# Neural Stem Cell Encapsulation within Hetero-Assembling Protein Hydrogels

Cheryl Wong Po Foo[2], Widya Mulyasasmita[1], Andreina Parisi-Amon[1], Ji Seok Lee[2], Sarah C. Heilshorn[2]

[1]Bioengineering, [2]Materials Science and Engineering, Stanford University, Stanford, CA

**Statement of Purpose:** Physical hydrogels, characterized by transient crosslinks and shear-thinning behavior, are ideal vehicles for minimally invasive cell transplantation. However, the assembly of polymers into physical hydrogels for cell encapsulation primarily relies on external environmental triggers, such as temperature sweeps from 4°C to 37°C for collagen and Matrigel; pH shifts from ~2.5 to 7.4 for PuraMatrix™; and cation concentration increases from 20 to 200 mM for alginate and peptide amphiphiles. In these systems, cells mixed with precursors in the sol phase are momentarily exposed to non-physiological conditions that not only are detrimental to cells and accompanying proteins, but also are difficult to reproduce in clinical settings. To address this, we harness the specific molecular recognition between two naturally evolved peptide domains to design two-component systems that hetero-assemble into physical hydrogels upon mixing at constant physiological conditions. This strategy enables simple cell encapsulation without requiring variations in pH, temperature, or ionic strength. Hydrogels resulting from the hetero-assembly of these two polymer types are collectively termed MITCH (Mixture-Induced Two-Component Hydrogels) (Fig 1). By means of modular specification of the primary amino acid sequence, the binding affinity ($K_d$) and the frequency (number of repeats per chain) of the association domains were precisely tunable. Coupled with simple polymer physics considerations, we demonstrate that such molecular-level design methodology affords hydrogels with controllable bulk viscoelastic properties.

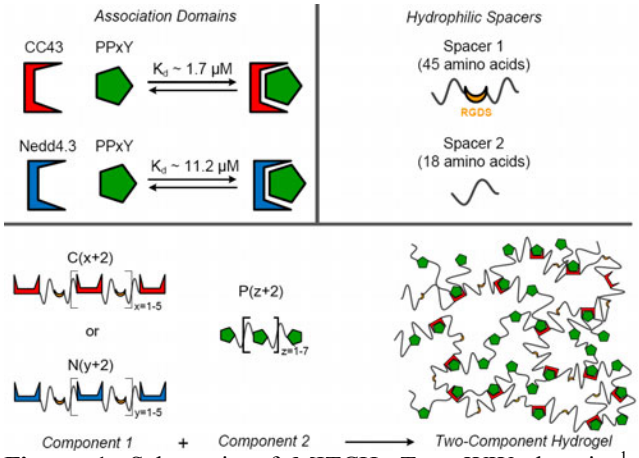


**Figure 1.** Schematic of MITCH. Two WW domains[1] (CC43 and Nedd4.3) bind to the same proline-rich peptide (PPxY). When linked by hydrophilic spacers,[2] the components separately form three protein polymer families: C[x+2], and N[y+2], and P[z+2]. A hydrogel is formed when Component 1 (either C[x+2], and N[y+2]) is mixed with Component 2 (P[z+2]) at constant physiological conditions.

**Methods:** Block-co-poly-peptides were synthesized using recombinant protein expression in *Escherichia coli*. Engineered proteins were purified by affinity column chromatography and assayed by circular dichroism, gel electrophoresis, amino acid analysis, and mass spectrometry. Binding affinity between the hetero-assembling domains was characterized by isothermal titration calorimetry. Hydrogel viscoelasticity (including shear-thinning and gel-recovery) was characterized using micro-rheology (tracking of the diffusive paths of embedded fluorescent microparticles) and oscillatory rheology. Neural stem cells were encapsulated within the hydrogels and assayed for viability (Live/Dead fluorescent staining), proliferation (metabolic assay), and differentiation (confocal microscopy of immunostaining).

**Results:** Circular dichroism and calorimetry verified that the association domains properly fold when fused to hydrophilic random coils on their C- and N-termini. Separately, the individual block-co-poly-peptides exhibit Newtonian fluid behavior and cannot self-assemble; however, upon simple mixing, the two polymers hetero-assemble via hydrogen bonding to form a percolating network at constant physiological pH, temperature, and ionic strength. Gels form within ~10 seconds, are shear-thinning and injectable, and re-form after removal of shear to their original viscoelasticity. The gels exhibit plateau storage moduli, G', in the range of 10-100 Pa, similar to common biological hydrogels such as Matrigel. Rheology measurements demonstrate that tuning the domain association energy and the domain repeat frequency provides a direct link between molecular-level design of the polymeric network and macroscopic material properties. Human endothelial cells, rat mesenchymal stem cells, and rat neural stem cells have been successfully cultured in three-dimensional MITCH gels. Furthermore, MITCH materials promote the growth and differentiation of rat neural stem cells into glial and neuronal phenotypes, with neurites often extending over hundreds of microns.

**Conclusions:** Applying biomimetic design principles and simple polymer physics considerations, we designed physical hydrogels whose viscoelastic properties are tunable through precise molecular-level engineering. These hydrogels form without the use of environmental triggers, are injectable, and are cell-compatible for use in cell encapsulation and cell delivery applications.

**References:**

1. Russ WP, Lowery DM, Mishra P, Yaffe MB, Ranganathan R. Nature 2005, 437:579-583.
2. Petka WA; Harden JL; McGrath KP; Wirtz D; Tirrell DA. Science 1998, 281:389-92.