In vivo response of strontium and zinc-based ionomeric cement implants in bone

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In this study the osteoconductive properties of strontium based ionomeric cements (ICs) named, LG125 and LG119, as well as zinc-based ICs, designated by LG130 and LG132, were compared. Wet ICs were surgically implanted into the femora of weaned Wistar rats for 4 weeks. To assess the percentage osseointegration the perimeter of the implant and the perimeter of bone in contact with the implant were measured using a pointer (the length of bone/implant interfacial contact). Osteoconduction was determined by taking six points at random around the perimeter of each ionomeric rod measuring the thickness of newly formed bone. The degree of osteoconduction was taken as the average thickness of new bone produced on the implant surface. It was found that osteoconduction was greatest in the strontium based IC implant LG125. From these studies it can be concluded that the composition LG125 might provide a useful purpose as a bone cement.

1. Introduction

Ionomeric cements (ICs) are formed by mixing a fluoroalumino-silicate glass with poly(acrylic) acid. The glassy phase acts both as a filler and as a source of cations to crosslink the polymer chains. The setting of ICs is based on a non-exothermic neutralization reaction, unlike that of poly(methylmethacrylate) (PMMA) which is exothermic. The most outstanding property of the IC is its adhesion to stainless steels and bone tissue [1]. It is believed that chemical bonds are formed between the calcium ions of bone and carboxyl groups from the polymer chain [1]. These attributes along with their apparent osteoconductive potential have led to the use of ICs as preformed implants and bone cements in otolaryngology [2–4]. The release of potentially osteoconductive ions (e.g. fluoride) [1, 4–8] and the biological effects of other ions (e.g. aluminum) have been linked with the biological response to ICs [9].

Strontium (Sr) has been reported to contribute to the increase in bone mass and volume when given at low doses, remineralizing skeletal lesions [10]. It seems that strontium directly suppresses bone resorption and has no deleterious effect on bone mineralization [11]. Strontium has also been reported [10–12] to have beneficial effects on bone formation in rodents and humans which results in increased trabecular volume.

Zinc was selected in the ICs for its osteoconductive properties [13], since it has been reported to have a direct stimulatory effect on bone mineralization *in vitro* and bone protein synthesis [13, 14]. Yamaguchi *et al.* [14] reported an increase in protein synthesis in bone cells, inducing bone formation [13]. Also, zinc deficiency may be a risk factor in the pathogenesis of osteoporosis [14]. The aim of this work was to compare bone growth resulting from IC implants with zinc and strontium.

2. Materials and methods

2.1. Materials

Two series of ICs were investigated. The first were strontium based ICs (all non-porous) and had the general formula, $4.5SiO_2$ $3.0Al_2O_3$ $1.5P_2O_5$ $(3.0-X)CaO$ $2.0CaF_2$ $XSrO$, where X is the mole fraction. Two types of ICs were defined: LG119 (low strontium, $X=1.5$) and LG125 (high strontium, $X=3.0$). The second series were zinc based ICs having the general

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formula $4.5SiO_2$ $3.0Al_2O_3$ $1.5P_2O_5$ $(3.0-X)CaO$ $2.0CaF_2$ XZnO, designating two types of ICs: LG130 (high zinc, $X=1.5$) and LG132 (low zinc, $X=0.75$). Controls used for this study were the IC LG26 with the general formula $4.5SiO_2$ $3.0Al_2O_3$ $1.5P_2O_5$ $2.0CaO$ $3.0CaF_2$, with no strontium or zinc, and the femur of rat bone with a drilled hole without cement.

2.2. Formulation of wet cements for surgical implantation

ICs were produced by mixing 1.0 g glass, 0.2 g poly(acrylic) acid (PAA), and 0.3 ml sterile water. The materials were mixed using a sterile spatula on a cool sterile tile. Prior to implantation, the cement was placed into dental tubing (Odus Full Tuben-transparent, Zurich, Switzerland), where the end of the tube was snipped off and the wet cement was squeezed into a drilled hole (2.0 m in length × 1.0 mm in diameter) in the mid-shaft of the left femur. The ICs were allowed to set *in vivo* after implantation.

