A Titanium Surface Enhances Mineralization From Bone Marrow Stromal Cells Earlier Than Cobalt Chrome

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INTRODUCTION: A successful cementless orthopaedic implant must have sufficient bone ingrowth into the material for stable fixation. It is commonly believed that titanium implants achieve better fixation *in vivo* and thus, the majority of orthopaedic and dental implants are comprised of titanium. Cobalt chrome is believed to have higher wear resistance and is most useful in joint replacements with articulating surfaces. Clinical evaluations between the two metals have not shown consistent significant differences, thus leading one to believe that there is no long-term effect of material composition on fixation. This discrepancy promoted us to investigate the early biological effects of bone formation on the two metals. The objective of this study was to evaluate the differences between polished titanium and polished cobalt chrome surfaces on bone mineralization *in vitro* in order to better understand the differences in the early biological response.

METHODS: Ti6A14V (25.4mm dia., 3mm thick) and CoCrMo disks (31.75mm dia., 3mm thick) were polished to a mirror finish and characterized using a surface roughness tester (Mitutoyo). Gamma irradiated disks were arranged in Petri dishes so that all samples for each time period were in one dish to assure cellular response was due to substrate composition and not a difference in cell density. Ti and CoCr samples were incubated in media alone to determine if mineral formation on the surfaces was cell-dependent. For bone marrow cell preparation, cells from the femora of Wistar rats were prepared according to established protocols. 20mL of the cell suspension was added to each dish and stored in a humidified incubator at 37°C and 5% CO_2. The medium was refreshed every 2-3 days and filtered through a 10K MWCO membrane prior to calcium (Ca) measurement using flame atomic absorption spectrophotometry (AAS). The cultures were maintained for 6, 12, 14, 19 and 26 days before fixation, staining, and/or biochemical assays. Samples for surface characterization were fixed in 0.1M sodium cacodylate buffer (pH 7.4) containing 3% gluteraldehyde and serially dehydrated. After drying, samples were analyzed using diffuse reflectance Fourier transform infra-red spectroscopy (FT-IR), thin-film x-ray diffractometry (XRD), and carbon coated prior to imaging with scanning electron microscopy (SEM) linked with energy-dispersive spectroscopy (EDS). Samples for Von Kossa were stained and imaged with digital photography. Cells for alkaline phosphatase (ALP) measurement were removed via trypsin digestion and lysed. For total protein measurement, cells were dissolved in 0.01M EDTA in lysis buffer and analyzed using a bicinchnoinic acid (BCA) total protein assay (Pierce). All results were normalized based on total surface area.

RESULTS and DISCUSSION: The roughness (Ra) of the Ti and CoCr disks was below 0.15µm for all the samples. AFM and SEM confirm the surfaces were smooth and free from defects. The Ti and CoCr control samples incubated in media alone through 26 days showed no evidence of CaP deposits and, therefore, all mineralization occurring in this experiment was cell-mediated.

After 12 days in culture, mineralized nodules on the Ti surface were present as indicated with Von Kossa staining (Fig 1a), but not on CoCr (Fig 1b). After 19 days, staining of the Ti surface shows more nodules and in greater density (Fig 1c) as compared to CoCr which is beginning to mineralize (Fig 1d). SEM-EDS results confirm CaP mineralization, collagen fibers, and distinct nodule formation at 14 days on Ti and at 19 days on CoCr. FT-IR shows the appearance of carbonate and phosphate at 12 days on Ti and XRD confirms the presence of carbonated apatite by 14 days. XRD shows the presence of carbonated apatite on CoCr at 19 days in culture. ALP activity for Ti increases dramatically through 12 days (Fig 2) in correlation with formation of mineralized nodules. ALP activity for CoCr increases slightly through 14 days (Fig 2), followed by nodule formation. Ca concentrations in the cell culture media start to drop sharply around 9 days along with mineral formation on the two surfaces (Fig 2).

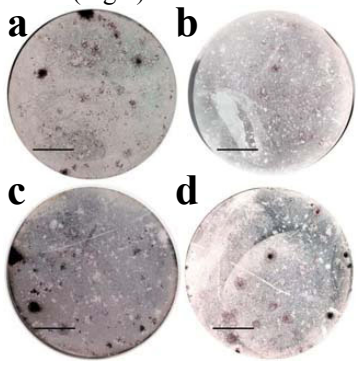

Figure 1. (Black) Areas of mineralized nodules on Ti (a) and the lack of on CoCr (b) after 12 days. Nodules become larger and denser n Ti (c) after 19 days at which point the beginnings of nodule formation are apparent on CoCr (d). Bar a&c= 0.6cm; b&d=0.8cm.

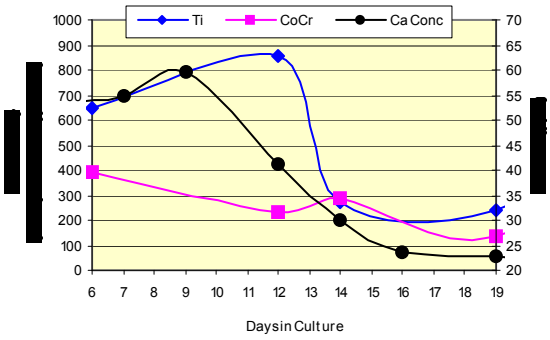

Figure 2. ALP activity for the Ti (blue) increases through 12 days in culture while the activity for the CoCr (pink) does not peak until 14 days in culture. The drop in Ca concentration (black) from the media occurs in concurrence with mineral formation.

SUMMARY: A titanium material is capable of enhancing bone marrow cells to produce more mineralized nodules and in greater density as compared to cobalt chrome. The onset of mineralization *in vitro* occurred at least one week earlier for the Ti surface.