Moldable Composite Bone Grafts Consisting of Calcium Phosphate Cement and Biodegradable Polymer Microspheres: Mechanical Properties and Controlled Release of Bioactive Molecules

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Introduction: We have developed novel bone grafts based on calcium phosphate cement (CPC). The powder component of CPC is an equimolar mixture of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA). The cement powder is mixed with water to form a workable paste that can be shaped to fit the contours of a wound. The paste sets within 30 minutes to yield microcrystalline hydroxyapatite, which is the predominant mineral component of bones. CPC is biocompatible and is used clinically to treat dental and craniofacial defects. However the growth of new bone around a CPC bone graft is usually observed only in areas adjacent to host bone. To enhance the ingrowth of bone into the CPC bone graft and the rate of CPC resorption, we have developed composite bone grafts made of CPC and poly(d,l-lactide-co-glycolide) [PLGA] microspheres, or poly(d,l-lactide) [PLA] microspheres, or other porogens. The new bone grafts are moldable, resorbable, and capable of the controlled release of bioactive molecules.

Experimental: PLGA (co-monomer molar ratio of 50:50, average molecular mass of 91,200 g/mol) was from Birmingham Polymers. PLA (average molecular mass of 2,140 g/mol) was from Boehringer Inglheim Chemicals, Inc. Polyvinyl alcohol (mol fraction of 88 % hydrolyzed, molecular mass of 25,000 g/mol) was from Polysciences. Fluorophore-labeled Protein A was from Sigma Chemical. TTCP [$Ca_4(PO_4)_2O$] was prepared as described by Takagi *et al.*[1] CPC powder was prepared by mixing equimolar amounts of TTCP (mass fraction of 72.9 %) and DCPA (mass fraction of 27.1 %). Microspheres were prepared in the manner described by Laurencin *et al.*[2] A paste was made from 0.1 g of PLGA microspheres [(0.17 to 0.36) mm in diameter, 0.6 volume fraction], 0.15 g of CPC, and 0.062 g of water. Disks (6.4 mm in diameter, 4.4 mm in height) for diametral tensile strength (DTS) and mass loss measurements were made by applying a 2.2 kg load on the paste in a mold at 37 ^{o}C. Disks (4 mm in diameter, 4.5 mm in height) for the release of fluorescent Protein A (a model for growth factors) were similarly prepared, using PLA microspheres [(0.17 to 0.36) mm in diameter, 0.6 volume fraction] or mannitol crystals [(0.12 to 0.25) mm length; 0.0, 0.35 or 0.60 volume fraction] as porogens and a Protein A solution (1.3 mg Protein A per mL of 4 mol/L urea solution). Disks for DTS and mass loss were stored at 37^{o}C in a saliva-like solution. Disks for Protein A release were stored in a phosphate buffered saline solution. The release of Protein A was measured with a spectrofluorometer.

Results: Scanning electron micrographs of PLGA and PLA microspheres showed that they were not agglomerated and were (0.17 to 0.36) mm in diameter. The X-ray diffraction pattern for a specimen of the PLGA/CPC composite showed that DCPA completely reacted while a small amount of TTCP remained, indicating the formation of calcium-deficient hydroxyapatite in the composite specimen. Figure 1 gives the DTS and the mass loss of the CPC/PLGA composite as a function of time.

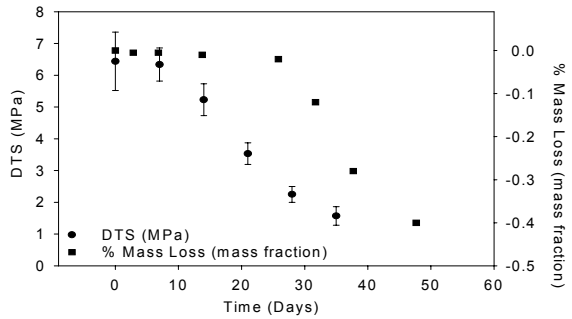

Figure 1. DTS and mass loss of CPC/PLGA composite in saliva-like solution at 37 ^{o}C.

Figure 1 shows very little decrease in the mass of the composite during the first four weeks, but shows a steady decrease in DTS during this period. There was little decrease in the mass probably because the degradation reaction was not sufficient to produce PLGA molecules that were low enough in molecular mass to diffuse out of the composite. However, the reduction in the average molecular mass of PLGA, together with the hydration-induced softening of PLGA microspheres, caused the decrease in DTS.

Although half the extractable Protein A was released from CPC/PLA discs in 140 h, the release of Protein A persisted even after 640 h. The release rate of Protein A from CPC/mannitol discs increased with the volume fraction of mannitol. Thus, the release of proteins from composite grafts can be modulated by the volume fraction and the dissolution rate of porogens.

We have described in this paper the degradation kinetics and mechanical properties of novel moldable, resorbable, composite bone grafts consisting of calcium phosphate cement (CPC) and poly(d,l-lactide-co-glycolide) [PLGA] microspheres, or poly(d,l-lactide) [PLA] microspheres, or other porogens. We have also reported that the release of proteins from composite grafts can be modulated by the volume fraction and the dissolution rate of porogens.

References:

1. Takagi S, Chow LC: *J Mater Sci: Mater Med* **11** pp 1-5, 2000.
2. Laurencin CT, Borden MD, Attawia MA, Ko F, Morril GM: *Polymer Preprints* **216 (2)** pp 73-74, 1998.