Effects of glass ionomer cements on bone tissue

P. LUCKSANASOMBOOL[1,3], W. A. J. HIGGS[2,3], R. J. E. D. HIGGS[1,2], M. V. SWAIN[2,3]*, C. R. HOWLETT[4]
[1]*Faculty of Medicine, University of Sydney*
[2]*Department of Mechanical and Mechatronic Engineering, University of Sydney*
[3]*Biomaterials Science Research Unit, Faculty of Dentistry, University of Sydney*
[4]*Bone Biomaterial Unit, School of Pathology, The University of New South Wales*
E-mail: mswain@mail.usyd.edu.au

In vivo biocompatibility of glass ionomer cements (GICs) was evaluated for use in orthopaedic surgery using a rat model and compared with conventional bone cement, Polymethyl methacrylate, PMMA. The unset GICs and PMMA were inserted into the marrow cavities of rat femora and retained *in situ* for various periods of time. The PMMA bone cement showed complete biocompatibility with no interference with reparative bone. The conventional GIC with smaller glass particles and lower powder/liquid ratio showed an initial minor toxic effect on rat bone tissue with later disturbance of adjacent bone formation. The conventional GIC with larger-size glass particles and higher powder/liquid ratio and resin-modified GIC showed more severe toxic effect on rat tissue with the resin-modified GIC affecting the rat bone tissue later. The causes of toxicity associated with the conventional GIC with larger glass particles and higher powder/liquid ration and the resin-modified GIC are thought to be related with the unreacted acid component of both materials and longer ongoing metallic ion release.

1. Introduction

Joint replacement improves the quality of life for patients suffering degenerative joint diseases of knees and hips. Bone cement (Polymethyl methacrylate), which is used to fix prostheses in the elderly, attracts little criticism because of acceptable long-term results [1]. Extensive research and sophisticated technology over the decades has all but eliminated mechanical failure of prostheses by breakage or material wear. However, implant-bone interface failure as typified by aseptic loosening is still the major long-term cause of revision surgery [2].

Polymethyl methacrylate cement (PMMA) has been used since 1961 as the major material for cemented prostheses. There have been few attempts to improve the quality of the material or to replace PMMA with any new material [3, 4]. In 1970, Charnley [5] had advocated that improved bone cements were needed. He strongly advised that the compound must have a biocompatibility to match the cured state of PMMA while enhancing the adhesion and fixation of the cement to bone. The undesirable features of PMMA are the exothermic curing and the local cytotoxicity of the monomer. Ideally these features should be removed, and the adhesive nature of the cement improved; the latter would ensure better physiological stress transfer from prosthesis to supporting bone [6].

Glass ionomer cements (GICs), introduced by Wilson [7] in 1970, have been used extensively in dentistry with excellent results. As these cements, (formed by an acid-based reaction between finely ground calcium fluoro-alumino-silicate glass and aqueous polyacrylic acid), bond chemically to bone and prosthetic materials [7, 8], they have the potential to achieve early stability of skeletal devices. GICs release osteoconductive (aluminum and fluoride) ions, which ensure their long-term stability [9, 10]. Although conventional GICs do not exhibit all of the satisfactory mechanical properties of acrylic bone cement, they can be modified extensively thereby fulfilling those features [11–13]. However, modification of GICs may alter their biocompatibility. GICs have been reported [14–19] to be biocompatible *in vitro* as well as being used extensively in dentistry for more than two decades though there have also been *in vitro* reports of biological incompatibility [20–25]. Others have shown that polymerized GICs have good biocompatibility, whereas deleterious effects do arise from their components and/or hydroxyethyl methacrylate (HEMA), resin used to modify them [16–18, 23, 25]. Recently, other deleterious effects have been observed in association with these cements [20–22, 24, 26–29]. These effects are considered to be a consequence of ion leaching, especially aluminum [27–29].

Conventional GICs have been used in dentistry with no or little adverse response from dental tissues [30, 31]. Their lower setting temperature and adherence to bone suggested possible potential in orthopaedic surgery

*Author to whom all correspondence should be addressed: Biomaterials Science Unit, Suite G11, National Innovation center, Australian Technology Park, Eveleigh, NSW 1430, Australia.

TABLE I

Materials	Powder	Particle size	Liquid	Consistency	P/L ratio
GIC L	Fluoroalumino silicate glass 95% Powdered polyacrylic acid 5%	Less than 15 μm	Polybasic carboxilic acid 10% Water 50%	Low	1.8g/1.0g
GIC H	Fluoroalumino silicate glass 95% Powdered polyacrylic acid 5%	Less than 25 μm	Polybasic carboxylic acid 10% Water 45%	High	3.6g/1.0g
S-430	Fluoroalumino silicate glass 100%	Less than 15 μm	Polyacrylic acid 30% HEMA 30% Methacrylate Resin 10% Water 30%	Low	1.8g/1.0g

providing their mechanical strength could be improved [32–34]. Resin-modified GIC was developed to improve the mechanical strength of the conventional GIC [35–37]. The aim of this study was to determine and compare the biocompatibility of these materials in amounts, which would be used in orthopaedic prosthetic joints (weight of cement to body weight), to commonly used orthopaedic cement, PMMA.

