**Organisation of the dermal matrix impacts the biomechanical properties of skin**

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What’s already known about this topic?

- Human skin offers protection against external environment via the reversible deformation of its structure; the stratum corneum and components of the dermal extracellular matrix imbue skin with these important biomechanical properties.
- Skin biomechanical properties are amenable to dynamic testing in vivo, using the non-invasive methods of cutometry and ballistometry.

What does this study add?

- This study combines in vivo testing of white Caucasian and black African skin with the histological assessment of skin biopsies in order to interrogate the relationship between underlying skin architecture and biomechanical function;
- Disruption to skin architecture, particularly loss of oxytalan fibres and effacement of rete ridges correlates with perturbation of biomechanical function.
Summary

Background: Human skin has the crucial roles of maintaining homeostasis and protecting against external environmental insults such as exposure to ultraviolet radiation. It also offers protection against mechanical trauma due to the reversible deformation of its structure. Skin biomechanical properties are amenable to dynamic testing using a wide range of non-invasive devices.

Objectives: To characterise the biomechanical properties of young, white Caucasian and black African/African-Caribbean skin from different anatomical sites; and to relate underlying skin architecture to biomechanical function.

Methods: Using cutometry and ballistometry, the biomechanical properties of buttock and dorsal forearm skin were determined in black African/African-Caribbean (n=18) and white Caucasian (n=20) individuals aged 18-30 years. Skin biopsies were obtained from a subset of the volunteers (black African/African-Caribbean: n=5; white Caucasian: n=6) and processed for histological and immunohistochemical detection of the major elastic fibre components and fibrillar collagens.

Results: We have determined that healthy skin from young African and Caucasian individuals has similar biomechanical properties (F3) in that skin is resilient (capable of returning to its original position following deformation; R1), exhibits minimal fatigue (R4) and is highly elastic (R2, R5 and R7). At the histological level, skin with these biomechanical properties is imbued with strong interdigitation of the rete ridges at the dermal-epidermal junction (DEJ) and candelabra-like arrays of elastic fibres throughout the papillary dermis. Dramatic disruption to this highly organised arrangement of elastic fibres, effacement of the rete ridges and alterations to the alignment of the fibrillar collagens is apparent in white Caucasian forearm and coincides with a marked decline in biomechanical function.

Conclusions: Maintenance of skin architecture – both epidermal morphology and elastic fibre arrangement – is essential for optimal skin biomechanical properties. Disruption to underlying skin architecture, as observed in young white Caucasian forearm, compromises biomechanical function.


INTRODUCTION

Human skin has the crucial roles of maintaining homeostasis and protecting against environmental insults, such as exposure to ultraviolet radiation (UVR). Skin also offers protection against external mechanical trauma, via the reversible deformation of its structure\(^1\); the stratum corneum and components of the dermal extracellular matrix (ECM) imbue skin with these important biomechanical properties. The fibrillar collagens (types I and III) have high tensile strength and form the main structural support for the skin, whilst components of the elastic fibre network, consisting of elastin, fibrillin-rich microfibrils and microfibril-associated proteins, are the primary effectors of elastic recoil following mechanical deformation. The collagen network and elastic fibre components work in concert to resist strain and enable the skin to return to its original shape\(^2\).

The biomechanical properties of skin are amenable to dynamic testing via a wide range of non-invasive techniques using the experimental methods of suction, torsion, traction or indentation\(^3,4\). The most common technique for evaluation of skin elasticity is the suction method using the commercially available Cutometer\(^5\) (Courage + Khazaka Electronic, Koln, Germany). This device uses a probe to apply a negative pressure to the surface of the skin for a defined time period. The degree of skin deformation into the probe is recorded before the skin is allowed to recover on removal of the suction. The pressure-release cycle is repeated and the ability of the skin to both resist the negative pressure and to return to its original shape can be determined. An additional, but infrequently used, dynamic test for skin elasticity is the ballistometer. In contrast to cutometry, ballistometry records and analyses the rebound pattern of a small hammer striking the skin’s surface\(^6\). An appreciation of skin biomechanics is important for our understanding of its normal functions. Numerous comparative studies have been undertaken to characterise these properties in both health\(^7,8\), disease\(^9\)\(^\text{-}\)\(^11\) and ageing\(^12,13\). However, in order to understand the consequences of both ageing and disease, it is essential to first appreciate the biomechanical properties of young, healthy skin.

