Surface Hydrophilicity as an Indicator of Implanted Microcapsule Biocompatibility

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Introduction

The transplantation of micro-encapsulated cells or tissues represents a promising treatment for a number of disorders (Orive 2003). Micro-encapsulation is an advantageous approach to cell transplantation because the microcapsule's semi-permeable membrane can provide immuno-protection to the grafted cells without requiring immuno-suppression. In particular, type 1 diabetes mellitus may be treated in the near future by transplanting clusters of insulin-secreting cells (i.e. the islets of Langerhans) that are first enclosed within protective hydrogel-based microcapsules. In this case, the viability and function of islet cells encapsulated within alginate-based microcapsules must be improved before the technique can be clinically applied on a regular basis.

One of the main factors influencing cell viability and function is the biocompatibility of the microcapsule in which the islets are enclosed. That is, an inflammatory or immune response to the microcapsule can lead to changes in the physiological environment, such as shifts in pH, which in turn can alter the microcapsule's ability to immuno-protect the encapsulated cells. Furthermore, a persisting immune response to the microcapsules usually leads to fibrotic overgrowth that can essentially suffocate the encapsulated cells.

The biocompatibility of implantable alginate-based microcapsules is determined by a number of inter-related factors (Orive 2006), including the chemical and physical properties of the microcapsule surface, the nature of the encapsulated tissue, and the environment at the implantation site. Here we focus on the possibility that the hydrophilic/hydrophobic balance at the microcapsule surface is a determinant of the short-term *in vivo* biocompatibility of the microcapsules. Surface hydrophilicity or hydrophobicity has been investigated to predict the biocompatibility of other biomaterial types (Lee 1998), but has not been explored extensively in the case of implantable hydrogel-based microcapsules.

Materials and methods

Materials. Sodium alginates had either a high guluronic acid (G) content (Protanal® LF 10/60, with 65-75% G, FMC Biopolymer, Norway) or an intermediate G content (Keltone® LVCR, ~ 40% G, International Specialty Products Corp, UK). The alginates were purified according to previously published protocols (Dusseault 2006; De Vos 1997). Poly-L-lysine (PLL) hydrobromide (Mw = 30, 200 Da MALLS) was purchased from Sigma (St-Louis, Missouri, USA). All other chemicals were purchased from Fisher Scientific Ltd (Napean, Ontario, Canada).

Bead and microcapsule preparation. Alginate droplets of sodium alginate (2.3 – 2.8% in 150 mM saline) were produced using an electrostatic drop generator. Barium alginate beads were produced by allowing the alginate droplets to gel in a 50 mM barium chloride solution for 30 minutes. Alginate-poly-L-lysine-alginate (APA) microcapsules were produced by gelling the droplets in a 100 mM calcium lactate solution for 30 minutes, followed by immersion in PLL (0.05% in saline) and alginate (0.23 – 0.28% in saline) solutions for 5 minutes each in order to form the membrane.

Evaluation of in vivo biocompatibility. For each sample, ~ 500 beads or microcapsules were injected into the peritoneal cavity of a C57BL/6 mouse. At 2 or 14 days after implantion, the samples were recuperated by peritoneal washing. Biocompatibility was evaluated in terms of the degree of cellular adhesion to the explanted microcapsules. This pericapsular reaction was quantified using a weighted scoring system (score 0 = no cells adhered, score 1 = partial coverage by cells, score 2 = complete coverage by cells).

Wettability studies. Wettability (i.e. hydrophilic/hydrophobic balance) was evaluated by measuring the contact angle of sterile water on the surface of thin films. The films were created by air-drying polymer solutions that were cast on glass microscope slides. Alginate films were made from 200 µL samples of alginate solutions (2.0% in sterile water). APA films were fabricated by mimicking the protocol for microcapsule fabrication, i.e. by immersing a thin film of (wet) alginate in calcium lactate, PLL and dilute alginate solutions respectively. Contact angles of 1.00 µL drops of sterile water were measured on the film surfaces using a VCA Optima System (AST Products, Inc., Billerica, MA, USA). For each film, readings were taken on at least 3 spots.

