ABSTRACT INTRODUCTION:
Enhanced tissue integration between bone and orthopedic implant devices is essential for implant fixation and longevity. An immunological response leads to fibrous encapsulation of metallic implants. Bioactive ceramics have the ability to make a direct bond with bone. Coating orthopedic implants with bioactive ceramics is an attractive mean of improving the in vivo performance of load bearing implants. Silica-calcium phosphate nano composite (SCPC) is a novel bioactive resorbable ceramic that has the ability to bond to bone and expedite bone formation. In our previous work, we have demonstrated the coating of bioactive SCPC on medical grade Ti-6Al-4V implants using electrophoretic deposition (EPD). The objective of the present study is to evaluate bone cell responses to the SCPC-coated implant material. In particular, the effect of the SCPC coating on gene expression is evaluated. Moreover, the release of cytokines from bone cells as a result of cell/material interaction is described.

METHODS:
Ti-6Al-4V discs (n = 5) were coated with SCPC as described elsewhere. Briefly, the discs were coated in 34% HNO3 and the EPD coating was carried out in 5% (w/v) SCPC suspension in ethanol for 30 sec at 50 V followed by thermal treatment at 800°C under argon. MC3T3-E1 osteoblast-like cells were seeded on SCPC-coated and uncoated Ti-6Al-4V discs at a cell density of 6 x 10^5 cells/sample and covered with 6 mL tissue culture medium (TCM) containing 10% fetal bovine serum, 0.05% gentamycin and 0.1% amphotericin-B in DMEM. Cells seeded on tissue culture polystyrene (TCP) served as control. The samples were incubated for 48 hrs at 37°C with 5% humidified CO2 following which the TCM was replaced and supplemented with 10^8 M dexamethasone and 50 µg/mL ascorbic acid. The samples were incubated for another 48 hrs and the total mRNA was extracted using the TRIZOL reagent. The purified mRNA from each sample type was pooled together and cDNA generated using random hexamer primers and reverse transcriptase. Polymerase chain reaction (PCR) was performed for the expression of mRNA encoding osteoblast phenotypic markers including collagen-I (Col-I), osteonectin (OSN), osteopontin (OPN) and osteocalcin (OCN) as well as the homekeeping gene glyceraldehyde 3-phosphate dehydrogenase (G3PDH) and the products were analyzed by agarose gel electrophoresis. Moreover, real-time PCR was performed to quantify gene expression levels. In addition, the morphology of the cells on SCPC-coated and uncoated Ti-6Al-4V samples was analyzed using SEM and EDX after 24 hrs and 96 hrs of incubation in TCM. In-situ release of interleukins IL-6 and IL-12 was measured to determine the cellular immune response as a result of cell/material interaction. The levels of IL-6 and IL-12 were measured in TCM using respective ELISA kits as per the manufacturer’s instructions (R&D Systems, MN). Statistical analysis was performed using student’s t-test with *p < 0.05, indicating a statistically significant difference.

RESULTS:
PCR analyses confirmed the expression of the osteoblast phenotypic markers Col-I, OSN, OPN and OCN in all samples. Real-time PCR analyses indicated greater up-regulation of Col-I, OSN and OPN on uncoated Ti-6Al-4V and TCP samples as compared to the SCPC-coated Ti-6Al-4V samples. However, OCN expression on SCPC-coated samples was increased by over 10 fold versus uncoated samples and this, in turn, was higher than that measured on TCP samples (Fig. 1). SEM analyses of the MC3T3-E1 cells attached to SCPC-coated Ti-6Al-4V samples incubated in TCM for 24 hrs showed that the cells transformed to acquire polygonal shape and formed multiple cellular processes. EDX analyses of the cell layer showed rapid Ca2+ assimilation as indicated by the greater Ca/P ratio. After 96 hrs incubation, the cells attached to the uncoated Ti-6Al-4V sample acquired “fibroblast-like” morphology, as indicated by their shape (Fig. 2a) while those attached to the SCPC-coated Ti-6Al-4V sample formed a confluent cell layer (Fig. 2b). The formation of extra-cellular matrix was observed on both samples; however matrix mineralization was limited to the SCPC-coated samples. As a result of cell/material interaction, the MC3T3-E1 cells released interleukins IL-6 and IL-12 into the tissue culture media. Significantly lower IL-6 levels (p<0.05) were produced by cells attached to SCPC-coated samples as compared to uncoated or TCP samples (Fig. 3). On the other hand, lower levels of IL-12 were present in the SCPC-coated sample than the uncoated Ti-6Al-4V sample, although this difference was not statistically significant (p > 0.05). Minimal IL-12 release was measured for the cells on TCP.

DISCUSSION:
The MC3T3-E1 osteoblast-like cells expressed osteoblast phenotypic markers Col-I, OSN, OPN, and OCN mRNA upon interaction with SCPC-coated Ti-6Al-4V samples. More importantly, the bioactive SCPC coating stimulated enhanced up-regulation of OCN and hence rapid mineralization of the extracellular matrix. On the other hand, cells on the uncoated Ti-6Al-4V samples showed a delayed response towards synthesizing mineralized matrix as indicated by the lower OCN levels. The up-regulation of OCN, in conjunction with the down-regulation of OPN, co-related well with the marked decrease in the expression of Col-I on the SCPC-coated samples. These results reflect a temporal variation in the gene expression pattern of osteoblasts wherein the levels of Col-I and OPN are lowered during OCN expression. It should be noted that osteocalcin is expressed only postproliferatively during the bone mineralization stage by the differentiating osteoblasts. Hence, an early expression of OCN is indicative of the strong stimulatory effect of SCPC on bone cell differentiation. Osteolytic reactions around orthopedic implants are promoted by the production of inflammatory and osteogenic mediators such as IL-6 and IL-12 by osteoblasts. In the present study, we showed that SCPC-coated Ti-6Al-4V samples stimulated significantly lower release of IL-6 as compared to the uncoated samples. The lower release of both IL-6 and IL-12 may enhance orthopedic implant fixation by limiting leukocyte recruitment and reducing the formation and activity of bone-resorbing osteoclasts that can lead to aseptic loosening. Experiments are currently ongoing to evaluate the role of other selected cytokines in the osteoblast immune response. Moreover, protein expression analyses are underway to correlate osteoblast gene expression with protein synthesis.

REFERENCES:

Osteoblast Gene Expression on a Novel Bioactive Ceramic Coating for Orthopedic Implants

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Fig. 1: Osteocalcin mRNA expression after 96-hr incubation.

Fig. 2: SEM images of the MC3T3-E1 cells on uncoated Ti-alloy disc and SCPC-coated Ti-alloy disc after incubation for 4 days. (a) The cells on the Ti-alloy surface acquired “fibroblast-like” morphology while (b) those on the SCPC-coated sample formed a dense confluent cell layer over the SCPC coating.

Fig. 3: Evaluation of IL-6 and IL-12 production at 96 hrs.

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