Historically, the majority of dermatology research has focussed on defining the properties of white Caucasian skin. However, optimal skin health is desirable for all individuals; hence a subspecialty of dermatology research has now emerged which involves understanding skin of colour. Skin of colour is a succinct and simple term to describe individuals with Fitzpatrick skin types IV–VI; this constitutes a wide range of racial and ethnic groups including Africans, African-Americans, African-Caribbeans, Hispanics, South-east Asians, Chinese and Japanese\(^14\). Recent comparative studies of young, photoprotected skin from black African and white Caucasian cohorts have attempted to understand
at the histological level, what is considered to be the defining characteristics of “healthy” skin in these diverse cohorts. These studies have identified differences in epidermal thickness and morphology, DEJ interdigitation and dermal ECM composition\textsuperscript{15-17}. Whilst these comparisons help to define histology of healthy skin, they provide no functional information on how it behaves biomechanically. Previous attempts to characterise skin biomechanics in white Caucasian and black African cohorts have been largely inconclusive due to contradictory findings between different studies\textsuperscript{18,19}. This lack of consensus has mainly arisen due to differences in the ages of participants, the anatomical sites tested and the disparate methods available to characterise skin biomechanics.

With this in mind, the aims of the current study were: 1) to characterise, using cutometry and ballistometry, the biomechanical properties of young, healthy skin from cohorts of black African/African-Caribbean and white Caucasian individuals; and ii) to relate these biomechanical properties to the underlying architecture of the epidermis DEJ and the organisation of the dermal extracellular matrix.

**MATERIALS AND METHODS**

**Participants and study design**

Young, healthy, white Caucasian (Caucasian; Fitzpatrick skin phototypes I-II; mean age 24.5 years; SD 3.3 years; males n = 6; females n = 14) and black African/African-Caribbean (African; Fitzpatrick skin phototype VI; mean age 23.1 years; SD 2.9 years; males n = 2; females n =16) volunteers were recruited to the study. Local ethical approval was obtained from the University of Manchester Research Ethics Committee (ref. 14161). Written informed consent was obtained from the participants and the study adhered to Declaration of Helsinki principles. Basic demographic information was collected and participants were asked to self-declare their ethnicity. Participants were provided with a standard soap to use for bathing and were requested not to apply any creams or lotions for at least 1 week prior to testing. Test sites were selected on the buttock (at the midpoint between the intergluteal cleft and the lateral border) and forearm (at the midpoint between the dorsal, proximal wrist crease and olecranon process). All buttock measurements were performed with participants in a prone position with their head positioned either in neutral or to the side, depending on the participants’ comfort. All forearm measurements were performed on the dorsal aspect, with the participants’ shoulder abducted to 45 degrees and the elbow flexed to 90 degrees and fully supported on a table top. All sites were washed with alcohol-free sterile wipes and when necessary, forearm hair was trimmed with scissors before the measurements were taken. A template, 3 cm in diameter was marked on the skin at the selected sites to ensure each instrument
was placed at the same anatomical location. Participants were left to acclimatise in a room without direct sunlight (temperature 24-25°C; relative humidity 43-45%) for 30 minutes prior to testing.

**Measurement of skin biomechanical properties**

**Ballistometer**

The ballistometer (Diastron Ltd., Andover, UK) was applied to three adjacent but non-overlapping areas at each anatomical test site. To ensure consistency of the data, a single investigator performed all ballistometer measurements (HKG). The ballistometer records four parameters: indentation; alpha; coefficient of restitution (CoR); and area (Fig 1a). Indentation is the peak penetration depth of the probe tip beneath the skin surface level (in mm), alpha is the rate of energy damping, CoR is the bounce height relative to the start height and area describes the area between the bounce profile and the skin zero datum (Table 1).

**Cutometer®**

The Cutometer® MPA580 (Courage + Khazaka Electronic) with a 4mm aperture probe (mode 1; 3 second suction followed by 3 second relaxation period, for a total of 10 cycles using a negative pressure of 450 mbar) was applied to three adjacent but non-overlapping areas of the test site, with removal of the probe between each area to ensure a higher reliability of the data\(^{20,21}\). To ensure consistency of the data, a single investigator performed all Cutometer® measurements (AKL). In addition, a 6mm diameter probe was used at the same test sites in a subset of participants (Caucasian, n = 8; African, n = 6). The Cutometer® works by creating negative pressure in the device and the skin is drawn into the aperture of the probe and after a defined time, is released. Inside the probe, skin deformation is determined by a non-contact optical measuring system. The resistance of the skin to the negative pressure and its ability to return to its original position are displayed as curves in real time during the measurement (Fig 1b). Application of suction to the skin induces an immediate deformation (Ue); followed by a slower viscoelastic deformation (Uv) until finally the total skin deformation (Uf) is reached (Fig 1c). Removing the suction induces an immediate retraction (Ur) followed by total skin recovery (final retraction, Ua). The ratio of the recovery to initial deformation quantifies the ability of the skin to elastically recoil. The Cutometer® software is able to determine additional properties of the skin by the calculation of a number of supplementary parameters. The most biologically relevant are defined as follows: R0, height of the first maximal skin deformation; R1, the ability to return to the original state (Uf-Ua); R2, gross elasticity of the skin, including creep and creep recovery (Ua/Uf); R5, the net elasticity, excluding creep and creep recovery (Ur/Ue); R6, the ratio between viscoelastic and elastic deformation (Uv/Ue) and R7, the
ratio of elastic recovery to the total deformation (Ur/Uf). Additional important parameters, such as skin fatigue (R4: as measured by the difference between the last minimal value and the first minimum value), hysteresis (R9: as measured by the difference between the last and the first maximal skin deformation) and the area covered by the skin deformation curve (F3) can also be assessed if the time-strain cycle is repeated multiple times (Table 1). Parameters of skin mechanical properties were calculated from the first completed cycle (unless otherwise stated) and represent the mean values from the three adjacent areas. R-values and the area parameter F3 were analysed using the instrument software.

