Influence of fibril taper on the function of collagen to reinforce extracellular matrix

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Collagen fibrils provide tensile reinforcement for extracellular matrix. In at least some tissues, the fibrils have a paraboloidal taper at their ends. The purpose of this paper is to determine the implications of this taper for the function of collagen fibrils. When a tissue is subjected to low mechanical forces, stress will be transferred to the fibrils elastically. This process was modelled using finite element analysis because there is no analytical theory for elastic stress transfer to a non-cylindrical fibril. When the tissue is subjected to higher mechanical forces, stress will be transferred plastically. This process was modelled analytically. For both elastic and plastic stress transfer, a paraboloidal taper leads to a more uniform distribution of axial tensile stress along the fibril than would be generated if it were cylindrical. The tapered fibril requires half the volume of collagen than a cylindrical fibril of the same length and the stress is shared more evenly along its length. It is also less likely to fracture than a cylindrical fibril of the same length in a tissue subjected to the same mechanical force.

Keywords: collagen; extracellular matrix; fibre reinforcing; tapered fibrils

1. INTRODUCTION

The extracellular matrix (ECM) is a biological composite material in which collagen fibrils provide reinforcement by resisting forces that tend to pull the tissue apart (Hukins & Aspden 1985). Collagen fibrils isolated from tissues of invertebrates (Trotter & Koob 1989; Thurmond & Trotter 1994; Trotter *et al.* 1994, 1998, 2000a) and vertebrates (Holmes *et al.* 1994, 1998; Birk *et al.* 1995, 1996; DeVente *et al.* 1997; Graham *et al.* 2000) have tapered ends. Tapered ends are also observed in collagen fibrils formed *in vitro* (Kadler *et al.* 1990; Holmes *et al.* 1992, 1996; Fertala *et al.* 1996). Measurements of axial mass distribution along these fibrils using dark-field scanning transmission electron microscopy have established that the taper is paraboloidal in shape (Holmes *et al.* 1992; Trotter *et al.* 1998, 2000a; Graham *et al.* 2000).

The purpose of this paper is to investigate how the taper influences the ability of collagen fibrils to reinforce the ECM. Collagen fibril lengths have been estimated as being from about 12–30 µm (vertebrates; DeVente *et al.* 1997) to 600 µm (invertebrate; Trotter & Koob 1989), which is low compared to the dimensions of most tissues. When a force is applied to the tissue, an interfacial shear stress will then be generated between the stiff collagen fibrils and the ECM macromolecules that surround them. By analogy with synthetic composite materials, this shear stress tends to stretch the fibrils; the restoring force in the stretched fibrils then balances the applied force (Hukins & Aspden

1985). The ECM contains a variety of macromolecules whose functions are primarily structural (proteoglycans (PG), hyaluronan, elastin, collagens) and other molecules that have accessory functions (mainly cell adhesion; Hukins *et al.* 1995). PG forms a hydrated gel that surrounds the fibrils (Hukins & Aspden 1985); in at least some tissues, molecules such as decorin and biglycan may have a mechanical role by being responsible for fibril–gel adhesion (Hedbom & Heinegaard 1993; Roughley & Lee 1994; Keene *et al.* 2000).

In general, stress transfer to the collagen fibrils can then be considered to occur by two physical mechanisms: elastic stress transfer (when the stress in the ECM, arising from the applied force, is low) and plastic stress transfer (when it is high) in which the PG-gel flows past the reinforcing collagen fibrils (Goh *et al.* 2004a). Eventually the applied force will be so high that the ECM and, hence, the tissue, ruptures. This paper is concerned with what happens before rupture, i.e. with first elastic and then plastic stress transfer. Although ECM is visco-elastic, it approaches elastic behaviour when loads are applied in time-scales much shorter than its relaxation time (see, for example, eqn 2 in Holmes & Hukins 1996); relaxation times typically have values of 10 min (tendon; Elliott *et al.* 2003) and 16 ± 8 min (intervertebral disc; Holmes & Hukins 1996). Following our previous work, elastic transfer is investigated by finite element (FE) modelling since there is no analytical theory for elastic stress transfer to non-cylindrical fibres (Goh *et al.* 2004b); plastic transfer is investigated theoretically (Goh *et al.* 1999). The present paper uses the theory and techniques developed previously to investigate how taper influences the ability of

