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Composite theory and the effect of water on the stiffness of horn keratin

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α-keratin is a hydrogen-bond dominated composite material. The dry α-keratin (0% regain) of the horns of an oryx has a stiffness of 6.1 GPa. Water interacts only with the amorphous matrix of α-keratin to break down structural hydrogen bonds and reduce stiffness to 4.3 GPa at 20% regain and 1.8 GPa at 40% regain. The effect of water on the stiffness of horn α-keratin is not modelled by the Voigt estimate at high to moderate regains. Water interacts probably with the disordered regions within the fibres which reduces the effective fibre length. As a result the reinforcing effect of the fibres is reduced and the stiffness and strength of hydrated horn keratin are less than that predicted by the simple Voigt estimate. If the Voigt estimate is modified to take into account a short fibre length of 40 nm, the stiffness and tensile strength of horn α-keratin can be modelled successfully.

1. Introduction

The stiffness or Young's modulus of composites can be determined from the stiffnesses or Young's moduli and proportions of their component materials. For example, the Young's modulus (E_c) parallel to the fibres in a unidirectional fibre composite is given by the Voigt estimate (e.g. [1])

$$E_c = E_f V_f + E_m V_m \quad (1)$$

where E_f = Young's modulus of fibres (Pa), E_m = Young's modulus of matrix (Pa), V_f = volume fraction of fibres and V_m = volume fraction of matrix.

The Voigt estimate of composite stiffness is applicable to composites with discontinuous fibres if the shear modulus of the matrix and/or length of the fibres are sufficient to transfer stress effectively to the fibres via the matrix [1]. Strength can be modelled similarly by substituting strength of fibres and matrix for their stiffnesses [1]. Insect cuticle is the only biological composite which has been modelled successfully using a modified Voigt estimate of composite Young's modulus or stiffness which takes into account a short fibre length [2].

The stiffness and strength of a short discontinuous fibre composite will be less than their Voigt estimates. This is because the load cannot be fed directly into the ends of the fibres as it can in a continuous fibre composite. Instead the matrix must shear at each end of the fibres to build up the fibre stress to the level found in the continuous fibre composite. The proportion of the fibre over which the stress is built up is dependent on the length of the fibre and the shear modulus of the matrix [3]. Therefore, the average fibre stress in a discontinuous fibre composite will always be less than the fibre stress of a continuous fibre composite of the same volume fraction of fibres. As a result stiffness and tensile strength will also be less.

α-keratin is a widespread biological composite consisting of stiff protein fibres and a pliant protein matrix [4]. The fibres or microfibrils are made up of eleven three-stranded α-helical chains, but the matrix is an amorphous assemblage of proteins [5]. α-keratin is a material dominated by hydrogen-bonds (H-bonds) although there are some covalent disulphide bonds in the matrix [4]. The stiffness of H-bond dominated materials is proportional to the density of effective H-bonds [6]. Water can disrupt these "structural" H-bonds to reduce the density of effective bonding, thereby reducing the stiffness of the material. In α-keratin water interacts only with the amorphous matrix and not with the crystalline fibres [7]. Most studies of the effects of water on α-keratin have used wool (e.g. [8–10]) and have concentrated on the mechanism which produces the characteristic sigmoidal sorption/desorption isotherms. Horn α-keratin shows similar sorption/desorption behaviour [11]. There are few data available on the effect of water on the mechanical properties of α-keratin (e.g. [8, 12]) and none exist for horn α-keratin.

In the experiments reported here the stiffnesses and strengths of dry (0% regain), wet (40% regain) and intermediate or fresh (20% regain) horn keratin were measured using the horns of an oryx, *Oryx g. gazella* (L.). Changes in stiffness and strength due to the effect of water were modelled using composite theory taking into account changes in the volume fraction and mechanical properties of the matrix caused by changes in hydration. The effect of water on the mechanical properties of the matrix were measured using a torsional pendulum. Stiffnesses were measured in three-point bending and strengths were measured in tension.

