A poroviscoelastic description of fibrin gels

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\textbf{A B S T R A C T}

The mechanical induction of specific cell phenotypes can only be properly controlled if the local stimuli applied to the cells are known as a function of the external applied loads. Finite element analysis of the cell carriers would be one method to calculate these local conditions. Furthermore, the constitutive model of the construct material should be able to describe mechanical events known to be responsible for cell stimulation, such as interstitial fluid flow. The aim of this study was to define a biphasic constitutive model for fibrin, a natural hydrogel often used for tissue engineering but not yet thoroughly characterized. Large strain poroelastic and poroviscoelastic constitutive equations were implemented into a finite element model of a fibrin gel. The parameter values for both formulations were found by either analytically solving equivalent low strain equations, or by optimizing directly the large strain equations based on experimental stress relaxation data. No poroelastic parameters that satisfactorily described the fibrin carrier behaviour could be found, suggesting that network viscoelasticity and fluid-flow time-dependent behaviour must be separately accounted for. It was demonstrated that fibrin can be described as a poroviscoelastic material, but a large strain characterization of the parameter values was necessary. The analytical resolution of the low strain poroviscoelastic equations was, however, accurate enough to serve as a reliable initial condition for further optimization of the parameter values with the large strain formulation.

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1. Introduction

Controlled mechanical induction of specific phenotypes in 3D carriers is limited by the difficulty of relating external loads to the cell local environments (Pedersen and Swartz, 2005). Finite element (FE) analysis of the constructs would allow calculating these local conditions believed to alter the cell behaviour, but relies on accurate constitutive models of carrier materials. As a biopolymer with natural cell attachment and hydrogel structure, fibrin is a promising support for cell mechanostimulation. It has been modelled with both physical polymer-chain (Storm et al., 2005; Henry and Nestler, 2004) and continuum models (Benkherouf et al., 2000). However, none of these models predicted fluid flow, shown to be an important cell mechanostimulator (Isaksson et al., 2006). Therefore, as a first step toward the use of fibrin for cartilaginous tissue engineering, this study aimed to assess biphasic constitutive models able to describe the compressive behaviour of a 3D fibrin carrier, up to 10% strain (Pelaez et al., 2008). Poroelastic and poroviscoelastic finite strain formulations were investigated and analytical small strain solutions were explored to find parameter values.

2. Materials and methods

Twelve fibrin specimens (8 mm diameter × 4 mm height) with 45 mg/ml fibrinogen and 1 U/ml thrombin were prepared from Baxter (Vienna, Austria) non-commercial products. Composition was chosen to ensure mechanical stability (Eyrich et al., 2007) and cell viability (Pelaez et al., 2008). Specimens were compressed in a saline bath (RT, 0.9% NaCl), between an electro-polished stainless-steel flat top plate and a bottom flat smooth glass support. Contact between samples and the top plate was established by lowering a precision actuator (PM-500C, Newport, Irvine, CA) until the reaction force, measured with a 5 mN resolution load cell (8432-2.5N, Messtechnik Schaffhausen, Neuhauen, Switzerland), reached 0.02 N. After fibrin relaxation, successive compressive strain steps of 2% were applied from 0% to 10% with loading ramps at 0.1 mm/s. For each load, full relaxation was assumed when the reaction force rate of change dropped below 5 mN/min.

Finite strain poroelastic and poroviscoelastic models expressed fibrin stress ($\sigma_{PS}$ or $\sigma_{PVE}$) as a sum of solid and fluid contributions. Solid stress was either...
purely elastic (\(\sigma_r\)) or a sum of elastic and viscoelastic contributions (\(\sigma_r + \sigma_v\)). Pure pressure, \(p\), represented fluid contribution

\[
\sigma_{pg} = \sigma_r + \sigma_v - Pf
\]

(1a)

\[
\sigma_{ps} = \sigma_r - Pf
\]

(1b)

\(I\) is the identity tensor. \(\sigma_r\) characterized the equilibrium behaviour of the gel and was described by a Neo-Hookean compressible model (Wilson et al., 2005):

\[
\sigma_r = E \ln \frac{q}{q_0} + \frac{2E}{3} (\frac{q^T q}{q_0^2})
\]

(2)

Fig. 1. Geometry and boundary conditions of the axisymmetric finite element model used for simulation of the stress relaxation experiments. \(p\) is the pore pressure and \(\dot{u}_f\) the fluid velocity vector.

