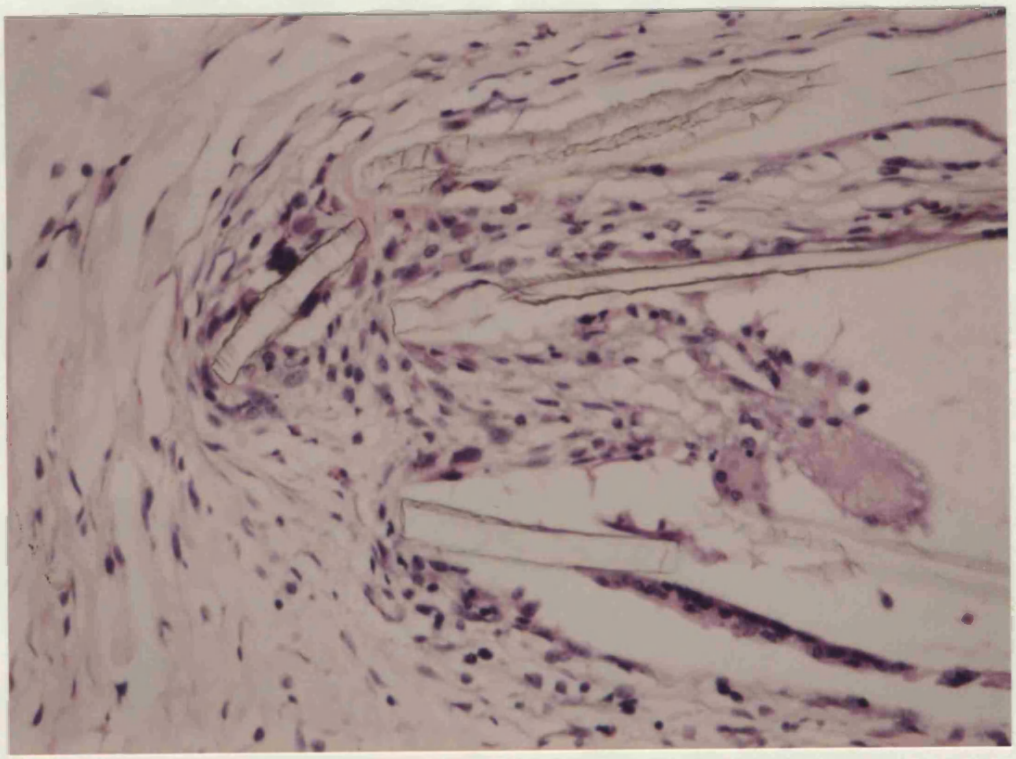


Fig. 3.27

Fig. 3.28 Transverse crack-like lines were observed on a number of fragments which can be clearly seen in several fragments in the previous photograph. It was thought that these represented stress lines in the material which occurred during manufacturing or subsequent surgical placement of the material. This photograph illustrates a number of fragments two weeks after placement. The fragment on the left side of the illustration has been severely bent, and therefore highly stressed. Some transverse lines are dimly visible on the two large fragments towards the bottom of the photograph. (Mag: x 6.3)

Fig. 3.29 When examined under polarised light, it can be seen that the fragments do exhibit some birefringence throughout their length. Birefringence is not increased where the material is bent and therefore stressed. The refrigence does not show a predeliction for one or other surface of the material. The transverse lines are highlighted in the two horizontal fragments towards the bottom of the photograph. These pieces are straight, and therefore unstressed. These lines therefore probably do not relate to the stressing of the material but rather to the internal crystaline structure of the material, or stresses induced during manufacture of the material, or during preparation of the specimen.

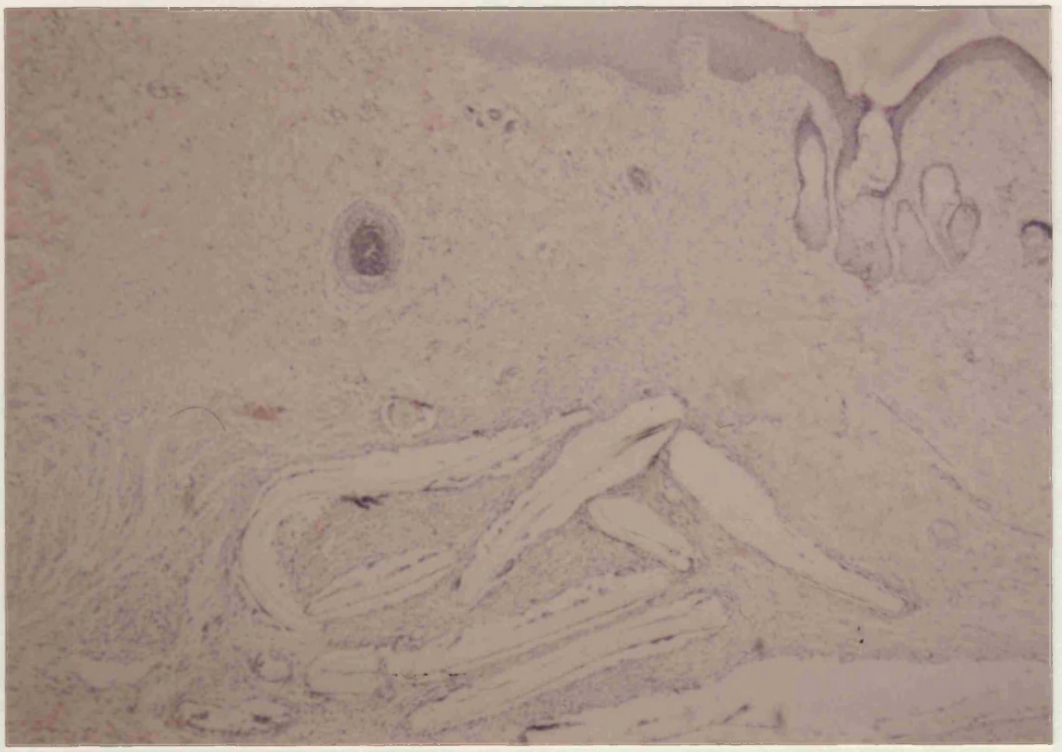


Fig. 3.28

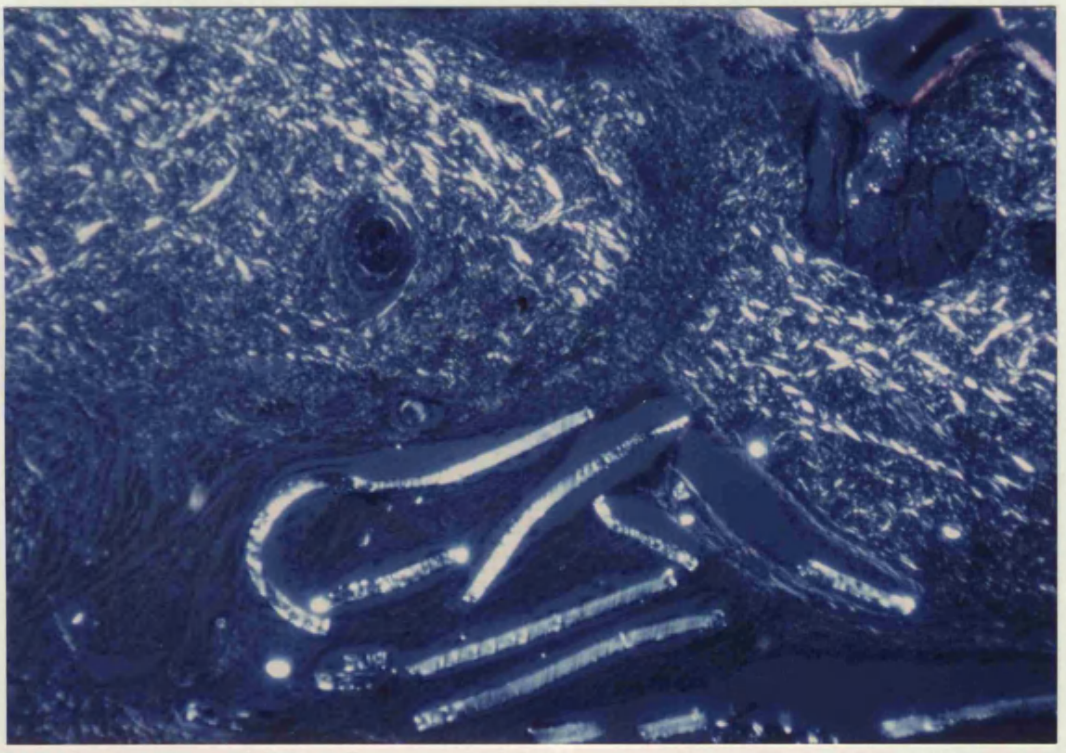


Fig. 3.29

Fig. 3.30 This photograph illustrates the residual fragments of PLA at 4 weeks post-operatively. In the region of the subcutaneous muscle layer which is at the lower extremity of the photograph, fibrous encapsulation is occurring. There is an absence of any fragments superficially. The narrow band of immature collagen extends from the fragments to the surface, and epithelial thickening marks the healed surgical site at the top of the photograph.

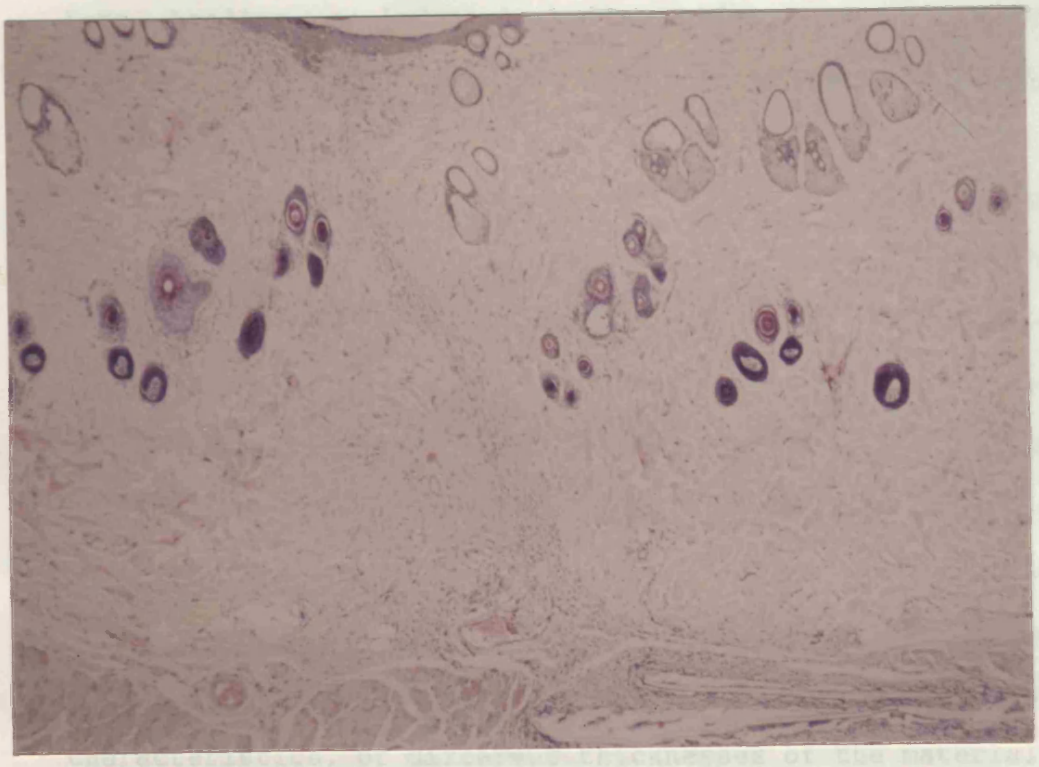


Fig. 3.30

3.5.4 DISCUSSION

The technique of guided tissue regeneration which utilises barrier membranes to prevent epithelial ingrowth into healing periodontal wounds has been developed relatively recently and a degree of success has been reported (Gottlow et al 1984).

In this study the tissue responses to the implantation in an animal model of degradable and non-degradable membranes was evaluated. A considerable variation in the response was observed between the individual animals in the study, between the different materials and even at different depths below the epithelial surface. In addition, a variety of responses were noted even for the same material. It is uncertain whether the responses were influenced by the different physical surface characteristics, or different thicknesses of the material.

Near the surface there was a varying degree of epithelial downgrowth, which was generally greater for the expanded PTFE membrane and to a slightly lesser extent the PTFE Millipore filter. Minimal downgrowth was observed with the biodegradable polymer materials. These characteristics may be related to the surface free energy, and the roughness of the materials. There is some evidence that this may be so in this study, but further work would need to be undertaken to confirm this hypothesis.

There was an absence of, or low incidence of, acute inflammatory reaction observed for all the experimental materials. Areas of the inflammation, when present, were

localised and most noticeable around the portion of the specimens nearest the surface. On the expanded PTFE specimens, where polymorphs were more common, these were found in greatest density at the surface and within the voids of the mesh. Contaminants from the external environment may be able to percolate within the voids of the specimens and thus stimulate an infiltration of leukocytes in the deeper portions of the wound.

The density of the fibrous tissue surrounding the implant varied. The PTFE membrane was associated with a tendency to greater inflammatory infiltrate and early capsule formation. It was uncertain whether this was as a result of the material being relatively hydrophobic, having relatively low surface energy, or whether it was associated with the surface roughness characteristic of the material. By contrast, the biodegradable polymer membranes were generally surrounded by a loose network of immature fibrous tissue in the form of predominantly granulation tissue. This may be related to the relative hydrophilicity of the materials, or their surface energy characteristics. The polymer membranes have smooth surfaces which may be an important factor in the different histological responses between these types of materials by comparison to the non-degradable membranes, especially the Gore-Tex PTFE. Thus, it could be argued that the histological differences between the Gore-Tex expanded PTFE and Millipore PTFE were due to the difference in surface roughness and porosity of the two forms of PTFE. The differences between the

Millipore PTFE and the biodegradable polymers, all of which had smooth surfaces and were cast membranes rather than a fibrillar woven mesh as in the Gore-Tex PTFE, could be attributed to differences in the surface free energy of the materials. The Filter PTFE was more hydrophobic and cell adherence to the material was virtually absent on histological observation, while the polymer materials which were more hydrophilic demonstrated better cellular adherance to the membrane surfaces.

The degree of absorption of the biodegradable specimens was very irregular and seemed often to occur at restricted locations causing a break in continuity of the membranes. In some specimens there were zones on one surface showing vertical clefts and erosion defects, whereas the opposite surface was intact (photograph 3.27). In the present study one surface of the membrane was formed against mica and was hence smooth, whereas the other surface was formed in a vacuum by solvent evaporation. It was not clear whether the differences in degradation on different surfaces could be associated with the characteristics of the one surface compared with the other. The influence of the surface characteristics of the membranes needs to be investigated further.

These differences in healing for experimentally implanted membrane specimens would concur with the varying degrees of success reported in previous studies into regeneration of periodontal tissues after surgery. The clinical studies have shown variation in such healing

characteristics as degrees of regeneration, organisation of fibrous tissue, epithelial down-growth and anchylosis (Gottlow et al 1986). Although some of the variation could be attributed to the moist environment, the numerous microorganisms within the oral cavity, and the presence of an interface between soft tissue and dental tissue at the site of the healing wound intra-orally, similar variations have also been found extraorally in the present study where such conditions did not prevail.

It became clear that the variations described above were not solely time dependant as originally thought. As a result, the hypothesis that variations in histological features would be similar at each time period of harvesting of the samples was not upheld by this investigation. Analysis of the data was undertaken to examine the histological characteristics of the tissue response to each material, rather than in relation to the length of time after placement, and this has suggested that epithelial migration was marginally influenced by the angulation of the material to the wound surface. Most migration occurred in the few samples at approximately right angles to the root surface. Less migration occurred with samples at an angle to the wound surface which tended to be greater at obtuse angles than at acute angles. Once again, a wide variation in these observations was noted, and similar differences were noted in sham sites which received no membranes at all. The published work, however, shows that the proportion of periodontal regeneration occurring

varies. Magnusson et al (1988) have shown that regeneration resulted in the healing of up to 46% of the initial periodontal defects using PLA membrane, whereas studies using Millipore filter showed about 25% regeneration. Nyman et al (1987) have shown in the human that variable amounts of new connective tissue attachment occurred with only a proportion of sites showing complete regeneration of new attachment. Pitaru et al (1988) have described three distinct types of healing, namely:-

(1) Partial regeneration of periodontal tissue in the apical half of defects

(2) Long epithelial attachment in the coronal quarter of the defects

(3) Connective tissue adhesion in the region between these two extremes.

Thus, a varied response must be anticipated when using techniques for achieving regeneration of periodontal tissues.

The presence of a superficial exudate and poor collagen maturation highlights the difference in tissue response of the Gore-Tex PTFE when compared to the other materials. Earlier in the study, the roughness and woven fibrillar texture of this material was considered to be one of the main factors in the different observed tissue responses. However,the Millipore -PTFE also showed poor wound maturation, albeit with a very mild tissue reaction. Thus, other factors like hydrophobicity, surface polarity and surface characteristics may play a part in determining the

overall cellular response to materials placed transcutaneously in maturing surgical wounds.

This study suffers from a number of disadvantages. A large number of materials were investigated in an animal model. As a result, relatively small numbers of samples could be obtained from each material. The problem was exacerbated by the high percentage of loss of samples from the implanted sites. As a result true statistical analysis of the results could not be performed, and only inferences drawn. With this in mind, the conclusions drawn from this study should be treated with great caution. However, these observations are greatly strengthened by comparison with other published work on the subjects of biodegradable and non-biodegradable materials transcutaneously.

A classic study was undertaken by Winter (1974) using a pig model to observe the tissue response to a number of blocks of different materials placed transcutaneously. In this study considerable downgrowth into the sub-epithelial dermis was observed. Millipore PTFE used in the Winter (1974) study showed the formation of an epithelial cuff at the surface which is remarkably similar to that observed with Millipore PTFE filter described in this study despite the fact that a different animal model was utilised. Variations in the degree of epithelial downgrowth were described, but this was minimal in the case of porous materials like PTFE and Hydron sponge. The study by Winter (1974) proposed an hypothesis that epidermal cells grow down the surfaces of the transcutaneous implants into the

dermis in an attempt by the epithelial cells to re-establish epithelial continuity. This does not occur with the porous materials because the porosity within the materials enables communication between the epithelial cells on either side of the material. This enables chemical diffusion through the implant to bring about inhibition of epithelial migration. This may explain the minimal epithelial downgrowth with the formation of a gingival crevice-like epithelial cuff observed with the PTFE filter in our study. Another observation in the Winter study was that the junction between implant and epithelium was unstable and susceptible to infection in the absence of fibrous tissue ingrowth, which in the present study occurred only in association with the porous implant. Again in the present study the PLA biodegradable materials, which showed little epithelial downgrowth, were associated with rapid degradation and superficial absorption of the material with fibrous tissue formation in the deeper part of the wound. Thus retention of the deeper fragments with exfoliation of the more superficial fragments resulting in early disappearance of these fragments from the wound were characteristically observed. Although the cellular responses to the implanted materials are not described in the text of the Winter (1974) paper, the photomicrographs show remarkably similar mild monocytic infiltrates in a narrow band adjacent to the implanted materials to those observed in this study.

The material which demonstrated very different

responses to the others was that of Gore-Tex PTFE. Schmidt and van Recum (1991) have emphasized the effect of surface topography of materials in relation to cellular responses of tissue cells. Surface texture has a major effect on tissue responses, although the actual nature of the texture differences which cause these effects are not yet clear. Nevertheless, earlier work has clearly established that surface geometry and texture of implanted materials are associated with increased cellular and enzymic activity (Salthouse, 1976). As the Gore-Tex membranes had rough surface textures due to the fibrillar woven nature of the material, this could be expected to contribute significantly to the increased inflammatory reaction to this material. Winter (1974) has also observed that woven materials like sutures enable percolation of contaminants into healing wounds as evidenced by the formation of abscesses in wound sites in which silk sutures are retained for prolonged periods of time. This may be an additional factor in the prolonged inflammatory infiltrate and exudate containing polymorphs which was observed with the woven, fibrillar Gore-Tex PTFE, and not with the Millipore, smooth surfaced PTFE.

Zislis et al (1989) have studied degradation characteristics of biodegradable polyesters like polylactic acid and polyglycolic acid. Biodegradation takes place by means of hydrolytic scission, mediated by a number of proteolytic enzymes. As a result of variations in cellular infiltrate, proteolytic enzymes in the vicinity of the

materials, and the concentration of degradation products at the material/tissue interface, variations in the adhesion of tissue cells could be discerned. Breakdown of the polymers occured by the formation of irregular enlarging pores and scattered surface cratering within the materials which resulted in retained fragments. Initially these polymers were associated with adherent white and red blood cells and fibrin clot, which were then replaced by leucocytes only and later by fibroblasts with collagen formation. The Zislis et al (1989) study was performed on rats, and is in agreement with the observations of this study. These workers were able to investigate the tissue responses more closely than in our study, as electron microscopy was employed to investigate the actual cellular interface with the materials. The Zislis (1989) study suffers from the same disadvantage as our study in that the number of samples which could be assessed was so small that tests for significance of results could not be applied. However, when the Zislis (1989) study is considered in conjunction with our investigation the observed variations in the biodegradation characteristics of the polymers used in our study are greatly strengthened.

3.7 CONCLUSIONS

It is concluded that the tissue responses to the membrane materials investigated were governed by a variety of factors which might include the healing characteristics of the individual host, the chemical composition of the material placed in the tissue, the physical and surface characteristics of the material, the structure and porosity of the material, and the depth to which the material is placed within the tissue. Within the limitations of this study, the statistical significance of these observed variations could not be established.

This investigation has demonstrated that healing typically occurs over a variable period of time. Healing was different with the polymer degradable membranes, and with both the non-degradable expanded PTFE and Millipore PTFE membranes. In addition, differences were noted between the two types of PTFE membrane used in this study. Nevertheless, a great deal of variation was noted between samples of the same materials, even within the same site. Although some of this variation can be attributed to different rates of healing in different animals and different sites, other factors may also play a part in altering the cellular response to the membranes.

CHAPTER 4

GENERAL DISCUSSION

4.1 INTRODUCTION

In the two parts of this study the responses of tissues to the placement of synthetic materials were evaluated, to establish what cellular interactions occurred with a number of materials when placed in surgical wounds. This was undertaken to establish whether any of the materials investigated might have the potential to enhance the healing and/or regeneration of tissues after surgery.

4.2 SYNTHETIC BONE IMPLANT MATERIAL

A synthetic hydroxy-apatite bone implant material was evaluated in human subjects, and it was found that although this material appeared to be biocompatible with the tissues, a variety of different histological responses occurred. It seemed as though, despite the fact that this material is described as being non-absorbable, absorption did in fact occur in some cases. This was not consistent, as in other sites either fibrous encapsulation or the deposition of osteoid on the crystal surfaces was observed. All of these occurrences were observed as occurring concurrently in the same histological section.

As this was a human study the causes of these histological responses could not be evaluated in greater detail. However, a number of clinical studies have also reported varying results on surgical placement of the same hydroxy-apatite implant material used in this study (Froum

et al 1982; Boyne & Flemming, 1982; Gamales et al 1986). Galgut (1990), using this material, has described changes which were observed on standardised radiographs over a three-year period. These changes were not consistent as they varied between different individuals, and even in different sites in the same individual. This variation in tissue response is not limited to this synthetic bone implant material alone. A number of studies (Amler, 1987; West & Burnstein, 1985; Wirthkin, 1987; Yukna, 1990), have demonstrated the limited and varied tissue responses to a whole range of synthetic bone implant materials. These materials have included "non-absorbable" hydroxy-apatite and "absorbable" tricalcium-phosphate in different forms (dense, porous, biphasic, coralline), plaster of Paris, pyrolytic carbon, zirconium oxide, and copolymer beads.

Clinical evaluation of sites in which the synthetic material had been placed showed no significant difference in healing or long-term stability between implanted and non-implanted sites. Thus, although great enthusiasm for the efficacy of the synthetic bone implant materials was expressed when they initially became available, the long-term results have been less than satisfactory.

It could therefore be concluded that the regeneration of bone in association with synthetic materials is limited, and varied tissue responses are observed irrespective of the type of material used. This may account for the unpredictable and varied results reported in clinical trials using these materials. However, it is possible that

other regenerative techniques or combination techniques using these bone implant materials in combination with other materials or regenerative techniques might enhance tissue regeneration, and improve predictability of regenerative procedures in clinical practice.

4.3 TISSUE REGENERATION USING BARRIER MEMBRANES

As the use of synthetic materials in the regeneration of bone has been shown to be unpredictable, other regenerative techniques aimed at exclusion of the epithelium from the healing wounds have been advocated. By preventing epithelial downgrowth along the root surfaces of teeth which had previously suffered periodontal infection and contamination using barrier membranes, it was proposed that selective repopulation of the root surfaces by cells derived from the periodontal ligament, and maturation of the granulation tissue in the surgical site, would result in regeneration of the periodontal attachment apparatus (Nyman et al 1982). A number of materials have been utilised for this purpose, and although enhanced regeneration of connective tissue attachment and bone have been shown to occur, the amount of regeneration achieved appears to be variable. The most extensively used material clinically is expanded polytetrafluorethyline manufactured by Gore-Tex. Although good results have been reported in the literature, some studies have indicated variable and inconsistent results with root resorption and anchylosis and without any regeneration occurring in some cases

(Claffey et al 1989; Stahl et al 1990). The Gore-Tex material has the additional disadvantage of being non-degradable and therefore more recent work has focused on the development of a degradable alternative membrane.

Collagen would seem to be a suitable alternative material albeit that this is a non-synthetic tissue derived substance. Although it is freely available, concerns about the use of tissue derived materials clinically have been expressed, and in addition, it may have the disadvantage of requiring an inflammatory response to remove this foreign protein from the wound before regeneration can proceed, possibly resulting in tissue necrosis, and delayed healing. Galgut (1990) has demonstrated that clinical results using collagen as a barrier membrane produce a variety of different tissue responses, which give rise to unpredictable results.

This study has therefore concentrated on the use of synthetically produced biodegradable polymers for potential use in regenerative procedures. The materials were evaluated against control sites which received no membrane materials, and also against polytetrafluorethyline non-degradable materials. As little is known of the tissue responses of these materials, particularly when placed transcutaneously, as would occur in the case of periodontal surgery, it was decided to investigate the tissue responses which occurred in an extraoral environment where complicating factors such as saliva and food contamination, functional forces, and contaminated tooth/root surfaces did

not occur.

This study has demonstrated that:-

(1) All of the non-degradable and degradable materials investigated are biocompatible and are associated with varying degrees of mild monocytic inflammatory infiltrates after placement

(2) Different healing responses were shown to occur with the degradable and non-degradable materials.

(3) The Gore-Tex woven PTFE membrane was associated with the most inflammation, which persisted as long as the membrane remained in situ. As there was a minimal inflammatory response to the Millipore PTFE filter, the persistence of inflammation was attributed to the physical characteristics of the Gore-Tex material which presents an uneven, rough, fibrillar surface to the tissues which results in irritation

(4) As the Gore-Tex PTFE membrane was associated with more epithelial downgrowth, prolonged presence of exudate containing both polymorphs and monocytes, and delayed wound healing with persistence of inflammatory cellular infiltrate in the surgical site, the use of this material in preference to others concurrently under investigation, should be questioned. In the light

of these results, there may be a possibility of secondary wound infection with subsequent tissue breakdown and the possibility of long-term inflammation in the tissues if fragments of the material are retained. Therefore, the possible disadvantages of this material need to be seriously considered.

(5) A highly variable tissue response was noted to all of the biodegradable polymeric materials investigated. This was attributed more to the variations in individual subjects or sites or factors within sites which regulate wound healing rather than factors attributable to the materials themselves.

(6) A frequent tendency for exfoliation of the materials was noted with all of the membranes. This perhaps indicates that epithelial downgrowth occurs initially in order to attempt to exfoliate the material. If this is not possible, attempts at degrading the material by means of monocytes and giant cells are seen on sections. These are more prevalent in the deeper layers of the lamina propria and submucosa. In the case of the biodegradable materials, absorption results in exfoliation of the superficial portion of the material in conjunction with the epithelial downgrowth, and degradation of the material by

the cellular infiltrate described before. When absorption of the material is not possible, an epithelial cuff-like structure is formed, which seals the wound and enables the material to be tolerated. If the material is physically irritant to the tissues, persistent inflammation with poor tissue maturation and healing results.

(7) Within site variability in histological sections has been shown with all of the materials investigated in this study. Although the reasons for this observed variation are unknown, it has been shown that this can be explained in part by different healing rates in different regions of the wound. This study has shown that the biodegradable membranes degenerate erratically and inconsistently, and this would account for the variations in observed histology. However, these two factors account for only part of the variation as Photograph 3.27 demonstrates that the membrane itself is subject to different types of degeneration, and the cells in association with residual fragments of the material are not consistent.

On the other hand, it may be argued that these observed variations pertain to the study design in which extraoral sites were used to place the membranes transcutaneously. A recent paper by Magnusson et al (1990)

has observed that substantial variation occurs in the amount of regeneration achievable when biodegradable Polylactic acid membranes are used intraorally. In this study, regeneration of only 50% was achieved, and this was not significantly different from the amount of regeneration which was achieved in sites which did not receive the membrane. In the previous study undertaken by this group (Magnusson et al 1988) using the same membrane, an average of 46% regeneration occurred in sites which received the membrane. Although this was greater than the regeneration which was achieved in sites which did not receive the membrane (12%) a wide variation in the amount of regeneration which occurred, was noted.

In the present study in which two different concepts have been investigated, the first using synthetic bone infill materials and the second, several types of membranes, a wide range of cellular responses were consistently observed and these variations are similar to the findings of other workers using the same or similar materials to those used in this study. The reasons for this wide variation in observed responses is unknown at the present time. Although different rates of healing may account for some of this variation, other factors must be present to account for such substantial differences, especially in respect of differences occurring within a single site. It is possible that cell growth and regulation factors may account for these changes, with different growth factors being secreted by different cells

in close proximity. Alternatively, a cascade of substances not unlike the complement cascade which is well known in the inflammatory response, may occur, resulting in apparently different histological responses being observed concurrently in adjacent sites. However, little is known about these factors and their effect on wound healing and tissue regeneration.

