EVALUATION OF GLASS-IONOMER CEMENTS FOR USE AS BONE SUBSTITUTES WITH REFERENCE TO THEIR VALUE FOR TREATMENT OF ATROPHIC ALVEOLAR BONE

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SUMMARY

Progressive resorption of alveolar bone occurs following loss of teeth and results in severe functional, social and aesthetic problems for those individuals who are unable to cope with dentures.

Existing biomaterials used to augment the jaws with the aim of restoring the alveolear bone to enable successful denture wear suffer from problems of migration and require complex surgical techniques to achieve success. The aim of this study was to investigate glass-ionomer (polyalkenoate) cements (GIC) as a class of materials for use as bone substitute and cements and compare them with currently used calcium phosphate ceramics.

Initial biological testing was undertaken using primary bone cell and organ cultures based on explants of rat calvarial bone. In vivo evaluation involved implantation of GIC, into or onto, the mid-shaft of rat femora and study of diffusion chambers containing GIC implanted into baboons (supplied by Prof L M Jonck).

In vitro certain formulations of GIC were colonised by bone cells which regained their phenotype and laid down a collagen-containing extra-cellular matrix, cells responded less favourably to unset and fluoride-containing GIC and material with a rough surface.
In vivo direct bonding of bone to GIC based on fluoro-alumino-silicate glass occurred, with a mineralised collagen-containing extra-cellular matrix being deposited on the surface of the GIC. Aqua Cem and a fluoride-free GIC showed incomplete osseointegration.

Study of GIC indicated that this ionomeric group of materials, which can be made to resemble bone biomechanically, have potential advantages over currently available calcium phosphate ceramic bone substitutes.

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I. INTRODUCTION

Ii. Alveolar atrophy

a. The Clinical Problem

Approximately 19% of the adult UK population has no natural teeth and must wear complete dentures to restore oral function. Complete dentures have maximum stability in the presence of well developed residual alveolar ridges. However, once the teeth are lost progressive alveolar resorption occurs resulting in atrophy of the bony ridge, instability of the associated denture and a decrease in the quality of life for the patient. (1)

Alveolar atrophy is a chronic, progressive, cumulative, irreversible disease. The rate at which alveolar bone loss occurs varies not only between individuals but within the same individual at different times. (1-3) In general however, rapid loss of alveolar bone occurs in most patients during the first year following extraction of the teeth and provision of dentures. The rate of mandibular ridge reduction in the first year postextraction is reported to be in the order of 12mm and then the process slows to an estimated 0.2mm/year throughout life. (4,5) The mandible is more susceptible to alveolar bone loss than the maxilla, resorption being up to four times greater. (4) Loss of alveolar bone leads to reduction of the physical dimension of the residual ridge both in height and width and follow a characteristic pattern. (6) Ultimately loss of bone reduces the available denture bearing area, the sulcus depth and by

altering the jaw relationships jeopardizes the successful provision of complete dentures.

The aetiology of alveolar resorption stems from the fact that the function of the alveolar bone is to support the teeth and once the teeth are lost the major functional stimulus for the presence of alveolar bone is lost. Gross mandibular atrophy has been described as a 'multifactorial biomechanical disease' resulting from a combination of anatomic, metabolic and mechanical factors. (7) The different rates of alveolar bone loss between the maxilla and mandible and between different patients is however poorly understood.

Local and systemic factors responsible for alveolar bone loss have been extensively investigated. (2) Systemic factors such as osteoporosis appear to play a role in some patients and osteoporosis of the mandible has been shown to be associated with marked alveolar bone loss. (8) Local factors however play a major role in the majority of patients. Pre-existing periodontal disease and surgical injury at the time of extraction are determinant factors in how much initial alveolar bone is available for denture support. Once dentures have been provided it is their use which appears to stimulate alveolar atrophy. The habit of continuous denture wearing (day and night) leads to significantly more ridge reduction than only diurnal use. (5,9) Pressure-produced resorption of underlying bone following ischemia of the overlying mucosa by the denture was proposed as early as 1956 by Lammie (10) while others have noted that excess local pressure especially related to lateral forces and

parafuctional activity such as denture clenching/grinding lead to gross porosity of the medullary bone of the ridge crest and subsequent bone loss. (2,11-13) Following tooth loss loading of the mandibular bone is reduced by up to two thirds of that recorded for dentate mandibles. (5) This relative disuse of the alveolar bone is also considered to be a major factor in subsequent alveolar bone atrophy and is further compounded should the dentures cause inflammation of the mucosa (11). Others have postulated that the dampening effect of the 'visco-elastic' mucoperiosteum on the transmission of force from the dentures to the bone reduces physiological stimulation and thus results in disuse atrophy. (12) Gross ridge resorption in the mandible has also been linked to a reduction in mandibular blood flow with increasing age. (14-16)
A wide range of local factors are implicated in the aetiology of alveolar atrophy. Even dentures of optimal design appear not to be without their problems as in one study excessive loading from 'well fitting dentures' was reported to be a major factor in alveolar bone loss. (17)

b. Current management of alveolar atrophy

Two basic methods exist for rehabilitation of edentulous patients with atrophic ridges following failure of convention prosthodontic techniques. The first involves insertion of permucosal implants. Techniques of this type have greatly improved of late, (18-22) but several factors prevent their universal application. Subjects

requiring treatment are predominantly elderly and have a poor reaction to the prolonged surgical procedures involved. They also find the complex follow-up demanding and only learn the necessary habits of meticulous oral hygiene with difficulty. Perhaps just as significant, for a section of the population which is mostly of retirement age, the operation is very costly. An alternative is ridge augmentation. Implants are placed beneath the mucoperiosteum and serve to replace lost alveolar bone and thus require no long-term maintenance. (23-25)

All materials in past and current use fail to fulfil the ideal criteria for this conceptually simple procedure. Use of plaster of Paris or autologous bone results in resorption of the implanted material and loss of gained ridge height. (26,27) More biomechanically acceptable materials such as silicone or polyethylene/hydroxyapatite composites tend to be biologically inert and, fail to form a firm bond with living bone, become susceptible to infection in the long term (28).

c. Bone substitutes for reconstruction of alveolar bone and for maxillofacial/reconstructive surgery

The use of biomaterials for reconstruction or replacement of hard tissues (bone/teeth) has been more successful than for soft tissues. Metals, ceramics, polymers and composites are commonly used in orthopaedic surgery and in reconstructive surgery of the head and

neck. Metals are mainly used for fracture fixation devices in the form of rods, pins, screws and plates. The use of metals for the replacement or reconstruction of bones has especially in the face fallen into disuse as the have been superceded by the ceramics and polymer/composite materials. To date however no ideal material exists, the ideal properties of an alveolar bone augmentation material have been defined by Frame et al 1987 (23) as:-

a) Easily carved and moulded
b) Adequate mechanical properties to support a denture
c) Biocompatible and stable
d) Bond firmly to bone and soft tissue
e) No adverse effects on adjacent bone

Hydroxyapatite is the most popular material used at present but does not fulfil all the above criteria. Dense block forms of hydroxyapatite are difficult to carve while porous block varieties are liable to become infected and exfoliate. (24,29,30) These problems have encouraged the use of hydroxyapatite in the particulate form.

However, difficulties with clinical placement remain, along with migration and loss of material from the implant site, and stress shielding due to the elastic modulus of ceramic materials being greater than bone. (25-31) Thus hydroxyapatite, although a widely used material with good biological potential for alveolar augmentation, can be said to be a technique-sensitive material because its success is not entirely predictable. One method of

solving the clinical problems associated with ceramic bone substitutes is to develop modified surgical techniques to overcome the limitations of the materials available. The development of reliable techniques for augmentation of the atrophic alveolar ridge using existing hydroxyapatite formulations by the author is given in Appendix II.

Iii. Bone substitutes and their biological reactions.

The development and use of bone substitutes requires knowledge of the nature of bone. Pritchard in 1956 described bone as - a specialised cementitious tissue characterised by cells (osteocytes) residing in cavities (lacunae) with long branches running in thin channels (canaliculi) embedded in a dense, hard matrix consisting of groups of collagen fibres within an amorphous boundary substance (cement) impregnated with calcium phosphatic complexes. (32) The above definition still serves well at the microscopic level. To understand how bone interacts with biomaterials consideration has to be given to the histo-cytological and histo-chemical reactions that take place following material implantation. The events that take place at the interfaces between bone and biomaterials are the subject of much debate. The majority of work in this field is essentially morphological in nature, as a result certain terms, and their employment are often critical in both the description and interpretation of the experimental results reported. As many of these terms have no universally agreed meanings a list of terms and an interpretation as understood by the author are given here in alphabetical order-

Apatite like: structure similar to the physiological apatite found in teeth and bone.

Bioactive: The ability of a material to actively influence the surrounding tissue and the development of a response from the

tissue. In the case of bone to aid the formation of a bone/material bond. "Chemical bonding" of bone with bioactive materials gives them their 'direct' attachment and interfacial strength which prevents interfacial fracture (33). Bioactive materials include bioactive glass (34), ceramics (35,36) and calcium phosphate materials. (37,38)

Biocompatibility: The ability of a material to induce/perform with, an appropriate host response in a specific application. The response should be such as to ensure the continued safe and effective performance of the material.

Bio-inert: Materials that do not interact with the tissues of the body; forming no direct attachment with body tissues. (33)

Biomaterial: 'a non-living material designed to interface with biological systems' (Consensus conference Chester 1988) also defined as ' any material to interact with a biological system to evaluate, treat, augment or replace any tissue, organ or function in the body' (European Society for Biomaterials 1991)

Bone bonding:- Interdigitation: intimate association of the collagen fibres, of the extra-cellular bone matrix, with a materials surface, especially finger like projections into the material (39).

Bone bonding:- Osseointegration A direct structural and functional connection between ordered, living bone and the

surface of a load carrying implant, as assessed at the light microscopical level; modified from P-I Branemark 1987 (18).

Brittle fracture: Sudden failure with minimal deformation of a material.

Osteoconduction/ive: a material acting as a scaffold to facilitate bone formation eg. "the properties of hydroxyapatite used in bone reconstruction are -' not due to the hydroxyapatite but - due to the cells and extracellular matrix immobilized on its surface',(33) - the appositional growth of bone from/on existing bone

Osteoinduction/ive agent that stimulates bone cells to produce new bone eg. bone morphogenic protein. (41) A process involving cellular changes and differentiation that is outside the expected normal behaviour of the involved tissue eg. undifferentiated mesenchymal cells stimulated to differentiate into osteoblasts and produce bone. (42)

Stress: (Mechanical) The force per unit area of cross section. Has basic dimensions of $M\ m^{-2}$ in SI units, and commonly stated as the derived unit of Pascals (Pa)

Strain: (Mechanical) The response of a polymeric material to an applied stress. A dimensionless term defined as the increase in length per unit length of the original specimen.

Tough fracture: Failure of a material following substantial deformation of a material in the form of necking

Young's modulus, (E): The ration of stress to strain. Has dimensions of force per unit area usually expressed in Pascals. Young' modulus is independent of the specific details of the test method being a property of the material. The smaller the value of E the more rubbery a material, and the higher the more stiff and less deformable a material

Following fracture of bone or implantation of material the trauma initially disrupts the normal blood circulation of the local bone and in the adjoining soft tissue. The central capillaries passing through the haversian systems are damaged as are local feeding vessels. Blood from the damaged vessels flows out to surround the implanted material and fill any cavities in the bone and soft tissue. Osteocytes cut off from the nutrient supply die and the bone undergoes necrosis as far as the nearest unaffected volkmann's canal. The degradation of tissue gives rise to free radicals which

exert a weak bacteriocidal effect and stimulate fibroblasts to proliferate and attract macrophages. Osteoclasts invade the dead bone and resorb necrotic cells and bone matrix. Complimentary to this osteogenic cells proliferate to begin the reconstruction of bone by elaboration of a non-mineralised collagenous matrix (osteoid), which subsequently undergoes calcification. (43) A material placed to act as a bone substitute or cement should be designed to aid the above process and materials that exert a toxic effect on the cellular processes of healing/repair are excluded from consideration. At present the calcium phosphate ceramics are the most widely used synthetic bone substitutes and bone cements based upon acrylic cement are widely used in the cementation of prosthesis.

a.Calcium phosphate ceramics

Calcium phosphate ceramics are probably the most biocompatible synthetic bone substitutes in current clinical use. They are used widely for jaw augmentation (24,29) tooth replacement (44) (although in this situation their application is limited by the poor biomechanical performance of the material), bone substitution (45) and middle ear reconstruction (46). The major advantage of this group of materials is their capability to become functionally integrated with natural bone without fibrous tissue encapsulation and with little inflammatory or foreign body response. (23,31,37-39,44-50) The inherently favourable response seen on implantation of the calcium phosphate ceramics, despite variations

in the chemical composition and material structure, is attributable to their being mainly composed to calcium and phosphate ions the commonest constituents of vertebrate hard tissue. (45,49)

The calcium phosphates bond firmly with bone, such that when subjected to mechanical force fracture occurs either in the material or bone and not at the bone/material interface. (50) The nature of the bone/material interface has been studied in a variety of animal models and in retrieved human implants and is the subject of debate. (27,39,44,48-55) The majority of workers have described a bonding zone of between 500A to 2000A wide between the surface of the calcium phosphate implant and the surrounding bone, which contains ground substance and is heavily mineralised but contains few collagen fibres. (53) The bonding 'zone' has thus been considered to be analogous to the lamina limitans of bone (53,54) or to the natural resting/cement line of bone. (56,57) Calcium phosphate ceramics appear thus to allow normal 'wound' healing following implantation on/in bone defects. Recent work studying the interfacial reaction of bone cells <u>invitro</u> has demonstrated that bone-derived cells, as the initial step in osteogenisis occurring on a foreign, but compatible surface, produce a highly mineralised layer composed of globular calcified afibrillar accretions approximately 1um in diameter. (58) It is suggested that this collagen free layer may act as a substratum (implant) conditioning layer prior to overt bone formation (57) and is analogous to the cement lines formed at discontinuities in natural bone. Others however using similar methods (57b) have noted an initial layer more

analogous to the natural resting line or lamina limitans of bone and this has been reported invivo. (46,53) Defining the ultrastructural and biochemical nature of the bone/ceramic interface is ongoing data available to date on the interaction of bone/ceramics does however gives a a good bench mark against which any new potential bone substitute/cement can be measured.

b.Glass-Ionomer Cement as a bone substitute

Glass-ionomer cement (GIC), was originally developed by A D Wilson and B E Kent in 1969 (59), and is now widely used as a dental restorative material. These novel cements are formed by the reaction of an aluminosilicate glass with poly(acrylic)acid. The acid degrades the glass releasing cations, which serve to crosslink the polyacid chains and form a hard ceramic-like cement. Most dental ionomer glasses contain significant quantities of phosphate and are similar in composition to the bioactive surface-active ceramics termed 'Bioglasses' and crystallized bioglass-ceramics developed as bone substitutes by Hench (60,61) and bases upon 'surface-bioactive' sodium-calcium-scilicate glass with the addition of phosphate. In aqueous media these glasses initially lose sodium ions by dissolution to form a surface film consisting of a phosphate-calcium gel enriched in SiO_2 ions, which with time changes to a polycrystaline layer of apatite agglomerates that can incorporate bone matrix. Recent studies at Thames Polytechnic have also shown that dental ionomer glasses (based on G338/G200) crystalise to an apatite phase and thus have the potential to be

well accepted biologically. (62,63) GICs also possess several characteristics that make them attractive both as a possible bone substitute and as a bone cement:-

i. In current dental use GICs are mixed and placed in a plastic, semi-polymerised state. Setting occurs by transfer of metal ions from the glass to the acid without the liberation of heat. (59) Thus, in contrast to acrylic cements, GICs are potentially ideally suited for delivery of drugs/hormones and do not have the potential for causing thermal damage to the implant bed on in situ setting. The ability to clinically mould and shape an implant material to conform to the bone topography and set to a required shape would also overcome many of the current problems of implantable ceramics.

ii. The ratio of glass to poly(acrylic acid) in a GIC can be varied to produce a wide range of cements, thus allowing the formulation of materials for specific applications. (59) Potentially, GIC can be produced that are biomechanically compatible with bone by matching factors such as modulus of elasticity.

iii. When freshly mixed, GIC are able to bond chemically to apatite.(59) This gives them an advantage over current materials which rely on a relatively slow cellular growth to establish an intimate integration or intimate bond between the material and the bone.

iv. Even when set, GIC are not inert but bioactive and release F^-, Ca^{2+}, PO_4^{2-}, and SiO_2 ions.

Fluoride in a low systemic dose has been shown to stimulate

bone formation. (64) The release of calcium ions may increase the degree of the supersaturation of the ion in the tissue fluid adjacent to the implant and the silicate ion might provide favourable sites for nucleation of apatite on the surface of the GIC as has been postulated to explain the bioactive response observed with bioactive glass-ceramics. (65) The release of ions from GIC and the negatively charged surface of the GIC due to the presence of carboxylic acid functional groups in the hydrogel matrix phase may act to confer an osteoconductive potential on this group of composite materials.

Potentially therefore, GICs not only fulfil the criteria for a successful alveolar bone substitute (23) but exceed them.

c. Evaluation of biocompatibility

The principle aim in the evaluation of biocompatibility is to determine if the biomaterial performs appropriately in a given situation. This definition necessarily involves the exclusion of materials that are known to or can be predicted to exert a toxic effect on cells. Toxicity being defined as, a negative or deleterious effect produced by an agent on normal cellular biochemical functions.

In vitro toxicity is manifest by :-

Cell death : observable by direct observation (light/electron microscopy). Cells round up - detach from the substratum, this is preceded by nuclear shrinkage (pyknosis) and degeneration/fragmentation of the cytoplasm.

Reduced Cell Adhesion : this occurs without cell death and leads to the detachment of monolayers or the failure of cell suspensions to adhere to the material/substratum.
In some circumstances reduced cell adhesion may be a desirable property of a material making it biocompatible - for example in haematological devices failure of platelets and other blood products to adhere is a positive attribute of the biomaterial.

Altered Cell Morphology : often a precursor of cell death/loss of vitality.
Manifest by increasing vacuolation of the cytoplasm - development of autophagosomes - accumulation of lamellar phospholipid membranes (demonstrated with electron microscopy). It should be noted that a certain degree of vacuolation is normal in in vitro representing intracytoplasmic lipid accumulation which usually decreases once cells form a confluent monolayer. (66)

Reduced Cell Proliferation : this can be a sensitive parameter of toxicity and can be quantified by cell growth curves: -

counting the number of cells in culture (following enzymatic treatment to yield a cell suspension) using a counting chamber with direct microscopy or in an automated particle counter. Alternative methods include measurement of total protein production (Lowry procedure Sigma Ltd UK) and MTT (3-[4,5 DIMETHYLTHIAZOL-2-YL]-2,5 diphenyl tetrazolium bromide) assay which utilises a tetrazolium salt cleaved by mitochondrial dehydrogenase in living cells in culture.

Biosynthetic Activity : it is possible that a biomaterial although not exerting an effect on cell proliferation in vitro may affect the production of biosynthetic products.

Alkaline phosphatase production by osteoblasts is an example of a product produced by osteoblasts that can be measured (Alkaline phosphatase Assay Sigma Ltd procedure 104/86)

As well as detection of toxic effects a more clinically meaningful use of in vitro testing is to create conditions which simulate the in vivo state and allow selective evaluation of biomaterials on cells biosynthetic function. (67) This type of in vitro test is designed not only to determine inert biocompatibility, involving the exclusion of negative effects of biomaterials on cells and tissues, but involves evaluation of positive or desirable stimulatory effects that biomaterials may have on cellular activity.

Two main methods exist in in vitro testing the direct or contact method and the indirect method. The direct method is the method of choice when evaluating the potential of metals or polymers for biointegration, as cells are allowed to interact directly with the surface of the material. (68) Indirect methods involve separating the material from the cells/tissue by a diffusion barrier. The agar overlay technique is an example of an indirect technique in which a monolayer of cells is covered by a thin layer of sterile agar through which products released from test material can diffuse to act upon the cells. (69) Although indirect tests are often simple to perfore data derived from them can be limited and is often similar to that which could be obtained from physical or chemical tests. (70)

To provide information on the tissue response to implanted material and allow assessment of any systemic effects prior to clinical use animal implantation and human studies are required. Initial in vitro evaluation can however provide useful information, which may enable more decerning use of in vivo tests and can sometimes provide information on material/tissue or cell interaction that is difficult to gain during in vivo implantation; where the complex in vivo response to implantation of materials can make the interpretation of results at a cellular level difficult. In vitro testing enables the response of the material to specific types of cells to be studied in isolation in a controlled environment. In vitro evaluation can be undertaken more rapidly than testing of materials in animals

and is relatively low in cost. A further advantage of in vitro techniques is that they reduce the need for, and enable a reduction in the amount of, animal testing that has to be undertaken. (71) In vitro testing has been shown in some cases to be a more sensitive method of determining material toxicity, although as with in vivo evaluation care has to be taken in the interpretation of results. It must be realized that in vitro, one is dealing with a closed system. Kawahara et al (72) demonstrated this, proposing that the in vitro cytotoxicity seen during the release of monomer from cold curing acrylic may not be a true guide to the in vivo response. It is known that monomer levels in autopolymerising acrylic decrease with post-cure time as the curing process continues and that in vivo (because there is potential for repair, which does not exist in vitro) the overall prognosis of the material may be different from that suggested by in vitro evaluation. (72)

Clinical materials in current use have not, on the whole, been specifically developed as biomaterials but came into clinical use because of their stability and corrosion resistance in the biological environment. The inert nature of such materials gives them good 'inert biocompatibility' in terms of low cytotoxicity and systemic toxicity. This level of 'biocompatibility' - (the material does not damage the patient) - or biological tolerance is now regarded as the minimum acceptable. When evaluating/designing materials for future clinical use the aim should be to develop materials which have positive interactions

with the tissues of the intended implant bed. The nature of the response of the cells of the intended implant bed to the implant material are crucial factors in determining both initial healing and any long term clinical success of an implant / implant material.

The in vitro approach enables the interaction of specific cell types with a material to be studied. Dental materials have been evaluated using a variety of cell types viz fibroblasts, epithelial cells, (73) pulp cells, (74) and osteoblasts. (75) When testing biomaterials for use as bone substitutes it is the response of the bone-forming cells, the osteoblasts, which are of the greatest interest as these cells are responsible for the formation of the bone-implant interface. Several groups of researchers have developed techniques using osteoblast cells for studying dental/orthopaedic implant materials. (39,51,75-82)

Materials intended ultimately for bony implantation should following in vitro testing be evaluated in an in vivo model that allows both cortical and cancellous bone to come into contact with the material. Initial evaluation can be undertaken using models that allow evaluation of the bone/material interface, without attempting to simulate the ultimate clinical use. (52,83)

Following initial in vivo testing of materials for use in jaw augmentation the use of a non-healing model is desirable in order to mimic the clinical situation in which alveolar bone does not reform once teeth are lost. (84,85)

Iiii. Hypothesis

Not only are current surgical techniques for restoration of alveolar bone inadequate but the biomaterials available are deficient. Numerous ceramic, glass and composite materials have been developed for implantation within the body over the last twenty years. Despite reported cases of stimulation of new bone formation by apatite-based materials, current materials have poor biomechanical properties with relatively low flexural strengths and toughness (28). The use of granulate material which, following ingrowth of bone, forms a 'bone/ceramic composite' is one method of improving the biomechanical properties of bone substitutes. (25) There remains however a real clinical need for improved materials for use as bone substitutes.

Glass-ionomer cements are a class of materials suitable for use as bone substitutes and bone cements.

Iiv. **Aims**

To investigate the potential of GICs as a possible class of materials for replacement of bone and for use as a bone cement. With the aim that GICs could then be developed for use as preformed implants for a variety of non-weight bearing situations such as alveolar augmentation, chin prosthesis, ear ossicles and skull plates.

Improved understanding of the interaction of GICs with the biological environment may also lead to the development of dental cements with improved properties and be of use in the development of improved cements for orthopaedic use.

The study was designed to assess the reaction of bone cells and the tissues of the implant bed to GICs of different formulations. A limited biomechanical evaluation was also undertaken.

The theoretical range of formulations of GICs is vast and thus this study concentrates on a limited number of representative GICs (chosen following consultation with Drs A.D Wilson and B Ellis formerly of the Materials Group, Laboratory of the Government Chemist). GICs were chosen to give one which was known to release fluoride ions (G338 laboratory of the Government Chemist) and one

known not to contain fluoride (Mp4 Pilkington PLC UK).

Biological evaluation was carried out using cell/tissue culture and by in vivo implantation.The nature of the initial GIC/cell-tissue interface was addressed, and particular attention paid to:-

1. The nature of initial healing and bone cell and migratory response.

2. Mineralization in the implant bed and the osteoconductive potential of GICs.

3. The stability of the implant material and its susceptibility to degradation.

4. The morphology and ultrastructure of the bone/implant interface.

5. The cellular response to GICs in set and paste form, and during the transition from paste to solid form.

The mechanical properties of an ideal bone augmentation material are critical, and it is here that the novel properties of GIC may allow an elastic match with bone which will prove to be superior to currently used materials. It is possible to produce compositions of GIC with a range of physical properties very different from those

normally associated with dental filling materials by varying the ratio of polymer (polyacrylic acid + water): to glass. (86,87) Following biological evaluation some of the GICs were investigated with a view to determining their mixing and handling properties, setting time, set strength, and adhesion to bone. The effect of formulation on these parameters was thus determined in a limited way.

II. MATERIALS AND METHODS

IIi. Materials

Four different GIC were evaluated. Two were commercial dental luting cements, Ketac Cem Radiopaque (Espe, W.Germany) and AquaCem (DeTrey, U.K.), both based on fluoroaluminosilicate glasses, reacted with a co-polymer of poly(acrylic)acid / maleic acid and poly(acrylic)acid respectively. The other two were:-

Mp4 (Pilkington, U.K.) composition (% wt)

SiO_2	30.8%
Al_2O_3	38.5%
CaO	28.6%
Na_2O	2.1%

G338 (Laboratory of the Government Chemist)

SiO_2	25.2%
Al_2O_3	14.2%%
CaF_2	12.8%
Na_3AlF_6	19.2%
$AlPO_4$	24.1%
AlF_3	4.5%

Both were reacted with poly(acrylic)acid L3(4) mol Wt 28,500 (Supplied by the laboratory of the Government Chemist, U.K.). The commercial materials were mixed as directed for dental use and the two other GICs were made up in volume fractions (vf) of 0.5 glass, 0.2 polymer, 0.3 sterile distilled water containing 10% tartaric acid to control the set.

The use of volume fractions to determine the ratio of glass:polymer:water to produce set GIC is standard practice. (86)

To calculate the amount of material in the GIC mix the vf is multiplied by the density; for this study the density of the glasses was taken as 2.5 and the polymer as 1 thus:

0.5vf glass = 1.25mg

0.3vf water = 0.3mg

0.2vf polymer = 0.2mg

Two types of set GIC were produced for evaluation.

1. Irregular pieces approximately 1mm by 1mm of GIC were produced by fracturing set material with a hammer.

2. Rods (nominally 2mm long, 1mm in diameter for implantation or 1mm by 1mm for tissue culture) were produced by allowing the material to set in silicone moulds. Set material was stored for one week in sealed containers at 100% humidity prior to use.

The ceramics evaluated for comparison were:

1. Densely sintered hydroxyapatite (Ha) granules; Calcitite 40-60 (Calcitek Inc U.S.A.)

2. Porous hydroxyapatite (Interpore International USA)

3. Tri-calcium phosphate (TCP) granules (Friedrichsfeld, W.Germany).

Sterilisation of materials

Glass and polymer powders received a radiation dose of not less than 25Greys (Swann Morton Services). Unset GIC and the components of GIC were subsequently handled and mixed under aseptic conditions. Set rods/pieces of GICs were also autoclaved prior to use.

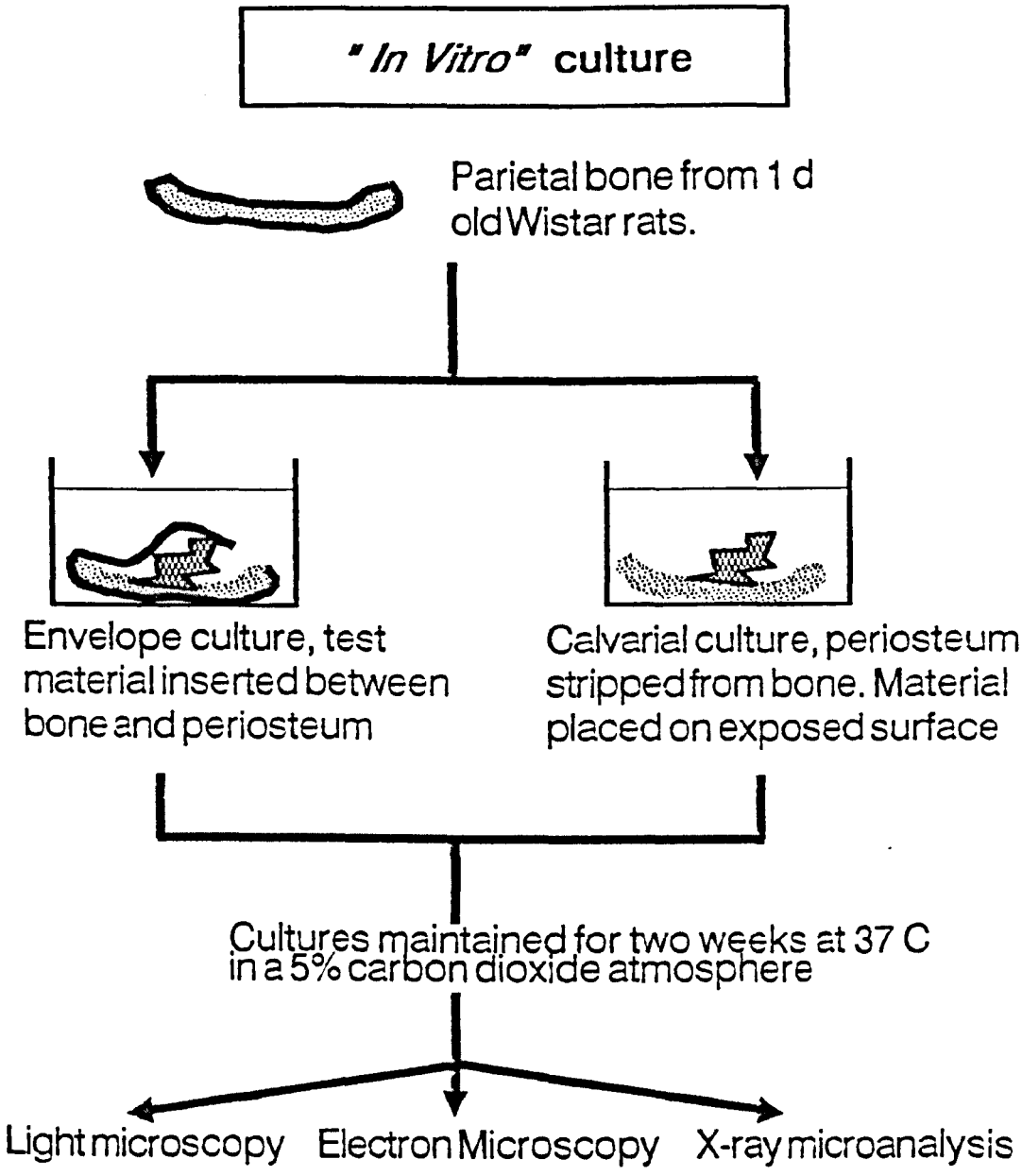
IIii. *In vitro* techniques Bone Organ / Tissue Culture

The culture methods used were based upon those originally developed by S. J Jones , A Boyde and J. E Davies. (39,51,75-78) Cells were harvested in a clean room from one day old inbred Wistar rats. Following dislocation of the neck the skin of the scalp was incised and reflected to expose the fronto-parietal, parietal and occipital bones of the calvarium. The calvarium was then removed and washed in phosphate buffered saline (PBS) containing 9% foetal calf serum. Calvaria were placed in a sterile petri dish containing RF10 HEPES medium (Gibco UK.) until all the required number of calvaria were harvested. Using a stereo-dissecting microscope the parietal bones were dissected off each calvarium, care being taken to discard any areas with sutures. The parietal squares were washed in PBS and stored in RF10 HEPES medium prior to undertaking two types of culture (Fig. 1):-

i. The first was a bone cell culture in which the endocranial and epicranial periostea were stripped from the bone and test material placed onto exposed endocranial side of the bone - 'calvarial culture'.

ii. The second was a bone organ culture in which the periostea were retained upon the bone, test material being introduced into the culture to lie between the surface of the bone and the overlaying periosteum on the endocranial surface - 'envelope culture'.

FIGURE 1.



Evaluation of GIC in tissue culture

The prepared cultures were placed individually into culture wells and maintained at 37°C in an atmosphere of 5% CO_2 for two weeks. Each well contained 2ml of Fitton-Jackson's modification of Bigger's medium (Gibco, U.K.) supplemented with 10% foetal calf serum, 20ul/ml 200mM glutamine, 10ul/ml penicillin/streptomycin (500i.u./ml), 25ul/ml HEPES 1M solution , 10ul/ml B-glycerol-2-phosphate 1M solution and 50ug/ml ascorbic acid, the latter two being made up and added to the media immediately prior to use. The media were replenished every two or three days and cultures stopped on the 14th day by removing the media and replacing them with 2.5% Glutaraldehyde in 0.1M Sodium Cacodylate buffer.
All cultures were undertaken on at least 5 occasions for each material in mixed batches of up to 20 cultures in 24-well multiwell plates.

Detailed technique for harvesting calvaria, preparing / undertaking cultures and preparing blocks

1. The day before harvesting, clean room tidied and inessential equipment removed.
2. Dissecting microscope swabbed with alcohol.
3. UV light switched on overnight
4. The following day sterile paper drapes were used to cover the bench in the clean room and all those entering donned a sterile gown hat/mask.
5. Instruments as follows were laid out:-

   No 4 watchmakers forceps x2,

Small curved and straight scissors x2

Small dissecting forceps non-toothed x 3

Sterile swabs

40ml of Chlorhexidine in Alcohol

3 sterile petri dishes for instruments

Tissue culture dishes x 5 for washes/storage

6. Culture media sufficient to set up 20 calvarial cultures warmed to 37°C.

Three separate media were used:-

a. 44ml **Washing solution** consisting of:-

4ml Foetal calf serum heat inactivated (Flow 29 101 54)

40ml Phosphate buffered saline (Dulbecco's) (Oxoid)

b. 23ml **Store media** consisting of:-

20ml RPMI 1640 media with HEPES (Flow 12 604 54)

200ul 200mM Glutamine

200ul Penicillin/Streptomycin 500units/ml

c. 46ml **Culture media** consisting of:-

40ml Fitton-Jackson modification of Bigger's media (Gibco 041 02591M)

4ml Foetal calf serum (Flow 29 101 54)

400ul 200mM Glutamine

400ul Penicillin/Streptomycin 500units/ml

2ml 1M HEPES

Just prior to use fresh beta-glycerophosphate and ascorbic acid

(Sigma tissue culture grade) were added to the culture media to give a concentration in the media of 10mM and 50ug/ml respectively.

7. The neonates were killed using an overdose of ether and dislocation of the neck. The scalp was then cleaned with chlorhexidine in 70% alcohol and the calvarium exposed by dissection. The calvaria were then removed and washed twice in two separate wash solutions prior to being placed in store media. Between animals, instruments were cleaned with chlorhexidine in alcohol. When all the calvaria had been harvested, the parietal bones were dissected out with the aid of the dissecting microscope and then passed through a third wash and placed in fresh store media.

8. For envelope cultures (which were prepared first) sutural areas were trimmed from the squares of parietal bones. The edge of the periosteum was carefully lifted up and the material under test slipped between the periosteum and the bone. As far as possible the same volume of material was used each time. Thus 2 particles of Ha or TCP or a set rod of GIC 1mm diameter, 1mm long were introduced. If the periosteum became detached while manipulating the bone, the parietal square was not wasted but used as a calvarial culture.

9. Calvarial cultures were prepared as above but the periosteum was removed from both sides of the bone and extra care taken to ensure that there were no sutural areas present, so as to minimise fibroblastic contamination of the culture.

10. When the parietal squares had been prepared they were placed in 24-multi-well plates, 2ml of culture media being placed in each well. Test materials were positioned on the calvarial squares after placement in the multi-well plate.

11. A record of the position of each culture/material was kept on standardised forms and the multi-well plates placed in a CO_2 incubator (5%) and kept at 37°C for up to 14 days. All cultures were set up on a Friday and the media changed on subsequent Mondays, Wednesdays and Fridays until the cultures were stopped by fixation. Media were changed and cultures inspected in a laminar flow cabinet. Old media were placed in bleach prior to discarding.

12. If a culture became contaminated during the experimental period the medium was removed and replaced with chlorhexidine in water.

13. Cultures were stopped and fixed for 2hrs by removing the medium and replacing it with 2% Gluteraldehyde in 0.1M Sodium Cacodylate made up as follows.

Solution 1. 0.2M Sodium Cacodylate

(4.28g Cacodylate (Sigma) in 100ml distilled water)

Solution 2. 0.2M HCL

Solution 3. 25% Gluteraldehyde EM grade (BDH Ltd)

20ml of fixative was made up using:-

10ml of solution 1 added to 0.6ml of solution 2, 2ml of solution 3 together with 8ml of distilled water. The pH was then adjusted using NaOH to lie between 7.2-7.4 .

14. Following fixation, calvarial cultures were carefully removed and placed in small containers and processed for scanning electron microscopy (SEM). Envelope cultures were kept in the multi-well and dried using 15 min changes of 30%, 40%, 50%, 60%, and overnight in 75%, alcohol followed the next day by 20 min changes of 90% and twice at 100% prior to embedding in LR White Hard Grade Resin (London Resin Co)

15. Following drying, cultures were removed from the multi-well plate and placed in small glass bottles. Infiltration was undertaken at 4°C using 4 changes of resin over a 4 day period. Following infiltration, cultures were placed in gelatin capsules with a paper label and then embedded in autopolymerising resin (LR White, Hard Grade, London Resin Co.) .

16. The blocks of specimens for transmission electron microscopy were ground to expose the material/cells and the whole block placed in 10% EDTA for up to 12 weeks, prior to processing.

IIiii. In vivo techniques

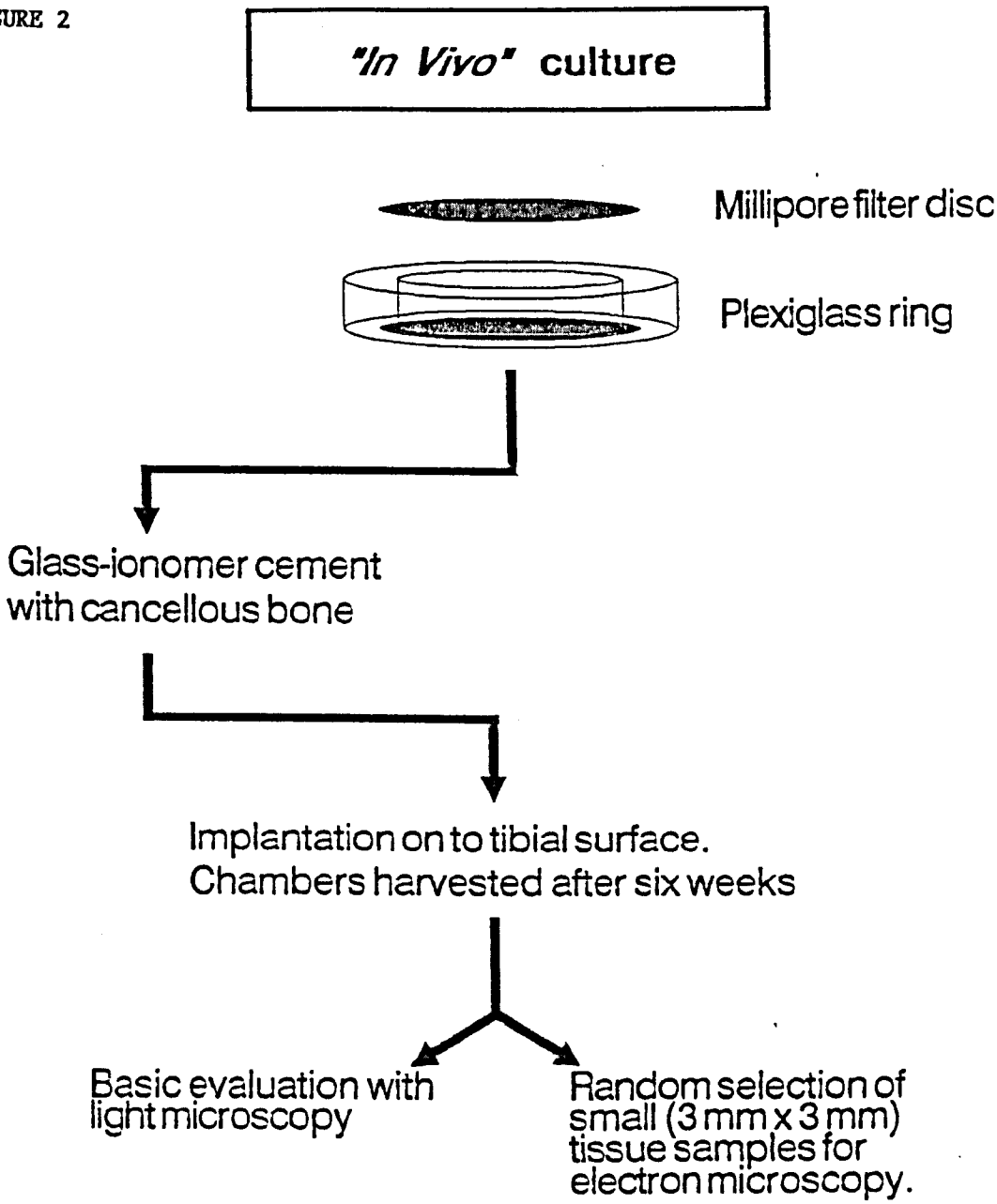
a. In vivo primate bone culture model.

This part of the study used material supplied by Prof L M Jonck of the Centre for Bone Biology, Department of Orthopaedics, University of Pretoria, South Africa. Details of the in vivo culture model used have been published by Jonck, Grobbelaar and Strating in 1989 (88) and are summarized in Figure 2. The aim of the technique is to simulate the heterogeneous cell and tissue fluid environment of the in vivo situation in an in vitro assay. The technique is based upon that described for the development of a quantitative assay for osteogenesis. (89-92)

Plexiglas diffusion chambers were prepared by gluing a millipore filter disc (pore size 0.45um) to one side of a 12.5mm diameter plexiglas ring (Figure 3). The chambers were pre-packed with additional membranes and sterilized with ethylene oxide, they were then stored for two weeks prior to use.

Three primate baboons (Papio ursinus) were anaesthetised with nitrous oxide and halothane via an endotracheal tube. Incisions 10cm long were made over the proximal half of the tibia and following retraction of the muscles of the anterior compartment the periosteum was elevated from the bone. Using a trephine with saline irrigation three circular holes were made in the cortical bone in order to accommodate the diffusion chambers in such a way in that they were flush with the surface of the bone.

FIGURE 2



Diffusion chambers implanted into *Papio ursinus*

Care was taken to ensure a good fit of the chambers so the inner membrane contacting the bleeding bone surface. Prior to placing the chambers a pouch was prepared in the anterior tibialis muscle overlying the implantation site to aid harvesting of the chambers.

Each chamber was loaded with 50mg of the set particulate GIC V-Os*.

A trephine was then used to remove a core of cancellous bone from the proximal end of the tibia (remote from the site prepared for placement of the diffusion chambers) and approximately 50mg of bone was placed adjacent to the GIC in the diffusion chamber. The chambers were then sealed off with one of the spare pre-packed membranes and implanted into the depressions on the surface of the tibiae.

After 42 days the chambers were harvested and fixed in gluteraldehyde.

Fixed specimens were received from Prof Jonck, and the plexiglas and millipore filters were removed prior to processing. (Figure 3)

* V-Os is a GIC produced by Espe (Germany) and has the same basic composition as Ketac Cem also produced by Espe but it does not contain a radiopacifier (lanthanum).

FIGURE 3

i. ii. iii.

Photographs (Magnification x2) of diffusion chambers

i. as received

ii. following removal of one membrane showing contents

iii. after removal of contents for processing.

Following removal from chamber the contents of the chambers were processed for light and electron microscopy (Section IIiv and v).

IIiii b.In vivo implantation of potential alveolar bone substitutes.

The four different GICs (section IIi) and densely sintered rounded hydroxyapatite (Ha) granules (Calcitite, USA.) were evaluated by in vivo implantation.

Each of the five materials and wet Ketac Cem, which was mixed and placed unset below the periosteum onto the surface of the bone, were implanted in a standardized manner into the midshaft of the femora of weaned (25 days old) inbred male Wistar rats (seven rats being used for each material) and healing allowed to take place for six weeks. In addition, implants of Ha, set Ketac Cem, and wet Ketac Cem were implanted for two and twelve weeks, using three rats for each material/time interval.

General anaesthesia was induced and maintained by inhalation of Halothane 3% and nitrous oxide 11t/min / oxygen 500ml/min via a nasal mask. The mask was constructed out of a plastic funnel with a piece of dental rubber dam material stretched over the mouth of the funnel to form a facial seal on the animal and incorporated a scavenging circuit.

Following cleaning of the skin over the right femur, the bone was exposed using sharp and blunt dissection and the periosteum reflected.

Wet GIC was then placed and allowed to set directly on the surface of the bone or, alternatively, under saline irrigation; a slow speed dental drill fitted with a round bur (number 3, Ash UK.) was used to

prepare up to two holes through the cortical plate into the marrow space. The diameter of the holes being matched to the diameter of the implants. Set rods of GIC were then placed into the holes to lie flush with the surface of the bone. In the case of Ha, two particles were placed into the prepared hole (Fig. 3). The overlying periosteum and soft tissues were replaced and the wound sutured using 4-0 Vycril sutures (Ethicon, Scotland).

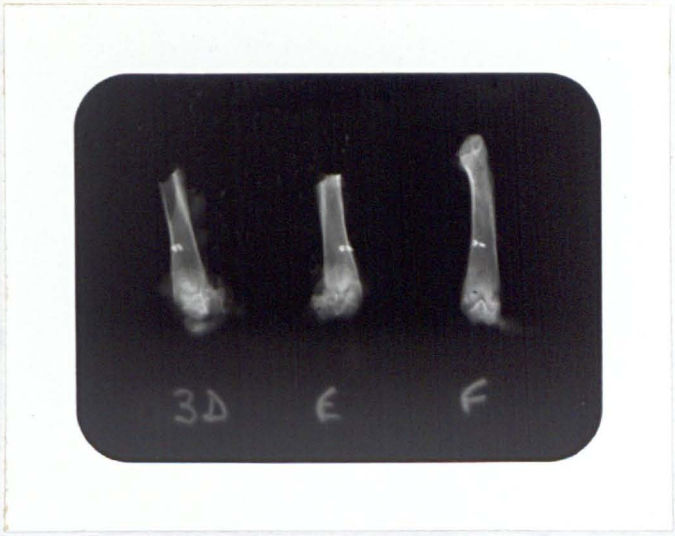
Post-operatively, wounds were inspected to monitor healing and rats were maintained on a standard laboratory diet.

Rats were killed, under anaesthetic, by perfusion of the vascular system with 14% gluteraldehyde buffered with 0.2M sodium cacodylate with 1mM of calcium chloride. The femora were then dissected out and stored for 24hrs in buffered 3% gluteraldehyde.

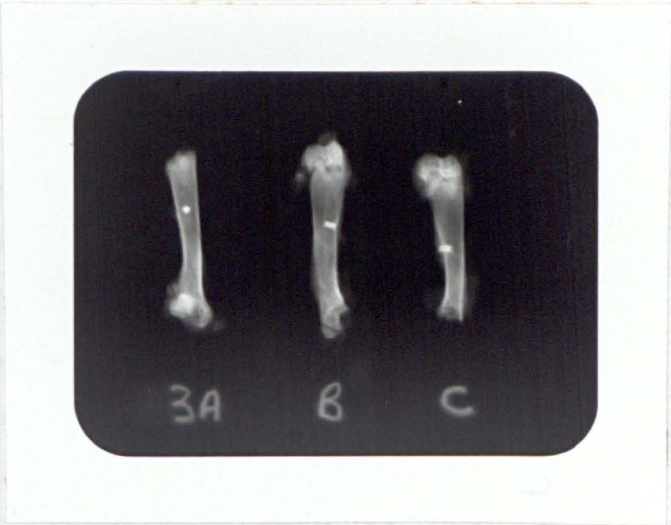
FIGURE 4

Radiographs of femora six weeks after implantation.

A. Two Ha (Calcitite) particles per femur.



B. One rod of GIC (Ketac Cem) per femur.



Detailed technique for implantation/anaesthetic and perfusion

Surgery was carried out under aseptic conditions, instruments were sterilised by steam autoclave and sterile gown hat/mask and gloves were worn.
Perfusions were undertaken in a fume cupboard using a non-sterile technique.

Implantation

1. Using 4% Halothane and 500ml/min of oxygen and 1000ml/min of nitrous oxide, anaesthesia was induced in 4 week weaned Wistar rats in a modified glass anaesthetic jar connected to a 'Salisbury' anaesthetic machine with an integral Halothane vaporizer calibrated up to 4%.

2. Following induction, rats were weighed and maintained via a nasal mask on 2.5% Halothane and 500ml/min of oxygen and 1000ml/min of nitrous oxide.

3. The skin over the right femur was washed with chlorhexidine in spirit and dried, care being taken to flatten the fur.

4. Using a No.15 blade the skin over the femur was incised and the bone exposed using Mackindo's scissors and dissecting forceps.

5. A self retaining apicectomy retractor (Thackaray) was placed and the periosteum divided with a scalpel.

6. Then, either a 1mm diameter hole was cut under saline irrigation using a dental drill through the cortical plate and an implant placed, or wet cement was run over the surface of the bone and allowed to set.
For some of the six week harvesting times, two implants were placed into two implant sites at least 4mm apart, to increase the number of implants available for histomorphometric analysis.

7. The wound was closed in layers using 4.0 vycryl (Ethicon) sutures.

8. Rats were placed in a clean cage and wounds monitored daily.

9. Records were kept using standard forms (appendix III).

Perfusion

1. Anaesthesia was induced and maintained as for implantation.

2. A midline abdominal incision was made with Y reliefs at the extremities.

3. The abdominal cavity was opened and the viscera moved laterally. The descending colon was clamped and tied off prior to cutting to expose the peritoneum.

4. The dorsal aorta lying superficial to the right vena cava was isolated and a plastic canula placed down the right side of the distal bifurcation. The canula was secured with a ligature and the vena cava cut.

5. Perfusion was undertaken with phosphate buffered saline until the leg blanched and continued with an infusion of 14% Gluteraldehyde solution followed by 3% Gluteraldehyde solution which was also used to store the femora overnight once they had been dissected out.

6. Fixative was made up using:-
A buffer of 0.2M Sodium Cacodylate Solution. with 1mM$CaCl_2$ consisting of:-
21.4g Sodium Cacodylate + 500ml distilled water + 20ml 0.2M HCl + 2ml of 10%$CaCl_2$ Solution.

3% fixative was produced by adding 6ml of 25% Gluteraldehyde to 19ml distilled water and 25ml of buffer.

14% fixative was produced by adding 28ml of 25% Gluteraldehyde to 22ml of buffer.

7. Following fixation, specimens for ground sections were washed in three changes of phosphate buffered saline and dehydrated using a graded series of ethyl alcohol consisting of one to two hours in 30%, 40%, 50%, 60% and overnight in 75% followed by one hour in 90%

and three hourly changes at 100% before embedding in resin (LR White, Hard Grade, London Resin Co.).

8. Specimens for TEM were decalcified,using 10% EDTA in 0.1 Trisma buffer, prior to dehydration as above.

Decalcifying solution was produced by dissolving 12.11gms of Trisma base (Sigma) in 900ml of distilled water. The pH was adjusted with HCL to pH 7.2-7.4 and the solution made up to 1000ml with distilled water. 100gms of EDTA (Disodium salt - Sigma) were then stirred in until dissolved.

Specimens were decalcified at room temperature for periods up to three months, the stage of decalcification being checked when necessary using radiographs. EDTA was replaced weekly and specimens shaken continuously during the day to speed up the decalcification process.

Embedding

Prior to embedding, specimens were cut using a small hand saw to remove unwanted tissue. Specimens were embedded over periods of up to 21 days using a minimum of four changes of resin at $4^{o}C$ and under reduced pressure. Specimens were placed into gelatin capsules and then, using fresh activated cold cured resin, polymerised blocks were produced. Labels were incorporated into each block to aid identification.

IIiv. Histological Methods

Undecalcified transverse sections 20-40um thick of the in vitro and in vivo specimens were produced. The resin blocks containing the specimens were initially reduced using a file and glass paper until the implant or area of interest was exposed. A dissecting microscope was used to examine the surface of the block during trimming. Following gross reduction the blocks were polished using precision lapping (Tessbourne Ltd). The lapping paper was fixed with water to a glass plate and blocks polished using light hand pressure moving in a circular motion over paper lubricated with distilled water. Aluminium silicate paper grit sizes 30um, 20um, 12um, 9um, 5um, 3um, 2um, 1um through to 0.5um were used. When the surface of the block was perfectly flat and smooth it was rinsed in distilled water and stained using surface stains.

Surface stains used were as described by C Maniatopoulos et al,. (89)

1. **Stevenel's blue** was made by mixing 75ml of distilled water, containing 1gm of methylene blue with 1.5g of potassium permanganate in 75ml of distilled water in a boiling water bath until the precipitate formed on mixing the two solutions redissolved. The stain was then allowed to cool and filtered.

