Staudinger Ligation for Dual Crosslinking of Alginate Hydrogels

Kerim M. Gattás-Asfura[1], Chris Fraker[1-2], Cherie L. Stabler[1-2]
[1]Diabetes Research Institute, Miller School of Medicine, [2]Department of Biomedical Engineering, College of Engineering, University of Miami, Miami, FL.

Statement of Purpose: Microencapsulation of cells using alginate-based hydrogels has been a long standing research interest in the field of tissue engineering. Commonly, alginate microbeads are formed via exposure to multivalent cations under mild aqueous conditions. Solely ionic crosslinking can result in mechanical instability, particularly in vivo. Hence, the development of schemes to permit the covalent stabilization of alginate hydrogel networks is of interest. In our laboratory, we have explored methods to achieve dual crosslinking via a chemoselective reaction scheme compatible with live cells, specifically the highly chemoselective Staudinger ligation scheme, which spontaneously results in amide bonding between azides and phosphine derivatives[2]. Alginate was functionalized with azide groups via short polyethylene glycol (PEG) linkers.[3] Four crosslinkers having 1-methyl-2-diphenylphosphino-terephthalate (MDT) end groups were synthesized, with varying number of functional groups, macromolecular structures, and material compositions. Herein, alginate-azide microgels (1±0.2 mm diameter) were stabilized with the variable crosslinkers and the chemical, physical, and cytotoxicity properties of the resulting microgels were studied and compared.

Methods: Azide-functionalization of alginate was performed as previously reported[3]. Four crosslinkers developed consisted of (**1**) linear PEG-MDT (M_w 3400), (**2**) a 4-arm PEG-MDT (M_w 11000), (**3**) a 4-arm PEG-MDT* (M_w 11000) with one Asp residue in between each of the PEG chains and the MDT group, and (**4**) linear alginate backbone functionalized with multiple MDT-PEG-NH_2 (M_w of PEG 3400). All MDT groups were covalently linked to the polymers via amide bonding utilizing carbodiimide chemistry. Polymers were characterized by ^{1}H-NMR, FT-IR, and/or mass spectroscopy. Protected Fmoc-Asp(OtBu)-NHS was used to assemble crosslinker **3** following solid-phase peptide protocols. ^{1}H-NMR and FT-IR revealed degree of modifications of 150 N_3/alginate and 15 MDT/alginate mol ratios for alginate-N_3 and crosslinker **4**, respectively. For hydrogel formation, alginate-azide (1.5% wt/v) was mixed with one of the crosslinkers **1-4** (1.5% wt/v) and preincubated for a set period of time. Microbeads were fabricated via drop-wise parallel air flow needle extrusion of mixed polymers into a 1.6% $BaCl_2$ bath. The capacity of the covalent crosslinks to enhance bead stability was evaluated via exposure to 5 washes in dH_2O and EDTA chelation. Microbeads containing cells were mixed with cells prior to extrusion and cultured in full media. Cell viability following encapsulation was assessed via MTT assay.

Results: FT-IR studies revealed slower reaction kinetics for alginate-azide, when compared to crosslinkers with equimolar amounts of PEG-MDT or PEG-N_3, respectively. Crosslinkers **1** and **2** formed cloudy solutions and phase separated when mixed with alginate-N_3 or in DMEM media. Introduction of the Asp residues or alginate backbone into crosslinker **3** and **4**, respectively, resulted in enhanced miscibility properties. A co-incubation time of (30, 20, or 15 min) prior to bead formation was required for adequate covalent stabilization of alginate-N_3 microgels when using crosslinker **1**, **2**, or **3** respectively. Longer co-incubation times resulting in enhanced viscosity of solution and difficulty in microbead fabrication, while shorter times resulted in leaching of crosslinker. Due to its alginate backbone, crosslinker **4** required no co-incubation time prior to bead fabrication. The dual crosslinking approach enhanced the stability of alginate beads, where beads exhibited signifcant decreases in swelling and resisted dissolution in EDTA (Fig 1). MTT assessment of MIN6 cells within microbeads found an initial detrimental effect on alginate-azide and **4** (up to 24% lower viability); however, the cells recovered and proliferated over time (Fig 2).

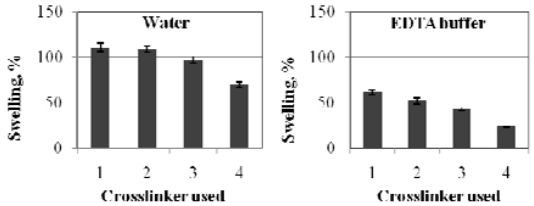

Fig 1. Diameter change of alginate-N_3/**1-4** microbeads

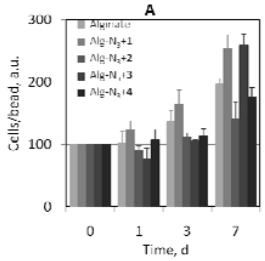

Fig 2. MTT assay of MIN6 cells-loaded microcapsules

Conclusions: Covalent stabilization of alginate microbeads was achieved via Staudinger ligation in the presence of live cells while maintaining viability and allowing for cell proliferation. We believe this dual crosslinking platform, with in situ covalent crosslinking achieved via chemoselective chemistry, will enhance the potential of alginate cellular encapsulation, particularly for long term use in vivo.

References

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