Other factors may also be of importance. Dehydration, contamination or secondary infection of the healing wound may occur, preventing regeneration from taking place. Tolmie et al (1988) have considered the problems and limitations of regeneration procedures in periodontal treatment, and have concluded that regenerative procedures will remain unpredictable due to a number of limiting factors which may include:-

1) Persistence of chronic inflammation after surgery

2) Inadequate surgical procedures and/or root debridement

3) Secondary infection or wound contamination which results in delayed healing and reduced regeneration

4) The types of cells which initially repopulate the wound, over which we have no control.

5) The spatial limitations within defects which govern the availability of cells in the vicinity for regeneration rather than repair.

Magnusson et al (1990) have shown, using polylactic acid

biodegradable membranes, that defect morphology determines the extent of achievable regeneration. Where infrabony defects are bounded by a bony wall which enables the blood clot and maturing granulation tissue to be supported and protected from disturbance during healing, no benefit is derived from the use of the membrane, whereas in dehiscence types of defects which have no bony walls, enhanced regeneration occurs. The limitations of regenerative procedures are clearly illustrated in the work by this group who have shown that on average only 50% regeneration occurred in those defects with bony support without using membranes and 46% regeneration occurred in sites without bony support using membranes to enhance healing.

Clinical difficulties in achieving predictable regeneration of tissues clinically are well documented. Claffey et al (1989) have attributed the variations in achieved clinical results to a number of factors which may include, the nature of the materials used, defect morphology, contamination of the healing wound, presence of residual inflammation after surgery, patient compliance with post-operative instructions, and the availability of an adequate blood supply carrying oxygen and nutrients to the maturing granulation tissue.

4.4 COMBINATION PROCEDURES

The question remains as to whether enhanced regeneration might be possible if a combination of both techniques are employed. This might be achieved by the

placement of synthetic bone implant material in an infrabony defect to act as a biodegradable infil or osteoconductive implant material, and the placement of a barrier membrane over the top of the implant to prevent epithelial ingrowth into the wound, minimising disturbance and contamination of the blood clot during maturation and healing.

In view of the observations made in respect of the limitations, variability and consequently predictability of procedures utilising the bone implant and the guided tissue regeneration techniques individually, it might be anticipated that similar difficulties would arise when the two techniques are combined. Very little published work is available to substantiate this hypothesis, but some indications of the possible outcome of such procedures have been described. Lekovic et al (1990) have demonstrated that although no statistical differences in soft tissue probing depth occurred between sites which received PTFE membrane alone, and sites which received PTFE membrane plus hydroxy-apatite, more defect fill, less recession, and more horizontal bone fill occurred in the sites which received the combination procedure than those which received the membrane alone. In another study (Galgut, 1990 Part 1, Part 2) a different biodegradable membrane was used with and without the addition of hydroxy-apatite bone grafting material. It was observed that although some sites showed good healing, others did not, and there was a tendency for those sites which demonstrated initially good healing to

show signs of breakdown with time. Thus, although there is some evidence that a combination of both procedures may result in enhanced regeneration of tissue, or minimal post-operative tissue loss due to recession, these procedures may well be unpredictable, and will in all probability remain so until such time as a better understanding of the regulatory factors in wound healing is gained.

CHAPTER 5

PROJECT REVIEW

5.1 CONCLUSIONS

Although the primary aim of periodontal treatment is the elimination of disease from the periodontal tissues in order to restore and maintain dental health, the problem of regeneration of tissues lost by the destructive disease process has remained. A number of attempts have been made to enhance regeneration by means of coronally positioned flap surgery, fibrous tissue grafts, free gingival grafts and autologous bone grafts. These techniques have been successful to some extent, but clinical difficulties have precluded their routine use.

Regenerative techniques using synthetic bone implant materials like hydroxy-apatite have been advocated, but results have not been as good, or as predictable as initially hoped. More recently, the technique of guided tissue regeneration has been shown to have the potential to regenerate the tissues of the periodontium. Although Gore-Tex PTFE membrane has been most extensively used, this membrane has several disadvantages and a number of other materials have been tried. Although some success has been reported, there is increasing evidence that this technique may not produce the consistent regeneration and maintenance of tissue of the periodontium initially thought possible.

Thus irrespective of the technique used to facilitate tissue regeneration after surgery, variable and unpredictable results have been achieved clinically. In

this study, the cellular interface with a number of materials was examined histologically. It was found that considerable variations in the rates of healing, cellular infiltrates, epithelial responses and fibrous tissue maturation occurred with all of the materials investigated, irrespective of whether they were synthetic bone infil materials, or degradable or non-degradable membranes.

The validity of the hypotheses on which this study was based have therefore only been partially upheld. Both bone implant materials and the biodegradable membranes investigated have been shown to be biocompatible, and may have the potential to enhance tissue healing. The physical characteristics of the materials used may affect the healing response but the factors determining the healing response are complex and may vary between different hosts, sites and areas within the same site. Variations in healing over time were more closely related to host factors than to the nature of the materials placed in the tissues.

It was therefore concluded that the observed variation in tissue responses to the placement of these materials was mainly due to factors in the wound site which regulate the nature and rate of the healing process. Other factors which determine the cellular response at the material interface may include, the individual host or tissue response to the surgical procedure and material placements, surface characteristics like roughness, hydrophobicity or polarity of the material, trauma, and secondary infection of the wound during healing.

5.2 SCOPE FOR FURTHER STUDY

Clearly with the present level of knowledge consistent and predictable regeneration of tissues will remain limited. As the regulatory mechanisms of healing tissues are better understood, it may be possible to integrate the primary factors initiating regeneration into or onto biocompatible synthetic materials like those investigated in this study. However, at the present time, too little is known of the factors which interact to achieve an observed pattern of healing in any given site.

Synthetic materials may also be useful as carriers of nutrients or chemotherapeutic agents into the sites of healing. Those materials which are biodegradable would be particularly suitable for this purpose, as the incorporated agents may be released over the period of time during which the membrane degrades.

One of the observations in this study has been the erratic degradation of the membranes. Further work is required to engineer the material so as to achieve more consistent and uniform degradation characteristics throughout its body. It would also be desirable to improve the physical handling characteristics to make the materials more pliable and less springy, and possibly to construct adhesive surfaces to ensure that the membrane remains *in situ* without the need for mechanical suturing after placement. This would enable easier and more successful placement of these materials in sites where they are to perform their function.

Materials used in this study.

Hydroxy-apatite: Durapatite/Periograf: Sterling-Winthrop Group Ltd., Guildford, U.K.

Gore-Tex e-PTFE: W.L. Gore Associates Inc., Flagstaff, Arizona, U.S.A.

Millipore PTFE Filter: Millipore Ltd., Harrow, Middlesex, U.K.

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A microcomputer system for the analysis of dental radiographs

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Br Dent J 1989; **167:** 135

A system for the digital storage and computer analysis of dental radiographs is described. The system is based on a popular microcomputer, of a type commonly used for other purposes in general dental practice. The analysis system includes methods to compensate for variations due to exposure and development in serial radiographs. Interactive software allows a detailed analysis of the radiograph, producing qualitative and quantitative data for diagnosis and monitoring. An example of its application is given.

The use of dental radiography for diagnostic purposes and for following up treatment is now established universally. The diagnostic assessment of radiographs is usually based on subjective visual interpretation, and linear measurements of the image are sometimes used as an adjunct.

There is a need to develop more accurate and objective techniques for measuring small changes in serial radiographs. Minor changes on radiographs are of importance when monitoring periodontal disease and caries, especially when the response to preventive treatment is being assessed. Accurate monitoring of bone lesions is also necessary in the management of various pathological conditions affecting the jaws and alveolar bone.

There have, in recent years, been several reports on methods and systems for the densitometric analysis of dental radiographs, as well as a number of descriptions of trials and applications. The methods involved the measurement of optical density values on the radiograph, usually by means of automatic television (TV) or scanning techniques. The exposed film was either scanned by a light source and detector, or placed on a light box and viewed by a TV camera, the output of which was transferred to a computer system via a suitable interface. In either case, the distribution of light transmitted through the radiograph was recorded as a set of numbers. These measuring systems were found to be more sensitive than the human eye when detecting small changes in optical density and allowed a greater amount of clinically significant information to be derived from the radiograph. For longitudinal studies, a method was used to standardise the radiographs to take account of variation in film development and exposure. One such technique involved obtaining an image of an aluminium or copper stepwedge superimposed on the radiograph.[1,2] Techniques have also been described to reduce errors associated with variations in alignment of the tube and film, and various devices have been used to enable reproducible series of radiographs to be taken of a given site. This technique has allowed the changes in relative density at selected areas in serial studies to be compared. In the early studies, standard densitometry techniques were used, and only limited areas were digitised.

Previous work has suggested that these techniques improve the quantitative estimation of changes in bone densities, for example following autogenous alveolar bone implants.[2] Subsequently, a television camera interfaced to a computer has been employed to digitise the whole of a radiograph. These more versatile analysis techniques have been verified; changes in bone thickness measured by image subtraction have been shown to correlate with independent measurements using radioactive iodine absorbtiometry.[3] It has also been shown that image subtraction techniques increased examiner agreement in estimating changes in periodontal bone, and also improved their diagnostic performance with regard to both accuracy and speed.[4,5]

The aim of the present study was to further develop a system based on a desktop microcomputer compatible with those that are already used for many purposes in current dental practice,[6] and to provide facilities for the standardisation and analysis of dental radiographs at a cost which would permit their widespread use.

Materials and methods

System hardware

The image analysis system is illustrated in figure 1. The equipment comprises an IBM PC-AT* compatible microcomputer fitted with a MATROX expansion card for real-time digitisation of a video image to a 512 by 512 pixel array with 256 grey levels. A CCAT solid state camera is used with a detector array of 604 × 576 cells (interlaced); the automatic gain control was disabled. The camera is mounted on a specially designed light box, which illuminates the film

*Details can be found at the end of the paper.

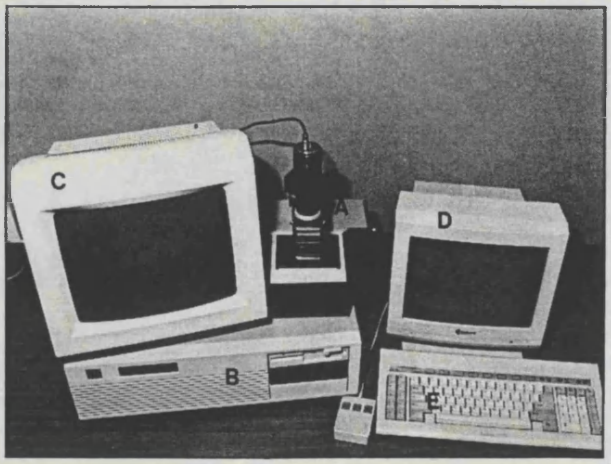

Fig. 1 The system. (A) The solid state camera/viewing box; (B) The microcomputer; (C) Image display monitor; (D) Computer monitor; (E) Keyboard and mouse.

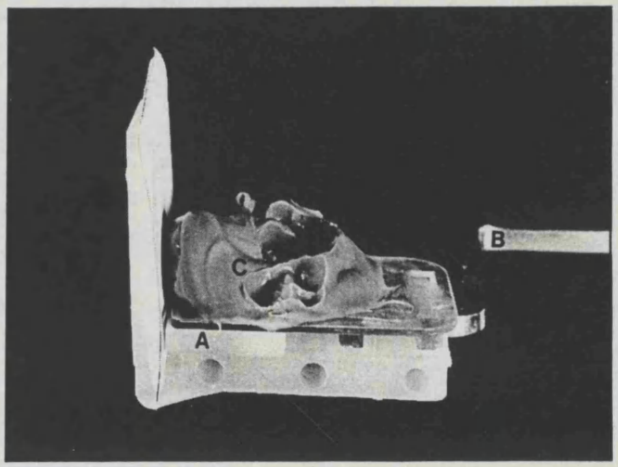

Fig. 2 Alignment device. (A) Wedge; (B) X-ray tube alignment device; (C) Bite block.

uniformly and without heating. The software for the system is written in 'C' and runs under the Microsoft Windows operating environment to provide a user-friendly interface.

Approximately 15 minutes are needed for an experienced user to digitise a pair of radiographs and analyse a single defect site on the two digitised images. Most analytical functions are virtually instantaneous once the image is digitised, making the system very interactive.

Radiographic techniques

The image analysis techniques are designed to work on dental radiographs taken under conditions of constrained alignment. A modified film holder is used (fig. 2), which enables reproducible radiographs to be taken, with very little change from conventional systems used in general dental practice. An aluminium stepwedge is included for use in contrast and exposure correction. This wedge can be seen in the clinical radiographs that follow. It is recommended that film development is standardised as far as possible, although this is much less critical than alignment as errors can be corrected.

The problem of reproducibility has been discussed elsewhere.[7] A study of the system resolution was therefore carried out, in which the approximate error levels for each part of the analysis process (misalignment at the time of the radiographic examination, exposure differences, different contrast and alignment at the time of digitising the images, poor contrast correction) were established by experimental work on dry skull radiographs. This work indicated that distance measurements could be made to an accuracy of ± 0·2 mm, and bone density measured to within ± 0·3 mm of aluminium equivalent (5–10% for typical interdental bone), provided that misangulation was limited to less than 3 degrees. The consistent values obtained from clinical studies lead us to believe that this level of accuracy is being achieved in practice.

Techniques for analysis

Despite the care taken in obtaining the radiographs, it is inevitable that some variations in geometry and exposure will occur between radiographs of the same site taken at different times. A major part of the processing of the digitised images is the application of procedures to compensate for these variations.

Registration

When radiographs from a serial study are to be compared, one of them is first selected as a standard. This radiograph is digitised, and the resulting image is used to bring the series into registration, the objective being to enable quick and easy comparisons to be made between the standard image and the video image of the other radiograph. The standard image is searched by the computer for contours at an estimated intensity corresponding to that of tooth enamel. These contours are stored and superimposed on the video picture from the camera, allowing the user to move the new radiograph so that the tooth boundaries are aligned. This image can be swapped with the standard image for finer spatial adjustment. The new radiograph can then be captured into the memory and filed on disk.

It is a more difficult task to achieve automatic modification to improve the registration of two digital images. It must be borne in mind that a radiograph is a two-dimensional projection of three-dimensional anatomy, and it is not possible to register exactly two such images without having multiple views available, as in computed tomography. However, some improvement in registration can be made with techniques such as template matching. This has been used in areas such as nuclear medicine,[8] and can be usefully applied to dental images. In this method, the operator marks regions containing structures which are expected not to have changed on the source image. The computer can then use the matrix of grey levels from each of these areas as a template, and search the new images for regions which most closely correspond to the templates. For reasons of simplicity, it is often assumed that a linear transformation (rotation, translation, and magnification) exists between two images. In this case, the coordinates from three template matches define the transformation. The coordinates of the best match can then be used to define a transformation from the source image to the new image. The sum of the absolute value differences between the pixels of the template and the pixels of the new image is often used as a measure of mismatch, and this quantity must be minimised for the best match. Erroneous matches do result (due to random noise and the 2D–3D problem mentioned above), but these are minimised by matching several templates and averaging the resulting transformations. Thus, six templates could be matched, and

a least-squares fit could be used to give the best linear transformation. It should be emphasised that the field of image registration is one where a good deal of research is underway, and several alternative methods are being investigated in the current project.

Contrast correction

Coarse correction for variations in contrast can be achieved at the time of digitising the new radiograph. Once the video image of the radiograph is aligned with the standard images, the user can adjust the camera gain so that the two images appear to have similar brightness levels, before digitising the new radiograph. However, this sort of correction is very crude, and there are two main methods for further correction with respect to contrast variation. One is to take the histograms of the grey level distributions of the two images, and to find a mapping from one to the other. This is a well-known image processing technique, and has been shown to reduce the contrast mismatch when applied to dental radiographs.[9] This procedure is applicable to radiographs without a stepwedge, but does not provide quantitative information. Another technique may be used if a stepwedge is present in the radiograph. This is to mark out the wedge steps, so that the computer can find the average grey level for each step, and to combine these levels with knowledge of the X-ray absorption and scattering behaviour of the wedge material thickness to produce a mapping from grey level to aluminium equivalent.[10,11] Both these methods of contrast correction have been applied in the current system.

Data presentation

Once the images have been normalised, the user must be able to analyse the data in a flexible manner. The methods implemented on the current system are:

(1) Graphical presentation of the bone density along a line between two points marked by the user. This line might, for instance, be along the middle of the interdental space.

(2) Calculation of the crestal height. These measurements are made by a new algorithm developed for this purpose. On each radiograph, the clinician marks the positions of the two CEJs on either side of the defect, and two points on the periodontal ligament. The computer calculates the grey-level histogram of the enclosed region, and by the method of Otsu[12] calculates a threshold to separate alveolar bone from empty space. The crestal height is calculated by tracking down from the midpoint between the CEJs to the midpoint of the periodontal ligaments until the threshold grey level is reached.

(3) Calculation of the mean density of an area or at a point marked by the user.

(4) Calculation of the total bone loss or gain within such an area.

(5) Contour mapping at a given bone density in a region marked by the user.

All these facilities/functions are selected by means of a mouse-operated menu system. The mouse is also used to mark points on the image. Output can be drawn on the screen for interactive use, sent to a printer for inclusion in patient records or stored as data for future research purposes.

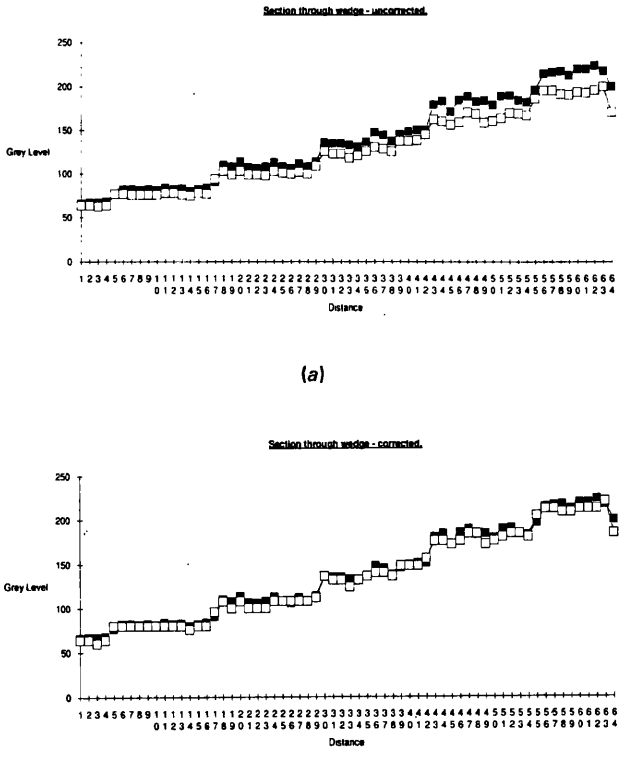

Fig. 3 Graph of grey levels of digitised stepwedge from two radiographs: (*a*) Before correction; (*b*) After correction.

Clinical programmes

The computerised radiograph analysis system is currently applied to several research projects. A sample study from one of these is presented, to demonstrate the application of the system. This study involves longitudinal radiographic analysis of the treatment of periodontal bone defects, comparing conventional flap surgery techniques with experimental treatment involving the implantation of sintered hydroxyapatite into the bone defects as an addition to the conventional treatment.

Results

Investigation of reliability of compensation procedures

The grey levels of the image of the stepwedge can be illustrated graphically (fig. 3*a*). The graphs show the grey levels of the digitised images of the same stepwedge taken from two clinical radiographs. Comparison between these and similar results from other paired films indicated that there were usually slight discrepancies between images of the wedge of equal thickness, giving different grey levels on the two graphs. The causes of these differences probably include a combination of factors, such as changes in the power supply to the x-ray machine and variations in the technique for developing the film.

If the data for the grey levels of each wedge increment are calculated to correct the two images, this information can be applied to match the grey scales for the whole of these paired images. Figure 3*b* illustrates the same sections as figure 3*a* after this matching, and shows that the differences between the two sets of grey levels have been corrected. This matching function can then be applied to areas of the image of clinical interest.

Diagnostic Aids

The application of radiographic assessment by computer to compare periodontal treatment procedures

The radiographs are shown to illustrate the results of control and test procedures carried out on the mesial aspects of the mandibular second and first molar teeth, respectively, for a study of the treatment of periodontal intra-osseous defects by either curettage at open flap surgery alone or the same procedure with the implantation of hydroxyapatite material (fig. 4*a* and *b*). In these and subsequent figures, the radiographs taken pre-operatively and immediately post-operatively are coded *a* and *b*, respectively; those taken at approximately 52 weeks are coded *c* and those taken at 104 weeks are coded *d*. The control series of radiographs show minimal change in interproximal bone density, whereas the test radiographs show a post-operative increase in height and density of the hard tissue interproximally, representing the implant material.

The computer-aided analysis of the radiographic images with matching of grey scales is illustrated by the graphs for the control treatment (fig. 5*a*, *b*, *c*, and *d*) and for the test treatment (fig. 6*a*, *b*, *c* and *d*). Each of the graphs represents a longitudinal section through the interproximal bone, taken from the contact point and passing apically through the estimated mid-line of the interproximal space, crestal bone, and trabecular interproximal bone. The measurements along the horizontal axis are in millimetres from the enamel cement junction, which is plotted at zero. The x-axis was scaled by converting all distances in proportion to a 1-cm scale derived from the standard incremental markings on the wedge. The changes at the one-year and two-year time intervals for the control site were minimal, with perhaps a small increase in crestal bone density as the length of time after surgery progressed. On the test side, immediately post-operatively, there was a clear and localised increase in density, corresponding to the site of the implant (fig. 6*a*, *b*). It is interesting that the implant material did not extend uniformly to the base of the lesion. This result has been found for the radiographs of many of the experimental sites, and the computer-aided analysis confirms the presence of a localised increase in density represented by a peak (P), and, further apically, there is a relative reduction in density represented by a trough (T). By 104 weeks, the increase in density subsequent to implant is more evenly distributed throughout the bone defect.

The computer-assisted analysis system can be used to confirm the previous qualitative analysis by quantitative measurement of the distance from the enamel–cement junction to the bone crest. A constant density value is first determined from the pre-operative radiograph, by calculating the mean density for the interproximal space on the radiograph and the mean density for the interproximal bone; the average is then taken. On the various radiographs, the distance from the enamel–cement junction to the point at which bone reaches the constant density is then measured. In figure 7, the changes in crestal bone height are shown for the previous radiographic series. There were minimal changes in bone height after surgery on the control site. However, there was a sustained increase in bone height (ie a reduction in the distance from the enamel–cement junction to the bone crest) on the site with the implant.

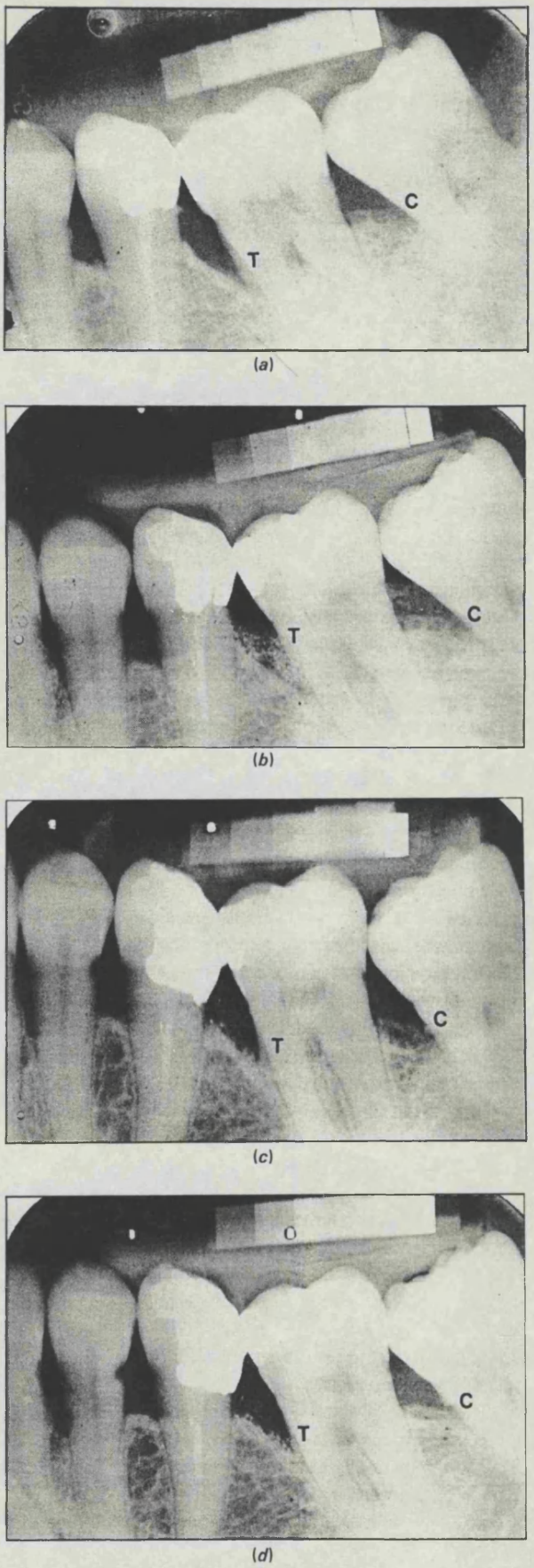

Fig. 4 Computer-generated images of a longitudinal series of radiographs of bone defect treated by curettage (control) and treated by hydroxyapatite (test). (*a*) Pre-operative; (*b*) Post-operative; (*c*) 52 weeks post-operative; (*d*) 104 weeks post-operative.

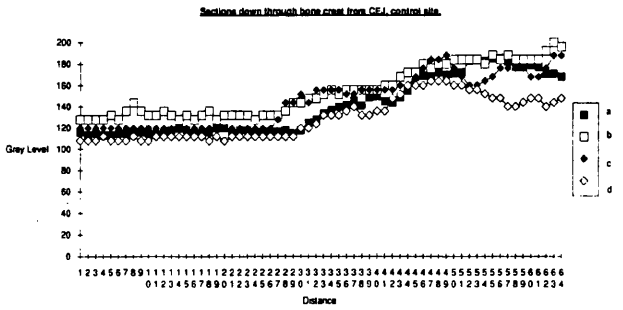

Fig. 5 Contrast corrected section through interproximal bone (control). (*a*) Pre-operative; (*b*) Post-operative; (*c*) 52 weeks post-operative; (*d*) 104 weeks post-operative.

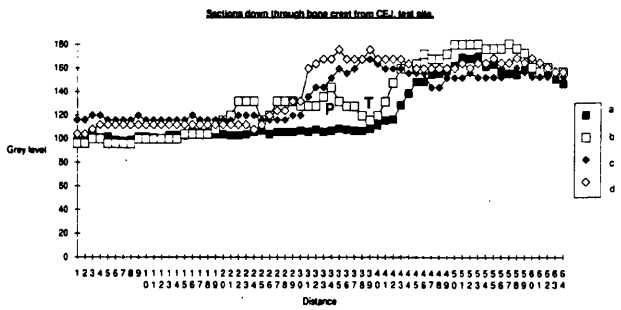

Fig. 6 Contrast corrected section through test site. (*a*) Pre-operative; (*b*) Post-operative; (*c*) 52 weeks post-operative; (*d*) 104 weeks post-operative.

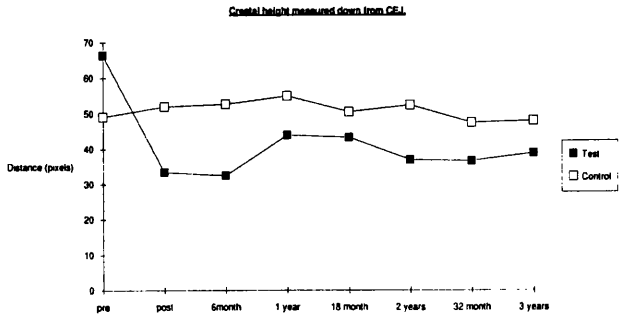

Fig. 7 Crestal bone height at constant density between test and control sites.

Discussion and conclusions

The system for computer analysis of radiographs includes comprehensive methods to compensate for discrepancies by means of a stepwedge and adjustment to match grey levels. The adaptation of the program to enable analysis to be performed on a microcomputer and the development of user-friendly, interactive software has been undertaken with the aim of introducing the system into general clinical use.

Programs have been developed for producing graphical representation of the bone density of given structures on radiographs. The analysis also enables qualitative data to be derived for the mean density over a selected area. A linear measurement may be determined from a given point (for example the enamel–cement junction) to another (for example the interproximal crestal bone defined by a selected density). Subtraction techniques may be used to represent changes visually, and the derived data may also be used to measure the area of change, and the mean density change over this area.

The system represents a significant innovation compared with previous computer-based methods, as the software is based on a microcomputer compatible with those already being used in many hospital departments and dental practices.

Acknowledgements

The authors wish to thank the Department of Health for financial support for the development of the system described. Thanks are also due to Miss Aurea Fenty for her help in preparing this paper, and to Mr Charles Day for the photography.

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IBM PC–AT compatible microcomputer: CAS Computer Point Ltd, London SE23.
Microsoft Windows: Microsoft Ltd, Reading, Berks.
Sintered hydroxyapatite: Durapatite, Sterling Winthrop

Computerized Densitometric Analysis of Interproximal Bone Levels in a Controlled Clinical Study into the Treatment of Periodontal Bone Defects With Ceramic Hydroxyapatite Implant Material

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THE AIM OF THIS CONTROLLED CLINICAL STUDY was to utilize computer-assisted densitometric analysis of radiographs to assess the effectiveness of treating periodontal osseous defects with a sintered hydroxyapatite implant material. It was found that over the 2-year period of the study for the osseous defects treated by the implant material, there was a gain in the height of the hard tissue relative to the cemento-enamel junction; this gain was statistically significant compared with the results for the control sites. *J Periodontol 1991; 62:44–50.*

Key Words: Periodontal disease/therapy; hydroxapatites; dental implants; computer-assisted diagnosis; radiography, dental.

There is the need in periodontal treatment to develop procedures that conserve periodontal tissue and increase the potential for repair and regeneration. More radical techniques, for example, those involving pocket eradication by the excision or apical repositioning of the soft tissue wall of the pocket, and osseous surgery to correct bone defects, result in a number of disadvantages. The associated recession with exposure of the root surfaces is often undesirable esthetically, hypersensitivity of the teeth to heat and cold is often a problem, and the exposed root surfaces are difficult for the patient to clean. The use of more conservative treatment procedures reduces post-surgical recession, thereby avoiding the complications described.

