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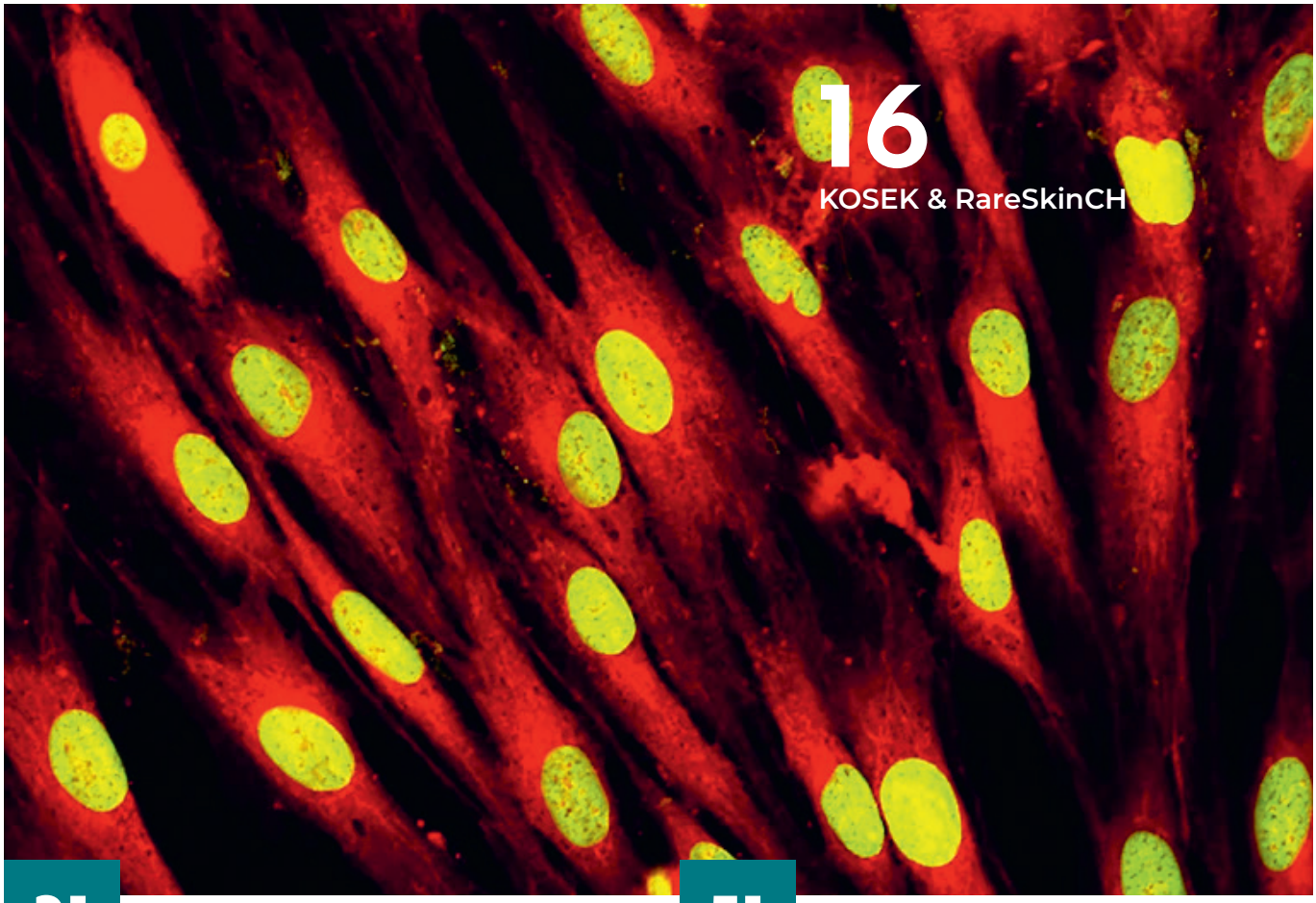

Société Suisse de Dermatologie et Vénéréologie
Società Svizzera di Dermatologia e Venereologia
Swiss Society of Dermatology and Venereology
Schweizerische Gesellschaft für Dermatologie und Venerologie

WHAT'S NEW

Shining a Light on Collagen Dynamics in Wound Healing

This section is a contribution from the SKINTEGRITY.CH interdisciplinary research consortium. The present work was performed by Dr. Paul Hiebert and colleagues, including SKINTEGRITY.CH Principal Investigators, Prof. Helma Wennemers and Prof. Sabine Werner.

