

Nanoscale Design of Protein Hydrogels for Stem Cell Transplantation

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Abstract – Current physical hydrogels require shifts in environmental conditions (e.g., pH, temperature) to initiate the sol-gel phase transition, which can be damaging to encapsulated cells. In response, we designed a new class of materials: Mixture-Induced Two-Component Hydrogels (MITCH), based on simple polymer physics considerations and biomimicry. MITCH materials are synthesized using protein engineering to create two block-copolymers with nanoscale precision. Upon mixing, nanoscale modules in the two components undergo molecular-recognition via hydrogen bonding to initiate gelation. Gel viscoelasticity is predictably tuned through nanoscale design of the copolymers. Adult neural stem cells encapsulated in MITCH materials undergo proliferation, differentiation, and neurite extension.

The assembly of polymers into physical hydrogels for cell encapsulation has been governed by the use of external triggers. In these systems, cells are mixed with precursor macromolecules in the solution phase under specific environmental conditions. Following this, cells are encapsulated by exposure to a sudden change in pH, temperature, or ionic concentration to induce a solution to gel phase transition either *in vitro* or *in situ*. For example, common triggers for cell encapsulation by physical hydrogels include temperature sweeps from 4°C to 37°C for collagen and Matrigel; pH shifts from 3.0 to 7.4 for PuraMatrix™; and cation concentration increases ranging from 20 to 200 mM for alginate and self-assembled peptide amphiphiles. These triggers can be irreversibly detrimental to the encapsulated cells and accompanying proteins; and furthermore, these environmental conditions can be difficult to reproducibly control in a clinical setting. To address this issue, we utilized the concept of protein-protein interactions between specific nano-domains in nature to design a Mixture-Induced Two-Component Hydrogel (MITCH) system. The two components each contain separate peptide domains that associate together upon mixing under constant physiological conditions. Therefore, the MITCH strategy is tailor-made to encapsulate cells and proteins without subjecting them to variations in pH, temperature, or ionic strength.

The synthesis of these precisely designed polymers was achieved using recombinant protein technology to encode each primary sequence in an exact modular genetic construct. The engineered proteins were expressed in *Escherichia coli* and purified via affinity chromatography. Circular dichroism was employed to verify that the association domains properly fold when fused to hydrophilic spacers on their C- and N-termini. Two separate MITCH systems (named N7:P9 and C7:P9) were synthesized utilizing nano-domains with ten-fold differences in binding affinity. The effective dissociation constants, K_d , of the repeating block-copolymers were measured by isothermal titration calorimetry to be $4.6 \pm 0.01 \mu\text{M}$ and $62 \pm 4.6 \mu\text{M}$, respectively. These assays demonstrate that the modular approach to protein engineering allows for the nanoscale design of families of polymers that physically bind through precise molecular-recognition interactions with tunable association energies.

Separately, the N7, P9, and C7 polymers exhibit Newtonian fluid behavior; however, upon simple mixing of the P9 polymer with either the N7 or C7 polymer, a viscoelastic hydrogel is formed at constant physiological pH, temperature, and ionic strength (5-10 wt%). Gels form within ~10 seconds, are shear-thinning and injectable, and re-form after removal of shear to their original viscoelasticity. Micro-rheology was performed via particle tracking of the Brownian motion of micron-sized fluorospheres embedded within the hydrogel networks. Through rational design of the nanoscale peptide modules, the polymer binding affinity and hence the resulting hydrogel viscoelasticity can be precisely tuned. These experiments provide a direct link between molecular-level design of the polymeric network and macroscopic material properties.

To evaluate the ability of our MITCH systems to encapsulate cells at constant physiological conditions, murine adult neural stem cells were pre-mixed with solutions of N7 or C7 prior to the addition of P9 to induce gelation. Confocal imaging of LIVE/DEAD stained samples showed high cell viability and uniform cell distribution five days after encapsulation, confirming the cytocompatibility of this novel encapsulation method and the lack of cytotoxicity of the MITCH materials. This uniform cell distribution further confirms the fast gelation kinetics of our hydrogels. Immunostaining of the 3D cultures demonstrates that adult neural stem cells maintain their neural multipotency upon encapsulation and that scaffolds can support cell spreading, cell differentiation into neuronal and glial phenotypes, 3D neurite outgrowth, and neurite branching. Due to their nanoscale design and synthesis, the MITCH materials can be easily optimized to further enhance cellular interactions. This novel MITCH encapsulation strategy is expected to improve cell viability during cell transplantation therapies for regenerative medicine.