Conservative treatment to reduce pockets aims to achieve one or more objectives: the formation of a long junctional epithelial attachment,[1] the repair of a bone defect,[2,3] or the regeneration of new periodontal ligament fibers with reattachment to cementum and bone.[4] The healing of the various tissues, epithelium, bone, and connective tissue occur independently and it is clear from current research that the mode of healing of the various tissue systems should be considered separately.

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The treatment of periodontal bone defects represents a particular problem, as after surgery the recurrence of pocketing is not uncommon consequent to the downgrowth of an epithelial lining into residual infra-bony defects. After successful surgery the most common result of conservative surgical treatment of bone defects is repair with formation of a long junctional epithelial attachment, a thin layer of epithelium being found attached to the root surface even in the presence of new bone.[5] The filling-in of a bone defect potentially improves the adaptation of the gingiva to the tooth, increases the support for the tooth, and reduces gingival recession. Clinically, repaired bone defects have been found to be stable.[6]

Reports on the filling-in of bone defects after periodontal treatment do not agree about the degree of bone repair that occurs. Although some workers have found a considerable degree of filling-in,[6,7] others have found residual bone defects after surgery.[8] Residual defects are more common when the lesion is wide and deep and when bony walls do not completely surround the lesion.[9] The unpredictability of filling-in of bone defects after periodontal surgery has resulted in the investigation of various types of bone grafting materials[2] and of synthetic implant materials.[10,11] The source of bone for grafting procedures has varied. The ideal material is an autograft, but cancellous bone is not available except in very limited amounts from intra-oral sites. If iliac crest bone marrow is to be used, the need for a preliminary

operation is a major disadvantage. The initial operation to obtain donor tissue must be timed separately from that of the actual periodontal surgery, the graft material then frozen, as research has shown that fresh marrow may cause root resorption. The use of allografts in osseous defects has been described; but there is a slight danger of transmission of infection, and also the possible disadvantages of an immune reaction or of the osteogenic component of the graft being destroyed during removal of the organic, antigenic material.[12]

The use of synthetic implant materials to overcome some of the difficulties in treating osseous defects caused by periodontal disease has been investigated by a number of workers. Various assessment techniques have been used including clinical measurements, reentry procedures, histological assessment, and radiographic measurement. The use of radiographs has advantages because hard tissue levels can be measured and because the assessment procedures are not invasive. There have been several studies that have described systems for the densitometric analysis of dental radiographs.[13–15] The technique involves the registration and storage of optical density values from the radiographs by means of television or scanning devices, the output being transferred via an interface to a computer system. The distribution of light transmitted through the x-ray is recorded as a set of numbers. These measuring systems were found to be more sensitive than the human eye when detecting small changes in optical density and allowed a greater amount of clinically significant information to be derived from the radiograph. For longitudinal studies, a method was used to standardize the radiographs to take account of variation in film development and exposure. One such technique involved obtaining an image of an aluminium or copper step-wedge superimposed on the radiograph.[13,14] Techniques have also been described to reduce errors associated with variations in alignment of the tube and film, various devices having been used to enable reproducible series of radiographs to be taken of a given site.[13] These advances have allowed the changes in relative density at selective areas in serial studies to be compared.

The aim of the present study was to investigate the effectiveness of treating periodontal osseous defects with an hydroxyapatite implant material using clinical assessment criteria and densitometric analysis of radiographs.

MATERIALS AND METHODS

Subjects for the Study

The subjects for the study were nine patients aged between 33 and 59 years (mean age 43.2; 6 females, 3 males) who had been referred to the Periodontal Department of University College and Middlesex School of Dentistry.

Prior to their admission to the study, a medical history was taken from each patient to ensure that they had no systemic disease that might influence their periodontal condition or contraindicate periodontal surgery. It was ascertained that they were not wearing a prosthesis or orthodontic appliance. All participants were prepared to attend for regular follow-up visits.

The clinical criteria for selection were that the patients should have completed a course of treatment involving root planing and plaque control, and that they should have achieved consistently low levels of plaque during the last few assessments. The duration of this preparatory phase varied depending on the response of the patient to the plaque control program, and on the rate of resolution of the inflammation.

For admission to the study each patient was required to have at least two periodontal osseous defects of similar radiological appearance to be used for test and control sites. Where a greater number of lesions were present the additional sites were also used in the study. The subjects gave informed consent to participate in the project.

Clinical Assessments

The initial assessment of the periodontal condition of each of the patients was carried out after the root planing and plaque control phase of the study. Thereafter further measurements were performed at 6 months, 1 year, and 2 years.

The assessment techniques were similar at each of these time intervals. The measurements were made at multiple sites on each experimental tooth at all surfaces involved by the bone defect: at the interproximal surfaces vertically below the contact points, at the line angles, at the points of maximum convexity, and, on multirooted teeth, at the entrances to the furcations. Single rooted teeth were thus measured at five sites on both vestibular and oral aspects, whereas lower molar teeth were measured at seven sites on both aspects.

The measurment criteria for plaque were based on a 0 score for the absence of plaque and a 1 for each surface where plaque was present. Similarly gingivitis was based on a score of 0 or 1, depending on whether or not the gingiva showed bleeding within 20 seconds after probing at the various sites. Pocket measurements were carried out with a Williams graduated periodontal probe[§] with a point diameter of 0.6 mm. The distance from the cemento-enamel junction (CEJ) to the gingival margin was also measured using the same probe to enable attachment level to be determined.

Reproducibility was determined prior to the study for the various measurement criteria by assessing six volunteers on two separate occasions with a time interval of at least 60 minutes between the examinations. The percentage reproducibility was 87% for plaque, 91% for gingivitis, 92% for pocket measurement, and 88% for the level of the gingival margins.

Radiographic Procedures

The radiographic procedures were carried out using a standardized technique. A commercially available film holder

[§]Hu-Friedy, Chicago, IL.

and tube aligning system‖ were modified to enable a removable film-locating impression to be incorporated in the occlusal block, together with an aluminum step wedge.[16] The locating impression was used to improve the reproducibility of the aligment of the film and x-ray tube to the teeth. Experiments on dry skulls by Albander[17] have demonstrated that the errors from radiographic techniques were minimized by the use of an impression compared with using an unmodified film holder. In the present study the protection of the patients from unnecessary radiation precluded the taking of repeat films at the same visit. Measurements of bone level on films taken at different times will be influenced by changes in bone height as a result of the effect of treatment, or possibly of continuing periodontal disease and, therefore, could not be used for repeatability assessments. Clinical assessment of the series of radiographs indicated that there were some discrepancies in exposure geometry, such as changes in the degree of interproximal overlap or changes in position of the teeth on the film which indicate that it is more difficult to achieve reproducible alignment under clinical conditions than on dry skulls. There is also the possibility that minor changes in the position of individual teeth in the arches as a result of their periodontal condition may have had an influence on the relocation of the impression. The use of an individual impression for the repeated measurement of each site was aimed at reducing variation in the film and tube alignment. Radiographs of test and control sites would seem to be equally susceptible to any residual error.

Periapical radiographs were taken preoperatively, postoperatively, and at 6-monthly intervals thereafter until the end of the study.

The radiographs were assessed densitometrically using a system based on a microcomputer that has been described previously.[16] In brief, the system comprised a solid state camera which was used to obtain a video image from a given radiograph, illumination being provided by a light box. The resultant image was digitized and stored on a microcomputer disc. The image of the aluminum step wedge, which was incorporated in the film holder, acts as a standard and the inevitable discrepancies in technique for exposing and developing of films in serial radiographs of the same site can thereby be corrected.

The level of the interproximal bone was measured for the various sites by means of a specially developed algorithm.[18] The digitized radiograph was viewed on the screen and the cursor was used to mark and record reference points. For each interproximal region to be assessed a quadrilateral "zone of interest" was marked out, bounded by the two cemento-enamel junctions and by two points marked in the periodontal ligament spaces. The level of these points was not critical, being ascribed to a level about 2 mm apical to the base of the bone defects. A calculation was then made by the computer program of the gray-level histogram of the enclosed region. From this calculation, the appropriate threshold gray level to delineate the height of interproximal bone was determined.[18] The interproximal bone level was then determined by means of the same program. Three measurements were made for each interspace, the first being taken along the line from the point midway between the cemento-enamel junctions to the midpoint between the periodontal ligament markers. The computer program also enables measurements to be taken at intermediate points along these lines, and two further measurements were taken at the equidistant, tertiary points along the above two reference lines. For each interproximal site the mean of these three values was used to represent the level of the hard tissue at that site.

The reproducibility of the measurements obtained by the computerized system for determining the bone levels was assessed by a second operator independently by remeasuring 15 interproximal sites previously measured as part of the main study. The second series of measurements was undertaken several weeks after the first series. It was found that the mean value for the first series of 15 recordings was 91.21 pixels and the mean value for the second series was 91.01 pixels (there are approximately 17 pixels per mm). The maximum difference between any pair of recordings was 1.5 pixels. No statistically significant difference was found between these series of measurements (paired students $t = 0.41$; $P = 0.68$).

The postoperative values were subtracted from the baseline values to give a value for change in hard tissue level from baseline. It was not possible to take into account method error when comparing these values, as errors were likely to have arisen from several sources including: technical errors in film alignment; variation in film exposure and development, partly allowed for by the densitometric compensation using the image of the aluminum step wedge; and errors in defining the level of the hard tissue. However, by automating the analysis system to a considerable degree, bias has been removed as much as possible enabling both procedures to be assessed in such a way that human decisions are reduced to a minimum. The system thus enabled the distance from the CEJ to the interproximal bone (or to a repaired bony defect) to be measured objectively.

Surgical Procedure

Prior to periodontal surgery, randomization was used to allocate the implant and control procedures to the various experimental sites.

The surgical treatment was carried out under regional or infiltration local analgesia using 0.2% lignocaine with adrenaline 1:80,000. A conservative inverse bevel incision was used retaining as much of the gingival tissue as possible. Any remaining deposits were carefully removed, and the exposed cementum surfaces were planed with a curette until they felt smooth and hard. The bone defects were curetted to remove granulation tissue. The surgical area was then washed with physiological saline and carefully in-

‖Rin XCP System, Elgin, IL.

Table 1: Change From Baseline in Level of Interproximal Bone Measured in Pixel Units From the CEJ for the Nine Patients (test sites)

Patient	6 Months	1 Year	2 Years
1	4.17	7.60	6.50
	8.87	9.90	16.20
2	22.10	21.67	23.23
	52.37	5.00	−3.00
3	10.40	4.87	2.33
	14.97	17.63	17.10
4	8.80	12.03	14.03
	1.50	13.33	14.67
	42.03	41.23	39.93
5	9.00	1.67	3.33
6	9.50	−0.90	1.17
7	25.60	16.03	25.00
8	−0.27	−0.67	−8.37
	0.40	−3.30	−1.60
9	−15.23	−11.43	−18.17

Table 2: Change From Baseline in Level of Interproximal Bone Measured in Pixel Units From the CEJY for the Nine Patients (control sites)

Patient	6 Months	1 Year	2 Years
1	4.33	3.20	0.17
2	−2.67	−2.67	−1.67
3	6.37	5.37	0.00
	−12.30	−20.10	−21.13
4	2.63	7.13	6.33
	2.03	12.87	18.20
5	−6.23	−4.40	−0.10
6	−5.43	0.63	−4.13
7	−5.67	−6.77	−3.63
8	1.50	−1.13	0.47
	−3.73	−2.70	−5.30
9	0.90	3.50	2.23

Table 3: Mean Values and Standard Deviations for Changes From Baseline in Level of Interproximal Hard Tissue, Measured in Pixel Units From the CEJ for Test and Control Sites, With Statistical Analysis (17 pixel units are approximately equivalent to 1 mm)

Time	Test		Control		Student's *t*		Student's *t* (paired)	
	Mean	SD	Mean	SD	t	*P*	t	*P*
6 Months	12.95	17.01	−2.24	5.10	2.84	0.007	2.92	0.016
1 Year	8.98	12.54	−0.96	8.21	2.26	0.021	3.07	0.014
2 Years	8.82	14.71	−0.46	8.95	1.83	0.061	3.16	0.012

spected to ensure that the procedures had been completed satisfactorily. The test sites were treated by the implantation of sintered hydroxyapatite particles.¶ The most satisfactory method of inserting the material into the defect was mixing it to a paste with some of the patient's own blood, which was withdrawn from the operative site in a syringe. The resultant mixture was taken to the site on the blade of a curette. The control defects were left to fill with a blood clot. The flaps were then sutured over the wound with interrupted sutures using 4/0 braided silk. Dressings# were placed over the surgical area for 1 week. Subsequently the dressings and sutures were removed and the teeth cleaned and polished. A 0.2% chlorhexidine gluconate mouthwash was prescribed for use twice a day for 1 week.

Postoperative clinical healing was satisfactory. There was a tendency for some of the implanted material to be shed during the first few weeks.

RESULTS

The serial radiographs of the sites at which bone defects had been treated were examined to ensure that the required anatomical landmarks could be discerned; those series that proved suitable were subjected to densitometric analysis. A total of 15 test and 12 control sites will subsequently be referred to as the experimental sites. As a result of having to reject three radiographic series, there were, by chance, fewer control series than test series.

Table 1 shows the results for the test sites of the analysis of the change from baseline in the height of the interproximal hard tissue, measured in pixel units from the CEJ. At the various sites and time intervals there were 35 analyses showing gain in hard tissue and only 10 showing a loss relative to baseline measurements.

Table 2 shows the changes from baseline of the interproximal bone height for the control sites. These results indicate that 16 of the analyses show a gain in height, whereas 18 show a loss.

The compiled data in Table 3 show that at all the time intervals there was a significant mean gain in pixel units of hard tissue height on the implanted sites, whereas there was a slight mean loss of bone height on the control sites. Statistical analysis was carried out by two methods; using all the data in an unpaired test, or restricting the data to the matched test and control sites and using a paired test. Both methods of statistical analysis indicated that the test sites showed a relative gain in hard tissue height compared with the control sites.

The clinical data in Table 4 present the mean values for the proportion of surfaces with plaque present and values for bleeding on probing; the mean values for pocket depth and level of attachment relative to the CEJ are also given. Satisfactory levels of plaque control in general were achieved, although there was some degree of variation both between individual subjects and for different time intervals. The amount of inflammation was found to decrease after the operative procedures, with minimal differences between the two procedures. The pocket depths were reduced by a similar extent for both procedures, the 24-month mean values being approximately half those at baseline. The clinical attachment level also decreased for both test and control sites, the results again being similar for the two procedures.

DISCUSSION

Several studies have assessed the results of treating periodontal bone defects with implant materials.[10,19,20] The pro-

¶Durapatite (Penograf), Sterling-Wintrop,
#Coe-Pak, Coe Laboratories, Chicago, IL.

Reprinted from *Journal of Oral Implantology*, Vol XVI/No. Three/1990

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Variations in Healing of Infrabony Defects Treated with Ceramic Bone-grafting Material Observed Radiographically for a Period of Three Years

Abstract

This study has observed the radiographic changes occurring after surgical treatment of areas of severe periodontal destruction with bone loss. Regions designated test sites were grafted with Periograf (Durapatite®) and compared with non-grafted control sites over a three-year period, by use of a standardized radiographic alignment technique. Variations in the healing of both the grafted and non-grafted sites were observed, and it was concluded that regeneration of infrabony defects which had received grafting material can occur over a protracted period of time, which may be different in different individuals, and in different parts of the mouth within the same individual. In addition, periodontal destruction may occur as a slowly progressive condition, or in acute bursts of activity at different times, and at different rates in different parts of the mouth. Furthermore, some defects may be associated with active destructive periodontal disease, while adjacent areas concurrently demonstrate healing radiographically.

Introduction

The use of ceramic bone-grafting materials in the treatment of severe infrabony defects has been intensively studied over the last few years. The ceramic implant materials are produced from two closely related materials: (1) hydroxyapatite, which is considered non-resorbable; and (2) tricalcium phosphate, which is the anhydrous form of hydroxyapatite, therefore closely related to it and considered to be resorbable. Both forms of the ceramic bone-grafting material have been used extensively in the treatment of periodontal disease. Although good results have been achieved clinically with each, the exact nature of the healing process which takes place within the grafted sites is not yet fully established.

Review of the Literature

Rhinelander *et al.* (1971) found that when non-degradable ceramic was implanted in bone, connective tissue proliferation occurred, and trabeculae of bone were deposited directly onto and into the ceramic material. Getter *et al.* (1972) and Cutright *et al.* (1972) observed that bio-degradable ceramic made from tricalcium phosphate was very well-tolerated by the tissues and was progressively eliminated and replaced by bone. Levin *et al.* (1974), using tricalcium phosphate in monkeys, reported that it showed good tolerance by the host tissue and some evidence of osteoid formation eight weeks after surgery. They found that the ceramic granules which were in contact with the bone were progressively degraded and replaced by osteoid tissue,

whereas when not placed against bone, the granules became embedded in soft tissue and did not form osteoid but rather underwent progressive degradation and replacement by fibrous tissue. Boyne and Fleming (1982) indicated (in rhesus monkeys) that hydroxyapatite (Durapatite, which, along with Periograf, is a registered tradename for hydroxyapatite marketed by Winthrop Pharmaceuticals Limited) showed connective tissue fibers encasing the crystals of graft material placed in the coronal parts of infrabony defects, whereas in the more apical regions, bone was deposited in association with the graft material. There was no evidence of inflammation as a result of the grafting material.

Froum *et al.* (1982) examined histologic sections derived from extracted teeth which had failed clinically after periodontal therapy in which Durapatite was placed. No signs of osteogenesis were found, and in all cases the granules of grafting material were surrounded by a dense collagen mat without evidence of inflammation. Meffert *et al.* (1985) observed that sites treated with ceramic hydroxyapatite were partially calcified at re-entry, which was performed nine months after initial placement. Clinically, these sites were found to be resistant to penetration with a periodontal probe and to removal with curette. Stahl and Froum (1986) used tricalcium phosphate ceramic in the treatment of infrabony defects which were removed up to eight months post-surgery by means of block section. The subsequent histologic investigation showed that the particles acted as "non-irritating fillers" embedded in collagen and showed no signs of resorption of the graft material. Ganales *et al.* (1986) performed re-entry of surgical sites treated with Durapatite and obtained tissue samples which were then subjected to histologic investigation. Of the 19 samples obtained in this manner, two showed crystals of graft material embedded in bone, while 17 showed crystals embedded in densely packed collagen fibers. No signs of inflammation were present in any of the samples.

Bowers *et al.* (1986) examined resorbable ceramic particles which had been implanted in intra-osseous defects by means of a re-entry procedure performed after one year. They found evidence of osteoid forming alongside and inside many of the ceramic particles. Some fragments of osteoid material were also observed in sites not in contact with the ceramic material. Kenney *et al.* (1986) used porous hydroxyapatite implants which were removed from human subjects at various periods up to six months after placement due to the failure of the clinical procedure. The porous hydroxyapatite used in this study was found to be associated with progressively more osteoid formation over time, with evidence of lamellar bone formation six months after placement.

It is difficult for a conclusion to be reached from the results of these histologic investigations of the tissue response to synthetic bone-grafting materials. Comparisons between the results are made difficult by several factors: Some have analyzed animal tissue, while others analyzed human tissue; different commercially available synthetic grafting materials are used in different studies; and what little human material was available had been derived from cases in which the teeth were extracted due to concomitant pathology, which may have interfered with physiological healing. Furthermore, histologic investigation of healing is limited, since the site from which the sample is derived is destroyed so that the section can be obtained. Therefore, histologic investigations cannot be used for the longitudinal assessment of healing of a specific site over time. The studies using sections taken at different time periods after the placement of the graft may not be valid because of variations among experimental sites, making longitudinal evaluation of these results open to question as well. Since histologic evaluation of the healing status of infrabony defects is limited, particularly in the human subject, Moskow and Lubarr (1983) suggested that radiographic evaluation of healing for synthetic grafting material placed into infrabony defects may be a suitable non-invasive method for assessing their status longitudinally. It would appear that this method can serve as a useful alternative in the assessment of long-term healing of grafted sites. The purpose of this study was to evaluate radiographically the healing of sites which were grafted with Durapatite ceramic grafting material over a period of three years.

Methods and Materials

The findings reported in this study are based on the radiographs of two patients who are part of a group of subjects participating in a controlled longitudinal study. The radiographs of these two individuals were selected because they are characteristic of the varied healing response observed on radiographs representing the entire group. Both individuals received a careful screening to ensure that they were medically acceptable and to establish that they would be available for long-term maintenance therapy and evaluation of post-operative healing. In order to be selected for the study, each individual had to have a minimum of two similar infrabony defects which were suitable for periodontal therapy—one for grafting and the other to serve as a control. Each subject received a course of initial therapy which included

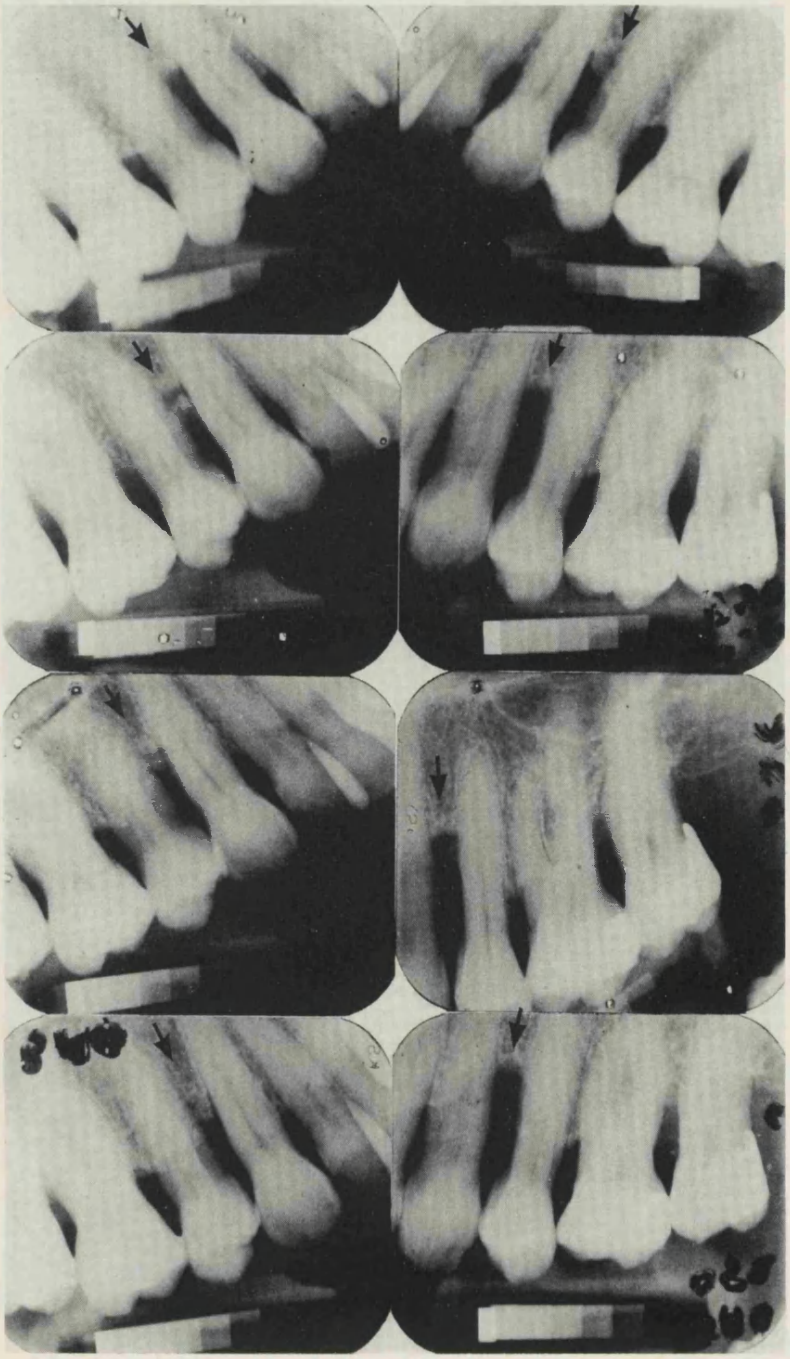

Figure 1. The healing responses in test and control sites in Case No. 1. (test, left; control, right) a1/a2, pre-operative; b1/b2, one week post-operative; c1/c2, six months post-operative; and d1/d2, one year post-operative.

oral hygiene instruction, and scaling and root planing under local anesthesia. An appointment was made one month after completion of the initial therapy so that the results of the initial therapy could be evaluated, and for confirmation that the level of oral hygiene maintenance justified periodontal surgery. Those individuals found suitable for the study then underwent surgical exposure of infrabony defects by means of the modified Widman procedure as described by Ramfjord and Nissle (1974), with care taken that as much of the gingival soft tissue as possible be preserved. Test and control sites were determined by means of randomization tables. Periograf (Durapatite)® synthetic bone-grafting material was then placed in the test sites only. The control sites were allowed to fill with a blood clot. The surgical wounds were closed by means of interrupted sutures, and periodontal packs were placed over all study sites. One week after the surgical procedure, packs and sutures were removed and a second appointment made one month post-operatively for preliminary

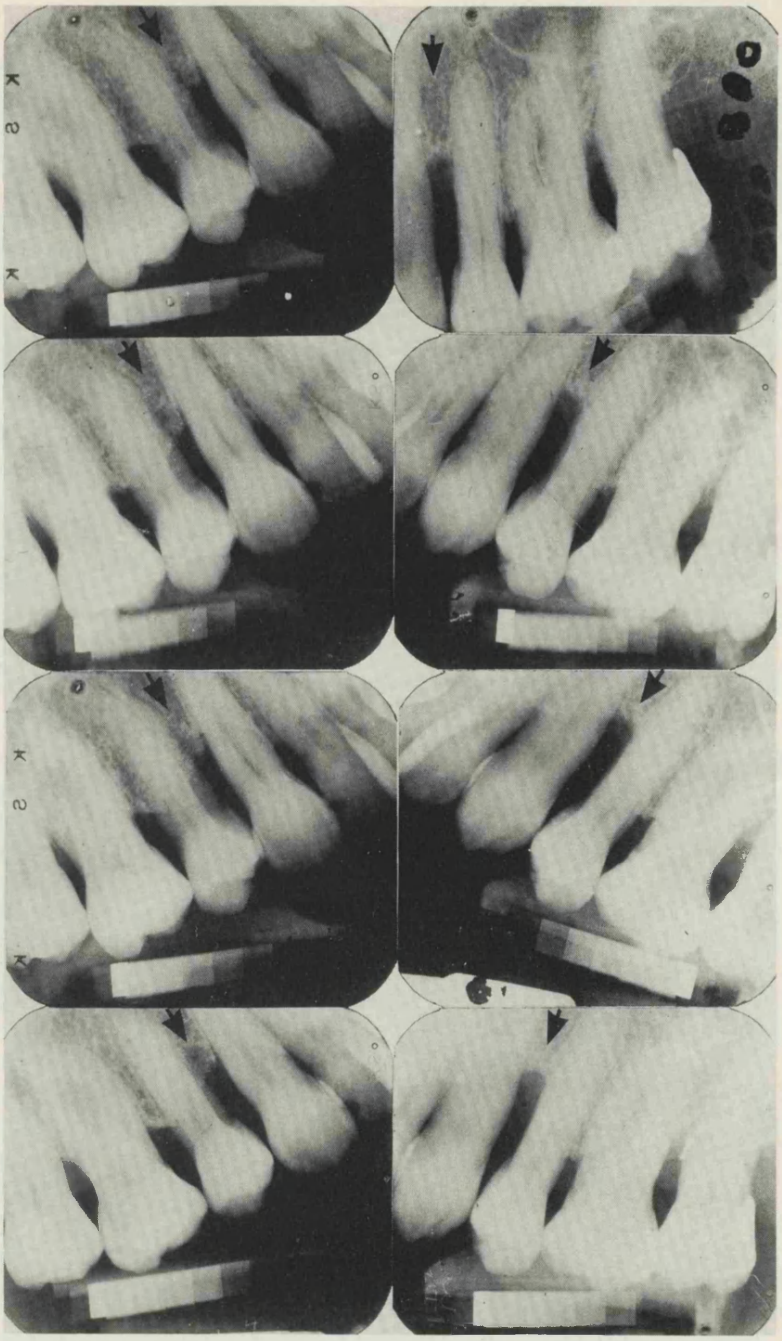

Figure 1 (continued). (test, left; control, right) e1/e2, 18 months post-operative; f1/f2, two years post-operative; g1/g2, 2½ years post-operative; and h1/h2, three years post-operative.

assessment and plaque removal. The patients were then seen at three-month intervals throughout the study for remotivation in oral hygiene and clinical evaluation of all the sites under investigation.

Radiographic assessment of the infrabony defects was performed prior to surgery, one week after placement of the grafting material, and six months thereafter, with use of the Rinn radiographic paralleling system adapted to incorporate an aluminum wedge and a custom-made plastic table on which an elastomeric impression of the occlusal surfaces of the individual patient's teeth could be registered. This ensured that standardized radiographic views of the defects would be obtained for the duration of the study.

Radiographic observations of healing in both the grafted and non-grafted sites are presented in these case studies.

