Review

How stiff is skin?

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Abstract

The measurement of the mechanical properties of skin (such as stiffness, extensibility and strength) is a key step in characterisation of both dermal ageing and disease mechanisms and in the assessment of tissue engineered skin replacements. However, the biomechanical terminology and plethora of mathematical analysis approaches can be daunting to those outside the field. As a consequence, mechanical studies are often inaccessible to a significant proportion of the intended audience. Furthermore, devices for the measurement of skin function *in vivo* generate relative values rather than formal mechanical measures, therefore limiting the ability to compare studies. In this viewpoint essay we discuss key biomechanical concepts and the influence of technical and biological factors (including the nature of the testing apparatus, length scale, donor age and anatomical site) on measured mechanical properties such as stiffness. Having discussed the current state-of-the-art in macro-mechanical and micro-mechanical measuring techniques and in mathematical and computational modelling methods we then make suggestions as to how these approaches, in combination with 3D X-ray imaging and mechanics methods may be adopted into a single strategy to characterise the mechanical behaviour of skin.

1. Introduction

The mechanical properties of skin mediate its ability to resist external forces whilst facilitating normal movement and maintaining a youthful appearance. In order to understand and ultimately prevent or reverse the mechanisms which drive aberrant mechanical remodelling (such as age-related frailty, pathological stiffening and the induction of wrinkles), it is vital to develop better approaches to characterising the mechanical behaviour of skin. In this viewpoint article we introduce the key biomechanical concepts, discuss the capabilities and limitations of current mechanical characterisation approaches and suggest potential future strategies.

2. Current state of knowledge

2.1 Biomechanics and skin

Unfortunately the terms used in materials science and biomechanics disciplines are often unfamiliar to scientists and clinicians outside the field of biomechanics, but an appreciation of this terminology is important to understanding mechanical investigations. The structure of skin is complex and as a consequence it behaves **anisotropically** when deformed. In contrast to isotropic materials (such as a rubber ball), anisotropic materials behave differently depending on the direction in which a force is applied. The area over which the force is applied is also important (contrast the effects of a needle and a finger applied with the same force to the surface of skin) and the force divided by area is known as the **stress.** This stress in turn may induce a change in the shape of material which is expressed as the **strain** (calculated as the change in dimension divided by original dimension) [1] (Fig. 1A). The goal of many mechanical investigations is to characterise the resultant **stress-strain curve**. Typically for biological materials, the initial regions of these curves are J-shaped (i.e. a low stress induces a large strain). From such curves it is possible to measure multiple functional attributes (formal material properties) including **stiffness** (the elastic or Young's modulus), **strength** (stress at fracture) and **extensibility** (strain at failure) (Fig. 1B). Because skin is mechanically anisotropic it is often essential to characterise its properties (i.e. stress-strain curve) along more than one direction [2, 3].

For accessible introductions to the general mechanics of materials and properties of biological materials the reader is referred to the excellent works of Professors Gordon and Gosline respectively

[4, 5]. Finally, and in contrast to many inorganic materials (such as steel and rubber), biological materials such as skin behave **viscoelastically**, so that the initial rapid deformation is followed by a slower subsequent deformation [6]. In order therefore to fully characterise the mechanical properties of skin it will be necessary to take into account anisotropy and viscoelasticity whilst measuring local stress and strain at multiple length scales and preferably in three dimensions (3D). A further challenge is to reconcile *in vitro* mechanical measurements with the non-invasive determination of skin biophysical properties *in vivo* in order to exploit biophysical knowledge of skin in the clinical environment [7].

Currently there is no single answer to the question "how stiff (or indeed strong or extensible) is skin". Measurements of skin stiffness vary by 3 orders of magnitude (from 1MPa to 1GPa) depending on the methods used, anatomical site and donor age, the length scale tested and the degree of hydration (Table 1) [3, 8-10]. Additionally collagen-rich human dermis (measured by macro-mechanical uniaxial testing) is apparently substantially stiffer than comparable collagen-rich tissues characterised by AFM indentation [11, 12]. Finally, in the case of non-invasive macro-mechanical methods, the measured parameters extracted from skin force displacement curves are relative or qualitative values and not readily relatable to formal mechanical material properties (eg., elastic modulus and resilience).

