Injectable controlled delivery system for adipogenesis of adipose derived stem cells

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Introduction: Current therapies for soft tissue reconstruction due to trauma or tumor resection include autologous tissue flaps and prosthetic implants. Both of which have disadvantages, including donor site morbidity, foreign body reaction, and implant migration. Injectable autologous cell-based therapies for soft tissue reconstruction are a promising alternative to the previous therapies. Adipose tissue is a plentiful source for autologous mesenchymal stem cells with low morbidity and the potential to differentiate into multiple lineages.[1]

Our objective was to maintain controlled delivery of adipogenic factors to induce differentiation of adipose derived stem cells into adipocytes. As such, we encapsulated dexamethasone (dex) and insulin in poly (lactic-co-glycolic acid) (PLGA). The in vitro release kinetics were determined. The ability of the released factors to induce differentiation was assessed.

Materials and Methods:

Encapsulation of adipogenic factors: Dexamethasone sodium phosphate was encapsulated in PLGA utilizing a single emulsion solvent extraction technique. Insulin was encapsulated in PLGA utilizing a double emulsion technique. The microspheres (MS) were lyophilized and characterized by SEM.

Release of adipogenic factors: To determine the in vitro release kinetics from the microspheres, 10 mg of microspheres were incubated in 1mL phosphate buffered solution at 37°C in a microcentrifuge tube. At each time point, the tubes were centrifuged and the supernatant collected and frozen at -80°C. The released dex was measured spectrophotometrically at 242nm (n=10). The released insulin was measured utilizing a commercially available ELISA kit (LINCO Research) (n=3).

Isolation of ASCs: ASCs were isolated from waste adipose tissue of human female patients as previously described.[2] Briefly, adipose tissue was minced and digested in a collagenase solution then centrifuged. The cellular pellet was resuspended in erythrocyte lysis buffer and centrifuged. The resulting pellet was then plated in ASC medium.

In vitro studies: ASCs were seeded at a density of $5*10^3$ASCs/mL in 12-well tissue culture plates. Once confluent, the ASCs were treated with one of the following medium groups in Table I with and without the addition of the corresponding microsphere groups (n=5).

Table I. ASC Treatment Groups

Medium	Microspheres
ASC medium	Dex MS + Insulin MS
Differentiation medium without dex or insulin	Dex MS + Insulin MS
Differentiation medium without dex	Dex MS
Differentiation medium without insulin	Insulin MS

The medium was changed every other day for 2 weeks. On day 14 the cells were fixed and stained for Oil Red O.

Results/Discussion: Dexamethasone and insulin were successfully encapsulated in PLGA and examined by SEM (Figure 1). The average diameter of the dex MS was 9.85 µm, and the average diameter of the insulin MS was 272.4 µm.

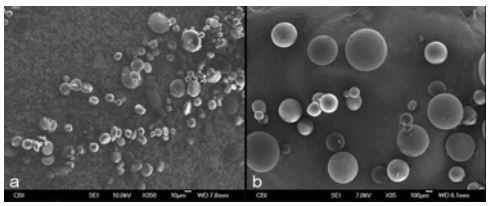

Figure 1. Scanning electron microscopy of dex MS (350x) (a) and insulin MS (35x) (b).

The controlled release of dex was maintained over 52 days in vitro (Figure 2a). The insulin was released over 18 days (Figure 2b).

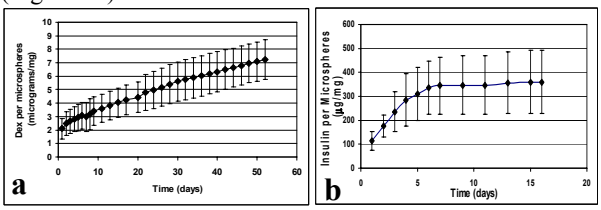

Figure 2. Cumulative release of dex (a) and insulin (b).

Adipogenesis was analyzed by Oil Red O staining. The groups with the addition of microspheres contained more lipids than their controls (Figure 3). Thus, the released dex and insulin induced adipogenesis of the adipose derived stem cells.

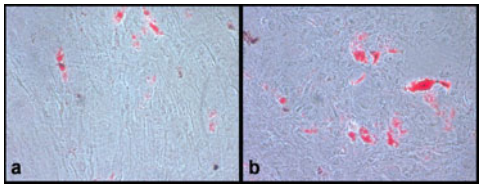

Figure 3. Oil Red O staining of cells treat with ASC medium alone (a) and ASC medium plus dex MS and insulin MS (b).

Conclusions: The need for an injectable system for soft tissue reconstruction remains a clinical problem. We have developed an injectable controlled delivery system that can locally release adipogenic factors that induce differentiation of ASCs. We are currently analyzing protein expression of PPAR-γ to confirm the adipogenesis. The injectable microspheres offer an alternative solution for soft tissue reconstruction.

References:

[1] Zuk PA, et al. Tissue Eng 2001; 7:211-228.
[2]Clavijo-Alvarez JA, et al. Plast Reconstr Surg 2006;118: 1132-1142.