2.3. Surgical implantation

The five ICs were implanted *in vivo* under anesthesia (Halothane 2.0%, May & Baker, UK) in oxygen 25.0% and nitrous oxide 75.0%, into the midshaft of the femur of groups of nine weaned (three-week-old) Wistar male rats. Rats were used as the healing process is faster in the bones of young vertebrates and the rates of bone deposition and absorption are rapid [15].

Under saline irrigation, a slow speed 2.0 mm length × 1.0 mm diameter tungsten carbide burr was used to drill a hole matched to the diameter of the implant through one cortex into the marrow space of the midshaft of the left femur. An amount of 2 mm^3 of the wet cement was placed to lie level within the outer surface of the bone, penetrating through the cortex into the marrow cavity. The overlying periosteum and soft tissues were replaced and the skin was sutured.

Post-operatively, wounds were inspected to monitor healing and rats were maintained on a standard laboratory diet. After four weeks of implantation femora were extracted, fixed in neutral buffered formalin for 48–72 h, and decalcified in 4 N formic acid for one week prior to trimming. Routine histological processing of the tissue sample was carried out by embedding it in paraffin wax. The bone specimen of 4.0 cm (length) × 1.5 cm (width) was sectioned along its main axis.

2.4. Light microscopy/histomorphometry

Five stepped serial sections of 7.0 µm thick, each separated by 70.0 µm (10 sections per sample) were observed for each of the five IC samples. Sections were cut from the implant bed in each femur using a rotary microtome (Leitz 1512, Germany). Sections were dewaxed by taking them through a series of xylene and rehydrated by passing through a graded series of alcohols of decreasing strengths and finally through water. Finally, these were stained using Harris hematoxylin and eosin Y, then dehydrated through an increased series of alcohols for 2 min, cleared in xylol and mounted in DPX.

The biological response to the different ionomeric implants was studied blind to the IC by covering the labels and the degree of osteoconduction (bony ingrowth) [16]. Percentage osseointegration (direct contact between a loaded implant surface and bone at the light microscopic level) [17] was determined using the transmission Nikon Optiphot 2 Microscope linked to an image analyzer system (Optimas 5.2, Biosoft, USA).

Osteoconduction was determined by taking six points at random around the perimeter of each IC implant, measuring the thickness of newly formed bone. An average of ten sections were analyzed to carry out measurements for each IC, to obtain the mean bone thickness for lamellar bone. For percentage osseointegration, the perimeter of the implant and the perimeter of bone in contact with the implant were measured using a pointer. Statistical analyses were undertaken using the one-way ANOVA.

3. Results

3.1. Histological assessment of *in vivo* surgical implantation

All forty-five operations healed uneventfully. The controls with no surgery (Fig. 1) showed normal marrow and mature cancellous trabeculae through the mid-shaft of the rat femur. In some cases, wet ICs such as LG125 were surrounded by a combination of woven and

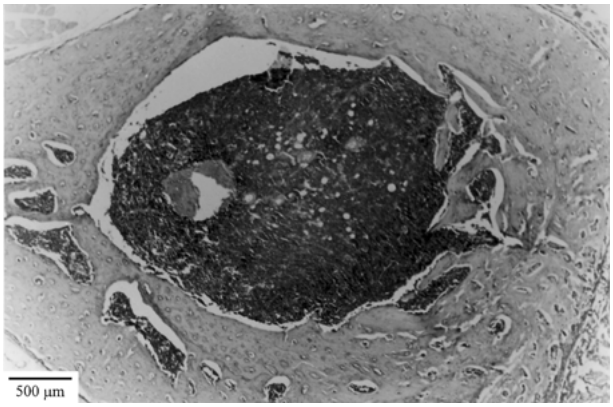

Figure 1 Transverse section through mid-shaft of femur showing extent of normal marrow and minimal cancellous trabeculae; Hematoxylin and Eosin.