2. **Van Gieson picro-fuchsin** was made by mixing 10ml of a 1% solution of acid fuchsin with 100ml of saturated aqueous picric

acid.

3. **Alizarin red S** was made by diluting 2g of alizarin red dye in 100ml of warm distilled water. The solution was allowed to cool and the pH adjusted to pH 7.2 using 0.2M ammonium hydroxide.

4. Staining methods:- The Stevenel's blue and a bottle of distilled water (for washing excess stain off the block) were placed in a water bath at 60°C. Using small containers, blocks were immersed in the warm stain for 15 mins. They were then removed and rinsed using the warmed distilled water and allowed to air dry. The surface was then counter-stained with either Alizarin red S or Van Gieson picro-fuchsin. A drop of Alizarin red S being pipetted onto the surface of the block and allowed to remain for 5mins before washing in distilled water or the block was placed in Van Gieson picro fuchsin again for 5 mins. before rinsing briefly in 100% ethyl alcohol and air drying.

The stained blocks were mounted on slides using 'glass bond' glue (Locktite UK.) applied to the flat stained surface. The glue was allowed to cure by placing the slide in a sunny place and then the block was cut off the slide. Cutting was undertaken using a diamond band saw (Exakt, Germany) to leave a section approximately 30-40u thick. The cut surface of the section was then polished and thinned again using precision lapping paper and finally a cover slip was placed to protect the section, mounted with Xam (BDH Ltd UK.) The stained surface of the section was then examined, being viewed through the slide rather than, as conventionally, through the cover slip.

IIv. Ultrastructural techniques

a.Scanning electron microscopy (SEM).

Specimens from in vitro 'calvarial' and in vivo cultures were studied in the SEM. For calvarial cultures the whole of the culture was processed in one piece if possible. For diffusion chambers, the contents were removed and divided into small pieces so that the surfaces of V-Os particles could be studied. Secondary fixation was undertaken with 2% aqueous osmium tetroxide for one hour followed by dehydration through a graded acetone series; specimens were then critical point dried from CO_2 using a Polaron Critical Point Drier and coated with a thin layer (approximately 20nm) of gold using a Nanotech Sputter Coater. SEM was undertaken using a Cambridge Stereoscan 600 (CS600) set at 25kv or a Hitachi S-2300 SEM (HS) - University of Birmingham Medical School.

Transmission electron microscopy (TEM)

Specimens from in vivo implantation were decalcified in 10% EDTA in Tris Buffer (Sigma UK.) for periods of up to four months depending on the bulk of the tissue (Section IIiv b). Radiographs of the bone/implants were used to determine the endpoint of decalcification.

Dehydration was carried out using ethanol. The in vitro samples being left for 15mins; in vivo samples for 1hour in 30, 50, 75, 95

and for three changes in 100% prior to embedding in LR resin as described for light microscopy.

Prior to TEM examination, blocks were trimmed under magnification using a razor and semi-thin sections cut using a glass knife mounted on an Reichert OmU3 and a Reichert-Jung ultracut E microtome. Sections were expanded using chloroform and 500nm sections were stained with 1% toluidine blue solution containing 1% borax.
The stain was produced by dissolving 1g of toluidine blue and 1g of sodium tetraborate in 100ml of water. The solution was filtered prior to use and placed on sections which had been dried onto glass slides on a hotplate ($75^{o}C$). Stain was applied for approximately 20secs until a gold line formed around the edge of the drop of stain. Excess stain was washed off using distilled water and a cover slip applied using Xam. Areas of interest were then identified and the blocks were usually trimmed further and 100nm sections cut using a diamond knife. The ultra-thin sections produced were picked up on, uncoated and collodion coated, 200 mesh copper grids. Specimens were stained for 15 mins with saturated uranyl acetate in 50% ethanol in a covered petri-dish. The grids were then washed in distilled water and stained for 5 mins with Reynold's lead citrate again in a closed petri-dish to avoid precipitation of lead carbonate on contact with atmospheric CO_2 . The grids were then washed again with distilled water and allowed to air dry prior to viewing using a Philips CM 10 transmission electron microscope set at 80kv.

It was difficult to determine the position of the Ha in the bone

following decalcification and some of the specimens obtained from in vitro culture were very fragile. Some of these specimens were processed without decalcification being treated as for the ground sections. TEM sections were then produced following trimming of the blocks where cutting was not possible the block was surface decalcified in EDTA (Section IIivb). This worked well but care had to be taken when cutting the blocks as the surface containing the specimens became porous and tended to absorb water from the reservoir on which ultra thin sections were floated. This caused difficulty in cutting and retrieving sections and caused some swelling of the tissue in the block.

The GIC was not removed by decalcification but softened. This made it easy to locate the implant site but more difficult to cut sections. To make cutting easier most of the GIC was carefully removed using a fine needle and saline irrigation under magnification. This resulted in the GIC at the tissue interface being preserved.

IIvi.TEM energy dispersive X-ray microanalysis (EDAX) methods

TEM energy dispersive X-ray microanalysis (EDAX) was used to confirm the presence and structure of the GIC at the bone interface. This was undertaken on conventionally fixed specimens (Section IIiv/v) and specimens fixed using a freeze substitution technique to avoid the translocation or movement of ions in the specimen that can occur with conventional preparative methods. (90,91)

Cryofixation and Freeze Substitution

To enable accurate EDAX analysis representative specimen were fixed using cold temperature methods.

At the time of harvesting in vivo specimens the area of the implant was cleaned of excess soft tissue and the tissue plunge frozen in pentane/methylcyclohexane over liquid nitrogen. In vitro cultures and material specimens were plunge frozen whole.

Specimens were transferred to plastic sintilation vials containing activated molecular sieve (type 3A, 5g) and absolute acetone (10ml). The vials were held in an aluminium block at -196°C during transfer. The large thermal mass of the block ensured that samples were warmed at a slow rate during freeze-substitution.

The aluminium block and vials were then placed in a freezer at -70°C for 10days following which they were removed and allowed to warm up to room temperature. Specimens were then washed in absolute acetone (three changes at 30min intervals) before conventional infiltration

and embedding in Spurr's resin.

The Spurr's mixture used consisted of:-

10g	ERL 4206	(epoxide)
6g	DER 736	(epoxide flexibilizer)
26g	NSA	(hardener)
0.4g	DMAE	(accelerator)

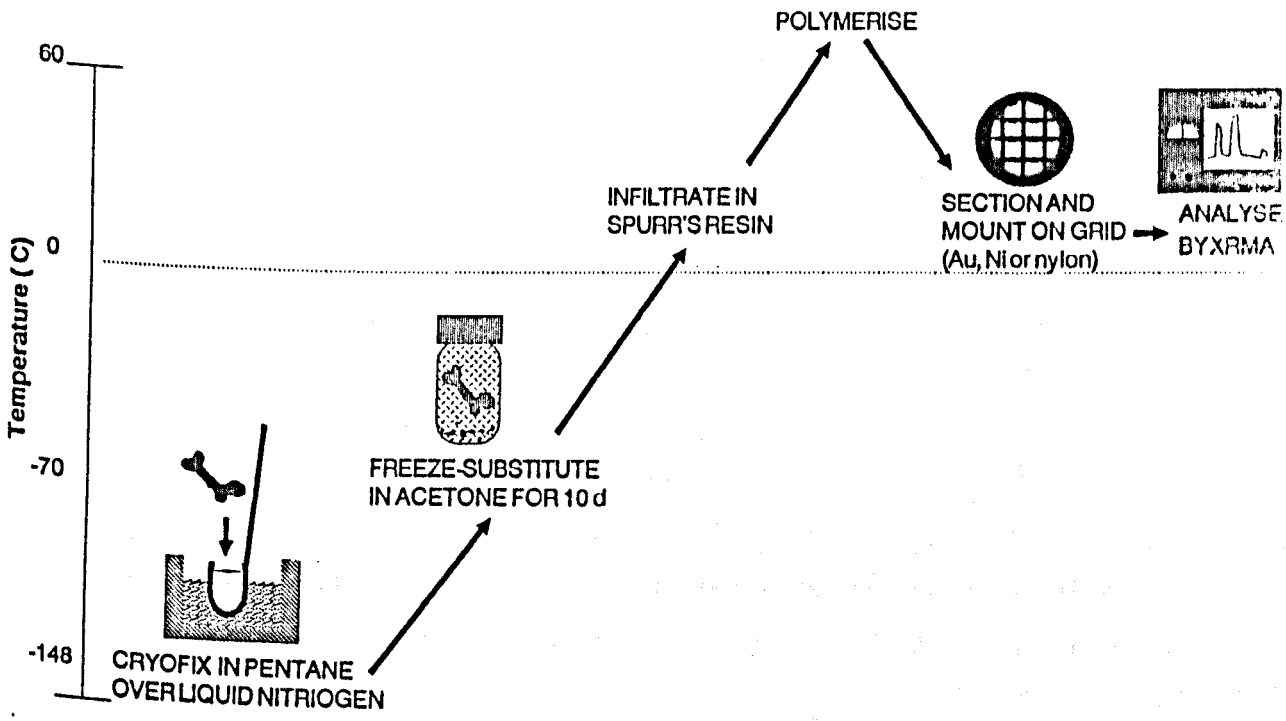
Sections 0.5-1.0um were dry cut with a diamond knife on an ultramicrotome, placed in a gold sandwich grid (100) mesh and examined in a Philips CM10 electron microscope operated at 100KV. Freezing quality and ice crystal damage were assessed in stained sections (Section IIv) cut from the block sequentially to the unstained sections used for EDAX.

For EDAX the specimen holder was tilted 12^{o} towards the X-ray microprobe. An energy dispersive spectrophotometer connected to a multichannel analyser (EDX 9800) was used to generate spectra. X-rays were collected using a 200nm probe for between 25 and 100 live seconds. Analyses were performed in regions of material that did not overlap so as to avoid signal contamination by stray X-rays.

Background radiation was determined in adjacent areas of resin and subtracted from the spectra to compensate for the effects of beam-spreading in the section.

The cold temperature cryofixation / freeze substitution technique is summarised in figure 5.

FIGURE 5



Summary of Cryofixation / freeze substitution technique for TEM energy dispersive X-ray microanalysis (EDAX).

IIvii. Histomorphometric techniques

The amount of new bone associated with implanted set GIC rods and HA particles was determined in order to evaluate the degree of osteoconductivity (amount of new bone formed adjacent to the implant) (92) and osseointegration (the length of bone/implant interfacial contact) (92-95).

The evaluation was undertaken using ground sections of set rods of GIC and particulate Ha following implantation for six weeks 7 implant sites being used for each material. Following fixation of the femora/implant (Section IIiiib) the implant site was located by visual inspection and or X-ray (Figure 4). The femora was cut transversely with a hand saw and a ground section taken through the mid point of the implant (Figure 6) sections were stained with Stevenel's blue/Alizarin red S (Section IIiv). Sections were then placed on a projecting microscope (Leitz Micro Promar) and projected onto a digitising pad (Summa Sketch +, Summa Graphics Ltd). The pad was linked to a Viglen microcomputer and using the Bioquant BQ system 4 (R & M Biometrics Inc. USA.) a cross section through the femur and the implant was traced, at x34 magnification. Thus the set rods of GIC were approximately 3cm wide when traced (1mm nominal real width). The amount of new bone formed around the implant was determined by drawing a line through the centre of the implant bisecting the femur (line **B**), two further lines were then drawn 3cm from and parallel to the first line and the area of bone enclosed by these lines **A**′ and **C** and the inner/outer cortical plates

taken as a guide to the amount of newly formed bone (Figure 6)

Measurements were made of the:-

Implant Perimeter Length P

Length of intimate Implant/Bone contact I

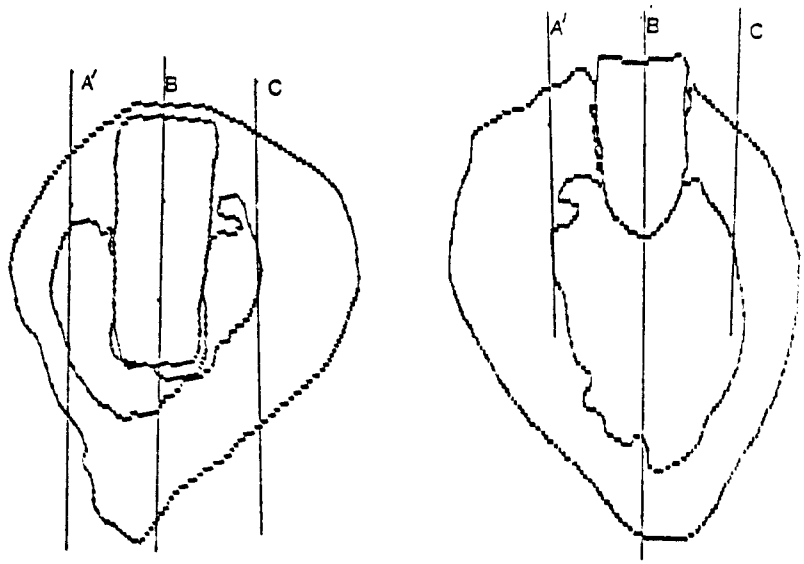
Amount of bone contained in the lines A′ and C around the implant A

The amount of bone adjacent to the implant was taken as A/P
(new bone around the implant per unit implant length)

% Osseointegration was taken as I/P x 100
(% bone/implant contact)

Statistical evaluation of results was undertaken using the Mann-Whitney U Test and the paired Student's t-test (two-tailed), to compare the % osseointegration and osteoconductivity for each group of implants.

FIGURE 6.



Schematic from two digitised ground sections showing tracings

IIviii. Mechanical test methods

Setting / Working time

To obtain baseline data and give a quantitative measure for the handling properties assessed clinically, the setting and working times of G338 and Ketac Cem were determined using the standard tests laid down in the ISO no. 7489 (1986) and BS no. 6039 (1981).

To carry out these tests a simple indenter was made (Figure 7). The base of the indenter was maintained at 37^{o}C to simulate the clinical situation. GIC was mixed at room temperature and then placed in the teflon mould resting on the warmed base of the indenter. (Figure 8) The GIC was indented every 10 seconds until the indenter failed to form a complete circle when examined under x4 magnification. For the working time the mass of the indenter was 28g and a flat rod 4mm in diameter was fitted to the end of the indenter. Once the indenter failed to form a complete circle the time was noted. The setting time was evaluated after increasing the mass of the indenter to 400g and using a flat rod 2mm in diameter to form the indent. Ten tests for the working and setting time were performed on each material.

Set Strength / Young's Modulus

A limited investigation of the mechanical properties of GIC relevant to their possible use as bone substitutes was undertaken using a four point bend test.

FIGURE 7.

Photograph of indentor resting on heated table.

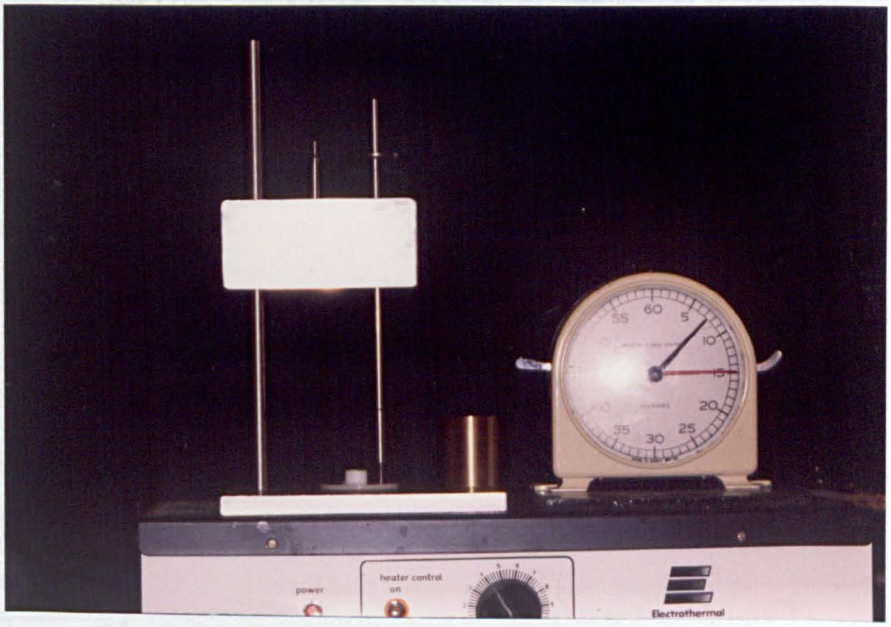
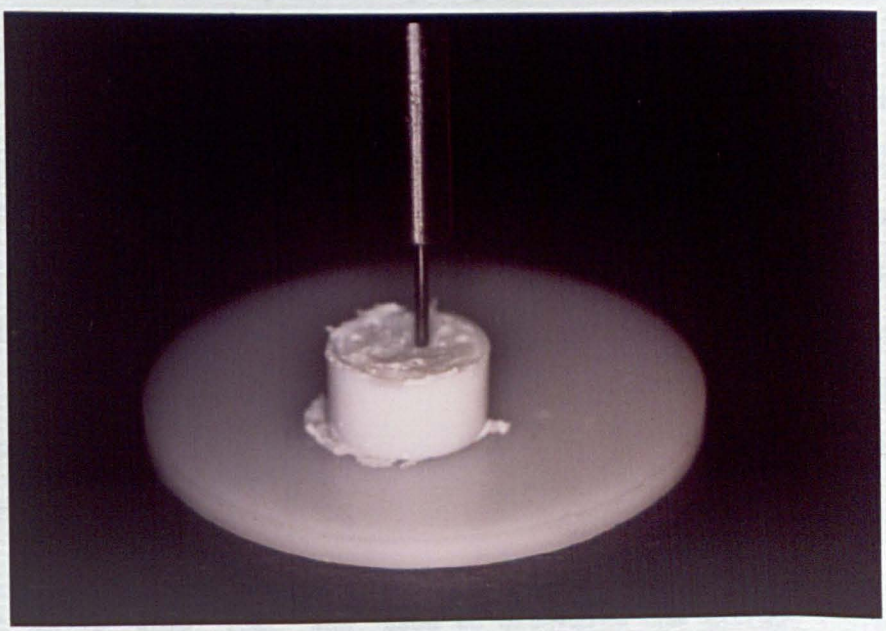


Figure 8.

Close up showing needle indenting partially set GIC.



The effect of varying the volume fraction of the glass component of the GIC was investigated. The properties of two formulations of Ha/GIC composites were also tested.

To undertake mechanical tests on GIC it was necessary to develop a method for testing small samples as conventional testing using standard size rods would have been both wasteful of material and expensive.

The method described was developed in conjunction with Mr C Oldfield (formerley of the Department of Materials, University of Sheffield) who was evaluating the fibre reinforcement of GIC and also required a mechanical test method that utilised small rods.

Rods of GIC nominally 4.6mm in diameter and 36mm long were produced using 1ml disposable plastic syringes. A batch of material sufficient to make 6 rods was mixed and loaded so as not to trap air in the material. Syringes were filled by rapidly moving the vertically held syringe barrel over the mixing pad to cause the GIC to be drawn up and fill the syringe. The method of loading the syringes in this way is similar to that used to load dental impression syringes. The material was mixed and allowed to set for 48hrs prior to testing. Rods were left enclosed in the barrel of the syringe, which was placed in a sealed jar with wet cotton wool to ensure 100% humidity prior to use.

A four point bend test was used to determine the maximum fibre stress (tensile stress) and maximum distortion of the rod before

fracture (strain) from these results the Young's modulus of the material under test could be calculated by dividing the stress by the strain.

Tests were carried out using a jig with the upper inner contacts 6mm apart and the lower outer contacts 20mm apart (Figure 9). The jig was placed in a Mayes SM 200 testing machine (British Calibration BS. 1610). The rods were loaded at a rate of 1mm per minute. The diameter of each rod was measured using a digital micrometer and the deflection in mm and force in Newtons at rod fracture were recorded.

The data were analysed using a computer program (Appendix I) which applied the equations given below to calculate the fracture toughness - maximum fibre stress / maximum fibre strain and Young's modulus.

Bending moment $M = \frac{E.I}{R}$

Where E = Young's modulus

I = The moment of inertia

R = Radius of rod.

This approximates to $M = E.I.\frac{d^2y}{dx^2}$

In the jig used the outer loading points were 7mm from the inner pair which were 6mm apart. The total distance between outer points being thus 20mm.

Taking P to equal the load applied in newtons and De to equal the deformation of the rod..

and where the moment of inertia $I = \frac{piR^4}{4}$

the above equation becomes....

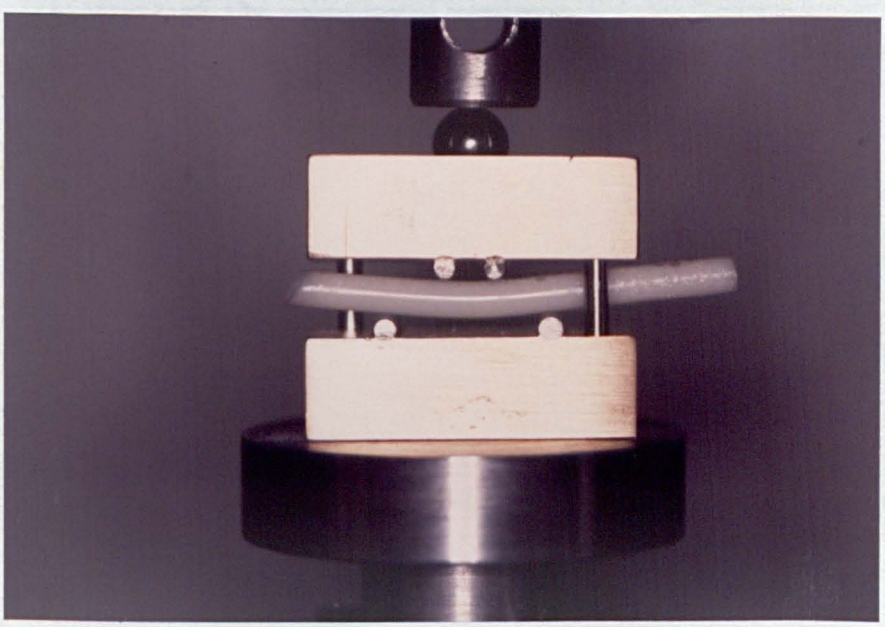
$$E = \frac{P \times 1.3066x10^{-7}}{De \times I}$$

FIGURE 9.

Photograph of jig used in four point bend tests.

Upper loading points 6mm apart, lower pair 20mm apart.

Sample shown Ketac Cem



III. RESULTS

IIIi. Results of In vitro evaluation.

Approximately 100 individual cultures were undertaken. Some wells (7%) became contaminated with bacterial or fungal growth and these cultures were discarded.

Of the remainder, a minimum of five calvarial and five envelope cultures were obtained for each material:-

Set GIC rods:- Mp4, G338, Ketac Cem, Aqua Cem.

Glass particles:- Ketac Cem, Mp4, G338.

Polyacrylic Acid (calvarial culture only), code E3(4) Laboratory of the government chemist.

Particulate Dense Ha. Calcitite 40/60, Interpore Ha./200.

Particulate TCP. Friedrichsfeld WG.

Light microscopy and TEM examination were undertaken on the envelope cultures. Cultures were embedded and each sample had at least one ground section and several semi-thin sections cut prior to TEM evaluation. The calvarial cultures were examined using the SEM.

Light microscopy

Cells in the envelope cultures containing ceramics or set GIC, except for the GIC Aqua Cem, were viable at the end of two weeks of culture. Fibroblast and osteoblast-like cells underwent differentiation, migrated onto the surface of the materials.
Ground sections of two week envelope cultures containing Calcitite Ha, Mp4 GIC and Interpore Ha are shown in Figures 10-12 and are representative of the appearance of the set materials in envelope cultures.

There was evidence of mineralisation/calcification occurring adjacent to some of the ceramic materials (Figures 10 and 13) and this resembled new bone morphologically.
Vital cells were seen associated with the GICs (Figure 14), except Aqua Cem where cells appeared non-vital and to have undergone degeneration (Figure 15).

FIGURE 10
Ground section Calcitite Ha 2wk envelope culture, area of calcification on superior surface arrowed. [original mag x20] Stevenel's blue / Alizaran red S.

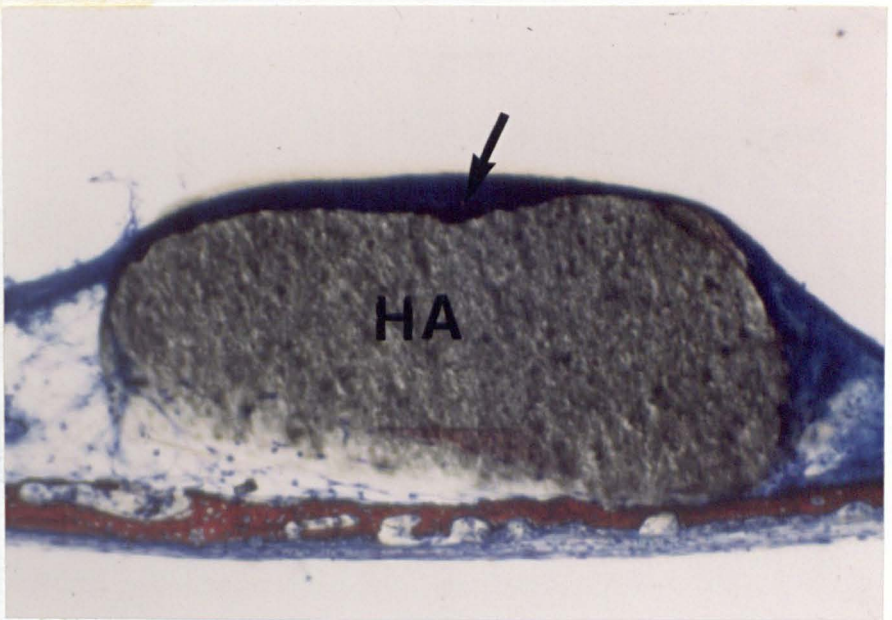


FIGURE 11
Ground section Interpore Ha 2 wk envelope culture, fibroblast-like cells are seen in the pores of the material [original mag x20] Stevenel's blue / Alizaran red S.

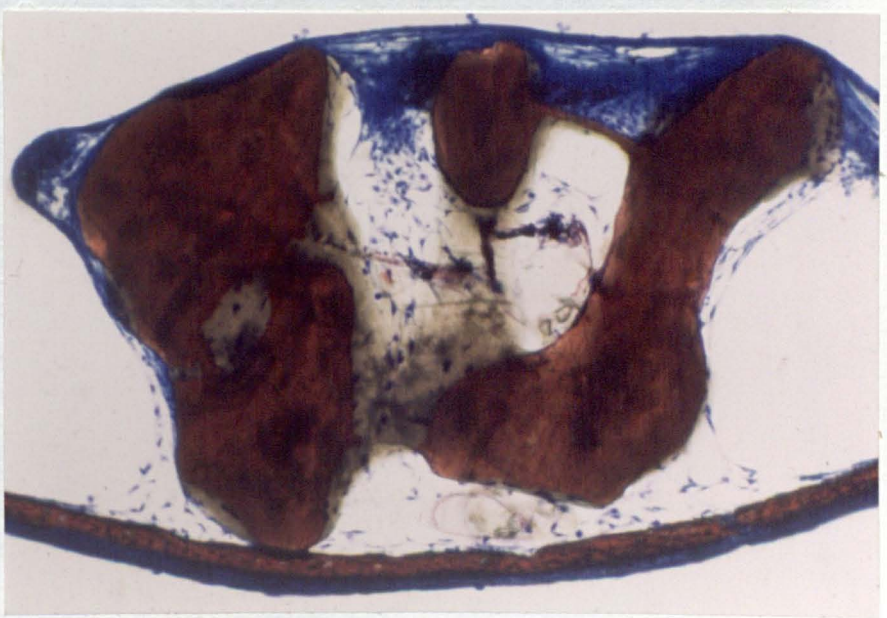


FIGURE 12
Ground section Mp4 GIC 2wk envelope culture, showing new cells around GIC [original mag x20] Stevenel's. blue / Van Gieson.

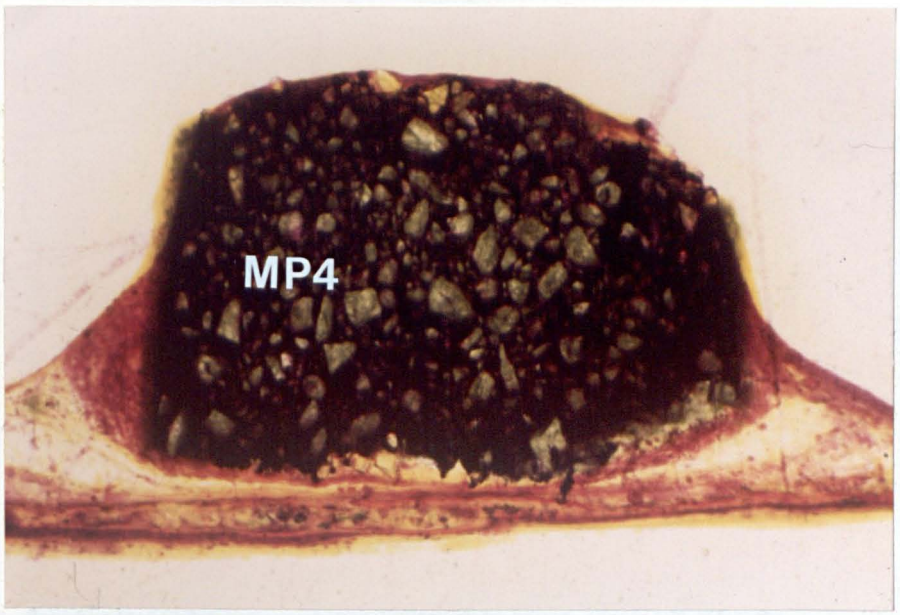


FIGURE 13
Semi-thin section through the middle of part of a 2wk envelope culture into which Tri-Calcium Phosphate (TCP) had been placed. Area of 'new bone' arrowed [original mag x200] Tol. blue

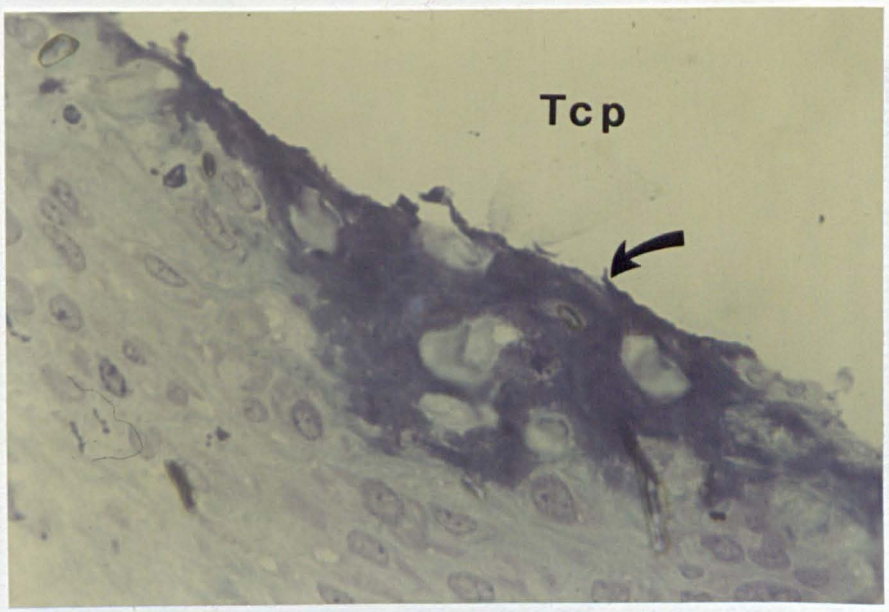


FIGURE 14
Semi-thin section of a 2wk envelope culture into which the GIC Mp4 had been placed. Vital fibroblast-like cells are seen adjacent to the surface of the GIC [original mag x 200] Tol. blue

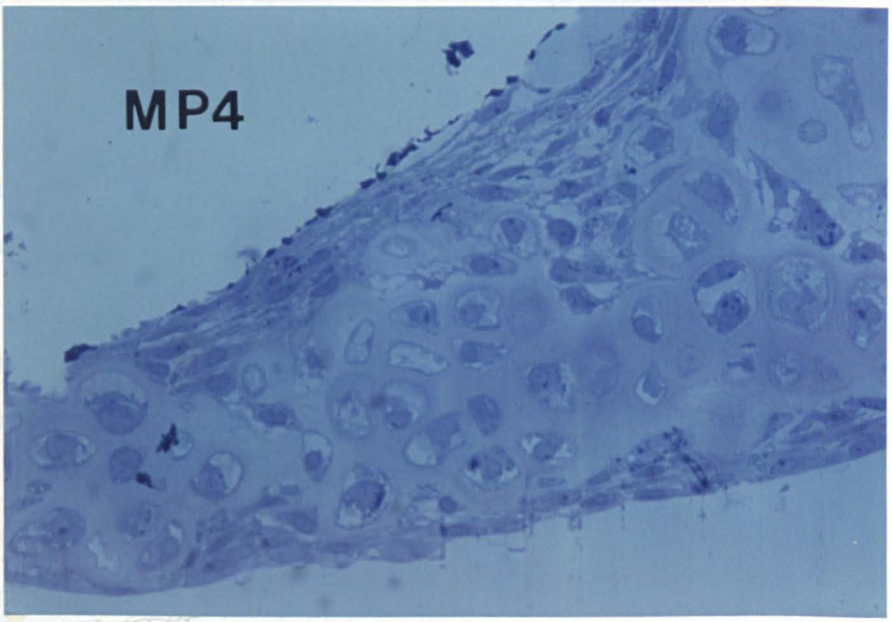
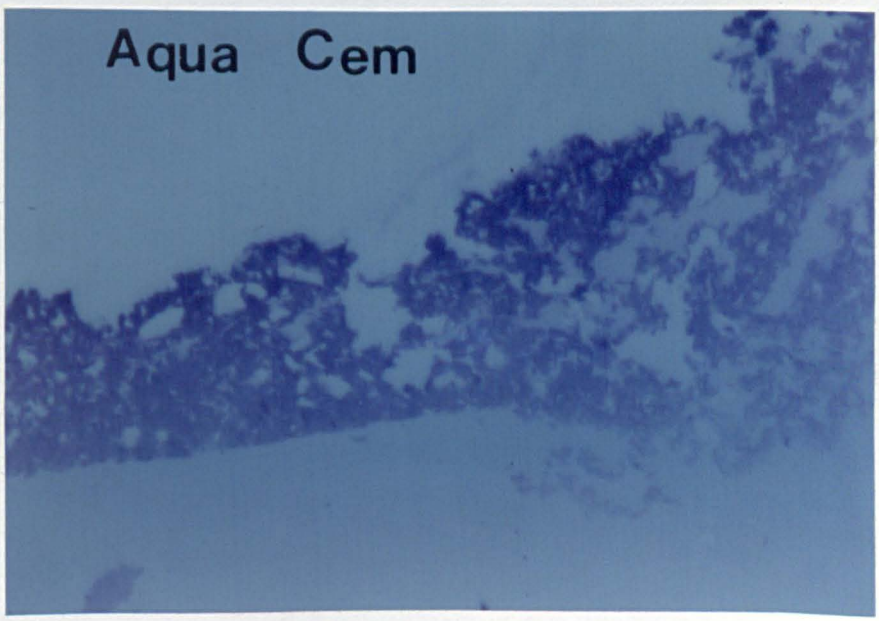


FIGURE 15
Semi-thin section through part of a 2wk envelope culture into which the GIC Aqua Cem had been placed, there is lysis of cells and no evidence of any remaining vital cells [original mag x200] Tol. blue



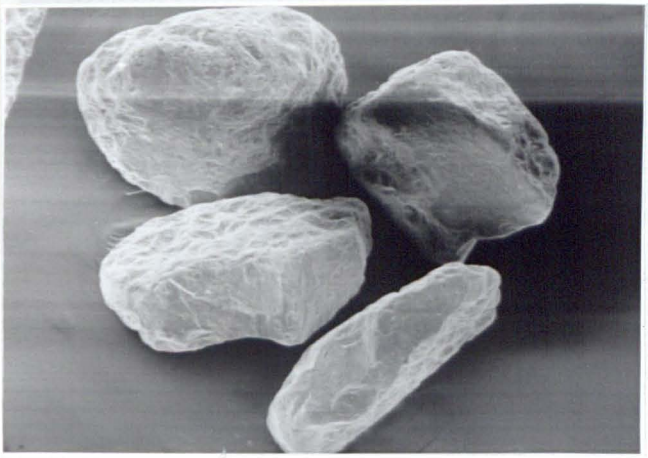
SEM examination of 'calvarial cultures'

Osteoblast-like cells, as evidenced by their polygonal morphology and tesselated appearance, migrated onto and held all the solid test materials to bone except for the GIC AquaCem which, despite repeated attempts, could not be retained in place on the calvarial bone during processing.

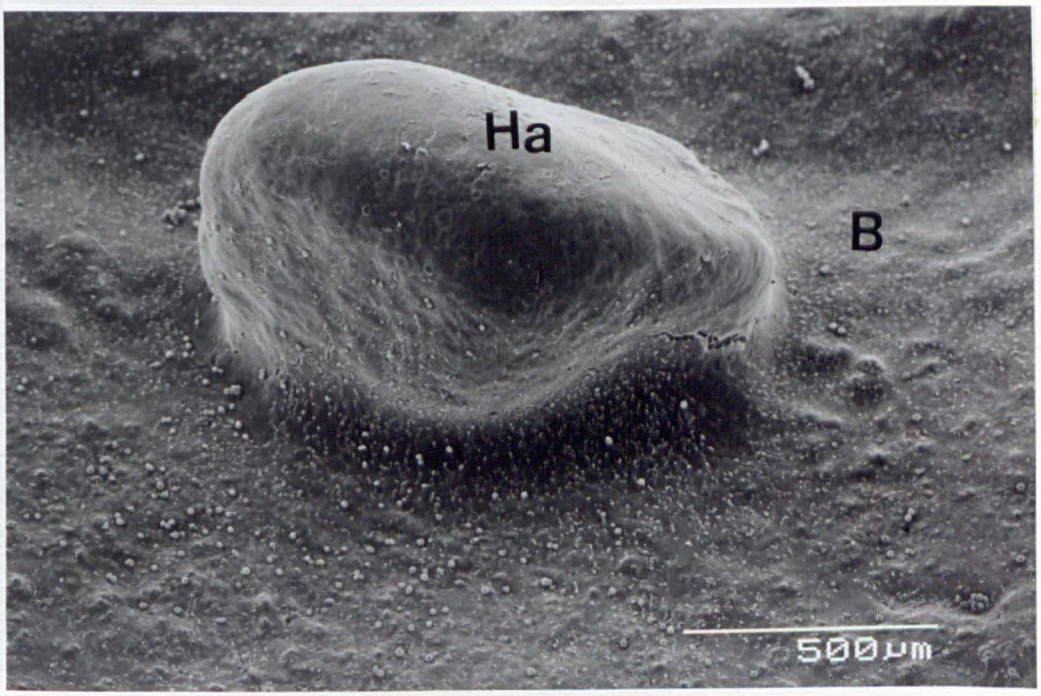
The two types of Ha responded in a similar manner and results apply to both types (Figure 16-19).

Osteoblast-like cells migrated onto the material from the calvarial bone (Figure 16). Figure 17 shows the cells at the junction of the bone and material, and demonstrates that there is no change in their morphological appearance as they move lying on bone to material. The detailed appearance of cells on the surface of the Ha ceramics can be seen in Figures 18 and 19, the polygonal, tessellated morphology of the osteoblast-like cells being clearly demonstrated.

FIGURE 16

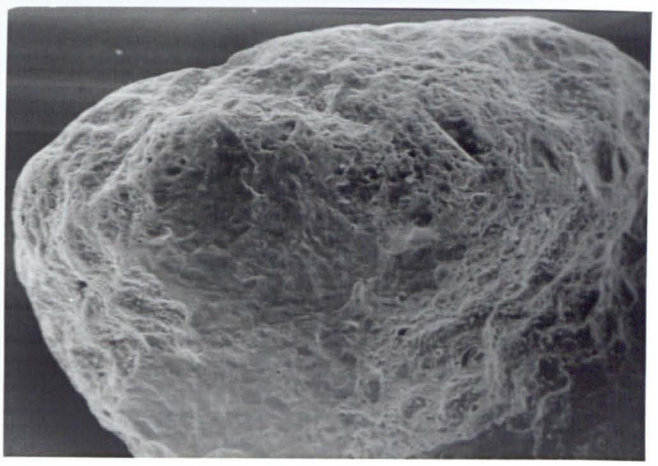


a. SEM (CS600) photomicrograph of particles of dense Ha (Calcitite) control. 20μm scale bar.

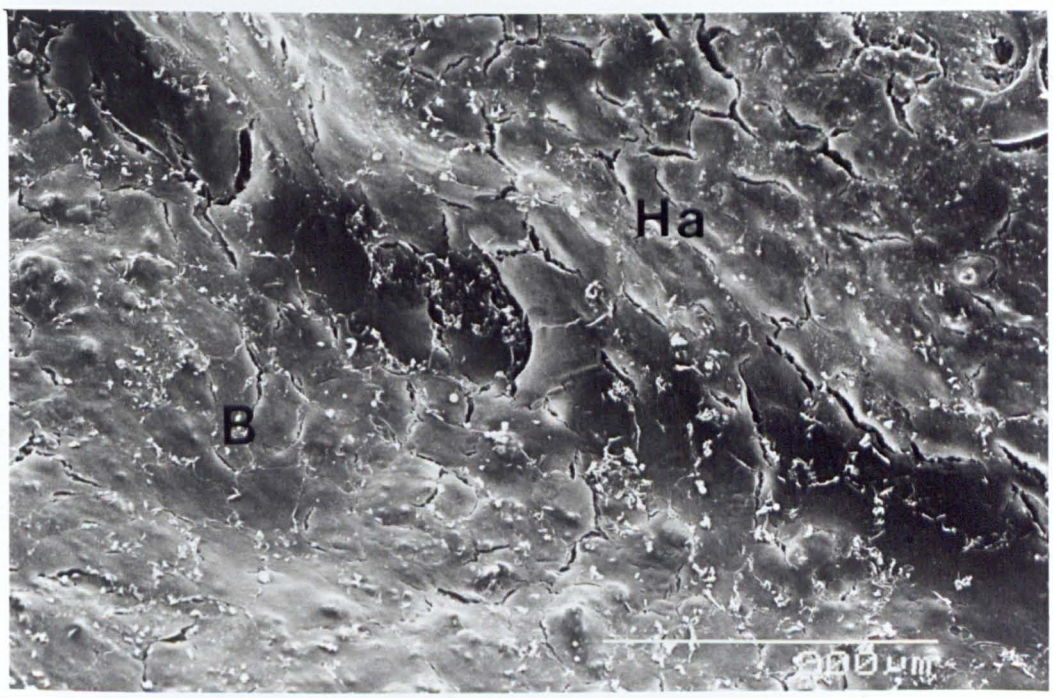


b. SEM (HS) photomicrograph of a particle of dense Ha (Calcitite) on calvarial bone after two weeks in culture. Cells have migrated from the calvarial bone (B) onto the material (Ha)

FIGURE 17

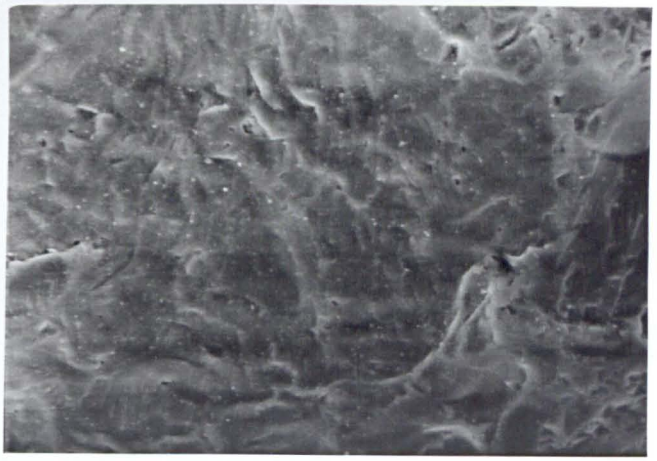


a. SEM (CS600) photomicrograph of particle of dense Ha (Calcitite) control. 80µm scale bar.

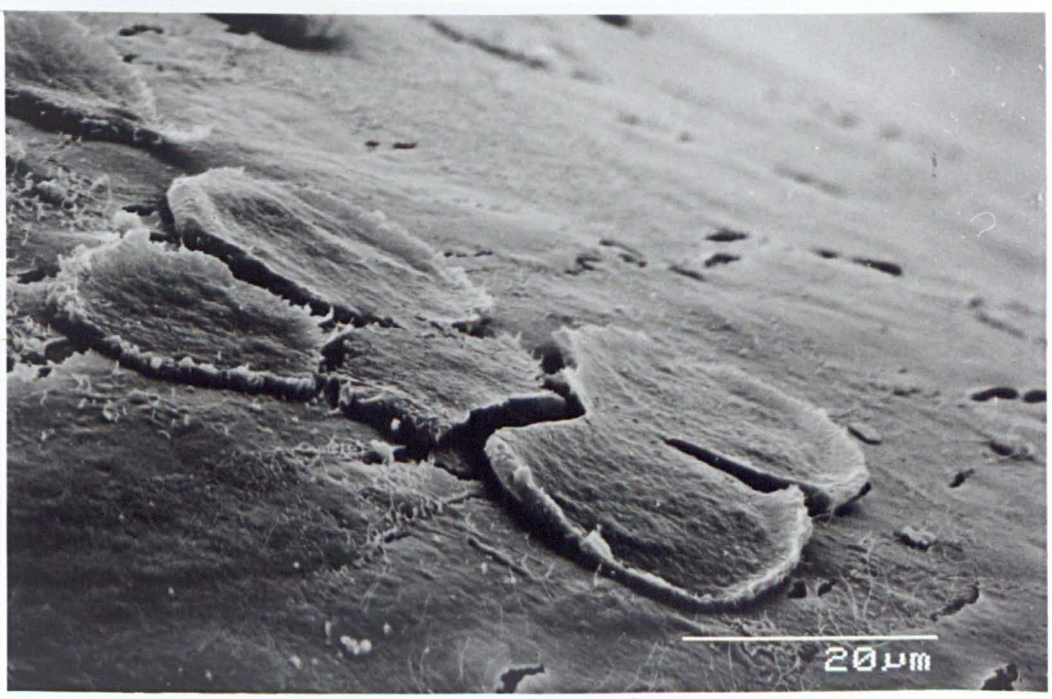


b. SEM (HS) photomicrograph of the junction of a particle of dense Ha (Calcitite) and calvarial bone following two weeks in culture. Cells have migrated from the calvarial bone (B) onto the material (Ha) and appear to have accepted the material 'as bone'

FIGURE 18

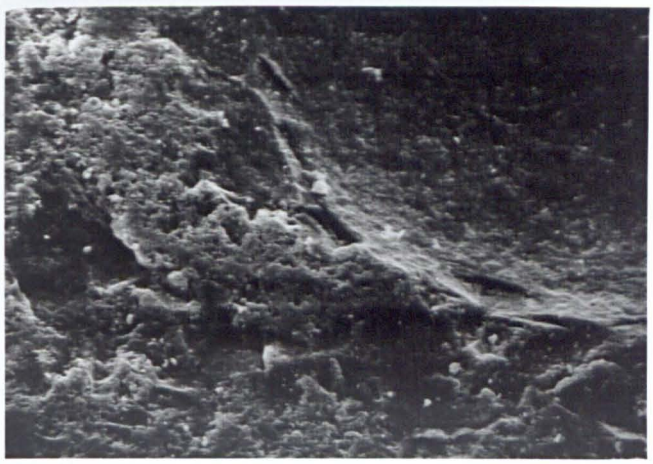


a. SEM (CS600) photomicrograph of the surface of a particle of dense Ha (Calcitite) control. 20μm scale bar.

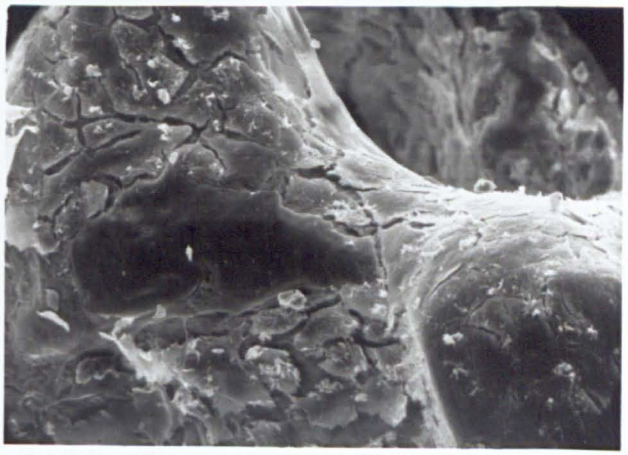


b. SEM (HS) photomicrograph of detail from the surface of the Ha particle shown in Figure 16 the tessellated polygonal cells exhibit an osteoblastic morphology.

FIGURE 19



a. SEM (CS600) photomicrograph of the surface of a particle of Interpore Ha control. 20μm scale bar.



b. SEM (CS600) photomicrograph of the surface of Interpore Ha particle after two weeks calvarial culture showing the material covered by tessellated polygonal cells as in Figures 18 and 17.

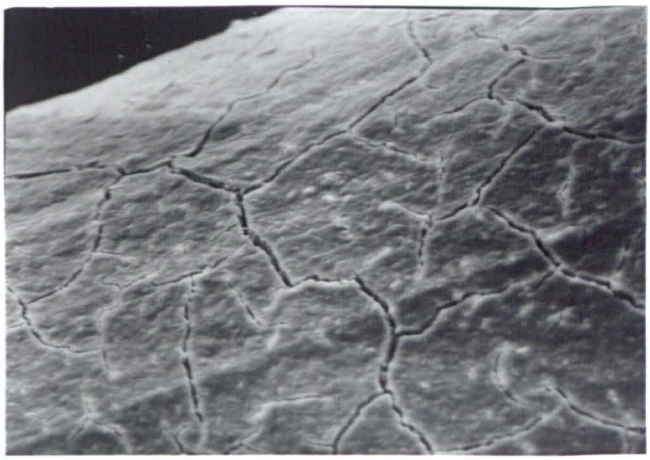
Cells which had migrated onto and colonised the smooth surfaces of the set rods of the GICs (Mp4, G338 and Ketac Cem) and the ceramic Tri-calcium phosphate (TCP) were also seen as a closely packed continuous sheet (Figures 20-23). An osteoblastic morphology was maintained with a polygonal tessellated appearance, similar to unmigrated cells on the surrounding calvarial bone (Figures 20 and 22). Parts of the superior surface of TCP particles were noted to be devoid of cells (Fig. 21) and these areas exhibited a rough surface topography.

Figure 20

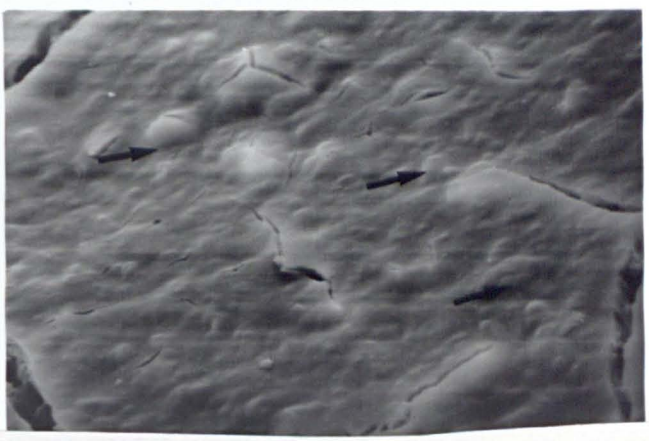
a. SEM (CS600) of a rod of Mp4 GIC control. Scale bar 200um.

b. SEM (CS600) of a set rod of GIC Mp4 after two week calvarial culture, side and end of rod showing migrated cells. Rods of G338 and Ketac Cem showed similar colonisation at two weeks

FIGURE 21



a. SEM (CS600) of the surface of Mp4 control, showing cracking of the surface due to dehydration. Scale bar 20um.

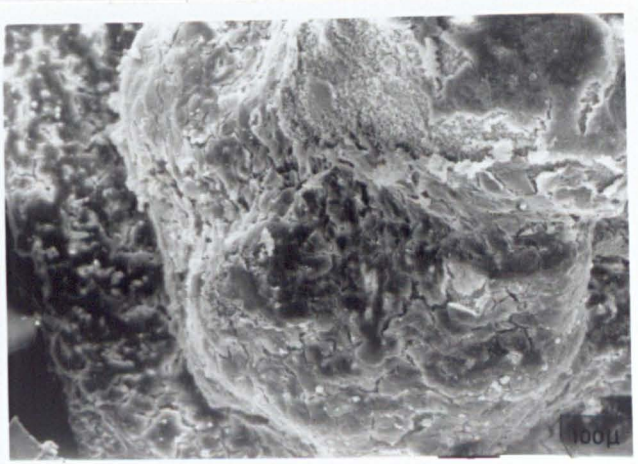


b. Detail from 21a individual glass particles (arrows) can be decerned in the matrix of the GIC. Scale bar 10um.

c. SEM (CS600) detail from figure 20b showing cells covering the surface of the rod of Mp4. Scale bar 40um.

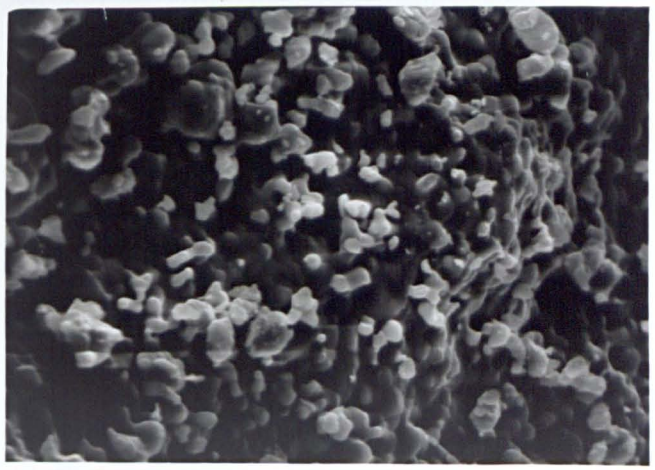
FIGURE 22

a. SEM (CS600) of a particle of TCP control. Scale bar 100um.

