Water-based fabrication and biological evaluation of calcite coatings on stainless steel

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Statement of Purpose: Calcium carbonate ($CaCO_3$) is one of the many resorbable biomaterials that has been clinically utilized for decades. Recent studies revealed that $CaCO_3$ plays a role as an intermediate precursor during the biomineralization process [1], suggesting the possibility that $CaCO_3$ can be used for the promotion of osseointegration. In order to investigate cell responses to calcite coatings in this study, these coatings were produced on stainless steel substrates, and the attachment of osteoblast cells to these coatings were evaluated.

Methods: Reagent grade $Ca(NO_3)_2$•$4H_2O$ and urea were mixed in ratios of 1:1, 1:2, and 1:3 and dissolved in 5 ml distilled water. 10wt% polyethylene glycol (PEG) or 0.1 N HCl was added to improve the phase purity of the coating. After aging in 80ºC oven for 1 hour, the calcium and urea containing solutions were spin coated on stainless steel 316 disks (15 mm diameter x 1 mm thick) followed by heating at 500 ºC for 1 hour. Coated samples were characterized using scanning electron microscope (SEM) and x-ray diffraction (XRD). ATCC CRL 1486 human embryonic palatal mesenchymal cells, an osteoblast precursor cell line, were used to evaluate cell attachment. The cells in Dulbecco's modified Eagle's medium were seeded on the disk at a density of 40,000 cells on each sample and incubated in a 5% CO_2 humidified incubator at 37˚C for 1, 2, 3 and 24 hours.

Results / Discussion: It was observed from this study that the formation of calcite crystals was dependent on the relative concentration of urea. The as-obtained coating was observed to consist of both calcite and un-reacted $Ca(NO_3)_2$ when $Ca(NO_3)_2$•$4H_2O$ /urea ratio was at 1:1 (Fig. 1). A pure calcite layer was obtained as the ratio of $Ca(NO_3)_2$•$4H_2O$ /urea was changed to 1:2.

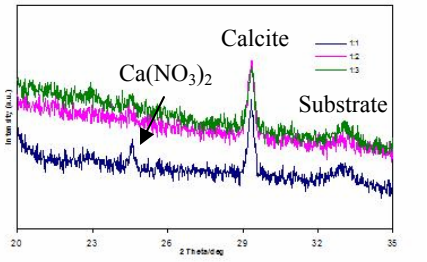

Fig. 1 XRD analysis of calcite coatings derived from different ratios of $Ca(NO_3)_2$ •$4H_2O$ /urea.

Prior to the addition of PEG or HCl to the solution, the un-reacted calcium nitrate was observed on as-obtained coatings. However, as shown in Fig 2, the addition of 10 wt% PEG and 0.1 N HCl improved the phase purity of calcite. The addition of PEG and HCl was suggested to induce a faster reaction rate between calcium nitrate and urea during the production of a calcium-urea complex, which subsequently resulted in the promotion in phase purity.

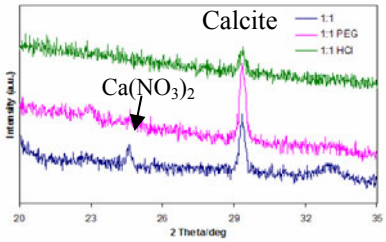

Fig 2. XRD analysis of calcite coatings derived from solutions with different additives.

SEM micrographs in Fig 3 show good dense coating coverage on the substrates.

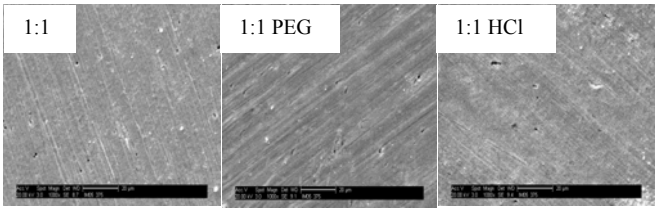

Fig. 3 SEM micrographs showing the morphology of calcite coatings derived from different solutions.

In vitro cell culture study indicates cell attachment on the coated surfaces within 2 hours of cell seeding, and cell spreading after 24 hours incubation.

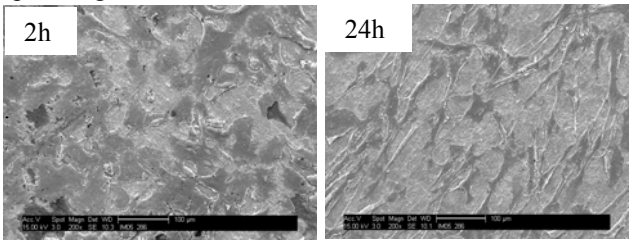

Fig. 4 SEM images for cell attached calcite coatings derived from 0.1 N HCl added solution.

Conclusions: Dense calcite coatings were fabricated on stainless steel 316 substrates using water based solutions. It was concluded that the ability to produce a pure phase calcite depended on the ratio of $Ca(NO_3)_2$•$4H_2O$ /urea and the presence of PEG and HCl in the solution. In addition, these calcite coatings exhibited excellent cell biocompatibility which promises a potential use as a coating on implant surfaces to enhance osseointegration.

Acknowledgement: This study is supported in part by NIH (1RO1AR46581).

References:

[1] S. Raz, et al, *Adv. Funct. Mater.*, 1997, 34, 430.