Release of Adenosine from Chitosan Films to Promote Healing of Diabetic Foot Ulcers

**INTRODUCTION:**
Diabetic foot ulcers affect approximately 15% of the almost 20.8 million people with diabetes mellitus, costing as much as $10.9 billion annually. In up to 65% of cases, diabetic foot ulcers have involvement of underlying bone. Of patients who suffer from diabetic foot ulcers, 20% end in lower extremity amputations, with a mortality rate of approximately 31% for diabetic patients five years post-amputation. Due to neuropathy, insufficient vascularization, and bone healing abnormalities associated with the disease, diabetic foot ulcers are difficult to treat. Although growth factors and cytokines have been found to improve wound healing, these treatments are expensive and clinically ineffective. Recent studies have found that a less expensive compound, adenosine, is also effective in promoting wound healing.

To improve the availability of adenosine during the healing process, a localized biomaterial delivery system can be utilized. Chitosan has been found to be an effective, biodegradable vehicle for local drug delivery. Chitosan constructs will be evaluated to determine if they can be used to deliver adenosine to fibroblasts and osteoblasts to promote growth and proliferation to enhance the healing of diabetic foot ulcers. In this study, elution of adenosine from chitosan films made with different solvent acids was measured and biological activity was tested in cell culture.

**METHODS:**

**Chitosan Film Fabrication.** Chitosan with 80% degree of deacetylation (DDA) (Dermaflux) was mixed with either 1% lactic acid or acetic acid containing 2 mM adenosine in 3 weight % chitosan. Films were cast into 9 mm diameter glass Petri dishes and placed into a 37°C oven for 24 hours to dry. The films were then neutralized in 2 M NaOH and washed in water.

**Elution.** Seven films from each acid solution were placed into 9 mm Petri dishes with 20 mL of PBS at 37°C. Samples were taken at 24, 48, and 72 hours. Elution media was completely replaced after each sampling. Concentrations of adenosine in eluate solutions were determined using HPLC technique.

**Activity Testing.** Normal human dermal fibroblasts (NHDF) (Cambrex) were plated in 96 well plates at 10^5 cells/cm² in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum. MC3T3 osteoblast-like cells were plated similarly in 96 well plates in alpha-MEM supplemented with 10% fetal bovine serum. After allowing the cells to attach overnight, cells were serum-starved for 24 hours prior to the addition of adenosine and eluates. Proliferation in response to known concentrations of adenosine and in response to eluates was measured using Cell-Titer Glo (Promega, Madison, WI).

**Statistics.** Statistical differences in elution profiles between similar samples were determined using ANOVA with Tukey’s Honest Significant Difference test. Statistical differences in activity were determined using a student t-test.

**RESULTS SECTION:**

Preliminary studies of adenosine biological activity showed dose-dependent increases in fibroblast proliferation in adenosine concentrations up to 500 µM (Figure 1). Concentrations of adenosine over 125 µM induced sharp increases in proliferation of MC3T3 osteoblast-like cells (Figure 2).

Release results of adenosine elution from the chitosan films showed an initial burst release of adenosine from lactic and acetic acid solvent films at 24 hours followed by significantly lower release after 48 hours (Figure 3). More total adenosine eluted from the chitosan films made with the acetic acid solvent than from films made with the lactic acid solvent. Samples were normalized to the initial weight of the chitosan films.

Initial analysis of activity of eluates indicates significant increases in proliferation from both sets of film samples tested (Figure 4).

**DISCUSSION:**
Chitosan films made with acetic and lactic acid solvents released adenosine at similar concentrations after 24 hours. However, films made with lactic acid had a higher release at 48 and 72 hours than did films made with lactic acid. Adenosine was found to promote growth of fibroblasts and osteoblasts in a dose-dependent manner, which could make this compound effective in promoting the healing of both bone and soft-tissue. Based on the results of the activity assay, both lactic acid and acetic acid films can release active adenosine.

In future studies, other methods of chitosan construct fabrication and adenosine loading will be evaluated. These characteristics will be modified to determine optimal chitosan composition and loading techniques for effective release in diabetic foot ulcers. Additional studies to evaluate collagen and cytokine production by the adenosine-exposed fibroblasts will be pursued.

**REFERENCES:**