# Design and Surface-Modification of Poly(Propylene Fumarate) Scaffolds for Mesenchymal Stem Cell Attachment and Differentiation

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**Statement of Purpose:** The current technology for MSC isolation necessitates a multi-step process. The use of modified biomaterial surfaces to enhance MSC enrichment from the bone marrow has shown some success; however, these technologies have limited capability for direct implantation. Our goal is to utilize three-dimensional, surface-modified poly(propylene fumarate) (PPF) scaffolds in order to simplify MSC isolation and implantation into a single-step system. We hypothesize that through covalent tethering of proteins and growth factors onto the PPF surface, we will be able to capture MSCs onto the scaffold surface and promote differentiation. Specifically, this study will investigate the design and characterization of porous PPF scaffolds fabricated using two methods – random pore architecture (RPA) using porogen leaching, and controlled pore architecture (CPA) using 3D printing methods. Additionally, we will investigate the impact of surface modification of PPF scaffolds on MSC adhesion. We hypothesize that surface modification will increase MSC attachment over unmodified surfaces, and the CPA scaffolds will see increased cell ingrowth and distribution.

**Methods:** For both designs, a pore size of 700µm was chosen based on previous results showing increased MSC osteogenesis in porous PPF scaffolds (Kim K, Biomaterials. 2011; 32(15): 3750–63). PPF was synthesized as described previously (Kasper FK, Nat Protoc, 2009;4(4): 518-25) and photocrosslinked using BAPO as an initiator. Two PPF:DEF weight ratios were tested – 56:44 and 66:33 wt%. For RPA scaffolds, the PPF:DEF mixture containing 0.5 wt% BAPO was homogenously combined with 70 wt% of salt porogen crystals and UV crosslinked for 2h. Discs were placed in water for 3 days to leach out salt porogen. For CPA scaffolds, 3D printed discs were fabricated from polymer resin consisting of PPF, DEF, BAPO, and attenuators using an envisonTEC Perfactory® additive manufacturing device, with which we can successfully print PPF with a resolution of 300µm. Scaffolds were designed with a theoretical porosity of 70% using SolidWorks software. All scaffolds were washed in a 50% acetone solution followed by washes in PBS. Scaffolds were sterilized prior to surface modification and MSC attachment. For modification, scaffolds first underwent aminolysis in a 10% w/v solution of hexamethylene diamine, followed by reaction with excess NHS in sodium bicarbonate buffer (pH = 8.5). Lastly, scaffolds were reacted with a solution containing ECM proteins. MSCs were seeded onto modified sterilized scaffolds and assessed using a live/dead assay to investigate cell viability and morphology.

**Results:** To estimate the actual porosity of the RPA scaffolds, the amount of salt leached from each group was assessed and shown in Table 1. Both groups resulted in scaffolds with porosities greater than the theoretical 70%. Macroscopic images of each formulation are shown in Figure 1.

| Group | PPF(wt%) | DEF(wt%) | Porogen Leached (wt%) |
|---|---|---|---|
| **1** | **56** | **44** | **71.2** |
| **2** | **66** | **33** | **74.8** |

Table 1. Calculated actual porosities based on the percent of porogen leached

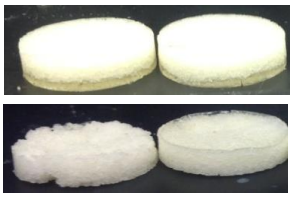

Figure 1. Macroscopic images of random pore architecture scaffolds fabricated with combination 1 (top) or 2 (bottom)

CPA scaffolds were 3D printed from SolidWorks designs in which a single layer of the scaffold is repeated in an offset porous arrangement (see Figure 2).

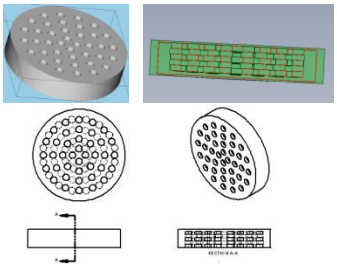

Figure 2. Software designs of controlled architecture scaffolds showing a repeating layer of offset pores to achieve the desired pore size and porosity (700µm and 70%)

PPF modified on the surface with fibronectin resulted in high levels of MSC adhesion. MSCs appeared fibroblast-like on the surface of the scaffolds, similar to the morphology of MSCs on tissue culture polystyrene. Modified scaffolds were found to have increased adhesion over unmodified scaffolds.

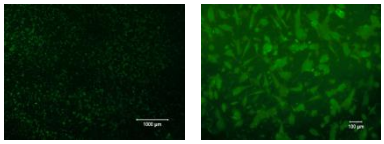

Figure 3. MSCs on modified PPF at 2.5x (left) and 10x (right) showing high levels of adhesion and spreading

**Conclusions:** Results showed successful fabrication of both RPA and CPA scaffolds with actual porosities near the desired percentage. Additionally, PPF scaffolds modified at the surface with ECM proteins increase adhesion and spreading of cells indicating that modified scaffolds can be used to facilitate MSC adhesion and subsequent 3D culture. These results will be used in future studies to investigate how the incorporation of growth factors at the PPF surface influences the differentiation of attached MSCs.