A Pre-Clinical Comparison of Healing Efficiency between InnovaMatrix® and a Commercially Available Dehydrated Human Amnion/ Chorion Membrane

Background:

Chronic wounds affect 2.4 to 4.5 million people in the United States alone and account for two to three percent of the health care budget[1-3]. These wounds fail to heal due to "prolonged or excessive inflammation, persistent infections, formation of drug-resistant microbial biofilms, and the inability of dermal and/or epidermal cells to respond to reparative stimuli"[4].

Currently, 4.3 to 7.3 million diabetics in the United States will develop foot ulcers in their lifetime. Once they do, these patients will be "significantly more likely to experience an infection (OR = 9.43; 95% CI 8.54-10.4), undergo an amputation (OR = 7.40; 95% CI 6.16-8.89), or experience a fracture (OR = 3.65; 95% CI 2.59-5.15) or fall (OR = 2.26; 95% CI 1.96-2.60)"[5-7]. Furthermore, "the 5-year mortality rate after ulceration [is] around 40%"[8]. Long-term management of chronic wounds is costly, with Medicare spending roughly 6.4 to 9.6 billion dollars annually treating diabetic foot ulcers[9]. The cost to treat a diabetic patient with a diabetic foot ulcer (DFU) versus the cost to treat a diabetic patient without a DFU is more than five times higher in the year after diagnosis, while costs for the treatment of the highest-grade ulcers are eight times higher than that of low-grade ulcers[10]. The use of advanced therapies, while sometimes initially costly, has been shown to reduce long-term costs, including reduced occurrences of foot complications and amputation rates[10]. One such advanced therapy in the treatment of chronic wounds is the use of amniotic membrane grafts. Human placental tissue has been documented to accelerate wound healing and reduce scarring while also being anti-inflammatory and antibacterial[11-13]. However, its effectiveness is impacted by human donor variability[14-16] and the presence of cells and cellular debris[17].

Convatec Triad Life Sciences, LLC. has introduced a solution to these problems in a medical device that retains the healing advantages of the placenta. This solution, InnovaMatrix®, is derived from porcine placental tissues. Unlike human donors, InnovaMatrix® donors are monitored for health, activity level, diet, age, and genetics. Further, InnovaMatrix® is a 510(k)-cleared medical device with stringent quality systems and design controls in place, leading to reliability, reproducibility, and safety in the final product. It is thoroughly decellularized, virally inactivated, and disinfected. Decellularizing the device facilitates the healing process by removing cells and cellular debris that would otherwise cause an M1-macrophage response[18-19].

Material and Methods:

To assess healing efficacy, InnovaMatrix® was compared to a commercially available dehydrated human amnion chorion membrane (dHACM) in a randomized, controlled trial. Using the chronic wound model developed by Stadler et al.[20], two Stage III pressure ulcers were induced on each rodent with 50mmHg of pressure caused by 10mm magnetic discs. For three consecutive days, the magnets were applied for 16 hours and then removed for eight hours. The ulcers were then surgically debrided with an 8mm biopsy punch to leave an approximate 1mm perimeter of ischemic tissue intact. The ischemic tissue helps to maintain the wound area and prevent contraction of the wound, which is common with excisional wounds in rodents.

The rodents (N=48) were divided equally into three groups to be studied at three different time points: 3-days, 7-days, and 14-days. Half of the rodents in each group received an application of InnovaMatrix® plus a transparent occlusive dressing on the right wound and only an occlusive dressing on the left wound. The other half of the rodents received an application of dHACM plus a transparent occlusive dressing on the right wound and only an occlusive dressing on the left wound. Following ulcer debridement and treatment with the membranes, each group of rodents was sacrificed at the different time points—either at Day 3, Day 7, or Day 14. After the sacrifice, the wounds were photographed, excised, and fixed, while the defect sites were bisected and processed for paraffin embedding. Staining of histologic sections of each defect was then performed with hematoxylin & eosin (H&E) to assess re-epithelialization. Finally, measurements of the gap between migrating epithelial fronts and CD31 were taken for assessment of neovascularization.

