Regulating Smooth Muscle Cells on Poly(ethylene glycol)-grafted Poly(ε-caprolactone) Networks

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Statement of Purpose: Although crosslinkable poly(ε-caprolactone) triacrylate (PCLTA) has its versatile controllability and potential usefulness,[1] the hydrophobicity of semi-crystalline PCLTA networks limits their tissue engineering applications. Here we photo-crosslinked methoxy poly(ethylene glycol) monoacrylate (mPEGA) with PCLTA at mPEGA compositions (ϕ_m) of 0-50% and different nominal mPEGA molecular weights of 350, 2000, and 10000 g/mol to modulate material properties and regulate primary rat aorta smooth muscle cells (SMCs).

Methods: Polymer preparation and photo-crosslinking were similar to our previous report.[1] Material properties such as water contact angle, friction coefficient, surface roughness, thermal and mechanical properties were characterized. Primary SMCs were cultured on the polymer disks and their adhesion, spreading, proliferation, differentiation, and gene expression were characterized according to our previous report.[2]

Results: The hydrophilicity of PCLTA network was greatly improved by grafting PEG chains, as indicated by a sharp decrease in the water contact angle. At the same ϕ_m, the network was more hydrophilic when grafted with longer PEG chains. Meanwhile, the ability of adsorbing serum proteins from culture media and surface frictional coefficient both decreased substantially with increasing ϕ_m or using longer mPEGA chains. Because mPEGA could polymerize by itself, PCLTA networks with tethered PEG chains had lower crosslinking density and consequently lower tensile moduli. This trend was more significant when mPEGA with a higher molecular weight was used. At 4 h and day 1 post-seeding, the number and spread area of attached SMCs increased when the PCLTA network was grafted with a small fraction of short PEG chains (Figure 1). For mPEGA350/PCLTA or mPEGA2000/PCLTA networks grafted with short PEG chains, SMC behavior showed a parabolic trend when ϕ_m increased and the attachment maximized at ϕ_m of 5% or 2%, respectively. Beyond that composition, SMC attachment and proliferation decreased sharply because of densely grafted PEG chains on the network surface. For mPEGA10000/PCLTA networks grafted with the longest PEG chains, the number of adhered SMCs was no longer enhanced but was reduced significantly even at a small ϕ_m and cells completely disappeared at ϕ_m of 7%. These trends indicated that a small fraction of short PEG pendant chains could enhance SMC attachment and proliferation by reducing surface hydrophobicity, while a high fraction of grafted PEG chains or the same fraction of long grafted PEG chains could demonstrate their well-known strong repulsive effect in preventing serum protein adsorption and cell adhesion. As shown in Figure 1c, stronger and more elongated focal adhesions with a larger size and a higher density were observed in SMCs on PCLTA networks grafted with 5% mPEGA350. Three contractile markers, smooth muscle myosin heavy chain (SM-MHC), smoothlin, and calponin showed higher expression levels on crosslinked mPEGA350/PCLTA with ϕ_m of 5%, which better supported SMC proliferation than lower or higher ϕ_m.

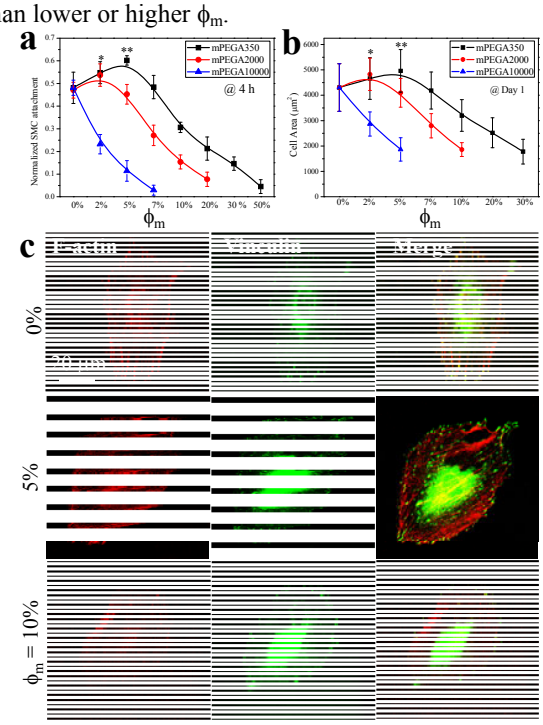

Figure 1. (a) SMC attachment at 4 h on crosslinked mPEGA/PCLTA disks. (b) SMC area at day 1. **: significant difference ($p < 0.05$) between mPEGA350/PCLTA (ϕ_m = 5%) and PCLTA (ϕ_m = 0%). *: significant difference ($p < 0.05$) between mPEGA2000/PCLTA (ϕ_m = 2%) and PCLTA (ϕ_m = 0%). (c) Confocal microscopic images of SMCs at day 1 with filaments and focal adhesions stained red and green using RP and vinculin antibodies, respectively.

Conclusions: mPEGA grafted chains on the surface of crosslinked PCLTA networks significantly reduced surface hydrophobicity, frictional coefficient, and serum protein adsorption. Longer mPEGA chains had a stronger effect in modulating these material properties. Grafting a small ϕ_m of short mPEGA pendant chains to PCLTA networks could enhance SMC attachment, proliferation and differentiation on the PCLTA substrates by reducing surface hydrophobicity. In contrast, a high ϕ_m or long mPEGA chains could significantly prohibit SMC adhesion and proliferation via strong repulsion to cell adhesion related proteins.

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References: 1. Cai L. *Polymer* **2010**, *51*, 164.
2. Cai L. *Biomaterials* **2010**, *31*, 4457.