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A pilot evaluation of an aluminum free glass ionomer cement using a sub-chronic osseous defect model in New Zealand white rabbits

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ABSTRACT

Until recently, the development of a clinical-viable aluminum-free glass ionomer cement has been limited by either (i) suitable working times for injection, but inadequate strength for clinical consideration, or conversely, (ii) acceptable strengths with inadequate working characteristics for practical utility. Recent studies, however, point to the inclusion of germanium in these materials as a promising step toward the balancing of clinical handling characteristics with mechanical properties, but no evidence yet exists as to their biocompatibility. In the present study a pilot group of New Zealand White rabbits was implanted with a novel aluminum-free zinc-silicate glass ionomer cement (Zn-Ge GIC) in a subchronic osseous defect model, in order to evaluate the host response to these materials, with a focus on bone healing and remodeling. The Zn-Ge GIC used in this pilot elicited a tissue response characterized by mild and incomplete fibrous encapsulation which did not appear to impair osteoconduction, or osseointegration, characterized by multifocal bone–biomaterial apposition. These data, together with the growing body of literature supporting the clinical utility of Zn-Ge GICs, indicate the significant potential of these materials for hard tissue augmentation.

1. Introduction

It has recently been shown that it is possible to design zinc-based aluminum-free glass ionomer cements (Al-free GICs) with performance characteristics suitable for percutaneous bone augmentation procedures [1]. This evolution in materials properties was enabled by the partial substitution of Si for Ge within a zinc-silicate GIC (Zn-Ge GICs) [1]; whereby the substitution, in the glass phase, augments the setting reaction of the contiguous cements by delaying but not hindering cross-link formation within the polysalt matrix [2]. In clinical terms, this improved chemistry provides Al-free GICs which, for the first time, offer clinically practical working times (c. 12 min) and strength characteristics (*e.g.* compressive strength >50 MPa) [3], a realization that enables expanded investigations for these materials. Prior to balancing these material properties, implantation studies with these materials were not possible due to the poor materials performance characteristics – specifically, the dichotomous nature of the interaction between setting reaction and compressive strength where Al-free GICs have either demonstrated (i) suitable working times for injection, but inadequate strength for clinical consideration, or conversely, (ii) acceptable strengths with inadequate working characteristics for practical utility [4–12]. Accordingly, there exist no published studies on the *in vivo* response to Al-free GICs and, as a consequence, our understanding of the biocompatibility for such materials remains limited, and in many instances conflicted. To date the published data relating to biological responses for Al-free GICs has focused largely on *in vitro* performance extrapolated from elution assays in cell culture. Interestingly, for Al-free GICs there exists evidence indicating that Zn release from the matrix (400 μM) is likely to cause acute cytotoxicity that may "…preclude their use in vivo" [13]. However, elsewhere it has been noted that the inclusion of Ge in Al-free GICs not only balances handling characteristics and mechanical properties, but also limits Zn release to a negligible level of <10 ppm *(31 days of incubation in simulated physiological conditions)* [14,15]. In addition to this data further *in vitro* studies of Zn-Ge GICs have illustrated that these materials are cytocompatible (cell viabilities $>80\%$) [16] and provoke no genotoxic response in AMES assays [17].

In order to more fully understand the biocompatibility of such materials, it is first necessary to examine in a pilot study the host

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response provoked as a result of implantation of these materials in a living and functional model. Results from such pilot studies can provide provisional evidence on the biocompatibility of new materials and, if appropriate, act as the basis for justification of more comprehensive studies in the future. Accordingly, the objective of this study is to implant a clinically viable formulation of a Zn-Ge GIC in a subchronic osseous defect model using New Zealand White (NZW) rabbits. This model represents a functional load-bearing *in vivo* anatomical model for evaluating bone healing and remodeling, and is accepted for the evaluation of osseointegration with respect to examining new bone cement materials [18].

2. Materials and methods

Zn-Ge GIC kits for this study comprised (i) glass powder (DG302) synthesized as per Dickey et al. [19], (ii) Polyacrylic Acid (PAA) powder (M_w = 12,500 g/mol, Advanced Healthcare Limited, UK), and (iii) sterile tissue culture water (Sigma Aldrich, Canada). All components of the kit were packaged separately. Both the glass and PAA powders were gamma irradiated at 25 kGy (Nordion, Canada). Sterilized cement kits (n = 4) were subsequently shipped directly from the sterilization facility to the preclinical testing facility (AccelLAB Inc., Canada) and stored at room temperature (below 25 °C, protected from light) prior to surgical use. Cements were formulated immediately prior to injection by blending the glass with a 50 wt% aq. solution of PAA at a powder: liquid ratio of 2:1.5.

