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The effect of humidity on the fracture properties of human fingernails

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SUMMARY

Fingernails are a characteristic anatomical feature of primates and their function is dictated by the environment in which they are utilised. The present study examined the mechanical properties of human fingernails as a function of relative humidity (RH) and the subsequent moisture content of the nail material. Nail clippings were stored at a range of RH values and then weighed in order to determine their moisture content. There was a non-linear relationship between the moisture content of nails and the RH of their local environment. The *in vivo* moisture content of nails, measured from 55% to 80% RH, was between 14% and 30%, similar to other keratinous materials such as claws, hooves and feathers. Cutting tests on the nail samples showed that the work of fracture was between 11 and 22 kJ m^{-2}, rising to a peak at 55% RH and falling at higher and lower humidities. At all RH values there was anisotropy within the nail between the proximal and lateral directions, the work of fracture being greater proximally. This anisotropy was greatest at 55% RH, at which point the proximal work of fracture was double the lateral value. These results suggest that the mechanical behaviour of human fingernails is optimised at *in vivo* conditions; they resist tearing most strongly under these conditions and particularly resist tearing into the nail bed. At more extreme humidity levels the fracture properties of the nail deteriorate; they are brittle when fully dry and fracture and split when wet.

Key words: fingernails, mechanics, moisture, fracture

INTRODUCTION

Fingernails are a characteristic feature of primates, and are homologous to the claws of most other mammalian species (Hamrick, 1998). Although the structure of fingernails has been comprehensively reported in the literature by primatologists and clinicians (Achten, 1981; Ditre and Howe, 1992; Dykyj, 1989; Le Gros Clark, 1936; Lewin, 1965; Lewis, 1954; Soligo and Muller, 1999), little attempt has been made to understand how they perform mechanically under changing environmental conditions.

The nail plate comprises three histological layers of keratinous tissue, which are deposited by the nail matrix at the base of the nail unit beneath the skin (Achten, 1981; Caputo et al., 1982). These layers are arranged in a sandwich-like structure as shown in Fig. 1A. The intermediate layer, in which the keratin fibres are oriented laterally, comprises approximately two-thirds of the thickness of the nail. It is enclosed by the thinner dorsal layer, which makes up approximately a quarter of the nail's thickness, and wraps round the edge of the intermediate layer to join up with the thinner ventral layer. In both of these outer layers the keratin fibres show no preferred orientation.

An earlier study (Farren et al., 2004), performed by carrying out cutting tests with instrumented scissors, showed that this design is admirably suited to limit and control fracture. The lateral orientation of the fibres in the thick intermediate layer ensures that the work of fracture is greater proximally than laterally, deflecting cracks laterally away from the nail bed and allowing self-trimming. The outer layers of the sandwich, meanwhile, protect the intermediate layer and prevent cracks forming at its edge. A disadvantage is that the nail layers tend to peel apart at their boundaries, particularly when wet (Farren et al., 2004).

One of the problems with this research, however, is that cutting tests were carried out when the nails were saturated with water. In life, nails will have intermediate humidity as they are moistened by the nail bed, and this will affect both their moisture content and their mechanical properties.

Fraser and Macrae (Fraser and Macrae, 1980) highlighted six main factors that determine the mechanical properties of filament–matrix composites such as the fingernail. These are: (1) the mechanical properties of the filaments; (2) filament length; (3) orientation and packing of the filaments; (4) the mechanical properties of the matrix; (5) the volumetric ratio of matrix to filaments; and (6) the adhesion of the matrix to the filaments. Most of these factors are determined by the morphology of the nail, and would remain invariant with a changing environment. Factors 4 and 6, however, may be expected to vary according to the hydration status of the nail. Indeed, in horses' hooves it has been found that the water content varies along the length of the hoof, resulting in differences in the mechanical properties measured in different regions (Bertram and Gosline, 1987). These authors suggested that the hoof is more hydrated close to the growth region, which allows it to be malleable, thus acting as a shock absorber. Hydration was shown to decrease distally, terminating in the hoof tip, which is stiffer than the growth region and also provides physical protection against abrasion (Bertram and Gosline, 1987).

