Proceedings of IMECE'03
2003 ASME International Mechanical Engineering Congress & Exposition
Washington, D.C., November 16-21, 2003
IMECE2003-43157

POLYMER-FILLED CALCIUM PHOSPHATE CEMENT: MECHANICAL PROPERTIES AND CONTROLLED RELEASE OF GROWTH FACTOR

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ABSTRACT

Novel calcium phosphate cement (CPC) was developed by incorporating pore-forming particulates (porogens) to induce macropores and proteins to stimulate bone growth. A paste was made from CPC powder (0.15 g, equimolar mixture of tetracalcium phosphate and dicalcium phosphate), biodegradable polymer microspheres [0.1 g, volume fraction of 0.6, (0.17 to 0.36) mm in diameter], and 0.062 g of water. Disks for determining diametral tensile strength (DTS) and mass loss were prepared from the paste in a mold at 37 °C. Disks for the release of a protein were similarly prepared using a solution of the protein, and biodegradable polymer microspheres or water-soluble crystals (mannitol or salicylic acid) as porogens. The disks were immersed at 37 °C in an aqueous solution in order to quantify the effects of the solution on DTS and mass loss, as well as the release of the protein into the solution. The initial DTS value for the disks was (6.4 ± 0.9) MPa. The release of the protein from the CPC/porogen disks persisted for at least 300 h. The release rate of Protein A from the CPC/mannitol disks increased with the volume fraction of mannitol crystals. At a fixed volume fraction of porogens, the release rate of TGF-β1 from the CPC/porogen disks increased with the dissolution rate of the porogens. Thus, the release of a protein from composite grafts consisting of CPC and porogens can be modulated by the volume fraction and the dissolution rate of the porogens.

INTRODUCTION

Novel bone grafts based on calcium phosphate cement (CPC) have been developed (Wang, 2003; Khatri et al., 2003). The powder component of CPC is an equimolar mixture of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA). The cement powder is mixed with an aqueous solution to form a paste that can be shaped to fit the contours of a wound. The paste sets within 30 min to yield microcrystalline hydroxyapatite, which is the predominant mineral component of bone. 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 new bone into the CPC bone graft and increase the rate of CPC resorption, improved composite bone grafts have been developed by incorporating bioactive molecules into CPC to stimulate bone growth and by incorporating porogens [e.g., poly(d,l-lactide-co-glycolide) microspheres, poly(d,l-lactide) microspheres, mannitol crystals, and salicylic-acid crystals] to induce macropores. The new bone grafts are moldable, resorbable, and capable of controlled release of bioactive molecules.

MATERIALS AND METHODS#

Poly(d,l-lactide-co-glycolide) (PLGA, co-monomer molar ratio of 50:50, average molecular mass of 91,200 g/mol) was obtained from Birmingham Polymers. Poly(d,l-lactide) (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 (Warrington, PA). Fluorophore-labeled Protein A was from Sigma Chemical. DCPA was from J. T. Baker Chemical Co. TTCP [$Ca_4(PO_4)_2O$] was prepared as described by Takagi *et al.* (2001). 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.* (1998). A paste consisting of 0.1 g of PLGA microspheres [(0.17 to 0.36) mm in diameter, volume fraction of 0.6], 0.15 g of CPC powder, and 0.062 g of water was made. For diametral tensile strength (DTS) and mass loss measurements, CPC/PLGA disks (6.4 mm in diameter, 4.4 mm in height) were made by applying a 20 N load on the paste in a mold while the mold was placed in a 37 °C humidity chamber for 24 h. For determining the release of fluorescent Protein A (a model for growth factors), CPC/PLA disks or CPC/mannitol disks (4 mm in diameter, 4.5 mm in height) were similarly prepared, using PLA microspheres [(0.17 to 0.36) mm in diameter, volume fraction of 0.6] or mannitol crystals [(0.12 to 0.25) mm in length, volume fraction of (0.0, 0.35 or 0.60)] as porogens and a solution of Protein-A (1.3 mg of Protein A per mL of 4 mol/L urea solution). The effects of a saliva-like solution on the DTS and mass loss were determined by measuring these properties after submerging the disks in the solution at 37 °C for various time intervals. The release of Protein A was determined by immersing the disks in a phosphate-buffered saline solution at 37 °C and measuring at various time intervals the amount of Protein A in the solution with a spectrofluorometer.

