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Microencapsulation of islets within alginate/poly(ethylene glycol) gels cross-linked via Staudinger ligation

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ABSTRACT

Functionalized alginate and poly(ethylene glycol) (PEG) polymers were used to generate covalently linked alginate–PEG (XAlgPEG) microbeads of high stability. The cell-compatible Staudinger ligation scheme was used to cross-link phosphine-terminated PEG chemoselectively to azide-functionalized alginate, resulting in XAlgPEG hydrogels. XAlgPEG microbeads were formed by co-incubation of the two polymers, followed by ionic cross-linking of the alginate using barium ions. The enhanced stability and gel properties of the resulting XAlgPEG microbeads, as well as the compatibility of these polymers for the encapsulation of islets and beta cells lines, were investigated. The data show that XAlgPEG microbeads exhibit superior resistance to osmotic swelling compared with traditional barium cross-linked alginate (Ba–Alg) beads, with a five-fold reduction in observed swelling, as well as resistance to dissolution via chelation solution. Diffusion and porosity studies found XAlgPEG beads to exhibit properties comparable with standard Ba–Alg. XAlgPEG microbeads were found to be highly cell compatible with insulinoma cell lines, as well as rat and human pancreatic islets, where the viability and functional assessment of cells within XAlgPEG are comparable with Ba–Alg controls. The remarkable improved stability, as well as demonstrated cellular compatibility, of XAlgPEG hydrogels makes them an appealing option for a wide variety of tissue engineering applications.

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1. Introduction

Microencapsulation of cells has been an extensively investigated approach for the immunoisolation of transplanted cells, where the goal is to protect foreign cells from the host immune system using biocompatible biomaterials. This technique has been applied to treat a wide variety of diseases, including the encapsulation of dopamine-producing adrenal chromaffin cells for Parkinson's disease [1] and parathyroid cells for hypocalcemia [2]. Much attention has been directed to microencapsulation of insulin-producing cells for treatment of Type 1 diabetes mellitus. Multiple studies have shown reversal of diabetes upon the transplantation of microencapsulated islets, illustrating the promise of this technique for retaining graft function in the presence of decreased immunosuppressive therapy [3–8].

Alginate has been the most widely used material for microbead formation. Alginate is a collective term for a family of polysaccharides derived primarily from brown algae [9]. Alginate molecules are linear binary co-polymers of β-D-mannuronic (M) and α-L-guluronic (G) acids, with a variation of sequential arrangement and composition, depending on their source [9,10]. The gelation of alginate beads is commonly achieved via the presence of divalent cations (typically Ca^{2+} or Ba^{2+}); however, an ionically bonded alginate hydrogel commonly lacks the stability to withstand the mechanical and chemical strains associated with life-long implantation, where microbead degradation and rupture slowly develop as a result of the slow exchange of cations with sodium ions under physiological conditions [11,12].

The development of methods for the formation of covalent bonds within the encapsulation polymer is an appealing option for improving microbead stability. Published reports have shown alginate modification, commonly via carboxylic groups on the alginate backbone, with functional groups capable of forming cross-links results in increased stability and reduced swelling of alginate-based hydrogels [13–18]. The introduction of photopolymerizable groups, such as a methacrylate group onto the alginate chain to permit covalent bond formation via photo-cross-linking, is the most common method [15–17,19–26]. Resulting hydrogels were shown to possess high stability and increased mechanical strength; however, photoinitiator solutions, as well as the free radical generation following initiation of bond formation, can lead to

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significant cell toxicity, thereby reducing the ease in translation to cell-based systems, particularly for cell types inherently sensitive to free radical stress [27–30].

With a focus on the development of covalent ligation schemes with high cell compatibility and chemo selectivity, the present study explored the utility of the Staudinger ligation scheme as a novel method for the formation of stable alginate-based hydrogels. Cell compatible, efficient, chemo selective and catalyst-free, Staudinger ligation has been used for numerous biological applications, from cell surface modification to protein detection, both in vitro and in vivo [31–35]. The reacting groups include an azide and a phosphine (e.g., triphenylphosphine) moiety with an electrophilic trap. To achieve hydrogel formation via covalent cross-linking, an alginate functionalized with azide groups, termed N_3-alginate or **[1]**, and a high molecular weight poly(ethylene glycol) (PEG) terminated with complementary phosphine groups, termed MDT–PEG or **[2]**, were recently developed, as shown in Fig. 1. PEG was selected as the cross-linker polymer, given its ease of functionalization, demonstrated cell compatibility and established use in islet encapsulation [36,37]. In a previous report, the capacity of these polymers to spontaneously form stable hydrogels after several hours was demonstrated [38]. Furthermore, instant gel formation and fabrication of microbeads were attained via incubation with divalent cations. In the latter case, once the azide-functionalized alginate and 1-methyl-2-diphenylphosphino-terephthalate (MDT) terminated PEG mixture was fabricated into microbeads via ionic interactions, covalent cross-links were subsequently formed *in situ* via Staudinger ligation. Therefore, a "gel and lock" system was created, where ionically cross-linked alginate/PEG hydrogels were "locked" into place through the additional formation of interlocking covalent linkages (as illustrated in Fig. 1) [38]. These resulting covalently cross-linked hydrogels are termed XAlgPEG.

