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A Comparison of Friction behaviour for *ex vivo* Human, Tissue Engineered and Synthetic Skin

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Abstract

Skin tribology is complex and *in situ* behaviour of skin varies considerably between test subjects. The main influencing factor, elasticity, varies due to structural and moisture differences. To find a more reliable test platform, for the first time, synthetic and biological (tissue engineered) substitutes were compared to *ex vivo* skin, epidermis and dermis.

Friction initially increased with rising hydration, before decreasing beyond a threshold for all samples. Friction for Synthetic skin and dermis increased at a similar rate to the other samples, but from a different starting point, and friction dropped at lower hydration. Tissue engineered skin could provide a reliable test platform, but the synthetic skin could only be used if the offset in the data is accounted for.

1 Introduction

Human skin tribology is a very diverse and complicated area of study. This is largely due to the complex structure of skin, its non-linear material properties and the fact that the surface condition and texture has influence (e.g. presence of fingerprint ridges or hair). It is also open to the environment and comes into contact with many different materials. It is very important to understand skin friction in particular though as this plays a critical role in many tasks of daily living [1] and leisure activities [2, 3] that involve gripping, feeling and manipulating objects; it dictates levels of comfort and how we feel about a product [4] and it affects how consumer products and medical devices interact with the skin as well as driving adverse effects such as skin reddening and irritation. It is also an important factor in assessing how well topical cosmetic and medical skin treatments are performing [5].

There are a number of comprehensive reviews of skin tribology [6-8]. All highlight that one of the major limitations in studies of skin tribology is the inherent variability between human test candidates. There is large scatter in almost all *in situ* measurements related to skin. Friction, for example, can vary between 0.1 and 2.5 for the same type of test [6]. This is a result of a number of causes, but ultimately comes down to variations in the elasticity in the skin that will dictate the contact area and deformability under a particular load. Skin is unlike traditional engineering materials in that it does not follow Amontons' law and friction varies with contact area and load. As elasticity rises, contact area also grows which increases the adhesive component of friction. Higher elasticity will also lead to a higher deformation component. For finger pad testing variations in contact area also result from the fact people

all have different ridge patterns [9]. The ridges also lead to a further friction mechanism occurring as the ridges interact with surface texture features, known as "interlocking" [10-13]. The changes in skin elasticity mainly result from variability in skin structure (Liu et al., 2015) [14] and different moisture levels [15, 16].

Quantifying the Young's Modulus of skin is not a straight forward process and examination of the literature gives a wide spread of data. Techniques such as tensile testing [17, 18], indentation [19, 20] and suction methods [21] are used. Data varies across the different measurement techniques for the same skin sample. The effective Young's Modulus in skin being loaded by a probe in a tribological test will also vary with applied load and also with probe diameter [22].

Young's modulus varies across all the different layers of skin with the stratum corneum being several orders of magnitude stiffer than measurements taken on the forearm (largely because of the soft underlying tissue) [23]. It also varies, as mentioned above, with moisture level. Tensile tests have shown a decrease from ≈1 GPa at 50% RH [17] to a value of ≈3 MPa in water [18]. Notably, while a great deal of data exists for forearm skin and *ex vivo* skin and different skin layers, little data exists for skin substitutes, especially engineered skin.

As a result of the variability observed during *in situ* skin friction testing and in skin properties such as elasticity, a great deal of attention is being paid to finding an alternative material to use as a test platform. There are two main options available: biological and synthetic substitutes.

Biological substitutes can be generated using tissue engineering. The main application for these processes is treatment of severe burns. When a person's body is severely burned, they may not have enough healthy skin remaining for healing to occur through skin cell regeneration within their own skin. In this case, tissue engineered skin provides a potential substitute. A lot of progress has been made regarding the construction of tissue engineered skin from a biological point of view [24, 25], but it has not been tested from a mechanical and tribological perspective.

Tissue engineered skin construction involves very complex processes and it is not accessible for every laboratory, thus, an alternative approach is to use a synthetic skin substitute. Development of synthetic skin substitutes has largely been driven by the need for a material for use in training of medical practitioners and a large range is available [26]. These materials are optimised for their mechanical properties, however, rather than their tribological response. Applications where friction has been a strong consideration have been in assessing skin textile interactions for improvement of comfort [27, 28] and topical skin formulations [29] or in understanding blistering [30, 31] and the development of artificial "sensing" fingers [32] or robot fingers [33, 34].

The aim of the work outlined in this paper was to determine whether biological and synthetic skin substitutes behave like *ex vivo* human skin in tribological tests. This was primarily to determine their viability as test platforms for assessing skin interactions with materials used to manufacture medical devices, consumer products and other objects that come into contact with skin. A particular focus was assessment of friction in changing moisture conditions as this has been identified as a weakness of some synthetic substitutes.

2 Experimental Details

Sliding friction tests were carried out on three different materials. *Ex vivo* skin was compared against tissue engineered (TE) skin, dermis and epidermis (obtained by splitting from the

dermis after incubation in sterile 1 M NaCl for 18 h at 37°C) and SynDaver^{TM}, a synthetic skin.

2.1 Specimen Preparation

The skin specimens used in the study are summarised in Table 1. Human skin samples were collected from consenting patients undergoing abdominoplasty. This type of surgery involves the removal of excess tissue from the middle and lower abdomen from patients with loose or sagging skin after pregnancy or major weight loss. All tissue was banked and used on an anonymous basis under Human Tissue Authority Research Tissue Bank Licence Number 12179. The samples were stored in a standard physiological solution (phosphate buffered saline, PBS) with antimicrobial agents at 4°C, for no longer than 12 hours before processing. Sample thicknesses were measured using micro-callipers or histology images.

Skin Type	Description	Thickness (mm)	Sources
Human skin	*Ex vivo* human abdominal skin	0.50	3 different donors
	Epidermis	0.08	4 different donors
	Dermis	0.42	2 different DED donors
Biological skin substitute	Tissue engineered (TE) skin	0.50	2 different donors
Synthetic skin substitute	SynDaver synthetic skin	11.0	Batch nb. 3370

Table 1. Sample description and variability of sources

The TE skin was prepared as previously described [35]. The human skin was incubated in 1M sodium chloride at 37°C for 12 hours before the epidermis was removed using forceps, leaving behind the de-cellularised de-epidermised dermis (DED). The epidermal samples were stored in PBS with antimicrobial agents at 4°C, and the DED samples were stored in tissue culture media (10% Green media) [36].

