Strontium (Sr) Doped Calcium Phosphate Coatings on Biodegradable Magnesium Alloys

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Statement of Purpose: Calcium phosphate (CaP) coatings have been studied to tailor the uncontrolled corrosion of Mg alloys while also enhancing their bioactivity. The use of aqueous techniques to deposit CaP coatings is attractive due to the simplicity and capability to coat complex structures. In the current study, AZ31 substrates were subjected to various pretreatment conditions prior to depositing Sr doped and undoped CaP coatings. It was hypothesized that the coating bioactivity and stability could be improved by incorporating Sr into the CaP coating structure. Increased Sr concentrations have been shown to promote osteogenic differentiation *in vitro* [1]. Strontium was selected since Sr supplements are known to increase bone density and are currently used to treat osteoporotic patients [2]. Various pretreatment conditions were also explored to determine the effect of pretreatment on coating structure and cytocompatibility.

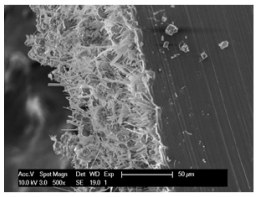

Figure 1. Cross section of Sr0-400. Scale bar is 50 µm.

Methods: A magnesium alloy containing 3% Al and 1% Zn (AZ31) was used as a substrate. Cleaned AZ31 was immersed in Na_2HPO_4 prior to heat treating in argon to either 350 or 400˚C for 10 h. CaP coatings were deposited by immersing pretreated substrates in solutions prepared with 0, 5, and 10% Sr/(Ca+Sr) mole ratios and a fixed (Ca+Sr)/P molar ratio at 70˚C for 48 h.

XRD, ATR FT-IR, SEM and EDX were used to characterize coating structure and composition. Corrosion protection was assessed using immersion tests, H_2 evolution, and potentiodynamic polarization (PDP) tests. *In vitro* cytocompatibility was evaluated with a mouse preosteoblast cell line (MC3T3-E1) and human mesenchymal stem cells (hMSCs). Cell viability was determined at 3 and 7 days prior to inducing osteogenic

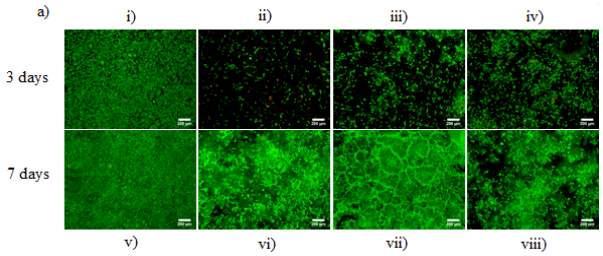

Figure 2. Live/dead staining on culture plastic, Sr0-350, Sr5-350, and Sr10-350 after 3 (i-iv) and 7 (v-viii) days of culture with MC3T3-E1 cells.

differentiation. The capability of coatings to promote differentiation was evaluated using qRT-PCR, the ALP assay, and a magnetic bead assay for quantitative analysis of osteocalcin (OCN) concentration.

Results: XRD patterns of CaP coatings indicated that pretreatment to 350˚C resulted in a lower vol. % of crystalline content. Elemental analysis using EDX confirmed the doping of Sr into CaP coatings. Coating thickness was approximately 50µm (fig. 1) despite varying pretreatment conditions and Sr concentrations. H_2 evolution and immersion tests both indicated improved corrosion protection for coated substrates in comparison to uncoated AZ31. However, little difference could be observed among the coated samples. PDP tests were used to further study coated samples. Corrosion current density for coatings prepared on substrates pretreated to 350˚C decreased upon increasing Sr content. However, the opposite trend was observed for coatings on substrates pretreated to 400˚C.

Cell attachment was initially low on coated samples in comparison to tissue culture plastic. However, after 7 days cell number greatly increased (fig. 2). The osteogenic differentiation of hMSCs cultured on coated samples was also studied. Significantly greater levels of ALP were expressed on coatings prepared on substrates pretreated to 350˚C (fig. 3a). However, OCN

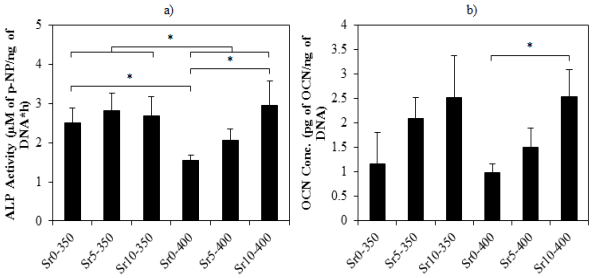

Figure 3. a) ALP activity and b) OCN concentration measured from hMSCs after 18 days of culture in osteogenic media normalized with respect to DNA concentration.

concentration was similar for both pretreatment conditions (fig. 3b) although in general increased OCN expression was observed upon increasing Sr content.

Conclusions: Pretreatment conditions and Sr concentration were found to greatly influence the vol. % of crystalline content in CaP coatings and their capability to provide corrosion control. Greater levels of osteogenic differentiation were observed on coatings prepared with increased amounts of Sr regardless of pretreatment conditions. Future studies will aim to incorporate Sr doped CaP nanoparticles into polymeric coatings to control the release of Sr and bioactive molecules to further improve the bioactivity of metallic implants.

References: [1] F. Yang, D. Yang, J. Tu, Q. Zheng, L. Cai, L. Wang, Stem Cells, 29 (2011) 981-991.

[2] P. Habibovic, J.E. Barralet, Acta Biomaterialia, 7 (2011) 3013-3026.