Protein-Engineered Two-Component Physical Hydrogels for 3D Stem Cell Encapsulation and Transplantation

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Statement of Purpose: Modular protein engineering design and recombinant DNA technology create monodisperse polymeric proteins that allow functional and structural domains to be encoded directly into the protein backbone, giving rise to biomaterials with tunable mechanical, bio-chemical, and degradation properties. We describe the design and characterization of physical hydrogels consisting of two protein block copolymers that hetero-assemble by specific recognition between two peptide association domains[1]. These hydrogels form by a simple mixing procedure (Fig. 1), allowing three-dimensional cell encapsulation at constant physiological conditions. The hydrogels can be dissolved by the addition of competitive-binding peptide domains, facilitating non-enzymatic gel dissolution and recovery of encapsulated cells. In addition, the hydrogels exhibited shear-thinning and rapid post-shear regelation, thus promoting suitability as scaffolds for stem cell injection.

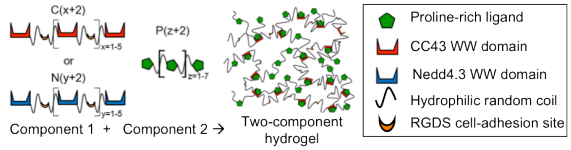

Figure 1. Schematic of the two-component protein hydrogel system.

Methods: The peptide association domains were chosen to be the WW domain and its proline-rich ligand, which interact by hydrogen bonding with a 1:1 stoichiometric ratio. Two WW domain variants (Nedd4.3 and CC43, abbreviated as N and C) that bind to the proline-rich PPxY peptide (abbreviated as P) with an order of magnitude difference in dissociation constant[2] were used to tune the interaction energy between the two hydrogel components. Multiple repeats of the association domains were linked by hydrophilic random coil poly-peptide spacers, creating families of block copolymers with varying domain frequency (repeats per chain) and binding affinity (Fig. 1). RGDS, a cell-binding sequence, was incorporated into the spacer to promote cell adhesion. The DNA constructs of the engineered proteins were cloned and expressed in *Escherichia coli* and purified via affinity chromato-graphy. The molecular weight and composition of purified proteins were verified by mass spectrometry and amino acid analysis, while domain folding and binding interactions were confirmed using circular dichroism and isothermal titration calorimetry. Hydrogels were formed by mixing various amounts of components 1 and 2, and competitive gel dissolution was achieved by adding soluble PPxY peptide. To study shear-thinning and self-healing properties, fully-formed hydrogels were extruded through 26-gauge syringe needles. Gelation, dissolution, and shear-thinning behaviors were studied by microrheology and bulk shear rheology. Murine neural stem cells (NSCs) were encapsulated in the hydrogels and assessed for viability and proliferation by Live/Dead and Picogreen DNA assays and for differentiation by immunocytochemistry.

Results: Microrheology measures material viscoelasticity by analyzing the Brownian motion of embedded tracer particles. In the logarithmic plot of mean square displacement (MSD) versus time, the slope α ranges from 0 for purely elastic to 1 for purely viscous materials. At 5 wt% and above, individually viscous C7 and P9 components underwent sol-to-gel phase transitions into C7:P9 hydrogels, whose viscoelastic properties were governed by the stoichiometric ratios of the binding domains (Figs. 2A and 2B). Lower functionality combinations of C3:P3, C3:P9, and C7:P3 did not form stable hydrogels even at 10 wt%, consistent with the percolation theory of gelation[3]. Substituting the weaker binding N7 component for C7 resulted in more compliant gels, indicated by higher MSD values in microrheology and lower storage modulus in bulk rheology (G'~50 Pa and 9 Pa for 10 wt% C7:P9 and N7:P9, respectively). By virtue of non-covalent crosslinks, fully-formed hydrogels could be dissolved by simple addition of PPxY peptide (Fig. 2C). Moreover, the gels were shear-thinning and self-healing when extruded through a syringe needle, thus supporting injectability (Fig. 2D). NSCs encapsulated in the hydrogels were viable and maintained differentiation potential into neuronal and glial phenotypes (Fig. 2E).

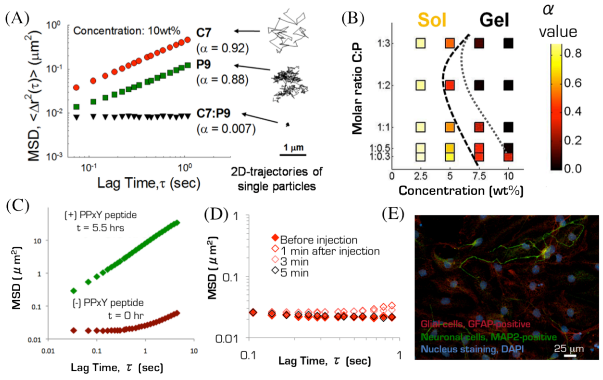

Figure 2. (A-D) Microrheology data of C7:P9 mixtures showing: (A) sol-gel transition; (B) sol-gel phase diagram; (C) gel dissolution by PPxY peptide addition; and (D) shear-thinning and self-healing upon shearing through a needle. (E) Confocal image of encapsulated NSCs in C7:P9 gel differentiated into both neuronal and glial phenotypes (red = glia (GFAP); green = neurons (MAP2); blue = nuclei (DAPI)).

Conclusions: Through rational specifications of domain binding strength and frequency, we designed recombinant protein polymers that hetero-assemble into hydrogels whose viscoelastic properties are tunable at the molecular level. These cyto-compatible hydrogels are shear-thinning and self-healing, thus acting as effective scaffolds for cell encapsulation and transplantation by injection.

References: (1) Wong Po Foo CW, et al. *Proc Natl Acad Sci* (2009) 106(52):22067-72.
(2) Russ WP, et al. *Nature* (2005) 437(7058):579-83.
(3) Semenov AN, et al. *Macromolecules* (1998) 31:1373.

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