Docket #: S08-065A

Hetero-assembling, tunable, and injectable hydrogels for cell encapsulation

Stanford researchers have designed a hydrogel system which allows for the easy encapsulation of cells and biomolecules without requiring external changes in environmental conditions or exposure to chemical crosslinkers. This fully biocompatible material is highly tunable and can encapsulate proteins, drugs, and/or cells at constant physiological conditions. Their unique structure makes them easily injectable (either pre- or post-gelation) and promotes maximum diffusion of biomolecules throughout the scaffold, two important criteria for cell encapsulation for both primary cell biology studies as well as cell transplantation for regenerative medicine. After injection, the material rapidly self-heals into a gel, making it suitable for use in cell and drug delivery applications and as a bio-ink for 3D printing of cell-laden structures.

This invention presents two new improved designs to the original Mixing-Induced Two-Component Hydrogel (MITCH) system described in Stanford Docket **S08-065** "Self-assembling, biocompatible hydrogel for cell and drug encapsulation"

Figure

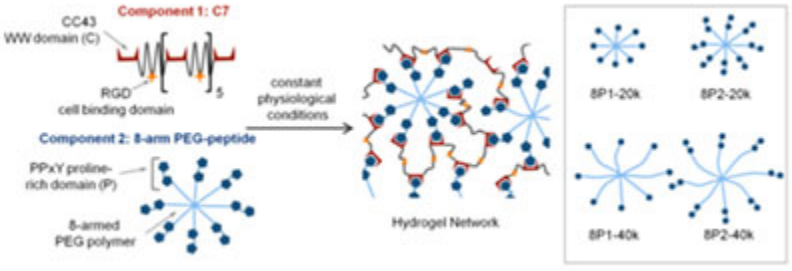

Figure description - Schematic of MITCH-PEG hydrogel formation. Component 1 is a recombinant protein copolymer bearing CC43 WW domains (denoted as C) and RGD cell-binding domains. Component 2 is an 8-arm PEG-peptide conjugate bearing complementary proline-rich peptide domains (denoted as P). Simple mixing of the

two components results in hydrogel network formation. Inset shows variants of the 8-arm PEG-peptide conjugate, created by varying domain repeat (P1 for one domain or P2 for two domains) and the PEG molecular weight (20 kDa or 40 kDa).

Stage of Research:

- **Proof-of-principle experiments completed** - In vitro cell culture studies and in vivo injections using a healthy murine model are complete and demonstrate improvement in functionality compared to the previously reported hetero-assembling hydrogel.
- Preliminary pre-clinical studies in a non-obese diabetic murine model of peripheral arterial disease and a rat model of spinal cord injury have demonstrated proof-of-concept.

Applications

- Research tool for in vitro cell culture studies
- Bio-ink for 3D printing of cell-laden structures
- Delivery vehicle for cells, proteins, and/or drugs in medical therapies
- Encapsulation material for cells, drugs, and proteins to protect cell viability during transplantation surgeries

Advantages

- Simple to use.
- Does not expose biological cargo to any chemical reactions.
- Improves cell viability during transplantation procedures.
- Fully defined chemical structure.
- Previously disclosed Mixing-Induced Two-Component Hydrogel (MITCH) system (Stanford docket S08-065) can encapsulate cells simply by mixing two components together at physiological conditions without needing any external triggers, such as chemical crosslinkers, ultraviolet irradiation, or sudden shifts in pH, ionic strength, or temperature.
- To optimize the hydrogel properties, this invention proposes two improved designs to the original MITCH: **MITCH-PEG and MITCH-PEG-PNIPAM.**
    - **MITCH-PEG** offers better tunability of hydrogel mesh size, viscosity, and shear thinning properties, which are highly pertinent to the mechanical

protection of cells during injection.
    - **MITCH-PEG-PNIPAM** further improves mechanical properties of the hydrogels by forming a secondary Poly(N-isopropylacrylamide) (PNIPAM) network, which can also sequester growth factors and increase cell retention time.

Publications

- Marquardt LM, Doulames VM, Wang AT, Dubbin K, Suhar RA, Kratochvil MJ, Medress ZA, Plant GW, Heilshorn SC. Designer, injectable gels to prevent transplanted Schwann cell loss during spinal cord injury therapy. Science Advances, 2020, 6(14):eaaz1039
- Foster A, Dewi R, Cai L, Hou L, Strassberg Z, Alcazar C, Heilshorn SC, Huang N. Protein-engineered hydrogels enhance the survival of induced pluripotent stem cell-derived endothelial cells for treatment of peripheral arterial disease. Biomaterials Science, 2018, 6:614-622
- Dubbin K, Hori Y, Lewis K, Heilshorn SC. Dual-Stage Crosslinking of a Gel-Phase Bioink Improves Cell Viability and Homogeneity for 3D Bioprinting. Advanced Healthcare Materials, 2016, 5(19):2488-2492
- Mulyasasmita, Widya, et al. Avidity-controlled hydrogels for injectable co-delivery of induced pluripotent stem cell-derived endothelial cells and growth factors. Journal of Controlled Release (2014).

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