Hierarchical macroporous multifunctional hydrogel formation via thermally-induced phase separation for neuronal regeneration

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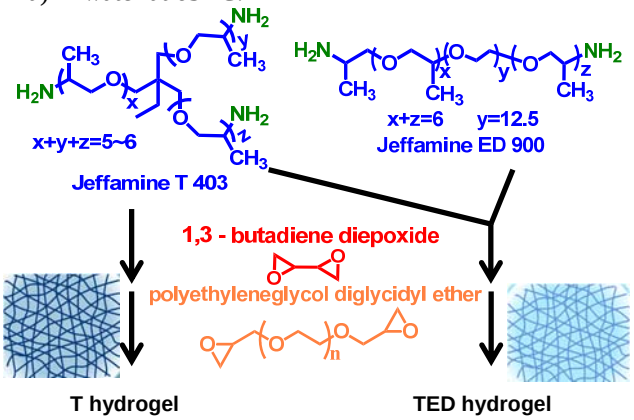
Statement of Purpose

Macroporous hydrogels with hierarchical structure show immense potentials for tissue engineering. Recently, there are increasing interests of integrating additional characteristics into hydrogels to expand their functional roles. In the context of neural regeneration, it is desirable to have hydrogels not only support neuron adhesion and neurite extension, but also capable of neural protection against hostile pathological surroundings. Toward this goal, we explored the amine-epoxy ring opening reaction to form thermal-responsive polymers and their subsequent use to prepare macroporous and multifunctional hydrogels.

Materials and Methods

A pre-polymer was first synthesized via step growth polymerization of Jeffamines and 1,3-butadiene diepoxide in aqueous solution (Scheme 1). The hydrogels were then prepared by reacting the pre-polymer with polyethyleneglycol diglycidyl ether (PEGDE) (Mn=500 Da) in water at 65 °C.



Scheme 1. Monomers for T hydrogel and TED hydrogel preparation.

The micro-morphology of the hydrogels was examined via scanning electron microscopy (SEM) and fluorescent microscopy. A stress-controlled rheometer was used to study the mechanical properties. The swelling behaviors were examined via monitoring both mass and volume changes. Cytotoxicity of the hydrogel against NIH3T3 fibroblasts was examined using a direct contact assay coupled with WST-1 evaluation. Chick cortical neurons were seeded on the hydrogels and cell growth was examined via calcein AM fluorescent staining.

Results and Discussion

Loading of Nile red dye into the hydrogel allowed visualization of the porous microstructure in a hydrated state (Fig. 1a). It also implied the feasibility to encapsulate hydrophobic neuroprotective drug molecules. The interconnected, particulate-bearing structure of the hydrogel was confirmed by SEM (Fig. 1a). To tune the hydrogel properties, Jeffamine ED 900 was introduced. With about 12 ethylene oxide repeating units, the aforementioned monomer considerably extended the distance between neighboring amine groups and therefore lead to a reduction of the crosslink density. The obtained TED hydrogel showed a storage modulus G′ of 200 Pa, nearly 15 times lower than that of the T hydrogel (Fig. 1b). The equilibrium swelling ratio for T hydrogel was around 6, while that for the TED hydrogel reached 16 (Fig. 1c). Both increase in softness and swelling ratio indicated the decrease of crosslink density. Direct contact assay showed that the cytocompatibility of T hydrogel was comparable with that of the negative PTFE film control (Fig. 1d). In addition, neuron adhesion and neurite extension was observed from neurons cultured on the hydrogels, warranting further study of these hydrogels as scaffolds for neural regeneration.

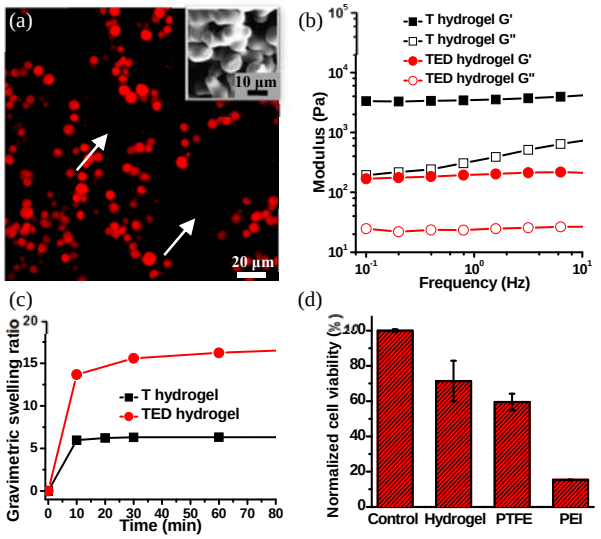


Figure 1. (a) Fluorescent and SEM (inset) images of T hydrogel, (b) frequency sweep of shear modulus and (c) kinetic swelling of T hydrogel and TED hydrogel, and (d) direct contact cell culture results with polytetrafluoroethylene (PTFE) and polyethylenimine (PEI) as negative and positive control, respectively.

Conclusion

The hierarchical macroporous and interconnect hydrogels were successfully fabricated utilizing thermal-induced phase separation phenomenon. Incorporation of a new comonomer to the original T hydrogel formulation resulted in TED hydrogel with a lower modulus and crosslink density. In addition, cytocompatibility of these hydrogels was demonstrated with both fibroblast and neuron cultures.

Acknowledgements

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