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Cell Contraction Forces in Scaffolds with Varying Pore Size and Cell Density

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Abstract

The contractile behavior of cells is relevant in understanding wound healing and scar formation. In tissue engineering, inhibition of the cell contractile response is critical for the regeneration of physiologically normal tissue rather than scar tissue. Previous studies have measured the contractile response of cells in a variety of conditions (e.g. on two-dimensional solid substrates, on free-floating tissue engineering scaffolds and on scaffolds under some constraint in a cell force monitor). Tissue engineering scaffolds behave mechanically like open-cell elastomeric foams: between strains of about 10 and 90%, cells progressively buckle struts in the scaffold. The contractile force required for an individual cell to buckle a strut within a scaffold has been estimated based on the strut dimensions (radius, r, and length, ℓ) and the strut modulus, E_s. Since the buckling force varies, according to Euler's law, with r^4/ℓ^2, and the relative density of the scaffold varies as (r/ℓ)^2, the cell contractile force associated with strut buckling is expected to vary with the square of the pore size for scaffolds of constant relative density. As the cell density increases, the force per cell to achieve a given strain in the scaffold is expected to decrease. Here we model the contractile response of fibroblasts by analyzing the response of a single tetrakaidecahedron to forces applied to individual struts (simulating cell contractile forces) using finite element analysis. We model tetrakaidecahedra of different strut lengths, corresponding to different scaffold pore sizes, and of varying numbers of loaded struts, corresponding to varying cell densities. We compare our numerical model with the results of free floating contraction experiments of normal human dermal fibroblasts (NHDF) in collagen-GAG scaffolds of varying pore size and with varying cell densities.

Keywords

collagen-GAG scaffolds; contraction; pore size; cell density

Introduction

Wounds to the skin heal by a combination of cell-mediated contraction of the wound followed by scar formation. Scar tissue is less flexible than normal dermis, leading to restricted range of motion at joints, along with physical disfigurement. Regeneration of
normal dermis in a wound requires inhibition of wound contraction [1]. Contractile cells have also been identified in transected peripheral nerve [1,2], injured anterior cruciate ligament [3], cirrhotic liver [4] and the conjunctiva [5].

The contractile response of cells has been measured using various methods. Initially, cells were seeded onto thin silicone membranes and the contractile forces were inferred from the resulting wrinkling patterns [6–8]. A more complete analysis of the cell force-deformation response was made possible by the introduction of latex microbeads embedded in or sprayed onto the surface of non-wrinkling substrates such as pre-stressed silicone or polyacrylamide. Measurement of bead displacement with microscopy and various image analysis tools allowed the corresponding cell forces to be calculated from the linear elastic response of the substrate using the Boussinesq equations [9-14]. Deformation of imprinted micropatterns has also been used to calculate cell-generated forces [15].

Cantilever-based measurement techniques were later developed to allow greater control of the substrate stiffness and to simplify mathematical computation. Various experimental designs have been used, including horizontal cantilevers micromachined on substrate surfaces [16,17], vertical posts arranged in regular arrays [18–20] and single cantilevers which either prod cells until detachment occurs or onto which cells can be seeded [21–23]. Although these methods offer advantages over 2D membranes (for instance, they allow higher cell seeding densities), they are not ideal: the in vivo environment is not accurately simulated, and forces can only be measured in the direction of cantilever bending.

Contractile forces have also been measured in three-dimensional tissue engineering scaffolds that more closely resemble the in vivo environment of the extracellular matrix (ECM). Typically, sensors measure the cell-induced scaffold deformation and the corresponding total contractile force and the population-averaged force per cell is calculated [24–31]. In contraction experiments using fibroblasts on collagen-GAG scaffolds, optical video microscopy indicated that the struts of the scaffold deform by bending and buckling in response to the forces imposed by individual cells. Measurement of the strut dimensions using optical microscopy and the strut modulus using atomic force microscopy allows calculation of Euler buckling loads in the range of 11 to 41 nN, corresponding to the individual cell force acting on a single strut in a three-dimensional matrix [32]. This method offers benefits over other reported methods: individual cell forces generated in 3D substrates can be calculated and it is computationally simple. The main limitation of this method is in estimating the appropriate end constraint factor for the strut.

Three-dimensional scaffolds for tissue engineering often have a microstructure and stress-strain response similar to that of open-cell foams (Fig. 1). One approach to modeling open-cell foams is to apply standard methods of structural mechanics to a periodic unit cell that packs to fill space, such as a tetrakaidecahedron. The Young’s modulus of this cell has been analyzed by applying a uniaxial stress normal to the top and bottom square faces of the tetrakaidecahedron in Fig. 1b [33]. By symmetry, each member of the unit cell either has zero force or an identical non-zero force. Their analysis gives equations relating the overall uniaxial stress applied to the unit cell to the overall uniaxial strain, as well as to the normal force in the load-bearing members. Applying these relationships to our collagen-GAG scaffolds, we find that at a strain of 10%, corresponding to the onset of buckling in the scaffolds [34], the normal force in the struts of the unit cell are between 20 and 45 nN for scaffold pore sizes of 96 to 151 μm, in the same range as that calculated by Harley et al., [32].