**Biopsy procurement and sample preparation**

Once all non-invasive measurements had been completed, 6mm punch biopsies were obtained from the two body sites in a subset of Caucasian (mean age 22.7 years; SD 3.6 years; males n = 2; females n = 4) and African (mean age 22.4 years; SD 2.0 years; females n = 5) participants. Each skin biopsy was obtained under 1% lignocaine local anaesthesia. At the time of procurement, biopsies were snap frozen in liquid nitrogen and stored at -80 °C. Biopsies were cryosectioned at 7 µm in a single run, using the same blade and the same cryostat settings.

**Antibodies and immunohistochemistry**

Mouse monoclonal antibodies were used to detect: tropoelastin (clone 10B8, dilution 1:150; Millipore (U.K.) Limited, Watford, U.K.); fibrillin-rich microfibrils (clone 11C1.3, dilution 1:1000; Neomarkers, Runcorn, U.K.); collagen I (clone 5D8, dilution 1:500; Abcam, Cambridge, U.K.) and; collagen III (clone 1E7-D7/Col3, dilution 1: 500; Abcam). Rabbit polyclonal antibodies were used to detect fibulin-2 (catalogue #HPA001934; dilution 1:500; Sigma Aldrich) and fibulin-5 (catalogue #HPA000848; dilution 1:500; Sigma Aldrich). Cryosections were fixed in ice-cold acetone or 4% paraformaldehyde (PFA) and hydrated in Tris-buffered saline (TBS). Primary antibody was applied for 1 hour at room temperature or 4°C overnight. Sections were washed in TBS prior to incubation in appropriate biotinylated secondary antibody (Vector Laboratories, Peterborough, U.K.). Antibody staining was visualized using a well-characterised immunoperoxidase reaction (VectaStain Elite ABC system; Vector Laboratories) using Vector SG® (Vector Laboratories) as the chromogen. For immunofluorescence staining, rabbit anti-mouse Alexa Fluor 488 secondary antibody was applied for 30 minutes at room temperature (Life Technologies, Paisley, U.K.).

**Picrosirius red staining for fibrillar collagens**

Cryosections were stained with picrosirius red (PSR; 0.1% sirius red F3BA in saturated aqueous picric acid at pH 2) for 1 hour followed by clearing in 0.1% acidified water. When visualised under cross-
polarised light, the resultant collagen-associated birefringence can be semi-quantitatively assessed against total tissue area. Quantitative analyses of collagen alignment (coherency) were conducted on picrosirius red stained images using a well-established methodology, OrientationJ\(^23\).

Microscopy, image analysis and statistical testing

Brightfield and fluorescence images were captured using a BX53 microscope (Olympus Industrial, Southend-on-Sea, U.K.) and image analysis was performed using ImageJ software\(^24\). DEJ convolution index was measured using the method described previously\(^17\). The length of fibrillin-rich microfibrils was determined for those fibres positioned a maximal distance of 100um of the DEJ. Statistical significance was determined using Student’s t-test or Mann-Whitney U test (IBM SPSS Statistics 20; IBM United Kingdom, Portsmouth, U.K.). Results were considered significant if \(P < 0.05\) (95% confidence level).