2.2 Macro-mechanics

The assessment of cutaneous mechanical properties has been used extensively in both in clinical and research settings to determine the severity and progress of disease, wound repair, extrinsic and intrinsic ageing and the success of therapeutic strategies. In contrast to many internal organs the macro-(sometimes termed gross)-mechanical properties of skin can be measured rapidly and non-invasively by devices which employ suction (cups or cutometer), indentation (ballistometer), and torsion (dermal torque meter). Other approaches to measure dermal properties include reviscometry, tonometry, adherence, elastometry and quantitative electrical characterisation methods such as dielectric and bioelectrical impedance [13-15]. Perhaps the most widely employed of these devices is the Cutometer® (Courage and Khazaka Electronic GmbH, Köln, Germany). On application of a negative pressure, the skin surface is deformed into the chamber via an aperture of known diameter. The degree of deformation is measured using a laser. The Cutometer® is now widely used in both

dermatology and aesthetic research settings and may be adapted to partially distinguish between epidermal and dermal mechanical contributions [16, 17].

The utility of these devices is clear: age-dependent mechanical remodelling for example can be quantified by torsion (elasticity and stretchability: [14]) and suction (tonicity, extensibility, elasticity and fatigability [18, 19] approaches. Additionally when suction and indentation methods are employed in the same study, there is often reasonable agreement between the methods as to the mechanical trends When used in combination with subsequent histological analysis (of the same skin site) instruments such as the Cutometer® and ballistometer can provide complimentary information on the influence of skin composition and ethnicity on mechanical behaviour [15, 20]. Using this approach we have shown that the Cutometer® alone can readily distinguish between the macro-mechanical behaviours of photo-protected buttock skin from young (18-30 year old) White Northern European and Black African volunteers. However, both instruments detected mechanical differences in the photo-exposed forearm skin of the same volunteers and the ballisotmeter clearly identified increased damping (of the bouncing probe) in White Northern European forearm skin. Other groups have also compared the ability of these two instruments to distinguish between the mechanical properties of differing skin sites such as the forehead, cheek and volar forearm [15]. Once again the differing measurements provided by the instruments yield complimentary mechanical insights (Figure 2).

Whilst such empirical observations are useful , the inability to extract formal mechanical properties from the output of these measuring devices and the difficulty in relating the contribution of complex and variable skin structures to the averaged mechanical behaviour of a poorly defined skin volume has hampered progress in understanding skin mechanics. A key goal should be to move beyond qualitative terms to describe the mechanical properties of skin (wrinkling, elasticity and pliability) to the extraction of formal mechanical parameters (such as elastic modulus and resilience) from non-invasive macro-mechanical testing methods in order to facilitate direct comparisons between studies. However, in order to establish the relative contribution of discrete skin regions (*stratum corneum*, epidermis, papillary and reticular dermis and hypodermis) to the mechanical behaviour of the organ it will be necessary to make localised measurements at the length scale of these structures and individual components (cells and fibres).

2.3 Micro-mechanics

The mechanical properties of biological tissues are length-scale dependent. In general measured stiffness increases as length scale decreases so that organs and tissues are less stiff than their component molecules [21]. In addition, cells sense the stiffness of their local (μm and nm) environment and the importance of local stiffness in mediating cell phenotype is clear from studies in cancer biology where stiffness influences cancer progression [22].

A number of methods have been used to characterise biological tissues, and specifically skin at μm length scales. Nanoindentation uses spherical and conical probes [23] to indent the surface of biological samples and measure the mechanical properties of discrete tissue areas. Using a spherical indenter with a radius of 500 μm, Geerligs and colleagues measured the stiffness of both the epidermis and *stratum corneum* as between 1 and 2 MPa [8], this contrasts values of 5-1000MPa when measured using fracture mechanics under varying hydration conditions [3]. The Young's modulus of human dermis, as determined using macro-mechanical tensile testing of ex vivo tissue (following dissection of the epidermis and hypodermis), ranges from 83MPa [9] to 70-160MPa (depending on orientation) [10].

To probe the mechanical properties of even more compartmentalised areas of tissue, smaller indenter sizes are required. AFM indentation uses probes with diameters of <10 μm resulting in high spatial resolution mechanical measurements. Using hydrated cryosections and indentation depths of less than 5% of the section depth it is possible collect many hundreds of force-extension curves and to relate local mechanical stiffness to tissue structure. Using this approach we have recently shown that high mammographic density (which is a key risk factor for breast cancer) is associated with the presence of mechanically stiff, large diameter collagen fibril bundles in the periductal region of the breast [11]. In order to understand the contribution of architecturally complex skin components to the macro-mechanical behaviour of skin it will be necessary to computationally model the behaviour of the composite structure.