The DED was used to make TE skin constructs (as shown in Figure 1) as described previously [36]. The cells, human keratinocyte and fibroblasts, were cultured (Figure 1a) until they reached 80% confluency. They were then seeded onto DED samples using stainless steel rings (Figure 1b). The rings were removed after 3 days and the tissue engineered constructs (dermis plus cells) were elevated to the air-liquid interface by transferring them to a stainless steel grid in a petri dish culture plate (Figure 1c).

Cultures were maintained for 14 days at 37°C and 5% CO_2 and the Green media was replaced every three days.

The TE skin structures were examined using histology (see Figure 2), to confirm that they had formed in an appropriate way. The histology sections showed that there was an underlying dermis with a fully reconstructed epidermis. Keratinocytes grown at the air‑liquid interphase had differentiated to form a stratified epithelium, which is similar to the stratum corneum.

Because the TE skin constructs are made from DED (equivalent of dermis) on the top of which cells were seeded (keratinocytes and fibroblasts), the DED was checked to see if it had a fully reconstructed stratified epidermis (i.e., the differentiated cells seeded on the top of DED)).

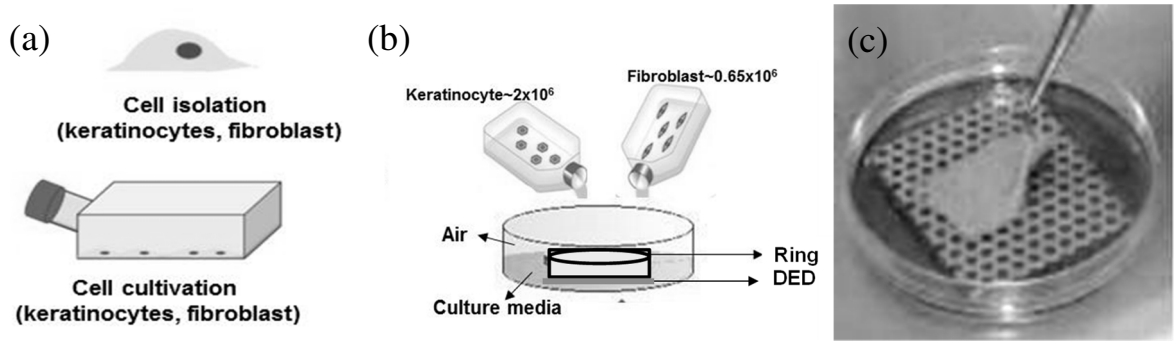


Figure 1. Preparation of Tissue Engineered (TE) Skin: (a) Cell Isolation; (b) Cell Seeding; (c) Air-Liquid Interface

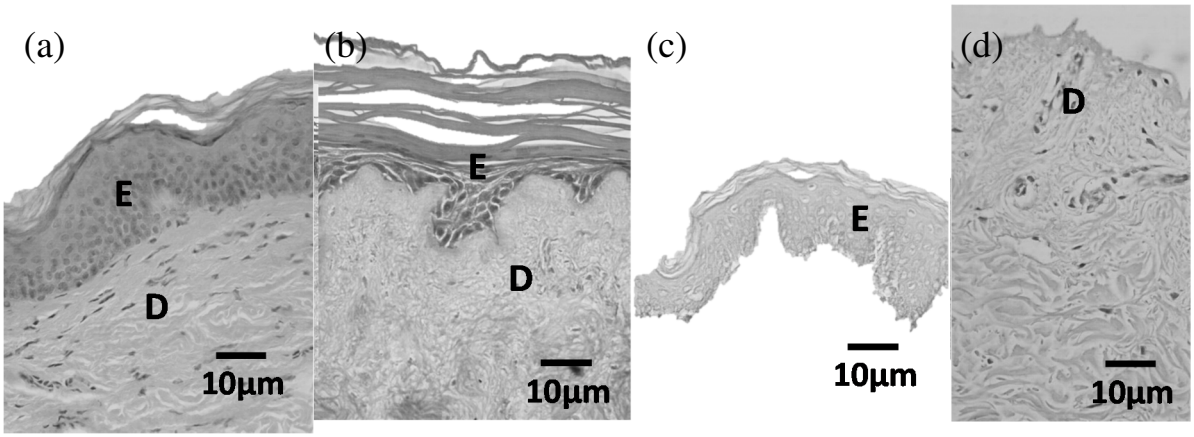


Figure 2. Hematoxylin and Eosin (H&E) Stain of: (a) Human Skin; (b) TE Skin; (c) Epidermis; (d) Dermis (the samples were fixed with 3.7% formaldehyde; after dehydration, the specimens were embedded in paraffin, and sectioned into 4 µm thickness pieces) (E – Epidermis; D – Dermis)

The synthetic substitute skin was purchased from SynDaver™ (model T-PLA-A-0010). It is made up of layers to mimic the skin (epidermis and dermis, 1 mm; fat tissue, 5 mm, and muscular tissue, 5 mm). It is typically used by medical professionals to practice surgery. This synthetic tissue is a patented SynTissue™ brand synthetic human tissue. It is claimed to be experimentally designed on the basis of physical tests performed on actual living tissue [37].

All the samples were kept in a hydrated state until testing: PBS was used for storage of human skin, epidermis and dermis (4°C), Green's media for tissue engineered skin (37°C) and distilled water in the case of synthetic skin (room temperature). Prior to testing, all samples were allowed to stabilise at room temperature for one hour. Surface images of each sample are shown in Figure 3 in dry and wet conditions (Corneometer measurements of 30 AU and 110 AU respectively – see Section 2.2 for details). TE skin and *ex vivo* skin looked very similar (as would be expected) so only one image is included. The surface of the *ex vivo* skin looks very similar to that used by Adams et al. [15]. Much more water is present on the surface of the "wet" dermis and synthetic skin.