2. Materials and methods

Four cements were used in this study. Conventional PMMA, Simplex® (Howmedica), was compared to three GICs; two conventional type GICs, GIC L and GIC H, and a resin-modified GIC, S-430. GIC H was a conventional type GIC modified by increasing the size of the glass particles and the powder/liquid ratio. S-430, a resin-modified GIC, was developed to use in orthopaedic surgery by adding HEMA (Table I). Fifteen female Spraque Dawley rats (Bioethical approval ACEC number 99/41, UNSW) aged 8–10 weeks, of an average weight of 208 ± 13.99 g, were anaesthetized using an intramuscular injection of 0.3 ml Hypnorm (Fluanisonum 10 mg/ml + Fentanylum 0.2 mg/ml) followed by intravenous injection of 0.06 ml of diazepam (2.5 mg/ml in 0.9% saline). The femoral-tibial joint was opened aseptically using medial para-patellar surgical incision. The patella was reflected and the femoral cavity entered by inserting a needle into the femur via the intra-condylar notch. Marrow was flushed out of the cavity with isotonic saline. At this point cements were mixed in a laminar flow cabinet using aseptic techniques and the powder/liquid ratio and mixing instructions according to each manufacturers recommendations.

Cement was introduced via a 16-gauge needle attached to a syringe until the marrow cavity was filled. The pressure required introducing the cement into, and to fill the marrow cavity was dependent on the viscosity of each cement. The patella and the joint capsule were repositioned and stabilized with two sutures and the skin closed with interrupted sutures. After surgery, each rat received, (a) Naloxone HCl 0.3 ml subcutaneously, (b) 3 ml of normal saline was injected subcutaneously, and (c) 0.25 ml of Penicillin intramuscularly. Two of the 15 rats were used as controls in which the entire operation was performed on the contra-lateral femur but without cement insertion.

The animals were euthanatized (using intra-peritoneal administered sodium pentobarbitone) at 1, 2 and 4 weeks. Harvested femora were immersed in 10% saline-buffered formalin (pH 7.2) for one week. Femoral blocks of tissue were prepared and dehydrated in ascending concentrations of ethanol. These specific areas from the femurs were immersed in monomeric methyl methacrylate (MMA) for two weeks. Subsequently blocks were positioned in polymerization mixture of MMA and accelerators. The resin cured longitudinal sections, approximately 120 μm thick, were sawn from these resin-embedded blocks. Sections were stained, free floating with either Methylene Blue and Basic Fuchsin; or 1% Toluidine Blue in Borax buffer; or Von Kossa or Picrosirius stain. After staining, sections were polished flat on one side and then fixed to a microscope slide for viewing.

3. Results

3.1. Complications

One rat died shortly after inserting the resin-modified GIC, S-430, into its marrow cavity. The autopsy revealed that the cement completely filled the marrow cavity and had exited the femoral cavity via the diaphyseal nutrient arterial entrance. This extrafemoral cement had permeated the perimyseal and epimyseal tissue planes of the enclosing musculature. The lungs were intensely congested and haemorrhagic and contained crystalloid material (Fig. 1). Another rat within the S-430 group developed dyspnoea after the operation. As its clinical status failed to improve within 48 h, it was euthanatized. At post mortem, there was pulmonary congestion and petechial haemorrhages but no resin was observed. Microscopically multiple pulmonary infarcts occurred throughout the parenchyma associated with intra-arterial birefringent material. All animals in the S-430 group developed a noticeable hyperventilation immediately following insertion of the cement into their femoral cavity. Ventilation returned to normal in about a quarter of an hour. The condition of all rats in each group, except the above-mentioned two, recovered well and gained weight.

3.2. Microscopic morphology

3.2.1. Viscosity and penetration of the cements

The penetration of the materials in the femoral cavity and the adjoining intertrabecular spaces of the distal epiphysis and metaphysis displayed considerable differences presumed in part to be the result of the viscosity of each cement. GIC L and S-430 (Fig. 2a) were less

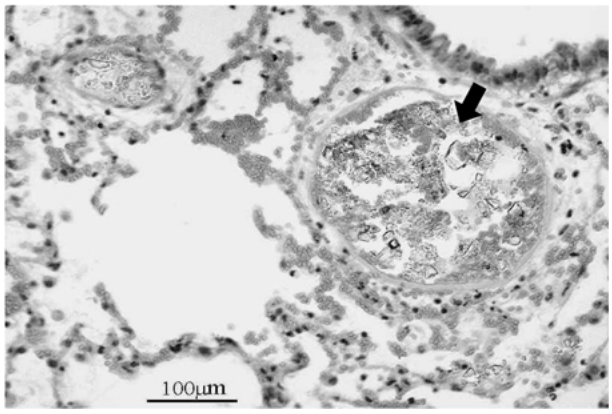

Figure 1 Intravascular crystaloid material appears to be the nidus for the fibrin thrombi (arrow) formed in the pulmonary parenchyma (H & E stain).

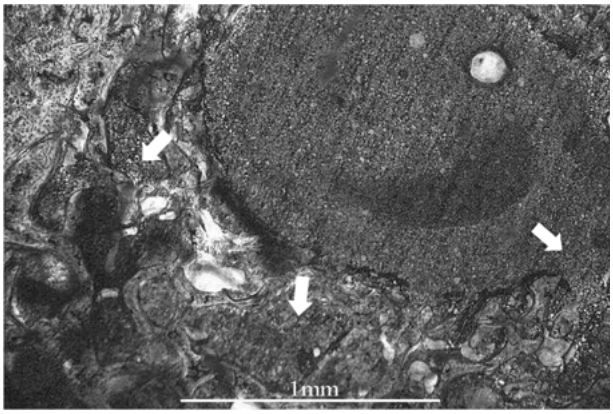

Figure 2a Spread of cement (S-430) out of the PMMA bolus (arrow) (S-430-one week; Methylene Blue and Basic Fuchsin stain).

viscous and injected easily into the cavity and consequently were found distributed through the intertrabecular spaces. In contrast, GIC H and PMMA (Fig. 2b), which were judged to be more viscous failed to penetrate to any extent into the intertrabecular spaces.

