

FIBRILLAR MECHANICS AND STRUCTURE OF FIBROTIC TISSUE USING IN SITU SYNCHROTRON X-RAY NANOMECHANICAL IMAGING

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Introduction

Keloids are an example of a fibrotic skin disorder linked to hypertrophic scarring [1], with abnormal extracellular matrix (ECM) composition in terms of collagen type and keloid-specific proteins. The biomechanics of the ECM is believed to be critical to progression of fibrotic disorders[2], but so far there is little known about the ultrastructure (fibrillar-level) biomechanics in keloid and scarring ECM. Here, we show how the combination of synchrotron small-angle X-ray scattering (SAXS), *in-situ* loading, and diffraction-based image correlation can quantify fibrillar strain, reorientation, and ordering in keloids under biomechanical loading [3].

Materials and Methods

Keloid samples were obtained from patients at the plastic surgery department at Barts NHS Health Trust with full ethical approval. Planar keloid sections were prepared (30-35×10-15×1mm³) with a double-scalpel setup and stored at -20°C till SAXS measurements. Samples were measured at the SAXS beamline I22 (Diamond Light Source, Harwell) using a Pilatus 2M detector (X-ray energy 14 keV; beam size 15 μm). Three types of experiments: scanning SAXS, *in situ* tensile loading with scanning SAXS, and scanning SAXS with sample rotation (texture) were carried out on different samples. The 2D SAXS patterns were azimuthally- $I(q)$ and radially- $I(\chi)$ averaged using the software DAWN (www.dawnsci.org). $I(q)$ and $I(\chi)$ profiles were analyzed [CITE] to extract ultrastructural parameters like fibrillar D-period (linked to fibril pre-strain), collagen peak intensity (fibrillar order), $I(\chi)$ peak position (fibril orientation).

Results and Discussion

SAXS images in Figure 1(A) shows peaks due to collagen fibrillar D-period and interfibrillar proteoglycan-rich phase. Under tensile loading, $I(q)$ profiles show peak shift (increased D-period/fibril strain) and profile narrowing (reorientation and intrafibrillar ordering) in $I(\chi)$ (Fig 1(C)). Mapping these parameters across the tissue (Fig 1(D)) show i) clear internal pre-strain gradients in native state and ii) appearance of highly-stress fibrillar-level bands across the tissue. Our results demonstrate proof-of-concept in the use of synchrotron X-ray nanomechanical imaging to understand the mechanobiology of fibrotic disorders.

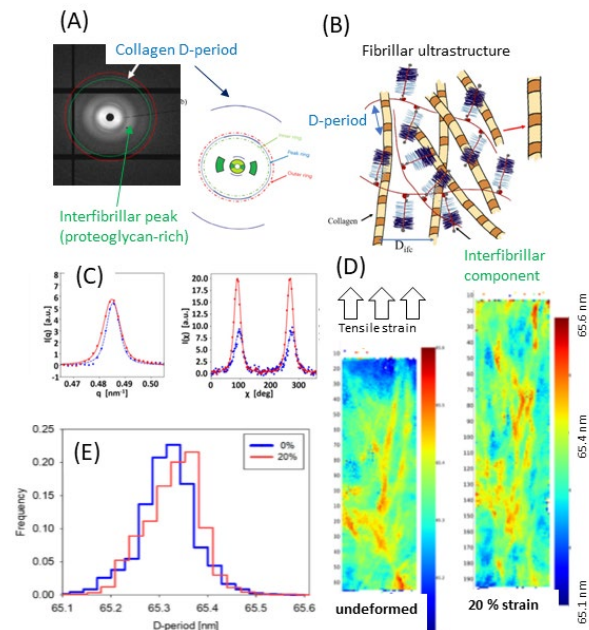


Figure 1: (A) Experimental (left) and schematic (right) SAXS image of keloid fibrotic tissue (B) Schematic representation of fibrillar ultrastructure of keloids (C) Left: radial $I(q)$ and right: azimuthal $I(\chi)$ profiles at selected keloid micro-anatomical location before (blue) and after (red) loading, (D) Representative map of fibril pre-strain, mapped across a keloid sample in (left) unstressed conditions and (right) under macroscopic tissue strains of 20%. (E) Histogram of fibril pre-strain across the unstressed (blue) and stressed (red) sample.

References

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3. Y. Zhang et al, Materials 15:1836 (2022).

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