Biomechanics of stem cells

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Abstract. Stem cells play a key role in the healthy development and maintenance of organisms. They are also critically important in medical treatments of various diseases. It has been recently demonstrated that the mechanical factors such as forces, adhesion, stiffness, relaxation, etc. have significant effects on stem cell functions. Under physiological conditions, cells (stem cells) in muscles, heart, and blood vessels are under the action of externally applied strains. We consider the stem cell microenvironment and performance associated with their conversion (differentiation) into skeletal muscle cells. Two problems are studied by using mathematical models whose parameters are then optimized by fitting experiments. First, we present our analysis of the process of stem cell differentiation under the application of cyclic unidirectional strain. This process is interpreted as a transition through several (six) stages where each of them is defined in terms of expression of a set of factors typical to skeletal muscle cells. The stem cell evolution toward muscle cells is described by a system of nonlinear ODEs. The parameters of the model are determined by fitting the experimental data on the time course of expression of the factors under consideration. Second, we analyse the mechanical (relaxation) properties of a scaffold that serves as the microenvironment for stem cells differentiation into skeletal muscle cells. This scaffold (surrounded by a liquid solution) is composed of unidirectional fibers with pores between them. The relaxation properties of the scaffold are studied in an experiment where a long cylindrical specimen is loaded by the application of ramp displacement until the strain reaches a prescribed value. The magnitude of the corresponding load is recorded. The specimen is considered as transversely isotropic poroelastic cylinder whose force relaxation is associated with liquid diffusion through the pores. An analytical solution for the total force applied to the cylinder in terms of the mechanical properties of the scaffold (longitudinal and lateral Young’s moduli, two Poisson’s ratios, and typical time of liquid diffusion) is used. The number of constant is then reduced to three by estimating the longitudinal Young’s modulus and one of Poisson’s ratios from an earlier experiment. Finally, three remaining parameters are estimated by fitting the relaxation curve corresponding to strain rate of loading of 0.01 s⁻¹. The developed mathematical solution is then tested by comparing the theoretical and experimental results for another strain rate of 0.0025 s⁻¹. The scaffold relaxation properties can be important for differentiation of stem cells inside the pores.

1. Introduction
Stem cells, under certain conditions, are capable of conversion (differentiation) into different type(s) of cells. Among other (biochemical) factors, the mechanical factors play a significant role in stem cell differentiation. The externally applied forces (stresses) and strains may direct or accelerate the process of stem cell differentiation [1]. In the case of skeletal muscle, cyclic unidirectional strains affect stem cell differentiation [2].

Here we model of the process of stem cells differentiation into skeletal muscle cells under the
action of cyclic strain of 10% magnitude and 0.5 Hz frequency. We describe this process as a transition through several stages and solve the corresponding system of nonlinear ODEs.

Another way to direct stem cell differentiation is to affect it by changing the surface or (and) bulk properties of the substrate. In the former case, the substrate surface has special topography that provides cell aligning in a prescribed direction [3]. The substrate/cell interaction can be changed by changing the bulk mechanical properties of the substrate. It has been demonstrated that stem cell differentiation can be directed toward brain cells, or muscle cells, or bone cells by switching from soft-to medium-stiff- to rigid substrates [4]. The viscous properties of the substrate have also a significant effect on stem cell differentiation which was recently demonstrated by using substrates of the same stiffness and different relaxation times [5].

Here we consider the relaxation properties of a novel porous scaffold developed for differentiation of stem cells [6]. We show that the relaxation properties of such scaffold can be associated with the diffusion of the liquid through the pores and explained by a biphasic poroelastic model. We simulate an experiment with ramp-displacement loading of a cylindrical specimen and estimate the parameters of the transversely isotropic poroelastic scaffold. We consider the modeling results for different strain rates and show that they are close to the experimental data.

