Physico-chemical changes of alginate-based immunoisolating microcapsules

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INTRODUCTION

Diabetes represents a major public health problem in industrialized countries (Kleinman J.C., 1988). Encapsulation of pancreatic islets is a promising approach for preventing or reversing complications associated with this disease. Encapsulation of pancreatic islets in a semipermeable membrane allows successful transplantation of not only human cells (allograft) but also of nonhuman cells (xenografts), stem cells or cells generated from adult precursors. Semipermeable membranes allow exchange of nutrients, metabolites and insulin but excludes the diffusion of hazardous immunoglobulins, complement and white blood cells. It protects pancreas islets from host immune response (Kizilel S., 2005).

A general problem in the field of encapsulation is the low reproducibility of the procedures. Some capsules have adequate performance of the up to periods of two years while others can not have an optimal functionality for periods longer than a few weeks. Many have been the efforts to improve the duration of the functional performance of capsules.

As of the first day of implantation in human or experimental animals many proteins and environmental changes occur in order to facilitate tissue repair associated with the mandatory surgery. Although this response is meant to maintain homeostasis, an undesired side-effect may occur. Proteins associated with tissue repair may adsorb to the surface of the membrane and form anchors for inflammatory cells. These proteins are very common proteins such as fibrinogen which are abundantly produced by cells in the vicinity of the capsules. Whether this anchoring of proteins results in a gradual increase of adsorption and cell adhesion depends on the capsule properties. Capsules have different susceptibility for environmental changes after implantation. Alginate-poly-l-lysine capsules produced from alginates with a high amount of guluronic acid (G, > 53% G) are more susceptible for inflammatory responses than alginates with an intermediate G content. As high-G capsules have superior properties over intermediate-G capsules, e.g. the capsules have a higher mechanical resistance, many strategies have been employed to improve the biocompatibility of these capsules. In an attempt to improve the biocompatibility the changes in capsule properties were studied in vivo. An event that is insufficiently realized is that the direct environment of the capsules changes directly after implantation. A pertinent change is a drop in pH as the consequence of a temporary inflammation process due to the mandatory surgery. Such a drop in pH can for instance induce changes in the charge density of the capsules and make the capsule more vulnerable for adhesion of proteins and cells. Capsules should be able to withstand these kind of environmental changes. In the present study we compared the zeta-potential of capsules with different composition and do proposals for improving the biocompatibility.

MATERIAL AND METHODS

Alginates: Alginates contain various amounts of guluronic acid (G)-chains and of mannuronic acid (M)-chains. Intermediate-G (Keltone LV) and high-G (Manugel) sodium alginates were obtained from Kelco International, London, UK. Purification of alginate was performed as described in detail elsewhere (De Vos P., 2006). Alginates were dissolved at 4^0C in Krebs-Ringer-Hepes (KRH) with an appropriate osmolarity to a solution with a viscosity of 4 cps. This viscosity is necessary for the

production of spherical droplets without any tails or other imperfections associated with bioincompatibility. For intermediate-G solutions we applied a concentration of 3%, and for the high-G we applied a 2% solution. The solutions were sterilized by 0.2 µm filtration.

Encapsulation: Capsules were produced according to a three step procedure. First, we produced rigid Ca-alginate beads, by converting an alginate solution into droplets using an air-driven droplet generator. The droplets were collected in a Ca-rich solution to gelify into rigid Ca-beads. Secondly, the Ca-beads were subjected to a procedure to form a semipermeable PLL-membrane. Finally, to cover incompletely bound PLL, the alginate-PLL beads were coated with alginate again. Capsules had a diameter of 600-700 µm. All procedures were performed under sterile conditions (De Vos P., 2006).

Capsules were injected into the peritoneal cavity. Upon peritoneal lavage, microcapsules were either freely floating and non-adherent, or adherent to the surface of abdominal organs. First, non-adherent microcapsules were retrieved by peritoneal lavage, and brought into a syringe with appropriate measures for quantification of the retrieval rate. Subsequently, the microcapsules adherent to the surface of abdominal organs, were excised and processed for histology. All surgical procedures were performed under isoflurane anesthesia.

Electrophoretic mobility: It was showed that zeta-potential measurement on capsules are good measures to quantify chances on the capsule's surface after implantation. The zeta potential was measured using the Electro Kinetic Analyzer (EKA, Anton Paar GmbH, Austria). The EKA operates according to the principles of streaming potential and includes a powder measuring cell, the electrolyte circuit, and a pair of Ag/AgCl electrodes. The electrolyte (10^{-3} M KCl solution) is forced through the measuring cell containing the sample. A pressure drop (ΔP) depending upon the flow resistance of the sample is detected across the measuring cell. The circulation of electrolyte through the cell results in a flow of ions (streaming current). The resulting potential difference (streaming potential, U_p) is detected by electrodes placed at each end of the cell. During a measurement, ΔP and U_p are recorded.
The zeta potential (ζ) was calculated using the equation as follows:

$$\zeta = \frac{Up * \eta * æ}{\Delta P * \varepsilon * \varepsilon_0}$$

In this equation η is the dynamic viscosity of the electrolyte solution, æ is its electrical conductivity, ε is the liquid permittivity, and ε_0 is the permittivity of free space.