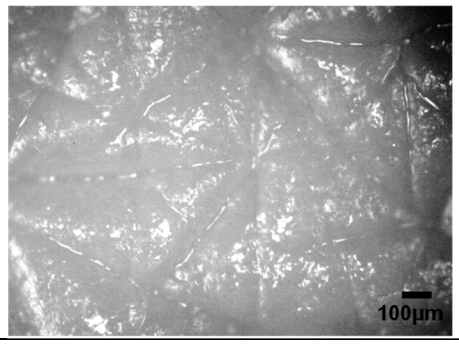
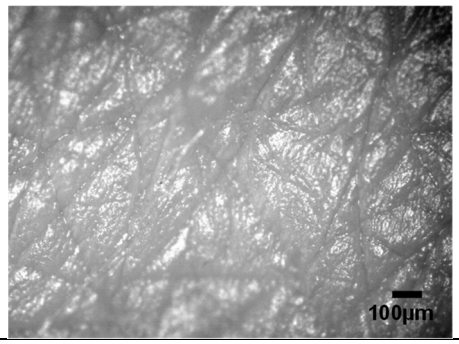
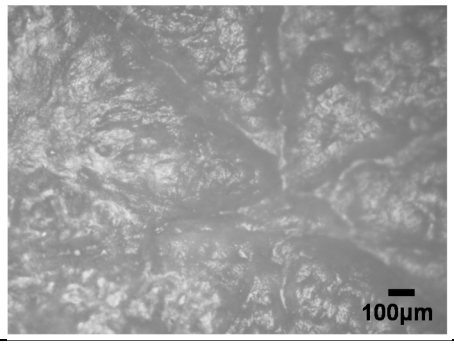
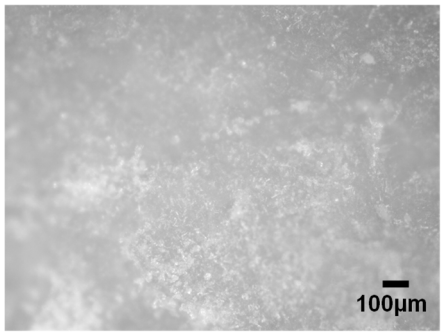
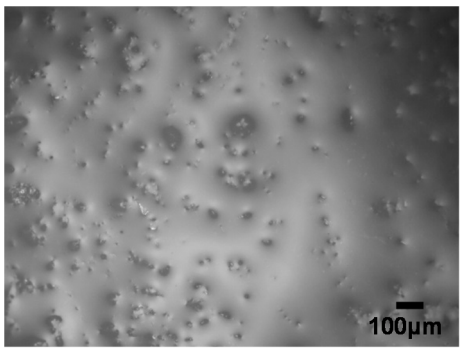
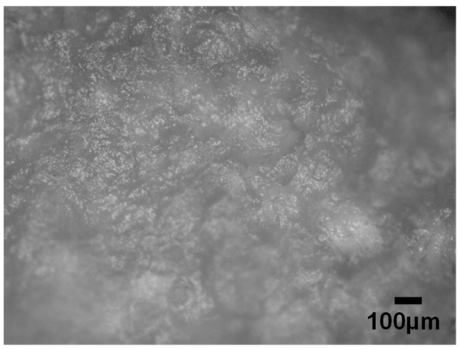
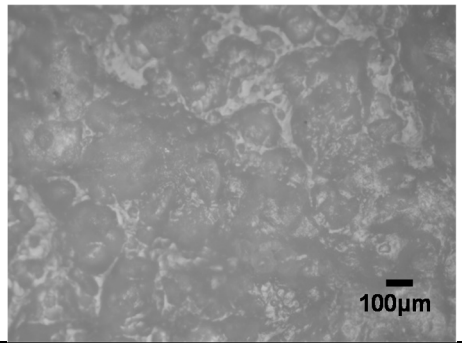
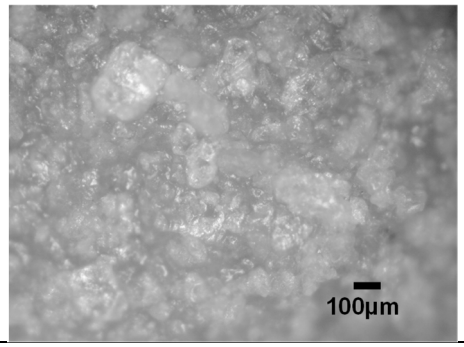
Sample	Wet (110 AU)	Dry (30 AU)
Human *ex vivo* skin		
Epidermis		
Dermis		
Synthetic Skin		

Figure 3. Optical Microscopy Images of the Specimen Surfaces in Wet and Dry Conditions (Corneometer measurements of 110 AU and 30 AU respectively – see Section 2.2 for details)

2.2 Friction Testing

In order to provide a stable base for friction testing and improve the consistency of the sample thicknesses (Table 1), the synthetic substitute skin was used as a base layer for the dermis, human skin and TE skin tests. This was glued onto sand paper attached to the rig. The epidermis was tested too, in order to provide a comparison that might validate the use of synthetic skin as a base layer. For these studies different skin donors were tested (Table 1).

Friction tests were carried out using a CETR-UMT 2 (Bruker) tribometer. The configuration used was a normally loaded probe (a 4 mm diameter stainless steel ball) being driven across the sample in a linear reciprocating fashion. The tests were completed at three different normal loads (5, 10 and 30 mN) at an average speed of 0.5 mm/s for six sliding cycles with a stroke of 10 mm (one cycle is twice the stroke). The normal load was stabilised for 20 s before testing. Figure 4 shows the measurement system which is composed of a double load cell (one for normal load and one for friction force). The kinetic coefficient of friction was calculated as the ratio between the friction force and the normal force. For each sample at least two repeats were carried out.