2. Mechanical (strain) effects on stem cell differentiation

2.1. Mathematical model [7, 8]

The focus of our model of stem cell differentiation toward skeletal muscle cells is the kinetics of expression of five typical factors (called PAX3, desmin, MyoD, myogenin, and MHC) as well as the effect of the applied strain. We formulate the model in terms of the number of cells in the distinct stages of differentiation which are determined by particular combinations of the expressed factors (figure 1). The model is described to the following system of ODEs:

\[
\frac{dn_0}{dt} = [(2r_0 - 1)p_0n_0 - d_0n_0]f(n_{tot}),
\]

\[
\frac{dn_i}{dt} = [(2r_i - 1)p_in_i - d_in_i + 2(1 - r_{i-1})p_{i-1}n_{i-1}]f(n_{tot}), \quad i = 1, 2,
\]

\[
\frac{dn_3}{dt} = [p_3n_3 - d_3n_3 + 2(1 - r_2)p_2n_2 - D_3(n_3, \varepsilon)]f(n_{tot}),
\]

\[
\frac{dn_3}{dt} = [p_3n_3 - d_3n_3 + 2(1 - r_2)p_2n_2 - D_3(n_3, \varepsilon)]f(n_{tot}),
\]

\[
\frac{dn_5}{dt} = [D_4(n_4, \varepsilon) - d_5n_5]f(n_{tot}).
\]

Here, the right-hand sides on equations (1)–(5) give the sums of fluxes that determine the rates of the cell numbers in stages 0, 1, . . . , 5, respectively, and \(n_0, \ldots, n_5\) are the cell numbers in these stages. The terms on the right-hand side of equation (1) are associated with division (proliferation rate, \(p_0, \text{day}^{-1}\)), self-renewal (self-renewal coefficient, \(r_0\)), and death (death rate, \(d_0, \text{day}^{-1}\)) in stage 0. The right-hand side in equations (2) are determined by division, (proliferation rates \(p_i, \text{day}^{-1}\)), self-renewal (self-renewal, coefficients \(r_i\)), and death (death rates, \(d_i\)) in the current (i) stage and by division (proliferation rate, \(p_i, \text{day}^{-1}\)) and differentiation (differentiation coefficient, \(1 - r_i\)) in the previous stage \((i - 1)\). The right-hand side of equation (3) is determined by division and differentiation in previous stage 2, proliferation, direct (without division) differentiation, and death in current stage 3. The right-hand side of equation (4) is determined by direct differentiation in previous stage 3 and direct differentiation and death in current stage 4. Finally, the right-hand side of equation (5) is determined by direct differentiation in previous stage 4 and death in current stage 5. The function \(f(n_{tot})\) (\(n_{tot}\) is the total cell number) describes the effects of stem cell density on their differentiation.
Figure 1. Multi-stage model of stem cell differentiation into skeletal muscle cells [8].

Direct differentiation in late stages 3, 4, and 5 is described by functions $D_3$ and $D_4$ that depend on the applied strains. Assuming that the applied strains initiate signalling that ultimately effect expression of the late factors, the direct differentiation functions take the following form:

$$D_3(n_3, \varepsilon) = \beta_3 \left( \frac{\varepsilon n_3}{\varepsilon + k_s^3} \right) \left( \frac{\varepsilon n_3}{\varepsilon + k_s^3} + k_3 \right)^{-1},$$

$$D_4(n_4, \varepsilon) = \beta_4 \left( \frac{\varepsilon n_4}{\varepsilon + k_s^4} \right) \left( \frac{\varepsilon n_4}{\varepsilon + k_s^4} + k_4 \right)^{-1},$$

where $\varepsilon$ is the magnitude of the applied strain and $\beta_3$, $\beta_4$, $k_s$, $k_3$, and $k_4$ are parameters estimated by fitting the experimental data.

2.2. Optimization of the model parameters

We estimate the parameters of our model by optimally fitting the experimental data on the differentiation into skeletal muscle cells of adipose-derived stem cells (stem cells derived from fat tissue of a patient). In this experiment, the stem cells are seeded on a flexible membrane to which a unidirectional cyclic strain of a magnitude 10% and frequency 0.5 Hz is applied. The goal of the strain application is to maximize the outcome of differentiated skeletal muscle cells which is quite low without the mechanical stimulation. In the experiment, the numbers of cells expressing the groups of factors that correspond to 6 stages of our model are measured at several moments of time (3, 7, 14, and 21 days). The result of the parameter optimization are presented in figure 2 where the time course of the computed $n_1, \ldots, n_5$, and $n_{tot}$ are shown vs. corresponding experimental points at the four moments of time.

2.3. Prediction of the differentiation time course for different strains

By using the estimated model parameters we are able to predict stem cell differentiation results for strains different from the experimental 10% as well as for the times different from 21 days. Figures 3 a–c present the computed results of the time course (up to 33 days) of cell numbers in all six stages for external strain of 3%, 9%, and 15%.
Figure 2. Optimization of the model parameters by fitting the experiment with the stem cell differentiation under the action of strain of 10% magnitude [8].