Results

The results are shown in Figs. 1, 2, and 3. Fig. 1 represents the healing typical of a successful case, and Figs. 2 and 3 show different areas from an-

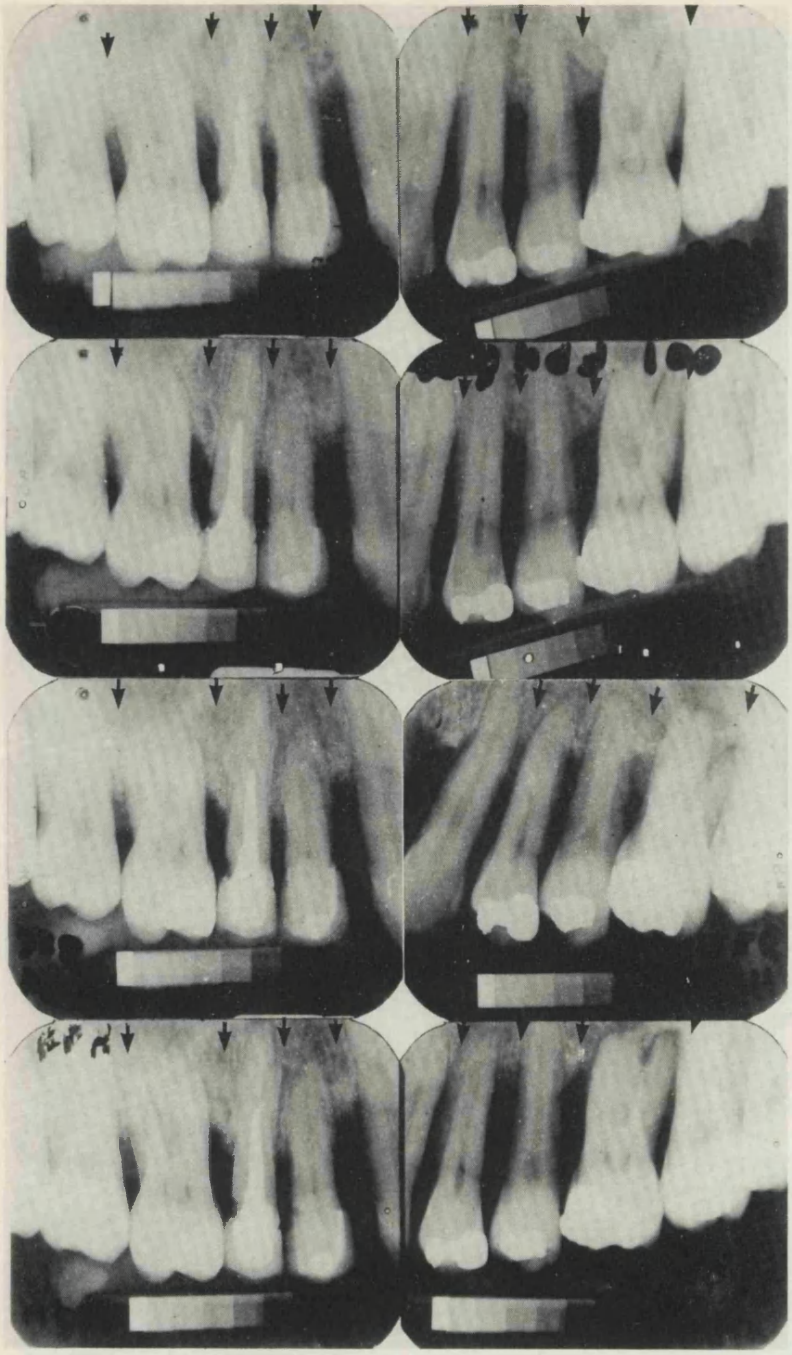

Figure 2. The healing responses in test and control sites in Case No. 1. (test, left; control, right) a1/a2, pre-operative; b1/b2, one week post-operative; c1/c2, six months post-operative; and d1/d2, one year post-operative.

other individual whose response to treatment was only partially successful.

Case No. 1

Case No. 1 was a Caucasian female in her middle fifties with good oral hygiene and two localized periodontal defects associated with the maxillary and right canines and premolars. The mirror-image defects are shown in Figs. 1a1 & 1a2 prior to surgery. Figs. a1 to f1 show the test side and a2 to f2 the control side at various time intervals. Radiographs 1b1 and 1b2 show the defects one week after surgery. On 1b2, little change is visible as compared with the pre-operative radiographs, while on 1b1, the grafting material is clearly visible near the base of the defect. This is seen as a radio-dense area which has not been placed fully in contact with the apical bony margin of the defect. Radiographs 1c1 and 1c2 at six months show that little change has taken place on the control side (1c2), whereas in the test side (1c1), the grafting material has diffused to the full extent of the intrabony defect. By one year (1d1, 1d2), little change has taken place on the control side, while

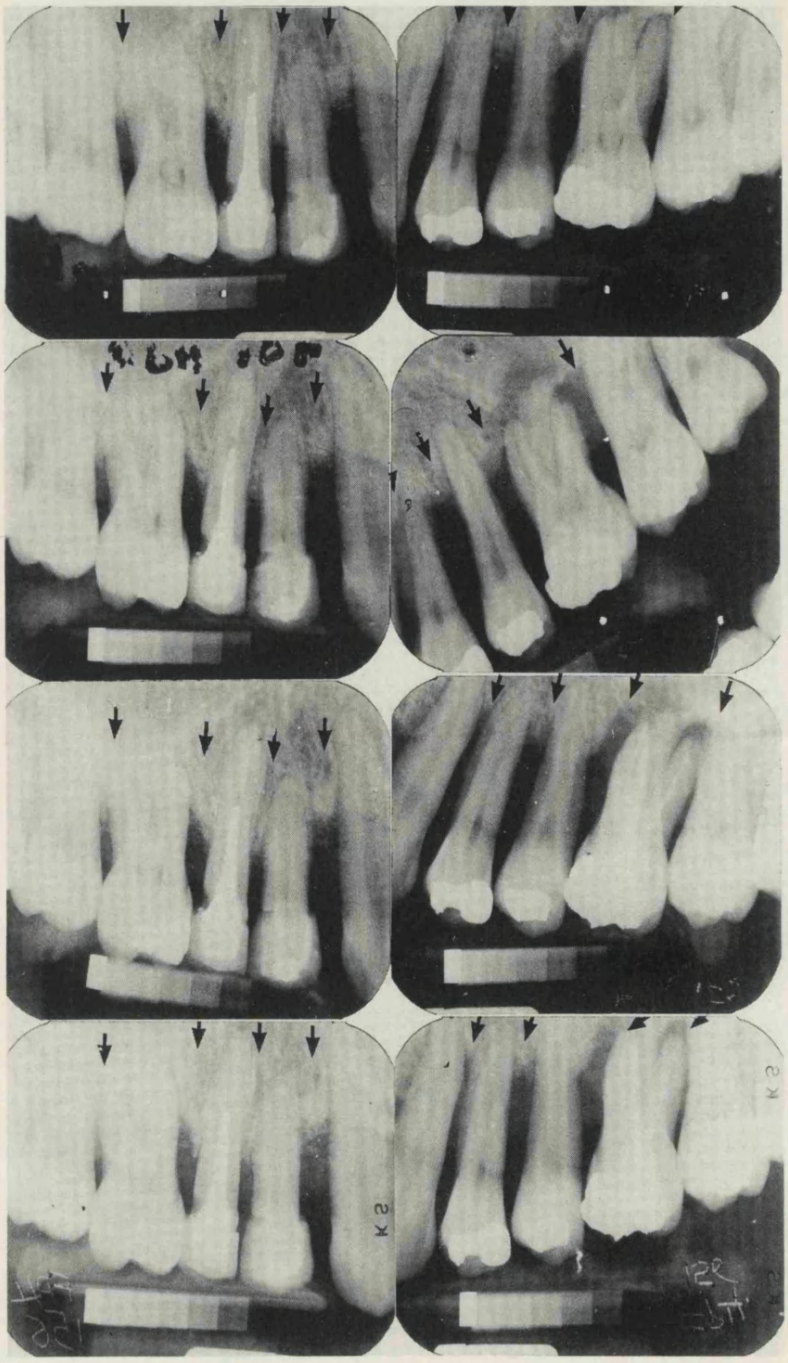

Figure 2 (continued). (test, left; control, right) e1/e2, 18 months post-operative; f1/f2, two years post-operative; g1/g2, 2½ years post-operative; and h1/h2, three years post-operative.

on the test side, there is evidence of some trabeculation and loss of granularity in the grafting material. By 18 months, no change has occurred in the control defect (1e2), while the test side (1e1) shows still further change with additional trabeculation occurring, further loss of granularity of material, some radiolucency in the area, and the appearance of what seems to be a new periodontal space on the distal aspect of the canine. By two years after surgery (1f1, 1f2), no change has occurred on the control side, whereas in the test area, evidence of trabeculation and loss of granularity is progressing, with the maintenance of a "periodontal space" distal to the canine. By 2½ years after surgery, increasing evidence of trabeculation is seen in the test side (1g1), with only minimal evidence of the characteristic granularity of the grafting material. The control side (1g1) shows no signs of any change in the bone. By three years, further bone regeneration is evident in the test region (1h1), with the formation of a lamina dura distal to the canine, indicating the deposition of

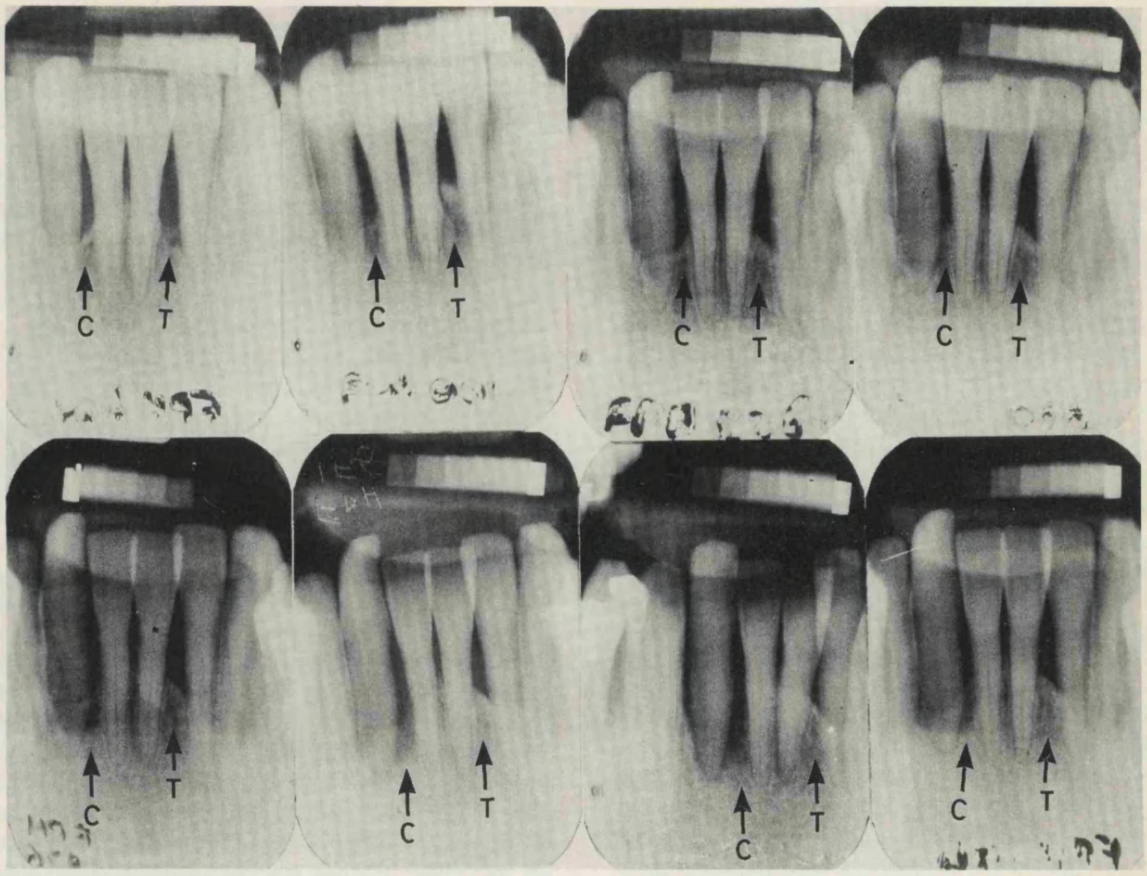

Figure 3. The healing responses in test and control sites in another region of the mouth in Case No. 2. (C = control, T = test) a, pre-operative; b, one week post-operative; c, six months post-operative; d, one year post-operative; e, 18 months post-operative; f, two years post-operative; g, 2½ years post-operative; and h, three years post-operative.

cortical bone lining the periodontal space on this tooth. Since the lamina dura does not continue over the crest of the interdental bone, it may be presumed that healing in this area is still not complete. The control side (1h2) still shows no change in the bone.

Case No. 2

Individual No. 2 was a Caucasian male in his middle fifties who presented for treatment with increasing mobility of his teeth and bleeding of his gingivae. The upper anterior teeth were considered so hopeless as to be extracted, and an immediate replacement prosthesis was fitted. Fig. 2 (a-h) illustrates the radiographs of the upper posterior quadrants through the study. In the preoperative radiographs (2a1, 2a2), extensive periodontal disease has occurred, and this resulted in extensive loss of supporting alveolar bone. Although the teeth were considered to have had hopeless prognoses, it was decided to attempt to treat those in the maxillae by means of surgical exposure of the defects, debridement, and placement of synthetic bone-grafting material in designated test sites. The maxillary right side was designated as the test region, and the upper left side was designated as the control. The radiographs taken at one week after surgery (2b1, 2b2) show evidence of retention of some of the grafting material (2b1), although clinically it was evident that some of the material had been exfoliated. The control site (2b2) showed no change in the levels of supporting bone one week after surgery. At six months post-operatively, no change has taken place in the grafting material in the test site (2c1), while at the control sites, further loss of bone, particularly between the premolars and the distal root of the molar, is clearly visible. By one year post-operatively, very little change has taken place in the grafted site (2d1), while in the control site (2d2), the bone loss seems to be stable in all areas except the distal root of the molar, which shows signs of periapical involvement. By 18 months after surgery, no change has taken place in sites with the grafting material (2e1), while the control site (2e2) shows little further change in the levels of bone.

At two years post-placement, the grafted site (2f1) shows no further change, while the non-grafted site (2f2) shows a sudden and increased level of bone loss, with a clear periapical radiolucency appearing at the distal root of the molar. The sudden increase in destructive activity can be seen clearly on radiograph 2g2, which was taken 2½ years after surgery. The test site (2g1), on the other hand, has remained stable throughout this period. By three years after placement of the grafting material, the destructive process on the control side (2h2) has not progressed, and this may be indicative of a quiescent period or a remission of destructive activity. The test area (2h1) still shows no change in the granularity of the grafting material.

Fig. 3 (a to g) shows the response to treatment in the lower anterior region of the same individual. Test and control defects are between the four incisors. With use of randomization tables, the left side was designated as the test site and the right was designated as the control. Pre-operative radiograph 3a shows that extensive, predominantly horizontal, bone loss has occurred on both sides. Radiograph 3b shows the two areas one week after surgery. The left has a radiopaque area of grafting material in the defect, whereas the right does not. Radiograph 3c shows the defect at six months after placement; in both cases, good healing has taken place. In the test area, evidence of some trabeculation within the grafted area is seen, while in the control, similar healing has taken place without the benefit of grafting material. By one year (Fig. 3d), both sites have remained stable. Eighteen and 24 months after surgery (radiographs, 3e,f), recurrence of the periodontal problem has occurred, with evidence of further extensive bone loss in the control area, while no further bone loss has occurred in the test site. By 2½ years (radiograph 3g), extensive bone loss is still occurring in the control area, with possible periapical involvement of this tooth, while no further degeneration has occurred in the test area. At the end of three years (radiograph 3h), some degeneration of the bone in the control area seems to be occurring, evidenced by increased radiolucency in the periapical regions and between the two incisors. Throughout the period of observation, continued evidence of loss of granularity of the particles of graft material, with trabeculation of bone, was visible in the test defect on the left side, which is characteristic of the healing process as described in patient No. 1.

Discussion

Case No. 1 may be described as a localized adult periodontitis which resulted in bone loss. Clinically, healing took place in both test and control areas whether or not grafting material was used. Although the grafting material is described as non-resorbable, the radiographs contain evidence of changes in the grafted areas over a period of three years. The material initially appears to be hypercalcific and granular, whereas over a protracted period of time, it appears to become more radiolucent. Subsequent trabeculation characteristic of new bone formation is noted. This seems to indicate that resorption of the material and its replacement with natural bone have occurred in healed periodontal defects. Furthermore, about one year after placement, some evidence of a periodontal space became visible (on the radiographs) that appeared to be more clearly defined with time. This might indicate a level of periodontal membrane regeneration over a period of time. These changes took place over prolonged periods of time, and it would appear that the regenerative process in periodontal therapy may occur very slowly. The assessment of healing as a result of the placement of synthetic bone grafting material probably should take place over a long time period. Since changes are still taking place three years post-operatively, it is not known how long the healing process will continue. It is clearly not complete at this stage.

In Case No. 2, a very different clinical result was obtained. After surgery, the tissues were thin and friable, with a significant tendency toward gingival bleeding. Despite regular post-operative maintenance at three-month intervals, recurrence of pockets occurred, presenting the features of a rapidly progressive type of periodontal destruction. In the series of radiographs presented in Fig. 2, the control series (2a to h) demonstrated the rapidly progressive nature of the condition. However, it is interesting to note that although some bone loss occurred immediately after surgery in the control side (b2), this remained stable for a period of 18 months. In the area of the left premolar/molar, a sudden "burst" of activity occurred, leading to a marked increase in bone loss from two to 2½ years after surgery. The area between the two molars, on the other hand, showed a slow, continuous pattern of degeneration throughout the length of the study. Thus, the degeneration of the supporting bone indicated different characteristics at different sites of the 1st molar. Mesially, a burst of activity occurred after a period of stability, whereas distally a continuous slow degeneration was tracked. This might indicate that different types of disease activity can occur in the same mouth, and even around the same tooth, concurrently. When the test areas were evaluated (2a to h), no changes occurred in the grafting areas, and no further degeneration of the tissues around the teeth was noted. This may offer evidence that the grafting material had a "buffer" effect in making the

tissues more resistant to breakdown. In contrast to Case No. 1, no changes in the granular characteristics of this grafting material took place over the three-year period. This might be because of the intractable nature of this case, which prevented active regeneration of the bone and resorption of the grafting material as seen in Case No. 1, due to the continued presence of clinical soft-tissue inflammation. Thus, the response of a defect in which bone-grafting material has been placed may vary, depending upon the conditions and environment that are present. When healing of the soft tissues is noted with no recurrence of the inflammatory process, repair of the underlying hard tissues may occur progressively over a period of time with the possible formation of osteoid-like material or bone and possibly the regeneration of a new periodontal space, while in the presence of continued inflammation, no regeneration can occur, due to the phenomenon of hyperemic decalcification, and the grafting material will remain *in situ* as a non-irritant "filler" which will not undergo any changes over time. This would explain the differing histologic findings described in the literature, in which, in some cases, the graft seems to be associated with bone, while in others, it is incorporated only in a fibrous mat. There is, unfortunately, no way of accurately assessing the histologic picture in human subjects so that the precise nature of tissue change observed in the radiographs can be established. However, Sapkos (1986) has observed that new osseous tissues associated with an apparently functional periodontal attachment, complete with Sharpey's fibers and new cementum, are possible in areas grafted with Durapatite grafting material. This may explain the observation of a periodontal space in the series of radiographs illustrating the healing in the first individual.

In the series illustrated in Fig. 3 (a to h), a similar picture is seen in the same patient. In the control area, stability of the defect occurs in the immediate post-surgical period, with a clear lamina dura visible. However, by one year post-surgery, some evidence of loss of bone is visible mesial to the right lateral incisor, which becomes increasingly noticeable by 18 months and progresses even more at two and 2½ years. Once again, this illustrates the "burst"-like nature of the disease process with a period of remission after surgery and an exacerbation approximately 18 months post-operatively. It is noteworthy that this sudden catabolic activity had not occurred at the same time as the burst which occurred in the maxillary left quadrant. This would be expected in terms of the "random burst" theory proposed by Socransky *et al.* (1984), in which bursts of activity may occur in different sites at different times at different rates in the same mouth. In the test area, the grafting material has been incorporated into the bone, and the defect has become stable with some loss of granularity. This is in contrast to the upper right quadrant, where no change in the granularity occurred over the entire period of study. Thus, the healing process may also be different in different parts of the mouth at the same time, which would tend to indicate that some sites are more susceptible to recurrence of periodontal disease than others. Thus, the disease process may progress at different rates in different sites in the same mouth, with a pattern of periods of quiescence followed by acute bursts of activity. Conversely, healing of the tissues in infrabony defects takes place over a long period of time. In different sites within the same mouth, differences in the type and rate of healing may be observed radiographically. This would explain the inconsistency in findings of histologic investigations in which some studies have indicated osteoid formation with synthetic bone-grafting material, while others have not.

Conclusions

From the two cases presented in this study, it can be seen that radiographic changes are visible in infrabony defects observed over a period of three years. It is therefore essential for the healing process to be studied over periods of three years or more when the healing of infrabony defects is being investigated, particularly in response to the placement of grafting materials.

Healing processes seem to be different in different individuals and in different sites in the same individual.

In the presence of recurrent periodontal disease, the grafting material may act as buffer to further degeneration and bone loss around an affected tooth, prolonging the useful life of that tooth in function.

In the presence of recurrent periodontal inflammation, the regenerative processes seem to be inhibited, with no changes occurring within the area occupied by the grafting material.

In the presence of healing and long-term stability of the soft tissues, healing of the supporting hard tissues can occur, with continuous evidence of bone formation occurring within the grafted area and the development of a new periodontal space along the root surface.

In the healed periodontal defect, progressive resorption of non-resorbable grafting material seems to be visible radiographically over a three-year observational period.

Assessment of the success or failure of infrabony defects treated with grafting material can be established only over prolonged periods of time, which

may be in excess of three years after placement of the grafting material.

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International Dental Journal (1990) 40, 333–338

A comparison between measurements made with a conventional perlodontal pocket probe, an electronic pressure probe and measurements made at surgery

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SUMMARY

This study compared clinical measurements using a manual periodontal probe and an electronic pressure probe using a 0.25 N force, and related them to measurement of the bone levels at the time of surgery. All measurements were taken from the cemento-enamel junction at specified sites. It was found that neither probing technique provided a means for accurately measuring bone level. In the presence of healed tissue, little difference was found between the two instruments; whereas the manual probe more closely approximated the actual level of bone in the presence of inflammation. It was therefore concluded that under the conditions of present study the manual probe depth measurements were the more applicable for routine clinical use.

Periodontal probing is one of the main methods of detection and assessment of periodontal disease used in clinical practice. However, the accuracy of these measurement techniques has been questioned[1–11]. Several studies have suggested ways in which improvements could be made[3,4].

It has been suggested that controlled force periodontal probes might improve the accuracy of probing depth measurements[12,13] by minimizing the variation in the probing forces applied by clinicians. The differences in the attachment level assessments could be as great as 2 mm[14]. The most appropriate probing force for clinical use was found to be 0.25 N force[15]. Several disadvantages of using constant pressure probes in the clinical assessment of probing depth have been described. These have included poor access to the molar areas, difficulty in detecting the end point of probe penetration, production of rotational and lateral forces causing instability of the probe tip[16], visual and tactile observation errors, and positional errors[5].

The aim of this study was to compare probing depth measurements obtained by means of a manual probe with an electronically controlled constant force probe, and to relate these to alveolar bone levels measured during surgical exposure of infrabony defects.

Materials and methods

Nine patients with advanced periodontal destruction received initial therapy, followed by periodontal surgery using the modified Widman procedure as described by Ramfjord and Nissle[17] to gain access to the infrabony defects. After surgery the flaps were sutured with interrupted sutures, and thereafter the subjects were placed on a 3-monthly regular maintenance programme.

Assessments of the periodontal pocket depths and attachment levels were undertaken pre-operatively and after 6 months, by measuring the degree of recession from the cemento-enamel junction to the free gingival

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margin, and the pocket probing depth from the free gingival margin to the point of resistance to probing. These figures were summed to obtain data for attachment loss. The pocket measurements were first made using a Vine Valley Research model 200 (Middlesex, NY, USA), electronic periodontal pressure probe, which was set to register a probing force of 0.25 N, as recommended in the literautre[15]. The measurements were then repeated using a standard Williams periodontal probe, with an identical probe tip to the electronic periodontal pressure probe. In addition, during surgical exposure, the distances from the cemento-enamel junction to the level of the alveolar bone were measured at the sites under investigation using the periodontal probe.

The measurements for the study were all undertaken at predetermined sites by the same investigator (PNG), who was experienced in periodontal probing techniques. A total of 949 sites were evaluated in the study.

The results were divided, according to the initial values obtained with the manual probe, into subgroups of mild (0–3 mm), moderate (4–5 mm) and severe (6 mm or greater), and an analysis of the data obtained with the two probes was performed using the Student's 't' test for paired data.

Results

Tables 1 and *2* give the mean values for pocket depth and attachment levels for mild, moderate, and severe pockets, before treatment and 26 weeks after surgical treatment. It can be seen from these tables that over the 26-week period a reduction in pocket depths and a gain in attachment occurred in all of the groups irrespective of whether this was assessed by means of the manual probe, or the electronic pressure probe. In all cases mean pocket depths and loss of attachment measurements using the electronic pressure probe were less than those using the manual probe.

Statistical analysis indicated that the differences between the measurements taken with the two instruments was significant at baseline, especially in the case of the moderate and severe groups. At 26 weeks after surgery the differences between the measurement techniques were much smaller, significance only being found between pocket depths in the severe group and loss of attachment in the moderate group ($P > 0.05$).

The mean values of pocket depth measurements and attachment level using manual and pressure probe immediately before periodontal surgery are shown in *Table 3*. These are related to corresponding values at the same sites taken at open flap surgery measured

Table 1 Mean (± SD) values for pocket depths derived using the manual and pressure probes for mild, moderate and severe pockets at baseline and 26 weeks after surgery

Weeks no.	*Classification*	*Manual probe*	*Pressure probe*
Week 0 (baseline)	Mild	1.57 ± 0.58*	1.14 ± 0.51*
	Moderate	3.89 ± 0.38**	2.16 ± 0.48**
	Severe	7.13 ± 0.72**	4.30 ± 1.19**
26 weeks	Mild	1.16 ± 0.47	0.84 ± 0.34
	Moderate	1.97 ± 0.58	1.36 ± 0.30
	Severe	2.81 ± 0.95*	1.91 ± 0.73*

Significance levels (manual *v.* pressure probe): * $P < 0.05$; ** $P < 0.001$.

Table 2 Mean (± SD) values for loss of attachment using the manual and pressure probes for mild, moderate and severe pockets at baseline and 26 weeks after surgery

Weeks no.	*Classification*	*Manual probe*	*Pressure probe*
Week 0 (baseline)	Mild	1.90 ± 0.72	1.43 ± 0.57
	Moderate (3.5–5.0 mm)	4.57 ± 0.70***	2.89 ± 0.37***
	Severe	7.86 ± 1.32**	5.11 ± 1.62**
26 weeks	Mild	1.98 ± 0.81	1.71 ± 0.78
	Moderate	3.42 ± 0.57*	2.79 ± 0.51*
	Severe	4.91 ± 1.09	4.01 ± 1.02

Significance levels (manual *v.* pressure probe): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

from the cemento-enamel junction to the level of the alveolar bone. The results for the manual probe were found to be higher than those for the pressure probe at this and at other time intervals. It can also be seen that they were closer to the values for the bone levels derived at periodontal surgery.

The data were divided into anterior and posterior segments to see whether the position of the teeth in the arch would influence the measurement techniques as a result of differences in ease of access (*Table 4*). In the anterior segments it was found that use of the manual probe resulted in deeper pockets being recorded than the pressure probe, and similarly the level of attachment was also found to be greater using the manual probe ($P > 0.001$).

However, the difference between the two techniques was found to be minimal in the posterior segments although there was a similar trend to that in the anterior regions. *Table 4* also shows that the manual probe measurements of the level of attachment more closely approximate the bone level measurements, especially in the anterior segments, although both probes underestimated the degree of bone loss. The manual probe clearly gave deeper pocket depth readings both in the anterior and the posterior sites. There was a highly significant difference between the two instruments in the anterior sites ($P > 0.001$), while there was no significant difference between the two instruments in the posterior sites.

In both the anterior and posterior sites, the manual probe measurements of the level of attachment more closely approximated the bone levels at the time of surgery than the pressure-controlled probes.

Discussion

The measurement of loss of attachment using the manual probe gave readings closer to the level of bone measured at surgery than the pressure-controlled probe (*Tables 3* and *4*). The mean values for the loss of attachment using the electronic pressure probe were 2.82 mm less than the surgical bone level measurements, whereas the values for the manual probe were 1.24 mm less than the surgical measurements. Simons and Watts[18] found that the probe tip of both manual and pressure probes penetrated to a point 1.2 mm coronally to the level of connective tissue attachment, both manual and pressure probes giving similar readings. In the present study the manual probe gave readings similar to the findings of these workers, but the pressure-controlled probe did not result in as great a degree of penetration.

In normal clinical use, the manual probe is probably introduced into the pockets with sufficient pressure and in such a manner as to displace or rupture the delicate inflamed epithelial tissue at the base of the pocket, or it may even come to rest within the coronal portion of the fibrous tissue corium as described in the literature[7,8,18]. Conversely, the pressure-controlled probe probably measured the anatomical base of the soft-tissue pocket more accurately, coming to rest on or in the long junctional epithelial tissue[8]. The difference of 1.58 mm between the two techniques is

Table 3 Mean (± SD) values of pocket depths and levels of attachment before surgery, related to mean (± SD) bone level derived at the time of surgery

	Pocket depth	*Attachment level*	*Bone level*
Manual	4.19 ± 0.59	5.15 ± 0.91	5.63 ± 2.54
Pressure	3.05 ± 0.71	3.57 ± 0.85	

Table 4 Data for anterior and posterior sites; pocket depths and levels of attachment using manual and pressure probes at baseline related to bone levels at surgery

	Anterior sites		*Posterior sites*	
	Manual	*Pressure*	*Manual*	*Pressure*
Pocket depth	4.57 ± 2.75*	2.75 ± 4.26*	3.8 ± 2.77	3.35 ± 4.92
Recession	0.76 ± 6.69		0.18 ± 0.60	
	Manual	*Pressure*	*Manual*	*Pressure*
Attachment level	5.33*	3.55*	3.98	3.53
Surgical bone level	5.67 ± 2.68		5.58 ± 2.40	

Significance levels (manual *v.* pressure probe): * $P < 0.001$.

approximately the width of the normal junctional epithelium. However, it could equally be argued that both the manual and the pressure-controlled probes were giving results that were inaccurate, as suggested by Watts[5].

The relatively large difference between manual and pressure-controlled probes was not evident in the post-treatment results. The various grouped data indicated that there was good resolution with mean residual crevice depths of less than 3 mm in all cases, the standard deviations for manual and pressure probes also being similar. Greater variation in the measurements may occur in inflamed tissue than in healthy tissue[10] and this concept offers an explanation for the closer approximation of measurements as the periodontal condition improved.

When these results are taken in conjunction with the observations that the manual probe gave results closer to the surgical measurements than the pressure-controlled probe, it was considered that measurements using the manual probe were more valid than the results using the electronic pressure probe at a force of 0.25 N.

