Harnessing the Regenerative Capability of Naturally Neotenic Salamanders (Ambystoma Mexicanum, the Axolotl) for Wound Management – A Porcine Wound Healing Model to Evaluate Safety and Effectiveness of Axolotl Wound Matrix

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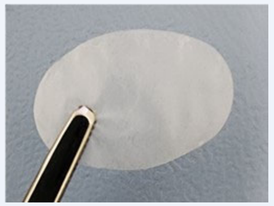
INTRODUCTION

The axolotl (*Ambystoma mexicanum*) has the unique ability to regenerate complex structures such as limbs, spinal cords, and entire organs[1,2,3] and is considered a model organism for the study of regeneration. With over 3,000 published studies over the past 150 years, axolotl regeneration is well characterized. All vertebrates possess the genetic program for limb regeneration within their genome[4].

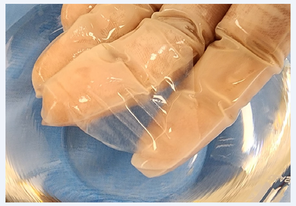
Regeneration in axolotls and tissue repair in humans involve the same basic mechanisms[5] and follow the same four phases of healing with some differences in timing. Axolotl ECM has earned the name "regeneration specific ECM".

Certain ECM factors support scar free healing in axolotl and during fetal wound healing in mammals. Additionally, fibrosis and cancer progression is different in animals that can regenerate. Regenerative salamanders develop tumors at a lower frequency than animals that do not have regenerative capability.[3]

A decellularized and sterile extracellular matrix (ECM) wound dressing derived from the dermis of the axolotl, has been designed and cleared by FDA as a xenograft collagen wound dressing for the management of a variety of full and partial thickness, chronic and acute wounds.



Sterile - Elliptical Shape



Naturally conforming when hydrated

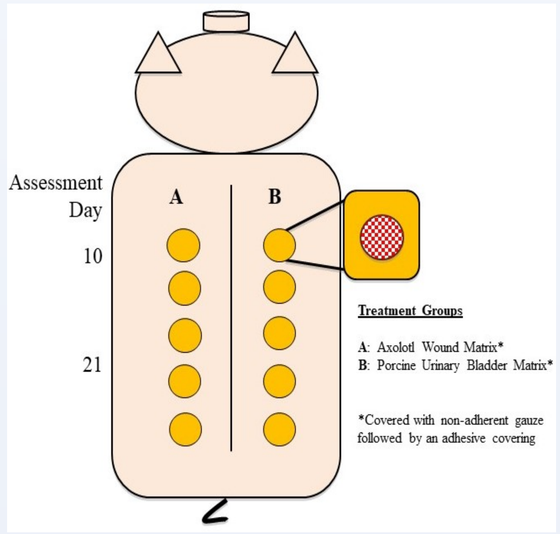
The axolotl acellular dermis collagen wound dressing device was studied in a full-thickness porcine wound healing model to evaluate safety and effectiveness compared to a porcine predicate wound dressing for its intended use in full thickness wounds. It is worth noting that the porcine device would be considered an allograft in a porcine *in vivo* study and the amphibian derived device would function as a xenograft.

The data generated from this study provided insight into whether application of an axolotl-based wound dressing could support wound healing that mimics the salamander's great ability to regenerate damaged tissues and to determine if the axolotl's regenerative capabilities could be harnessed for human medical use.

METHODS

A total of 6 animals were utilized in this study. Each pig underwent a surgical procedure to create 10 full thickness dermal wounds per animal measuring 10mm in diameter through the paravertebral skin, with 5 wounds on each side of the midline. The incisions were made through the skin, the dermis and epidermis. The subcutis was removed and the underlying lumbar fascia was exposed. Wound margins were separated by at least 4 cm from each other.

The axolotl dermis Wound Matrix (WM) or a product derived from porcine Urinary Bladder Matrix (UBM), was delivered onto the wound bed. Each wound was dressed with a non-adherent gauze followed by an adhesive covering. Dressing changes were performed every 7 days to mimic worse case clinical conditions and reapplication of axolotl dermis and UBM was performed weekly at 0, 7, and 14-day intervals. Wound inspections were performed between dressing changes to assess immunological response. A protective shirt was used to further protect the wounds.

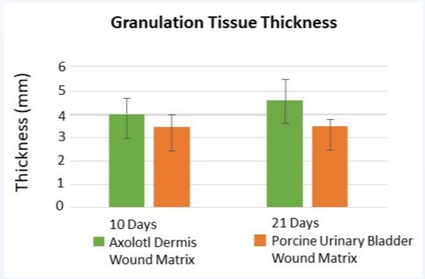


The *in vivo* portion of the study was 10 and 21 days utilizing 3 animals per time period. Wound healing was assessed by Draize scoring, dimensional wound measurements, epithelial gap, and histopathology analysis.

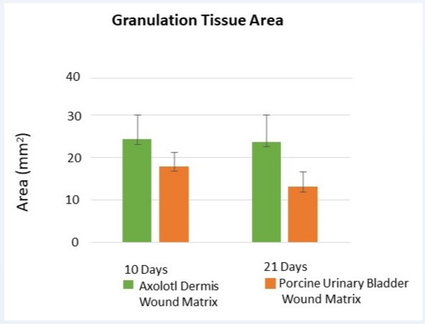
RESULTS

Draize scoring and wound measurements were similar between the axolotl dermis WM and the porcine UBM test groups. Observations of all operative sites showed no adverse clinical effects for both test groups at 10 and 21 days post-operatively. There was no inhibition of wound healing or epithelialization for the axolotl dermis WM test group vs. porcine UBM test group.

There were some differences in the quality of the healed tissue as determined by the following granulation tissue results at both time points for granulation thickness are area:



At 10 days post-operatively, the mean granulation tissue thickness of the axolotl dermis WM test group was 16% greater than porcine UBM and increased to 33% after 21 days.



The mean granulation tissue area was 36% more for the axolotl dermis WM test group in comparison with porcine UBM at 10 days and increased to 81% more area at 21 days post-operatively.

As the graphs indicate, the granulation tissue response to the axolotl dermis wound matrix was significantly higher that the reaction to the porcine urinary bladder matrix. Both, granulation tissue thickness and granulation tissue areas were higher at 10 days and 21 days for the axolotl wound matrix treated areas vs. the porcine urinary bladder matrix.

DISCUSSION

The full-thickness dermal wound healing study in pigs demonstrated that both the axolotl dermis wound matrix and the porcine urinary bladder matrix met safety and effectiveness substantial equivalence for an FDA cleared wound management device. However, there were some differences which may provide some insight into the benefits of a neotenic species for use in wound care. Findings such as increased neovascularization, lack of mineralization in the wound bed in the axolotl dermis group compared to the predicate group need to be further evaluated. The granulation tissue response to the axolotl dermis wound matrix was markedly greater than the porcine urinary bladder matrix.

A hallmark of the tissue regeneration response in salamanders is a prolonged granulation phase in comparison with the normal wound healing found in mammals[2].

The prolonged granulation phase allows significant time for tissue organization leading to a regeneration response.

In contrast, the human response is characterized by a stunted granulation phase which can lead to scar formation and an inferior wound repair.

Therefore, the data generated in this animal study suggests that axolotl-derived tissue used as a wound covering device should be studied further as it may promote a prolonged granulation phase by acting as a covering, which allows significant time for tissue organization and remodeling leading to the human skin's regeneration response.

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