RESULTS

Young buttock skin has optimal biomechanical properties

Biomechanical properties of photoprotected buttock skin

The biomechanical properties of buttock skin were determined using the Cutometer\(^\circ\) with a 4mm aperture probe. Time-strain curves generated using this regime (Fig 2a) indicated that the biomechanical properties of buttock skin of young African and Caucasian individuals were similar and this was further confirmed by the finding that the overall curve shape (F3 envelope), and total deformation (R0) and immediate deformation (Ue) were not significantly different between the two cohorts. Comparison of biologically relevant parameters identified that residual deformation (R1) values were small for both cohorts and indicate that buttock skin was resilient and capable of returning to its original position following deformation. Similarly, for both cohorts, minimal skin fatigue (R4) and hysteresis (R9) were detected following the 10 repetition cycles. Three parameters that measure different aspects of skin elasticity (R2, R5, R6 and R7) were not significantly different between the cohorts. In particular, gross elasticity (R2) values for both cohorts indicated that buttock skin, regardless of ethnicity was highly elastic (African: 0.92 ± 0.01 a.u.; Caucasian: 0.92 ± 0.01 a.u.; a value of 1 is defined as being perfectly elastic) (Table 2). In a subset of volunteers, a larger 6mm aperture probe was further used to measure the biomechanics of buttock skin and data from this probe were in full agreement with the initial 4mm probe data for all parameters analysed (Table 2). Skin biomechanical testing of the buttock using the ballistometer (Fig 2b) identified no significant differences in indentation, alpha, CoR or area between the two cohorts (Table 2). The ballistometer parameter CoR is a measure of skin elasticity, where a measurement near 1 indicates
For both cohorts, the buttock values were again comparable (African: 0.82 ± 0.004 a.u.; Caucasian: 0.82 ± 0.006 a.u.) and showed high elasticity.

**Biomechanical properties of young forearm skin are not comparable between African and Caucasian cohorts**

**Biomechanical properties of photoexposed dorsal forearm skin**

Skin biomechanical properties were further determined from extensor forearm, an anatomical site often photoexposed. Cutometer® time-strain curves indicate that differences in the biomechanical properties of skin were apparent in the forearm of the two cohorts (Fig 3a). In particular, Caucasian skin could not be deformed to the same extent as African skin (R0; P < 0.05) and overall curve shape was significantly different between the cohorts (F3 envelope; P < 0.0001). A comparison of the individual parameters confirmed that in Caucasian skin, immediate deformation (Ue; P < 0.01) was significantly decreased, there was a decline in the ability of skin to return to its original position (R1; residual deformation, P < 0.0002) and increased fatigue was noted (R4; P < 0.0001). In contrast, African skin exhibited significantly higher elastic properties (R2, R5 and R7; P < 0.0001) than Caucasian skin (Table 2) at this photoexposed body site. There were however, no differences noted in viscoelastic properties (R6) or hysteresis (R9) between the cohorts. In a subset of volunteers the 6mm probe was again employed to measure the gross elasticity of forearm skin; as with the buttock, the data from this probe was in full agreement with that measured using the 4mm Cutometer® probe (Table 2). Although direct comparison of the cutometry data for buttock and forearm is not permissible for absolute parameters such as F3, R1 and R4, those that are expressed as ratios can be compared between different anatomical sites. In African participants, values for the elasticity parameters R2, R5 and R7 showed a modest decrease (-5.4%, -6.0% and -8.9%, respectively) when forearm was compared to buttock. However, for Caucasian volunteers, these same parameters showed a more dramatic reduction in elasticity (-19.0%, -29.5% and -35.1%, respectively).

Data from the ballistometer identified that there was a significant decrease in indentation, CoR and area values in the forearm skin of Caucasians as compared to Africans. A significant increase in alpha was also indicative of Caucasian skin being a more energy damping, less elastic tissue than that of African skin (Fig 3b and Table 2). Hence, in African skin, it appeared that only subtle variations in biomechanical properties existed at the two anatomical sites and were largely due to differences in the total deformation permitted by the skin, whilst parameters such as elasticity were comparable. However, in Caucasian individuals, skin’s biomechanical properties were markedly different between the two sites. Therefore it appears that optimal biomechanical properties are achieved when
following repetitive deformations, skin can continually return to its original shape without the onset of fatigue. According to this definition, photoexposed Caucasian forearm skin does not appear to behave in a mechanically optimal manner.

**Flattening of the dermal-epidermal junction and loss of dermal matrix organisation impact on the biomechanical function of dorsal forearm skin**

A subset of the volunteers who participated in the biomechanical testing study also consented to provide skin biopsies from their buttock and forearm. Biopsies were processed for immunofluorescent detection of the fibrillar collagens using antibodies raised against mature collagen I (Fig 4a-d) and III (Fig 4e-h). The overall intensity of collagen I and III immunostaining was significantly reduced in the papillary dermis of Caucasian forearm compared with African forearm (P < 0.001). The abundance of organised fibrillar collagen visualised by PSR staining did not differ significantly between Caucasian and African forearm and buttock (Fig 4i-l). When assessed for alignment of mature collagen fibres however, the fibrillar collagen was more aligned in the forearm of Caucasian subjects (0.21 ± 0.01 a.u.) as compared to African participants (0.14 ± 0.02 a.u.; P < 0.001). No significant difference in alignment of fibrillar collagens was observed between Caucasian and African buttock (Fig 4m-p).

