Developing electrospun meshes to model the lymph node stromal cell network

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Statement of Purpose: As a secondary lymphoid organ, the lymph node (LN) mediates adaptive immune responses to specific antigens.[1] LN function is highly dependent on its architecture, with controlled cellular organization into discrete niches guiding the direction and magnitude of immune responses.[2] Developing cellular models that replicate the LN microenvironment will be beneficial for investigations into how LN functions are modulated by immune events such as vaccination and infection. In the LN, stromal cells form networks and secrete chemokines which physically support and chemically direct immune cell migration respectively.[2] Existing models of lymphoid organs use bulk hydrogels[3] where the stromal network forms spontaneously, thus leaving little room for tuning stroma morphology to better mimic *in vivo* conditions. Electrospinning is a tool of choice for developing tissue engineering scaffolds owing to the user control it offers over mesh properties.[4] In addition, the morphology of electrospun meshes bears a structural resemblance to the porous and open nature of the LN architecture. Here, we have cultured human LN stromal cells on electrospun meshes as a first step towards developing an artificial stromal network to study cellular interactions in the LN.

Methods: Polyethylene glycol-norbornene (PEG-NB) meshes were electrospun from a solution of 8-arm PEG-NB (~20 kDa, 10% w/v), polyethylene oxide (5% w/v), Irgacure 2959 (0.05% w/v) and 0.6 [thiol]:[norbornene] stoichiometric ratio (dithiothreitol). Spinning conditions were: 16G needle, collection distance of 15 cm, flow rate of 0.8 mL/hr, 10-14 kV positive voltage (applied to needle) and 6 kV negative voltage (applied to collector). Fibers were collected for 30 min and crosslinked with 365 nm light (10 mW/cm^2 intensity) under a nitrogen atmosphere for 15 min. Meshes were tagged with a thiolated carboxyfluorescein peptide (100 µM), irradiated at 10 mW/cm^2 for 2 min and imaged using a Leica DMi8 widefield microscope to visualize mesh morphology. To enhance cell attachment, 1 mM thiolated RGD (GCGYGRGDSPG) was conjugated to the fibers by crosslinking at 10 mW/cm^2 for 2 min. Alternatively, meshes were modified with poly-L-lysine (PLL) by incubating plasma treated meshes in PLL solution (0.1 mg/mL) overnight at 37 °C. Meshes were sterilized with UV light radiation for 1 hour prior to cell seeding.

The stromal cells used were primary human lymphatic fibroblasts (ScienCell) maintained in fibroblast media supplemented with 2% fetal bovine serum (FBS), fibroblast growth factor and Pen/Strep. Cell viability was assessed with the lactate dehydrogenase (LDH) assay after 4 days of culture. Impact of fiber alignment on cell adhesion was investigated by comparing stromal cells cultured on random and aligned meshes after 7 days. Aligned meshes were generated by spinning meshes onto a rotating mandrel (350 rpm). Cell adhesion on the scaffolds was visualized using a Zeiss AxioZoom microscope.

Results: Fluorescein labelling revealed the intricate fiber networks in the electrospun PEG-NB meshes (Fig. 1a). To assess the impact of mesh surface chemistry on cell adhesion and viability, we cultured stromal cells on PEG-NB/RGD and PEG-NB/PLL meshes. Cells seeded on PLL meshes (Fig. 1b) were clumped and had fewer branches compared to cells seeded on RGD meshes, which spread well (Fig. 1c). After 4-day culture, we measured cell viability and found that LDH release from cells seeded on either mesh was minimal and comparable to the 2D well controls, indicating that the cells were viable (Fig. 1d). Next, we cultured cells on randomly spun or aligned meshes to test the effect of fiber orientation on cell adhesion. Stromal cells seeded on randomly spun meshes (Fig. 1e) spread in a random fashion while cells seeded on aligned meshes (Fig. 1f) align with topography, highlighting the importance of interplay of nanofibrous cues and cellular architectures in models of the LN.

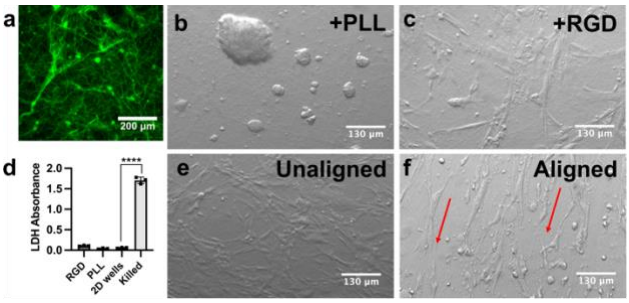

Figure 1. Electrospun mesh characterization, stromal cell adhesion and viability on meshes. (a) Fluorescein labelled meshes (green) showing the network of fibers, scale bar = 200 µm**.** Brightfield images of stromal cells on PEG-NB/PLL (b) and RGD (c) meshes, scale bar = 130 µm. (d) Stromal cell viability from the LDH assay after 4-day culture on PEG-NB meshes compared to live controls in tissue culture plates (2D wells). Low signal indicates better viability. 1-way ANOVA, ****p<0.0001. Brightfield images of stromal cells on random (e) and aligned meshes (f). Red arrows indicate direction of alignment.

Conclusions: We have established the basis for the first model that recapitulates the reticular network of the LN *in vitro* using electrospun PEG-NB hydrogel meshes. Stromal cell adhesion was enhanced on RGD-functionalized meshes and dictated by mesh alignment. Cell viability on the electrospun meshes was similar to that of controls in 2D wells. Future work will explore co-culture of lymphocytes and stromal cells towards building a more biomimetic LN model for mechanistic studies.

References:

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