PS-10

DAEGU FATIMA HOSPITAL

Immunohistochemical Analysis of Bovine Amniotic Membrane in Non-Surgical Wound Healing

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Introduction

Prolonged wound healing remains a difficult challenge in rehabilitative medicine, posing various limitations that delay the process and result in poorer outcomes. Thus, an effective, non-surgical wound healing method could help expedite rehabilitation, without surgical intervention. Bovine amniotic membrane, which contains various proteins, growth factors and extracellular matrix components, is known to promote cell proliferation and tissue regeneration. This study aims to investigate the wound healing potential of bovine amniotic membrane through immunohistochemical analysis.

Methods

Mouse excisional wound splinting models using 8-week-old male C57BL/6 mice were divided into 4 groups: negative control (Group A), positive control using DuoDERM (Group B), amnion (Group C) and amnion with compression (Group D). For 14 days, 10mm diameter DuoDERM and amnion were attached to the wounds once daily, in their respective groups. Wound tissue from day 10 were immunohistologically analyzed for specific wound healing markers via H&E and trichrome staining: alpha-smooth muscle actin (α-SMA), collagen type III (Col3), collagen type I (Col1), CD4, SMAD1/5/8 and SMAD 2/3. Stained images were analyzed via Image J and the equation in Figure 1 was used to determine the number of positive cells.

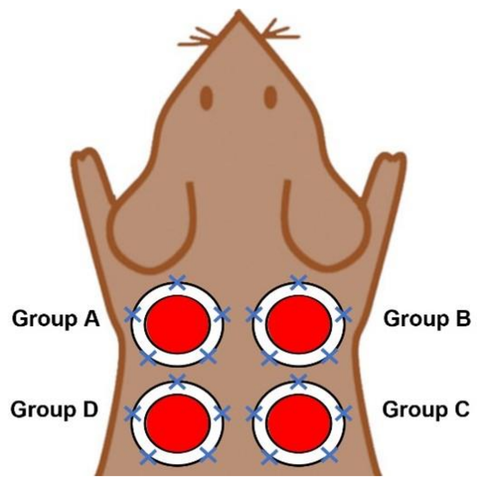

Figure 2. A schematic representation of the mouse excisional wound splinting model.

Result

H&E staining exhibited more organized epidermal and dermal layers with fewer inflammatory cells in groups C and D, indicating more advanced wound healing. Trichrome staining showed more structured and organized collagen networks in groups C and D, consistent with enhanced tissue strength and integrity. Decreased CD4 levels in groups C and D are suggestive of a potential anti-inflammatory effect of bovine amniotic membrane treatment. Quantitative analysis showed elevated levels of α-SMA, Col3, SMAD 1/5/8 and SMAD 2/3 in groups C and D, indicative of an active wound remodeling phase and modulation of the TGF-β signaling pathway. Comparative assessment of Col1 levels revealed no significant changes across all groups.