Next, immunohistochemical analysis of the elastic fibre components tropoelastin (Fig 5a-d), fibrillin-rich microfibrils (Fig 5e-h), fibulin-2 (Fig 5i-l) and fibulin-5 (Fig 5m-p) was performed on skin biopsies from the buttock and forearm of black African and white Caucasian volunteers. In African buttock, Caucasian buttock and African forearm the elastic fibres were arranged in distinctive candelabra-like arrays, connecting the oxytalan fibres of the DEJ to the elaunin fibres of the superficial papillary dermis. However, immunohistochemical staining of Caucasian forearm skin identified depletion of fibrillin-rich microfibril architecture at the DEJ (Fig 5h), accumulation of elastotic material that was immunopositive for tropoelastin (Fig 5d) and fibulin-2 (Fig 5i) and diminished staining of fibulin-5 (Fig 5p) in the papillary dermis. Similarly, whilst African buttock, Caucasian buttock and African forearm were largely indistinguishable from one another with regard to interdigitation of the rete ridges at the DEJ (convolution index; African buttock: 2.24 ± 0.14 a.u.; Caucasian buttock: 1.72 ± 0.07 a.u.; African forearm: 1.55 ± 0.07 a.u.), Caucasian forearm had a flattened appearance and exhibited significant effacement of the rete ridges (convolution index: 1.09 ± 0.01 a.u.; P < 0.0001).

Using an X-Y-Z plot the relationship between epidermal morphology (as measured by DEJ convolution), fibrillin-rich microfibril architecture (as measured by the mean length of microfibril
bundles at the DEJ) and skin biomechanical properties (F3 parameter) were explored (Fig 6). Using this visualisation method African and Caucasian buttock and African forearm all share similar biomechanical properties and exhibit the architectural features of strong rete ridge interdigitation and arborizing fibrillin-rich microfibrils at the DEJ (mean length; African buttock: 35.90 ± 1.64µm; Caucasian buttock: 37.39 ± 0.10µm; African forearm: 31.80 ± 0.60µm). However, Caucasian forearm does not share these properties with the other groups. In Caucasian forearm, effacement of the rete ridges, combined with significant truncation of fibrillin-rich microfibril bundle length at the DEJ (mean length; Caucasian forearm: 13.65 ± 0.48um; P < 0.0001) was strongly associated with a marked decline in in vivo biomechanical function.

DISCUSSION

In this study, we established that maintenance of skin architecture – DEJ convolution, elastic fibre arrangement and basket-weave fibrillar collagen – appears to be essential for maintaining appropriate biomechanical behaviour. Using the non-invasive techniques of cutometry and ballistometry at different anatomical sites in cohorts of young Caucasian and African individuals, we show that healthy skin is resilient, exhibits minimal fatigue and is capable of returning to its original position following deformation. At the histological level, skin with these biomechanical properties exhibits strong interdigitation of the rete ridges at the DEJ and a highly organised arrangement of candelabra-like arrays of elastic fibres connecting the papillary, reticular and deep dermis. Dramatic disruption to this highly organised arrangement of elastic fibres, alignment of fibrillar collagens and effacement of the rete ridges is apparent in white Caucasian forearm and is associated with a significant decline in skin’s biomechanical function.

Although it remains difficult to assign skin biomechanical parameters to any single structural element of the dermis, the strength of this current study was in the additional collection of skin biopsies from a subset of volunteers, allowing interrogation of the relationship between the tissues’ biomechanical properties to the underlying composition of the dermal ECM. In agreement with previous studies, histological assessment of dermal ECM from buttock samples identified significant differences in the abundance of elastin, fibrillin-rich microfibrils, fibulin-5 and fibrillar collagens between cohorts; however, no difference was noted in biomechanical function for any of the parameters measured. This suggests that in terms of skin resilience, there appears no functional advantage in having an over-abundance of these proteins i.e. there is a limit to how much skin can be deformed and increasing the presence of specific ECM components has little further effect on this property.
Previous studies have identified several biomechanical parameters that change as a result of chronic sun exposure or photoageing; including a simultaneous decrease in total deformation (R0) and immediate deformation (Ue), deterioration of skin elasticity (R2, R5 and R7)\textsuperscript{25}, an increase in the prevalence of viscoelastic over elastic parts of skin deformation (R6) and the onset of skin fatigue (R4 and R9)\textsuperscript{13}. These changes are detrimental to skin biomechanical function and are thought to occur as a result of skin thickening and the accumulation of dystrophic elastin, termed “solar elastosis”\textsuperscript{26}. In the current study, many of these biomechanical parameters are also significantly altered in the dorsal forearm of young Caucasian individuals. After multiple deformations, Caucasian forearm progressively loses the ability to recover to its original position, shows larger fatigue and each subsequent curve has a lower amplitude and lower elastic retraction than observed in African forearm. Clinically, the dorsal forearm of our young Caucasian cohort did not exhibit any overt hallmarks of chronic sun exposure, such as skin laxity or hyperpigmentation. However, it was clear that sun exposure - even at this relatively young age - had caused profound flattening of the DEJ and remodelling of the dermal elastic fibre network, and to a lesser extent, the fibrillar collagen matrix. In contrast, photoexposed dorsal forearm of young African individuals was virtually indistinguishable from buttock skin at both histological and biomechanical levels. Whilst we cannot control for an individual’s lifestyle choices regarding habitual sun exposure, it is clear that epidermal pigmentation protects skin architecture to such an extent that biomechanical function is unaffected in individuals with higher Fitzpatrick phototypes.