Nails are primarily composed of keratin, which is a complex coiled-coil protein (Creighton, 1997). Sequence studies of mammalian filament proteins have shown that they possess a regular pattern of hydrophobic residues, which favours the formation of a coiled-coil rope-like structure (Crick, 1953), composed of segments around 15 nm in length (Crewther et al., 1978; Gough et al., 1978). The mechanical properties of the nail will depend on how these filaments are bound to the matrix material. Analysis of these proteins has revealed a pentapeptide repeat, which suggests that they are stabilised by disulphide bridges (Dopheide, 1973; Elleman et al.,

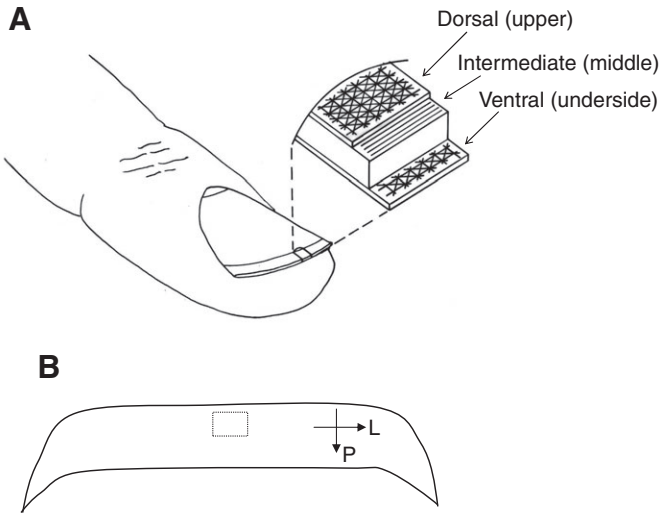

Fig. 1. (A) Schematic diagram of a human fingernail showing the dorsal (upper), intermediate (middle) and ventral (lower) layers. (B) Schematic diagram of a nail clipping where the dotted lines indicate an example of a sample taken for clipper testing and L and P are the lateral and proximal directions, respectively.

1973). The adhesion between the matrix and the keratin fibrils occurs in mammalian material between high sulphur regions in the former and low sulphur regions in the latter, where the net charges are of opposite sign (Crewther and Dowling, 1960; Gillespie and Simmonds, 1960) and it is thought that this adhesion mechanism will be disrupted by the presence of moisture. Disruption of the disulphide bridges is hypothesised to alter the mechanics of the nails, particularly the ability to cut them along the fibre direction.

This study was designed to investigate the effect of relative humidity (RH) on the moisture content and fracture properties of nails; effects that will have important implications for nail care and maintenance.

MATERIALS AND METHODS

Specimen selection

Human fingernail clippings were taken from 10 healthy young adult human subjects using nail clippers, and were stored in bags prior to being transferred to the RH controlled environments. The specimens were placed into a random pool and not individually tested from each of the 10 subjects. Some inter-digit variation may occur, but a much larger population of specimens would be required to study this, and this is left as a topic for future investigation.

Moisture content control

The use of saturated salt solutions has been a common method for controlling the RH of biological samples (Winston and Bates, 1960). Small containers must be used in order to store the samples (<1 litre capacity), and a fixed physical barrier must be present to prevent contact between the salt solution and the sample (Winston and Bates, 1960).

The saturated salt solutions used for this study were prepared by adding powdered salts to boiling, distilled water until no more solid could dissolve. These solutions were then thoroughly mixed using a magnetic stirrer and allowed to cool before use. A small volume (100 ml) of each solution was then poured into a 0.25 l container. Fingernail samples were placed above the solutions on pieces of non-absorbent cotton wool. The range of solutions used to control humidity, along with the RH values obtained, are reported in Table 1.

