Ultraviolet Functionalized Surface Treatment of 3D Printed PEEK resulted in Calcium Phosphate Layer Formation

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Statement of Purpose: Treatment for intervertebral disc degeneration often involves the complete removal of the disc and the fusion of two adjacent vertebrae via a biocompatible implant [1]. The implant must be capable of both supporting the mechanical forces experienced by the spine, as well as promoting bone growth. After one year, polyetheretherketone (PEEK) cages have demonstrated between 84% [2] and 100% [3] fusion, but PEEK is also a bioinert polymer that is not ideal for osseointegration [3]. Surface modification has the potential to maintain the positive bulk mechanical properties of PEEK while increasing bioactivity. Ultraviolet (UV) light assisted functionalization of PEEK is one possible method because PEEK's benzophenone groups generate free radicals when exposed to UV radiation; these free radicals have been shown to allow the successful grafting of PEEK to styrene and polyacrylic acid [4]. We investigated the use of UV-assisted functionalization in facilitating nucleation and growth of a calcium phosphate layer to 3D printed PEEK and measured the response of murine preosteoblasts seeded on their surface.

Methods: PEEK samples with dimensions of 10x10x1 mm were obtained via fused filament fabrication [1]. 2X Simulated body fluid (2XSBF) was prepared according to a method described by C. Tas [5]. PEEK samples were submerged in 2XSBF. The samples were placed under a 2W/cm^2 UV-A light for six hours. Samples were then placed in a water bath set to 37°C for 72 hours. After functionalization, the PEEK samples were washed with DI water and dried. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) (Nicolet Nexus 870 Mid-IR spectrometer) was performed on the functionalized samples, control PEEK samples that were not treated, and control samples that were soaked in SBF for 72 hours but were not exposed to UV light (n=3). Images of the surface of the functionalized samples and controls were obtained using a scanning electron microscope (SEM) (Zeiss Supra 50VP). Regarding *in vitro* testing, 30,000 MC3T3-E1 cells were seeded onto sterilized PEEK samples that were functionalized and PEEK samples that were not. Tissue culture plastic acted as a positive control (n=6). MTT and ALP assays acted as markers for cell proliferation and osteogenic activity, respectively, and were performed at 7 and 14 days. Normalized ALP was calculated to measure osteogenic activity relative to cell number. ANOVA with Tukey post-hoc analysis was used to measure statistical differences between groups.

Results: The samples exposed to UV for six hours and soaked in 2XSBF for 72 additional hours had notable peaks at 560 and 600 cm^{-1}, and between 1000 and 1100 cm^{-1}, which indicates the presence of a phosphate group [6]. FTIR results for the non-functionalized PEEK control samples and the samples that were not exposed to UV but were soaked in 2XSBF showed a peak at 1489 cm^{-1}, indicative of an aromatic C-C bond only, not representative of a calcium phosphate coating (Fig. 1D). SEM images of samples soaked in 2XSBF and exposed to UV displayed distinct nodule topography consistent with calcium phosphate coatings (Fig. 1C). In contrast, SEM images of control PEEK samples show a relatively smooth surface with some linear features as a typical result of the printing process (Fig. 1A). Samples soaked in 2XSBF, but not exposed to UV light did not show calcium phosphate structures and were similar in appearance to control PEEK samples (Fig. 1B). SEM images of functionalized samples showed cell adhesion to calcium phosphate structures on surface (Fig. 1E&F). MTT, ALP, and normalized ALP results were not significantly different between functionalized and non-functionalized samples.

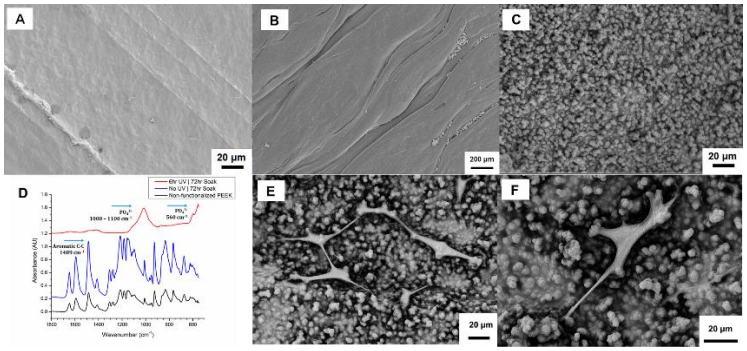

Figure 1: SEM images of (A) control PEEK, (B) no UV+2XSBF, and (C) UV+2XSBF; (D) FTIR results; and (E&F) cells adhered to UV+2XSBF samples after 7 days of culture.

Conclusions: The appearance of calcium phosphate structures on samples exposed to UV light and soaked in 2XSBF, but not on samples soaked only in 2XSBF suggests that the UV light is necessary for the formation of calcium phosphates on the surface of PEEK. A potential limitation of functionalization process is that it is unlikely that all calcium phosphate structures formed are crystalline, and instead are likely an array of amorphous calcium phosphates. X-ray diffraction testing could be used in the future to verify the degree of crystallinity. While *in vitro* results do not suggest that bioactivity is increased in samples with calcium phosphate surface, they do demonstrate that cells are capable of attaching to the modified surface. Adjustments to the cell culture protocol, such as investigating longer time points, utilizing primary human osteoblasts, and using osteogenic medium with ascorbate and β-glycerophosphate could be used to further explore potential mechanisms.

References: [1] C Basgul, *J Mater Res* (2018). [2] DP Mittal, *Int J Orthop* (2017). [3] YC Chou, *J Clin Neurosci* (2008). [4] A Yousef, *React Funct Polym* (2014). [5] AC Tas, *Biomaterials* (2000). [6] L Berzina-Cimdina *InTech* (2012).