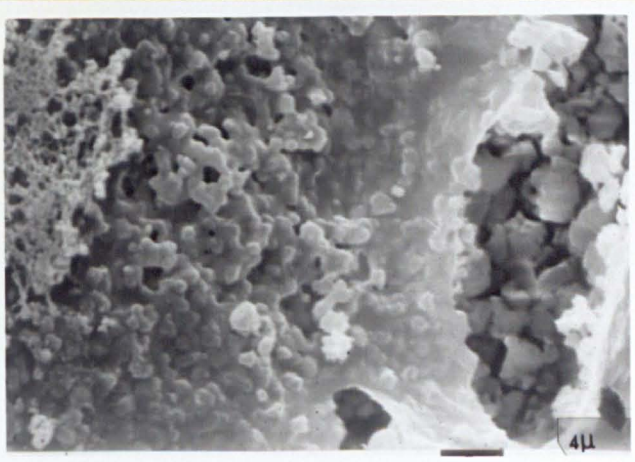


b. SEM (CS600) of a particle of TCP after two week calvarial culture showing partial coverage of cells superior surface has not been colonised. Scale bar 100um.

FIGURE 23



a. SEM (CS600) of TCP particle control. Scale bar 5um.



b. SEM detail from superior surface of Figure 22c. Rounding of the crystallite structure of the TCP can be seen, indicating possible physico-chemical breakdown of the material in the tissue culture conditions. Scale bar 5um.

In areas where fracture of the GIC had left an irregular surface, cells were loosely packed and 'migrating osteoblasts' exhibited a more fibroblastic morphology with multiple pseudopodial and filopodial cellular extensions anchoring cells to the GIC and maintaining contact between adjacent cells (Figure 24).

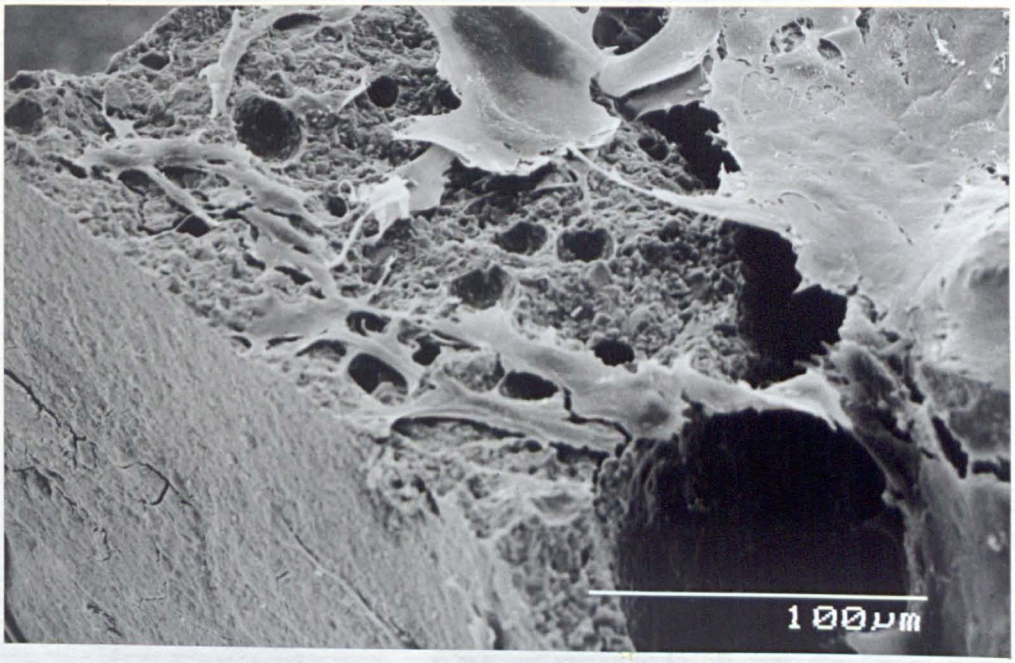
The placement of set AquaCem, freshly mixed Ketac Cem and the glass component of Ketac Cem into culture resulted in cell death in the immediate proximity of the materials and cell migration from the surrounding areas had not occurred at two weeks (Figure. 25).
In calvarial cultures in which Aqua Cem was placed, no material was retained following processing despite repeated attempts. It appeared that cells had not migrated onto the material. Examination of sites from which AquaCem had been lost revealed, compared to control bone, loss of the collagenous extracellular matrix (ECM) from the surface of the bone which appeared non-vital (Figure 25).

Cultures contaminated with Mp4 glass and the poly(acrylic)acid E3(4) behaved as control cultures grown without test material over the two week period (Figure 26).

Examination of the calvarial cultures of Ketac Cem, Aqua Cem and G338 produced evidence that the cause of the cell death associated with these GICs was due to diffusion of a cytotoxic leachant from the GIC. (Figure 27)

FIGURE 24.
SEM (HS) photomicrographs of the **A** rough and **B** smooth surface of a fractured piece of Ketac Cem GIC after 2wk culture showing 'migratory osteoblasts' exhibiting a more fibroblastic morphology. Cells are in close apposition to the material surface and are linked to contiguous cells by long cytoplasmic processes.

A



B

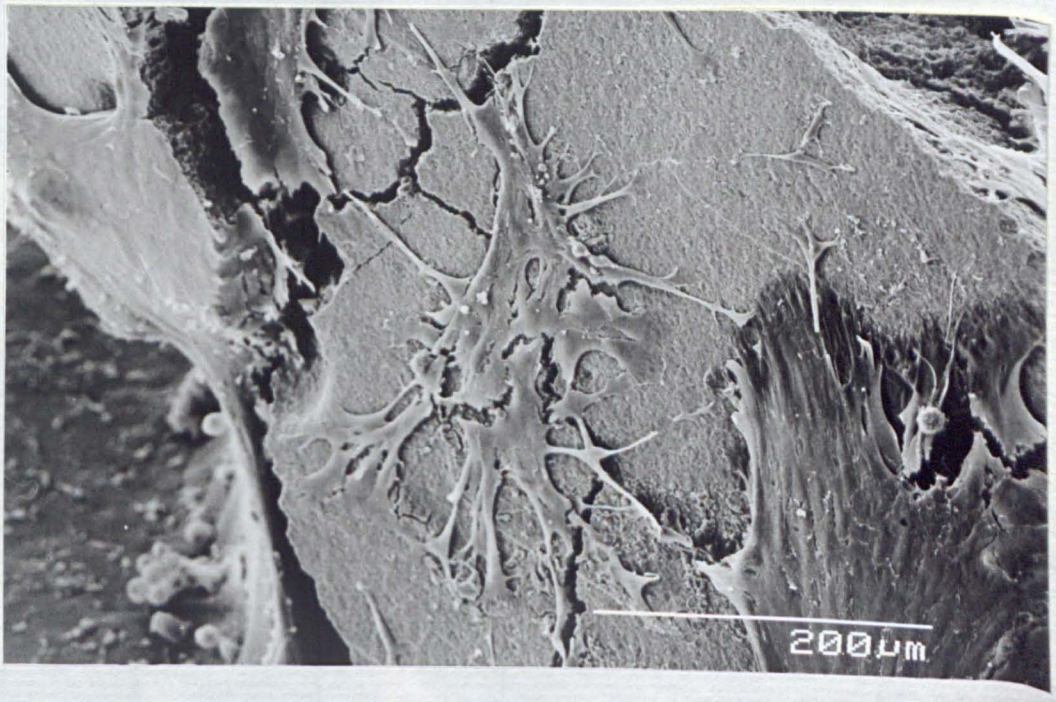


FIGURE 25
SEM (CS600) photomicrographs of calvarial bone cultured for two weeks and on which Aqua Cem GIC had been placed :

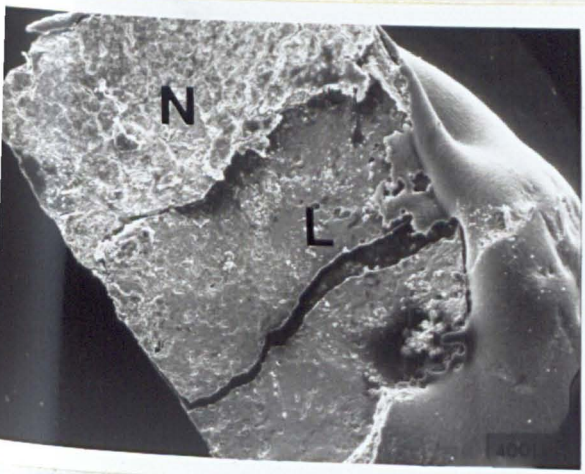
a. bone with area from which Aqua Cem had been lost , showing lack of cellular cover (L) and normal appearance of adjacent bone (N);

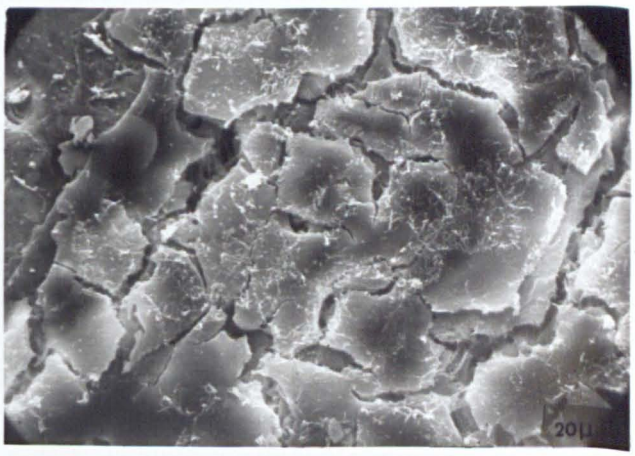
b. detail of osteoblast-like cells from area (N);

c. area (L) showing non-vital bone where Aqua Cem had rested;

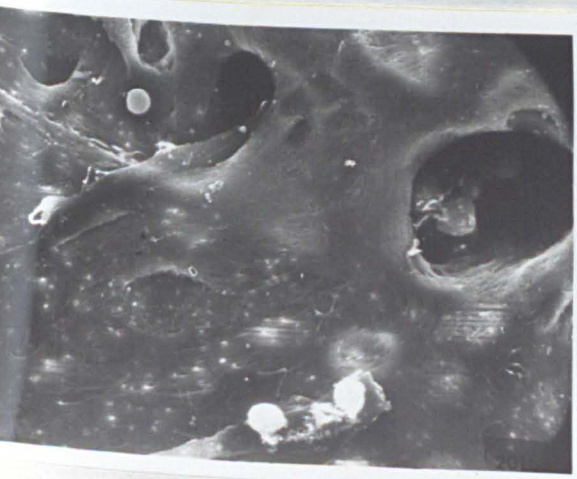
d. fixed, uncultured vital bone stripped of periosteum for comparison with **c.**

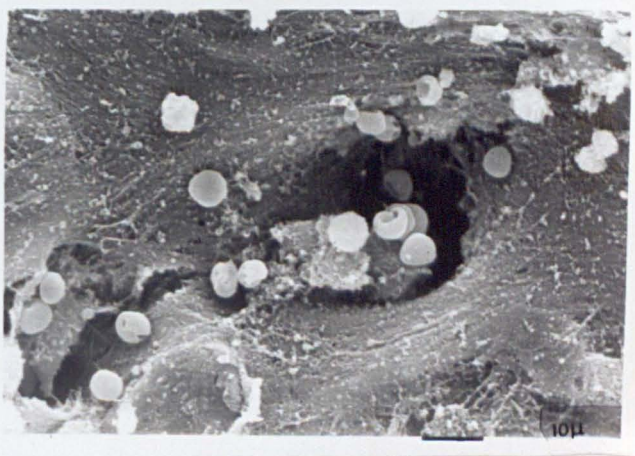
e. Culture contaminated with the particulate glass component of Ketac Cem; no evidence can be seen of cell migration onto the material or of vital cells.





a b





c d

25 e

FIGURE 26

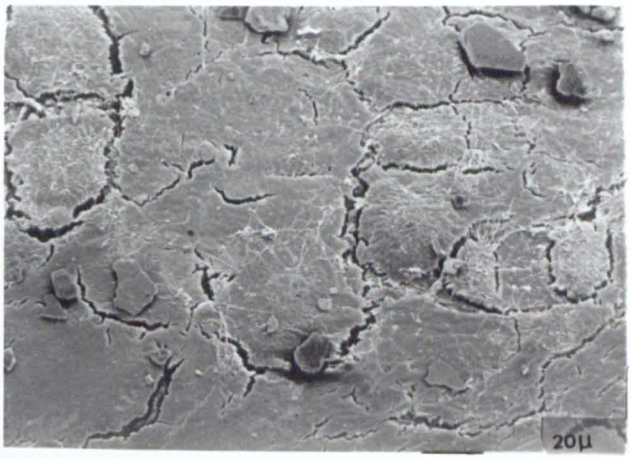
SEM (CS600) photomicrographs of host bone cultured for two wks:

a. control; no material in culture;

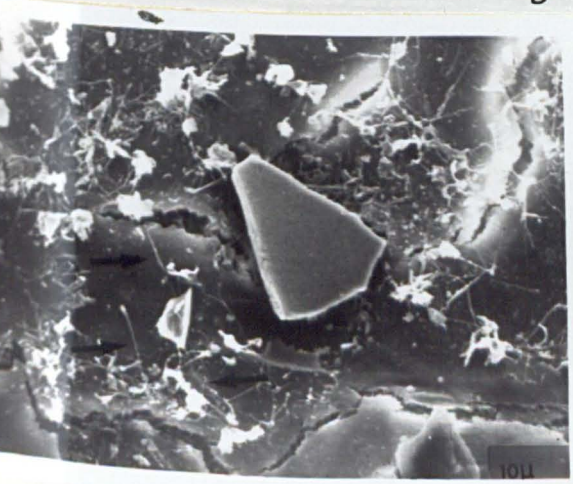
b. Culture contaminated with Mp4 glass; note vital cells producing collagenous ECM (arrowed) adjacent to a glass particle in centre of field;

c. culture contaminated with poly(acrylic)acid E3(4) some of which has precipitated onto the surface of the cells during processing; the cells appear normal

a



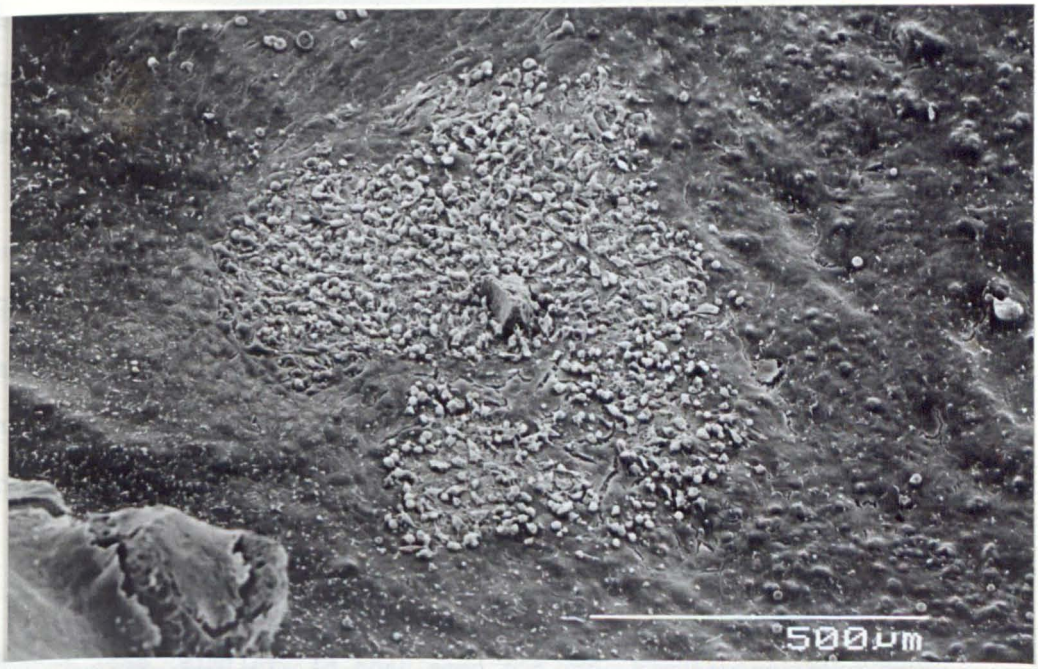
b



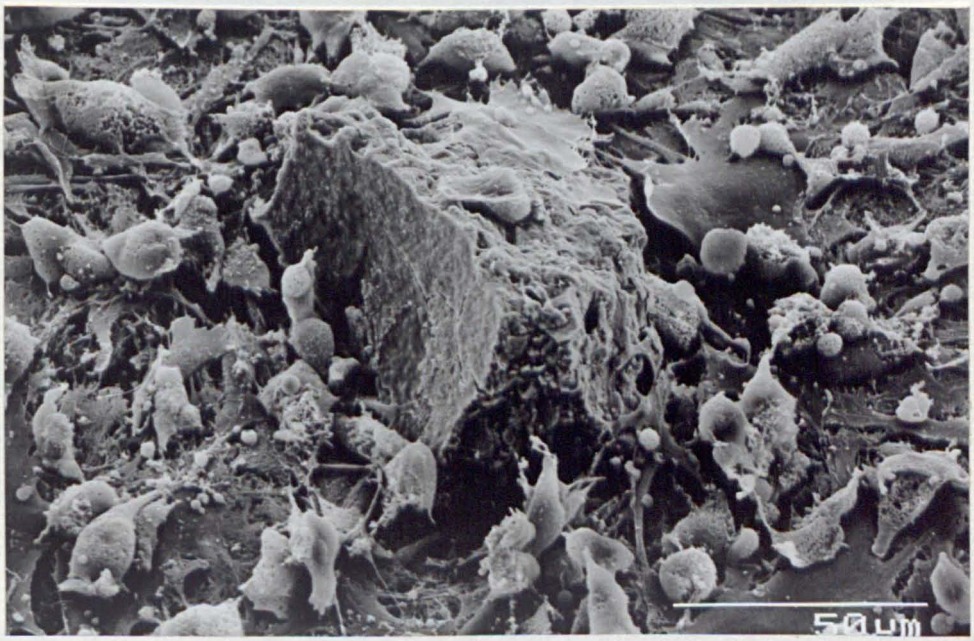
c

FIGURE 27.
SEM (HS) photomicrographs of **A** , an isolated fragment of Ketac Cem GIC (after 2wk culture) on host bone surrounded by a zone of dead cells. **B** detail showing vital and dead cells around fragment.

A



B



TEM examination of 'envelope cultures'

Cells in contact with all test materials except the GIC AquaCem (Figure 28) remained viable throughout culture.

Cells were seen to be intimately associated with the surface of the test materials onto which a collagen containing ECM was elaborated (Figures 28-32).

The GIC/tissue interface was similar for Mp4, G338 and Ketac Cem. Collagen fibres exhibited an alternating layered configuration which, in close proximity to the GIC, became random with fibres interdigitating with the surface of the material (Figure 29).

The interface between the ceramics (Ha - Calcitite/Interpore and TCP) and the tissue showed two configurations (Figure 30 and 31). The first was similar to that seen for GIC, consisting of an ECM containing randomly orientated collagen fibres which interdigitated with the surface of the ceramic. The other type of interface was seen was associated with Ha and consisted of a narrow electron dense zone dividing the ECM from the Ha (Figures 30 and 31). In places, where mineralization of the ECM had been noted on ground sections there was also evidence at the ultrastructural level, where decalcification was incomplete, that the ECM had been minieraled (Figures 30-32). In the case of TCP, some of the elaborated tissue resembled bone morphologically (Figure 32).

There was however no convincing evidence of mineralization in any of the two week cultures of GIC.

FIGURE 28

TEM photomicrographs of two week 'envelope cultures' into which GIC was placed.
a Set AquaCem - the material has broken down; spaces occupied by particles (arrowed) are seen surrounded by lysed cells / debris [Scale bar 30um]

b Glass component of Ketac Cem, glass particles (arrowed) are surrounded by vital cells exhibiting prominent nuclei, rough endoplasmic reticulum [Scale bar 12um].

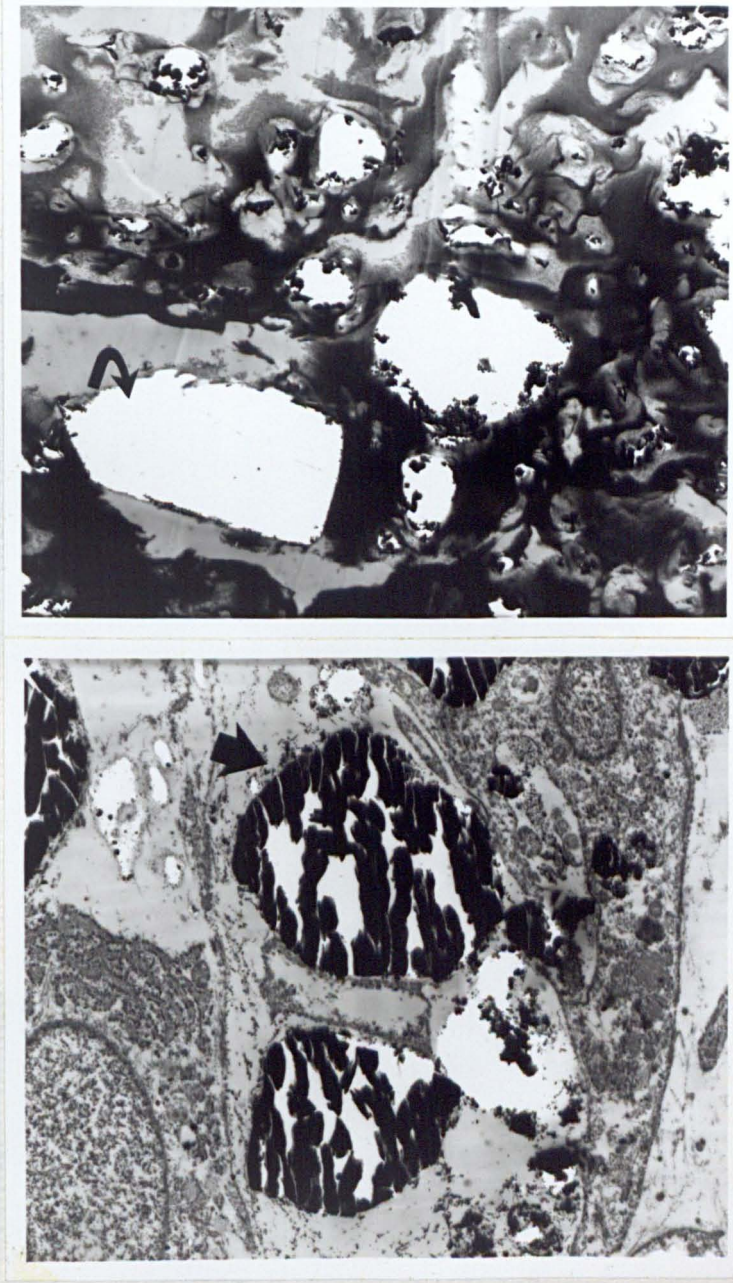


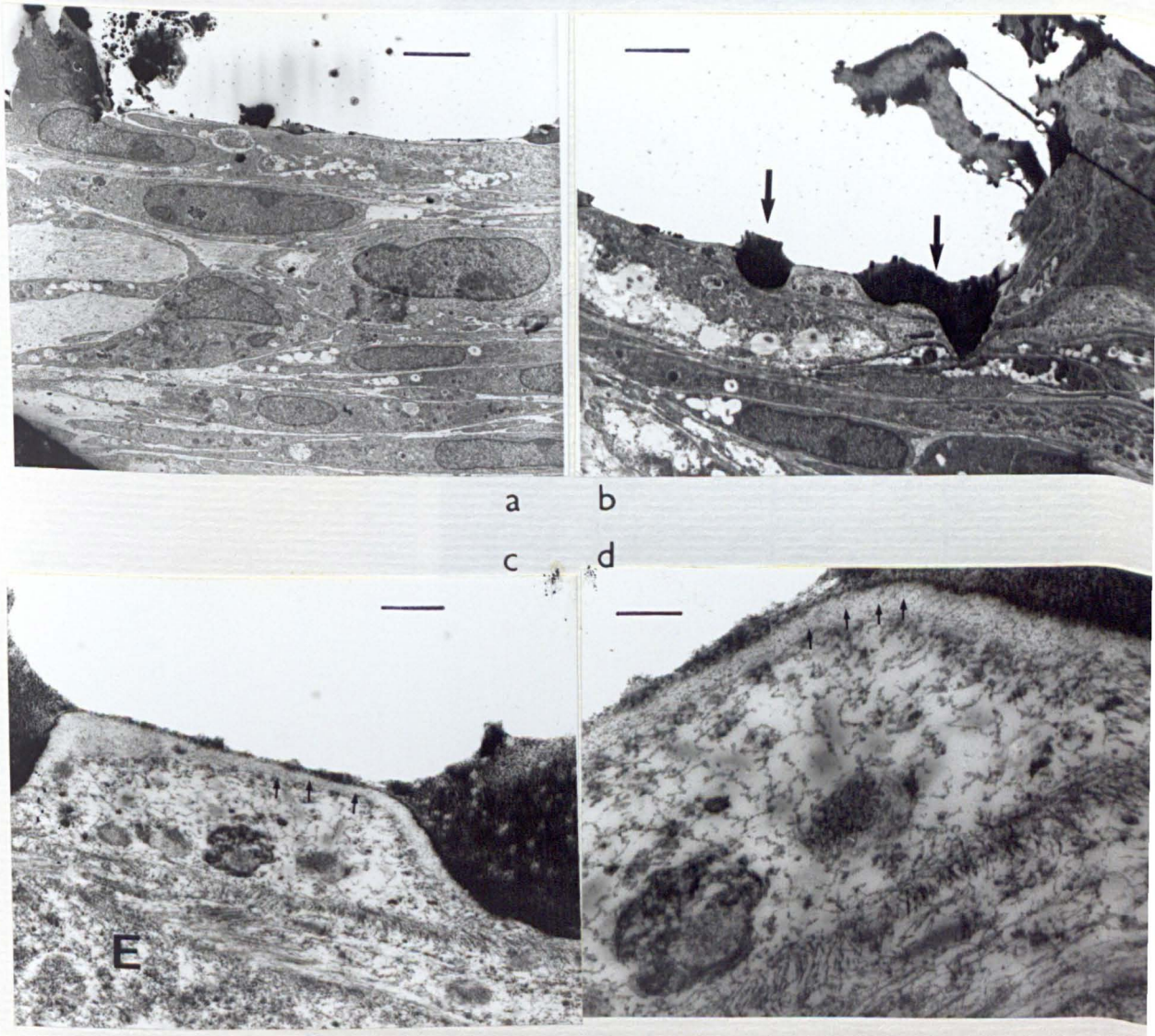
FIGURE 29

TEM photomicrographs of two week 'envelope cultures' into which set GIC based upon Mp4/E3(4) had been placed:

a Section through whole of cellular layer apposed to upper surface of GIC (the periosteum) showing close apposition of fibroblast-like cells to the GIC retained fragments (arrowed) [Scale bar 22um].

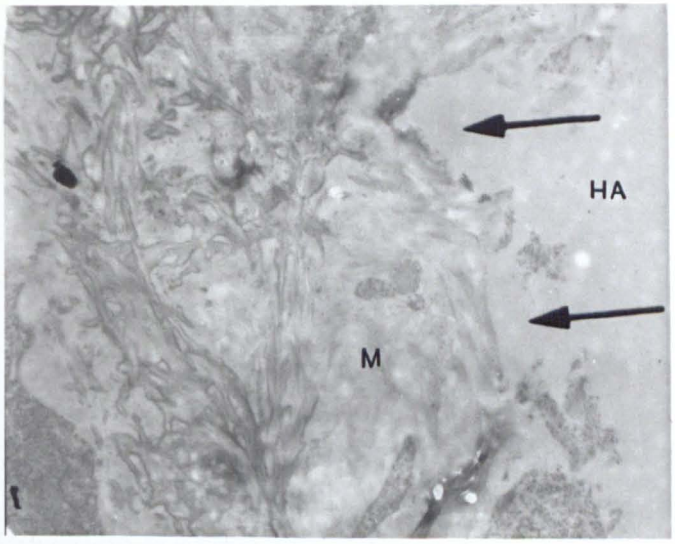
b Interface of cells and Mp4 GIC (arrowed) [Scale bar 17um].

c & d Higher power of interface [Scale bars 3um and 1um respectively] showing layered arrangement of collagen in ECM (E) becoming random at the material interface with interdigitation of fibres, across a final less dense zone [arrowed], into the Mp4 GIC, fragments of which remain in contact with the tissue.

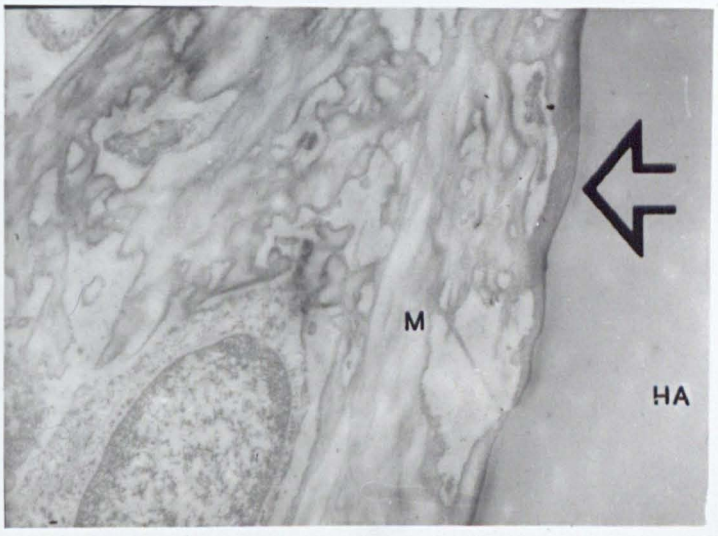


FIGURES 30

a. TEM photomicrograph of two week 'envelope cultures' into which the ceramic Ha (Calcitite) was placed.
Interface exhibiting interdigitation of mineralized collagenous ECM [M] with Ha (Ha), which was lost on decalcification, a cellular process [C] can be seen [Scale bar 5um].

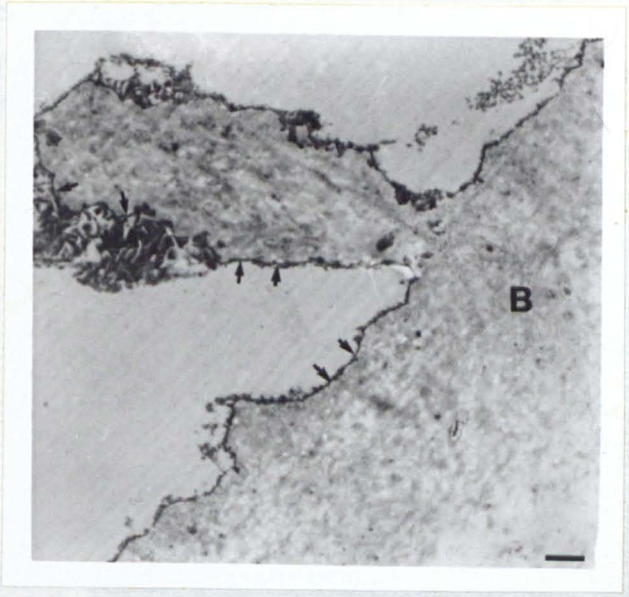


b. TEM photomicrograph of two week 'envelope cultures' into which the ceramic Ha (Calcitite) was placed.
Interface exhibiting electron dense layer [arrowed] between mineralization ECM [M] Scale bar 5um.



FIGURES 31

a. TEM photomicrograph of two week 'envelope cultures' into which the ceramic Ha (Interpore) was placed.
Interface exhibiting electron dense layer [arrowed] between Ha and tissue [B]. Scale bar 1um



b. TEM photomicrograph showing detail from 'a'. Scale bar 0.5um

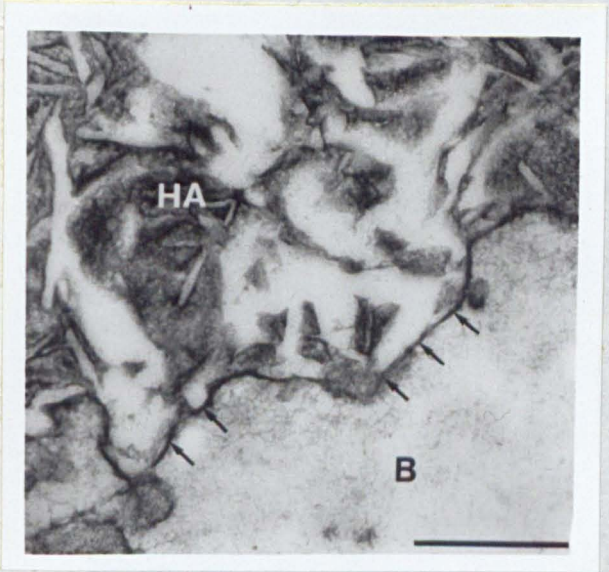


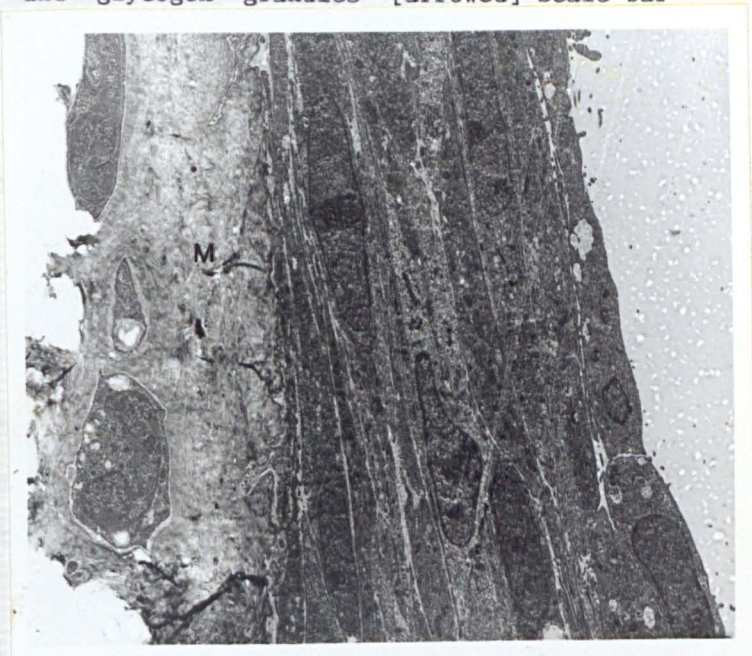
FIGURE 32

TEM photomicrographs of two week 'envelope culture' into which the ceramic TCP was placed.

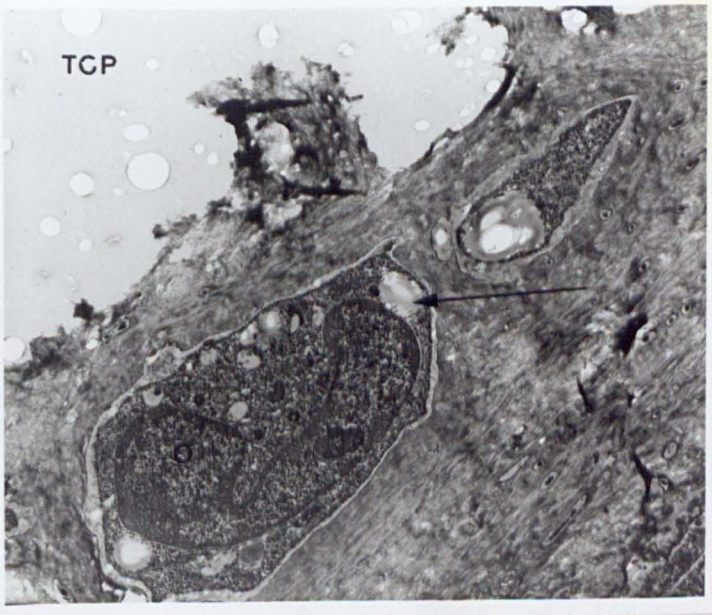
a. Section through whole of cellular layer on upper surface (periosteal) of material, showing fibroblast-like cells and ECM (M) containing an osteocyte like cell adjacent to the TCP which has been lost. Scale bar 22um

b. Detail of interface showing close apposition of partly mineralized ECM (M) and TCP. The osteocyte-like cell (O) exhibits numerous mitochondria and glycogen granules [arrowed] Scale bar 0.1um.

a



b



IIIii. Results of *In vivo* primate bone culture model.

Light microscopy

Ground sections showed that the diffusion chambers were completely filled with loosely arranged, variably cellular connective tissue in which glass particles and transplanted bone chips could be discerned.

Examination of decalcified semi-thin sections (6um) stained with hematoxylin and eosin (Figure 32) confirmed the presence of a cellular and partly collagenous connective tissue filling the diffusion chamber, as reported by Jonck (88); the transplanted cancellous bone appeared non-vital as judged by the absence of osteocytes in lacunae.

In some places, the transplanted bone chips and glass particles were associated with areas of increased cellularity in the adjacent stroma (Figs. 33,34 and 35). Even allowing for the effects of prolonged fixation, the cells involved presented an osteoblast-like morphology and were supported in a matrix of homogeneous, eosinophilic material resembling osteoid. Staining with hematoxylin and Van Gieson confirmed the collagenous nature of this matrix (Figure 35) and the appearances were considered consistent with those of newly-formed, metaplastic woven bone.

Elsewhere (Figure 36), the connective tissue supporting the V-Os implant appeared less cellular and became more condensed and hyaline in character.

As evaluated in toluidine blue stained sections (Figure 37), the

interface between V-Os particles and stroma was characterised by the presence of cells exhibiting a fibroblast-like morphology which were intimately associated with the implant surface.

FIGURE 33

Contents of diffusion chamber comprising V-Os particles (g) and transplanted bone chips (b) supported in a matrix of variably cellular connective tissue; a focus of metaplastic bone formation (m) is evident adjacent to both the glass and bone. Hematoxylin & Eosin, x 25.

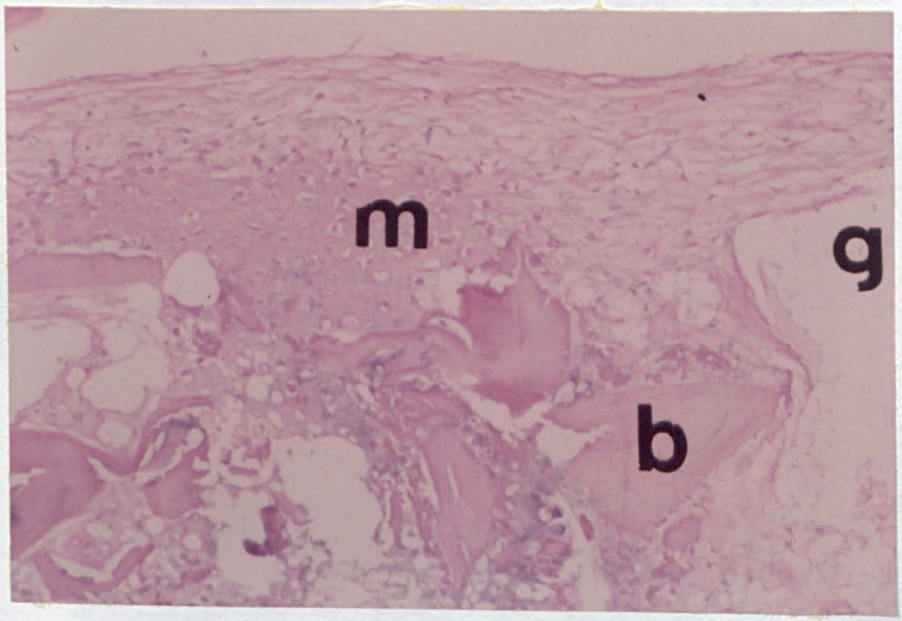


FIGURE 34

Detail from figure 33 showing metaplastic ossification in continuity with a fragment of non-vital transplanted bone (b). Hematoxylin & Eosin, x 125.

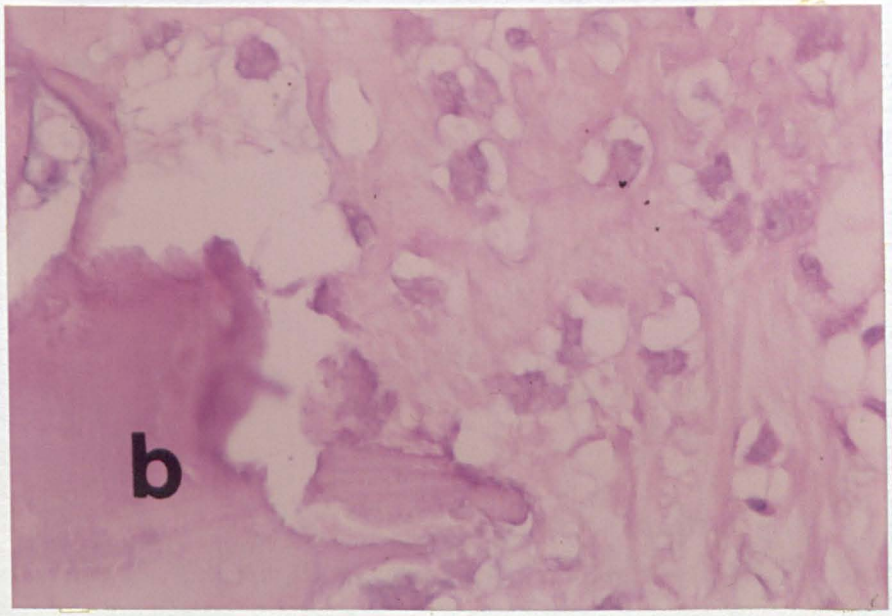


FIGURE 35

Legend as for figure 33, the Van Gieson stain demonstrates the collagenous nature of the metaplastic bone (m) matrix. Hematoxylin & Van Gieson, x 25.

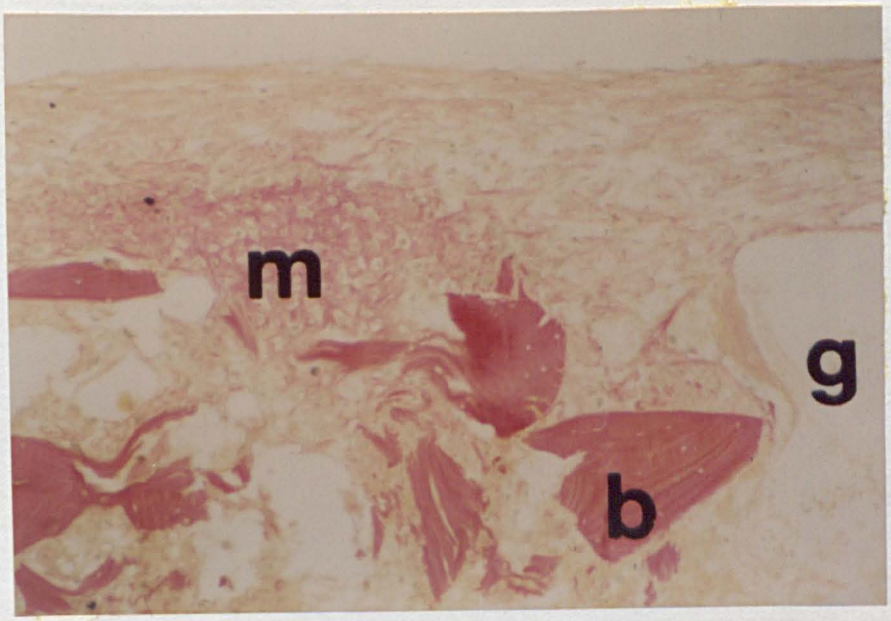


FIGURE 36
Contents of diffusion chamber comprising V-Os particle (g), transplanted bone chips (b) and stromal connective tissue (c); in places the interface between glass particle and stroma comprises condensed, hyalanised collagen (arrows). Hematoxylin & Eosin, x 25.

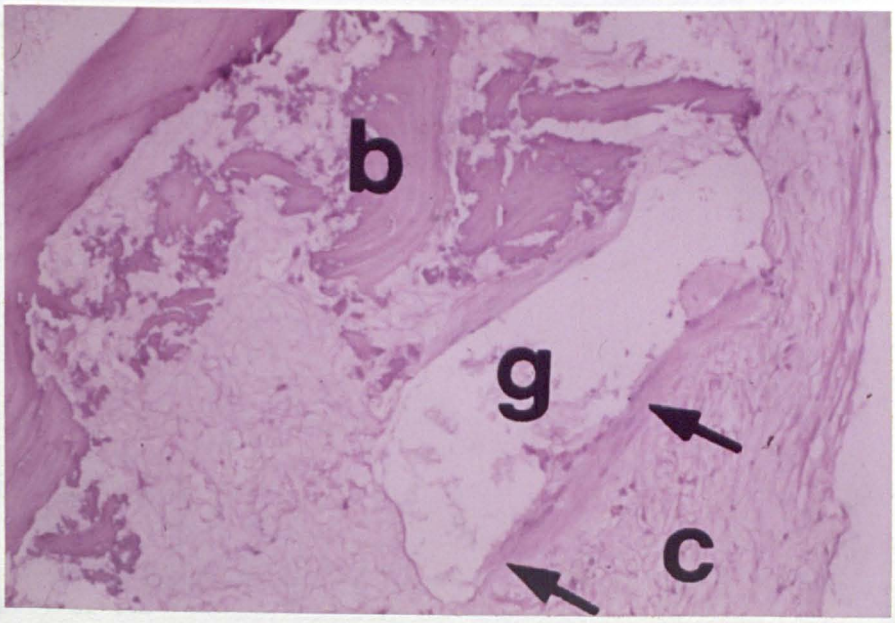
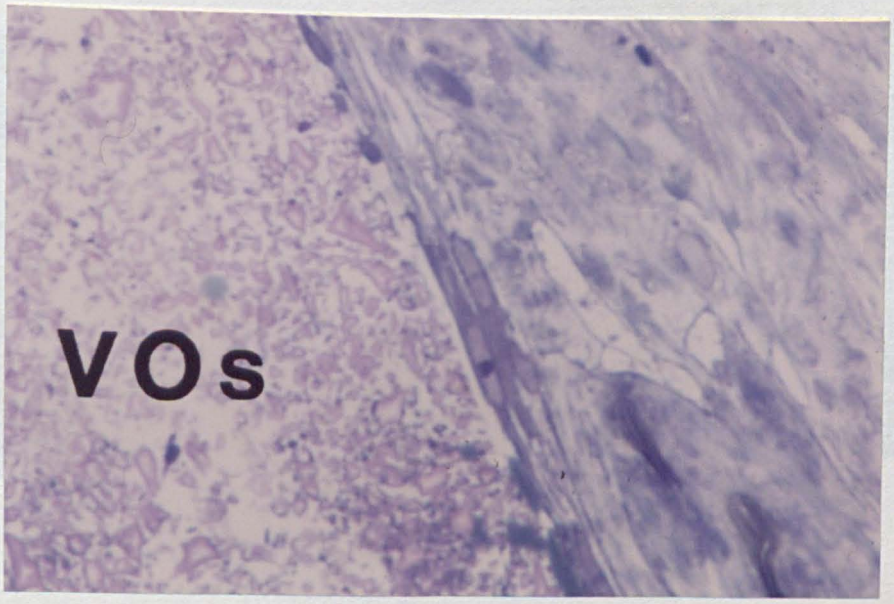


FIGURE 37
Interface between glass particle (V-Os) and stromal connective tissue; fibroblast-like cells and collagen fibres (not visible) are orientated parallel to the implant surface. Toluidine blue, x 250.



Ultrastructural SEM examination of diffusion chambers

SEM examination of the disrupted contents of the diffusion chambers revealed granules of GIC V-Os embedded in a fibrous connective tissue (Figures 38-40). Part of the surfaces of the V-Os granules were colonised by polygonal cells exhibiting a tessellated arrangement (Figure 39) Other areas were devoid of cells (Figures 38 and 39) and in places fibroblast like cells were noted (Figure 40).

FIGURE 38

a. SEM (CS600) photomicrograph of a V-Os granule control. scale bar 200um.

b. SEM (CS600) of part of the contents of a diffusion chamber, a piece of V-Os can be seen. Scale bar 200um.

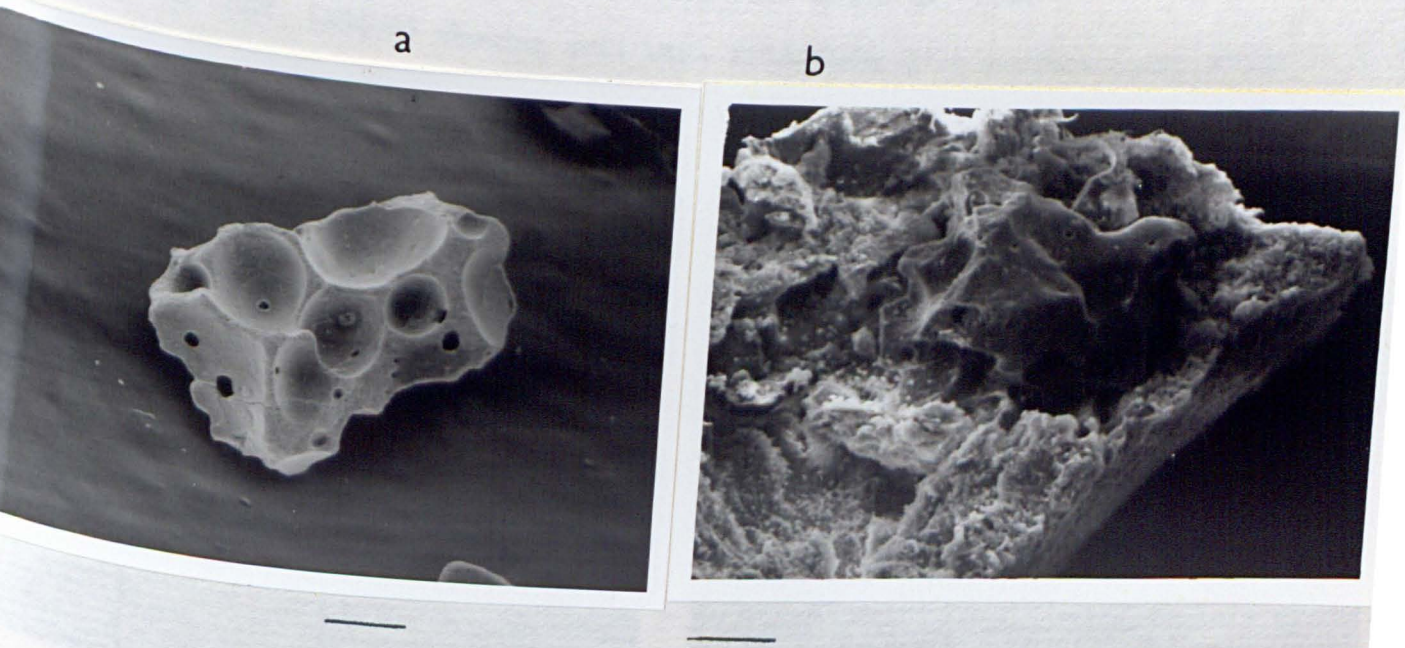


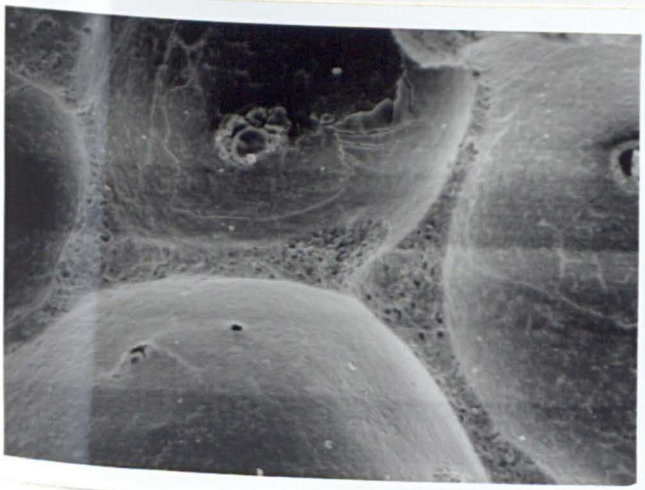
FIGURE 39

a. SEM (CS600) of a V-Os granule control. Scale bar 80um.

b. SEM (CS600) Showing V-Os granule with osteoblast like cell **arrowed** adherent to the surface of the glass.

