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Polyelectrolyte microcapsules for immune protection of pancreatic islets toward the clinical transplantation

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INTRODUCTION AND OBJECTIVES

The encapsulation technology has found numerous areas for which the basic research has already been translated to the large scale applications. The BRG has significantly contributed to these achievements within its 20 years history. One encapsulation field, however, has not reached the final stage yet that is the diabetes treatment by encapsulated islets of Langerhans. This is mainly due to multitude parameters in terms of biomaterials, islets, encapsulation and transplantation protocols, immunology, possible other factors and still lacking knowledge, which of those parameters are the most critical (Halle 2009).

The encapsulation community keeps in tackling these hurdles and in progressing towards the final goal, i.e., the identification of both islet source and suitable encapsulation technology. The multidisciplinary and open approach should be employed in order to effectively utilize the state-of-the-art knowledge. These principles are represented by The Chicago Diabetes Project (www.chicagodiabetesproject.org), which mission is to find the functional cure for diabetes by a global cooperation among the teams of diverse expertise.

The aim of this contribution is to highlight the main results for one of the microencapsule tested in this project, which is based on polyelectrolyte complexation between polyanions sodium alginate (SA) and cellulose sulfate (CS) with the polycation poly(methylene-co-guanidine) (PMCG) (Lacik 1998). The contribution outline will include selection and characterization of polyelectrolytes through detailed characterization of microcapsules to the *in vivo* studies using various animal models.

MATERIALS AND METHODS

Polyelectrolytes.
High viscosity sodium alginate (SA-HV) (SP 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 polyelectrolytes were characterized by ^{1}H NMR, size-exclusion chromatography and other analytical techniques.

Microcapsule preparation.
PMCG microcapsules were prepared using the principles described previously (Lacik 1998) except for some concentration changes in the polymer solutions. Typically 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) provided a continuous 1-step encapsulation process (Lacik 2006). The reaction time of about 40 s was typically selected for the polyelectrolyte complexation. Equilibration of membrane composition was obtained by treatment with 50 mM sodium citrate in 0.9 % NaCl solution pH 7.4 for 10 min. The additional coating layer was made with 0.1 % CS in 0.9 % NaCl solution for 10 min.

Microcapsule characterization. A number of characterization techniques were used including determination of mechanical properties, molecular weight cut-off, surface topography and chemistry, and permeation of fluorescently labeled solutes of different molecular weight. The complement activation response induced by PMCG microcapsules was assessed by the human whole blood assay together with several other types of microcapsules (Rokstad 2011).

In vivo studies. Empty PMCG microcapsules and microcapsules with encapsulated human islets were transplanted to rodent animal models (nude mice, Balb/C mice, Wistar rats), and the empty microcapsules were implanted to baboons (Qi 2011). A histology evaluation was used to characterize the retrieved microcapsules.

RESULTS AND DISCUSSION

Figure 1 shows the representative optical microscopy image of PMCG microcapsules with the diameter of ~ 700 μm and the membrane thickness of a few tens of micrometers. The size cut-off of PMCG microcapsules can be adjusted to the range between 2 and 20 nm with the target permeability being at the limit of IgG permeation. The rupture strength is around 10 g/capsule. Various ultrapure alginates next to the non-certified SA-HV alginate were tested in order to improve the biocompatibility of PMCG microcapsules. The experience says that any minor modification of the encapsulation protocol in terms

of polyelectrolyte properties and selection is mostly connected with the need to adjust the encapsulation process.

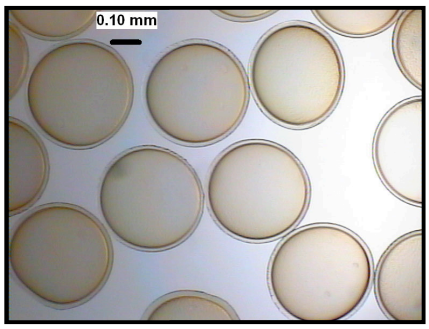

Figure 1. PMCG microcapsules

The human whole blood assay was introduced to determine the inflammatory response of various microcapsules (Rokstad 2011), which gives an indication of the microcapsule biocompatibility in terms of the human immune system. As an example, Figure 2 shows an amount of soluble terminal complement complex and monocyte activation in the presence of different microcapsules. Overall, the PMCG microcapsules stimulate the complement and leucocyte activation, which is not seen for the alginate beads. Therefore, the current version of PMCG microcapsules should be optimized in order to to minimize the stimulation of the human immune system recognized by this assay.

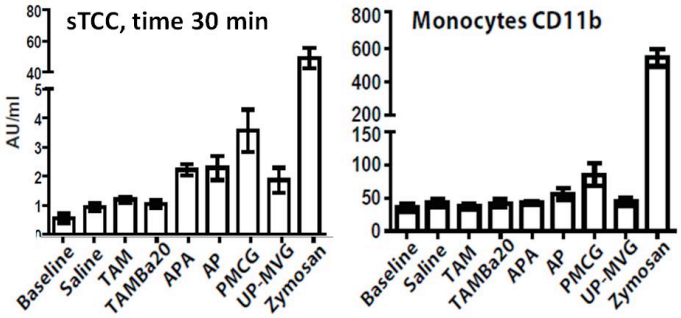

Figure 2. Terminal complement complex activation (sTCC, after 30 min of incubation) and monocyte activation as measured by CD11b expression for various microcapsules and controls tested in the human whole blood assay (Rokstad 2011)

The *in vivo* studies showed that encapsulated islets transplanted to the peritoneal cavity of diabetic nude mice, Balb/C mice and Wistar rats can control the blood glucose levels for 200, 90 and 70 days, respectively. Along this line, the degree of overgrowth determined by histology was increasing.

Interestingly, the empty PMCG microcapsules have shown a good biocompatibility after implantation to the peritoneal cavity of baboons by using a laparoscopic implantation (Qi 2011). The microcapsules were free-floating and did not exhibit a significant overgrowth at least four months after implantation.

CONCLUSION

The PMCG microcapsules have been proposed for immune isolation of transplanted islets of Langerhans about 15 years ago. It is the polycation-containing microcapsule type with several advantages with respect to adjustment of its properties and continuous encapsulation process. The presence of polycation (PMCG), on the other hand, may represent a disadvantage in terms of biocompatibility (Rokstad 2011 and references therein). It is unknown if microcapsules made in the absence of polycation and based solely on the gelled alginate beads represent the final microcapsule material to be used in clinics (Tuch 2009), since the fibrosis observed might have been due to the viability of the encapsulated islets being only 75%.
Certainly it is important to continue studies, in the multidisciplinary way, into all the directions with the potential for success until a proper biomaterial is identified for the clinics.

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