Electrospun Chitosan Fibers for Insulin Delivery

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Introduction: Currently, the overwhelming majority of insulin therapy patients rely on insulin delivery through subcutaneous injection. Patient non-compliance with this mode of delivery is a major problem with potentially fatal side effects. Oral insulin delivery is the most attractive alternative route in terms of both patient comfort and potential risks, but is complicated by processes in the GI tract. Natural polysaccharides such as chitosan (CS) have received much interest as inexpensive means to facilitate drug transport across the oral mucosa, but surface area must be maximized. In contrast to nanoparticles, electrospinning is a more versatile technique for rapid production of solid nanomaterials. Previously this lab has developed gelatin based fibers capable of insulin delivery across the buccal mucosa, but these fibers are not stable under physiologic conditions without cross linking. (Xu et al., Pharm Res. 2015;32: 275-285.) In this work we have developed electrospun chitosan fibers stable in aqueous solution under physiologic conditions. Insulin released from the scaffolds retains bioactivity through the electrospinning process. We believe this material can serve as an affordable solid platform for oral insulin delivery.

Methods: All materials were purchased from Sigma-Aldrich, St. Louis MO unless otherwise stated, Electrospun fiber mats were generated using low molecular weight chitosan dissolved in hexafluoroisopropanol (HFIP) at a concentration of 8mg/mL. Poly (ethylene oxide) (PEO) (900kDA MW) was then added to the solution at weight ratios of 1:1, 2:1, or 4:1 CS:PEO. Human insulin was dissolved in the solution 48 hours prior to scaffold fabrication at a 20:1 CS:Insulin ratio. Scaffolds were spun using a custom electrospinning apparatus using the following parameters adjusted slightly to account for differences in total solution concentration and humidity: 15-20kV, 17cm airgap distance, 2-3.5mL flowrate, 18ga. blunted needle. Scaffolds were air dried at least 72 hours to remove residual solvent before any further testing. Insulin release experiments were performed in PBS at 37°C. A sandwich ELISA assay (CalbioTech, Spring Valley CA) was used to quantify insulin concentrations. 10mg fiber samples were hydrated in PBS at 37 °C, removed at specified time points and lyophilized to quantify degradation under physiologic conditions. Cell tests were performed using 3T3-L1 preadipocyte cells (ATCC). Insulin bioactivity was assessed by exposing preadipocytes to either 6 hour fiber release medium or growth medium containing dissolved insulin (200µIU/mL) for 10 minutes, then lysing the cells and performing a western blot for Akt-1 and p-Akt.

Results: Electrospinning of pure chitosan was not successful due to the high surface tension of chitosan solutions. The addition of PEO enabled production of smooth fibers, but mixed beading due to fiber rupture was observed at polymer concentrations less than 20% (wt/wt). A mixed population of sub-micron (~200 nm) and larger (1-2 µm) fibers were seen under SEM for high PEO content blends. ELISA results showed insulin release over narrow time periods (<6 hr) was highly dependent on polysaccharide concentration. 4:1 CS:PEO fibers released significantly more insulin in 6 hours than both other blends, and achieved approximately 100% release. The lower CS ratio blends were abandoned for any further experiments. Degradation measurements showed 4:1 CS:PEO fibers lost about 15% of their total mass within the first 15 minutes of hydration, but no significant mass loss over the next 6 hours. (Fig. 1) SEM images (not shown) confirmed fiber morphology was partially intact after degradation, with some regions beginning to melt. Akt1/p-Akt western blots showed 3T3-L1 preadipocyte cells exposed to fiber release media had a 3.5 fold higher Akt activation level than cells treated with fresh media (Fig. 2). This indicates insulin is not denatured sufficiently during the electrospinning process to cause loss of bioactivity.

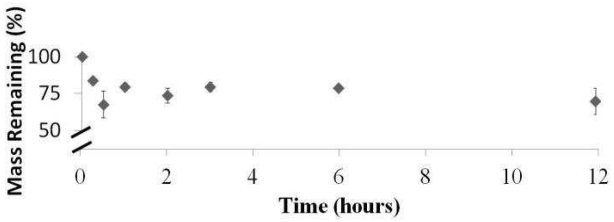

Figure 1: Chitosan fibers are stable under physiologic conditions.

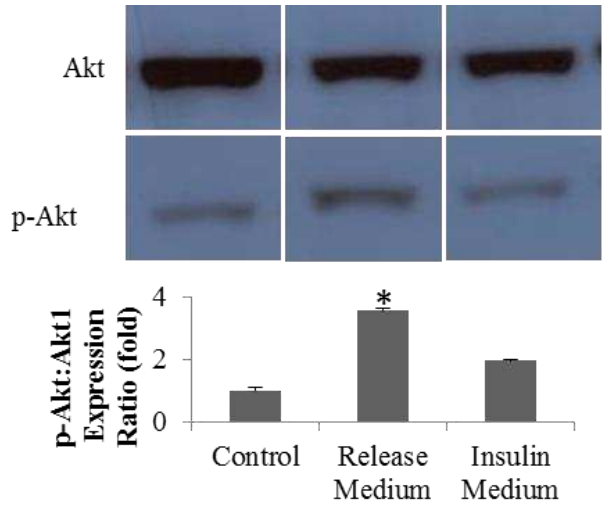

Figure 2: Insulin released from fiber scaffolds maintains bioactivity. Cells treated with fiber release medium for 10 minutes showed increased Akt activation.

Conclusions: In this work it has been demonstrated that insulin containing electrospun chitosan fibers can be generated without surfactant using a minimum of 20% PEO. Fibers produced with a 4:1 CS:PEO ratio release all loaded insulin within 6 hours. The fibers are stable under physiologic conditions for at least this time scale. Akt activation experiments have shown the bioactivity of dissolved insulin is not impaired by the electrospinning process. Future work will seek to independently confirm insulin bioactivity results by inducing 3T3-L1 preadipocyte differentiation and lipid accumulation. Experiments will also determine the transport kinetics of released insulin across the buccal mucosa.

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