Scale bar 100um

a



b

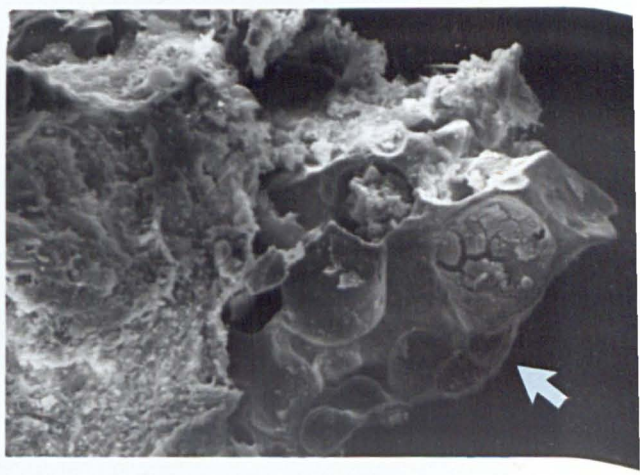
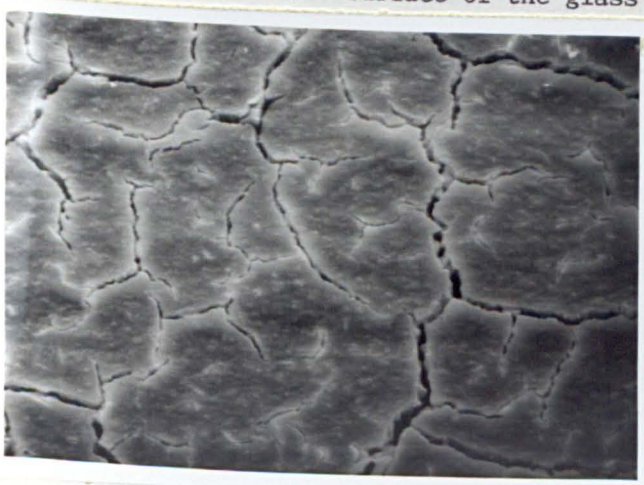
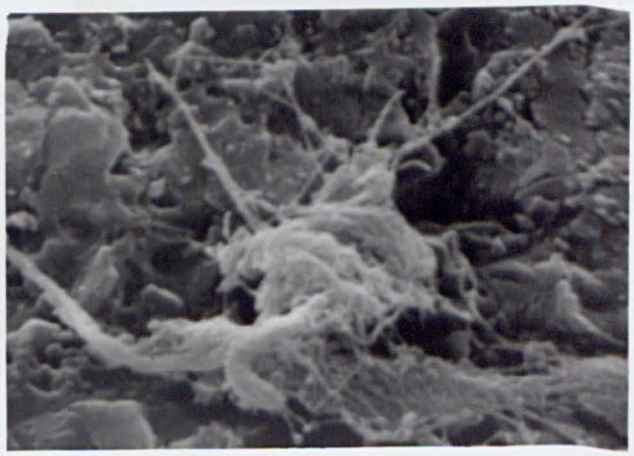


FIGURE 40

a. SEM (CS600) of V-Os granule control. Scale bar 20um

b. SEM (CS600) showing cell with fibroblast-like morphology in close contact with the V-Os implant. Cytoplasmic processes anchoring the cell to the surface of the glass can be seen. Scale Bar 5um.





a

b

TEM examination of diffusion chambers

The evaluation of the contents of diffusion chambers using the TEM was compromised due to the overlong fixation (9weeks) which the tissue had undergone, this resulted in loss of intra- and extra cellular detail.

The interface between the V-Os granules and the extracellular matrix consisted of either a direct contact of collagen fibres with the surface of the material, the fibres at the interface lying at right angles to the surface rather that parallel with it (Figure 41), or of a thin (60-700nm) granular electron dense zone which had been deposited on the surface of the V-Os (Figure 42).

FIGURE 41

TEM photomicrograph showing intimate relationship of collagen fibrils and surface of V-Os implant (**arrows**). Glass particle (G), hydrogel matrix (H). Scale bar 2um

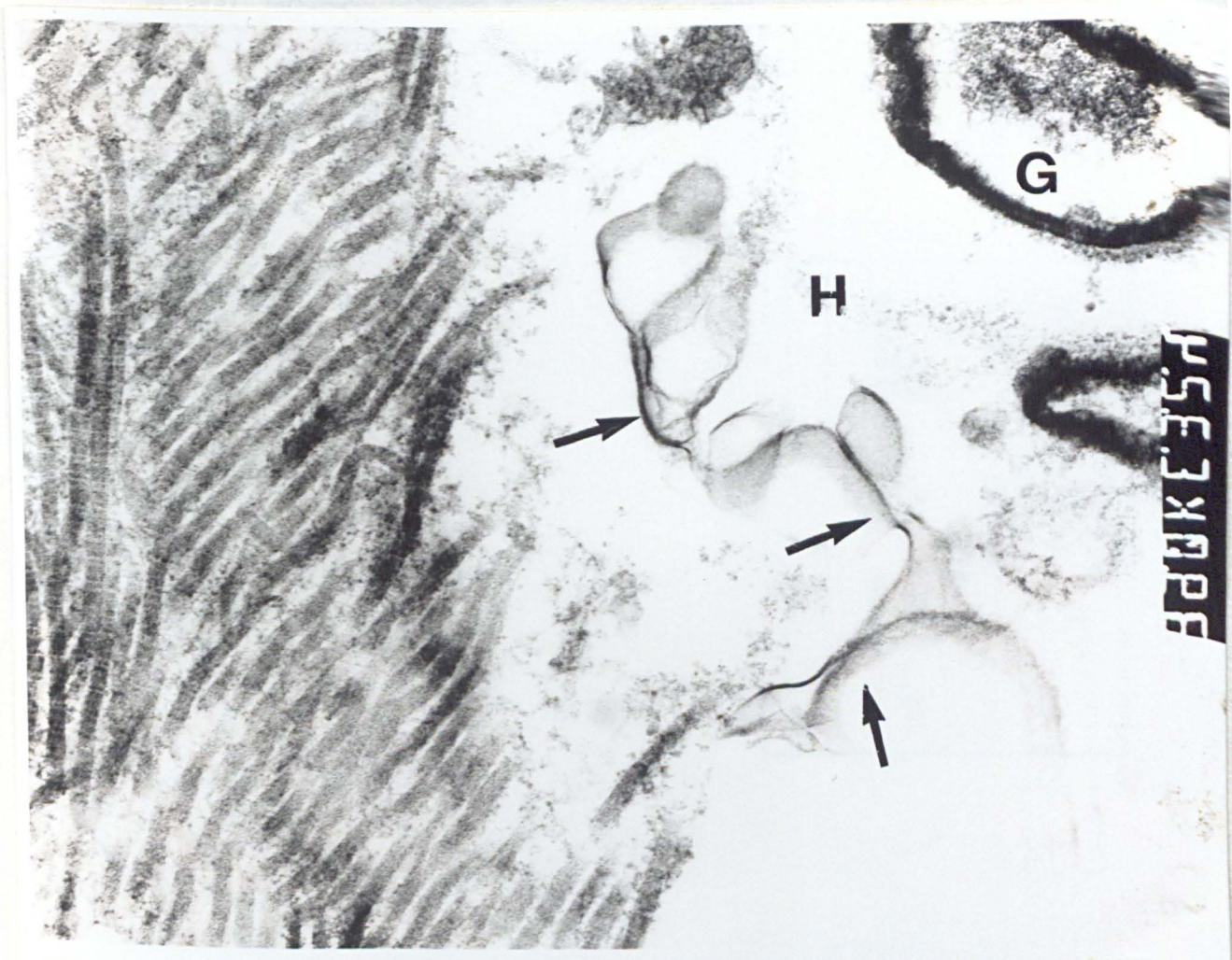
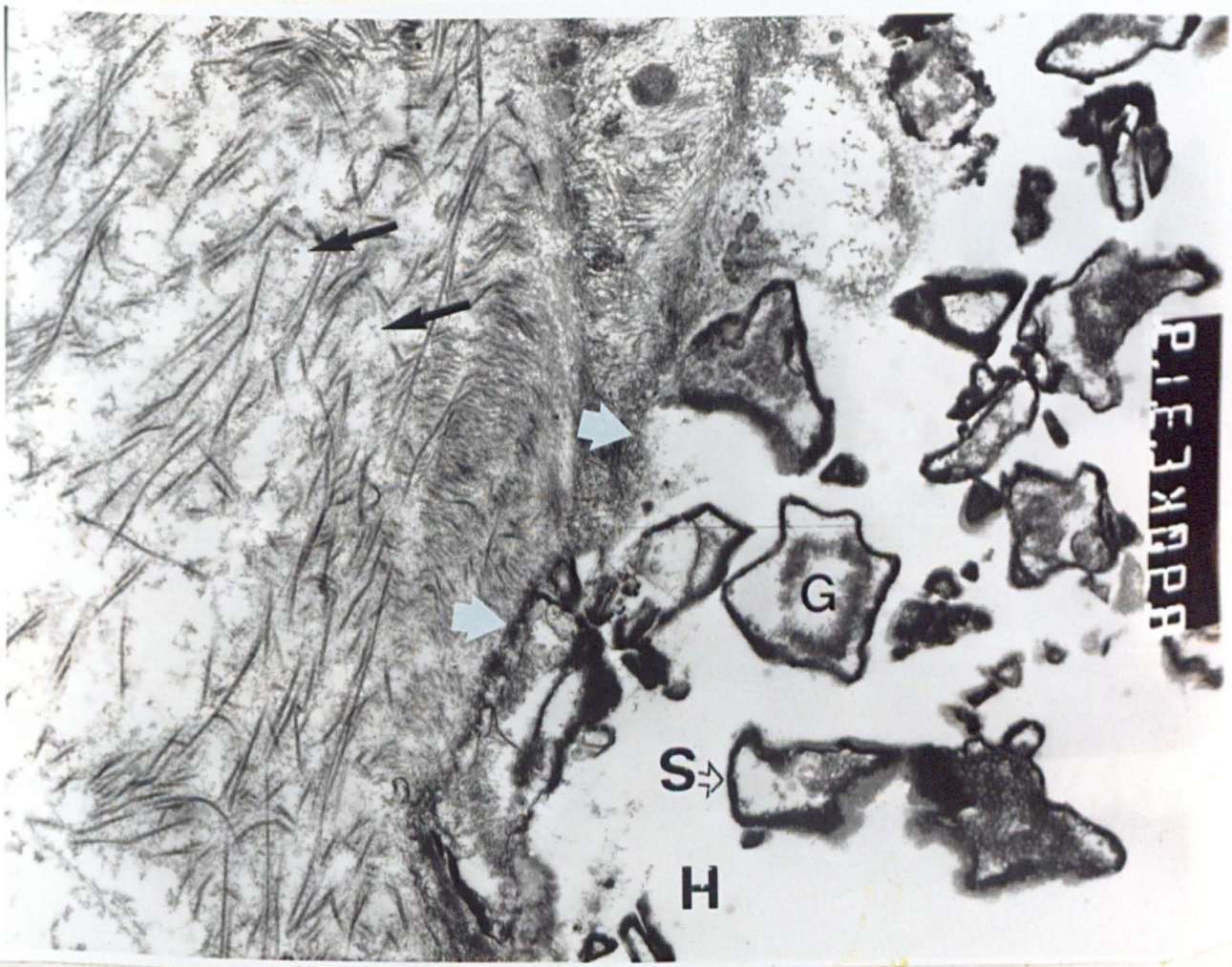


FIGURE 42

TEM photomicrograph of interface V-Os implant and collagen-containing extra-cellular matrix, a granular zone is seen at the interface (**white** arrows). Collagen fibrils with typical 64nm banding are seen and proteoglycan particles **(black arrows)** can also be determined. Individual glass particles (G) surrounded by silicacous hydrogel sheath (S) can be seen in the hydrogel matrix (H) of the V-Os implant. Scale bar 6um



IIIiii. **In vivo results (Implantation)**

Of the 54 operations all healed uneventfully except for one where Ketac Cem had been placed; in this case at day three wound breakdown/infection was noted but this resolved following topical application of chlortetracycline and further healing was uneventful.

Light microscopy

At two weeks the Ha implants were seen to be embedded in new bone which was in intimate contact with the surface of the material (Figure 43). By twelve weeks Ha projecting into the marrow space was completely encapsulated by a shell of bone which was in continuity with, and appeared to have grown down from, the endosteal surface of the cortex (Figure 44).

The responses to set Ketac Cem and G338-based GICs were similar to that for Ha except that a more pronounced periosteal reaction was noted at two weeks (Figure 45). At both six and twelve weeks, the normal marrow anatomy was restored and the architecture of the bone surrounding the GIC showed a regular trabecular pattern with evidence of normal remodelling and no intermediate layer separating the material from the bone (Figs. 45-48).

After six weeks, Mp4-based GIC, and to a lesser extent AquaCem, exhibited a less intimate relationship with the bone, there being interposition of fibrous and/or marrow tissue between the proliferating cancellous and cortical bone and the material (Figure

50).

The application of wet Ketac Cem to the pre-drilled bur hole proved technically difficult (when mixed as directed for a dental cement, the mix was too fluid). Examination of the sections showed that the majority of the material had remained on the surface of the bone. At two weeks the Ketac Cem followed the surface contour and was intimately related to the underlying bone, which exhibited a thin, darker staining layer at the interface with the Ketac Cem (Figure 51). By six weeks there was evidence of extensive periosteal reaction and subperiosteal bone resorption /remodelling and the intimate relationship between the bone and the Ketac Cem seen at two weeks was no longer present along the entire interface. The remodelling and proliferation of new bone was still evident at twelve weeks, as shown in Figs. 52 and 53.

FIGURE 43

Ground section of femur with Ha (Calcitite) implant at two weeks, showing integration of Ha particle in cortex and a thin shell of new bone (orange) surrounding separate particle in marrow. (Original mag. x10; Stevenel's blue/van Gieson).

FIGURE 44

Ground section of femur with Ha implant at twelve weeks, showing Ha particles intimately covered with a thin shell of new bone (red) and surrounded by normal marrow (blue). (Original mag. x20; Stevenel's blue/Alizarin red S).

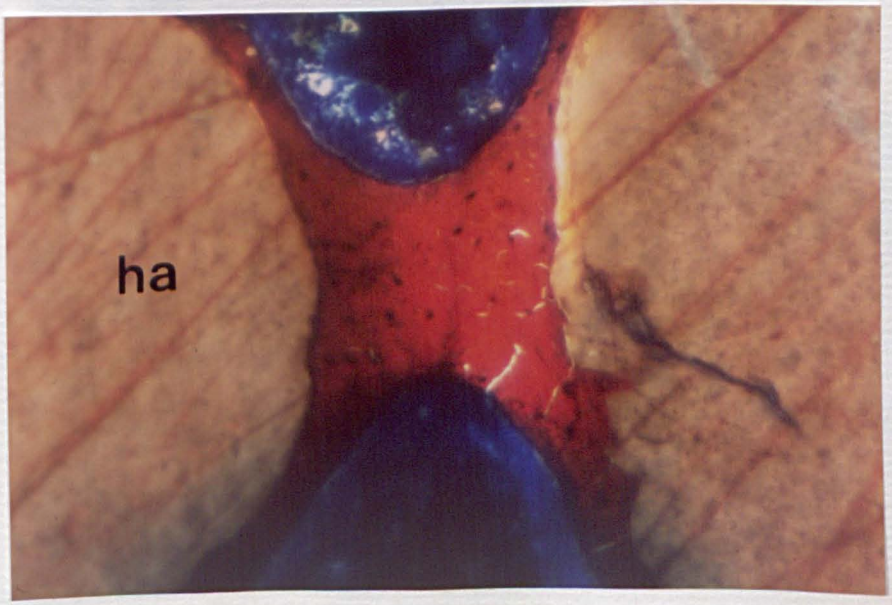


FIGURE 45
Ground section of femur with Ketac Cem (kc) implant at two weeks, showing periosteal reaction (arrow) and proliferation of bone (red) to cover surface of material. (Original mag. x10; Stevenel's blue/Alizarin red S).

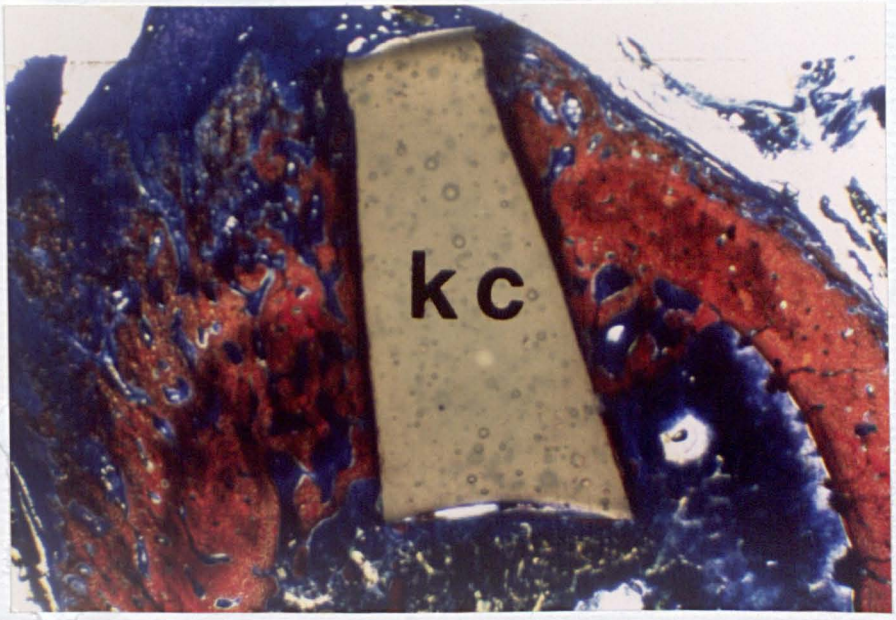


FIGURE 46
Ground section of femur with Ketac Cem (kc) implant at six weeks, showing integration of Ketac Cem and restoration of normal surrounding bony architecture (red). (Original mag. x10; Stevenel's blue/Alizarin red S).

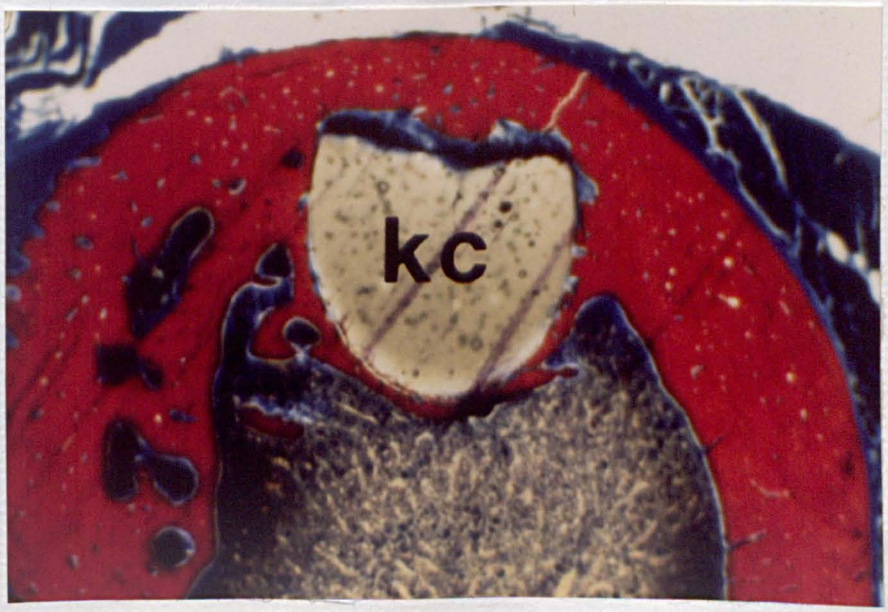


FIGURE 47
Ground section of femur with Ketac Cem (kc) implant at twelve weeks, showing complete integration of Ketac Cem with bone. (Original mag. x10; Stevenel's blue/van Gieson)

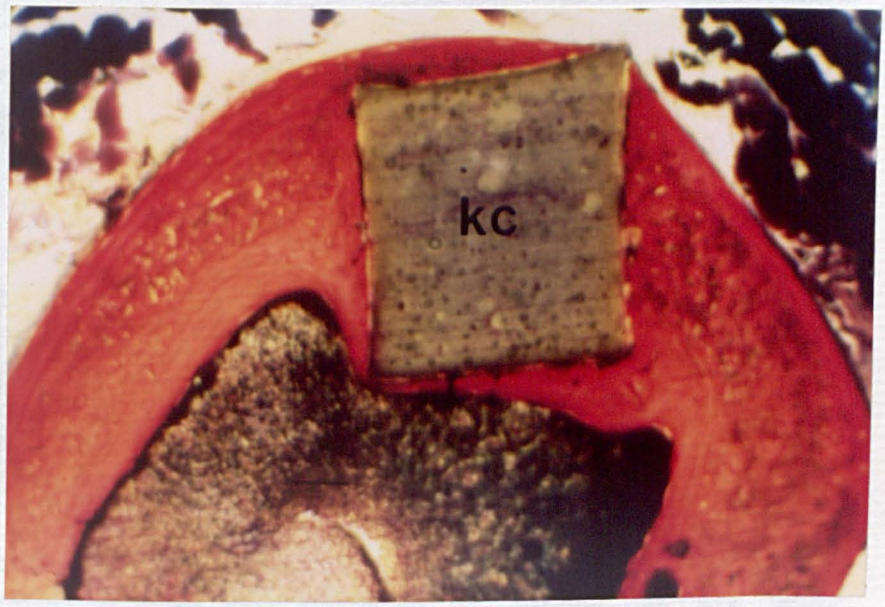


FIGURE 48
Ground section of femur with G338 implant at twelve weeks, showing integration of G338. (Original mag. x10; Stevenel's blue/van Gieson)

FIGURE 49
Semi-thin section of femur with Ketac Cem (kc) implant at six weeks, showing interface of Ketac Cem with bone. (Original mag. x200; Toluidine blue)

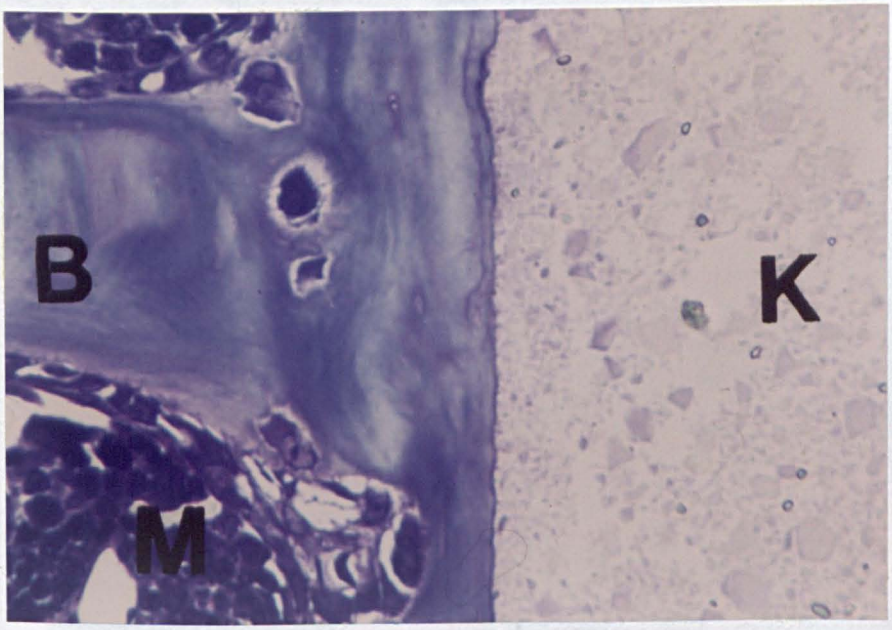


FIGURE 50
Ground section of femur with Mp4-based GIC implant at six weeks, showing minimal downgrowth of endocortical bone around implant and interposition of non-calcified cellular tissue (blue) between bone (red) and implant. (Original mag. x10; Stevenel's blue/Alizarin red S).

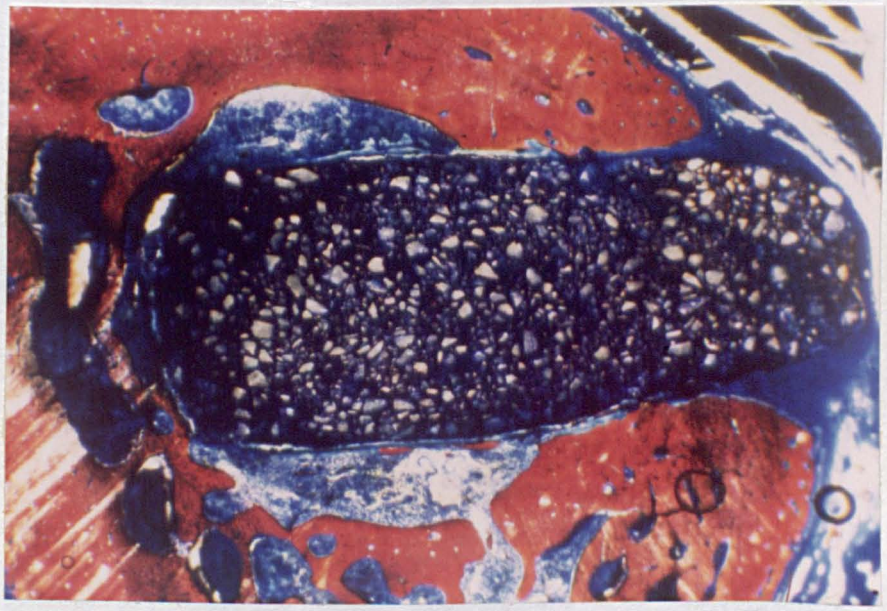


FIGURE 51
Ground section of femur two weeks following placement of wet Ketac Cem (K) onto surface of the bone. The GIC is intimately related to the surface of the bone, a darkly staining line being visible at the interface (arrow). (Original mag. x25; Stevenel's blue/van Gieson)

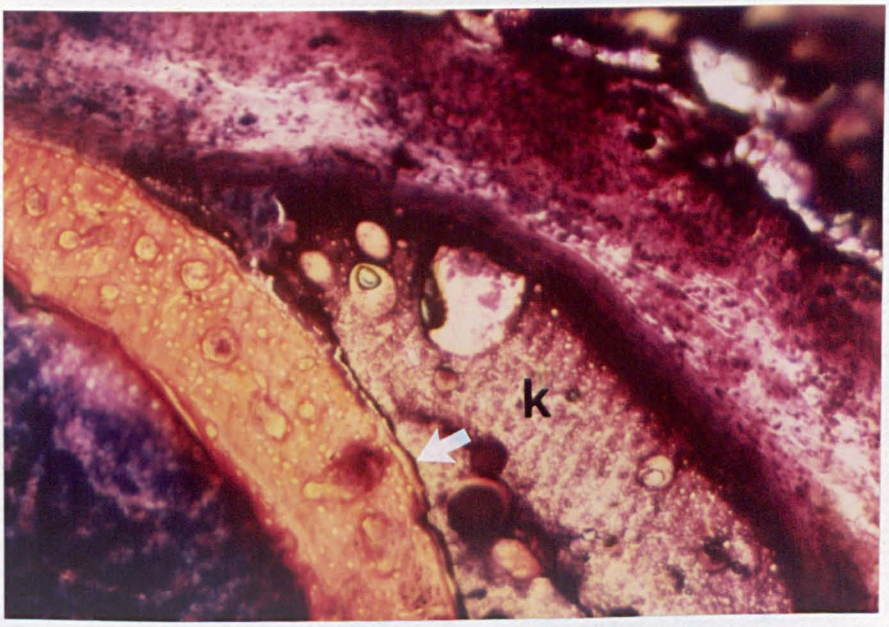


FIGURE 52
Ground section of femur twelve weeks following placement of wet Ketac Cem (kc) onto surface of the bone. The periosteum has reformed (p) and new bone (n) has proliferated at the edge of the implant. Extensive remodelling at the interface of the implant and surface of the bone is occurring and Howship's lacunae containing osteoclasts can be seen (arrows). (Original mag. x25; Stevenel's blue/van Gieson)

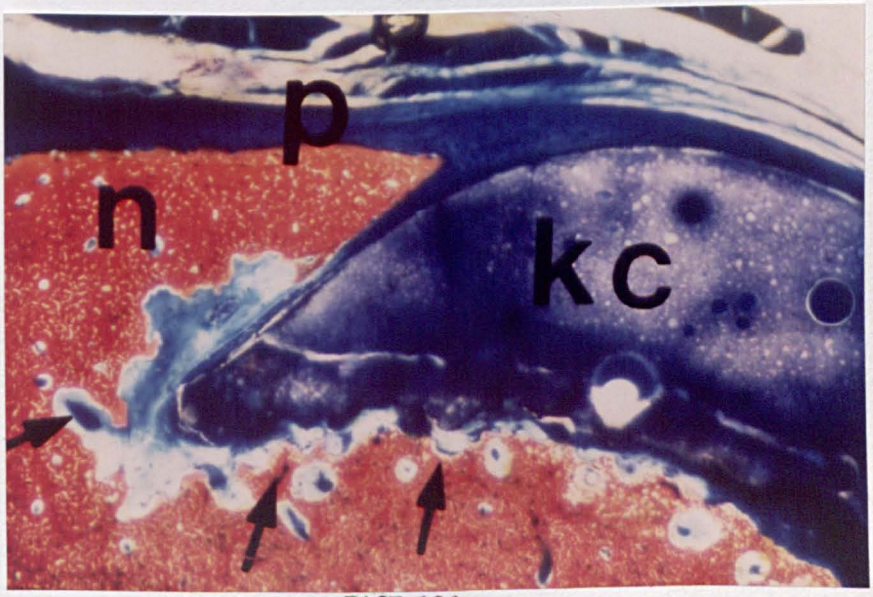
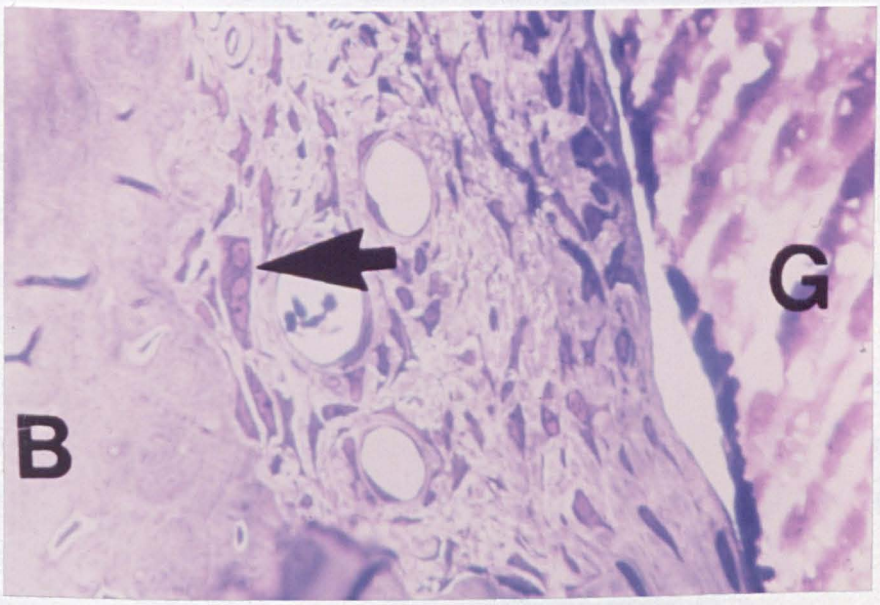


FIGURE 53

Semi-thin section of femur twelve weeks following placement of wet Ketac Cem (G) onto surface of the bone. Remodelling at the interface of the implant and surface of the bone (B) is occurring, osteoclasts (arrow). (Original mag. x200; Toluidine Blue)



Ultrastructural

The ultrastructure of GIC was characterised, X-ray microanalysis (EDAX) confirming the elemental structure (Figure 54).

The GIC/Tissue interface was preserved in both decalcified and undecalcified sections. The apparently intimate association between GIC implants (Ketac-Cem and G338) and bone seen at the light microscope level was confirmed at the ultrastructural level (Figures 56-58). A collagen-containing extracellular matrix was laid down directly upon the surface of the GIC and there was evidence of mineral deposition (Figure 56 and 59).
At the interface, the direction of the incorporated collagen fibres were noted to change from being orientated parallel to the material surface to become randomly arranged (Figure 57) with some interdigitating with cracks in the GIC (Figure 59).

The ultrastructure of the bone/GIC interface following placement of wet GIC and allowing it to set *in situ*, confirmed the light microscopic findings (Figs. 50 and 51) and at six weeks there was evidence of a zone of non-vital bone which was undergoing degeneration (Figure 60) and concomitant repair (Figure 58).

FIGURE 54

Cross-section through Ketac-Cem GIC showing glass particles (G) surrounded by siliceous hydrogel (arrows) set in a hydrogel matrix (M). Low magnification and high magnification scale bar 1um.

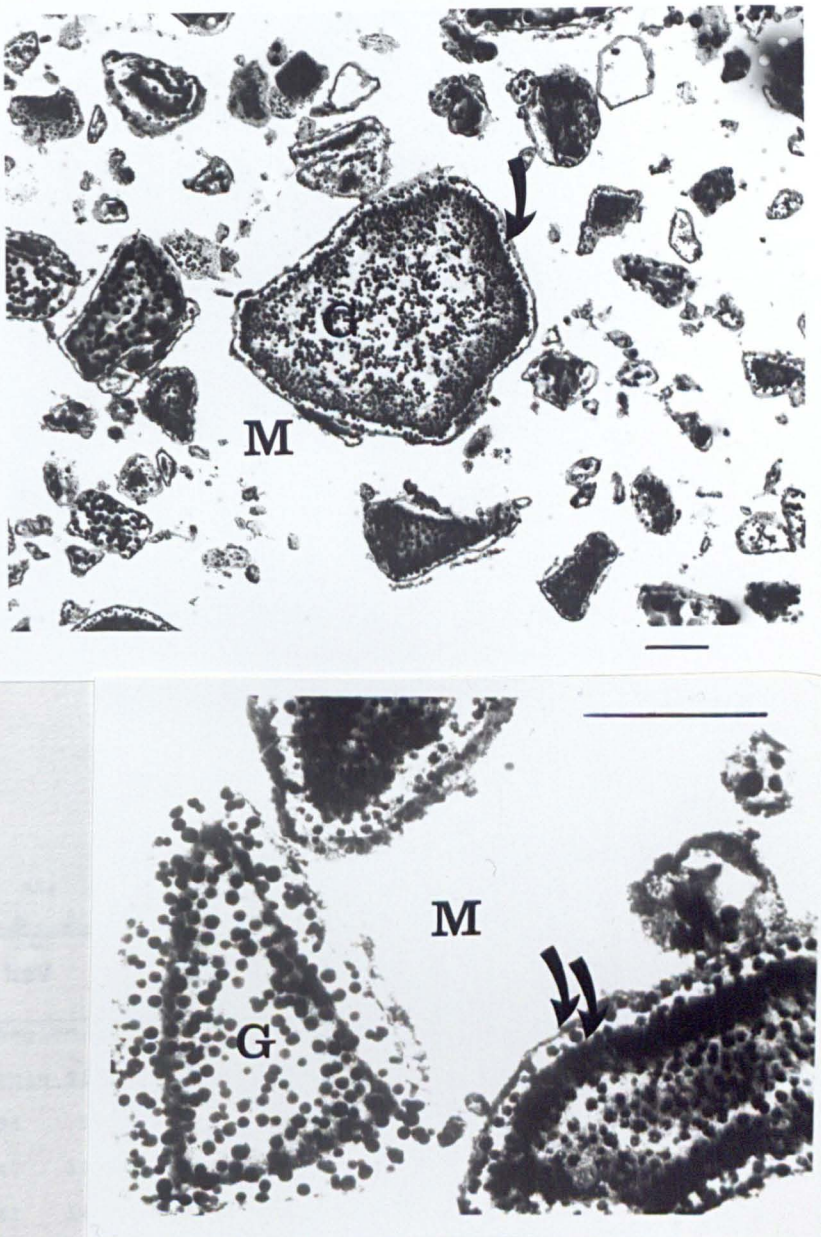
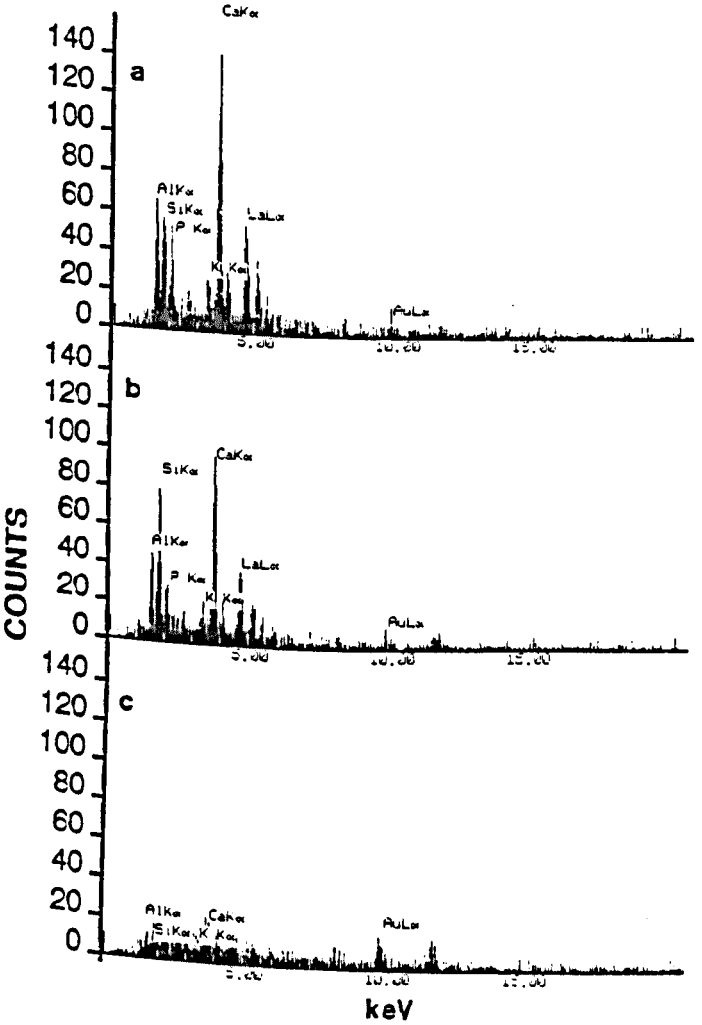


FIGURE 55

Energy spectra from different regions of set Ketac-Cem (figure 54).
a. Spectrum showing composition of glass particle.
b. Spectrum from region of siliceous hydrogel surrounding glass particle
c. Spectrum of hydrogel matrix between glass particles.



Element	Region/counts per second		
	Glass particle	Si-rich layer	Matrix
Na	1.93 ± 0.54	0.66 ± 0.39	0.18 ± 0.16
Al	29.01 ± 6.47	10.95 ± 2.90	0.40 ± 0.04
Si	30.88 ± 4.41	14.54 ± 2.46	0.61 ± 0.34
P	12.64 ± 2.14	6.68 ± 1.35	0.88 ± 0.42
Ca	22.99 ± 4.04	12.87 ± 2.17	0.76 ± 0.52

Relative detection of elements in different regions of set glass ionomer cement (G338). Results are the arithmentic mean of six analyses (± standard deviation) after subtraction of background radiation.

FIGURE 56

TEM photomicrograph of the interface between Ketac Cem and newly-formed bone after 6 weeks implantation and partial decalcification.

Presumptive osteocyte (O), exhibiting eccentric nucleus and glycogen granules (arrow), incorporated into a collagen-containing extracellular matrix (M), areas of mineralization can be seen (small arrows) and decalcified areas appear 'watermarked' (W). Ketac Cem (G S H) was retained during processing. (Original mag. x 6,600).

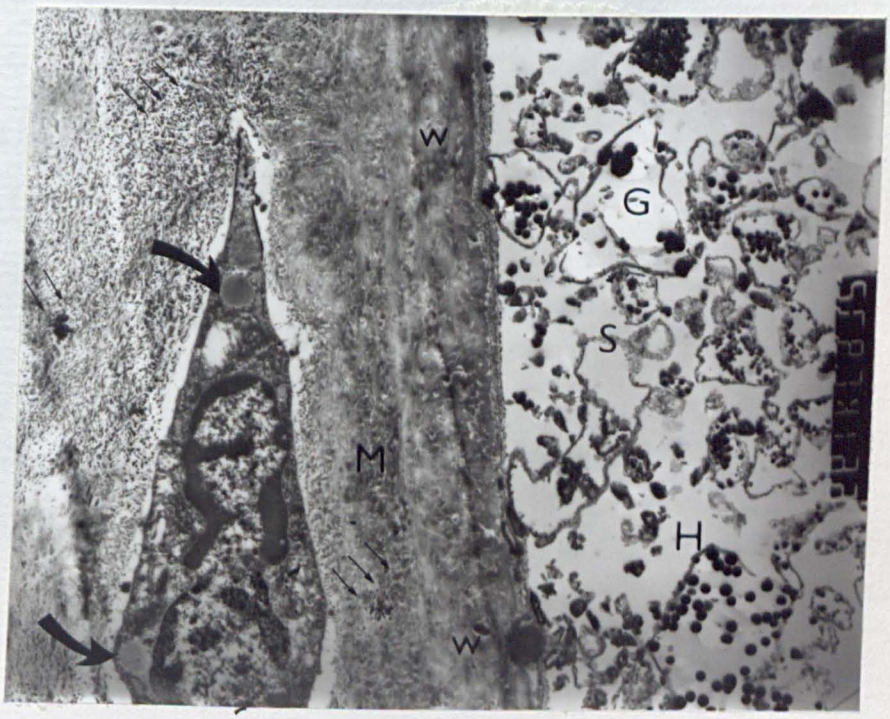


FIGURE 57

Detail from (Figure 56) showing orientation of collagen fibres (arrows) with the surface of Ketac Cem (KC) (Original mag. x21,000).

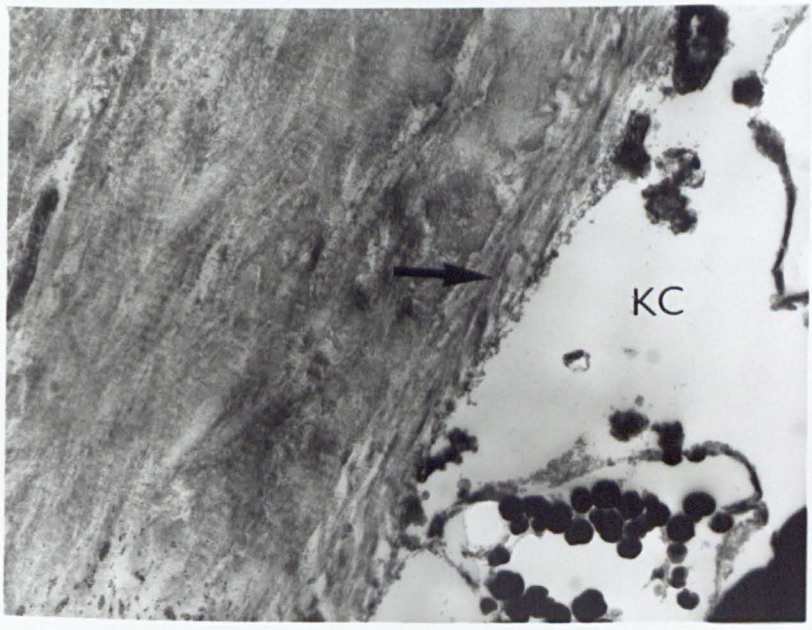


FIGURE 58

TEM photomicrograph of the interface between Ketac Cem (KC) (placed when wet) and bone after 6 weeks implantation.

The disrupted interface appears to be undergoing remodelling with a cell **(C)** embedded in a collagenous extra-cellular matrix. (Original mag. x28,500).

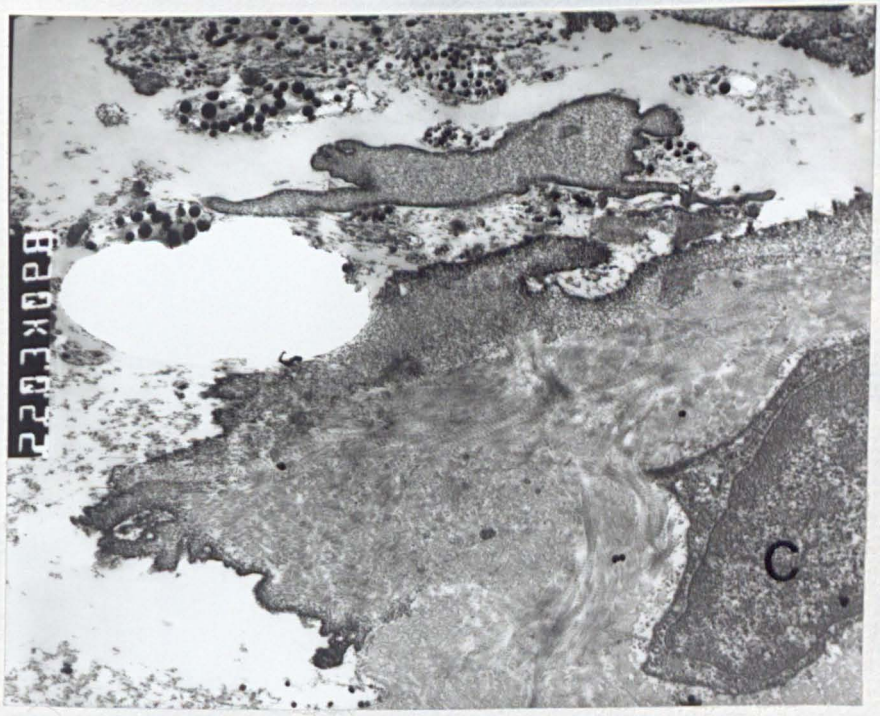
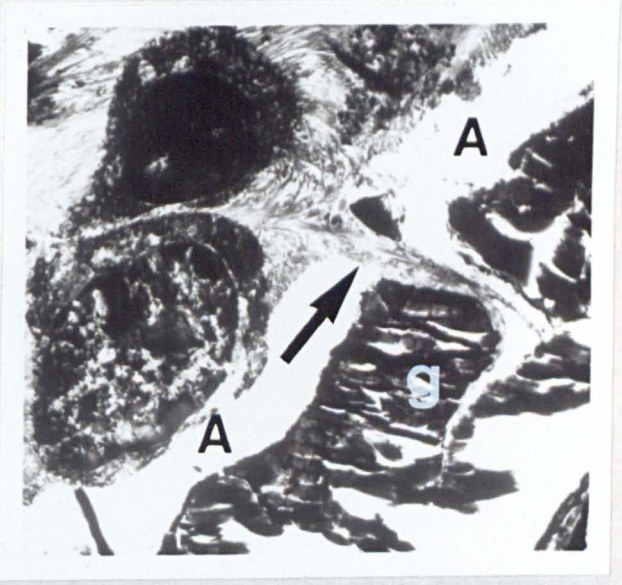


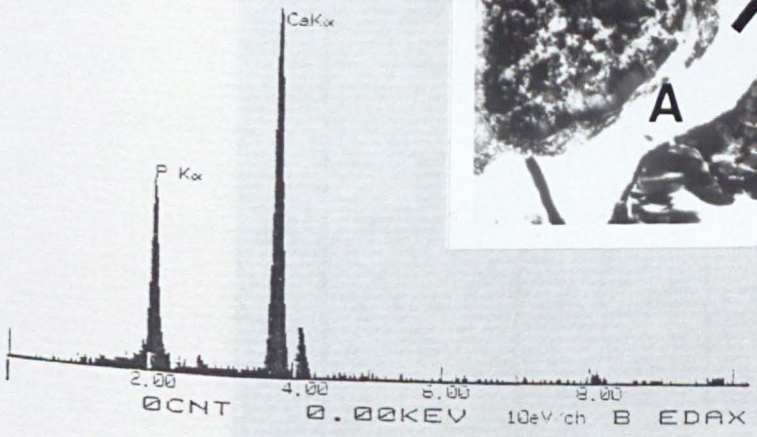
FIGURE 59

TEM photomicrograph of an undecalcified cryo-fixed specimen following two weeks implantation of G338 (Original mag. 11,000)

A freeze artifact is present (**A**), but despite this a finger-like projection of the collagen fibres of the extra-cellular matrix into a crack in the GIC (**G**) can be seen (arrow).

a. EDAX spectra of cortical bone remote from the implant site (control)
b. EDAX spectra from mineralised extra-cellular matrix (bone) formed on the surface of G338. calcium and phosphorous peaks are present together with aluminium and silicon.





b

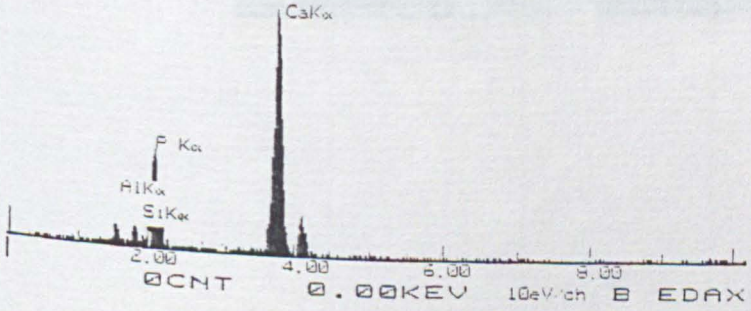
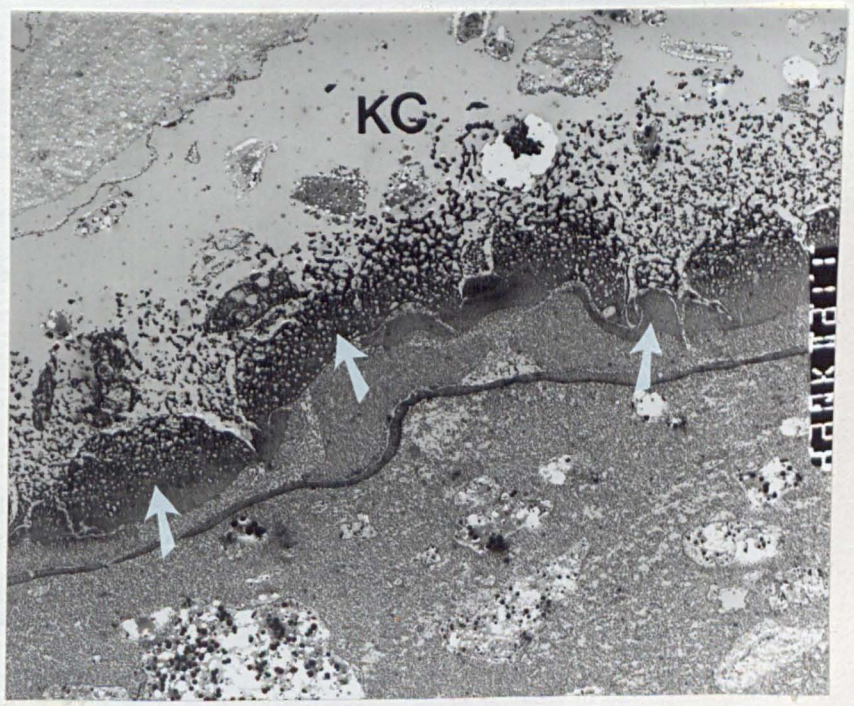


FIGURE 60

TEM photomicrograph of the interface between Ketac Cem (KC) (placed when wet) and bone after 6 weeks implantation.

An electron dense zone of tissue (arrows) undergoing breakdown is seen at the interface. (Original mag. x 11,800).



IIIiv. Histomorphometric results.

There were differences in the amount of new bone in contact with the implanted materials - length of the bone/implant interfacial contact (osseointegration), and in the amount of bone formed adjacent to the implants (osteoconduction) as assessed after six weeks implantation (Tables I and II).

The results for the rods of Mp4, G338, Aqua Cem and Ketac Cem are directly comparable; however, those for particulate Ha although included are not strictly comparable as the shape of the implant and any biomechanical effects may have been different with the particulate material.

Key to Tables

Implant Perimeter length mm = P

Length of intimate implant/bone contact mm = I

Amount of new bone round implant = A

% Osseointegration = I/P x 100

Osteoconductivity = A/P

(New bone / unit length)

TABLE I.

Shows the % osseointegration and the degree of osteoconductivity of Ha (Calcitite), and the GICs Mp4, Ketac Cem, G338 and Aqua Cem, at six weeks.

Material	P	I	A	I/Px100	A/P
Ha	2.9	1.93	0.86	82.5	0.3
	6.3	5.08	1.57	80.4	0.24
	4.5	3.84	1.93	85.3	0.42
	4.68	4.68	2.45	100	0.53
	5.05	5.05	1.72	100	0.34
	2.93	2.25	0.11	76	0.03
	2.68	2.13	1.02	79	0.38
Mean				86	0.32
S.D.				9.8	0.1

Material	P	I	A	I/Px100	A/P
Mp4	5.27	2.2	1.2	41	0.23
	6.48	0.95	0.81	14	0.16
	4.76	0.63	1.89	13.2	0.39
	6.18	2.59	1.51	41	0.24
	7.28	0	0.79	0	0.10
	6.65	0.44	0.69	6	0.10
	7.71	1.26	1.5	17.5	0.20
Mean				18.9	0.19
S.D.				16.1	0.10

Material	P	I	A	I/Px100	A/P
Ketac Cem	3.83	2.92	1.90	76	0.49
	3.47	3.41	1.38	98	0.39
	7.34	3.74	1.55	50.9	0.21
	8.16	7.03	3.02	86	0.37
	6.44	6.13	1.76	95	0.27
	7.94	7.94	3.25	100	0.41
	5.97	4.46	2.54	77.7	0.42
Mean				83.3	0.37
S.D.				17.2	0.09

continued-

(Table I continued)

Material	P	I	A	I/Px100	A/P
G338	6.65	5.46	2.32	82	0.34
	4.21	3.48	1.28	82	0.3
	5.38	3.77	2.01	70	0.37
	5.0	2.99	2.02	60	0.68
	6.01	4.6	1.84	77	0.40
	5.98	2.35	0.64	39	0.10
	10.76	5.84	3.54	54	0.32
Mean				66.3	0.35
S.D.				16.11	0.17

Material	P	I	A	I/Px100	A/P
Aqua Cem	6.75	3.92	1.25	58	0.17
	5.7	4.6	1.28	80	0.22
	5	1.38	0.78	27	0.15
	6.55	2.04	1.42	31	0.21
	7.4	3.01	0.69	40	0.09
	6.33	6.21	1.04	98	0.16
	6.82	5.06	1.31	74	0.91
Mean				58.3	0.17
S.D.				27	0.04

TABLE II

Summary of Mean and S.D for each material at six weeks implantation N=7.

Material	% Osseointegration I/Px100	Osteoconductivity A/P
Ha	86 +/- 9.8	0.32 +/- 0.1
Mp4	18.9 +/- 16.1 *	0.19 +/- 0.1 ***
Ketac Cem	83.3 +/- 17.2	0.37 +/- 0.09
G338	66.3 +/- 16.1	0.35 +/- 0.17
Aqua Cem	58.3 +/- 27 **	0.17 +/- 0.04 ***

* Rods of Mp4 were significantly less well integrated than other materials using the paired Student's t-test and Mann Whitney U test yielding P values of <0.005 and < 0.04 respectively.