Magnusson and Listgarten[2], using a periodontal probe which could be operated as a manual probe as well as a constant pressure probe, observed high levels of correlation between probing measurements taken with the instrument in these two modes of operation, indicating that probing pressure was not the cause of reduced accuracy, but rather observational and positional differences occurring as a result of using two different instruments. The subjective clinical observations of the operator using both instruments was that the shank of the electronic pressure probe was large and bulky and difficult to place accurately in some parts of the mouth, especially where the teeth were bulky with increased convexity of the crowns. Errors also occurred as a result of an inability to read some of the pocket depths accurately when the measurement sites were obscured by the shank of the instrument. Another problem encountered was a loss of tactile sensation which occurred with the pressure-controlled probe, as a result of which there were false readings associated with irregularity and roughness of the root surfaces. In addition, the bulky handle section of the pressure probe tended to be fouled against the occlusal surfaces of the teeth, particularly in deep defects. This was particularly relevant in the posterior sites, resulting in readings unrelated to actual depths of the defects. On the other hand, using the manual probe, greater tactile sensation enabled the probe to be introduced into the pocket beyond the level of any small amount of sub-gingival calculus and tooth surface aberrations.

As the manual probe gave readings closer to those of the actual surgical defects, especially in the anterior segments (*Table* 4) it probably gave more valid measurements compared with the pressure-controlled probe. The standard deviation was greater for the electronic pressure probe than the manual probe. This higher variation of readings with the electronic pressure probe could be interpreted as indicating reduced accuracy with this probe.

The manual probe was found to be a more versatile instrument than the electronic pressure probe as a result of its compactness, lightness and increased tactile sensibility.

Accessibility of the sites may be a factor in determining the accuracy of both pocket-depth measuring instruments, as described by Watts[5]. It was found that in both anterior and posterior sites, the manual probe produced readings closer to the actual depth of the surgical defects, and that the difference between the two instruments was highly significant. The differences between the two instruments may indicate that they measure different end points – the manual probe measuring close to the fibrous tissue attachment as described in a previous study[18], while the pressure probe measured the coronal level of the junctional epithelial attachment[18], or part way into the junctional epithelium[8]. It may be that the 0.25 N standardized force is not adequate to penetrate the pockets to the same extent as the manual probe, as the pressure-controlled probe gave consistently lower readings in both the anterior and posterior sites.

Although a variety of probing forces have been evaluated[15], the ideal level of probing force to be applied has not been established, and it could be that higher standard probing forces may be required to get equivalent measurements between manual and pressure probes. In addition, the experienced clinician is aware of the variation in the resistance to probing of the tissues at the base of the pocket that occurs in different sites and different individuals. Sensitive tactile perception allows variation in the duration and line of application of forces in response to the consistency of the tissues in clinical practice. It may be that there is no standard routine or force that can be specified that would produce consistent results in all circumstances, and that over-reading or under-reading of pocket depths may occur in an instrument having a fixed probing force. However, the disadvantage of variation in the force applied to the probe is clearly a problem with any manual instrument.

Conclusions

Our findings show that the measurement of pockets with a manual probe was found to relate more closely and more consistently to the bone levels at the initial assessment, where there was more inflammation, especially in the anterior segments where access was good.

For the assessment of healthy tissue, there was little or no significant difference between the measurements obtained with the two instruments and there was little or no significance between the readings in shallow crevices whether these were associated with inflam-

mation or not. The greatest differences occurred for untreated, deeper pockets.

The greatest difficulty in using the pressure-controlled probe was found in the posterior, less accessible segments. This may be a result of the sensitivity of the pressure-controlled probe to root surface irregularities and its increased bulkiness, often making accurate clinical assessments of pocket depths difficult.

It is therefore concluded that in routine clinical practice, and in studies that involve a single, experienced operator, it is unnecessary to utilize a standardized pressure probe, and that the use of this probe may even contribute to greater inaccuracies in the data compared with a manual probe.

F

COMPARAISON ENTRE LES MESURES PRISES AVEC UNE SONDE CLASSIQUE À CUL DE SAC PARODONTAL, UNE SONDE ÉLECTRONIQUE ET CELLES PRISES PAR LE CHIRURGIEN

RÉSUMÉ

Cette étude compare les mesures cliniques prises avec une sonde parodontale manuelle et celles données par une sonde électronique à pression utilisant un courant de 0.25 N avec les mesures des niveaux osseux effectuées lors de l'acte chirurgical. Toutes ces mesures ont été prises à partir de la jonction cément-émail à des endroits bien précis. On a trouvé qu'aucune des techniques de sondage ne donnait des mesures exactes du niveau osseux. Sur du tissu cicatriciel, il n'y avait que peu de différence entre les deux instruments; par contre, la sonde manuelle s'approchait davantage du niveau osseux réel lorsqu'il y avait inflammation. D'où l'on conclut que, dans les conditions actuelles, l'utilisation de la sonde manuelle pour mesurer la profondeur des culs de sac restait le système le plus commode en pratique clinique courante.

D

EIN VERGLEICH ZWISCHEN MESSUNGEN MIT EINER KONVENTIONELLEN PA-SONDE, EINER ELEKTRONISCHEN DRUCKSONDE UND MESSUNGEN BEI DER CHIRURGISCHEN BEHANDLUNG

ZUSAMMENFASSUNG

In dieser Untersuchung werden klinische Messungen mit einer manuellen PA-Sonde und einer elektronischen Drucksonde mit 0.25 N Kraft mit den Messungen des Knochenniveaus bei der chirurgischen Behandlung verglichen. Alle Messungen wurden an spezifischen Stellen der Schmelz/Zementgrenze vorgenommen. Es wurde festgestellt, daß keine Sondenmethode ein Mittel zur genauen Messung des Knochenniveaus darstellt. Bei ausgeheiltem Gewebe wurde wenig Unterschied zwischen den beiden Instrumenten ermittelt, während bei einer Entzündung die manuelle Sonde dem tatsächlichen Knochenniveau am nächsten kam. Daraus wurde gefolgert, daß unter den Bedingungen der vorliegenden Studie die Taschenmessung mit der manuellen Sonde für die routinemäßige klinische Anwendung geeigneter sei.

E

COMPARACION ENTRE MEDICIONES HECHAS CON UNA SONDA DE BOLSA PERIODONTAL CONVENCIONAL, UNA SONDA DE PRESION ELECTRÓNICA Y MEDICIONES HECHAS DURANTE LA CIRUGIA

RESUMEN

Este estudio comparó las mediciones clínicas utilizando una sonda periodontal manual y una sonda de presión electrónica utilizando 0.25 N de fuerza, con las mediciones de los niveles de hueso

durante la cirugía. Todas las mediciones se tomaron desde la unión amelocementaria en sitios específicos. Se halló que ninguna técnica de sondaje proporcionó un medio para una medición del nivel óseo con precisión. En presencia de tejido sano, se encontró una pequeña diferencia entre los dos instrumentos; mientras que la sonda manual se aproximó con mayor precisión al actual nivel de hueso en presencia de inflamación. Por consiguiente se llegó a la conclusión que bajo las condiciones del presente estudio las mediciones de profundidad con la sonda manual eran más aplicables para el uso clínico rutinario.

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Histological evaluation of biodegradable and non-degradable membranes placed transcutaneously in rats

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Galgut P, Pitrola R, Waite I, Doyle C and Smith R: Histological evaluation of biodegradable and non-degradable membranes placed transcutaneously in rats. J. Clin Periodontol 1991; 18: 581–586.

Abstract. The aim of this study was to assess histologically the response to membrane materials that might be used for guided tissue regeneration procedures, using a transcutaneous model on the dorsal surface of the rat. The materials included expanded polytetrafluoroethylene (PTFE) membrane (Gore-Tex), PTFE Millipore filter, biodegradable polylactic acid (PLA) and polyhydroxybutyrate/polyhydroxyvalerate copolymer membranes. 70 implants were placed in 10 rats under general anaesthesia. At 2 weeks after the operative procedure, the specimens were assessed histologically for the composition of the tissue surrounding the material, the types of cell in the infiltrate and the degree of absorption particularly in the superficial tissues. PTFE was associated with greater epithelial downgrowth and a mainly polymorphonuclear infiltrate. PLA showed a high incidence of absorption, with a mainly monocytic infiltrate. Considerable variation was found between these extremes. It was concluded that the healing responses associated with the membranes were complex and varied, and further work is required to establish the factors which govern the variation in the healing response. The findings are of relevance to regenerative procedures which rely on the guiding influence of artificial membranes on the healing tissues. The PLA material was well tolerated and was gradually absorbed. Further research may enable this material to form the basis of a one stage guided tissue regeneration procedure.

Key words: membranes; histology; guided tissue regeneration.

Accepted for publication 19 July 1990

Healing of the periodontal tissues after surgery is predominantly by means of the formation of a long junctional epithelium rather than by means of regeneration of the periodontal tissues. When new fibrous tissue attachment to root surfaces exposed by experimental periodontal defect formation does occur, it has been shown that this tissue is derived from periodontal ligament cells present at the margins of the defects (Karring et al. 1985), the migration of these cells being a pre-requisite for the formation of new attachment on denuded root surfaces (Isidor et al. 1986). Lindskog et al. (1987) have demonstrated that the first stage in healing of a denuded root surface was the formation of a fibroblast layer; subsequently the formation of new cementum and fibre attachment was possible, by means of differentation and proliferation of cells at the periphery of the wound.

Techniques aimed at excluding gingival connective tissue and epithelium by the placement of barrier membranes beneath surgical flaps prior to replacement, have been introduced. A variety of membranes have been used for this purpose, including Millipore filter (Nyman et al. 1982), polytetrafluoroethylene (PTFE) (Gore-Tex*) (Gottlow et al. 1984, Becker et al. 1987, Nyman et al. 1987) and polylactic acid film (PLA) (Magnusson et al. 1988). These studies have shown that the use of such membranes results in substantial regeneration of new periodontal ligament.

Although the potential for regeneration of periodontal tissues has been demonstrated with these materials, little is known of the detailed histological responses to these membrane materials during healing. The aim of this study was to evaluate, using an animal model, the histological response to various materials placed transcutaneously in the dorsal surface of rats. The materials tested were millipore filter, PTFE membrane and biodegradable membranes of different molecular weights, thicknesses and surface energies.

Materials

This study was carried out in two parts. In the 1st part of the study membranes of Polylactic acid (PLA) (molecular weight 50,000 g/mol) were manufactured by the solution casting method onto a mica surface to a thickness of 0.02 mms, 0.04 mms, and 0.07 mms respectively.

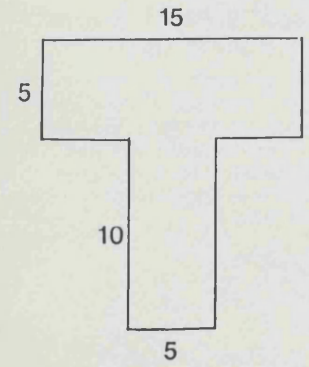

Fig. 1. Dimensions of the implant specimens (mm).

In the 2nd part of the study, the membranes were similarly prepared, but PLA was made from a higher molecular weight material (200,000 g/mol) than in the first part. A copolymer of poly hydroxybutyrate 80% – hydroxyvalerate 20% (P(HB-HV)) was also made. The membranes were of standardised thickness as specified. Both of these polymers are biodegradable, but the PLA is more hydrophilic, potentiating cell attachment and growth; whereas the P(HB-HV) copolymer is relatively more hydrophobic (i.e., less polar) with associated reduction in potential for cell attachment and growth. The cellular response to these membranes was compared to Millipore filter** of pore size 0.5 mu and commercially available PTFE periodontal membrane material*, which are both relatively hydrophobic.

The polyester films of PLA and P (HB-HV) were cast from solutions in chloroform with respective concentrations of 2% and 10% weight/volume. The castings were made against mica which was freshly cleaved to ensure that the casting surface was atomically smooth and superclean. After slow and controlled evaporation of the solvent, all films were vacuum dried at room temperature, and their surface free energies were determined at a standardised temperature of 22.5°C ± 1°.

The determination of the contact angle/surface free energy values for the various materials is the subject of another study (Pitrola et al. 1990). In brief three methods of evaluation were used; the geometric mean, the harmonic mean and Fowkes' Method. The average value of these three methods was then calculated for each of the membranes under investigation.

The membrane strips for the animal study were cut into a T-Shape as shown in Fig. 1. The samples were then placed in individual containers and sterilized by means of 2.5 Mrads of gamma irradiation.

* Gore-Tex (W.L. Gore Association, Inc., Flagstaff, Arizona, USA).

** Millipore Filter: Millipore Ltd., Harrow, Middlesex, UK.

Method

A total of 10 adult, male, Wistar rats weighing between 250 to 300 g each were anaesthetised, using Hypnoval 0.05 ml/100 g weight. Their dorsal fur was shaved and the skin was sterilised by washing with a 0.5% solution of chlorhexidine. Incisions of about 0.75 cm long were made through the cutaneous tissue parallel to the long axis and 1 cm on either side of the midline. Between six to eight incisions were made on each animal having assigned the materials by random allocation. A single test membrane was placed in each incision so that the broader section of the T-shaped membrane protruded from the wound, so as to prevent the sample being incorporated into the healing wound by epithelial overgrowth. Selected incisions received no membrane so as to act as controls.

After placement of the membranes horizontal, cross-over mattress sutures were used to close the wounds and retain the specimens. 2 weeks later the animals were sacrificed and the dorsal skin with the samples was excised. Sections containing the specimens were then cut and stained with Haematoxalin and Eosin prior to histological examination.

Results

A small number of specimens showed epithelial downgrowth, which was predominantly associated with the expanded PTFE and PTFE millipore filter, with the expanded PTFE showing fibrous condensation around the material in the deeper regions.

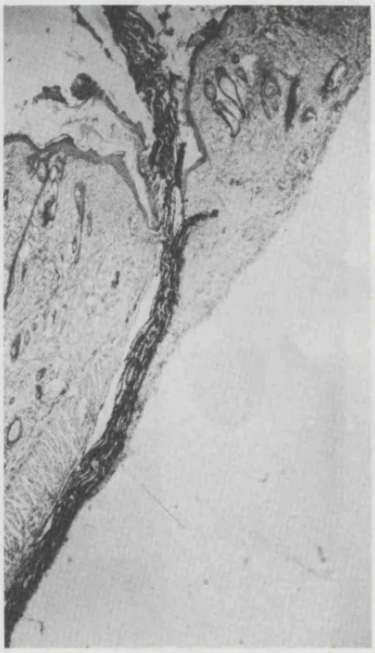

Fig. 2. Photo-micrograph of the tissue response to Gore-Tex expanded PTFE membrane.

The cellular response to the material was varied, with a predominantly monocytic response to all of the materials. There was however a greater tendency for the persistence of acute inflammatory polymorphonucleocytes with the expanded PTFE material than any of the other materials.

Similarly the absorption of the membranes was varied showing retention of fragments of the biodegradable membranes with regions of resorption in some areas and not in others. It is interesting to note that in some of the expanded PTFE specimens, fragments were retained in the deeper tissues. As the material could not be absorbed, it is possible that the membrane was cleaved by invasion of the inflammatory

Table 1. Surface free energies of the various polymers (for smooth surfaces)

Polymer	Surface free energy (mJm^{-2})		
	Dispersion component	Polar component	Total
PLA[a]	32.5 ± 2.0	5.0 ± 0.4	37.5 ± 1.8
PLA[b]	33.7 ± 1.7	5.2 ± 0.3	38.9 ± 1.4
expanded PTFE	18.1 ± 1.0	0.4 ± 0.2	18.5 ± 0.8
PTFE[c]	18.6	0.5	19.1
P(HB-HV)	36.9 ± 1.5	3.4 ± 0.7	40.3 ± 1.0

[a]Poly (L-lactic acid) of molecular weight 50,000 g mol^{-1}.
[b]Poly (L-lactic acid) of molecular weight 200,000 g mol^{-1}.
[c]Derived from the literature (Owens & Wendt 1969).

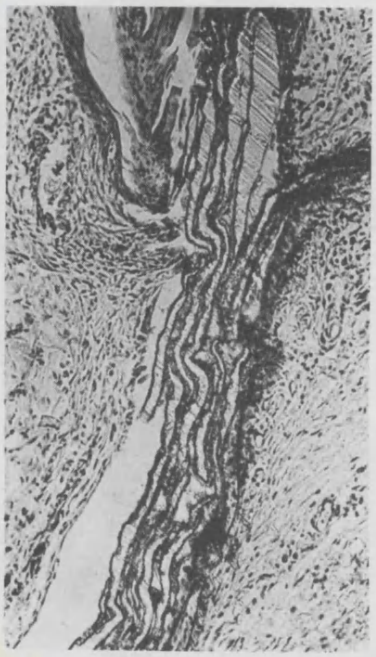

Fig. 3. High power view of the tissue interface with the Gore-Tex membrane.

cells, with subsequent exfoliation of the superficial portion of the material.

The contact angle/surface free energy values for the various materials under investigation are presented in Table 1. From this table, it can be seen that the surface free energy of the biodegradable materials is much higher than the PTFE materials. The surface free energy is subdivided into dispersion and polar components. A higher polar component is indicative of a higher level of hydrophilicity. It can be seen that the PLA and P(HB-HV) materials are more hydrophilic than the PTFE materials, with P(HB-HV) being less hydrophilic than PLA. It would therefore be anticipated that cellular attachment will be best in association with PLA, reduced with P(HB-HV) and greatly reduced with the PTFE materials.

Examples of representative histological sections for the various materials are shown in Figs. 2–8. The sections were assessed to determine the following.

(1) The degree of epithelial cell proliferation from the wound entrance alongside the implanted material.

(2) In the deeper parts of the wound, the presence of loose disorganised fibrous tissue or evidence of fibrous condensation with early capsule formation around the implant.

(3) The type of cellular infiltrate in proximity to the material.

(4) The histological assessment of the types of cellular responses associated with the placement of the materials are presented in Table 2.

In total, 17 samples were found to be absent at the time of histological assessment. In general at many of the experimented sites there was evidence of the sutures having been abraded away, and it seemed likely that some of the specimens were lost as a result of the animals having rubbed the operated sites against the cages. The specimens from non-degradable materials showed a greater number of lost samples that the absorbable ones. From Table 2, it can be seen that a total of 54 specimens were subjected to histological evaluation.

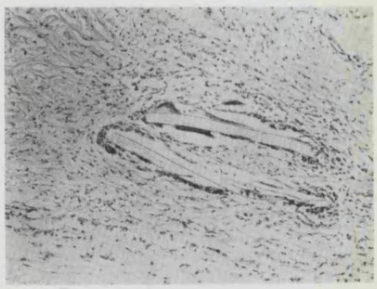

Fig. 6. High power view of the tissue response to retained fragments of biodegradable polymer (PLA).

Epithelial downgrowth was found most commonly and to the greatest extent with the PTFE membrane material (Figs. 2, 3) and to a lesser degree with the millipore specimens (Fig. 4). The biodegradable materials in general were associated with relatively minor degrees of epithelial downgrowth. The specimen shown in Fig. 5 representing about the greatest extent of proliferation.

In the deeper parts of the wound the non-biodegradable PTFE materials, often tended to show a relatively dense fibrous tissue condenstation surrounding the implant (Fig. 3). This early capsule formation contrasted with the relatively unstructured granulation tissue generally surrounding the degradable materials (Fig. 5); however, it was found that there was considerable variation in

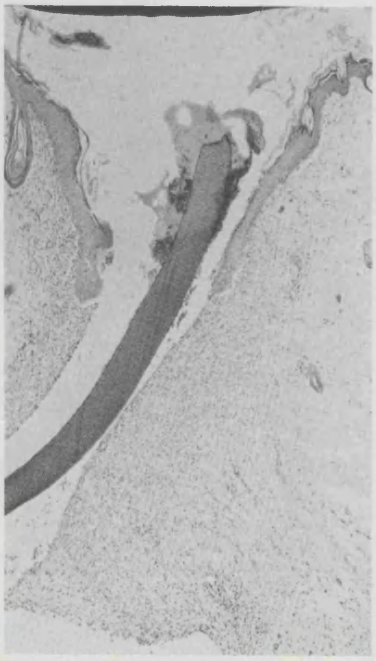

Fig. 4. Photo-micrograph of the tissue response to Millipore PTFE filter material.

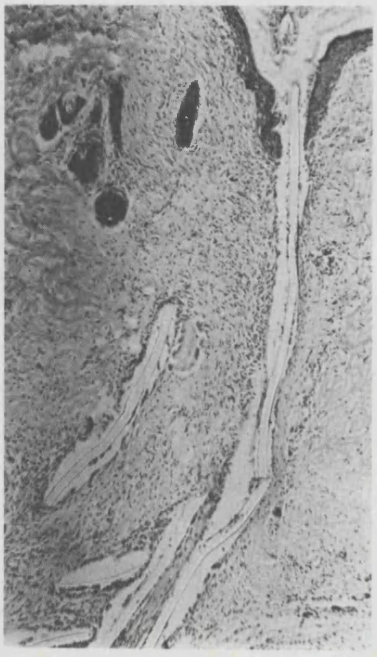

Fig. 5. Photo-micrograph of the tissue response characteristic of biodegradable polymers (P(HB-HV)).

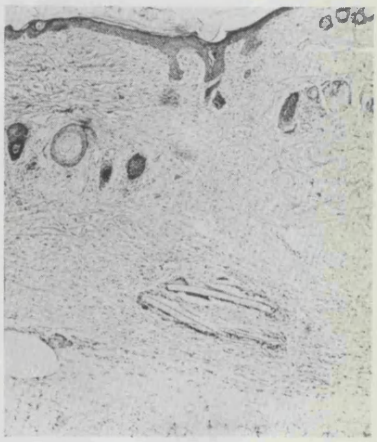

Fig. 7. Photo-micrograph showing characteristic degradation of PLA with retention of fragments of the material in the deeper tissues.

Fig. 8. High-power view of the tissue response to the retained fragments.

response between the specimens, even for the same material.

The type of cellular infiltrate was also found to vary considerably both between and within specimens and between the various materials. In general, sites at which absorption was occurring were populated mainly by monocytic cells, some of which were vacuolated. Giant cells were also seen, but these were found less commonly (Fig. 8). Polymorphonuclear leukocytes (PMN) were not found to any great extent in proximity to the implants. Localised infiltrations were found on occasion near the entrance of the wound. In the case of the PTFE membrane material an inflammatory infiltrate was sometimes found to have penetrated into the meshwork of the implant in the upper part of the wound, the majority of these cells being PMN leukocytes.

Areas of absorption were observed on the PLA and P(HB-HV) specimens, these being most frequently seen on the sections that were thinnest. The insertion and suturing of the implant strips at the time of operation seems to have resulted in distortion or folding of the material in the wound, especially for the thinner specimens. The presence of sharp bends in the material was frequently found to be associated with a greater tendency for more rapid absorption to occur. Thus, in Fig. 5 and 6, what were probably originally regions of bending of the strips, now appear as separate laminae. Continuing absorption can be seen at various sites on these laminae, most commonly at either end or on the surface. The regions where absorption was occurring were typified by small superficial erosions and fine-crack-like defects, vertical to the surface. The extent and distribution of the absorption varied markedly from one position to another on the specimens. Often only one surface was involved, the opposite aspect appearing intact (Figs. 6, 8). There was some difficulty in determining whether a localised, apparent discontinuity in a strip was caused by absorption or was caused by curvature of the strip resulting in an artefact from the plane of section. Examination of serial sections demonstrated that the membrane fragments were not artefactual defects. In Fig. 7, a superficial proliferation of epithelial cells was probably indicative of the site of the wound entrance. No sign of the strip can be seen in the loose immature fibrous tissue deep to this epithelial proliferation until the two lamellae near the base of the wound. This feature was characteristic of the majority of the sections of the biodegradable membranes. These fragments of the implant material show the various signs of absorption referred to above.

Discussion

The technique of guided tissue regeneration which utilises barrier membranes to exclude gingival connective tissue and prevent epithelial ingrowth into the healing periodontal wounds has been developed relatively recently and a degree of success has been reported (Gottlow et al. 1984). The published work however shows that the proportion of periodontal regeneration occurring is variable (Magnusson et al. 1988, Nyman et al. 1987). Pitaru et al. (1988) have described 3 distinct types of healing namely:

(1) partial regeneration of periodontal tissue in the apical half of defects;

(2) long epithelial attachment in the coronal quarter of the defects;

(3) connective tissue adhesion in the region between these two extremes.

Thus, a varied response must be anticipated when using techniques for achieving regeneration of periodontal tissues.

In this study, the tissue responses to the implantation in an animal model of degradable and non-degradable membranes was evaluated. A considerable variation in the response was observed between the individual animals in the study, between the different materials and even at different levels along a single implant. In addition a variety of responses were noted even for the same material. It is uncertain whether the responses were influenced by the different physical surface characteristics, or different thicknesses of the material.

Near the surface, there was a varying degree of epithelial downgrowth, this in general being greater for the expanded PTFE membrane and to a slightly lesser extent the PTFE millipore filter. Minimal downgrowth was observed with the P(HB-HV) and the PLA materials.

Table 2. Summary of the histological responses of the tissues to the implanted membranes

Material	Specimen not retained	Epithelial downgrowth	Fibrous tissue condensation	Monocytes	PMNs	Fragments of strip retained	Multiple areas of resorption	Total no. of retained specimens
PLA 02 (low density)	2		1	1	2	1		6
PLA 02 (high density)	1	1		7	1	7	7	9
PLA 04	3		1	2	1	3	2	6
PLA 07	2		1	3	2	2	2	6
P HB-HV	1			7		7	7	8
PTFE Millipore filter	4	2	1	2	1			5
Expanded PTFE membrane	4	3	3	4	3	3		6
Controls	(8)							(8)
exclusive totals	17	6	7	26	10	23	18	54

These characteristics may be related to the surface free energy, and the roughness of the materials but further work would need to be undertaken to confirm this hypothesis. It was noted that those materials tending to show the greatest degree of epithelial downgrowth also were found to show the highest incidence of unretained specimens, indicating a higher susceptibility to exfoliation of these materials.

There was an absence of, or low incidence of acute inflammatory reaction observed for all the experimental materials. Areas of inflammation when present were localised and most noticeable around the portion of the specimens nearest the surface. On the expanded PTFE specimens, where PMN Leukocytes were slightly more common, these were found in greatest density within the voids of the mesh (Fig. 3). Contaminants from the external environment may be able to percolate within the voids of the specimen and hence cause an infiltration of leukocytes in the deeper portions of the wound.

The density of the fibrous tissue surrounding the implant varied. The PTFE membrane was associated with a tendency to greater inflammatory infiltrate and early capsule formation it was uncertain whether this was as a result of the material being relatively hydrophobic, having relatively low surface energy, or whether it was associated the surface roughness characteristics of the material. The biodegradable materials were generally surrounded by a loose network of fibrous tissue in the form of immature granulation tissues.

The degree of absorption of the biodegradable specimens was very irregular and seemed often to occur at restricted locations causing a break in countinuity of the strip. In some specimens there were zones on one surface showing vertical clefts and erosion defects, wheres the opposite surface was intact (Fig. 8). In the present study, one surface of the membrane was formed against mica and was hence smooth, whereas the other surface was formed in a vacuum by solvent evaporation. It was not clear whether this could be associated with the characteristics of the one surface compared with the other. The influence of the surface characteristics of the membranes needs to be investigated further.

These differences in healing for experimentally implanted membrane specimens would concur with the varying degrees of success reported in previos studies into regeneration of periodontal tissues after surgery. The clinical studies have shown variation in such healing characteristics as degrees of regeneration (Gottlow et al. 1984, Nyman et al. 1987, Magnusson et al. 1988) organization of fibrous tissue Nyman et al. (1987), and partial epithelial downgrowth (Pitaru 1988). Although some of this variation could be attributed to the moist environment, the numerous microorganisms within the oral cavity, and the presence of an interface between soft tissue and hard tissue at the site of the healing would in the mouth, similar variations have also been found extraorally in the present study where such conditions did not prevail.

It was therefore concluded that the healing response was governed by a variety of factors which might include the healing response of the individual host, the chemical composition of the material placed in the tissue, the physical and surface characteristics of the material, the thickness and porosity of the material, and the depth to which the material is placed within the tissue. Further research is needed into these various factors, in the development of an ideal material to achieve predictable tissue regeneration for the treatment of supporting tissue loss in periodontitis.

Acknowledgements

We are most grateful to Alison Garvey for her patience in preparing the typescript for this paper. We are also very appreciative of the work in preparing the histological sections by Sheila Barnett and Sarah Iriving, Department of Research in Plastic Surgery, Mount Vernon Hospital.

Zusammenfassung

Histologische Bewertung von biologisch abbaubaren und nicht abbaubaren Membranen, die transkutan bei Ratten gelegt wurden

Das Ziel dieser Studie war es, die Reaktion auf Membranmaterialien, die für die gesteuerte Geweberegeneration Verwendung finden könnten, histologisch zu bestimmen. Es wurde ein Transkutanmodell auf der Rückenoberfläche der Ratte benutzt. Die Materialien waren. gestreckte Polytetrafluoräthylen-Membran (PTFE) (Gore-Tex), PTFE Millipore-Filter, biologisch abbaubare Polygalaktin-Säure (PLA) und Polyhydrobutyrat/Polyhydroalerat-Kopolymer-Membranen. Unter Vollnarkose wurden in 10 Ratten 70 Implantate gesetzt. Zwei Wochen nach den operativen Maßnahmen wurden die Proben histologisch verarbeitet. Die Zusammensetzung des das Material umgebenden Gewebes, die Zellarten des Infiltrates und der Grad der Absorption, besonders in den oberflächlichen Geweben, wurden untersucht. PTFE war mit einem größeren epithelialen Tiefenwachstum und einem hauptsächlich polymorphkernigen Infiltrat verbunden. PLA zeigte ein hohes Auftreten von Absorption, verbunden mit einem hauptsächlich monozytären Infiltrat. Zwischen diesen Extremen gab es beträchtliche Variationen. Es wurde geschlossen, daß die Heilungsvorgänge in Verbindung mit den Membranen komplex und variabel waren und daß weitere Arbeiten notwendig sind, um die Faktoren, die die Variation des Heilungsvorganges hervorrufen, zu ermitteln. Diese Ergebnisse sind von Relevanz für regenerative Maßnahmen, die sich auf den steuernden Einfluß von künstlichen Membranen auf heilende Gewebe stützen. Das PLA-Material wurde gut toleriert und wurde schrittweise absorbiert. Weitere Forschung könnte es ermöglichen, daß dieses Material die Grundlage eines einzeitigen Vorgehens bei der gesteuerten Geweberegeneration bildet.