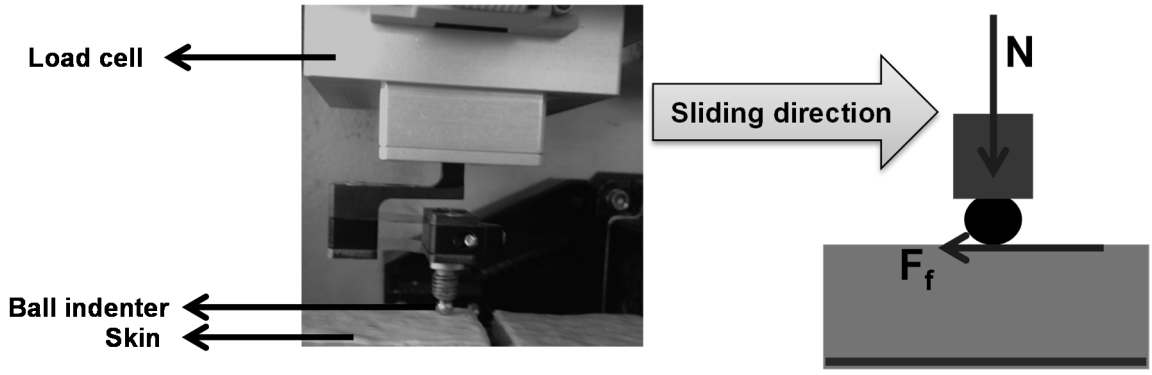


Figure 4. Measurement System for Friction Force (N – Normal Load; F_f – Friction Force)

A Corneometer 825 (Courage-Khazaka Electronics) was used to measure hydration levels (as described previously by Palma et al. [38] and Waranuch et al. [39]). This instrument has a handheld probe and measures moisture based on the capacitance of skin due to its properties as a dielectic medium. The probe sensor area is 49 mm² and the measurement depth is 10-20 μm below the skin surface to avoid the influence of deeper skin layers. A spring in the probe head maintains constant pressure on the skin to improve reproducibility. Hydration levels, measured in "arbitrary units" (AU) (maximum measurement level was 110 AU for fully hydrated samples), were taken along the length of the samples at five different points, to provide mean values. Samples initially soaked in PBS solution (in their fully hydrated state) were allowed to dry over a long period during which they were tested at regular intervals to give data for a range of hydration levels. The tests were performed in a laboratory with at a temperature of 19-23°C and relative humidity of 50-60%. The mean of 5 measurements along the length of the samples was taken as the hydration level. Hydration means with variations of more than 10% were not taken into consideration. The hydration level was measured before and after friction tests. Full details, including specimens, loads used and the hydration levels tested, of all the friction tests carried out are given in Table 2.

Number of Friction Tests, Normal Load Applied and Hydration Level Range (AU)				
Sample	Description	30 mN	10 mN	5 mN
Human skin	*Ex vivo* human abdominal skin (HL: 60-110)	17	35	16
Biological skin substitute	Tissue engineered skin (HL: 30-100)	27	30	24
	Skin epidermis (HL: 40-110)	27	26	22
	Skin dermis (HL: 40-100)	10	17	12
Synthetic skin substitute	SynDaver synthetic skin (HL: 20-120)	69	42	38

Table 2. Description of Friction Tests Carried out

3 Results and Discussion

3.1 Effect of Hydration Level on Skin Friction and Proposed Mechanisms for Observed Behaviour

The graphs in Figure 5 show the friction data measured across a range of hydration levels for each material at the three loads used. The spread in the data is high, but in line with friction measurements taken at different hydration levels by Hendriks & Franklin [10].

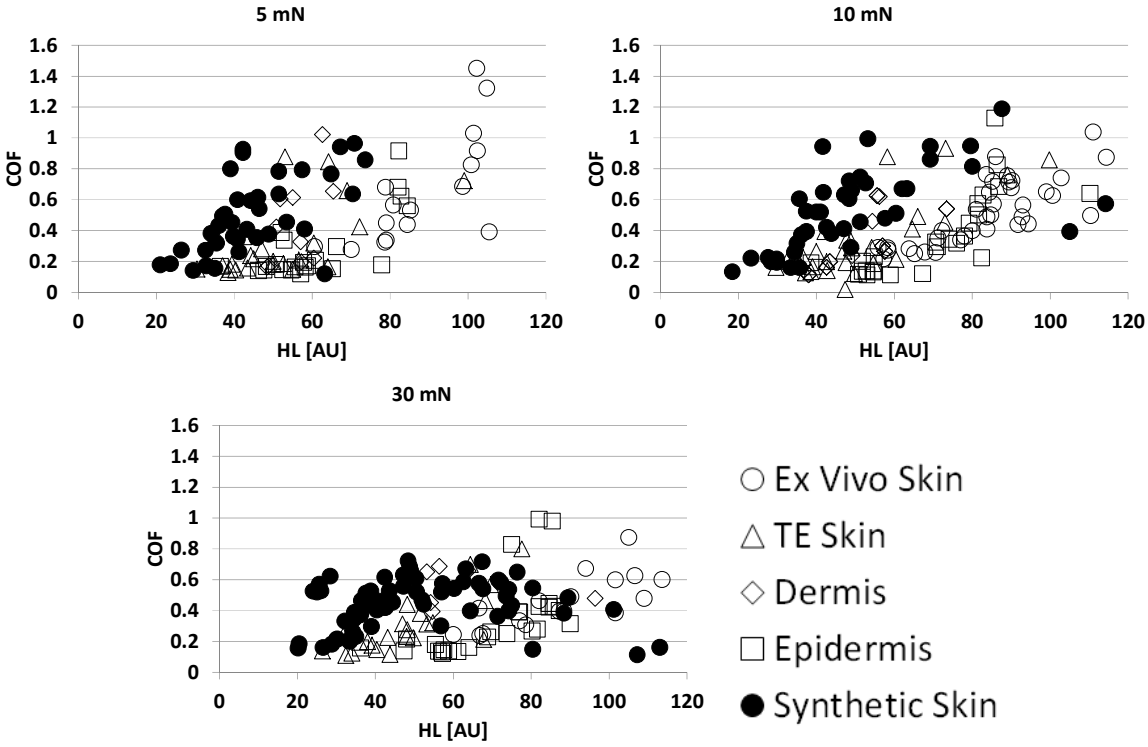


Figure 5. Coefficient of Friction (COF) versus Hydration level (HL) for the Five Specimens at Loads of 5 mN, 10 mN and 30 mN

As hydration levels increase for each of the materials, the friction generally rises. In some cases past a certain point the friction then decreases. This behaviour is similar to that seen in previous studies [15, 16]. It is also noticeable that the friction levels are lowest at the highest applied load. The effects of load will be explored in more detail in the next section, but aside from the effect load has on its own it will also influence the levels of moisture in the interface, for example at higher loads more liquid may be squeezed out of the specimen.

The *ex vivo* skin, TE skin and epidermis all give a similar response. The synthetic skin and dermis friction values, however, while having a similar gradient to the other specimens, are slightly offset to the left, i.e., the values climb at the same rate from a lower "dry" hydration level. The friction levels of the synthetic material and the dermis also drop at a lower hydration level. This is most noticeable at the highest applied load of 30mN.

