Application Note

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Mechanobiology of wound healing

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Revolutionizing wound care with the Pavone: mechanical forces as potential biomarkers in wound healing

by Optics11 Life

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Mechanical properties of wound sites significantly influence the healing rate and quality of newly formed tissue, making mechanics a potential label-free biomarker for tissue regeneration. Here, we introduce the Pavone - a high-throughput mechanical screening platform for thoroughly characterizing epidermal cells and supporting physiologically relevant in vitro models for wound healing.

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1 INTRODUCTION

Injuries to the skin's normal barrier function heal through a progressive cellular response involving fibroblasts, macrophages, endothelial cells, and keratinocytes to restore the skin's integrity. During wound healing, mechanical forces are crucial to skin regeneration. Changes in tissue mechanical properties, such as stiffness and viscosity, affect cell behavior and wound healing quality[1,2].

Characterizing the mechanical properties of the skin, particularly at cell length scales, is becoming increasingly relevant in regenerative medicine, mainly because the mechanics of cells and their microenvironment regulate various biological processes involved in wound healing[3]. However, conventional methods for mechanically characterizing tissues, such as an atomic force microscope (AFM), are complex and time-consuming. They often require sample preparations that compromise the tissue's native mechanical properties.

In this context, we introduce the Pavone as a powerful instrument to mechanically characterize cells, tissues, spheroids, organoids, and biomaterials in a nondestructive manner. This innovative technology can assay the mechanical properties of cells and their surroundings in health and disease conditions and elucidate the mechanical responses triggered by disturbances with the potential to inspire novel therapeutic strategies.

2 OPTICS11 LIFE NOVEL TECHNOLOGY

Pavone is a mechanical screening platform for novel research applications, such as disease modeling, drug screening & delivery, regenerative medicine, and tissue engineering. More specifically, it can monitor the mechanical environments in healthy, injured, repaired, and fibrotic tissues[4]. Moreover, it characterizes the influence of mechanical properties on biological processes linked to tissue healing, such as collective cell migration[5,6] and wound contraction[7]. By applying this technology to developing human skin equivalents[8] and organoids[9], scientists can more accurately model the physical microenvironment of native tissues. Besides, Pavone supports the development of scaffolds[7,10] and drug delivery systems[11]. The mechanical properties of these materials are crucial to ensure compliance with injured tissue *in vivo*.

This application note describes the mechanical properties of a bladder tumor and surrounding areas using Pavone technology.

3 MECHANICAL CHARACTERIZATION OF KERATINOCYTES

Pavone is a powerful tool for characterizing the mechanical features of epidermal cells in wound healing. Keratinocytes, the predominant cells in the epidermis, are responsible for the skin's protective barrier function. Keratin cytoskeleton remodeling is essential for cell-cell and cell-matrix adhesion, modulating cell motility during wound healing[12,13]. Here, we investigated the influence of two bioactive peptides on keratinocyte mechanical properties using Pavone.

Bioactive peptides can influence the expression of cytoskeletal and cell-extracellular matrix interacting proteins, which are crucial for tissue remodeling and wound healing[13]. In this study, human keratinocyte cell lines (HaCaT) were treated with two distinct bioactive peptides (BP): BP1 (5 µg/ml) or BP2 (0.5 µg/ml). Untreated cells containing only culture medium were included as a control. After five days, HaCaT cells' stiffness and topography were measured using the Pavone.

Cells' mechanical properties were assessed using a probe with a stiffness of 0.2 N/m and a tip radius of 9.5 µm. The indentation depth was 3 µm. All indentations were performed in cell culture medium. To determine Young's modulus and surface topography, the contact point was identified using Optics11 Life DataViewer software. A Hertzian model with a Poisson's Ratio of 0.5 was fitted to the load vs. indentation curve corresponding to the first 1000 nm of indentation. Any indentation with a fit for the Hertzian model below an R2 value of 0.80 was excluded from further analysis.

Our findings revealed that exposure of HaCaT cells to BP1 and BP2 decreased cell stiffness and proportionally to the concentration of the investigated peptide (4.25 ± 1.08 KPa for cells treated with BP1, 3.18 ± 1.00 KPa for cells treated with PB2, and 5.50 ± 1.61 KPa for untreated cells) (Figure 1). Exposure to the peptides, particularly BP1, also influenced the cells' morphology, which became rounded, unlike the typical flat appearance of untreated HaCaT. It might indicate cytoskeletal rearrangements that align with cell stiffness changes (data not shown).

Additionally, by mapping the surface of the untreated HaCaT cell monolayer, we could visually reconstruct the 3D topography of distinct cellular compartments, especially the cytoplasm (thicker area) and the cell-cell junctions (thinner area) (Figure 2).

