Confined compression of collagen hydrogels

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Abstract

Reconstituted collagen hydrogels are often used for in vitro studies of cell-matrix interaction and as scaffolds for tissue engineering. Understanding the mechanical and transport behaviours of collagen hydrogels is therefore extremely important, albeit difficult due to their very high water content (typically > 99.5%). In the present study the mechanical behaviour of collagen hydrogels in confined compression was investigated using biphasic theory (J. Biomech. Eng. 102 (1980) 73), to ascertain whether the technique is sufficiently sensitive to determine differences in the characteristics of hydrogels of between 0.2% and 0.4% collagen. Peak stress, equilibrium stress, aggregate modulus and hydraulic permeability of the hydrogels exhibited sensitivity to collagen content, demonstrating that the technique is clearly able to discriminate between hydrogels with small differences in collagen content and may also be sensitive to factors that affect matrix remodelling. The results also offer additional insight into the deformation-dependent permeability of collagen hydrogels. This study suggests that confined compression, together with biphasic theory, is a suitable technique for assessing the mechanical properties of collagen hydrogels.

Introduction

Reconstituted type I collagen hydrogels seeded with cells are often used in studies of cell-matrix interaction (Elsdale and Bard, 1972; Bell et al., 1979; Grinnell, 2003) and as scaffolds for tissue replacement therapies (Auger et al., 1998; Zaulyanov and Kirsner, 2007). Since the mechanical properties of a substrate are known to affect the behaviour of cells within it (Discher et al., 2005), it is essential that effective methods for the mechanical characterisation of collagen-based hydrogel scaffolds be developed.

In the present study we investigated the technique of confined compression. Collagen hydrogels may be considered biphasic, consisting of a loosely connected network of collagen fibrils (solid phase) filled with a large excess of interstitial fluid (fluid phase, typically > 99.5%). Under compressive loads the applied stress is initially supported by the fluid pressure, and then transferred to the fibril network (Chandran and Barocas, 2004); the rate at which this occurs being determined by the permeability and stiffness of the collagen matrix. Biphasic theory (Mow et al., 1980) has been used to characterise cartilaginous tissues (Ateshian et al., 1997; Iatridis et al., 1998; Périé et al., 2005), which may be considered to be similar in composition to collagen hydrogels. It was therefore hypothesised that biphasic theory may also be suitable to analyse the mechanical behaviour of collagen hydrogels.

A biphasic theory proposed by Barocas and Tranquillo (1997) has previously been applied to collagen hydrogels in confined compression (Knapp et al., 1997), although it can only be used in conjunction with data from rheological and unconfined compression testing. In contrast, the linear biphasic theory of Mow et al. (1980) allows estimation of the aggregate modulus (stiffness modulus in confined compression, H_A) and hydraulic permeability (k) of a material in confined compression – based solely on the stretch ratio (λ) and corresponding stress response – by appropriate fitting of the numerical solution for Eq. (1).

$$\frac{\partial^2 u}{\partial z^2} = \frac{1}{H_A k}\frac{\partial u}{\partial t}$$

(1)

In (1) $u = u(z, t)$ is the displacement of the solid phase in the direction z at time t. It should be noted that the aggregate modulus, H_A, is related to the Young's modulus, E, of the tissue by:

$$H_A = \frac{E(1-v)}{[(1+v)(1-2v)]} \qquad (2)$$

where v is the Poisson's ratio, and that k may be a function of stretch, i.e. $k = k(\lambda(z, t))$. While later models incorporated large strains and non-linear elasticity (Holmes, 1986; Holmes and Mow, 1990), it has been suggested that at low strain amplitudes collagenous tissue is better described by the linear model (Périé et al. 2005). Furthermore, the linear model has been successfully applied to 2% agarose gels (Gu et al., 2003), but to date the theory has not been applied to reconstituted collagen gels which characteristically contain less than 0.5% collagen.

This paper aims to characterise hydrogels of between 0.2% and 0.4% collagen to ascertain whether the linear biphasic model is sufficiently sensitive to determine differences in stiffness and permeability between gels. If successful, this technique may be considered validated to identify differences in gel behaviours associated with tissue growth.

Materials and Methods

Collagen (type I) was prepared from rat tail tendon and hydrogels (1 ml volume) set in standard 24-well tissue culture plates according to Elsdale and Bard (1972). Samples of diameter 16 mm, radially confined in the 24-well plates, were compressed with a porous platen (316L sintered steel mesh, $k = \sim6 \times 10^{-7}$ m^4/Ns) of diameter ~15.9 mm, attached to a 10 N load cell (Honeywell, NJ, USA). The load cell was displacement controlled via a BOSE ElectroForce® 3200 Test Instrument (BOSE, UK) and accompanying WinTest® software, which was also used for data acquisition of the stress response.