Cutometry and ballistometry are useful methods that describe related, but not identical, aspects of skin biomechanics\textsuperscript{27}. The differences in measuring principle suggest that cutometry predominantly measures skin elasticity, whilst ballistometry predominantly measures stiffness\textsuperscript{28}. The Cutometer® is often used with a probe aperture of 2mm\textsuperscript{29,30}, thought to measure the properties of the epidermis, whereas an 8mm probe is useful for full-thickness measurements of the skin including the hypodermis\textsuperscript{8,31}. However, there is evidence that differences exist in skin thickness between males and females\textsuperscript{32} and between African and Caucasians with regards to stratum corneum cell compaction\textsuperscript{33} and epidermal thickness\textsuperscript{17}. In order to mitigate these differences, it was important for the current study to ensure that measurements were being made that would better reflect the biomechanical properties of the dermal, as opposed to the epidermal, compartment; therefore assessments were predominantly made using the 4mm aperture probe\textsuperscript{34}. In a subset of participants, the 6mm aperture probe was used, which should reflect the biomechanical properties of the papillary and reticular dermis together. The data obtained from both apertures were remarkably similar, indicating the robustness of the technique.
The results presented here provide a detailed insight into the remarkable similarities in biomechanical properties of healthy skin, and explore the histological features and changes to biomechanical function that arise as a consequence of sun exposure. Our data suggests that it is the highly organised arrangement and not abundance *per se*, of elastic fibres, in combination with the strong rete ridge interdigitation at the DEJ that have the greatest impact on skins’ biomechanical function. An appreciation of these biomechanical properties is important for our understanding of skin during both health and disease and provides knowledge of the intimate link between the maintenance of skin architecture and function. The objective, quantitative assessment of skin biomechanical properties, combined with histological assessment of underlying ECM architecture, has further research application for the examination of both the clinical progression of skin ageing and the development of effective treatment options for improving skin laxity.
FIGURE LEGENDS

Figure 1. Measurement of biomechanical properties of the skin
Graphical representation of the biomechanical properties obtained from application of the ballistometer to the skin (a). Application of the Cutometer® in mode 1 generates time strain curves that represent the F3 envelope function (b) and absolute parameters of mechanical function (c).

Figure 2. Graphical representations of the biomechanical properties of young buttock skin
The biomechanical properties of buttock skin were determined using the Cutometer® with a 4mm aperture probe (a) and the ballistometer (b). Curves generated using these devices indicate that the biomechanical properties of buttock skin of young African and Caucasian individuals are similar.

Figure 3. Graphical representations of the biomechanical properties of young forearm skin
A comparison of skin biomechanical properties was also determined for the forearm. Cutometer® time-strain curves using the 4mm aperture probe (a) and ballistometer curves (b) indicate that differences in the biomechanical properties of skin are apparent in the two cohorts.

Figure 4. Fibrillar collagens in buttock and forearm skin
Immunohistochemical analysis of the fibrillar collagens was performed using antibodies raised against mature collagens I (a-d) and III (e-h). The overall intensity of collagen I and III immunostaining was reduced in the papillary dermis of Caucasian forearm compared with African forearm. Picrosirius red staining for organised fibrillar collagen viewed under polarised light (i-l) and assessed using OrientationJ (m-p). The fibrillar collagen was significantly more coherent in Caucasian forearm (p) compared to African forearm (o). Scale bar: 50µm.

Figure 5. Immunohistochemical detection of elastic fibres in buttock and forearm skin
Tropoelastin (Figure a-c), fibrillin-rich microfibrils (e-g), fibulin-2 (i-k) and fibulin-5 (m-p) are arranged in distinctive candelabra-like arrays in the superficial papillary dermis of African buttock, Caucasian buttock and African forearm. Whereas, Caucasian forearm skin showed depletion of elastic fibre architecture at the dermal-epidermal junction (d, h, l & p), accumulation of elastotic material that was immunopositive for tropoelastin (d) and fibulin-2 (l) and diminished staining of fibulin-5 (p). Scale bar: 50µm

Figure 6. Association between biomechanical properties and skin architecture
An X-Y-Z plot reveals that African buttock, Caucasian buttock and African forearm share similar biomechanical properties (F3 parameter) and architectural features as measured by DEJ convolution and fibrillin-rich microfibril bundle length at the DEJ. However, Caucasian forearm does not share these properties with the other groups.