The 100% RH samples are different to the immersed samples previously studied (Farren et al., 2004) in that the properties of the immersed samples depend on their ability to absorb moisture. The samples that were immersed in water were generally thicker than any of the samples analysed in our study, which suggests that they took on much more water. Hence it was anticipated that the properties of these nails would be different to those of the nails in our study. In order to assess the moisture content of the nails, as a function of RH, 20 test samples were clipped and weighed immediately, equilibrated sequentially using the different RH environments, weighed again, and finally oven dried at 80°C for a period of 5 days. The *in vivo* water content and the water content at the different RH values were then calculated by comparing the mass of freshly clipped nail specimens and the mass of nails exposed to varying hydration conditions with that of the oven-dried samples. Tests were conducted to verify that the nails had reached equilibrium by weighing the nails over time. It was found that the mass of the nails did not change after they were exposed to the humidity environments for longer than 48 h.

Clipper cutting tests

Cutting tests for estimating the fracture properties of plant and animal tissue (Eichhorn et al., 2007; Farren et al., 2004; Lucas and Pereira, 1990; Vincent, 1992) have usually involved placing a pair of scissors between the platform and the crosshead of a universal testing machine, and recording the work done to fracture or cut a specimen. The main limitation of this technique is that the frictional forces generated during the closing of the blades are not constant, thereby increasing the variability of the data (Bonser et al., 2004). In order to overcome these difficulties a pair of Boots (Nottingham, UK) toenail clippers were placed within the jaws of an Instron model 4301 Universal Testing machine, with the lower blade held rigid, as described by Bonser and colleagues (Bonser et al., 2004). The clippers have approximately a 25 mm radius of curvature. These clippers are normally operated by depressing a lever attached to a spigot proximal to the blades. This was removed, so that the force could be applied directly to the upper lever of the clippers by the compression platen of the testing machine. In order to calibrate this set up, the blades were first positioned approximately 1.4 mm apart, which was taken as the zero gauge length. They were then gradually closed until they met, at which point the load started to increase sharply. The machine was subsequently stopped, and lower extension limits set at this point. The set up was then calibrated to calculate the work required to close the blades without any sample in place. The jaws of the clippers were opened, and the crosshead of the Instron, with a 1 kN load cell mounted into it, was lowered at a speed of $8.3\times10^{-5}\,m\,s^{-1}$, causing the blades to close.

In preliminary experiments, load–displacement traces of scissors closed without a sample in place were found to vary from one

Table 1. Solutions and desiccators used to obtain relative humidity conditions

Solutions/desiccators	Relative humidity (%)
Distilled water	100
Potassium chloride	85
Sodium nitrite	65
Calcium nitrate	55
Magnesium chloride hexahydrate	33
Silica gel	0

experimental run to another. This was found not to be the case with clippers. The method of cutting is entirely different with clippers in that different fracture modes are likely to be generated compared with the test using scissors. This may result in differences in comparing mechanical data from scissors and clippers.

After calibration, the nails were tested by placing samples between the blades and repeating the cutting procedure previously described. Test pieces about 3 mm×3 mm were cut from the nail clippings using sharp scissors (see Fig. 1B). It was not possible to obtain larger sample sizes than this in the proximal direction. A crosshead speed of 8.3×10^{-5} m s^{-1} was found to be effective in making a clean cut through the samples. Each sample was cut in both the lateral direction (perpendicular to the growth axis) and the longitudinal/proximal direction (parallel to the growth axis). Thirty nail samples were tested at each humidity level, and in each cut direction. Samples were placed within the jaws of the clipper and tested within 4 min, after which time significant moisture regain is reported to occur (Schulz et al., 2002).

The force required to cut through the samples was recorded by the instrumentation software, and the energy was calculated as the integral of the force–displacement curve. The energy required to cut through the sample was then calculated as the total energy minus the energy required to close the clippers without a sample in place. In order to calculate the cross-sectional area of the cut, their lengths were measured using callipers, and then this value was multiplied by the mean thickness of the nail. The thickness of the nails was measured using a micrometre screw gauge calliper, taking three measurements per sample. The energy required to cut the sample was then divided by the cross-sectional area to give a value for the work of fracture.

Scanning electron microscopy

In order to observe the fracture surface of a torn sample of nail to see the form of the fibrous structure, samples were mounted onto aluminium stubs, with carbon tabs attached, and gold coated using an Edwards S150B Sputter Coating Unit for a period of 20 s. Images of the torn surfaces were then obtained using a Topcon 300 Series scanning electron microscope (SEM). Samples were placed within the SEM chamber, and scanned using a beam of energy of 5 keV.

