Single-use Automated Microcurrent Electrical Stimulation Therapy Accelerates Re-epithelialization in an *In-vitro* Human Skin Wound Model

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ABSTRACT

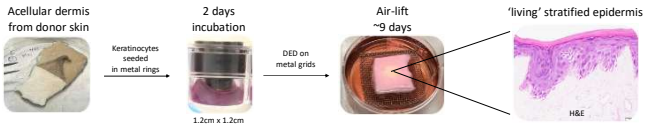
The clinical use of **Single-use Automated Microcurrent Electrical Stimulation Therapy (SAM-EST)** has been shown to **reduce pain and stimulate healing in chronic wounds** that are thought to be devoid of endogenous bioelectric microcurrents[1,2].

The aim of this study was to **evaluate the effect of the Accel-Heal device (AH) on wound closure** using an ***in-vitro*** 3D De-Epidermized Dermis-Human Skin Equivalent (DED-HSE) model[3].

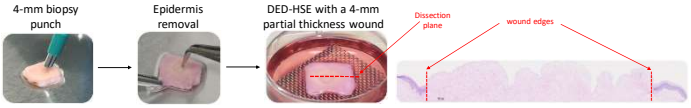
Our data showed that Accel-Heal treatment **improved re-epithelialization** (keratinocyte migration and proliferation) as well as **enhanced the attachment** of newly-formed stratified epidermis to the wound, **coherent with clinical findings**.

METHODS

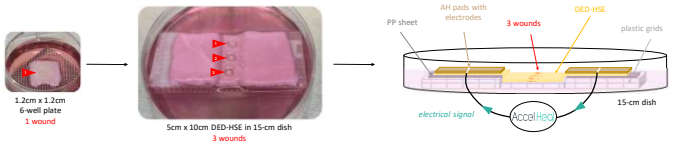
(A) PREPARING THE DE-EPIDERMIZED DERMIS-HUMAN SKIN EQUIVALENT (DED-HSE)



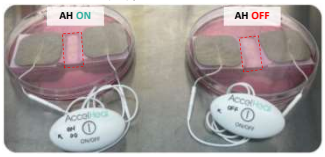
(B) CREATING A WOUND MODEL



(C) OPTIMIZING WOUND MODEL IN A LARGE FORMAT



Accel-Heal device (AH) placed on each side of the wounds

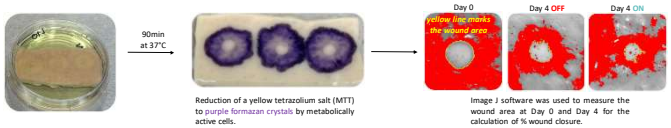


Key points for optimization:

- ✓ Use large skin tissues from matched-donors
- ✓ Avoid microcurrent electrical leakage
- ✓ Culture in a sterile environment
- ✓ Keep the AH pads dry
- ✓ Use same donor skin for ON and OFF comparison
- ✓ Assess wound healing at different time points
  - Day 0: to assess quality of DED-HSE & wounds
  - Day 4: to measure wound closure
  - Day 7: to assess re-epithelialization

(D) ASSESSING WOUND CLOSURE (MAIN READOUTS)

(I) MTT staining: to assess cell viability and proliferation, and measure wound closure

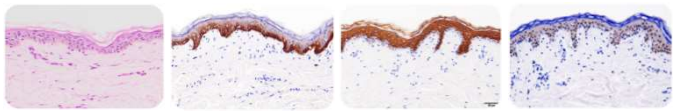


Reduction of a yellow tetrazolium salt (MTT) to purple formazan crystals by metabolically active cells.

Image J software was used to measure the wound area at Day 0 and Day 4 for the calculation of % wound closure.

(II) Histological analysis

- ➢ Hematoxylin & Eosin (H&E): to assess cell migration & re-epithelialization
- ➢ K14, K10, and P63 Immunohistochemistry (IHC): to assess cell proliferation & differentiation



H&E
Hematoxylin stains the nucleus, while eosin stains proteins in the cytoplasm, allowing the visualization of different cell types and structures of skin layers.

Keratin 14 (K14)
Proliferation marker that stains mitotically active basal layer cells that are dividing. K14 protein provides structural support in basal keratinocytes.

Keratin 10 (K10)
Marker of differentiation that stains post-mitotic keratinocytes. It is expressed in the suprabasal layers of the epidermis.

Tumor protein p63 (p63)
Nuclear stem cell marker that regulates both proliferation and differentiation. It also regulates cell adhesion and formation of the basement membrane.