2. Experimental methods

2.1. Preparation of test-pieces

The oryx horns were obtained *post mortem* and stored in a domestic freezer until required. Each horn was cut

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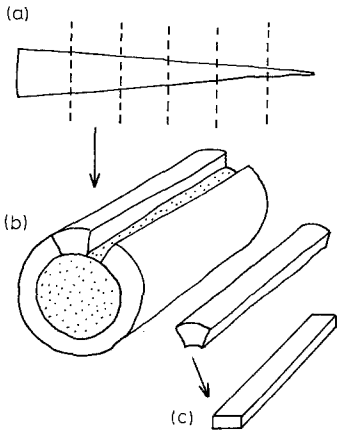

Figure 1 The preparation of test-pieces from the horn of an oryx. (a) The horn is cut up into sections 80 mm long, (b) longitudinal radial cuts produce strips of horn α-keratin which are machined square (c) on a milling machine.

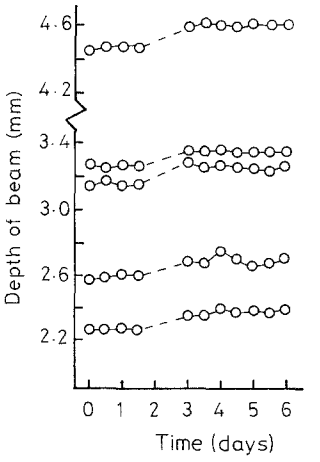

Figure 2 The swelling of horn α-keratin in water. Swelling is complete after three days.

into sections approximately 80 mm long. A series of longitudinal radial cuts was made into each section of horn to produce strips of keratin 80 mm × 15 mm × 10 mm. The strips were milled using an edge cutter until square. No problems were encountered concerning the overheating of the keratin during milling. The above procedure is summarized in Fig. 1. The test-pieces so produced represent intermediate or fresh horn. No special storage conditions were needed for fresh horn because stiffness was measured immediately after the test-pieces had been prepared.

2.2 Measurement of stiffness

All the test-pieces from both horns were tested as fresh material in three-point bending using an Instron 4202 testing machine with an output to a chart recorder. Tests were carried out at room temperature at an arbitrary loading speed of 2 mm min^{-1} and a bending span of 45 mm. The gradient of the force–deflection curve produced by the chart recorder for each test was used to calculate bending stiffness or Young's modulus in bending using standard beam formulae (e.g. [13]).

The test-pieces were weighed so that changes in water content (regain) of the keratin could be measured. The test-pieces were immersed in distilled water for one week to produce wet horn. Measurements of the changes in dimensions of the test-pieces made twice daily show that horn keratin undergoes a rapid and complete swelling after about three days (Fig. 2). The test-pieces were retested and reweighed as described above to measure the stiffness of wet horn. The test-pieces were then put in an oven at 130° C for 24 h to produce dry horn. The test-pieces were retested and reweighed. The test-pieces from only one pair of horns were used in the above procedure to minimize between-sample and between-animal variations in stiffness.

2.3. Torsional pendulum

Test-pieces of fresh, wet and dry horn keratin approximately 110 mm × 3 mm × 3 mm in size were prepared from the horns of an oryx as described above. They were mounted in a compound torsional pendulum and left to equilibrate for 15 min. The angular scale was zeroed at the equilibrium position. An angular displacement of 15° was applied to the test-pieces and the period of oscillation was recorded for up to 10 cycles. This was repeated twice more for each test-piece to test for repeatability of results. The tests were carried out at room temperature in air.

The shear or storage modulus of the matrix (G_m) which represents the elastic component of the viscoelasticity of the horn keratin was calculated as follows (e.g. [14]):

$$G_m = I\left(\frac{4\pi^2}{T^2} - \frac{1}{T'^2}\right)\left(\frac{L}{K}\right) \quad (2)$$

where I = inertia of bar and masses of torsional pendulum (42 000 kg mm^2), T = period of oscillation (sec), T' = period of oscillation of apparatus without specimen (110.5 sec), L = length of test-piece (m) and

$$K = ab^3\left[\left(\frac{16}{3}\right) - 3.36\left(\frac{b}{a}\right)\left(1 - \frac{b^4}{12a^4}\right)\right] \quad (3)$$

where a and b are the transverse dimensions of the test-pieces and a is greater than b [14].