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Figure 1. Model of a collagen fibril embedded in a coaxial cylinder of gel in the ECM. Here, L denotes the half-length of the fibril and r_o its radius at the fibril centre, O. L_m and r_m denote the half-length and radius, respectively, of the ECM model. The complete model can be obtained by rotating by 360° , about the line OD, and reflecting in OB. Replacing the paraboloidal profile by a rectangle leads to a model in which the fibril is cylindrical.

collagen fibrils to reinforce the PG-gel in the ECM. In order to investigate stress transfer, we consider a single collagen fibril and its surroundings (Goh *et al.* 1999, 2004a). Thus the purpose is to investigate the effect of fibril shape and not to model aspects of the mechanical behaviour of the ECM that might involve fibril–fibril interactions. We need not consider specific interactions between the collagen fibril and the PG-gel at the molecular level, because the fibril is so large (radius about 30 nm; Hukins *et al.* 1995) compared to the dimensions of the surrounding macromolecules that they effectively form a continuum.

2. METHODS

(a) The model

Figure 1 shows the model that has been used previously to determine the stress in a non-cylindrical fibre in a fibre-reinforced composite material (Goh *et al.* 2004a). In the context of this paper, the Z -axis is defined to be along the axis of a collagen fibril whose centre is at $Z=0$ and whose ends are at $Z=-1$ and $Z=+1$. Because of the symmetry of the model, we need only consider the axial stress distribution, σ_z , along a fibril from $Z=0$ to $Z=1$. The fibril is defined to have a length of $2L$ and a radius, at its centre, of r_o . We define the axial ratio of the fibril to be $q=L/r_o$. The response of the model to an applied force also depends on the Young's modulus of the fibril, E_f , and its surroundings, E_m .

(b) Validity of the model

At first sight, our model is restricted to fibrils whose tapers are identical at both ends. Such symmetric tapers are observed in collagen fibrils from ligaments of sea urchin (*Eucidaris tribuloides*) and sea cucumber (*Cucumaria frondosa*) (Trotter & Koob 1989; Trotter *et al.* 1998, 2000a). Asymmetric fibrils are observed *in vitro* (Kadler *et al.* 1990; Holmes *et al.* 1992, 1996; Fertala *et al.* 1996) and in developing tissues (Birk *et al.* 1995, 1996; Graham *et al.* 2000) where one end has a long slender taper (α -tip) and the other a shorter taper (β -tip). However, our model enables us to draw general conclusions about the effects of tapered fibrils which can be applied, separately if need be, to both ends, even if their tapers are not the same. Similarly, the results from the model can be applied to understanding the function of the tapered ends of fibrils

that are cylindrical in the middle (Trotter *et al.* 1998, 2000a,b). The principles also apply to tapers in which the slope changes and so have to be described as a combination of two paraboloids (Trotter *et al.* 1998, 2000a); the results from our model can be applied to each portion of the taper separately.

Our model is restricted to fibrils that are oriented in the direction of the tensile force applied to the tissue. Collagen fibrils in the ECM are oriented in the direction in which the tissue has to withstand tensile stress (Hukins 1984; Hukins & Aspden 1985). In some soft tissues collagen fibrils are further oriented by the application of tensile stress (Hukins *et al.* 1990).