As hydration levels increase, a number of possible mechanisms exist that can explain the increasing friction levels [16]. The most dominant though is the plasticisation/softening of the skin as it absorbs the applied water which leads to a greater contact area between the probe and the skin and hence greater adhesive friction occurring [15, 16] (see Figure 6). It might be expected that the *ex vivo* skin, TE skin and epidermis would respond in the same way, due to this mechanism, when hydrated as they have similar material properties.

The dermis and the synthetic skin have a lower modulus than the other skin specimens (unpublished data) which could explain the higher friction levels that they give for the same hydration condition (which means that they look off-set from the other specimens). Initially as hydration is increased their elasticity changes at a similar rate, but it appears to be able to absorb less water than the skin samples as its friction dips earlier. At the point the friction drops it is likely that enough liquid is present for partial film of water to form (see Figure 6). Some evidence for this is shown in Figure 3, where much more liquid is visible on the fully wetted dermis and synthetic skin than the other specimens.

At higher loads it is likely that for all specimens that there will be more liquid on the surface as the probe will squeeze more out. It is clear at 30mN though, that far more is being squeezed out of the synthetic skin, as its dip is well before the other specimens. The dermis behaves in the same way. In actual skin the epidermis would be there to act as a barrier to water loss. Once this is removed more water can be squeezed out.

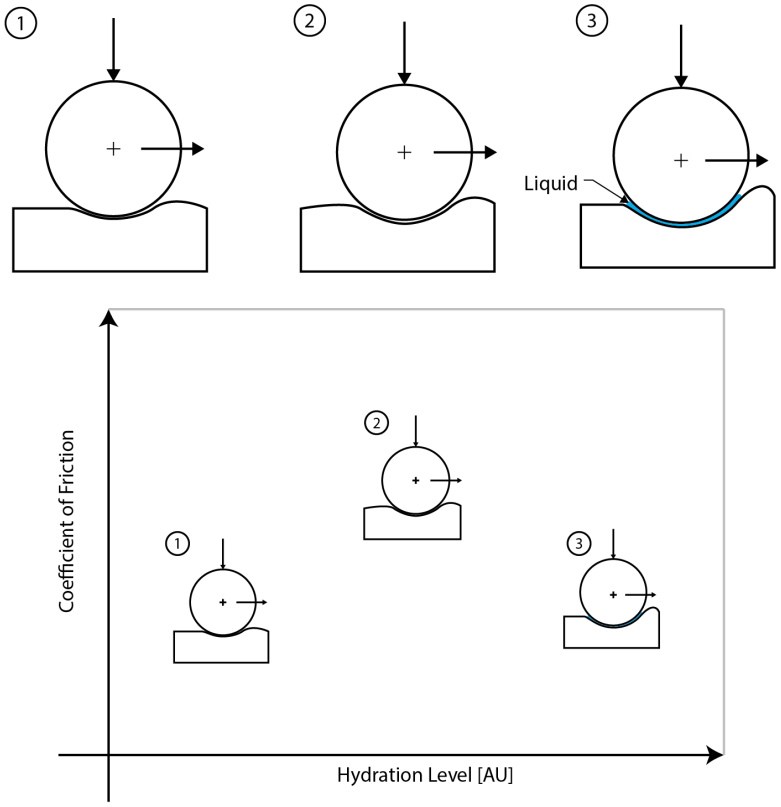


Figure 6. Proposed Hydration Mechanisms (contact area increase as hydration increases leads to rising friction coefficient, but eventual film formation leads to a friction drop)

It should be noted that, as shown in Figure 7, there were no major differences in measuring the hydration level before and after the friction tests as observed for *ex vivo* skin specimen 1 tests at 30 mN. This was the same for the other specimens.

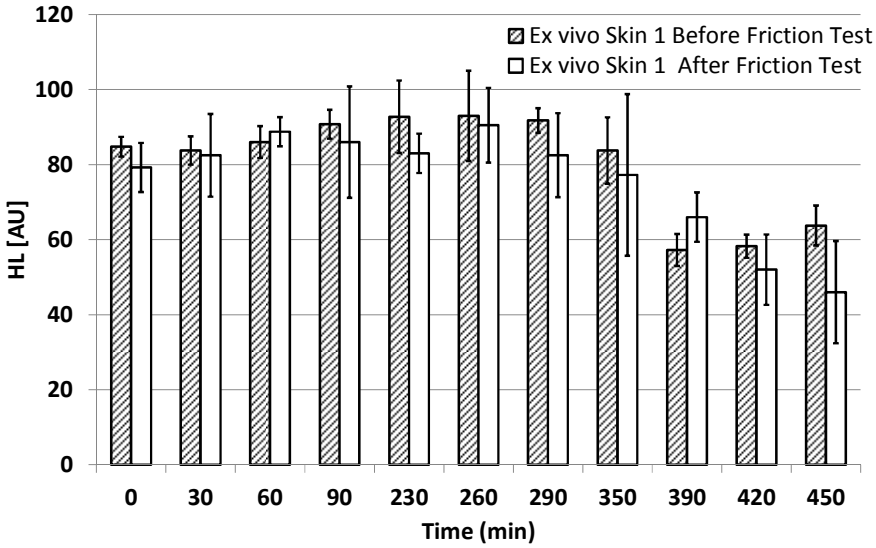


Figure 7. Differences between Hydration Level before and after each Friction Test for *ex vivo* Skin at 30 mN

3.2 Effect of Normal Force on Friction

In order to demonstrate the effect of changing normal load, the friction data from Figure 5 has been replotted in Figure 8.

There is some evidence of a slight reduction in friction coefficient as load increases for the *ex vivo* human skin. This has been observed in previous skin friction testing [22]. The fact this trend is not so clear for the other materials may be due to the lack of data at higher hydration levels.

