Comparison of Chitosan-Coated Titanium via Two Coating Methods: Evaluation of Protein Release Profiles

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Statement of Purpose: Osseointegration is the ability of an implant to form a direct interface with bone without intervening fibrous tissue. It is a prerequisite for functional implant loading and for the long term success of the implant. Bioactive coatings and their controlled release of proteins have been investigated as methods for enhancing the integration of implants.

The biopolymer chitosan, a de-acetylated derivative of chitin, has been investigated as a bioactive coating to enhance osseointegration due to its demonstrated biocompatibility, controllable degradation, and osteogenic properties[1]. Previous research has demonstrated that chitosan can be bonded to titanium through silane-glutaraldehyde reactions using different solvents for depositing the silane molecules[2]. The solvent has been shown to influence the attachment of the chitosan to the metal surface which could play a role in osseointegration[2]. However, the effect the solvent may have on the coating's protein release profile has not been investigated.

The method used to coat the titanium with chitosan could influence the protein release profile of the coating as well as the mechanical bond strength. Therefore, the objective of this study was to compare the protein release profiles of chitosan-coated titanium in which two different solvents (toluene and an acidified ethanol-water solution) were used for depositing the silane molecules.

Methods:

Titanium preparation: A 1200 grit finish on the titanium (grade 4) coupons was achieved by wet sanding with a series of 180, 400, 600, 800, and 1200 grit SiC papers. The coupons were sonicated for 10 minutes each in ethanol (70% by volume), acetone, ethanol (70%), and de-ionized water in succession. Following sonication, the coupons were placed in a 30% nitric acid for 30 minutes at room temperature for passivation as previously described[1].

Coating procedure: The titanium coupons were divided into two groups (n=5) in which Group 1 was coated as previously described by Bumgardner et al.[1] in which the solvent used to dissolve the 3-Aminopropyltriethoxy-silane (APTES) (United Chemical Technologies, Bristol, PA, USA) was an acidic ethanol-water solution[1] and a 1wt% chitosan solution (Vanson, WA, 87.4% DDA) was solution cast. Group 2 was coated as previously described by Martin et al[2] in which toluene was used to dissolve the APTES and the same chitosan solution as group 1 was solution cast.

Elution study: Protein A labeled with fluorescein isothiocyanate (FITC) (molecular weight 42 kDa; Sigma Chemical Company, USA) was used as a model protein[3] to obtain a protein release profile of the chitosan coatings. A 10µg/mL protein A solution in 1X sterile PBS was prepared and 100µL was allowed to absorb overnight into the chitosan coated titanium coupons. The coupons were rinsed in 1X sterile PBS and then the release rate in vitro was estimated by suspending the coupons in 2 ml of 1X sterile PBS at 37°C. At 12 hrs, 1, 2, and 5 days, the release of Protein A-FITC was estimated by measuring the fluorescence intensity of PBS solution with a fluorescence microplate reader (BioTek FLx800™, Vermont, USA). Fluorescence values were compared to standards which were incubated and ran at each time point with samples.

Results: The measurements of the release of protein A-FITC showed that there was a burst release from both groups of coatings in the first 12 hours. In group 1, the protein recovery appears to be higher than protein added which could be due to an artifact of the measurement or an effect of time on the fluorescence of the protein A-FITC in solution. In group 1, it appears as if there is a gradual release of protein A-FITC after the initial burst but in group 2, it appears as if protein A-FITC is not released after the first 12 hrs. After 5 days, group 2 had not released 100% of absorbed protein which could indicate that more protein remained in coating at Day 5 or that not all the protein was initially absorbed.

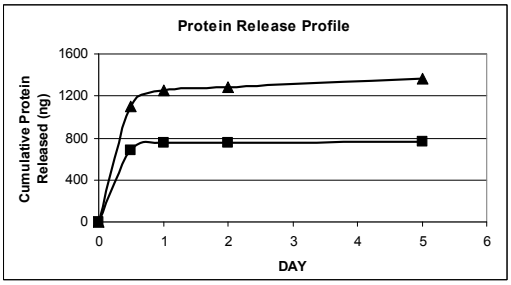

Figure 1. Release profile of Protein A-FITC from the two groups of chitosan coated titanium (n=5). Group 1 (▲). Group 2 (■).

Conclusions: It is appears that the solvent used during the coating procedures to deposit the silane molecules does influence the absorption and release of protein A. However, since the results indicate a recovery higher than loading, the results may not be reliable to make any conclusions. Additional experiments should and will be completed to verify these results. Also different methods to absorb the protein will be investigated. Effects of the coating method on cell attachment and growth will also be investigated

References:

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