Shape Memory Polymer Recovery Activates Nano-scale Polyelectrolyte Multi-layer Topographical Wrinkles

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Statement of Purpose: Currently, there remains a need to understand the synergistic effects of surface topography and chemistry on cell behavior in order to improve implant biocompatibility and to understand cell-surface interactions. In the recent past, shape memory polymers (SMPs) have been used to induce cell alignment *in vitro*,[1] while polyelectrolyte multilayers (PEMs) have been studied for their impact on substrate wettability and cell compatibility.[2] The present work uniquely combines these approaches by using an SMP to compress PEM coatings to a useful wrinkled state through a buckling phenomenon. In particular, wrinkle patterns are formed by heat-induced shrinkage of SMPs that are first spin-coated with PEMs. Unlike many static cell culture substrates, this new system can be used to explore changes in cell alignment and motility dynamically with only a small change in temperature. We report here the effects of wrinkle wavelength and amplitude on surface properties and endothelial cell-surface interactions for vascular implant applications.

Methods: A shape memory polymer, crosslinked poly(*tert*-butyl acrylate-co-butyl acrylate), was synthesized by UV-initiated polymerization and crosslinking of a mixture of *tert*-butyl acrylate and butyl acrylate monomers, the crosslinker tetraethylene glycol dimethacrylate (TEGDMA), and a photoinitiator 2,2-dimethoxy-2-phenyl acetophenone (DMPA) for 1 h in a glass mold. Rectangular specimens were strained uniaxially to varying degrees and fixed in that state using their shape memory characteristics. PEMs were then prepared on the SMPs using sequential spin-coating of poly (sodium-4-styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) following priming with poly(ethylene imine) (PEI) and washing with deionized water between steps. This spin-coating process was repeated to yield 10 bilayers. A range of spin-speeds was used for later comparison. Next, the SMP substrates were heated to 50 °C, recovering the fixed strain and wrinkling the PEM coatings. Roughness, water contact angle, and optical transmittance were measured with AFM, a goniometer, and an optical microscope, respectively. Coating thickness and wrinkle wavelength and amplitude were determined by analysis of the AFM data. PEM wrinkle integrity in PBS was monitored for 48 h. Bovine aortic endothelial cells (passage 4-6) were seeded at 25,000 cells/cm^2 onto PEM-coated SMPs (10 bilayers spin-coated at 3000 rpm). The cells were grown to confluence for 3 d, followed by fixing with 4% paraformaldehyde. For imaging, cell nuclei were stained with Sytox Green while actin filaments were stained with Alexa Fluor 568 Phalloidin. Images were taken with a fluoresence microscope at 20x magnification. Comparisons were made between groups with and without PEM coatings and with PEMs wrinkled by shape memory recovery.

Results: A variety of wrinkled PEM patterns were prepared to study the effects of amplitude, topography, and PEM chemistry on endothelial cell attachment. We observed a decrease in wavelength with increasing recovery strain for all PEM spin coating conditions, along with increasing wrinkle wavelength with spin coating speed for all recovery strains (Figure 1A). Further, we observed an increase in wrinkle amplitude (whether root mean square height (Sq) or arithmetic mean height (Sa)) with increasing recovered strain (Figure 1B). Concerning wettability, SMP-induced wrinkling was found to increase hydrophobicity, as shown in Figure 1C. This observed hydrophobicity change, triggered by shape memory recovery, impacted endothelial cell attachment and morphology, with endothelial cells attaching and spreading more favorably than without coating and with an effect of recovery strain that is currently under investigation.

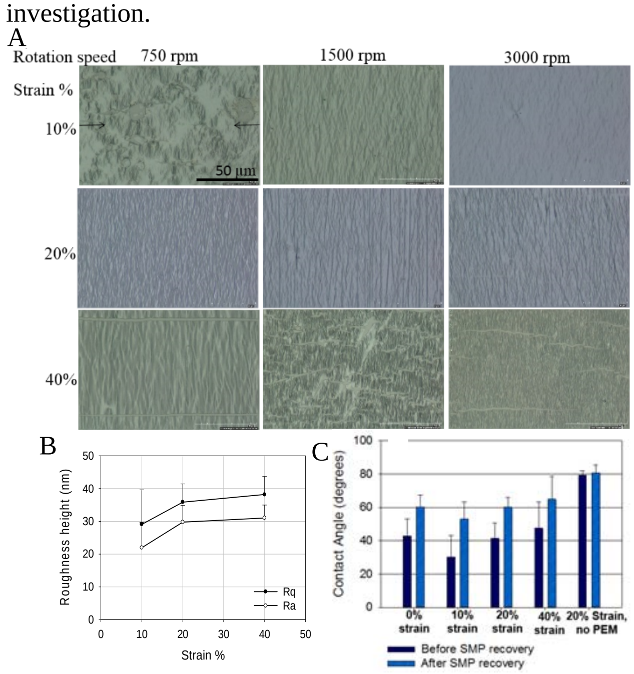

Figure 1. Material Characterization of PEM coated SMPs. A) Optical microscopy of PEM wrinkle patterns. The arrows indicate direction of compression. The scale bar is the same for all pictures. B) Substrate roughness analysis after SMP recovery. C) Contact angle analysis after SMP recovery.

Conclusions: We have introduced a new dynamic cell culture system combining SMPs with PEMs. We observed that shape recovery of the SMP induces wrinkles and that this topographical change impacts wettability and cell attachment and spreading. We are presently studying the effects of wrinkle wavelength, amplitude, and PEM terminating charge on endothelial cell attachment and alignment.

References: (1) Yang, P. et.al. *Soft Matter,* 2013, 9, 4705 (2) Kidambi, S et.al. *Tissue Engineering* (2007) 13:8, 2105