Results and Discussion

Effect of alginate purity. Alginates having a high guluronic acid content (HiG), yet varying in degree of purity, were compared. As shown in Fig 1a, alginate films produced from the unpurified alginate was significantly ($p < 0.005$) less hydrophilic (contact angle 53.6°) than each of the purified alginates (contact angles 30.9° to 41.6°). Upon implantation, there was a stronger reaction to barium alginate beads that were produced from those alginates that were less hydrophilic, as compared to the beads produced from more hydrophilic alginates (Fig 1b). There was a direct correlation between the severity of the pericapsular reaction at 2 days ($R = 0.67$) or 14 days ($R = 0.79$) after implantation and the measured contact angles on the corresponding alginate films.

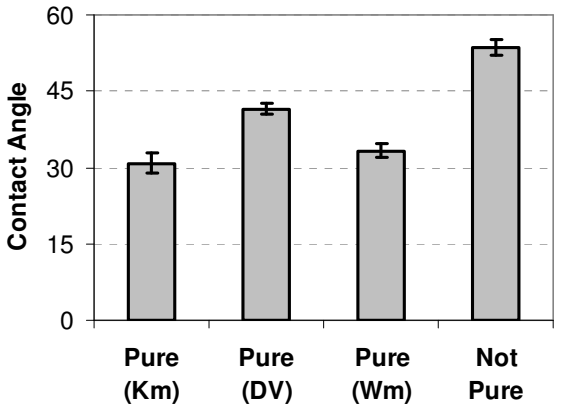

Fig 1a : Contact angle of water on films cast from HiG alginate solutions.

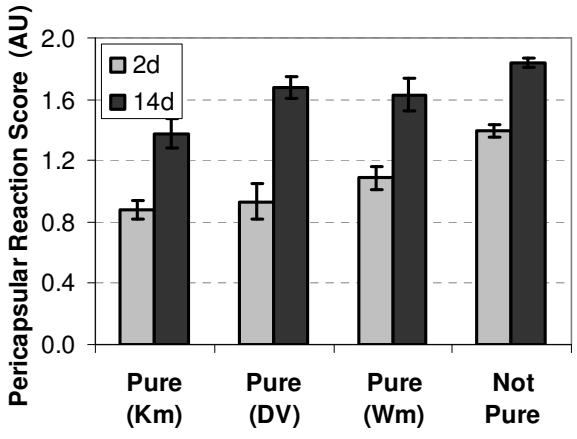

Fig 1b : Pericapsular reaction to barium alginate beads (HiG) 2 and 14 days after implantation

These results demonstrate that for alginates of the same source, wettability is a good indicator of the purity and corresponding *in vivo* biocompatibility of the alginates. Detailed discussions about the influence of contaminants on alginate wettability have recently been published (Tam 2006).

Effect of alginate composition. Alginates having an intermediate G content (IntG) were compared to those having a high G content, the samples being purified or non-purified. Recent results using the IntG alginate (Fig 2a) confirm that purifying the alginate has an effect of increasing its hydrophilicity, which is measured by a lower contact angle. Furthermore, the IntG alginate was less hydrophilic than the HiG alginate in the case of the unpurified polymers. When the biocompatibility of the barium alginate beads was evaluated (Fig 2b), we found that for each alginate type the purified sample (and thus the more hydrophilic) tended to induce a less severe pericapsular reaction. Despite the purity level, both IntG alginates were much more biocompatible than the HiG alginates.

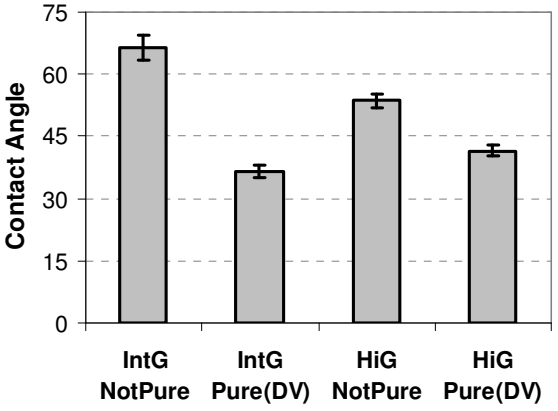

Fig 2a : Contact angle of water on films cast from alginate solutions.

