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In vivo response of ionomeric cements: effect of glass composition, increasing soda or calcium fluoride content

K. K. JOHAL, G. T. CRAIG, A. J. DEVLIN, I. M. BROOK*
University of Sheffield, Biomaterials Group, School of Clinical Dentistry, Claremont Crescent, Sheffield. S10 2TA UK

R. HILL
University of Limerick, Department of Materials Science, National Technological Park, Limerick, Ireland

The *in vivo* response of two defined groups of set ionomeric cements (ICs), were evaluated following implantation in the midshaft of three week old Wistar rat femora for four weeks. New bone formation was associated with all the IC implants, the amount of new bone increasing with increasing sodium or calcium fluoride content of the basic glass component. Previous work has shown that there is a link between glass composition and ion release, fluoride ion release increasing as the sodium or fluoride content of the glass increases. It thus appears that in the series studied improved bone formation associated with the ICs was mediated by increased fluoride ion release.

1. Introduction

Ionomer cements are hybrid glass polymer composites formed by the neutralization reaction of a basic ion leachable inorganic glass and an organic polyelectrolyte (polyacrylic) acid. Their properties include a rapid snap set, high compressive strength, adhesion to enamel and dentine, and the release of potentially osteoconductive ions such as fluoride and calcium [1]. Additional properties that make them attractive bone substitutes include a non-exothermic setting reaction, chemical adhesion to bone and metals, and the ability to mould and shape the cement at the implant site with minimal setting shrinkage [2].

Ionomer cements are established restorative dental materials and more recently have been used as preformed implants and cements in otolaryngology and cranial surgery [3, 4]. Further development of this group of materials for orthopaedic use is being undertaken [2, 5].

Clinical success of biomaterials depends largely upon the structure, composition and stability of the bone/implant interface achieved. The purpose of this study was to evaluate the *in vivo* response of bone to six different formulations of ionomer cements based upon two defined series of ionomeric glasses. The first series had a constant fluoride content but increasing soda content and the other an increasing calcium fluoride content.

2. Materials and methods

2.1. Materials

Six fluoroaluminosilicate glass-based ionomer cements were used in this study. The first series of ionomer cements were sodium based LG2, LG6, and LG63 (Department of Materials Science, University of Limerick, Ireland). They had the following chemical structure, $\mathbf{W}SiO_2$. $\mathbf{Y}P_2O_5$. Al_2O_3. $(\mathbf{1\text{-}Z}Na_2O,)$ **CaO**. CaF_2 where **W**,**Y** and **Z** are the mole fractions and where **Z** for ionomer cements LG2 = 0, LG6 = 0.1 and LG63 = 0.2. The second series of ionomeric cements (LG26, LG27 and LG30) with a Ca : P ratio of 1.66 were based on the general formula (**P**) SiO_2(**Q**) Al_2O_3(**5-X**) CaO $1.5P_2O_5$(**X**) CaF_2. With the production of glasses having a larger content of modifying oxide (CaO) the mole fraction of silica and alumina were increased to ensure glass formation. As the fluoride content of the glass varied by the addition of CaF_2, the CaO content present was influenced by the variable "**X**" where **X** in LG26 = 2, LG27 = 1 and LG30 = 0. The glass LG2 can be considered to be in both series having no soda and a value of **X** = 0.75.

2.2. Formation of implant rods

Ionomeric cements were produced using a ratio of 1.0 g glass, and 0.2 g freeze dried mercaptan free polyacrylic acid (Advanced Healthcare, UK) and