2.4. Tensile tests

Test-pieces of fresh, wet and dry horn keratin approximately 150 mm × 15 mm × 2 mm in size were prepared from the horns of an oryx as described above. Tabs of aluminium approximately 40 mm × 15 mm × 2 mm were folded lengthways in half and fixed to each end of the test-pieces using Araldite epoxy resin adhesive. The tabs ensured that the test-pieces were not damaged by clamping and that the load was fed smoothly and evenly into the ends of the test-pieces.

The test-pieces were mounted in the Instron and loaded at 2 mm min^{-1} until complete failure. Tensile strength was calculated using standard formulae (e.g. [13]).

3. Experimental results

3.1. The effect of water on the bending stiffness of horn keratin

Fresh horn has a mean stiffness of 4.3 GPa (n = 10) and a regain of approximately 20% (Table I). This is within the range of tensile and compressive stiffnesses of other α-keratins at intermediate relative humidities

TABLE I The effect of water on the bending stiffness of horn keratin and Voigt estimates of composite stiffness

	Dry	Fresh	Wet
Regain (%)	0	20	40
Mean bending stiffness (GPa) (standard error)	6.1 (0.16)	4.3 (0.09)	1.8 (0.09)
Range of stiffnesses (GPa)	4.8 to 6.8	3.8 to 4.9	1.4 to 2.3
E_f (GPa)	6.1	6.1	6.1
E_m (GPa)	6.1	3.1	0.9
V_f	0.61	0.56	0.53
V_m	0.39	0.44	0.47
G_m (GPa)	2.3	1.1	0.9
Voigt estimate of bending stiffness (GPa)	6.1	4.8	3.6
Modified estimate of bending stiffness assuming a fibre length of 40 nm (GPa)	6.1	3.8	1.9

which vary between 2.3 GPa for human hair and 5.8 GPa for porcupine quill to give a mean of 4.0 GPa [4]. The stiffness of fresh horn is increased by more than 40% when it is dried to 0% regain. The mean stiffness of dry horn is 6.1 GPa (n = 8) (Table I). The stiffness of dry horn is similar to the tensile and compressive stiffnesses of Cotswold wool (5.6 GPa), horsehair (6.5 to 6.8 GPa) and porcupine quill (6.7 GPa) at 0% relative humidity [4].

The stiffness of fresh horn is decreased by almost 60% when it is hydrated to 40% regain. The mean stiffness of wet horn is 1.8 GPa (n = 10) (Table I). The stiffness of wet horn is similar to the tensile stiffnesses of human nail (1.8 GPa), human hair (1.5 GPa) and Cotswold wool (2.0 GPa) [4].

3.2. The effect of water on the shear modulus of the matrix

The shear modulus of the matrix of fresh horn was 1.1 GPa (n = 2, standard error (s.e.) = 0.001). Unfortunately, test-pieces were difficult to prepare so that the data are rather limited. The shear modulus of the matrix of wet horn as measured in the torsional pendulum as 0.3 GPa (n = 2, s.e. = 0.026).

It was not possible to measure the shear modulus of dry horn due to catastrophic failure of the test-pieces when the slightest torque was applied to them. An estimate of the shear modulus, $(G_m)_{dry}$, of dry horn can be made as follows (e.g. [4]):

$$(G_m)_{dry} = \frac{E_{dry}}{2(1 + \nu)} \quad (4)$$

where E_{dry} = stiffness of dry horn keratin (6.1 GPa) and ν = Poisson's ratio of dry horn.

The use of this equation assumes that the material is isotropic, which horn keratin is not. However, it does give a rough idea of the shear modulus of dry horn.

A Poisson's ratio of 0.23 has been measured for sheep horn [15] and a Poisson's ratio of 0.4 was measured repeatedly for a single test-piece of oryx horn [16]. In view of the paucity of data concerning the Poisson's ratio of horn keratin, an arbitrary value of 0.3 was used to give a $(G_m)_{dry}$ of 2.3 GPa. The actual value of Poisson's ratio used in Equation 4 in this case will not affect greatly the calculated shear modulus. For a variation in Poisson's ratio of between 0.2 and 0.5, the shear modulus will vary between 2.5 and 2.0 GPa.

