Poly(ethylene glycol)-grafted Poly(propylene fumarate) Networks for Regulating Surface Physicochemical Characteristics and MC3T3 Cell Behavior

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Statement of Purpose: Injectable poly(propylene fumarate) (PPF) is a promising candidate for orthopaedic applications because of its excellent biocompatibility, mechanical properties, osteoconductivity, biodegradability, and handling characteristics.[1-2] In this study, PPF was modified by photo-crosslinking with methoxy poly(ethylene glycol) monoacrylate (mPEGA) at seven mPEGA compositions from 0% to 30%. Unlike PPF crosslinked with PEG dimethacrylate,[1] mPEGA dangling chains on the surface of crosslinked PPF did not influence the swelling ratio in water while they increased surface hydrophilicity greatly. Bulk properties such as thermal, rheological and mechanical properties of crosslinked mPEGA/PPF blends have been also investigated and correlated with surface characteristics in order to investigate their roles in modulating mouse MC3T3 cell adhesion, spreading, proliferation, and differentiation.

Methods: mPEGA was synthesized using mPEG of 350 $g.mol^{-1}$ purchased from Sigma (St. Louis, MO) and acryloyl chloride in the presence of K_2CO_3. PPF (M_n=1820 $g.mol^{-1}$, M_w=3410 $g.mol^{-1}$) was synthesized in our laboratory as described earlier.[2,3] mPEGA and PPF were dissolved in methylene chloride and fully dried to prepare mPEGA/PPF blends with the mPEGA weight compositions (ϕ_{mPEGA}) from 0% to 30%. Polymer disks were fabricated via photo-crosslinking (Figure 1) as described previously.[3]

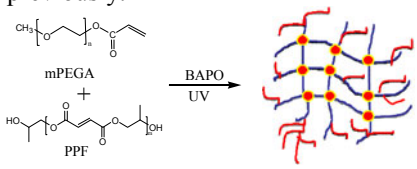

Figure 1. Photo-crosslinking of mPEGA/PPF.

Results: The water contact angles decreased progressively from 87° for crosslinked PPF to 74°, 65°, 45°, 18° on the disks of crosslinked mPEGA/PPF blends with ϕ_{mPEGA} of 5%, 10%, 20% and 30%, indicating mPEGA dangling chains could greatly improve surface hydrophilicity. However, protein adsorption and mechanical properties decreased with more grafted mPEGA chains due to their known repellent effect[4] to proteins and lower crosslink density, respectively. No swelling in cell culture medium was observed for all the samples. All crosslinked mPEGA/PPF blends showed no cytotoxicity in 4 days for MC3T3 cells. MC3T3 cell attachment, proliferation and differentiation (Figure 2) all had a parabolic trend that maximized at ϕ_{mPEGA} of ~5%, suggesting an effective method for modifying cell responses of PPF. Cell images also showed consistent results as most spread-out phenotype and fastest proliferation were found at ϕ_{mPEGA} of 5%. Surface and bulk properties both played important roles in tuning cell behavior as the improved hydrophilicity with sparsely grafted mPEGA chains might be responsible for the improved cell responses. Decrease in cell responses at higher ϕ_{mPEGA} can be interpreted by less protein adsorption and mechanical strength.

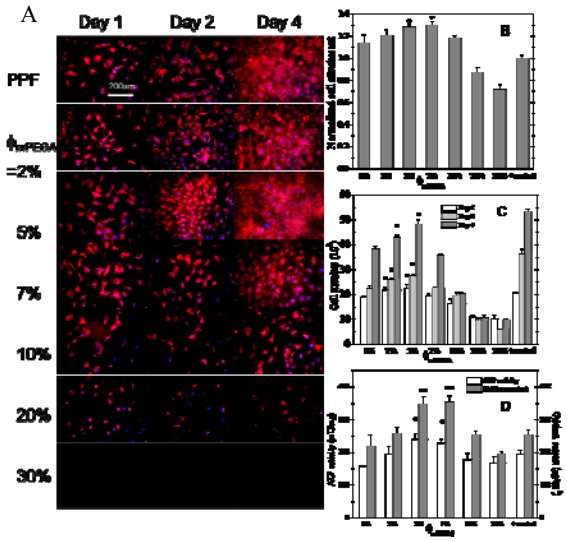

Figure 2. A: MC3T3 cell morphology (red: rhodamine-phalloidin, blue: DAPI) of MC3T3 cells on crosslinked mPEGA/PPF disks at day 1, 2, and 4 post seeding. The scale bar of 200 μm is applicable to all. B: Normalized MC3T3 cell attachment after 4 hr. C: MC3T3 cell number at day 1, 2, and 4. D: ALP activity and calcium content at day 7 on crosslinked mPEGA/PPF disks post seeding, compared with cell-seeded TCPS as the positive (+) control. *·**, p<0.05 compared to crosslinked PPF.

Conclusions: We have developed a series of miscible, injectable, and photo-crosslinkable mPEGA/PPF blends with controllable bulk and surface characteristics. MC3T3 cell attachment, proliferation and differentiation reach maximum at the mPEGA composition of 5-7% and the trend can be closely correlated with their surface and bulk properties. Besides revealing that mPEGA dangling chains may enhance cell responses by increasing hydrophilicity when their fraction on the hydrophobic surface is small, the present study also offers a new method of improving the wettability and performance of the scaffolds made from this widely used bone-tissue-engineering biomaterial, PPF.

References:

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