RESULTS

Moisture content of nails

The data obtained for the moisture content of the nails as a function of RH are presented in Fig. 2. They show that varying the environmental RH had an effect on the moisture content of the nails, and that this relationship is non-linear. The highest water content of 64% (±3.4% s.d., N=10) of dry mass was obtained by placing the nails over distilled water, and the lowest of 6% (±2.0% s.d., N=10) by placing them over silica gel. The *in vivo* moisture content of the nails was between 14% and 30% of dry mass (mean=19.6±5.0% s.d., N=10, equivalent to hydration of between 55% and 80% RH).

Clipper cutting tests

A typical load–displacement curve for a nail sample is shown in Fig. 3A. The initial linear increase seen in the data (up to ~1 mm) was due to the clippers coming together and being strained before the cut was made; the peak in force occurred when the nail was being cut.

The work of fracture of nail samples using the clipping technique varied between 11 and 22 kJ m^{-2} (see Fig. 3B). It is apparent from the figure that humidity had a significant effect on the work of fracture, in both the lateral and proximal directions ($F_{5,114}$=12.12, P<0.001), reaching a maximum at 55% RH and falling at both low and high humidity. The nails showed anisotropy in the work of fracture, at all levels of humidity, on average having a work of fracture 1.5 times higher in the proximal compared with the lateral direction. Furthermore, anisotropy, defined as the ratio between the proximal and lateral works of fracture, also differed significantly between humidity levels ($F_{5,53}$=15.73, P<0.001). The anisotropy ratio (the proximal to lateral work of fracture ratio) was nearly equal to 2 at 55% RH, which is much larger than at all other humidity levels.

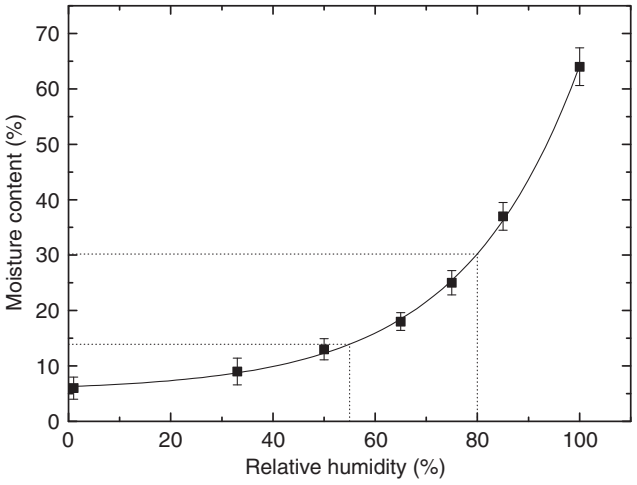

Fig. 2. Moisture content of human fingernails as a function of the relative humidity (RH). Error bars are standard deviations from the mean. Data were fitted using an exponential relationship of the form $y=A_1\exp(x/t_1)+y_0$ where A_1=0.8% RH, t_1=23.2% RH and y_0=5.5% RH; R^2=0.9.

DISCUSSION

The results show that it is possible to control the percentage water content of fingernails using saturated salt solutions. The *in vivo* moisture content of the nails was variable, but was nevertheless in agreement with the findings of other researchers (Schulz et al., 2002) who showed that the moisture content ranged between 10% and 60%. The values are also similar to the moisture content of other keratinous structures. Bertram and Gosline showed that the moisture content of hoof wall varied considerably at different RH levels (Bertram and Gosline, 1987), but a study by Leach on hoof wall showed that it had an *in vivo* moisture content of 17–24% (Leach, 1980). Ostrich feathers, another predominantly keratinous material, have been shown to contain 11% moisture at 50% RH, but claw material from the same species had only 4.5% moisture at the same RH (Taylor et al., 2004). Differences between our data and those of other workers are probably due to morphological differences between samples.

