Assembly of PEG Microgels into Porous Cell-Instructive 3D Scaffolds via Thiol-Ene Click Chemistry

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Statement of Purpose: Hydrogels are of broad interest for tissue engineering because their polymer networks provide 3D support to cells that mimics the native tissue environment. Synthetic hydrogels presenting tissue microenvironmental cues, such as bioactive molecules, topography, and substrate elasticity, have been proposed as scaffolds that recapitulate cell-instructive features of the natural extracellular matrix (ECM). However, an important and often overlooked challenge in developing 3D cell-instructive hydrogels is that conventional hydrogels are porous on the nanoscale and, therefore, constitute a restrictive barrier that must be degraded to permit cell spreading, migration, and deposition of new ECM. These crucial cellular processes can be decoupled from degradation by introducing microporosity, and using microgels as building blocks for microporous hydrogels is a particularly promising strategy. Here, we introduce a microporous hydrogel platform prepared by photopolymerizing electrosprayed poly(ethylene glycol) (PEG) microgels and aim to dictate cellular behaviors in a 3D milieu independently from scaffold degradation.

Methods: Multifunctional PEG-norbornene (PEG-Nb, 4-arm) and PEG-dithiol (PEG-DT, 3,400 Da) were used to fabricate microgels via thiol-ene photopolymerization and submerged electrospraying. Cell adhesive peptide, CGRGDS, was also incorporated in the microgels utilizing the thiol groups of cysteine. The ratio of thiol to norbornene groups was controlled to 0.75:1 so that there would be free norbornene groups available for subsequent photopolymerization of the microgels into 3D scaffolds. Different molecular weights of PEG-NB (5 and 20 kDa) were used to tune the stiffness of microgels. The size of resulted microgels was 200 μm on average. As-prepared microgels were added into a mold with PEG-DT and photoinitiator and assembled into microporous scaffolds via photopolymerization. Human mensenchymal stem cells (hMSCs) were incorporated during the assembly process for 3D cell encapsulation.

Results: The morphology and inner structure of the scaffolds was characterized by confocal microscopy. A 3D rendering image of fluorescent-labeled microgel scaffolds indicated that microgels were successfully linked together and revealed inner pores that were several hundred microns in size (Fig. 1a). Incubating the scaffolds with high molecular weight fluorescent-labeled dextran verified pore interconnectivity (Fig. 1b).

Following the characterization of scaffold inner structure and microporosity, we seeded hMSCs into microgel scaffolds with varying stiffness. It is well understood that increasing matrix stiffness in 2D culture can induce enhanced cell spreading and mechanosensing. For 3D culture in conventional hydrogels, however, this spreading trend with stiffness is opposite because the higher crosslinking density leads to more restrictive environment for cells to spread. In our microgel scaffolds, we found that hMSCs spread well along the surface of microgels in PEG5 scaffolds (36 kPa) after 72h culture, while they stay rounded in PEG20 scaffolds (8 kPa) (Fig. 1c). We also evaluated hMSC mechanosensing in the scaffolds by immunostaining for Yes-associated protein (YAP) after 72h culture. YAP relative nuclear intensity was significantly higher for hMSCs cultured in PEG5 scaffolds than in PEG20 scaffolds (Fig. 1d,e). Due to the 3D permissive environment provided in microgel scaffolds, we achieved comparable spreading and mechanotransduction results to what has been observed in 2D culture. This is an important step forward in the development of 3D cell-instructive scaffolds.

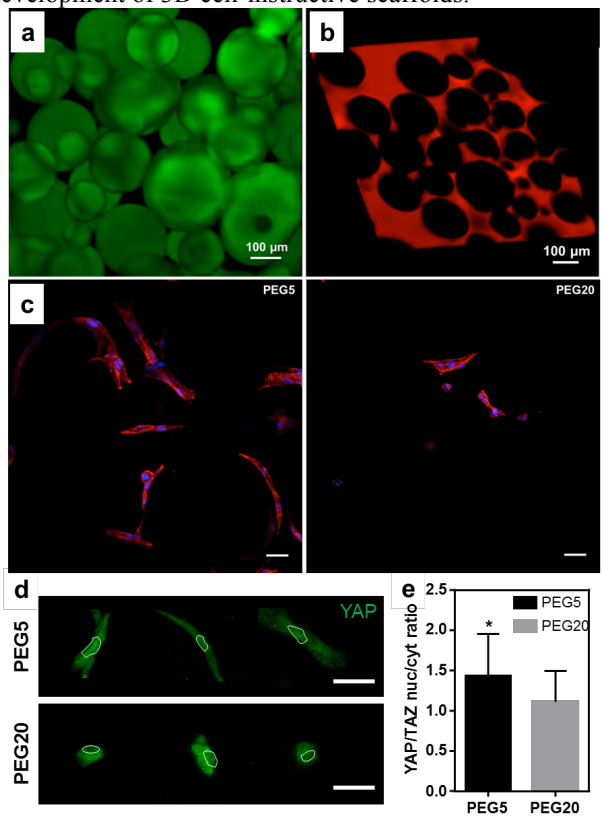

Figure 1 – a) Z-stack image of a PEG5 microgel scaffold labeled with Alexa Fluor 488-succinimidyl ester (top-down view of a 3D reconstruction); b) Inner pore structure of microgel scaffolds visualized by fluorescent-labeled dextran; c) hMSC spreading within PEG5 and PEG20 microgel scaffolds at 72h. Red represents F-actin staining and blue represents nucleus; d) Representative images of hMSC YAP nuclear localization; e) Quantification of YAP nuclear/cytosolic ratio. The scale bar is 50 μm. *: $P < 0.05$

Conclusions: 3D porous hydrogel scaffolds were prepared by assembling PEG microgels via thiol-ene photopolymerization. The interconnected micropores provide a permissive environment allowing cellular processes, such as spreading and mechanosensing. Future studies will investigate the use of these scaffolds as cell-instructive materials for hMSC delivery and bone tissue engineering.