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Paul Hiebert. Collagen synthesis and remodelling is a critical aspect of wound repair, necessary for restoring the structural integrity and functionality of tissues following injury. Visualizing this process is important because it allows researchers and clinicians to understand the dynamics of tissue repair, identify areas of active collagen formation, and detect abnormalities that may lead to chronic wounds or excessive scarring. Accurate and real-time visualization tools are needed to monitor these processes and develop targeted therapeutic interventions; however, existing tools have been limited in their ability to provide direct and specific insights into these collagen dynamics. A new collaborative study by researchers at SKINTEGRITY.CH introduces a synthetic collagen peptide sensor (referred to as Probe 1) that enables the visualization of collagen formation and remodelling during wound healing, offering unprecedented insights into these dynamics in both spatial and temporal dimensions.

Tissue repair involves the dynamic remodelling of the extracellular matrix (ECM), with collagen being a primary structural component. Lysyl oxidases (LOX) are crucial for collagen maturation as they trigger cross-linking within collagen fibrils through oxidation of lysine to allysine residues. Abnormal LOX activity is associated with various pathologies, including fibrotic and malignant diseases, making it essential to monitor this process accurately. Probe 1 selectively binds to sites of LOX-mediated collagen cross-linking, thereby illuminating areas of active collagen formation and remodelling. This probe showed high selectivity for newly forming and remodelling collagen, particularly at the wound site, and not in matured fibrillar collagen found in unwounded skin. This specificity was evident in both mouse and human wound samples.

The effectiveness of Probe 1 is due to its three key elements *(Figure 1):* 1) the collagen mimetic peptide, which provides selectivity for accessible collagen molecules undergoing assembly or remodelling; 2) the anchor, an aminooxy group that reacts chemoselectively with aldehydes produced by LOX activity, ensuring the probe remains bound to the target site; and 3) the LOX-responsive masked fluorophore, which lights up upon reaction with LOX, allowing real-time visualization of LOX activity. In our study, Probe 1 provided clear visualization of collagen remodelling at different stages of wound healing, while the control probe, which lacked the collagen mimetic peptide, showed broader and non-specific staining, underscoring its importance for selective binding.

At day 14 post-injury, when closure of excisional mouse wounds is complete and active remodelling and scarring are occurring, Probe 1 selectively stained the wound margins and interior, highlighting areas of active collagen synthesis and remodelling. In contrast, at day 5 post-injury, prior to complete wound closure, Probe 1 primarily stained outside the wound near the periphery, indicating the initiation of collagen remodelling events adjacent to the wound itself prior to wound closure. This information is important because it provides insight into the spatial and temporal dynamics of collagen remodelling during wound healing, beginning at the wound edges prior to wound closure, and subsequently progressing inward. Understanding these early stages can be crucial for developing targeted therapies to optimize wound healing and minimize scarring.

Our study also explored the probe's

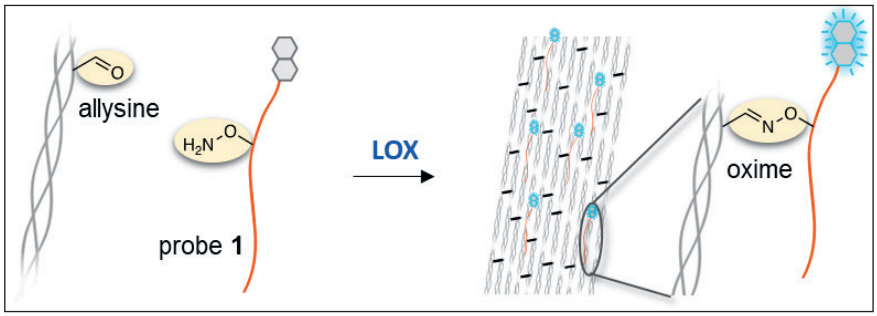

Figure 1: Depiction of the chemoselective reaction of the anchoring group of Probe 1 with LOX-generated allysine residues in newly forming/remodelling collagen. The collagen mimetic peptide (orange) allows for selective binding to collagen, while the attached fluorophore (blue) lights up when reacting with LOX.

performance in transgenic mouse models with altered wound healing dynamics. In mice expressing a constitutively active mutant of the transcription factor Nrf2 (caNrf2) in fibroblasts, wound healing completes faster. However, the skin of these mice exhibits reduced collagen content, making it weaker and more prone to tearing. In 14-day wounds from these mice, Probe 1 staining was significantly reduced, suggesting that the accelerated healing process in caNrf2 mice is coupled with suboptimal collagen synthesis and remodelling, potentially compromising the integrity of the healed tissue. Conversely, mice overexpressing the highly diffusible cytokine activin A in keratinocytes also exhibit accelerated wound closure but have increased production of ECM. Here, Probe 1 staining was significantly increased, reflecting enhanced collagen synthesis and remodelling. These findings highlight the probe's sensitivity to variations in collagen dynamics, making it a valuable tool for studying different mouse models with various genetic modifications or the effect of pharmacological compounds. This sensitivity allows it to be used in studies related to wound healing mechanisms and potentially other disease processes, providing critical insights into the underlying biological pathways.