where \(J\) is the determinant of the deformation gradient tensor, \(E, v\) and \(E\) are, respectively, Poisson’s ratio and undeformed Young’s modulus of the porous solid. \(\sigma_r\) characterized the transient fluid-like response of the solid. Since continuum \(\pi\) theory of linear poroelasticity assumes almost incompressible solid grains, \(\sigma_r\) was only associated to deviatoric deformations (Suh and DiSilvestro, 1999). An upper convected Maxwell (UCM) model was adapted (Baaijens et al., 2005)

\[
\sigma_{v} = \frac{1}{2} \mu \dot{e}_d + \frac{1}{2} \lambda \varepsilon_3 + \frac{1}{2} \mu \dot{e}_d T + \frac{1}{2} \lambda \varepsilon_3 T
\]

(3)

where \(G_v\) and \(\lambda\) are porous solid shear modulus under steady simple shear and relaxation time, respectively. \(p\) was related to fluid velocity, \(\dot{u}_f\), through Darcy’s law

\[
\dot{u}_f = -\kappa \nabla p
\]

(4)

where \(\kappa\) is the hydraulic permeability of the biphasic material. Equivalent small strain expressions of (2) and (3) were, respectively

\[
\sigma_{ps} = \frac{E}{1 - 2v} \ln (\frac{q}{q_0}) + \frac{E}{2(1 + v)} \left( \frac{q^T q}{q_0^2} - 1 \right)
\]

(5)

\[
\frac{\varepsilon}{\varepsilon_0} = \frac{1}{2} \frac{\mu}{\varepsilon_0} + \frac{1}{2} \frac{\lambda}{\varepsilon_0} = 2G_v \frac{\varepsilon}{\varepsilon_0}
\]

(6)

where \(\varepsilon\) is the linear strain tensor. \(E, v,\) and \(G_v\) were analytically determined from the experimental measurements. \(\kappa_{\text{analytic}}\) was estimated from (5) after complete relaxation at 4% strain

\[
\kappa_{\text{analytic}} = f \frac{h_0}{A_d d_0}
\]

(7)

where \(f\) is the measured reaction force, \(A_d\) and \(h_0\) are respectively the undeformed cross-section and height of the sample, and \(d_0\) the 4% strain actuator displacement. Viscoelastic parameters were estimated from the 4% strain relaxation data by solving (6) and replacing \(\sigma_{ps}\) in (1a) with (5). Subsequent time derivation and linearization gave

\[
\ln \left( \frac{\varepsilon(t)}{\varepsilon(t_0)} + \frac{\varepsilon(t)}{\varepsilon(t_0)} \right) = \ln \left( \frac{4\mu_0 G_v (1 + v)}{3\mu_0} (d_0\varepsilon(t_0) + d_2) \right) - \frac{t}{\tau_4}
\]

(8)

where \(\tau_4\) is the relaxation start time at 4% strain, and \(d_2\) the strain actuator displacement at 2% strain. Hypothesizing that during relaxation, polymer–chain viscosity and fluid effects may have different time scales, at some point, \(A_d (\varepsilon(t)/\varepsilon(t_0))\) could become insignificant in (8). Then, time evolution of \(\ln (\varepsilon(t)/\varepsilon(t_0))\) would temporally almost depend on a unique constant, i.e. \(\lambda\)

\[
\ln \left( \frac{\varepsilon(t)}{\varepsilon(t_0)} \right) = \frac{-t}{\tau_{\text{analytic}}} + H
\]

(9a)

with

\[
H = \ln \left[ \frac{4\mu_0 G_v k_{\text{analytic}} (1 + v)}{3\mu_0} (d_0\varepsilon(t_0) + d_2) \right]
\]

(9b)

Linear regression on (9a) returned \(k_{\text{analytic}}\). \(G_{v,\text{analytic}}\) was then calculated from (9b). The error when passing from (8) to (9a) was estimated by re-evaluating \(k_{\text{analytic}}\) from (9b) with \(G_{v,\text{analytic}} = 1.2G_{v,\text{analytic}}(2(1 + v))\) (Baaijens et al., 2005). \(\sigma_{v,\text{analytic}}\) was computed from the loading ramp data to 4% and 6% strain. Assuming that under fast linear displacements, radial fluid velocity, \(\dot{u}_f\), and piston velocity, \(\dot{u}_p\),