Data/Results:

All surgeries were performed without complications, and all animals recovered and survived until their respective sacrifice points. The ischemic wounds were consistent and reproducible. The ischemic wound areas consisted of cold, hard skin and were surgically debrided using a biopsy punch to create uniform wound beds.

Samples collected from the three day post-surgery group all maintained their relative circular shapes with little to no contracture. All of the no-treatment Control wounds developed large fibrotic masses between the skin and muscle tissue, whereas the wounds treated with either InnovaMatrix® or the commercial dHACM graft did not have such masses. Re-epithelialization trended higher in the wounds treated with InnovaMatrix® versus those treated with either the dHACM graft or the no-treatment Controls, although the difference was not statistically significant.

Samples from the seven day post-surgery group all showed evidence of wound healing, but the wounds treated with InnovaMatrix® were generally smaller than those treated with either the dHACM commercial graft or the no-treatment Controls. Wounds in the dHACM and Control groups remained larger and circular, while the wounds treated with InnovaMatrix® varied in appearance, with some having an elliptical shape and others showing a more irregular but circular shape.

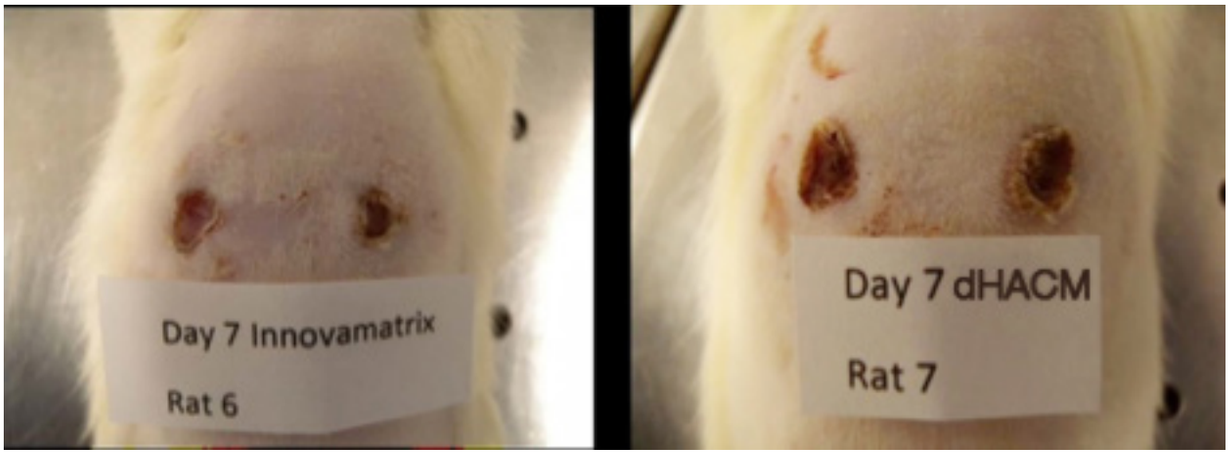

Figure 1: Representative images of wounds at Day 7. No-treatment Controls are on the left of each animal. Test articles are on the right[15].

Histologically at seven days post-surgery, wounds in both treatment groups had highly granulated tissue that primarily consisted of dermal fibroblasts with mononuclear cells at the border of the wounds and the eschar. Also, epithelial cells could be seen growing under the eschar at the wound edges.

Conversely, the no-treatment Control wounds were filled with granulation tissue and were less cellular when compared to the wounds in the treatment groups and contained primarily mononuclear cells. It was also observed that the wounds treated with InnovaMatrix® had a statistically significant greater re-epithelialization than the Controls, while the wounds treated with dHACM did not exhibit a statistically significant difference from their no-treatment Controls.

Samples from the 14 day post-surgery group showed that almost all wounds were nearing complete closure and were mostly stellate in shape. There were areas of re-epithelialization around all wounds as evidenced by newly formed skin that lacked hair follicles. The no-treatment Controls still had areas of redness at the centers of the wounds that were larger than what was found in wounds treated with either InnovaMatrix® or the dHACM commercial graft.