* To whom correspondence should be addressed

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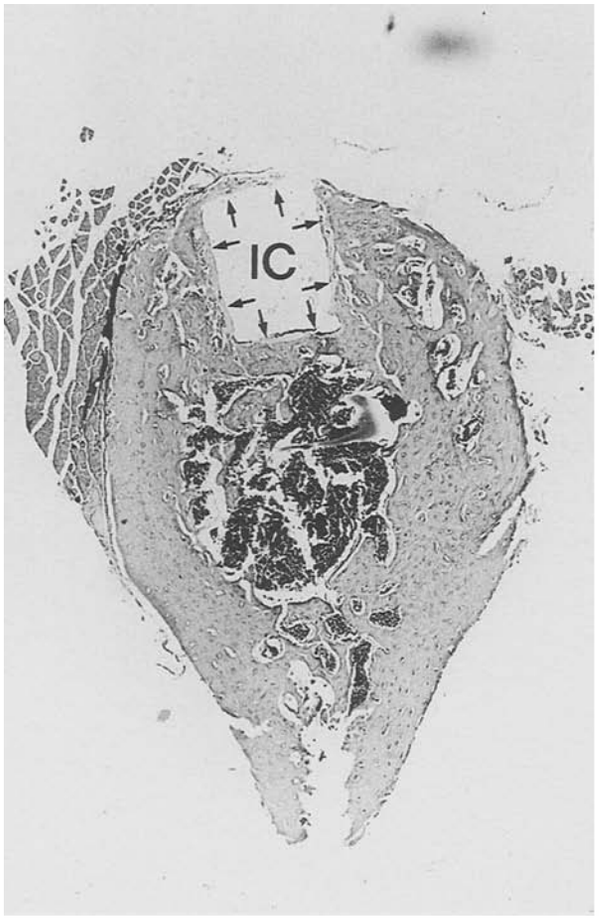

Figure 1 Transverse section of femur showing a rod of ionomeric cement (IC) completely surrounded by bone; arrows depict six points around the perimeter at which osteoconduction might be evaluated; haematoxylin and eosin × 16.

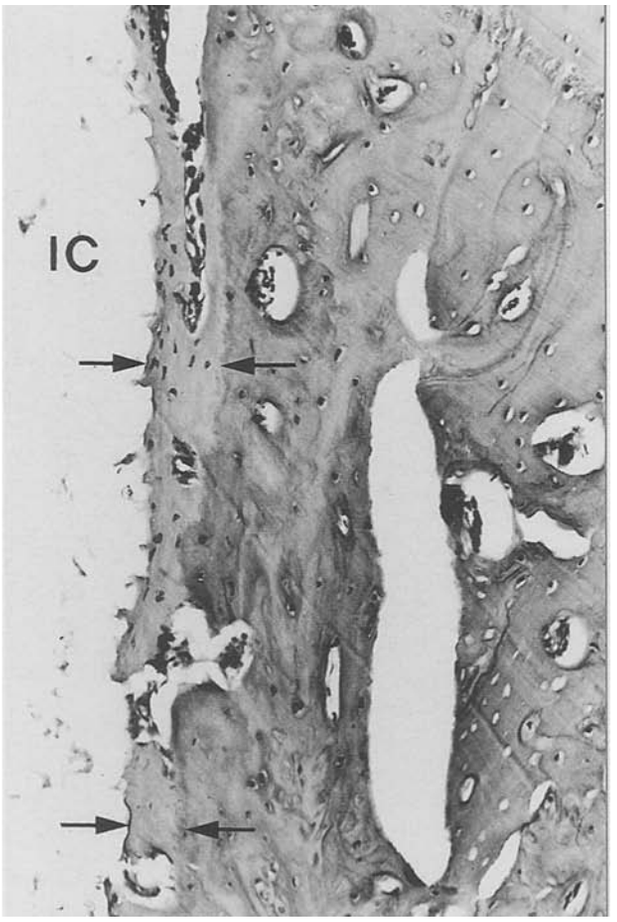

Figure 2 Interface between ionomeric cement (IC) and bone; the opposing arrows demarcate the thickness of newly formed bone and its measurement formed the basis for evaluating osteoconductive potential; haematoxylin and eosin ×162.

0.3 ml sterile non-pyrogenic water. Smooth rods (nominally 2 mm length × 1 mm diameter) were produced by placing unset material in silicone moulds. The rods were cured for at least 5 h at 37 °C at 100% relative humidity. The rods were then steam sterilized returned to room temperature and stored overnight at 100% humidity prior to implantation.

