O8-5 **Insulin microcapsules in alginate dressing provide controlled bioactive delivery**

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INTRODUCTION AND OBJECTIVES

Skin is an important, living barrier that is designed to protect internal tissues and organs. Injury-resulting in an open wound, requires rapid restoration.

Insulin, has been shown to improve the rate of healing in a variety of situations. Studies by Greenway (2008) showed that insulin accelerates wound closure by stimulating keratinocyte migration. In our previous study (Hrynyk 2010), we demonstrated that insulin could be encapsulated within PLGA microspheres using a solid-in-oil-in-water (S/O/W) suspension technique, providing extended term (25 days) controlled release of bioactive insulin. However, a method of dispersing the microparticles in a hydratable wound dressing matrix was required.

In this study, we present the incorporation of insulin-loaded PLGA microspheres into alginate-PEG foam matrices, formed by a freeze-drying method. Both high M (A-M) and G (A-G) alginates at different concentrations, along with various molecular weight PEGs were tested to determine effects on foam physical properties, insulin release kinetics and insulin bioactivity. The results demonstrate a potential for alginate-PEG foams incorporating the controlled release microspheres as a viable wound dressing for topical insulin delivery and accelerated wound healing.

MATERIALS AND METHODS

PLGA (*L*,*G* 50:50, 17 kDa) (Purac). Crystalline human recombinant insulin, poly(vinyl alcohol) (PVA), poly(ethylene glycol) (PEG) (Sigma Aldrich). Protanal LF 10/60 and 10/60LS (Pronova). Human Insulin ELISA kit (Mercodia). Human keratinocytes (HaCaT) (CLS Inc.).

Preparation of PLGA Microparticles – A 100 mg batch of microparticles containing 5% crystalline insulin were prepared by dissolving 1 mg PLGA into 1 mL dichloromethane. The suspension was then added drop-wise into 30 mL 5% PVA solution. An impeller mixer stirred the resulting emulsion under constant mixing at 430 rpm for 1 min. The emulsion was transferred to 400 mL distilled water stirring at 180 rpm for 10 h on ice. Microspheres were then collected by vacuum filtration.

Preparation of Alginate Foam Disks – Approximately 5 mg of insulin loaded-PLGA microparticles were combined into i) 2% and 4% A-M, ii) A-G and iii) 2% A-M 0.1-10% 1.45 and 10 kDa PEG solutions cast into 24 well plates. The plates were frozen for up to 1 h at -80°C, prior to being lyophilized overnight.

Foam Morphology– Alginate foam sections were examined by SEM (JEOL 840, USA).

Tensile Testing – Foam disks were cut into 5 x 20 mm rectangular strips and subjected to a strain of 2 mm/min (TA Instruments, USA). The maximum stress (MPa), Young's modulus (MPa) and percent elongation of fracture were calculated.

Insulin In Vitro Release – Alginate foam disks were placed into netwell inserts and lowered into chambers filled with solution A (142 mM NaCl, 2.5 mM $CaCl_2$) Samples were incubated at 35°C on a flat orbital shaker rotating at 60 rpm and insulin assayed in the supernatant using ELISA.

Insulin Bioactivity: Scratch Assay – HaCaT cell suspensions were seeded onto 24 well plates (Costar; Lowell, USA), and were confluent at 48h. A scratch was made using a 10 µL pipette tip. Cells were washed, and treated with (i) fresh insulin; (ii) insulin released from foams; or (iii) from placebo foams. Scratch closure was measured using microscopically over 48h.

RESULTS AND DISCUSSION

A-M and A-G alginate foam disks prepared from 2-4% solutions were analyzed using SEM (Figure 1A-D). Two distinct morphologies were observed. A-M foams exhibited corrugated and open cell structures, versus A-G foams which had more layered sheets of alginate. Little effect of alginate concentration on foam morphology, however, the appearance of microspheres could be seen embedded in the foams.

Alginate foam disks were prepared with solutions of 2% A-M and 0.1-10% 1.45 and 10 kDa PEG and analyzed using SEM (Figure 2A-F). PEG resulted in bulking of the open foam cell structure.

Tensile testing was performed on alginate and alginate-PEG foams. (Tables 1 and 2). A-M foams containing 1% 1.45 kDa PEG were more flexible and stronger, versus other PEG-free and alginate-PEG preparations.

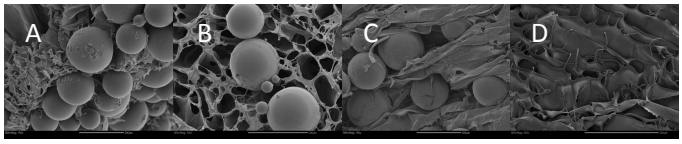

Figure 1. Cross-sectional SEM images of alginate foam wound dressings prepared with 2% A-M (A), 4% A-M (B), 2% A-G (C), and 4% A-G (D) alginate foam disks loaded with insulin-PLGA microparticles.

