Bioactive Microparticle Assembly in Microchanneled Hydrogel for Guided Vascular Network Formation

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Statement of Purpose: Controlling spatial organization of drug-releasing microparticles in a three-dimensional matrix has been long sought to guide the growth direction and spacing of tubular networks; however, it still remains a grand challenge. This study demonstrates that a simple uniaxial freeze drying of a hydrogel loaded with microparticles temporally increase freezing-induced shear stress on and subsequently align microparticles in resulting microchannels, similar to the glacier-induced moraine formation. This nature-inspired process with the hydrogel loaded with poly(lactide-co-glycolic acid) (PLGA) microparticles releasing vascular endothelial growth factor (VEGF) resulted in a material that stimulated vascular growth into microchannels and further recovered perfusion in an ischemic hindlimb. We believe this process would be broadly useful in modifying the microstructure and properties of various hydrogel systems.

Methods: RGD-Alginate (Mw ~ 250,000 g/mol) sterilized via filtration was dissolved in 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) buffer at a concentration of 2 %(w/v). The alginate solution was sequentially mixed with PLGA microparticles, 1-hydroxybenzotriazole (Hobt), 1-ethyl-3-(3-dimethylaminopropyl) carodimide (EDC) and adipic acid dihydrazide (AAD). PLGA microparticles were encapsulated with VEGF via double emulsification. The pre-gelled mixture was cured, and incubated in DI water at room temperature for 12 hrs. Then, the microchanneled cryogel was created by placing the gel disk on top of a copper plate with controlled temperatures. The frozen sample was freeze-dried to introduce microchannels through the gel matrix. In contrast, the microporous gel was prepared via sequential freezing of the alginate gel in a copper container, lyophilization, and rehydration.

Results: The uniaxial freeze-drying of the hydrogel resulted in PLGA microparticles aligned with the anisotropically aligned microchannels, as confirmed with scanning electron microscope (SEM) image (Figure 1). PLGA microparticles were also partially embedded in the channel wall (Figure 1a-2). In contrast, the isotropic freeze-drying of the gel kept random distribution of PLGA microparticles in the resulting cryogel with poorly interconnected micropores (Figure 1b-1). In addition, microparticles were mostly separated from the cryogel matrix (Figure 1b-2).

According to the analysis of VEGF released from a hydrogel, the microchanneled gel released more VEGF than the microporous gel, because of the higher level of particle retention and superior permeability.

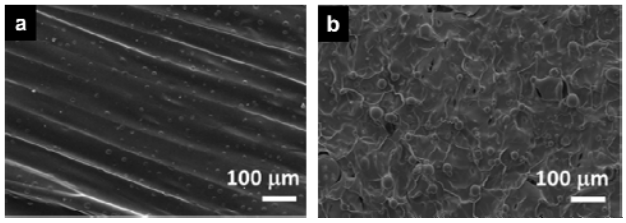

Figure 1. SEM images of PLGA microparticle distribution in the alginate hydrogels prepared by uniaxial freezing (a) and isotropic freezing (b).

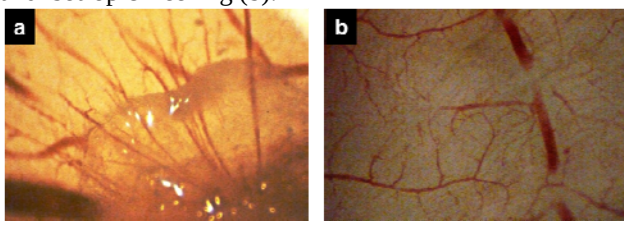

Figure 2. Optical top-view images of vascular networks formed through and around microchanneld (a) and microporous (b) hydrogel matrix implanted on Chick Chorioallantoic Membrane (CAM).

Therefore, the gel with microchannels aligned with VEGF-releasing microparticles significantly enhanced vascularization through the gel matrix (Figure 2). In addition, the gel implanted in an ischemic hindlimb improved perfusion recovery compared with the microporous cryogel in which VEGF-releasing microparticles were randomly distributed.

Conclusions: Our results demonstrate that drug-releasing microparticles can be spatially organized in a microchanneled hydrogel by uniaxial freeze-drying process. The resulting hydrogel was advantageous in controlling VEGF release into microchannels, thus promoting vascularization through microchannels and perfusion recovery in ischemic tissue. Overall, the fabrication process developed in this study will greatly contribute to taking both fundamental biological studies and clinical treatments to the next level.

References: Adv. Healthcare Mater. 2014, DOI: 10.1002/adhm.201400153.