2.4 Modelling

The complex and non-linear interplay of skin structures and physical phenomena present us with tremendous challenges at an experimental and modelling level. [7, 24-26] also highlighted the need for tight integration of modelling, instrumentation and imaging. As of 2017, throughout many industries, advanced physics-based numerical simulations, typically relying on finite element [27] and/or meta-modelling techniques [28], are used in the rational design of products intended to interact with the skin (e.g. razors and skin stimulation devices). At a more fundamental level, and as hypothesis-driven research tools, mathematical and computational models of the skin are developed to shed light on the biophysical complexity of skin physiology [25, 29-36] and to unravel particular mechanobiological aspects associated with diseases and the ageing process. Mathematical and computational models offer the ability to deconstruct complexity by varying one parameter at once, and therefore allow the simulation of many “what if” scenarios to gain a mechanistic and quantitative understanding in such a complex biophysical system. They can also assist in the design of physical experiments by optimising equipment use (for example, by simulating expected mechanical loads and response).

3. Future developments

3.1 Material properties, macro-mechanical testing and modelling

Mathematical and computational models of the skin have now reached a high level of sophistication, and can capture a wide range of relevant biophysical processes from elasticity and viscoelasticity, through growth and remodelling to damage, ageing and thermoelasticity [25]. The major hurdle limiting the applicability and wide-spread adoption of these models is the scarcity and *relevance* of captured experimental data. There are several fundamental questions associated with this observation [25] that should be points of focus for future research efforts: i) use of modelling approaches to extract formal mechanical properties from in vivo testing data, ii) characterisation and reconciliation of differences between measurements made in the *in vivo* and *ex vivo* biophysical environments, iii) statistical models to account for intra- and inter-individual variability, iv) the sensitivity of skin biophysical properties to external environmental conditions, v) the exploitation of full-field measurement macroscopic characterisation techniques which can provide rich data [37, 38].

With regards to integrate methodologies it remains unclear what the best strategies and methodologies might be to integrate multi-modality and multiscale imaging, characterisation and modelling techniques. Data mining and machine learning techniques [39] are likely to play an increasing role in the future to make sense of large and complex heterogeneous data sets, whether they originate from physical or computer experiments, expert knowledge (e.g. anatomists, clinicians, nurses, vets) or from any other means (e.g. patient's observations, shamanic knowledge). Multi-variate and multiscale data-based and/or physics-based statistical models of biological tissues built from the results of machine learning (i.e. meta-models) [28, 40] could then replace computationally expensive physics-based finite element models, and be used to predict a variety of scenarios and outcomes. For example, one could ask: What should be the optimal locations/lengths/types of surgical skin sutures in complex reconstructive surgery procedures such as face transplant [41] and what might be the effect of ethnicity and age? The answers to these questions will depend on many factors and hence are likely to be statistical distributions rather than single deterministic values.

3.2 Micro-mechanics and correlative microscopy

Correlative microscopy employs complimentary techniques to characterise differing structural, compositional and mechanical characteristics of the same tissue [42]. Modern AFMs may be mounted on optical microscopes with control software which tightly integrates optical (μm scale) and AFM (nm scale topography and nm/μm scale mechanics). These approaches have the potential to directly relate microscale biomechanical measurements with local tissue micro-architecture. Recent work in rat tail skin for example has shown that AFM indentation is able to detect significant differences in the elastic moduli of the epidermis (MPa range) and the more compliant (less stiff) dermis (kPa range) [43]. Current work in our laboratory is using an analogous technique but applied to human skin, assessing both photoexposed and photoprotected sites in young and old volunteers to investigate the effects of chronological age and sun exposure on dermal micromechanical properties.