The non-linear relationship between the moisture content of nails and RH may be due to diffusion characteristics; the data are of a typically Fickian nature – a greater amount of water is taken up by the nails as the RH is increased. This may reflect the transition from nails containing mainly tightly bound water to containing both tightly and loosely bound or surface water. It may be the case that the keratin fibres expand with increasing RH, giving rise to an increase in the available surface area to which the water can bind. Saturation may also occur, whereby the internal structure is fully saturated and therefore the surface of the nail takes on more water. The data

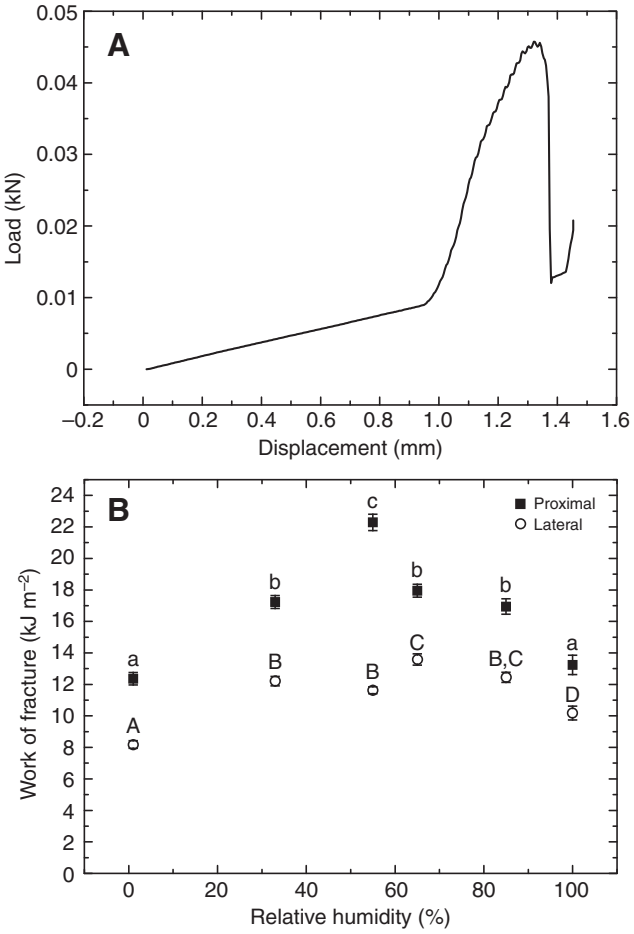

Fig. 3. (A) A typical load–displacement curve for a clipper test and (B) the work of fracture values for nail samples tested in both the proximal and lateral directions at a number of humidity levels. Groups denoted by the same character are not significantly different from each other at $P<0.05$ according to Tukey's *post-hoc* tests. Error bars are standard deviations from the mean.

indicate that the RH levels of 55–80% are close to what one might expect *in vivo*; a moisture content of greater than 63% is only likely to be reached if the nails are fully immersed in water.

The clipper test that we used provides a reliable technique for estimating the work of fracture, albeit in a cutting mode rather than generating controlled crack growth. Preliminary tests have shown that the amount of energy required to close the clippers is consistent from test to test, and this is therefore an improvement on scissor cutting used in previous studies (Eichhorn et al., 2007; Farren et al., 2004; Lucas and Pereira, 1990; Vincent, 1992) in which friction between the scissor blades during closure may have resulted in inconsistency and experimental error. The test proved useful for nails conditioned in the range 33–100% RH. When testing nails conditioned at 0% RH it was found that the mode of failure was extremely rapid, and was therefore difficult to detect using the equipment. It was possible to obtain reliable data in the proximal direction at 0% RH, although there was a different fracture process; brittle fracture occurred leaving a powder rather than cleanly cut surfaces. The most likely reason for the brittle fracture of the nails at 0% RH is a desiccation of the protein matrix, leading to crack growth along many interfaces where typically water was present prior to drying.

The force–displacement trace (Fig. 3A) itself shows some small steps during the fracture of the nail. These are due to the low sampling rates used to collect the data. It can be seen that the steps occur during the initial loading period, before the sample is being tested, and so are nothing to do with the fracture of the specimen itself.