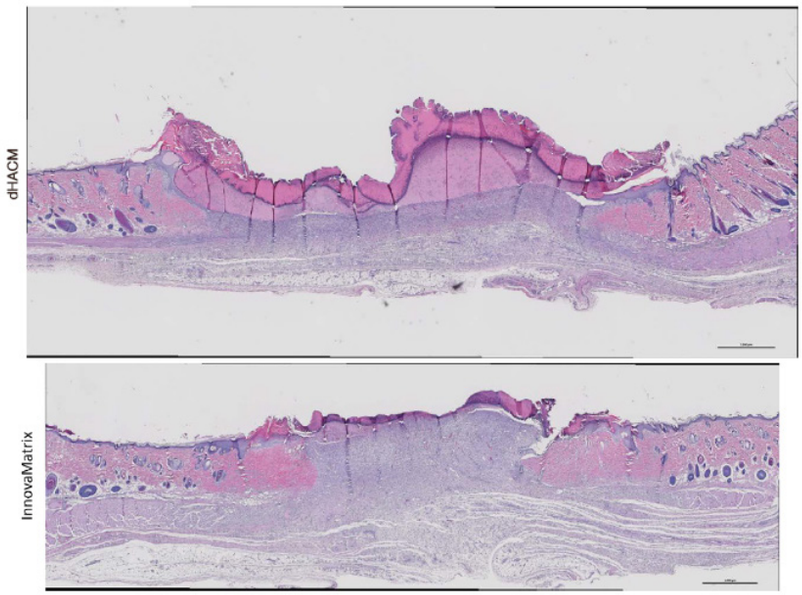

Figure 2: At Day 7, histology revealed new epithelial cells growing at the wound edges for both InnovaMatrix® and dHACM commercial graft. Scale bar = 1000um (1mm)[15].

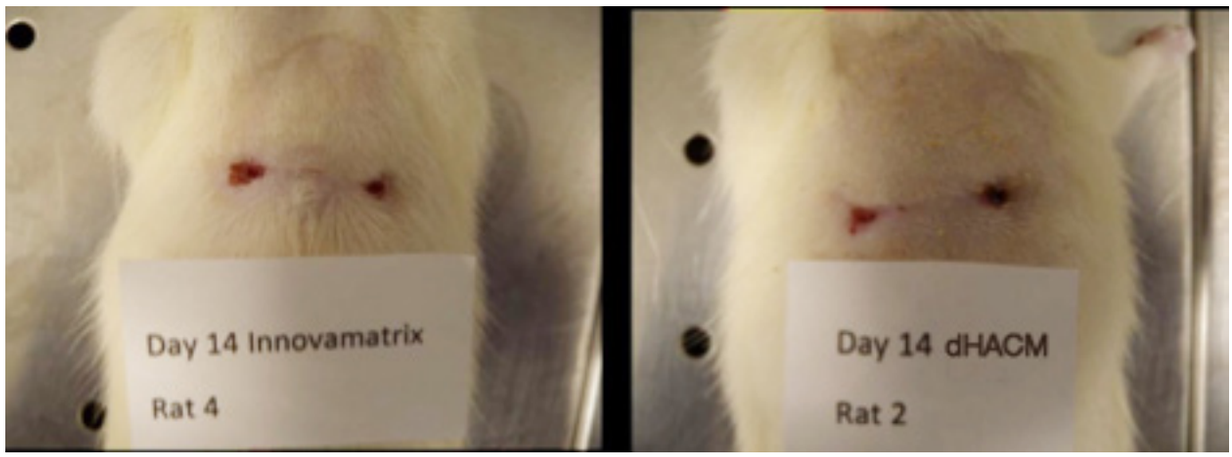

Figure 3: Representative images of wounds at Day 14. No-treatment Controls are on the left of each animal. Test articles are on the right[15].