If not otherwise mentioned, the pH of the electrolyte solution was kept at 7.0 since this is the physiological pH to which capsules are exposed *in vivo*. During the assessment of the streaming potential, the temperature was kept at 25 ^{o}C (De Vos P., 2006).

RESULTS AND DISCUSSION

Biocompatibility was quantified by studying the number of capsules affected by inflammatory cells at one month post implant in rats. In recipients of high-G capsules, we found large numbers of capsules adherent to the surface of the abdominal organs and virtually all were overgrown by inflammatory cells. This was totally different with intermediate-G capsules where the fast majority of capsules where freely floating in the peritoneal cavity. Obviously, the biological responses against the capsules was determined by the type of alginate applied.

The number of freely floating capsules with overgrowth was larger with high-G alginate capsules (48.8 ± 10.1%) than with intermediate-G alginate capsules (2.7 ± 0.4%). In a previous study, we have shown that high-G capsules bind more proinflammatory poly-l-lysine than intermediate-G alginate. This has subsequently, been interpreted to be the causative factor for the difference in the biological response since more PLL may imply a higher positive charge density at the capsule surface and therefore a lower degree of biocompatibility, De Vos P. (2006). Unfortunately, it was far from simple to assess the charge density of a capsule surface. We have applied many technologies (gold labeling experiments, light and electron microscopy, and immunochemistry) without success since the fragile features of capsules do not allow the required processing for these procedures. Therefore, we finally have assessed the streaming potential to calculate the zeta-potential which can be used as a measure for the charge density at the capsule surface. This was done at physiological pH but also at 5.4 which reportedly is the pH that can occur in vivo when an inflammatory response occurs.

Intermediate-G and high-G capsules showed minor differences in zeta-potential at a physiological pH. However, the lowering of pH to 5.4 induces an increase of the zeta-potentials of the intermediate-G and high-G capsules ($P < 0.05$) but not of the intermediate-G capsules. The increase in zeta-potential was more pronounced with intermediate-G than with high-G alginates, De Vos P. (2006).

This illustrates that seemingly minor changes in capsule composition (12% difference in G-content) has an extreme effect on the physico-chemical properties of the capsules and its biocompatibility. In order to overcome these issues we propose to change the capsules composition and to create stable polymer brushes on the capsule surface. Polymer brushes consist of end-tethered (grafted, anchored) polymer chains stretched away from the substrate to avoid overlapping.

The behavior of ultra thin polymeric layers is strongly dependent on the grafting density, the molecular weight and chemical composition of the polymer chains. Polymer brushes are typically synthesised by two different methods, physisorption and covalent attachment. Polymer physisorption normally involves absorption of block copolymers onto a substrate, where one block interacts strongly with the surface and the other block forms the brush layer, Belder, G. F. (1997). The disadvantages of physisorption include thermal and solvolytic instabilities, poor control over polymer chain density and complications in synthesis of suitable block copolymers. Tethering of the polymer chains to the surface is one way to surmount some of these disadvantages. Covalent attachment of polymer brushes can be achieved by either “grafting to” or “grafting from”. In the “grafting to” approach end-functionalized polymer chains are grafted directly to a solid substrate via a chemical reaction of the end-groups with complimentary groups on the grafting surface, Minko S. (2006). This technique often leads to low grafting density and low film thickness. To overcome this problem, the “grafting from” approach can be used and has generally become the most attractive way to prepare thick, covalently tethered polymer brushes with a high grafting density, Zhao, B. (2000). The “grafting from” technique involves the immobilizing of initiators onto the substrate followed by in situ surface initiated polymerization to generate the tethered polymer brush. As the chains are growing from the surface, the only limit to propagation is diffusion of monomer to the chain ends, thus resulting in thick tethered polymer brushes with high grafting density.

Recent advances in polymer synthesis techniques has given rise to the importance of “living” free radical polymerization, as it provides a number of advantages over traditional free radical techniques, Matyjaszewski, K. (2000). The main advantages that a “living” free radical system

provides for polymer brush synthesis are control over the brush thickness, via control of molecular weight and narrow polydispersities, and the ability to prepare block copolymers by the sequential activation of the dormant chain end in the presence of different monomers, Husseman, M. (1999). Thus, the nature of the polymerization process permits structural characteristics of the grafted polymer brush to be readily varied and controlled.

Numerous “living” free radical techniques have been used to produce polymer brushes, these include atom transfer radical polymerization (ATRP) (Husseman M., 1999; Ejaz M., 2000), reversible addition-fragmentation chain transfer (RAFT) (Baum M., 2002), and nitroxide mediated polymerization (Husseman M., 1999). Probably the most common “living” radical technique to produce polymer brushes is ATRP.

CONCLUSIONS

Minor changes in the capsule composition has strong effects on the physico-chemical properties of the capsule’s surface. This can have major effects on the biocompatibility of the capsule’s. Building polymer brushes on the surface may overcome the variations in biocompatibility and allows inclusion of materials with biocompatibility issues but more desirable mechanical or stability properties in the capsule construction.

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