2.3. Implantation

For each of the six materials a single rod was implanted, under anaesthesia (Halothane 2% (May and Baker, UK); in oxygen 25% and nitrous oxide 75%), into the midshaft of the femora in groups of five weaned inbred Wistar rats. Under saline irrigation a slow speed 1 mm diameter Tungsten Carbide burr was used to cut a hole matched to the diameter of the implant, through one cortex into the marrow space. Implants were placed to lie level with the surface of the bone penetrating through the cortex into the marrow cavity. The overlying periosteum and soft tissues were replaced and the wound sutured, antibiotics were not used. Post-operatively, wounds were inspected to monitor healing and rats were maintained on a standard laboratory diet. After four weeks, animals were sacrificed and the femora removed, fixed in neutral buffered formalin, and decalcified in 4 N formic acid for one week prior to trimming, routine histological processing and paraffin wax embedding.

2.4. Light microscopy/histomorphometry

Five stepped serial sections 7 μm thick, each separated by 70 μm, were cut from the implant bed in each femora using a rotary microtome, mounted on glass slides and stained using haematoxylin and eosin. The biological response to the different ionomer implants was studied by determining the degree of osteoconduction and percentage osseointegration, using a transmission microscope linked to an image analyser system (Optimas 5.1, Biosoft, USA). Osteoconduction was determined by taking six points at random around the perimeter of each ionomeric rod and measuring the thickness of new bone formed (Figs 1, 2 and 3). The degree of osteoconduction being taken as the average thickness of new bone produced on the implant surface. Percentage osseointegration was determined by measuring the proportion of the total implant perimeter in contact with bone (Figs 1, 4–6). Statistical analysis was undertaken using the Unistat statistical package (University software, UK) and by applying Student's t-test.

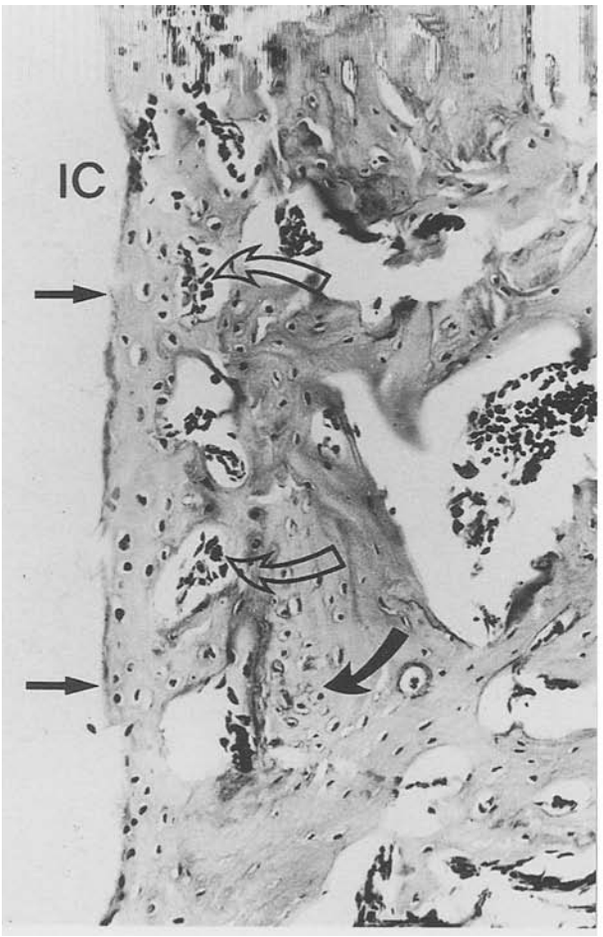

Figure 3 Interface (straight arrows) between ionomeric cement (IC) and newly formed bone; note its cellularity and the chondroid appearance seen focally (curved arrow); osteoblasts line marrow spaces adjacent to the interface (open arrows); haematoxylin and eosin ×162.

