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X-Ray Microanalysis as a Tool for Analyzing Stem Cell Differentiation

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Cell culture serves as an important tissue model in research labs, allowing the effects of a treatment to be examined in a reproducible, relatively inexpensive controlled environment. Although 2D cell culture is most common, it does have drawbacks including limited cell-cell interactions and a possible disconnect between cellular behavior *in vivo* and *in vitro*. One method that is gaining popularity in response to these issues is culturing cells on a 3D matrix, or scaffold. The polycaprolactone (PCL) scaffolds available through 3D Biotek are non-toxic, have well-defined pore size and fiber diameter and are free of animal-derived material. In addition, the PCL scaffold is biodegradable, meaning that the scaffold can be introduced into an *in vivo* system from an *in vitro* system to examine the true effects on an organism. This study involves the use of scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) to analyze the differentiation of mesenchymal stem cells (MSC) into osteoblasts on PCL scaffolds. Differentiation would be indicated by the presence of osteoblast nodules composed of calcium phosphate, a main component of bone tissue.

PCL matrices were coated with human fibroblast (hFB) or MSC extracellular matrix (ECM), seeded with the corresponding cell line and cultured in growth media containing osteoblast differentiation factors. Matrices were removed from growth media and placed in SEM fixative (5% glutaraldehyde in PBS) at one week intervals for five weeks. The PCL matrices were post-fixed with 2% osmium tetroxide, dehydrated in a graded series of ethanol, critical point dried, mounted and coated with carbon. Samples were imaged with an FEI Quanta 200 3D and both quantitative analysis as well as elemental mapping were performed for each sample.

Analysis of the control hFB and the MSC samples showed increased cell coverage on the surface of the scaffold at the later time points of the series, as was expected. Upon analysis by EDS, it was also observed that at the later time points, specifically 5 weeks growth, there was no elemental signature for calcium or phosphorous on the element map or quantitative analysis of the hFB cells treated with osteoblast differentiation factors (Figure 1). Quantitative analysis of MSCs grown on the scaffolds after 5 weeks did show elemental signatures for both calcium and phosphorous (Figure 2A). Elemental mapping showed that the largest concentrations of calcium and phosphorous were localized to regions containing nodules observed during SEM imaging (Figure 2B).

This study was successful in showing that scanning electron microscopy, paired with EDS, can be used to determine stem cell differentiation for cell lines in which signature elements are characteristic of the differentiated cells, such as osteoblasts.

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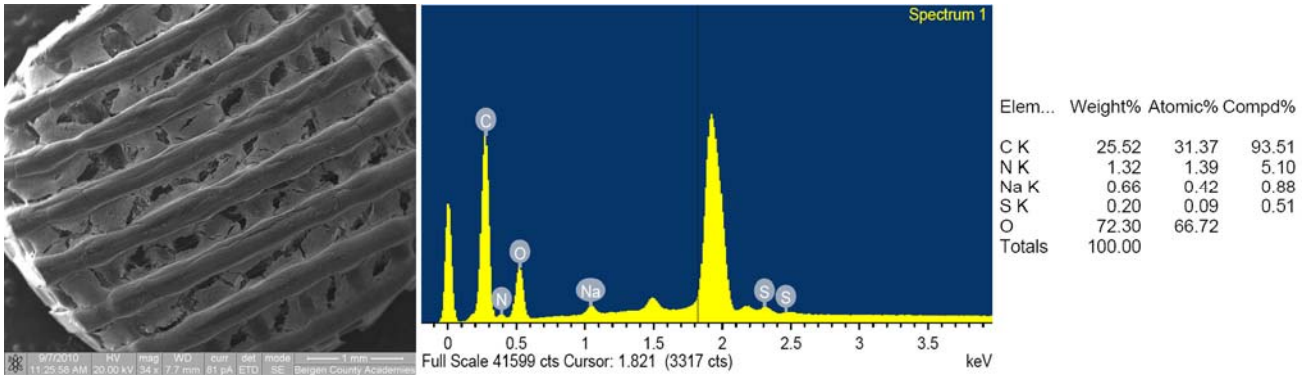

Figure 1: Week 5 scaffold containing hFB cells. Quantitative analysis of scaffold with the elements detected (C, N, Na, S & O).

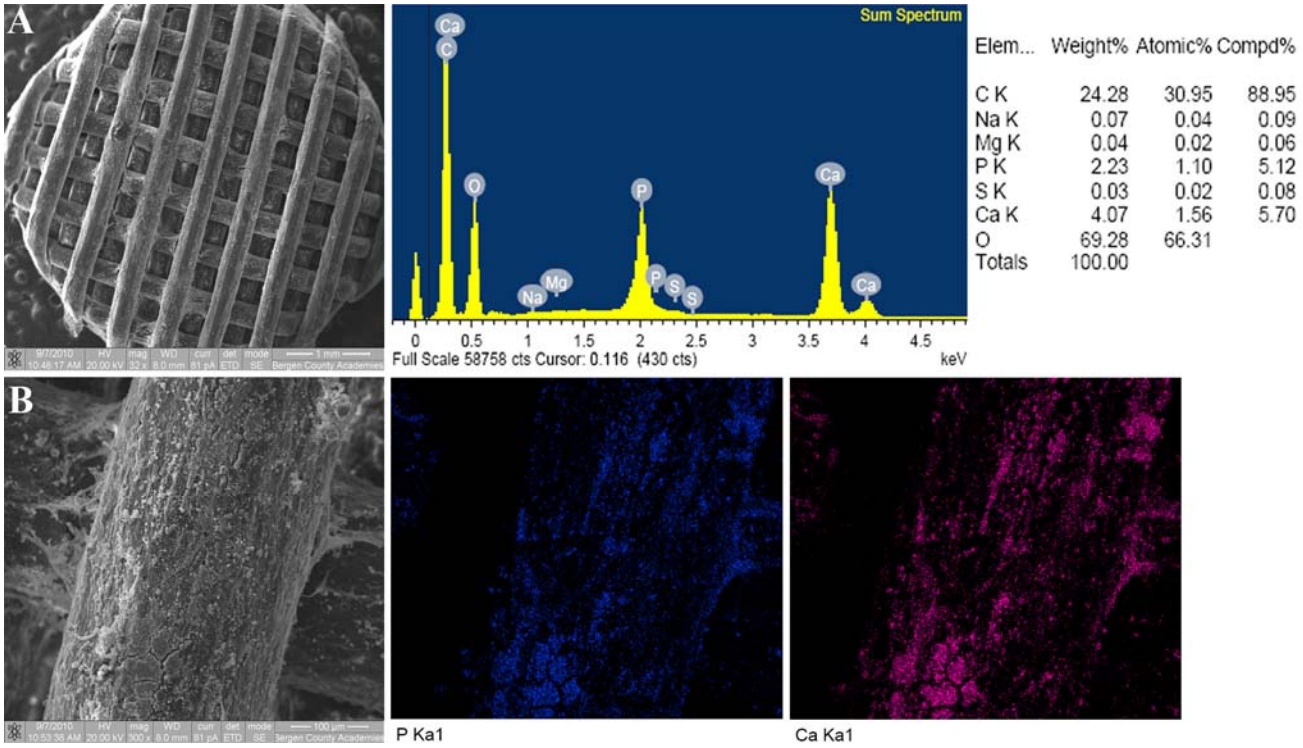

Figure 2: Week 5 scaffold containing MSCs. (A) Quantitative analysis of scaffold covered in differentiated cells, and the elemental makeup of the region (C, Na, Mg, P, S, Ca & O). (B) Elemental maps of nodules on a scaffold fiber.

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