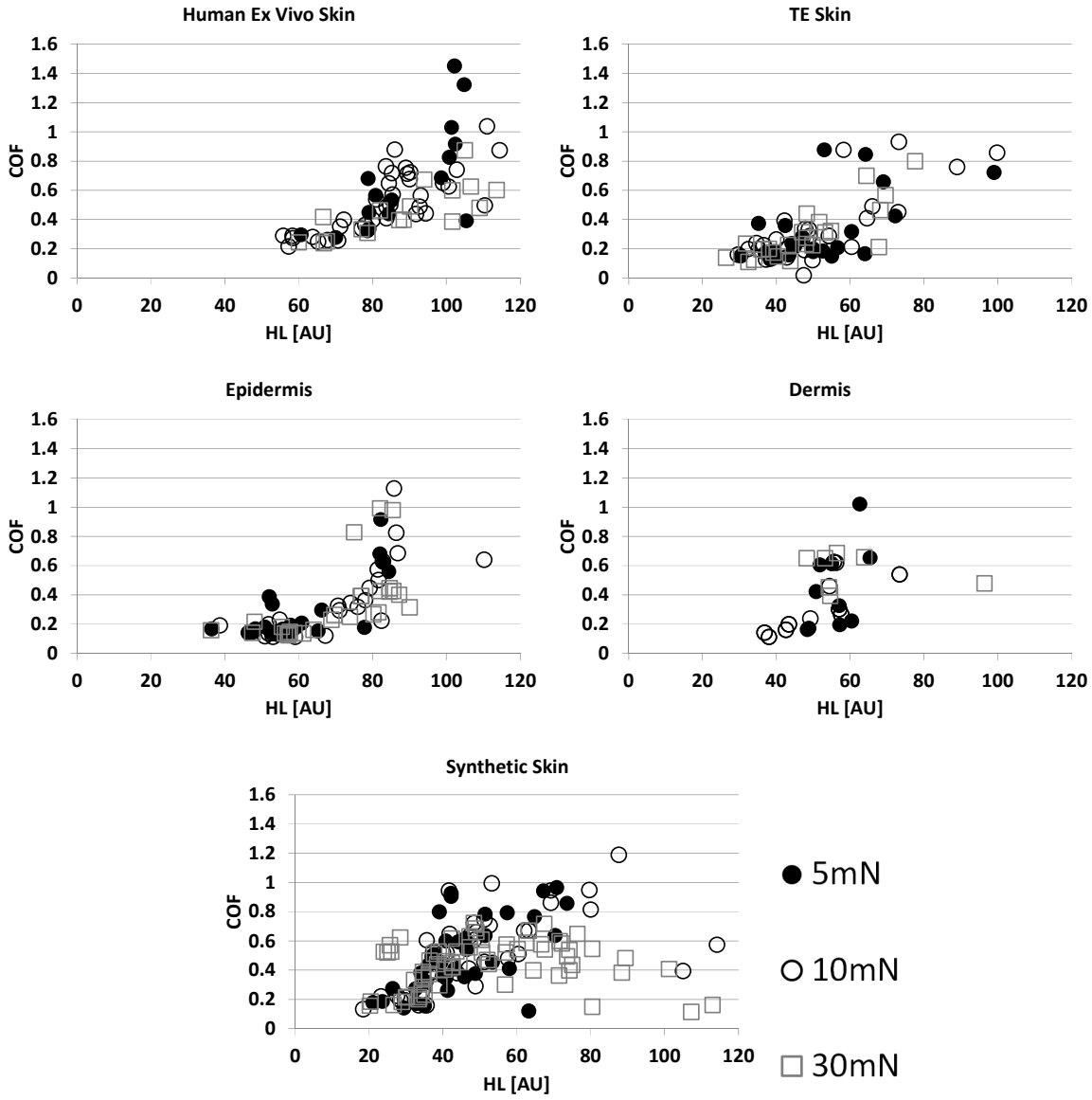


Figure 8. Coefficient of Friction versus Hydration Level for three different Normal Loads for the Five Specimen Types

3.2.3 Patient Variability

Measuring the coefficient of friction of human skin presents several challenges concerning consistency and repeatability of results. As mentioned earlier, one of the major issues is human variability. As stated in Table 1, *ex vivo* skin and skin used for epidermis and dermis preparation and for the TE skin came from a variety of donors. In Figure 9, the results are

plotted by donor and the different samples of the synthetic skin are compared (it should be noted that these were a different set of tests to those shown in Figures 5 and 8. As can be seen there is very good consistency between donors.