Résumé

Evaluation histologique de membranes biodégradables et non dégradables en implantation transcutanée chez le rat

Cette étude avait pour but l'évaluation histologique de la réaction envers des matériaux qui pourraient servir pour les membranes employées dans les techniques de régénération tissulaire guidée; l'étude était basée sur un modèle transcutané, sur la surface dorsale du rat. Les matériaux utilisés comprenaient une membrane (Gore-Tex) de polytétrafluoroéthylène expansé (PTFE), un filtre Millipore de PTFE, et des membranes biodégradables d'acide polylactique (PLA) et de copolymère de polyhydroxybutyrate-polyhydroxyvalérate (P(HB-HV)). Chez 10 rats, 70 implants ont été mis en place sous anesthésie générale. Une évaluation histologique des spécimens a été pratiquée 2 semaines après l'intervention; elle concernait la composition du tissu environnant le matériau, les types cellulaires dans l'infiltrat et le degré de résorption, particulièrement dans les tissus superficiels. On a constaté l'association de PTFE avec une pénétration épithéliale plus importante et un infiltrat principalement polynucléaire. Avec PLA, il y avait une forte incidence de résorption, avec un infiltrat principalement monocytaire. Entre ces extrêmes, on constatait une variation considérable. En conclusion, les réactions de guérison associées aux membranes étaient complexes et variées; pour établir les facteurs qui gouvernent les variations de la réaction de guérison, des travaux ultérieurs sont nécessaires. Ces résultats sont importants pour les techniques de régénération basées sur le guidage des tissus en voie de guérison sous l'influence des membranes. PLA était bien toléré, et était graduellement résorbé. Il est possible que des recherches ultérieures per-

mettent de baser sur ce matériau une technique de régénération guidée des tissus en une seule étape.

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Guided tissue regeneration: observations from five treated cases

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In this clinical investigation, a biodegradable collagen material was used in membrane and paste form to assess its suitability for use in guided tissue regeneration after surgery. A variety of healing responses in only the soft tissues were noted, ranging from gross necrosis to enhanced healing. The problems and limitations of regeneration procedures are discussed to explain the reasons for the observed healing of the tissues. (Quintessence Int *1990;21:713–721.*)

Introduction

Regeneration of the tissue lost to destructive periodontal disease has long been the aim of periodontal therapy. Traditional therapy, aimed at debridement of the root surfaces and removal of granulation tissue and pocket lining epithelium, results only in minimal regeneration of the periodontium in the most apical 1 to 3 mm and the margins of the deepest pockets. The remainder heals by means of a long junctional epithelium.[1] It has been demonstrated in vivo that reformation of a fibroblast layer on denuded root surfaces with the formation of new cementum and fiber attachments is possible and that the new cells are derived from the differentiation and proliferation of cells in the periphery of the wound to form precementoblasts.[2] The cells of newly regenerated fibrous attachment to root surfaces that have been exposed by experimental pocket formation are derived from periodontal ligament cells present at the margins of the defect.[3] These cells are a prerequisite to the formation of new attachment on denuded root surfaces.[4] Although regeneration of fibrous attachment is possible, Listgarten and Rosenberg[5] have shown histologically that regeneration does not occur in humans because the downgrowth of epithelial cells into the healing wound forms a long junctional epithelium. A technique, termed *guided tissue regeneration*, has been developed whereby a physical barrier membrane is introduced to prevent epithelial cells from entering a surgical site. A number of membranes have been used under soft tissue flap for this purpose, including millipore filter, polytetrafluoroethylene (Teflon), a collagen-coated barrier membrane (Biobrane), and lyophylized collagen.[6–11]

Recent research into polytetrafluoroethylene membranes that have been expanded to increase porosity has demonstrated their potential for encouraging coronal migration of periodontal ligament cells and attachment of new bone, cementum, and periodontal fiber in periodontal defects with poor regenerative capacity.[12]

Collagen heterograph materials have long been used as biologic dressings extraorally and intraorally. Porcine skin has been used as a biologic dressing in periodontal mucogingival grafting procedures, and so has lyophylized allergenic collagen derived from processed human dura mater.[13] Clinically a statistically significant decrease in probing attachment levels and greater reduction in pocket depths are observed, together with less gingival recession and significant bone regeneration, in those sites in which the material is used.[13] Histologic examination has shown that the implant remodels completely and is replaced gradually by enzymic breakdown and fibroblast infiltration to form new collagen. New bone formation occurs underneath the implant, but not within it, and the implant acts as a barrier membrane, preventing downgrowth of epithelium into the healing surgical wound.

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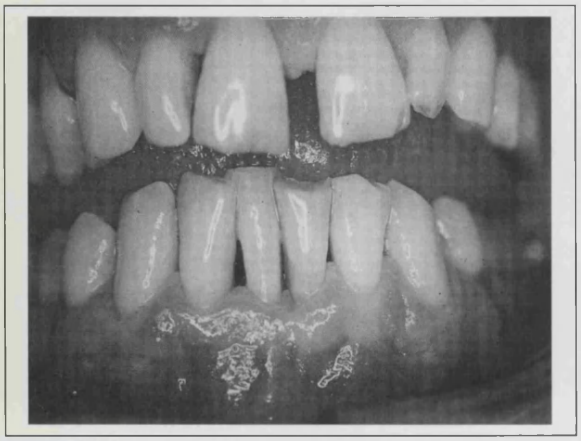

Fig 1a Preoperative view of the patient in case 1.

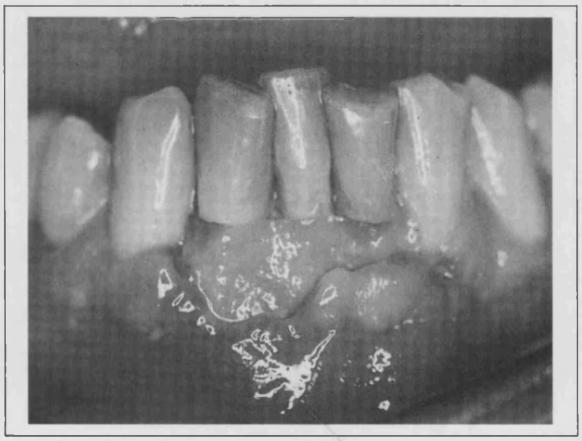

Fig 1b Postoperative view showing the membrane in place and gross tissue necrosis.

The aim of this paper is to evaluate the clinical use of biodegradable collagen as a barrier to epithelial downgrowth, utilizing the principles of guided tissue regeneration, to enhance the healing of the soft tissues after surgery.

Method and materials

All of the patients in the study were referred for treatment for chronic periodontal destruction. They received a course of initial therapy that consisted of oral hygiene instruction and scaling and root planing, followed by a period of time to ensure that good oral hygiene was maintained. Replaced flap surgery was performed to expose the periodontal defects and to facilitate debridement of the root surfaces. To encourage regeneration of the periodontal tissues after surgery, by exclusion of the epithelium from the soft tissue flaps, lyophilized, sterilized porcine dermis heterograft (Zenoderm, Ethicon Ltd) was placed under the surgical flaps before they were replaced, using the principles of guided tissue regeneration. Photographic records were taken before, during, and after surgery to evaluate the response of the soft tissues to the material. In two patients the degree of postoperative recession was evaluated 1 week and 1 month postoperatively by observing the height of the free gingival margins relative to the cementoenamel junctions of the teeth. Because this was a study of short duration, designed to evaluate the soft tissue responses after surgery, no attempt was made to evaluate the degree of bony filling of the defects by radiographic means. Pocket depth readings were not taken so soon after surgery, to prevent damage to the healing tissues.

Case 1

The patient was a 46-year-old white man who required surgery in the mandibular anterior region. The collagen membrane was placed across the mandibular anterior teeth so as to protrude above the replaced soft tissue flap (Fig 1). The soft tissue flap was sutured with horizontal mattress sutures to draw the soft tissue as coronally as possible and to achieve close adaption of the flap, the membrane, and the underlying tissues. Although the material was reconstituted according to the manufacturer's instructions, it did not become pliable enough to adapt closely to the root surfaces.

Case 2

The patient was a 53-year-old white man. In this patient the membrane was placed over the infrabony defect, but under the flap between the mandibular right lateral incisor and canine and over the flap as a biogenic dressing between the mandibular right canine and premolar. The membrane had been cut into thin strips for interdental placement (Fig 2).

Case 3

As a result of the difficulties related to the placement of the membrane in the previous cases, it was decided to use the same material in paste form so that it could be closely adapted to the root surfaces and placed interdentally before the flap was replaced. The patient was a 37-year-old white man, who required periodontal surgery. Lyopaste was placed around the periodontal defects between the maxillary right canine and lat-

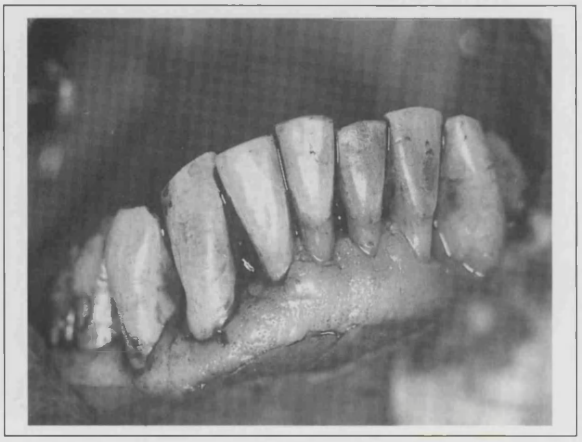

Fig 2a Preoperative view of the patient in case 2.

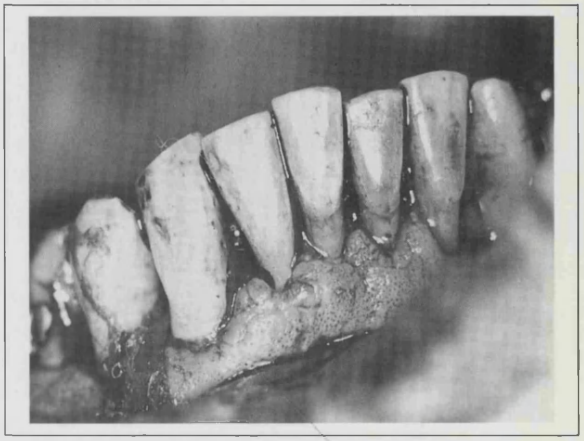

Fig 2b Surgical placement of the membrane between the canine first premolar, over the surgical flap, and between the lateral and incisor and canine, under the surgical flap.

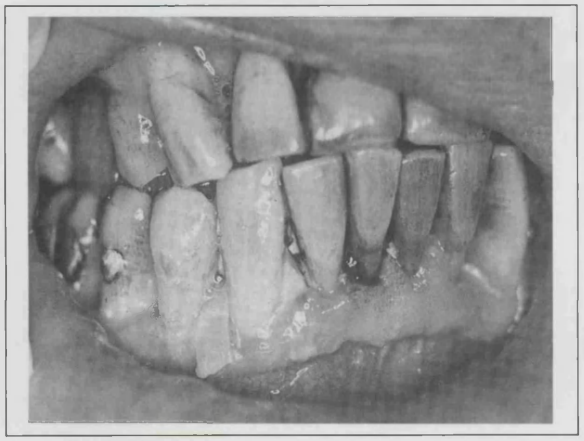

Fig 2c Exfoliation of the membrane.

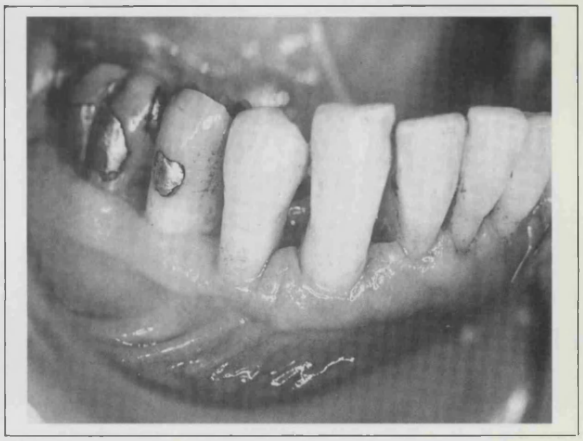

Fig 2d Healing subsequent to removal of the membrane.

eral incisor, the central incisors, the left central and lateral incisors, the left canine and first premolar, and the left first and second molars, and in the furcation of the maxillary left first molar. No material was placed between the maxillary right central and lateral incisors, so that a direct comparison could be made with the sites that received the material. The material was closely adapted into the defects and around the root surfaces so as to protrude coronal to the soft tissue flap (Fig 3a). The procedure is illustrated in Fig 3.

Case 4

The patient was a 38-year-old Asian man. Extensive buccal recession had occurred on the maxillary right canine and first premolar. After surgical exposure and debridement, the defects as well as the buccal surfaces of the teeth were filled with lyophilized collagen paste. This was placed to extend beyond the coronal margin of the soft tissues. To cover these defects, a laterally sliding flap was carried to the surgical site using the attached gingival tissue from the region of the central and lateral incisors (Fig 4).

Case 5

The patient was a 55-year-old white man. The initial phase of treatment was completed, and then surgery was performed. Lyophilized collagen paste was placed between the maxillary left central and lateral incisors. The teeth on either side (ie, between the central incisors and between the lateral incisor and canine) acted as controls. Because the defects were shallow, no filling

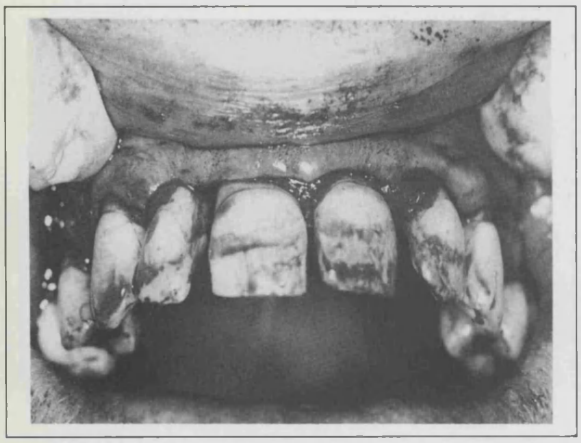

Fig 3a Placement of the paste and replacement of the flap during surgery in the patient in case 3.

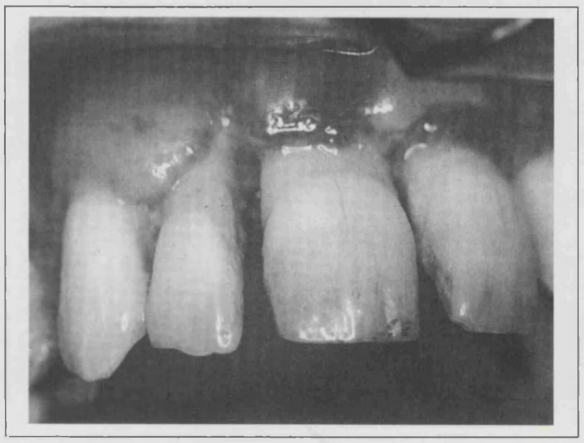

Fig 3b Healing of the maxillary right region 1 week postoperatively.

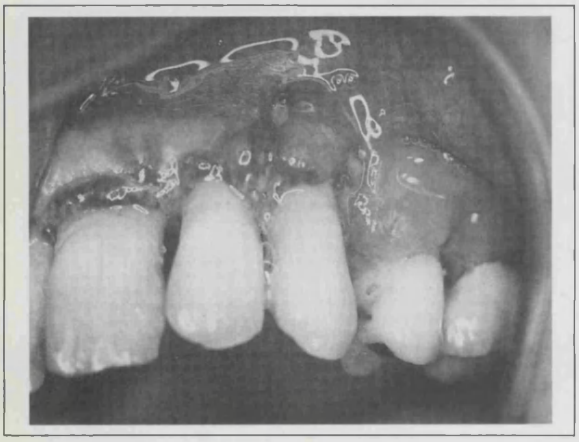

Fig 3c Healing of the maxillary left region 1 week postoperatively.

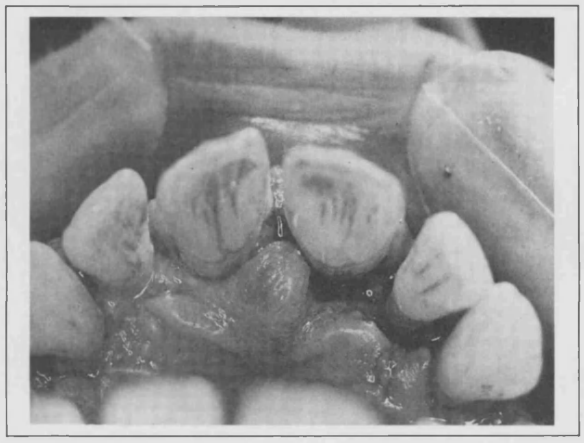

Fig 3d Palatal view of the healing 1 week postoperatively.

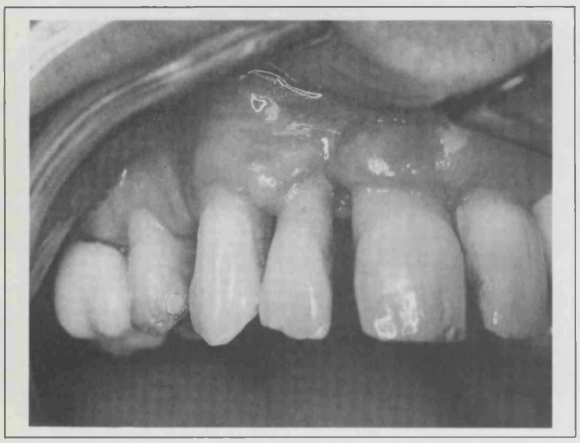

Fig 3e Maxillary right region 1 month postoperatively.

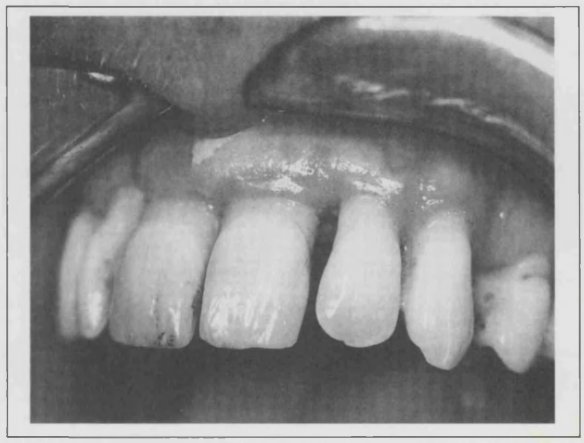

Fig 3f Maxillary left region 1 month postoperatively.

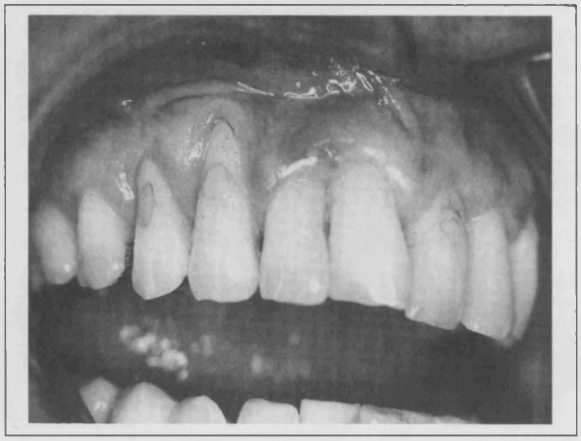

Fig 4a Preoperative view of the patient in case 4.

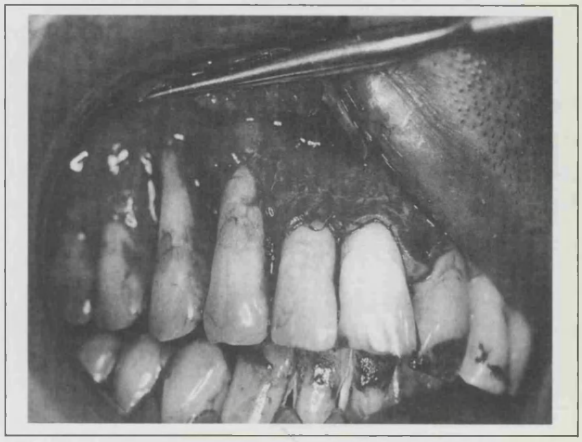

Fig 4b Surgical exposure of the defects reveals extensive bone loss.

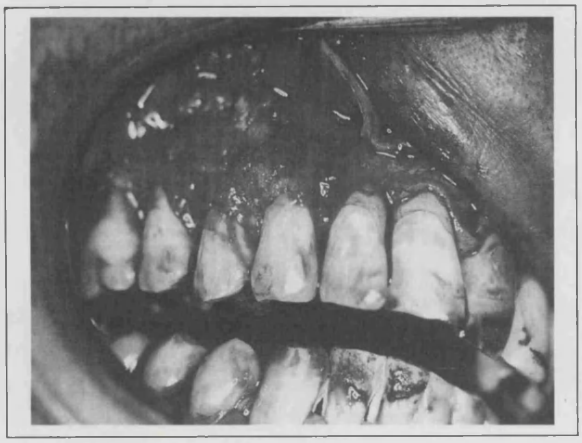

Fig 4c Paste placed in the defects prior to replacement of the flap.

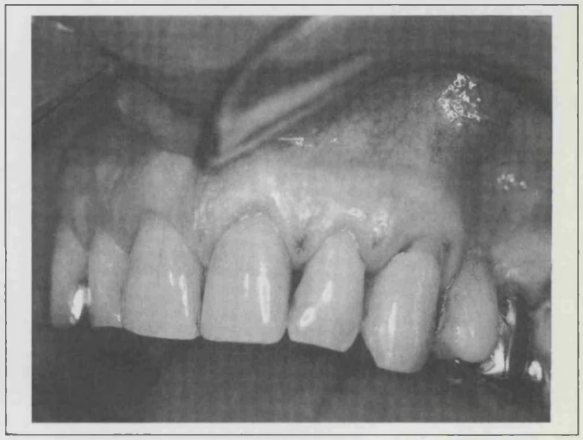

Fig 5a Preoperative view of the patient in case 5.

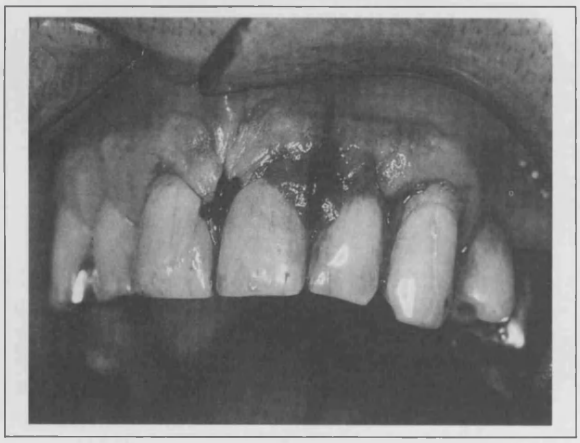

Fig 5b Replaced flap and lyophilized paste in situ.

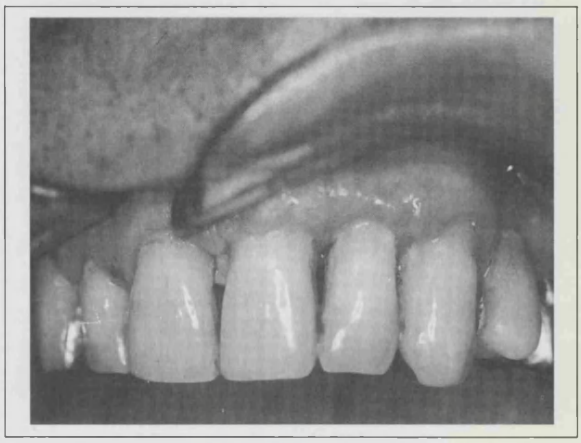

Fig 5c Postoperative healing of the site.

of infrabony defects was attempted. The material was therefore used only for epithelial exlusion during healing (Fig 5). Pocket depths were recorded preoperatively and 1 month postoperatively. Recession was evaluated preoperatively and 1 week and 1 month postoperatively to assess differences between sites that received the materials and sites that did not.

Results

Five cases have been presented in which a biodegradable collagenous membrane or paste was used to inhibit epithelial downgrowth into healing periodontal defects after surgery. In each case a different healing response was achieved.

Case 1

A collagen membrane was used as a barrier to epithelial downgrowth from the surgical flap. Necrosis of the tissue overlying the membrane occurred within 1 week of placement, and the membrane was removed to prevent further destruction of the tissues. The membrane was found to be well retained and could not be easily dislodged from the underlying tissue.

Case 2

The membrane placed over the surgical wound to act as a biological dressing and wound sealer resulted in no advantage in postoperative healing. In the site in which the membrane was placed as a barrier membrane underneath the flaps, the material was exfoliated from the healing wound during healing, because of recession of the soft tissues (similar to that in case 1). The membrane was therefore removed, and normal healing without further recession followed.

Case 3

Despite the presence of poor postoperative healing, acute hypersensitivity, and subsequent poor oral hygiene, the sites that received the lyophilized paste material healed more quickly and better with less loss of attachment than did those that had not received the material. Buccally on the maxillary left central incisor there was hardly any exposure of the root, whereas on the maxillary right central incisor there is about 1.5 mm of exposure of the root. Thus, in spite of delayed healing with some postoperative inflammation in all of the sites (see Fig 3b), those sites that received the lyophilized material healed without the extensive loss of tissue that occurred in sites that did not receive the material. The postoperative tissue recession and interdental cratering on tooth 12 was extensive (see Fig 3b) compared to the minimal tissue loss and interdental cratering on the sites that received the material (see Fig 3c). The same results are also evident on the palatal aspects of the teeth (see Fig 3d). Figures 3e and 3f show the surgical site 1 month after surgery. Healing was still not complete, but there was more inflammation buccally between the right central and lateral incisors than there was on the maxillary left side, which had received the lyophilized material.

Case 4

Lyophilized collagen paste, which was used to fill an extensive infrabony defect and to form a barrier to epithelial downgrowth, resulted in good healing of the tissues initially, but breakdown subsequently occurred with loss of attachment to approximately presurgical levels.

Case 5

The lyophilized collagen paste was used as a barrier to prevent epithelial downgrowth under the replaced flap after surgery. There was no substantial difference in postoperative recession or pocketing between the sites that received the paste and those that did not (Tables 1 and 2).

Discussion

The potential for regeneration of periodontal membranes by using nondegradable membranes has been shown.[6,11] These membranes have several disadvantages:

1. A second procedure is necessary to remove the membrane.
2. The second procedure results in additional cost and inconvenience for the patient.
3. If any recession of the soft tissue flap overlying the membrane occurs, it is possible that the membrane will be exposed during the healing phase. This may act as a nidus for infection, particularly in the collar region of the comercially available membrane.
4. The technique as described and the availability of the comercially available membranes are such that only single teeth can be treated concurrently; this

Table 1 Degree of recession in test* and control sites (mm)

Test sites				Control sites			
Tooth No.	Pre-operative	1 Week post-operative	1 Month post-operative	Tooth No.	Pre-operative	1 Week post-operative	1 Month post-operative
21	1	1	3	21	0	2	3
	1	3	4		0	1	2
22	1	4	3	22	0	2	2
	0	2	2		9	3	2
				23	0	3	3
					0	2	2
21	0	1	2	21	0	0	0
	0	2	3		0	0	0
22	0	2	3	22	0	1	2
	0	1	2		0	2	3
				23	0	2	3
					0	1	2
Total	3	16	22	*Total*	9	19	24

* Test sites were those sites that received the lyophilized material.

Table 2 Degree of pocketing in test* and control sites (mm)

Test sites			Control sites		
Tooth No.	Preoperative	1 Month post-operative	Tooth No.	Preoperative	1 Month post-operative
21	3	1	21	3	1
	4	2		3	1
22	3	2	22	3	1
	3	1		3	1
			23	4	1
				4	1
21	4	3	21	5	4
	4	2		3	4

* Test sites were those sites that received the lyophilized material.

is not necessarily the clinical presentation of periodontal defects.

5. The procedure demands that the membrane be totally submerged under the soft tissue flap, which is sometimes difficult to achieve clinically.
6. It is a time-consuming and demanding procedure.
7. The material itself is extraordinarily expensive.

A biodegradable barrier membrane would overcome many of these disadvantages. It this study, biodegradable collagen has been used to evaluate the potential for enhanced healing using the principals of guided tissue regeneration. Although the potential for enhanced healing has been demonstrated in some of the individuals presented in the study, the results were

highly variable. Assessment after treatment of the patient in case 5 revealed no difference between the degree of postoperative pocketing or recession in the sites that received the grafting material and the degree of pocketing or recession in the sites that did not. Aukhil et al[8] have shown in beagle dogs that a collagen-coated barrier membrane is capable of inducing coronal migration of periodontal ligament progenitor cells and up to 2.94 mm of new attachment during healing. However, some of their specimens showed the development of long junctional epithelium, root resorption, and, in one specimen, an inflammatory response with the production of giant cells. Other workers[14,15] have also observed a varied healing response to collagen membranes used for guided tissue regeneration in periodontal tissues. Although the collagen membranes show a capacity to encourage regeneration of periodontal tissues, three different healing types have been observed:

1. Partial regeneration of periodontal tissues, including new bone, cementum, and periodontal ligament
2. Long junctional epithelial attachment
3. Connective tissue adhesion

Although histometric analysis showed that these different healing responses occurred concurrently, no postoperative differences in pocket depths were observed clinically in the sites that received the membranes as opposed to the sites that did not.

It is not possible in a human clinical study to establish whether healing took place as a result of the formation of a long junctional epithelium or regeneration of the periodontal membrane. However, in a number of sites, particularly when the lyophilized collagen paste was used as a separating medium between the flap and the granulation tissue, healing was apparently enhanced. However this was not uniformly observed in all sites, and initially good healing was susceptible to subsequent breakdown in one case. Therefore, several observations, which concur with other published research, can be made from this study.

Variable healing responses have been shown in different sites and in different individuals. The factors that govern the nature, and therefore the predictability, of the healing response are unknown at this time.[16]

Necrosis tends to occur on the margins of the soft tissue flap. As organic collagenous material undergoes biodegradation by enzymic digestion and inflammatory reaction of the tissues, this may well result in inflammatory damage and necrosis in the healing tissues as well. Other biogradable materials, such as polylactic acid, have been used for the purposes of guided tissue regeneration.[17] Although some success has been reported with polylactic acid membranes, some necrosis at the flap margins was evident during healing. Therefore, the necrotic change in the overlying soft tissue may not be due entirely to the nature of the material, but possibly may be a result of other factors, such as blood supply to the flap during healing, individual host response, contamination of the surgical site during healing, or other factors.