Table 1

Summary of the small strain analytical parameter solutions and finite strain-optimized parameter values for the poroviscoelastic (Eq. (1a)) and the poroelastic models (Eq. (1b))

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Definition</th>
<th>Model</th>
<th>Calculation method</th>
<th>Value</th>
<th>Relative difference between analytical and optimized values (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E) (MPa)</td>
<td>Poroviscoelastic</td>
<td>(E_{\text{analytic}}) (Eq. (7))</td>
<td>0.022</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(E_{\text{optimized}}) (Eq. (7))</td>
<td>0.019</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poroelastic</td>
<td>(E_{\text{analytic}}) (Eq. (7))</td>
<td>0.022</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(E_{\text{optimized}}) (Eq. (7))</td>
<td>0.024</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>(\lambda) (s)</td>
<td>Poroviscoelastic</td>
<td>(\lambda_{\text{analytic}}) (Eq. (9a))</td>
<td>77</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(\lambda_{\text{optimized}}) (Eq. (9b))</td>
<td>59</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>(G_v) (MPa)</td>
<td>Poroviscoelastic</td>
<td>(G_{v,\text{analytic}}) (Eq. (9b))</td>
<td>0.0086</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(G_{v,\text{optimized}}) (Eq. (9b))</td>
<td>0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\kappa) (mm(^3)/N s)</td>
<td>Poroviscoelastic</td>
<td>(\kappa_{\text{optimized}}) (Eq. (12))</td>
<td>0.24</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(\kappa_{\text{analytic}}) (Eq. (11))</td>
<td>0.39</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>
were similar, (4) gave

\[ p = 0.45 \frac{r_0 \Delta d}{h_0} \]  

(10)

where \( r_0 \) and \( h_0 \) are the undeformed radius and height of the specimen, and 0.45 describes nearly isochoric deformations. Poroelastic permeability was given by replacing (5) and (10) in (1b)

\[ k_{\text{PE}}^{\text{analytic}} = \frac{0.45 r_0 \Delta d}{h_0} \left( \frac{h_0}{A_0} \right) \frac{r}{C_0} \]  

(11)

Solving (6) for constant strain rates and replacing \( \sigma_{ss}^v \) in (1a) with (5) and (10), gave

\[ k_{\text{PVE}}^{\text{analytic}} = \frac{0.45 r_0 \Delta d}{h_0} \left( \frac{h_0}{A_0} \right) \frac{r}{C_0} \left( \frac{1 + \frac{1}{C_0} \frac{e^{\frac{d}{C_0}}}{C_0}}{1 + \frac{1}{C_0} \frac{e^{\frac{d}{C_0}}}{C_0}} \right) \]  

(12)

Small strain parameter solutions for (1a) and (1b) were verified by simulating the compression experiment with an axisymmetric FE model (ABAQUS 6.5-6, Simulia, Maastricht, The Netherlands) with four-node bilinear pore pressure elements (Fig. 1). Finite strain parameter solutions were also determined by optimizing the small strain solutions with a nonlinear unconstrained minimization of the quadratic residual between simulated and experimental reaction forces (MATLAB 7.0.1, The Mathworks, Bern, Switzerland).

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Fig. 2. Graphic representation of Eq. (9a) including linear regression for the relaxation period at 4% strain.

Fig. 3. Graphic representations of the poroviscoelastic (a, b) and poroelastic (c, d) hydraulic permeabilities (Eqs. (11), (12)) as a function of time during the loading ramps to 4% (c) and to 6% (d) strain.
3. Results

Table 1 summarizes the small strain analytical parameter values. During 4% strain relaxation, linear regression on $\ln(\frac{q_f}{q_t})$ was nearly perfect (Fig. 2) and returned a relaxation time of 77 s compared to 81 s given from (9b) with $G_v^{analytic}E^{1.2(\frac{E^{analytic}}{2(1+n)})}$. $\rho E^{analytic}$ then only had a slight effect in (8), which validated (9a). The most linear part of the displacement ramps to 4% and 6% strain (Fig. 3 c, d) gave a nearly constant $k_{PVE}^{analytic}$ value (Fig. 3 a, b). Although $k_{PE}^{analytic}$ varied substantially during loadings (Fig. 3 e, f), only 10% variations were calculated during linear displacements.

