Evaluation of Electrosprayed Chitosan Coatings with Incorporated Calcium Phosphate Nanoshells

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Statement of Purpose: Electrospraying has been utilized to coat 3D titanium (Ti) surfaces with chitosan (CH). [1] These coatings have shown potential to enhance implant integration into bone and used to locally deliver therapeutic such as antibiotics to prevent infection. Calcium Phosphate (CaP) is a major component of bone and shown to improve osteointegration of implants. This work aims to evaluate the method to incorporate CaP nanoshells into electrosprayed (ES) CH coatings on Ti implants to improve implant integration and deliver therapeutic and orthopedic drugs. [2, 3]

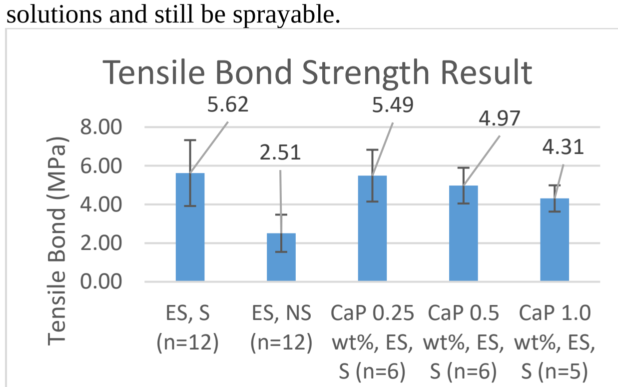
Methods: *Ti Sample Preparation and Ethanol Silanation:* Ti samples (12.7 x 63.5 x 2 mm) were wet polished then cleaned to insure a clean and smooth sample surface. After cleaning, samples received a NaOH treatment to enhance surface hydroxide formation. Hydroxylated Ti samples underwent a triethoxsilylbutyraldehyde (TESBA) surface treatment to enhance chitosan surface attachment.

Synthesis of CaP Nanoshells: CaP nanoshell were made via calcium phosphate condensation reaction around carbon nanosphere precursors. Precipitant was washed until material turned light grey. Material dried and ground with the resulting powder then baked to remove carbon nanosphere precursors.

Electrospraying: A 1 wt% CH (95% DDA, 300-700kDa, HMC, Germany) solution was made by dissolving CH powder in 0.5% acetic acid. A 95% ethanol-5% 2-propanol solution was mixed with the CH solution in a 1:3 ratio to reduce the viscosity and surface tension of the CH solution to make it sprayable [1]. CaP powder was incorporated into the electrospraying solution at 0.25, 0.5, 1.0 wt% CaP to determine optimal combinations. Solutions were ultrasonicated to break apart any CaP aggregates. Solution was sprayed using a previously developed single capillary electrospray setup with specific spray parameters optimized for each solution. After being sprayed, coatings are neutralized in 0.25 M phosphate buffer solution then rinsed and dried.

Evaluation and Testing: Fourier-transform infrared (FTIR) spectroscopy was used to evaluate samples after polishing and cleaning, after NaOH treatment, and after silanation. Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS) were used to examine surface morphology, and amount and dispersal of CaP Nanoshells in chitosan coatings. Tensile testing of the Ti coatings, a roughened aluminum stud was attached to the coatings using GorillaWeld epoxy. Specimen were loaded into an Instron 3300R test frame using custom fixtures and tested in tension using 5kN load cell at 0.5mm/min. Test specimen were examined visually to determine failure mode. Test groups were; A, B] control groups of electrosprayed coatings on silanated and non-silanated Ti, and C-E] electrosprayed coatings with 0.25, 0.5, 1.0 wt% CaP on silanated Ti. *In Vitro* testing was performed on the chitosan coatings using W2017, preosteoblast mouse bone marrow stromal cells. Cells were seeded on the Ti control and test chitosan-CaP coated specimen (9.5 mm diameter disks) in 48 well plates at $3x10^4$ cells/well in 0.5 ml of medium and allowed to attach overnight. Viability was measured after 1, 3, and 5 days after seeding. Viability of the cells was determined using the Cell Titer glo assay. There were three replicates per group for each time point. Data was then analyzed statistically using two-factor ANOVA with replication.

Results: Results of FTIR showed an increase in hydroxides on the Ti surface after NaOH treatment. Silane deposition was confirmed by -Si-O and –C=O peaks from the TESBA molecule. All prepared coatings appeared as slightly transparent and smooth with CaP incorporated coatings having a slight grey tint. Initial results have shown that all wt% of CaP can be incorporated into solutions and electrosprayed. Using SEM/EDS to evaluate the interface between the chitosan coating and the titanium substrate show the CaP particles to be incorporated throughout the full depth of the chitosan coating. ANOVA and post-hoc testing showed silanated (S) coatings had significantly higher adhesion properties than the non-silanated (NS) but there was no statistically significant effect of the amount of CaP nanoshells on coating adhesion strengths. For *In Vitro* testing, viability and growth of W2017 cells were measured. All coatings had positive cell growth and showed statistical difference from each other, but no group could be determined as superior. Results indicate that our currently utilized electrospraying technique can be utilized to incorporate up to 1.0 wt% CaP into chitosan solutions and still be sprayable.



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