Physical characteristics of the membrane are important, as was demonstrated by case 1. The collagen membrane was too rigid to be closely adapted to the tooth surface. The lack of adaptation may have led to contamination and poor healing of the tissues. When the material is placed interdentally, it is possible for the healing tissue to undermine the membrane and cause it to be exfoliated (as in case 2). This may be as a result of an inability to achieve a close seal and apposition between the membrane and the tooth surfaces, enabling contamination. Alternatively, it may relate to factors in the host response to a foreign material.

In the cases presented in this study, the technique of guided tissue regeneration was not limited to a single tooth, as was the case in other studies. The degree of tissue breakdown that occurred in some sites indicated that there may be limitations to the extent to which this technique can be applied clinically. Tolmie et al[18] listed the possible limitations of regenerative procedures:

1. Persistance of chronic inflammation
2. Inadequate surgical procedures
3. Contaminating infection, which results in reduced healing
4. The types of cells that repopulate the wound initially
5. The spatial limitations within the defect that govern the availability of cells necessary for regeneration rather than repair

Factors that determine the limitations of guided tissue regeneration may be dependent on individual regenerative capacity. This would include such factors as the individual host response, healing characteristics of the individual, immune reactivity, and the nature of the tissues. Other factors might include the friable or fibrous consistency of the tissues, the blood supply to the flap, and the blood and nutrient supply from the underlying tissue in the surgical bed.

Conclusions

Although biodegradable collagen has been shown to have potential to aid healing after surgery in some cases, it is probably not the best material for use as a membrane or barrier in the technique of guided tissue regeneration.

The results of this study and others indicate that predictability of the procedure may be questionable: Different healing responses may occur in different individuals and in different sites within the same individual.

Further research is necessary to determine the factors that govern and regulate the healing and regenerative responses rather than those that result in resorption and necrosis of tissues after surgery; to establish the characteristics of the membrane or barrier that is best suited for guided tissue regeneration techniques; and to determine the physical limitations in terms of multiple sites within a surgical procedure that may limit regenerative procedures.

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Oxidized cellulose mesh

II. Using hydroxy-apatite bone grafting material in the treatment of infrabony defects

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Biodegradable oxidized cellulose mesh has been used in conjunction with hydroxyapatite synthetic bone grafting material to enhance retention of the graft material and to improve post-operative healing of infrabony defects treated surgically. Initially a good healing response was achieved, but there was a tendency for the sites to relapse in the 8 wk following surgery. A tendency to relapse in reconstructive dentistry is well known, and the possible factors which limit the amount of regeneration of tissues are considered. Although there appear to be limitations on the regeneration possible in tissues, the factors that determine these limitations are poorly understood at present.

Keywords: Biodegradation, membranes, cellulose

Regeneration of tissues lost as a result of destructive periodontal disease has long been the aim of periodontal therapy. When the lost tissue includes supporting alveolar bone, management of the osseous deformities remains problematical. If the deformity is resected, excessive removal of supporting bone is often necessary to re-establish normal alveolar anatomy. If regenerative procedures are attempted, the results are often unpredictable[1]. Some bone infill occurs after surgical debridement but may be incomplete[2]. Ellegaad and Loe[3] observed that most two- and three-walled defects showed the greatest potential for regeneration, provided no chronic inflammatory process or contamination of the maturing granulation tissue occurred[4].

The treatment of one-wall and broad two-wall defects which have little or no inherent regenerative capacity remains a problem for the clinician. A variety of autografts and allografts have been utilized over the years to enhance the potential for bone regeneration. More recently, synthetic bone grafting materials have been developed for this purpose. Regeneration of bone using these materials has been variable, with no single material or mixture of different materials resulting in complete regeneration of the lost bone[5]. Reported problems have included lack of new bone formation, and encapsulation and exfoliation of the graft material.

The aim of this clinical investigation was to see if enhanced retention of a synthetic bone grafting material with improved soft tissue healing could be achieved when placing a biodegradable oxidized cellulose mesh over a synthetic bone grafting material placed in infrabony defects before surgical flap replacement.

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MATERIALS AND METHODS

Synthetic hydroxyapatite bone grafting materials (Periograf®: Sterling Winthrop, Guildford, UK) was placed in infrabony defects which had been surgically exposed by inverse-level flap reflection and meticulous debridement of the root surfaces. The grafting material was mixed in accordance with the manufacturer's instructions and gently compacted into the infrabony defects. A layer of contoured resorbable oxidized cellulose mesh (Surgicel®: Johnson & Johnson, Slough, UK) was placed over the material to protect the area of the oral environment, to stabilize the graft material, and to prevent epithelial down-growth into the graft material with subsequent exfoliation of the material. It was allowed to gel with the blood oozing from the surgical wound, before replacement of the soft tissue flaps. The soft tissue was sutured using horizontal mattrass sutures to avoid disturbing and contaminating the grafted sites by the suture material. The patients were advised to use chlorhexidine mouthwash for 1 wk post-operatively in addition to gentle toothbrushing. Sutures were removed 1 wk post-operatively. Two individuals are described in this investigation.

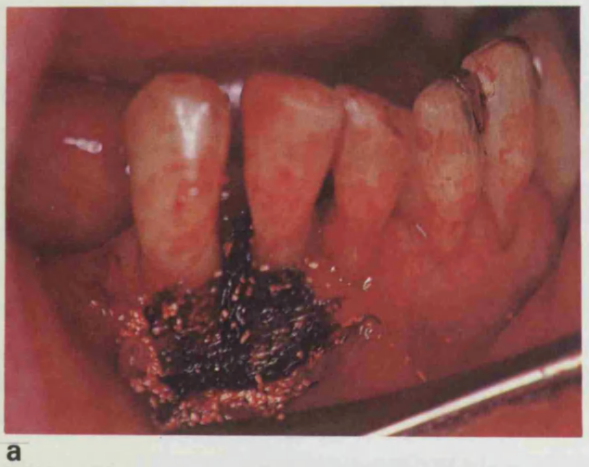
Individual 1. Caucasian male, 58 years old who required surgery in the 3/ region where a deep infrabony defect was present. Extensive bone loss had occurred interdentally and buccally. Hydroxyapatite bone grafting material (Periograf)

was placed in the defect, and an oxidized cellulose dressing (Surgicel) was placed over the graft. Healing was uneventful initially, but over a 2 month period some loss of tissue height occurred.

Individual 2. Caucasian male, 51 years old who required surgery in the /345 region of his mouth due to residual deep pockets with extensive infrabony defects associated with recurrent abscess formation. The region was exposed by creating buccal and palatal flaps, and a relieving incision was placed anteriorly to the /3. After root debridement hydroxyapatite bone grafting material (Periograf) was placed in the infrabony defects. Extensive bleeding occurred during surgery, making placement of the grafting material difficult. Resorbable oxidized mesh (Surgicel) was placed over the graft material and contoured interdentally and facially around the teeth to seal the grafted site from the oral environment.

RESULTS

Individual 1. 1 wk post-operatively, the tissue was healing slowly with no loss of the bone grafting material although some superficial sloughing could be seen over the surgical site. By 8 wk post-operatively, however, loss of tissue height had occurred with recession of the soft tissues, exfoliation of most of the graft material and characteristic interdental cratering where epithelial ingrowth had occurred (*Figure 1b*).



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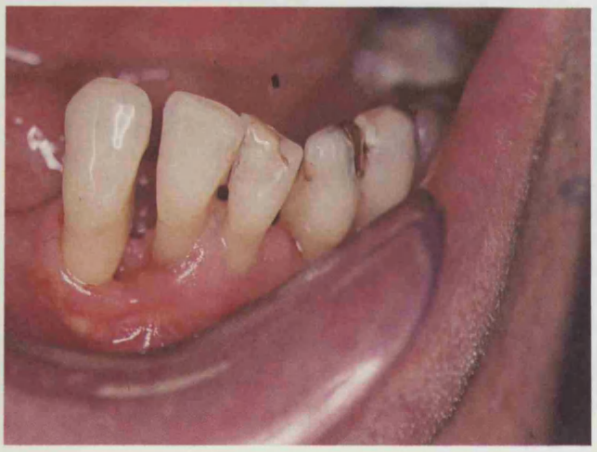

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Figure 1 (a) Placement of bone graft and oxidized cellulose mesh. (b) 8 wk post-operatively, showing recession and exfoliation of most of the graft material.

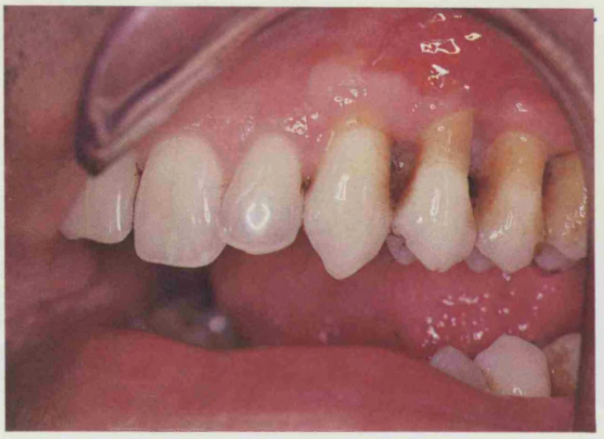

Figure 2 8 wk post-operatively, showing that some relapse has occurred although healing was satisfactory.

Individual 2. The tissues healed well, although there was some evidence of soft tissue sloughing over the buccal root of the /4 region and the mesh was not completely resorbed by 1 wk post-operatively. By 8 wk post-operatively soft tissue shrinkage had occurred, with loss of most of the attached gingiva in this area, although a great deal of the graft material was retained (*Figure 2*), and the tooth was functional.

DISCUSSION

Guided tissue regeneration techniques have been shown to enhance new attachment formation with minimal post-operative recession[6]. Skoag[7] has demonstrated the efficacy of surgical oxidized cellulose mesh to act as a scaffold to enhance healing in reconstructive surgery. He emphasized the difficulties in achieving and maintaining long-term stability of maxillary reconstruction. The tendency for relapse in oral surgery is in agreement with the observations of the present study. While good initial healing occurred in all individuals investigated, some sites were susceptible to breakdown. This is difficult to explain, because hydroxyapatite is known to be biocompatible, as is the surgical mesh. The factors governing healing and regeneration of tissues are unknown[8], but suggestions[9] include, the materials used, the defect morphology, contamination of the healing wound, the presence of residual inflammation after surgery, patient compliance with post-operative instructions and the availability of adequate blood supply with oxygen and nutrients, to the maturing granulation tissue. Skoag[7] has postulated that not only is a stable blood clot necessary for regeneration of bone and soft tissue, but also that the periosteum plays a crucial role in the stimulation of new bone and soft tissue regeneration. Thus the tendency for relapse of the tissues may be related to the materials placed in the surgical site, or be directly related to a number of poorly understood factors which govern wound healing. This would imply that there may be limitations to the extent of regeneration that is possible. Although Gottlow *et al.*[6] have not reported any relapse or necrosis of tissue, other authors[9] have demonstrated wide variation in the healing response and degree of regeneration of tissues using guided tissue-regenerative techniques. In some sites, good healing took place without the presence of adjunctive membranes, whereas in other sites poorer healing and the development of a long junctional

Insolubilized properties of UV-irradiated CO_3apatite–collagen composites

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To examine the response to biological hard tissues, a carbonate-containing hydroxyapatite with chemical composition and crystallinity similar to those of bone was synthesized at pH 7.4 and 60°C. The apatite powder was mixed with collagen solution, whose antigenicity had been removed by enzymatic treatment, and formed into apatite–collagen pellets. After insolubilization by UV-irradiation for 4 h, the composites showed remarkably reduced disintegration and maintained their shape under 3.6 MPa of stress after 1 wk incubation in 0.9% NaCl solution. They showed good biocompatibilty when implanted beneath the periosteum cranii of rats. The UV-irradiated sample kept its features well and was packed with newly created material 3 wk after implantation.

Keywords: Composites, collagen, hydroxyapatite

Recently, a number of biomaterials for bone replacement have been studied. Hydroxyapatite is especially useful as an implantable material because of its good biocompatibility with surrounding tissues[1]. Apatites have been applied in dentistry to fill up resorbed bone after tooth extraction and in defective areas of mandibular bone[2, 3].

However, most commercial hydroxyapatites are sintered, and their chemical properties differ somewhat from those of biological apatites, which are composed of carbonate apatite formed at physiological temperature.

Furthermore, conventional block-type hydroxyapatite is difficult to process into complex form, and granular-type hydroxyapatite, which is injected into the resorbed place with NaCl solution, does not readily retain the required form. To obtain better handling properties, Harvey *et al.*[4] mixed hydroxyapatite with a microfibrillar collagen haemostatic agent and found that this mixture enhanced the surgical manipulation of implants. Hahn *et al.*[5] suggested that hydroxyapatite–collagen implants may be effective in reducing ridge height loss in rats. In our previous study[6] carbonate-apatite with chemical composition and crystallinity similar to those of human bone was synthesized and mixed with collagen solution. This apatite–collagen composite can be formed into complex shapes. As a pellet, the composite was biocompatible with soft tissues. In addition, UV-irradiated samples kept their shape for longer.

In this study, to examine their insolubilized properties and biocompatibility with hard tissue, namely, bone, apatite–collagen composites were implanted beneath the periosteum cranii of rats.

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MATERIALS AND METHODS

Apatite synthesis

Carbonate-containing hydroxyapatite, CO_3apatite, with chemical composition and crystallographic properties similar to those of bone was synthesized at 60 ± 1°C according to the previous method[7]. 500 ml of 200 mM $Ca(CH_3COO)_2 \cdot H_2O$ and 500 ml of 120 mM $NH_4H_2PO_4$ containing 60 mM $(NH_4)_2CO_3$ were fed into 1 l of 1.3 M CH_3COONH_4 solution with automatic stirring. pH was maintained automatically at pH 7.4 ± 0.2 with concentrated NH_4OH solution. The precipitate was washed well with distilled water and dried at 60°C for 2 d.

X-ray diffraction was employed to identify precipitates. Measurements were made on a Rigaku Denki X-ray Diffractometer at 35 kV and 23 mA. Differential thermal analysis (DTA) was performed with 10 mg samples in a Rigaku Denki Thermoflex apparatus.

Calcium concentrations were determined by atomic absorption spectrophotometry. Total phosphate concentrations were determined by the UV-spectrophotometric method of Eastoe[8]. Carbonate concentrations were determined by the method of Conway[9].

Preparation of apatite–collagen composites

Lyophilized collagen derived from calf skin (Nippi Co. Ltd, Japan), whose antigenicity had been removed by enzymatic treatment, was dissolved in 10^{-3} N HCl solution at 0.5%. Collagen solutions were neutralized with 0.05 N NaOH, then mixed immediately at 10% of collagen by dry weight with

epithelial attachment were observed with membranes placed under the surgical flaps.

This variable response to regenerative procedures, especially with the addition of synthetic bone grafting material in defects with poor inherent regenerative capacity requires further investigation to establish the limitations of regenerative procedures, what factors determine the limitations placed on tissue healing and regeneration, and what materials are most appropriate to enhance the regeneration of the tissues during healing.

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Oxidized cellulose mesh

I. Biodegradable membrane in periodontal surgery

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Regeneration of tissues after surgery has been demonstrated using a variety of membranes placed after surgical exposure of periodontal defects. In this study, biodegradable oxidized cellulose mesh was used in human subjects to improve post-operative healing. Healing was good, with all pockets less than 3 mm post-operatively. Less recession occurred in some of the sites which received the mesh, but this was not significantly different from the sites which did not receive the mesh. A varied healing response was noted in different sites and in different individuals. A number of factors may determine the nature and extent of the healing response, but these are ill defined. Histological investigation of the healing response of the tissues was not possible in this clinical study. The study has shown that oxidized cellulose mesh has the potential to enhance healing after surgery, and it may he useful as an alternative biodegradable material for use in the technique of guided tissue regeneration.

Keywords: Biodegradation, membranes, cellulose

Regeneration of the periodontal ligament following periodontal disease has long been the ultimate goal of periodontal therapy[1]. A major area of recent investigation has advocated the insertion of synthetic membranes into surgical wounds before closure to facilitate selective cell recolonization of tooth root surfaces by cells derived from organizing granulation tissue, rather than those of epithelial origin. It is thought that exclusion of epithelial cells and gingival tissue fibroblasts selectively allows repopulation of the surgical site by periodontal ligament progenitor cells on the root surface[2-6]. The potential for enhanced regeneration of the destroyed periodontal ligament has been demonstrated using a variety of biodegradable and non-biodegradable membranes.

REVIEW OF THE LITERATURE

Terranova and Wikesjo[7] have reviewed those organic factors which potentiate wound healing. They have described enhanced adherence of cells of mesenchymal origin to root surfaces, in the presence of rapidly deposited non-specific extracellular matrix components. This predominantly fibrin-containing matrix has been shown to inhibit epithelial downgrowth and it promotes mesenchymal adherence. It is derived predominantly from the blood clot in the healing wound. Therefore, to achieve enhanced regeneration of the periodontal ligament, in addition to exclusion of the epithelial cells from the surgical wound, it is essential to facilitiate the formation of the fibrin matrix with the fibroblast growth factors necessary for rapid repopulation of the wound by the periodontal membrane progenitor cells.

Oxidized cellulose (Surgicel®: Johnson & Johnson, Slough, UK) is commercially available resorbable haemostatic dressing in the form of a knitted fibrous mesh. When in contact with blood, the mesh changes to a gelatinous mass which is incorporated in the blood clot to form a blood/membrane continuum. *In vivo* studies have shown that the material is resorbed when placed in surgical wounds, and in addition, antibacterial activity of the material has been demonstrated without deleterious effects to the healing process[8]. This material has been shown to potentiate osseous and soft tissue regeneration in congenital maxillary cleft reconstructive surgery, when used as a subperiosteal graft before flap closure[9].

The aim of this clinical investigation was to evaluate biodegradable oxidized cellulose with respect to tissue healing after surgical debridement of periodontal defects, using the technique of guided tissue regeneration.

MATERIALS AND METHODS

Three patients who had received an initial phase of treatment which included oral hygiene instruction, scaling and root planing were selected for the trial because of the presence of deep residual pocketing which required surgery. Replaced flap surgery was undertaken to expose the periodontal defects to enable the removal of granulation tissue and root

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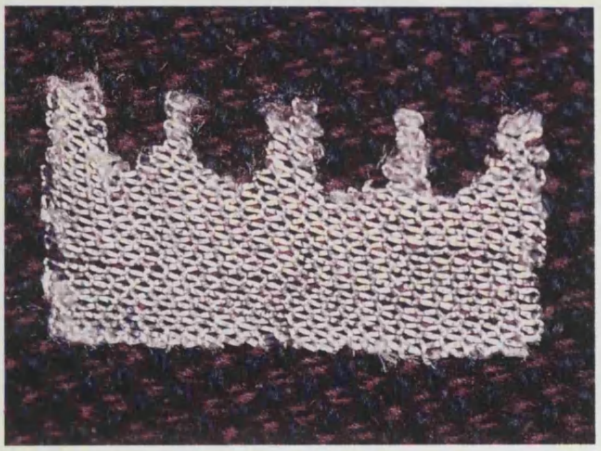


Figure 1 Contoured surgical dressing to enable placement of the membrane interdentally.

surface debridement. The oxidized cellulose membrane (Surgicel) was contoured to approximate the anatomy of the facial and interdental regions (*Figure 1*). To prevent premature degradation of the membrane, it was held in its inner protective package during contouring. After debridement the membrane was placed bucally and palatally over the surgically exposed alveolar bone but underneath the soft tissue flap, and the extensions of the material teased interdentally. The mesh was placed over the bony margins of the defect and up to the teeth surfaces in such a way as to protrude approximately 1 mm coronally to the soft tissue flap so as to preclude epithelial down-growth into the healing wound and form a seal interdentally and around the facial surfaces of the teeth.

After placement of the mesh, the soft tissue was replaced over the surgical site and sutured with horizontal mattress sutures below the mucogingival line to avoid contamination of the healing wound by the passage of the suture material through surgical site. After 1 wk the sutures were removed.

Before surgery, and 1 and 8 wk post-operatively, the levels of soft tissue recession were assessed by placing a Williams periodontal probe parallel to the long axes of the teeth at the mesial and distal line angles, midlingual and midbuccal points on each tooth.

The soft tissue heights were measured using the millimetre scale engraved on the probe to establish the distance between the soft tissue margins and the cemento-enamel junctions of the teeth.

Patient 1: 53-year-old Caucasian female who required flap surgery for elimination of residual pockets in the 6431/1 region. Surgical exposure revealed that the bone loss around the 1/ was predominantly horizontal, and after debridement, the mesh was wrapped around the tooth from the palatal surface around the distal surface to the buccal surface as described earlier. In the 64/ region the mesh was placed interdentally over the infrabony defect and fanned out buccally and palatally to occlude the defect. No mesh was placed at the 1/1 or mesially on the 3/ which served as controls.

Patient 2: 50-year-old Caucasian male who had residual pocketing in the /123 region. Flap surgery was performed, and the mesh was placed between the /23 as described earlier. No mesh was placed between the /12 or distally on /3 which acted as controls.

Table 1 Results of Student's t test on pooled recession data for the group of individuals investigated in this study

	Test/Control	No.	Mean (mm)	Range (mm)	SD	Student's *t*
Pre-operative	Control	17	1.65	−1 → 4	1.41	0.06
	Test	19	1.68	0 → 3	1.29	n.s.
1 wk	Control	17	2.47	1 → 3	1.01	0.94
post-operative	Test	19	1.63	0 → 4	1.28	n.s.
2 month	Control	17	3.24	1 → 6	1.30	1.46
post-operative	Test	19	2.84	0 → 5	1.46	n.s.

n.s., not significant

Patient 3: 71-year-old Caucasian male requiring surgical pocket elimination due to poor healing and recurrent abscess formation in the region of 76/ furcation areas. An inverse bevel flap was raised and all of the granulation material was removed. Copious bleeding occurred which ceased almost immediately after placement of mesh and replacement of soft tissue flap. The mesh was placed over the furcation of the 6/ and interdentally between the 76/ but not in the furcation of the 7/, mesially on the 6/ or distally on the 7/ which acted as controls.

RESULTS

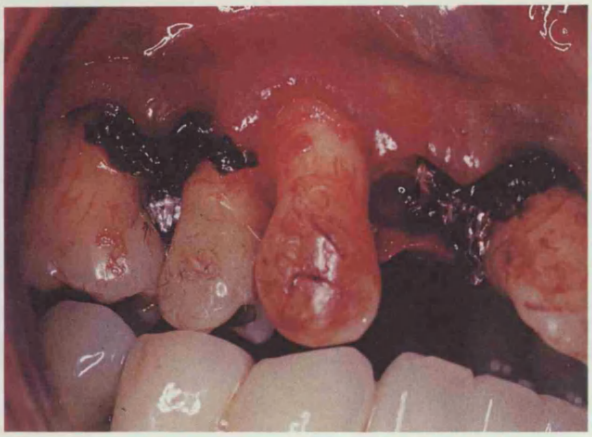
The results of placement of this membrane under the soft tissue flaps at the time of periodontal surgery are illustrated for each individual in the study, and summarized in *Table 1*.

Healing was good in all cases, and no abnormal signs of inflammation, resorption, or necrosis of the soft tissue flap were observed clinically 1 wk after placement of the mesh. Pocket depth measurements taken 8 wk after surgery showed that all pockets were less than 3 mm, with no difference noted between those sites which received the mesh and those which did not.

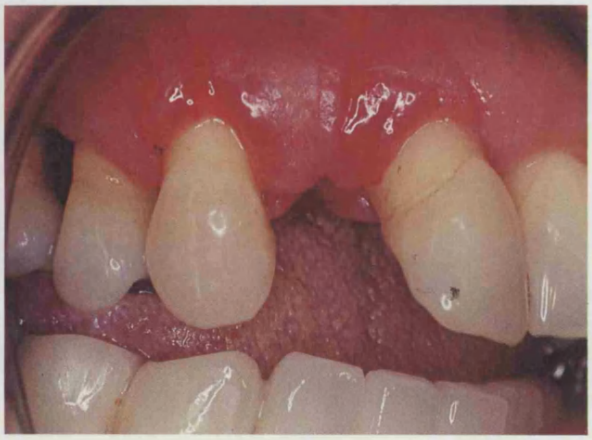
Patient 1 (Figure 2). Healing was good with no post-operative necrosis of the soft tissue. In the 64/ region, some interdental cratering was observed, which was typical of healing surgical wounds without the placement of the mesh. The site which received the mesh (1/ distally) shows the same degree of residual inflammation post-operatively as the one which did not receive it (3/ mesially).

Patient 2 (Figure 3). Post-operative healing was good with no signs of necrosis of the tissues or inflammation. Interdental cratering was visible 1 wk post-operatively which is characteristic of healing of surgical wounds after periodontal therapy without the placement of occlusive membranes. Similar interdental cratering was noted interdentally in the areas which had received the mesh, and those which had not.

Patient 3 (Figure 4). Post-operative healing was good, with no signs of post-operative erythaema, oedema or cratering when observed clinically 1 wk post-operatively. There was a slight tendency to bleed on the mesial surface of the 6/ (which received the mesh) than the mesial surface of the 7/ (which did not). Soft tissue recession was greater in the



a



b

Figure 2 Patient 1: (a) Surgical dressing in place before flap replacement. (b) 1 wk post-operatively.

furcation of the $\overline{7/}$ (which did not receive the mesh), than the furcation of the $\overline{6/}$ (which did receive the mesh).

Table 1 shows the results of a Student's *t* test for significant differences between the test and control sites pre-operatively, 1 wk post-operatively, and 8 wk post-operatively. It can be seen that no significant differences were present between the two groups pre-operatively or post-operatively at 1 and 8 wk. Numerically less recession occurred in the sites which received the mesh than in those which did not, but the differences between the test and control sites were not significant.

DISCUSSION

Good healing was observed in all individuals in this investigation with an absence of any deleterious effects on the tissue in which the mesh was placed. Some sites showed evidence of minor post-operative bleeding and other sites showed residual inflammation or slower than average healing of the tissues after surgery. However, as no significant differences could be seen between those sites which received the dressing, and those which did not, these differences were attributed to the differences in the healing response between individuals, rather than to any deleterious effects from placement of the mesh in the surgical site.

In some sites the presence of the mesh was associated with good healing, in others it made no difference, and in others it took longer to resorb with delayed wound closure as a result. The factors governing the variability of the healing between different surgical sites cannot be determined, and this is in agreement with the observations of other workers[10].

From *Table 1* it can be seen that less recession occurred after surgery in the sites which received the mesh than those which did not. Although this difference was not statistically significant, it is clinically noticeable in most sites. Therefore, the placement of a membrane or mesh of this type under the replaced flap, to enhance regeneration of tissue, and in addition to stabilize and seal the blood clot to prevent contamination of the healing tissue from the oral environment, has the potential to reduce post-operative recession of the tissues. Histological investigation of the healing response could not be undertaken in this clinical study, and further work is needed in this area to determine exactly what healing response is occurring and whether or not inhibition of epithelial down-growth occurs.

SUMMARY

1. The placement of a biodegradable membrane in surgical sites to achieve guided tissue regeneration offers many advantages over non-degradable membranes, and the oxidized cellulose mesh used in this study has been shown to offer the potential of a biodegradable membrane

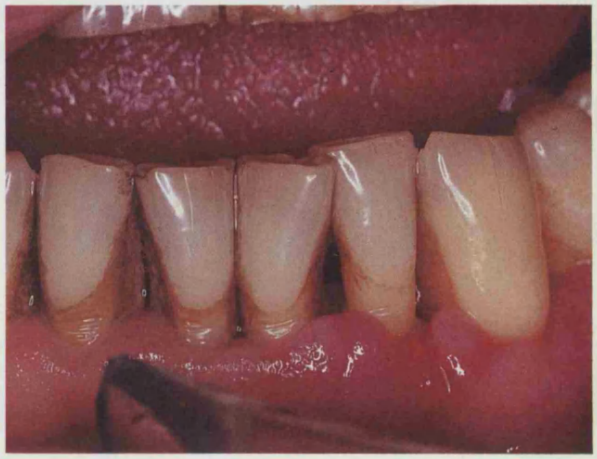


Figure 3 Patient 2: 1 wk post-operatively.

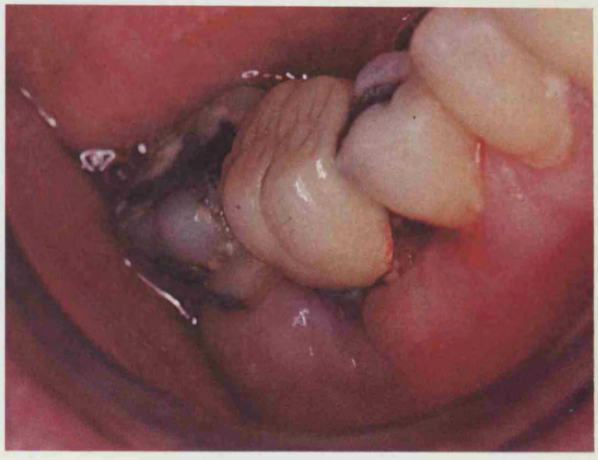


Figure 4 Patient 3: 1 wk post-operatively.

for this purpose. In addition to its biodegradable properties, its haemostatic and bacteriostatic properties are clinically advantageous.

2. The technique of placement is easy and it requires no specific suturing technique, as once incorporated in the blood clot it becomes self-supporting in the surgical site.
3. Although good healing was generally observed, variation in the healing response between different sites and different individuals was observed, and more work needs to be done into those factors which govern the healing and regeneration of tissues in different sites.
4. Histological investigation of the healing response could not be undertaken in this study to establish whether inhibition of epithelial down-grown occurs. Further work is required to establish whether or not this material facilitates inhibition of epithelial down-growth, and enhanced regeneration of the tooth-supporting structures.

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A 4-year controlled clinical study into the use of a ceramic hydroxylapatite implant material for the treatment of periodontal bone defects

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Galgut PN, Waite IM, Brookshaw JD and Kingston CP: A 4-year controlled clinical study into the use of a ceramic hydroxylapatite implant material for the treatment of periodontal bone defects. J Clin Periodontol 1992; 19: 570–577.

