Evaluation of Chitosan Coated on Titanium to Deliver VEGF-121 and Enhance Saos-2 Cell Mineralization In Vitro

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Statement of Purpose: Clinical success of dental implants is believed to be dependent on the early osseointegration of the device.[1] Vascularization of peri-implant tissue is a very important parameter both for the remodeling of the bone and for the preservation of a dental implant after its insertion.[2] Therefore, improving angiogenesis in the tissues surrounding a dental implant may provide an effective method to enhance early osseointegration and improve implant success rates.

Vascular endothelial growth factor (VEGF) is a positive regulator of angiogenesis and has been reported to not only affect endothelial cells, but also indirectly induce proliferation and differentiation of osteoblasts. The method for the local delivery of VEGF can be addressed with an arising biopolymer known as chitosan. 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].

The objective of this study was to evaluate the use of 87.4% deacetylated chitosan films, bonded to titanium via a silane-glutaraldehyde process, to deliver VEGF-121 locally and to the evaluate osteoblastic cells response to the coatings *in vitro*.

Methods:

Titanium preparation: A 1200 grit finish on the titanium coupons (grade 2, 1.3cm x 1.3cm x 0.1cm) was achieved by wet sanding with a series of SiC papers. The coupons were ultrasonically cleaned and then passivated in a 30% nitric acid for 30 minutes as previously described[3].

Coating procedure: The titanium coupons were chemically modified through a silane-glutaraldehyde method and a 1wt% chitosan (87.4% DDA, Vanson) solution was solution cast as previously described.[3,4] After drying for 7 days, coupons were gas sterilized.

Protein loading: Human vascular endothelial growth factor-121 (VEGF-121; PeproTech; Rocky Hill, NJ) was reconstituted in sterile PBS at 0 ng/mL, 200 ng/mL, 500 ng/mL, and 1µg/mL concentrations. Coated titanium coupons were hydrated with 100µL of one of the four solutions, loading each coating with either 0ng (C0), 20ng (C20), 50ng (C50), or 100ng (C100) of VEGF-121. The samples were incubated for 24 hours at room temperature and rinsed with sterile PBS to remove unabsorbed protein.

Elution study: Samples (n=4) were covered with 2 mL of PBS and incubated at 37°C. The solution was sampled and replaced over 8 day period with collected eluates frozen at -20°C. After 8 days, the concentration of VEGF-121 was quantified with VEGF ELISA kit (PeproTech; Rocky Hill, NJ) according to manufacturer's directions.

Mineralization study: Saos-2 osteosarcoma cells (ATCC, Manassas, VA) were grown in complete medium of HyQ McCoy's 5a supplemented with 10% Fetal Bovine Serum (Hyclone, Logan, UT), and 1% antibiotic. Chitosan coated titanium samples loaded VEGF as well as passivated, uncoated titanium controls (n=4) were seeded with 10^4 cells/cm^2 (passage five) in 2 mL of mineralizing medium (complete medium additionally supplemented with 5µM ascorbic acid and 10mM β-glycerophosphate). Over 28 days, cultures were evaluated for cell growth via quantification of DNA, expression of ALP for bone phenotype expression, and secretion of osteocalcin and deposition of Ca as indicators of bone matrix production.

Results: Elution study showed VEGF to be released over 3 day period with a large burst release of ~75% of protein being released during first 12 hours (Figure 1). Released VEGF did not show influence on ALP or osteocalcin (results not shown), but did influence cell growth (Figure 2) with continuous proliferation throughout 28 days. Calcium deposition significantly increased when titanium was coated with chitosan, but it was not further enhanced by the addition of VEGF into the coatings (Figure 3).

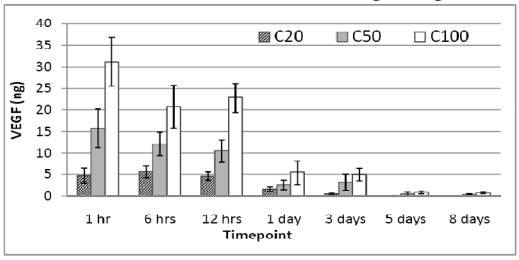

Figure 1. VEGF-121 released over 8 day period.

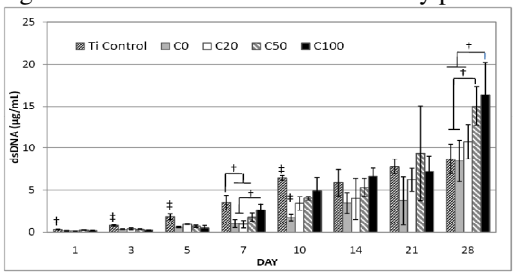

Figure 2. Saos-2 cell growth via DNA quantification.

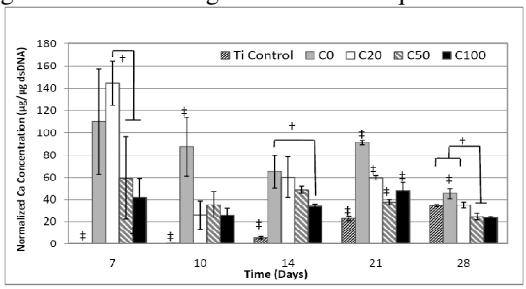

Figure 3. Calcium deposition on chitosan coatings.

Conclusions: Chitosan coatings loaded with VEGF show potential to influence proliferation of Saos-2 cells, but more research needs to be done with other VEGF concentrations as well as modifying the release of protein from coatings to determine potential of coatings.

References:

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