3.3. The effect of water on the tensile strength of horn keratin

The tensile strength of fresh horn is 122 MPa (n = 38, s.e. = 2.9). These data include results from notched specimens because it had been established that fresh horn is a notch-insensitive material [16].

The tensile strength of dry horn is 137 MPa (n = 4, s.e. = 5.7). Few data are available due to the extreme notch-sensitivity of dry horn. The tensile strength of wet horn is 56 MPa (n = 13, s.e. = 4.0). This sample includes also notched specimens because of the notch-insensitivity of wet horn [16].

4. Composite theory

The Voigt estimate of composite stiffness was applied to bending stiffness assuming that the stiffness of horn keratin is similar in tension and compression. This seems reasonable considering the similarity between bending stiffness of horn keratin and the tensile stiffness of other keratins (seen above) [4]. It was assumed also that the fibres were unidirectional and parallel to each other.

The parameters needed for the calculation of the Voigt estimate of bending stiffness are given in Table I. The stiffness of both the fibres $(E_f)_{dry}$ and the matrix $(E_m)_{dry}$ at 0% regain are assumed to be equal because the stiffness of the matrix approaches that of the fibres with decreasing regain (see [4]). The stiffness of the matrix at the higher regains ($(E_m)_{fresh}$, $(E_m)_{wet}$) was calculated using Equation 4 and the experimental shear moduli. A Poisson's ratio of 0.5 was assumed for the fully hydrated random matrix which was assumed to represent an isotropic, incompressible material. This gives an $(E_m)_{wet}$ of 0.9 GPa. The Poisson's ratio of stiff biological materials is characteristically less than 0.5 and greater than 0.2 [17]. Therefore, it is assumed that the Poisson's ratio of stiff keratin matrix in fresh horn is 0.4 to give $(E_m)_{fresh}$ = 3.1 GPa. In this case quite large variations in Poisson's ratio do not affect greatly the calculated matrix stiffness.

The proportions of fibrous proteins (V_f) varies from 0.82 in cow horn to 0.88 in sheep horn [12]. There are no data available for oryx horn. There is a model for wool [18] which shows that 26% of the fibrous proteins is amorphous so that V_f could be as low as 0.61 to 0.65 in dry horn if the same model were to apply to horn keratin. The microfibrils are identical in all mammalian hard keratins so that this assumption is

probably reasonable [12]. The volume of the test-pieces of fresh horn was 20% greater than the volume of the test-pieces of dry horn. The weight of the test-pieces of fresh horn was also 20% greater than for dry horn. Wet horn test-pieces were 40% greater in volume than dry horn test-pieces. Therefore, V_m should be 20% greater in fresh horn and 40% greater in wet horn compared to dry horn. This reduces V_f to the values given in Table I assuming that the α-helical regions of the fibres do not take up any water [7].

The Voigt estimate of the stiffness of dry horn will be the same as the experimental result because it is assumed that the stiffnesses of the matrix and fibres are identical [4]. The Voigt estimate of the stiffness of fresh horn is greater than the mean experimental result (Table I). The estimate is 12% greater than expected, but well within the range of experimental results. The Voigt estimate for the stiffness of wet horn keratin is 100% greater than the mean experimental result and exceeds the greatest stiffness recorded experimentally (Table I). Therefore, the Voigt estimate does not model the stiffness of horn keratin at high to moderate regains.

5. The effect of water on the stiffness of a discontinuous fibre composite

The overestimate of composite stiffness of fresh and wet horn keratin by the Voigt model could be due to the inability of the matrix to shear sufficiently to transfer stress effectively to the fibres, given their length. There is a length at the end of the fibres where the matrix shear reaches a maximum to transfer stress to the fibres. This is known as the transfer length of the fibres or the critical fibre length (e.g. [1]). In the case of fresh and wet horn, water has hydrated the matrix to such an extent that the transfer length of the fibres is a significant proportion of the total length of the fibres. This leads to a significant reduction in the reinforcing effect of the fibres and consequently in stiffness and strength of the composite. In discontinuous fibre composites with very long fibres and/or a very large matrix shear modulus the transfer lengths of the fibres is negligible compared to the length of the fibre, so that the average fibre stress is close to that expected for a continuous fibre composite. Only in this case can the simple Voigt model be used.