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Figure 1

(a) 

Height

\[ k = \text{start height} \]

\[ \text{area} \]

\[ \alpha = \text{rate of decay} \]

Time

Indentation

(b) 

Deformation

\[ \text{F3} \]

Time

(c) 

suction (3 seconds) relaxation (3 seconds)

\[ U_f = \text{delayed deformation} \]

\[ U = \text{total deformation} \]

\[ U_e = \text{immediate deformation} \]

\[ U = \text{immediate retraction} \]

\[ U_{\text{final}} = \text{final retraction} \]

Deformation

Time

\[ R_1 = \text{residual deformation} \]
Figure 2

(a) Deformation (mm) over time (seconds) for white Caucasian and black African.

(b) Height (mm) over time (milliseconds) for white Caucasian and black African.
Figure 4

<table>
<thead>
<tr>
<th></th>
<th>Buttock</th>
<th>Forearm</th>
</tr>
</thead>
<tbody>
<tr>
<td>African</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Collagen I**
  - (a) Butttock African
  - (b) Butttock Caucasian
  - (c) Forearm African
  - (d) Forearm Caucasian

- **Collagen III**
  - (e) Butttock African
  - (f) Butttock Caucasian
  - (g) Forearm African
  - (h) Forearm Caucasian

- **PSR polarised**
  - (i) Butttock African
  - (j) Butttock Caucasian
  - (k) Forearm African
  - (l) Forearm Caucasian

- **PSR orientation**
  - (m) Butttock African
  - (n) Butttock Caucasian
  - (o) Forearm African
  - (p) Forearm Caucasian
Figure 5

<table>
<thead>
<tr>
<th>Buttock</th>
<th>Forearm</th>
</tr>
</thead>
<tbody>
<tr>
<td>African</td>
<td>Caucasian</td>
</tr>
<tr>
<td>tropoelastin</td>
<td></td>
</tr>
<tr>
<td>fibrillin-1</td>
<td></td>
</tr>
<tr>
<td>fibrillin-2</td>
<td></td>
</tr>
<tr>
<td>fibrillin-5</td>
<td></td>
</tr>
</tbody>
</table>
Cutometer parameters:

- F3 = surface area that envelopes the curves (the larger, the better)
- R0 (Uf) = height of the first maximal skin deformation
- R1 (Uf-Ua) = residual deformation i.e. can skin return to original position? (the smaller, the better)
- R2 (Ua/Uf) = gross elasticity (closer to 1 = skin is perfectly elastic)
- R4 = skin fatigue (difference between last minimum value and first minimum value)
- R5 (Ur/Ue) = net elasticity (closer to 1 = more elastic)
- R6 (Uv/Ue) = viscoelastic to elastic ratio
- R7 Ur/Uf = elastic recovery (closer to 1 = more elastic)
- R9 = hysteresis (difference between the last and the first maximal skin deformation)

Ballistometer parameters:

- Indentation = the peak penetration depth of the probe tip beneath the skin surface level (in mm);
- Alpha = the rate of energy damping (large values indicate non-elastic damping materials);
- Coefficient of restitution (CoR) = the bounce height relative to the start height (large values indicate high elasticity of the sample);
- Area = the sum of the area under the curve described by the probe vs. the resting level of the surface of the skin.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Caucasian (mean ± SEM)</th>
<th>African (mean ± SEM)</th>
<th>Significance?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>65.5 ± 2.0 mm</td>
<td>63.0 ± 1.2 mm</td>
<td>NS</td>
</tr>
<tr>
<td>Ue</td>
<td>0.92 ± 0.04 mm</td>
<td>0.85 ± 0.02 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R0</td>
<td>1.16 ± 0.04 mm</td>
<td>1.11 ± 0.02 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R1</td>
<td>0.10 ± 0.01 mm</td>
<td>0.08 ± 0.01 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R2</td>
<td>0.92 ± 0.01 a.u.</td>
<td>0.92 ± 0.01 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R4</td>
<td>0.17 ± 0.02 mm</td>
<td>0.16 ± 0.01 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R5</td>
<td>0.86 ± 0.02 a.u.</td>
<td>0.83 ± 0.01 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R6</td>
<td>0.27 ± 0.01 a.u.</td>
<td>0.31 ± 0.01 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R7</td>
<td>0.57 ± 0.01 a.u.</td>
<td>0.63 ± 0.01 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R9</td>
<td>0.11 ± 0.001 mm</td>
<td>0.09 ± 0.004 mm</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>107.7 ± 4.7 mm</td>
<td>103.0 ± 6.0 mm</td>
<td>NS</td>
</tr>
<tr>
<td>Ue</td>
<td>1.52 ± 0.08 mm</td>
<td>1.34 ± 0.07 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R0</td>
<td>1.87 ± 0.09 mm</td>
<td>1.75 ± 0.09 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R1</td>
<td>0.11 ± 0.02 mm</td>
<td>0.07 ± 0.01 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R2</td>
<td>0.94 ± 0.01 a.u.</td>
<td>0.96 ± 0.01 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R4</td>
<td>0.21 ± 0.02 mm</td>
<td>0.14 ± 0.01 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R5</td>
<td>0.84 ± 0.02 a.u.</td>
<td>0.88 ± 0.03 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R6</td>
<td>0.24 ± 0.02 a.u.</td>
<td>0.30 ± 0.01 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R7</td>
<td>0.68 ± 0.01 a.u.</td>
<td>0.68 ± 0.02 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R9</td>
<td>0.13 ± 0.007 mm</td>
<td>0.12 ± 0.01 mm</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>0.78 ± 0.01 mm</td>
<td>0.77 ± 0.01 mm</td>
<td>NS</td>
</tr>
<tr>
<td>Alpha</td>
<td>0.02 ± 0.0006 a.u.</td>
<td>0.02 ± 0.0004 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>CoR</td>
<td>0.82 ± 0.006 a.u.</td>
<td>0.82 ± 0.004 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>Area</td>
<td>124.9 ± 4.9 mm</td>
<td>119.4 ± 3.7 mm</td>
<td>NS</td>
</tr>
</tbody>
</table>

