**Introduction**

Ideal bone cements should be non-toxic, osteoconductive, osteointegrative, have suitable mechanical properties and porosity, and should be easy to apply in an operating room setting. Recently low temperature setting calcium phosphate cements have received a great deal of attention as bone substitutes due to their structural and compositional similarity to the mineral component of bone. Our laboratories have developed novel physiological temperature setting composite bone cements that have superior mechanical and osteogenic characteristics. The aim of the present study was to evaluate the in vivo response of two novel polyphosphazene-calcium deficient hydroxyapatite composites after implantation in rabbit tibial defects.

**Materials & Methods**

**Synthesis of Polyphosphazene-Calcium Deficient Hydroxyapatite Precursors:** The polyphosphazene used in this study was poly[bis(ethyl alamine)]phosphazene (PNEA). The hydroxyapatite precursors were prepared by an emulsion technique. Two calcium deficient hydroxyapatite-polyphosphazene composites with different Ca/P ratios (PNEA-CDHA – Ca/P: 1.5; PNEA-CDSHA – Ca/P: 1.6) were synthesized from the precursors.

**Tibial Defect Model and Cement Injection:** All procedures were approved by the Institutional, Animal Care and Use Committee. Thirty-four healthy New Zealand white rabbits were randomly divided into three different groups with 6 rabbits per group per time point that received the composite bone cements and 5 rabbits per group per time point for the control. An incision of 10 mm was made to expose the proximal and medial tibia. A 5 mm unicortical defect was made by drilling burr (Synthes, USA) just medial to the tibia tuberosity. The muscle and skin were closed and the same procedure was repeated on the contralateral limb. At predetermined time points (4 and 8 weeks) all animals were sacrificed and the limbs were excised for further analysis.

**Histology:** The tibia from the rabbits were excised immediately after sacrifice and placed in methanol (Fisher Scientific, USA) for 24 hours at 4°C and afterwards stored at room temperature for 1 week. The limbs were embedded in glycol methyl acrylate (PolySciences, USA) for 3 weeks (n=3). The blocks were polymerized and sectioned 100µm inside the implant to obtain 5µm thick sections using a microtome and mounted on glass slides. The samples were stained with silver nitrate to demonstrate calcified bone (von Kossa stain) followed by a hematxoylin and eosin counterstain.

**Micro CT:** Tibial segments were scanned at 25 µm voxel resolution using a cone beam µCT system (GE Healthcare BioSciences, London, Ontario) to assess micro-densitometry. To analyze differences in local mineralization, a color map was applied to a representative slice for each specimen (Alpha-Blend Plug-in, GEMS Microwin View Version 1.1.8). The lower and upper limits for this color map were standardized across all samples to encompass the entire range of gray scale values.

**Pull Out Test:** The soft tissue, bone marrow, and the periosteum were removed before the mechanical testing. The diaphysis region was sectioned longitudinally along the long axis of the tibia. The samples were mounted onto a fixture and the implants were pushed out of the bone using a Uniaxial mechanical testing machine (Instron, MA).

**Results & Discussion**

Fig. 1A, and B show the calcified bone formed on the surface of PNEA-CDHA and PNEA-CDSHA respectively after 4 weeks of implantation. Osteoblast cells were found along the periphery of the new bone that was formed. Figs 2A, and B shows the deposition of osteoids by the osteoblast cells on the two matrices at the end of 4 weeks. At 8 weeks, the contact between the bone and the implant was difficult to identify since new bone had started to form from the sides of the defect into the implant. The PNEA-CDHA group after 8 weeks demonstrated the formation of bone and, cellular infiltration predominantly of osteoblast and few osteoclasts were observed.

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