Selective cell patterning on photoactive electrospun meshes

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Statement of Purpose: The development of 3-dimensional (3D) constructs for high throughput chemotherapeutic drug screening is necessary for the rapid discovery of novel therapeutic agents.[1] We have developed a polymeric non-woven electrospun nanofiber mesh that transitions from being hydrophobic (135° water contact angle) to hydrophilic (0°) after ultraviolet (UV) light exposure (365 nm) for the purposes of pattering an MCF7 breast cancer cell line on the hydrophilic regions of the mesh.[2]

Methods: Nonwoven electrospun 7:3 poly(ε-caprolactone) (PCL) (70,000 to 90,000 g/mol) to poly(glycerol 12-(1-(2-nitrophenyl)ethoxy)-12-oxododecanoic acid-co-caprolactone) (PGC-C12-NPE; 20,127 g/mol; PDI=1.63) copolymer meshes were spun using a modified electrospinning procedure for PCL alone.[3, 4] The UV activated deprotection of our PGC-C12-NPE polymer was analyzed by exposing the electrospun meshes (~10 mg) to various doses of UV light and observing the effects on the H-NMR spectrum of the dissolved polymer (Varian 400 MHz VNMRS) and the water contact angle (Kruss DSA100 contact angle goniometer) of the mesh. A 1.59 mm in diameter photomask was used to selectively expose a region of the mesh to UV light to create a hydrophilic deprotected island surrounded by hydrophobic protected polymer. MCF7 cells were applied to meshes with and without UV light exposure (365 nm, 23.04 J/cm^2) and cultured for 24 hours (37°C, 5% CO_2, humidified incubator). A CellTracker ™ green live cell dye was used to detect living cells on the meshes by imaging with a confocal microscope (Leica DMI6000 B) and a 488 nm laser with a Chroma ET bandpass 525/50 filter to capture the 529 nm wavelength emission from the fluorescent cell tracker dye.

Results: The deprotection of the 1-(2-nitrophenyl) ethyl (NPE) photoactive protecting group results in the exposure of a carboxylic acid group on each side chain within the 7:3 PCL:PGC-C12-NPE polymeric meshes. The groups are cleaved exponentially with increasing UV (365 nm) dose (J/cm^2) as illustrated in Figure 1. The deprotection was correlated with a transformation from a hydrophobic (135°) to hydrophilic (0°) water contact angle on the surface of the meshes (Figure 1). Using a 1.59 mm in diameter photomask to create hydrophilic islands within the hydrophobic meshes resulted in MCF7 cell patterning within the hydrophilic islands (Figure 2 (right)) and MCF7 repulsion from the unexposed hydrophobic regions (Figure 2 (left)).

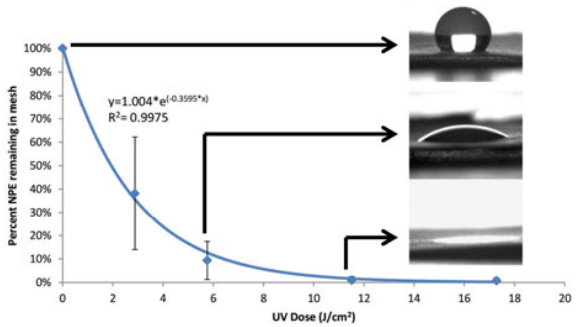

Figure 1: UV activated deprotection of the NPE protecting group occurs exponentially with the dose of UV energy (J/cm^2). The NPE deprotection results in a transformation from hydrophobic (135°) to hydrophilic (0°) water contact angles. (Avg±SD, n=3)

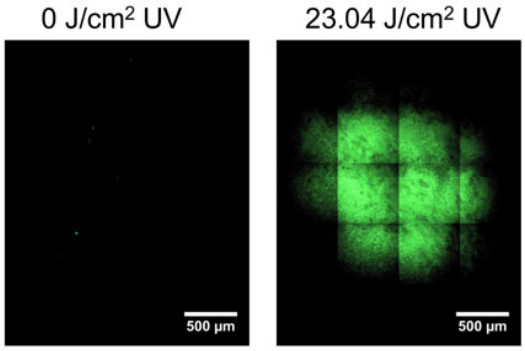

Figure 2: Effects of UV exposure on cell adhesion to the meshes after a 24 incubation. The meshes were exposed to 0 (left) or 23.04 J/cm^2 (right) of UV light through a 1.59 mm in diameter photomask. Cells were stained with a live CellTracker ™ green fluorescent dye. (Representative z-slice from n=3 meshes)

Conclusions: We successfully created a 3D photoactive polymeric mesh that transforms from a hydrophobic to hydrophilic mesh when exposed to UV light. The change in hydrophobicity affected MCF7 binding such that the cells were patterned into a circular shape, conforming to the dimensions of a photomask used to create the hydrophilic island within the hydrophobic mesh. The selective binding is presumably due to more favorable protein adsorption to the hydrophilic regions of the meshes compared to the hydrophobic regions. Currently, the pore size and lack of chemical stimuli prevent the MCF7 cells from penetrating deep into the meshes. Future work will investigate the mechanism of the selective cell binding and improve this technique to create more controlled 3D cell patterns for high throughput drug discovery.

References:

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