(c) Assigning values to the parameters of the model

In order to implement the model, we require values of E_f/E_m . Measurements of E_f range from 10^8 to 10^{10} Pa (Cusack & Miller 1979; Ashby *et al.* 1995). To estimate E_m , we note that the nucleus pulposus of intervertebral disc, which contains a low proportion of randomly oriented collagen, has a Young's modulus of 10^4 to 10^5 Pa (Iatridis *et al.* 1997; Leahy & Hukins 2001); the hydrated gel in digital flexor tendon appears to have a Young's modulus of the order of 10^5 Pa (Hooley & Cohen 1979). Thus the upper and lower limits of E_f/E_m appear to be between 10^3 and 10^6 . This approach implies that the components are isotropic and is reasonable for the continuum surrounding the fibril, which is composed of a gel that is expected to have random macromolecular orientations. Collagen fibrils are cross-linked assemblies of oriented rod-like molecules (Hukins *et al.* 1995) and are, therefore, expected to have anisotropic properties. There is inadequate information to model their anisotropy. In any case, such a model would be more complicated than is required to investigate the influence of taper on the ability of collagen fibrils to reinforce the ECM.

Values of q range from 550 for fibrils from the medial collateral ligaments of young rats (DeVente *et al.* 1997) to 3500 for sea urchin ligaments (Trotter & Koob 1989). Difficulties in isolating intact fibrils, especially from vertebrates (Kadler *et al.* 2000; Trotter *et al.* 2000b) mean that the range may be much wider. At the bottom end q was reduced to 200. However, q was not increased at the upper end for two reasons. One was the difficulty of performing an FE analysis when q is very large. The other reason is that, at the upper limit of q , the taper is so slight, except near the tip, that the fibre closely resembles a cylinder with tapered ends, as described in the previous section; this result can be inferred intuitively from figure 1.

(d) Modelling elastic stress transfer

Elastic stress transfer was investigated by FE modelling as described previously (Goh *et al.* 2004a). However, previous work used an educational software package (ANSYS UNIVERSITY HIGH version 5.4, Ansys Inc., Houston, PA) that did not allow the range of q and E_f/E_m values required here to be investigated. The work reported here used a more versatile version of the software (ANSYS STRUCTURAL version 6.0, Ansys Inc., Houston, PA).

(e) Modelling plastic stress transfer

Plastic stress transfer is characterized by a constant value for the interfacial shear stress, τ (Goh *et al.* 1999). Then σ_z can be calculated, for cylindrical fibrils and fibrils with a parabolic taper, from τ and q , for Z in the range 0 to $+1$, using the appropriate formulae that do not depend on E_f or E_m . (Goh

Figure 2. Tensile stress distribution along paraboloidal (continuous line) and cylindrical (dashed line) collagen fibrils for elastic stress transfer for: (a) $q=3500$, $E_f/E_m=10^6$; (b) $q=200$, $E_f/E_m=10^6$; (c) $q=3500$, $E_f/E_m=10^3$; (d) $q=200$, $E_f/E_m=10^3$. In each case, σ_z/σ_c , the fibril stress divided by the applied stress, is plotted from the fibril centre ($Z=0$) to one end ($Z=1$).

et al. 1999). These analytical calculations yield the same results as an FE model with constant τ (Goh *et al.* 2000).

The advantage of this analytical approach is that the results for all values of q and all loading conditions can be plotted on a single graph, for a given fibre shape by plotting $\sigma_z/\tau q$ against Z (Aspden 1994; Goh *et al.* 1999, 2000).

At the molecular level, a constant value for τ implies a constant number of interactions per unit area at the fibril surface (Aspden 1994; Goh *et al.* 1999). A constant number of interactions per unit area is to be expected if the macromolecular composition does not change along the length of a fibril, e.g. if the number of decorin molecules in a D-period of the collagen fibril remains constant (Hedbom & Heinegaard 1993; Keene *et al.* 2000). In plastic stress transfer, the interfacial stress involves overcoming these intermolecular forces at the interface between the collagen fibril and its surroundings.

