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Research paper

3D nanomechanical evaluations of dermal structures in skin

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A R T I C L E I N F O

Article history:
Received 31 July 2015
Received in revised form
20 October 2015
Accepted 17 November 2015
Available online 28 November 2015

Keywords:
Skin
AFM
Nanomechanics

A B S T R A C T

Skin is a multilayered multiscale composite material with a range of mechanical and biochemical functions. The mechanical properties of dermis are important to understand in order to improve and compare on-going in vitro experiments to physiological conditions, especially as the mechanical properties of the dermis can play a crucial role in determining cell behaviour. Spatial and isotropy variations in dermal mechanics are thus critical in such understanding of complex skin structures. Atomic force microscopy (AFM) based indentation was used in this study to quantify the three dimensional mechanical properties of skin at nanoscale resolution over micrometre length scales. A range of preparation methods was examined and a mechanically non-evasive freeze sectioning followed by thawing method was found to be suitable for the AFM studies. Subsequent mechanical evaluations established macroscale isotropy of the dermis with the ground substance of the dermis dominating the mechanical response. Mechanical analysis was extended to show significant variation in the elastic modulus of the dermis between anatomical locations that suggest changes in the physiological environment influence local mechanical properties. Our results highlight dependence between an isotropic mechanical response of the dermal microenvironment at the nanoscale and anatomical location that define the variable mechanical behaviour of the dermis.

1. Introduction

Skin is a complex biological composite material that performs a variety of functions including serving as a barrier to the external environment and transferring mechanical loads. The complexity of skin is of continuing interest particularly for developing synthetic skin equivalents (Grover et al., 2012; Wang et al., 2012) as well as from more fundamental studies into understanding the biological optimisation used to give flexibility and toughness (Groves et al., 2013). Anisotropic

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http://dx.doi.org/10.1016/j.jmbbm.2015.11.017
1751-6161/

fibrous components at relatively small length scales within skin are of significant interest as their organisation is critical in defining potentially complex mechanical anisotropy within bulk skin (Gahagnon et al., 2012; Gerhardt et al., 2012; Groves et al., 2013; Ní Annaidh et al., 2012b). The relationship between resultant mechanical performance, structural development and resultant organisation within skin has been identified as a considerable challenge due to this mechanical complexity that exists (Delalleau et al., 2006). Skin is structured as a multicomponent composite, as found in many biological materials with a mechanical function, while providing an environment for cells to grow and replenish the skin. From a structural mechanics approach, skin is a layered composite consisting of the dermis and the epidermis (Silver et al., 2003). The dermis makes up a higher volume fraction of the skin compared to the epidermis, and is mainly comprised of collagen fibres, elastic fibres and ground substance of proteoglycans and glycoproteins (Silver et al., 2001). The fibrous collagen forms the main structural component of the dermis while the ground substance is an amorphous matrix that surrounds the collagen. These components comprise the dermal extracellular matrix (ECM) that dermal fibroblasts occupy and maintain.

The complex three dimensional (3D) microenvironment that fibroblasts are exposed to within the dermis is key to understanding the behaviour and activity of the cells (Lo et al., 2000; Rehfeldt et al., 2007; Saez et al., 2005; Solon et al., 2007). Mesenchymal stem cell fate has also been shown to be directly influenced by the mechanical environment used to culture stem cells (Engler et al., 2006). The stiffness of the microenvironment or ECM is therefore critical in determining the behaviour of the cell and is expected to vary according to the 3D organisation found in the dermis.

