Surface Characterization and Investigation of Protein Adsorption for the Optimization of Microcapsule Biopeformance

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Statement of Purpose: Microcapsules for cell therapy protect transplanted therapeutic cells from host immune rejection without requiring immunosuppression. Such microcapsules are commonly composed of alginate, which is complexed with a polycation to form the capsule's protective membrane. Alginate-Poly-L-lysine-Alginate (APA) microcapsules have performed well in animal models, as an absence of fibrotic overgrowth is now achievable. However, the bioperformance of APA microcapsules between laboratories, and often between trials, remains difficult to reproduce. Furthermore, if microcapsule biocompatibility is not optimal, then inflammation that is induced by the implantation surgery can persist or develop into an immune response that reduces viability of the encapsulated cells.

Controlling the biological response to the implanted microcapsules, thus improving bioperformance reproducibility and minimizing the severity of inflammatory/immune response, requires a clear understanding of the relationship between the microcapsule and the biological response at the molecular level. The author's approach to achieving this is to investigate the relationships between (i) the surface properties of APA microcapsules, (ii) the adsorption of host proteins to the capsule surface, and (iii) the cellular response to the capsules. Results concerning the first two aspects are presented here, while the latter is currently being investigated. This approach has only recently been introduced as a tool for optimizing the bioperformance of alginate-based microcapsules for cell therapy.

Methods: Physico-chemical characterization of the APA microcapsule surface was performed using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR), X-ray Photoelectron Spectroscopy (XPS) and Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS). These techniques have analytical depths that range from 1-2 nm to 3 µm, allowing a complete picture of the membrane structure.

The adsorption of immunoglobulins (IgG, IgM and IgA) from human serum to APA microcapsules *in vitro* was investigated by fluorescence labelling. IgG and IgM are known to mediate complement activation when adsorbed to foreign bodies. Ig adsorption was measured quantitatively using a fluorescence microplate reader, and observed qualitatively using an inverted fluorescence microscope. The effects of each the alginate chemical composition and purity and the presence of PLL on the extent of Ig adsorption were investigated.

Results/Discussion: Although the fabrication process of APA microcapsules suggests that there exists a distinct outer layer of alginate, surface investigations indicated that the exposed surface actually consists of an alginate-PLL complex (Figure 1). Furthermore, ATR-FTIR studies indicated that the complexation efficiency between the alginate and PLL is not optimal. This may present a threat to the microcapsule biocompatibility since unbalanced positive charges of the PLL are reputed to induce inflammatory reactions.

Figure 1. Representative distribution of molecules at the APA microcapsule surface (ToF-SIMS)

Experimental results clearly demonstrated that immunoglobulin adsorbs to the APA microcapsule surface *in vitro*. The chemical composition and purity of the alginate had an observable effect of the extent of adsorption, in a manner that corresponded to their previously observed *in vivo* performance. The presence of PLL had a significant impact on adsorption, as adsorbed Ig reached negligeable amounts in the absence of PLL (Figure 2). This observation corresponds with published studies concluding that PLL is responsible for the observed immunogenicity of APA microcapsules, thus supports the view that Ig adsorption plays a role in mediating the immune response to APA microcapsules.

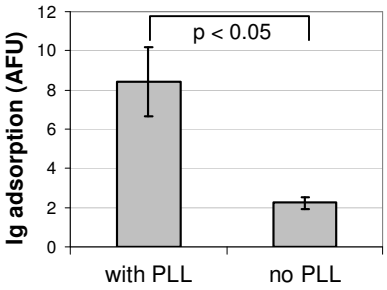

Figure 2. Adsorption of immunoglobulin on microcapsules with and without a PLL membrane

Conclusions: An investigation of the surface properties of APA microcapsules in relation to the adsorption of host proteins is being pursued in order to explain and optimize the bioperformance of APA microcapsules for cell therapy. Results to date suggest that exposure of PLL at the capsule surface plays a role in the adsorption of immunoglobulin, which may in turn be mediating an immune response to these microcapsules.