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Static and dynamic nanomechanical properties of human skin tissue using atomic force microscopy: Effect of scarring in the upper dermis

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ABSTRACT

Following traumatic injury, skin has the capacity to repair itself through a complex cascade of biochemical change. The dermis, which contains a load-bearing collagenous network structure, is remodelled over a long period of time, affecting its mechanical behaviour. This study examines the nanomechanical and viscoelastic properties of the upper dermis from human skin that includes both healthy intact and scarred tissue. Extensive nanoindentation analysis shows that the dermal scar tissue exhibits stiffer behaviour than the healthy intact skin. The scar skin also shows weaker viscoelastic creep and capability to dissipate energy at physiologically relevant frequencies than the adjacent intact skin. These results are discussed in conjunction with a visual change in the orientation of collagenous fibrils in the scarred dermis compared with normal dermis, as shown by atomic force microscopy imaging.

1. Introduction

Skin, the most voluminous organ in the human body, is a complex multilayered biomaterial consisting of a compact keratin-rich epidermis overlying a dermis consisting of a collagenous and elastin fibril phase within an extracellular matrix (ECM). The ECM is rich in proteoglycans (PG), providing hydration throughout the tissue. The functions of skin are manifold and include barrier protection, heat regulation, sensory reception and transpiration, while being sufficiently supple that it will allow for biomechanical mobility and locomotion [1]. While the outer layer of skin (epidermis) is an avascular multilayered structure containing mostly keratinocytes and minor subpopulations of melanocytes, Langerhans cells and few Merkel cells, the dermis layer consists of a collagenous connective tissue containing hair follicles, blood vessels, and sweat and sebaceous glands. The upper (papillary) dermis layer is densely packed with bundles of collagen fibres, which include those fibrils that provide attachment between the epidermis and dermis. The lower (reticular) dermis by contrast is much less fibrillar and merges with a hypodermis (also known as subcutis) that connects with a muscle layer and then to underlying bone.

Given its strategically important location at the interface with an often noxious external environment, the skin is imbued with a remarkable regenerative and repair capacity, e.g., as in wound healing. Upon receiving a trauma, the repair process gets started with platelet aggregation at the wound site to stop bleeding, via the formation of a fibrin clot. Subsequently, this fibrin clot forms a matrix for hosting a variety of cell types, predominately fibroblasts that dissolve the fibrin clot while depositing collagen. In the final phase of wound healing, the composition of the ECM changes over a number of months [2]. It has been shown recently that silver nanoparticle treatment on excisional wounds had a positive effect on the acceleration of the healing process with improved tensile properties, matching those of the normal skin [3].

Disfigurement of the skin by scars is associated with significant psychological and potential functional burden, which may lead to a significant decrease in quality of life [4]. Scars are normally classified according to their clinical behaviour and appearance: normotrophic, hypertrophic and keloidal. Although the histological distinction between these scar types remains controversial, Fourier imaging has shown clear differences in the collagen morphology within these scar types [5]. Skin wounds on early mammalian foetuses heal perfectly with no scars, whereas wounds to adult mammals result in scar formation [6]. Experimental studies suggest that specific anti-TGF-beta therapeutic strategies can improve scar formation in adult wound repair and fibrotic diseases [7]. Comparison between fetal and post-natal wound healing has revealed differences in inflammatory response, cellular mediators, cytokines, growth factors and ECM modulators [8].

Atomic force microscopy (AFM) studies on skin have been carried out to examine the adhesion, friction and wear at the nanolevel, where it was shown that skin cream reduces surface roughness

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and increases surface hydrophilicity [9]. Dynamic nanoindentation work on porcine stratum corneum showed a much reduced modulus and an increase in viscoelastic dissipation following its hydration [10]. Geerligs et al. [11] examined the nanomechanics of viable epidermis and stratum corneum, and showed that these layers exhibited reasonably similar moduli values of 1.1 ± 0.2 and 2.6 ± 0.6 MPa, respectively [11]. These measurements were carried out using a large spherical indenter and in a loading direction normal to the skin surface. Crichton et al. used AFM force spectroscopy to measure the elastic and viscoelastic (stress relaxation) properties of the epidermis and dermis of murine skin using small (1.9 μm dia.) and large (6.62 μm dia.) spherical probes [12]. Other medical uses of AFM imaging and force spectroscopy techniques include novel insights into the detection and recognition of osteogenesis imperfecta [13] and osteoarthritis [14].

Clinical uptake of tissue engineered skin substitutes for wound healing has been limited by often poor integration with the host tissue, in conjunction with wound contraction and scar formation. A successful tissue scaffold should exhibit similar mechanical and physical characteristics to the host tissue, while providing a suitable surface chemistry and structure to facilitate proliferation and differentiation of cells [15]. This study aims to examine the nanomechanical properties and viscoelastic nature of the upper (papillary) dermis of human skin in healthy and scar skin tissue using AFM nanoindentation with the aim to understand how its function may be altered and reduced in damaged skin.