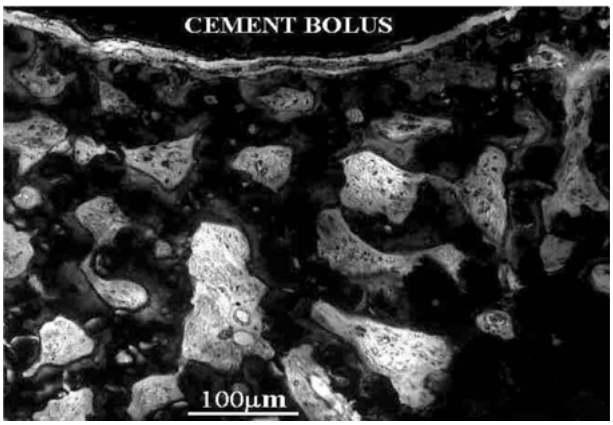

Figure 2b No cement spread out of the GIC H bolus (four weeks; Methylene Blue and Basic Fuchsin stain).

3.3. Reaction of skeletal tissues

3.3.1. PMMA: Week one

There is a marked proliferation of subperiosteal cells in which foci and trabeculae of osteoid and woven bone occur. Osteocytes occupy most of the lacunae in the diaphyseal corticalis and the metaphyseal trabeculae, however, some endosteal and metaphyseal lacunae adjacent to the bolus of PMMA are empty. Haemorrhagic necrosis of the marrow is a prominent feature particularly close to the PMMA. Small nests of erythyroid and myeloid cells occur centrifugally in the marrow cavity and the metaphysis (Fig. 3).

3.3.2. Week two

The subperiosteal tissue is circumferentially thickened by an outer layer of oval plump cells covering a trabeculated layer of mixed woven and lamellar bone often jutting from the corticalis. The trabeculae are covered by plump osteoblasts. The marrow cavity contains occasional remnants of necrotic debris adjacent to the PMMA. The polymer is enclosed in a vascularized mesenchymal tissue in which foci and islands of woven bone are distributed nearest to PMMA, while newly formed trabeculae of lamellar bone arises from the endosteum. The metaphyseal intertrabecular spaces are filled with vascularized fibrous mesenchymal tissue (Fig. 4).

3.3.3. Week four

The periosteal tissues are thickened with oval cells, occasional cartilaginous foci but mostly trabeculae of adult bone. These trabeculae are thicker than at week two and are continuous with the diaphyseal corticalis. Osteoclasts occur in Howships lacunae along the corticalis and the newly formed bone. In the marrow cavity the PMMA is encircled by a thin rim of fibrous tissue supported by a cuff of trabecular bone, extending from the endosteum (Fig. 5). Minimum osteoid seams are observed. Pockets of myeloid and erythyroid cells are clustered within the intertrabecular spaces of the new bone, metaphysis and epiphysis.

3.4. Glass ionomer cements: Week one

3.4.1. GIC H

The subperiosteal tissues are moderately thickened by proliferating mesenchymal cells and differentiated foci and islands of osteoid and woven bone. Most osteocytic

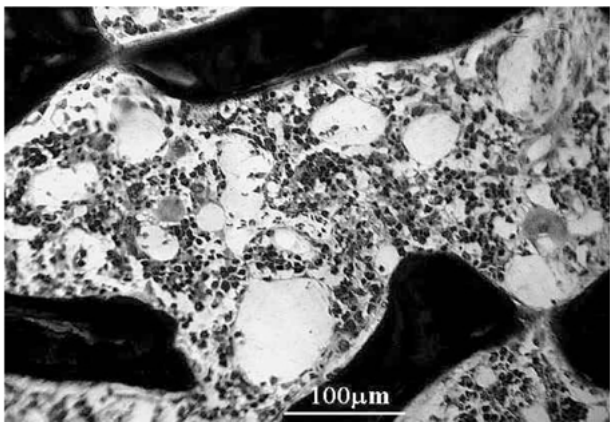

Figure 3 Nests of erythyroid and myeloid cells in the marrow cavity and the metaphysis (PMMA; one week; Toluidine Blue stain).

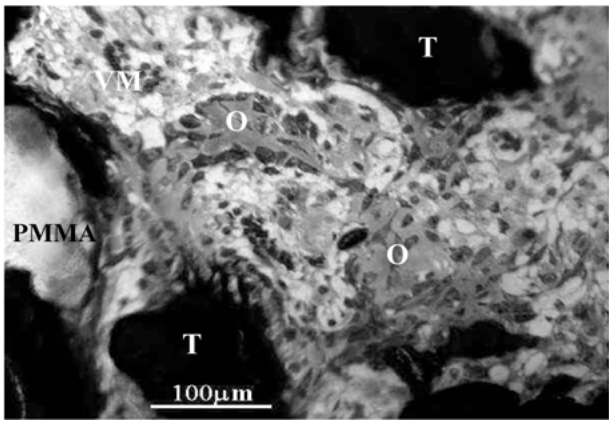

Figure 4 Intertrabecular spaces are filled with vascularized mesenchymal tissue (VM) and osteoid (O) (T = trabecular bone) (PMMA; two week; Toluidine Blue stain).

lacunae contain osteocytes although a few are empty close to the cement. Haemorrhagic necrosis of soft tissue in the marrow, metaphysis and epiphysis is prominent.

3.4.2. GIC L and S-430

The periosteal and subperiosteal tissues are mildly thickened by proliferating cells but minimal matrix has formed. The cement has widely dispersed throughout the marrow cavity and the metaphyseal intertrabecular spaces. Pronounced haemorrhagic necrosis of the marrow in the medullary cavity and metaphysis has occurred. A few spindle cells have survived in those areas furthest from the cement. Intertrabecular spaces contain a mix of necrotic pyknotic and a few viable cells with the implantation of GIC L and S-430 (Fig. 6).