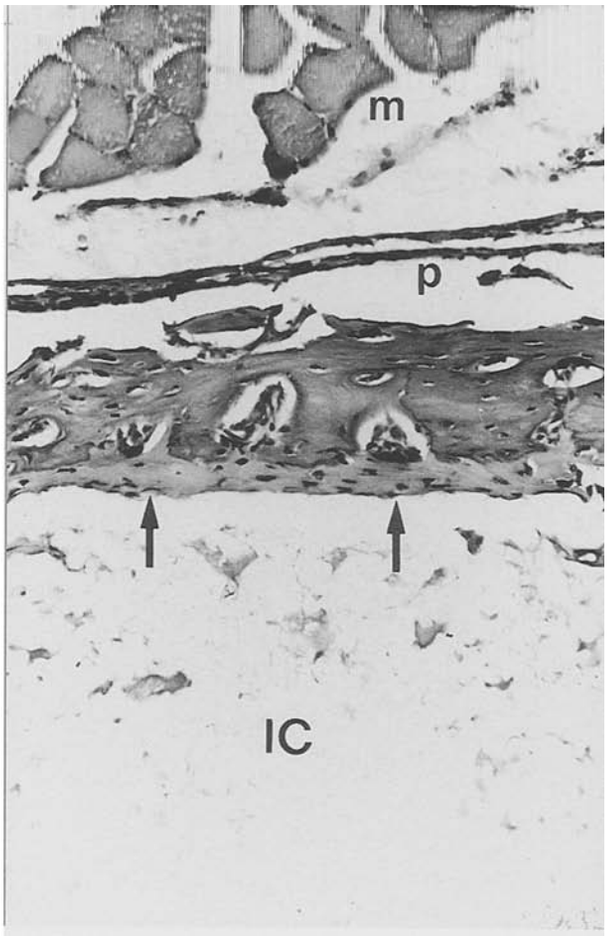

Figure 4 Partly remodelled cortex and new bone (arrows) at the periosteal interface of the implant (IC); p = periosteum; m = voluntary muscle; haematoxylin and eosin ×162.

3. Results

3.1. Histological assessment

Of the 36 implant sites all healed uneventfully. All the ionomeric cements exhibited formation of new bone on their surface and generally new bone was formed in continuity with the implant surface from the cortical bone at the surgical site into the marrow space (Fig. 1). In most cases, the interface between implant and host was characterized by a layer of notably cellular and, apparently, variably mineralized mature bone (Figs 2 and 3); in places the cellularity of the newly formed bone resulted in a chondroid appearance: marrow spaces close to the interface were lined by prominent osteoblasts. Even as judged subjectively, the layer of new bone varied in thickness for different ionomeric cements. In all cases, the periosteal end of the implant was covered by a layer of partly remodelled cortical bone (Fig. 4). Similarly, that portion of each implant which projected endosteally (Fig. 5) was separated from the adjacent vital marrow tissue by a thin layer of variably mineralized woven bone (Fig. 6).

3.2. Histomorphometry osteoconduction and osseointegration

Increased levels of new bone formation were observed as the soda or calcium fluoride content of the glass component of the ionomer cements increased. There was a significant difference in the mean thickness of new bone (degree of osteoconduction) formed adjacent to the soda containing glass based series this being a mean of 0.12 mm for LG63 greatest soda content, 0.07 mm for LG6 and 0.005 mm for LG2 (no soda). The difference between LG63 and LG2 being significant ($t = 5.2$, $df = 8$, $P < 0.007$). In the second series significantly more new bone formation was associated with the ionomer cement LG26 (highest fluoride content) compared to the ionomers in the soda series or other ionomeric cements in the same series ($t = 2.16$, $df = 8$, $P < 0.04$). The mean new bone formation (osteoconduction) observed in the calcium fluoride series being: 1.55 mm for LG26, 0.08 mm for LG27 and 0.07 mm for LG30 with the difference between LG26 and LG27/LG30 being significant ($t = 4.5$, $df = 8$, $P < 0.002$).