RESULTS & DISCUSSION

(A) GOOD CELL VIABILITY AND IMPROVED WOUND CLOSURE IN AH-TREATED WOUNDS

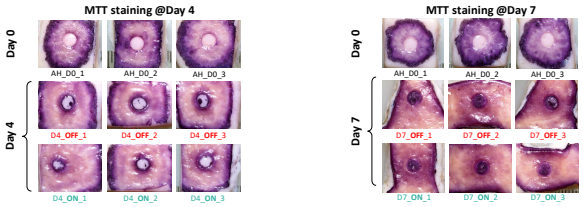


Figure 1: MTT staining (purple color) demonstrated good cell viability and metabolic activity of wounded DED-HSE over 7 days of treatment with AH. Wounds of DED-HSE treated with AH device (ON) seemed to close faster than non-treated samples (OFF).

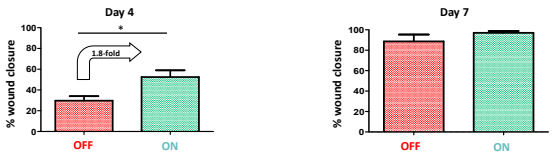


Figure 2: Wounds closed significantly faster with the use of AH (Day 4, ON) compared to non-treated wounds (Day 4, OFF). Although most wounds fully healed at Day 7, re-epithelialization on AH-treated wounds seemed more efficient than non-treated skin (see Figure 3). n=3 for each condition, unpaired t-Test, two-tailed, *p-value ≤0.050. Results were reproduced in at least 3 independent experiments, using skin from different donors.

(B) ENHANCED WOUND CLOSURE AND RE-EPITHELIALIZATION IN AH-TREATED WOUNDS

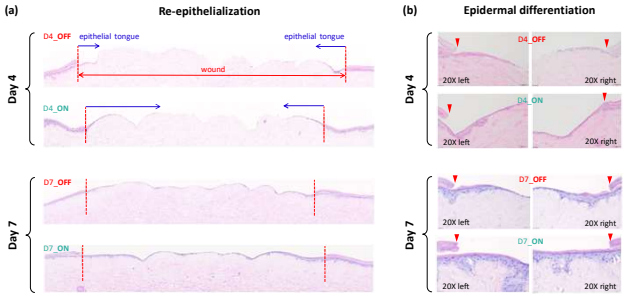


Figure 3: (a) H&E staining and full image scan of the DED-HSE showed longer epithelial tongues on Day 4 AH-treated wounds (ON) compared to OFF, confirming enhanced wound closure upon EST. On Day 7, all wounds had almost reached full closure. **(b)** 20X obj. images showed markedly thicker and well-stratified epidermis in AH-treated wounds (ON) compared to non-treated skin (OFF). Red dash lines and arrows mark the wound edges.

(C) INCREASED CELL PROLIFERATION & DIFFERENTIATION IN AH-TREATED WOUNDS

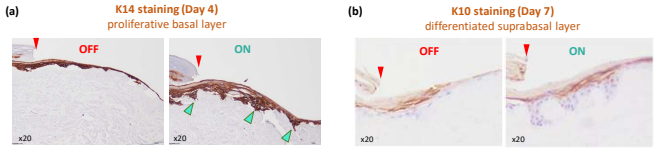


Figure 4: **(a)** K14 immunostaining revealed thicker epidermis and higher proliferative activity when AH was ON compared to OFF. Deeper cell projections (green arrows) suggested stronger attachment of the newly formed epidermis to the wound (under investigation). **(b)** K10 images showed advanced cell differentiation and stratification of the suprabasal layer of the epidermis upon treatment with AH devices. Red arrow in each image marks the wound edge.

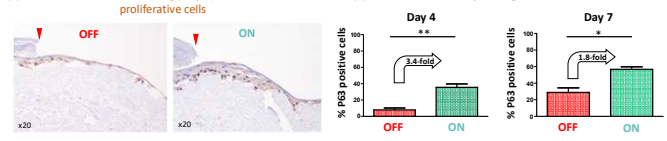


Figure 5: **(a)** Thicker neo-epidermis and higher number of P63-stained cells were observed in AH-treated wounds (ON) compared to non-treated (OFF). **(b)** P63-expressing cells were significantly higher in the newly formed epidermis treated with AH ON compared to OFF at Days 4 and 7, confirming that EST stimulated keratinocyte proliferation from the first days of treatment. % of P63 positive cells was calculated based on total number of cells. n=3 for each condition, unpaired t-Test, two-tailed, *p-value ≤0.05, **p-value <0.01. Results were replicated in at least 3 independent experiments, using skin from different donors.

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CONCLUSION

- We **optimized an *in-vitro* wound model** to evaluate the effects of **Accel-Heal Electrical Stimulation Wound Therapy (EST)** on wound closure/healing.
- We demonstrated that **Accel-Heal EST**:
  - ✓ Maintained good cell viability & metabolic activity
  - ✓ **Improved** keratinocyte **migration & re-epithelialization** of the wound
  - ✓ **Enhanced** epidermal **proliferation, differentiation and attachment** to dermis.
- Our research study **backs up clinical findings** and **strongly supports the use of Accel-Heal devices** in patients with **hard-to-heal wounds**.