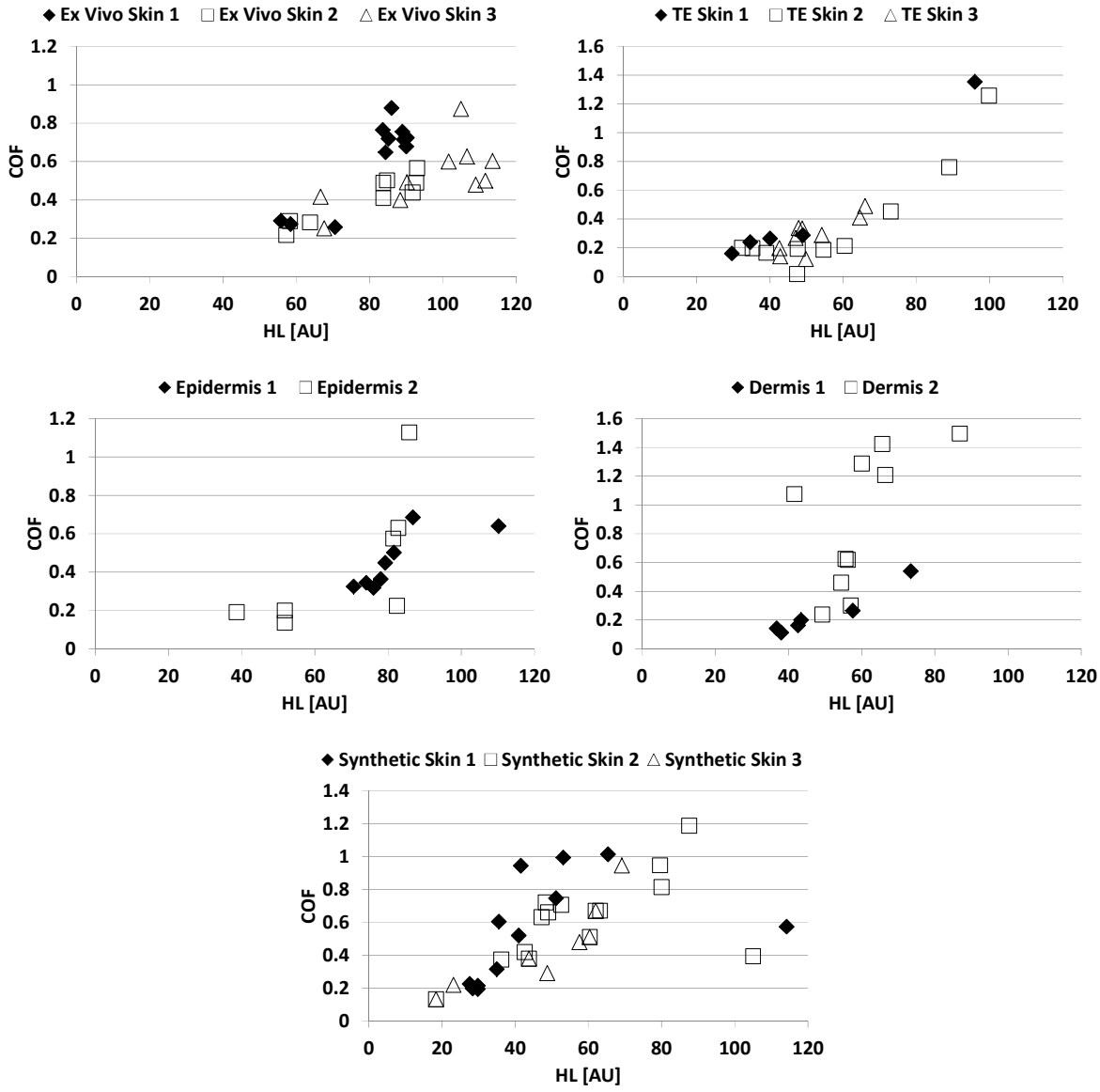


Figure 9. Plots of Patient/Sample Variability for Tests at 10mN

4 Skin Friction Modelling

Skin friction modelling using the approach proposed by Adams et al. [15] for *in vivo* forearm skin, was carried out to see whether similar trends were seen and to provide a comparison of *in vivo* and *ex vivo* results.

As mentioned earlier above, total friction force can be defined as a two term model (Equation 1) [40]. For a situation where a spherical probe is sliding across a skin surface, the adhesion is the result of interfacial shear, and deformation is the result of energy dissipation by sub-surface visco-elastic deformation (see Figure 10 and Equation 1).

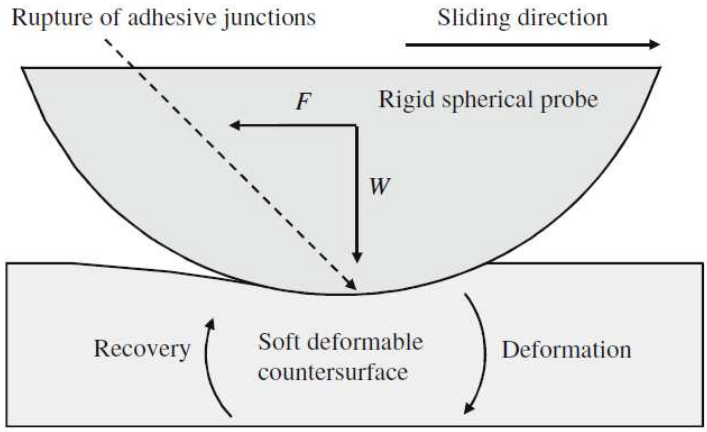


Figure 10. A Schematic of a Rigid Spherical Probe Sliding on a Soft Counter-surface (from Adams et al. (2007))

$$F_{total} = F_{def} + F_{adh} \quad (1)$$

The deformation component, defined originally by Greenwood and Tabor [41] and experimentally validated by Adams et al. [15] is:

$$F_{def} = \beta \left(\frac{9}{128R}\right)^{2/3} \left(\frac{1-\nu^2}{E}\right)^{1/3} W^{4/3} \quad (2)$$

where β is the visco-elastic hysteresis loss fraction, R is the probe radius, ν is the Poisson's ratio, E is the effective Young's modulus of the skin and W is the normal force. This is usually ignored, however, as its contribution to the overall friction force has been shown to be very low for this contact configuration at relatively low loads on skin [15, 22]. For the testing outlined in this work using a 4 mm diameter probe and using values from the literature for skin properties: β = 0.24 and ν = 0.49 [42] (Johnson et al., 1993); E = 100kPa [22] and W = 30 mN, F_{def} = 0.017, an order of magnitude lower than the total dry skin friction force expected.

The interfacial, or adhesive, component is given by:

$$F_{adh} = \tau A \quad (3)$$

where A is the contact area and τ is the interfacial shear strength. Despite the roughness seen on the *ex vivo* skin samples shown in Figure 4, it is anticipated that the reduction in elastic modulus and the swelling that occurs when skin is wet would cause it to be smooth within a probe contact [15]. This means that a Hertz approximation for contact area might be appropriate:

$$A = \pi \left(\frac{3WR}{4E^*}\right)^{2/3} \quad (4)$$

It has been noted in work on thin organic films that the interfacial shear strength is related to the mean contact pressure, p, as follows [43]:

$$\tau = \tau_0 + \alpha p \quad (5)$$

where τ_0 is the intrinsic interfacial shear strength, α is a pressure coefficient and $p = W/A$.

Adams et al. [15] applied this approach to water soaked skin to obtain the following equation for the adhesive force (from Equations 3, 4 and 5). This was an extension of the Wolfram

[44] adhesion model for smooth contacts to take account of wetted skin where the stratum corneum has plasticised and become very soft:

$$F_{adh} = \pi\tau_0 \left(\frac{3R}{4E^*}\right)^{2/3} W^{2/3} + \alpha W \quad (6)$$

The friction coefficient can then be calculated by dividing by W:

$$\mu_{adh} = \pi\tau_0 \left(\frac{3R}{4E^*}\right)^{2/3} W^{-1/3} + \alpha \quad (7)$$

Using E = 40 kPa (value for wet skin used by Adams et al. [15]) and ν = 0.49, area, A, was calculated, using Equation 4, and then values for p were derived for each of the loads used in the current testing, 5, 10 and 30 mN. Using average friction force data for the *ex vivo* skin samples at 100 AU (for wet) and 40 AU (for dry) to calculate values for τ, a plot was created to derive values for τ_0 and α, as shown in Figure 11.