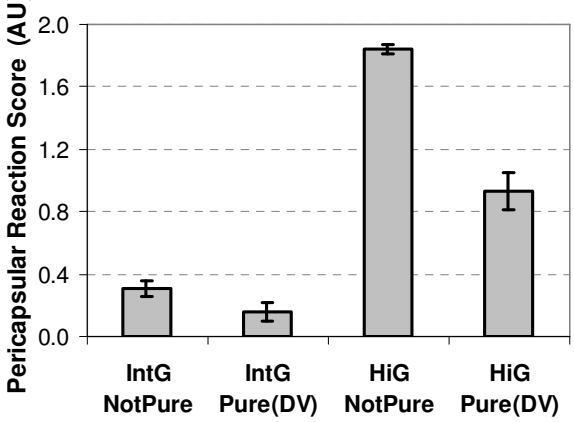

Fig 2b : Pericapsular reaction to barium alginate 2 days after implantation.

These results confirm that hydrophilicity is an indicator of alginate purity when the source of the alginate is common. Otherwise, it appears that the guluronic acid content has a stronger influence on the adhesion of immune cells to the implanted alginate beads than the surface hydrophilicity.

Effect of poly-L-lysine membrane.

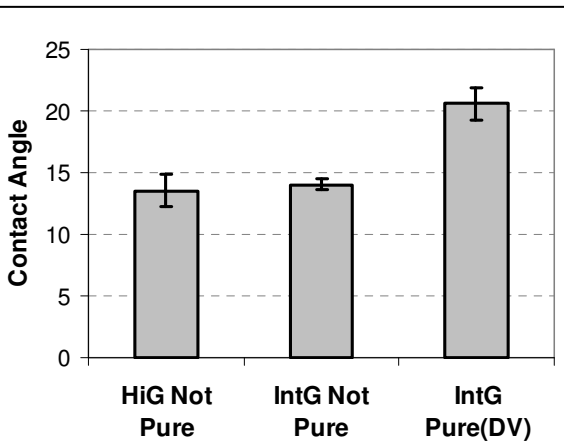

Fig 3a : Contact angle of water on tri-layered APA films.

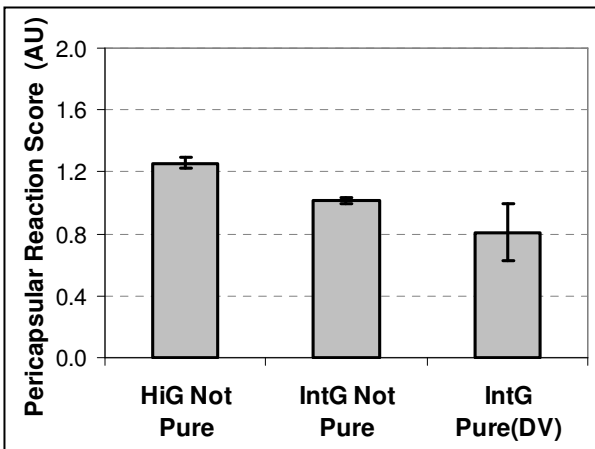

Fig 3b : Pericapsular reaction to APA microcapsules 2 days after implantation.

Tri-layered films of alginate and PLL were cast onto microscope glass slides in order to produce a 2-dimensional surface that imitates the chemical make-up the APA microcapsule surface. As can be seen in Fig 3, the APA film made from purified IntG alginate showed the least hydrophilicity (contact angle 20.6°) when compared to the unpurified samples (14.0° for IntG and 13.6° for HiG), yet the corresponding microcapsules were the most biocompatible (mean score 0.81). A comparison of the pericapsular reactions to the samples also demonstrates that, in the case of APA films or microcapsules, there is an inverse correlation between hydrophilicity and biocompatibility.

The measured surface wettability may have been influenced by either the presence of the polycation forming a complex with the alginate (Tam 2005), or by the salt in the solutions which can interfere with the readings. To test these hypotheses, we also measured the hydrophilicity of films of alginates (no PLL) dissolved in saline at concentrations identical to the solutions used for microcapsule fabrication. The results (not shown) indicated that, when dissolved in saline, the alginate wettabilities follow the same tendency as seen in Fig 3, with the purified IntG alginate being the least hydrophilic. Further studies must be completed to confirm these hypotheses.

Conclusions

The wettability of alginate films is observed to be a good indicator of the polymer purity. Hydrophilicity is also strongly influenced by the guluronic acid content of the alginate. The hydrophilicity of the alginate is directly correlated with the biocompatibility of the corresponding barium alginate beads, although this effect may be overriden by the influence of alginate chemical composition. With the introduction of a polycationic membrane, the hydrophilicity of the surface appears to be inversely correlated with the biocompatiblity of the APA microcapsules.

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