TCP Particles as a Sustained Release Carrier of Osteogenic Proteins

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Statement of Purpose: Beta-tricalcium phosphate (β-TCP), a bioresorbable ceramic material, has been extensively used as a platform for osteogenic growth factor delivery and bone regeneration due to its attractive osteoconductive properties. However, previous study has shown that the TCP granules have burst release and low delivery efficiency of growth factor [1]. The objective of this study is to develop a sustained release formulation of TCP for effective delivery of osteoinductive factors with reduced initial burst by surface modification of the β–TCP particles with acid treatments or apatite coatings.

Methods: Beta-TCP granules were purchased from Synthes Spine (CO, USA). Acid treatments were achieved as following: the surfaces of the TCP particles were modified by surface etching in citric acid solution: 0.2 g of TCP particles were soaked in 30 ml of 10% citric acid aqueous solution (pH 1.56) for 120 s. Surface modification by creating apatite coatings with simulate body fluid immersion approach as described somewhere else [2-3]. The morphology of the TCP microparticles was observed using scanning electron microscopy (SEM). To examine the feasibility of TCP particles as protein carriers, recombinant human Nel-like molecul (Nell-1), osteogenic protein, was lyophilized onto the particles. The release kinetics of recombinant human Nell-1 protein from surface etched or apatite-coated and un-coated TCP particles was compared using the 3-(4-carboxybenzoyl) quinoline-2-carboxaldehyde (CBQCA) protein assay to determine if surface etching or apatite coating can reduce initial burst release. The bioactivity of the Nell-1 released from TCP microparticles was assessed by monitoring alkaline phosphatase (ALP) expression in the murine chondrogenic cell line ATDC-5 (Riken Cell Bank, Japan).

Results: The morphology of the surfaces of both non-treated TCP and citric acid or apatite coated TCP particles were shown in Fig.1.The citric acid etched or apatite coated TCP particles have more surface area compared with untreated TCP particles, which is favorable for protein adsorption and may be the reason of reducing initial burst.

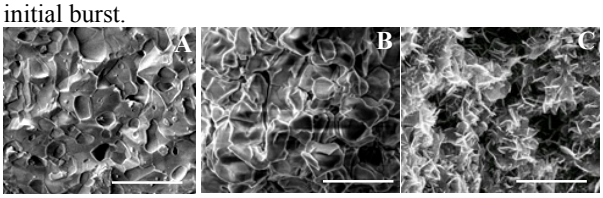
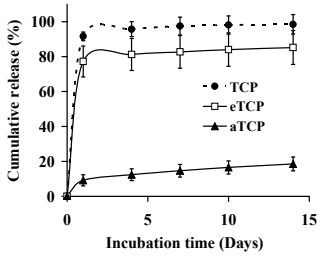
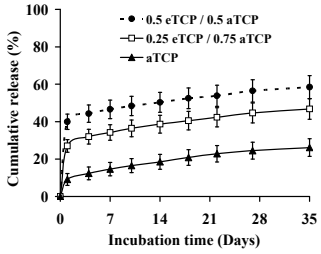


Fig.1. Scanning electron micrographs of the surfaces of (A) untreated TCP, (B) citric acid etched and (C) apatite coated TCP particles (scale bar = 10 um).

The release of Nell-1 (Fig. 2a) from untreated particles exhibited a rapid burst release profile (90%) for all experimental particles sizes. Approximately 80% of the initially loaded Nell-1 was release from acid-etched particles during the first day, followed by slow release during the subsequent incubation time. In contrast, the burst release of Nell-1 was significantly reduced by the apatite coating. The apatite coating increased the protein retention capacity of the particles. It is also possible that high surface due to the plate-like morphology of the particles presented more binding surfaces for non-specific protein adsorption. In addition, the bioactivity of Nell-1 was preserved during the loading procedure onto TCP microparticles. Due to the burst release of Nell-1 of apatite coated TCP particle, to modulate the releasing profile, we combined citric acid etched and apatite coated TCP particles at different ratios. As shown in Fig. 2b, by increasing the ratios of acid etched TCP to apatite coated TCP, the burst release rates were increased.



(a)



(b)

Fig.2. In vitro release of Nell-1 from (a) untreated, citric acid etched and apatite coated TCP particles in phosphate-buffered saline (PBS) and from (b) mixture of acid etched TCP and apatite-coated TCP particles in PBS.

Conclusions: TCP microparticles modified with biomimetic apatite coatings provided sustained delivery of osteogenic proteins. This apatite-coated TCP formulation might offer the potential on osteoinductive therapeutics.

References:

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