3.4.3. Week two

In the thickened subperiosteal zone of each ionomer cement, there was a mixture of osteoid calcified woven and lamellar bone, some of the latter bone displaying pronounced osteoid seams. More osteoid was commonly observed with all GIC materials (Fig. 7). A thick band of haemorrhagic necrotic debris enclosed each implanted cement. Surviving islands of mesenchymal cells occurred close to the endosteum and/or in the intertrabecular spaces furthest from metaphyseal residing cement.

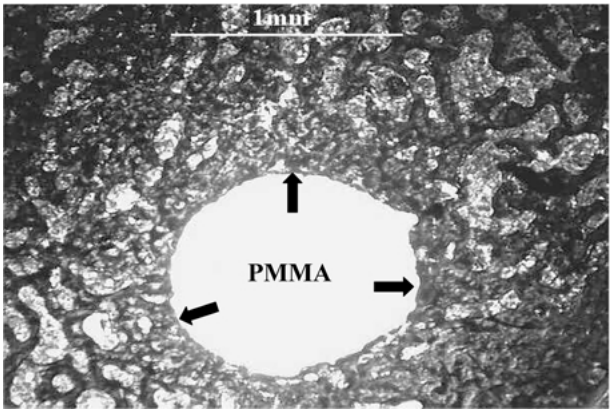

Figure 5 Cuff of trabecular bone (arrow) encircled PMMA bolus at week four (Methylene Blue and Basic Fuchsin stain).

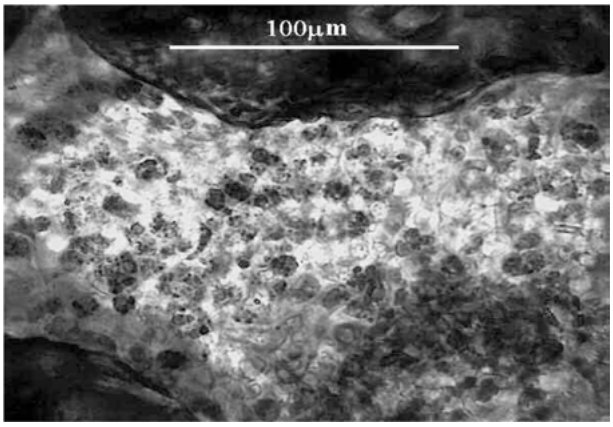

Figure 6 Intertrabecular spaces containing a mix of necrotic pyknotic and a few viable cells is the feature of GIC L and S-430 in the first week (GIC L; one week; Methylene Blue and Basic Fuchsin stain).

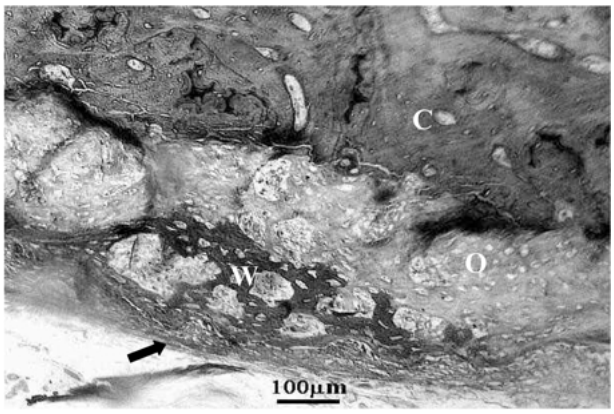

Figure 7 Thickened subperiosteal (arrow = periosteal) area of GIC commonly has a mixture of osteoid (O) and calcified woven bone (W) (GIC L; two weeks; Methylene Blue and Basic Fuchsin stain).

In rats treated with GIC H more cells and cartilaginous islands were observed than those injected with S-430. Moreover in the latter group there were large foci of subperiosteal basophilic and metachromatic fibrillary extracellular matrix with few nucleated cells (Fig. 8). The latter pattern is suggestive of ongoing necrosis with minimal cellular survival and proliferation.

3.4.4. Week four

Circumferentially, the subperiosteal zone had a mixture of osteoid and woven bone with considerably more osteoid and poorly calcified bone associated GICs (Fig. 9) than PMMA. Osteoclastic resorption pits were observed particularly on the outer diaphyseal surface. A layer of necrotic material enclosed each type of GIC and little to no new bone occurred either in the marrow cavity or in the metaphysis. Although more viable intertrabecular tissue was encountered at week four in the GIC L and GIC H groups, when compared to these groups at week two.

In contrast, those animals receiving S-430 had less proliferative fibrovascular tissue from endosteal margins and metaphyseal intertrabecular spaces at four weeks

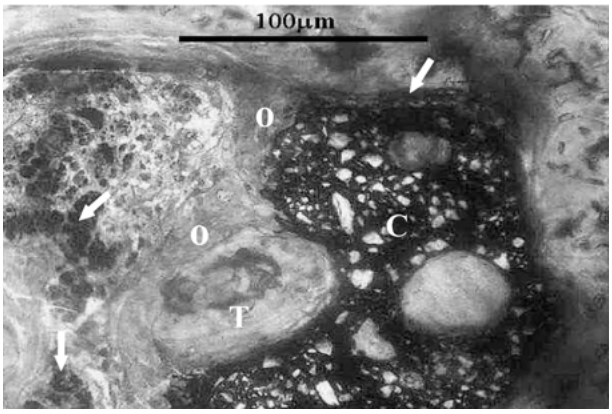

Figure 8 Basophilic and metachromatic fibrillary extracellular matrix (arrow) scatters within intertrabecular spaces. (GIC L; four weeks; Methylene Blue and Basic Fuchsin stain) (C = cement, T = trabecular bone and O = uncalcified osteoid).