The results for the percentage osseointegration of the ionomeric cements showed a similar trend as that seen for osteoconduction with the difference between the calcium fluoride and soda series being significant (LG26 versus LG63 $t = 4.5$, $df = 8$, $P < 0.002$). The soda based ionomeric cements following the order LG63 > LG6 > LG2 (being 68%, 61% and 37% respectively). The difference between LG2 and LG63 being significant ($t = 37.71$, $df = 8$, $P < 0.0001$). The calcium fluoride based glasses following the order of

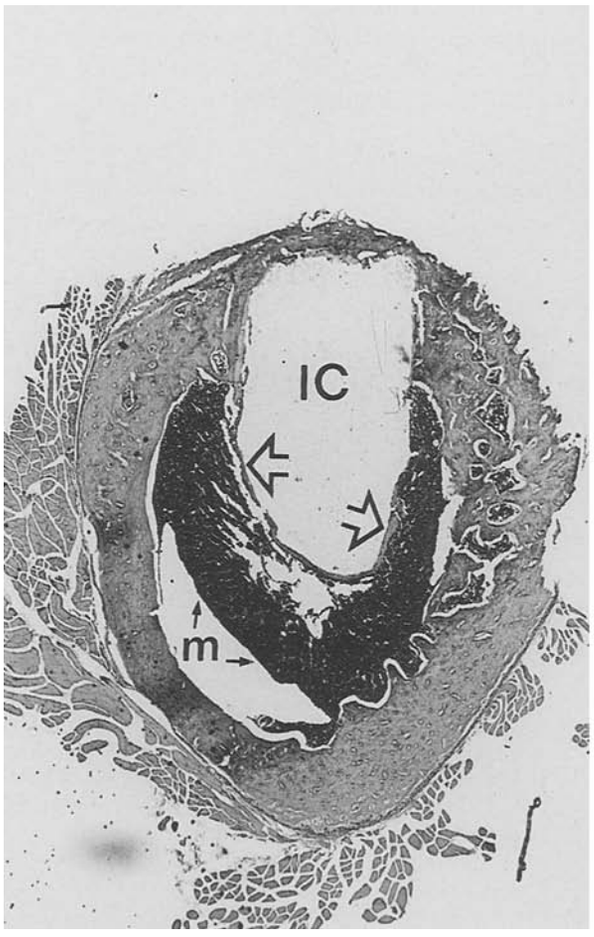

Figure 5 Implant (IC) projecting into marrow cavity and separated from vital marrow (m) by a thin layer of new bone (open arrows); haematoxylin and eosin ×16.

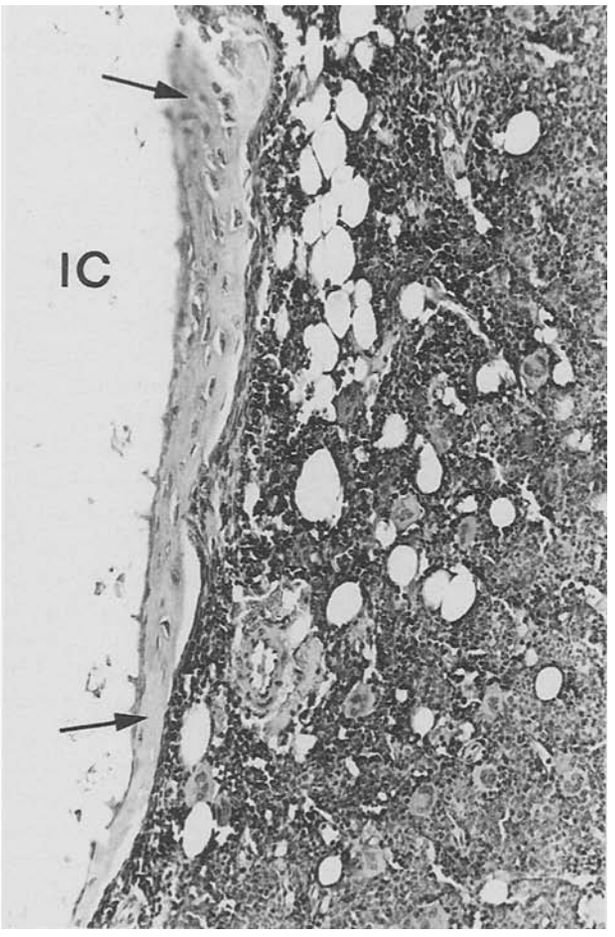

Figure 6 Detail from Fig. 5 showing a thin layer of partly mineralized woven bone (arrows) separating the implant (IC) from vital marrow tissue; haematoxylin and eosin ×162.