** Rods of Aqua Cem were also less well integrated than rods of Ketac Cem, G338 and Ha particles and this was just significant when evaluated using the paired Student's t-test, Ha and Ketac Cem $P< 0.03$ and for G338 $P< 0.3$.

*** Rods of Aqua Cem and Mp4 were less 'osteoconductive' than rods of G338, Ketac Cem and Ha particles and this was just significant when evaluated using the paired Student's t-test $P< 0.02$

IIIv. Results / Discussion of mechanical evaluation

Setting / Working time

The mixing, setting and working times required for the surgical placement of GIC for use as a bone substitute in maxillofacial surgery were studied. Results of indentation studies are given in Table III and were carried out only on those GICs which appeared promising from the point of view of their biological evaluation (G338 and Ketac Cem). No attempt was made during this study to modify the setting/working time of cements except by the addition of tartaric acid (TA) to slow and sharpen the set of Mp4 (not shown) and G338-based GICs as, without the addition of TA at 10% concentration to the water, these GICs were unworkable.

TABLE III

Working / Setting Time Test

Times are in seconds from the START of mixing.

n=10

		Mean	S.D.
G338	Working time	73	4.7
	Setting time	133	8.5
Ketac Cem	Working time	182	15.5
	Setting time	222	9.5

For use as a bone substitute in maxillofacial surgery, in such situations as alveolar augmentation, the GIC should, ideally, mix to a paste which can be loaded into a syringe, or should be encapsulated for injection placement. Encapsulation and machine mixing would help to standardise the mix and is usually more rapid than hand mixing in effect allowing for a longer 'placement time'. At delivery the GIC should be of sufficiently high viscosity not to flow at the shear stresses employed in manipulation/placement, preventing undue spreading of material and enabling accurate placement at the implant site. Once placed, contamination by body fluids/blood will rapidly occur so a snap set is required. In achieving the above aims the mixing and working times are not critical but should be of sufficient length to allow manipulation. Under simulated surgical conditions this was in the order of 200 seconds, which is close to the setting time of Ketac Cem (Table III). However, the actual setting time needs to be known accurately so that placement can be timed to be followed immediately by a snap set.

Addendum.

Since this study was completed Ionos GmbH & Co KG have produced an encapsulated ionomeric cement based on Ketac Cem with a setting time of 210 seconds (manufacturer's data)

Set strength / Young's modulus

Maximum fibre stress and Young's moduli of rods of GIC nominally 4.6mm diameter 36mm long determined using four point loading.

TABLE IV

4 point bend tests carried out on Mayes SM 200 testing machine (British Calibration BS1610)

	Material	**Maximum Fibre Stress** M.Pascals +/- S.D.		**Young's Modulus** G.Pascals +/- S.D.		
1.	**Mp4** E3(4) TA vf .5 .2 .3	9.5	0.74	0.95	0.059	n=6
2.	**Mp4** E3(4) TA vf .33 .22 .45	12.7	0.68	0.99	0.032	n=5
3.	**Mp4** E3(4) TA vf .22 .35 .45	7.1	2.1	0.65	0.14	n=5
4.	**Mp4** E3(4) TA 10%Ha vf .31 .21 .43 .05	11.0	1.2	0.87	0.08	n=6
5.	**Mp4** E3(4) TA 20%Ha vf .3 .2 .4 .1	10.8	1.4	0.89	0.2	n=5
6.	**G338** E3(4) TA vf .5 .2 .3	18.2	3.3	3.0	0.49	n=6
7.	**Ketac Cem**	11.0	2.9	3.75	1.7	n=8

Key
vf = volume fraction
E3(4) code for polyacrylic acid used (The Laboratory of The Government Chemist)
TA = Tartaric Acid

The Young's moduli of the GIC evaluated ranged from 0.9 to 3.75 G.Pascals (Table IV). These values are within the range obtained for cortical and cancellous bone of 10-25Gpa and 0.09-0.2 GPa. respectively (97), although they are not the best values that can be obtained by modification of GIC formulation (Data, Laboratory of The Government Chemist).

The maximum tensile fibre stress of GIC in the series studied (18.2MPa) was, however, much less than that reported for bone at 50-150 Mpa. (97)

The samples tested were all cured for 48hrs and it is known that with time the set strength of GIC can be expected to improve as the setting reaction is a continuous process. Recent work on a G338-based bone cement has shown that, after 25 days in store under physiological conditions, the maximum fibre stress doubled and showed further improvement at 100 and 225 days; in contrast , the set strength of acrylic cement (Simplex bone cement) did not improve on storage (98). Set strength is, however, less relevant for the non-weight bearing role envisaged for GICs in maxillofacial surgery. Even when used to provide increased support for a removable denture, this will be tissue borne and the ability of the soft tissues to bear the stress of mastication will be the limiting factor. Indeed, it could be argued that bone substitutes intended for use in alveolar augmentation should be matched biomechanically to the soft tissues, rather than the underlying bone and this could be achieved using GICs. However, it is important for the GIC to maintain its integrity under masticatory load. The protective effect of overlying soft tissues and the ability of GIC to osseointegrate mean that it is difficult to predict

the actual strength and modulus required for clinical success. The main advantage of GICs is that it is possible by modifying the volume fractions of the various components, to alter their biomechanical properties (Table IV).

Increasing the volume fraction of glass material 2 compared to material 3 (Table IV), leads to an increase in strength until the volume fraction of glass is sufficiently great such that not all the glass is wetted and a porous material with lower strength results; (material 1. Table IV); these observations are in accordance with other reported work. (59,63,87)

Interestingly, the addition of particulate Ha to GIC (materials 4 and 5. Table IV) did not appear to affect the set strength greatly indicating the the Ha may have not purely acted as a filler but may have reacted with the polyacid. It has recently been proposed that during production part of the glass phase of commercial GICs changes to Ha during the melt (63)

IV. DISCUSSION

IVi. Biological evaluation of Glass-Ionomer Cement

Previous evaluation of the biocompatibility of GIC has concentrated on their dental applications. Existing commercial glass-ionomer dental cements have given promising biocompatibility results in cell culture and animal studies. (88,99-104) Biological evaluation has concentrated on the in vitro (100,101) and in vivo (74,102-104) responses of the dental pulp to GICs and in these studies the biocompatibility of GICs compares favourably with those of other types of dental filling material. (88,100) The clinical response of bone/periodontium to GICs has also been compared to traditional endodontic materials in investigation of GICs as possible endodontic sealers and again GICs compare favourably with other endodontic materials in current clinical use. (103,104) However, although a GIC (V-Os Espe W. Germany) has been evaluated as a possible cement for orthopaedic implants the ultrastructure of the bone/material interface has not been studied,(88,99) and no other GICs have been studied in the role of a possible bone substitute.

The biological evaluation of a new group of materials for clinical use is helped if there is an existing material or group of materials with which they can be compared. It is also useful if there are standard biological models in which the materials can be evaluated.

Unfortunately, the existing tests for evaluation of bone substitutes are concerned mainly with the concept of toxicity and are not appropriate for studying the detailed interfacial relationship between bone and the implanted material.

In this study the initial biological evaluation of GIC was undertaken in vitro using cell types which would be encountered by the materials in the in vivo situation. (80) Two in vitro models were used, both of which used primary cells, thus the problems of using cells which may have lost their phenotype following enzymatic digestion and/or multiple passaging was avoided. (76) Osteoblast cell lines that have been treated in such a way tend to form multiple layers on the test material rather than a monolayer. This compromises the evaluation of the material and does not recreate the in vivo situation because mineralised extracellular matrix is then produced in the intracellular spaces and not at the material interface. (76) In contrast the calvarial system allows the majority of cells to stay in their normal in vivo environment and has been shown to reproduce some of the known in vivo responses to ceramic implants and bioglass. (39,51,75,76) The calvarial system was thus felt to be suitable for evaluating the initial response of bone to GICs, the 'envelope' culture being particularly appropriate as a bone organ culture model with periosteal cells, bone cells and bone being present in the culture.

It is accepted that ideally, human/primate cells should be used for

materials intended for human use although it can be difficult to obtain primary cells and there are ethical problems in testing donors for infections such as Hepatitis B which is a precondition for the use of human cells in our laboratories. A novel in vivo culture system in a primate model - (Papio ursinus) closely related both evolutionary and haematologically to man was used in addition to the in vitro evaluation. (88) Although the results of studying the GIC V-Os in this model have been previously reported using light microscopy, evaluation at an ultrastructural level has not been previously undertaken. (88)

In vivo evaluation was undertaken to determine the initial response of bone and the interface achieved between bone and different GICs, and compare this with hydroxyapatite currently in clinical use. A model involving implantation into immature rat femora was thus undertaken. (52,105) These models allow rapid assessment of the initial response/healing of bone around the implanted material and study of the bone implant interface. (52,83,105-107) Healing in these model occurs rapidly as the animals are growing and the bone still remodelling.

IVii. *In vitro* evaluation.

The *in vitro* model used was based upon the ability of osteoblasts to migrate, colonise and lay down collagen fibrils on denuded bone, and on foreign material placed on the surface of bone in culture. (108)

Several research groups have used this phenomenon to evaluate the biocompatibility of calcium phosphate ceramics and bioglass. (39,78,51,80-82,109,110) By careful choice of culture conditions and tissues, together with the addition of ascorbic acid and B-glycerol-2-phosphate to culture media, *in vitro* bone formation can be induced. (57,58,82,111)

In the current study, the responses of bone cells to materials in current use clinically in Oral and Maxillofacial Surgery as bone substitutes (Hydroxyapatite and Tricalcium phosphate) were undertaken concurrently with the evaluation of novel GICs. This allowed a comparison to be made between materials in successful clinical use with the GICs and afforded an opportunity to further evaluate the interaction of these ceramics with bone, attention being paid to the ultrastructure of the bone/material interface.

The response of the bone cultures to Ha and TCP as seen in Figures 10,11,13,16-19 and 22 were similar to the responses reported by other authors using similar in vitro models and confirms the suitability of these materials for use as bone substitutes. (39,51,76-82,109,105,111) Osteoblast-like cells were seen to colonised the ceramic particles, regain their phenotype and their ability to produce ECM containing collagen that in places appeared morphologically to be new bone/osteoid (Figures 13,30-32). The typical tessellated appearance of polygonal osteoblasts can be seen in figures 18 and 19b and the difficulty of distinguishing the cells lying on bone from those situated on the Ha or TCP is demonstrated by figure 17.

The interface produced between the cells and ceramics in the envelope cultures was either analogous to the normal lamina limitans of bone (54) (Figures 13,30b and 31) or direct contact of the collagen fibres of the ECM with the surface of the ceramic (Figs 30a and 32). Both types of interface were noted in association with the Ha ceramic but only the second type was seen associated with the TCP ceramic.

The lamina limitans has been reported to be due to the laying down of organic material on the surface bone on completion of mineralization and is seen in the walls of osteocyte lacunae. (54)The finding of an electron dense zone at ceramic/bone implant interfaces has been described by other research workers at the

interface of bone/Ha in vivo (53,112). The electron dense lamina limitans-like interface has been reported to be a less active, more matured interface, which occurs when mineralization is complete. (53,54) It is, however, possible that the electron dense layer is an artifact produced by demineralisation of bone since all our material for TEM was subject to some decalcification, as has been the material of others who have reported this feature (53,54,112). However figure 31 clearly shows the lamina limitans like zone in both fully decalcified and partly mineralised areas of the section, which tends to refute the artifact hypothesis.

The direct contact of the collagen fibres of the ECM and the ceramic implants is seen in figures 13,30a and 32 and here there is absence of a lamina limitans like zone. A possible explanation for this finding is loss of cell anchorage, upon which cell growth and migration is dependent, caused by dissolution of the ceramic by the culture fluid. (Ref 81, figure 23) In the more protected 'envelope culture' where the material was placed under the periosteum, new bone formation occurred on the surface of TCP ceramic, (Figure 32) though without the demarcation of a lamina limitans like zone, this may indicate perhaps a more bioactive rather than an inert interface. (34,53,81,112)

The response of the bone cultures to GIC showed more variation than that seen to the ceramics. This can be explained by the more bioactive nature of GICs and by their more complex composite formulation. GICs are formed by the reaction of an acid soluble

ionomeric glass with an aqueous solution of polyalkenoate acid. The acid chemically attacks the glass particles, leaching metal cations (Al^{3+}, Ca^{2+}, and Na^{+}) out into the matrix. Gelation of the GIC is thought to occur through the reaction of these cations with the acid groups on the polymer chains and is shown diagrammatically in figure 61. (59,63) In the unset state, before gelation of the GIC has occurred, free polyalkenoate acid and metal cations will be available to react with the cells in the bone culture and this could explain the toxic response seen to freshly mixed and wet, Ketac Cem and Aqua Cem (figures 15,25,28a). When GIC were mixed and allowed to set against a mould, a smooth surface was produced composed of a matrix-rich layer containing few glass particles. (59) The set rods of GIC were produced in this manner and the smooth surfaces of the GIC rods made from the glasses Mp4, G338 and Ketac Cem were colonised and induced a response from the bone cultures identical to that of Ha/TCP ceramics. The typical response to the smooth surface of GIC is seen in figures 12,14,20 and 21) and can be compared to the similar response seen for the Ha ceramics (Calcitite and Interpore) (Figures 10,11, and 16-19). Osteoblast-like cells migrated onto and colonisation of smooth surfaces of the GICs and behaved in a similar manner to that seen associated with normal bone and the ceramics studied here. The appearance of migratory bone cells (pre-osteoblasts) and their close association to the surface of the GIC Ketac Cem is seen in figure 24b.

On fracturing GIC, more glass particles are exposed to the cells. Some of the irregularly fractured surfaces of GIC were not colonised

by cells (Figures 24a) and in the case of the fluoride-containing GICs Ketac Cem, G338 and Aqua Cem, this appeared to be a result of inhibition of cell growth rather than an inability to colonise an irregular surface, as was observed for certain areas of TCP. Figure 27 shows a zone of cell lysis around a small particle of GIC, the appearance being analogous to the zone of inhibition produced on a bacteriological plate by diffusion of antibiotic from a test paper disc. The GIC Aqua Cem, which is more soluble than G338 or Ketac Cem-based GIC (Personal communication A.D Wilson), provoked cellular breakdown in set form (Figures 15,25 and 28a).

The glass components of G338, Mp4 and Ketac Cem were evaluated separately to study the response of cells to the glass phase of the GICs. A toxic response was induced by the G338 and Ketac Cem glass particles (Figure 25e) similar to that provoked by the more soluble Aqua Cem (where breakdown of the material released free glass particles) was seen (Figure 28a). However, it seems that the response is dose-related, or relies on an interaction with the culture media. In 'envelope cultures' contaminated with Ketac Cem glass particles there appeared to be no evidence of cytotoxicity (Figure 28b). In contrast to the other GICs studied, the Mp4-based GIC (a fluoride-free GIC), and Mp4 glass when studied in isolation, produced little or no cellular death (Figures 26b). The polyacrylic acid component of the GICs under test was also added to cultures and in the small quantities used there was no qualitative evidence of inhibition of cell growth (Figure 26c).

Previous *in vitro* studies of GICs based on fluoro-alumino-silicate

glasses have reported a mild toxic effect and it has been suggested that the unfavourable response may be due to a leachate, possibly fluoride, (100,101,113,114) which is known to be released from set GIC. (59 and 115-117) The lack of any toxic response to set rods or fractured particles of Mp4/E3(4) GIC or MP4 glass particles alone, which do not contain fluoride, supports this hypothesis.

The interface between GICs and the tissues in vitro 'envelope culture' was similar to the direct contact of collagen fibres of the ECM with the materials surface as seen for the ceramics (Figures 29) and similar to the reported interface of the bioactive glass in vivo. (75)

The in vitro response to freshly mixed GIC was also studied, as the placement of GIC in the unset or partly set state is of clinical interest. Previous studies using human pulp cells growing in vitro immediately adjacent to the material have demonstrated that fresh GICs are toxic to pulp cells. (114) Although fluoride release may again be responsible for this effect, in the unset state it is possible that free metal ions could be released from the surface of the glass into the culture media. Contamination of the GIC by an aqueous environment would exacerbate this effect. (59, 115-117) In this study, freshly mixed GIC and set Aqua Cem were similarly toxic to cells. However it has been reported the osteoblasts at the edges of the bone culture are able to survive the effects of the fresh GIC but do not migrated over the material at two weeks. (118) This may be been due to continued inhibition of cell growth/division or due to the few cells surviving the initial contact with the unset GIC.

The GIC Aqua Cem produced a toxic response in both types of culture causing cell death immediately adjacent to the material. Although the general composition of Aqua Cem and Ketac Cem (both type II GIC luting cements) is similar, because both are commercial materials their exact compositions were unknown. It is assumed that the combination of glass and alkenoic acid in Aqua Cem was sufficiently different from Ketac Cem to produce a different, and in the case of Aqua Cem toxic, effect. The more soluble nature of Aqua Cem may also contribute to its less favourable biocompatibility.

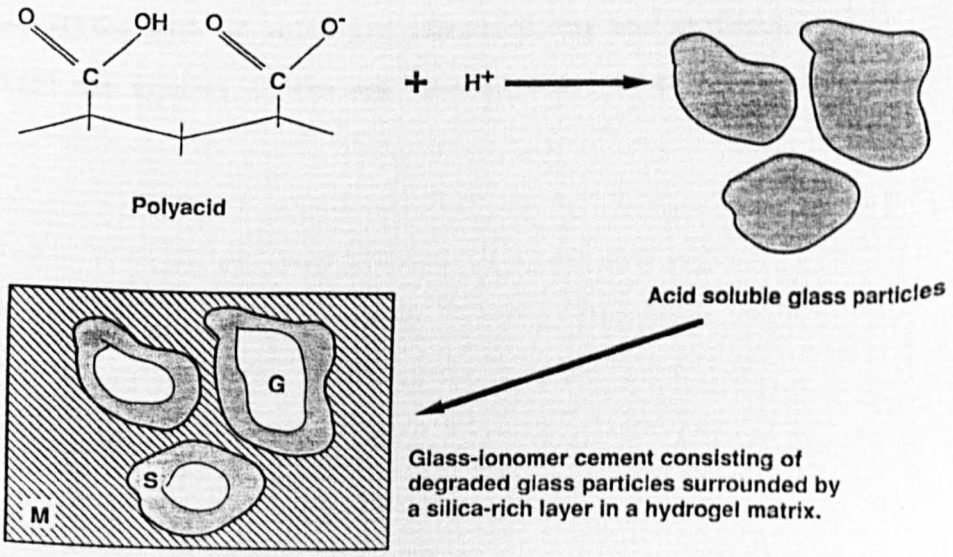
IViii. **Characterization and X-ray microanalysis of glass-ionomer cements.**

Examination of the TEM sections from the diffusion chambers and invivo model revealed that it was possible to retain the GICs during processing (Figures 42 and 56).

The existing structural model of GICs suggests that they consist of a glass core sheathed by a siliceous hydrogel matrix. (59,119) Visible support for this model has been provided only by three scanning electron microscope studies. (119-121)

Figure 61.

The relationship between the setting reaction and structure of a glass-ionomer cement. GICs are hybrid materials consisting of inorganic glass particles in an insoluble hydrogel matrix; formed from the neutralisation reaction of basic glass powder and polyalkenoate organic acid.



The cohesive forces which maintain the structure of the GIC matrix are presumed to be a mixture of ionic cross-links, hydrogen bridges and chain entanglements. (59) The theoretical structure of GICs is largely based upon this reaction sequence and SEM studies of fracture surface morphology. (119-121) The ultrastructure of the GIC Ketac-Cem is shown in figure 54, and the physical arrangement of the material is in accordance with the theoretical model in figure 61. (59, 119-121) The elemental spectra for G338 and Ketac-Cem are shown in figure 55 and a similar except that lanthanum was detected throughout Ketac-Cem, it being added to render to radiopaque for dental use. The XRMA analysis confirmed that ions from the glass phase are present in the matrix of the GIC aluminium, calcium and silica being detected. (Figure 55) Ion mobility during the setting of GICs and subsequently probably account for the tissue responses seen produced by GICs. (63,122-124) The movement of ions from set G338 GIC implanted into a rat femora is demonstrated in figure 59. Unfortunately fluoride could not be detected by our XRMA TEM but calcium and silica both of which are important for the nucleation of apatite (125) are present in the new bone adjacent to the GIC.

IViv. *In vivo* primate bone culture model.

The response of bone cells to set particulate V-Os GIC (set Ketac-Cem) in diffusion chambers implanted into the primate model has been described previously. (88) The chambers were for the most part filled with collagenous extra-cellular matrix interspersed with fibroblast-like cells. (Figures 33-37 and 40) This is similar to that seen on culturing human bone marrow cells in diffusion chambers implanted into the rat in the absence of test materials. (126) In places osteoblast-like cells were seen lining the surface of the GIC (Figures 37 and 39b). The biological response was thus similar to that seen for the GICs Mp4, G338 and Ketac Cem in the *in vitro* culture models using rat calvaria. In the diffusion chambers examined, the presence of the GIC V-Os (similar to Ketac Cem) did not inhibit active tissue proliferation and differentiation. Histologically, the V-Os appeared inert, being well tolerated by the newly-formed tissue within the chamber. Unlike Jonck (88) who reported the evaluation of these chambers at the light level we did not observe evidence of cartilage formation. This could be attributable to the few chambers examined or to differences in interpretation, as extensive evidence of metaplastic bone formation was found within the body of the chambers (Figures 34 and 35). This new bone formation appeared to be associated with both the pieces of transplanted autogenous bone and the GIC V-Os particles. The transplanted bone itself was non-vital but appeared to be acting in an osteoconductive manner (Figures 33 and 35).

SEM examination revealed osteoblast-like cells on the surface of the V-Os with a similar morphological appearance to those seen on the ceramics Ha/TCP and smooth surfaces of GICs placed in the calvarial culture model (Figures 16-22 and 38-40), and similar to that reported by others of rat osteoblasts which have colonised hydroxyapatite / bioglass. (75,39,51,82 and 109) Thus, it appears that the primate cells had accepted the surface of the GIC V-Os implant as a 'bone substitute'.

TEM examination of the contents of diffusion chambers was hampered by the overlong fixation time. Careful examination, however, showed that a collagen-containing extra-cellular matrix was elaborated directly onto the surface of V-Os granules, the interface being a direct contact of collagen fibres with the surface of the GIC V-Os, with the fibres running at right angles to the surface (Figure 41 and 42), in places an indistinct granular zone was also noted, however it was not a clear as that seen in the in vitro culture model (Figure 42). The GIC/biological interface was thus similar to that seen in envelope culture model for Mp4-based GIC (Figure 29) and TCP/Ha (Figures 30-32).

IVv. *In vivo* implantation

The *in vitro* and *in vivo* tissue/organ culture studies showed that certain formulations of GIC were well tolerated by living cells, with evidence that some GICs allow attachment of osteoblasts cells becoming well spread and forming an intimate bond with the GIC in a similar manner to the reactions reported for Ha ceramics and bioactive glasses studied in similar biological models. (51,72,74,81,109 and 127) However, *in vitro*, GICs based upon fluoro-alumino-silicate glasses appeared to release a mildly cytotoxic leachate. This finding has also been noted by others who also observed that the toxic effect of the leachate rapidly diminished with time, as the cement fully sets. (100,101 and 105) The nature of the toxic agent has not been defined but has been postulated to be fluoride ions released from the GIC. (124,128)

The GIC Mp4 (alumino-silicate glass-based) gave subjectively the best biological response in the *in vitro* evaluation of the GIC tested. Mp4 could thus be postulated to be the basis for development of a GIC for *in vivo* implantation. However the *in vivo* evaluation of the different GICs using the rat femora model gave unexpected results. Qualitative evaluation of the *in vivo* implantation of set rods of GIC showed that the Ketac Cem and G338 rapidly became osseointegrated (Figures 47-49). Set rods of the GIC Mp4 and to a lesser extent Aqua Cem integrated less well than the other GICs or Ha (Figures 50, 43 and 44). The qualitative *in vivo* evaluations were confirmed by the histomorphometric analysis which,

even given the limited number of implants examined (7 for each material), showed statistically significant differences between the percentage osseointegration and percentage osteoconductivity of Mp4 and Aqua Cem compared to the GICs Ketac Cem, G338 and Ha (calcitite) the latter materials being superior to the former (Table II). These poor histomorphometric results for Mp4 can be accounted for by the fact that rods of Mp4 were in the main at six weeks surrounded by a thin layer of fibrous tissue rather than by bone (Figure 50).

An explanation for this difference between *in vivo* and *in vitro* responses can be derived by considering the action of fluoride on bone. Jonck et al. (88,99) proposed that slow fluoride release from a GIC-based bone cement may have a beneficial effect on *in vivo* osteogenesis, in a manner similar to the stimulation of osteoid formation by fluoride therapy in osteoporosis. (129) It has also been shown that fluoride administered via the drinking water to chicks and rats stimulates bone formation at low doses but inhibits it at higher doses. (64,123) This can be explained by the the formation of fluorapatite in the presence of fluoride ions rather that normal apatite pushes the dynamic state of bone formation/resorption to the left flourapatite being less easy to resorb at higher doses fluoride ions may act as an enzyme poison and thus inhibit bone cells directly. (64) It thus appears that there is an optimum dose of fluoride for stimulation of bone formation and, in a relatively closed *in vitro* environment, the more toxic effects of fluoride ions may be manifest. (100,101,105,110,113, and 114) In the *in vivo* situation, the leaching of fluoride may stimulate osseointegration and could account for the better response

seen for the fluoro-alumino-silicate GICs, Ketac Cem and G338, compared to the GIC-based Mp4 glass. The poor response to Aqua Cem could be explained by breakdown of this more soluble GIC and release of large amounts of fluoride from the increased surface area of exposed glass particles, or from release of metal ions from the matrix phase of the cement.

The differences seen in vitro and in vivo support the theory that inertness in a material may not be the ideal criterion for optimum biocompatibility. (68)

The biological response to Ha (Calcitite) and the GICs G338/Ketac Cem was similar (Figures 43-49) although subjectively it appeared that more bone was formed around the GICs compared to the Ha implants. Histomorphometric analysis although reflecting this trend did to reach significance with the small number of implants examined.

The experimental design can also be criticized, in that to compare Ha with GIC the same shape of implant should have been be used. Rods of GIC and particles of Ha were used in this study - the latter possibly being more mobile during the healing phase and thus reducing the amount of new bone formed around them. The better biomechanical compatibility of GIC compared to Ha (97) and the reduced stress shielding achieved with GIC (Young's moduli in the range 0.95-3.75 GP) compared to that of Ha (Young's moduli in the range 48-80 GP) would also have an effect (positive) on the response of the bone to GIC.

Thus, although the methods used were able to detect a difference between GICs, it is not possible to draw firm conclusions regarding the osseoconductivity of GICs as compared to Ha except in so far as that in this study, fluoride containing GICs (G338 and Ketac Cem) induced a similar response when implanted into rat femora to the ceramic Ha (Calcitite) and the response to Ha was as reported by others using similar models and following human implantation. (29,47,52,and 112)

Ultrastructure of the bone/GIC interface

The osseointegration of GIC with bone seen at the light level (Figures 46-49) was confirmed on TEM examination (Figures 56,57,59). Direct bonding of the bone matrix to the GIC Ketac Cem, without any intervening layer has been is clearly demonstrated in figures 56 and 57. The observed interface comprised of collagen fibres running at right angles into the material's surface and appeared as described for bioglass implanted into rabbit femora (130) and to the interface material/tissue interface reported for glass-ceramics, Ha and titanium. (34,53,95,107,112 and 127) It was also noted that in places where there were pores in the surface of the GIC implants there was a direct interdigitation of collagen fibres with the material (Figure 59).

In the limited number of GIC/bone interface sections examined, no evidence was seen of the thin electron dense or amorphous/granular zone which has been reported to separate bone matrix from

biomaterials used as bone substitutes. (34,53,112 and 131) The significance, or indeed absence, of this zone, which is thought to represent the lamina limitans of bone and was also noted in the in vitro evaluation of Ha (Figures 30b and 31), is not known. The lamina limitans is thought to occur on cessation of active bone formation, its thickness depending upon, and giving an indication of, the rate at which mineralization of the bone proceeds; a broad lamina limitans forms when calcification is slow. (53,54,112 and 132) The layer has thus been described as 'time dependant'. (133) Absence of a lamina limitans may thus indicate that active bone formation or resorption is occurring; however, examination of the specimens of set GIC rods at 6 and 12 weeks showed there was no evidence of osteoclastic activity and mineralised tissue was in intimate contact with the implant surface. It has been suggested that the presence of a lamina limitans-like zone may indicate that incomplete bonding has occurred. (130) Thus the absence of a lamina limitans like zone in the case of materials such as bioglass, tricalcium phosphate and the GICs based upon fluoro-alumino-silicate glasses as reported here, may indicate the formation of a more dynamic, but possibly less stable interface. (34,130,134 and 135) The in vivo evaluation of the bone/GIC bond, however, places GICs in a similar position to the 'Bioglasses', TCP and Ha ceramics such as 'Interpore' in that they form a strong bioactive bond with bone based on an intimate interdigitation of the collagen fibres of the mineralised ECM of bone with the material's surface.

The results of the EDAX examination of the in vivo bone/GIC (G338) interface using cryofixation undecalcified sections confirmed the

presence of mineralised tissue adjacent to the GIC implants seen at the light level using ground sections. EDAX also enabled identification of the components of the GIC (Figures 54,55 and 59). Evidence was also obtained for the movement of ions across the bone/GIC interface, aluminium and silicon being detected adjacent to the implant but not in bone remote from the implant site (Figure 59). It is likely that other ions including fluoride, calcium and phosphate are also exchanged. However, because of limitations associated with the EDAX available for use in this study elements with atomic weights below sodium could not be detected and the movement of phosphate and calcium ions wold be masked by the calcium and phosphate normally present in bone. The movement of ions does however support the theory that as with bioglasses GICs form a dynamic (bioactive) bond with bone rather than an ankylotic or inert bond. (34,36,44,55,134-137)

Evaluation of GIC set 'in situ'

One of the proposed advantages of a bone substitute based on GIC as compared with ceramics in current clinical use, is the potential for in situ gellation. (63) In this respect, the response to implantation of wet Ketac Cem is of interest. It appears that an initial intimate bond was formed with the bone, possibly due to chemical adhesion of the GIC to the bone apatite and adsorption of poly(acrylic)acid, by hydrogen bonding, onto the collagen matrix (Figure 51). (124 and 128) By six weeks, however, the intimate relationship had been lost and extensive bone remodelling was occurring (Figures 52,53,58 and 60) The surface of the bone below

the cement exhibited numerous areas of resorption as manifest by the presence of osteoclasts in Howship's lacunae (Figures 52 and 53)

A constant feature of the interface between wet GIC/bone at the ultrastructural level was a dark band at the main interface of the bone/GIC and this appeared to represent dead bone undergoing resorption. This zone had been noted by Jonck et al (88) at the light level and was attributed to the absorption of polyacid into the bone matrix.

The cells in the zone adjacent to the interface were however, active and there was evidence that new bone formation was occurring (Figs.52 and 58).

This sequence of events may indicate that, as seen in vitro, wet GIC causes a mild toxic response and although initial adhesion occurs (Figure 51) the release of free metal ions and the acidity/irritability of the alkenoic acid may produce damage; (Figures 52 53 and 60) the remodelling seen at six and twelve weeks reflecting repair of damaged tissue and the attempted establishment of a new bone/GIC interface.

V. CONCLUSION

Evaluation of GICs *in vitro* and *in vivo* has been undertaken.

In vitro the GIC Mp4 and set Ketac Cem show favourable biological responses as potential bone substitutes when compared to Ha and TCP ceramics, both of which are used successfully in clinical practice. GICs provided a surface for bone cell attachment, spreading, and deposition of extracellular matrix.

The role played by the fluoride component of GICs in determining the biological responses seen to GIC was demonstrated to be of importance.

In vitro there was evidence that the fluoride-containing GICs exhibited mild toxicity. However *in vivo* GICs formed an intimate bond with living bone. The ability of some GICs to leach fluoride appeared to enhance osseoinduction/integration.

The implantation of wet GIC initially produced an intimate bone/GIC interface but this was not stable.

The work reported here thus partially supports the hypothesis that:- **'Glass-ionomer cements are a class of materials suitable for use as bone substitutes and cements'** in that GICs based on fluoro-alumino-silicate glasses show potential for use as bone substitutes but further work is required before the potential of GICs for use as bone cements can be assessed.

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APPENDIX I

i. Program for analysis of mechanical four point bend test.

ii. Record cards for *in vivo* and *in vitro* studies.

```
  10 REM CALCULATION OF FRACTURE
 20 REM TOUGHNESS FROM FOUR-POINT
 30 REM BEND TESTS
 40 CLS
 50 C=1E3
 60 INPUT"NAME SAMPLE";A$
 70 NU=0:EAC=0:MSAC=0:MFAC=0:AA=0
 80 MF=0:MS=0:E=0:ESQ=0:MSSQ=0:MFSQ=0
 90 INPUT"DO YO WANT YOUR RESULTS PRINTING YES=2, NO=3";X
100 VDU(X)
110 PRINT A$
120 PRINT"Beam span, L=20mm":L=20
130 INPUT"Beam Diameter, d / mm",Di
140 R=Di/2
150 PRINT"Beam Radius, r =";R;"mm"
160 INPUT"Depth of notch, c / mm",Cu
170 INPUT"Deflection, (delta) / mm",De
180 INPUT"Load, P / N",P
190 Di=Di/C:Cu=Cu/C:De=De/C:R=R/C
200 REM MOMENT OF ENERTIA
210 I=(R↑4*3.14159)/4
220 REM YOUNGS MODULUS
230 ET=P*1.3066E-7:EB=De*I:E=ET/EB
240 REM MAXIMUM FIBRE STRESS
250 MFT=P*(20E-3-6E-3):MFB=R↑3*3.14159:MF=MFT/MFB
260 REM MAX STRAIN
270 MS=MF/E
280 IF Cu=0 THEN 310
290 PRINT"Notch Length / Diameter=";Cu/Di
300 PRINT"Normalised Diameter-Notch length=";(Di-Cu)/Di
310 PRINT"Maximum Fibre Stress =";MF/C/C;"MPa"
320 MFAC=MFAC+MF
330 NU=NU+1
340 MFSQ=MFSQ+(MF*MF)
350 PRINT"Maximum Fibre Strain =";MS*100;"%"
360 MSAC=MSAC+MS
370 MSSQ=MSSQ+(MS*MS)
380 PRINT"Young's modulus, E = ";E/C/C/C;" GPa"
390 EAC=EAC+E
400 ESQ=ESQ+(E*E)
410 PRINT"Log E = ";LOGE
420 PRINT
430 VDU3
435 AA=0
440 INPUT"Still the same composition (No=1)";AA
450 VDU(X)
460 IF AA=1 THEN 470
465 PRINT:GOTO130
470 PRINT:PRINT "Summation of data for composition:-":PRINT A$
480 PRINT"Average maximum Fibre Stress =";MFAC/NU/C/C;"MPa"
490 SDMF=((MFSQ-(MFAC↑2/NU))/(NU-1))↑0.5
500 PRINT"Standard deviation =";SDMF/C/C;"MPa"
510 PRINT
520 PRINT"Average maximum Fibre Strain =";MSAC/NU*100;"%"
530 SDMS=((MSSQ-(MSAC↑2/NU))/(NU-1))↑0.5
540 PRINT"Standard deviation =";SDMS*100;"%"
550 PRINT
560 PRINT"Average Young's modulus, E = ";EAC/NU/C/C/C;" GPa"
570 SDE=((ESQ-(EAC↑2/NU))/(NU-1))↑0.5
580 PRINT"Standard deviation =";SDE/C/C/C;" GPa"
590 PRINT
600 VDU3:GOTO60
610 REM
```

I.B Dept Oral/Maxfac Surgery

Date..... Expt code..... Date of Birth.....

Multiwell Plate layout

A1.....A2.....A3.....A4.....A5.....A6.....

B1.....B2.....B3.....B4.....B5.....B6.....

C1.....C2.....C3.....C4.....C5.....C6.....

D1.....D2.....D3.....D4.....D5.....D6.....

Type of culture Envelope.'E'... Open.'O'...

Materials

Sterilization proceedure

Culture medium..... Total culture time.....

Medium changes

1.	2.	3.	4.	5.	6.
7.	8.	9.	10.	11.	12.
13.	14.	15.	16.	17.	18.

****

Processing

Fixation date ..... Fixative .....
Buffer wash.....

Dehydration in Ethanol 10% 20% 30% 40% 50% 70% 74op Absol 1 2 3

Embeding resin..... Time 1 2 3

SEM .....

Decalcification.....

Ground section .....

Notes

Date of Surgery _____ **Total Time** _____ **End Date** _____

Dept of Oral/Mf Surgery University of Sheffield IMB PPL 50/00604

Identification Code [_____]

S Dawley M/F DOB. [ ][ ][ ].
Wistar M/F
Implant site / Number Femora [ ]. ST [ ]. Jaw [ ].

1. wt. 1 _____ gms. wt. 2 _____ gms.

Processed for Light H&E [ ] formalin
GROUND [ ]
SEM TEM [ ]
EDAX [ ]

2. wt. 1 _____ gms. wt. 2 _____ gms.

Processed for Light H&E [ ] formalin
GROUND [ ]
SEM TEM [ ]
EDAX [ ]

3. wt. 1 _____ gms. wt. 2 _____ gms.

Processed for Light HE [ ] formalin
GROUND [ ]
SEM TEM [ ]
EDAX [ ]

Material _____

Sterilisation AC.[ ] EO.[ ] ALCOHOL.[ ] Irradiation [ ].

Healing NOTE

Perfused Y/N Date _____

APPENDIX II

CLINICAL EVALUATION AND DEVELOPMENT OF OPERATIVE TECHNIQUES FOR PLACEMENT OF HYDROXYAPATITE IMPLANTS.

i. Local Alveolar Augmentation with Hydroxyapatite.

ii. Mandibular Ridge Augmentation.

iii. Maxillary Ridge Augmentation.

i. Local Alveolar Augmentation with Hydroxyapatite.

The two published papers included in this section describe the development of surgical techniques to improve ridge contour prior to the placement of fixed bridges.

The techniques described have been adopted widely and are taught to Dental Practitioners attending postgraduate courses.

The open techniques described have the advantage of increasing the amount of soft tissue cover as well as augmenting the alveolar ridge. However, the use of an open operation increases the morbidity of the procedure compared to 'tunnel or closed' operations, and are thus best limited to those cases involving one or two dental units where severe alveolar atrophy has occurred. More extensive 'local' augmentation may, if only an increase in ridge width or elimination of undercut is aimed for, be carried out using a tunnel technique. Alternatively the use of silicone 'spacers' as described for mandibular augmentation may be used.

The Use of Particulate and Block Forms of Hydroxylapatite for Local Alveolar Augmentation

I.M. Brook, B.D.S., M.D.S., F.D.S./
D.J. Lamb, B.D.S., M.D.S., F.D.S.

When replacement of a missing tooth or teeth is being planned, excessive resorption of alveolar bone may complicate the clinical problem. A simple, esthetic, fixed restoration can often be more easily made after local augmentation of the defect with hydroxylapatite via a palatally extended flap.

When a tooth is lost the supporting bone resorbs. If resorption is excessive and the resulting alveolar defect is visible, it can pose a problem. Sometimes a removable prosthesis is suggested as the only way to replace the missing tooth and alveolar bone. Patients often dislike this type of restoration (on both functional and esthetic grounds). A fixed prosthesis is usually preferred. Appearance is rarely ideal, because as a result of the degree of bone loss a pontic must either be longer than the adjacent natural teeth or must be inclined lingually to make contact with the tissues in an abnormal position. If the patient has a high lip line, these esthetic shortcomings may be objectionable. The solution is to restore the lost bone with a suitable implant material before making a conventional fixed restoration.

Hydroxylapatite has proved successful for augmentation of edentulous ridges prior to full denture prosthetics,[1] in periodontal surgery for replacing lost bone,[2] and for inhibiting alveolar resorption when placed in recent extraction sockets (as in the form of root replica implants).[3] Most recently, its use has been advocated for augmentation of local alveolar defects prior to fixed bridgework.[4]

During the process of manufacture, hydroxylapatite powder is sintered to a dense, microporous form which, although hard and brittle, is suitable for implantation.[5] Using this material in the form of blocks or rounded particles, local augmentation can be carried out. The two forms have different indications for use and require slightly different preoperative and operative techniques for best results.

This article describes a useful technique that allows local alveolar defects to be restored with hydroxylapatite. A palatally extended flap allows direct visualization and accurate implant placement. The modified flap design allows the necessary soft tissue cover for the augmented area without prejudice to sulcus depth.

Technique

Particulate hydroxylapatite. Whenever particulate hydroxylapatite (Calcitite 40-60, Calcitek Inc.) is used, case management is concerned with prevention or control of particle migration. The technique described here involves raising a buccally based flap and takes place in two stages:

Stage 1. At the first visit, using a diathermy point or scalpel, a semilunar incision is made down to bone, around the buccal periphery of the area to be augmented (Fig. 1). Over the next few weeks, as the incision heals, the resultant scar will act to hold down the deepest part of a surgically produced pocket at this site, and will prevent buccal migration of implanted particles.

Stage 2. Three weeks later a buccally based mucoperiosteal flap is raised. The incision is started at least 5 mm palatal to the defect and extended around the labial margin of the teeth adjacent to the space (Fig. 2). A pocket is created over the defect and filled with wetted hydroxylapatite particles using a sterile amalgam carrier or a curette (Fig. 3). After replacing the flap to check that the contour has been restored it is sutured into place. The flap design allows increased soft tissue cover while preserving the depth of the buccal sulcus. Any residual gap at the palatal margin is left to granulate (Fig. 4). Although the design of this flap is found to limit particle loss, further precautions should be taken. If the patient has a denture it is first adjusted to fit (which usually involves removing the labial flange) and then lined with a periodontal pack (Coe-pack, Coe Laboratories, Inc.) over the site of operation. Alternatively if there is no den-

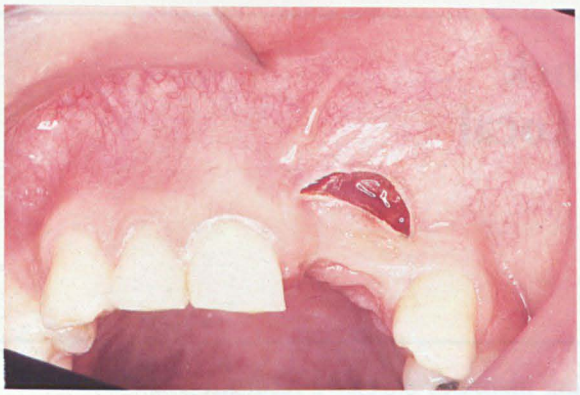

Fig. 1 Incision around the buccal margin of area to be augmented. The tissues are not raised but left to allow scar formation.

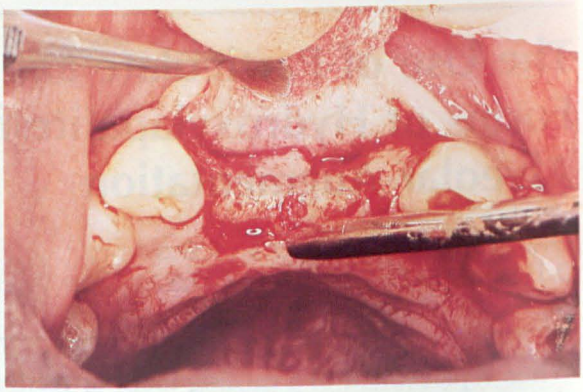

Fig. 2 Palatally extended flap designed to gain access to the alveolus and create a subperiosteal pocket.

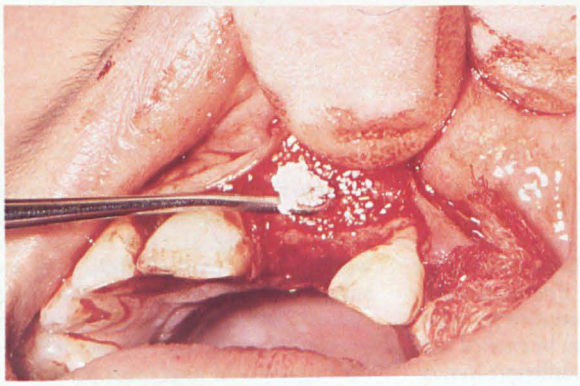

Fig. 3 Placement of hydroxylapatite particles.

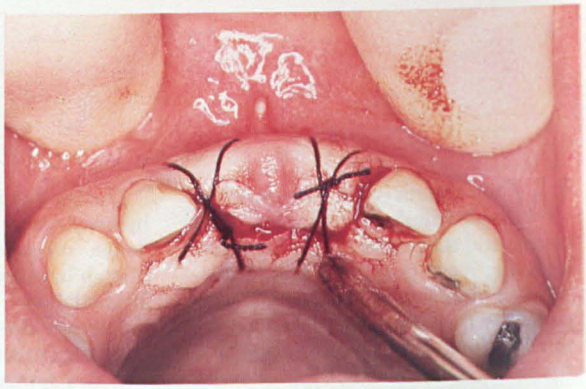

Fig. 4 Flap sutured following placement of block hydroxylapatite. The small defect at the palatal margin is due to the increased size of the augmented ridge that is left to granulate.

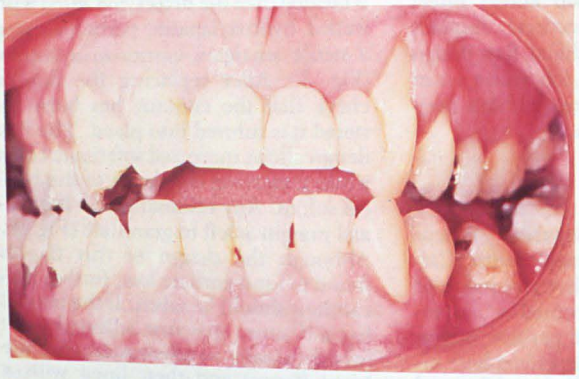

Fig. 5a Preoperative appearance prior to augmentation.

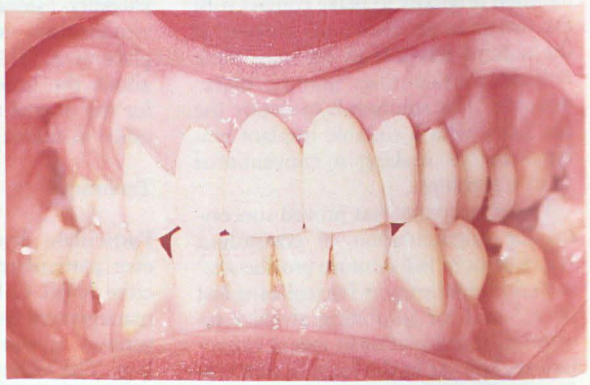

Fig. 5b Postoperative appearance 6-month postaugmentation with a fixed restoration in place (a lateral periodontal flap repair to tooth 11 has also been undertaken).

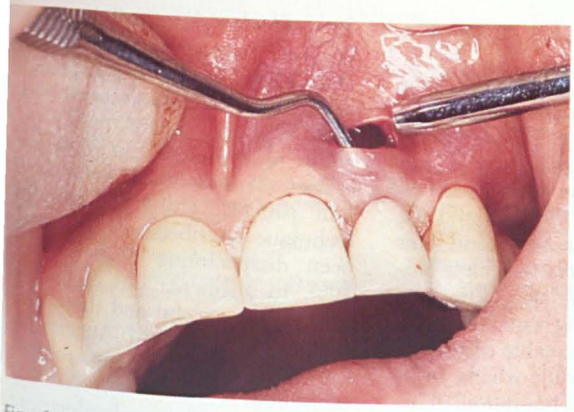

Fig. 6a Buccal incision for placing hydroxylapatite particles via a tunnel technique.

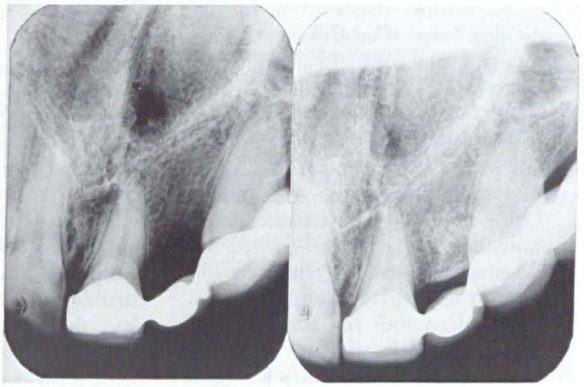

Fig. 6b Pre- and postoperative radiographs of hydroxylapatite particles inserted via a subperiosteal tunnel for augmentation of area beneath a pontic.

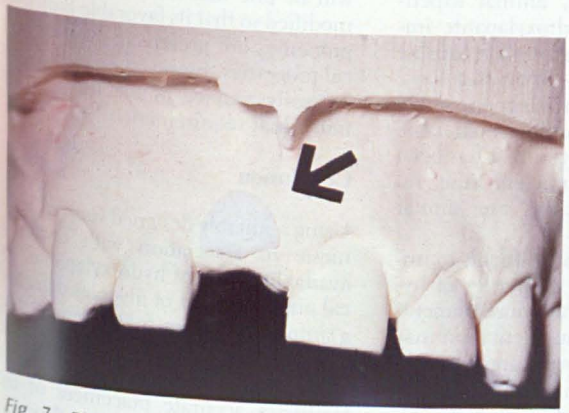

Fig. 7 Block of hydroxylapatite carved to restore the alveolar contour.

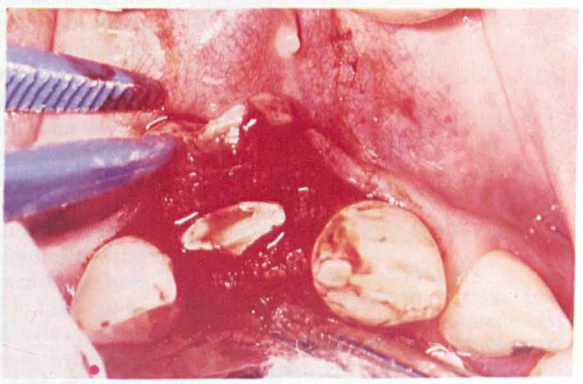

Fig. 8 Placement of hydroxylapatite block.

ture the implant site is protected with a periodontal pack attached to the teeth alongside.

Finally, a five-day course of penicillin, given orally, is prescribed (erythromycin instead of penicillin for sensitive patients) and an appointment made for one week later. At this time the sutures are removed. If the healing is progressing satisfactorily an appointment is made for three weeks later, when—if the ridge is firm—restorative procedures can be started.

This technique is best utilized for replacement of a single tooth. If used for more than one or two teeth, the degree of augmentation possible is disappointing as the central part of the flap is unsupported (Figs. 5a and 5b). A modification of the technique is possible when a bridge is already in position and bone resorption has caused an unsightly gap to appear at the cervical margin of the pontic. In this instance the particles are inserted blind via a tunnel procedure. An incision is made high in the buccal sulcus above the site. After raising a tunnel, hydroxylapatite particles are introduced with a sterile amalgam carrier and molded into shape (Figs. 6a and 6b).

Block hydroxylapatite. The use of a solid block of hydroxylapatite minimizes the opportunity for implant migration and, by supporting the flap, prevents loss of contour due to collapse. Block hydroxylapatite is therefore indicated for augmentation when a wide defect exists due to the loss of several teeth. While it is unnecessary to make a limiting scar at a preliminary visit, the hydroxylapatite blocks must be shaped beforehand to fit a model of the implant site. The hydroxylapatite can be cut with a diamond disk and, while it need not be an accurate fit against the model, it must be capable of bridging over the defect and restoring the normal contour of the model (Fig. 7). The preparation time is sometimes prolonged because hydroxylapatite is brittle and tends to chip unexpectedly. After preparation, the shaped pieces are ultrasonically cleaned, autoclaved, and retained until required.

At the time of operation a flap is raised that is the same shape as that used for the particulate form. The defect is exposed and the shaped implant accurately placed under direct vision

(Fig. 8). Further stages are as for the particulate form. The area is protected by a periodontal pack and an antibiotic is prescribed. When the defect is especially deep or the hydroxylapatite blocks do not fit well, a small amount of the particulate form can be used to obturate any defects.

Using dense hydroxylapatite, local augmentations have been carried out at 28 sites in 16 patients who were followed up for 5 to 34 months. Maintenance of contour has been assessed by study models and in all cases the ridge/pontic relationship has been maintained. Radiographic follow-up has revealed no detectable loss of any material.

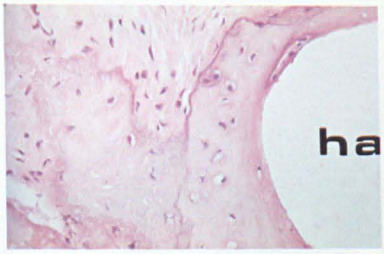

Fig. 9 Photomicrograph of decalcified section of human alveolus 18 months after augmentation with Calcitite 40-60 (hematoxylin-eosin × 450). ha = space once occupied by hydroxylapatite particle, now bordered by newly formed metaplastic bone arising from stromal cellular connective tissue (Courtesy of Dr. G. Craig).

Discussion

In the past, the results following augmentation of alveolar bone have proved disappointing. While short-term results using autogenous[6] or homografted bone[7] have been good, resorption has always followed and the long-term results have been poor. This has caused researchers to seek a biocompatible, nonresorbable substitute capable of integrating with bone. To date the most acceptable discovery has been hydroxylapatite which, in its dense form, will resist resorption.[1] This is not to imply that the material is osteogenic—implants of hydroxylapatite into the soft tissues of experimental animals show no evidence of new bone formation. However, animal experiments show that hydroxylapatite implants in subperiosteal sites are capable of acting in an osteo-conductive way,[5] that is they can act as a biocompatible obturating material onto which bone can be laid. More recently it has been shown that, given sufficient time, results in human beings are similar (Fig. 9).