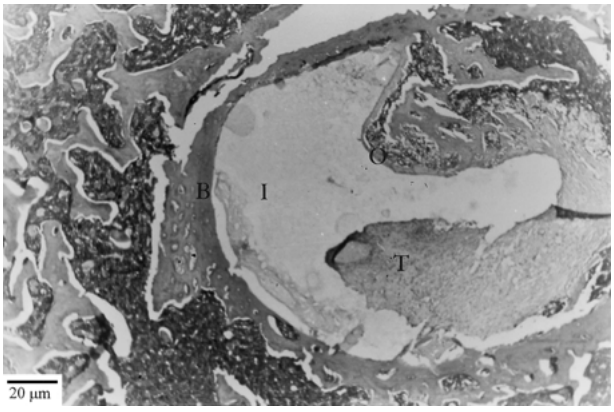

Figure 2 LG119 (I) surrounded by a combination of mature bone (M), osteoid (O) and cellular osteogenic connective tissue (C); Hematoxylin and Eosin.

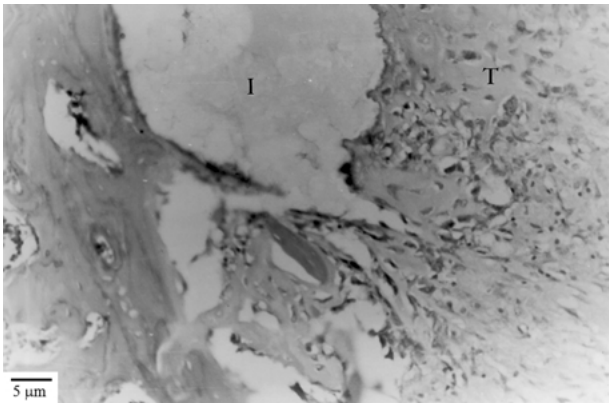

Figure 3 Interface between the wet cement LG119 (I) and notably cellular connective tissue (C); Hematoxylin and Eosin.

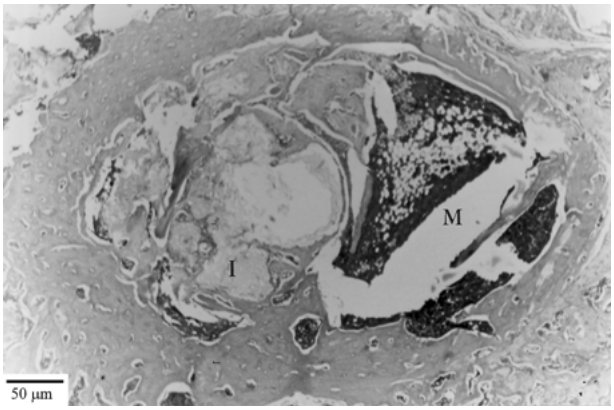

Figure 5 Particles of the wet cement LG125 (I) surrounded by newly formed woven bone and separated from the adjacent healthy marrow tissue (M); Hematoxylin and Eosin.

mature bone with partly mineralized osteoid situated at the periphery (Fig. 2). LG119 wet IC implants appeared to evoke an extensive endosteal reaction and subendosteal bone resorption where the osteogenic cells were contained.

At four weeks the IC implants were surrounded by combinations of mature bone, osteoid and osteogenic connective tissue. Osteogenic cells were found on all non-resorbing surfaces of living bone (Fig. 3). The interface between these wet ICs is demonstrated with LG119 showing cellular osteogenic connective tissue (Fig. 4).