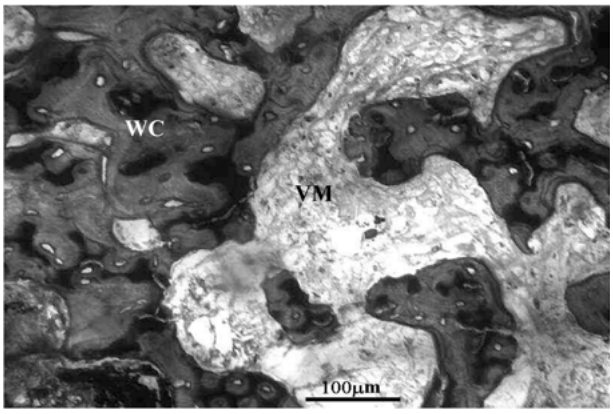

Figure 10 Intertrabecular spaces of S-430 at week four show less proliferative vascular mesenchymal tissue (VM) than at week two. (four weeks; Methylene Blue and Basic Fuchsin stain) (WC = woven calcification).

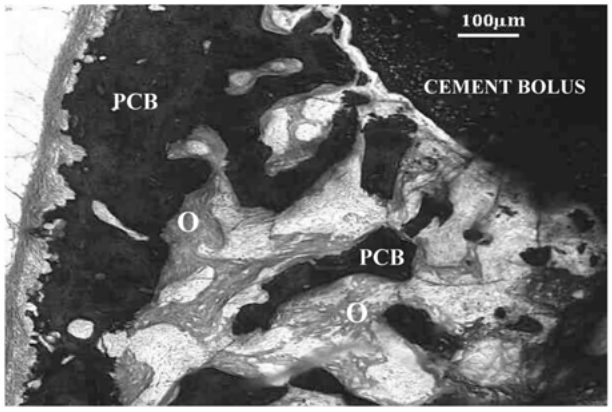

Figure 9 Subperiosteal zone reveals poorly calcified bone (PCB) and abundance of osteoid (O) (GIC L; four weeks; von Kossa & Masson stain).

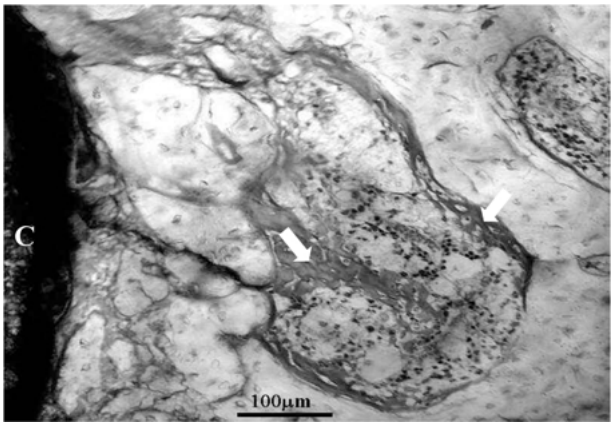

Figure 11 Intracortical soft tissues display necrosis admixed and osseous surfaces are covered by thick acellular basophillic material (arrow) (S-430; four weeks; Toluidine Blue stain) (C = cement bolus).

than at week two (Fig. 10). Furthermore, most extra- and intra-cortical soft tissues display coagulative necrosis admixed and encompassing necrotic osteoid seams and trabeculae. Some osseous surfaces are covered by thick acellular basophilic material mimicking somewhat grown and distorted cement lines (Fig. 11). Surviving spindle cells are located in regions distant to the cement.

GIC L implantation was associated with more numerous and extensive areas of osteoid compared with that observed with other glass ionomer and PMMA cements. In animals receiving the experimental S-430, surfaces of the diaphyses and osseous trabeculae of metaphyses and epiphyses were noticeably remodeled and considerable intraosseous soft tissue was apparent frequently coating trabecular bone.

In summary, the sections from femora containing the four different cements displayed different reactions. Simplex PMMA affected the proliferating cells of the bone and marrow least of all and appeared not to interfere with the regeneration of the bone and marrow. The expected cellular regeneration and differentiation of fibrovascular tissue adjacent to endosteum and in the inter-trabecular spaces occurred promptly.

The microscopic pattern of cellular reaction with GIC L suggested an initial toxic effect on the cells of the marrow, which appeared to abate; although the healing phase in comparison to that associated with PMMA is sluggish.

S-430 and GIC H were associated with patterns of tissue response suggestive of on going tissue and cellular necrosis. GIC H appears to have affected the cells earlier than did S-430 but S-430 appeared to be causing considerably more necrosis at four weeks. All tested GICs caused an increase in the amount of osteoid and poorly calcified bone. Erythroid and myeloid regeneration only occurred in those rats injected with PMMA.

4. Discussion

Two rats were eliminated from this study because of severe complications, which appeared to be directly associated with unset S-430 cement. Macroscopically crystalline material was observed in the peri-femoral soft tissues of the rat that died during anaesthesia, while birefringent material occurred in the pulmonary micro-vascular tree of the rat with persistent pulmonary distress. Presumably monomeric resin reached the venous circulation causing cardio-respiratory failure in the former while in the latter this material formed nidi for the numerous fibrin thrombi, causing micro-pulmonary infarcts. Hyperventilation occurred immediately in all rats receiving an intrafemoral injection of S-430. This sign, which settled in about quarter of an hour, may have been caused either by glass ionomers and/or the resin,

HEMA. As glass ionomers polymerize they form an acid-base reaction between the calcium fluoro-alumino-silicate glass and aqueous poly-acrylic acid and the pH of the mixture is extremely acid for over 15 min [38], arguably excessive hydrogen ions reaching the circulation might be eliminated by hyperventilation. However, hyperventilation was not noticed when GIC L and GIC H materials were injected suggesting that hydrogen ion release was not a major contributor to the observed tachypnoea and dyspnoea. Although as S-430 cement has a lower viscosity (Fig. 2a) and a longer setting time than conventional GICs, the volume of unreacted acid (and HEMA plus fine glass particles) reaching the circulation could be greater than the conventional GICs (GIC L and GIC H) thus accounting for the observed differences. An alternative and more likely explanation is that the resin monomer, HEMA caused the cardiovascular collapse as well as the transient hyperventilation. Certainly, MMA has been documented as causing these signs [5, 39].