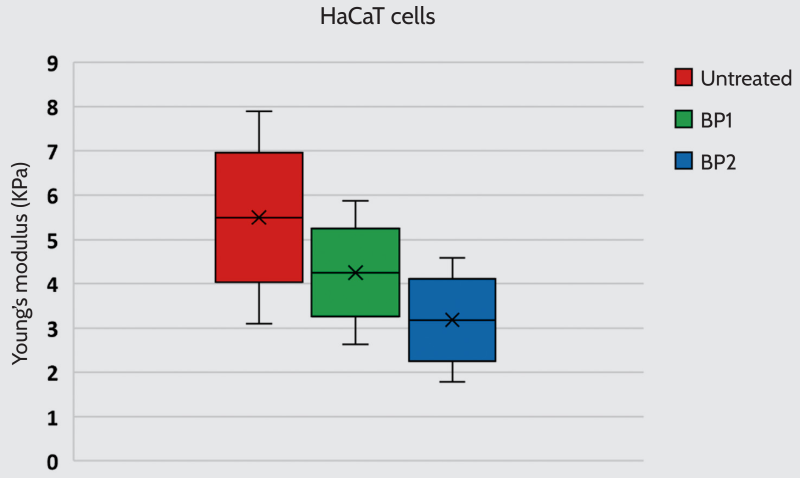

Figure 1

Young's modulus of HaCaT cells. Cells were treated with two bioactive peptides (BP): BP1 (5 μg/ml) or BP2 (0.5 μg/ml). Untreated cells were included as a control.

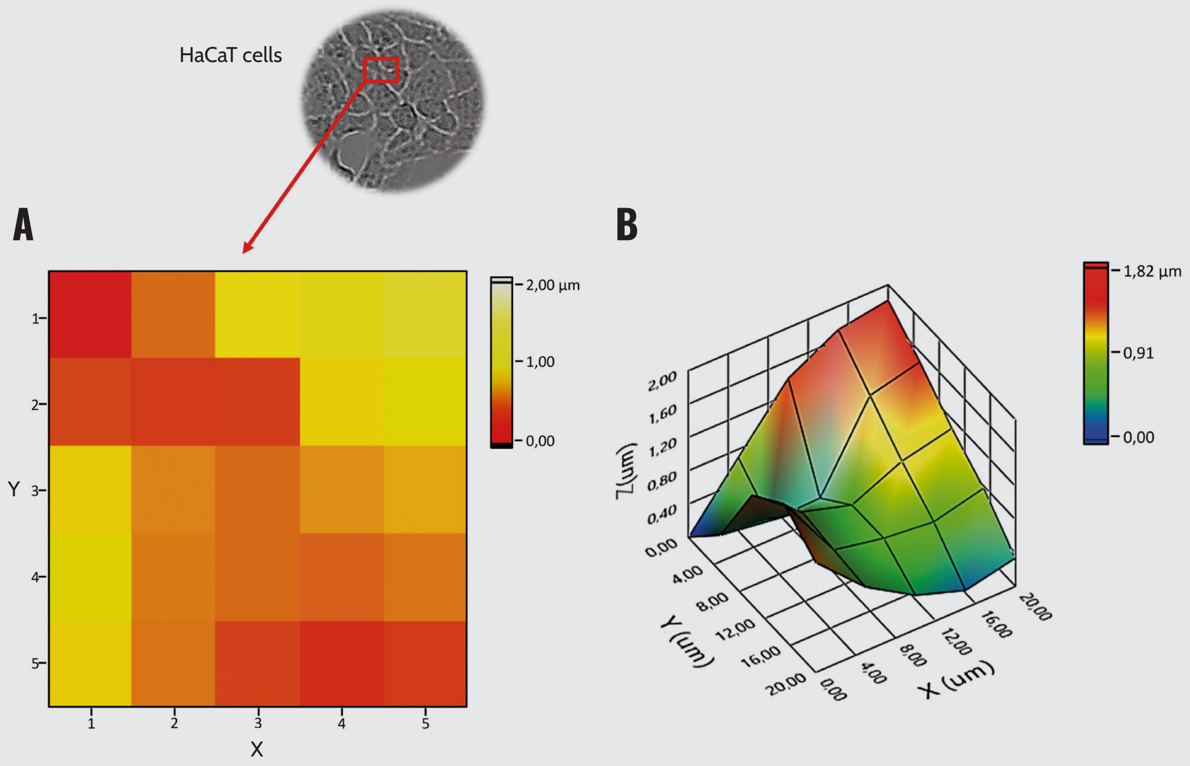

Figure 2

HaCaT cells topography. 2D surface plot **(A)** and 3D surface topography **(B)**.

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CONCLUSION

- Pavone is a powerful tool to measure the mechanical properties of cells involved in wound healing, specifically keratinocytes.
- Pavone revealed how potential treatments influence cell stiffness, providing significant insights into the mechanics of keratinocytes with potential applications to skin regeneration and wound healing research.

The mechanical properties of wounded tissues affect the rate and quality of healing, making mechanics a potential label-free biomarker for tissue regeneration.

In this context, Pavone can support novel wound care approaches by fostering a better understanding of tissue regeneration and healing and developing physiologically relevant in vitro models.

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