Anatomical Effects in the Development of a Delayed Wound Healing Model

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Introduction

ACell, Inc. currently markets multiple configurations of its Urinary Bladder Matrix (UBM-ECM) product, called MatriStem®. This material is derived from the decellularized basement membrane and tunica propria layers of the porcine bladder and consists of extracellular matrix proteins including collagens, glycosaminoglycans, and growth factors, which provide both a structural and biological framework for tissue healing [1, 2, 3].

Although the mechanism of action behind ECM-mediated constructive remodeling is not yet fully understood, it has been hypothesized that these ECM-derived biomaterials act as *in situ* bioactive regenerative templates, serving as substrates for progenitor cell infiltration and differentiation. The ECM biomaterial can be considered a concentrated version of the body's own natural scaffold that occurs after injury. The body is hypothesized to act as a bioreactor, providing additional site-specific biomechanical and biochemical cues that guide cell differentiation. Studies have demonstrated the chemo-attractive effects of UBM-ECM biomaterial breakdown products on progenitor cells [4].

In June 2012, ACell, Inc. received a Space Medicine and Related Technologies Commercialization Assistance Program (SMARTCAP) award from the National Space Biomedical Research Institute (NSBRI) to develop a novel MatriStem UBM gel formulation which can be easily administered to wounds in space. The development of a novel gel formulation of MatriStem UBM technology that preserves the bioactivity and vulnerary properties of the biomaterial would provide astronauts with a powerful new tool to combat lacerations and abrasions incurred during flight missions. Although existing MatriStem products have the potential to improve healing in space, current dressing configurations would be difficult to administer in low gravity and maintain position under high activity levels. Promising prototypes have been developed (Figure 1) and are undergoing continuing characterization. In order to mimic the delayed healing in space, a novel model of ischemic wound healing in rats has been developed which will be used to test the *in vivo* efficacy of the gel prototypes.

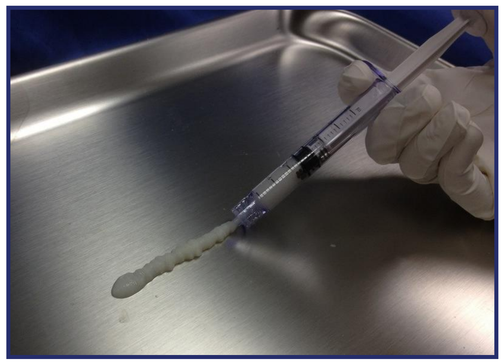

Figure 2: Gel Prototype application following reconstitution (7% w/v, 100mM NaOH)

Methods

A delayed healing model of ischemic wound sites with intrinsic controls was developed to investigate the vulnerary properties of a novel gel form of the ECM-derived MatriStem wound dressing. A second study was initiated to 1) evaluate the validity of the use of the lateral control wounds on the ischemic model and 2) determine if there were systemic effects of the bipedical flap on the healing of the lateral control wounds in the first study. Models were adapted from previously published methods [5, 6, 7].

Study 1: Four 8.0mm wounds were created in 16 Sprague-Dawley rats, down to but not through the anterior fascia of the panniculus carnosus muscle layer of the skin, 2.0cm down from the cranial edge of the dorsal midline of each rat. The location and size of the wounds were optimized in 3 preceding studies. A bipedical skin flap was created using two linear incisions in the cranio-caudal direction, measuring 8.0cm long and 3.0cm wide. The bipedical flap was then lifted from the underlying tissue and a 6.0cm x 4.0cm pre-cut sterile silicone sheet was placed underneath the flap. The silicone was cut shorter than the bipedical flap to avoid fluid buildup of the wound and buckling of the silicone sheet. Silicone sheets were sutured to the flap and native tissue. Ischemic wounds (within the flap) were treated with the appropriate saline volume (20µl) on Day 0. Wounds outside the flap were considered internal controls for all animals (n=16). All wounds were covered with a sterile non-adherent silicone dressing (Mepitel, Molnycke) followed by a sterile occlusive dressing (Renasys, Smith & Nephew). Digital photography, wound measurements, and dressing changes occurred on Days 0, 3, 7, 10, and 14, and Days 17 and 21 as needed.

Study 2: Four 8.0mm wounds were created in 10 Sprague-Dawley rats, down to but not through the anterior fascia of the panniculus carnosus muscle layer of the skin, 2.0cm down from the cranial edge of the dorsal midline of each rat. The pattern and location of the four wounds were identical to those in Study 1 with the exception that no bipedical skin flap was created. Wounds in the center where the flap would be were treated with saline as previously performed. Outside wounds were left untreated. These control treatments replicated those in Study 1.

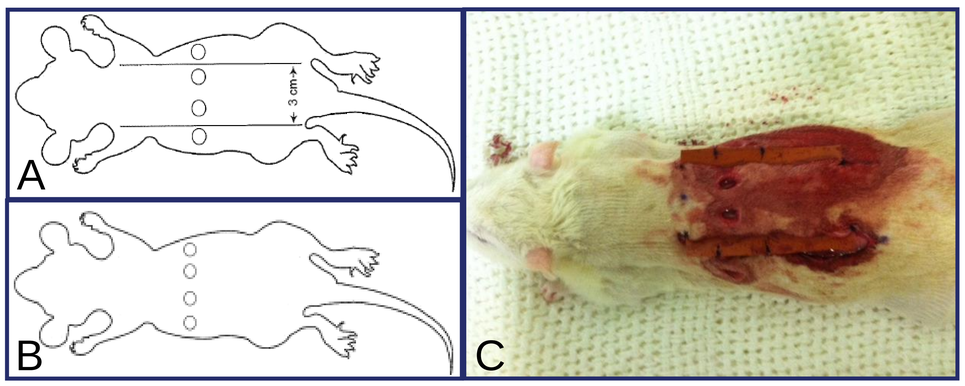

Figure 2: Design schematic of Study 1 (A) and Study 2 (B). Photograph of ischemic wound healing model with bipedical flap and silicone sheet (C).