The stiffness (E_c') of a discontinuous fibre composite is a modification of the Voigt model and can be calculated as follows [3]:

$$E_c' = E_f V_f \left(1 - \frac{\tanh(\beta L)}{\beta L}\right) + E_m V_m \quad (5)$$

where

$$\beta = \left(\frac{2G_m}{E_f r^2 \ln(R/r)}\right)^{1/2} \quad (6)$$

and

$$\ln\left(\frac{R}{r}\right) = 0.5 \ln\left(\frac{2\pi}{3^{1/2} V_f}\right) \quad (7)$$

It is assumed here that the fibres are packed hexagonally. Symbols are as in Equation 1 except G_m = matrix shear modulus (Pa), r = fibre radius (3.65 nm), L = half fibre length (m) and R = interfibre distance (M).

The estimated composite stiffness as calculated by Equation 5 depends on the length of the fibres. However, there are very few data concerning the length of the microfibrils of α-keratin. Fibres have been traced for between 500 nm [12] and 1000 nm [19]. Both of these lengths overestimate the composite stiffness of wet horn.

A fibre length of 40 nm (i.e. L = 20 nm) gives a very good estimate of the stiffness of both fresh and wet horn. At 20% regain the modified Voigt estimate is 3.8 GPa compared to the experimental mean of 4.3 GPa (Table I). At 40% regain the modified Voigt estimate is 1.9 GPa compared to the experimental mean of 1.8 GPa (Table I). It would seem that the effective fibre length of horn α-keratin is approximately 40 nm.

A model has been proposed for wool which shows that every 15 nm along the protofibrils making up the microfibrils is a 5 nm disordered or amorphous region [18]. These disordered regions are staggered with respect to each other and it is assumed that this model applies also to horn keratin. The disordered regions have been included in the volume fraction of the matrix. Therefore, when water is taken up by the matrix the disordered regions of the protofibrils will also be hydrated so that the integrity of the fibres will be affected. It is possible that this is the mechanism by which the effective fibre length is reduced to about 40 nm to give the experimentally observed stiffnesses.

6. The tensile strength of a discontinuous fibre composite

The tensile strength of discontinuous fibre composites can also be modelled using a modified Voigt estimate which takes into account the length of the fibres and the shear modulus of the matrix [3].

The tensile strength (σ_t') of a discontinuous fibre composite is calculated as follows [1]:

$$\sigma_t' = \sigma_f V_f \left(1 - \frac{L_c}{2L}\right) + \sigma_m' V_m \quad (8)$$

where σ_f = tensile strength of fibres (Pa), σ_m' = stress in matrix at failure (Pa), L = half fibre length (20 nm) and L_c = transfer length of fibres (m). The use of this equation assumes that L_c is less than L. Therefore, the transfer lengths of the fibres must be calculated to see if it is appropriate to apply this equation. The transfer length of the fibres at different regains is calculated as follows [13]:

$$L_c = \frac{\sigma_f r}{2\tau_i} \quad (9)$$

where

$$\tau_i = E_f e_m \left(\frac{G_m}{2E_f \ln(R/r)}\right)^{1/2} \frac{\sin h(\beta L)}{\cos h(\beta L)}$$

The symbols are the same as above (see Equations 5 to 7) except that e_m is the strain in the composite at the point of failure.

The transfer lengths are given in Table II assuming a fibre length of 40 nm. At all regains the transfer lengths are less than the 20 nm half fibre length so that in all cases the effective fibres can be loaded to their tensile strength of 137 MPa. Therefore, it is

TABLE II The data used to calculate the transfer lengths of the fibres and the tensile strengths of horn keratin at different regains

	Dry	Fresh	Wet
Regain (%)	0	20	40
E_f (GPa)	6.1	6.1	6.1
σ_t (MPa)	137	122	56
e_m	0.022	0.028	0.031
G_m (GPa)	2.3	1.1	0.3
ln (R/r)	0.89	0.92	0.96
r (nm)	3.65	3.65	3.65
L (nm)	20	20	20
τ_i (MPa)	62	53	28
L_c (nm) (c.f. 20 nm)	4.0	4.7	8.9
Modified Voigt estimate of tensile strength (MPa)	137	98	65

appropriate to use Equation 8 to calculate the expected tensile strength of horn keratin at different regains.