Eight collagen hydrogel samples were made at each of three collagen concentrations (0.2%, 0.3% and 0.4% (w/v)). Hydrogels were detached from the sides of the tissue wells

immediately prior to testing, and the bathing fluid removed. The hydrogel surfaces were found by lowering the platen until a pre-load of 0.01 N (0.05 kPa) was achieved, and then resting the system until the stress response equilibrated. The sample thickness was deduced from the displacement applied to reach this tare load, resulting in an average thickness of 4.79 ± 0.06 mm. Samples were then compressed by 5% at 0.5%/s and held for 300 seconds. Biphasic theory (Lai and Mow, 1980; Mow et al., 1980), utilising an exponential decrease in permeability with strain, $k(\lambda) = k_0 e^{M(\lambda-1)}$ (Lai and Mow, 1980), where M is a non-dimensional non-linear permeability coefficient describing the loss of permeability with compression, was fitted to the ramp and hold phases using a Nelder-Mead scheme (Riches, 2011). The tissue parameters H_A, k_0 and M, in addition to the peak and equilibrium stresses, were compared by analysis of variance (ANOVA) followed by Tukey's test, using SPSS (IBM, Armonk, NY) statistical analysis software.

Results

Compression testing of the collagen hydrogels provided stress responses with well defined ramp, peak and hold phases (Fig. 1). The stress responses in Fig. 1 are indicative of biphasic behaviour, with both peak stresses (p < 0.001) and equilibrium stresses (p = 0.016) demonstrating significant increases with increasing collagen content (Fig. 2a, b). The final 30s of the hold phase saw a less than 10Pa change in stress (~0.02g).

The zero-strain hydraulic permeability, k_0, decreased with increasing collagen concentration (p = 0.015), and was in the region of 10^{-10} m^4/Ns for 0.3% collagen hydrogels (Fig. 2c). H_A conversely increased with collagen content (p = 0.008), and was approximately 1 kPa for 0.3% collagen hydrogels (Fig. 2d). The nonlinear permeability coefficient M was significantly different to zero (p = 0.002), and also tended to increase with collagen content albeit non-significantly (Fig. 2e).

Discussion

To validate the technique and theory, compression tests were performed on collagen hydrogels of three different collagen concentrations. Similar experiments on materials with a

higher solid content have previously been performed using slower strain rates than adopted in the present study in order to avoid overly straining the surface layer of the material (e.g. Périé et al, 2006; Soltz and Ateshian, 1998; Wang et al, 2011). However, we propose that the high permeability of the collagen hydrogels allows relaxation to proceed quickly enough such that fast strain rates do not cause overly high localised solid strains, with no surface damage evident upon removal of the platen.

Both peak and equilibrium stresses demonstrated significant increases relative to collagen content, suggesting that these parameters may be used successfully to characterise mechanical differences in hydrogel properties, allowing for easy and direct comparison between various samples tested in the same way. Additionally, the main biphasic parameters of H_A and k_0 also varied with collagen content, clearly characterising the physical properties of stiffness and permeability in each case.

Measurement of H_A is important as scaffold stiffness can influence cell behaviour (Discher et al., 2005; Engler et al., 2006; Helary et al., 2010). Furthermore, the majority of commonly used methods for mechanical characterisation of soft biological tissues allow calculation of Young's modulus (Anseth et al., 1996), which can be used to compare tissue characteristics even where different testing methodologies have been utilised. Indeed, other studies using the same and alternative techniques have reported stiffness moduli of similar orders of magnitude to those calculated in the present study (e.g. Lee et al., 2001; Gu et al., 2003; Harley et al., 2007). Measurement of tissue permeability is also important to determine localised fluid flux, which through viscous shear forces, elicit cellular responses (Glossop and Cartmell, 2009; Trappmann et al., 2012; Wang et al., 2011). Furthermore, while the transport of small metabolites in collagenous tissues is dominated by diffusion (Urban et al., 1978; Roberts et al., 1996), convection may play an important role in the transport of enzymes and hormones (Roberts et al., 1996; Ferguson et al., 2004; Riches and McNally, 2005). Despite its potential significance, the hydraulic permeability of collagen hydrogels has received little attention prior to this study.

Increasing the collagen content in hydrogels results in a greater fibril density while maintaining a relatively constant fibril diameter (Roeder et al., 2002). It was therefore hypothesised that as collagen content increases k_0 should decrease, because a larger collagen content, and hence fibril density, would increase the viscous drag on the fluid flow. As expected, k_0 decreased with increasing collagen content (Fig. 2c), showing that the technique

can be used to differentiate between hydrogels of varying permeabilities, even where the permeabilities are very high: collagen gels tested in the present study demonstrated k_0 more than 1000× greater than 2% agarose gels (Gu et al., 2003). The stiffness of collagen hydrogels in tension increases relative to collagen content (Krishnan et al. 2004, Roeder et al. 2002), and consequently a larger fibril density should also translate to increased compressive matrix stiffness. Accordingly, the present results demonstrated significant increases in H_A relative to collagen content in hydrogels (Fig. 2d). It is interesting to note that, although not statistically significant, M also increases with collagen content, i.e. the loss of permeability with strain becomes more severe (Fig. 2e). This may be because hydrogels with a higher collagen content experience larger variations in absolute solidity following compression (a 5% increase in the solidity of a 0.4% collagen gel would, in absolute terms, be twice as much as for a 0.2% gel), and this disparity in changes in solidity with strain would manifest itself into a greater loss of permeability with strain for more concentrated hydrogels.

