Enhancement of Mineralization From Bone Marrow Cells on a Nano-Structured Titanium Surface

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Statement of Purpose: Surface texture and composition have been identified as two important elements affecting fixation of implants in bone. There is a growing interest in adding a nanoscale-textured surface on implants for enhancement of its integration to bone. The nano-textured surface is believed to effectively influence the function of adherent bone-forming cells and thereby to improve bone apposition on the surface. A novel technology has recently been developed to create nanoscale roughness on titanium by using a mild sodium fluoride solution instead of a strong acid solution. The objective of this study is to investigate the effect of a nanoscale textured titanium surface on mineralization from rat bone marrow cells and a commonly used cell line. This study is aimed to demonstrate the ability of our nano-textured titanium surface to promote mineralization of two different cell types to develop a more clinically relevant investigation.

Methods: Polished and surface nano-structured Ti6A14V disks (31.75mm diameter, 3mm thick) were characterized using a surface roughness tester (Mitutoyo), atomic force microscopy (AFM), and scanning electron microscopy (SEM) linked with energy-dispersive spectroscopy (EDS). Sterilized disks were arranged in Petri dishes so that all samples for each time period were in one dish in order to assure the cellular response was due to the substrate texture and composition and not a difference in cell density. Unoccupied areas of the Petri dishes were used as control. Ti6A14V samples without cells were incubated 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 and 20mL of the cell suspension added to each dish. For the cell lineage, MC3T3-E1 cells were plated at a density of $1x10^5$ cells/cm^2 with and without the addition of β-glycerophosphate (β-GP) on the two substrates. Cultures were stored in a humidified incubator at 37°C and 5% CO_2 and the medium refreshed every 2-3 days. The cultures were maintained for 7, 11, 14, 18 and 21 days (28 days for MC3T3 cells) before fixation, staining, and biochemical assays. Samples for surface characterization were fixed in 0.1M sodium cacodylate buffer (pH 7.4) containing 3% gluteraldehyde, serially dehydrated and dried at room temperature. 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 SEM-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 prior to measurement.

Results / Discussion: The roughness (Ra) of the Ti substrate increased from 0.07±0.01 µm to 0.20±0.03 µm after the nano-scale engineering process in NaF. AFM and SEM confirm the formation of a nano-scale textured surface and EDS confirmed that there was no fluoride detected on the surface. The Ti control samples incubated in media alone showed no evidence of Ca or P deposits and, therefore, all mineralization occurring in this experiment was cell-mediated.

For the bone marrow cells, SEM-EDS results show CaP mineralization at 11 days on both the polished and nano-textured surfaces. Von Kossa staining indicates a strong influence of the nano-textured surface as the coverage of the mineralized nodules on the polished samples is less than the nanoscale surface beginning at 11 days through 21 days (Fig 1). ALP concentration increases at 11 days, in correlation with the mineralized nodules seen with SEM and Von Kossa staining. FT-IR and XRD confirm the presence of a carbonated apatite on both surfaces beginning at 11 days (Fig 2). SEM morphological examination revealed that at earlier time points (7-11 days), cells on the polished surface are flattened and spread out, whereas cells on the nano-textured surface are more spherical in shape. This unique difference in morphology may indicate that cellular activity is dependent upon morphology and therefore, by altering the surface texture, the biological function of bone-forming cells can be enhanced.

For the MC3T3 cell line, SEM-EDS results show no mineralization through 28 days on either the polished or nano-textured surfaces. ALP concentration peaks by 21 days for samples with and without β-GP. The samples with β-GP had a two-fold increase in concentration which may indicate that the addition of β-GP is necessary for mineralization to occur. FT-IR and XRD (Fig 2) do not demonstrate any mineralization on either the polished or nano-textured substrate through 28 days. SEM morphological examination of the MC3T3 cells revealed a similar pattern of attachment and growth as the bone marrow cells. Therefore, the cells respond similarly to the different substrates, but the cell line cannot be induced to mineralize in less than 28 days with or without β-GP.

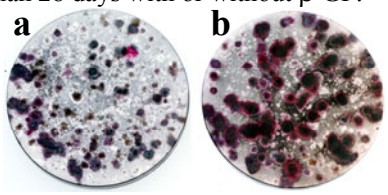

Figure 1: Von Kossa staining of polished (a) and nano-textured samples (b) with (black) areas of mineralization after 21days.

Figure 2: HA peaks are not detected with XRD (20-45°) on the polished (blue) and nano-textured (green) substrates with MC3T3 cells after 28 days. HA peaks are present on the polished (gray) and nano-textured (red) substrates with bone marrow cells after 21 days.