Enhanced Wound Healing in Horses and Dogs Using Crosslinked Hyaluronic Acid-Based Hydrogel Films

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Statement of Purpose: Large animals tend to get severe wounds on their legs that can be difficult to treat, particularly on the forelegs of horses where the wounds develop a large amount of granulation tissue, commonly called "proudflesh". Hyaluronic acid (HA) is a component of the extracellular matrix that has been implicated as having a major role in the wound healing process. While HA solutions have previously been used to accelerate wound healing with some success, they have not proven successful in many large animal species, including horses. This may be due to the fact that the HA solution does not remain in place for extended periods of time. Here, a crosslinked HA-based hydrogel film was created that could be applied to a wound and would be slowly degraded, thereby supplying HA to the wound bed over longer time periods. We have used these hydrogel films to examine their effect on the rate and quality of wound healing in both horses and dogs.

Methods: Hydrogels were created by mixing a 2.5% (w/v) solution of thiolated carboxymethylhyaluronic acid with a 4% (w/v) solution of poly(ethylene glycol) diacrylate (MW 3400) in a 4:1 volumetric ratio. The resulting solution was poured into 24x37x3mm molds and allowed to gel. For films cast on gauze, non-stick gauze pads were placed on top of the hydrogel 10 minutes after mixing. The molds were then placed in a 40 ºC oven for 24 hours. At this point, the hydrogels were dried into a thin film, and the films were removed from the molds. Films were then sterilized using H_2O_2 gas (Sterrad) and stored at room temperature.

Full-thickness wounds were created on the front lower legs of horses (20x30mm, n=8) or dogs (20mm diameter, n=10). One leg of each animal received a hydrogel film (film alone for horses, film on gauze for dogs) while the contralateral leg received chlorhexadene. The legs were then bandaged. Wounds were re-treated at 8 and 17 days for horses and 7 and 14 days for dogs (more often as needed if bandages were removed by the animal). Images were captured of all wounds immediately after wounding, at scheduled re-treatment times, and at final bandage removal (26 and 21 days for horses and dogs, respectively). Wound size was determined using image analysis software.

Results/Discussion: Crosslinked hydrogel films were created using thiolated carboxymethylhyaluronic acid and poly(ethylene glycol) diacrylate. These HA-based films were used to treat full-thickness wounds on the lower legs of both horses and dogs and compared to wounds treated with a standard antibacterial treatment. As shown in Figure 1, both treated and control wounds contracted initially, creating a larger wound area than that traced on

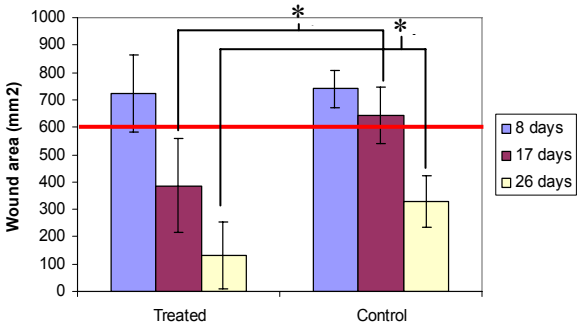

Figure 1. Area of treated and control wounds on lower front legs of horses. *p < 0.005

the skin. For horses, at 8 days the size of the treated and control wounds were not significantly different. However, the control wounds were already showing signs of granulation tissue, indicated by a yellow color, while the treated wounds had no granulation tissue. By 17 days, the treated wounds were significantly smaller than control wounds. This trend continued at 26 days. Additionally, upon gross examination, the treated wounds appeared to be re-epithelializing while the control wounds did not (see Figure 2). Similar results were observed with the dogs. At

Figure 2. Treated (left) and control (right) wounds of a horse at 26 days.

7 days, there was no significant difference in wound healing between treated and control wounds, while treated wounds were significantly smaller than control wounds at 14 and 21 days. Gross examination of the wounds on dogs also showed the treated wounds to be re-epithelializing.

Conclusions: Crosslinked hydrogel films using a modified hyaluronic acid were used to treat wounds on the lower legs of horses and dogs. The films were found to accelerate the rate of wound healing, without scarring, in these animals compared to a standard antibacterial treatment. Importantly, these films may help to eliminate the formation of proudflesh on horses. Additionally, hydrogels using the same crosslinked modified hyaluronan are under examination for other applications in animals, including post-surgical adhesion prevention of tendons and in the abdomen.