Diabetic Complications Consortium

Application Title: Targeting Cathepsin K to Promote Healing of Diabetic Wound
Principal Investigator: Sreejayan Nair

1. Project Accomplishments:

Specific Aim 1. Determine the role of cathepsin K in diabetic wound healing in a clinically-relevant porcine model.

Results: First, with the help of our collaborator Dr. Liechty, we established the porcine diabetic wound healing model. Five female Yorkshire pigs (3-months old) were rendered diabetic by a single intravenous injection of streptozocin (75 mg/kg). Four weeks after induction of diabetes, 10 full-thickness wounds measuring 2.54 x 2.54 cm were created on the back of the diabetic and nondiabetic pigs. Each wound was injected intradermally with 1mL of PBS along the circumference of the wound on day zero and again on days three, seven, and 14 after wounding. Animals were euthanized 14 or 21 days after injury and tissue was harvested for future analysis. Wound sizes were measured using NIH Image J and expressed as percent wound closure for each wound separately. As shown in Figure 1, there was significant delay with wound closure in diabetic wounds as only 50% re-epithelialization was observed at 14 days compared to 80% in non-diabetic wounds. On day 21, the wounds in the diabetic pigs exhibited a 70% re-epithelialization in contrast to 95% re-epithelialization of the wounds in the non-diabetic pigs.

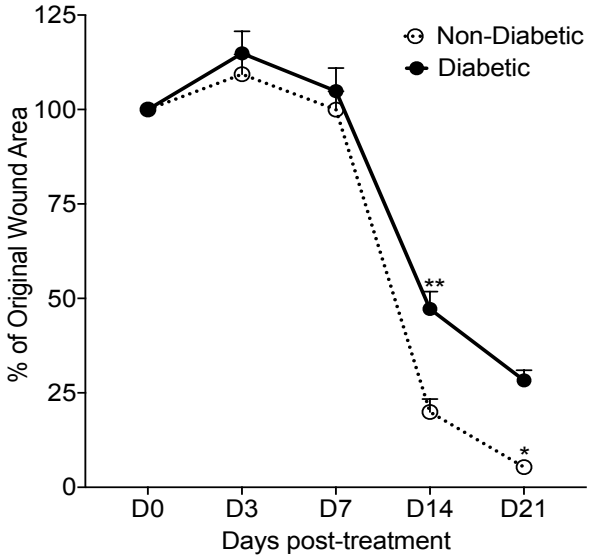

Figure 1. Diabetic pig model for wound-healing. Pigs (3-4 months old) rendered diabetic with intravenous injection of streptozotocin (STZ, 75 mg/Kg). Four weeks following STZ injections, 10 full-thickness (one-inch-square, ~300-μmeter thickness) wounds were created in two columns on each side of the dorsal thoracolumbar midline. Wounds were treated with intradermal injections of phosphate-buffered saline on day 0, 3, 7, 14 and 21 and wound area was determined. Data are represented as mean ± SEM *$p<0.005$ or **$p<0.001$, n=10 wounds.

Next, using this established model, we treated diabetic wounds with cathepsin K inhibitor-II (a peptide inhibitor of cathepsin K) and compared the area of the wound with those treated with PBS). The inhibitor was tested at a dose of (100 ng/mm^2/wound). Percent of original wound area in the vehicle-treated wounds at day 21 was 30.5 ± 2.7 (mean ± SEM, n= 8), whereas treatment with cathepsin K inhibitor-II high-dose and low-dose at 21 days was ± 0.6 (p=0.05, n=8). Figure 2 shows the percent of original wound area on days 0, 3, 7, 14 and 21.

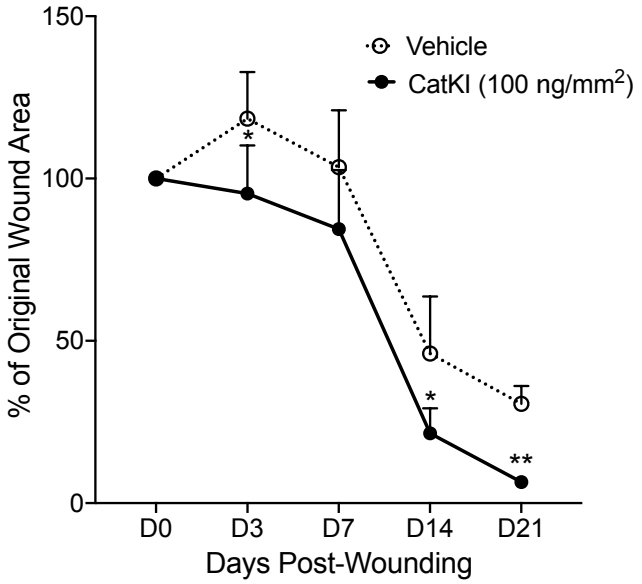

Figure 2. Diabetic pig model for wound-healing. Pigs rendered diabetic with intravenous injection of streptozotocin (STZ, 75 mg/Kg). Four weeks following STZ injections, 10 full-thickness (one-inch-square, ~300-μmeter thickness) wounds were created in two columns on each side of the dorsal thoracolumbar midline. Wounds were treated with intradermal injections of phosphate-buffered saline on day 0, 3, 7, 14 and 21 and wound area was determined. Data are represented as mean ± SEM *p<0.005 or **p<0.001, n=10 wounds.

Summary of accomplishments: By creating a larger dorsal wound area at the initial time point of injury, we have designed an improved model of chronic diabetic wounds in a large animal model. This model has incomplete wound closure at 21 days post-injury whereas previous models had fully re-epithelized by day 18. The establishment of our chronic diabetic wound model is clinically relevant and may be used to test novel therapeutics for chronic diabetic wounds in a large animal model These results demonstrate that pharmacological inhibition of cathepsin K resulted in a significant improvement in diabetic wound healing, suggesting that targeting cathepsin K may offer a therapeutic option of treatment of diabetic wounds.

Specific Aim 2. Validate the role of dysregulated cathepsin K in diabetic wound healing using recombinant cathepsin K and cathepsin K siRNA.
Results: None

2. Publications:

None