Electrospun Photoclickble Thio-ene Poly(ethylene glycol) Hydrogel Microarrays for Stem Cell Fate Optimization

Sadhana Sharma[1], Michael Floren[2,3], Wei Tan[2], Stephanie Bryant[1]
[1]University of Colorado, Department of Chemical and Biological Engineering, Boulder, CO. 80309
[2]University of Colorado, Department of Mechanical Engineering, Boulder, CO. 80309
[3]University of Trento, Department of Material Science & Industrial Technologies, Trento, TN. ITALY

Statement of Purpose: Several recent studies have demonstrated that cellular microenvironments such as ligand-activated cell-matrix interactions and/or matrix physical properties such as elasticity and geometry have significant role in directing the differentiation processes in stem cells (Lutolf, 2009; Wingate, 2012). Microarrays have emerged as an important tool for studying stem cell processes in a high-throughput manner (Gobba, 2011). Nevertheless, most of the existing ECM arrays being either 2-D or shallow 3-D are not able to capture the effects of biophysical and chemical cues on stem-cell fate completely. Here, we report the development of a microarray platform based on electrospun nanofibrous photoclickable thiol-ene poly(ethylene glycol) hydrogels. Thiol- ene polymerizations proceed by an orthogonal, step-growth mechanism where one thiol reacts with one ene leading to a highly homogenous distribution in crosslinks, thus imparting a good control over substrate elasticity (Hoyle, 2010). Furthermore, it allows for the subsequent modification of the already prepared electrospun hydrogel substrates with ECM molecules such as peptides with high reactivity and specificity.

Methods: *Preparation and characterization of electrospun thiol-ene hydrogel platform:* Four-arm Poly(ethylene glycol) norbornene (PEGNB; MW: 5 kDa) was prepared as described elsewhere (Roberts, 2013). PEGNB (5-10 wt%), polyethylene dithiol (1 kDa; thiol: ene = 0.9), poly(ethylene oxide) (3-7 wt%; MW: 400 kDa), and Irgacure 2959 (0.1 wt%) were dissolved in DI water. Electrospun hydrogels were prepared by using a custom set up using a 14-mm syringe at 30 kV. Needle-to-collector distance (20- 26 cm) and flow rate (0.4 – 1.2 ml/hr) were varied as desired. Electrospun fibers were collecled on a glass slide (25mm X 75mm) previously modified with 3-(mercaptopropyl) triethoxysilane. Substrates were subsequently exposed to UV (352 nm light) with an average intensity of 5 mW/cm^2 for specific time points. Scanning electron microscopy was used to examine the microstructure of the electrospun hydrogel substrates in both dry and hydrated states. For hydrating, samples were soaked in deionized water for 1 or 24 hours. Hydrated samples were shock frozen in liquid nitrogen and lyophilized for 48 hours for SEM imaging. Image J was used to measure fiber diameter. Elastic properties of the electrospun hydrogels were characterized using parallel plate rheometry. *Microarray printing:* Microarrays were prepared using a 2470 Aushon arrayer. A printing buffer consisting of 1% glycerol and 0.2% Triton X-100 was utilized for Alexa Fluor® 488 or Alexa Fluor® 546 -C_5 maleimide printing. Prepared microarrays were stored at 4°C in a humid environment for 24 hours before confocal imaging.

Results: In this study, we developed electrospun hydrogel platform using thiol-ene chemistry. Fig 1A shows the fabrication process. The diameter of the electrospun nanofibers ranged from 200-600 nm in the dry state. There was a 2 to 4-fold increase in fiber diameter when substrates were soaked and imaged after lyophilization. The elastic modulus of the substrates ranged from 1- 5 kPa. Fig 1B shows the schematic of combinatorial printing of ECM molecules on our substrates. As a proof of concept, we have demonstrated that Alexa Fluor® 488 and Alexa Fluor® 546 -C_5 maleimide dyes can be selectively printed with high specificity (Fig 1C). To this end, our studies indicate that our platform is highly tunable and we can create substrates with different elastic properties by varying the molecular weight, weight %, and thiol: ene ratio. Further studies will be conducted to achieve substrates with higher elastic modulus to cover the entire range of elasticity, and printing ECM molecules relevant for stem cell differentiation.

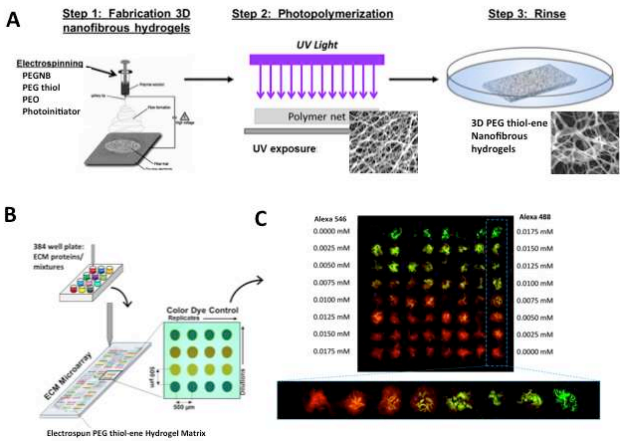

Figure 1. (A) Schematic of the fabrication of 3D-electrospun nanofibrous thiol-ene PEG hydrogels (with SEM images- dry and wet scaffolds), (B) Schematic of microarray preparation by combinatorial printing of ECM molecules on the substrates, (C) Representative example showing microarray with varying concentrations of Alexa Fluor® 488 and Alexa Fluor® 546 -C_5 Maleimide.

Conclusions: We have developed a highly tunable platform with 3-D nanofibrous hydrogels to facilitate high-throughput combinatorial screening of engineered microenvironments for optimizing stem cell differentiation.

References: Gobaa S., Hoehnel S., Roccio M., Negro A., Kobel S., Lutolf M.P., *Nat Methods* 2011, 8(11), 949-55.
Hoyle C.E., Bowman C.N., *Angew Chem Int Ed Engl.* 2010, 49(9), 1540-73.
Lutolf M.P., Gilbert P.M., Blau H.M., *Nature* 2009, 462 (7272), 433-41.
Roberts J.J., Bryant S.J., *Biomaterials* 2013, 34(38), 9969-79.
Wingate K., Bonani W., Tan Y., Bryant S.J., Tan W., *Acta Biomater.* 2012, 8(4), 1440-9.