Novel Alginate-Poly(ethylene glycol) Hydrogel for Immobilization and Delivery: Synthesis and Physical Properties Assessment

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INTRODUCTION

Considerable progress in the fields of immobilization/encapsulation biotechnology has been made during recent years as a result of extensive research that has been carried out. The technology is based on the entrapment of biologically active substances within a semipermeable membrane, providing protection of the cells from mechanical stress and host's immune system, and preventing rejection of the implanted materials. This protection could potentially allow transplantation (allo- or xenotransplantation) without the need for immunosuppression.
In the last three decades, especially after the pioneering study of Lim (Lim. F., 1980) , alginate (alg) based hydrogel has become the most studied system for cell immobilization/transplantation. Alg is anionic polysaccharide composed of homopolymeric regions of β-D-mannuronic (M-block) and α-L-guluronic (G-block) acids interspaced with regions of mixed sequence. Alg based hydrogels are obtained mostly by extruding sodium alginate solution into a gelation bath containing calcium or barium ions. However, these hydrogels frequently suffer from mechanical stability deficiency, durability issues, and permeability drawbacks. To overcome these problems, the most utilized approach is the subsequent coating of the initially formed alg bead with polycations to form a polyanion-polycation complex semi-permeable membrane. Nevertheless, the main issue of this coating system is the lack of biocompatibility. For instance, poly-L-lysine (PLL), which the most commonly used polycation for coating purposes, has been identified as the main capsule component responsible for the fibrotic overgrowth often seen on implanted alg-PLL-alg microcapsules, via activation of the host macrophage (Juste. S.,2005). Others *in vivo* biocompatibility drawbacks were attributed to non-specific protein adsorption on the material surface. This adsorption is believed to be the initial event when a material comes into contact with a biological environment, and the adsorbed protein layer will influence the subsequent biological reactions including platelet adhesion and activation, as well as permeability characteristics by reducing the diffusion of oxygen and nutrients that are essential for cell survival. Therefore, understanding the interaction between proteins and material surfaces is critical, and control of protein–surface interactions continues to be an important factor for consideration in the design of biocompatible surfaces.
Considerable number of researchers have explored surfaces that are resistant to non-specific protein adsorption or enhance specific protein selectivity. Sawhny and Hubbell (Sawhny A.S., 1992) demonstrated that polyethylene glycol (PEG) is able to form proteins-repellent surfaces, which enhance the biocompatibility of the implanted material by minimizing cell adhesion. They have described the use of a graft copolymer of Poly(l-Lysine) (PLL) and methoxy poly(ethylene glycol) to increase the biocompatibility of alg-PLL microcapsules.
Taking these considerations into account, we have developed a novel alginate-poly(ethylene glycol) hydrogel (alg-PEG). The method takes advantage of a reaction of multiarm vinyl sulfone-terminated poly(ethylene glycol) (PEG-VS) and thiol containing cross linker via Michael-type reaction (also called conjugate addition), while alg is gelled by ionotropic effect in presence of calcium ions. The surrounding alg layer gives a spherical shape and retains the integrity of the formed hydrogel. Alg-PEG hydrogel is formed in one step under physiological conditions (Fig.1).

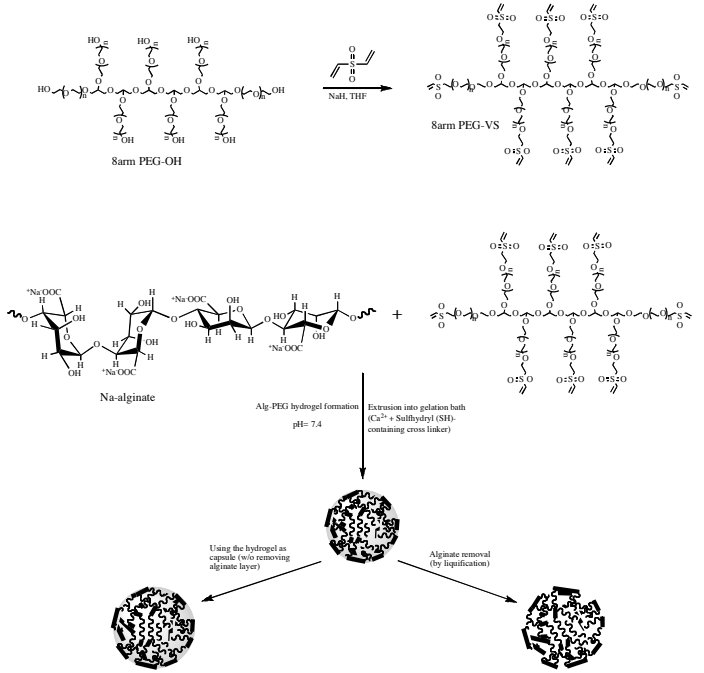

Fig.1. Schematic representation of the followed approach for alg-PEG hydrogel formation.

