Microcapsule design for encapsulation of islets of Langerhans: An ongoing effort

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INTRODUCTION AND OBJECTIVES

Diabetes treatment by the insulin-producing cells encapsulated in microcapsules or another type of semipermeable membrane is considered to be a future immunosuppression-free therapy for controlling the blood glucose levels of diabetic patients. Recent clinical trials demonstrate the safety and limited functionality of transplanted human (Jacobs-Tulleneers-Thevissen 2013, Tuch 2009, Basta 2011) and porcine (www.lctglobal.com) islets. However, the freedom from insulin injections after transplantation of encapsulated islets to humans has not been achieved. There are several challenges connected with the microencapsulation based therapy related to the islets, biomaterials, permselectivity, insulin release kinetics, and packing density (Weir 2013). In designing the microcapsules for human use, the biocompatibility in terms of an inflammatory response is one of the most critical issues to be resolved. This is essential for both the functional transplant and the safety of patient. A number of principles for preparation of microcapsules exist (Lacik 2013), however, the biomaterial that convincingly meets the criteria for clinical application is not yet available.

This contribution deals with the design of microcapsules for encapsulation of islets linking the design of microcapsules with immunological approach. The polyelectrolyte-complex based microcapsule is made of sodium alginate, cellulose sulfate and poly (methylene-co-guanidine) (Lacík 1998). We have been tailoring this microcapsule to be tolerated in humans applying the human whole blood assay (WBA) as an effective short-time screening assay for predicting the inflammatory potential of microcapsules (Rokstad 2011, Rokstad 2013a). In relation to these studies, also other microcapsule types will be discussed as well as the general principles targeting the clinical trials.

MATERIALS AND METHODS

Sodium alginates (SA) high-viscosity SA (ISP Alginates) and ultrapure alginates UP-LVG, UP-LVM and UP-MVM (Novamatrix), sodium cellulose sulfate (CS) (Acros Organics), poly(methylene-co-guanidine) hydrochloride (PMCG) (Scientific Polymer Products Inc.) were used for microcapsule formation. The acronym “PMCG” is used in the text below referring to this microcapsule type. The photo-sensitive copolymer photo-switchable from polycationic to polyzwitterionic form, based on the recently published principle (Sobolčiak 2013) was used for covalent crosslinking of polyelectrolyte-complex membrane and, in parallel, formation of zwitterionic layer on the microcapsule surface.

PMCG microcapsules were prepared using the principles described previously (Lacík 1998). The polyanion solution contained 0.90 % alginate, 0.90 % CS in 0.9 % NaCl and the polycation solution contained 1.2 % PMCG, 1 % calcium chloride, 0.9 % NaCl, with the pH of both solutions adjusted to 7.4. The droplets of polyanion solution formed by air-stripping at the flow rate of about 0.6 ml/min were falling to a multi-loop reactor (Anilkumar 2001). The reaction time of about 40 s was typically selected for microcapsule formation by polyelectrolyte complexation. The additional coating layer was made either with 0.1 % CS or 0.1% heparin or 0.1 % of photo-sensitive copolymer, all in 0.9 % NaCl solution. Microcapsules were characterized by various methods providing their physico-chemical characteristics.

A human whole blood model as described in (Rokstad 2013b) previously established for evaluation of the inflammatory potential of microcapsules (Rokstad 2011, Rokstad 2013a) were used for the evaluation of the modified PMCG microcapsules.

RESULTS AND DISCUSSION

The representative PMCG microcapsules are of ~ 600 - 800 mm diameter and the membrane thickness of around 40 mm. The molecular weight cut-off of PMCG microcapsules can be adjusted in the large range with the target being at the limit of IgG. The rupture strength is around 5-10 g/capsule.

The human WBA was employed to determine the ability of various microcapsules to provoke an inflammatory response (Rokstad 2011, Rokstad 2013a). The empty PMCG microcapsules were tolerated after the intraperitoneal transplantation to baboons (Qi 2011) with the absence of overgrowth reactions. In the baboon WBA, these microcapsules did not show significant stimulation of complement. However, in human WBA PMCG microcapsules resulted in formation of terminal complement complex (sTCC), release of cytokines and activation of leukocytes compared to the control or alginate microbeads. This finding led to a series of modification steps in terms of polymer chemistry and the protocol for preparation, which resulted in representative data shown in Figure 1.

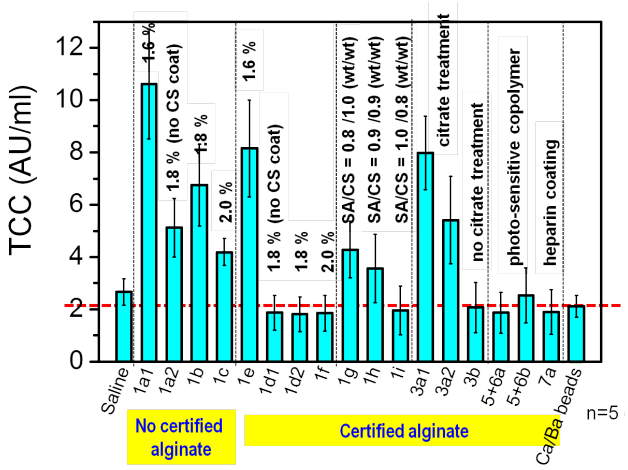

Figure 1: Effect of experimental conditions used for preparation of PMCG microcapsules on formation of terminal complement complex (TCC) in plasma after incubation for 240 min compared to the control Ca/Ba alginate beads. The numbers refer to the total concentration of polyanions in the solution.

These data indicate that utilization of GMP-certified sodium alginates instead of the *in house* purified ones (microcapsules 1a1, 1a2, 1b) in combination with (i) increased polyanion concentration (1f), (ii) increased ratio of SA to CS in solution of polyanions (1i), (iii) the absence of citrate treatment (3b), (iv) using photo-sensitive copolymers leading to zwitterionic structure (5+6a), and (v) heparinization of surface (7a) suppress the activation of complement to the level of alginate microbeads. Thus, Figure 1 points at the ability to correlate the microcapsule preparation conditions with the complement activation when using the human WBA.

In addition, a recent study shows that polycation-containing microcapsules activate inflammatory cytokines and growth factors as a consequence of complement activation (Rokstad 2013a). Intuitively, the polycation-containing microcapsules may be considered as potentially non-suitable due to stimulation of the inflammatory response. Our data suggest that the encapsulation protocols should be directed towards masking or neutralizing the presence of polycations. In this process, the activation potential can be revealed using the WBA.

CONCLUSIONS

The response of the immune system is highly complex and currently it is difficult to identify the principal reasons why the transplants do/do not fail from the point of view of biomaterial characteristics and biocompatibility (Rokstad 2013c). Nevertheless, the primary focus, after the feasibility studies in the animal model, should be the microcapsule design for human recipient. A direct link between the steps used in designing the microcapsules and human recipient is deemed to be represented by the human whole blood assay. In this contribution, the sensitivity of this assay is demonstrated for PMCG microcapsules. This strategy is recommended to be considered in designing other devices aimed at the immunoprotection of encapsulated cells in moving with this technology towards the clinics.

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