Calcium Phosphate Coating of EBM Manufactured Three-Dimensional Titanium Scaffolds via Alkali Heat Treatment

Nathan J. Castro, Malcolm N. Cooke, Ph.D.
College of Engineering, University of Texas at El Paso, El Paso, Texas 79902.

Statement of Purpose: The focus of this study was to investigate the surface treatment of Ti-6Al-4V (Ti64) titanium porous scaffolds, manufactured by electron beam melting (EBM), using a method of rapid calcium phosphate deposition to produce an osteoconductive substrate. Recent studies have shown enhanced cell adhesion on surface-treated titanium implants by a series of surface modification techniques. The reported method used a commercial β-tri-Calcium phosphate (β-TCP) as the calcium phosphate source for the scaffold surface modification. Ti64 scaffolds were treated in 1M and 5M Potassium hydroxide (KOH) followed by heating to 600ºC to reduce surface morphology alteration while producing an oxide layer for calcium phosphate deposition. Treated scaffolds were post-treated, by soaking in 25mM [Ca^{2+}] β-TCP solution at 37°C for 4 hours. Scaffolds were seeded with rat bone marrow stromal cells (MSCs), cultured in supplemented media for 24 hours and examined by scanning electron microscopy. Preliminary results show excellent cell adhesion, bridging and spreading of the MSCs suggesting the surface treatment process produced a calcium-rich, osteoconductive substrate on the Ti64 scaffolds.

Methods: *Scaffolds:* Ti64 scaffolds, (16.4mm dia. X 4mm thick) were manufactured using an ARCAM EBM machine (Arcam, Mölndal, Sweden) courtesy of the W.M. Keck Center (University of Texas at El Paso, El Paso, Texas) using a particle size of 30 - 50μm. Scaffolds were sterilized by autoclaving.

Scaffold Preparation: The porous Ti64 scaffolds were ultrasonically cleaned (VWR 150D, West Chester, PA 19380) in acetone, 97% ethanol, and distilled water respectively for 10 minutes each.

Alkali and Heat Treatment: 500 mL alkali solutions of 1M and 5M KOH were prepared with distilled water. Ti64 scaffolds were alkali treated by submersion in KOH at 60ºC for 24 hours, Followed by washing in distilled water and then oven dried overnight at 40ºC. Scanning electron micrographs were taken prior to and after treatment to monitor changes in surface morphology. After alkali treatment, the scaffolds were placed in an oven and heated to 600ºC and left at temperature for 1 hour and allowed to furnace cool.

Calcium phosphate coating: Based on an ion concentration of 2.5mM Ca^{2+} found in blood plasma [1], a high ionic concentration of Ca^{2+} (25mM) was used. β-TCP ($Ca_3O_8P_2$) (Sigma-Aldrich, St. Louis, MO 63178) was employed as the calcium phosphate source. The scaffolds were post-treated at 37°C for 4 hours.

Cells: Rat bone marrow stromal cells were extracted from the femora of male Wistar rats (approx. 350g), cultured in standard media (α-MEM with 15% fetal bovine serum) prior to being used for scaffold seeding. The cells used in this study were of passage 4.

Cell seeding: Prior to cell seeding, the scaffolds were pre-wet in standard media and incubated overnight. Scaffolds were seeded with rat MSCs at a concentration of 5 x 10^5 cells/scaffold in supplemented media (standard media, 40μg/mL penicillin, 20μg/mL gentamycin, 40nM dexamethasone, 110μM ascorbic acid, 4mM sodium-β-glycerophosphate) and incubated for 24 hours at 37°C, 5% CO_2 and 95% humidity.

SEM preparation: Scaffolds were fixed in gluteraldehyde and gold sputter-coated under argon for 60 seconds prior to viewing using scanning electron beam microscopy (S-4800 UHR FE-SEM, Hitachi, Pleasanton, CA).

Results: The formation of an amorphous titanate layer was formed after the alkali treatment, and crystallization of the oxidative layer occurred after heat treatment. Uniform calcium deposition was observed on the scaffold surface after treatment with β-TCP. The crystalline titanate layer provided the mechanism for calcium adsorption. After a 24 hour incubation period in supplemented media, osteogenic-like cells were observed on the scaffold surfaces and within the pores. Cells were well attached, polygonal with well defined and extended cell processes. Cells were clearly observed on the surface and within the internal spaces of the scaffold (Figure 1). Cell attachment processes appeared to be directed towards areas of higher concentrations of calcium deposits, although this was not quantified.

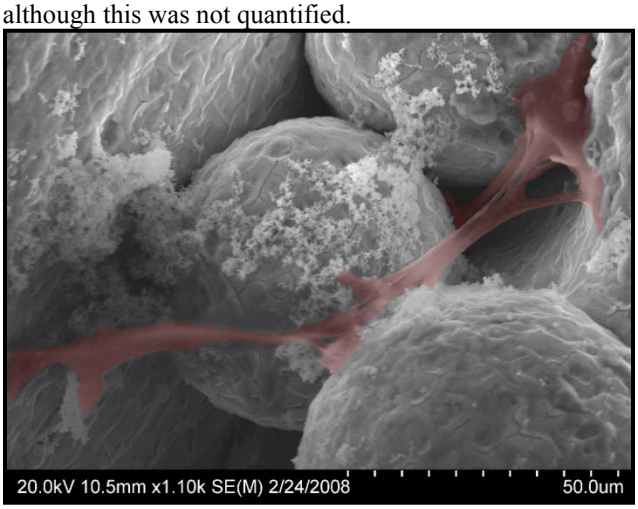

Figure 1. Scanning electron micrograph of an osteo-like cell on alkali heat treated β-TCP deposited Ti64 porous scaffold after 24 hour incubation.

Conclusions: The present experimental study shows that bone-derived cells are able to attach and spread on Ti64 porous scaffolds produced by the ARCAM EBM process after alkali, heat treatment and calcium deposition. The treatment produced a uniform, calcium-rich osteoconductive surface substrate that facilitated cell attachment. Extended cell culture studies are needed to determine; rate of proliferation, cell differentiation pathways and rate of extra-cellular matrix deposition.

References:

1. Serro, A.P., Biomaterials, 2003:**24**:p. 4749-4760.