In some places, the wet ICs with a high zinc concentration, such as LG130, were surrounded by compressed cellular connective tissue with minimal evidence of new bone apposition (Fig. 5). There was suspect macrophage activity in the surrounding woven bone. The surface folds are active in phagocytosis. In the case of large objects, e.g. other cells, the folds spread over and surround the object to be phagocytosed. LG26, the control material, was surrounded by acellular connective tissue (Fig. 6).

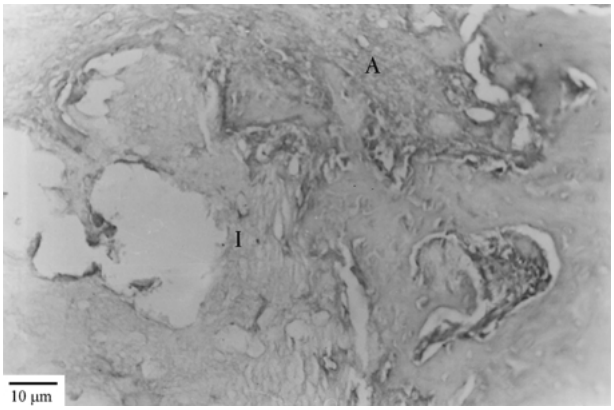

Figure 6 The wet cement LG26 (I) surrounded by acellular connective tissue (A); new bone formation is present peripherally; Hematoxylin and Eosin.

3.2. Histomorphometric results

There were differences in the amounts of osteoconduction and osseointegration with all the implanted ICs assessed after four weeks (Table I).

TABLE I Summary of mean and SD of each wet ionomeric cement at four weeks implantation, $N = 9$

Ionomeric cement tested	Osseointegration/%	Osteoconduction/mm
LG26	67.1 ± 1.9	0.06 ± 0.02
LG119	56.2 ± 3.8	0.05 ± 0.02
LG125	77.0 ± 2.6	0.08 ± 0.01
LG130	72.4 ± 3.3	0.05 ± 0.03
LG132	42.5 ± 1.3	0.05 ± 0.01

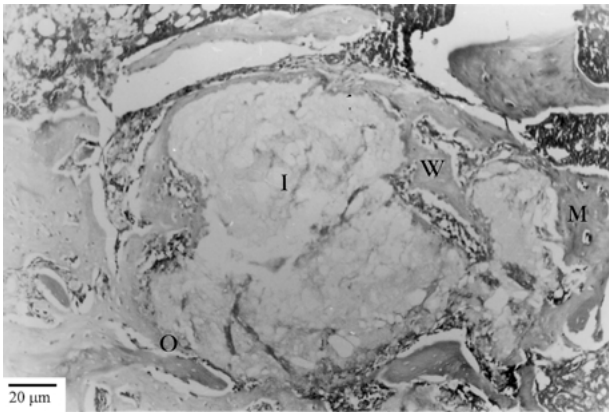

Figure 4 LG125 (I) surrounded by a combination of woven (W) and more mature bone (M) with partly mineralized osteoid (O) situated more peripherally; Hematoxylin and Eosin.

3.3. Statistical analysis of percentage osseointegration

Increased levels of new bone formation were observed as the strontium or zinc content of the glass component of the ICs increased. There was a significant difference in the mean osseointegration formed adjacent to the strontium based ICs. The one-way ANOVA (analysis of variance) test showed that the P value was < 0.0001 $(N = 9)$. This was considered extremely significant for all osseointegrative values. ANOVA assumes that the data are sampled from populations with identical standard deviations (SDs). This assumption is tested using the method of Bartlett. Bartlett statistics showed a value of 9.8 and a P value of 0.04; this suggests differences among the SD's. Intermediate calculations using the

Fisher (F) test gave a statistically significant value of 230.9 at a 95.0% confidence interval where the P value was < 0.05. The Tukey–Kramer multiple comparisons test showed that all groups had P values < 0.05, hence all groups of osseointegration were statistically significant. The same test showed that for LG119 vs LG125 the P value was < 0.05 and that for LG125 vs LG130 the P value was < 0.05.