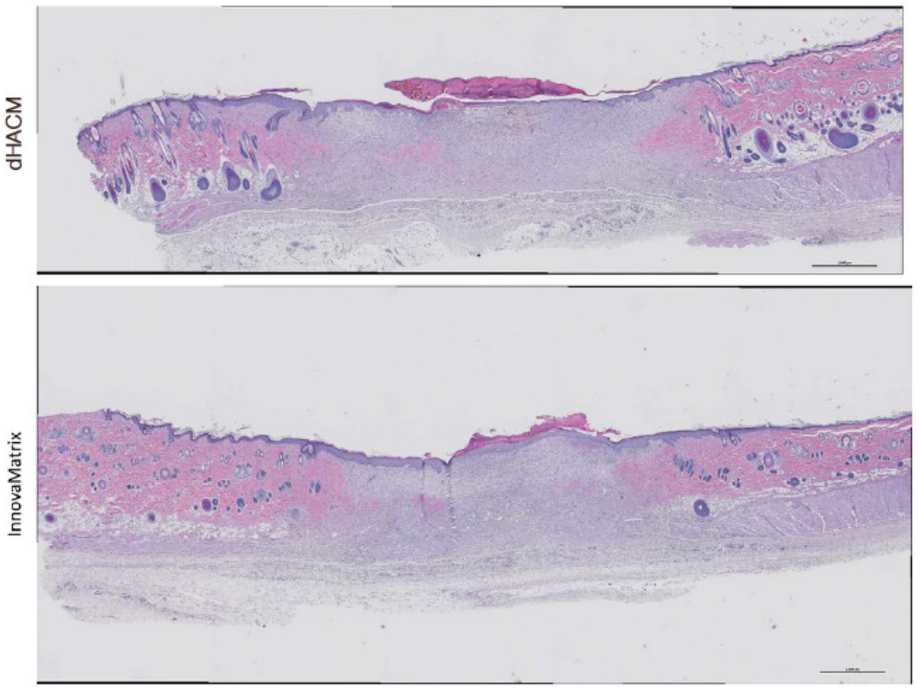

Figure 4: At Day 14, histology revealed more complete re-epithelization in wounds treated with InnovaMatrix® than those treated with dHACM commercial graft, as evidenced by the continuous layer of light purple. Scale bar = 1000um (1mm)[15].

Histologically at Day 14, the no-treatment Control wounds had not completely re-epithelialized and were primarily composed of cellular granulation tissue, which is similar to what was observed in the Control wounds at seven days post-surgery. In contrast, wounds treated with dHACM had more complete re-epithelialization and the granulation tissue was less cellular with areas of organized extracellular matrix beginning to form. In the wounds treated with InnovaMatrix®, re-epithelialization was more extensive, and the epithelial layers were similar in thickness to the surrounding uninjured tissues. Further, the granulation tissues of these wounds were less cellular than what was observed at Day 7, and the tissues contained areas of organized extracellular matrix that had a similar density to the adjoining native dermis.

At 14 days post-surgery, the wounds treated with InnovaMatrix® had statistically significant greater re-epithelialization (83.79% ± 15.49%) than what was observed in the wounds treated with the dHACM commercial graft (70.51% ± 17.28%) or the no-treatment Control wounds (67.81% ± 2.95%). Also, three out of the eight wounds treated with InnovaMatrix® had closed by this time, whereas only one wound treated with the dHACM commercial graft had closed.

At each time point, the CD31 stained sections from three random areas with the wound were imaged at 32X magnification for each of the groups. The images were processed using QuPath Image Analysis, which counted all CD31 stained blood vessels but excluded single stained cells not associated with a lumen. For the analyses, the wounds treated with either InnovaMatrix® or dHACM were compared to their contralateral Controls to account for individual animal variability. The results showed that wounds treated with InnovaMatrix® trended higher in number of blood vessels when compared to contralateral controls at Day 3 and Day 14, while dHACM trended higher only at Day 7. While the number of vessels was not statistically different between any of the groups, it was noted that the diameters of the blood vessels in wounds treated with InnovaMatrix® were larger than those found in the wounds treated with the dHACM commercial graft, as seen in the CD31 stains of the central parts of the wounds.

