Preliminary Evaluation of *In Vivo* Degradation and Biocompatibility of Chitosan Sponges

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Statement of Purpose: Musculoskeletal injuries are some of the most prevalent injuries in both civilian and military populations and their infections can be difficult to treat, often resulting in multiple surgeries and increased costs.[1] The presence of debris, necrotic tissue, and fixation devices in these traumatic injuries creates an environment where infection due to the formation of biofilm is very likely.[2] Local antibiotic delivery can overcome some of the issues associated with systemic delivery, including sub-bactericidal antibiotic concentrations in avascular zones of wound sites, by providing high levels of antibiotics directly to the injured tissue.[3] A porous chitosan sponge has previously shown to prevent *S. aureus* biofilm infection *in vivo*.[4-5] Due to manufacturing and material changes, the chitosan sponge *in vitro* degradation has drifted from previous research. Our objective was to formulate chitosan sponges and evaluate the effects of a new buffering procedure on *in vivo* degradation and biocompatibility properties.

Methods Chitosan sponges were manufactured by dissolving 1% (w/v) chitosan in a 1% (w/v) acid solution and casting the solutions into containers. Sponges were frozen at -20°C, lyophilized, neutralized in 0.6 M sodium hydroxide, and washed with water. Buffered chitosan sponges were soaked in 0.25 M acetate buffer at a pH of 5.6 or 4.6 for 30 minutes, and excess buffer was removed. Neutral and buffered chitosan sponges were refrozen at -20°C, and lyophilized. *In vivo* analysis of the degradation and biocompatibility of the sponges was conducted using a rat back muscle pouch model. In this model, two 1.5 cm incisions were made through the skin on each side of the midline. In each incision, a 1.25 cm pouch was created in the latissimus dorsi muscle using blunt dissection. One sterilized cylindrical implant, 9.5 mm in diameter and approximately 5 mm thick, were implanted bilaterally in each muscle pouch. Test groups included the neutralized chitosan sponge, both pH 5.6 and 4.6 buffered chitosan sponges, and a positive control absorbable gelatin sponge (Gelfoam®, Pfizer, New York, NY). Each rat received 1 of each implant, randomized among the 4 sites. After implantation, the incision was closed with sutures and 2 staples. Ten rats were sacrificed at day 4 and 10 time points (n = 10), and 1 satellite rat was sacrificed on day 14, 21 and 28 time points (n = 1). After sacrifice, the implanted region and surrounding tissue were excised and placed in 10% formalin buffered phosphate (FBS). After 2 days in FBS, tissue sections were bisected across the implant for histological evaluation (paraffin-embedded and H&E stained). Percentage of implant and fibrous tissue in the defect was quantified using BIOQUANT® software. Tissue inflammatory response was assessed on a scale of 0-5 and the average score of 3 blinded reviewers was determined.

Results: After 4 days of implantation, all chitosan sponges and 6 of 10 gelatin sponges were remaining in the defects (Fig.1). After 10 days, 3 of 10 gelatin sponges and all neutral chitosan sponges were remaining, while 9 of 10 of both buffered chitosan sponges were still partially present in the tissue. Both types of buffered chitosan sponges were not present in tissue at 14 and 21 days, but both sponges were partially visible in the day 28 tissue sample. Tissue with the pH 5.6 buffered sponges exhibited lower amounts of fibrous tissue and slightly lower inflammatory response at day 4 and 10, compared to the pH 4.6 buffered sponges.

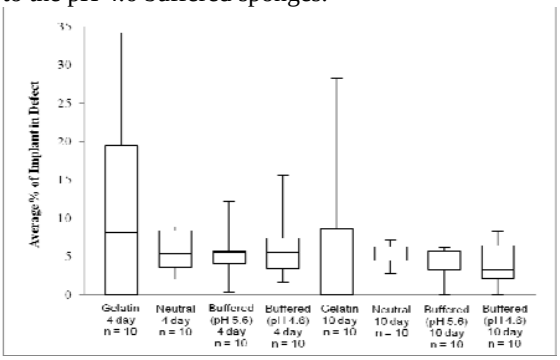

Figure 1. Average % of implant remaining in the muscle pouch defect in rats after 4 and 10 days (n = 10)

Conclusions: Results indicate that adding the buffering step to the chitosan sponge manufacturing does slightly increase *in vivo* degradation in the muscle pouch model, as compared to neutral sponge degradation. However, no significant differences in degradation could be seen between the two buffered sponge types. Based on these results, the complete *in vivo* degradation timing of the sponges was not clearly defined and additional extended *in vivo* research is needed. A limitation of this study is the method of sponge degradation quantification; some of the sponges might have been lost in the histological processing. Another *in vivo* rat muscle pouch model is planned with the same sponges, but with degradation time points of 10 and 28 days (n = 10) and histological and mass based degradation measurements. The results of this preliminary study helped to establish an initial time frame of chitosan sponge degradation, useful for future *in vivo* drug delivery studies and determining a target length of time for clinical applications.

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