Maximum difference between analytical and optimized poroviscoelastic parameter values was 39% (Table 1). However, $k_{PE}^{optimized}$ and $k_{PVE}^{analytic}$ differed by more than 200%. Small strain analytical parameter values overestimated the poroviscoelastic reaction forces above 6% strains (Fig. 4a), but finite strain optimization allowed correct predictions up to 10% strain (Fig. 4 c). With the analytical parameter values, the poroelastic model returned equilibrium reaction forces similar to the experimental ones, but underestimated peak forces (Fig. 4 b). Finite strain optimization did not improve poroelastic predictions (Fig. 4 d).

4. Discussion

While the poroviscoelastic formulation with optimized parameter values allowed reproducing the carrier behaviour, the poroelastic model was never able to predict transient responses. Poroelastic modelling with small strain analytical parameters produced accurate equilibrium forces but underestimated peak forces, as already reported with linear poroelastic model applied to articular cartilage (Suh and DiSilvestro, 1999). However, fewer adjustable poroelastic parameters than used for poroviscoelastic modelling could explain less accurate predictions after optimization. Divergence of $k_{PE}^{optimized}$ from $k_{PE}^{analytic}$ and the wavy shape of $k_{PVE}^{analytic}(t)$, both suggest that a strain-dependent permeability could improve poroelastic predictions. Such a model was included in (1b) with a permeability function successfully used for cartilage non-fibrillar matrix (Wilson et al., 2005) and agarose (Gu et al., 2003)

$$k_{PE} = k_0M^{13}$$

where $k_0$ is the undeformed permeability, and $M$ a positive constant. Although the use of (13) in (1b) gave similar number of poroelastic and poroviscoelastic parameters, poroelastic predictions were not improved. This was actually consistent with the opacity of our gels. Fibrin gels with reduced light transmission have more lateral aggregations (Okada and Blomback, 1983), resulting in more viscous fibrin networks (Weisel, 2004).

As in other poroviscoelastic models used for cartilage (Huang et al., 2001; Setton et al., 1993), our viscoelastic solid modelling was based on the principle of superposition. It consisted of a nonlinear Neo-Hookean spring in parallel with a nonlinear Maxwell element. However, unlike cartilage models, the present model had a one-mode instead of a two-mode relaxation function, which was motivated by the frequency- (Gerth et al., 1974) and strain (Roska and Ferry, 1982) independent transient times found for ligated fibrin. However, Benkherrou et al. (2000), without having modelled fluid pressure, reported that two Maxwell elements were necessary to predict the transient behaviour of fibrin with more than 1 mg/ml fibrinogen. According to the insignificance of $A_0(\frac{cp}{ct})$ in (8) and $k_{PVE}$ values $1 \times 10^5$ times
higher than for articular cartilage, poroviscoelastic simulations returned a product $A_p\beta$ always at least 100 times lower than $f$. This suggests that $f$ was more controlled by the high fibrinogen content of the solid phase than by fluid pressurization. With maximum 3 mg/ml fibrinogen, the soft gels used by Benkerhorou et al. (2000) probably had permeability values similar or lower than $k_{\text{PVE}}$ (Van Gelder et al., 1995; He et al., 2005) which could have allowed enough fluid pressurisation to induce an apparent relaxation mode. Thus, with high fibrinogen content, a single relaxation mode appears reasonable and has the advantage to reduce by one number of parameters, compared to other poroviscoelastic models developed for cartilage (Huang et al., 2001; Setton et al., 1993).

As expected, small strain analytical parameter solutions only allowed accurate poroviscoelastic predictions at low strains. At larger strains, many physical simplifications could explain the failure of $J_{\text{analytic}}$, $E_{\text{analytic}}$, and $k_{\text{PVEanalytic}}$ in modelling gel response, but $E_{\text{analytic}}$ came directly from (5) and alone contributed to overestimating the equilibrium forces. Thus, small strain equivalences for analytical parameter calculation might not be reliable to describe fibrin up to 10% strain. Parameter value optimization from the finite strain equations appears then necessary. Nonlinear unconstrained optimizations have, however, limited capacities and their consistency depend on the quality of the initial values (Conn et al., 1997). Fortunately, the analytical small strain parameter values were accurate enough to induce only slight optimization adjustments. Continuum poroviscoelastic cartilage models (Setton et al., 1993; Huang et al., 2001) would have led to more complicated parameter determinations, and using the simplest material-specific equations was preferable.


Conflict of interest statement

None of the authors have any financial and/or personal relationships with other people or organisations that could inappropriately influence (bias) this work.

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