3.3 3D X-ray imaging and digital volume correlation

In common with other tissue and organs, skin is organised in three dimensions (3D) and yet the mechanical measurements either integrate the responses of whole tissue volumes (regardless of structural differences) or map the mechanical stiffness in two dimensions (AFM of cryo-sections). We have recently shown that phase contrast μCT using laboratory X-ray sources can successfully resolve key anatomical structures in chemically fixed, yet unstained tissues and organs (rat aorta and human skin) [44]. Recently we have also shown (unpublished data) that: i) synchrotron phase contrast μCT can rapidly (within 10 minutes) resolve μm-scale structures in whole native rat intervertebral disc (like the dermis, this tissue is rich in fibrillar collagens and other ECM proteins). The technique of digital volume correlation combined with sequential deformation of skin has the potential to map structure in response to strain in 3D at μm scale resolutions [45, 46].

4. Conclusions

Skin is our prime interface with the external world, and the medium through which we have many daily interactions be they social, neural, biological, mechanical, thermal, chemical or electromagnetic. As a consequence the skin is the subject of considerable research effort in cosmetics and pharmaceutics, personal care products, sports equipment and consumer electronics [25]. Developing improved strategies to characterise the mechanical properties of skin is an important step in the quest to maintain and restore optimal skin function. These strategies will of necessity be multidisciplinary (Figure 3) and will draw on both well-established techniques (such as non-invasive mechanical testing and histology) alongside newly developed mathematical and imaging approaches. The ultimate goal will be to develop a high-resolution 3D finite element model of skin mechanical behaviour.

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Figure legends

Figure 1. Mechanical terminology and stress-strain curves. (A) The measurement of material stiffness is a key step in the characterisation of tissue mechanics. For a material with an initial length l_o and cross sectional area A, the force F induces a change in dimension e (in this case an extension). From these parameters the stress and strain can be calculated and in turn (if stress is proportional to strain), the elastic modulus. High modulus values indicate a stiff material. (B) Idealised force-displacement curve for a biological material stressed to failure. Commonly, biological materials will undergo an initial large extension (tow region), followed by a potentially reversible elastic extension (in which stress is proportional to strain), an irreversible plastic deformation and finally failure (post-yield and fracture) [47].

Figure 2. Comparative macro-mechanical behaviour of forehead, cheek and volar forearm skin measured by Balisotmeter and Cutometer®. (A and B) Balistometer measurements of coefficient of restitution (CoR) and area under the curve differed for the three anatomical sites. (C and D) The Cutometer® parameter R2 (gross elasticity) clearly distinguished between the three sites but R7 (firmness) was similar between forehead and cheek. The figure was adapted with permission from Woo et al. 2014 and the reader is referred to the orginal paper [15] for a full discussion of the data.

Figure 3. A multi-disciplinary approach to characterising skin mechanics. In order to understand and model skin mechanical properties it will be necessary to draw on techniques from disparate disciplines which operate both *in vivo* and *ex vivo* and at macroscopic and microscopic length scales. Macroscopically skin structure (such as epidermal thickness) varies with anatomical site and can be assessed by *in vivo* imaging techniques (ultrasound [Illustrated], optical coherence tomography and confocal microscopy). The macro-mechanical behaviour of skin can be assessed in vivo by indentation (Ballistometer curve illustrated), suction or torsion but modelling approaches will be required to extract formal mechanical parameters. These in vivo approaches may be complemented by ex vivo tensile testing. The micro-structure and composition of skin can be assessed by conventional histology and immunohistochemistry in 2D and in 3D by microCT. Measurement of skin micro-mechanical properties currently requires the use of *ex vivo* techniques such as atomic force microscopy indentation. In the future it may be possible to apply digital volume correlation approaches

in combination with microCT imaging to map 3D strain in skin biopsies. The 3D microCT images of skin are reproduced with permission from the publishers of Newton et al. 2016 [48].

Table 1. Estimating tissue stiffness.

Study	**Tissue**	**Technique**	**Stiffness (MPa)**
Geerligs et al. [6]	Human *stratum corneum*	*In vivo* indentation (500μm spherical probe)	1-2
Wu et al. [7]	Human *stratum corneum*	*Ex vivo* fracture mechanics (varying hydration)	5-1000
Annaidh et al. [8]	Human dermis	Uniaxial tensile testing	83±34
Ottenio et al. [9]	Human dermis	Uniaxial tensile testing	70-160
McConnell et al. [10]	Human breast	AFM indentation (1μm radius probe)	0.2-0.6
McConnell et al. [10]	Rat tendon	AFM indentation (1μm radius probe)	0.86±0.9
Desai et al. [11]	Murine liver	AFM indentation (1μm diameter probe)	0.0015-0.060

The apparent stiffness of human skin compartments is dependent on the technique used and the length scale over which the measurements are made. Tissues such as the dermis, breast and fibrotic liver are all rich in fibrillar collagen and yet estimates of stiffness range from 0.06-0.86MPa (6-86kPa).

