Formation of cell-encapsulating gel with azide-modified hyaluronic acid and water-soluble cyclooctadiyne

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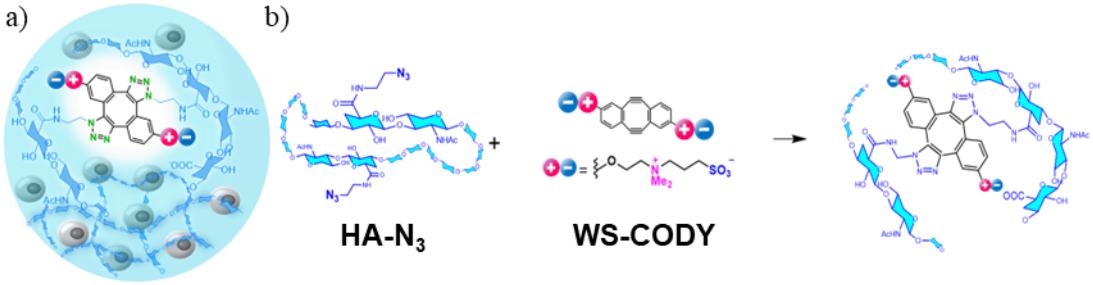

Figure 1: (a) Schematic representation of the hydrogel structure encapsulating cells, formed by crosslinking azide-modified hyaluronic acid (HA-N_3) with water-soluble cyclooctadiyne (WS-CODY). (b) Depiction of the chemical crosslinking reaction between HA-N_3 and WS-CODY.

Cell encapsulation technologies have emerged as key tools for replicating the intricate interplay between cells and their extracellular matrices, driving advances in organ model development and pharmaceutical screening. However, traditional approaches have been limited by the inherent cell adhesion properties of the cell types used. To overcome this limitation, we have developed novel extracellular scaffolds by combining azide-modified hyaluronic acid (HA-N_3) with water-soluble cyclooctadiyne (WS-CODY)[1], which serves as a bioorthogonal reagent for crosslinking two azide groups in aqueous solutions (Figure 1).

In this study, we demonstrated that mixing WS-CODY (1 mM) with HA-N_3 (0.75%) and a cell suspension, followed by its introduction into a hemispherical Teflon mold, yields a homogeneous cell-encapsulating hydrogel, limited only by the confines of the mold. The gel matrix forms independently of the native adhesion capabilities of the cells and exhibits permeability to small molecules and proteins. In addition, we have shown that our encapsulation protocol not only promotes cell proliferation, but also ensures robust cell viability; over 90% of encapsulated cells remain functional after 72 hours, with cell survival confirmed after 20 days (Figure 2). Notably, the scaffold can be dissolved using hyaluronidase, allowing gentle recovery of the encapsulated cells without compromising their integrity. Through this methodology, we propose a versatile scaffold that can be seamlessly integrated with different cell types, increasing the utility of cell encapsulation in biomedical applications.

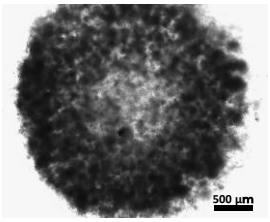

Figure 2: Image of the spheroid of PC-9 cells using our encapsulation method, at day 20.

Reference
[1] K. Kitagawa, F. Satoh, M. Tera, et al. (2023) Ion-Pair-Enhanced Double-Click Driven Cell Adhesion and Altered Expression of Related Genes *Bioconjugate Chem. 34 (4),* 638-644.