The above analysis of the tetrakaidecahedral unit cell applies an overall uniaxial stress to the top and bottom faces of the tetrakaidecahedron and finds the internal forces in each of the
members. In a scaffold, cells randomly adhere to the struts, applying contractile forces to the struts in a more random manner than in the above analysis. Here, we perform a finite element analysis to simulate cell-imposed forces on the struts of a scaffold. We perform a parametric study of the effect of the pore size, as well as the cell density, on the contractile response. We compare the finite element results with measurements of the contractile response of normal human dermal fibroblasts (NHDFs) on collagen-GAG scaffolds of varying pore size and with varying cell densities.

**Analysis: Finite element modelling**

The collagen-GAG scaffolds are foam-like materials with interconnected pores and a relative density of about 0.006, allowing models for open-cell foams to be used to analyze their mechanical behavior. We begin by considering a simple dimensional argument [35]. The force, $F_{cr}$, required to buckle a strut of length, \( \ell \), and radius, \( r \), in an open-cell foam made from a material with a Young’s modulus, \( E_s \), is, from Euler’s buckling equation:

$$F_{cr} = \frac{n^2 E_s I}{\ell^2} \propto \frac{E_s r^4}{\ell^2}$$

(1)

where \( n \) is an end constraint factor (e.g. for a pin-ended column, \( n = 1 \)) and \( I \) is the moment of inertia of the cross-section (e.g. \( I = \pi r^4/4 \) for a solid circular cross-section). The relative density, \( \rho^*/\rho_s \), (the ratio of the foam density, \( \rho^* \), to that of the solid strut material, \( \rho_s \)) is proportional to \((r/\ell)^2\) so that:

$$F_{cr} \propto E_s \left(\frac{\rho^*}{\rho_s}\right)^2 \ell^2$$

(2)

The relative density of the scaffold is equivalent to the volume fraction of solids. For scaffolds of constant relative density, with a geometry similar to that of an open-cell foam, we expect that the force required to buckle a strut increases with the square of the strut length, \( \ell \), or pore size, \( d \).

A tetrakaidecahedral unit cell is often used to represent the geometry of open-cell foams as it packs to fill space and has a morphology similar to that of low-density foams [33,36,37]; we use the tetrakaidecahedral unit cell here (Fig. 1). The scaffold struts were modeled as homogeneous, linear elastic, isotropic solid cylinders of radius, \( r \). The strut length, \( \ell \), can be related the scaffold pore size, \( d \), by assuming that the internal volume of the tetrakaidecahedron is similar to that of a spherical pore with the same diameter [38]:

$$\ell = \frac{d}{2.78}$$

(3)

For an open-cell tetrakaidecahedron with struts of circular cross-section, the strut radius \( r \) can be related to the relative density, \( \rho^*/\rho_s \), and length, \( \ell \), by the relation [35]:

$$\frac{\rho^*}{\rho_s} = 3.34 \left(\frac{r}{\ell}\right)^2$$

(4)
One model was created for each of the four scaffold pore diameters: 96, 110, 121 and 151 μm. The strut radius for each model was chosen so that the relative density of an infinite array of tetrakaidecahedral cells matched that of the scaffolds ($\rho_s/\rho = 0.006$) (Table 1). The Young’s modulus of the solid strut material was taken to be 5.28 MPa, based on the experimental results of Harley et al. [34] for hydrated collagen-GAG scaffolds. The Poisson’s ratio of the solid was assumed to be 0.33.

A tetrakaidecahedral model was created in Solid Works (Dassault Systèmes Solid Works Corp., Concord MA). Abaqus (Hibbitt, Karlsson, & Sorenson, Pawtucket RI) was used to perform the finite element analysis. Point loads were eccentrically applied to the ends of each strut, on the surface of the strut, to simulate previously observed cell adhesion and contraction (Fig. 2). Each model representing a different pore size was analyzed first with one “cell” attached, then with two, and so on, with up to 32 “cells” attached. The “cells” were randomly attached to the struts, with the condition that no strut had more than 1 “cell” attached. We assumed that all the cells contracted equally, and so applied the same force to each strut. The Abaqus buckling analysis was used to determine the critical buckling load of the structure at each cell density (i.e. the load at which the response first becomes non-linear).

**Experimental Methods and Materials**

**Collagen Scaffold Fabrication**

Collagen-GAG scaffolds with a uniform pore structure were fabricated using a standardized lyophilization process [39]. In this process, a slurry was prepared by mixing 3.6g type 1 microfibrillar collagen isolated from bovine tendon (Integra Life Sciences, Inc. Plainsboro, NJ) with 600 ml 0.05 M acetic acid (Glacial Acetic Acid, Mallinckrodt Chemical Co, Paris, KY) mixed with distilled deionized water to achieve a pH of 3.2. The solution was blended for 3 hours at 15,000 rpm at a constant temperature of 4°C. Halfway through the blending process, when the collagen was solubilized, 0.32g of chondroitin-6 sulfate, the GAG component, was added. Once blending was complete, the slurry was degassed with a vacuum to remove large bubbles. 66 ml of the degassed slurry was pipetted into a square aluminum mold of dimensions 125mm × 125mcm × 38 mm. Bubbles found in the solution were pushed to the edge of the pan with a pipette tip. The molds were then placed in a freeze dryer at room temperature. During the freeze-drying process, the slurry was controllably cooled to one of four final freezing temperatures: -40°C, -30°C, -20°C, and -10°C to produce scaffolds of four mean pore sizes (96, 110, 121 and 151 μm, respectively) [39]. Once the slurry was frozen, a vacuum (< 300 Torr) was applied for 17 hours to sublimate the acetic acid ice crystals. The remaining collagen-GAG content formed a highly porous scaffold with a uniform pore microstructure. The average relative density of the scaffolds was 0.00630 (assuming a solid density of 1.3 g/cm$^3$, similar to collagen); there was no significant difference in relative density between scaffolds of different pore sizes. The average thickness of the dry scaffolds was 3mm. This thickness decreased by typically less than 10% when the scaffold was hydrated.

Dehydrothermal treatment (DHT) was used to crosslink the matrices to increase their mechanical properties. The matrices were placed in a vacuum oven at 50m Torr for 24 hours at 105°C. This process also served to sterilize the scaffolds.

Excess slurry that was not immediately used to make scaffolds was stored at 4°C. Slurry that had been stored for more than 2 weeks was re-blended and degassed again before use.
Cell Culture

Normal human dermal fibroblasts (NHDFs) were obtained frozen from Cambrex/Lonza Walkersville, Inc (Walkersville MD). Prior to culturing the frozen cells, 15 ml of FGM-2 media supplemented with 2% FBS, rhFGF-B, insulin, and gentamicin/amphotericin-B (FGM-2 bullet kit, Lonza Walkersville, Inc., Walkersville, MD) were pipetted into a T75 culture flask. The flask was placed in an incubator (37°C, 5% CO\textsubscript{2}, 95% relative humidity) for at least 30 minutes to allow the media to equilibrate. The cells were then thawed, gently pipetted into the culture flask, and placed in the incubator. Media was replaced every 2 days. The cells were passaged when they were 90% confluent. NHDFs between passages 2 and 4 were seeded into matrices.

Normal human dermal fibroblasts were used as they are primary cells that should exhibit contractile behavior similar to that of cells active in wound healing. Our preliminary experiments (data not shown) indicated that they are more contractile than the commonly used Swiss 3T3 cell line.

Cell Viability and Proliferation

A preliminary assay to determine NHDF adherence, viability and proliferation within the collagen-GAG scaffolds was performed with the 96 μm pore size scaffold. This assay was designed to check that the number of cells stabilized within a short time relative to the duration of our contraction experiments. Cells were seeded in matrices at three different densities: 10\textsuperscript{5}, 5 \times 10\textsuperscript{5} and 10\textsuperscript{6} cells per matrix. The matrices were digested every two days after seeding and the number of cells was counted. Each sample was briefly washed in PBS to remove any non-adherent cells and then placed in a pre-warmed (37°C) dispase solution (2.525 mg/ml) to digest the matrix. The samples were shaken gently every 5 minutes to facilitate degradation of the matrix. After the matrices were completely digested, the cells were counted using a hemacytometer; all cell counts were done within 30 minutes after the matrices were digested. Trypan blue was used to assess cell viability. Four to six counts were performed for each sample.

Matrix Seeding and Contraction Measurements

To measure cell-mediated matrix contraction, NHDFs were seeded onto matrices of 4 different pore sizes (96, 110, 121 and 151 μm). Biopsy punches were used to cut six millimeter diameter samples from 3mm thick dry sheets of matrix. Each sample was placed in one well of a low-adhesion 6-well plate. Phosphate buffered saline solution (PBS) was gently pipetted into each well to hydrate the matrices. After 30 minutes of hydration, the PBS was removed and replaced with FGM-2 supplemented with 2% FBS, rhFGF-B, insulin, and gentamicin/amphotericin-B (FGM-2 bullet kit, Lonza Walkersville, Inc., Walkersville, MD). The matrices were placed in an incubator for at least 30 minutes to allow the matrices to equilibrate to cell-culture conditions. Prior to cell seeding, the media was pipetted out of the wells. Blotting paper, sterilized in the autoclave on the dry cycle for 20-30 minutes, was used to remove excess fluid in and around the scaffolds.

While the matrix samples were hydrating and equilibrating, a cell suspension was prepared. NHDFs were trypsonized and suspended in media. After the matrices had been blotted, 10 μl of the cell suspension was pipetted onto one side of each matrix. After 10 minutes, the matrices were flipped over and 10 μl of the cell suspension was pipetted onto the other side. The cells were incubated for 2 hours to allow the cells to adhere to the matrix, after which 3 ml of media was added to each well. Matrices of each pore size were prepared with the following cell concentrations: 100,000 cells per matrix sample, 500,000 cells per matrix, and 1 million cells per matrix. 100,000 cells per matrix corresponds to 1180 cells/ mm\textsuperscript{2}, or, for 100 μm pores, 0.62 cells/ pore. Six samples were tested for each combination of pore size.
and cell density. The matrices were kept in the incubator for the duration of the experiment. The media was changed every 2 days. The diameter of the matrix was measured every two days using a measurement template: the matrix diameter was determined by comparing it to known diameters printed on the template. The contraction of non-seeded controls was also measured.

The contraction experiments were stopped when the matrix deformation reached a steady state (i.e. became constant) or when the contraction became so large that it was not possible to measure further deformation. At the end of each contraction test, the number of adherent cells on each sample of matrix was measured, as described in the preliminary study on cell attachment and proliferation.

Estimation of the cell force from the matrix strain in the free-floating contraction experiment

We assume that the cells contract the scaffold uniformly in all three principal directions, as the cells are randomly distributed in the scaffolds and the scaffolds are roughly isotropic. To estimate the force that the cells apply to produce the matrix strain, we calculate the equivalent externally applied hydrostatic pressure required to produce the matrix strain (with no cells present) and estimate the force in each strut corresponding to that hydrostatic pressure [40].

Since the scaffold is roughly isotropic and we are considering hydrostatic loading, the strains in all three principal directions (radial, circumferential and through the thickness) are identical. The dilatation, ε, or volume change, ΔV/V₀, can be expressed in terms of the scaffold strain ε, or, using Hooke’s law, in terms of the scaffold modulus E, Poisson’s ratio, ν, and the applied hydrostatic pressure, p:

\[
e = \frac{\Delta V}{V} = 3\varepsilon = 3\frac{(1-2\nu) p}{E}
\] (5)

taking compressive strain to be positive. Using a dimensional argument, the force on a single member within the scaffold is proportional to \( p\ell^2 \), or

\[
F \propto p\ell^2 = C \frac{E\varepsilon}{3(1-2\nu)} \ell^2 \quad \text{for} \quad 0 \leq \varepsilon \leq \varepsilon_{el}^c
\] (6)

where C is a constant related to the pore geometry of the scaffold, which is found experimentally. For pores of a consistent geometry, C is independent of the pore size. The Young’s modulus of the scaffolds used in this study, in the hydrated state, has previously been measured to be independent of the pore size: \( E = 208 \text{ Pa} \) [34]. We take \( \nu = 0.33 \). For a given strain, the force on a single member within the scaffold is proportional to \( \ell^2 \).

The above equation is valid for the linear elastic regime (for strains up to about 10% for the collagen-GAG scaffolds). In the stress plateau regime (for strains between about 10% and 90%), the same analysis gives:

\[
F = C \left[ \frac{E\varepsilon_{el}}{3(1-2\nu)} \ell^2 + \frac{E_c (\varepsilon - \varepsilon_{el})}{3(1-2\nu_c)} \ell^2 \right] \quad \text{for} \quad \varepsilon > \varepsilon_{el}^c
\] (7)
where, $E_c$ is the slope of the scaffold stress strain curve in the plateau regime ($E_c = 92$ Pa), $\nu_c$ is the Poisson's ratio in the plateau regime (we take this to be zero, as in uniaxial compression, in the plateau regime, there is essentially no lateral expansion of a foam) and $e_{el}$ is the dilation at the transition between the linear elastic and stress plateau regimes: for a uniaxial strain at the beginning of strut buckling of 10%, $e_{el} = 0.27$.

Results

Finite element analysis

The buckling force per cell, $F_{cell}$, calculated from the Abaqus buckling analysis of a single tetrakaidecahedron, increased with the square of the pore size, $d$, for all cell seeding densities modeled, consistent with eqn (2) (Fig. 3). The best-fit curve for each cell density is:

$$F_{cell} = C_1 d^{2.00}$$

(8)

where $F_{cell}$ is the buckling force/cell in nN, $C_1 = 0.0190$ for 1 cell attached, $C_1 = 0.00260$ for 10 cells attached, $C_1 = 0.00140$ for 20 cells attached, $C_1 = 0.000900$ for 30 cells attached and $d$ is the pore size in microns ($R^2 = 1$ in all cases).

For a given pore size, the buckling force per cell, $F_{cell}$, for a single tetrakaidecahedron, decreased as the number of cells per pore, $N_pore$, increased (Figs. 3, 4a). The best-fit curve for the different pore sizes is:

$$F_{cell} = C_2 (N_pore)^{-0.88}$$

(9)

where $F_{cell}$ is the buckling force/cell in nN, $C_2 = 176$ for $d = 96\mu m$, $C_2 = 231$ for $d = 110\mu m$, $C_2 = 280$ for $d = 121\mu m$ and $C_2 = 436$ for $d = 151\mu m$ ($R^2 = 0.9957$ for all cases). We note that $C_1$ depends on cell density and varies with $N_{pore}^{-0.88}$, while $C_2$ depends on pore size and varies with $d^2$. Combining eqn (8) and (9), we find:

$$F_{cell} = 0.0190 \frac{d^2}{N_{pore}^{0.88}}$$

(10)

where $F_{cell}$ is in nN and $d$ is in $\mu m$.

The total force, $F_{total}$, (the sum over all buckled struts) increases with the number of cells/pore up to a roughly constant value at about 12 cells/pore (Fig. 4b). The tetrakaidecahedron has 36 struts, so that the total force reaches the constant value when roughly 1/3 of the struts are buckled.

The results can be extended to simulate a matrix specimen of given dimensions by calculating the number of pores in the matrix specimen (the volume of the matrix, $V_{matrix}$, divided by the volume of a single tetrakaidecahedron, $11.31\ell^3$), multiplying this by the number of cells/pore, $N_{pore}$, and dividing by 3 since each strut is shared between 3 tetrakaidecahedra. The number of cells/matrix is then:
For our matrix specimens, cylinders 6mm in diameter and 3mm high,

\[ N_{\text{matrix}} = \frac{1}{3} V_{\text{pore}} N_{\text{pores}} \]  

(11)

where \( \ell \) is the edge length of the tetrakaidecahedron and \( d \) is the pore size in \( \mu \)m.

The buckling force per cell, \( F_{\text{cell}} \), can then be plotted against the number of cells per matrix, \( N_{\text{matrix}} \) (Fig. 4c). The best-fit curve, found using a power law fit in Matlab, is:

\[ F_{\text{cell}} = C_3 (N_{\text{matrix}})^{-0.88} \]  

(13)

where \( F_{\text{cell}} \) is the buckling force per cell in nN and \( C_3 = 2.71 \times 10^6 \) for \( d = 96 \) \( \mu \)m, \( C_3 = 2.47 \times 10^6 \) for \( d = 110 \) \( \mu \)m, \( C_3 = 2.33 \times 10^6 \) for \( d = 121 \) \( \mu \)m and \( C_3 = 2.03 \times 10^6 \) for \( d = 151 \) \( \mu \)m (\( R^2 = 0.9982 \) in all cases). Combining eqn (10) and (12), we can also write:

\[ F_{\text{cell}} = \frac{5.26 \times 10^7}{N_{\text{matrix}}^{0.88} d^{0.64}} \]  

(14)

where \( F_{\text{cell}} \) is in nN and \( d \) is in \( \mu \)m. Equation (14) gives values of \( C_3 \) within 5% of those of the best-fit power curves. We note that the sensitivity of \( F_{\text{cell}} \) to pore size is much lower in eqn (14) than eqn (10), explaining the collapse of the 4 curves in Fig. 4a nearly onto a single curve in Fig. 4c. The results in Fig. 4c are well described by a single curve:

\[ F_{\text{cell}} = 1.37 \times 10^6 (N_{\text{matrix}})^{-0.83} \quad (R^2 = 0.986) \]  

(15)

Finally, the total force can be plotted against the number of cells per matrix (Fig. 4d). The result is similar to Fig. 4b: the total force reaches a maximum when about 1/3 of the struts are buckled.

**Experimental Results**

**Cell viability and proliferation**—For each of the seeding densities, the number of cells in each matrix increased over the first four days and then reached a roughly constant number that was significantly higher (\( p<0.1 \)) than the initial seeding density (Fig. 5). The percentage of cells that initially adhered to the matrices increased with the seeding density. About 20% of the seeded cells initially adhered to the scaffolds at the lowest density, while the percentage increased to 29% and 34% at the two higher densities, respectively. The difference between these percentages of initially adherent cells is not significant (\( p>0.1 \)). Furthermore, for each time point, there is no significant difference between the percentage of adherent cells at each initial seeding density.

To model the cell proliferation and predict the number of adherent cells at any given time point in the contraction experiments, the data were fit to an exponential curve of the form (Fig. 5):

\[ N(t) = N_0 e^{k t} \]  

where \( N(t) \) is the number of cells at time \( t \), \( N_0 \) is the initial number of cells, and \( k \) is the proliferation rate constant. The best-fit curve is shown in Fig. 5.
where C is the number of cells on the desired day, \( C_0 \) is the initial number of cells, t is the time in days, and \( \tau \) is a time constant. The \( C_0 \) and \( \tau \) values were obtained by fitting the experimental data to equation (16) using the least squares fitting function in MATLAB. The \( C_0 \) values for the \( 10^5, 5 \times 10^5, \) and \( 10^6 \) seeding densities were 62,000, 245,000, and 454,000, respectively, while the \( \tau \) values were 0.154, 0.0600, and 0.0600 days, respectively. Although this experiment was only performed on 96 μm pore size scaffolds, it was assumed that similar behavior would be observed in the other scaffolds.

Contraction experiments—The net strain in the matrix resulting from the contractile forces in the cells is plotted against time in Fig. 6. The net strain is the displacement in a cell seeded matrix at a given time minus the displacement in the unseeded matrix at the same time point, divided by the initial diameter of the cell seeded matrix. The net strain increased with time, to strains of up to 60%. The strains in the 110 μm scaffolds were lower than those in the scaffolds of other pore sizes. In the 151 μm scaffolds strains of 60% were reached at much shorter times than in the other scaffolds (15 days as compared with 40 days).

In compression, collagen-GAG scaffolds behave like a foam (Fig. 1) [34]. The members of the scaffold initially bend, giving a linear elastic regime. At sufficiently high stresses, one layer of struts within the scaffold collapses by elastic buckling, followed by the progressive collapse of neighboring layers, giving the stress plateau regime. Once the scaffold has completely collapsed, opposing members touch and load each other directly and the stress rises sharply, giving rise to the densification regime. Hydrated collagen-GAG scaffolds initially buckle at a strain of 10% [34]. The buckling force per cell, \( F_{\text{cell}} \), corresponding to a measured scaffold net strain of 10%, calculated using eqn (6), assuming \( C = 1 \), is plotted against pore size in Fig. 7. The number of cells per pore was determined by counting the cells in each matrix with a hemacytometer and dividing by the average number of pores in each scaffold. To plot the data for a single isolated pore (to compare with the finite element results of Fig. 3a), the cell number was then multiplied by 3 to account for the fact that each strut is shared by three pores. The best-fit curve to the data, found using a power law fit in Matlab, is:

\[
F_{\text{cell}} = C_4 d^3
\]  

(17)

where \( F_{\text{cell}} \) is the buckling force/ cell in nN, \( d \) is the pore size in microns, \( C_4 = 5.51 \times 10^{-6} \) for 3 cells attached, \( C_4 = 4.13 \times 10^{-6} \) for 4 cells attached, \( C_4 = 2.75 \times 10^{-6} \) for 6 cells attached, \( C_4 = 1.84 \times 10^{-6} \) for 9 cells attached and \( C_4 = 1.65 \times 10^{-6} \) for 10 cells attached (\( R^2 = 0.999 \) in all cases). The experimental buckling force per cell increases with the cube of the pore size for all cell densities, rather than with the square of the pore size given by the dimensional analysis (eqn 2) and the finite element analysis (eqn 8).

The buckling force per cell, at the onset of buckling (eqn 6, at 10% strain) is plotted against cell density in Fig. 8. The number of attached cells in the scaffold at the time corresponding to 10% strain was calculated from Fig. 6 and eqn (16). The number of pores in a matrix sample was found from the volume of the sample divided by the volume of one pore \( (V_{\text{pore}} = 11.31 \ell_d^3 \) for a tetrakaidecahedral pore). The number of attached cells per pore, \( N_{\text{pore}} \), was then determined by dividing the number of attached cells by the average number of pores. To account for the fact that each strut is shared by three pores, this number was multiplied by 3 to find the number of cells in a single, isolated pore. Similar to the FEM
results, for a given pore size, the buckling force per cell, for a single, isolated pore, decreases as the cell density increases (Fig. 8a). However, the force values are lower, ranging from 1-55 nN instead of 8-429 nN as given by the finite element analysis. The best-fit curve for the buckling force per cell for a single isolated pore, for each pore size, is:

\[ F_{cell} = C_5 \left( N_{pore} \right)^{-1} \]  

(18)

where \( F_{cell} \) is the buckling force per cell in nN, and \( C_5 = 14.6 \) for \( d = 96 \mu m \), \( C_5 = 21.9 \) for \( d = 110 \mu m \), \( C_5 = 29.2 \) for \( d = 121 \mu m \), and \( C_5 = 56.7 \) for \( d = 151 \mu m \) (\( R^2 = 1 \) in all cases). Equations (17) and (18) can be combined to give:

\[ F_{cell} = \frac{1.64 \times 10^{-5} d^3}{N_{pore}} \]  

(19)

The total force exerted by all of the cells combined does not vary with the cell density, but does vary with the pore size (Fig. 8b). We note that the total force is equal to the force per cell (eqn 19) multiplied by the number of cells per pore, \( N_{pore} \), or:

\[ F_{total} = F_{cell} N_{pore} = 1.65 \times 10^{-3} d^3 \]  

(20)

where \( F_{cell} \) is in nN and \( d \) is in \( \mu m \).

When the buckling force per cell, \( F_{cell} \), is plotted against the number of cells per matrix, \( N_{matrix} \) (Fig. 8c), the curves from Fig. 8a collapse onto one curve described by the best-fit equation to the data (\( R^2 = 1 \)):

\[ F_{cell} = 8.86 \times 10^5 (N_{matrix})^{-1} \]  

(21)

where \( F_{cell} \) is in nN. This can also be obtained by combining eqn (19) and eqn (12).

The total force in the matrix does not vary with pore size or cell density (Fig. 8d). The total force is given by (combining eqn 19 and 11):

\[ F_{total} = F_{cell} N_{pore} \frac{V_{matrix}}{3V_{pore}} = 0.886 mN \]  

(22)

The independence of pore size is expected, since the force is determined at the same strain value (10%) for each scaffold and the modulus of the scaffolds is independent of the pore size [34]. Increasing the cell density reduces the time required for the cells to contract the matrix by a given strain, but the force required remains constant.

At the end of the experiments, the NHDFs had contracted the matrices to strains of greater than 10% (Fig. 6) (i.e. into the collapse plateau regime). The total force exerted by the cells on the scaffolds in this regime was calculated using eqn (7), again assuming \( C = 1 \). The number of cells in an isolated pore, \( N_{pore} \), and the number of cells per matrix, \( N_{matrix} \), were calculated as previously described. Similar to the results at the onset of buckling, the force per cell decreases as the number of cells per pore, \( N_{pore} \), increases (Fig. 9a). The best-fit curves for each pore size are:
where $F_{cell}$ is the force per cell in nN. The total force exerted by all of the cells in the pore does not vary with the cell density, but does increase with the pore size (Fig. 9b), analogous to Fig. 8b.

When the force per cell is plotted against the total number of cells in the matrix (Fig. 9c), the resulting curves can be described by the equations:

\begin{align}
F_{cell} &= 41.2(N_{pore})^{-0.81} \text{ for } 96 \mu m \text{ pore size } \left( R^2 = 0.9265 \right) \\
F_{cell} &= 80.4(N_{pore})^{-1.01} \text{ for } 110 \mu m \text{ pore size } \left( R^2 = 0.9839 \right) \\
F_{cell} &= 147(N_{pore})^{-1.27} \text{ for } 121 \mu m \text{ pore size } \left( R^2 = 0.9149 \right) \\
F_{cell} &= 222(N_{pore})^{-1.04} \text{ for } 151 \mu m \text{ pore size } \left( R^2 = 0.9810 \right)
\end{align}

This data can be fit to a single curve:

\begin{equation}
F_{cell} = 4.04 \times 10^{6}(N_{matrix})^{-1.02} \left( R^2 = 0.989 \right)
\end{equation}

When the total force is plotted against the number of cells in each matrix (Fig. 9d), the result is similar to Fig. 8d; in general, the total force does not vary with seeding density or the matrix pore size.

The results at the end of the contraction experiment, shown in Fig. 9, follow the same trends as those at initial buckling (at 10% strain), shown in Fig. 8. The different time points and different strains at the end of the test give somewhat more variability to the results.
The force per cell, for a single, isolated pore, is plotted against the total number of cells per matrix in Fig. 10 for the finite element results (at the onset of buckling) (eqn 15) and the experimental data, both at the onset of buckling (eqn 21), and at the conclusion of the experiment (eqn 25). All of the data follow the same trend and can be described by similar curves. The experimental data at the onset of buckling differ from the results predicted by FEA by a factor of 12, while the experimental data at the end of the experiment differ by a factor of 3.

**Discussion**

The finite element model applies forces to individual struts in the tetrakaidecahedron, simulating the forces that individual cells apply to struts in tissue engineering scaffolds. This gives a better physical representation of the loads applied to scaffolds by cells than foam models that are loaded on the external edges. The models capture a number of the features of measurements of cell contraction in tissue engineering scaffolds.

Cells contract tissue engineering scaffolds primarily through buckling (and some bending) of the struts of the scaffold [41]. The finite element model indicates that the buckling force per cell, $F_{cell}$, decreases with the number of cells per pore, $N_{pore}$, and, for a given $N_{pore}$, it increases with increasing pore size (Fig. 4a). The distinct curves for models with different pore sizes collapse onto a single curve if $F_{cell}$ is plotted against the total number of cells in the model, $N_{matrix}$ (Fig. 4c); here we extended our model results to represent the number of cells in the cylindrical specimens of scaffolds used in the experiments. Our experimental results for the cell contractile forces at 10% strain in the scaffold (Fig. 8a,c), corresponding to strut buckling when the unseeded scaffold is loaded externally in a uniaxial compression test, show exactly the same trends. Quantitatively, we find, for the finite element analysis, that the buckling force per cell varies with the number of cells on a single isolated tetrakaidecahedral pore as: $F_{cell} \propto N_{pore}^{-0.88}$ (Fig. 4a, eqn 9) while the experimental contraction results indicated that $F_{cell}$ varies with $N_{pore}^{-1}$ (Fig. 8a, eqn 18). The exponents are similar; the difference is probably caused by edge effects associated with analyzing only a single isolated tetrakaidecahedron.

If, instead of the buckling force per cell, $F_{cell}$, we plot the total force, $F_{total}$, (the sum of the forces in all the cells) against either the number of cells per pore, $N_{pore}$, or the total number of cells in a scaffold specimen, $N_{matrix}$, we find that, for the finite element model, at low cell densities, the total force increases until it reaches a roughly constant value when roughly 12 cells are contracting each pore, or roughly 1/3 of the struts in the tetrakaidecahedron are loaded (Fig. 4b, d). The value of the constant force at cell densities above this depends on the pore size. Again, our experimental results at 10% strain (Fig. 8b, d) show the same trend of constant total force, although the initial increase in force at low cell densities is not observed. For the finite element analysis $F_{cell} \propto N_{matrix}^{-0.88}$ (Fig. 4c, eqn 13) while for the contraction experiments, $F_{cell} \propto N_{matrix}^{-1}$ (Fig. 8c, eqn 21); again we expect the difference in exponent to be related to modeling only a single isolated tetrakaidecahedron. Interestingly, all of these trends are also seen at the end of the experimental measurements of cell contraction (Fig. 9).

The collapsed curves of force per cell plotted against the number of cells per matrix are compared in Fig. 10. Over the range of cell densities in this study (up to about 700,000 cells/matrix or 8250 cells/mm$^3$) the buckling force/ cell calculated in the finite element analysis (Fig. 4c) is larger than that obtained in the contraction experiments at 10% strain (Fig. 8c) by a factor of about 12, suggesting that the constant $C$ in eqn (6) is about 12. We note that since the exponent in the finite element analysis differs slightly from that found in the
experiments (0.88 vs. 1), this value is not exactly a constant, but varies from about 10 for 50,000 cells/ matrix to 15 for 700,000 cells/ matrix. We expect that a more complete finite element analysis, for a larger array of tetrakaidecahedra, would give an exponent of 1, as is found experimentally, giving a constant value for C.

Both dimensional analysis (eqn 2) and finite element analysis (eqn 8) indicate that the buckling force per cell, $F_{cell}$, increases with the square of the pore size, $d$, while the contraction experiments indicate that it increases with the cube, $d^3$ (eqn 17). While the reason for this is not entirely clear, we note that previous studies have shown that cell attachment increases with the specific surface area available within the scaffold (or $1/d$) [38,42]. In the finite element analysis, the number of cells attached to the model is varied independently of the pore size. In the contraction experiments, however, the number of cells attached to the scaffold is expected to vary as $1/d$.

Conclusions

The contraction experiments indicated that the contractile force required to buckle the struts in the scaffold varies with the cube of the pore size, $d^3$, rather than with $d^2$, as predicted by the dimensional analysis and the finite element analysis; the reason for this difference is unclear. In spite of this difference, the trends observed in the cell contraction experiments are also reflected in the finite element results. First, the buckling force per cell decreases with increasing numbers of cells per pore and with decreasing scaffold pore size. Second, both the data from the cell contraction experiments as well as the finite element results collapse onto a single curve when the buckling force per cell is plotted against the total number of cells per matrix. Third, the total force applied by the cells in the contraction experiment is roughly independent of the number of cells per pore or per matrix; this result is duplicated in the finite element results when there are cells on more than 1/3 of the struts. Finally, the trends observed for the buckling force (at 10% strain in the scaffold) are also observed for the force at the end of the contraction experiment (at strains of up to 60%).

Acknowledgments

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References


Fig. 1.
(a) Scanning electron micrograph of a collagen-GAG scaffold (reprinted from Pek et al., 2004, with permission from Elsevier).
(b) Tetrakaidecahedral model of the structure.
(c) Compressive stress-strain curve for the collagen-GAG scaffold (courtesy of Brendan Harley).
(d) Compressive stress-strain curve for an open-cell polyurethane foam (reprinted from Gibson and Ashby, 1997, with permission).
Fig. 2.
(a) Optical micrographs of a dermal fibroblast buckling a strut in a collagen-GAG scaffold. Scale bar 50 μm. (b-e) Schematic of cell buckling the scaffold strut (reprinted from Freyman et al., 2001b, with permission from Elsevier.)
Fig. 3.
Finite element results for buckling force per cell plotted against pore size, for different numbers of cells attached. The buckling force varies with the square of the pore size (eqn 8).
Fig. 4.
(a) Finite element results for the buckling force per cell, on a single, isolated tetrakaidecahedron, plotted as a function of the number of cells attached per pore, for 4 different model pore sizes. The equations for the best-fit curves are given in the text (eqn 9).
(b) The total buckling force plotted as a function of the number of cells attached per pore.
(c) The buckling force per cell plotted as a function of the number of cells per matrix. The equations for the best-fit curves are given in the text (eqn 13).
(d) The total buckling force plotted as a function of the number of cells per matrix.
Fig. 5. NHDF adhesion and proliferation with the 96 micron pore size collagen-GAG scaffolds. Data points indicate the average cell counts for all matrices seeded at a given cell density on a given day. Bars indicate one standard deviation. Lines show the best-fit curves for the equation $C = C_0 \left(1 - \exp\left(-\frac{t}{\tau}\right)\right)$. $C_0$ and $\tau$ are given in the text.
Fig. 6.
Cell contraction results. Net strain in the scaffolds plotted against time for (a) 96 µm (b) 110 µm (c) 121 µm and (d) 151 µm pore size scaffolds.
Fig. 7.
Experimental results for buckling force per cell plotted against pore size, for different number of cells attached. The best-fit equations are given in the text (eqn 17). Individual data points for each pore size are plotted. The average number of cells per pore is indicated with arrows next to the corresponding data points. The lines are best-fit curves extrapolated from the data set.
Fig. 8.
(a) The force per cell plotted as a function of the number of cells attached per pore, for 4 different scaffold pore sizes at the point the scaffolds buckle. The best-fit equations are given in the text (eqn 18).
(b) The total force plotted as a function of the number of cells attached per pore.
(c) The force per cell plotted as a function of the number of cells per matrix. The best-fit curve for the cumulative data is (eqn 21) \( F_{cell} = 8.86 \times 10^3 N_{matrix}^{-1} \), \( R^2 = 1 \).
(d) The total force plotted as a function of the number of cells per matrix: \( F_{total} = 0.886 \text{mN} \).
Fig. 9.
(a) The force per cell plotted as a function of the number of cells attached per pore, for 4 different scaffold pore sizes at the end of the experiment. The best-fit equations are given in the text (eqn 23).
(b) The total force plotted as a function of the number of cells attached per pore.
(c) The force per cell plotted as a function of the number of cells per matrix. The best-fit curve for the cumulative data is (eqn 25) $F_{cell} = 4.04 \times 10^6 N_{matrix}^{-1.02}$, $R^2 = 0.989$.
(d) The total force plotted as a function of the number of cells per matrix.
Fig. 10.
The force per cell plotted against the number of cells per matrix. The graph shows the finite element results (eqn 15, denoted FEA) as well as the experimental results, both at the onset of buckling (at 10% strain) (Exp10%□) and at the end of the experiment (ExpEnd).
Table 1
Scaffold strut lengths and radii for finite element modeling ($\rho^*/\rho_c=0.006$)

<table>
<thead>
<tr>
<th>Pore size, $d$ ($\mu$m)</th>
<th>Strut length, $l$ ($\mu$m)</th>
<th>Strut radius, $r$ ($\mu$m)</th>
</tr>
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<tbody>
<tr>
<td>96</td>
<td>34.5</td>
<td>1.47</td>
</tr>
<tr>
<td>110</td>
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<td>1.68</td>
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<tr>
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<td>43.5</td>
<td>1.85</td>
</tr>
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<td>151</td>
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<td>2.31</td>
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