In the present study, in contrast to Erbe *et al.*'s [27] results, no cardio-respiratory side-effects were observed after intra-femoral implantation of PMMA cement; a circumstance which others have attributed as the cause of death [5, 39]. The initial reports of cardiovascular collapse used earlier PMMA cements in which the formulation and possible release of monomer was less rigorously controled. In the present study, the PMMA product (Simplex) is a recent formulation, which on mixing has a high viscosity and a short polymerization time. Such characteristics are supported by the limited extent of the observed interdigitation of the PMMA into the trabecular bone about the prepared cavity.

The microscopic features of marrow and reparative bone to the injected PMMA over four weeks is similar to that described by Willert and Puls (1972) [cited from Feith [39], 1975] viz. the initial phase of necrosis and haemorrhage; the phase of reparation characterized by the presence of proliferation of vascularized undifferentiated mesenchymal cells, nucleated marrow cells, and multinucleated giant cells, and the final phase of stabilization. The latter phase has commenced by week four in the present investigation but is incomplete; for although new bone interfaces with the PMMA, it is only trabecular and appears to be still in the remodeling phase. In general, rodent laboratory animals heal faster than other larger mammals and man. The inert nature of polymerized PMMA as observed by others [27] is reflected in the present investigation as the rate and nature of osteogenesis in the presence of PMMA was similar to those control rats where only the surgical technique was performed.

In contrast, all glass ionomers at week one had more cellular necrosis in the intertrabecular spaces proximate to the cement mass than PMMA. Indeed, the advanced and apparent persisting cellular necrosis has not previously been reported by others [9, 27–29]. However, these microscopic features may merely reflect an early stage of the response by bone and its marrow as other laboratories terminated their experiments at later time points [27–29]. Regardless of the length of the study, it has previously been noted that there was a significant difference in the tissue response associated with the incorporation of either set GICs or whether they were injected in a fluid state [28]. Furthermore, some of the cements used in this study were different from previous investigations and as such may account for the observed differences. Similar detrimental effects on calcification of osteoid and bone as previously reported were encountered [9, 27–29]. These results suggest that although there appears to be an increased early cellular toxicity by GIC L compared to PMMA, this initial toxicity of GIC L could be tolerated, minimized and/or eliminated particularly as previously investigators showed no significant toxicity of the GICs at that early time points [9, 27–29].

On the other hand, GIC H caused more severe and persisting necrosis than GIC L in the first week, an effect, which appeared to be abating by week four. Notable differences between these conventional GICs are that GIC L has a lower initial viscosity and contains more soluble glass components than GIC H and therefore different levels and timing of toxicity can be expected. GIC H containing the larger glass particles and the higher powder/liquid ratio (Table 1) which would indicate that the glass solubility of GIC L is much higher than GIC H [40–42]. This together with a greater penetration into the trabecular bone structure might expect that the initial intraosseous tissue damage by GIC L was greater. But from our results, the damage by GIC H was greater. This may possibly be the effect of the unreacted acid, which was greater in GIC H than GIC L by the increase in the powder/liquid ratio [43] and the increase in the size of the glass particles causing the reduction of the surface area to react with the acid component. The higher powder/liquid ratio of GIC H increased freeze-dried acid component, which was not neutralized and cross-linked due to lesser overall surface area of the glass particles, dissolved into the surrounding tissues greater from GIC H than from GIC L causing more initial damage. Once GIC is set the rate of release of the major (aluminum and fluoride) ions leaching would be expected to decrease and the tissues commence to repair through active cellular recovery. This was seen from the result of GIC L. However, as intra-femoral necrosis is present at four weeks it is presumed to be ongoing and resulting from a continuous release of soluble acid in GIC H. Our results agree with the *in vitro* studies of Doherty [22] and Müller *et al.* [21], which showed that toxic substances were leached from conventional GIC.

The initial toxic effect with S-430 cement appeared to be less than encountered with GIC H. However, the pattern of repair at week two followed extensive widespread necrosis at week four is suggestive of a delayed release of toxic components. As the initial effect by S-430 on the tissues was maximal at week one it was presumed to be toxic factors released due to lowered solubility of the glass in the resin-modified GICs as previously reported [41, 42, 44]. However, regardless of the initial effect overall the biphasic pattern of necrosis, a distinctly different pattern to that for GIC H, is indicative of either the release of toxic factors contained within the GIC either modified by the physical presence of the resin or the HEMA is toxic per se as reported *in vitro* [16–18, 23, 25] or these two elements combine to produce an additive effect.

Tissue toxicity caused by GICs is very complex.