Conflict of interests

The authors have declared no conflicting interests.

Author contributions

HKG, JCM, GL and MJS wrote the manuscript. All authors have read and approced the final manuscript.

References

1. Sherratt M J. Tissue elasticity and the ageing elastic fibre. Age 2009: **31**: 305-325.
2. Kvistedal Y A, Nielsen P M F. Estimating material parameters of human skin in vivo. Biomech Model Mechanobiol 2009: **8**: 1-8.
3. Wu K S, van Osdol W W, Dauskardt R H. Mechanical properties of human stratum corneum: Effects of temperature, hydration, and chemical treatment. Biomaterials 2006: **27**: 785-795.
4. Gordon J E. Structures: Or Why Things Don't Fall Down: Da Capo Press: 2003.
5. Gosline J, Lillie M, Carrington E, et al. Elastic Proteins: Biological Roles and Mechanical Properties. Philosophical Transactions: Biological Sciences 2002: **357**: 121-132.
6. Akiyama Y, Yamamoto Y, Doi Y, et al. Analysis of viscoelasticity of human skin for prevention of pressure ulcers. Journal of Mechanics in Medicine and Biology 2008: **8**: 33-43.
7. Jor J W Y, Parker M D, Taberner A J, et al. Computational and experimental characterization of skin mechanics: identifying current challenges and future directions. Wiley Interdisciplinary Reviews: Systems Biology and Medicine 2013: **5**: 539-556.
8. Geerligs M, van Breemen L, Peters G, et al. In vitro indentation to determine the mechanical properties of epidermis. Journal of Biomechanics 2011: **44**: 1176-1181.
9. Annaidh A N, Bruyere K, Destrade M, et al. Characterization of the anisotropic mechanical properties of excised human skin. Journal of the Mechanical Behavior of Biomedical Materials 2012: **5**: 139-148.
10. Ottenio M, Tran D, Annaidh A N, et al. Strain rate and anisotropy effects on the tensile failure characteristics of human skin. Journal of the Mechanical Behavior of Biomedical Materials 2015: **41**: 241-250.
11. McConnell J C, O'Connell O V, Brennan K, et al. Increased peri-ductal collagen micro-organization may contribute to raised mammographic density. Breast Cancer Res 2016: **18**.
12. Desai S S, Tung J C, Zhou V X, et al. Physiological Ranges of Matrix Rigidity Modulate Primary Mouse Hepatocyte Function in Part Through Hepatocyte Nuclear Factor 4 Alpha. Hepatology 2016: **64**: 261-275.
13. Lee K C, Dretzke J, Grover L, et al. A systematic review of objective burn scar measurements. Burns and Trauma 2016: **4**.
14. Agache P G, Monneur C, Leveque J L, et al. Mechanical-properties and youngs modulus of human-skin in vivo. Arch Dermatol Res 1980: **269**: 221-232.