Figure 3. Predicted time course of stem cell differentiation for different strain magnitudes by using optimized model parameters 3% (a), 9% (b), and 15% (c).

3. Mechanics of a fibrous/porous scaffold for stem cell differentiation

3.1. Force relaxation experiment

A novel fibrous porous scaffold is designed for stem cells differentiation into skeletal muscle cells via cell alignment along the fiber direction, tuning by the scaffold mechanical properties, and application of the external strain [6]. The stem cells are located inside the pores of the scaffold. The relaxation properties of the scaffold have been demonstrated to be important to stem cell differentiation, and we focus on the experiment that helps estimate such properties. In this experiment, a cylindrical specimen is a subject to ramp displacement following by a regime of constant strain, and the corresponding horizontal force is recorded. The specimens are located in the liquid solution moving in the porous specimen in response to the applied tensile strain (figure 4).
3.2. A poroelastic model to interpret the force relaxation experiment

We assume transverse isotropy of the specimen properties and axisymmetry of the stress-strain distribution. We propose a poroelastic mechanism where the applied force relaxation is associated with the fluid diffusion inside the specimen in response to its tensile deformation. A solution for the compression of a short transversely isotropic poroelastic cylinder is available [9], and it can be adjusted to our case of the extension of a long poroelastic cylinder. Thus, we use the following function describing the time course of the force $P(t)$ applied to the cylinder:

$$P(t) = E_3\dot{\varepsilon}_0 t + E_1\dot{\varepsilon}_0 t_g F_1(t_g, E_1, E_3, \nu_{21}, \nu_{31}) \quad \text{for } 0 \leq t \leq t_0,$$

$$P(t) = E_3\dot{\varepsilon}_0 t_g - E_1\dot{\varepsilon}_0 t_g F_2(t_g, E_1, E_3, \nu_{21}, \nu_{31}) \quad \text{for } t \geq t_0,$$  

where:

$$F_1 = \Delta_3 \left( \frac{1}{8} - \sum_n \exp \left( -\alpha_n^2 \frac{t}{t_g} \right) \{ \alpha_n^2 [\Delta_2^2 \alpha_n^2 - \Delta_1 (1 + \nu_{21})]^{-1} \right),$$

$$F_2 = \Delta_3 \sum_n \left[ \exp \left( -\alpha_n^2 \frac{t}{t_g} \right) - \exp \left( -\alpha_n^2 \frac{t - t_0}{t_g} \right) \right] \{ \alpha_n^2 [\Delta_2^2 \alpha_n^2 - \Delta_1 (1 + \nu_{21})]^{-1} \},$$

$$\Delta_1 = 1 - \nu_{21} - \frac{2\nu_{31}^2 E_1}{E_3}, \quad \Delta_2 = 1 - \nu_{31}^2 \frac{E_1}{E_3}, \quad \Delta_3 = 1 - \frac{2\nu_{31}^2 \Delta_2}{\Delta_1}.$$  

Here $E_1$ and $E_3$ are the transverse and longitudinal Young’s moduli, $\nu_{21}$ and $\nu_{31}$ are in-transverse plane and transverse plane/out-of-plane Poisson’s ratios, $t_g$ is a typical time of fluid filtration through the pores, and $\alpha_n$ are the roots of a characteristic equation [9].

3.3. Estimation of the scaffold mechanical properties

We reduce the number of parameters of the proposed poroelastic model to three by extracting Young’s modulus, $E_3 = 30$ kPa and Poisson’s ratio, $\nu_{31} = 0.3$ from the previously reported independent experiment [6]. The remaining three parameters are estimated by fitting the current relaxation experiment. Figure 5 shows the theoretical solution with optimal values of the parameters ($E_1 = 11.5$ kPa, $t_g = 38.8$ s, and $\nu_{21} = 0.77$) vs. the experimental data in the case of strain of 10% and strain rate of 0.01 s$^{-1}$. We finally test the model by checking its results against the data of relaxation experiment for a different strain rate of 0.0025 s$^{-1}$ (figure 6).
Figure 5. Optimization of the poroelastic model parameters by fitting the data of the force relaxation experiment for ramp displacement loading (strain rate of 0.01 s\(^{-1}\)) following by a constant strain regime (strain of 10%).

Figure 6. Modelling results for the optimal parameters vs. the data of the force relaxation experiment for the ramp displacement loading with the strain rate of 0.0025 s\(^{-1}\).

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