Controlled Release of Transforming Growth Factor from Composite Bone Grafts Consisting of Calcium Phosphate Cement and Biodegradable Particulates

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Introduction: We have recently described novel composite bone grafts based on calcium phosphate cement (CPC).[1] 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 medium to form a workable 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 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 the 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 biodegradable polymer microspheres or water-soluble crystals, which serve as macropore-forming particulates. The new composite bone grafts, which are moldable, resorbable and osteoconductive, can be made osteoinductive by adding to the grafts growth factors, such as transforming growth factor-β1 (TGF-β1) and bone morphogenetic proteins (BMP). The purposes of this research were to measure the controlled release of TGF-β1 from the composite bone grafts and to demonstrate that we can modulate the release rate of a growth factor by controlling the dissolution rate of the pore-forming particulates.

Experimental[#]: CPC powder was prepared by mixing equimolar amounts of TTCP (mass fraction of 72.9 %) and DCPA (mass fraction of 27.1 %).[1] Salicylic acid crystals and mannitol crystals were sieved to yield crystals of (0.12 to 0.25) mm length. A paste was made from 0.0577 g of salicylic acid crystals (0.4 volume fraction) and 0.1923 g of CPC (0.6 volume fraction), and 0.062 g of 4 mmol/L hydrochloric acid (HCl) containing bovine serum albumin (BSA, mass fraction of 1 %). The liquid component also contained 200 ng of TGF-β1 reconstituted in 4 mmol/L HCl. The paste consisting of CPC and mannitol was similarly prepared, using 0.1905 g of CPC powder (0.6 volume fraction), 0.0595 g of mannitol crystals (0.4 volume fraction), and 100 ng of TGF-β1 in the liquid component. Three disks (6.4 mm in diameter, 4.4 mm in height) were made by applying a 22 N load on the paste in a mold at 37 °C for 4 h. 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 in an incubator. At specific time intervals, a 200 μL aliquot was collected from each PBS solution, and then each disk was separately immersed in 1 mL of fresh PBS solution. The collected aliquots were stored at -40 °C. The amount of TGF-β1 in each of the aliquots was analyzed with an ELISA (enzyme-linked immunosorbent assay) kit (Quantikine Kit, R&D Systems, MN).

Results: The ELISA analysis of the aliquots collected at time intervals yielded the amount of TGF-β1 released as a function of time. Figure 1 shows the optical density, which is proportional to the amount of TGF-β1, as a function of time in hours, for the release of TGF-β1 from the composite made of calcium phosphate cement and salicylic acid crystals. Each error bar, an estimate of the standard uncertainty, is the standard deviation of the mean value derived from three measurements.

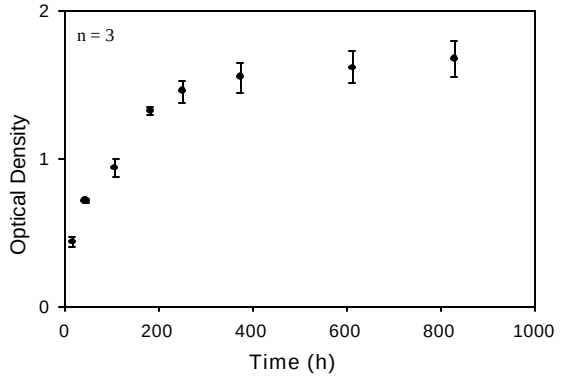

Figure 1. Optical density against time for release of TGF-β1 from CPC/(salicylic acid) composite.

There have been reports wherein a growth factor was introduced by encapsulating it into biodegradable polymers. In contrast, our method is simpler and provides control release of a growth factor.

The release of TGF-β1 from CPC/mannitol composite was also monitored. Mannitol dissolved in PBS faster than salicylic acid, and hence the initial rate of the release from the CPC/mannitol composite was faster.

Thus we have demonstrated that the release of a growth factor can be modulated by controlling the dissolution rate of the pore-forming particulates.

References:

1. Wang FW, Khatri CA, Hsii JF, Hirayama, S, Takagi S: Biomedical Engineering: Recent Developments. J Vossoughi (Editor), Medical and Engineering Publishers, Inc, pp 37-38, 2002.

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