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Engineering membrane scaffolds with both physical and biomolecular signaling

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ABSTRACT

We report on the combination of a top-down and bottom-up approach to develop thin bioactive membrane scaffolds based on functional elastin-like polymers (ELPs). Our strategy combines ELP cross-linking and assembly, and a variety of standard and novel micro/nanofabrication techniques to create self-supporting membranes down to ~500 nm thick that incorporate both physical and biomolecular signals, which can be easily tailored for a specific application. In this study we used an ELP that included the cell-binding motif arginine–glycine–aspartic acid–serine (RGDS). Furthermore, fabrication processes were developed to create membranes that exhibited topographical patterns with features down to 200 nm in lateral dimensions and up to 10 μm in height on either one or both sides, uniform and well-defined pores, or multiple ELP layers. A variety of processing parameters were tested in order to optimize membrane fabrication, including ELP and cross-linker concentration, temperature, reaction time and ambient humidity. Membrane micro/nanopatterning, swelling and stiffness were characterized by atomic force microscopy, nanoindentation tests and scanning electron microscopy. Upon immersion in phosphate-buffered saline and an increase in temperature from 25 to 40 °C, membranes exhibited a significant increase in surface stiffness, with the reduced Young's modulus increasing with temperature. Finally, rat mesenchymal stem cells were cultured on thin RGDS-containing membranes, which allowed cell adhesion, qualitatively enhanced spreading compared to membranes without RGDS epitopes and permitted proliferation. Furthermore, cell morphology was drastically affected by topographical patterns on the surface of the membranes.

1. Introduction

Within many tissue engineering and regenerative medicine approaches, the role of the scaffold is essential and continues to increase in importance primarily because of our growing ability to create materials with high precision and bioactivity [1–3]. In vivo, scaffolds may serve as smart matrices for minimally invasive surgeries [4]; constructs that recruit, support and guide cell growth [5]; or materials that provide both structure and delivery of bioactive molecules [1]. Likewise, biomimetic structures that recreate in vitro the complex natural environment have tremendous implications for the design of novel therapies [2], drugs [6] and tissue engineering scaffolds [7], while decreasing the need for in vivo testing. Nonetheless, while significant advances have been made, there is still great interest in improving the complexity of current scaffolds, primarily because the in vivo environment is likely to benefit from the orchestrated presentation of a mixture of physical and biomolecular signals with spatio-temporal control [8].

It is well known that scaffolds exhibiting precise biomolecular signals, such as peptides, proteins and growth factors, are able to elicit particular biological responses. Peptides, such as arginine–glycine–aspartic acid–serine (RGDS) [9], IKVAV [10] or AL-KRQGRTLYGFGG [11], have been incorporated within scaffolds and shown to directly promote cell adhesion, neuronal differentiation and bone cell growth, respectively. Furthermore, they can be used to recruit other bioactive signaling molecules, such as heparin [12] and growth factors. Physical properties have also been shown to affect and even direct cell behaviors and biological processes both in vitro and in vivo. For example, topographical patterns have been used to facilitate cell adhesion [13], direct cell migration [14], enhance cell proliferation [15] and control cell differentiation [16]. Recent work by Engler and co-workers [17] has generated widespread agreement that surface stiffness can also be used to affect cell phenotype. Other physical properties, such as scaffold porosity [18] and permeability [19], are also known to play a key role in modulating and guiding cell growth. In vivo, the mechanisms that

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