A Poroviscoelastic Description of Fibrin Gels for use in Tissue Engineering

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Introduction: In vitro engineered tissues often have inadequate mechanical properties. Appropriate mechanical stimulation of cells may improve the local mechanical conditions inside the gel and the mechanical properties of those tissues. In vitro, 3D hydrogels are frequently used as scaffolds for tissue engineering. Although such cell support is promising, the local mechanical conditions inside the gel are hardly predictable from the applied external loading and boundary conditions[1,5]. One alternative would be to use numerical models to calculate these local conditions, but this would require an accurate constitutive modelling of the carrier. Fibrin is a biological polymer commonly used as scaffold for 3D culturing of different cell types. Compared to naturally occurring fibrin gels, high fibrinogen content is often required in vitro to ensure sufficient stability for long-term experiments[6]. The mechanics of such strengthened networks are however poorly described. In this study, we hypothesized that fibrin can be well described by a poroviscoelastic constitutive model and the parameter values for a particular gel formulation are demonstrated.

Materials and Methods: A poroviscoelastic model was used to describe the mechanical response of the gel as the superposition of an elastic stress, a viscoelastic stress, and a fluid pore pressure. The constitutive equation involved four unknown parameters, namely the Young's modulus (E), the viscoelastic relaxation time (λ), the viscoelastic shear modulus (G′), and the hydraulic conductivity (κ). Stress relaxation tests at successive unconfined compressive strains (2%, 4%, 6%, 8%, and 10%) were conducted on 6 cylindrical constructs with 45mg.mL⁻¹ fibrinogen and 1U.mL⁻¹ thrombin. A finite element model was used to find the best parameter values. Initial parameter values for the optimization process that minimized the difference between experimental and predicted reaction forces were extracted from the stress relaxation results at low strain, i.e. 4% strain. Initial value for E was given by the equilibrium stiffness measured after relaxation. λ was estimated by using the low strain exponential form of viscoelastic stress, given by the Maxwell relaxation function. A time derivation of the measured reaction force allowed suppressing the time-independent terms and a subsequent linearization of the exponential curve gave a straight line whose slope was -1/λ. Influence of pore pressure was verified by double checking the estimated λ at the origin of the linear regression. G′, initial value was set equal to the low shear strain modulus and the initial guess for κ was computed assuming that under unconfined compression, fast displacement ramps led to similar fluid and solid motions. The pore pressure could then be analytically integrated from Darcy’s law and κ was expressed at low strain as a function of the measured reaction forces and imposed displacements. A uniqueness study of the optimized parameters allowed checking the consistency of the procedure and the quality of the initial guess. It consisted of four sessions of 11 optimization runs where each of the four initial estimates was examined over a range of 4 orders of magnitude, i.e. x₀.01 to x₁₀₀.

Results: Stiffness computed from the equilibrium forces showed no significant variations from 2% to 10% strain and an initial guess of 0.02 MPa was taken for E. At 4% strain, both the slope and the origin of the linearized reaction force time derivative returned an initial λ of 77s. During displacement ramps from 2% to 4% and from 4% to 6% strain, κ expressed from the reaction forces and axial displacements was nearly constant until fluid velocity and displacement rate could not be considered equal anymore. An initial guess of 7x10⁻⁷mm.s⁻¹ was taken. Over the 44 optimization runs, 10 sets of parameters were obtained but just one was strictly repeated in each session of initial parameter variations (Fig. 1).

Discussion: According to the poroviscoelastic assumption, reaction force measurements showed that neither damage nor plasticity occurs over the studied strains. Uniqueness study suggests that the most accurate set of optimized parameters was the one that was repeated several times in each optimization session. It could be obtained directly from the experimental based initial parameters and led to accurate predictions of the fibrin scaffold reaction forces. This shows both the robustness of the optimization procedure and the quality of the specific initial guess. In some optimizations, the initial parameter values were in the range of those reported for a low fibrinogen content gel similar to blood clots. This led to unstable results highlighting the importance of the material specific initial values. In general, the optimized parameters were consistent with the available literature data. E was comparable to the values of 12kPa and 34kPa found respectively for gels with 22mg.mL⁻¹[3] and 35mg.mL⁻¹[4] fibrinogen. The optimized G′ was close to the 7.7kPa computed from an extrapolation of a reported empirical relation between shear stiffness and fibrinogen concentration[5]. A fair comparison can also be made between λ and the characteristic time of 100s identified for the primary relaxation process in fibrin strips[6]. Finally, at small strains, estimated initial κ was nearly constant which agreed with experimental observations on plasma networks[7]. For the first time, the constitutive behaviour of fibrin was explicitly studied. The proposed poroviscoelastic model was able to predict the compressive behaviour of an undocumented fibrin scaffold whose mechanical parameters could be quantified. The determination of a reasonable initial guess was necessary for finding the parameter values and the method presented in this study could be easily transferred to other non-standardized hydrogels whose compositions must be adapted for use in tissue engineering.


Figure 1: Results given by the unique set of optimized parameters repeated over the four sessions of 11 optimization runs

<table>
<thead>
<tr>
<th>Optimized parameters</th>
<th>Value (MPa)</th>
<th>G′ (MPa)</th>
<th>λ (s)</th>
<th>κ (mm.s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>1.92x10⁻²</td>
<td>1.00x10⁻²</td>
<td>5.91x10¹</td>
<td>3.03x10⁻⁶</td>
</tr>
</tbody>
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