15. Woo M S, Moon K J, Jung H Y, et al. Comparison of skin elasticity test results from the Ballistometer(®) and Cutometer(®). Skin Res Technol 2014: **20**: 422-428.
16. Diridollou S, Berson M, Vabre V, et al. An in vivo method for measuring the mechanical properties of the skin using ultrasound. Ultrasound Med Biol 1998: **24**: 215-224.
17. Smalls L K, Wickett R R, Visscher M O. Effect of dermal thickness, tissue composition, and body site on skin biomechanical properties. Skin Res Technol 2006: **12**: 43-49.
18. Couturaud V, Coutable J, Khaiat A. Skin biomechanical properties: in vivo evaluation of influence of age and body site by a non-invasive method. Skin research and technology : official journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI) 1995: **1**: 68-73.
19. Ryu H S, Joo Y H, Kim S O, et al. Influence of age and regional differences on skin elasticity as measured by the Cutometer (R). Skin Res Technol 2008: **14**: 354-358.
20. Langton A K, Graham H K, McConnell J C, et al. Organisation of the dermal matrix impacts the biomechanical properties of skin. Br J Dermatol 2017.
21. Akhtar R, Sherratt M J, Cruickshank J K, et al. Characterizing the elastic properties of tissues. Mater Today 2011: **14**: 96-105.
22. Sherratt M J, McConnell J C, Streuli C H. Raised mammographic density: causative mechanisms and biological consequences. Breast Cancer Res 2016: **18**.
23. Li J, Thostenson E T, Chou T W, et al. An investigation of thin-film coating/substrate systems by nanoindentation. J Eng Mater Technol-Trans ASME 1998: **120**: 154-162.
24. Li W. Modelling methods for In Vitro biomechanical properties of the skin: A review. Biomedical Engineering Letters 2015: **5**: 241-250.
25. Limbert G. Mathematical and computational modelling of skin biophysics-A review. Transactions of the Royal Society A-Mathematical Physical and Engineering Sciences 2017.
26. Silver F H, Siperko L M, Seehra G P. Mechanobiology of force transduction in dermal tissue. Skin Res Technol 2003: **9**: 3-23.
27. Belytschko T, Liu W K, Moran B. Nonlinear Finite Elements for Continua and Structures: Oxford/Wiley: 2000.
28. Forrester A I J. Black-box calibration for complex-system simulation. Philos Trans R Soc A-Math Phys Eng Sci 2010: **368**: 3567-3579.
29. Flynn C, Taberner A J, Nielsen P M F, et al. Simulating the three-dimensional deformation of in vivo facial skin. Journal of the Mechanical Behavior of Biomedical Materials 2013: **28**: 484-494.
30. Flynn C, McCormack B A O. Simulating the wrinkling and aging of skin with a multi-layer finite element model. Journal of Biomechanics 2010: **43**: 442-448.
31. Gefen A. How do microclimate factors affect the risk for superficial pressure ulcers: A mathematical modeling study. J Viab 2011: **20**: 81-88.
32. Leyva-Mendivil M F, Lengiewicz J, Page A, et al. Skin Microstructure is a Key Contributor to Its Friction Behaviour. Tribology Letters 2017: **65**.
33. Leyva-Mendivil M F, Lengiewicz J, Page A, et al. Implications of multi-asperity contact for shear stress distribution in the viable epidermis – An image-based finite element study. Biotribology.
34. Leyva-Mendivil M F, Page A, Bressloff N W, et al. A mechanistic insight into the mechanical role of the stratum corneum during stretching and compression of the skin. Journal of the Mechanical Behavior of Biomedical Materials 2015: **49**: 197-219.
35. Zoellner A M, Tepole A B, Kuhl E. On the biomechanics and mechanobiology of growing skin. Journal of Theoretical Biology 2012: **297**: 166-175.
36. Zoellner A M, Holland M A, Honda K S, et al. Growth on demand: Reviewing the mechanobiology of stretched skin. Journal of the Mechanical Behavior of Biomedical Materials 2013: **28**: 495-509.
37. Avril S, Bonnet M, Bretelle A-S, et al. Overview of identification methods of mechanical parameters based on full-field measurements. Experimental Mechanics 2008: **48**: 381-402.
38. Pierron F G, Grediac M. The Virtual Fields Method: Springer: 2012.
39. Barber D. Bayesian reasoning and machine learning: Cambridge University Press: 2012.
40. Forrester A, Sobester A, Keane A. Engineering design via surrogate modelling: a practical guide John Wiley & Sons: 2008.
41. Lantieri L, Grimbert P, Ortonne N, et al. Face transplant: long-term follow-up and results of a prospective open study. Lancet 2016: **388**: 1398-1407.
42. Burnett T L, McDonald S A, Gholinia A, et al. Correlative Tomography. Sci Rep 2014: **4**.
43. Kao A P, Connelly J T, Barber A H. 3D nanomechanical evaluations of dermal structures in skin. Journal of the Mechanical Behavior of Biomedical Materials 2016: **57**: 14-23.

