Clicking Mesenchymal Stem Cells on Hydrogel Surfaces: Towards Applications in Wound Healing

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Statement of Purpose: Human mesenchymal stem cells (hMSCs) are self-renewing, multipotent cells able to differentiate into multiple tissue-forming cell lineages. hMSCs have immunomodulatory and antiflammatory functions and are also capable of releasing antimicrobial peptides and proteins.[1] Due to these properties, hMSCs have great therapeutic potential; particular attention has been focused on wound healing applications of these cells. Wounds are often complicated by infection. However, use of hMSCs as antimicrobial agents is hampered by low cell survival after injection to the wound site.[2,3]

To address this complication, our work focuses on developing an antimicrobial material that covalently anchors hMSCs to a polymeric hydrogel scaffold made from gellan gum. Gellan is a natural polysaccharide able of rapid gelation and due to its biocompatibility and biodegradability gellan hydrogels have great potentiality in tissue engineering and regenerative medicine.[4] hMSCs were covalently bound to gellan using a "strain-promoted click chemistry" synthetic strategy.[5] The successful binding of azide-bearing cells to the dibenzocyclooctyne (DBCO)-functionalized gellan was demonstrated and an increased viability of the clicked cells was observed.

Methods: Materials: Gelzan™ and DMT-MM were purchased by Sigma, DBCO-amine from Click Chemistry Tools and DBCO carboxyrhodamine and *N*-azidoacetyl mannosamine tetraacylated (Ac4ManNAz) from Kerafast. hMSCs and growth media were purchased from Lonza.

Hydrogel functionalization: Purified gellan[6] was dissolved in H_2O/DMSO with 0.5 equivalents of DBCO amine and 5 equivalents of DMT-MM and left for 4 hours at 40°C. The conjugate was then dialyzed against water and characterized by Nuclear Magnetic Resonance (NMR).

Hydrogel preparation: Gellan hydrogels were prepared on 48-well plates using non-purified raw gellan with or without DBCO-gellan at different concentration.

Cell glycoengeneering: hMSCs (12,000 cells/cm^2) were treated with 25 or 50 µM of Ac4ManNAz for 72 hours, and then with DBCO carboxyrhodamine (0 to 100µM) for 1 hour. Cells were analyzed by fluorescence microscopy.

Cells clicked on gellan hydrogel: Azide-hMSCs were seeded on hydrogel surfaces (10,000 cells/cm^2) for 24 hours, then cells were fixed and F-actin and nuclei were stained and analyzed by fluorescence microscopy.

Results: NMR confirmed gellan functionalization with a DBCO moiety through an amidation reaction on the carboxylic groups of the polymer (Fig. 1A). Mixing this functionalized gellan with raw gellan, a sequence of hydrogels with varying concentrations of the DBCO functional group were prepared. The approach investigated to create our hybrid material involves two steps: first hMSCs are glycoengineered to express azide moieties on their surface, and second, the azide-functionalized cells are clicked to the DBCO gellan. Preliminary studies examining the covalent attachment of a DBCO-fluorophore to azide-containing MSCs was qualitatively (Fig. 1B) and quantitatively (Fig. 1C) examined with varying concentrations of DBCO-fluorophore. The same approach was then translated to the gellan hydrogels (Fig. 1D). Azide-containg hMSCs were incubated on the surface of DBCO modified gellan hydrogels. After one day, the hybrid materials were analyzed using fluorescence microscopy. On non-DBCO functionalized hydrogels (Fig. 1E left), hMSCs formed large aggregates of rounded cells; this was expected based on gellan's known lack of cell adhesion capabilities. In the presence of DBCO (Fig. 1E right), cells tended to interact with the gellan surface and become spread, likely due to the chemical reaction between hMSCs and gellan. A cytotoxicity assay confirmed greater cell survival with cells seeded on the DBCO-gellan compared to unmodified gellan.

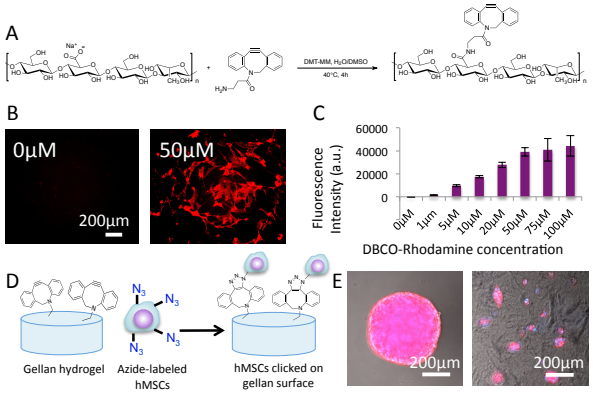

Figure 1. A) Synthetic scheme of gellan DBCO functionalization. B, C) Effect of DBCO-fluorophore concentration on azide-hMSC. D) Scheme of gellan functionalization with hMSCs. E) Fluorescence microscope images of hMSCs seeded on raw gellan (left) or DBCO-gellan (right).

Conclusions: We have developed a unique method to covalently attach hMSCs to hydrogels by click chemistry. Functionalized DBCO-gellan was used to prepare these clickable hydrogels. Incubation of azide-functionalized hMSCs with these hydrogels allowed the cells to attach to and spread on the surface of the material, suggesting effective covalent anchorage to the polymer chain. Using this approach, this functionalized material can serve as a depot of hMSC-released bioactive factors including various antimicrobial peptides that can aid in combating microbial infections. We are currently studying 3-D encapsulation of MSCs in these hydrogels and examining antibacterial efficacy of the cell-functionalized materials.

References:

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