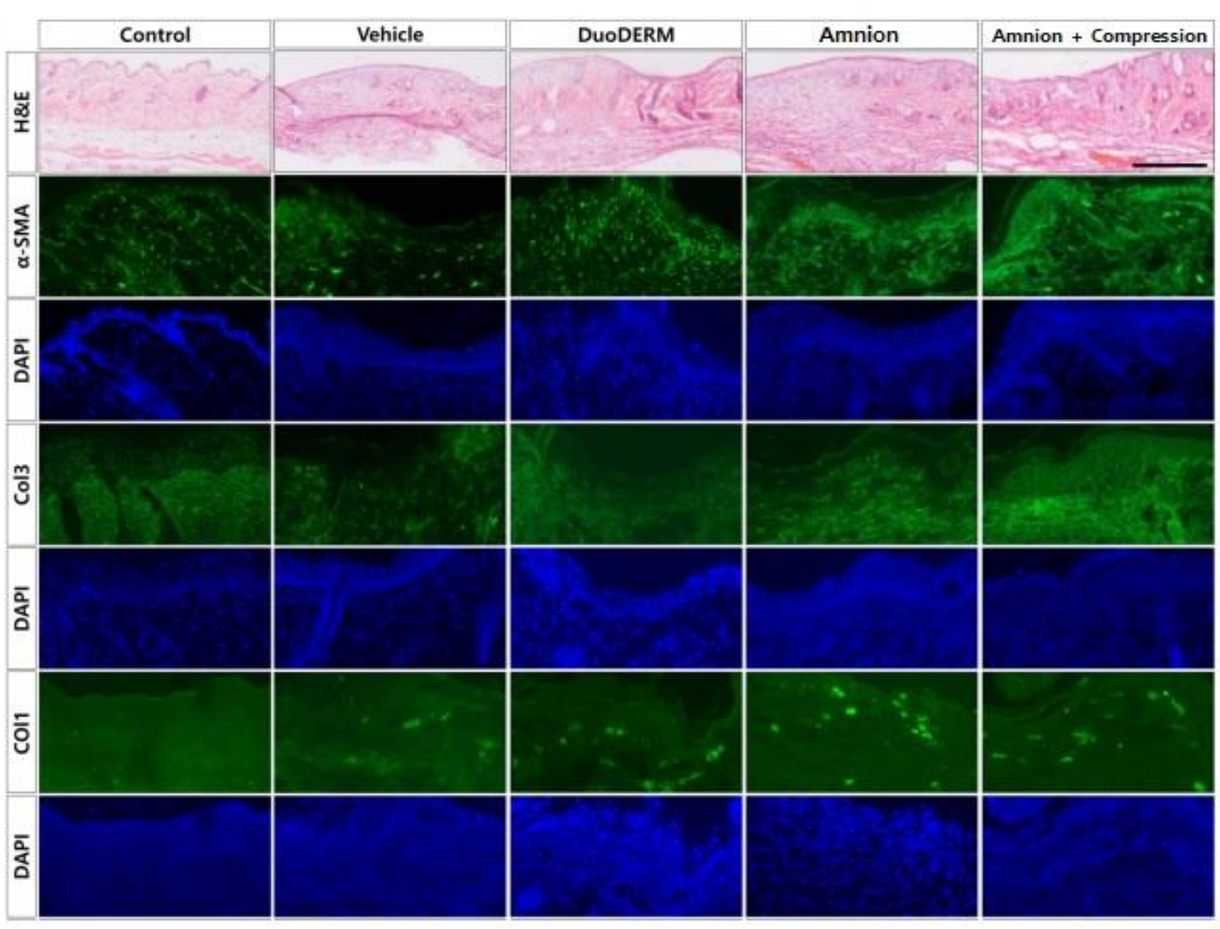

Figure 3. H&E staining for immunohistochemical analysis of wound healing markers α-SMA, Col3 and Col1. All microscopic images are accompanied by a scale bar representing 200μm.