Results

Results were analyzed using calibrated digital caliper measurements at each time point. Measurements were taken across the widest and narrowest part of each wound. The area of each wound was then calculated by using the equation for an ellipse. Areas of the wounds were verified using Aranz Silhouette™ Advanced Wound Assessment and Management System. The calculated area from digital calipers and the Aranz system were not statistically different. All data reported herein was taken using digital calipers. Wound area was measured and analyzed as the percent of the original size of the wound. Digital photographs with sterile rulers were also taken to record gross inflammatory response.

Study 1: On average, ischemic wounds showed delayed healing when compared to non-ischemic internal control wounds at Days 3, 7, 10, 14, and 17 days (Figure 3). The data suggested that the model could be used for ischemic wounds with two internal controls outside of the bipedical flap for each rat.

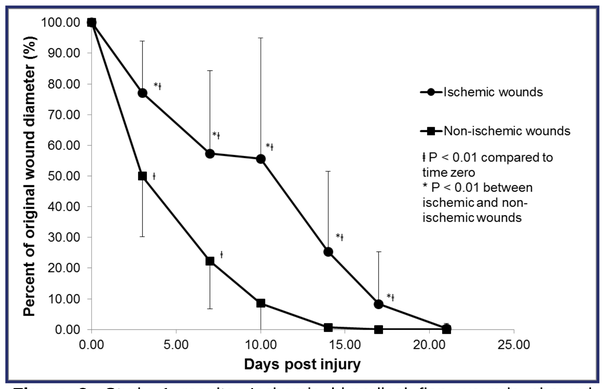

Figure 3: Study 1 results: Ischemic bi-pedical flap wounds showed delayed healing compared to outside control wounds.

Study 2: The rate of healing of the 2 lateral wounds were compared to the 2 medial wounds to determine whether the outside wounds could serve as controls for the ischemic wound model. Results showed that the outside wounds healed much faster than the inside wounds, even with adequate vascularization (Figure 4). These data suggest that there are positional effects that vary with the anatomy of the rat, which means that the lateral wounds are inadequate controls for the medial ischemic wounds. Data from the medial wounds of each study were then compared to determine whether the ischemic flap was delaying wound healing at all (Non-ischemic inside wounds from Study 2 and Ischemic inside wounds from Study 1). Based on the data, the silicone bi-pedical flap model causes delayed healing of the ischemic wounds (Figure 5).

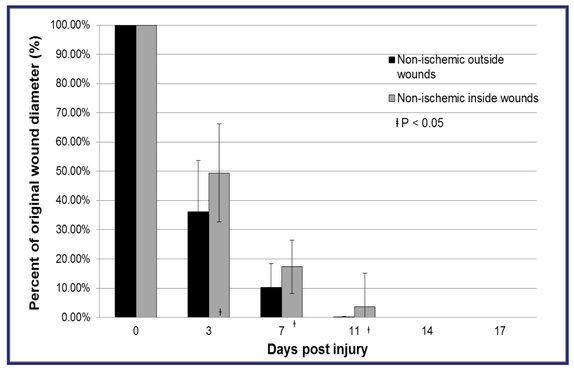

Figure 4: Study 2 results: Non-ischemic medial wounds showed delayed healing when compared to outside non-ischemic wounds, showing anatomical effects of the position of the wound on the rat dorsum on the rate of healing.

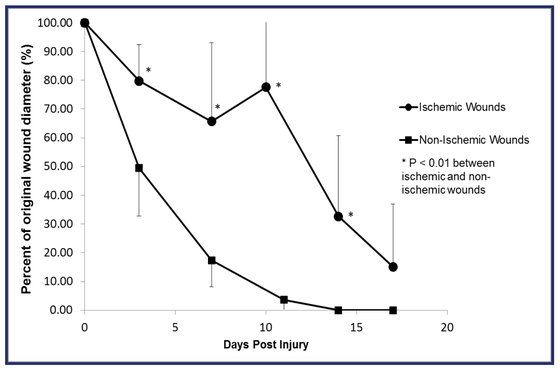

Figure 5: Data from Ischemic and Non-ischemic wounds located at anatomically equal positions on the dorsum of the rat suggesting that the bipedical flap causes delayed healing of ischemic wounds.

Conclusions

There are currently no universally accepted and reproducible animal models of tissue ischemia for *in vivo* analysis of various products or conditions on wound healing parameters. A refined ischemic wound healing model has been developed based on the anatomical and tissue healing effects seen in previous studies [5, 6, 7]. Although the skin of the rat heals mostly through contraction, in contrast to human skin, the model and model refinement represent a cost effective and easily reproducible model for wound healing studies [6, 8].

It has been shown that ischemic flap models show a gradient of wound healing based on their anterior position along the back of the animal, however based on these results there are also ischemic gradient effects along the width of the dorsum [7]. Positional effects of wounds are a significant parameter to evaluate before finalizing any wound model. It is important to consider where the model may have deficiencies in order to save time and resources on future studies. In order to verify and expand on this ischemic wound healing model, future studies may be conducted to verify the results using bi-pedical flap ischemic wounds compared to non-ischemic wounds in the same position along the dorsum of the rat. Controls for future studies must account for positional effects. Another possible study would include the same anatomical location of the wounds using splints with or without silicone sheets to limit the effect of contractility, thus causing the wound to heal primarily through granulation tissue and re-epithelialization which is more clinically relevant to human wound healing.

References

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This work is supported by the National Space Biomedical Research Institute through NASA NCC 9-58.