Poly(vinyl alcohol)-Heparin Biosynthetic Hydrogel Microspheres for Cell Encapsulation

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Statement of Purpose: Encapsulation of cells within hydrogel microspheres is a promising technology for the treatment of diseases such as diabetes. Biosynthetic polymers have been applied in other areas of tissue engineering and combine the mechanical and chemical stability of synthetic components with the bioactivity of a biologic. Applying these polymers to microcapsule formation remains a challenge. However they could address one of the main limitations of current microencapsulation systems preventing their translation into the clinic- that they simply act as a passive barrier to the immune system and cells eventually lose function. By covalently incorporating a biologic into a synthetic hydrogel such as poly(vinyl alcohol) (PVA), these biosynthetic microcapsules should not only be mechanically superior to the common alginate capsules, but also be able to actively support cell function and viability.

Therefore, the aims of this study were to fabricate PVA microspheres and covalently incorporate a biologic using a cytocompatible process to improve long term success of microencapsulation systems. Heparin has been identified to be important for beta-cell differentiation and insulin secretion (Takahashi I. Biochem Biophys Res Commun. 2009;383;1;113-118) and as beta-cells are the eventual target of this research, heparin was chosen as the biologic for this study. A submerged electrospray technique was developed and used with UV photopolymerisation to fabricate the microspheres.

Methods: PVA (16kDa, 98% hydrolysis) and heparin (porcine intestinal mucosa, 15kDa) were methacrylated with ICEMA (Bryant SJ. Macromolecules. 2004;37(18); 6726-6733) and GMA (Nilasaroya A. Biomaterials. 2008; 29(35);4658-4664) respectively. Macromer solutions of 20wt% PVA and 19wt% PVA / 1wt% Heparin in PBS with 0.05wt% photoinitiator (I2959) were prepared with or without cells. Microspheres were fabricated from these solutions by dispersion in oil with a custom-built submerged electrospray apparatus (applied voltage 4kV and flow rate 0.1ml/min) and subsequent exposure to UV light (365nm, 30 mW/cm^2, 3 minutes). Microspheres were isolated by washing several times in PBS. L929 murine fibroblasts were encapsulated in microspheres (1x10^6 cells/ml) and cultured in complete media for 28 days. Spheres were characterized for mechanical properties (compression testing at 10µm/s) and cell viability (Live/Dead assay- Calcein AM and Propidium Iodide) at various time points.

Results: The submerged electrospray technique in combination with UV photopolymerization was able to produce smooth, spherical microspheres. The size of the spheres could be tailored by changing the applied voltage and flow rate. The conditions used in this study produced spheres ~400µm in diameter. The compressive modulus was 350±99kPa and 360±95kPa for PVA and PVA-Heparin spheres respectively without cells after 1 day incubation in PBS to remove the sol fraction. This is comparable with the bulk compressive modulus of PVA hydrogel of 312±65 kPa. The compressive modulus of PVA-Heparin spheres with encapsulated L929 cells (Fig. 1) was slightly, but not significantly, lower than PVA spheres and neither changed significantly over 28 days.

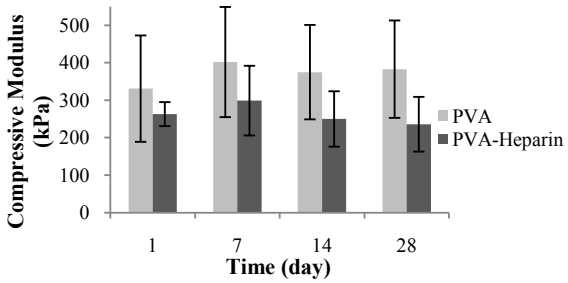

Figure 1. Compressive modulus of microspheres encapsulating L929 cells over 28 days in culture

Viability of the L929 cells in the microspheres over 28 days in culture (Fig. 2) was shown to remain high in both systems, but was significantly higher in PVA-Heparin spheres at time points after 7 days.

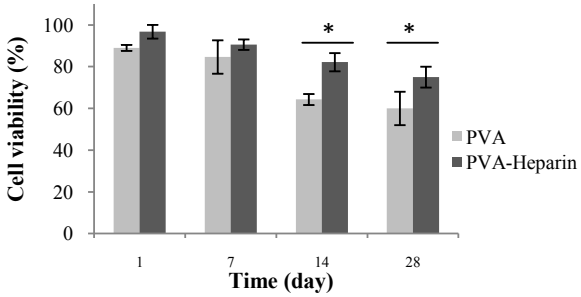

Figure 2. Viability of L929 cells encapsulated in PVA and PVA-Heparin Microspheres over 28 days in culture, * $p<0.05$

Conclusions: Submerged electrospray was able to produce microspheres, and the biologic heparin was successfully covalently incorporated. These biosynthetic microspheres retained good mechanical properties and supported cells in culture for 28 days. Heparin improved long term cell viability compared to PVA alone, with the benefits possibly due to heparin being able to bind and present growth factors to cells.

This system could be used for many cell encapsulation applications to improve outcomes by prolonging cell survival and function. Future work will utilize this system to encapsulate beta cells for the purpose of diabetes treatment and study the effect of heparin on viability and glucose stimulated insulin secretion.

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