MATERIAL AND METHODS

Sodium alginate (LV Kelton, lot N° 46198A) was obtained from Kelco, Chemical Company, San Diego. Multiarm PEGs were purchased from Shearwater Polymers (Huntsville, AL). PEG-VS were synthesized in one step by coupling PEG-OH with an excess of divinyl sulfone. The degree of functionalization of PEG was determined using ^{1}H-NMR, and was found to range from 81 to 98 %. Alg-PEG beads were prepared at 37 °C employing a coaxial airflow droplet (Ceausoglu I., 2002). PEG-VS was dissolved at desired concentration in alg solution and leaved on a shaker at 4°C over night until complete dissolution. The solution was then extruded into the gelation solution containing the cross linker. For instance, to form 5 % (w/v) hydrogel, 50 mg PEG-VS (8arm, 40 KDa, 98 % functionalized) was dissolved in 956 µL alg stock solution, mixture solution was then extruded into a gelation bath containing the corresponding amount of cross-linker, and incubated at 37°C. Gelation occurs within a few minutes, however, beads were incubated for additional 3 hours to achieve optimal PEG cross-linking.

RESULTS AND DISCUSSION

Ideally, the stoichiometric ratio (r) that equals the molar ratio of SH and VS should be equal to 1. However, in our case no PEG hydrogel was formed using this amount, and only hydrogels formed with stoichiometric ratio r > 2 kept their spherical shape after alg dissolution. In order to optimize the stoichiometric ratio (r), we have prepared different alg-PEG beads by varying r. The effect of the stoichiometric ratio was investigated using the swelling degree (S_w), and mechanical resistance as indexes. The optimal r corresponds to the minimal swelling degree (Fig.2). Bead size can be monitored either by changing the airflow, syringe diameter, or pump speed. Good sphericity and dispercity were obtained (less than 5 %, expressed as relative standard deviation of bead diameter).

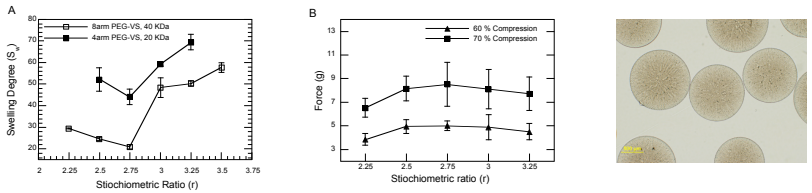

Fig.2. Left: Swelling degree (A) and Mechanical resistance (B) as a function of r. Right: Alg-PEG beads hydrogel formed following the described method.

The MWCO was assessed using the ingress diffusion of labeled dextran standards. Two types of hydrogel formed from PEG-VS having different arm length. Results have shown that hydrogel formed using 8arm PEG-VS, 40 KDa allows the diffusion of the dextran 150 KDa, while this latter is completely excluded when using 8arm PEG-VS, 10 KDa. Informations about pores distribution were obtained from inverse size exclusion chromatography (Brissova M., 1996). The half-width half-maximum value (HWHM) has been found to be 0.25. Mechanical resistance of our alg-PEG has been assessed using a Texture Analyzer (Stable Microsystems TA.XA 2i).

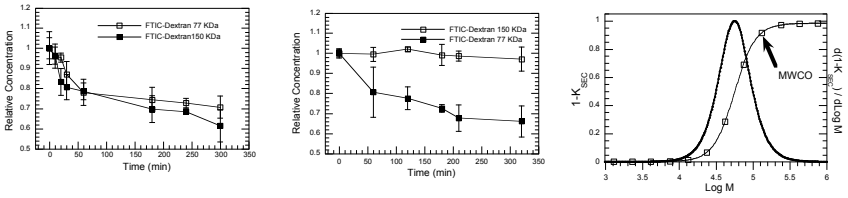

Fig.3. Diffusion of labeled dextran into alg-PEG hydrogel. Left: Hydrogel formed using PEG 40 KDa, middle: hydrogel formed using PEG 10 KDa. Right: ISC curve for hydrogel formed using PEG 10 KDa.

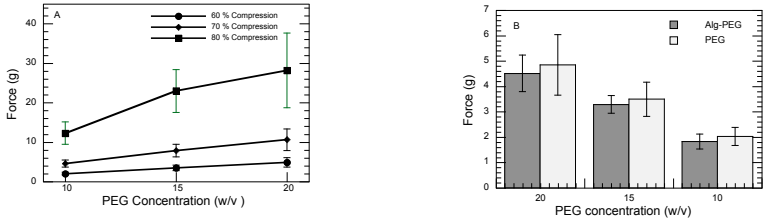

Fig.4. Mechanical resistance as a function of PEG concentration (A), and the effect of alg layer on mechanical stability at 60 % (B).

Hydrogels have shown very distinct mechanical properties depending on the hydrogel density. Generally, mechanical resistance increased with PEG concentration. This was predictable since the mechanical resistance depends on the quantity on the compressed material. However, surprisingly this resistance has shown to be independent of the presence of alginate. This might be explained by the higher absorption of water, which compensates the mechanical resistance of the dissolved alg hydrogel.

CONCLUSIONS

The synthesis of a novel alg-PEG hydrogels was performed allowing formation of hydrogels under physiological conditions. The sensitivity of the hydrogel to the preparation conditions including stoichiometry, PEG concentration, and dissolution of alg layer was investigated. It was shown that the hydrogel swelling could be tailored either by changing the PEG concentration or by dissolving the outer alg layer. Mechanical resistance has been shown to be independent of the presence of alg layer. Permeability studies have shown quite uniform distribution of pores along the hydrogel. Moreover, the MWCO can be tuned by changing the arm length.

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