Attempts to understand a macroscopic mechanical environment have led to studies investigating the mechanical properties of whole skin in response to various loading conditions. These macroscopic tests often utilise various experimental techniques including tension, compression and suction to establish the elastic modulus, yield strength and ultimate tensile strength of whole skin. Testing of whole skin indicates the resultant elastic modulus lies between 5.5 kPa and 83.3 MPa, depending on the evaluation method used (Diridollou et al., 2000; Gahagnon et al., 2012; Gąsior-Głogowska et al., 2013; Geerligs et al., 2011; Groves et al., 2013; Hendriks et al., 2003; Jachowicz et al., 2007; Ottenio et al., 2014; Wang et al., 2013; Yuan and Verma, 2006; Zahouani et al., 2009). Uniaxial tensile testing of whole skin shows a dependence of the elastic modulus to the direction of the applied load and the alignment of the collagen fibre network of the dermis in the sample, indicating some degree of anisotropy in the dermis (Gąsior-Głogowska et al., 2013; Ní Annaidh et al., 2012b). These macroscopic tests provide insight into the bulk properties of skin in response to extreme loading conditions, but do not give any indication of the microenvironment within the bulk material that cells are exposed to. Therefore, reducing the length scale examined provides some measure of mechanical properties relevant to the loading conditions cells are exposed to. Measurements of the stiffness of tissues have been carried out using atomic force microscopy (AFM) to examine the mechanical properties at small length scales (Crichton et al., 2013; Last et al., 2009). AFM allows for mechanical characterisation at the nanoscale and can provide high spatial resolution of the surface properties of the material. Many biological materials have been examined by AFM in order to determine the mechanical properties (Hang and Barber, 2011; Hang et al., 2014), including AFM indentation of the dermis (Grant et al., 2012). Such an approach has provided a 2D mechanical map through a thickness cross section of skin or upper dermal surface after removal of the epidermal layers. These studies have provided mechanical information by reporting the elastic modulus of the probed area. The range of reported elastic modulus varied from 0.77 kPa to 322 kPa for human dermal tissue and 6.43 kPa–11.62 MPa for mouse ear dermal tissue (Achterberg et al., 2014; Crichton et al., 2011; Grant et al., 2012; Petrie et al., 2012). Crichton et al. (2011) characterised the distribution of collagen fibres within the probed area indicating the contribution of collagen fibres within the region to the measured elastic modulus. AFM indentation of upper dermal tissue from a human donor to depths of hundreds of nanometres resulted in a measured elastic modulus of 322 kPa, and also reported higher elastic modulus values in the megapascal range potentially influenced by collagen fibres in the probed region (Grant et al., 2012). Previous studies have commented on the possible contribution of the collagen fibres to the elastic modulus of the dermis, with the elastic modulus of collagen fibres reported by indentation being 1–2 GPa (Heim et al., 2006), where potential contact of indenter with the collagen fibre bundles increasing the measured elastic modulus of the dermis (Grant et al., 2012).

The importance of the directionality of the collagen network within the dermis on resultant skin mechanics have led to extensive experimental and numerical modelling methods. Uniaxial testing of human back skin excised from different orientations of the Langer's lines showed significant differences in the measured elastic modulus, with samples tested parallel to the Langer's lines showing a higher elastic modulus than samples tested perpendicular to the lines (Ní Annaidh et al., 2012b; Ottenio et al., 2014). Tensile testing was also modelled using finite element methods to produce a numerical model to predict the anisotropic behaviour of human and murine skin (Groves et al., 2013; Ní Annaidh et al., 2012a). Numerical models showed significant contribution of the alignment of the collagen fibres within the dermis to the mechanical behaviour of skin. These experimental and numerical results consider macroscopic scale testing and applying a large strain to skin with the assumption that the collagen fibres will be exposed to the loading conditions. However, no studies currently report on the effect of the direction of the applied load on the mechanical properties of the dermis at the nanoscale, where directionality could have an effect on the microenvironment experienced by cells within the dermis. This work aims to explore the 3D mechanics of the dermis by utilising AFM indentation in conjunction with a range of tissue preparation methods to establish a comprehensive understanding of the elastic behaviour of the dermis through relationships between directional nanoscale mechanical measurements and macroscopic mechanical properties. A particular focus is on evaluating the spatial 3D mechanical microenvironment in order to understand the response of the tissue to the directionality of the loading conditions.