All surgical procedures and animal husbandry adhered to protocols approved by the Institutional Animal Care and Use Committee of an AAALAC and CCAC-certified preclinical testing facility (AccelLAB Inc., Canada). Consistent with the nature of a pilot study, three male NZW rabbits (8 months old; 3.5–4.1 kg, Covance Research Products, USA) were acclimated to the facility (16 days), monitored at least twice per day, and were maintained in conformance with the standards of the *Guide for the Care and Use of Laboratory Animals* (e.g. certified high fiber diet, hydration, housing, etc.). Temperature and humidity were monitored, and the light cycle was controlled by an automated system. Each animal was identified by a unique study number tattoo. In order to create the defect site, each animal was tranquilized (subcutaneous administration of acepromazine); anesthesia induction was achieved with propofol injected intravenously. Each animal was intubated (presence of passive balloon ventilation), and isoflurane in oxygen was administered to maintain a surgical plane of anesthesia. Intravenous warm fluid therapy (Lactate Ringer's) was utilized throughout the procedure at a rate of ~10 mL/kg/h. Prior to surgical creation of the defect, blood samples were collected for baseline clinical pathology including blood hematology and serum biochemistry analysis prior to heparin administration and prior to initiation of intravenous fluid therapy. Whole blood was analyzed immediately, while plasma and serum samples were kept frozen (in a −80 °C freezer). Burprenorphine (0.05 mg/kg, IM) and Rimadyl/carprofen (4 mg/kg, SC) were administered during preparation for surgery as preemptive analgesia, and animals were given long action penicillin (Duplocillin, IM, 0.25 mL/kg or 75,000 UI/kg). Cylindrical defects (n = 2 per animal, 4 mm × 10 mm) were drilled, bilaterally, in distal femoral medial condyles. The depth was standardized using a custom-made stopper fixed at 10 mm. Surgical site preparation, animal positioning and monitoring was performed as per O'Connell et al. [20].

Once the surgical defect had been created, Zn-Ge GIC cement pastes were prepared and injected into the designated defects (Animal 1 and 2, Table 1). Defects in Animal 3 were left open and untreated (sham/control). Injection of the cement paste into the defect was distal to proximal, and started exactly five minutes after commencement of mixing of Zn-Ge GIC. Gauze was used to cover the area around the defect, keeping it free from excess material while the cannula was positioned into defect. A 1 cc syringe was filled with cement and delivered to the site *via* an 11G vertebroplasty cannula (Cook Medical, Canada). The stylet was re–inserted to extrude cement paste remaining in the 11G cannula. Before closure, residual material around the defect site was removed and a collagen membrane (ConFORM Collagen Membrane; ACE Surgical Supply Co., USA) was cut to size and applied over the opening of all defect sites. Sites were then surgically closed over the defects. All animals were allowed to recover and monitored as per O'Connell et al. [20].

Table 1
Defect site labelling.

Animal #	Implant site designation	Defect side
1	Zn-Ge GIC-01-L	left-side
	Zn-Ge GIC-01-R	right-side
2	Zn-Ge GIC-02-L	left-side
	Zn-Ge GIC-02-R	right-side
3	SHAM-L	left-side
	SHAM-R	right-side

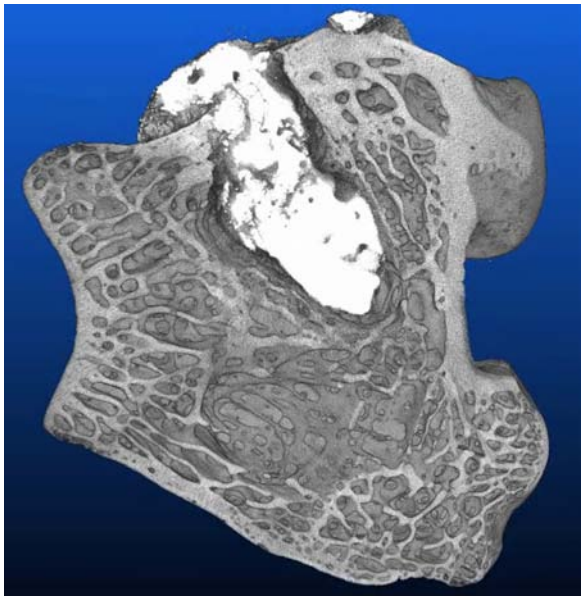

Fig. 1. MicroCT revealed irregular cement geometry but confirmed complete filling of femur defects and showed evidence of interdigitation between the implants and the surrounding cancellous bone.

At 8 weeks, and prior to euthanasia, blood samples were again collected from each animal for hematology and serum chemistry. All animals were then weighed, tranquilized, and euthanized according to AccelLAB standard operating procedures (*i.e.* a lethal injection of veterinary euthanasia solution Euthanyl 240 mg/mL, rapid IV bolus was utilized). All defect sites were dissected, scanned using microCT, and then processed and sectioned for histological evaluation and stained with Goldner's Trichrome (GT) as per O'Connell et al. [20]. The histology sections were evaluated by the Study Pathologist, and graded according to cell type and responses. Areas of inflammation, encapsulation, osteoconduction, and osseointegration (bone–biomaterial contact) were also identified, with a focus on the identification of an adverse host–material response (when present).

3. Results and discussion

All animals maintained general good health throughout the