3.4. Statistical analysis of osteoconduction

The results for the degree of osteoconduction adjacent to the strontium and zinc containing glass based series were analyzed using the ANOVA one-way test where the P value was 0.0078, considered very significant for all osteoconductive values. Variation among the mean column was significantly greater than expected by chance. The Tukey–Kramer multiple comparison test showed that for LG119 vs LG125 the P value was < 0.05 and for LG125 vs LG130 the P value was < 0.05. Bartlett statistics showed a value of 12.9 and $P = 0.113$ being considered statistically significant.

4. Discussion

4.1. *In vivo* evaluation of ionomeric cements

Following the comparison of strontium and zinc based IC implants, LG125 (strontium-based IC) had the greatest amount of osseointegration (77.0%) and the greatest amount of osteoconduction (0.08 mm) from all other ICs used in this study, as shown in Table I. LG132 (zinc based IC) had the least amount of osseointegration (42.5%) and the least amount of osteoconduction (0.05 mm). LG125 had the greatest amount of new bone, which might be related to the three mole fractions of strontium oxide, whereas LG119 had less bone growth, was less osseointegrative and had less strontium (mole fraction of 1.5) than LG125. LG125 and LG26 have similar chemical formulae with the exception of the former having an excess of three mole fractions of strontium. Hence, it might be said that strontium was an osteoconductive stimulating factor in these ICs and that LG125 could have the most appropriate chemical formulae to obtain the optimal osteoconduction as a wet IC *in vivo* implant [5] from all the ICs used in this study.

Strontium has shown encouraging osteoconductive results when given at low doses [18, 19] as shown in Table I. LG125 contained two mole fractions of calcium fluoride, which could indicate that the fluoride release [6] was an additional factor in the positive osteoconductive result of LG125. It has been reported that low doses of both strontium and fluoride increase the number of bone forming sites and vertebral bone volume in rats, not having detectable adverse effects on the mineral profile, bone mineral chemistry or bone matrix mineralization [19]. In contrast it has been found that high doses of strontium induce defective bone mineralization [12]. However, the strontium based IC LG125 did not cause such perturbing effects on mineralization, hence the concentration (three mole fractions) is proposed to be appropriate. The deleterious effects of strontium on bone mineralization may result from the reduction in the intestinal absorption of calcium [20]. Hence, the positive effects of strontium depend on the dose released by the ICs. However, in this present study mineralization did take place as can be shown in Fig. 5.

In addition, high doses of fluoride may alter bone mineralization [21] and have no beneficial effects on bone mineral density [22]. These adverse effects could be associated with changes in the mineral profile as a result of the substitution for hydroxyl groups in the apatite lattice of bone [23]. Moreover, zinc has been reported to play an important role in the growth and stimulation of bone formation [24–26] and this theory is accepted by the positive osteoconductive results of LG130 and LG132, when compared to the control IC LG26 (Table I). LG130 was significantly better integrated with bone than LG132. This could originate from the fact that LG130 had a greater zinc oxide mole fraction (1.5) than LG132 (0.75). In general, it has been observed that the ions released from a set IC relates to the cement formulation [7]. However, the low amount of osteoconduction with zinc-based ICs could result as zinc has been reported to be a potent inhibitor of osteoclastic bone resorption *in vitro* [25]. Although zinc has been shown to be important for the normal function of bone cells, however there is no causative relationship between acute zinc deficiency and decreased osteoblast number and activity in rat bone [26]. This might indicate that zinc was not the dominant ion influencing the amount of bone formation and integration.

5. Conclusions

From this study it might be concluded that LG125 was the most osteoconductive wet IC implant containing a high mole fraction of strontium. All ICs were osteoconductive. It can also be concluded that this composition provides a useful and promising bone cement. The high zinc based IC LG130 was exceptionally better integrated than the low strontium-based IC LG119 and equally osteoconductive to LG119 and LG132.

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