Bone Bioactive Ceramic Coatings: The Synergistic Effects of Surface Roughness and Material Chemistry

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Statement of Purpose: Bioactive ceramic coating on orthopedic implants modifies both the surface chemistry and the topography of the device. The dynamic nature of bioactive ceramic surfaces, characterized by dissolution-precipitation reactions to and from the solution, as well as the inherent surface roughness of the implant have been shown to influence osteoblast behavior and the bone-bonding ability of the implant.[1] The objective of this study is to investigate the concomitant effects of the surface roughness of Ti-6Al-4V implant material coated with a bioactive silica-calcium phosphate nanocomposite (SCPC50)[2] and its dynamic microenvironment on modulating osteoblast activity. Temporal variations in cell morphology, spreading, and cytoskeletal organization as well as the ionic dissolution products have been evaluated. In addition, large-scale transcriptional modifications have been determined via high-throughput RNA sequencing and bioinformatics based approaches.

Methods: SCPC50 (in mol%, 40.68% CaO, 20.34% P_2O_5, 19.49% Na_2O and 19.49% SiO_2) was coated on Ti-6Al-4V discs by electrophoretic deposition using a voltage of 50V for 30 or 60s in a 10 wt% SCPC50/ethanol suspension followed by thermal treatment of the coated substrates at 800˚C/1hr under argon.[3] The average roughness (R_a) of the SCPC50-coated and uncoated samples was analyzed at a resolution of 40µm x 40µm using atomic force microscopy (AFM) in tapping mode (n=3). MC3T3-E1 osteoblast-like cells were seeded on coated and uncoated substrates (n=3) and the changes in cell morphology, cell number, and cell area were assessed by a combination of SEM and image analyses after 4, 8, and 24 h. Moreover, F-actin organization was visualized by confocal microscopy using a fluorescent rhodamine phalloidin probe. Broad changes in gene transcription were evaluated by high throughput RNA sequencing using an Illumina Hiseq 2000 platform followed by *in silico* sequence alignment. The ionic composition of the tissue culture medium incubated with the SCPC50-coated samples was measured using inductively coupled plasma optical emission spectroscopy (ICP-OES).

Results: AFM analysis showed that the R_a of SCPC50-coated Ti-6Al-4V substrate increased significantly ($p<0.05$) from 217.8 ± 54.5 nm to 284.3 ± 37.3 nm as the coating duration increased from 30 to 60s. Cells attached to the SCPC50-coated substrates of either roughness exhibited greater cell-to-cell contact and a more polygonal shape than the cells attached to the uncoated substrate at all time-points. After 24 h, the cell density on the 30s SCPC50-coated substrate was 1.7-fold higher than that on the 60s SCPC50-coated substrate. In addition, cells attached to the latter substrate showed a significant ($p<0.05$) 2-fold decrease in surface area as the incubation time increased from 4 h to 24 h, while those attached to the 30s SCPC50-coated or uncoated substrate showed comparable surface area during the same period. Cells attached to the SCPC50-coated substrates demonstrated multiple focal contacts after 4 h; however, they showed poor F-actin organization after 24 h. On the other hand, cells attached to uncoated substrates showed minimal focal contacts but exhibited well defined, parallel F-actin filaments oriented along the long axis of the cell. Gene sequence analyses showed that cells grown on SCPC50-coated substrates expressed higher levels of cell adhesion molecules, anchor proteins, extracellular matrix (ECM) components, the expression of which increased with increasing R_a (Table 1). ICP-OES analyses showed significantly higher Ca uptake by the 60s SCPC50-coated substrate as compared to the 30s SCPC50-coated substrate; however, the former substrate released significantly more ($p<0.05$) P and Si than the latter.

Gene name (Entrez)	Ti-alloy	SCPC50 (30s)	SCPC50 (60s)	Role
integrin α-7	14	452	646	cell adhesion
laminin	0	101	148	ECM protein
titin	4	509	678	elasticity
ladinin 1	0	87	226	anchoring filament

Table 1: Sequence alignment and gene expression data for cells attached to uncoated and SCPC50-coated substrates

Conclusions: Results of the study highlight the importance of both the surface roughness and the chemistry of a bioactive ceramic coating in modulating osteoblast responses. An increase in R_a of the SCPC50-coated substrate was accompanied by a decrease in cell attachment, cell spreading, and cell density; however, the cytoskeleton organization was largely unaffected. The decrease in cell density on the rougher 60s SCPC50-coated substrate as compared to that coated for 30s correlates well with an enhanced Ca-uptake and a greater release of P and Si by the former substrate, which may provide cues for early onset of cell differentiation. Our preliminary gene expression analysis of cells grown on SCPC50 coated and uncoated surfaces revealed marked differences in the level of expression of specific mRNAs under each growth condition. Our results indicate that increased surface roughness enhances upregulation of key osteogenic factors responsible for cell signaling and ECM synthesis. In conclusion, the synergistic effects of bioactive ceramic coating roughness and material dissolution stimulate early cell differentiation.

References:

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