Artacent Aura Shows Healing in a Full Thickness Wound Model: a Tri-Layer Human Placental Membrane Graft

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Introduction

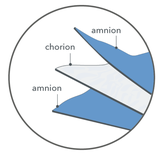
Wound coverings derived from dried human placental membranes have been successfully used in a wide array of wound healing applications. The wide multitude of processing methods currently being used has resulted in a diverse market of placental wound coverings. These wound coverings differ not only in their composition, but also in their growth factors. One of the products on the market is ArtacentTM AC, a tri-layer graft composed of chorion sandwiched between two layers of amnion. Another similar product is ArtacentTM Aura, which has the same product composition as Artacent AC, but is constructed in a frame-like shape that is designed to heal larger wounds from the outside in. In this study, we used a novel full thickness wound model with a large 2 x 2 cm defect in a Sprague Dawley rat to differentiate re-epthalization rate between Artacent AC and Artacent Aura. In addition, histologic analysis of Hematoxylin and Eosin (H&E) and immunohistochemical staining for transforming growth factor ß (TGF- ß[2] in Artacent Aura is conducted to evaluate the re-epithelialization process to better understand and compare the performance of these wound coverings during the healing process.

Materials & Methods

MATERIALS

Artacent AC
Tri-layer graft composed of chorion sandwiched between two layers of amnion, square or rectangular shape

Artacent Aura
Tri-layer graft composed of chorion sandwiched between two layers of amnion, frame-like shape



METHODS

A total of two 2x2 cm full thickness wounds were created on the dorsal side of each Sprague Dawley rat. This study used large wounds to create a challenge model, in which the wounds do not completely heal in the timeframe of the experiement. Samples were randomized into 18 implantation sites across 9 animals. Implants were harvested at two time points, 4 days and 7 days. Explants were then grossly assessed for healed wound geometry, remodeling, and re-epithelialization. Implants were fixed in neutral buffered solution (NBS) and were sent for histology. Samples were stained with H&E where cell nuclei appear purple and extracellular matrix is stained pink. Stained cross sections were evaluated for surface re-epithelialization and granulation tissue formation. Each histological image was rated using a numerical scale to represent the amount of re-epithelialization and granulation tissue formation. Granulation tissue formation was rated using a scale of 1-3 and re-epithelialization was rated using a scale of 1-5.

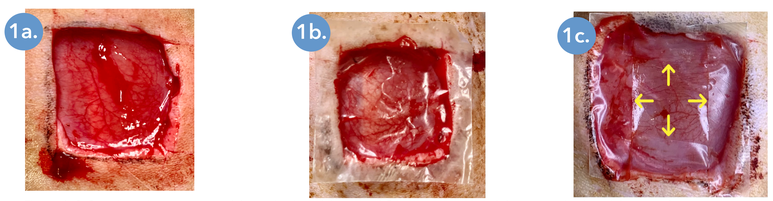


Figure 1: 2x2 cm full thickness wound (a), application of Artacent AC in the wound bed (b), and application of Artacent Aura to the edges of the wound (c).

Statistical Analysis

Statistical analysis on all results was performed using one-way ANOVA. Statistical significance ($p<0.05$) is indicated by an asterisk.

Results

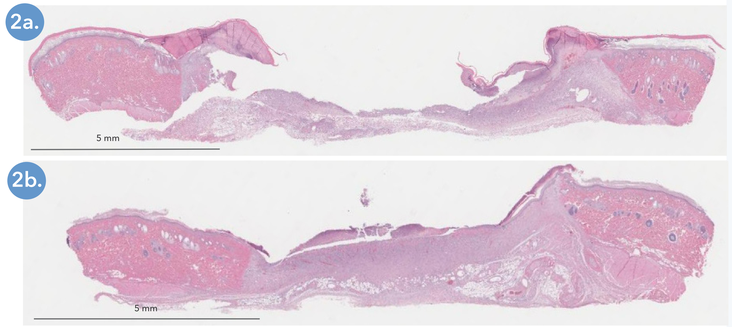


Figure 2: Example of wound of 4 days after Artacent application (a) and at 7 days (b). Partial granulation tissue formation and reepithelialization compared to increased healing at 7 days.

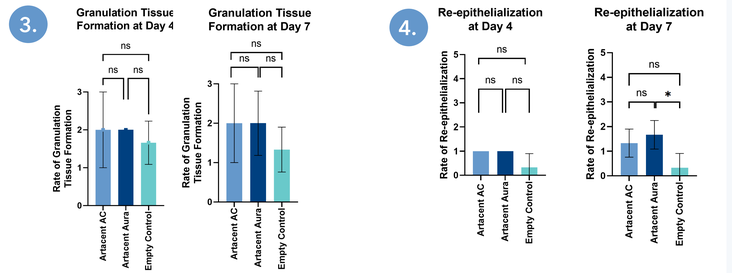


Figure 3: Granulation tissue formation at 4 days and 7 days. Ratings are based on a scale of 1-3.