2. Methods

2.1. Tissue samples

Skin samples were obtained from a 59-year-old male who presented for elective plastic surgery to remove excess tissue, which included an established scar of several years' standing. Full consent was obtained from the ethics committee of the clinic and university for the use of this tissue in research. Skin specimens containing a central scar of 2 cm and adjacent normal skin of 3 cm on either side of the scar were frozen upon arrival in the laboratory (within 6 h of surgery) and stored at −80 °C until used. One-centimetre skin specimens from the centre of the scar and also from the centre of the adjacent tissue were collected for cryo-sectioning and placed onto metal chucks using O.C.T. embedding compound (Agar Scientific, Essex, England) and sectioned in a cryostat (CM1510, Leica Microsystems, Wetzlar, Germany).

Skin specimens were sectioned with thickness 10 μm and collected onto polylysine-coated microscope slides. Ten sections each of normal and adjacent scar tissue were stored in a −80 °C freezer and brought to room temperature before use.

2.2. AFM Scanning and nanomechanics

Microscope slides with the skin sections were placed on the sample stage of the MFP-3D AFM instrument (Asylum Research, Santa Barbara, USA) before placing ~1 mL of ultrapure water (18.4 MΩ cm) on the surface. Following laser alignment of the v-shaped silicon nitride cantilever (Hydra6 V series; AppNano, Santa Clara, USA), the cantilever was then calibrated for its laser sensitivity and spring constant (k = 0.32 N m^{-1}) using the thermal method [16] after 1 h equilibration time. Using the AFM optical microscope (×10), the cantilever was moved above the dermis (~200 μm proximal to the epidermis/dermis boundary) (Fig. 1). Only interfollicular dermis was assessed, to avoid any confounding influence of skin appendages (e.g., hair follicles and other glands). As the lower dermis (reticular) contains sweat and sebaceous glands as well as blood vessels and hair follicles, this region was avoided so that the nanoindentation results are not affected by any underlying sub-surface anatomical structures.

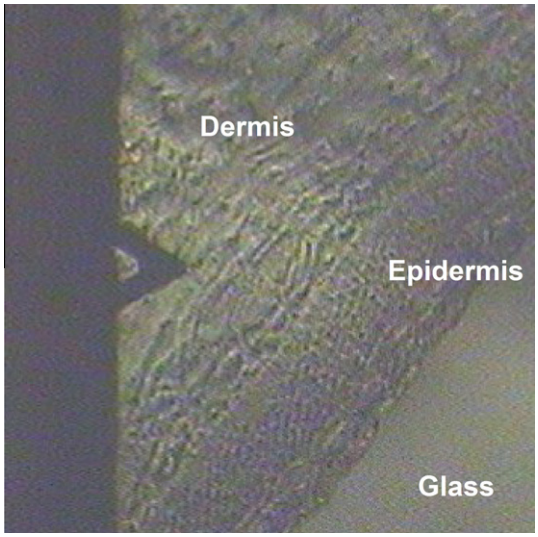

Fig. 1. AFM optical image showing the location of the AFM cantilever (length = 100 μm) in the upper dermis region, close to the epidermis boundary.

Following scanning of the dermis using an intermittent contact mode (AC mode), nanomechanical measurements was made by pressing the cantilever into the dermis in an organized array (32 × 32). Each indentation was at a maximum load of F_{max} = 20 nN and a tip velocity of 4 μm s^{-1}. Three random regions near the dermis/epidermis boundary were scanned and indented for each of the 10 skin sections per tissue type. Elastic modulus was then estimated using a linear elastic based theory for a cone:

$$F = \frac{2}{\pi}\left[\frac{E}{1-\nu^2}\right]h^2 \tan\alpha \quad (1)$$

In this expression, ν is Poisson's ratio, taken to be 0.5 (i.e., incompressible), h is the indentation depth, and α is the half cone angle of the AFM probe. For this, the manufacturer's nominal value of 36° was used, which was independently confirmed by scanning a standard calibration sample.

Each indentation had a 3-s hold at maximum load, as applied by continuous feedback to maintain cantilever tip deflection. During this time, the viscoelastic biological tissue will undergo creep deformation. The amount of indentation creep during this hold period is recorded to give a measure of the tissue's viscoelasticity.

Dynamic indentation on the dermis was carried out to examine damping properties of the upper dermis of the healthy and scarred skin tissue types. A single 10 × 10 force map was taken at a random location in the upper dermis. Following loading (F_{max} = 20 nN, tip velocity 4 μm s^{-1}), the skin was given 10 s at F_{max} to eliminate creep before then undergoing 3-s periods of oscillatory motion at 4, 3, 2 and 1 Hz, each separated by a 3-s hold. The force/indentation vs time curves are extracted and fitted to a sinusoidal curve ($y(t) = A\sin(\omega t + \delta)$), ω is the angular frequency, and δ is the phase difference. The measured strain (indentation) lags behind the applied stress (force), as expected for a viscoelastic material. The phase change (δ) between the applied load and the measured indentation is recorded for each frequency. A phase change of $\delta = 0$ would indicate a pure elastic response, and a phase change of $\delta = \delta/2$ indicates a pure viscous response.

ID	Title	Pages
389	Static and dynamic nanomechanical properties of human skin tissue using atomic force microscopy: Effect of scarring in the upper dermis	7