

The syndecan-1 binding domain released by cleavage of laminin-332 impacts dermal repair during wound healing

Alejandro Castillo¹, Silène Vincent^{1,2}, Viktoriia Gross³, Anatole Chessel³, Marine Montmasson¹, Bernard Ragaru⁴, Benjamin Herbage⁴, Didier Pin², Marie-Claire Schanne-Klein³ and Patricia Rousselle¹

¹ Laboratoire de Biologie Tissulaire et Ingénierie Thérapeutique, CNRS Université Lyon 1, 7 Passage du Vercors, 69367 Lyon, France. ² Campus Vétérinaire de Lyon / VetAgro Sup – Unité de Dermatologie, 1 avenue Bourgelat 69280 Marcy l'Etoile, France ³ Laboratoire d'Optique et Biosciences, Ecole Polytechnique, CNRS, INSERM, Institut Polytechnique de Paris, 91128 Palaiseau, France. ⁴ Symatase, les Troques, 69630 Chaponost, France.

Keywords. Laminin, epithelialization, granulation tissue, inflammation.

Background. Laminins (LM) are heterotrimeric glycoproteins comprised of one α , one β , and one γ -chain that play a fundamental role in basement membrane architecture and function in human skin. The five C-terminal laminin globular (LG) modules of several α chains are modified by proteolysis to generate a shortened LG1-3 containing LM α chain and a secreted LG4-5 tandem which harbours binding sites for heparan sulphate proteoglycans including those carried by the cell surface receptor syndecan-1. The LG4-5 module within the full-length α 3 chain of LM-332 (α 3 β 3 γ 2) or released from it, was reported to promote the migration of epidermal keratinocytes during the epithelialization phase of wound repair. Here, we have investigated the effect of a peptide mimicking the syndecan-1 binding site within the α 3 chain LG4-5 module on wound healing.

Materials & Methods. The efficacy of the LM peptide was evaluated *in vitro* using primary human keratinocytes and *in vivo* in a model of partial thickness wounds in pigs. Hyaluronic acid based formulations were created as dressings to carry the peptide to the wounds. The follow-up and evaluation of wound closure included macroscopic observations and microscopic analysis of biopsies taken at different times. Imaging tissue sections stained or labelled with antibodies assessed characterization of both the regenerated epidermis and the repaired dermis. Second Harmonic Generation (SHG) Microscopy revealed the 3-dimensional architecture of the collagen fibrils in the repaired dermis.

Results.

A peptide-induced acceleration of epidermal closure was found *in vitro*. *In vivo*, the examination of the biopsies at D7 and 12 revealed a significant enhancement of epidermal thickness confirmed by the increased expression of the Ki67 proliferation marker, suggesting an advanced regenerative process in the presence of the peptide. The treated wounds surprisingly revealed a thinner granulation tissue populated by less CD163⁺ and more CD206⁺ macrophages; accompanied by a reduced fibroblasts expression of α SMA. SHG microscopy revealed a significant and consistent difference in the entropy of the collagen distribution between normal dermis and granulation tissue whether the wound was treated with the peptide or not; revealing no defects in the repair process.

Conclusions.

The syndecan-1-binding peptide has most likely expedited the inflammatory process in its resolution phase either through its induced-fostered epidermal regenerative process or through a syndecan-1 related and yet unknown fibroblast- or inflammatory cells-derived mechanism.