At present it appears difficult to improve the biological properties of hydroxylapatite and future developments in the field of bone augmentation may well involve improved surgical techniques to prevent implant migration. The physical properties of hydroxylapatite will also be improved to allow easy modification of its shape to suit surgical needs. At present only one form of hydroxylapatite (Interpore 200, Interpore International) can be carved at the chairside. This is a form derived from coral and its interconnected porous structure imparts sufficient friability for it to be cut to shape with a scalpel. This form has been used in four patients. Using the placement technique described here, results have been disappointing (Figs. 10a and 10b). In augmentations at five sites, two were successful and three had to eventually be removed. Anaerobic infection was diagnosed on microbiological testing.

With the continuing development of implantable forms of hydroxylapatite the ideal form required for present requirements will one day be found. It will be one that has been structurally modified so that its favorable biological properties are preserved. Its mechanical properties, however, will allow it to be easily adapted or machined to the individual requirements.

Conclusion

Using a suitably designed surgical technique in association with currently available forms of hydroxylapatite, local augmentation of alveolar defects is a simple and successful technique. Use of the palatally extended flap allows the alveolus to be inspected directly which facilitates accurate placement of the implant. On replacing the flap, increased soft tissue cover is provided to the implanted area. □

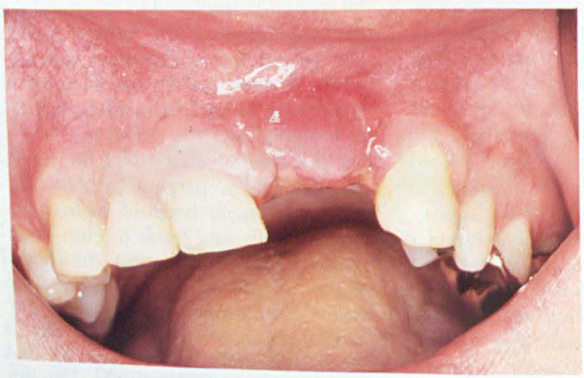

Fig. 10a Sinus formation over porous hydroxylapatite implant 2 months postplacement.

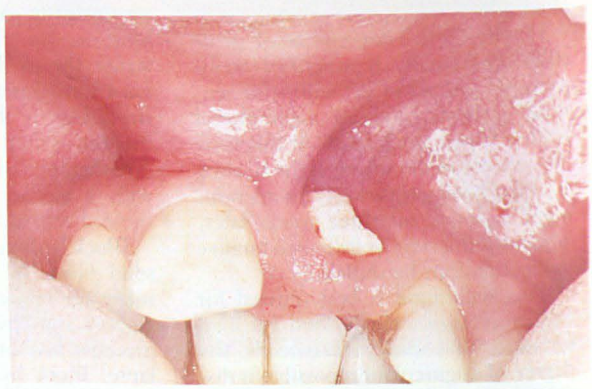

Fig. 10b Porous hydroxylapatite exfoliating 3 months postplacement.

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Erratum

In the article "Osseointegration and Facial Prosthetics" by Parel, Holt, Brånemark, and Tjellstrom the wrong column headings were set in Table 1. The corrected Table 1 is reprinted below.

Table 1 Summary of experience with osseointegrated fixtures in Göteborg and University of Texas

	Fixtures placed		Fixtures removed		Abutments removed	
	Göteborg	San Antonio	Göteborg	San Antonio	Göteborg	San Antonio
1977	3	0	0	0	1	0
1978	10	0	0	0	0	0
1979	4	0	0	0	0	0
1980	25	0	0	0	0	0
1981	12	0	0	0	0	0
1982	60	2	2	1	3	0
1983	84	24	0	2	0	0
1984	45	37	0	0	0	0
Total	**243**	**63**	**2**	**3**	**4**	**0**

Prosthodontics—The State of the Art 1987

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Dr. S. Hobo, *Tokyo, Japan*
Dr. B. Bergman, *Umea, Sweden*
Dr. I. Klineberg, *Sydney, Australia*
Dr. W. Klotzer, *Marburg, West Germany*

FRIDAY, SEPTEMBER 18
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Dr. T. Maruyama, *Osaka, Japan*
Dr. J. Pameijer, *Amsterdam, The Netherlands*
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Free Communication: Research in Prosthodontics
Table Clinics: Titles of Table Clinics will be published in the final program.

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Prosthodontics: Education

Lecturers
Dr. H. Tsuru, *Hiroshima, Japan*
Dr. L. Crawford, *Sydney, Australia*
Dr. R. McGregor, *Glasgow, United Kingdom*
Dr. J. DeBoever, *Gent, Belgium*
Dr. J. Preston, *Los Angeles, California*

Free Communication: Clinics in Prosthodontics
Treatment Planning: The following clinicians will discuss different treatment methods for the same case: Dr. H. Tanaka, *Morioka, Japan,* Dr. E. Budtz-Jörgensen, *Copenhagen, Denmark,* Dr. P. Fuchs, *Munich, West Germany,* Dr. F. Neurohr, *New York, New York,* Dr. F.J. Kratochvil, *Los Angeles, California,* Dr. G.F. DiFebo, *Bologna, Italy*

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Implantology

Correction of local alveolar defects by implantation of hydroxyapatite: a preliminary study

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Br Dent J 1986; **161:** 68

Preliminary results are presented of five patients for whom local alveolar bone loss has been corrected by implantation of hydroxyapatite. By utilising a palatally based flap, increased soft tissue can be made available to cover the restored ridge. The technique appears applicable when replacement of a missing tooth by a bridge is being considered and an alveolar defect compromises good appearance.

Hydroxyapatite (HA) is a commonly occurring mineral. It is found in the skeleton of most vertebrates and constitutes up to 65% of the mass of cortical bone and 98% of dry dental enamel. Elsewhere in nature it is found in large deposits as the skeletal remains of coral and is mined as a principal source of phosphate for a variety of uses. After extensive studies, it has been shown to be biocompatible,[1,2] and continuity between implanted HA and bone is quickly established.

Dense HA has been used for total and partial alveolar ridge correction utilising a 'tunnelling' submucous vestibuloplasty approach[3,4] and for filling periodontal defects.[5] In the form of manufactured root replicas it has been placed in extraction sockets, in order to reduce alveolar resorption.[6]

This paper presents a technique for restoring local defects of the alveolus by use of a rounded form of polycrystalline HA (Calcitite)* prior to restoration of missing teeth. Calcitite is available in two sizes of particle, Calcitite 20–40 and 40–60 mesh; in this study the finer 40–60 mesh was used.

After loss of a tooth, alveolar resorption will follow. When resorption is excessive, replacement of a missing anterior tooth by a bridge can result in poor appearance. Either the cervical margin of the pontic must be inclined lingually to achieve the correct relationship of the cervical margin with the resorbed ridge, or the pontic must be elongated axially (fig. 1). In neither case will the appearance be acceptable to a demanding patient or to one with a high 'smile line'. The alternatives of adding pink porcelain to the cervical margin of the pontic or making a partial denture can be equally unacceptable on the grounds of aesthetics or oral hygiene difficulties. The ideal treatment would often be to correct the ridge deficiency with HA and restore the missing unit or units with a conventional bridge.

Technique

Following routine history, examination and radiography, alginate impressions are taken of the dental arches and the alveolar deficiency assessed by means of study models. The area to be corrected is waxed up on the model (fig. 2), and an idea gained not only of the necessary extent of any implant required to produce an ideal contour, but also the amount of any implant material needed.

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

The surgical procedure is undertaken in two stages. First the buccal extension of the future implant is limited by creating a band of fibrous tissue on the alveolus. This is achieved by drawing a diathermy needle or scalpel point around the planned buccal periphery of the implant. (In two cases this preliminary procedure was combined with other pre-implant procedures: fraenectomy and apicectomy.) The incision is taken down through periosteum to bone, but at this stage no attempt is made to raise the tissues. When healing is complete and the blood supply from the buccal tissues re-established (normally after two weeks), the second stage is undertaken.

A one-sided full-thickness flap based on the buccal tissues is raised starting at least 5 mm palatal to the alveolar crest (fig. 3). The incision is carried forwards around the buccal gingival margins of the teeth adjacent to the area to be corrected (fig. 4). A subperiosteal pocket is created over the defect and the alveolar deficiency inspected directly. The pocket is filled with HA (fig. 5) until repositioning the flap shows that the alveolar contour has been restored.

The particles of HA are transferred to the implant site with a spoon excavator or a clean, sterile amalgam carrier and pressed carefully into place. Manipulation is made easier if the particles are made to adhere to one another by moistening them with saline or sterile water. If they become very wet and uncontrollable, a dry dental cotton wool roll is used to remove excess blood.

The flap is then replaced over the corrected alveolus. Owing to the increased ridge size, the flap will be seen to have advanced buccally and a denuded area left palatally (fig. 3). The flap is sutured in its new position, and if a single tooth is missing, a periodontal dressing can be placed over the denuded area and retained by the standing teeth. Alternatively, if the patient has a partial denture, then, after suturing, the denture can be relieved over the implant site and relined with soft liner or periodontal dressing.

Post-operatively, patients are instructed to maintain good oral hygiene with a soft toothbrush, and the use of a warm saline mouthwash is recommended. A five-day course of phenoxymethyl penicillin is prescribed in all cases.

Sutures are removed on the seventh day, and restorative procedures begun when the area is clinically healed and firm.

Case Report

Differential diagnosis

Juvenile periodontitis and chronic osteomyelitis were considered in the differential diagnosis. The patient's age, the relative absence of periodontal disease in relation to the teeth on the left side, and the radiographic appearance of the right mandible were features considered to be against the diagnosis of juvenile periodontitis. Although the history, clinical findings and radiographic appearance were suggestive of chronic osteomyelitis, the distribution of the lesions was unusual and no predisposing factors were found.

Comment

Eosinophilic granuloma of bone is a benign disorder, but in the multifocal form, and depending on the site, may be responsible for considerable morbidity. In adults the prognosis is excellent, and the presence of additional bone lesions or subsequent generalised disease is rare. However, a full physical examination and a skeletal survey with either radiographs or radioisotope scanning are necessary to detect any additional lesions at the initial presentation. After treatment which is usually in the form of surgical curettage followed by irradiation, the patient should be followed up for several years. It has been suggested that physical examination and radiographic skeletal survey should be carried out when indicated at three monthly intervals during the first year and after this the interval can be increased if the patient remains disease-free.[5] Local irradiation was not considered in this case because of the accessibility of the site for clinical and radiographic examinations and for surgery in case of recurrence of the disease.

Acknowledgements

I am grateful to Professor R. O'Neil for his permission to report this case and to Professor P. G. Isaacson for the photomicrograph.

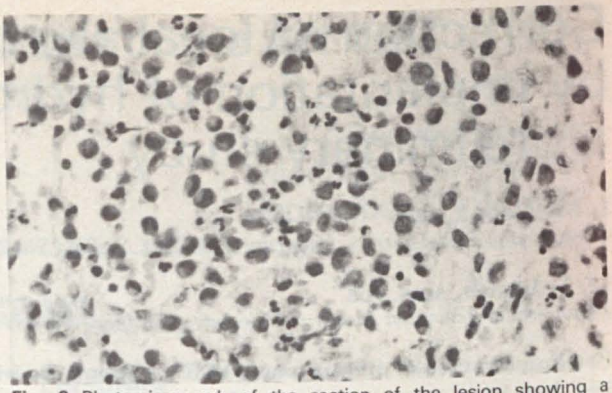

Fig. 2. Photomicrograph of the section of the lesion showing a collection of histiocytes with deeply folded nuclei and eosinophils (H. and E. × 600).

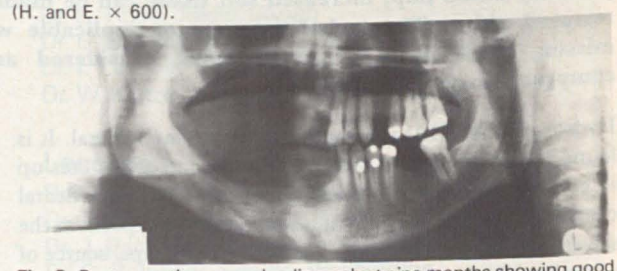

Fig. 3. Post-operative panoral radiograph at nine months showing good healing and no evidence of recurrence.

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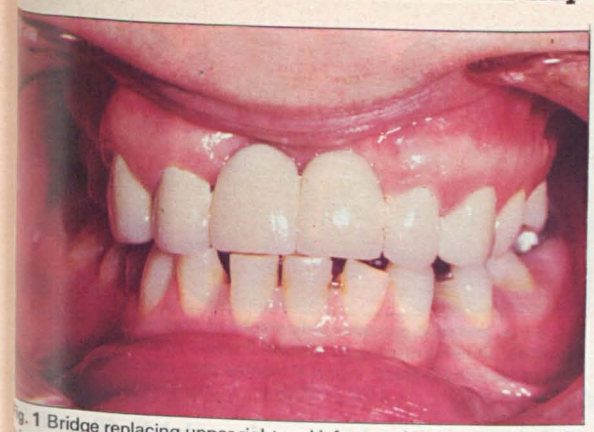

g. 1 Bridge replacing upper right and left central incisors and showing ial elongation of the pontic to achieve normal pontic/mucosa eationship after excess alveolar resorption.

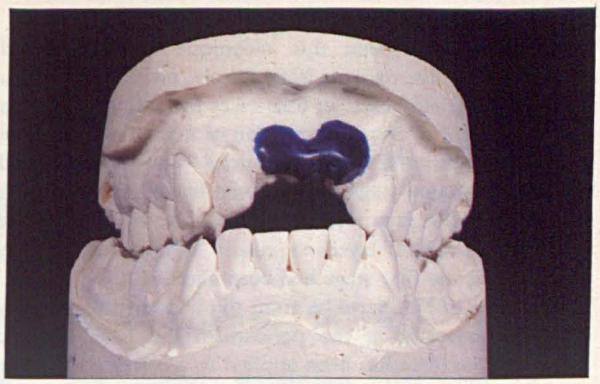

Fig. 2 A waxed-up model showing the planned extent of the HA implant.

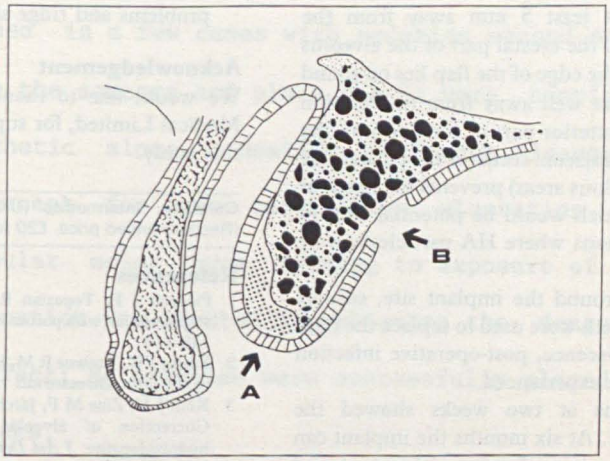

Fig. 3 Diagrammatic sagittal section through an augmented alveolus. A = area of resorbed alveolus; B = denuded area of bone post-operatively; dotted area = HA implant particles in place.

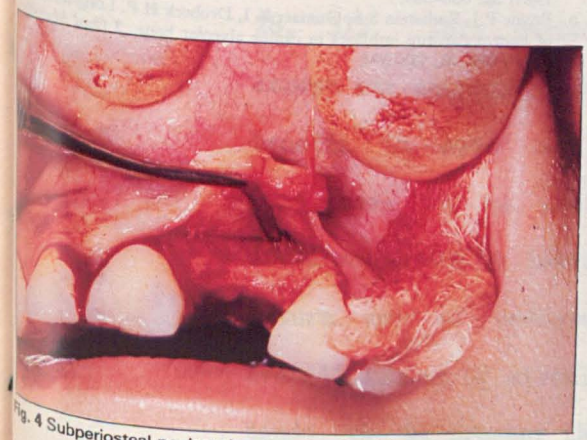

Fig. 4 Subperiosteal pocket showing alveolar defect.

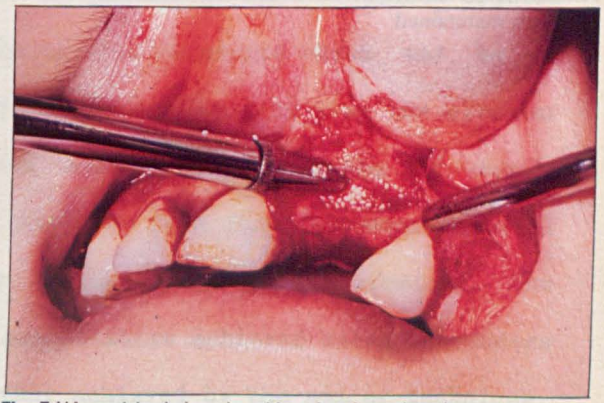

Fig. 5 HA particles being placed in subperiosteal pocket.

Discussion

Under local anaesthetic this technique has been used successfully on five patients (four female, one male) with an age range of 21–45 years. In four cases the second stage was undertaken two weeks after creation of a scar in the buccal sulcus. It is important that the buccal tissues have healed because the second stage flap is based on the buccal tissues and relies on this area for its blood supply. In one case the second stage was delayed to ensure that an apicectomy performed at the first stage had been successful.

Migration of HA particles from the implant site can be a problem, especially if the periosteum has been elevated excessively or force has been applied to a corrected ridge in the immediate post-operative period. For this reason our operative technique is designed principally to prevent implant migration.

The scar created in the first stage acts as a barrier to HA particle migration in the early stages of healing. Additionally, it prevents the flap being raised too far buccally and aids creation of a localised subperiosteal pocket. In the same way, siting the palatal incision at least 5 mm away from the maximum palatal extension of the crestal part of the alveolus ensures that, when replaced, the edge of the flap lies on sound bone and the HA particles are well away from the incision line. Finally, extending the anterior part of the flap into the buccal gingival crevice of the adjacent teeth (or the palatal side of the alveolar crest in edentulous areas) prevents the need for buccal relieving incisions which would be potential sites of wound breakdown and locations where HA particles can be lost.

To achieve a good seal around the implant site, sutures passing round the adjacent teeth were used to replace the flap. No problems of wound dehiscence, post-operative infection or obvious particle loss were experienced.

Post-operative radiographs at two weeks showed the implanted HA to be in place. At six months the implant can still be seen, although some loss of contrast has occurred which may be due to new bone ingrowth (fig. 6).

Restoration of the missing teeth was undertaken 4–6 weeks later when the ridge was firm. After initial follow-up times ranging from 16 to 22 months, all corrected areas have remained firm and sympton free and the new alveolar contour has been maintained.

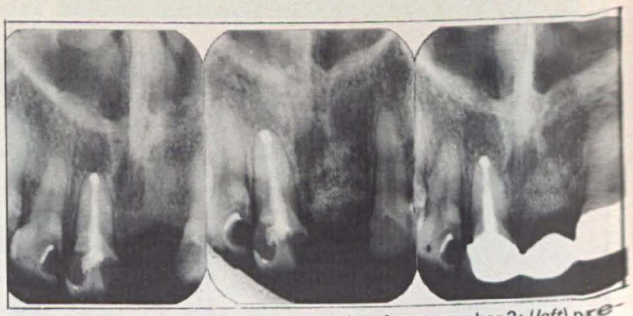

Fig. 6 Pre- and post-operative radiographs of case number 3: (*left*) pre-operative, showing missing |1; (*centre*) 2 weeks post-operative showing implant particles in place; (*right*) 6 months post-operative.

Conclusions

(1) Subperiosteal implantation of rounded 40–60 mesh HA particles appeared to be a successful means of correcting local alveolar bone loss.

(2) Implant migration could be prevented effectively by a two-stage operation and a suitably designed incision.

(3) During preliminary follow-up, patients experienced no problems and ridge shape was maintained.

Acknowledgement

We would like to thank Intermedics (UK) and Quantum Medical Limited, for supplying the implant material used in this study.

Calcitite: Intermedics (UK) Ltd, Welwyn Garden City, England. (Recommended price, £20 for 1-gram phial.)

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ii. Mandibular Ridge Augmentation.

Since compilation of the original papers, in the period 1988-1991, the technique has been extended for partial augmentation of the maxilla. A total of 26 patients have undergone alveolar augmentation at 49 sites. Placement of spacers was achieved in all patients under local anaesthesia combined in a few cases with sedation second stage procedures to remove the spacers and place the Ha were completed under local anaesthetic alone. Healing following placement of spacers was good except for one case where ulceration occurred in the mandibular molar area leading to exposure of the spacer. This complication was treated by relieving the denture and suturing the defect; Ha granules were successfully placed after a delay of two weeks.

An alternative to tissue spacers is that of tissue expanders Quayle A.A., et al (1990) Brit J Oral and Maxillofacial Surgery 28, 375. This technique is similar to that of tissue spacers except the anterior mandible is also augmented which may be of benefit in some patients. However, in this area the alveolar bone tends to be preserved due to the presence of muscle attachments and by leaving this area free from implanted material future use of endosseous implants is not compromised. The overall amount of alveolar augmentation achieved using expanders tends to be greater than that achieved by the spacer technique,

however, there is less control over the final shape of the augmented ridge.

The spacer technique thus has a useful place in the procedures and techniques that are currently available for alveolar ridge augmentation.

J Oral Maxillofac Surg
47:331-335, 1989

Two-Stage Combined Vestibuloplasty and Partial Mandibular Ridge Augmentation With Hydroxyapatite

IAN M. BROOK, BDS, MDS, FDS,* AND DAVID J. LAMB, BDS, MDS, FDS†

The preliminary results of a new two-stage technique for partial augmentation of the edentulous mandibular ridge with hydroxyapatite are reported. The technique, which has been applied to five patients, can be performed under local analgesia, and involves initial placement of temporary silicone spacers, together with submucous vestibuloplasty to preserve sulcus depth. The spacers are later replaced by dense particulate hydroxyapatite, and the connective tissue capsules that remain after removal of the spacers allow control of particle migration and minimize the amount of hydroxyapatite required.

Introduction

The support of an adequate residual alveolar ridge is helpful for denture stability. Following loss of the teeth, however, alveolar resorption begins and continues throughout life. Hence, with increasing longevity, the problems of alveolar atrophy in the elderly are becoming more common.[1] To help alleviate the difficulties experienced by the elderly in adapting to the edentulous state, there is a need for procedures that improve denture stability. A number of surgical techniques have been described, ranging from relative increases in ridge height by sulcus deepening[2] to augmentation of the residual alveolar ridge by materials as diverse as autogenous bone,[3] alloplastics,[4] or biocompatible ceramics. Of the latter, hydroxyapatite appears to be the most suitable, and its use in the form of dense particles,[5] porous blocks,[6] or within a collagen matrix[7] have been documented.

The major drawback to such procedures is that they are most often necessary in a section of the population that is elderly and often receiving complex drug therapy; consequently, in this group prolonged surgery under general anesthesia carries a high morbidity. Further, when hydroxyapatite is used in particulate form, a variety of additional implant-stabilizing techniques have been suggested as being necessary to minimize the problem of particle migration and the subsequent loss of part of the gained ridge height. These include complex surgical methods,[5] special suturing techniques,[8] complex splinting,[9] mixing hydroxyapatite particles with bone, plaster,[10] or fibrin,[11] and the use of vicryl or collagen tubes,[12] and submucosal tissue expanders.[13]

The present work describes a simple two-stage technique for ridge augmentation using dense particulate hydroxyapatite (Calcitite, Calcitek Inc, San Diego). It can be performed under local analgesia, is designed to minimize particle migration in the postoperative phase, and appears useful for treatment of the elderly. The technique is most appropriate when treating the common pattern of mandibular alveolar resorption where loss takes place principally in the molar/premolar region, leaving a concave deficiency. Particulate hydroxyapatite is used to fill the posterior defects, and the untouched bone in the incisor and retromolar pad regions provides pain-free support to the patient's modified denture in the postoperative phase. In this way, pressure on the augmented site is avoided, collapse of the implant is minimized, and appearance is preserved.

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Technique

Preparatory Work

At a preliminary visit, the nature of the operation is explained, the condition of the denture-bearing mucosa is noted, and any soft tissue pathology is corrected. The patient is prescribed a chlorhexidine antiseptic mouthwash for the week before the first operative stage and advised to wear the denture as little as possible during this period. Preoperative panoramic radiographs are taken to assess the relationship between the mental foramina and the planned augmentation.

An alginate impression is taken of the patient's mandibular ridge, a model is prepared, and, after identifying the sites for augmentation on the lingual side of the residual ridge, the sites are built up with wax to the extent of the proposed ridge form. The wax replicas are removed and duplicated twice in room-temperature vulcanizing silicone elastomer (Dow Corning Europe SA, Brussels). One set of silicone duplicates is replaced on the model prior to surgery to check that the form is correct, and is used later when modifying the denture to form a splint for the augmented site (Fig 1). The other, after being ultrasonically cleaned in distilled water for ten minutes, is autoclaved in preparation for implantation.

First Stage

The bilateral vestibuloplasties[2] and placement of silicone spacers are performed under infiltration and regional block analgesia. Vertical incisions are made in the canine region from the crest of the ridge into the reflection of the buccal sulcus, extending only through mucosa. Submucosal tunnels are created posteriorly on the buccal side of the ridge back to the retromolar pads. The canine incisions are now carried through the periosteum and extended down the lingual side of the ridge. Before the subperiosteal dissection is performed, the mental nerve is identified by direct visualization. After extending the subperiosteal tunnel posteriorly to the retromolar pads, the periosteal layer separating the two tunnels is incised along the crest or lingual side of the ridge, and the periosteum/buccinator muscle attachment is displaced inferolaterally. The autoclaved silicone implants are now inserted (Fig 2), and sulcus depth is evaluated. Should there be inadequate sulcus depth or tension over the silicone spacers, further stripping of muscle attachment is undertaken, including, if necessary, sectioning of the mylohyoid attachment. Adaptation of the silicone spacers to the ridge is usually good and can be improved by careful compression of the tissues to reduce dead space.

The patient's lower denture is hollowed out until the denture fits easily over the model with the duplicate silicone implants in place. The denture is then relined using a suitable soft lining material (Total Soft, Coe Industries, Chicago), with the unaugmented regions acting as a guide to the original position and the upper denture inserted to ensure even occlusal contact. When set, the lining is trimmed and cleaned. An appointment is made for suture removal seven to ten days later, and the patient is instructed to wear the dentures for appearance only during this time.

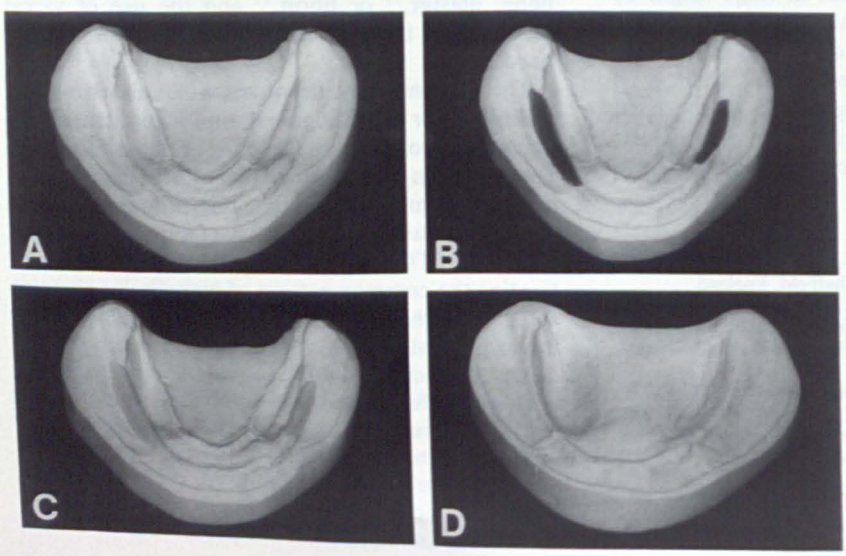

FIGURE 1. Use of silicone spacers. *A*, Model of mandible prior to augmentation. *B*, Model augmented with wax to desired height. *C*, Silicone spacers on model. *D*, Model of mandible 6 months after operation.

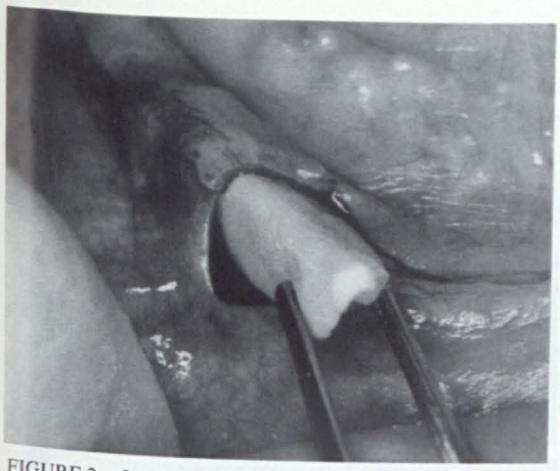

FIGURE 2. Insertion of silicone spacer into subperiosteal tunnel.

After suture removal, the occlusion of the dentures is evaluated, and another appointment made for 3 to 5 weeks later, so that the second stage can be carried out. During this time the silicone implants become less mobile, and the dentures may be worn for function.

Second Stage

At the second stage the implant site should be firm and pain free. After local analgesia is established, the silicone implants are exposed and removed via incisions in the canine region. Care is taken not to open the tissue planes or enlarge the spaces formed by connective tissue condensation around the silicone. Dense hydroxyapatite particles are packed firmly into the patent space left by the silicone, using either a suitable syringe or a clean, sterile amalgam carrier. The incisions are closed as before and an appointment made for suture removal. The patient is advised to wear the dentures for appearance only during the following week, and an antiseptic mouthwash is again prescribed.

Sutures are removed seven to ten days later, after which denture function may be carefully resumed. Some loss of initial ridge height occurs during the next few weeks, and the lower denture is relined as necessary with a semipermanent material (Total Hard, Coe Industries, Chicago). Replacement dentures are inserted 3 months after the augmentation procedure.

Records

To record the achieved degree of augmentation, panoramic radiographs were taken preoperatively, immediately postoperatively, and 6 months postoperatively. All panoramic radiographs were traced and lines drawn tangential to the lower borders of the mandible on either side. Perpendiculars were erected from the tangents, passing through the deepest concavities of the upper border of the ramus, and the original (d) and augmented (D) ridge heights were measured with calipers to the nearest 0.5 mm (Fig 3). It is well known that consecutive panoramic radiographs of the same subject may show differing degrees of vertical magnification,[14] and to allow for this, where differences in ridge height were detected, a corrected augmentation height (D_c) was calculated as follows:

$$D_c = D \cdot d_o/d_n$$

Where D = augmented ridge height, d_o = original ridge height (preoperative), and d_n = original ridge height (postoperative).

Results

To date, the operation of two-stage combined vestibuloplasty and mandibular ridge augmentation with hydroxyapatite has been performed for five patients (three male, two female) with resorbed mandibular ridges who had been referred for consultant prosthodontic opinion after complaining of inability to tolerate loose lower dentures. The results are summarized in Table 1. Immediately after surgery, the mean increase in ridge height in the deepest part of the mandible was 6.0 mm (range 1.5 to 10.0 mm), although over the following 6 months some loss of augmented ridge height was recorded (mean 1.0 mm, range 0 to 2.5 mm).

Although the augmented ridges have been firm and no problems have been encountered during prosthodontic rehabilitation, complete integration of the hydroxyapatite with the residual ridge (as judged by the radiograph) has taken place bilaterally in only two cases (Figs 4A and 4B). Immediately after the operation, all subjects showed clear

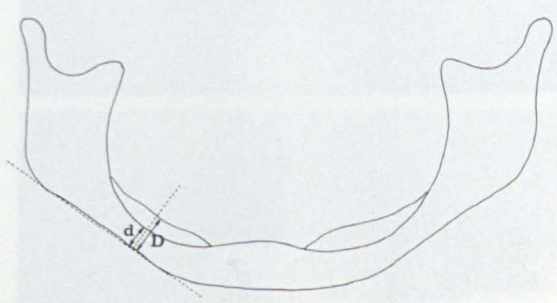

FIGURE 3. Tracing of postoperative panoramic radiograph. (d), Original mandibular ridge height; (D), augmented mandibular ridge height.

Table 1. Summary of Changes in Right (R) and Left (L) Posterior Ridge Heights Following Augmentation

Patient	Pre-operative Height (mm)		Post-operative Height (mm)		6-Month Height (mm)		% Loss Over 6 Months	
	R	L	R	L	R	L	R	L
1	14.5	15	23	25	22	22.5	4.3	10
2	20	27.5	24.5	29	23.5	28.5	4.1	1.7
3	10	10	17	18	16	16	5.9	11
4	13.5	14	20	19	19	19	5	0
5	15.5	14	19	19	19	18	0	5.2

separation between the residual ridge and the implant; the width of the separation zone decreased with time (Figs 5A, 5B, and 5C). The most significant complication has been disturbance of sensation in the distribution of the mental nerve, which was reported unilaterally by two patients; however, spontaneous resolution occurred within 3 months. Satisfactory full dentures were eventually made for all patients, with a marked improvement in function and comfort.

Discussion

Loose lower full dentures are a difficult problem to resolve for patients with severe mandibular resorption who consequently find denture control difficult. The problem might be expected to become more common as patients retain their teeth longer, losing them at an age when they find greater difficulty in adapting to the new situation. Unfortunately, the age at which the problem arises is usually one at which there is also a relatively greater risk of postoperative morbidity if a general anesthetic is given. Hence, the majority of ridge augmentation procedures should not be undertaken lightly. For such patients a less traumatic two-stage procedure, as described here, may be performed under local analgesia. The degree of augmentation achieved compares favorably with similar, but more extensive, operations.

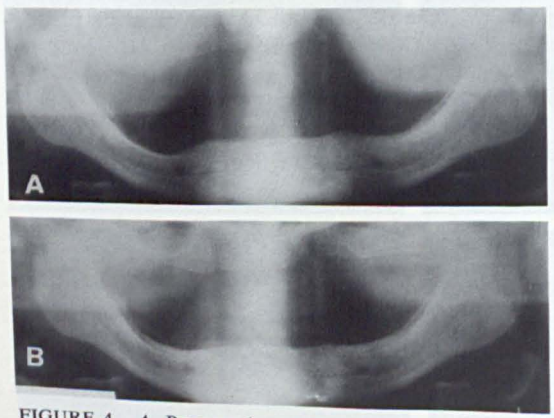

FIGURE 4. *A*, Preoperative panoramic radiograph (case no. 2). *B*, Postoperative panoramic radiograph showing apparent integration of hydroxyapatite and bone (case no. 2).

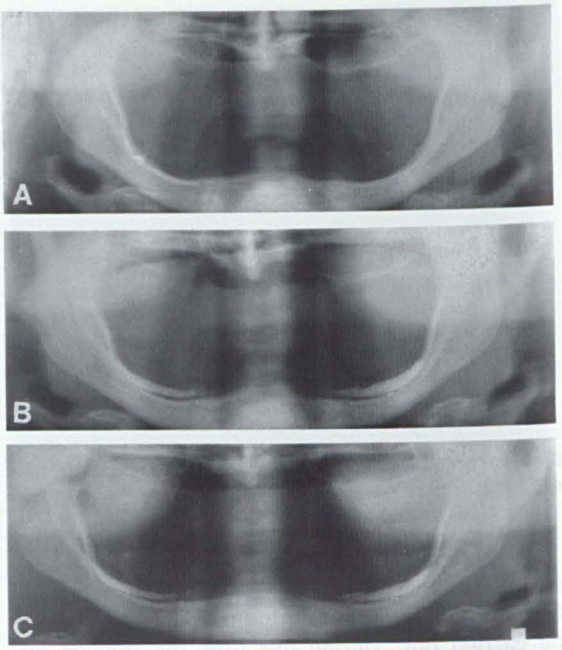

FIGURE 5. *A*, Preoperative panoramic radiograph (case no. 5). *B*, Panoramic radiograph immediately after operation. A radiolucent zone separates the implanted hydroxyapatite from the body of the mandible (case no. 5). *C*, Panoramic radiograph 6 months after operation. The radiolucent zone between the implanted hydroxyapatite and the body of the mandible has narrowed (case no. 5).

The two-stage procedure has another advantage. In all augmentations involving implantation of particulate hydroxyapatite, migration of particles is a complication unless stringent preventive measures are taken.[5,7-9,11,13] Edentulous augmentations by a tunneling technique are especially prone to this complication because, to secure adequate access, the tunnel has to be larger than the projected implant, and peripheral spread of the implanted material can follow. In the case of mandibular augmentations, a proportion of the material spreads lingually and buccally, with some loss of height. To minimize migration, various temporary splinting techniques have been described, but none have been completely successful.

When block forms are substituted to avoid the problems of migration associated with particulate hydroxyapatite, different problems may arise. Although the implant remains stable, dehiscence may

occur either because of difficulties in achieving soft tissue cover, or because the implant surface is sharp and irregular.[10,15] Furthermore, if the porous form of block is used, not only is dehiscence a problem, but bone penetration of the matrix may be incomplete, due possibly to stress shielding.[16]

The technique described here is a new approach to the problem. By first inserting a silicone duplicate of the implant, the tissues are allowed to encapsulate a space that is a precise reproduction of its final form. While the process of encapsulation is occurring, the denture can be modified to the future shape. Consequently, when inserted, the final implant is supported firmly on all sides and there is little particle migration.

It has been emphasized in the past that successful mandibular augmentation with hydroxyapatite depends more on the shape and position of the material than the amount of material implanted.[17] The technique described, while achieving moderate increases in ridge height, also allows augmentation at a critical site, resulting in improved denture function.

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TECHNICAL NOTE

Fabrication of custom made tissue spacers for use in tissue augmentation

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SUMMARY. **A method for producing custom made tissue spacers for use as an aid to accurate placement and to prevent migration of particulate bone substitute materials is outlined.**

INTRODUCTION

Difficulties with precise placement and early migration of particulate material used during procedures to augment the atrophic alveolus can contribute to poor results and continued prosthetic problems (Desjardins, 1985). Preformed solid implants are easier to place and more resistant to displacement; however they are difficult to shape at the time of surgery and suffer from problems of dehiscence of the overlying mucosa and subsequent infection (Brook & Lamb, 1987).

Inflatable tissue expanders (Quayle *et al.*, 1990) and solid tissue spacers (Brook & Lamb, 1989) enable prdictable improvements in both ridge height and sulcus depth. Placement of the spacer creates a fibrous tissue encapsulated pocket, which on removal of the spacer, can be filled with particulate bone substitute material, confining it to the required site.

METHOD

Dental stone models are produced from alginate impressions of the area of alveolus to be augmented. The extent of the augmentation is drawn on the model (Fig. 1). Separating medium is then applied to the model and wax used to recontour the alveolus to the desired shape (Fig. 2). The wax pattern is smoothed and removed from the model and invested in a two part artificial stone mould. Wax is then eliminated using boiling water and when cool the mould is coated with 'Medimold' wax sealant, which when dry, can be polished to a high sheen using dental napkins. The required quantity of silicone elastomer is mixed and catalysed according to the manufacturers instructions and air removed under vacuum (70 cms of mercury for 30 min). The elastomer is then carefully loaded into the mould which is closed and vulcanised under pressure in a drying cabinet for 30 min at 75°C.

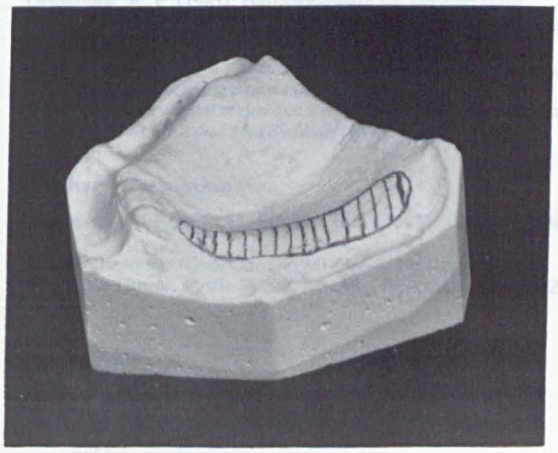

Fig. 1–Study model with a deficient right maxillary alveolus marked, prior to recontouring with wax.

Fig. 2–Restoration of the alveolar contour in wax.

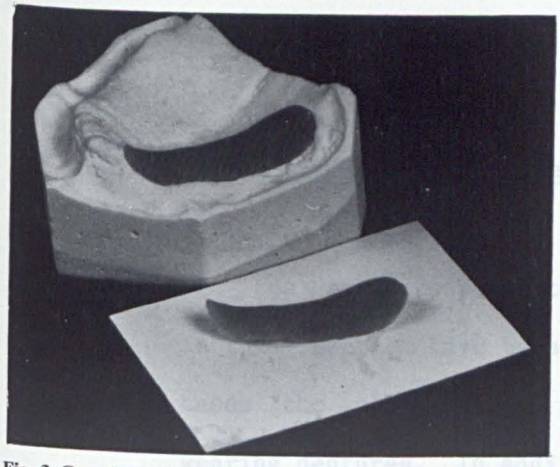

Fig. 3–Completed silicone spacer and duplicate spacer located on the study model.

After vulcanisation the mould is cooled and the silicone spacer carefully removed. Following minimal finishing using scissors and silicone trimming burs, the spacer is cleaned ultrasonically in distilled water for 10 min and then autoclaved prior to use. A completed spacer for augmentation of the right maxillary alveolus is shown in Figure 3.

DISCUSSION

Tissue spacers are placed using tunnelling techniques. During placement, soft tissues may be widely stripped and muscle attachments freed to gain good access and ensure tension-free soft tissue cover at the implantation site. When used for alveolar augmentation, sulcus deepening is usually required to ensure that ultimately an increase in clinical ridge height is achieved and this can best be undertaken at the time of insertion of the tissue spacer (Brook & Lamb, 1989).

Spacers are left in place for 3 to 4 weeks after which they are carefully removed leaving a fibrous tissue lined pocket into which particulate material can be placed to permanently augment the alveolus. It is helpful if a duplicate spacer is made and supplied with the original study cast (Fig. 3), because this allows orientation of the spacer at the time of surgery and acts as an aid to adjust the patients existing denture to the new ridge shape. The denture is relieved until it fits loosely over the study model with the duplicate spacer in place, prior to placing a soft lining and fitting in the mouth.

More than 30 spacers have been used for a variety of total and partial alveolar augmentation procedures, and predictable improvements in ridge form have been achieved in all cases. Custom made tissue spacers for use with particulate bone substitute material can be produced simply and economically in the maxillofacial laboratory.

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Further information

'Medimold': Polymed Ltd, PO Box 184, Cardiff, C5 3YZ, UK.

Silicone elastomer: Dow Corning MDX 4–4210, Dow Corning Ltd., Avco House, Castle Street, Reading, Berkshire, RG1 7DZ, UK.

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iii. Maxillary Ridge Augmentation.

The ten patients whose treatment is reported in the paper bound in this section have been followed up for six years and in all cases the alveolus has remained firm and they are successfully wearing dentures. In addition to the ten cases reported in the paper, five further patients received maxillary augmentation with combined Collagen/Hydroxapatite biograft material, placed using a tunnel technique. Of these five, two patients had inadequate post-operative improvement in clinical ridge height and required secondary vestibuloplasy. Of the remaining three patients, an improvement in ridge width and elimination in buccal undercut was achieved rather than an improvement in ridge height.

The collagen/hydroxyapatite biograft material is no longer easily available in the United Kingdom and seven patients have successfully undergone augmentation of the anterior maxilla with Ionomeric granules (Ionomeric microimplant, Ionos GmbH & Co KG Germany) using the open technique described, initial results being favourable.

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Management of the atrophic anterior maxilla by combined hydroxyapatite augmentation and vestibuloplasty: A pilot study

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SUMMARY. **A technique is described to overcome the problem of the grossly resorbed maxillary alveolus and improve denture wear, by combining augmentation and increased soft tissue cover of the alveolar ridge. Manipulation and placement of the hydroxyapatite was facilitated by use of a hydroxyapatite/collagen composite (HAC) block. The procedure has been carried out in ten patients where extensive resorption contraindicated the use of permucosal implants. Six cases have been followed up for 2 to 3 years. After some initial compaction of HAC the improvement in ridge height has been maintained and long term improvement in denture function obtained.**

INTRODUCTION

The atrophic, fibrous, anterior maxillary alveolus can be a major prosthetic problem. There is often insufficient remaining bone for permucosal implants and if the mobile fibrous tissue is excised to provide a firm denture foundation, all resistance to antero-posterior movement of the denture is lost and the resulting instability is a source of constant complaint by the patient. A more satisfactory alternative is to augment the atrophic ridge. Hydroxyapatite in particulate or block form has been widely used for alveolar augmentation (Rothstein *et al.*, Kent, 1986; 1984; Frame & Brady, 1987) but is not ideal. If implanted in the form of particles, early or late migration is a common problem (Desjardins, 1985). Precise particle manipulation is difficult and a vestibuloplasty is often required. While dense block forms of hydroxyapatite resist migration, they are difficult to shape at the time of surgery, and porous blocks appear susceptible to infection and dehiscence (Brook *et al.*, Brook & Lamb 1987a; 1987).

Predictable improvements in both ridge height and sulcus depth have been reported following partial mandibular augmentation, using a two stage tissue spacer technique to overcome the placement and migration difficulties of particulate hydroxyapatite (Brook & Lamb, 1989), and Lew (1985) has reported a successful technique for combining particulate hydroxyapatite augmentation of the severely resorbed maxilla with increasing the area of soft tissue support for the denture. We report a modification of the latter technique designed specifically to deal with the problem of the flabby atrophic anterior maxilla and technically simplified by utilisation of HAC blocks.

METHODS

Patients were selected from those referred to the Charles Clifford Dental Hospital, Sheffield, for the problem of complete denture instability, which on examination was seen to result from gross atrophy of the anterior maxilla. Criteria for treatment were failure of conventional prosthetic techniques and insufficient remaining alveolar bone for the placement of permucosal implants.

After recording the patients' judgements, pre-operative assessment of dentures was made by a prosthodontist who assessed qualitatively the retention of the dentures and their resistance to displacement, and the quality of the supporting alveolus and soft tissues. Any pathology of the soft tissues including denture induced hyperplasia was corrected prior to surgery. Lateral cephalometric radiographs were used to measure alveolar bone height and assess implant placement prior to surgery and at 14 days, 3, 12, 24 and 36 months after surgery. Ridge height, defined as the distance from the maxillary plane to the ridge crest, was determined at two points by tracing lateral cephalometric radiographs. Two parallel lines running at right angles to the maxillary plane and 10 mm apart were constructed, the first line passing through the midpoint of the anterior nasal spine. The mean height of the anterior maxillary ridge was taken as the average of these two heights.

On a pre-operative study model the area to be augmented was outlined by the prosthodontist and the alveolar ridge built up in wax to imitate the post-insertion size of an appropriate HAC block. The waxed up model was dulicated in plaster of Paris and a clear acrylic surgical plate, extended to the depth of the original sulcus reflection, was made to fit the

new ridge shape. The buccal edge of the plate was finished with either a soft silicone flange or trimmed 2 mm short of the sulcus reflection to allow space for a soft lining to be applied at surgery. An antiseptic mouthrinse (Corsodyl, ICI, UK.) was used three times a day for 3 days before surgery.

The operation was a modification of that of Lew (1985) and involved combined augmentation/vestibuloplasty undertaken by an open flap technique. The initial incision was made through mucosa and submucosa in the sulcus extending to the distal ends of the area to be augmented (normally the anterior third of the alveolus). The extent that this incision was taken into the lip was determined by the amount of soft tissue required to allow mucosal cover of the implant and alveolar crest (Fig. 1). Full thickness vertical incisions were made from the distal ends of the sulcus incision up on to the crest of the ridge. A split flap was raised and just buccal to the crest of the ridge, the periosteum was incised to bone. The full thickness flap was developed palatally, care being taken to restrict the dissection to the planned palatal extent of the desired augmentation. The surgical technique is shown diagramatically in Figure 2. Thinning of the flap to reduce any fibrous tissue at the crest of the ridge was undertaken by placing the flap under tension and making short mesiodistal incisions on its undersurface, taking care not to perforate the mucosa. In this manner not only was the flap thinned but an increase in bucco-palatal dimension was achieved. The labial edge of the flap (split thickness mucosa) was repositioned crestally

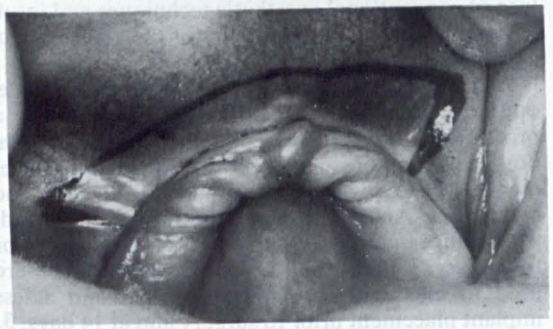

Fig. 1 – Outline of sulcus incision.

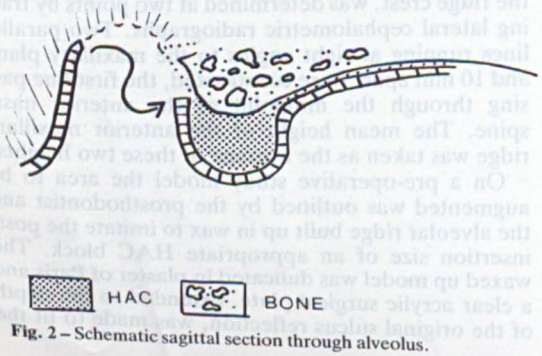

Fig. 2 – Schematic sagittal section through alveolus.

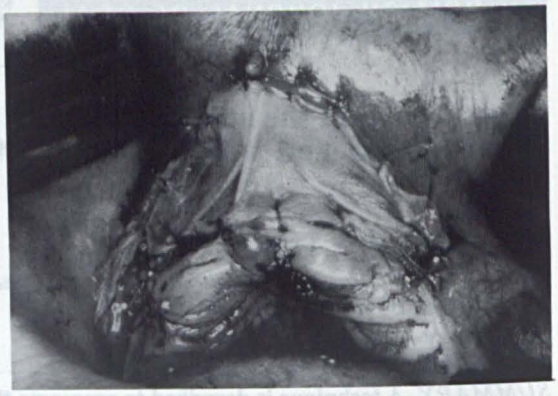

Fig. 3 – Split thickness skin graft prior to fitting of surgical splint.

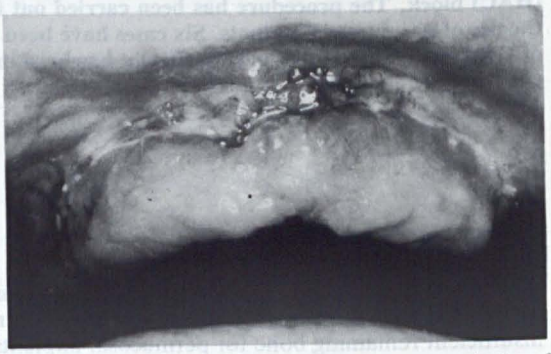

Fig. 4 – Healing at 14 days in a patient where laser vaporisation of the raw labial tissue had been undertaken.

and held with stay sutures to the edge of the incised periosteum thus creating a pouch. A dry curved HAC block was trimmed as required with a scalpel and inserted into the pouch. Further sutures were placed along the mucosal-periosteal junction to close the pouch and the surgical plate tried in. The HAC became mouldable on wetting so allowing it to conform to the alveolar ridge and overlaying mucosa adjacent to the fitting surface of the plate. Any excess HAC was extruded from the distal incisions prior to their suture.

Crestal advancement of the buccal mucosa left a raw muscle/periosteum bed labially. Using cotton pledgets any loose tissue was displaced apically. Extension of the vestibule and trimming of the anterior nasal spine was undertaken when necessary. The raw area was either covered with a split thickness skin graft (Fig. 3) or subjected to vaporization with a defocused CO_2 laser on a low power setting to produce a surface coagulum and allow healing with preservation of sulcus depth (Brook & Lamb, 1987b) (Fig. 4). The surgical plate was fitted and fixed using one or two palatal screws (Champy, Martin, Germany) placed well away from the augmented area.

Postoperatively all patients received a 7 day course of phenoxymethylpenicillin and a topical

Table 1 – Change in anterior maxillary alveolar ridge height: *=No change, –=Missing data.

Patient	Pre-op	Post-op	% gain	Mean Ridge Height mm 3 m	6 m	12 m	24 m	36 m	% shrinkage
1	6.3	11.3	80%	9.7	9.7	—	*	*	15%
2	4.5	10.5	134%	8.5	8.5	*	*	*	19%
3	10	14.5	45%	12.7	12.5	*	*	—	14%
4	10.5	13.5	28%	12	12	*	—	*	12%
5	7.5	12	60%	12	10.5	*	*	*	12.5%
6	5	8.3	66%	7.6	7.6	*	—	*	9%
Mean	7.3	11.6	68%	10.4	10.1	10.1	10.1		13.6%
SD	2.5	2.2	36	2.1	1.9	1.9	1.9		3.3

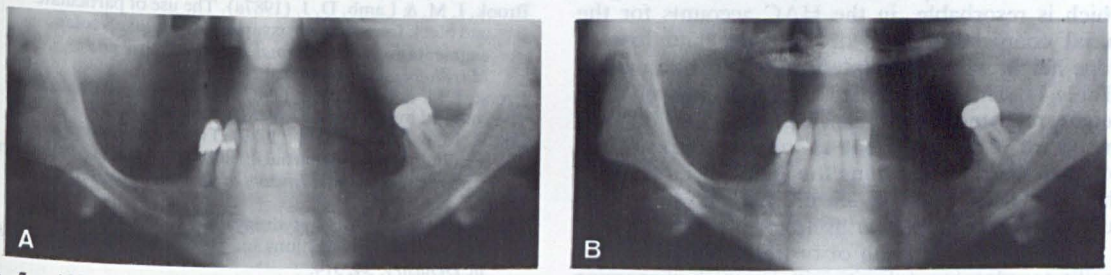

Fig. 5 – (A) Pre-operative radiograph. (B) Radiograph taken 18 months after augmentation of anterior maxilla.

analgesic mouthrinse (Difflam, Riker Laboratories, UK.). The surgical plate was removed 7 to 10 days after surgery and the fitting surface and labial extension of the original denture modified to fit the new ridge using a semi-permanent soft liner (Total Soft, Coe Laboratories, USA.). The patient was advised to wear the denture for appearances only. During the next few weeks denture function was gradually resumed. New dentures were constructed when the soft tissues were completely healed and the ridge firm (approximately 6 weeks after surgery).

RESULTS

The procedure has been performed in 10 patients, six of whom (2 female, 4 male) with mean age of 53 y (SD=8.5 y), have been followed up for 24 to 36 months. The results presented pertain to these six cases, three of whom had augmentations with skin grafts and three with laser vaporisation. Splints were placed in all patients for 7 to 14 days. On removal of the splint a dehiscence of the wound was noted in two patients, one on the crest of the ridge and the other at the skin graft/mucosa junction. In both cases a small area of HAC was exposed. Treatment consisted of relief to the denture in this area, following which healing by granulation occurred. The augmented ridge form was maintained in both cases.

The mean length of time that the patients had worn an upper complete denture was 26 y (SD= 13.8 y). Although all patients had initially worn dentures successfully, increasing problems with denture wear had occurred with time and on presentation all rated the comfort, fit, speech, appearance, ability to chew food and general satisfaction as poor or average. At 6 months postoperatively, all patients rated the same factors as average or good and this improvement was maintained at 12, 24 and 36 months. The prosthodontist rated the initial stability and retention of the maxillary denture as poor, but at 3 months after surgery the same factors were rated average or good and all but one case was rated as excellent at 6 months. The improvement was maintained at 12, 24 and 36 months. Changes in mean anterior maxillary ridge height are shown in Table 1. HAC blocks adapted well to the topography of the residual alveolar ridge and there was no radiographic evidence of migration of hydroxyapatite. Clinically all implants were firmly retained at the original site. Typical pre- and post-operative radiographs are shown in Figure 5.

DISCUSSION

The fibrous ridge in the anterior region of the atrophic maxilla has been an intractable problem that resists treatment by prosthodontic means alone. The technique reported enables improvements in ridge height and stability to be achieved, while maintaining, or if required deepening, the labial vestibule.

The use of particulate hydroxyapatite in procedures where vestibuloplasty and augmentation are combined can lead to early migration of hydroxyapatite away from the intended site and thus loss of ridge form (Harle, 1985). In the cases reported the combination of block HAC with its improved handling (Mehlisch *et al.*, 1987), together with the careful use of sutures to form a tunnel into which the hydroxyapatite is placed (Lew, 1985) enabled accurate placement and minimal migration of the hydroxyapatite with predictable improvement in ridge form and denture function.

HAC blocks are a combination of rounded dense

particulate hydroxyapatite in a matrix of bovine fibrillar collagen which on placement hydrates and softens, allowing the block to conform to the underlying bone and overlaying soft tissue. In contrast, blocks of porous hydroxyapatite have a rough surface which can traumatise the underside of the mucosal flap leading to ulceration, infection and loss of the implant (Frame & Laird, 1987; Brook *et al.*, 1987). HAC, like particulate hydroxyapatite alone, does not appear to be susceptible to infection and the dehiscence of the incision seen in two patients healed uneventfully. The incorporation of collagen, which is resorbable, in the HAC accounts for the initial compaction of hydroxyapatite and loss of ridge height which amounted to 19% in one patient (Table I). This is similar to the initial loss of ridge height reported by Mehlisch *et al.*, (1987). It is important to take account of the resorption of the collagen matrix in planning the augmentation and in the construction of new dentures which will require relining at about 3 months following surgery.

Although the addition of bovine collagen to HA facilitates placement, limits initial particle dispersion and may stimulate bone formation (Moore *et al.*, 1990), it is antigenic and is potentially capable of inducing a hypersensitivity reaction. However animal studies with implanted bovine Type I collagen have shown it to be a poor stimulator of the immune system (Moore *et al.*, 1990). This finding was confirmed in a study of 77 patients receiving HAC implants (Melisch, 1989) in that despite five patients having antibodies to bovine collagen before surgery and a further five developing antibodies following placement of HAC, no local or systemic hypersensitivity response occurred.

As with the majority of procedures aimed at improving denture function the technique is best used in those patients who have successfully worn conventional complete dentures in the past, but where progressive bone atrophy has made manipulation of their dentures increasingly demanding. For these patients alveolar augmentation can re-establish stomatognathic function and facilitate conventional denture construction. The technique is not suitable, however, for those patients who are unable to tolerate removable dental prostheses. For these patients permucosal implants and a fixed prosthesis may be required. It is important that these two groups of patients are identified before treatment is commenced because the use of an inappropriate implant technique can prejudice the use of the alternative.

Acknowledgements

This study was supported by Trent Regional Health Authority Grant No 122. Ethical scrutiny was provided by the Ethics Committee, Royal Hallamshire Hospital, Sheffield. The patients reported are part of a multi-centre study into the use of HAC coordinated by Professor van Reck, Universitaires St. Pierre Libre de Bruxelles.

Further information

The surgical technique described in this report is available on a video 'Augmentation of the Anterior Maxilla Open Flap Technique—hydroxyapatite/Collagen Biograft' from the University of Sheffield Television Service, Sheffield S10 2SZ Tel (0742) 750222 Cat No. C61 (1988). Collagen Hydroxyapatite, was used on a named patient basis, and was supplied as Maxill-HAP, Zimmer Surgical Specialities, Dunbeath Rd, Swindon. SN2 6EA. UK.

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APPENDIX III

Publications arising from thesis

In vitro interaction between primary bone organ cultures, glass–ionomer cements and hydroxyapatite/tricalcium phosphate ceramics

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Primary organ cultures derived from neonate rat calvaria were maintained for 2 wk and used to study the *in vitro* response of osteoblast and periosteal cells to the component and composite forms of three different glass–ionomer (polyalkenoic) cements, comparing them to densely sintered hydroxyapatite and tricalcium phosphate ceramics. Qualitative analysis by scanning and transmission electron microscopy revealed that osteoblasts colonized all the solid test materials, although there was a less favourable response to materials with a rough surface topography and to unset and fluoride-containing glasses. On solid materials migrated cells maintained their tessellated morphology and exhibited numerous micro-appendages anchoring them to the surface of the test materials. A collagen-containing extracellular matrix was elaborated on to the ceramics and set glass–ionomer cements, except for one (AquaCem). Mineralization of the extracellular matrix was seen adjacent to hydroxyapatite and tricalcium phosphate ceramics, that adjacent to the latter morphologically resembling bone.

Keywords: Biocompatibility, bone, osteoblasts, glass-ionomer cements

Glass–ionomer cements (GICs), developed by Wilson and Kent[1] in 1969, are widely used as dental restorative materials. They are formed by reaction of an inorganic base (aluminosilicate glass) with an organic polyelectrolyte (alkenoic acid) such as poly(acrylic)acid. Setting occurs by the transfer of metal ions from the glass to the acid. GICs are therefore hybrid materials and, when set, can be considered as a composite of glass particles in a hydrogel binding matrix[2].

Evaluation of the biocompatibility of GICs has concentrated on their dental applications. The *in vitro*[3,4] and *in vivo*[5,6] response of the dental pulp to GICs has been compared to that of other types of dental filling material and, when used as an endodontic sealer, the clinical response of bone–periodontium has been compared to traditional dental materials[7,8]. However, although a GIC (Ketac-O, Espe, Germany) has been evaluated as a possible cement for orthopaedic implants[9], none has been studied in the role of a possible bone substitute. The purpose of this *in vitro* study was to compare the initial responses of osteoblasts and bone to GICs and representative ceramic materials currently used as bone substitutes.

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MATERIALS AND METHODS

Materials

Three different GICs were evaluated. Two were commercial dental luting cements Ketac Cem Radiopaque (Espe, Germany) and AquaCem (DeTrey, UK), both based on fluoroaluminosilicate glasses of general composition SiO_2, Al_2O_3, AlF_3, CaF_2, NaF and $AlPO_4$, reacted with a copolymer of poly(acrylic)acid/maleic acid and poly(acrylic)acid, respectively, and the third was a fluoride-free glass Mp4 (Pilkington, UK) composition by mass SiO_2 30.8%, Al_2O_3 38.5%, CaO 28.6% and Na_2O 2.1%, reacted with poly(acrylic)acid L3(4) mol wt 28 500 (Laboratory of the Government Chemist, UK). The commercial materials were mixed as directed for dental use and the fluoride-free GIC was made up in volume fractions of 0.5 Mp4, 0.2 E3(4) and 0.3 sterile distilled water containing 10% tartaric acid to control the set.

The set materials were fractured with a hammer and irregular pieces of material approximately 2 × 1 mm selected. Smooth rods (nominally 2 mm long, 1 mm in diameter) were produced by placing unset material in silicone moulds. The set material was stored for 1 wk at

100% humidity before use. Evaluation of fresh GICs involved placing 0.2 ml of freshly mixed unset Ketac Cem directly into the culture. The unreacted glass components of Ketac Cem and Mp4 together with the poly(acrylic)acid E3(4) were similarly tested. The ceramics evaluated were densely sintered rounded hydroxyapatite (HA) granules (Calcitite, USA and one supplied by the Materials Advice and Research Centre, University of Sheffield, UK) and tricalcium phosphate (TCP) granules (Friedrichsfeld, Germany). The set GICs and ceramics were immersed in absolute alcohol, air dried aseptically and, just before placement into the culture system, washed in phosphate buffered saline (PBS) containing 10% fetal calf serum. Unset GICs and the components of GICs were handled and mixed under sterile conditions.

Methods

Tissue culture. Killed 1-day-old inbred Wistar rats were used as the source of primary cells. Small squares of suture-free parietal bone were harvested aseptically and two types of culture undertaken on at least 5 occasions for each material in mixed batches of up to 20 cultures in a multiwell plate:

1. A calvarial culture in which the endocranial and epicranial periostea were stripped from the bone and test material placed on to the layer of exposed osteoblasts on the endocranial side of the bone[10].
2. An envelope culture in which the periostea were retained upon the bone, the test material being introduced into the culture to lie between the surface of the bone and the overlaying periosteum on the endocranial surface[11].

The prepared cultures were maintained at 37°C in an atmosphere of 5% CO_2 for 2 wk in Fitton-Jackson's modification of Bigger's medium (Gibco, UK) supplemented with 10% fetal calf serum, 20 μl/ml 200 mM glutamine, 10 μl/ml penicillin/streptomycin (500 i.u./ml), 25 μl/ml HEPES 1 M solution, 10 μl/ml β-glycerol-2-phosphate 1 M solution and 50 μg/ml ascorbic acid.

Preparation for microscopy. Specimens were first fixed by replacing the culture medium with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2–7.4) for 24 h. Calvarial cultures were secondarily fixed with 2% aqueous osmium tetroxide for 1 h, followed by dehydration through a graded acetone series, critical-point dried from CO_2 (Polaron critical point drier) and coated with a thin layer (approximately 20 nm) of gold (Nanotech sputter coater) for scanning electron microscopy (SEM) (Cambridge Stereoscan 600, set at 25 kV). Envelope cultures were dehydrated through a graded ethanol series and embedded in resin (L.R. White, hard grade, UK). Blocks were ground to expose the tissue and decalcified in 10% EDTA in Tris buffer. Thin sections for transmission electron microscopy (TEM) were stained with saturated uranyl acetate in 50% ethanol and Reynold's lead citrate and viewed on copper grids coated with collodion in amyl acetate using a Philips CM 10 transmission electron microscope set at 80 kV.

RESULTS

SEM examination of calvarial cultures

Osteoblast-like cells migrated on to and confined all the solid test materials (*Figures 1, 2* and *3*) to bone except for the GIC AquaCem (*Figure 4*) which, despite repeated attempts, could not be retained in place on the calvarial bone during processing. The two types of HA responded in a similar manner and results for HA apply to both. Cells which had migrated on to and colonized the smooth surfaces of the set GICs (Mp4 and Ketac Cem) and the ceramics HA and TCP were closely packed in a continuous sheet. Osteoblastic morphology was maintained with a polygonal tessellated appearance, similar to unmigrated cells on the surrounding calvarial bone (*Figures 1, 2* and 3).

In areas where fracture of the GICs had left an irregular surface (*Figures 2b* and *3*), cells were loosely packed and exhibited a fibroblastic morphology with multiple pseudopodial and filopodial cellular extensions anchoring cells to the GICs and maintaining contact between adjacent cells. The placement of set AquaCem (*Figure 4*), freshly mixed Ketac Cem (*Figure 5*) and the glass component of Ketac Cem (*Figure 6d*) into culture resulted in cell death in the immediate proximity of the materials and cell migration from the surrounding areas had not occurred at 2 wk. In sites from which AquaCem had been lost, the underlying bone appeared non-vital with loss of the normal collagen and extracellular matrix (ECM) covering (*Figures 4c* and *d*). Cultures contaminated with Mp4 glass and the poly(acrylic)acid E3(4) behaved as control cultures grown without test material over the 2 wk period (*Figures 6a, b* and c).

TEM examination of envelope cultures

Cells in contact with all test materials except the AquaCem (*Figure 7a*) remained viable throughout culture. Cells were seen to be intimately associated with the surface of the test materials on to which collagen-containing ECM was elaborated (*Figures 8, 9* and *10*). The GICs (Ketac Cem and Mp4)–tissue interface was similar for both materials. Collagen fibres exhibited an alternating layered configuration (*Figures 8b* and *c*) which, in close proximity to the GICs, became random with fibres interdigitating with the surface of the material (*Figure 8d*). The GIC AquaCem appeared to have broken down in culture and glass particles were seen interspersed between lysed cell debris (*Figure 7a*). However, cultures contaminated with the particles of the glass component of Ketac Cem reacted in a similar manner to the set material (*Figure 7b*). The interface between the ceramics (HA and TCP) and the tissue showed two configurations. The first was similar to that seen for GICs consisting of an ECM containing randomly oriented collagen fibres which interdigitated with the surface of the ceramic. In places, however, mineralization of the ECM was noted (*Figures 9a* and *10*); convincing evidence of mineralization was not noted in any of the 2 wk cultures of GICs. In the case of TCP, some of the elaborated tissue resembled bone morphologically (*Figure 10*). The other type of interface seen was associated with HA and consisted of a narrow electron-dense zone dividing the mineralized ECM from the HA (*Figure 9b*).

DISCUSSION

Several characteristics of GICs make them attractive as a possible bone substitute:

1. In current dental use, GICs are mixed and placed in a plastic state, subsequently setting *in situ*. The ability to mould and shape an implant material clinically to conform to the bone topography and set to a required shape would

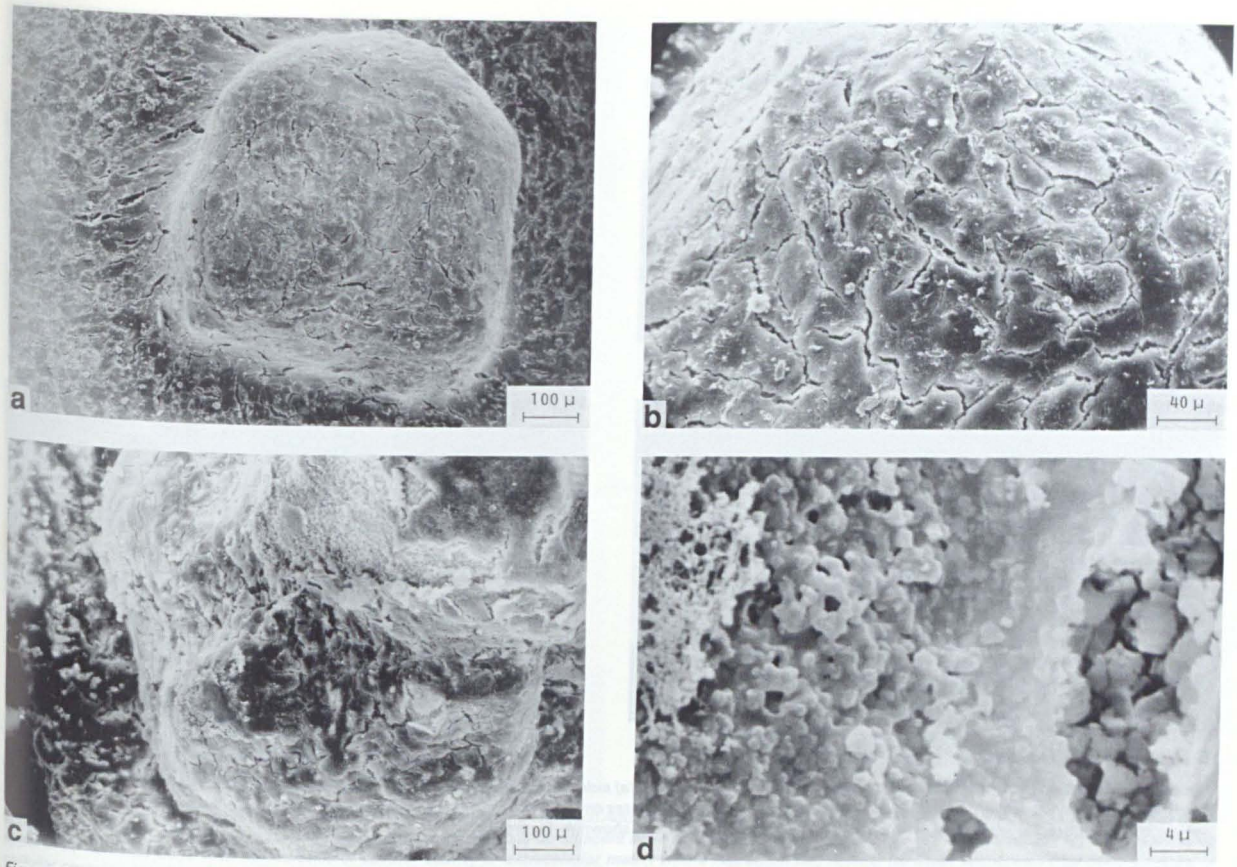

Figure 1 SEM photomicrographs of calcium phosphate ceramics after 2 wk in culture: (a) HA (University of Sheffield) particle covered by a continuous sheet of cells which have migrated from the adjacent host bone; (b) detail from HA surface (Figure 1a), showing tessellated polygonal cells which exhibit an osteoblast-like morphology; (c) TCP particle only partly covered by migrated cells from host bone; (d) upper surface of TCP devoid of cells; the exposed TCP exhibits a vermiform appearance suggestive of a physico-chemical breakdown.

overcome many of the current problems of implantable ceramics[12].

2. The ratio of the glass and the polyelectrolyte components of GICs can be varied to produce a wide range of cements, allowing the formulation of materials for specific applications[2]. GICs can therefore be produced that are biomechanically compatible with bone by matching a factor such as the modulus of elasticity[13].
3. When freshly mixed, GICs are able to bond chemically to apatite[14]. This gives them an advantage over current materials which rely on cellular growth to establish an intimate integration or bioactive interface between the material and the bone.
4. Even when set, GICs are not inert and their ability to leach ions[2], including fluoride, which in low systemic dose stimulates bone formation[15], may confer an osteoconductive potential upon them.

Initial evaluation of GICs as a bone substitute was undertaken using an *in vitro* model, in which the cell types encountered by the materials mirror the *in vivo* situation[16]. Such models are based on the ability of osteoblasts to migrate and colonize denuded bone and foreign material[17] and have been used to evaluate the interaction of calcium phosphate ceramics and bioactive glass in the biological environment[10, 11, 16, 18–20]. By careful choice of culture conditions and tissues, together with the addition of ascorbic acid and β-glycerol-phosphate to culture media, *in vitro* bone formation can be induced[11, 21].

The response of the bone cultures to HA and TCP, as seen in *Figures 1, 9* and *10*, was similar to the responses reported by other authors[11, 16, 18–20] and confirms the suitability of these materials for use as bone substitutes with osteoconductive properties. Osteoblast-like cells were seen to colonize the ceramics, maintain their phenotype and produce a collagenous ECM. The resulting interface was either analogous to the normal lamina limitans of bone[22] (*Figure 9b*), which has also been noted at the interface of bone/HA *in vivo*[23], or an interdigitation of collagen fibres with the ceramic; the former type appears to be a less active, matured, interface than the latter which seems to occur when mineralization is complete[22, 23]. In calvarial culture, osteoblasts did not colonize the whole surface of TCP particles (*Figures 1c* and *d*). A possible explanation for this finding implicates loss of cell anchorage, upon which cell growth and migration is dependent, resulting from dissolution of material by the culture fluid[18]. In the more protected envelope culture, where the material was placed under the periosteum, new bone formation occurred on the surface of the TCP though without the demarcation of a lamina limitans (*Figures 10a* and *b*), perhaps indicating a more bioactive rather than an inert interface.

The response of the tissues to GICs was more varied. When GICs are mixed and allowed to set against a mould, the smooth surface produced will be composed of a matrix-rich layer containing few glass particles[2]. This part of the GIC test specimen was responded to favourably and the smooth surfaces of both Ketac Cem and Mp4/E3(4) were colonized

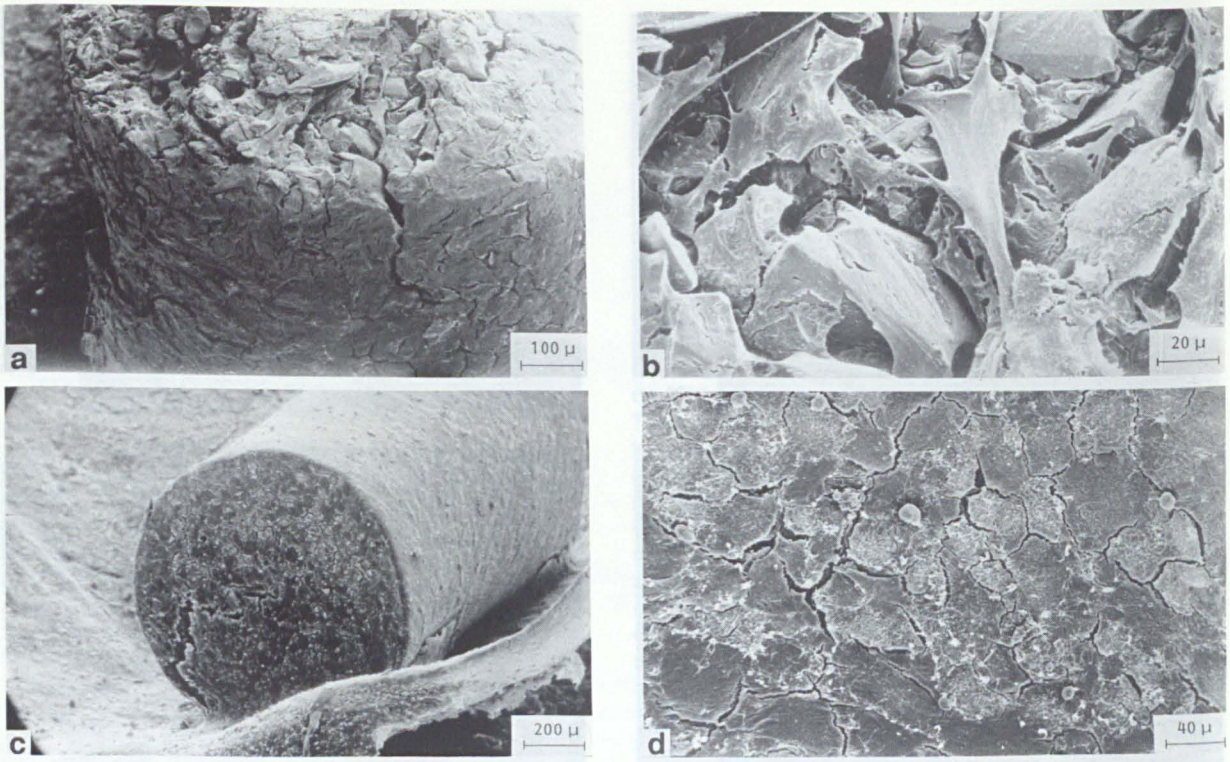

Figure 2 SEM photomicrographs of smooth rods of GIC after 2 wk culture: (a) side and fractured end of an Mp4-based GIC rod showing migrated cells; (b) detail from Figure 2a *showing actively migrating cells with long cytoplasmic processes on top of the rod; (c) host bone and rod of Ketac Cem covered by migrated cells as seen with HA* Figure 1a*; (d) Typical osteoblast-like cells on surface of rod shown in* Figure 2c.

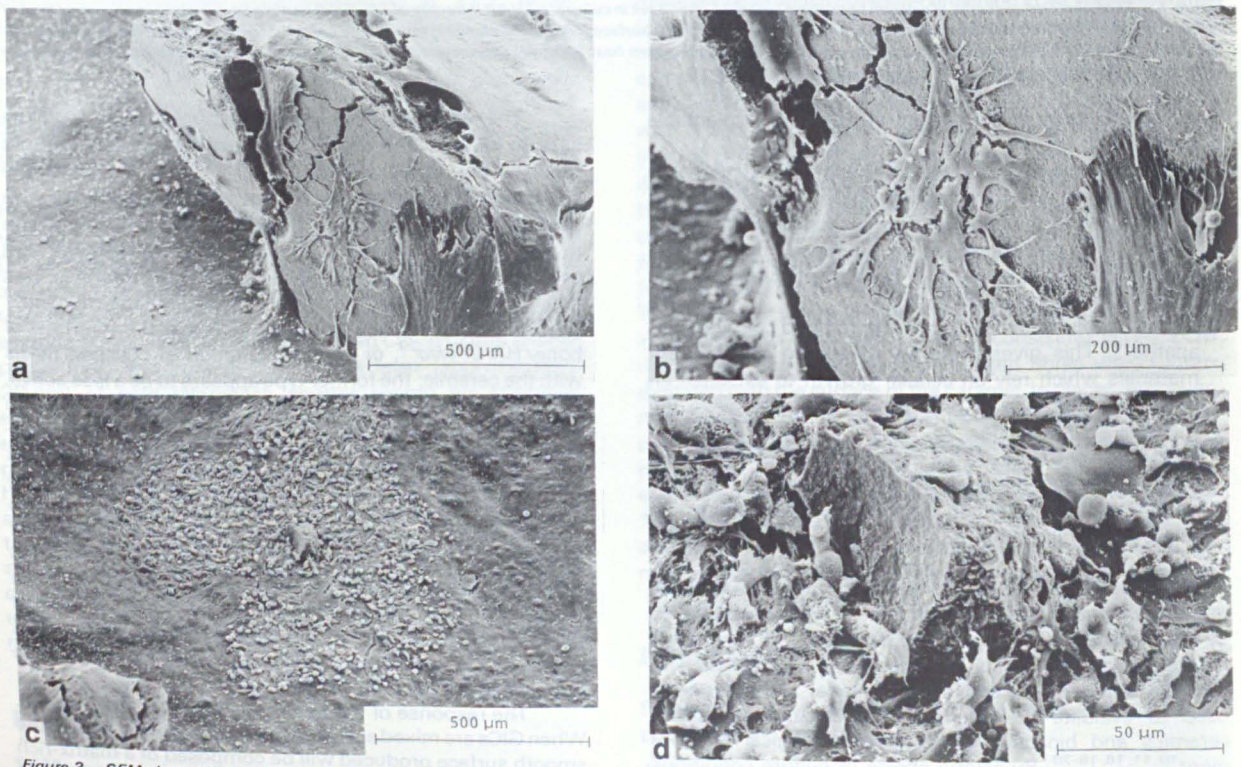

Figure 3 SEM photomicrographs of a rough fractured particle of Ketac Cem and host bone after 2 wk in culture: (a) host bone with particle of Ketac Cem partly covered by migrated cells; (b) actively migrating cells on a smooth part of Ketac Cem demonstrating close apposition of cell processes to material and contiguous cells; (c) isolated fragment of Ketac Cem on host bone surrounded by a zone of dead cells; (d) dead and vital cells round Ketac Cem fragment seen in Figure 3c.

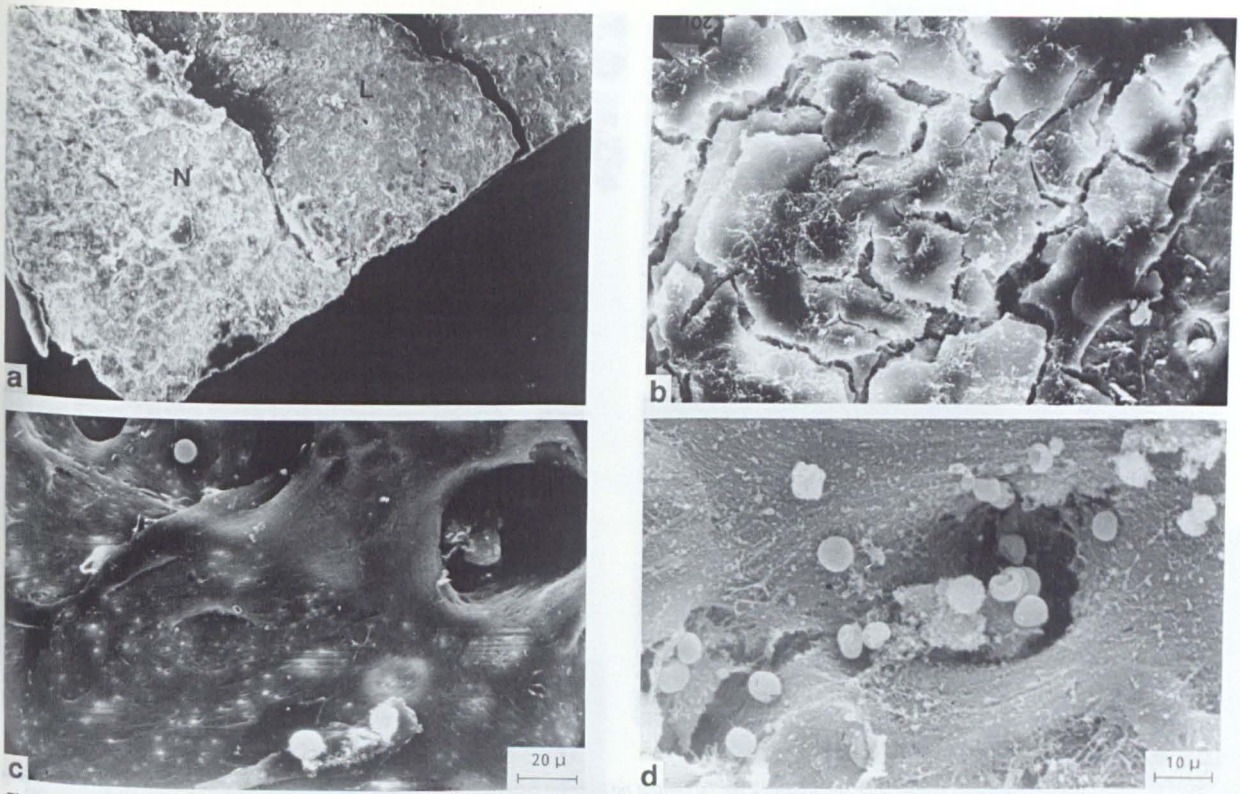

Figure 4 *SEM photomicrographs of host bone cultured for 2 wk and on which AquaCem had been placed: (a) bone with the area from which AquaCem has been lost, showing lack of cellular cover (L) and normal appearance of cells on adjacent bone (N); (b) detail of osteoblast-like cells from area (N); (c) area (L) showing apparently non-vital bone with no evidence of cellular activity or extracellular matrix where AquaCem had rested; (d) fixed, uncultured vital bone stripped of cellular covering but retaining the extracellular matrix for comparison with* Figure 4c.

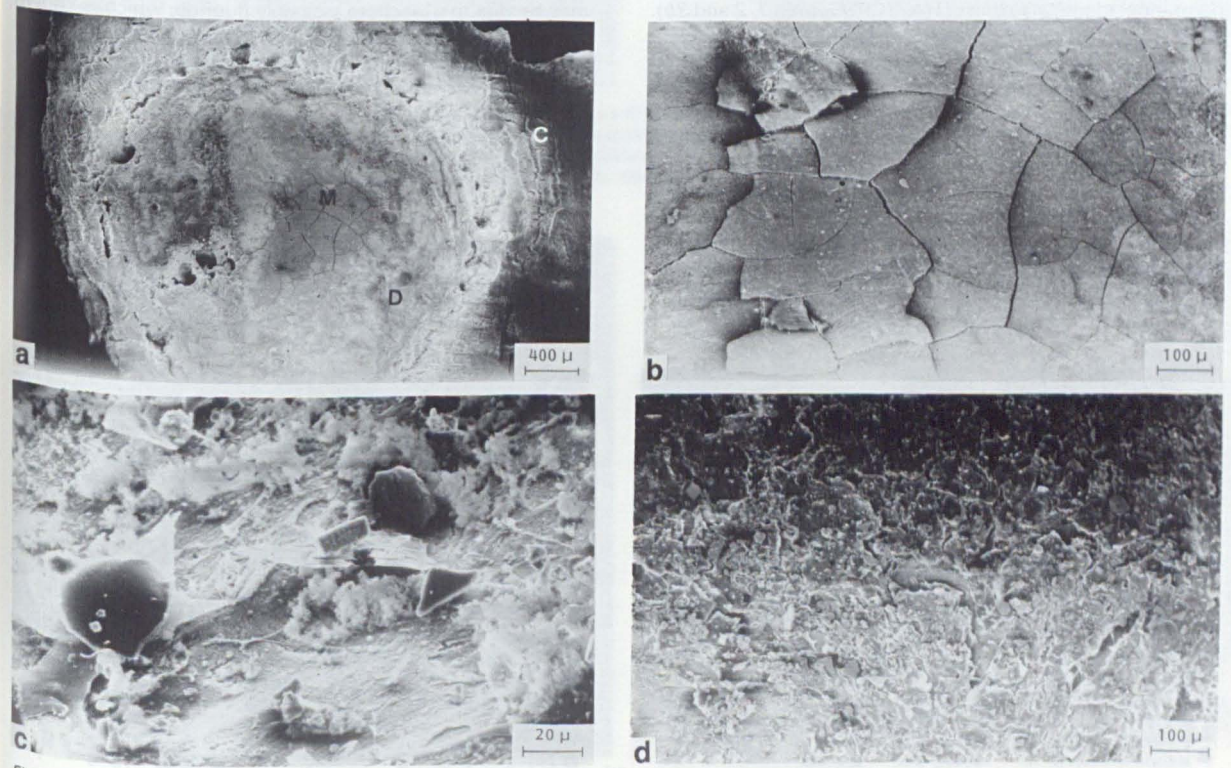

Figure 5 *SEM photomicrographs after 2 wk culture of host bone on to which a drop of freshly mixed Ketac Cem had been placed: (a) three distinct zones can be identified: (1) centrally smooth surface of material (M), (2) material covered by cellular debris (D), and (3) peripherally vital cells on host bone (C); (b) central zone (M) showing surface of Ketac Cem (cracks are processing artefacts); (c) zone D showing glass particles and cell debris; (d) zone C showing cells on host bone.*

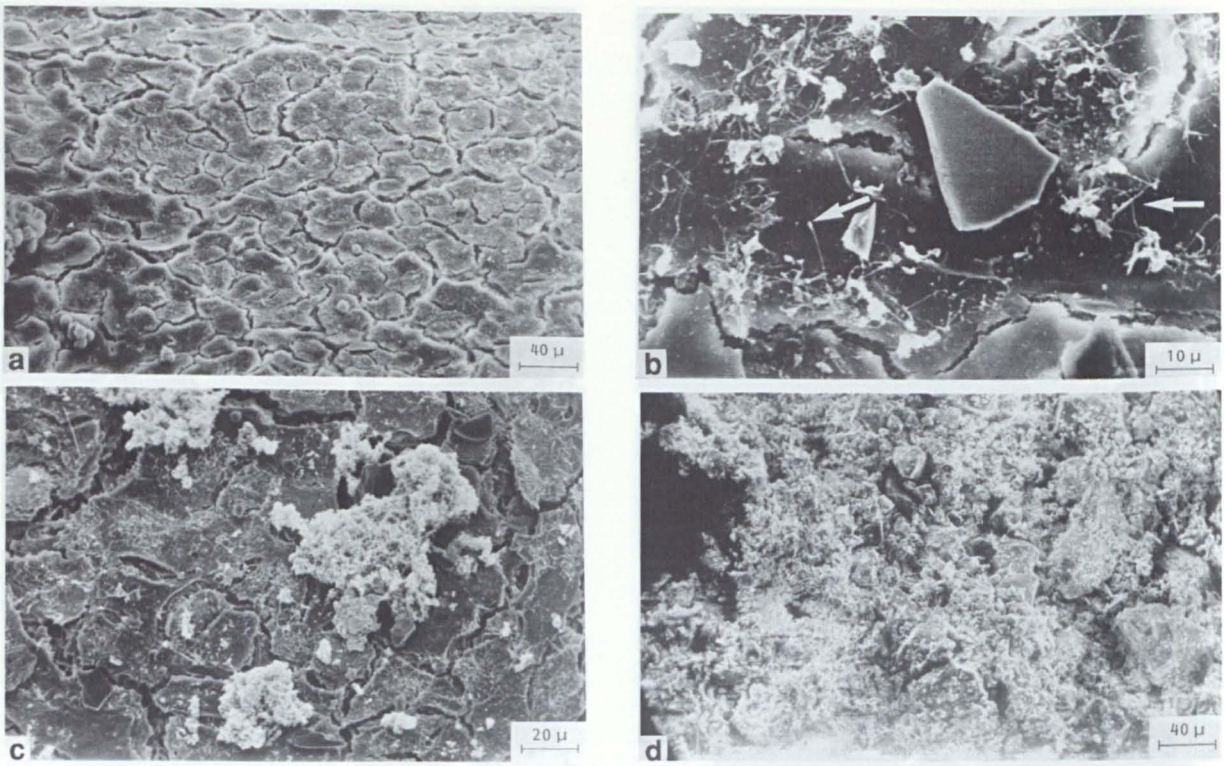

Figure 6 SEM photomicrographs of host bone cultured for 2 wk: (a) control, no material in culture; (b) culture contaminated with Mp4 glass, vital cells producing collagen ECM (arrowed), with a glass particle in centre of field; (c) culture contaminated with poly(acrylic)acid E3(4) some of which has precipitated on to the surface of the cells during processing; the cells appear normal; (d) culture contaminated with the particulate glass component of Ketac Cem; no evidence of cell migration on to the material or vital cells can be seen.

in a manner identical to that of HA/TCP (*Figures 1, 2* and *3b*). On fracturing the material, more glass particles were exposed to the cells. Some of the irregularly fractured surfaces of Ketac Cem were not colonized (*Figures 3c* and *d*) and this appeared to be a result of inhibition of cell growth rather than an inability to colonize an irregular surface, which was observed for certain areas of TCP (*Figures 1c* and *d*) and Mp4 (*Figure 2b*). However, it seems that the response is dose related, or relies on an interaction with the culture media, as in envelope cultures contaminated with Ketac Cem glass particles, there appeared to be no cellular embarrassment (*Figure 7b*). Previous *in vitro* evaluations of GICs[3,4] have noted a similar mild toxic effect and have suggested it may be due to a leachate, possibly fluoride, which is known to be released from set GICs[2]. The lack of any toxic response to set fractured Mp4/E3(4) GIC or Mp4 glass particles, which do not contain fluoride, supports this hypothesis (*Figures 2a, b* and *6b*).

In envelope culture, a direct interdigitation of collagen fibres was seen with the smooth surface of Ketac Cem and Mp4 GIC (*Figure 8*), a finding similar to that seen for the ceramics TCP and HA and the reported interface of the bioactive glass 45S5 (Nippon Kogaku k.k., Japan) *in vitro*[10].

In vitro freshly mixed GIC has been shown to be toxic to human pulp cells growing immediately adjacent to the

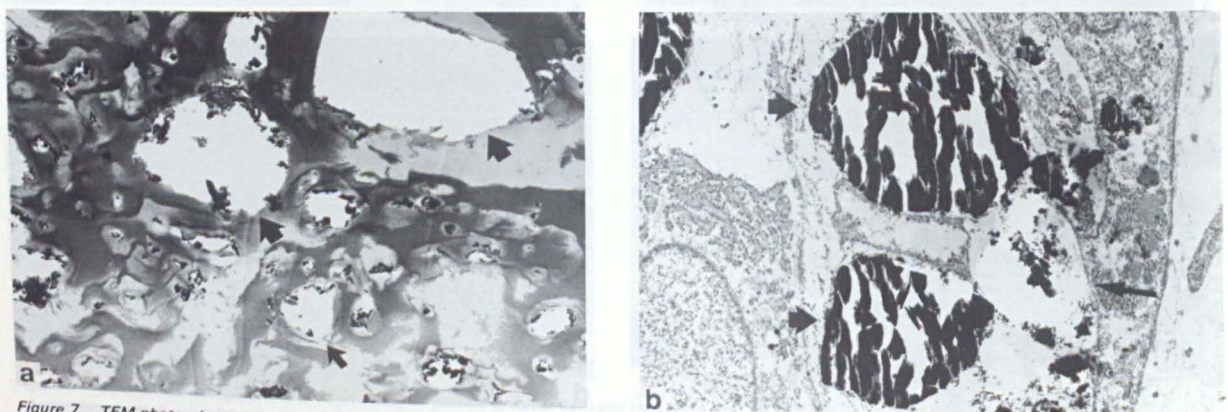

Figure 7 TEM photomicrographs of 2 wk envelope cultures into which GIC was placed: (a) set AquaCem (AQ); the material has broken down, individual glass particles/space occupied by particles (arrowed) are seen surrounded by lysed cells/debris; (b) glass component of Ketac Cem, glass particles (arrowed) are surrounded by vital cells exhibiting prominent nuclei, rough endoplasmic reticulum and numerous mitochondria.

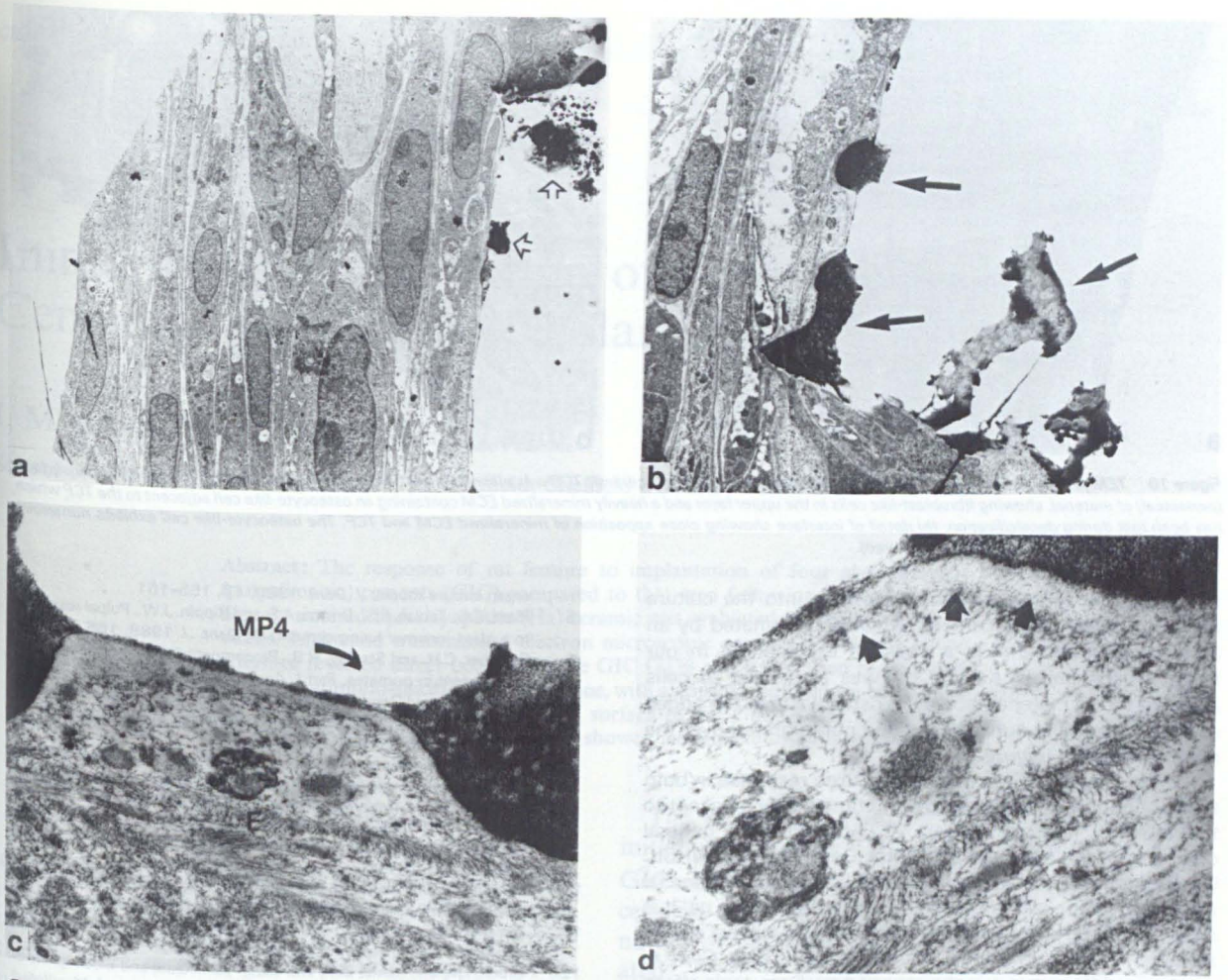

Figure 8 TEM photomicrographs of 2 wk envelope cultures into which set GICs based upon Mp4/E3(4) had been placed: (a) section through whole of cellular layer apposed to upper surface of the GIC (the periosteum) showing close apposition of fibroblast-like cell to the GIC-retained fragments (arrowed); (b) interface of cells and Mp4 GIC (arrowed); (c, d) higher power of interface showing layered arrangement of collagen in ECM (F) becoming random at the material interface with interdigitation of fibres, across a final less dense zone (arrowed), into the Mp4 GIC, fragments of which remain in contact with the tissue.

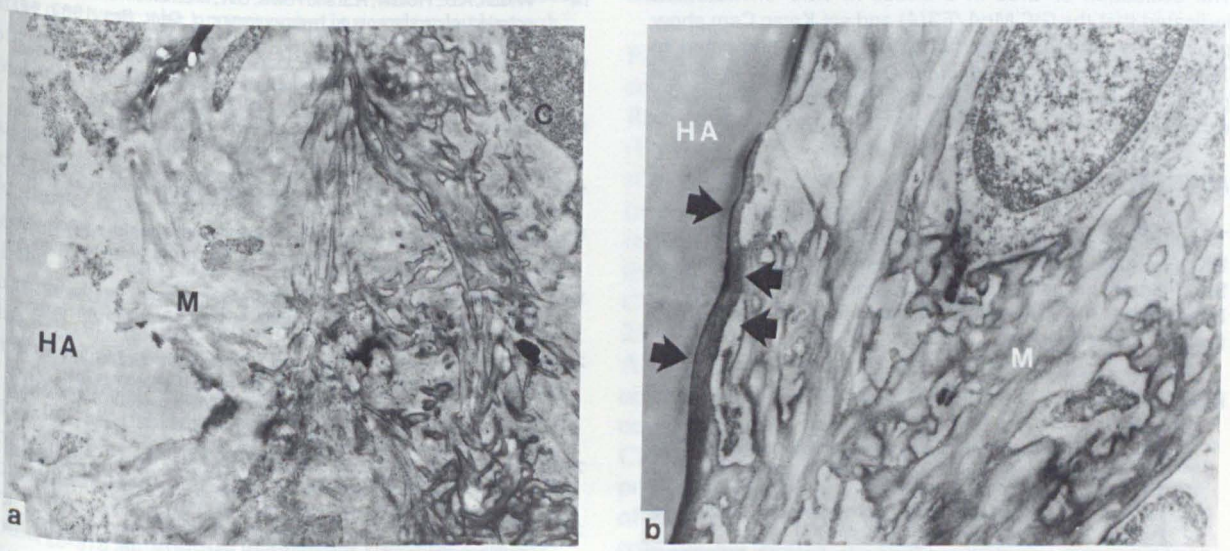

Figure 9 TEM photomicrographs of 2 wk envelope cultures into which the ceramic HA (calcitite) was placed: (a) interface exhibiting interdigitation of mineralized collagenous ECM (M) with HA (HA), which was lost on decalcification, a cellular process (C) can be seen; (b) interface exhibiting electron dense layer (arrowed) between mineralization ECM (M) and HA.

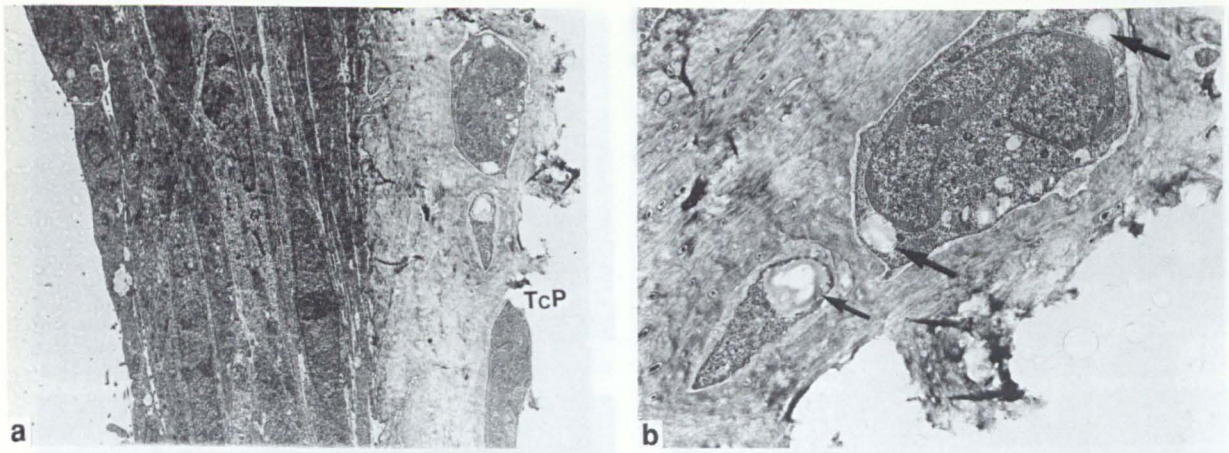

Figure 10 TEM photomicrographs of 2 wk envelope culture into which the ceramic TCP was placed. (a) section through whole of cellular layer on upper surface (periosteal) of material, showing fibroblast-like cells in the upper layer and a heavily mineralized ECM containing an osteocyte-like cell adjacent to the TCP which has been lost during decalcification; (b) detail of interface showing close apposition of mineralized ECM and TCP. The osteocyte-like cell exhibits numerous mitochondria and glycogen granules (arrowed).

material[3]. The release of free metal ions into the culture media from the unset cement, when contaminated by an aqueous environment[2] is a possible explanation. In our study, freshly mixed Ketac Cem was also toxic to cells adjacent to the material; however, osteoblasts at the edges of the bone culture survived but had not migrated over the material at 2 wk (*Figure 5*).

The GIC AquaCem produced a toxic response in both types of culture causing cell death immediately adjacent to the material (*Figures 4* and *7*). Although the general composition of AquaCem and Ketac Cem (both type II GIC luting cements) is similar, because both are commercial materials, their exact compositions are unknown to us. It is assumed that the combination of glass and alkenoic acid in AquaCem was sufficiently different from Ketac Cem to produce a different and, in the case of AquaCem, toxic effect.

CONCLUSION

The evaluation of GICs in a closed *in vitro* environment indicated that the GIC Mp4/E3(4) and set Ketac Cem show promise as potential bone substitutes when compared to TCP and HA, both of which are used successfully in clinical practice. There was evidence that fluoride-containing glasses exhibit mild *in vitro* toxicity.

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Initial In-Vivo Evaluation of Glass-Ionomer Cements for Use as Alveolar Bone Substitutes

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Abstract: The response of rat femora to implantation of four glass-ionomer (polyalkenoic) cements (GIC) compared to that seen following implantation of densely sintered hydroxyapatite (Ha) ceramic was evaluated for periods up to 12 weeks. Light and transmission electron microscopic analysis of the GIC/bone interface revealed direct bonding of the GIC G338 and Ketac Cem (both based on fluoro-alumino-silicate) glasses to bone, with a mineralized collagen-containing extra-cellular matrix deposited on the surface of the GIC. AquaCem and the fluoride-free GIC based on MP4 glass showed incomplete osseointegration.

INTRODUCTION

In the UK, approximately 20% of the population over the age of 16 are edentulous and need to wear full dentures. To provide the dentures with the stability necessary for good function, support of a substantial alveolar ridge is required. Although this is the situation immediately after extraction of teeth, progressive resorption of alveolar bone follows and while rapid resorption usually ceases after one year, the process continues slowly throughout life resulting in severe functional, social and aesthetic problems for many individuals.[1,2]

Over the past few years our group has developed surgical techniques for utilizing currently available ceramics. However, none of the materials presently available have the ideal biomechanical properties needed for restoration of the atrophic alveolar bone of the jaws. They also suffer from problems related to their surgical manipulation and often a two-stage surgical procedure is required to achieve a satisfactory clinical result.[3]

Glass-ionomer cements (GIC)[4] are widely used as dental restorative materials, their biocompatibility being comparable to, or better than conventional dental fillings and endodontic sealers.[5–8] It has recently been proposed that GICs could be formulated to produce a biomechanically-matched bone substitute[9] and used as an orthopaedic cement.[10]

The purpose of this study was to evaluate the initial in-vivo response of bone to four different GICs and compare this to hydroxyapatite (Ha), a ceramic currently used as a bone substitute. The nature of the bone/GIC interface was also evaluated.

