

Appendix S1.

SUPPLEMENTARY METHODS

The trial was approved by The Committees of Health Research Ethics in the Capital Region of Denmark (H-16045754).

The 2 wound models used were:

1. Standardized lancet wounds (1.5-mm wide and 2.0-mm deep) were made 2-cm apart using a paediatric blood collection device (BD Microtainer[®], 366594; Becton Dickinson, Franklin Lakes, NJ, USA). The spring-loaded surgical blade was released when the device was pressed onto the skin. A sterile non-woven swab was applied for 10 min to stop bleeding.
2. A fractional ablative CO₂ laser (UltraPulse[®], Lumenis, San Jose, CA, USA) was set to treat an area of 7×7 mm with a density of 10% and 20 mJ pulse energy, resulting in uniform wounds and channels penetrating the skin to a depth of ~0.8 mm, as assessed by optical coherence tomography (OCT).

Dressings were not used.

Erythema measurements

Wound erythema was assessed from digital images (20×) containing a 5-mm calibration scale and taken with a handyscope (FotoFinder Systems GmbH, Bad Birnbach, Germany) connected to an iPhone 5SE (model A1723). The erythematous area (mm²) was quantified using ImageJ software (NIH, Bethesda, MD, USA).

Collection of skin surface proteins by adhesive tape

The surface proteins of the skin and laser wounds were obtained using Sebutape[®] (29 mm×19 mm; Cu-Derm, Dallas, TX, USA)

adhesive tape. The tape was applied to the skin/laser wounds for 1 min and then kept at -80°C. Phosphate-buffered saline (PBS) (1 ml) was added, and the tape was subjected to 2 freeze-thaw cycles, sonicated for 10 min and centrifuged at 14,000×g for 15 min at 4°C.

K6A expression in cultured normal human epidermal keratinocytes

Normal human epidermal keratinocytes (NHEKs) were seeded at 5×10⁴ cells/well in 24-well tissue culture plates coated with type I collagen (Corning[®] 356236; Corning, Bedford, MA, USA) and cultured in 1 ml keratinocyte growth medium-2 (PromoCell, Heidelberg, Germany) containing 0.06 mM CaCl₂ (low calcium, 5 wells) to promote proliferation or in 1.40 mM CaCl₂ (high calcium, 5 wells) to promote differentiation. After 5 days of culture, the cellular morphology was examined through an inverted phase contrast microscope (Zeiss Primovert, Carl Zeiss GmbH, Göttingen, Germany). NHEKs cultured in low-calcium condition grew in monolayers without displaying stratification, whereas NHEKs stratified at high calcium concentrations. The conditioned medium was aspirated and concentrated 10× by Amicon[®] Ultra-0.5 centrifugal filter device (Merck KGaA, Darmstadt, Germany), and the cells were lysed in 0.5 ml radioimmunoprecipitation assay (RIPA) buffer. Cell lysates and concentrated media were analysed on K6A levels and cell lysates were also analysed on total protein contents.

Enzyme-linked immunosorbent assay analyses

Enzyme-linked immunosorbent assay (ELISA) kits were used to measure IL-1α (DY200; R&D Systems, Minneapolis, MN, USA) and K6A (MBS2022731; MyBioSource, San Diego, CA, USA). Total protein was quantified with a Pierce[™] BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA).