REVIEW

Whole cell mechanics of contractile fibroblasts: relations between effective cellular and extracellular matrix moduli

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While much is known about the subcellular structures responsible for the mechanical functioning of a contractile fibroblast, debate exists about how these components combine to endow a cell with its form and mechanical function. We present an analysis of mechanical characterization experiments performed on bio-artificial tissue constructs, which we believe serve as a more realistic testing environment than two-dimensional cell culture. These model tissues capture many features of real tissues with the advantage that they can be engineered to model different physiological and pathological characteristics. We study here a model tissue consisting of reconstituted type I collagen and varying concentrations of activated contractile fibroblasts that is relevant to modelling different stages of wound healing. We applied this system to assess how cell and extracellular matrix (ECM) mechanics vary with cell concentration. Short-term and long-term moduli of the ECM were estimated through analytical and numerical analysis of two-phase elastic solids containing cell-shaped voids. The relative properties of cells were then deduced from the results of numerical analyses of two-phase elastic solids containing mechanically isotropic cells of varying modulus. With increasing cell concentration, the short-term and long-term tangent moduli of the reconstituted collagen ECM increased sharply from a baseline value, while those of the cells decreased monotonically.

Keywords: cellular biophysics; contractile fibroblasts; viscoelasticity

1. Introduction

Dissecting the mechanical function and extracellular matrix (ECM) interactions of fibroblast cells is important for understanding wound healing (Tomasek *et al.* 2002; Gabbiani 2003; Desmouliere *et al.* 2005), pathologies such as fibrosis (Wakatsuki *et al.* 2004) and thermal therapies (Xu *et al.* 2008). An understanding

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One contribution of 9 to a Theme Issue ‘Multi-scale biothermal and biomechanical behaviours of biological materials’.

of these functions and interactions may also be of importance for molecular-level treatments for diseases involving aberrant responses of cells to mechanical stress (e.g. Ingber 2003).

The mechanics of a fibroblast is understood to be largely a function of three major protein structures: microtubules, which play a dominant role in directing tensile forces during cell division; actin filaments, which conduct forces to the ECM through integrin assemblies at focal adhesion sites and can form contractile stress fibres in conjunction with myosin; and intermediate filaments, which function as highways for directed vesicle movement and contribute to cellular mechanics at very large strains of the order of 20–50% (Janmey *et al.* 1991; Wang & Thampaty 2006). Recent evidence shows important roles for collagenase Brownian ratchet ‘motors’ in the form of TIMP-2/MMP-2 assemblies on the cell surface (Saffarian *et al.* 2004), and some hypothesize as well a role for cytoplasmic pressure gradients (Mitchison *et al.* 2008).

However, the system by which these subcellular proteins combine to endow a fibroblast with shape control and mechanical function is very much an open topic of debate (Discher *et al.* 2009). One model is the ‘tensegrity’ approach, in which microtubules act as internal equilibrators of tensile forces provided by actin cables (Ingber 1993). While our and other experiments agree that actin stress fibres exert tensile forces (Wakatsuki *et al.* 2000; Zahalak *et al.* 2000; Kumar *et al.* 2006), the disruption of microtubules results in an increasing force exerted by cells (Kolodney & Elson 1995). This indicates either that microtubules act in parallel with stress fibres to directly oppose contraction or that actin stress fibres do not rely on the compressive resistance from microtubules for their function (note that the increase in contraction is explained by the release of Ca^{2+} stores associated with microtubule disruption and the associated increased phosphorylation of the myosin regulatory light chain).

A different picture of the cell is one in which actin stress fibre tension is equilibrated by compressive resistance from the ECM, much like tension in bicycle wheel spokes (stress fibres) is equilibrated by compression in the rim (ECM). An embodiment of this picture through the Deshpande model (Deshpande *et al.* 2006) involves cells whose structure and mechanical function are mediated by stress fibre attachments to the ECM at focal adhesion assemblies. Our own experiments in three-dimensional tissue constructs are consistent with the picture of cell forces being directly proportional to stress fibre density (Nekouzadeh *et al.* 2008). Stress fibres assemble or disassemble in a two-dimensional culture in response to increased or reduced quasi-static mechanical loading and are mediated by the size of focal adhesions in a way that is predictable by the Deshpande model (Pathak *et al.* 2008). The dynamics of the focal adhesion assemblies are central to this functioning and may in fact drive cell architecture (e.g. Nicolas *et al.* 2008). A potential nanoscopic mechanism for the regulation of stress fibre density through force sensing by focal adhesion assemblies has recently been identified (Lee *et al.* 2007; del Rio *et al.* 2009) in which cryptic domains for vinculin binding within talin have been observed to open at physiologic levels of force, thereby allowing the recruitment of additional stress fibres.

Mechanical models for fibroblast function are difficult to test. The central challenge is that fibroblast cells in their natural environment are compliant relative to the surrounding ECM, making mechanical contributions of cells in

a natural tissue environment difficult to delineate. At the other extreme is testing cells on a two-dimensional substrate. Although accurate schemes exist for estimating cell tractions (Munevar *et al.* 2001; Franck *et al.* 2007), cell architecture changes fundamentally when cells are placed in a two-dimensional system. Measuring cellular response to mechanical stimuli poses an even greater challenge, as the natural structural system of a cell is difficult to probe in two dimensions. One of the earliest approaches to this was poking of a cell using an instrument like an atomic force microscope (AFM) developed in the 1970s (McConnaughey & Petersen 1980), and AFM poking of cells is now commonplace (Petersen *et al.* 1982). Additional schemes include displacement of magnetic beads connected to extracellular binding ligands, micropipette aspiration, and microrheology (Guilak *et al.* 1999; Mahaffy *et al.* 2000; Fabry *et al.* 2001; Georges & Janmey 2005). Each of these suffers from uncertainty owing to lack of connection to the natural structural network of a cell, and the reported values for the effective ‘elastic modulus’ of a cell range from 0.0002 to 0.1 MPa using these approaches. Although the patterning of cells onto substrates containing magnetically actuated, micropatterned ligand patches on elastic posts presents excellent data on cell forces, whole cell moduli cannot be deduced readily from these experiments (Sniadecki *et al.* 2007).

To avoid these problems, we study fibroblast cells in bio-artificial tissue constructs, which offer an *in vitro* environment that mimics the three-dimensional cellular environment of a natural tissue (e.g. Bell *et al.* 1979; Kolodney & Wysolmerski 1992; Asnes *et al.* 2006; Griffith & Swartz 2006). Contractile fibroblast cells within a collagen gel can remodel and compact the collagen ECM around them, resulting in a stiffening of the tissue construct (Isenberg & Tranquillo 2003; Pryse *et al.* 2003; Marquez *et al.* 2006*b*). Because these tissue constructs capture the strong interaction between cell and ECM components, these systems are preferable to studies of isolated cells. We have estimated effective elastic moduli ranging from 0.1 to 1 MPa for contractile fibroblasts that populate these tissue constructs sparsely (Marquez *et al.* 2005*a,b*).

Here, we develop an improved procedure for estimating the mechanics of cells and ECM in a bio-artificial tissue construct. Our procedure involves comparing mechanical tests performed on tissue constructs with subsequent tests performed on nominally identical tissue constructs, but with cells disassembled through the action of a detergent (deoxycholate). The procedure is demonstrated by establishing a relationship between the cell concentration and mechanical properties of cells and ECM in a tissue construct. The interdependence of contractile fibroblast concentration, ECM remodelling and mechanics is particularly important to the study of wound healing (Enever *et al.* 2002). Based on observations that cell shape and forces change as a result of collagen remodelling and collagen fibril density, we hypothesized that cell moduli would vary with the ECM modulus, which in turn would vary with cell concentration (Pizzo *et al.* 2005; Marquez *et al.* 2006*b*). We additionally hypothesized that, independent of ECM moduli, the moduli estimates of activated cells tested in a three-dimensional tissue construct would exceed those of cells in a two-dimensional culture.

We apply the analytical approaches in this paper to experimental data published elsewhere. The current work is distinct from our earlier work through a delineation of the mechanical contributions of cells and ECM to

tissue construct mechanics that enables the estimation of effective, isotropic, incremental (tangent) moduli of cells and ECM at specific loading levels. We summarize the experimental data in the next section and then describe the analytical procedures used to delineate ECM and cellular effective elastic moduli from the overall mechanical response of the tissue construct.

2. Background

The experimental data analysed involved the mechanical response of a tissue construct to rapid stretching of tissue constructs containing either (i) activated cells or (ii) voids remaining after cells were eliminated with deoxycholate.

Briefly, tissue construct specimens were synthesized from fibroblasts extracted from 10-day-old chicken embryos and from neutralized rat-tail collagen, as described elsewhere (Wakatsuki *et al.* 2000). Cultured fibroblasts were mixed with Dulbecco's modified Eagle's medium (DMEM) and type I rat-tail collagen at prescribed concentrations. The pH was brought to neutral using NaOH. Then, 1 ml of cell suspension was poured into the space between inner (9.5 mm diameter) and outer (14.9 mm inner diameter) cylinders of a Teflon mould.

After 3 days of culture in DMEM enriched with 10 per cent foetal calf serum, ring-shaped tissue construct specimens compressed to a measured fraction of their original cross-sectional area. Specimens beginning with 1 million cells ml^{-1} of cell suspension solution remodelled to a density of approximately 11 million cells ml^{-1} over these 3 days (Marquez *et al.* 2006b). Specimens were stretched by prescribed amounts and at prescribed strain rates, relative to their approximately 15 mm initial lengths (circumference of the inner diameter, flattened), on a mechanical testing apparatus while immersed in either serum-rich (HEPES-buffered DMEM, pH 7.4, with 3% foetal bovine serum) or deoxycholate (0.05% w/v deoxycholate in PBS, pH 7.4, 40 min exposure prior to testing) baths maintained at 37°C. Note that this level of exposure to deoxycholate eliminates the cellular contribution from the mechanical response to the tissue construct, but does not affect the mechanical properties of the ECM (Pryse & Elson submitted). Specimens were pre-conditioned with a stretch of 10 per cent over 150 s, and then returned to their reference length at this same rate. After a 3600 s relaxation period, they were stretched rapidly by a gravity loading (over 10–15 ms) first to a nominal strain of $\varepsilon = 0.02$, and then, after a 3600 s relaxation period, to a nominal strain of $\varepsilon = 0.08$. Conditions were designed so that inertial effects could be neglected (Nekouzadeh *et al.* 2005).

The isometric force following this second stretch was monitored at 200 Hz for 3600 s, over which time the force dropped from a post-stretch peak as a logarithmic function of time (figure 1). 'Short-term' and 'long-term' moduli (isometric force divided by the initial cross-sectional area, measured using confocal microscopy) at times of 10 ms and 3600 s post-stretch, respectively, were reported for both the intact tissue constructs (tested in a serum-rich bath) and the tissue constructs with cells removed (a 'deoxycholate-treated construct' tested in a deoxycholate bath; Marquez *et al.* 2006b). Here, we interpret these data to estimate short-term and long-term elastic moduli for the cells and ECM in these tissue constructs.

Figure 1. Logarithmic relaxation curves following stretches of a ring-shaped tissue construct specimen to strains of 2% and 8%. Specimens were stretched rapidly by a gravity loading (over 10–15 ms) first to a nominal strain of $\epsilon = 0.02$ (filled circles), and then, after a 3600 s relaxation period, to a nominal strain of $\epsilon = 0.08$ (open circles).

3. Methods

(a) Analytical techniques

The treatment of the tissue constructs by deoxycholate was for a period sufficiently long to depolymerize the nearly all cytoskeletal components in the cells, leaving behind an ECM containing fluid-filled voids (deoxycholate-treated construct). To identify the effective isotropic tangent moduli of the cells and ECM, two mechanics challenges had to be addressed: (i) subtracting the effects of fluid-filled voids from the overall mechanical response of deoxycholate-treated construct and (ii) determining composite properties from those of a composite's constituents.

Both problems have a long history in the field of composite mechanics. For the first problem, determining the short-term and long-term tangent moduli of interest required estimation of the ratio of the observed elastic modulus of the deoxycholate-treated construct to the effective tangent modulus of the ECM itself. Bounding theorems dictate limits on the elastic response of the collagen as a function of the mechanical response of the deoxycholate-treated construct (appendix A). Estimates like those of Ramakrishnan & Arunachalam (1993) and Monte Carlo simulations like those of Roberts & Garboczi (2000) incorporate specific void shapes, and predict that the modulus of a deoxycholate-treated construct will disappear at a void (cell) volume fraction sufficiently large that void clusters coalesce, eliminating continuity in the collagen. Accurate estimates do not exist for composites containing dense concentrations of ellipsoidal or spherical inclusions. Therefore, in this work, Monte Carlo simulations of deoxycholate-treated constructs were performed to establish the relation between the observed elastic moduli of the deoxycholate-treated constructs and the effective moduli of the ECM in these specimens.

For the second problem, the starting point was Eshelby's (1957) exact solution, which models the stiffening of the ECM by dilute concentrations of cells. For slightly higher cell concentrations, interactions between cells must be considered.

Figure 2. Representative FE discretizations of cells within FE meshes of tissue constructs containing (a) a low cell concentration and (b) a high cell concentration, in which the same number of cells is now confined to a smaller volume.

The extensions of Budiansky (1965), Hill (1965), Mori & Tanaka (1973) and Chen & Cheng (1996) model this stiffening. For very high cell concentrations, above the percolation threshold at which cells form a mechanically contiguous network (Bug *et al.* 1985*a,b*), composite mechanics are well approximated with the rule of mixtures (Marquez *et al.* 2005*a,b*). However, all of these analytical approaches fail for intermediate cell concentrations with a volume fraction greater than approximately 10 per cent but too low to reach the percolation threshold (Genin & Birman 2009). Therefore, bounds and numerical simulations were used to relate the observed moduli of tissue constructs to the estimated tangent moduli of the cells and ECM.

(b) Numerical models

To estimate the effective elastic moduli of tissue constructs over a broad range of material parameters and cell volume fractions, we performed Monte Carlo simulations involving repeated finite-element (FE) analyses using a commercial software package (ADINA v.7.5.2, Cambridge, MA, USA). We began by simulating void-containing matrices representing deoxycholate-treated constructs to develop relations between the observed elastic moduli of the deoxycholate-treated constructs and the effective moduli of the ECM in these specimens. Then, we simulated tissue constructs containing cells whose isotropic elastic modulus was compliant or stiff relative to that of the ECM to relate the observed moduli of tissue constructs to the estimated tangent moduli of the cells and ECM.

(i) Physical and finite-element models

A cube-shaped region of a linear elastic tissue consisting of ECM and cylindrical cells or voids was modelled (figure 2); the cylindrical shape is a first-order approximation of the spindle-like morphology that cells adopt in these tissue constructs (Zahalak *et al.* 2000). The positive y -face of the cube was constrained

to undergo uniform displacement imposed by a unit force, f , in the y -direction. The negative x -, y - and z -faces were constrained from displacing in the x -, y - and z -directions, respectively. The remaining faces were unconstrained. All faces were free of shear tractions. These boundary conditions provided mirror symmetry on two faces of the cube, and simulated an infinitely long repeating structure in the y -direction.

Each of these cube-shaped regions enclosed 27 randomly oriented cells whose centres were spaced uniformly on a $3 \times 3 \times 3$ grid (figure 2); cell concentrations were varied by adjusting the prescribed grid spacing. The choice of a uniform orientation distribution of cells was motivated by Wakatsuki *et al.* (2000) who observed random cell alignment throughout the bulk of tissue constructs similar to those we studied. Cells were assigned an aspect ratio of just over 12:1 (length:diameter) based upon experimental measurements described in §3c; as all simulations involved continuum models, the absolute length scale was arbitrary. The spacing between cells decreased for higher cell densities. The FE meshes were refined in the vicinity of the largest strain gradients, which occurred near the cell ends. Both the ECM and cells were modelled with three-dimensional parabolic tetrahedral elements to prevent Poisson's ratio locking effect (e.g. Szabo & Babuska 1991).

The cells and ECM were assigned a range of linear elastic, isotropic material properties. Both ECM and cells were taken to be nearly incompressible, with a Poisson's ratio of $\nu = 0.49$. Small strain theory was used, which was appropriate for the levels of strain (approx. 0.08) in our experiments. Dynamic reorientation of cells in response to loading (Zemel *et al.* 2006) was neglected as this is believed to occur over a time scale that is long compared to that associated with our experiments. While some postulate significant spatial clustering of cells in tissue constructs of this character (Evans & Barocas 2009), our confocal microscopy images did not reveal such clusters; hence, the cell distribution was modelled as uniform throughout the specimens.

(ii) Monte Carlo simulations and construct elastic modulus

The Monte Carlo simulations involved repeated FE analyses of models containing different random orientation distributions of cells, prescribed according to a uniform orientation distribution. For each simulation we estimated the infinitesimal displacement of the positive y -face, Δl_t , resulting from application of a unit force f over this face. We then computed the effective elastic modulus of the tissue construct, E_t ,

$$E_t = \frac{f}{A_t} \frac{l_t}{\Delta l_t}, \quad (3.1)$$

where A_t is the area of the y -face and l_t is the model length in the y -direction. Four FE simulations were conducted for each set of parameters to estimate the elastic modulus.

(c) Experimental methods

In addition to the data reported in Marquez *et al.* (2006b), a measure of the cell concentration was needed. The cell volume was computed from measurements of the average length and width of cells in constructs at different cell concentrations,

with the cell shape modelled as ellipsoidal. The cell dimensions were measured using confocal microscopy of cells stained with Cell Tracker C2927 (Invitrogen, Carlsbad, CA, USA). A small fraction of cells died and became spherical in appearance. Cell concentration, N , was related to cell volume fraction ϕ_2 by

$$N = \frac{\phi_2}{V_c}, \quad (3.2)$$

where V_c is the average cell volume measured ($3350 \mu\text{m}^3$). N represents the number of cells per unit volume in a specimen. A dimensionless cell concentration, C , has also been identified as important in describing the mechanics of tissue constructs (Marquez *et al.* 2005*a,b*):

$$C = Nl_c^2, \quad (3.3)$$

where l_c is the average length of the long axis of a cell. C can be interpreted as a measure of the ratio of cell length to cell spacing, b : $C = (l_c/b)^3$ (Marquez *et al.* 2005*b*).

4. Results

We began our Monte Carlo analyses by simulating a deoxycholate-treated construct and establishing how the overall modulus of the deoxycholate-treated construct scaled with void volume fraction and the actual effective modulus of the ECM. Then, we simulated a tissue construct containing either relatively stiff or relatively compliant cells and estimated relationships between the mechanical response of the tissue construct and the moduli of its constituents. Results of Monte Carlo simulations were checked against homogenization bounds (appendix A). Finally, we interpreted the experimental measurements using estimates based on the Monte Carlo simulations.

(a) Monte Carlo simulations

The objective of the first set of Monte Carlo simulations was quantification of the way the observed elastic modulus of deoxycholate-treated constructs, E_p , varied with respect to void (cell) volume fraction, ϕ . The trends observed in the results of the Monte Carlo simulations fit a one-parameter empirical model for $E_p(\phi)$ proposed by Pabst *et al.* (2005) for porous ceramics:

$$\frac{E_p(\phi)}{E_m} = (1 - \phi) \left(1 - \frac{\phi}{\phi_o} \right), \quad (4.1)$$

where E_m is the elastic modulus of the ECM and ϕ_o can be interpreted as the pore volume fraction beyond which the ECM is no longer continuous. The fitting (dashed line in figure 3 corresponding to $m = 0$, where $m = E_c/E_m$, in which E_c is the elastic modulus of the cells; $E_c = 0$ in the case of a void), accomplished using least squares, yielded $\phi_o = 0.872$ with a correlation coefficient $R^2 = 0.998$ and a maximum standard deviation with respect to its mean value of 14 per cent.

The next set of Monte Carlo simulations aimed to provide a framework for estimating the effective linear elastic, isotropic, homogeneous modulus of a cell given the effective linear elastic ECM modulus E_m , estimated as above, the

Figure 3. FE simulations of the ratio of tissue elastic modulus, E_t , to ECM modulus, E_m , for $m \leq 1$. Error bars represent the standard deviation of four FE simulations and dashed lines correspond to the two-parameter fitting function (equation (4.2)).

effective modulus E_t of the tissue construct, and the composition of the tissue construct. Since published estimates of the effective elastic modulus of a living cell range over six orders of magnitude (Marquez *et al.* 2005a), the framework had to be calibrated over a similarly broad range of moduli.

Results of Monte Carlo simulations for elastic moduli E_t of tissue constructs containing relatively compliant cells ($m = E_c/E_m = 0.05, 0.10, 0.25$ and 0.50) followed approximately

$$\frac{E_t(\phi)}{E_m} = 1 + (1 - m) \left(\frac{1 + \alpha m}{1 + \beta m} \right) \left(\frac{E_p(\phi)}{E_m} - 1 \right), \quad (4.2)$$

where $E_p(\phi)/E_m$ is as given by equation (4.1), and $\alpha = 11.57$ and $\beta = 15$ are dimensionless scaling parameters obtained by least-squares fitting; α and β determine the degree to which compliant cells reduce the modulus of a tissue construct relative to the effective modulus of the ECM. The correlation coefficient of this fitting (dashed lines $m > 0$ in figure 3) for all simulations was $R^2 = 0.9997$ and the maximum relative standard deviation among simulations was 5 per cent. Note that equation (4.2) reduces to equation (4.1) when $m = 0$ (voids) and simplifies to 1 when $m = 1$ (cells and ECM with identical mechanical response).

Overall elastic moduli from simulations for tissue constructs containing relatively stiff cells ($m = 10, 100$ and 1000) showed a greater standard deviation, especially for the stiffer cells (figure 4). This was due to the variation of cell orientations within the unit of repetition: in these simulations, the overall moduli of tissue constructs were greatly influenced by the stiffest phase (in this case the cells). Data for all simulations fell within the two-point homogenization bounds (dashed lines in figure 4); these bounds are rigorous for a composite material containing cylindrical inclusions like that considered (e.g. Milton 2002).

Figure 4. FE simulations (filled circles) of the ratio of tissue elastic modulus, E_t , to ECM modulus, E_m , for (a) $m = 10$, (b) $m = 100$ and (c) $m = 1000$. Error bars indicate the standard deviations of four FE simulations, solid lines correspond to the three-point bounds for overlapping spheres, dashed lines represent two-point bounds and dotted lines show the estimates for the elastic modulus. Shaded portion, Hashin-Shtrikman bounds.

Figure 5. (a) Short-term (10 ms post-stretch) and (b) long-term (3600 s post-stretch) moduli of ECM (triangles) and cells (circles) in a tissue construct, calculated from experiments. The trend lines for entire tissue constructs (dash-dotted lines) and deoxycholate-treated constructs (dotted lines) are curve fits; the trend lines for ECM (solid lines) and cellular (dashed lines) moduli are derived mathematically from the curve fits for entire tissue constructs and deoxycholate-treated constructs.

Also shown are the three-point bounds corresponding to overlapping spheres (solid lines in figure 4, calculated assuming an incompressible tissue construct, $E_e = 3G_e$); the mean values fall within these solid lines. The mean between these solid lines provided an estimate that coincided well with the average values from the Monte Carlo simulations (dotted line in figure 4). For this fit, $R^2 = \{0.975, 0.967, 0.846\}$ for $m = \{10, 100, 1000\}$.

(b) ECM and cell mechanical properties

Short-term and long-term ECM elastic moduli increased monotonically with cell concentration (triangles and solid lines in figure 5). At concentrations below 10 million cells ml^{-1} , the moduli of the ECM were nearly indistinguishable

from those measured moduli of the deoxycholate-treated construct (dotted lines in figure 4, visible only at high cell concentration); however, the difference grew to about one order of magnitude around 140 million cells ml^{-1} . The ECM elastic moduli increased exponentially by nearly four orders of magnitude over the range of cell concentrations studied, a change far above any that could be accounted for by the approximate order of magnitude decrease in the cross-sectional area over this range of cell concentrations. The curve fits for the ECM and overall tissue construct moduli (dot-dashed lines in figure 5) followed

$$E(C) = E_0 + E_i C^\gamma, \quad (4.3)$$

where E_0 is a baseline elastic modulus, and parameters E_i and γ represent the amplitude and scaling of the stiffening of the ECM by the action of cells as a function of dimensionless cell concentration C .

The elastic moduli of the cells decreased monotonically as the cell concentration increased (circles and dashed lines in figure 5). At low cell concentrations, cells were much stiffer than the ECM; cell and ECM elastic moduli were approximately equal near a cell concentration of $N = 55$ million cells ml^{-1} . For the lowest cell concentrations studied, elastic moduli for the cells could not be found: estimates diverged, indicating that a fundamental assumption of the model was violated at the lowest cell concentrations. The dashed line connecting the high cell concentration data points is not a curve fit, but rather a prediction formed by combining the fitting from equation (4.1) for the ECM with the curve fits in figures 2 and 3. Short-term elastic moduli of both cells and ECM were about three times higher than long-term elastic moduli.

5. Discussion

Contractile fibroblasts actively modify their environment by remodelling the ECM and secreting ECM and regulatory proteins. This work studied the changes in cell and ECM moduli produced by the combination of these factors. The results indicate that a relatively high concentration of cells is required to stiffen the ECM and the tissue construct; therefore, significant cell migration and/or proliferation must occur *in vivo*. Another important result is that cell modulus decreases as the ECM modulus and cell concentration increases, indicating that cells play an important mechanical role in the early stages of remodelling. In the later stages, their role focuses more on stiffening the ECM, as can be inferred from the exponential rise in the modulus seen in the experiments. The decrease in the cell modulus is most likely induced by a combination of both mechanical and molecular signalling based on the high mechanical and biochemical sensitivity exhibited by fibroblasts.

(a) Limitations

The methodology presented for estimating isotropic cell and ECM elastic moduli from tests on tissue constructs involved the application of linear elastic solutions to interpret tests on activated tissue constructs and on tissue constructs

with cells removed. Several limitations of this approach must be considered when interpreting the results. First is the well-known limitation of applying linear kinematics and a linear constitutive relationship to model a biological tissue (Prager 1969). In the experiments analysed in this paper, strain levels were sufficiently small to warrant application of linear kinematics; however, the moduli must be recognized to be average tangent moduli (e.g. Hill 1950) of cells and ECM at these strain levels. Second is the assumption of isotropy as a first-order estimate. Since collagen fibres follow a uniform distribution within analogous tissue constructs (Zahalak *et al.* 2000), isotropy is a reasonable approximation for the ECM. For the elongated cells, Eshelby's solution suggests that the assumption of isotropy provides a reasonable estimate of the tangent modulus for stretching along the axis of a cell (Marquez *et al.* 2006a). Third is the assumption of homogeneity. Estimates of cell moduli could not be found for the lowest cell concentrations studied, in which the lowest degree of spatial homogeneity in ECM remodelling would be expected, suggesting that homogeneity is an appropriate assumption only at moderate to high levels of remodelling.

The predicted tangent moduli represent the short-term and long-term mechanical responses, which embody the entire range of moduli exhibited by tissue construct constituents, but miss the intermediate time-varying behaviour. The short-term moduli are a reasonable approximation to the instantaneous modulus that would arise in a three-parameter viscoelastic solid description of the cells and ECM, since the time scale of measurement was appropriate compared with the time scale of loading. The long-term moduli cannot with confidence be interpreted as fully relaxed moduli, since the logarithmic decay of the isometric force in the experiments continued to the end of the 3600 s data-logging interval.

The homogenization models used to estimate moduli from tests on tissue constructs and deoxycholate-treated constructs also have limitations. Estimates of moduli for tissue constructs containing the lowest cell concentrations were the least reliable. For cell concentrations $N < 10$ million cells ml^{-1} , effective cell moduli could not be found in the context of the models. This uncertainty stems from three sources. First, Monte Carlo estimates rest on the assumption of homogeneity in the ECM, which might be violated by pockets of disproportionately or anisotropically remodelled ECM in the vicinity of cells, as in chondrons in articular cartilage (e.g. Guilak & Mow 2000). While no such factor has been identified, further study is needed, especially for tissue constructs containing low cell concentrations, in which large intercellular distances may exacerbate effects of spatial variations in remodelling. Second, scatter in Monte Carlo results was largest when cell moduli exceeded the ECM moduli, as was the case for lower cell concentrations; although estimates fell within elastic bounds, the bounds are very broad in these cases. Third, because the cell contribution to the overall tissue mechanics was low for sparse cell distributions, experimental uncertainty had the greatest effect on modulus estimates at low cell concentration.

The results presented are for a prescribed culture interval (3 days). This interval directly influences the degree of ECM remodelling and the final cell concentration: shorter culture periods result in lower final cell concentrations and lower degrees of ECM remodelling. The effects of remodelling interval on measured moduli and observed trends must be characterized in future work.

(b) Cell modulus is higher when measured in a tissue construct

The results reinforce earlier observations that the cell modulus is much greater when measured in the three-dimensional *in vitro* environment of the tissue constructs we studied (Marquez *et al.* 2005a,b, 2006b). We believe that this is due to loading cells through their natural structural networks in these three-dimensional tissue constructs, as opposed to probing the membrane as must be done when testing cell mechanics in two-dimensional cultures. Our predictions indicated that cell moduli decreased appreciably with increasing cell concentration to levels as low as 10 kPa for the highest cell concentrations attainable in this system, suggesting that cells remodel in response to changes in their mechanical environment; although earlier work showed that the overall cellular contribution to tissue construct mechanics increases with increasing cellular volume fraction (Marquez *et al.* 2006b), this is due to a diminished contribution from each member of a larger population of cells. These changes could be a combination of active and passive effects. However, all moduli measured were much higher than those associated with a cell expressing an internally equilibrated tensegrity architecture.

(c) Cell modulus is a strong function of cellular environment

The observation of a reduction in the cell modulus with increasing cell concentration can be viewed with confidence, especially for the highest cell concentrations: estimates of cell moduli were the best for the high cell concentrations ($N > 55$ million cells ml^{-1}) because the scatter in the Monte Carlo simulations was very small for cases in which the cells were more compliant than the ECM, and any spatial variations in mechanical properties can be expected to diminish rapidly beyond the ‘percolation threshold’ of between 20 and 30 million cells ml^{-1} , at which cells form a contiguous network (Marquez *et al.* 2005b). Since the Monte Carlo estimates for the ratio between the actual elastic modulus of the ECM and the measured tangent modulus of the deoxycholate-treated construct show very little scatter, the estimates of ECM tangent moduli and hence cell moduli contain little error from this source. The results suggest that cell crowding or rises in the ECM modulus could be external signals for cells to decrease their modulus.

Cell populations in these tissue constructs were observed to increase or decrease in size to approach the percolation threshold, at which a contiguous steric network of cells is predicted (Marquez *et al.* 2006b). The observation in this work that the cell and ECM effective moduli converge at the percolation threshold bears further investigation in the context of observations of Discher *et al.* (2005) on cellular adaptation to the ECM modulus.

6. Conclusion

We presented a scheme to study the mechanical processes involved in fibroblast-driven ECM remodelling. By estimating the short-term and long-term tangent moduli of activated contractile fibroblasts and reconstituted collagen ECM from experimental measurements on bio-artificial tissue constructs, we described the physiology of this process. Results obtained through this scheme indicated that

ECM moduli increased exponentially as cell concentration increased. Cell moduli decreased as the modulus of the ECM increased, and as cell concentration increased, with the two converging at the cell concentration corresponding to the percolation threshold. The methodology is most accurate for higher cell concentrations. Future work is needed to better characterize ECM morphology in order to check the validity of the assumption of matrix homogeneity at lower cell concentrations.

This work was supported in part by the National Institutes of Health through grant HL079165, and by the Center for Materials Innovation at Washington University in St Louis. The authors thank Mr Adam C. Nathan for helpful discussions, and Dr Kenneth Pryse for helpful discussions and assistance with the experiments.

Appendix A. Homogenization bounds

We summarize here formulae for homogenization bounds that are referenced in the article.

(a) Two-point bounds

The tightest bounds on the properties of tissue constructs with isotropic constituents that can be obtained without detailed information about microstructure are the two-point bounds of Hashin & Shtrikman (1963) for the effective bulk modulus, K_e , and effective shear modulus, G_e . Walpole (1966) generalized these bounds to n -phase isotropic composites, relaxing some limiting conditions of the earlier bounds. These generalized bounds can be expressed as

$$\left(\sum_n \phi_n (K_{\min}^* + K_n)^{-1} \right)^{-1} - K_{\min}^* \leq K_e \leq \left(\sum_n \phi_n (K_{\max}^* + K_n)^{-1} \right)^{-1} - K_{\max}^* \quad (\text{A } 1)$$

and

$$\left(\sum_n \phi_n (G_{\min}^* + G_n)^{-1} \right)^{-1} - G_{\min}^* \leq G_e \leq \left(\sum_n \phi_n (G_{\max}^* + G_n)^{-1} \right)^{-1} - G_{\max}^*, \quad (\text{A } 2)$$

where

$$K_{\min}^* = \frac{4}{3} G_{\min}, \quad (\text{A } 3)$$

$$K_{\max}^* = \frac{4}{3} G_{\max}, \quad (\text{A } 4)$$

$$G_{\min}^* = G_{\min} \left[\frac{9K_{\min} + 6G_{\min}}{6(K_{\min} + 2G_{\min})} \right] \quad (\text{A } 5)$$

and

$$G_{\max}^* = G_{\max} \left[\frac{9K_{\max} + 6G_{\max}}{6(K_{\max} + 2G_{\max})} \right], \quad (\text{A } 6)$$

in which ϕ_n , K_n and G_n are the volume fraction, bulk modulus and shear modulus, respectively, of phase n . Subscripts max and min stand for the maximum and minimum values of the variable to which they are attached. We considered phase 1 as the ECM and phase 2 as the cell or voids.

(b) *Three-point bounds*

Tighter bounds can be obtained using detailed statistical information about microstructure. Using admissible strain fields and minimum energy principles, Beran & Molyneux (1966) and McCoy (1970) obtained three-point bounds on the effective bulk modulus and the effective shear modulus for two-phase composites. Later, Milton & Phan-Thien (1982) improved McCoy's (1970) shear-modulus bounds. The simplified form (Milton 1981*a,b*) of the three-point Beran & Molyneux (1966) bounds on K_e of isotropic two-phase composites is a long but straightforward function of the shear and bulk moduli of the two phases, the volume fractions ϕ_1 and ϕ_2 and two parameters ζ_2 and η_2 that characterize the spatial distribution of the two phases (e.g. Torquato 1991):

$$\langle K \rangle - \frac{\phi_1 \phi_2 (K_2 - K_1)^2}{\langle \tilde{K} \rangle + (4/3) \langle G^{-1} \rangle_\zeta^{-1}} \leq K_e \leq \langle K \rangle - \frac{\phi_1 \phi_2 (K_2 - K_1)^2}{\langle \tilde{K} \rangle + (4/3) \langle G \rangle_\zeta}. \quad (\text{A } 7)$$

Three-point Milton–Phan-Thien bounds on G_e for isotropic two-phase composites are given by

$$\langle G \rangle - \frac{\phi_1 \phi_2 (G_2 - G_1)^2}{\langle \tilde{G} \rangle + \mathcal{E}} \leq G_e \leq \langle G \rangle - \frac{\phi_1 \phi_2 (G_2 - G_1)^2}{\langle \tilde{G} \rangle + \Theta}, \quad (\text{A } 8)$$

where

$$\mathcal{E} = \frac{\langle 128/K + 99/G \rangle_\zeta + 45 \langle 1/G \rangle_\eta}{30 \langle 1/G \rangle_\zeta \langle 6/K - 1/G \rangle_\zeta + 6 \langle 1/G \rangle_\eta \langle 2/K + 21/G \rangle_\zeta} \quad (\text{A } 9)$$

and

$$\Theta = \frac{3 \langle G \rangle_\eta \langle 6K + 7G \rangle_\zeta - 5 \langle G \rangle_\zeta^2}{6 \langle 2K - G \rangle_\zeta + 30 \langle G \rangle_\eta}. \quad (\text{A } 10)$$

In both equations, for any property material b ,

$$\langle b \rangle = b_1 \phi_1 + b_2 \phi_2 \quad \text{and} \quad \langle \tilde{b} \rangle = b_1 \phi_2 + b_2 \phi_1, \quad (\text{A } 11)$$

$$\langle b \rangle_\zeta = b_1 \zeta_1 + b_2 \zeta_2 \quad \text{and} \quad \langle b \rangle_\eta = b_1 \eta_1 + b_2 \eta_2 \quad (\text{A } 12)$$

and

$$\zeta_1 = 1 - \zeta_2 \quad \text{and} \quad \eta_1 = 1 - \eta_2. \quad (\text{A } 13)$$

Calculation of ζ_2 and η_2 is computationally expensive, and values have been reported for only a limited number of microstructures at this time. Values range between $0.15\phi_2 < \zeta_2 < \phi_2$ and $0.5\phi_2 < \eta_2 < \phi_2$ (Torquato 1991).

The nearest approximation to a tissue containing distributed cells for which three-point parameters are available is a distribution of overlapping spheres of phase 2 ($\zeta_2 \sim 0.6\phi_2$ and $\eta_2 \sim 0.7\phi_2$; Schulgasser 1976; Torquato & Stell 1983; Torquato *et al.* 1985).

References

- Asnes, C. F., Marquez, J. P., Elson, E. L. & Wakatsuki, T. 2006 Reconstitution of the Frank-Starling mechanism in engineered heart tissues. *Biophys. J.* **91**, 1800–1810. (doi:10.1529/biophysj.105.065961)
- Bell, E., Ivarsson, B. & Merrill, C. 1979 Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential *in vitro*. *Proc. Natl Acad. Sci. USA* **76**, 1274–1278. (doi:10.1073/pnas.76.3.1274)
- Beran, M. J. & Molyneux, J. 1966 Use of classical variational principles to determine bounds for the effective bulk modulus in heterogeneous media. *Q. Appl. Math.* **24**, 107118.
- Budiansky, B. 1965 On the elastic moduli of some heterogeneous materials. *J. Mech. Phys. Solids* **13**, 223–227. (doi:10.1016/0022-5096(65)90011-6)
- Bug, A. L. R., Safran, S. A., Grest, G. S. & Webman, I. 1985*a* Do interactions raise or lower a percolation threshold? *Phys. Rev. Lett.* **55**, 1896–1899. (doi:10.1103/PhysRevLett.55.1896)
- Bug, A. L. R., Safran, S. A. & Webman, I. 1985*b* Continuum percolation of rods. *Phys. Rev. Lett.* **54**, 1412–1415. (doi:10.1103/PhysRevLett.54.1412)
- Chen, C. H. & Cheng, C. H. 1996 Effective elastic moduli of misoriented short-fiber composites. *Int. J. Solids Struct.* **33**, 2519–2539. (doi:10.1016/0020-7683(95)00160-3)
- del Rio, A., Perez-Jimenez, R., Liu, R., Roca-Cusachs, P., Fernandez, J. M. & Sheetz, M. P. 2009 Stretching single talin rod molecules activates vinculin binding. *Science* **323**, 638–641. (doi:10.1126/science.1162912)
- Deshpande, V. S., McMeeking, R. M. & Evans, A. G. 2006 A bio-chemo-mechanical model for cell contractility. *Proc. Natl Acad. Sci. USA* **103**, 14 015–14 020. (doi:10.1073/pnas.0605837103)
- Desmouliere, A., Chaponnier, C. & Gabbiani, G. 2005 Tissue repair, contraction and the myofibroblast. *Wound Repair Regen.* **13**, 7–12. (doi:10.1111/j.1067-1927.2005.130102.x)
- Discher, D. E., Janmey, P. & Wang, Y. L. 2005 Tissue cells feel and respond to the stiffness of their substrate. *Science* **310**, 1139–1143. (doi:10.1126/science.1116995)
- Discher, D., Dong, C., Fredberg, J. J., Guilak, F., Ingber, D., Janmey, P., Kamm, R. D., Schmid-Schönbein, G. W. & Weinbaum, S. 2009 Biomechanics: cell research and applications for the next decade. *Ann. Biomed. Eng.* **37**, 847–859. (doi:10.1007/s10439-009-9661-x)
- Enever, P. A., Shreiber, D. I. & Tranquillo, R. T. 2002 A novel implantable collagen gel assay for fibroblast traction and proliferation during wound healing. *J. Surg. Res.* **105**, 160–172. (doi:10.1006/jsre.2002.6372)
- Eshelby, J. D. 1957 The determination of the elastic field outside an ellipsoidal inclusion and related problems. *Proc. R. Soc. Lond. A* **241**, 376–396. (doi:10.1098/rspa.1957.0133)
- Evans, M. C. & Barocas, V. H. 2009 The modulus of fibroblast-populated collagen gels is not determined by final collagen and cell concentration: experiments and an inclusion-based model. *J. Biomech. Eng.* **131**, 101014. (doi:10.1115/1.4000064)
- Fabry, B., Maksym, G. N., Butler, J. P., Glogauer, M., Navajas, D. & Fredberg, J. J. 2001 Scaling the microrheology of living cells. *Phys. Rev. Lett.* **87**, 148102. (doi:10.1103/PhysRevLett.87.148102)
- Franck, C., Hong, S., Maskarinec, S. A., Tirrell, D. A. & Ravichandran, G. 2007 Three-dimensional full-field measurements of large deformations in soft materials using confocal microscopy and digital volume correlation. *Exp. Mech.* **47**, 427–438. (doi:10.1007/s11340-007-9037-9)
- Gabbiani, G. I. 2003 The myofibroblast in wound healing and fibrocontractive disease. *J. Pathol.* **200**, 500–503.
- Genin, G. M. & Birman, V. 2009 Micromechanics and structural response of functionally graded, particulate-matrix, fiber-reinforced composites. *Int. J. Solids Struct.* **46**, 2136–2150. (doi:10.1016/j.ijsolstr.2008.08.010)

- Georges, P. C. & Janmey, P. A. 2005 Cell type-specific response to growth on soft material. *J. Appl. Physiol.* **98**, 1547–1553. (doi:10.1152/japplphysiol.01121.2004)
- Griffith, L. G. & Swartz, M. A. 2006 Capturing complex 3D tissue physiology *in vitro*. *Nat. Rev. Mol. Cell Biol.* **7**, 211–224. (doi:10.1038/nrm1858)
- Guilak, F. & Mow, V. C. 2000 The mechanical environment of the chondrocyte: a biphasic finite element model of cell–matrix interactions in articular cartilage. *J. Biomech.* **33**, 1663–1673. (doi:10.1016/S0021-9290(00)00105-6)
- Guilak, F., Jones, W. R., Ting-Beall, H. P. & Lee, G. M. 1999 The deformation behaviour and mechanical properties of chondrocytes in articular cartilage. *Osteoarthritis Cartilage* **7**, 59–70. (doi:10.1053/joca.1998.0162)
- Hashin, Z. & Shtrikman, S. 1963 A variational approach to the theory of the elastic behaviour of multiphase materials. *J. Mech. Phys. Solids* **11**, 127–140. (doi:10.1016/0022-5096(63)90060-7)
- Hill, R. 1950 *The mathematical theory of plasticity*. Oxford, UK: Clarendon Press.
- Hill, R. 1965 A self-consistent mechanics of composite materials. *Mech. Phys. Solids* **13**, 213–222. (doi:10.1016/0022-5096(65)90010-4)
- Ingber, D. E. 1993 Cellular tensegrity: defining new rules of biological design that govern the cytoskeleton. *J. Cell Sci.* **104**, 613–627.
- Ingber, D. E. 2003 Mechanobiology and diseases of mechanotransduction. *Ann. Med.* **35**, 564–577. (doi:10.1080/07853890310016333)
- Isenberg, B. C. & Tranquillo, R. T. 2003 Long-term cyclic distention enhances the mechanical properties of collagen-based media-equivalents. *Ann. Biomed. Eng.* **31**, 937–949. (doi:10.1114/1.1590662)
- Janmey, P. A., Euteneuer, U., Traub, P. & Schliwa, M. 1991 Viscoelastic properties of vimentin compared with other filamentous biopolymer networks. *J. Cell Biol.* **113**, 155–160. (doi:10.1083/jcb.113.1.155)
- Kolodney, M. S. & Elson, E. L. 1995 Contraction due to microtubule disruption is associated with increased phosphorylation of myosin regulatory light chain. *Proc. Natl Acad. Sci. USA* **92**, 10 252–10 256. (doi:10.1073/pnas.92.22.10252)
- Kolodney, M. S. & Wysolmerski, R. B. 1992 Isometric contraction by fibroblasts and endothelial cells in tissue culture: a quantitative study. *J. Cell Biol.* **117**, 73–82. (doi:10.1083/jcb.117.1.73)
- Kumar, S., Maxwell, I. Z., Heisterkamp, A., Polte, T. R., Lele, T. P., Salanga, M., Mazur, E. & Ingber, D. E. 2006 Viscoelastic retraction of single living stress fibers and its impact on cell shape, cytoskeletal organization, and extracellular matrix mechanics. *Biophys. J.* **90**, 3762–3773. (doi:10.1529/biophysj.105.071506)
- Lee, S. E., Kamm, R. D. & Mofrad, M. R. 2007 Force-induced activation of talin and its possible role in focal adhesion mechanotransduction. *J. Biomech.* **40**, 2096–2106. (doi:10.1016/j.jbiomech.2007.04.006)
- Mahaffy, R. E., Shih, C. K., MacKintosh, F. C. & Käs, J. 2000 Scanning probe-based frequency-dependent microrheology of polymer gels and biological cells. *Phys. Rev. Lett.* **85**, 880–883. (doi:10.1103/PhysRevLett.85.880)
- Marquez, J. P., Genin, G. M., Zahalak, G. I. & Elson, E. L. 2005a Thin bio-artificial tissues in plane stress: the relationship between cell and tissue strain, and an improved constitutive model. *Biophys. J.* **88**, 765–777. (doi:10.1529/biophysj.104.040808)
- Marquez, J. P., Genin, G. M., Zahalak, G. I. & Elson, E. L. 2005b The relationship between cell and tissue strain in three-dimensional bio-artificial tissues. *Biophys. J.* **88**, 778–789. (doi:10.1529/biophysj.104.041947)
- Marquez, J. P., Genin, G. M. & Elson, E. L. 2006a On the application of strain factors for approximation of the contribution of anisotropic cells to the mechanics of a tissue construct. *J. Biomech.* **39**, 2145–2151. (doi:10.1016/j.jbiomech.2005.06.010)
- Marquez, J. P., Genin, G. M., Pryse, K. M. & Elson, E. L. 2006b Cellular and matrix contributions to tissue construct stiffness increase with cellular concentration. *Ann. Biomed. Eng.* **34**, 1475–1482. (doi:10.1007/s10439-006-9160-2)
- McConnaughey, W. B. & Petersen, N. O. 1980 Cell poker: an apparatus for stress-strain measurements on living cells. *Rev. Sci. Instrum.* **51**, 575–580. (doi:10.1063/1.1136256)

- McCoy, J. J. 1970 On the displacement field in an elastic medium with random variations of material properties. *Recent Adv. Eng. Sci.* **5**, 235–254.
- Milton, G. W. 1981*a* Bounds on the electromagnetic, elastic, and other properties of two-component composites. *Phys. Rev. Lett.* **46**, 542–545. (doi:10.1103/PhysRevLett.46.542)
- Milton, G. W. 1981*b* Concerning bounds on the transport and mechanical properties of multicomponent composite materials. *Appl. Phys. A* **26**, 125–130. (doi:10.1007/BF00616659)
- Milton, G. W. 2002 *The theory of composites*. Cambridge, UK: Cambridge University Press.
- Milton, G. W. & Phan-Thien, N. 1982 New bounds on the effective moduli of two-component materials. *Proc. R. Soc. Lond. A* **380**, 305–331. (doi:10.1098/rspa.1982.0044)
- Mitchison, T. J., Charras, G. T. & Mahadevan, L. 2008 Implications of a poroelastic cytoplasm for the dynamics of animal cell shape. *Semin. Cell Dev. Biol.* **19**, 215–223. (doi:10.1016/j.semcdb.2008.01.008)
- Mori, T. & Tanaka, K. 1973 Average stress in matrix and average elastic energy of materials with misfitting inclusions. *Acta Metallurgica* **21**, 571–574. (doi:10.1016/0001-6160(73)90064-3)
- Munevar, S., Wang, Y. & Dembo, M. 2001 Traction force microscopy of migrating normal and H-ras transformed 3T3 fibroblasts. *Biophys. J.* **80**, 1744–1757. (doi:10.1016/S0006-3495(01)76145-0)
- Nekouzadeh, A., Genin, G. M., Bayly, P. V. & Elson, E. L. 2005 Wave motion in relaxation-testing of nonlinear elastic media. *Proc. R. Soc. A* **461**, 1599–1626. (doi:10.1098/rspa.2004.1437)
- Nekouzadeh, A., Pryse, K. M., Elson, E. L. & Genin, G. M. 2008 Stretch-induced force-shedding, force recovery, and cytoskeletal remodeling in contractile fibroblasts. *J. Biomech.* **41**, 2964–2971. (doi:10.1016/j.jbiomech.2008.07.033)
- Nicolas, A., Besser, A. & Safran, S. A. 2008 Dynamics of cellular focal adhesions on deformable substrates: consequences for cell force microscopy. *Biophys. J.* **95**, 527–539. (doi:10.1529/biophysj.107.127399)
- Pabst, W., Tichá, G., Gregorová, E. & Týnová, E. 2005 Effective elastic properties of alumina–zirconia composite ceramics. Part 5. Tensile modulus of alumina–zirconia composite ceramics. *Ceramics-Silikáty* **49**, 77–85.
- Pathak, A., Deshpande, V. S., McMeeking, R. M. & Evans, A. G. 2008 The simulation of stress fibre and focal adhesion development in cells on patterned substrates. *J. R. Soc. Interface* **5**, 507–524. (doi:10.1098/rsif.2007.1182)
- Petersen, N. O., McConnaughey, W. B. & Elson, E. L. 1982 Dependence of locally measured cellular deformability on position on the cell, temperature, and cytochalasin B. *Proc. Natl Acad. Sci. USA* **79**, 5327–5331. (doi:10.1073/pnas.79.17.5327)
- Pizzo, A. M., Kokini, K., Vaughn, L. C., Waisner, B. Z. & Voytik-Harbin, S. L. 2005 Extracellular matrix (ECM) microstructural composition regulates local cell-ECM biomechanics and fundamental fibroblast behavior: a multidimensional perspective. *Appl. Physiol.* **98**, 1909–1921. (doi:10.1152/japplphysiol.01137.2004)
- Prager, W. 1969 On the formulation of constitutive equations for living soft tissues. *Q. Appl. Math.* **27**, 128–132.
- Pryse, K. M. & Elson, E. L. Submitted. Mechanical effects of deoxycholate, triton, cytochalasin D, and latrunculin A, on reconstituted collagen and fibroblast cells.
- Pryse, K. M., Nekouzadeh, A., Genin, G. M., Elson, E. L. & Zahalak, G. I. 2003 Incremental mechanics of collagen gels: new experiments and a new viscoelastic model. *Ann. Biomed. Eng.* **31**, 1287–1296. (doi:10.1114/1.1615571)
- Ramakrishnan, N. & Arunachalam, V. S. 1993 Effective elastic moduli of ceramic materials. *J. Am. Ceram. Soc.* **76**, 2745–2752. (doi:10.1111/j.1151-2916.1993.tb04011.x)
- Roberts, A. P. & Garboczi, E. J. 2000 Elastic properties of model cell-free ceramics. *J. Am. Ceram. Soc.* **83**, 3041–3048. (doi:10.1111/j.1151-2916.2000.tb01680.x)
- Saffarian, S., Collier, I. E., Marmer, B. L., Elson, E. L. & Goldberg G. 2004 Interstitial collagenase is a Brownian ratchet driven by proteolysis of collagen. *Science* **306**, 108–111. (doi:10.1126/science.1099179)
- Schulgasser, K. 1976 On the conductivity of fiber reinforced materials. *J. Math. Phys.* **17**, 382–387. (doi:10.1063/1.522904)

- Sniadecki, N. J., Anguelouch, A., Yang, M. T., Lamb, C. M., Liu, Z., Kirschner, S. B., Liu, Y., Reich, D. H. & Chen, C. S. 2007 Magnetic microposts as an approach to apply forces to living cells. *Proc. Natl Acad. Sci. USA* **104**, 14 553–14 558. (doi:10.1073/pnas.0611613104)
- Szabo, B. A. & Babuska, I. 1991 *The finite element method*. New York, NY: John Wiley & Sons.
- Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C. & Brown, R. A. 2002 Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat. Rev. Mol. Cell Biol.* **3**, 349–363. (doi:10.1038/nrm809)
- Torquato, S. 1991 Random heterogeneous media: microstructure and improved bounds on the effective properties. *Appl. Mech. Rev.* **44**, 37–76. (doi:10.1115/1.3119494)
- Torquato, S. & Stell, G. 1983 Microstructure of two-phase random media. III. The n -point matrix probability functions for fully penetrable spheres. *J. Chem. Phys.* **79**, 1505–1510. (doi:10.1063/1.445941)
- Torquato, S., Stell, G. & Beasley, J. D. 1985 Third-order bounds on the effective bulk and shear moduli of a dispersion of fully penetrable spheres. *Lett. Appl. Eng. Sci.* **23**, 385.
- Wakatsuki, T., Kolodney, M. S., Zahalak, G. I. & Elson, E. L. 2000 Cell mechanics studied by a reconstituted model tissue. *Biophys. J.* **79**, 2353–2368. (doi:10.1016/S0006-3495(00)76481-2)
- Wakatsuki, T., Schlessinger, J. & Elson, E. L. 2004 The biochemical response of the heart to hypertension and exercise. *Trends Biochem. Sci.* **29**, 609–617. (doi:10.1016/j.tibs.2004.09.002)
- Walpole, L. J. 1966 On bounds for the overall elastic moduli of inhomogeneous systems I. *J. Mech. Phys. Solids* **14**, 151–162. (doi:10.1016/0022-5096(66)90035-4)
- Wang, J. H.-C. & Thampatty, B. P. 2006 An introductory review of cell mechanobiology. *Biomech. Model. Mechanobiol.* **5**, 1–6. (doi:10.1007/s10237-005-0012-z)
- Xu, F., Wen, T., Lu, T. J. & Seffen, K. A. 2008 Skin biothermomechanics for medical treatments. *J. Mech. Behav. Biomed. Mater.* **1**, 172–187. (doi:10.1016/j.jmbbm.2007.09.001)
- Zahalak, G. I., Wagenseil, J. E., Wakatsuki, T. & Elson, E. L. 2000 A cell-based constitutive relation for bio-artificial tissues. *Biophys. J.* **79**, 2369–2381. (doi:10.1016/S0006-3495(00)76482-4)
- Zemel, A., Bischofs, I. B. & Safran, S. A. 2006 Active elasticity of gels with contractile cells. *Phys. Rev. Lett.* **97**, 128103. (doi:10.1103/PhysRevLett.97.128103)