METHODS

Materials

Four different GICs were evaluated. Two were commercial dental luting cements: Ketac Cem Radiopaque (Espe, Seefeld, FRG) and AquaCem (DeTrey, UK), both based on fluoro-alumino-silicate glasses reacted with a co-polymer of poly-(acrylic)acid/maleic acid and poly(acrylic)acid respectively. The other two GICs were of known formulation: G338 (Laboratory of the Government Chemist, London, UK) composition by mass SiO_2 25·2%, Al_2O_3 14·2%, CaF_2 12·8%, AlF_3 4·5%, $AlPO_4$ 24·1%, Na_3AlF_6 19·2%, and a non fluoride-containing glass, Mp4 (Pilkington, Ormskirk, UK), composition by mass SiO_2 30·8%, Al_2O_3 38·5%, CaO 28·6%, Na_2O 2·1%, both reacted with poly(acrylic)acid L3(4) mol. wt 28 500 (Laboratory of the Government Chemist, London, UK). The commercial materials were mixed as directed for dental use and the G338/Mp4 based GICs were made up in volume fractions of 0·5 glass, 0·2 L3(4),

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and 0·3 sterile distilled water containing 10% tartaric acid to control the set. Smooth rods (nominally 3 mm long, 1 mm in diameter) were produced by placing unset material in silicone moulds. Freshly mixed Ketac Cem was also evaluated.

The ceramic evaluated concurrently was densely sintered rounded hydroxyapatite (Ha) granules (Calcitite, Palo Alto, USA). Set GIC and the Ha was sterilized using ethylene oxide and stored for one week prior to use. Freshly mixed GIC was handled and mixed under sterile conditions.

Implantation

Each of the five materials and freshly mixed Ketac Cem were implanted in a standardized manner into the midshaft of the femora of weaned inbred male Wistar rats (three rats being used for each material) and healing allowed to take place for six weeks. In addition, implants of Ha, set Ketac Cem, and fresh Ketac Cem were implanted for two and 12 weeks, also using three rats for each material/time interval. General anaesthesia was induced and maintained by inhalation of Halothane and nitrous oxide/oxygen. Following cleaning of the skin over the implant site, the bone was exposed using sharp and blunt dissection.

Under saline irrigation a slow speed dental drill fitted with a round bar (number 3, Ash, Bristol, UK) was used to cut a hole matched to the diameter of the implant. Implants were placed to lie flush with the surface of the bone. In the case of Ha, two particles were placed into the prepared hole. The overlaying periosteum and soft tissues were replaced and the wound sutured. Post-operatively, wounds were inspected to monitor healing and rats were maintained on a standard laboratory diet. Rats were killed under anaesthetic, by perfusion of the vascular system with 14% gluteraldehyde buffered with 0·2 M sodium cacodylate with 1 mM of calcium chloride. The femora were then dissected out and stored for 24 h in buffered 3% gluteraldehyde.

PREPARATION FOR MICROSCOPY

Transmitted light

Specimens were dehydrated through a series of graded ethanols and embedded in resin (L. R. White Hard Grade, London, UK). Ground (undecalcified) sections 20–40 μm thick, were produced through the centre of the long axes of the implants, stained with Stevenel's Blue and counter stained with either Alizarin red S or Van Gieson picrofuchsin.[11]

Ultrastructural

Blocks containing Ha and set Ketac Cem were surface decalcified in 10% EDTA in Tris Buffer (Sigma, UK) prior to cutting semi-thin sections which were stained with toluidine blue. From these sections, areas showing the implant/tissue interface were selected and processed for transmission electron microscopy (TEM). Ultra-thin sections were stained with saturated uranyl acetate in 50% ethanol and Reynold's lead citrate and viewed on copper grids coated with collodion in amyl acetate using a Philips CM 10 transmission electron microscope set at 80 kV. The GIC was not removed by surface decalcification and TEM energy dispersive X-ray microanalysis (EDAX) was used to confirm the presence and structure of Ketac Cem at the bone interface.

RESULTS

Of the 36 operations, all healed uneventfully except for one where Ketac Cem had been placed: at day three wound breakdown/infection was noted, but this was resolved following topical application of chlortetracycline.

Light microscopy

At two weeks the Ha implants were seen to be embedded in new bone which was in intimate contact with the surface of the material (Fig. 1). By

Fig. 1. Ground section of femur with Ha implant at two weeks, showing integration of Ha particle in cortex and a thin shell of new bone surrounding separate particle in marrow. (Original magnification × 10; Stevenel's blue/van Gieson).

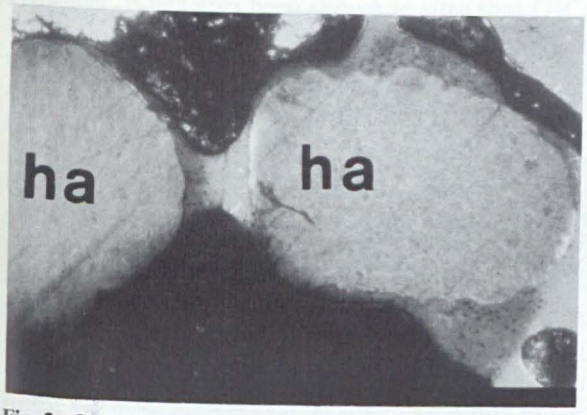


Fig. 2. Ground section of femur with Ha implant at 12 weeks, showing Ha particles intimately covered with a thin shell of new bone and surrounded by normal marrow. (Original magnification × 20; Stevenel's blue/Alizarin red S.)

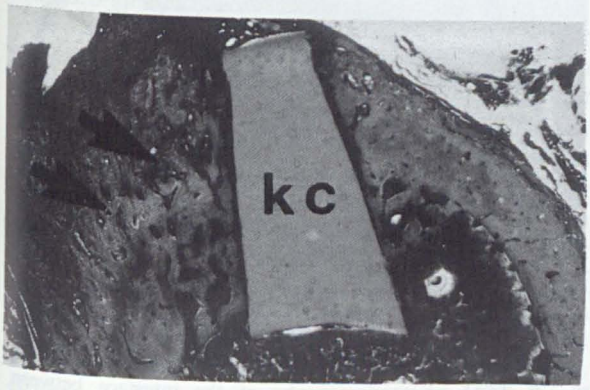


Fig. 3. Ground section of femur with Ketac Cem (kc) implant at two weeks, showing periosteal reaction arrows and proliferation of bone to cover surface of material. (Original magnification × 10; Stevenel's blue/Alizarin red S.)

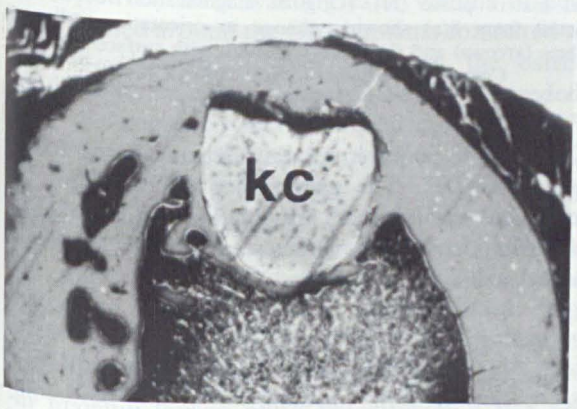


Fig. 4. Ground section of femur with Ketac Cem (kc) implant at six weeks, showing integration of Ketac Cem and restoration of normal surrounding bony architecture. (Original magnification × 10; Stevenel's blue/Alizarin red S.)

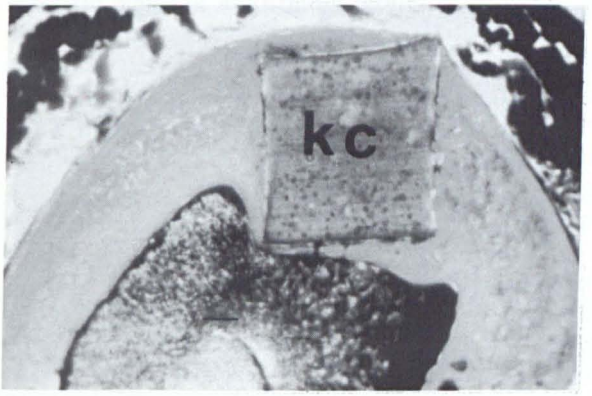


Fig. 5. Ground section of femur with Ketac Cem (kc) implant at 12 weeks, showing complete integration of Ketac Cem with bone. (Original magnification × 10; Stevenel's blue/van Gieson.)

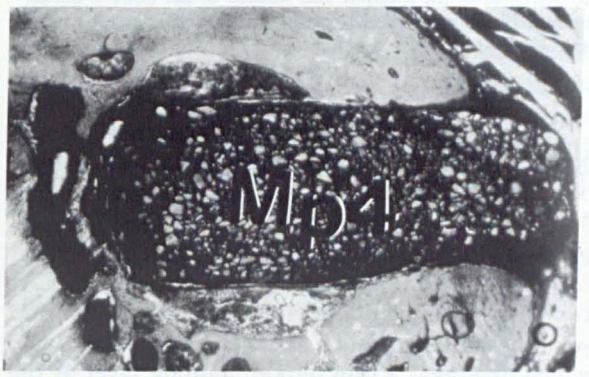


Fig. 6. Ground section of femur with Mp4-based GIC implant at six weeks, showing minimal downgrowth of endocortical bone around implant and interposition of non-calcified cellular tissue between bone and implant. (Original magnification × 10; Stevenel's blue/Alizarin red S.)

12 weeks Ha projecting into the marrow space was completely encapsulated by a shell of bone in continuity with, and appearing to have grown down from, the endosteal surface of the cortex (Fig. 2).

The responses to set Ketac Cem and G338-based GIC were similar to that for Ha except a more pronounced periosteal reaction was noted at two weeks (Fig. 3). At both six and 12 weeks, the normal marrow anatomy was restored and the architecture of the bone surrounding the GIC showed a regular trabecular pattern, with evidence of normal remodelling and no intermediate layer separating the material from the bone (Figs 4 and 5).

After six weeks, Mp4-based GIC, and to a lesser extent AquaCem, exhibited a less intimate relationship with the bone, there being interposition of fibrous/marrow tissue between the proliferating cancellous and cortical bone and the material (Fig. 6).

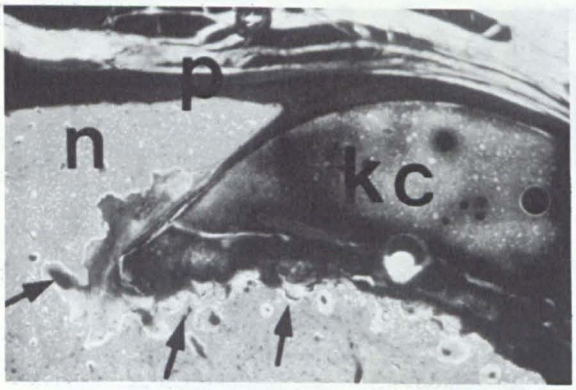


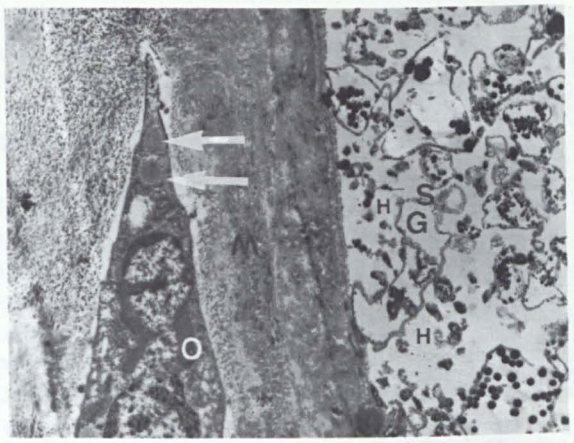
Fig. 7. Ground section of femur at 12 weeks following placement of fresh Ketac Cem (kc) onto surface of the bone. The periosteum has reformed (p) and new bone (n) has proliferated at the edge of the implant. Extensive remodelling at the interface of the implant and surface of the bone is occurring and Howships lacunae containing osteoclasts can be seen (arrows). (Original magnification × 25; Stevenel's blue/van Gieson.)

The application of fresh Ketac Cem to the predrilled bur hole proved technically difficult (when mixed as directed for a dental cement, too fluid a mix was produced). Examination of the sections showed that the majority of the material had remained on the surface of the bone (Fig. 7). At two weeks the Ketac Cem followed the contour and was intimately related to the underlying bone, which exhibited a thin darker staining layer at the interface with the Ketac Cem. By six weeks there was evidence of extensive periosteal reaction and subperiosteal bone resorption/remodelling, and the intimate relationship between the bone and the Ketac Cem seen at two weeks was no longer present along the entire interface. The remodelling and proliferation of new bone was still evident at 12 weeks, as shown in Fig. 7.

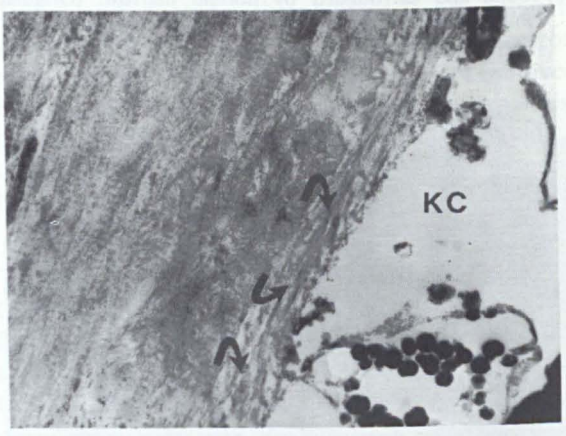
Ultrastructural

The apparently intimate association between implants and bone seen at the light microscope level for Ha, Ketac Cem and G338-based GIC was confirmed at the ultrastructural level by TEM as seen in Fig. 8 (a) and (b). A mineralized collagen-containing extracellular matrix was laid down directly upon the surface of the GIC. At the interface, the direction of the incorporated collagen fibres changed from being orientated parallel to the material surface, and were randomly arranged with some interdigitating directly with the GIC which was retained during processing (Fig. 8 (a) and (b)).

The presence of GIC was confirmed by EDAX analysis which revealed the presence of aluminium and silicone peaks in what were considered to be glass particles. The appearance of the Ha-bone interface was similar, except that in places an electron dense zone was seen at the interface between Ha and the bone.



(a)



(b)

Fig. 8. TEM photomicrographs of the interface between Ketac Cem and newly-formed bone after six weeks implantation. (a) Presumptive osteocyte (O), exhibiting eccentric nucleus and glycogen granules (arrows), incorporated into a mineralized collagen-containing extracellular matrix (M); Ketac Cem retained during processing comprises glass particles (G), siliceous hydrogel sheathing glass core (S), and hydrogel matrix phase (H). (Original magnification × 6600.) (b) Detail from 8(a) showing change in orientation collagen fibres (arrows) and their interdigitation with surface of Ketac Cem (KC) (Original magnification × 21 000).

DISCUSSION

Set GICs, investigated using several different in-vitro techniques, have been shown to be well tolerated by living cells,[12,13] with evidence that certain of them promote osteoblastic activity and form an intimate bioactive bond with bone cells.[9,14]

However, it has also been shown *in vitro* that GICs based upon fluoro-alumino-silicate glasses release a mildly cytotoxic leachate, the effect of which rapidly diminishes with time as the cement fully polymerizes.[9,15,16]

It is not known what component/s of GICs are responsible for the mild in-vitro cytotoxicity reported. In freshly mixed GIC, the release of free metal ions and the acidity/irritability of the alkenoic acid have been implicated,[12,15] as has fluoride which is known to leach rapidly from the surface and then gradually from the bulk of set GIC.[17] In this context Mp4, an alumino-silicate based GIC, was included in the present study as this material produced no in-vitro inhibition of cell growth in a previous investigation.[9] However, qualitative examination of the ground sections showed that Mp4 (Fig. 6) integrated less well than GIC based on fluoro-alumino-silicate glasses (Figs 1–5 & 7).

Jonck *et al.*[10,14] proposed that slow fluoride release from a GIC-based bone cement may have a beneficial effect on in-vivo osteogenesis in a manner similar to the stimulation of osteoid formation by fluoride therapy in osteoporosis.[18] It has also been shown that fluoride administered via the drinking water of chicks and rats stimulates bone formation at low doses but inhibits it at higher doses.[19,20] It thus appears that there is an optimum dose of fluoride for stimulation of bone-forming cells and in a relatively closed in-vitro environment toxic effects may be seen.[9,12–16] In the more dynamic in-vivo situation, the leaching of fluoride may stimulate osseointegration and could account for the better response seen for the fluoro-alumino-silicate GICs, Ketac Cem and G338, compared to the GIC based on Mp4 glass.

Subjectively there was a greater volume of new bone formed around the GIC implants compared to the Ha. However, further work on the osteoconductivity of GIC compared to Ha is needed. Also the improved biomechanical compatibility[21] and reduced stress shielding achieved with the GIC (Young's moduli in the range 0·95–3·75 GP) compared to that of Ha (Young's moduli in the range 48–80 GP) have to be considered. Direct bonding of the mineralized collagenous extracellular bone matrix to the GIC Ketac Cem, without any intervening layer, was seen on TEM examination (Fig. 8). This observation is as described for bioglass implanted into rabbit femora,[22] and is also similar to other reports for glass-ceramic and Ha bone interfaces.[23–25]

In the limited number of GIC/bone interface sections examined, no evidence was seen of the thin electron dense or amorphous/granular zone which has been reported to separate the bone matrix from the biomaterial.[5,22,25,26] The significance or indeed absence of this zone, which is thought to be the lamina limitans of the bone,[27] at a bone/implant interface, is not known. The lamina limitans is thought to occur on cessation of active bone formation and is attributed to absorption of organic material onto the bone matrix, its thickness depending upon the rate at which mineralization proceeds; a broad lamina limitans forming on gradual slowing of calcification.[25–27] Absence of a lamina limitans may thus indicate that active bone formation or resorption is occurring: however in the specimens of set GIC reported here there was no evidence of osteoclastic activity, and mineralization appeared complete right up to the implant surface. It has been suggested that the presence of a lamina limitans like zone may indicate that incomplete bonding has occurred.[22] Thus, its absence in the case of materials such as bioglass, tricalcium phosphate and the GIC Ketac Cem reported here may indicate the formation of a more dynamic, but possibly less stable interface.[9,22,24]

One of the proposed advantages of a bone substitute based on GIC compared to ceramics in current clinical use is the potential for in-situ polymerization.[9] In this respect the response to implantation of fresh Ketac Cem is of interest. It appears that an initial intimate bond was formed with the bone, possibly due to chemical adhesion of the GIC to the bone apatite and adsorption of poly(acrylic)acid by hydrogen bonding onto the collagen matrix.[28] By six weeks however, the intimate relationship had been lost and extensive bone remodelling was seen. This sequence of events may indicate that as seen *in vitro*, fresh GIC causes a mild toxic response.[9,12,15,16] The remodelling at six and 12 weeks served to repair damaged tissue and establish a new bone/GIC interface.

Further work to characterize the GIC/bone interface is required and also investigation into the initial and longer term response of bone to freshly mixed GIC. This initial study has however, demonstrated that certain GICs can form an intimate bond with living bone, and the ability of some GICs to leach fluoride seems to enhance this process.

ACKNOWLEDGEMENTS

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Bone cell interactions with a granular glass-ionomer bone substitute material: *in vivo* and *in vitro* culture models

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The interface between bone and a synthetic bone substitute constructed from glass-ionomer cement (ionomeric microimplant) was studied in diffusion chambers implanted in a primate baboon model (*Papio ursinus*) and in *in vitro* primary bone organ cultures derived from neonate rat calvaria. In both models osteoblast-like cells colonized the surface of the implant producing a collagenous extracellular matrix. An electron-dense bonding zone similar to that reported for hydroxyapatite and titanium was seen in both models but was a more constant feature of the tissue/implant interface in calvarial culture.

Keywords: Bone cement, osteoblasts, glass-ionomer cement, bone-implant interface

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The ultimate clinical success of a biomaterial intended for use as a bone substitute (excluding mechanical failure) depends largely upon the structure, composition and stability of the bone/implant interface.

A glass-ionomer cement[1], ionomeric microimplant granules (Ionos Medizinishche Produkte GmbH & Co. KG., D-8031 Seefeld/Oberbay, Germany), formed from the reaction of a fluoroaluminosilicate ion-leachable glass (composition by percentage weight: silicon as SiO 35%, aluminium as Al_2O_3 30%, calcium as CaO 15%, fluorine 10%, sodium as Na_2O 3% and phosphorous as P_2O_5 7%) with an organic polyelectrolyte consisting of a copolymer of polyacrylic/maleic acid (50% aqueous solution) has been developed for use as a granular bone substitute[2, 3].

The aim of this study was to characterize and compare the interface achieved between 0.5 mm granules of the ionomeric microimplant bone substitute, sterilized by γ-irradiation, in an *in vivo* primate baboon culture model and an *in vitro* rat calvarial bone culture models. In addition, this approach enabled a comparison to be made of the two culture techniques for studying the response of bone cells to potential biomaterials.

METHODS

In vitro culture using primary bone cell and organ cultures was based on explants of inbred neonate Wistar rat calvaria[4, 5]. The interaction of ionomeric microimplant granules with rat osteoblast cells was studied by placing granules on to small explants of suture-free parietal bone, harvested from 3-d-old Wistar rats, from which the periostea had been removed. Ionomeric microimplant granules were also studied in bone organ culture. Again explants of calvarial bone was used but in this case introducing granules to lie between the surface of the bone and the intact periosteum. Individual cultures were placed in multiwell plates and maintained at 37°C in an atmosphere of 5% CO_2 for 2 wk in Fitton-Jackson's modification of Bigger's medium (Gibco, UK). The media was supplemented with 10% fetal calf serum, 20 μl/ml 200 mM glutamine, 10 μl/ml penicillin/streptomycin (500 units/ml), 25 μl/ml HEPES 1 M solution, 10 μl/ml β-glycerol-2-phosphate 1 M solution and 50 μg/ml ascorbic acid, the last two being made up and added immediately before use. The medium was replenished every 2 or 3 d and cultures stopped on the 7th or 14th day by replacing the media with 2.5% gluteraldehyde in 0.1 M sodium cacodylate buffer.

In vivo culture was undertaken using a primate bone culture model[2]. Plexiglas diffusion chambers containing ionomeric microimplant granules and chips of transplanted autogenous tibial bone were implanted into depressions created on the surface of baboon tibiae (*Papio ursinus*). After 42 d the chambers were harvested and fixed in gluteraldehyde. The tissue was removed from the diffusion chamber before processing.

Preparation for microscopy

The primary bone cell calvarial cultures and individual ionomeric microimplant granules retrieved from the

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diffusion chambers by blunt dissection were examined using scanning electron microscopy (SEM). Specimens were secondarily fixed with 2% aqueous osmium tetroxide for 1 h followed by dehydration through a graded acetone series, critical point dried from CO_2 (Polaron Critical Point Drier) and coated with a thin layer (approximately 20 nm) of gold (Nanotech Sputter Coater). SEM was undertaken using a Cambridge Stereoscan 600 set at 25 kV.

Transmission electron microscopy (TEM) was used to examine the interfacial response of ionomeric microimplant granules in both the calvarial bone organ cultures and the diffusion chamber. Specimens were partly decalcified in 10% EDTA in Tris buffer and dried using a graded ethanol series before resin embedding (L.R White hard grade, London Resin Co. UK). Thin sections were stained with saturated uranyl acetate in 50% ethanol and Reynold's lead citrate and viewed on copper grids (some of which were coated with collodion in amyl acetate) using a Philips CM 10 transmission electron microscope set at 80 kV.

RESULTS

SEM examination of diffusion chambers and bone cell cultures

Polygonal cells exhibiting a tessellated arrangement colonized the surface of ionomeric microimplant granules retrieved from the diffusion chambers (*Figure 1*). These cells exhibited a similar osteoblastic morphology to the cells of the calvarial bone which had migrated on to the granules placed in the rat bone cell culture model (*Figures 2* and *3*).

TEM examination of diffusion chambers and bone organ cultures

The diffusion chambers were filled with cellular and partly collagenous connective tissue, pieces of transplanted cancellous bone which appeared non-vital as judged by the absence of osteocytes from lacunae and granules of ionomeric microimplant. In places, transplanted bone chips and glass particles were associated with areas of increased stromal cellularity; the cells involved presented an osteoblast-like morphology and were supported in a collagenous matrix. The interface between the ionomeric granules and the extracellular matrix consisted of either a direct contact of collagen fibres with the surface of the material or of a thin (60–750 nm) granular electron-dense zone which had been deposited on the surface of the ionomeric microimplant. In places, collagen fibres ran through this zone to the surface of the material (*Figure 4*).

The rat calvarial cells in contact with the ionomeric microimplant maintained their vitality and laid down a collagenous extracellular matrix on the surface of the ionomeric microimplant granules (*Figures 5a*). The condensed granular electron-dense zone seen at only some parts of the ionomeric microimplant/tissue interface in the diffusion chambers was a more constant feature of the interfacial region in the calvarial model (*Figure 5b*).

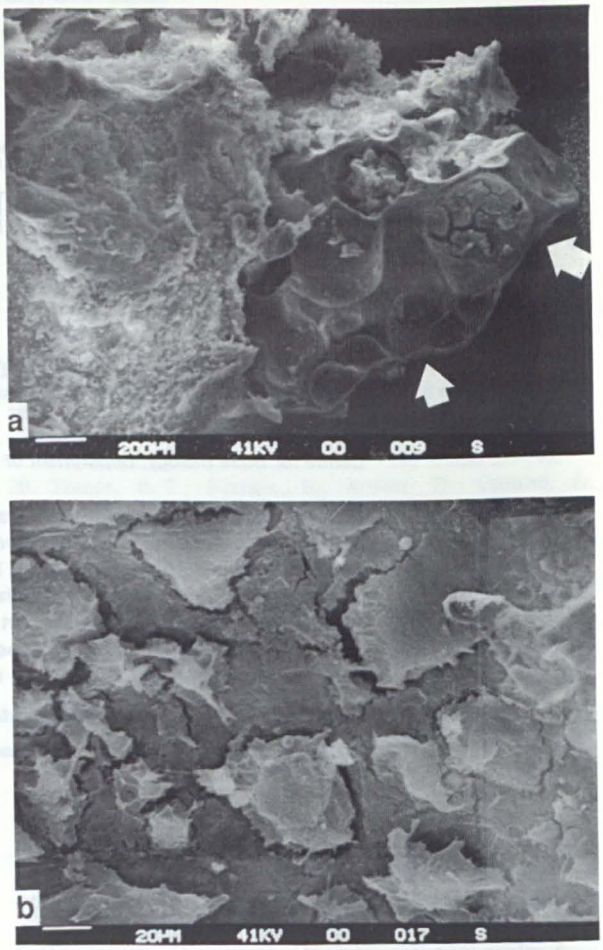

Figure 1 SEM photomicrographs of part of the contents of a diffusion chamber following 42 d implantation *Papio ursinus*. **a** Following dissection, a granule of ionomeric microimplant (arrows) has been exposed and cells adherent to depressions in the surface of the material can be identified (field width 2 mm). **b** Detail from **a**, showing polygonal cells which exhibit a tessellated 'osteoblast-like' morphology in close contact with the ionomeric microimplant. Cytoplasmic processes link adjacent cells and anchor cells to the surface of the implant which appears to be covered by an organic layer of ground substance (field width 200 μm).

DISCUSSION

Glass ionomer-based bone substitutes offer several advantages over ceramic or bioactive glass-based materials. They can be moulded into a variety of shapes, set without an exotherm on mixing, are chemically adhesive and have osteoconductive potential[1, 2, 5–9].

The favourable biological response reported for certain GICs[2, 3, 5, 9] probably depends upon the significant quantities of phosphate contained in the glass phase and their similarity in composition to the bioactive surface-active ceramics termed 'Bioglasses' and crystallized bioglass-ceramics[1, 10]. It has also been reported that the glass phase crystallizes readily to an apatite phase during production of ionomeric glasses[6]. It must be remembered, however, that glass-ionomer cements are a range of

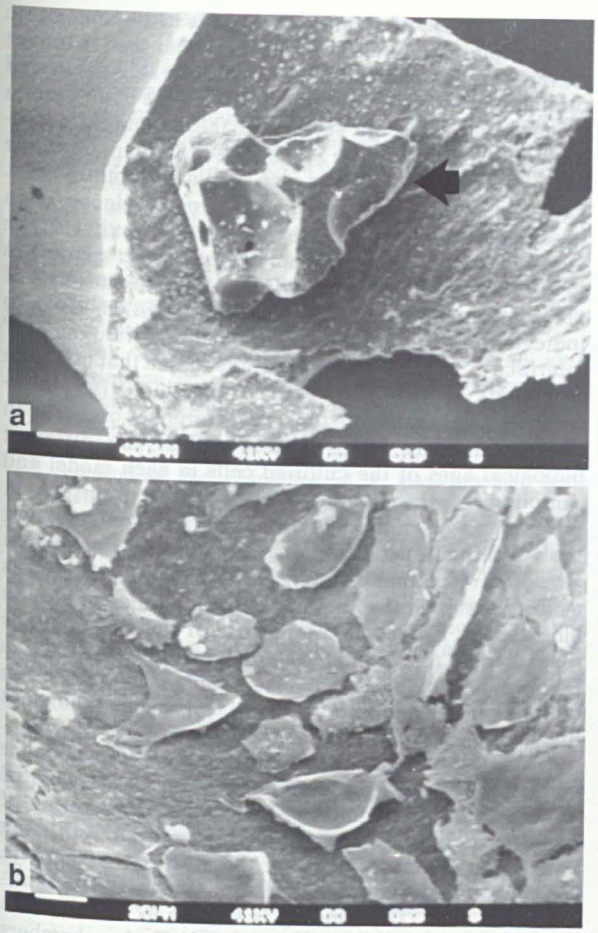

Figure 2 SEM of a 7 d rat calvarial derived bone cell culture: **a** granule of ionomeric microimplant (arrow) on calvarial bone (field width 3 mm); **b** detail from upper surface of granule in **a**, showing sheet of cells colonizing the surface and exhibiting similar morphology to those seen in *Figure 1b* (field width 200 μm).

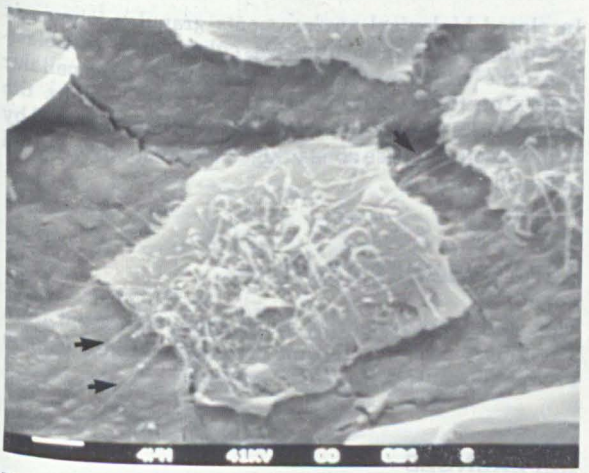

Figure 3 High-power SEM of polygonal cell which had migrated from the calvarial bone to lie on the surface of a ionomeric microimplant granule. The cell appears to be active and producing collagen fibres which anchor it to the surface of the material (arrows) (field width 40 μm).

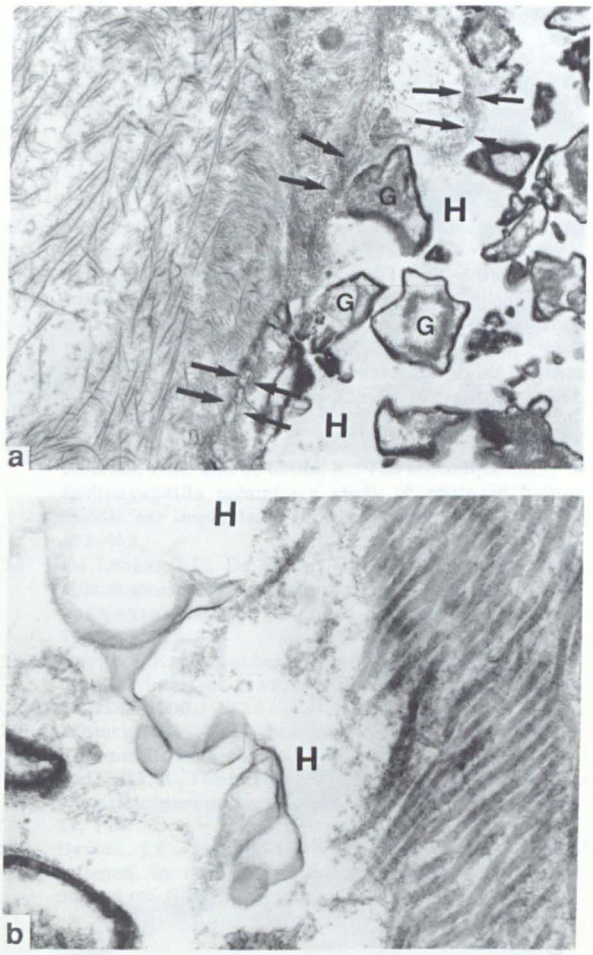

Figure 4 TEM of the interface between ionomeric microimplant and collagen-containing extracellular matrix filling diffusion chamber. **a** Collagen fibrils arranged in a layers, adjacent to the implant, the glass phase G and hydrogel matrix H can be identified. A narrow 60-400 nm wide condensed electron-dense zone is present in places (arrows) (field width 10 μm). **b** High power of interface; here the condensed zone appears absent and there is an intimate relationship between collagen fibrils and the hydrogel matrix (H) forming the surface of ionomeric microimplant granule (field width 2.4 μm).

materials and that their physical and biological properties are very composition dependent.

The histological response to ionomeric microimplant granules placed in diffusion chambers implanted into primates has been reported previously[4]; detailed examination of the ultrastructure of the resulting tissue/implant interface and the response of calvarial bone cultures to ionomeric microimplant material formed the basis for the present study.

The response of bone cells to granules of ionomeric microimplant in the calvarial explant model used for this study was similar to that seen for ceramic and bioactive glass materials developed for use as bone substitutes and studied under similar conditions[11–16]. Calvarial-derived bone cells appeared to accept the surface of the granules as 'bone', migrating from the calvarial bone to colonize the surface of the granules (*Figures 2* and *3*). This

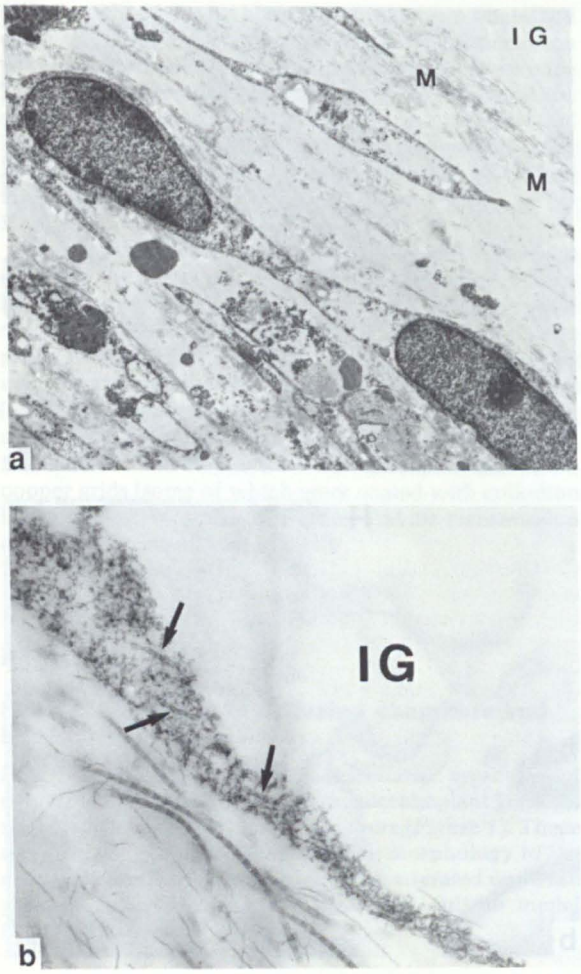

Figure 5 TEM of rat calvarial periosteum lying over a granule of ionomeric microimplant (IG) after 14 d in culture. **a** Section through periosteum; fibroblast-like cells have deposited a collagenous extracellular matrix (M) on the surface of the IG which has been lost during processing (field width 30 μm). **b** Detail from **a**, showing the granular electron-dense zone 60–400 nm wide crossed by randomly oriented collagen fibres (arrows) and by amorphous material at the tissue/ionomeric microimplant granule interface (IG) (field width 2.4 μm).

acceptance of granules as bone was confirmed by the TEM examination, in that cells maintained their osteoblast-like phenotype and elaborated a collagen-containing extracellular matrix on to the implant surface (*Figure 5a, 5b*). Detailed examination of the tissue/material interface in calvarial culture revealed the presence of an intermediate bonding zone which, when present, consisted of a thin electron-dense layer 60–750 nm wide. This layer resembled the bonding zone reported at the bone/hydroxyapatite interface both *in vitro* and *in vivo*[5, 17, 18] although it appeared to have some structure and contain some randomly oriented collagen fibres (*Figure 5b*). This latter feature was similar to that reported for the *in vitro* and *in vivo* bone–titanium interface, where collagen fibres formed during culture are incorporated in a 'cement line' bonding zone on the surface of the metal[19, 20].

The response of primate bone cells to the ionomeric microimplant granules was similar to that seen in the rat calvarial culture model. The osteoblast-like cells associated originally with the transplanted autologous bone were in places seen lining the surface of the ionomeric microimplant granules (*Figure 1*). The tissue/implant interface examined using TEM was also similar in both models. However, there was less convincing evidence of a intermediate bonding zone in *in vivo* culture, the interface consisting of collagen fibres in direct apposition to the surface of the granules (*Figure 4*). The presence of an electron-dense bonding zone has been reported to indicate the formation of a stable 'lamina limitans like' interface[17, 18], while others have postulated that this zone represents a more inert rather than 'bioactive' interface[5, 15, 16]. It is probable that the differences observed in the two models reported here reflect the different biological ages of the cultured cells in each model and sampling of the ionomeric/bone tissue interface at different stages of maturity. It is realized, however, that the variations seen could also be due to species or *in vitro/in vivo* culture differences. The baboon model was a closer match to the intended clinical application of the biomaterial. However, comparison of the two methods reveals that the biomaterial under study provoked a favourable biological response from the bone cells in both models; the *in vitro* calvarial test system being less invasive than the *in vivo* method.

Ultrastructural examination of the diffusion chamber contents confirmed the previously reported histological observations, no inhibitory effect upon proliferation of bone tissue by ionomeric microimplant material being noted. The chambers were for the most part filled with collagenous extracellular matrix interspersed with fibroblast-like cells. This finding is similar to that seen on culturing human bone marrow cells in diffusion chambers implanted into the rat in the absence of test materials[21].

CONCLUSION

In closed biological environments granular ionomeric microimplant bone substitute was colonized by osteoblast-like cells which produced a collagenous extracellular matrix on to the surface of the material. *In vitro*, the bonding of tissue on to the granules was characterized by a thin electron-dense zone bridged by randomly oriented collagen fibres.

ACKNOWLEDGEMENTS

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COMMUNICATION

Further observations on high impact strength denture-base materials

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Previous studies have shown that high impact strength can be conferred on denture-base poly(methyl methacrylate) polymers by modification with acrylic-terminated butadiene-styrene block copolymers, and that the acrylic end-group was necessary for effective reinforcement. It is now shown that, by solvent extraction studies, grafting of the copolymer occurs both with acrylic-terminated and non-terminated block copolymers. It is therefore concluded that the mode of grafting is different, and some possible mechanisms are discussed.

Keywords: Dental materials, PMMA copolymers, mechanical properties

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The development of a new type of high impact strength denture-base resin and the reinforcing mechanisms have been described previously[1, 2].

The basis of the reinforcement is acrylate-terminated butadiene-styrene block copolymers (Macromers, Revertex Ltd, Harlow, UK) of relatively low mol wt (~30 000), but narrow molecular weight range.

Three main observations[2] were made during the development and evaluation of these materials:

(1) Denture-base poly(methyl methacrylate) (PMMA) is fabricated by a dough technique whereby a suspension (bead) polymerized PMMA is mixed with methyl methacrylate (MMA) monomer to form a dough, which is moulded in a plaster of Paris mould and subsequently heated to effect polymerization. The heating process is usually a cycle starting at 70°C and subsequently increased to 100°C. There is sufficient residual benzoyl peroxide (~0.75% w/w) in the bead polymer to effect polymerization with the dough.

Reinforcement was obtained only if the Macromer was incorporated in the bead polymer. This was done by simply dissolving the requisite amount of Macromer in MMA, then carrying out suspension polymerization with benzoyl peroxide as the initiator. *A priori*, the bead polymers prepared are graft copolymers (see below).

(2) Reactive acrylate end-groups are needed to impart reinforcement, i.e. there was no increase in impact strength with corresponding Macromers without end-groups.

(3) The impact strength and Young's modulus of systems containing the same amount of rubber phase depended critically on the detailed blends used. The bead phase could comprise a physical mixture of conventional PMMA powder, with a powder of the modified PMMA powder.

(4) Ball-milling the polymer powder enhanced the impact strength significantly.

(5) Reinforcement could not be achieved by incorporating the Macromer in the monomer phase.

These results must therefore be critically dependent upon detailed differences in structure. Whilst it seemed likely that the modified bead polymer was a graft polymer, this had not so far been established. Likewise, it was not established whether grafting occurred with the non-terminated Macromer.

MATERIALS AND METHODS

Materials

The systems studied comprised copolymers described in (1) above of an acrylic-terminated butadiene-styrene block copolymer (Macromer 2M/6/378, Revertex Ltd) with MMA, prepared as a bead polymer in suspension[3]. These bead copolymers could be blended with a conventional PMMA bead polymer, and the resulting powder bead doughed with MMA monomer, then cured by the usual techniques of denture base processing. A range of materials was studied, with Macromer contents of up to 30% w/w of the bead phase, prepared as described previously[1, 2].

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Characterisation of the ultrastructure of glass-ionomer (poly-alkenoate) cement

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Set glass-ionomer cements were sectioned with a diamond knife and examined in the transmission electron microscope. Their appearance was in accordance with the theoretical structure of these materials, close examination revealing glass particles surrounded by a siliceous layer set in a hydrogel matrix. The elemental composition of each region was determined by X-ray microanalysis (energy dispersive). The results of microanalysis supported the ultrastructural observations, with ions that originated from the glass particles being detected throughout the matrix of the set cement. It was suggested that the mobility of these ions in the matrix phase was important in determining the biocompatibility and adhesive properties of glass-ionomer cements.

Glass-ionomer cements (GICs) are novel polymer glass composites, currently used as restorative materials in dentistry. Their properties include a rapid set, high compressive strength, adhesion to enamel and dentine and fluoride release.[1] It has recently been suggested that they may find wider clinical applications as bone substitutes for maxillofacial surgery or cements for hip joint replacement.[2-4] Additional properties that make them attractive bone substitutes include a non-exothermic setting reaction, adhesion to bone and metals, biomechanically matched formulations that may be easily moulded and shaped at the implant site with no shrinkage on setting, and bioactivity due to release of osteoconductive ions.[4,5] However, despite the importance of these materials we have only a limited understanding of their structure. The existing structural model suggests that GIC consist of a glass core sheathed by a siliceous hydrogel matrix.[6] Visible support for this model was provided by three limited studies carried out using the scanning electron microscope.[7-9]

Successful development of GIC with wider clinical applications requires an understanding of their composition and structure, and how these factors affect the biocompatibility of the material with bone. The aim of this investigation was to characterise the ultrastructure and composition of set GIC in the transmission electron microscope.

Materials and methods

Two GICs were evaluated in this study. The first was a commercial dental luting cement, Ketac Cem Radiopaque,* based on a fluoroaluminosilicate glass of general composition SiO_2, Al_2O_3, AlF_3, CaF_2, NaF, LaF_3 and $AlPO_4$ reacted with a copolymer of poly(acrylic) acid/maleic acid. The other GIC was G338 glass of general composition SiO_2, Al_2O_3, AlF_3, CaF_2, Na_3AlF_6 and $AlPO_4$ reacted with poly(acrylic) acid.

Material for X-ray microanalysis (XRMA) was quench frozen in pentane over liquid nitrogen and substituted in acetone over a molecular sieve. Material was freeze-substituted to avoid the translocation or movement of ions that has been associated with conventional preparative methods.[10,11] Freeze-substitution was carried out at −70°C for 10 days before material was allowed to warm up to room temperature. Specimens were washed in three changes of acetone before conventional infiltration with Spurr's resin and final embedding. Sections (0·5 to 1·0μm) were dry cut with a diamond knife on an ultramicrotome, placed in a gold sandwich grid (100 mesh) and examined in the transmission electron microscope (TEM, Philips CM10). X-ray microanalysis was carried out at 100 kV for 25 live seconds using a 200 nm probe and a 12° tilt on the specimen holder. Analyses were performed in regions of material that did not overlap so as to avoid signal contamination by stray X-rays. Background radiation was determined in adjacent areas of resin and subtracted from the spectra to compensate for the effects of beam-spreading in the section. An energy dispersive spectrometer connected to a multichannel analyser was used to collect spectra.

Material was also conventionally prepared for examination of ultrastructure in the TEM. Ultrathin sections (50–80 nm) sections were examined in the TEM to determine the structure of set GIC.

Results and discussion

The GIC are formed by reaction of an acid soluble aluminosilicate glass with an aqueous solution of a polyacid. The acid chemically attacks the surface of glass particles, leaching metal cations (Al^{3+}, Ca^{2+}, and Na^{+}) out into the matrix. Gelation of the cement is thought to occur through the reaction of these cations with the acid groups on the polymer chains. Consequently, set GIC are a hybrid material consisting of inorganic glass particles in an insoluble hydrogel matrix. This process is summarised in figure 1. The cohesive forces which maintain the structure of this matrix are presumed to be a mixture of ionic cross-links, hydrogen bridges and chain entanglements. The theoretical structure of GIC was based largely upon this reaction sequence and studies of fracture surface morphology.[7-9] Previous

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

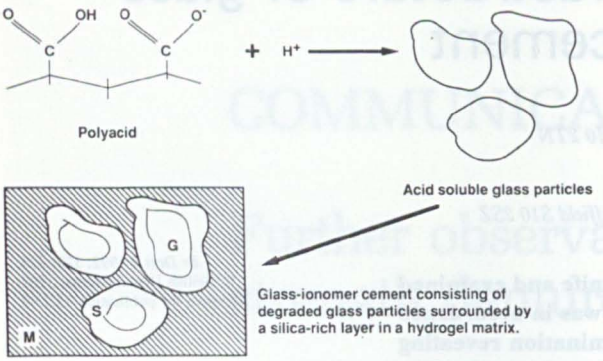

Fig. 1 Relationship between setting reaction and structure of glass-ionomer cement.

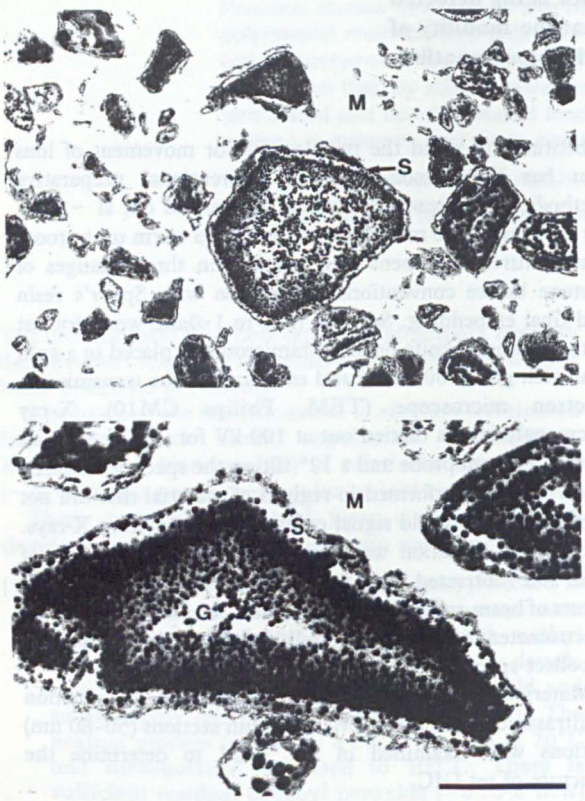

Fig. 2 Cross-section through glass-ionomer cement showing glass particles (G) surrounded by siliceous hydrogel (S) set in a hydrogel matrix (M). (a) Low magnification. (b) High magnification. Transmission electron photomicrographic; scale bar 1 μm.

microanalytical studies used the scanning electron microscope to characterise dental materials.[9] However, spatial resolution is superior in the TEM because of its smaller probe size and reduced beam spread in the specimen. In addition, use of cryofixation and freeze-substitution ensured that changes in structure and composition of the GIC were minimised.[10,11]

Low and high power transmission electron photomicrographs show the ultrastructure of set Ketac Cem (fig. 2). Glass particles (G) are clearly seen surrounded by a siliceous layer (S) set in hydrogel matrix (M). The glass core and siliceous sheath are seen more clearly at the greater

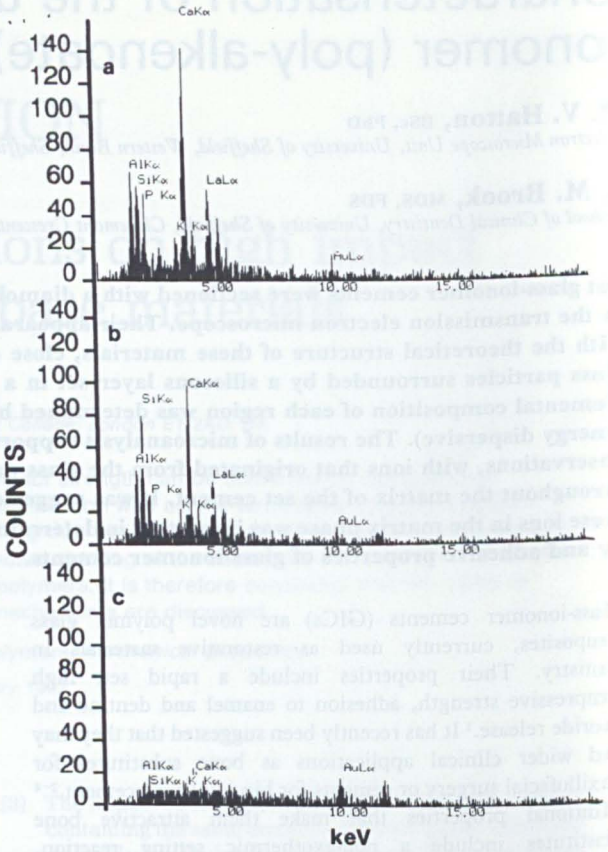

Fig. 3 Energy spectra from different regions of set Ketac Cem. (a) Spectrum showing composition of glass particle in set glass-ionomer cement. (b) Spectrum from region of siliceous hydrogel surrounding glass particle. (c) Spectrum of hydrogel matrix between glass particles. Spectra obtained using a 200 nm probe for 25 live seconds at 100 kV.

magnification. The arrangement of glass particles shown by these micrographs is in accordance with the theoretical model for the structure of GIC.[6]

The elemental composition of the glass particle, siliceous layer and matrix was determined by XRMA. Typical spectra for each region are shown in figure 3. The glass core was particularly rich in aluminium and calcium, while the outer layer of the glass has been depleted of metal ions and is proportionately rich in silicon. Both GICs investigated produced similar energy spectra, except that lanthanum was detected throughout Ketac Cem as it is added to this particular GIC by the manufacturer to render it radiopaque. The matrix component produced the lowest rate of X-ray collection and, as it was of predominantly organic composition, consisted of the least detectable mass. However, these results indicated that a low concentration of silicon ions was present in the matrix phase as well as aluminium and calcium. Wilson's original model does not account for the presence of silicon in this region and it may be that this ion also takes part in the setting reaction of GIC. Relative detection of the elements in different regions of set GIC are given in Table I. Ion mobility during the setting reaction and in the set cement is important to the adhesive properties of GIC and probably accounts for the biocompatibility of these materials.

This study demonstrated that glass-ionomer cements may

be sectioned for ultrastructural and microanalytical examination in the TEM. The results confirmed that the structure of set GIC was close to Wilson's theoretical model. In addition, there was evidence for the leaching of silicon from the glass particles during the setting reaction. This study increases our understanding of these highly effective dental materials and will be of further value in the development of novel ionomeric bone substitutes.

Acknowledgement

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Table I Relative detection of elements in different regions of set glass-ionomer cement (G338). Results are the arithmetic mean of six analyses (± standard deviation) after subtraction of background radiation.

Element	Region/counts per second		
	Glass particle	Si-rich layer	Matrix
Na	1·93±0·54	0·66±0·39	0·18±0·16
Al	29·01±6·47	10·95±2·90	0·40±0·04
Si	30·88±4·41	14·54±2·46	0·61±0·34
P	12·64±2·14	6·68±1·35	0·88±0·42
Ca	22·99±4·04	12·87±2·17	0·76±0·52

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Ketac Cem Radiopaque: Espe, Germany
G338 glass: Laboratory of the Government Chemist, UK

Statement by advisors and declaration concerning access.

This thesis is submitted under the general regulations for higher degrees of the University of Sheffield for the degree of Phd by Mr I M Brook under regulation 8; University Staff Candidate holding a full time appointment.

As a staff candidate Mr Brook had no formal supervisors, however, Dr G T Craig (Department of Oral Pathology) and Mr D J Lamb (Department of Restorative Dentistry) have acted as Internal Advisors for this study.

Dr G T Craig and Mr D J Lamb have also contributed to the publications arising from this work and were thus cited as co-authors. However, they certify that the work reported here was undertaken solely by the candidate and has not been presented by him for any other degree.

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