The Voigt estimate of the tensile strength of dry horn will be the same as its mean experimental value (137 MPa) because it is assumed that the fibres and matrix have the same tensile strength at 0% regain (Table II) [4].

The tensile strength of fresh horn is calculated as 98 MPa from the modified Voigt estimate compared to the mean experimental strength of 122 $\pm$ 6 MPa (Table II). Therefore, the model underestimates by about 20% the actual tensile strength. The modified Voigt estimate of the tensile strength of wet horn is 66 MPa, which is greater than the experimental mean of 56 $\pm$ 9 MPa (Table II). However, the upper limit of the mean (65 MPa) is nearly as great.

Unfortunately, the modified Voigt model does not seem to be able to predict the tensile strength of horn keratin at different regains as well as the stiffness of horn keratin at the same regains given the assumed effective fibre length of 40 nm. However, the tensile strength of dry horn is particularly prone to error because it is a notch-sensitive material. Therefore, the mean tensile strength may be an underestimate. Further work must be done to replace any assumptions with real data wherever possible in order to test the accuracy of the models further.

Another possible cause of reduced stiffness and strength at high regains could be the disruption of the parallel unidirectional orientation of fibres due to the extreme plasticization of the matrix. Any fibres that are not oriented in the direction of the load will not be stressed so that the effective volume fraction of the fibres would be reduced [1].

7. Conclusion

The effect of water on the stiffness of α-keratin of an oryx can be modelled using a modified Voigt estimate of composite stiffness which takes into account the shear modulus of the matrix and the effective length of the fibres. When the matrix is hydrated its shear modulus is too low to transfer stress effectively to the fibres of the α-keratin so that the simple Voigt model always overestimates stiffness and strength.

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References

1. B. HARRIS, in Proceedings of Symposium of the Society for Experimental Biology No. 34, Leeds University, September 1979, edited by J. F. V. Vincent and J. D. Currey (Cambridge University Press, Cambridge, 1980) p. 37.
2. R. F. KER, DPhil thesis, University of Oxford (1977).
3. A. KELLY, "Strong solids" (Oxford University Press, Oxford, 1973).
4. R. D. B. FRASER and T. P. MACRAE, in Proceedings of Symposium of the Society for Experimental Biology No. 34, Leeds University, September 1979, edited by J. F. V. Vincent and J. D. Currey (Cambridge University Press, Cambridge, 1980) p. 211.
5. R. E. DICKERSON and I. GEIS. "The structure and action of proteins" (W. A. Benjamin, Menlo Park, California, 1969).
6. A. H. NISSAN, *Macromolecules* **9** (1976) 540.
7. M. DRUHALA and M. FEUGHELMAN, *Colloid Polym. Sci.* **252** (1974) 381.
8. J. B. SPEAKMAN, *J. Soc. Chem. Ind.* **49** (1930) 209.
9. I. C. WATT and J. D. LEEDER, *Trans. Faraday Soc.* **60** (1964) 1335.
10. S. ROSENBAUM, *J. Polym. Sci. C* **31** (1970) 45.
11. G. KING. *Trans. Faraday Soc.* **41** (1945) 479.
12. R. D. B. FRASER, T. P. MACRAE and G. E. ROGERS. "Keratins: their composition, structure and biosynthesis" (Thomas, Springfield, Illinois, 1972).
13. R. C. STEPHENS, "Strength of materials" (Arnold, London, 1970).
14. R. J. ROARK and W. C. YOUNG, "Formulas for stress and strain", 5th Edn (McGraw-Hill, London, 1975).
15. F. L. WARBURTON, *J. Text. Inst.* **39** (1948) 297.
16. A. C. KITCHENER, PhD thesis, University of Reading (1985).
17. S. A. WAINWRIGHT, W. D. BIGGS, J. D. CURREY and J. M. GOSLINE, "Mechanical design in organisms" (Arnold, London, 1976).
18. M. FEUGHELMAN, *J. Macromol. Sci.-Phys. B* **16** (1979) 155.
19. G. E. ROGERS, *Ann. N.Y. Acad. Sci.* **83** (1959) 378.

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