Calcium phosphate coatings enhance stability of β-tricalcium phosphate biomaterials and serve as templates for binding and release of growth factors

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Statement of Purpose: Beta tricalcium phosphate (β-TCP) is an attractive ceramic for bone tissue repair because of its osteoconductivity and osteointegration. β-TCP has also a high affinity to growth factors and thus can also serve as a carrier material. However, compared to other ceramics, β-TCP has a fast rate of degradation. In the current study, β-TCP granules were mineral coated via heterogeneous nucleation in aqueous solution using simulated body fluids (SBF) with the aim of improving the stability of β-TCP. An additional goal was to use the coating as a carrier for controlled release of recombinant human vascular endothelial growth factor (rhVEGF), and a modular peptide version of bone morphogenetic protein 2 (mBMP2). The SBF formulations had varying concentrations of CO_3^{2-} as impurities such as CO_3^{2-} tend to increase the solubility of biological apatites. We hypothesized that the mineral coatings formed would have different dissolution dynamics, which would dictate biomaterial dissolution and growth factor release.

Methods: β-TCP granules were mineral coated by incubation in modified SBF with 4.2 mM, 25mM, or 100mM HCO3 at 37 ºC. Modified SBF is a solution similar in ionic composition and temperature to blood plasma. The mineral formed on the material was characterized using scanning electron microscopy (SEM), and Fourier transform infrared (FT-IR) spectroscopy. We assessed the stability of β-TCP with or without the mineral coating by measuring the amount of calcium released into PBS using a colorimetric assay. Binding and release studies were conducted using ^{125}I-labeled rhVEGF (Perkin Elmer; Boston, MA), and carboxyfluorescein labeled mBMP2. Mineral coated β-TCP granules were incubated at 37 ºC in solutions of either a rhVEGF or mBMP2. After binding, β-TCP granules were incubated in Dulbecco's modified Eagle's medium (DMEM) at 37 ^{o}C. At specific time points, the release media were collected and replaced with fresh medium. The amount of released growth factor was determined by measuring the radioactivity of the released medium for rhVEGF release; or by measuring fluorescence intensity for mBMP2.

Results: Our results demonstrate that we could form coatings on β-TCP biomaterials with different morphologies (Figure 1), and dissolution properties (Figure 2). The presence of a biomineral coating stabilizes the β-TCP, as evidence by the longer rate of dissolution for coated β-TCP (Figure 2). As the CO_3^{2-} content increased in the coating, the dissolution of the coating was faster than, but not as fast as, the rate of uncoated β-TCP. In addition, we showed release of VEGF over the short term (10 days) and mBMP2 over the long term (6 weeks) from the same biomaterial, which indicates that it is possible to temporally control release of multiple factors (Figure 3).

Conclusions: We present a novel approach to improve the stability of β-TCP granules using a biomineral coating. The coating also serves as a carrier for rhVEGF and mBMP2 and exhibits distinct release kinetics.

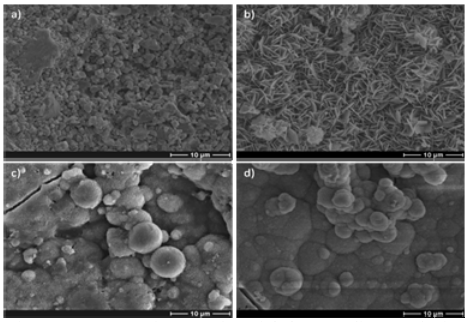

Figure 1. SEM micrographs of β-β-TCP discs a) before incubation in mSBF, b) After incubation in mSBF with a carbonate concentration of 4.2mM, c) 25mM HCO3, and d) 100mM CO3. Morphology of mineral is affected by the extent of carbonate substitution.

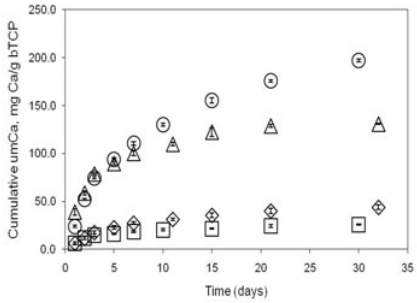

Figure 2: Release of calcium ions from uncoated (○) and coated βbeta-β-TCP after incubation in mSBF with 4.2mM (□), 25 (◊), and 100mM (△)HCO3. The presence of the mineral coating improves the stability of β-β-TCP. In the mineral coated groups, as we increased the HCO3 concentration in mSBF the rate of dissolution increased as well.

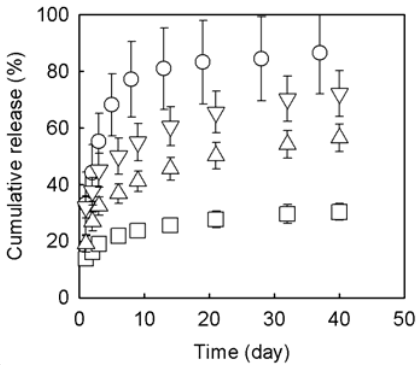

Figure 3: Cumulative release of VEGF (○) and mBMP2 (□, △, ▽) from mineral coating formed on β-β-TCP granules by incubating in mSBF with different carbonate concentration; 4.2 mM (○, □), 25 mM (△) and 100 mM (▽). rhVEGF was released during the first 10 days, whereas the mBMP2 was released over longer periods of time (greater than 40 days). The release of mBMP2 correlated with the rate of dissolution of the mineral coating.