ABSTRACT INTRODUCTION: Calcium phosphate (CaP) based bone cements are currently used clinically due to problems associated with autologous bone grafting, such as donor site morbidity and limited supply, and allogeneic bone grafting, such as potential for disease transmission. There are two main types of CaP cements – brushite cements and apatite cement. Each have benefits and limitations -brushite cements are resorbable in vivo but have poor strength, while apatite cements are stronger but are poorly resorbed. Brushite cements also have the disadvantage of undergoing transformation into hydroxypatite and becoming poorly resorbable in the body.

In order to overcome the limitations, and with the aim of developing a cement with the advantages but none of the disadvantages of current CaP cements, a new type of brushite-based cement has been developed. These novel cements have previously been reported with studies on their formulation, in vitro resorption and physical characterisation examined.

Recently, we have examined biologically mediated resorption of these cements in vitro and have demonstrated the cell mediated resorption of the cements in vitro using a macrophage cell-line differentiated into an osteoclast-like cell. These cements have exhibited higher compressive strength (25 MPa) than those reported for existing brushite cements. In vitro degradation tests have shown that the novel cements do not transform into hydroxypatite (HA), unlike existing brushite cements. In degradation tests in bovine serum, the novel cement lost considerably more mass than comparable samples aged in phosphate buffered saline indicating that a component in serum played a role in cement degradation. Proteins such as phosphatase enzymes were postulated as most likely to be involved in the process.

To date all work on these new cements have been performed in vitro. The work described here is the first in vivo study carried out on these new CaP bone cements. Two formulations of novel cement were tested in a sheep model for 3, 6 and 12 months. One cement was developed for optimal strength (termed hi-str CaPP cement) and contains 5-10% wt pyrophosphate, whilst the other was formulated to contain a high level of pyrophosphate (hi-conc CaPP). These were compared to a standard brushite cement control for resorption, tissue reaction and new bone formation.

METHODS: Cement formulations – The cements were produced by slowly mixing the powder component into the liquid in the proportions described in Table 1.

<table>
<thead>
<tr>
<th>Cement Type</th>
<th>Liquid component</th>
<th>Powder component</th>
<th>P/L ratio</th>
<th>% CaPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>High conc CaPP cement</td>
<td>100mL pyrophosphoric acid + 780mL water</td>
<td>Dicalcium pyrophosphate</td>
<td>2.25 g/ml</td>
<td>40%</td>
</tr>
<tr>
<td>High Strength Low</td>
<td>540 mg polyphosphoric acid - 720 mg water</td>
<td>E- TCP</td>
<td>2.25 g/ml</td>
<td>5-10%</td>
</tr>
<tr>
<td>Standard Brushite</td>
<td></td>
<td>E- TCP</td>
<td>1.75 g/ml</td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 1: Formulations for the 3 cement types investigated.

Fabrication into plugs – The cement pastes were quickly transferred to a PTFE cylindrical split mould, with dimensions of 6.4mm diameter and 12mm long. The mould was over-filled and the paste tapped down in order to remove any air bubbles from the cement and any excess removed with the flat edge of the spatula. The cements were left to set for at least 1 h at 37°C and then removed from the mould. Any samples with obvious surface defects were discarded at this stage. Prior to implantation samples were washed in distilled water and air dried, sealed into sterilisation bags and sterilised by gamma irradiation (26 kGy).

Implantation in vivo – Prior to any in vivo work commencing all work was authorised in accordance with the local ethical review process and satisfied local laws and regulations required. A total of 18 sheep were operated on. Before the procedures each sheep was put under general anaesthesia. Each sheep received 2 implants into defects drilled into the medial side of the proximal tibia in each hind leg. Defects were 6.4mm diameter and 12 mm deep. Implants were pushed fitted into the defects according to a randomisation schedule and the soft tissue closed. A total of 12 samples per treatment group were implanted, 4 samples per time-point (3, 6 and 12 months). Fluorescent bone markers were administered intravenously at various stages during the study.

Histological analysis of tissue: At the 3, 6 and 12 month time-points the 6 sheep were humanely euthanised and biopsy samples taken. Each sample was bisected transversely into two halves. One half was decalcified and processed for wax embedding and subsequently H&E staining. The other half processed for resin embedding and analysed for fluorescent bone marker staining and alizarin red staining for new bone growth. The cements harvested from the bone at each time-point were analysed by X-ray diffractions to determine composition.

RESULTS: Twelve months histology results for the 3 cement types are shown in fig 1 (representative images). The histology images show that the hi-strength CaPP cement (fig.1a,b) has undergone a large amount of degradation, with the degraded cement being replaced by bone. The high concentration CaPP cement (Fig.1c,d) had undergone little degradation in 12 months and the standard brushite (fig.1e,f) had degraded unevenly with new bone formed in discrete areas where the cement had degraded. The X-ray analysis showed that the control brushite samples at 3, 6 and 12 months contained some βTCP but no brushite. It was suspected to be mainly amorphous apatite in composition. However brushite and βTCP was detected in the hi-strength CaPP cement, indicating that the new cement had remained in the brushite phase.

DISCUSSION: This work is the first in vivo study reported on a new type of CaP cement containing calcium pyrophosphate. Histology data showed that the hi-strength CaPP cement degraded at a higher rate than the control (standard Brushite) cement and was replaced by bone. X-ray analysis showed that it did not undergo phase transformation, unlike the control. It is postulated that the pyrophosphate content in the cement acts as an inhibitor of the phase transformation of the brushite phase. Previous work has shown that these CaPP cements can exhibit higher compressive strengths than existing brushite cements, whilst this study shows that it possesses degradation properties superior to HA and existing brushite cements.

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