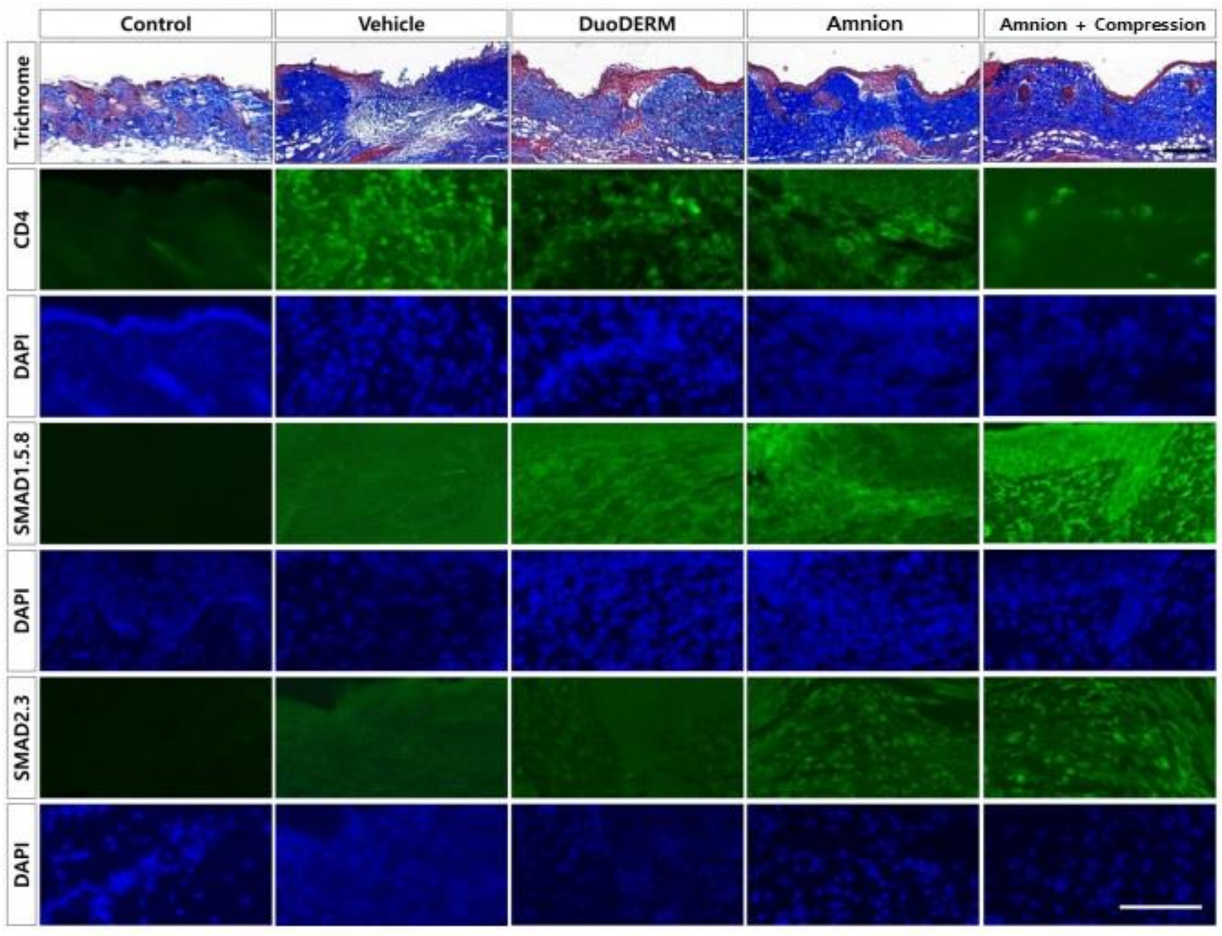

Figure 4. Trichrome staining for immunohistochemical analysis of wound healing markers CD4, SMAD 1/5/8 and SMAD 2/3. All microscopic images are accompanied by a scale bar representing 200μm.

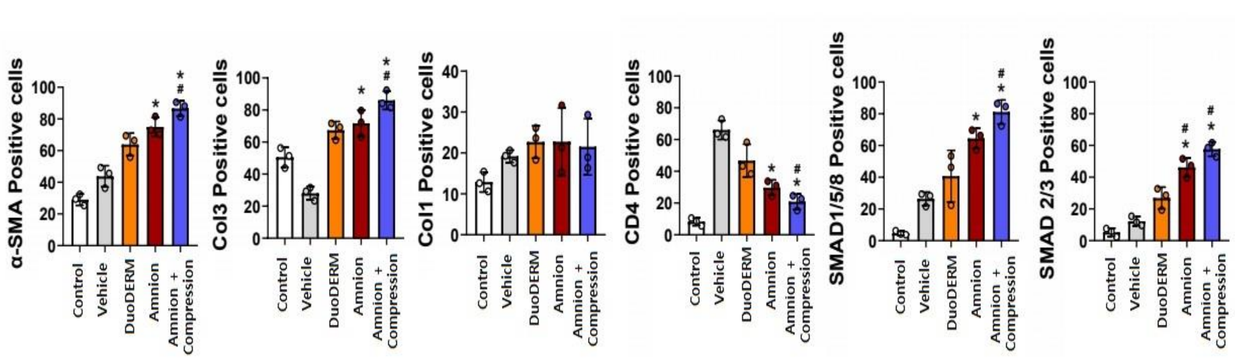

Figure 5. Quantitative analysis and comparative assessment of wound healing markers α-SMA, Col3, Col1, CD4, SMAD 1/5/8 and SMAD 2/3. Data are expressed as the mean ± SD. *= P < 0.05, ** = P < 0.01, and ***= P < 0.001 versus Vehicle control, # = P < 0.05, ## = P < 0.01, and ##= P < 0.001 versus DuoDERM.

Conclusion

In this study, we investigated the potential efficacy of bovine amniotic membrane as a non-surgical intervention for wound healing in rehabilitative medicine. Immunohistochemical analysis showed more organized tissue organization and collagen networks in the amnion groups, indicating accelerated wound healing and stronger tissue. Furthermore, reduced inflammatory response was evidenced by decreased CD4 levels in the amnion groups, suggestive of a potential immunomodulatory effect. However, increased *α-SMA levels* and activation of TGF-β signaling also are indications of a potential fibrotic response. Therefore, monitoring for prolonged fibrosis or scar formation will be needed despite the beneficial aspects of accelerated wound closure and reduced inflammation.