Conformal Encapsulation of Stem Cells Using Modified Hyaluronic Acid

Jack Whitewolf[1], Chris Highley[1,2]
Departments of [1]Biomedical Engineering and [2] Chemical Engineering, University of Virginia, Charlottesville, VA, USA

Statement of Purpose: Micro-scale encapsulation of cells have been used since the 1980s to facilitate cell therapy[1]. Most prominently, islets of Langerhans have been microencapsulated in varying formulations of polymers to facilitate transplantation[1,2]. More recently, groups have developed techniques to encapsulate cells in nano-scale coatings conformal to the cell surface[3]. Conformal encapsulations are deterministic and, unlike many microencapsulation techniques, typically do not rely on microfluidic devices, allowing facile generation of large amounts of encapsulated cells with high efficiency. The encapsulations are versatile and can be modified to protect cells from their physical environment; direct cell signaling using functional ligands; improve cell survival after transplantation; and curb immune response by size-selective permeability[3].

The functionalities provided by conformal encapsulation might be translated to stem cell therapy, where protection of transplanted cells and control over their phenotype after implantation may help address challenges in therapeutic application[4,5]. However, few have explored the encapsulation of neural stem cells (NSCs), and—despite its wide use in designing hydrogel materials for cell encapsulation in 3D hydrogel—there exist no examples of hyaluronic acid (HA)-based hydrogels in conformal encapsulation. Here, we utilized HA, a natural, non-immunogenic disaccharide present in the ECM, to encapsulate NSCs and mesenchymal stromal cells (MSCs). By functionalizing HA localize to cell membranes and introducing reactive chemical moieties that crosslink through click chemistry, we were able to achieve conformal encapsulation on NSCs and MSCs. We assessed the relationship between coating and cell encapsulation time and viability, as well as the potential for HA functionalization on the external surface of the coating to direct multicellular assembly.

Methods: To design an HA derivative that would conform to cell surfaces, lipid-modified HA (lipHA) was synthesized via BOP-mediated conjugation of dipalmitoyl phosphatidylethanolamine. LipHA is subsequently thiolated using 3,3 dithiodipropionic acid through Boc_2O chemistry. Separate batches of HA were thiolated or functionalized with maleimide, via Boc_2O or BOP coupling to yield HASH or MaHA, respectively. After purification, conformal encapsulation of NSCs or MSCs was achieved via layer-by-layer coating of the cells. Here, SCs were first incubating in lipHASH for 30 minutes, then cells washed with PBS to eliminate excess lipHASH. Hydrogels were then built by layers: alternately adding MaHA, washing with PBS, then adding HASH, washing with PBS. HA-derivatives were fluorescently tagged via fluorophores with thiol or maleimide functionalities.

Results: HA materials were successfully modified and characterized. Up to 4 layers of encapsulation were successfully accomplished on both NSCs and MSCs. The viability of cells and cell egress were tracked over a 3-day period. We observed that for both NSCs and MSCs, increasing the number of layers increased the time cells stayed within the conformal hydrogels (Fig. 1a). Both NSCs and MSCs maintained their viability after encapsulation, although NSC viability decreased over time as the number of encapsulation layers increased (Fig. 1b).

By combining cells with complementarily reactive outer layers on their coatings, we could direct multicellular assemblies of cells in suspension (Fig. 1c). Cells within conformal hydrogels that had a thiol-modified outer layer could be patterned onto norbornene modified HA gel through photochemistry (Fig. 1d) with high (100 µm) spatial resolution.

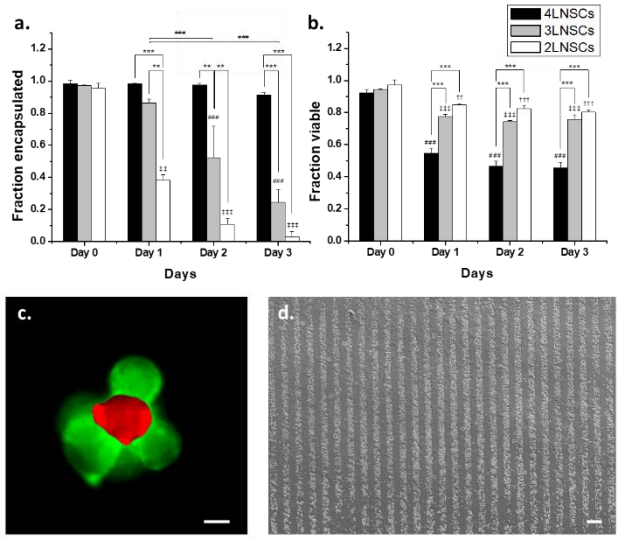

Figure. *a) The fraction of NSCs stayed in encapsulation and b) their viability over 3 days. 4 layers, 3 layers, and 2 layers of encapsulations are put onto NSCs and assessed. Two-way ANOVA and Tukey's multiple comparison was used to calculate statistical significance. c) Assembly of NSCs using maleimide coating (red) and thiol coating (green). Scale bar: 5 µm d) NSCs photo-patterned onto norHA surfaces. Scale bar: 200 µm*

Conclusion: We have introduced a new material platform that allows NSCs and MSCs to be encapsulated in conformal HA hydrogels. The encapsulation process using modified HA did not negatively affect NSC and MSC viability, although NSCs were observed to be sensitive over time to stable confinement in conformal hydrogels with increasing layers. MSCs were not significantly affected and were observed to more rapidly exit hydrogel coatings. This technology offers unique opportunities to control both cellular microenvironments and multicellular assemblies that might support cell survival and phenotype in therapies or impact biofabrication.

References: 1. O'Shea, Geraldine M., and A. M. Sun. Diabetes 35.8 (1986): 943-946. **2.** Fan, Mei-Ying, et al. Diabetes 39.4 (1990): 519-522. **3.** Lee, Hojae, et al. Advanced Healthcare Materials (2021): 2100347. **4.** Koh, IlKyoo, et al. ACS Biomaterials Science & Engineering 6.2 (2020): 813-821. **5.** Choi, Daheui, et al. Chemistry of Materials 29.5 (2017): 2055-2065.