Abstract. 10 patients with chronic adult periodontitis who had >1 tooth with infra-bony pockets were treated at the test defects by periodontal flap procedures with implantation of hydroxylapatite particles; the control defects were treated by the same surgical procedures but without the implant. A total of 58 test defects and 59 control defects were treated. Each defect had measurements carried out at given sites on the involved tooth surfaces, the sites being considered for subsequent tabulation purposes under the category of shallow (<3 mm) moderate (3–6 mm) and deep (>6 mm) initial pocket depths. There were 146 and 152 shallow sites, 216 and 241 moderate sites and 140 and 133 deep sites, at test and control sites, respectively. Measurements of recession, probing pocket depths and probing attachment levels were made at 6 months and 1, 2, 3 and 4 years. At all sites over the period of the study, for the moderate and deep initial pockets there was a significant reduction in probing depths and an increase in the probing attachment levels. At the 4th year of assessment for the initially deep pockets, the reduction in probing depths was significantly greater for the sites treated with the implant material. In view of the difficult clinical problem posed by the treatment of teeth with deeper periodontal bone defects, further research using either this type of implant material or similar material should be considered.

Key words: periodontal bone defects; implants; hydroxylapatite.

Accepted for publication 4 July 1991

The current trend in periodontal surgery is to use techniques that conserve periodontal tissue and increase the potential for healing by the formation of a long junctional epithelial attachment, new attachment or bone regeneration. These techniques contrast with previous procedures that were designed to eradicate pockets by excision or repositioning of unsupported soft tissue and by recontouring of any associated osseous defects.

The ideal result of periodontal surgery would be the regeneration of new periodontal ligament attached to regenerated bone and new cementum, thus achieving a return to the original anatomical relationship. However, the degree of new bone formation is uncertain even with guided tissue regeneration procedures.

There have been a number of clinical studies in the use of synthetic alloplastic materials as implants in periodontal osseous defects. Relatively few of these studies have included control procedures. Some of the longer term studies have included surgical re-entry procedures during follow-up; whereas reentry measurements provide very useful data on hard tissue contour and the retention of the implant in the bone defects, the secondary surgical procedure is liable to influence the subsequent follow-up measurements.

The objective of the present study was to carry out a long-term, controlled, clinical study into the use of a ceramic hydroxylapatite implant material in the treatment of periodontal bone defects. No further surgical treatment was carried out after the initial procedures. The study was designed to include regular follow-up visits for scaling and maintenance of plaque control.

Material and Methods

Subjects for the study

The subjects for the study were 10 patients aged between 33 and 59 years who had been referred to the Periodontal Department of University College and Middlesex School of Dentistry, London, for the treatment of chronic adult periodontitis. Their mean age was 42.5 years; there were 7 females and 3 males.

Prior to their admission to the study, a medical history was taken from each patient to ascertain that they had no systemic disease that might influence their periodontal condition or contraindicate periodontal surgery. It was as-

certained that they were not wearing a prosthesis or orthodontic appliance. All subjects were prepared to attend for regular follow-up visits.

The clinical criteria for selection were that the patients should have completed a course of treatment involving root planing and plaque control, and that they should have maintained an excellent standard of oral hygiene with consistently low levels of plaque during the last few assessments. The duration of this preparatory phase varied, depending on the response of the patient to the plaque control programme and on the rate of resolution of the inflammation.

For admission to the study, each patient was required to have at least 2 periodontal osseous defects of similar radiological appearance to be used for test and control sites. Where a greater number of lesions were present, these were also used in the study. The subjects gave informed consent to participate in the project.

Surgical procedures and maintenance

Prior to periodontal surgery, random allocation was used to assign the implant or control procedure to the various experimental sites.

The surgical treatment was carried out under regional or infiltration local analgesia using 0.2% lignocaine with adrenaline 1:80.000. A conservative inverse bevel incision was used retaining as much of the gingival tissue as possible. Any remaining deposits were carefully removed, and the exposed cementum surfaces were planed with a curette until they felt smooth and hard. The bone defects were curetted to remove granulation tissue. The surgical area was then washed with physiological saline and carefully inspected to ensure that the procedures had been completed satisfactorily. The test sites were treated by the implantation of sintered hydroxylapatite particles*. It was found that the most satisfactory method of inserting the material into the defect was by mixing it to a paste with some of the patient's own blood, which was withdrawn from the operative site in a syringe. The resultant mixture was taken to the bone defect on the blade of a curette. The control defects were left to fill with a blood clot. The flaps were then sutured over the wound with interrupted sutures using 4/0 braided silk. Periodontal dressings** were placed over the surgical area for 1 week. Subsequently the dressings and sutures were removed and the teeth cleaned and polished. A 0.2% chlorhexidine gluconate mouthwash was prescribed for use 2× a day for 1 week.

Postoperative clinical healing was satisfactory. There was a tendency for some of the implanted material to be shed during the first few weeks.

The patients were followed up with scaling and plaque control maintenance visits for the first 3 months after surgery at 2–4 week intervals, and thereafter at 3-monthly intervals.

Clinical assessments

The initial assessment of the periodontal condition of the patients was carried out after the root planing and plaque control phase. Thereafter during the study further measurements were performed at six months and at 1, 2, 3 and 4 years.

At the above time intervals, the measurements were made for each osseous defect, at all the involved sites, along the vertical axes of the teeth. As the bases of the pockets were found to be very irregular, the measurements were made at multiple sites for each bone defect: at the interproximal surfaces with the probe resting against the contact points, at the line angles, at the points of maximum convexity of the root and on multirooted teeth at the entrances to the furcations. Single rooted teeth thus could potentially be measured at five sites on both vestibular and oral aspects, whereas lower molar teeth could be measured at 7 sites on both aspects according to the number of sites involved.

The measurement criteria for plaque was based on zero being scored for the absence of plaque and one being scored for each surface where plaque was present. Similarly, gingivitis was scored zero or one on the basis of whether the gingiva showed bleeding within 20 s after probing at the various sites. Pocket measurements were carried out with a Williams graduated periodontal probe* with a point diameter of 0.62 mm. The distance from the enamel-cement junction (ECJ) to the gingival margin was also measured using the same probe.

All the measurements throughout the study were carried out by one examiner (PNG). Reproducibility was determined prior to the study for the various measurement criteria by assessing 6 volunteers on two separate occasions with a time interval of at least 60 min between the examinations. The % reproducibility for measurement by site was 92% for pocket measurement, 88% for recession of the gingival margins, and 89.5% for the level of attachment.

Analysis of the data

The data for the test and control defects were ordered separately. It was considered that the initial depth of pockets would influence the response to treatment. Inspection of the range of measurements for the original data indicated that the baseline figures could be divided into three categories: shallow pockets (<3 mm), moderate pockets (3–6 mm) and deep pockets (>6 mm). For each of the assessment criteria and at all the time intervals, the data were tabulated with respect to the baseline categories of shallow, moderate and deep pockets. The mean values for these grouped categories of pocket depths for each patient were taken as the units for the statistical analysis (Blomqvist 1985).

Statistical analysis was carried out using the paired Student *t*-test to compare test and control procedures, and the Multiple Range Test for the longitudinal changes within procedure (Duncan 1955).

Results

A total of 58 test defects and 59 control defects were treated. At the test and control defects there were 146 and 152 shallow sites, 216 and 241 moderate sites and 140 and 133 deep sites, respectively. The objective had been to achieve defects with similar degrees of involvement for test and control procedures, by randomly allocating the treatments to matched pairs of defects. There were, however, found to be minor differences in the distribution of the severity of defect between the test and control procedures at baseline. E.g., considering the gingival recession data at both shallow and deep sites, the mean value for recession was greater at the sites to be

* Durapatite (Periograf). Sterling-Winthrop, Guildford, UK.

** Coe-Pak, Coe Laboratories, Chicago, USA.

* Hu-Friedy, Chicago, USA.

treated by the test procedure than by the control procedure (Table 1). This finding also in turn influenced the values for loss of probing attachment at baseline there being a trend for more loss of attachment at future test than control sites (Table 5). Further analysis was carried out to provide data of change from baseline for all the measurements to compensate for the unequal distribution at the pre-surgical assessment (Tables 2, 4 and 6).

Results for gingival recession

After surgery at each of the assessment intervals for both the test and control procedures, there was a statistically significant increase in gingival recession compared to baseline (Table 1), the probability levels with the multiple range test being either <0.05 (indicated by the broken lines) for some of the control data or <0.01 (indicated by the continuous lines) for the remainder of the comparisons. In general, there was more recession for deeper initial pockets than for the shallow ones, the initial postoperative increase in recession ranging from 0.73 mm for shallow initial pockets to 1.48 mm for the deep initial pockets (Table 2). At successive follow-up assessments the change in recession tended to increase progressively being greatest at the four year assessment, when the values ranged from 1.21 mm to 1.95 mm. As can be seen in Table 2, there was no statistically significant difference between test and control procedures at any of the time intervals (Student's *t*-test, $P>0.05$).

Results for probing pocket depths

For both the test and control surgical procedures at the follow-up assessments after treatment compared with the baseline measurements, there were decreases in the probing pocket depths (Table 3). These decreases were not statistically significant for shallow pockets, but were significant for moderate and deep pockets ($P<0.01$). At the control sites for both the moderate and deep pockets the post-surgical mean values showed relatively minor changes during the follow-up period of the study. At those sites treated with the implant material however for the initially moderate and the initially deep pockets there was a trend for further reductions in probing pocket depths during the follow-up period.

The reductions in mean probing pocket depths at the various time intervals for the two procedures ranged from 0.17 mm to 5.00 mm, being greatest for the initially deep pockets (Table 4). Comparing the results for the two procedures it was found at the fourth year assessment that for the deep pockets the mean reduction in probing pocket depth for the test procedure of 5.00 mm was significantly greater than the mean value of 4.20 mm for the control procedure ($P<0.05$).

Results for the loss of probing attachment from ECJ

For shallow initial depths of pockets there were post-operative losses in probing attachment level at both the test and control sites which were significant at the $P<0.05$ level (Table 5). At 6 months after surgery, the mean loss of probing attachment for both experimental procedures was approximately 0.5 mm, subsequently there were further losses of attachment for test and control procedures, the results at 4 years after surgery for loss of probing attachment being respectively 0.90 mm and 1.09 mm (Table 6).

In contrast, for both moderate and deep pockets, there was a gain in probing attachment after surgery for the test and control procedures. For the moderate pockets, this gain was significant at the $P<0.05$ probability level for some time intervals, whereas for the deep pockets this gain was significant at the $P<0.01$ level at all post-operative assessments. During subsequent follow-up at the control sites, there was a trend for the initial gain to diminish; however at the test sites the gain was maintained or increased.

Comparing the results of the 2 procedures (Table 6), it was found that, at the 4-year time interval for the change in the attachment level after surgery, the difference between the test and control procedures for the initially deep pockets approached statistical significance ($P=0.058$), the difference being just over 1 mm.

Discussion

The use of synthetic implant materials to overcome some of the difficulties in treating osseous defects caused by periodontal disease has been investi-

Table 1. Mean and standard deviation for recession from ECJ for 10 patients at the various time intervals for different initial depth of pockets and for test and control procedures

Pockets	Shallow initial <3 mm		Moderate initial 3–6 mm		Deep initial >6 mm	
Time interval	Test mean (SD)	Control mean (SD)	Test mean (SD)	Control mean (SD)	Test mean (SD)	Control mean (SD)
Baseline	0.45 (0.27)	0.22 (0.20)	0.50 (0.55)	0.46 (0.54)	0.70 (0.71)	0.35 (0.55)
6 months	⌐ 1.18 (0.57)	⌐ 1.03 (0.45)	⌐ 1.54 (0.86)	⌐ 1.23 (0.59)	⌐ 2.18 (1.13)	⌐ 1.63 (1.05)
1 year	1.24 (0.64)	0.98 (0.63)	1.35 (0.67)	1.22 (0.69)	2.04 (0.89)	1.70 (1.21)
2 years	1.30 (0.59)	1.13 (0.45)	1.49 (0.67)	1.26 (0.50)	2.22 (1.19)	1.63 (1.43)
3 years	1.46 (0.52)	1.36 (0.50)	1.66 (0.64)	1.49 (0.74)	2.40 (1.21)	1.90 (1.36)
4 years	⌙ 1.69 (0.50)	⌙ 1.50 (0.72)	⌙ 1.90 (0.60)	⌙ 1.67 (0.78)	⌙ 2.44 (0.76)	⌙ 2.31 (1.36)

Analysis of longitudinal changes with time

Data within brackets is significantly different to values in the same column outside the brackets at probability levels: ⌐ $P<0.05$ and [$P<0.01$.

Table 2. Mean and standard deviation for change in recession from baseline for 10 patients at the various time intervals for different initial depth of pockets and for test and control procedures

Pockets	Shallow initial <3 mm		Moderate initial 3–6 mm		Deep initial >6 mm	
Time interval	Test mean (SD)	Control mean (SD)	Test mean (SD)	Control mean (SD)	Test mean (SD)	Control mean (SD)
6 months	0.73 (0.55)	0.80 (0.34)	1.03 (0.56)	0.77 (0.30)	1.48 (0.68)	1.28 (0.72)
1 year	0.80 (0.57)	0.75 (0.52)	0.84 (0.44)	0.76 (0.27)	1.33 (0.39)	1.35 (0.87)
2 years	0.86 (0.48)	0.91 (0.41)	0.98 (0.39)	0.80 (0.24)	1.51 (0.69)	1.28 (1.16)
3 years	1.01 (0.39)	1.14 (0.50)	1.15 (0.34)	1.03 (0.40)	1.69 (0.07)	1.55 (1.04)
4 years	1.24 (0.51)	1.28 (0.65)	1.40 (0.39)	1.21 (0.54)	1.74 (0.59)	1.95 (1.03)

Analysis of differences between procedures
No significant difference at any time interval.

Table 3. Mean and standard deviation for depths of pockets for 10 patients at the various time intervals for different initial depth of pockets and for test and control procedures

Pockets	Shallow initial <3 mm		Moderate initial 3–6 mm		Deep initial >6 mm	
Time interval	Test mean (SD)	Control mean (SD)	Test mean (SD)	Control mean (SD)	Test mean (SD)	Control mean (SD)
Baseline	[1.76 (0.16)	[1.77 (0.17)	3.80 (0.30)	3.69 (0.32)	7.40 (0.91)	7.10 (0.61)
6 months	[1.59 (0.25)	[1.44 (0.41)	[2.09 (0.76)	[1.99 (0.41)	[2.98 (0.71)	[3.02 (0.79)
1 year	1.64 (0.30)	1.56 (0.36)	2.12 (0.53)	2.15 (0.31)	3.07 (0.69)	3.37 (0.81)
2 years	1.43 (0.33)	1.33 (0.32)	1.92 (0.55)	1.95 (0.56)	2.69 (1.10)	3.03 (1.18)
3 years	1.52 (0.50)	1.40 (0.51)	1.84 (0.60)	1.88 (0.51)	2.50 (0.88)	2.89 (0.97)
4 years	[1.42 (0.40)	[1.58 (0.75)	[1.78 (0.36)	[2.19 (0.76)	[2.39 (0.70)	[2.90 (1.12)

Analysis of longitudinal changes with time
Data within brackets is significantly different to data in the same column outside the brackets at probability levels: [$P<0.05$ and [$P<0.01$.

Table 4. Mean and standard deviation for change (reduction) in pocket depths from baseline for 10 patients at the various time intervals for different initial depth of pockets and for test and control procedures

Pockets	Shallow initial <3 mm		Moderate initial 3–6 mm		Deep initial >6 mm	
Time interval	Test mean (SD)	Control mean (SD)	Test mean (SD)	Control mean (SD)	Test mean (SD)	Control mean (SD)
6 months	0.17 (0.24)	0.33 (0.39)	1.72 (0.60)	1.71 (0.50)	4.42 (1.28)	4.08 (0.88)
1 year	0.12 (0.26)	0.20 (0.39)	1.68 (0.44)	1.55 (0.48)	4.33 (1.01)	3.73 (0.70)
2 years	0.33 (0.32)	0.44 (0.34)	1.89 (0.38)	1.74 (0.56)	4.70 (1.28)	4.07 (1.22)
3 years	0.24 (0.57)	0.37 (0.58)	1.96 (0.67)	1.81 (0.58)	4.90 (0.92)	4.21 (1.06)
4 years	0.34 (0.42)	0.18 (0.83)	2.02 (0.56)	1.50 (0.71)	5.00 (0.81) *	4.20 (0.83)

Analysis of differences between procedures
* Statistically significant difference ($P<0.05$).

Table 5. Mean and standard deviation for loss of attachment for 10 patients at the various time intervals for different initial depth of pockets and for test and control procedures

Pockets	Shallow initial <3 mm		Moderate initial 3–6 mm		Deep initial >6 mm	
Time interval	Test mean (SD)	Control mean (SD)	Test mean (SD)	Control mean (SD)	Test mean (SD)	Control mean (SD)
Baseline	2.21 (0.31)	1.99 (0.22)	4.31 (0.72)	4.15 (0.56)	8.10 (1.09)	7.45 (0.99)
6 months	2.77 (0.70)	2.46 (0.70)	3.62 (1.06)	3.22 (0.42)	5.16 (0.96)	4.65 (1.29)
1 year	2.89 (0.63)	2.54 (0.90)	3.47 (0.59)	3.36 (0.79)	5.10 (0.96)	5.07 (1.45)
2 years	2.73 (0.56)	2.46 (0.65)	3.40 (0.78)	3.21 (0.79)	4.91 (1.21)	4.67 (1.78)
3 years	2.98 (0.65)	2.76 (0.84)	3.50 (0.56)	3.37 (0.92)	4.90 (0.89)	4.79 (1.48)
4 years	3.11 (0.56)	3.08 (1.35)	3.69 (0.53)	3.86 (1.32)	4.83 (1.07)	5.21 (1.80)

Analysis of longitudinal changes with time
Data within brackets is significantly different to data in the same column outside the brackets at probability levels: $[P<0.05$ and $[P<0.01$.

Table 6. Mean and standard deviation for change (gain) in attachment from ECJ from baseline for 10 patients at the various intervals for different initial depth of pockets and for test and control procedures

Pockets	Shallow initial <3 mm		Moderate initial 3–6 mm		Deep initial >6 mm	
Time interval	Test mean (SD)	Control mean (SD)	Test mean (SD)	Control mean (SD)	Test mean (SD)	Control mean (SD)
6 months	−0.56 (0.69)	−0.48 (0.65)	0.68 (0.62)	0.93 (0.55)	2.94 (1.60)	2.80 (1.17)
1 year	−0.68 (0.59)	−0.55 (0.88)	0.84 (0.52)	0.79 (0.59)	3.00 (1.12)	2.38 (0.88)
2 years	−0.52 (0.61)	−0.47 (0.59)	0.90 (0.53)	0.94 (0.51)	3.19 (1.33)	2.79 (1.43)
3 years	−0.77 (0.64)	−0.78 (0.80)	0.81 (0.60)	0.78 (0.63)	3.21 (0.82)	2.66 (1.05)
4 years	−0.90 (0.66)	−1.09 (1.35)	0.62 (0.58)	0.29 (0.90)	3.27 * (1.16)	2.24 (0.96)

* Approaching statistically significant difference ($P=0.058$).

gated by a number of workers. In a recent review, it was found that varying degrees of success had been reported in clinical studies of treatment of periodontal bone defects with alloplasts (Waite & Galgut 1987). The implant materials that have been used most extensively are hydroxylapatite and tricalcium phosphate: both materials have been found to be well tolerated by the local tissues when used in periodontal bone defects. The local response to the implant varies according to its chemical composition, its structural characteristics for example the density and porosity, and the type of host tissue adjoining the implant. It has been found that hydroxylapatite is degraded at a relatively slower rate than tricalcium phosphate (Han et al. 1984). More recently, implant materials with a porous structure have been introduced, it being considered that the presence of pores and interconnections might enhance the deposition of new bone; however relatively few studies have reported on the use of porous materials up to the present.

The present study represents a 4-year study of patients who had periodontitis with osseous defects, which were treated surgically by curettage of the defects at open flap surgery for the control procedures, or the same treatment combined with and hydroxylapatite implant material for the test procedures. Previous research on the same subject has been reported (Yukna et al. 1984; Yukna et al. 1985; Yukna 1989; Yukna et al. 1989). The present study used a modified protocol: first in order to avoid possible interference with the continuity of follow-up, this study did not include a re-entry procedure at any stage; secondly the defects were measured at defined sites and these sites were analysed independently according to the initial pocket depths within subjects; thirdly the present research was continued for a 4-year period; and fourthly a densitometric radiographic study was undertaken on a sample of the radiographs over a two-year period this report being the subject of another paper (Galgut et al. 1991).

Recession

A significant increase in gingival recession was found after both surgical procedures, and there was a gradual further increase in recession as the study progressed. A review by Lindhe & Nyman (1987) concluded that irrespective of

the type of therapy, including non-surgical procedures, about 50% of the reduction in pocket depth as a result of treatment can be explained by recession of the gingival margins. At the commencement of this study, it had been considered possible that the implant material might result in less postoperative recession, and this would clearly have been an advantage where aesthetics were important. However, at the 4-year assessment, the degree of recession after treatment was similar for both test and control procedures, ranging from 1.2 to 2.0 m depending on the initial depths of pockets. Yukna et al. (1984, 1989) also found similar mean values for recession postoperatively for both hydroxylapatite treated sites and controls. It can be concluded that hydroxylapatite implant material is unlikely to influence the degree of recession after periodontal surgery.

Pocket depths

In the present study, there was a significant reduction in probing pocket depth after surgery for all categories of site and for both procedures. The reduction was greatest for the initially deep pockets, and this finding is in agreement with the review by Pihlstrom et al. (1983). For the deeper pockets, there was a trend for further improvement on the implanted sites during the follow up period. At the 4-year assessment for the sites with initially over 6 mm pocket depths, there were significantly shallower pockets for the implanted compared with the control defects. Previous 3- and 5-year studies of hydroxylapatite implants in periodontal bone defects also found that there were statistically significant differences between test and control procedures but only at the later periods of the study (Yukna et al. 1984, 1989). There seems to be agreement that differences between the implant treated and the control sites are evident mainly in the long-term.

Level of probing attachment

In previous longitudinal studies of periodontal disease, the poor correlation found between loss of attachment and such other clinical criteria as pocket depths, bleeding on probing and gingival oedema and colour change emphasise the importance of long term measurement of level of probing attachment for assessing the results of periodontal treatment (Halazonetis et al. 1989, Vanooteghem et al. 1987).

The findings in the present study that surgical treatment of initial pockets of less than 3 mm resulted in a significant mean loss of probing attachment of up to about 1 mm is in agreement with other workers (Lindhe et al. 1982, Pihlstrom et al. 1983). For the initially moderate and deep pockets, the implanted sites tended to continue to maintain the improvement in probing attachment levels compared with baseline, or in the case of the deeper pockets to show further improvement. However, the level of probing attachment at the control sites began to deteriorate as the follow-up progressed. By the 4th year for sites with initially deep pockets the difference between test and control sites approached significance being at the $P=0.058$ level.

It was of interest that these differences in probing attachment level for deeper pockets became more apparent later on in the study. One of the main objectives of periodontal treatment is long-term stability of the attachment level, hence the later results in a study are of particular importance.

Yukna et al. (1984, 1989) did not find that there were any differences in probing attachment levels. The re-entry procedure at one year after surgery included in most if not all the studies by Yukna and co-workers may have influenced the follow-up measurements.

Implications of the results

This study has indicated that some of the clinical measurements may be influenced by the treatment of initially deeper periodontal bone defects with hydroxylapatite implant materials. The results should be considered in the light of previous studies into histological responses to such implants. It has been found that during the early stages after surgery hydroxylapatite implant particles become surrounded by fibrous tissue (Froum et al. 1982, Moskow & Lubarr 1983). Longer-term studies have shown that after about 6–18 months the particles may undergo localised areas of resorption, and regions were found where bone or osteoid material had formed around the implant particles. In one case, a collagenous bone matrix had enveloped the entire implant (Sapkos 1986, Galgut et al. 1990). The appearances in these studies were very variable. An important general finding was an absence of inflammatory cells in the vicinity of the hydroxylapatite particles, suggesting that the material was well tolerated by the tissues. There is no data available to compare the histological amount of new bone formed between sites treated with implant materials and control sites treated by curettage alone.

A previous radiographic study into a sample of the bone defects treated in the present study utilised computer aided densitometric measurement. Over the 2-year period of the study, the sites treated with the implant material showed a significant gain in the interproximal height of radio-opaque substance compared to the control sites, as determined by a computerised analysis system. The results indicated that the radio-opaque implant material was retained within the bone defects postoperatively.

The results of the present study can also be considered in relation to the conclusions of a review article on periodontal therapy by Lindhe and Nyman (1987). The results confirm the hypothesis that probing attachment loss frequently occurs following treatment of sites with initially shallow pockets, and that gain of probing attachment generally results following treatment of deep pockets. The authors observe that this gain in attachment is generally a false gain, the dentogingival epithelium during healing migrating close to the original preoperative level on the root surface. The apparent gain in attachment is the result of resolution of gingival inflammation with collagen fibres replacing the inflammatory infiltrate within the tissues. In the present study at sites where bone defects have been treated with implant materials, the incorporation of the implant particles in the fibrous tissue repair could hypothetically aid in supporting the sulcular epithelium and junctional epithelium against the root surface and hence increase the resistance to penetration by the probe.

The present results should also be considered in the light of recent work on the use of various membrane materials to guide regeneration of periodontal tissue over areas of cementum previously involved by periodontal disease. The objective of the technique is to restrict the proliferation of both epithelial cells and gingival connective tissue and to encourage the proliferation of periodontal ligament cells into the surgical wound area by providing a protected site. The various studies have

shown a gain of several mm of new periodontal ligament attachment, however relatively less predictable gain in bone height has been found in the relatively short-term studies reported to date (Gottlow et al. 1986). The ideal procedure for the treatment of a bone defect would achieve a return to structural integrity of the hard tissue and the formation of new periodontal ligament attachment. The use of implanted materials in conjunction with guided tissue regeneration procedures might be a subject for future research.

Conclusion

A possible rôle for the use of hydroxylapatite implant materials for the treatment of deeper infrabony defects is supported by the present study. During the latter part of this four year study it was found that initial pockets of more than 6 mm in depth showed a greater degree of reduction in probing pocket depths and an increase in probing attachment levels. In view of the difficult clinical problem posed by the treatment of teeth with deeper osseous defects, further research using either this type of implant material or similar forms should be considered.

Acknowledgements

The authors wish to express their gratitude to Mrs Christine Tham for her care and patience in preparing the typescript for this paper.

Zusammenfassung

Eine kontrollierte klinische 4-Jahresstudie über die Verwendung eines keramischen Hydroxylapatit-Implantmaterials zur Behandlung von parodontalen Knochendefekten

Bei zehn Patienten mit chronischer Erwachsenen-Parodontitis, die mehr als einen Zahn mit einer infraalveolären Knochentasche aufweisen mußten, wurden die Testdefekte durch eine Lappenoperation mit Implantation von Hydroxylapatit-Granulat behandelt. Die Kontrolldefekte wurden mit der gleichen Technik, jedoch ohne Implantation behandelt. Insgesamt wurden 58 Test- und 59 Kontrolldefekte behandelt. Bei jedem Defekt wurden an den einbezogenen Zahnflächen an bestimmten Stellen Messungen durchgeführt. Zum Zweck der Einteilung wurden drei Kategorien der initialen Taschentiefe gebildet: flach (< 3 mm), mittel (3–6 mm) und tief (> 6 mm). Bei den Test- bzw. Kontrollflächen gab es 146 und 152 flache, 216 und 241 mittlere sowie 140 und 133 tiefe Flächen. Messungen der Rezessionen, der Sondierungstiefe und des klinischen Attachmentniveaus wurden nach 6 Monaten, 1, 2, 3 und 4 Jahren durchgeführt. Während des Untersuchungszeitraums wurde an allen Zahnflächen mit mittleren und flachen Taschen eine signifikante Reduktion der Sondierungstiefe und Zunahme des klinischen Attachmentniveaus beobachtet. Für die initial tiefen Taschen, die mit Implantatmaterial behandelt wurden, war die Reduktion der Sondierungstiefe zum Zeitpunkt der 4-Jahresmessung signifikant höher. Im Hinblick auf die schwierigen klinischen Probleme, die mit der Behandlung von tiefen parodontalen Knochendefekten verbunden sind, sollten weitere Untersuchungen entweder mit diesem oder einem ähnlichen Material erwogen werden.

Résumé

Etude clinique contrôlée sur 4 ans concernant l'utilisation d'un matériau pour greffe en céramique d'hydroxyapatite dans le traitement des lésions osseuses parodontales

Chez 10 patients atteints de parodontite chronique de l'adulte et présentant des poches intra-osseuses sur plus d'une dent, le traitement des lésions test a été fait par interventions à lambeaux parodontaux avec greffe de particules d'hydroxyapatite; les lésions témoins (control) étaient traitées par les mêmes méthodes chirurgicales, mais sans greffe. Au total, 58 lésions test et 59 lésions témoins ont été traitées. Dans chacune des lésions, des mesures ont été pratiquées en certains sites déterminés sur les surfaces dentaires touchées, ces sites étant considérés pour les enregistrements ultérieurs comme appartenant à une des catégories suivantes: poches initiales peu profondes (< 3 mm), modérées (3–6 mm) ou profondes (> 6 mm). Le nombre de sites test et témoins était pour les sites peu profonds (shallow) respectivement 146 et 152, pour les sites modérés (moderate) respectivement 216 et 241, enfin pour les sites profonds (deep) respectivement 140 et 133. Des mesures de la récession, de la profondeur des poches au sondage et du niveau de l'attache au sondage ont été pratiquées à 6 mois et à 1, 2, 3 et 4 ans. Pour tous les sites il s'est produit dans les poches ayant une profondeur initiale modérée ou profonde une réduction significative des profondeurs de poches au sondage et une augmentation des niveaux d'attache au sondage au cours de la période de l'étude. Lors de la 4ème année d'observation, pour les poches initialement profondes, la réduction des profondeurs de sondage était significativement plus élevée pour les sites traités avec l'hydroxyapatite. Etant donné le problème clinique difficile que pose le traitement des dents ayant des lésions parodontales osseuses profondes, des recherches ultérieures avec ce type de matériau devraient être envisagées.

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