The work of fracture values determined in this study (~11–22 kJ m^{-2}) are higher than those previously published by Farren and colleagues of ~3–6 kJ m^{-2}, which were found using a scissor apparatus (Farren et al., 2004). These differences may be due to the fact that Farren and colleagues kept their samples wet until testing took place (Farren et al., 2004), and so it would be expected that lower work of fracture results would be obtained. The data are, however, in reasonable agreement with values obtained by Pereira and colleagues of ~6–17 kJ m^{-2} (Pereira et al., 1997). It is interesting to note that Pereira and colleagues found that fingernails were 'tougher' (higher work of fracture) in the lateral direction (Pereira et al., 1997), which is the opposite of what we have found in our study. It seems counter-intuitive that it would take more energy to cut between fibres, in the lateral direction, than across fibres in the proximal direction.

Samples soaked in water were tested using the clippers and a value of 8.35±1.95 kJ m^{-2} (N=7) was found for their proximal work of fracture. This value is slightly larger than values obtained using scissors on fully immersed samples (Farren et al., 2004), but well below the value found using clippers at 100% RH (about 13 kJ m^{-2}). This suggests that the results are broadly similar to those obtained using scissors, but a different value was expected given that the fracture mechanisms of the nail are thought to be different with clippers than with scissors. The value obtained for soaked nails is, however, similar to those of Pereira and colleagues, who used sharp scissors to cut their tissue (Pereira et al., 1997).

The present data show that humidity influences the work of fracture of nails, particularly when cut in the proximal direction. Nails were found to be much more resistant to cutting in the proximal direction at all humidity levels ($F_{5,54}$=98.66, $P<0.001$), particularly at 55% RH, where the work of fracture was found to be nearly twice that in the lateral direction. A SEM image of a nail torn (using a trouser tear test) laterally is shown in Fig. 4. The fibres are predominantly oriented in the lateral direction, and so it is conceivably easier to fracture the sample in this direction. In contrast,

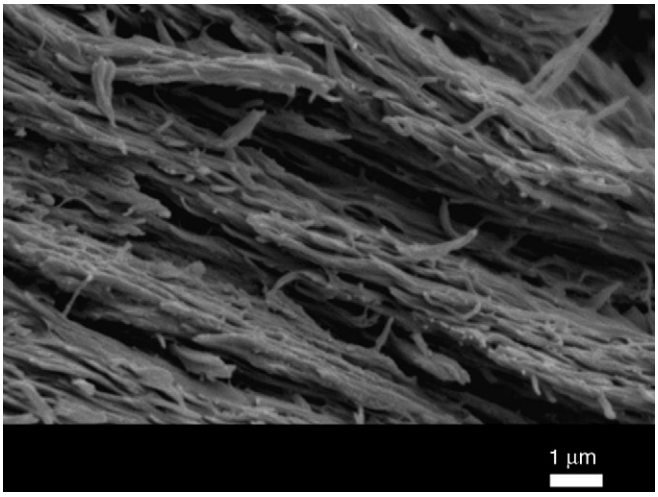

Fig. 4. Scanning electron microscope image of a torn nail sample fractured in the lateral direction.

cutting proximally requires fibre fracture, which takes more energy than breaking the interfaces between fibres. The mechanism by which fracture occurs more readily at 55% RH in the lateral direction may be plasticisation of the matrix material that binds the fibres together. This matrix material is known to contain a large number of sulphur bridges, which connect the matrix to the fibres (Crewther and Dowling, 1960; Gillespie and Simmonds, 1960). It is therefore thought that a breakdown of these sulphur bridges may lead to a plasticisation of the matrix. A study of the relative sulphur content of nails between mammalian species may therefore prove insightful as to their relative ability to resist fracture and their habitat and behavioural characteristics (e.g. foraging, etc.). The large fracture anisotropy at 55% RH suggests that cracks are very unlikely to propagate longitudinally, towards the nail bed. This is advantageous in terms of preventing irreversible damage to the nail unit, and shows that at this RH (which is within the *in vivo* region) the nails behave in a mechanically optimal manner.

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