3. RESULTS

(a) Elastic stress transfer

The effect of a paraboloidal taper, compared with a uniform cross-section, is to reduce the stress at the fibril centre (figure 2). From this value it then rises to the end of the fibril where it has to fall rapidly to zero (assuming no force transmission across the fibril ends). In contrast, axial stress in the uniform fibril falls monotonically from its maximum at the fibril centre (figure 2). The variation of stress in the paraboloidal fibril, along most of its length is more uniform than that in the fibril without a taper, *i.e.* more of the fibril carries an appreciable stress. This effect can be seen in all four graphs representing the full range of values for q and E_f/E_m : (a) both high, (b) q low and E_f/E_m high, (c) q high and E_f/E_m low and (d) both low. The effect is less obvious in (c) (high q and low E_f/E_m). The shapes of the curves for the cylindrical fibril are those predicted by theory, indicating that the FE model is capable of providing reasonable results (Goh *et al.* 2004a). There is

Figure 3. Tensile stress distribution along paraboloidal (continuous line) and cylindrical (dashed line) collagen fibrils for plastic stress transfer. In each case, $\sigma_z/\tau q$, the fibril stress divided by q and the interfacial shear stress, τ , is plotted from the fibril centre ($Z=0$) to one end ($Z=1$).

no analytical theory that can be applied to stress transfer to a tapered fibril; this is the reason for using FE modelling to investigate elastic stress transfer.

(b) Plastic stress transfer

Figure 3 shows that for a cylindrical fibril, the stress rises from zero (at its ends) to a maximum value at its centre. For a fibril with a paraboloidal taper the stress rises more rapidly near the ends but less steeply near the centre. The overall result is that tapering the fibril leads to a somewhat more even stress distribution along the fibril length and that the stress at the centre of the fibril is reduced. Indeed the stress at the centre of a cylindrical fibril is 1.5 times that at the centre of a tapered fibril.

4. DISCUSSION

One advantage of having tapered collagen fibrils (resembling paraboloids) rather than their not being tapered (*i.e.* cylindrical) is to make more effective use of the collagen

synthesized by the cells in the tissue. For a tapered fibril, the axial stress distribution is more uniform along its length. This greater uniformity enables greater use of the full length of the fibril in reinforcing the ECM. The volume of a cylindrical fibril is twice that of a paraboloidal fibril (Trotter *et al.* 1994). Thus half as much collagen is required to make a paraboloidal fibril as a cylindrical fibril of the same length. This volume factor combined with the more uniform stress distribution, means that collagen is more effective in reinforcing ECM if it is used to make tapered fibrils rather than cylinders.

A second advantage of tapered fibrils is that they are less likely to fracture than cylindrical fibrils of the same length. When the force applied to a tissue is low, stress will tend to be transferred to its collagen fibrils elastically; increasing the force will lead first to plastic stress transfer and, eventually, to failure (see §1). During plastic stress transfer, the stress in a fibril is greatest at the centre. Thus the fibril centre is a potential site for failure. Failure is more likely in a cylindrical fibril, than in a paraboloidal fibril of the same length, because the tensile stress at its centre is 1.5 times as great.

Because elastic interfacial shear stresses are greatest near the ends of a tapered fibril, the transition from elastic to plastic behaviour will occur first near its ends. This will provide a fail-safe mechanism in that the risk of damage due to plastic behaviour or the fibril strength being exceeded in this regime will be limited to the fibre ends. This mechanism will maintain the length of a fibril for as long as possible during failure rather than suffering an immediate halving in length that may occur in a cylindrical fibril.

As a result of this second advantage, the growth of tapered fibrils is less likely to be disrupted by high mechanical forces applied to a tissue. It has been suggested that fibrils grow by the binding of collagen molecules to the surface, near their centres (Holmes *et al.* 1992, 1996); this accretion process leads to additional binding sites that are propagated so that the fibrils simultaneously grow in length and diameter (Trotter *et al.* 2000a,b). Fracture of the fibrils, at their centres, would then disrupt this growth process.

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