This study sought to evaluate the potential of these XAlgPEG microbeads for cellular encapsulation, specifically for insulin-secreting cells. The stability, diffusional properties and porosity of the resulting gels were compared with those of standard barium ion cross-linked alginate, termed Ba–Alg. High guluronic alginate and barium ion cross-linking was selected for comparison, given that this alginate type and gelation ion commonly results in a highly stable ionically-linked microbead [39]. The viability and function of insulin-secreting cells, both cell lines and islets, within these microbeads were evaluated and compared with standard Ba–Alg microbeads. The significance of this novel alginate–PEG hydrogel in tissue engineering, particularly in the development of a bioartificial pancreas, is discussed.

2. Materials and methods

2.1. General reagents

Sodium alginate (PRONOVA UP MVG, M_w = 300 kDa, M_w/M_n = 1.87, DP_n of 28, Batch # FP-504-03) was purchased from NovaMatrix. The content and distribution of M and G units were determined by ^{1}H NMR spectroscopy and provided by the manufacturer. The fraction of G units (F_G) was 0.6875. The fractions of diads F_{GG}, $F_{MG/GM}$, and F_{MM} were 0.58, 0.11 and 0.20, respectively. The fractions of triads F_{GGG}, $F_{MGG/GGM}$, F_{MGM} were 0.54, 0.043 and 0.066, respectively, with a $N_{G>1}$ of 14.52. The 1-ethyl-(dimethylaminopropyl) carbodiimide hydrochloride (EDC) was from Advanced Chemtech. Other solvents and chemical reagents were purchased from Sigma–Aldrich at the highest purity available.

2.2. Polymer synthesis

Alginate–PEG-N_3, termed N_3-Alg or **[1]**, was fabricated as previously described [38]. In brief, 50 mg sodium alginate was dissolved with 14 mg *N*-hydroxysuccinimide (NHS) and 62 mg

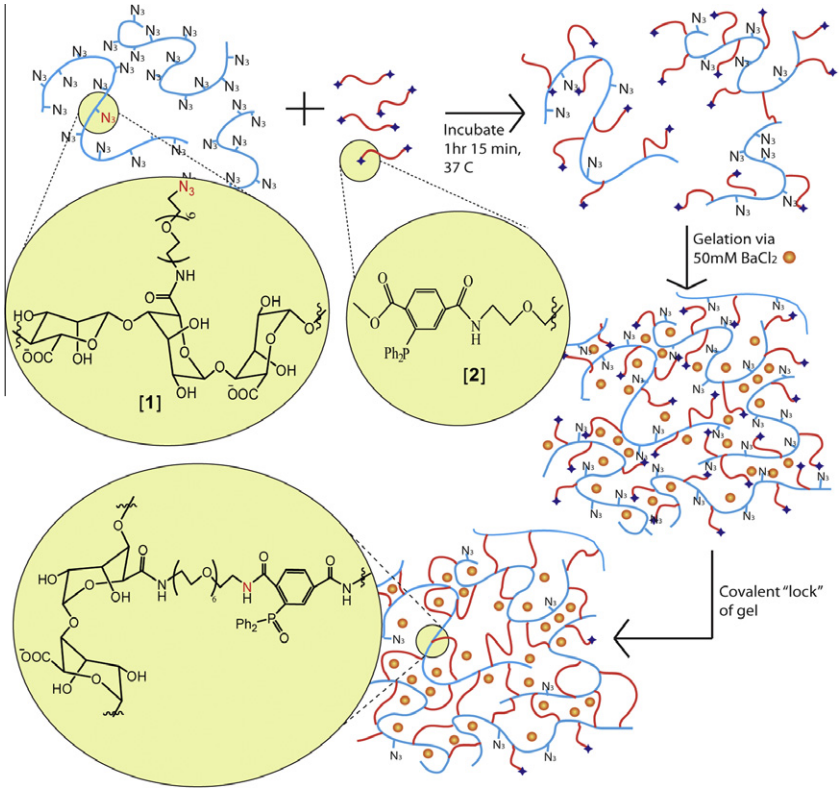

Fig. 1. Schematic representation of XAlgPEG microbead fabrication. Azide-functionalized alginate polymer **[1]** is pre-mixed with MDT-PEG polymer **[2]** for 1 h 15 min prior to cellular incorporation and instantaneous gel formation via subsequent exposure to divalent cations (Ba^{2+}). Additional cross-linking of gel is achieved via Staudinger ligation.

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