Membrane scaffolds exhibiting biochemical and physical signals for tissue engineering

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The use of thin bioactive membrane scaffolds in tissue engineering and regenerative medicine could have many applications *in vivo*, directly replacing or stimulating tissues, and *in vitro*, facilitating well-controlled studies of cell-cell communication or nutrient permeability. It is well known that both biochemical (specific epitopes) [1,2] and physical (topography, porosity, stiffness) [3,4] signals can be used to control and guide cell behavior.[5] In this work we report on the use of a top-down/bottom-up approach to develop thin self-supporting bioactive membranes exhibiting both biochemical and physical signals specifically designed to elicit a specific biological response.

Elastin-Like Polymers (ELP) containing the cell adhesive epitope arginine-glycine-aspartic acid-serine (RGDS) were synthesized using standard recombinant protein production techniques and cross-linked with 1,6-hexamethylene-diisocyanate (HMDI). The ELP membranes were fabricated by a drop-casting/evaporation technique using a spin-coater to precisely control membrane thickness. Membranes were fabricated with a variety of nano and micro topographical patterns on either one or both sides, uniform and well-defined through-holes, and exhibiting multi-layers. Membrane swelling and stiffness were characterized by atomic force microscopy (AFM), nanoindentation tests, and scanning electron microscopy (SEM). The membrane biocompatibility and bioactivity were assessed by *in vitro* culture using rat mesenchymal stem cells (rMSCs) and quantifying cell adhesion, morphology, and proliferation.

Membranes were reproducibly fabricated with thicknesses varying between 500 nm – 100 µm depending on the fabrication conditions, exhibited sufficient structural integrity to be handled and sutured, and served as *in vitro* cell culture substrates. Membranes were also fabricated comprising topographical features with heights ranging between 500 nm and up to 10 µm. Optical, inmuno-fluorescence, and scanning electron microscopy demonstrated that rMSCs adhered on the ELP membranes exhibiting a spread morphology and well-defined actin cytoskeleton. However, cell adhesion tests did not reveal a significant increase in cell number after 4 hrs of incubation between membranes comprising the RGDS epitope and those that did not exhibited the epitope ($p>0.05$). Nonetheless, cells were observed to spread faster on the RGDS membranes compared to those not exhibiting RGDS. Furthermore, cell morphology was qualitatively affected by the different topographical features present on the membrane's surface.

We have developed a variety of fabrication techniques based on micro and nanotechnologies to create thin self-sustained membranes of controlled thickness that comprise bioactive epitopes and a variety of topographical and structural components that could be fine-tuned to stimulate specific biological processes. These structures could potentially serve as thin bioactive, biomimetic, multifunctional, and biodegradable scaffolds for a variety of applications in tissue engineering and regenerative medicine.

References

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Figures

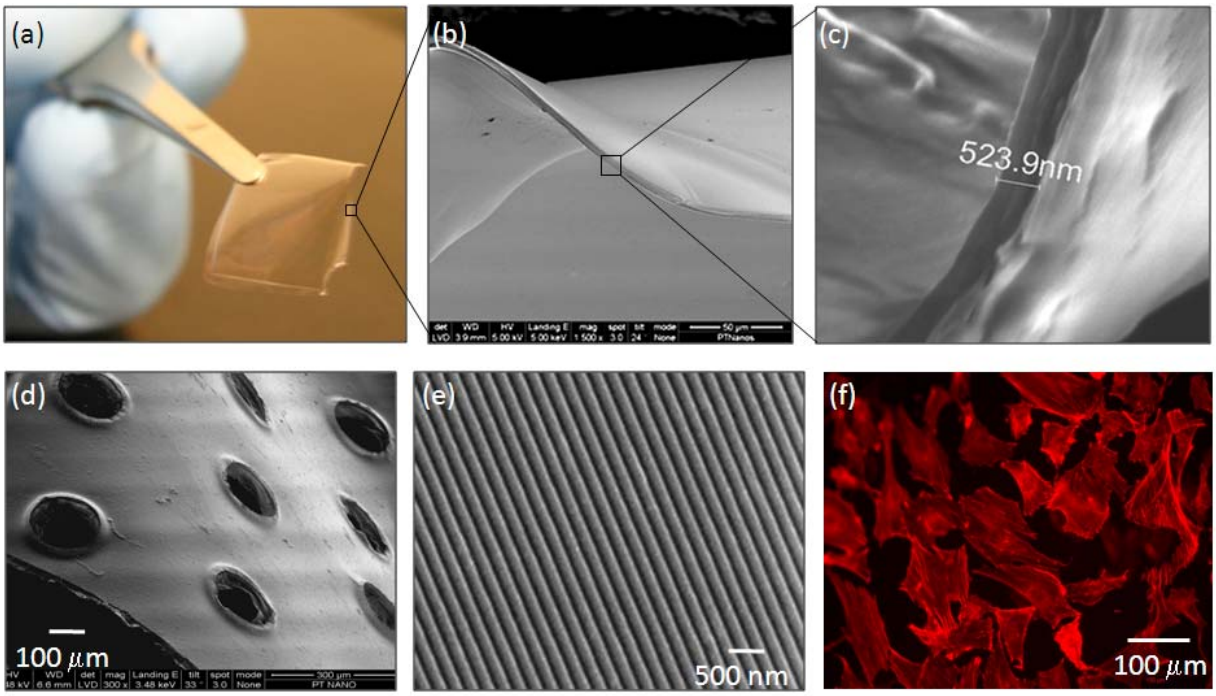

Figure 1: (a-c) Self-sustained membrane scaffolds exhibiting a controlled thickness. The membranes are made from Elastin-like Polymers (ELP) comprising the RGDS epitope to promote cell adhesion and a variety of topographical and structural components including (d) pores and (e) micro/nanotopographies that can be designed to provide biochemical and physical stimuli to (f) mesenchymal stem cells.