Further investigations using second harmonic generation (SHG) imaging, which specifically identifies mature collagen fibres, confirmed that Probe 1 did not co-localize with mature fibrillar collagen, confirming its selectivity for newly forming collagen and remodelling sites *(Figure 2)*. Its subsequent application in human wound samples further validated its clinical potential.

The practical implications of these findings are significant. Probe 1 offers a powerful tool for researchers to track collagen formation and remodelling in various wound healing models. Its ability to provide real-time insights into the dynamics of collagen synthesis and remodelling facilitates the identification of key areas of ECM changes and aids in targeted therapeutic interventions. In clinical settings, the application of Probe 1 in human wound samples demonstrates its potential as a diagnostic tool. It could be used to assess the wound healing progress and to tailor treatments to individual patients.

The introduction of Probe 1, therefore, represents a valuable addition to the toolkit of both wound researchers and clinicians. By enabling precise visualization of collagen remodelling, this novel sensor enhances our understanding of tissue repair processes and opens new avenues for drug testing.

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Reference

Hiebert, P., Antoniazzi, G., Aronoff, M., Werner, S., & Wennemers, H. (2024). A Lysyl Oxidase-Responsive Collagen Peptide Illuminates Collagen Remodeling in Wound Healing. Matrix Biology, 128, 11-20. https://doi.org/10.1016/j.matbio.2024.02.006

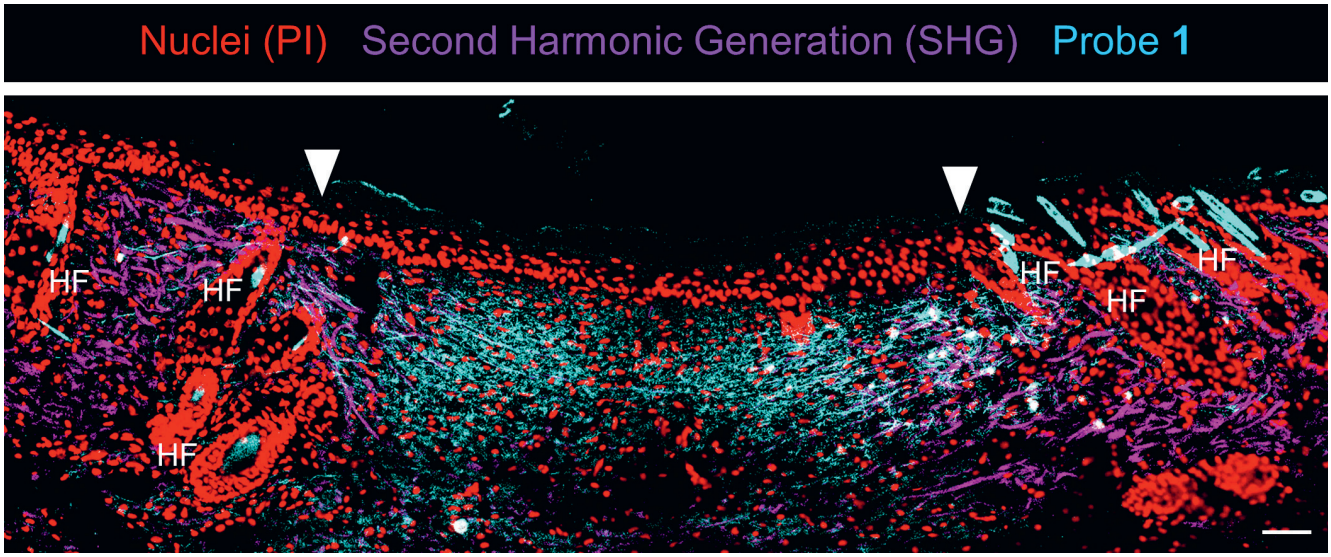

Figure 2: Pinpointing collagen remodelling events in wound tissue. Representative image of a 14-day mouse excisional skin wound stained with Probe 1 (blue). Nuclei were counterstained with propidium iodide (PI; red). White arrowheads indicate the wound margins. Note the blue autofluorescence of the hair within hair follicles (HF). The tissue section was imaged using multi-photon microscopy and second harmonic generation (SHG) to visualize mature fibrillar collagen (magenta). Scale bar: 100 µm.