Assessment of healing of 3D reconstructed wound skin in response to a wound care solution over 7 days

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Introduction

Wound healing is a fundamental process that re-establishes tissue integrity and skin barrier function. To support advanced wound care in chronic and acute wounds, wound wash, debridement and irrigation solutions are essential to protect and clean the wound. Effective wound care solutions are a key non-surgical tool, to support natural wound healing and reduce bacterial load. This study tracked wound healing in response to, a wound care solution[1], using a full thickness 3D reconstructed wounded skin tissue model.

Methodology

Wounded full thickness tissue models containing viable cells were treated with either the wound care solution[1] or DPBS every 48 hours for 7 days. On days 1, 2, 5 and 7 models were processed for immunostaining, fluorescent confocal microscopy, ELISAs and measurement of wound closure. Pro-inflammatory markers (IL-1β, TNF-α, IL-6) were quantified using an ELISA. ANOVA was used to determine statistical differences.

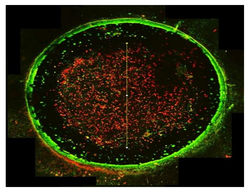

***Figure 1**. Confocal images of wound care solution[1] - treated Day 1 models. Green = Cytokeratin-14, Red = Vimentin.*

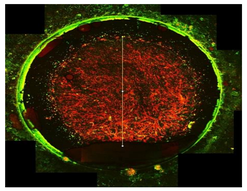

***Figure 2**. Confocal images of DPBS-treated Day 1 models. Green = Cytokeratin-14, Red = Vimentin.*

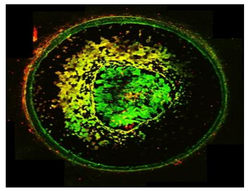

***Figure 3.** Confocal images of wound care solution[1]- treated Day 5 models. Green = Cytokeratin-14, Red = Vimentin.*

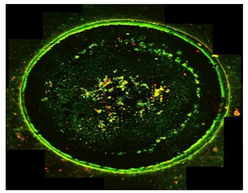

***Figure 4.** Confocal images of DPBS-treated Day 5 models. Green = Cytokeratin-14, Red = Vimentin.*

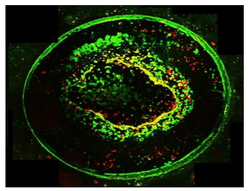

***Figure 5.** Confocal images of wound care solution[1] - treated Day 7 models. Green = Cytokeratin-14, Red = Vimentin.*

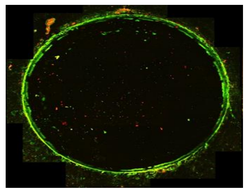

***Figure 6.** Confocal images of DPBS-treated Day 7 models. Green = Cytokeratin-14, Red = Vimentin.*

Interleukin-1β (IL-1β)

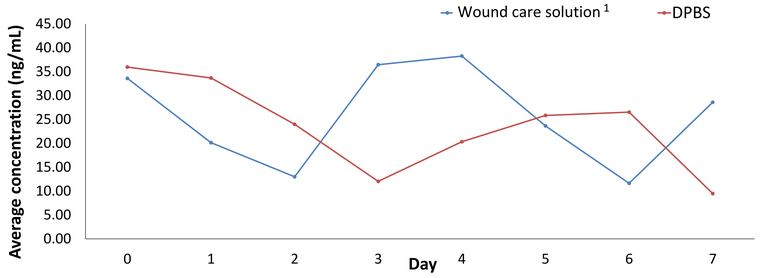

***Figure 7.** Average quantification data for interleukin-1B by ELISA following application of Wound care solution [1] or DPBS from Day 0 to Day 7.*

Tumor necrosis factor-α (TNF-α)

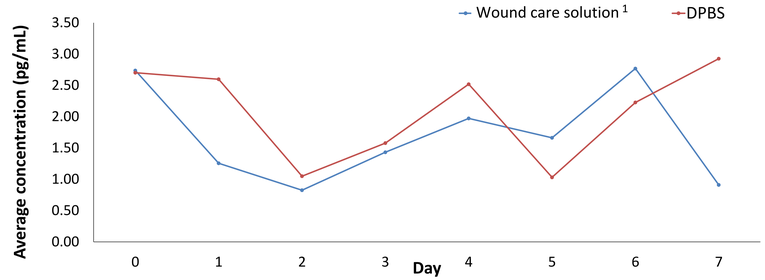

***Figure 8.** Quantification data for tumor necrosis factor-α (TNF-α) by ELISA following application of Wound care solution [1] or DPBS from Day 0 to Day 7.*

Interleukin-6 (IL-6)

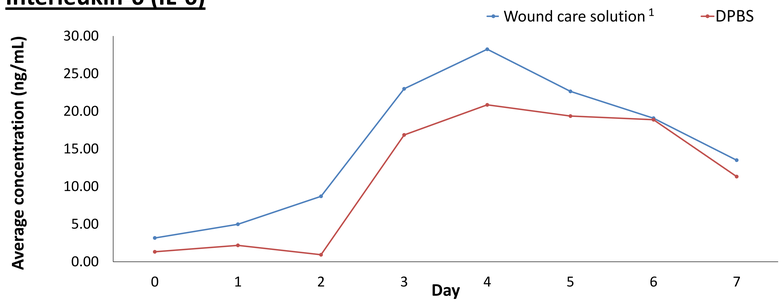

***Figure 9.** Quantification data for* interleukin-6 (IL-6) by ELISA following application of Wound care solution [1] or DPBS from Day 0 to Day 7.

Results

Following 2 days treatment, the average wound diameter of models treated with Wound care solution [1] was significantly reduced compared to controls, indicating an increased wound closure response. At Days 5 and 7, models treated with a wound care solution[1] showed complete coverage of the wounded area with fresh keratinocytes leaving no exposed dermal fibroblasts visible. Tissue models treated with Wound care solution [1] produced comparable levels of IL-1β, TNF-α and IL-6 to models treated with DPBS.

Successful and timely wound healing requires a balance of both fibroblast and keratinocyte growth. A lack or imbalance of either cell type can lead to increased inflammatory responses and prolonged wound healing[2]. The wound model used mimics this scenario closely by removing the top keratinocyte skin layer of a full thickness *in vitro* human skin model, exposing the dermal fibroblast layer underneath and can therefore provide an indication of performance in patients.

Conclusions

In conclusion, Wound care solution [1] showed better wound closure versus the control DPBS and did not induce significant pro-inflammatory effects.

Future work may focus on the investigation of pro-inflammatory markers using a macrophage cell line, such as RAW 264.7 cells. Anti-inflammatory cytokines such as IL-4 and IL-13, could also be assessed following application of Wound care solution[1]

References

1. Wound care solution refers to Bactiguard Wound Care HYDROCYN aqua® *This project was funded by Bactiguard*

2. Wiegand, C., Hipler, U. C., Elsner, P., & Tittelbach, J. (2021). Keratinocyte and Fibroblast Wound Healing In Vitro Is Repressed by Non-Optimal Conditions but the Reparative Potential Can Be Improved by Water-Filtered Infrared A. Biomedicines, 9(12), 1802.

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