Different stages of cement setting have different pH levels and are associated with a varying ability to leach ions resulting in toxicity [38, 45]. The toxicity of the GICs is attributable to an initial low pH level and continued leaching of ions from the glass particles [21, 45]. Ions released from GICs may be toxic per se or react with other ions and/or a biological pathway. In the latter circumstance these interactions may either exacerbate or suppress the toxicity. For instance, fluoride ion release, which occurs, is dependent on the sodium content of the cement [14], while the toxicity of aluminum ion release is reduced in the presence of silicon, as the latter complexes aluminum [46]. Regardless of the above-identified mechanisms the tissue damage may be due to the release of other minor ions or complexes, which may form. Alternatively some of the ions released may interact as cofactors in cellular or tissue cascades resulting in waves of necrosis [15].

Of all the ions leaching from GICs (F^-, Si^{4+}, Al^{3+}, Na^+ and Zn^{2+}), aluminum has been reported as the most bioincompatible, as it disturbs bone formation at many stages [47–52]. The effects of aluminum ions on bone are dependent on its local concentration and the stage of osseous healing. Aluminum can form complexes with DNA and RNA causing clastogenic and mitodepressive effects on proliferative osteoblasts during healing [47, 51, 52]. For instance, aluminum inhibits hydroxyapatite initiation, formation and growth *in vitro* [49]; impairs calcification of bone and has a direct effect on osteoblastic activity [48, 50]. In contrast to aluminum, fluoride stimulates the proliferation and alkaline phosphatase activity of osteoblasts in a dose-dependent manner [24], but in excessive amounts causes fluorosis. Less information is available regarding the release of other ions from GICs. Our histological results for GIC L were characterized by abundant and excessive osteoid as well as poorly calcified bone features associated with the effects of both excessive fluoride and aluminum ions. Previous studies [24] and the pathology of fluorosis have shown that excessive fluoride stimulates an osteoblastic response in which abundant poorly calcified bone and osteoid are formed. Aluminum also disturbs the mineralization process of osteoid [47, 52]. As both these ions are excessively released in the present experiment, it is not surprising to encounter considerable osteoid and poorly mineralized bone at four weeks. Others have also reported such findings (for instance, at one year the major reaction to GIC was poorly calcified bone and osteoid [27]). The features of disturbed calcification of the bone and its matrix were not prominent at four weeks in GIC H and S-430 groups because of the extensive tissue and cellular necrosis. Therefore, little to no extracellular matrix is being formed. The significant penetration throughout the trabecular bone by the low viscosity S-430 together with its slower setting time and the presence of HEMA may account for lower initial concentrations of toxic elements. However, this widespread dispersion may account for a more significant longer-term additive effect.

5. Conclusion

Of all the glass ionomer materials, GIC L caused minor initial toxicity and later bioincompatibility by disturbing normal bone formation, which was similar to previous observed effects of aluminum ions. GIC H and S-430 caused more severe toxicity. The cause of this greater toxicity is not identified but is postulated to be due to the combination and/or reaction of unreacted acid component and leached ions with or without the superimposed effect of HEMA, the resin in S-430 used to modify the conventional GIC. It may be concluded that in developing a GIC for use in orthopaedic surgery, the components of the powder and the selected components of the glasses, which contribute to the leaching of ions, are as important as reducing the aluminum content of the glasses [27, 29, 38, 53], which can disturb normal bone formation. Also it is concluded that, the resin, which is used to modify the physical properties, must be biocompatible. The results also show the influence of the viscosity of the cement in terms of penetration into the bone structure and into the circulation system.

Acknowledgment

We would like to thank Ms. Barbara James, Bone Biomaterial Unit, School of Pathology, UNSW, for all her help with the animal preparation and the staining of the tissue. W. A. J. Higgs acknowledges the support on an ARC Spirit Grant.

References

1. M. A. R. FREEMAN and R. E. TENNANT, *Instructional Course Lectures* **18** (1991).
2. E. A. SALVATI, M. H. HUO and R. L. BULY, *Instructional Course Lectures* **40** (1991).
3. J. L. GILBERT, D. S. NEY and E. P. LAUTENSCHLAGER, *Biomaterials* **16** (1995) 1043.
4. L. M. JONCK and C. J. GROBBELAAR, *Clin Mater.* **6** (1990) 323.
5. JOHN CHARNLEY, ''Acrylic Cement in Orthopaedic Surgery'' (E & S Livingstone, Edinburgh and London, 1970).
6. R. J. E. D. HIGGS and W. A. J. HIGGS, in ''Advances in Osteoarthritis'', edited by Tanake and Hamanishi (Springer-Verlag, Tokyo, 1999) p. 253.
7. J. W. MCLEAN and A. D. WILSON, *Br. Dent. J.* **136** (1974) 269.
8. P. LUCKSANASOMBOOL, W. A. J. HIGGS, R. J. HIGGS and M. V. SWAIN, *Proceedings of 58th Annual Scientific Meeting*, October 11–16, 1998, Australian Orthopaedic Association & ANZORS (1998).
9. D. H. CARTER, P. SLOAN, I. M. BROOK and P. V. HATTON, *Biomaterials* **18** (1997) 459.
10. I. M. BROOK and P. V. HATTON, *ibid.* **19** (1998) 565.
11. C. W. B. OLDFIELD and BRYAN ELLIS, *Clin. Mater.* **7** (1991) 313.
12. G. J. PEARSON, *ibid.* **7** (1991) 325.
13. ELEANOR A. WASSON and JOHN W. NICHOLSON, *Clin. Mater.* **8** (1991) 125.
14. U. MEYER, D. H. SZULCZEWSKI, R. H. BARCKHAUS, M. ATKINSON and D. B. JONES, *Biomaterials* **14** (1993) 917.
15. J. LEWIS, L. NIX, G. SCHUSTER, C. LEFEBVRE, K. KNOERNSCHILD and G. CAUGHMAN, *ibid.* **17** (1996) 1115.
16. A. OLIVA, R. F. DELLA, A. SALERNO, V. RICCIO, G. TARTARO, A. COZZOLINO, S. D'AMATO, G. PONTONI and V. ZAPPIA, *ibid.* **17** (1996) 1351.
17. G. P. TARTARO, S. D' AMATO, A. ITRO, G. CAROTENUTO, A. GALLO and L. NICOLAIS, *J. Mater. Sci.: Mater. Med.* **7** (1996) 431.
18. G. LEYHAUSEN, M. ABTAHI, M. KARBAKHSCH, A. SAPOTNICK and W. GEURTSEN, *Biomaterials* **19** (1998) 559.
19. S. STEA, M. CERVELLATI, D. CAVEDAGNA, L. SAVARINO,

