Chemical Modifications of Sodium Alginate for the Microencapsulation of Islets of Langerhans

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INTRODUCTION

Type I diabetes is an autoimmune disease causing the destruction of pancreatic ß-cells responsible for the glucose regulation by insulin secretion. Replacement of islet of Langerhans is an attractive option for patients in order to avoid insulin injection and complications associated with type I diabetes (Borg 2011). Pancreatic islet transplantation showed promising results but had varying long-term success (Merani 2006), was associated with inflammatory reactions (Weir 2013), and needs administration of immunosuppressive drugs. Thus, microencapsulation of cells could be an improvement. Islets of Langerhans could be isolated from the host system upon entrapment in hydrogel microspheres or multilayer systems, and would so be protected from severe immune destruction.

Hydrogels prepared from the biopolymer sodium alginate (Na-alg), which is composed of 1-4 linked ß-D-mannuronic acid (M) and its epimer α-L-guluronic acid (G), are widely used for cell microsencapsulation due to the biocompatibility and preparation under mild conditions of pH and temperature suitable for living cell encapsulation (Pawar 2012). However, for alginate hydrogels prepared by electrostatic cross-linking with divalent cations, frequently lack of sufficient mechanical properties especially for long-term *in vivo* applications was reported.

In the present project, we propose to chemically modify Na-alg (Mahou 2012) with the aim to improve the mechanical properties and permebility of its hydrogels for the microencapsulation of islets of Langerhans. Our goal is to covalently functionalize Na-alg by grafting poly(ethylene glycol) (PEG) chains on the backbone. The so modified molecules will allow for the fast formation of an electrostatically cross-linked hydrogel and additional chemical cross-linking by disulfide bridge formation, thus reinforcing the physical stability and permeability of the microspheres. Na-alg of different molar masses was modified, characterized and used as material for the preparation of microspheres.

MATERIALS AND METHODS

Na-alg (Kelton LV, batch No 46198A; Kelton HV, batch No 61650A) was purchased from Kelco Chemical Company, San Diego, USA and was used for the chemical modification.

Ingress/egress diffusion of labeled dextran was used to evaluate the permeability of the hydrogels. Size and size distribution were obtained from microscopical images using an Olympus AX70 microscope connected to an Olympus DP70 color digital camera. Olympus DP Manager software (Olympus, UK) was used for image analysis. A texture analyzer (model TA-XT2i, Stable Micro Systems, Godalming, UK) equipped with a force transducer of a resolution of 1 mN and the software Texture Expert v.1.16 was used to study mechanical resistance of the microspheres. *In vivo* studies were performed in a mouse model.

RESULTS AND DISCUSSIONS

Two strategies were envisaged for the pegylation of LVM and HVM alginates. Both of them rely on the conservation of the gelation characteristics of Na-alg but involve covalent grafting of heterobifunctional PEG without consuming the carboxylate groups of the monomeric units required for the ionic complexation.
In the first approach (Fig. 1), Na-alg 1 was first converted into the tetrabutylammonium (TBA) salt of alginic acid 2 to increase its solubility in organic solvents, so allowing access to different organic reactions (Pawar 2011).

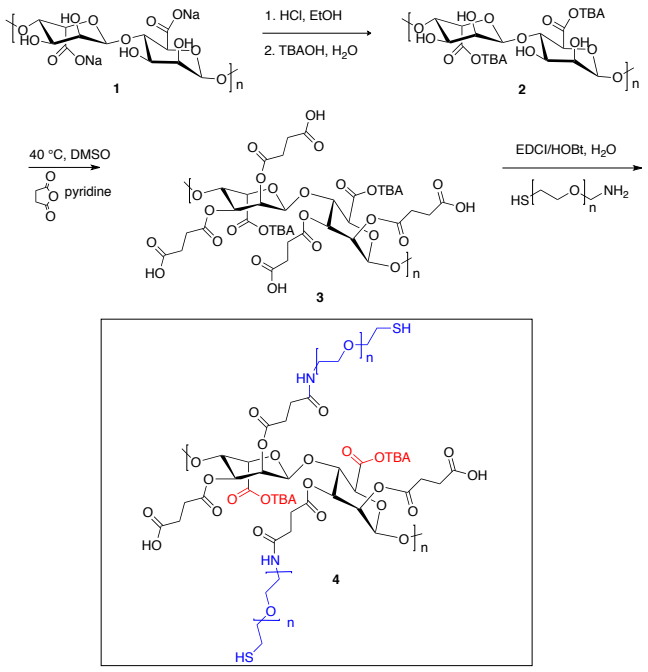

Figure 1: First route for the synthesis of PEG grafted sodium alginate

Insertion of new carboxylate groups was achieved via the reaction of the TBA-alginate with succinic anhydride in the presence of pyridine at 40 °C. Finally, the resulting polymer 3 was coupled to a modified PEG chain containing an amino group for the coupling reaction and a terminal thiol group for the additional cross-linking to afford the final compound 4.

In the second approach, Na-alg 1 was first oxidized in the presence of sodium metaperiodate at 4 °C to provide the corresponding aldehyde 5 (Fig. 2). This modification provides reactive aldehyde groups suitable for further chemical modification. Reductive amination by treatment with the same heterobifunctional PEG as used for the first approach, afforded the final compound 6.

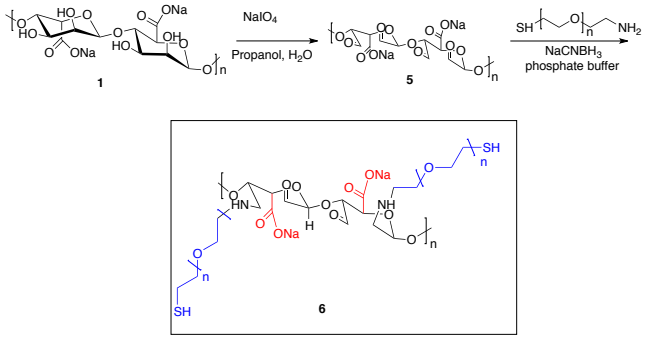

Figure 2: Second route for the synthesis of PEG grafted sodium alginate

Two different Na-alg derivatives (Fig. 1 and Fig. 2) containing both PEG chains and terminal thiol groups, but having a different chemical structure, were obtained in a fast and efficient manner. The chemical structures were confirmed by spectroscopic methods. In both cases, the carboxylate groups were maintained free for the gelation process. Polymers 3, 4 and 6 of LVM and HVM were tested for their ability to form microbeads at different concentrations. In addition to the degree of grafting, the concentration determines the permeability, swelling and the mechanical resistance to compression. In addition to the physical properties of the novel one-component hydrogel microspheres, we will present the results of *in vitro* and *in vivo* studies, which are ongoing.

CONCLUSION

Microencapsulation of islets of Langerhans represents a promising approach for the treatment of type I diabetes, in particular if xenotransplantion is the final intention. Despite continuous progress in the field, improvement of the encapsulation materials is needed to accelerate the translation to clinical application.
We propose two modified Na-alg structures, which allow for preparing hydrogel microspheres. These are composed of one-component polymer networks formed by fast electrostatic cross-linking of the carboxylate groups combined with covalent cross-linking of reactive PEG end groups. Differently to the modified Na-alg reported in (Mahou 2012), the hydroxyl groups of the monomeric units and not the carboxylate groups were grafted by heterobifunctional PEG, so maintaining the original gelling capacity of the Na-alg. The degree of grafting and the concentration of the polymer are the main parameters, which can be used to modify and optimize the hydrogel properties including permeability and mechanical properties.

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