In Vitro Analysis of Novel Porous PEEK Orthopedic Biomaterial

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Statement of Purpose: Polyetheretherketone (PEEK) was one of the first thermoplastic biomaterials to be approved and used for spinal and orthopedic implants. Because of its mechanical properties, it was preferred for specific implants to reduce the effect of stress shielding. However, because it traditionally is inert and non-porous, PEEK has suffered from low cell adhesion and lack of cellular ingrowth. A new porous form of the PEEK thermoplastic with approximately 25% porosity and pores ranging up to 200 microns in diameter has been produced. We have evaluated this new material through *in vitro* culture with rat mesenchymal stem cells (MSCs), to assess cellular interactions and differentiation. Also, we used scanning electron microscopy (SEM) to assess the morphological state of cells during osteogenic differentiation.

Methods: Constructs of non-porous PEEK were obtained from Invibio Inc. (Blackpoole, UK). Polymer sections were cut to 7.25 mm (W) x 13.5 mm (L) x 4.25 mm (H). After ethylene oxide sterilization, polymer constructs where placed in cell culture media under vacuum to displace the air in the pores. They were then seeded with $5x10^5$ cells/0.1 mL each. Constructs were cultured for 1, 3, 7, 14, and 21 days in osteogenic media. At each time point, seeded constructs were collected. SEM samples were rinsed, fixed, dehydrated, and sputter coated. All other scaffolds were ground, sonicated, and frozen to lyse cells before being assayed. Assays quantified DNA, alkaline phosphatase (ALP) activity, calcium deposition, and the level of osteopontin. Each experiment was done with n=4.

Results: Quantification of cellular DNA showed that the mean cell density did not change significantly over the experimental time course. The activity of the intracellular enzyme alkaline phosphatase, an early marker of osteoblastic differentiation, rose briefly, followed by a significant reduction at three weeks. The level of osteopontin stayed approximately the same throughout the experiment. Calcium was also shown to significantly increase between days 7 and 21 of culture (Figure 1). SEM image analysis shows control (unseeded sections) as well as cells cultured from days 3, 7, 14, and 21 *in vitro*. Over time, cells are shown to become less rounded and the matrix progressively more calcified as the cells become more differentiated down the osteoblastic lineage. In addition, specific elements were imaged with SEM, showing carbon, calcium, and phosphorus. Calcium and phosphorus, the components of hydroxyapatite, were seen to increase from 7 days to 21 days. In addition, the areas rich in calcium also appear to have the most differentiated cell morphologies. Compressive testing was performed on different samples of PEEK: dry uncultured, a wet, uncultured sample, and samples which had been cultured for 21 days. The initial deformation for the cultured samples was higher under a low stress than for the other samples. However, the elastic modulus of all samples was approximately the same after a stress of 0.5 MPa.

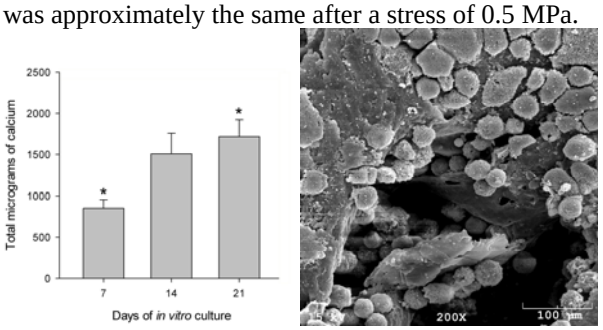

Figure 1: (left) Calcium content of constructs after varying days of culture. (right) SEM image at 200x of cells after 14 days of culture.

Conclusions: This study evaluated cell viability, growth and differentiation on porous PEEK. Scaffolds were seeded with MSCs to mimic conditions where the implant would be abutting the native bone marrow and the marrow stromal cells. The contact angle of pure PEEK is 80 degrees, which is less hydrophilic than ideal, which may have played a role in the reduced initial cell adhesion and proliferation. However, this is also indicative of early cellular differentiation, which is characterized by a period of reduced proliferation. This is supported by the drop in ALP activity, which decreases as cells commit to final differentiation. Further, the levels of osteopontin remained constant throughout the culture period, suggesting cells were already in the initial stages of differentiation. The amount of calcified matrix increased dramatically, shown through both assays and SEM imaging. In addition, the morphology of the cells was shown to be less rounded, indicating a more osteoblastic cell shape. SEM images also show stronger cell-cell interactions and increasing amounts of ECM. Biomechanical analysis shows the PEEK's initial compression may have been influenced by the ECM, which was less stiff than the rest of the material. The cells in this study were seen to rapidly differentiate to an osteoblast-like phenotype, with a degree of cell invasion into pores, as noted through SEM imaging. As such, porous PEEK may significantly improve the bonds between the implant and native tissue, with less probability for capsule formation and stress shielding concerns. Overall, the new porous PEEK biomaterial shows promising cellular interactions that may lead to improved *in vivo* tissue/biomaterial integration. As such, *in vivo* analysis would be an important next step.

References:

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