E. CENNI and A. PIZZOFERRATO, *J. Mater. Sci.: Mater. Med.* **9** (1998) 141.
20. W. R. HUME and G. J. MOUNT, *J. Dent. Res.* **67** (1988) 915.
21. J. MÜLLER, W. HÖRZ, G. BRUCKER and E. KRAFT, *Dent. Mater.* **6** (1990) 35.
22. P. J. DOHERTY, *Clin. Mater.* **7** (1991) 335.
23. P. SASANALUCKIT, K. R. ALBUSTANY, P. J. DOHERTY and D. F. WILLIAMS, *Biomaterials* **14** (1993) 906.
24. A. J. DEVLIN, P. V. HATTON and I. M. BROOK, *J. Mater. Sci.: Mater. Med.* **9** (1998) 737.
25. S. BOUILLAGUET, J. C. WATAHA, M. VIRGILLITO, L. GONZALEZ, D. R. RAKICH and J.-M. MEYER, *Dent. Mater.* **16** (2000) 213.
26. I. M. BROOK, G. T. CRAIG and D. J. LAMB, *Clin. Mater.* **7** (1991) 295.
27. M. ERBE, R. L. VAN DYCK-ERBE and H. J. SCHMITZ, *J. Mater. Sci.: Mater. Med.* **7** (1996) 517.
28. M. C. BLADES, D. P. MOORE and P. A. REVELL, *ibid.* **9** (1998) 701.
29. E. ENGELBRECHT, G. VON FOERSTER and G. DELLING, *J. Bone Joint Surg.* (Br) **82-B** (2000) 192.
30. D. A. FELTON, C. F. COX, M. ODOM and B. E. KANOY, *J. Prosthetic Dentistry* **65** (1991) 704.
31. N. SIX, J.-J. LASFARGUES and M. GOLDBERG, *J. Dentistry* **28** (2000) 413.
32. W. A. J. HIGGS, P. LUCKSANASOMBOOL, HIGGS R. J. and SWAIN M. V., *J. Bone Joint Surg.* (Br) **81(B)**: Supp I (1999).
33. W. A. J. HIGGS, P. LUCKSANASOMBOOL, HIGGS R. J. and SWAIN M. V., *J. Mater. Sci.: Mater. Med.* **12** (2001) 1.
34. W. A. J. HIGGS, P. LUCKSANASOMBOOL, HIGGS R. J. and SWAIN M. V., *Biomaterials* (in press) (2001).
35. R. E. KOVARIK and M. V. MUNCY, *Am. J. Dentistry* **8** (1995) 145.
36. J. F. MCCABE, *Biomaterials* **19** (1998) 521.
37. C. A. MICHELL, W. H. DOUGLAS and Y.-S. CHENG, *Dent. Mater.* **15** (1999) 7.
38. D. C. SMITH and RUSE, *J. Am. Dent. Assoc.* **112** (1986) 654.
39. R. FEITH, *Acta Orthopaedica Scandinavica Supplementum* **161** (1975) 10.
40. M. ROTHWELL, H. M. ANSTICE and G. J. PEARSON, *J. Dentistry* **26** (1998) 591.
41. M. S. BAPNA and MUELLER, *J. Oral Rehabilitation* **26** (1999) 786.
42. S. M. BERTACCHINI, P. F. ABATE, A. BLANK, M. F. BAGLIETO and R. L. MACCHI, *Quintessence International* **30** (1999) 193.
43. H. J. PROSSER, D. R. POWIS, P. BRANT and A. D. WILSON, *J. Dentistry* **12** (1984) 231.
44. K. YOSHIDA, M. TANAGAWA and M. ATSUTA, *J. Oral Rehabilitation* **25** (1998) 285.
45. M. S. BAPNA and H. J. MUELLER, *ibid.* **21** (1994) 577.
46. A. D. WILSON, D. M. GROFFMAN and A. T. KUHN, *Biomaterials* **6** (1985) 431.
47. A. K. ROY, A. SHARMA and G. TALUKDER, *Cytobios* **66** (1991) 105.
48. Y. EBINA, S. OKADA, S. HAMAZAKI, Y. TODA and O. MIDORIKAWA, *Calcified Tissue International* **48** (1991) 28.
49. N. C. BLUMENTHAL and A. S. POSNER, *ibid.* **36** (1984) 439.
50. WILLIAM G. GOODMAN and JEANENNE O'CONNOR, *Kidney International* **39** (1991) 602.
51. L. S. KIDDER, G. L. KLEIN, C. M. GUNDBERG, P. K. SEITZ, N. H. RUBIN and D. J. SIMMONS, *Calcified Tissue International* **53** (1993) 357.
52. C. G. BELLOWS, J. N. HEERSCHE and J. E. AUBIN, *ibid.* **65** (1999) 59.
53. K. K. JOHAL, G. T. CRAIG, A. J. DEVLIN and I. M. BROOK, *J. Mater. Sci.: Mater. Med.* **6** (1995) 690.

Received 23 January
and accepted 13 April 2001