Three CPC/(salicylic acid) disks for the release of transforming growth factor-β1 (TGF-β1) were prepared in the manner similar to that described for the release of Protein A. In this case, the solid component consisted of 0.0577 g of salicylic-acid crystals [(0.12 to 0.25) mm in length, volume fraction of 0.4] and 0.1923 g of CPC (volume fraction of 0.6), while the liquid component consisted of 0.062 g of 4 mmol/L hydrochloric acid (HCl), bovine serum albumin (BSA, mass fraction of 1 %), and 200 ng of TGF-β1 reconstituted in 4 mmol/L HCl. Three CPC/mannitol disks for the release of TGF-β1 were prepared in the same manner except for 100 ng of TGF-β1 in the liquid component and the use of mannitol crystals [(0.12 to 0.25) mm in length, volume fraction of 0.4]. Each disk was then separately immersed in 1 mL of a phosphate-buffered saline (PBS) solution (0.2 mol/L) containing an antibiotic (mass fraction of 1%) and stored at 37 °C. At various time intervals, a 200 μL sample was collected from each PBS solution, with each disk then separately immersed in 1 mL of a fresh PBS solution. The collected samples were stored at

-40 °C. The amount of TGF-β1 in each of the samples was analyzed with an ELISA (enzyme-linked immunosorbent assay) kit (Quantikine Kit, R&D Systems, Minneapolis, MN).

RESULTS

Scanning electron micrographs of PLGA and PLA microspheres showed that they were not agglomerated and measured (0.17 to 0.36) mm in diameter. The X-ray diffraction pattern of a CPC/PLGA disk that was removed from the mold 24 h after filling the mold with the paste showed that DCPA completely reacted while a small amount of TTCP remained, indicating the formation of calcium-deficient hydroxyapatite in the disk. There was very little decrease in the mass of the disk during the first four weeks. The 24 h and three-week DTS values for the disks were 6.4 MPa and 3.5 MPa (with uncertainties, representing the estimated standard deviations, of 0.9 MPa and 0.3 MPa, respectively). There was little decrease in the mass because the degradation reaction was not sufficient to produce PLGA molecules that were low enough in molecular mass to diffuse out of the disk in four weeks. 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 of the extractable Protein A was released from CPC/PLA disks in 140 h, the release of Protein A persisted even after 640 h. The release rate of Protein A from CPC/mannitol disks increased with the volume fraction of mannitol crystals. Thus, the release of a protein from composite grafts can be modulated by the volume fraction of porogens. The ELISA analysis of the samples collected at various time intervals yielded the amount of TGF-β1 released as a function of time. Figure 1 shows the optical density at 450 nm, which is proportional to the amount of TGF-β1 released from the CPC/(salicylic acid) composite disks, as a function of time in hours. Each error bar, an estimate of the uncertainty, indicates the value of the standard deviation derived from three measurements. The initial rate of the release of TGF-β1 from the CPC/mannitol composite disks (not shown) was faster than that of the release from the CPC/(salicylic acid) composite disks because mannitol crystals dissolved faster to yield macropores than salicylic-acid crystals. Thus, the release of a protein from composite grafts can be modulated by the dissolution rate of porogens.

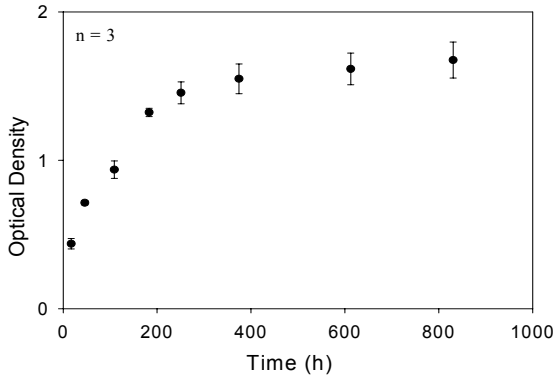

Figure 1. Optical density at 450 nm as a function of time for the release of TGF-β1 from CPC/(salicylic acid) composite disks.

Controlled release of a growth factor has often been accomplished by encapsulating the growth factor in biodegradable polymer microspheres, e.g., by double-emulsion techniques. In this simple and novel method based on the strong affinity of a growth factor with hydroxyapatite in the composite bone graft, the release of the growth factor was controlled by adding the growth factor into the liquid component of the CPC paste.

We have described in this paper the degradation kinetics and mechanical properties of novel, moldable, resorbable, composite bone grafts consisting of calcium phosphate cement and poly(d,l-lactide-co-glycolide) microspheres. In addition, we have described a simple method to control the release of a growth factor from the composite bone grafts and demonstrated that the release of the growth factor can be modulated by the volume fraction and the dissolution rate of porogens.

ACKNOWLEDGEMENTS

This work was supported by NIDCR through Interagency Agreement Y1-9006-03.

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