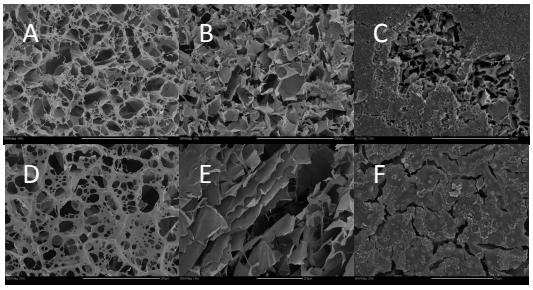

Figure 2. SEM images of 2% A-M alginate foam disks prepared with 1.45 kDa PEG at concentrations of 0.1 (A), 1 (B) and 10% (C). Images of 2% high M alginate dressings prepared with 10 kDa PEG at concentrations of 0.1% (D), 1% (E) and 10% (F).

Table 1. Tensile strength of A-M and A-G foams.

	2A-M	4A-M	2A-G	4A-G
Young's Modulus (MPa)	1.0 ± 0.2	9.4 ± 5.3	1.0 ± 0.7	4.3 ± 2.8
Max Stress (MPa)	0.06	0.25	0.04	0.16
Elongation at Fracture (%)	5.3 ± 0.7	4.5 ± 0.7	5.4 ± 1.1	6.2 ± 2.1

Table 2. Tensile strength of alginate foams prepared from 2% A-M alginate solutions containing 0.1-10% PEG 1450 Da and 10 kDa.

	PEG 1.45 kDa		PEG 10 kDa	
% w/v PEG	0.1	1	0.1	1
Young's Modulus (MPa)	5.0 ± 2.1	2.2 + 0.7	0.3 + 0.3	0.6 + 0.3
Max Stress (MPa)	0.13	0.03	0.01	0.02
Elongationat Fracture (%)	5.1 + 1.3	2.41 + 0.5	12.0 + 3.0	4.5 + 0.9

Insulin release kinetics revealed the A-M and A-G foams resulted in a burst release of insulin, followed by sustained release for up to 21 days (Figure 3). Incorporation of PEG reduced the burst and maintained zero order kinetics for up to 10 days (Figure 4)

Bioactivity of released insulin was measured using a HaCaT scratch assay to determine the effect of stimulation on cell migration and proliferation (Figure 5A-D). Alginate-PEG foams were able to maintain bioactivity for up to 10 days, versus PEG-free alginate foams which experienced earlier loss of bioactivity.

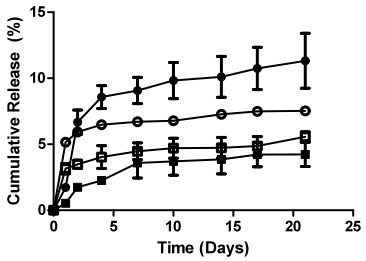

Figure 3. Insulin release kinetics from alginate foams prepared from 2% (⊖) and 4% (⊖) A-M and 2% (⊟) and 4% (⊟) A-G alginate foam disks.

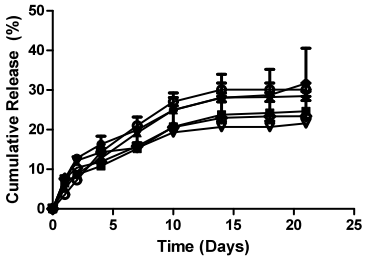

Figure 4. Insulin release kinetics from alginate foams prepared from a 2% A-M alginate solution with 0.1% (⊖), 1% (⊟) and 10% (▲) 1.45 kDa PEG and 0.1% (▼), 1% (◆) and 10% (⊖) 10 kDa PEG.

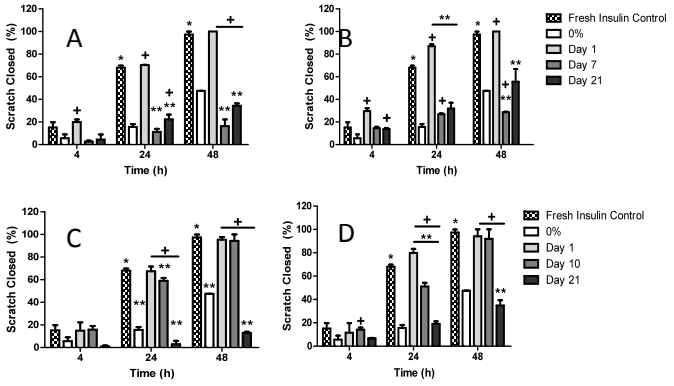

Figure 5. HaCaT cell scratch assays evaluating bioactivity of insulin released from 2% A-M (A), A-G (B), 2% A-M; 1% PEG 1450 Da (C), and 1% PEG 10 kDa (D) alginate foam disks from days 1, 7, 10, and 21.

CONCLUSIONS

PEG-alginate foam disks showed promise as future wound dressings with the strongest and most flexible matrices, capable of delivering bioactive insulin for up to 10 days. In vivo assays are in progress and results will be presented at the conference.

REFERENCES

- Greenway S.E. et al. (1999) *Topical insulin in wound healing: a randomised, double-blind, placebo-controlled trial.* Journal of Wound Care 8 526-528.
- Hrynyk M. et al. (2010) *Sustained prolonged topical delivery of bioactive human insulin for potential treatment of cutaneous wounds.* Int. J. Pharm. 398 146-154.