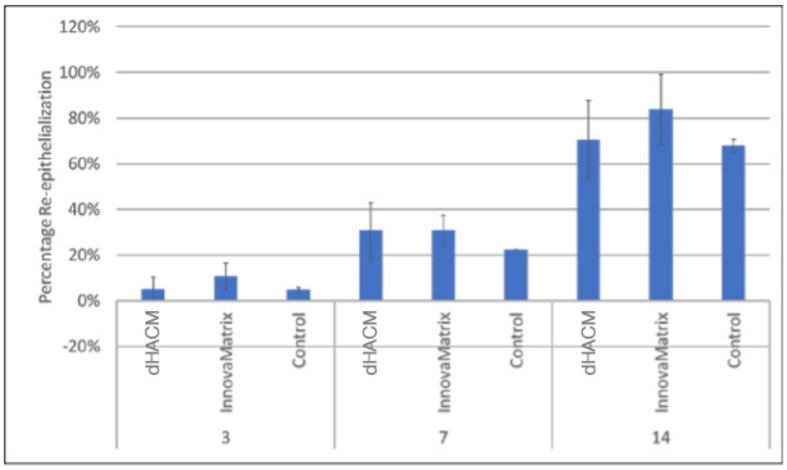

Figure 5: Wounds treated with InnovaMatrix® showed statistically significant greater re-epithelialization on Day 7 vs. Control and again on Day 14 vs. dHACM and Control[15].

Conclusion:

In this animal study, InnovaMatrix® promoted wound healing that was favorable to dHACM at all three of the evaluated time points: 3-days, 7-days, and 14-days post-surgery. Additionally, wounds treated with InnovaMatrix® exhibited greater re-epithelialization and wound closure than those treated with either the dHACM commercial graft or the no-treatment Controls, demonstrating a statistically significant difference in percentage of re-epithelialization at the final 14-day timepoint. The CD31 stain revealed that wounds treated with InnovaMatrix® had more mature blood vessels with larger diameter lumens when compared to those that were treated with dHACM. These metrics of healing support the use of InnovaMatrix® as a therapy for chronic wounds.

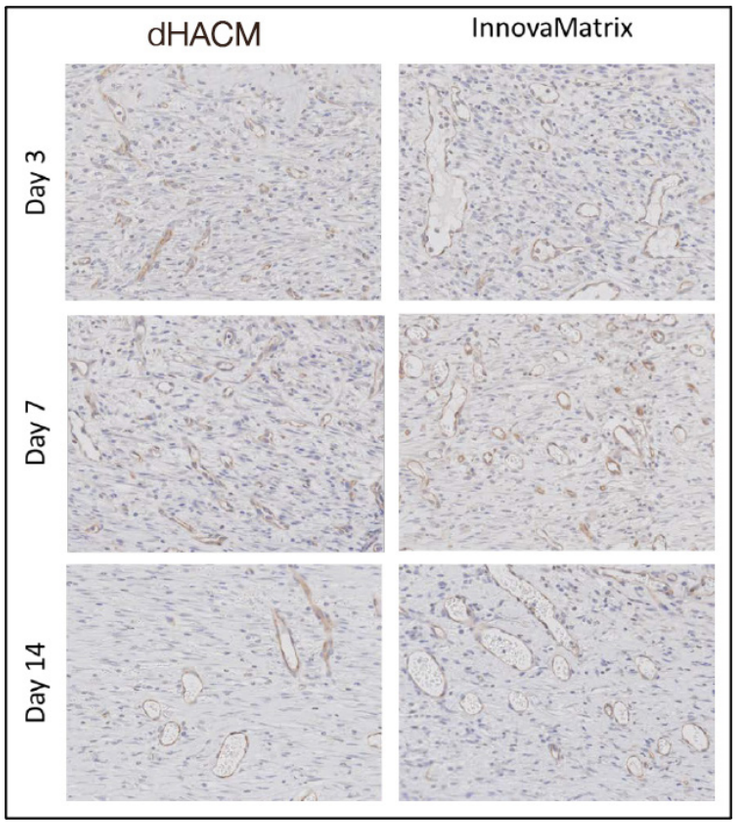

Figure 6: Images of neovascularization at Days 3, 7, and 14. dHACM commercial graft-treated wounds are on the left and InnovaMatrix®-treated wounds are on the right. Images are stained with CD31[15].

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