Particle hydrogels lead to dramatic decrease in gliosis and promote NPC migration after stroke

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Statement of Purpose: The extracellular matrix (ECM) provides tissues with the mechanical support, space, and bioactive signals needed for homeostasis or tissue repair after wounding or disease. Hydrogel based scaffolds that can match the bulk mechanical properties of the target tissue have been extensively explored as ECM mimics. Although the addition of microporosity to hydrogel scaffolds has been shown to enhance cell/tissue-material integration, the introduction of microporosity often involves harsh chemical methods, which limit bioactive signal incorporation and injectability. Particle hydrogels are an emerging platform to generate *in situ* forming microporous scaffolds[1,2]. In this approach, microgel (μgel) particles are annealed to each other to form a bulk scaffold that is porous due to the void space left by the packed μgels. We hypothesized that using particle hydrogels for brain repair after stroke would decouple hydrogel degradation and cellular infiltration, leading to a decreased inflammatory response and overall less cytotoxic environment.

Methods: Particle hydrogels were produced using a microfluidic water-in-oil emulsion. An acrylate functionalized hyaluronic acid backbone was mixed with an enzymatically cleavable dithiol cross-linker and quickly partitioned into μgel building blocks. The particle hydrogels were purified by repeated centrifugation and washing with PBS buffer. μgel building blocks anneal into a scaffold with addition of the activated enzyme, factor XIII. A middle cerebral artery occlusion (MCAo) stroke model in mice was used. Particle hydrogels were injected 5 days post stroke and samples were collected 10 days post hydrogel injection (Fig. 1A). Particle hydrogels were compared to injection of nano-porous hydrogel and the sham condition of no injection.

Results: Through atomic fluorescent microscopy (AFM) and instron mechanical testing we demonstrated that the local scaffold stiffness and overall scaffold stiffness are statistically similar to the stiffness of the cerebral cortex (~1000Pa). Further, with microfluidics we are able to impose a stringent control on the physical homogeneity of the individual μgels. Moreover, through diffusion of fluorescently labeled high molecular weight dextran we are able to confirm interconnected porosity. After 10 days *in vivo,* analysis of the brain's inflammatory response was done by assessing the astrogliosis and microgliosis (Fig 1B). In the porous hydrogel, the astrocytic scar was observed to be drastically decreased when compared to the nano-porous hydrogel and sham condition (43μm vs 234μm and 325μm, respectively). Similarly, the percent of microglia occupying the infarct area was found to be dramatically reduced in porous hydrogel than the nano-porous hydrogel and sham (19% vs 58% and 50%, respectively). The vasculature of all three conditions was analyzed by staining for GLUT1. Although no vascular infiltration into the infarct was observed for any of the conditions, we did observe more vascularization in the peri-infarct area of the porous hydrogel condition (22%) than the other two conditions (both 6%). To assess the potential for neural repair we analyzed the NPC migration to the infarct and we observed DCX and Nestin positive NPCs present in the porous scaffold (Fig 1C). To our knowledge this is the first report of NPCs migrating all the way to and infiltrating into the infarct. Very little migration of NPCs was seen in the nano-porous hydrogel condition and the sham condition. Although NPCs were present in the porous hydrogel, there were no mature neurons present in the hydrogel 10 days post injection.

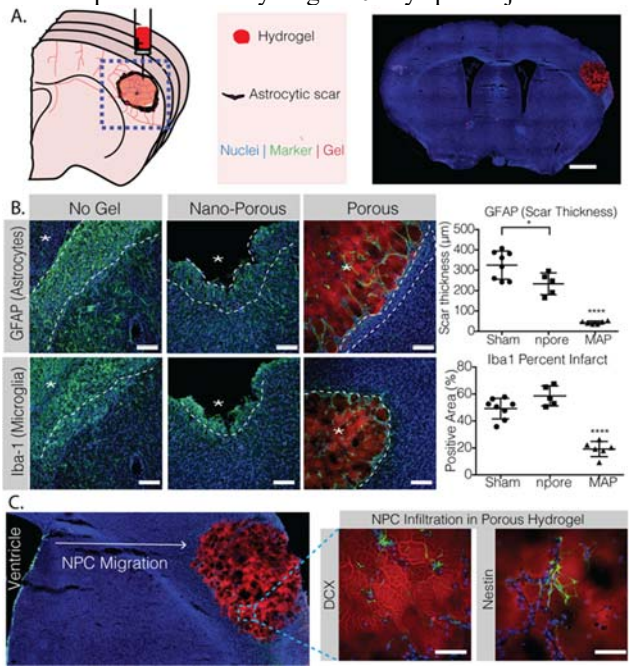

Figure 1. A) Schematic of hydrogel injection into stroke cavity and full section image with porous hydrogel (scale: 500μm). B) Decreased astrocytic scar and gliosis in porous hydrogel condition compared to nano-porous and sham (scale: 100μm). C) NPC migration from ipsilateral ventricle into porous hydrogel. DCX and Nestin positive NPCs observed in the porous hydrogel (scale: 50μm).

Conclusions: Particle hydrogels are capable of decreasing the inflammatory response, increasing the vasculature, and promoting NPC migration in brain post stroke compared to chemically identical non-porous hydrogels. Particle hydrogels are injectable, display interconnected porosity, and can be tuned to match tissue stiffness.

References:

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