Figure 4: Re-epithelialization at 4 days and 7 days. Ratings are based on a scale of 1-5.

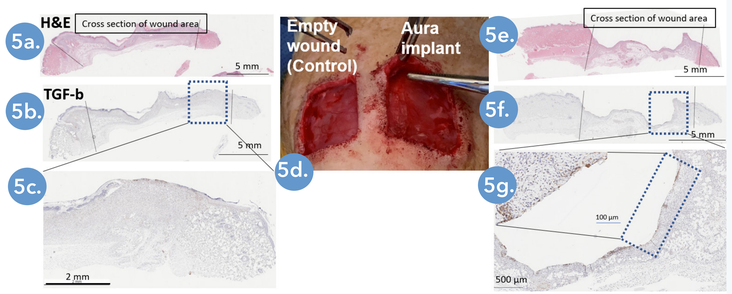


Figure 5: Empty wound at time of creation and implantation of Artacent Aura into the wound bed (d). Comparison of H&E staining (a) and TGF-beta staining (b and c) of empty wound explant at 4 days. Comparison of H&E staining (e) and TGF-beta staining (f and g) of Artacent Aura explant at 4 days.

Discussion

To assess wound healing using various wound coverings, 2x2 cm full thickness wound in Sprague Dawley rats was proposed as a challenge model. Different time points of 4 days and 7 days were evaluated and showed the proposed model can differentiate between different treatment groups at the chosen time points (Figure 2). Histological analysis shows more completed re-epithelization on day 7 and partial healing at day 4. The analysis of day 4 data shows better quantification at this time point compared to day 7. During wound healing, the formation of granulation of tissue occurs first, followed by reepithelialization. Granulation tissue formation and re-epithelialization is accelerated by the addition of a human placental membrane product to the wound bed (Figure 3&4).

In addition, there is a significant difference between the empty wound and implantation of both Artacent AC, while there is no significant difference in re-epithelialization, between Artacent AC and Aura ($P>0.05$). These results demonstrate that Artacent Aura maintains the wound healing efficacy of Artacent AC, even though it covers a smaller area of the wound. In addition, histological analysis of H&E and TGF- ß were assessed to evaluate the re-epithelization process. TGF- ß is one of the regenerative growth factors that immune cells secrets as a powerful antiinflammatory function to regulate the immune response [2]. TGF- ß stimulates most of the processes of wound healing, along with other growth factors, and is a major profibrotic factors [3-5]. As shown in Figure 5, TGF- ß was observed at the edge of the Aura implantation site and, over time, moves towards the center of the wound Figure 5e-g). This was also observed in the control empty wound where no implant was placed (Figure 5a-c). This finding shows that TGF- ß can potentially be used as a powerful immuno-histochemistry (IHC) biomarker to assess where wound healing re-epithelization occurs at the wound site.

Conclusions

- No significant adverse effect were observed after implantation of Aura.
- There is a significant difference in re-epithelization between the empty negative wounds and sites to which Artacent AC were implanted.
- No significant differences were observed between Artacent AC and Artacent Aura after day 7, which demonstrates that both products aid in wound healing, while Artacent Aura covers a larger wound area with a smaller amount of wound covering.
- This study demonstrates that wound healing takes place from the outer edge of the wound towards the center.
- Granulation of tissue forms first, then reepithelization occurs from the outer edge of the wound towards the center.
- TGF- ß is one of the regenerative biomarkers that contributes in wound healing, and can be observed at the edge of the wound 4 days after implantation of Artacent.

REFERENCES

1. McQuilling JP et al, Int Wound J. 2019;16:827-840.
2. Prud'homme, G. Pathobiology of transforming growth factor ß in cancer, fibrosis and immunologic disease, and therapeutic considerations. Lab Invest 87, 1077-1091 (2007).
3. Hyytiainen M, Penttinen C, Keski-Oja J . Latent TGF-beta binding proteins: extracellular matrix association and roles in TGF-beta activation. Crit Rev Clin Lab Sci 2004;4:233-264.
4. Ruiz-Ortega M, Rodriguez-Vita J, Sanchez-Lopez E, et al. TGF-beta signaling in vascular fibrosis. Cardiovasc Res 2007;74:196-206.
5. Leask A . TGFbeta, cardiac fibroblasts, and the fibrotic response. Cardiovasc Res :207-212.

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