Mineralization from Bone Marrow Cells on a Biomimetic Nano-crystalline Apatite Coating: An SEM Study

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Introduction: Biomimetic nano-crystalline apatite (BAp) coating formed in an aqueous solution mimicking physiological solution has been found to be osteoconductive. This BAp coating is capable of enhancing bone formation and forming a chemical bond with bone. It is generally believed that bone formation directly on bioactive ceramics, including calcium phosphates, is related to modification of ceramic surfaces in the physiological environment and their subsequent interactions with osteoblasts and osteo-progenitor cells in order to induce bone mineralization. In this study, we cultured rat bone marrow cells on BAp coating for up to 3 weeks to investigate their interactions leading to the formation of new apatite mineral and the mineralization of extracellular matrix *in vitro*.

Methods: BAp coating was formed at 45°C on Ti6Al4V disks (31.75mm diameter, 3mm thick) by soaking the disks in an aqueous coating source solution consisting of NaCl, KCl, $CaCl_2$, K_2HPO_4, $MgCl_2$, Na_2SO_4, and $NaHCO_3$, which are all present in the body. This process has been described in our previous publications. Bone marrow cells were harvested from the femora of Wistar rats according to our established protocols. The cell suspension obtained from 2 legs (20mL) was used for 6 disks in each 100mm^2 Petri dish. The dishes were stored in a humidified incubator at 37°C and 5% CO_2. Alpha-MEM was supplemented with FBS, 50μg/ml ascorbic acid, 10mM β-glycerophosphate and 10^{-8}M Dexamethasone and the culture medium refreshed every 2-3 days. The samples were removed after 7, 11, 14, 18 and 21 days and fixed in 0.1M sodium cacodylate buffer (pH 7.4) containing 3% gluteraldehyde at 4°C. The samples were then dehydrated in a series of ethanol and dried at room temperature. The samples were then characterized by FT-IR, thin-film XRD and carbon coated prior to the SEM study.

Results / Discussion: After 7 days in culture, the samples had three-dimensional cells on the coating surface (Figure 1a). These cells produced a biological substance that attached to the BAp coating (Figure 1b). A string of spherical minerals (Figure 1b) identified as apatite formed on the biological matrix. Apparently, a granular mineral formed on nano-porous BAp coating, as shown in Figure 1c. This indicates that apatite mineral began to form on the cellular matrix during the first week in culture. Close examination reveals that the matrix penetrated into the pores of the BAp coating and partially blocked them (Figure 1d). Thus, cellular attachment can be enhanced on the nano-porous BAp coating by allowing the extracellular matrix to grow into the coating pores, leading to additional mechanical interlocking.

Between 7 and 11 days, as shown in Figure 2a, the BAp coating was fully covered with the newly formed mineral layer. The initial nano-pore feature of the BAp coating was no longer observed after 11 days in culture because of the formation of new apatite mineral by the biological matrix. At that time, web-like patterns of the mineralized matrix started to form on the top of the newly developed granular minerals (Figure 2b). These areas may further develop into mineralized nodules, as shown in Figure 3. Apparently, the mineralized tissue formed on top of the apatite mineral layer that developed earlier on BAp coating. This mineralized tissue could be visualized using Von Kossa staining.

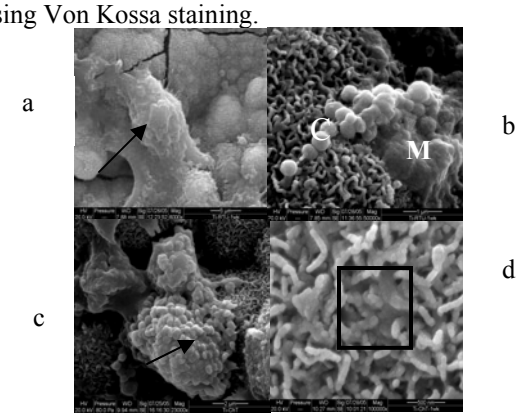

Figure 1: 7 days in culture (a) a cell on the BAp coating (b) matrix and mineralized collagen fiber (c) granular mineral (d) BAp coating was partially blocked with matrix.

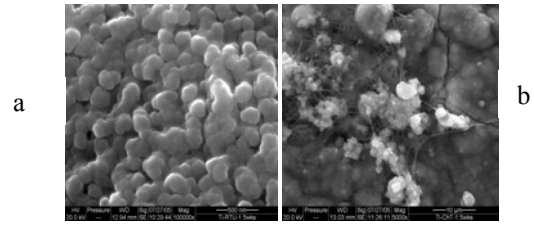

Figure 2: 11 days in culture (a) BAp porous structure was covered with newly formed mineral layer (b) web-like mineralized matrix starts to form on the apatite mineral layer earlier developed on BAp coating .

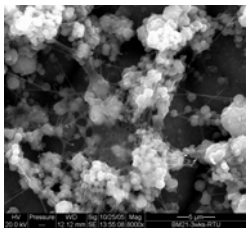

Figure 3: 21 days in culture: mineralized tissue formed on top of the apatite mineral layer.

Conclusions: Mineralization from rat bone marrow cells begins with the formation of apatite mineral on extracellular matrix attached to the BAp coating. This apatite mineral covers the BAp coating before the mineralization of extracellular matrix occurs. The subsequent mineralization of extracellular matrix leads to the formation of mineralized nodules that can be visualized using Von Kossa staining.