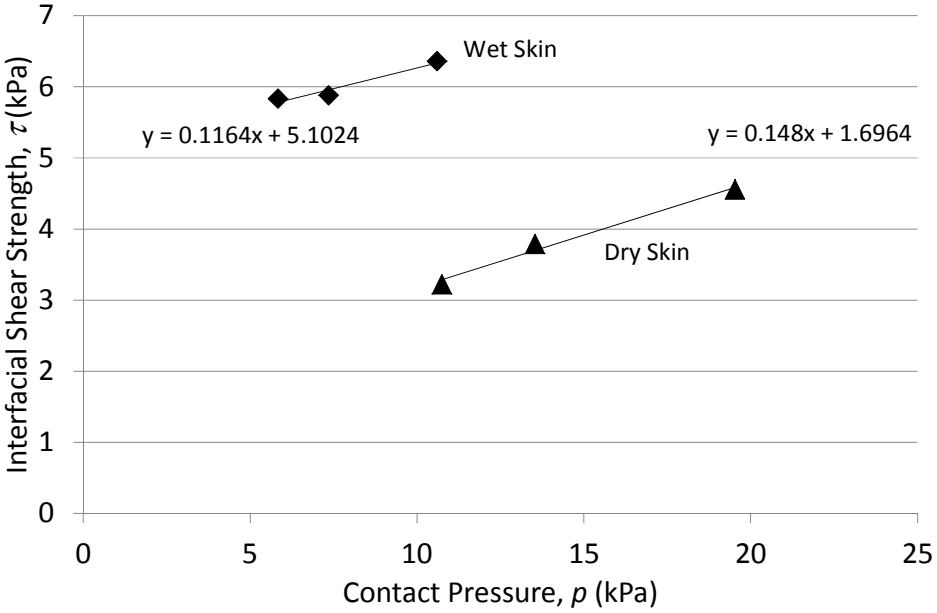


Figure 11. Interfacial Shear Strength versus Pressure for *ex vivo* Skin

The values of α and τ_0, 0.1164 and 5.1 kPa respectively, compare well with the values derived for wet forearm skin derived by Adams et al. [15]. This is a promising result and may indicate that *ex vivo* skin treated in the right way can give behaviour representative of *in-vivo* skin. There are a number of reasons why the behaviour would be slightly different, including the difference in temperature and the storage medium used for the *ex vivo* tissue.

In work of Adams et al., wet values were compared to literature values for dry glassy organic polymers, for which values for τ_0 of a few MPa have been noted, which are several orders of magnitude higher, and α values of 0.1 to 1 [45]. The assumption was made that skin values would be similar in dry conditions, and that the addition of water therefore reduces the interfacial shear. However, the friction coefficient rises with increasing hydration levels. The only explanation for this must be an increase in the contact area. In that case the assumption of a Hertzian contact area is probably invalid, at least for the wet case. This would not be a surprise as during the tests in the current study the probe seemed to compress into the material quite heavily and there was a bow wave seen (similar to that observed in forearm testing by Kwiatkowska et al. [22]), which would increase the contact area.

Using the same approach to calculate α and τ_0 for friction tests in dry conditions (around 60 AU, see Figure 5) using E = 100 kPa and ν = 0.49 gives values of 0.148 and 1.696 kPa respectively (see Figure 11). If it is assumed that the contact area is non-Hertzian, F_{adh} can be given by Equation 8:

$$F_{adh} = \tau_0 A + \alpha W \quad (8)$$

Assuming that α and τ_0 stay the same for wet skin as calculated for dry and taking the average value of F_{adh} for 5 mN at 100 AU, A can be determined and is 2.7 times higher than the Hertzian approximation. This seems plausible.

If it is assumed that E for "dry" TE skin, dermis and synthetic skin are proportionally lower by the same amount as the shear moduli shown in Figure 9, and the values of α and τ_0 are the same, the friction forces can be calculated as: 0.46, 0.49 and 1.21. These seem reasonable approximations when compared with the experimental values shown in Figure 9.

Equation 9, for the minimum film thickness, h_c, in a sliding IEHL (Isoviscous Elasto-Hydrodynamic Lubrication), contact (where sufficient pressure is generated in the converging region of a contact for the surfaces to become separated by a thin film of liquid) was derived by Johnson et al. (1997) for the case when ν = 0.49:

$$h_c = 1.43(\eta V)^{0.64} R^{0.80} E^{-0.42} W^{-0.22} \quad (9)$$

where η is the lubricant viscosity and V is the sliding velocity. This equation was used to obtain and approximate relationship for the coefficient of friction μ_{IEHL} [46]:

$$\mu_{IEHL} = 1.51(\eta V)^{0.36} R^{-0.134} E^{-0.247} W^{-0.113} \quad (10)$$

Putting in values of, h_c = 34 nm, which represents the minimum fluid film thickness required to fully separate the surfaces. This is considerably lower than the roughness of the surfaces used. For full film separation therefore, at the conditions used the surface roughness of the skin specimens were much lower than its unloaded state. μ_{IEHL} = 0.0025, which is

Predicted μ_{IEHL} for a smooth surface contact (using values of E = 40kPa and η = 1 mPas for tests with the 2 mm radius probe used in this work at 0.0005 m/s and 5 mN) was 0.0025 whih is lower than that seen in the tests, so it is likely that rather than full film separation mixed or boundary lubrication was occurring. This is supported by the calculation of the EHL film thickness (34 nm) which is less than the surface roughness.

5 Conclusions

This work was aimed at determining whether biological and synthetic skin substitutes behave like *ex vivo* human skin in tribological tests. This was primarily to determine if they could be used as a reliable test platform in tribological studies to assess skin interactions with materials used to manufacture medical devices, consumer products and other objects that come into contact with skin. The work has provided the first tribological assessment of tissue engineered skin.

For all specimens, the coefficients of friction initially increased with increasing hydration level, before decreasing beyond a threshold. *Ex vivo* skin, TE skin and epidermis showed similar characteristics. The friction for the synthetic skin and dermis, however, showed a similar rate of increase in friction as hydration increased, but from a different starting point, and the friction dropped at a lower hydration level. This was due to their reduced capability to hold water (in the dermis the outer epidermis usually holds the water in). The TE skin could provide a reliable test platform, as long as a sub-layer of synthetic skin is used.

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