44. Walton L A, Bradley R S, Withers P J, et al. Morphological characterisation of unstained and intact tissue micro-architecture by X-ray computed micro- and nano-tomography. Sci Rep 2015: **5**: 14.
45. Bay B K. Methods and applications of digital volume correlation. J Strain Anal Eng Des 2008: **43**: 745-760.
46. Midgett D E, Pease M E, Quigley H A, et al. Regional Variations in the Mechanical Strains of the Human Optic Nerve Head. In: Korach C S, Tekalur S A, Zavattieri P, eds. Mechanics of Biological Systems and Materials, Vol 6. New York: Springer, 2017: 119-127.
47. Beadle N, Burnett T L, Hoyland J A, et al. A novel ex vivo model of compressive immature rib fractures at pathophysiological rates of loading. Journal of Mechanical Behavour of Biomedical Materials 2015: **51**: 154-162.
48. Newton V L, Bradley R S, Seroul P, et al. Novel approaches to characterize age-related remodelling of the dermal-epidermal junction in 2D, 3D and in vivo. Skin Res Technol 2017: **23**: 131-148.

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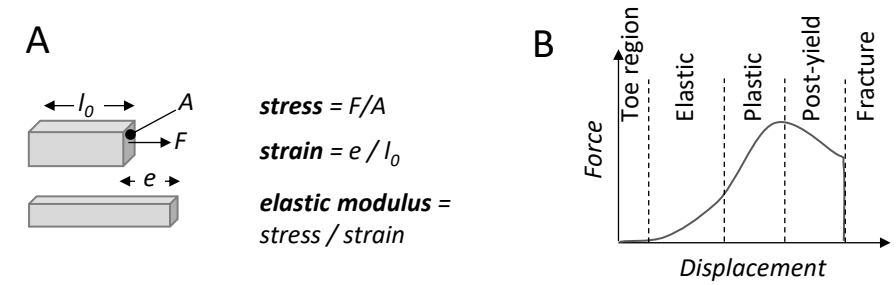
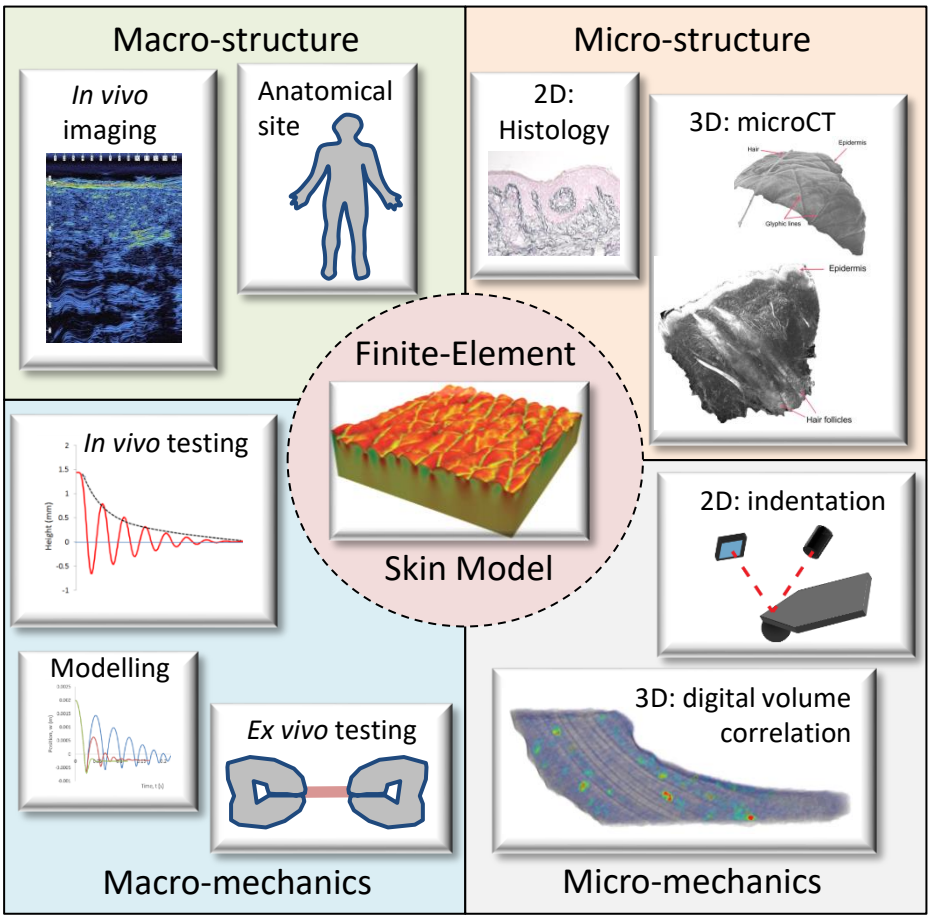


Graham et al. Figure 2



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