LG26 > LG27 > LG30 (being 80.8% > 78.2% > 70.6% respectively). The difference in integration between LG26 and LG30 being significant ($t = 1.953$, $df = 8$, $P < 0.05$).

4. Discussion

Semi-quantitative histomorphometic analysis and histological evaluation revealed that there was a more positive response from the surrounding bone associated with the ionomeric cement implants in the calcium fluoride series as compared to those in the soda containing series. The responses seen appeared to be correlated with the composition of the ionomer glasses. Increasing the fluoride content in the calcium fluoride series (LG30 to LG26) was associated with increased bone formation and confirms the results seen by ourselves and others as to the beneficial effect of this ion in appropriate concentration on bone formation [5–7]. Work previously reported on this series of glasses has however, demonstrated that increasing the soda content of ionomeric cements increases the rate and amount of fluoride release from the cement matrix [6]. This has been proposed to be due to increasing the mobility of fluoride ions in the matrix and by facilitating an ion exchange mechanism between cement and the environment. The results from studying ion release from ionomeric cements [6] taken together with those of the present study demonstrate that while the total amount of fluoride present is an important determinant of biological response, the mobility of ions within the cement matrix and their release characteristics are significant factors affecting the induced biological response.

In the calcium fluoride series as well as increasing fluoride content in order to ensure glass formation the mole fraction of alumina was increased throughout the series with LG26 containing 3 mole fractions, LG27 with 4 mole fractions, and LG30 containing 5 mole fractions. The amount of new bone was thus negatively correlated with alumina content, LG30 being associated with the least amount of new bone formation. Increasing aluminium ion release from ionomeric materials has been demonstrated to be negatively correlated with the *in vitro* response of osteoblasts [7]. The effect of aluminium ions on bone metabolism is however an area of continuing debate [8]. Aluminium ions have been shown to reduce new bone formation [9], at high concentrations inhibit bone mineralization [10, 11] and there is evidence that aluminium can enhance the mobilization of calcium from bone by a cell-independent mechanism [12]. In contrast, low concentrations of aluminium have been reported to stimulate the proliferation and differentiation of osteoblasts *in vitro* [10]. While *in vivo*, administration of aluminium has been shown to increase

bone volume by positively influencing trabecular networking in the axial skeleton [9]. Such enhancement of bone histogenesis contrasts with the effects of other pharmacologic agents that solely alter the thickness of existing trabecular plates or rods within the vertebral spongiosa [14, 15].

The results of this study show that biological response is reduced with increased alumina content associated with reduced fluoride content. Thus, the effects of aluminium and fluorine are likely to be dependent on the concentration of their ions. The beneficial effects of fluoride are thought to be due to promotion of osteoblastic activity and increasing trabecular bone density [7]. However, the effects of fluoride, like aluminium, appear to be dose-dependent *in vitro* [16, 17] and *in vivo* [18, 19]. The formulation of defined ionomeric cements enables controlled evaluation of the parameters that affect the biological response of bone to ionomeric cements. The biocompatibility and composition of ionomeric cements are of great importance because they need to be in direct contact with bone for any chemical adhesion to occur. The model ionomeric cement formulations studied here induced a favourable biological response from the tissues of the implant bed. Improved osseointegration and osteoconduction being associated with increased calcium fluoride or soda content and reduction in alumina content. Our previous work [6] demonstrated a link between glass composition, and ion release, with fluoride ion release increasing as the sodium or calcium fluoride content of the glass increases. Improved osseointegration and osteoconduction appear to be mediated by increased fluoride ion release.

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