Resorption of Biomimetic Apatite by Osteoclasts Cultured From Bone Marrow Cells With and Without Vitamin D

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Statement of Purpose: Osteoporosis and osteolysis associated with wear debris of orthopedic implants are the result of imbalance between bone formation and resorption related to the activities of osteoclasts (OC). The success in treating osteoporosis and osteolysis requires a comprehensive understanding of factors that control osteoclast activities reflected in resorption of bone mineral. We have recently developed a novel technology by mimicking mineralization of bone that allows a biomimetic apatite (BAp) coating to grow on the surface of titanium implants. This BAp coating is substantially similar to bone mineral in composition and structure. Owing to this similarity, BAp coating could be used to evaluate the activities of osteoclasts in order for us to understand the stability of the coating in the body, and also to investigate the factors that could influence osteoclast functions. It is generally believed that Vitamin D can facilitate differentiation of bone marrow cells into osteoclasts and enhance the activities of osteoclasts. The objective of this study is to investigate the resorption of BAp coating by osteoclasts cultured from rat bone marrow cells with and without Vitamin D. This work is a precursor of the study aimed at seeking a better solution to treat osteoporosis and implant related osteolysis.

Methods: Biomimetic nanocrystalline apatite (BAp) coating was grown on grit-blasted Ti6A14V disks (25.4mm diameter, 3mm thick). The coated disks were analyzed using diffuse reflectance Fourier transform infra-red (FT-IR) spectroscopy, thin-film x-ray diffractometer (XRD), and scanning electron microscopy (SEM). Bone marrow cells were prepared from the femora of Wistar rats according to our established protocols. Sterilized disks were arranged in Petri dishes so all samples for each time period were in one dish to assure the cellular response was due to the substrate and not a difference in cell density. The BAp disks were inoculated with 30mL cell suspension, stored in a humidified incubator at 37°C and 5% CO_2. Bone marrow cells were cultured in the presence and absence of vitamin D (supplemented 24 hours after cell preparation) and all cultures maintained for 7 days. Samples for surface characterization were fixed in 0.1M sodium cacodylate buffer (pH 7.4) containing 3% glutaraldehyde, serially dehydrated and dried at room temperature. After drying, samples were gold coated and analyzed using SEM. Cells were removed from the disks via trypsin digestion for resorption pit characterization. Five areas of each disk were examined by SEM and at each area, five sites of interest (500x) were imaged. Samples for TRAP staining were fixed in 0.1M sodium cacodylate buffer (pH 7.4) containing 2.5% glutaraldehyde and 2% paraformaldehyde. A TRAP kit (Sigma) was used to stain for TRAP-positive cells.

Results / Discussion: FT-IR, XRD, and SEM analysis shows that the BAp coating is a nanocrystalline, nano-porous, calcium-deficient apatite containing Mg^{2+}, and CO_3^{2-} ions. The bone marrow cells cultured in the presence of Vitamin D did not exhibit a greater number of differentiated osteoclasts after one week in culture. The samples without vitamin D (Figure 1a) had approximately 2 times more TRAP-positive cells than the samples with vitamin D (Figure 1b). Figure 2a is an SEM image of an osteoclast in a resorption lacuna with an extended cell body and ruffled border. After removal of adhered cells (Figure 2b), resorption pits were uncovered with the maximum dimension up to 100µm. The resorption pits usually present circular or curved shapes on the BAp surface. The chance of finding an area (500x) containing resorption pits on the samples without vitamin D was 84% compared to a substantially lower value of 44% on the samples with vitamin D. The samples without vitamin D had 5 times more resorption pits and were larger in size than the samples without vitamin D.

Even though there were a fewer number of osteoclasts on the samples with vitamin D, these samples had a greater number of cells and the cell morphology was different from that of the cells without vitamin D. SEM morphological examination of the samples without vitamin D exhibits a more clustered orientation of the cells (Figure 3a). Resorption pits can are usually observed on the outer edges of the cell clusters. For the samples with vitamin D, cells are arranged randomly across the surface of the BAp disk (Figure 3b). Most of the cells are approximately the same size and are more flattened in appearance. This may indicate that the vitamin D encourages other cell types to proliferate more readily from the bone marrow. Other cell types may also inhibit the growth of osteoclasts from bone marrow.

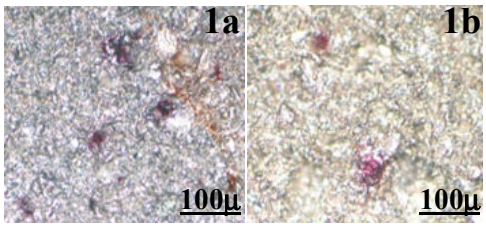

Figure 1: TRAP-positive osteoclasts without (a) and with (b) vitamin D after 7 days.

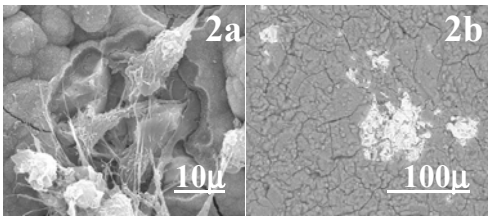

Figure 2: SEM of an osteoclast in a pit (a) and pits after cell removal (b) without vitamin D.

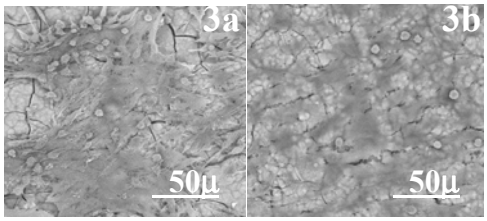

Figure 3: SEM of cell morphology without (a) and with (b) vitamin D.