**BIOMECHANICAL PROPERTIES OF FOREARM SKIN**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Caucasian (mean ± SEM)</th>
<th>African (mean ± SEM)</th>
<th>Significance?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>30.0 ± 1.4 mm</td>
<td>40.9 ± 1.9 mm</td>
<td>NS</td>
</tr>
<tr>
<td>Ue</td>
<td>0.50 ± 0.02 mm</td>
<td>0.58 ± 0.03 mm</td>
<td>**</td>
</tr>
<tr>
<td>R0</td>
<td>0.68 ± 0.02 mm</td>
<td>0.78 ± 0.03 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R1</td>
<td>0.17 ± 0.02 mm</td>
<td>0.10 ± 0.01 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R2</td>
<td>0.74 ± 0.03 a.u.</td>
<td>0.87 ± 0.01 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R3</td>
<td>0.28 ± 0.02 mm</td>
<td>0.18 ± 0.01 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R5</td>
<td>0.61 ± 0.03 a.u.</td>
<td>0.78 ± 0.01 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R6</td>
<td>0.38 ± 0.01 a.u.</td>
<td>0.35 ± 0.01 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R7</td>
<td>0.44 ± 0.02 a.u.</td>
<td>0.58 ± 0.01 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R9</td>
<td>0.08 ± 0.003 mm</td>
<td>0.08 ± 0.003 mm</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>51.2 ± 4.0 mm</td>
<td>71.6 ± 3.2 mm</td>
<td>NS</td>
</tr>
<tr>
<td>Ue</td>
<td>0.77 ± 0.04 mm</td>
<td>0.92 ± 0.04 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R0</td>
<td>1.10 ± 0.05 mm</td>
<td>1.29 ± 0.05 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R1</td>
<td>0.25 ± 0.02 mm</td>
<td>0.12 ± 0.02 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R2</td>
<td>0.77 ± 0.03 a.u.</td>
<td>0.90 ± 0.02 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R3</td>
<td>0.37 ± 0.03 mm</td>
<td>0.21 ± 0.03 mm</td>
<td>NS</td>
</tr>
<tr>
<td>R5</td>
<td>0.59 ± 0.03 a.u.</td>
<td>0.75 ± 0.05 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R6</td>
<td>0.44 ± 0.03 a.u.</td>
<td>0.41 ± 0.02 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R7</td>
<td>0.41 ± 0.02 a.u.</td>
<td>0.54 ± 0.04 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>R9</td>
<td>0.11 ± 0.005 mm</td>
<td>0.10 ± 0.01 mm</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>0.78 ± 0.01 mm</td>
<td>0.77 ± 0.01 mm</td>
<td>NS</td>
</tr>
<tr>
<td>Alpha</td>
<td>0.02 ± 0.0006 a.u.</td>
<td>0.02 ± 0.0004 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>CoR</td>
<td>0.82 ± 0.006 a.u.</td>
<td>0.82 ± 0.004 a.u.</td>
<td>NS</td>
</tr>
<tr>
<td>Area</td>
<td>68.9 ± 3.6 mm</td>
<td>80.9 ± 4.3 mm</td>
<td>NS</td>
</tr>
</tbody>
</table>