The results presented in this study suggest that confined compression, together with biphasic theory, is a suitable technique for assessing the mechanical properties of collagen hydrogels and other inherently weak hydrated tissues. It must be noted, however, there are other biphasic models which may elucidate the mechanical behaviour of collagen hydrogels equally or better. In particular, collagen can be considered a viscoelastic solid at the fibril level (Shen et al., 2011), and as such, a poroviscoelastic model (e.g. Suh and DiSilvestro, 1999) may be more suited to the characterisation of collagen hydrogels. Nonetheless, material properties and experimental stress values exhibited sensitivity to collagen content and therefore, this technique, depending on the tissue, may be sensitive to small changes in mechanical properties attributable to variations in the amount of synthesis or degradation of collagen or other macromolecules.

Acknowledgements

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Figure Legends

Figure 1: Average compressive stress (n = 8) in response to ramp-hold compressive strain (0.5%/s) for collagen hydrogels of different concentrations.

Figure 2: Variation in average peak compressive stress (a), equilibrium compressive stress (b), k_0 (c), H_A (d) and M (e) with collagen concentration. Error bars indicate one standard error of the mean with * implying $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.
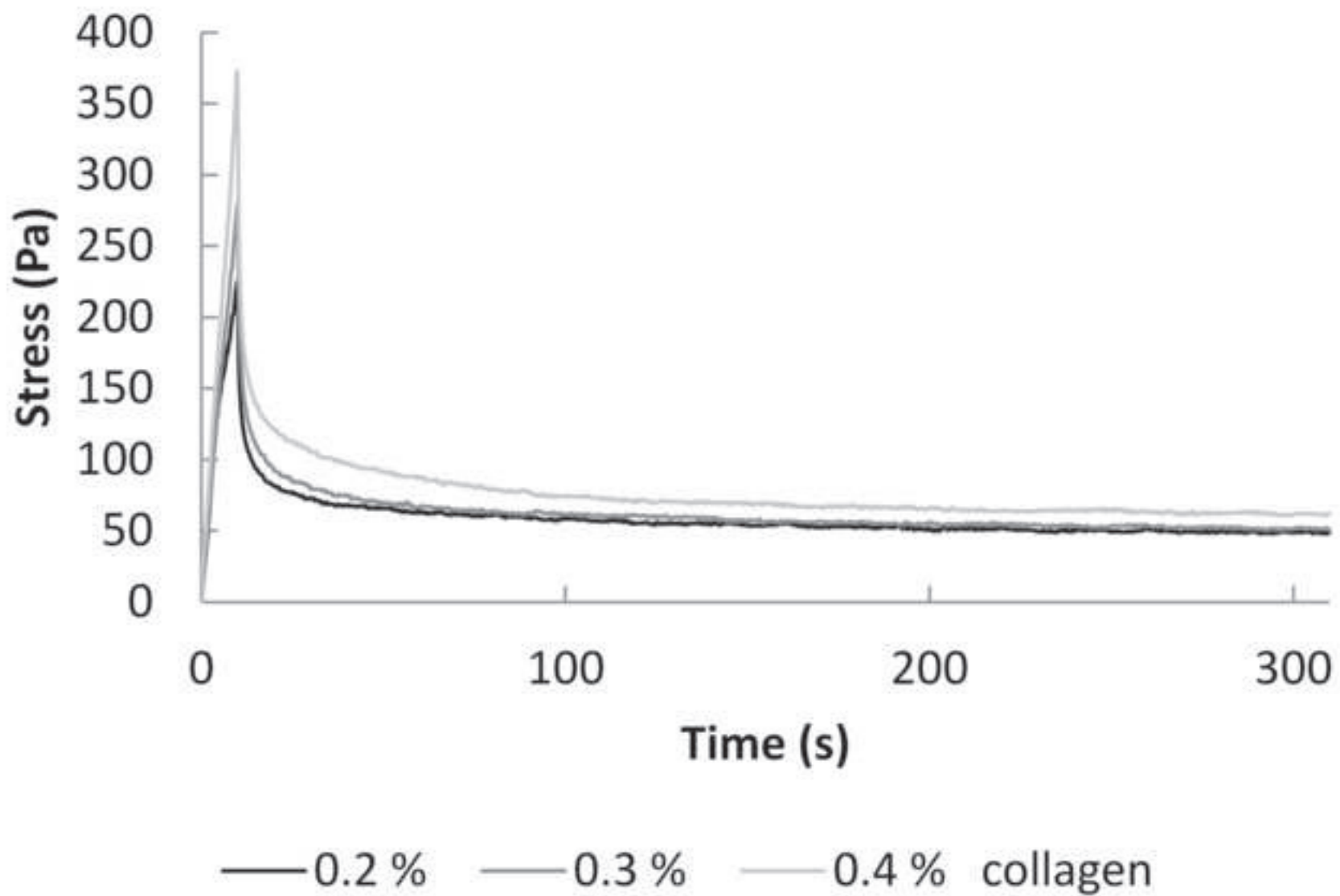
Figure 1
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