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Fixing Sore Knees: Mechanics of Articular Cartilage

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1. Introduction

Articular cartilage is a mechanically strong tissue located between the synovial fluid and subchondral bone commonly found in knees or hips preventing bone contact. Its strength is due to its depth dependent zonated structure composed of a network of Extracellular Matrix (ECM) with a superficial zone, middle zone and deep zone as shown in Figure 1 [1]. It mainly consists of its own native cartilage cells (chondrocytes) which maintain the kinetic equilibrium of the cartilaginous matrix and a type of protein called collagens which act to connect the tissue with a variety of different densities and orientations. This arrangement allows the tissue to withstand a high stress or pressure from any movements and activities, and mean that it acts a lubricating surface in the joint.

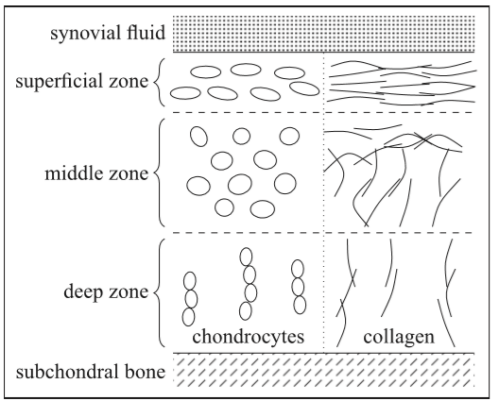


Figure 1: Schematic diagram of the zonated, depth dependent structure of articular cartilage[2].

However, cartilage has a low regeneration capacity for self-repair, so that when it degrades or gets damaged it can lead osteoarthritis. To solve this issue, a recent tissue engineering approach has developed that involves creating an implant composed of mesenchymal stem cells (MSCs) and chondrocytes to replace the damaged tissue. This implant will ideally mimic natural cartilage, with the seeded cell of undergoing chondrogenic differentiation upon treatment which could turn the mesenchymal stem cells into chondrocytes. In particular, this process is mediated by transforming growth factor-beta (TGF-β) which provides appropriate biochemical cues within the scaffold to direct the cells to promote new cartilage-specific Extracellular Matrix (ECM) formation within this little three-dimensional (3D) porous scaffold. It is specifically this process that is regulated by

transforming growth factor-beta, and so this plays an important role in both natural cartilage and in an approaches to engineer artificial cartilage.

A schematic of chondrogenic differentiation is given in Figure 2. It begins with the secretion of TGF-β from chondrocytes, which then bind rapidly with ECM for storage. Under an activation process, activated TGF-β is released from ECM stored state and can freely diffuse, which in natural cartilage then promotes chondrocytes to synthesis ECM components. For tissue engineering approaches, the activated TGF- β also drives the MSCs to differentiate into chondrocytes within the implant until the initial MSC population is fully differentiated.

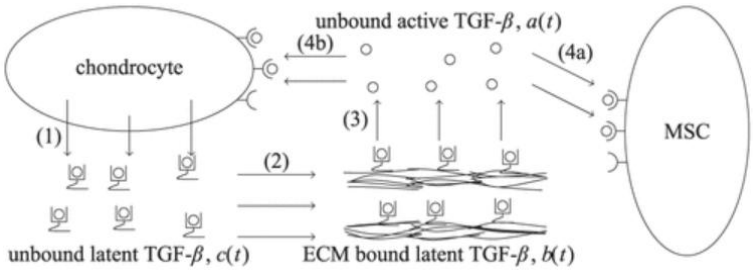


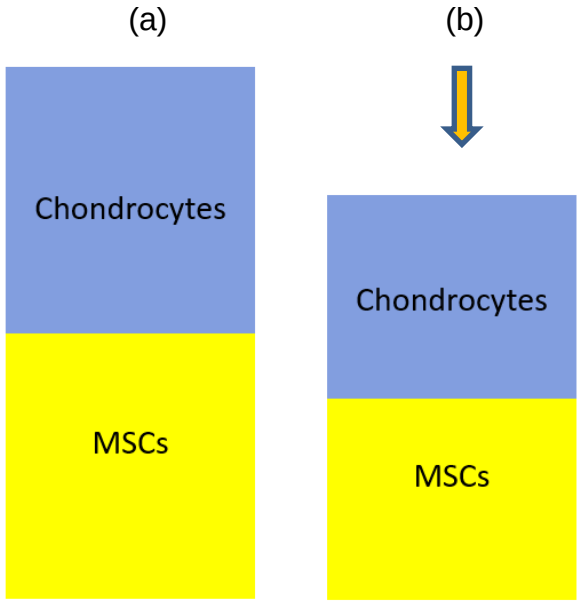
Figure 2: Simplified schematic of life cycle TGF-β[1].

To maximize the yield of chondrocytes in tissue engineering approaches, the scaffolds are given both chemical stimulation (ie. TGF- β) and mechanical stimulation. Chen et al. [1] built a mathematical model to simulate the responses of the scaffold under different chemical stimulation and cell seeding strategies. This included varying the initial concentration of TGF-β, the initial densities of the MSCs and chondrocytes, and the relative depths of the two layers. This study revealed that all these factors affected the long-time composition of the tissue engineering construct. The base case is defined as a layer MSCs lying below a layer of chondrocytes. It was discovered that the initial density of the seeded chondrocytes must be above a critical value to trigger the differentiation process. There was similarly a threshold value above which the exogenous of TGF-β could be used to trigger the chondrogenesis process without chondrocytes. The investigation of the case with both exogenous TGF-β and chondrocytes revealed that a reducing the depth of the chondrocyte layer (that is, seeding the available cells more

densely) meant that a lower concentration of exogenous TGF-β was required to trigger chondrogenesis.

This report is focused on extending the model of [2] to include the role mechanical stimulation on the scaffold, which is due to the interaction of the ECM and stored state TGF-β. From Hinz [3], when the ECM is pulled apart by applying a strain (that is, stretched) for a certain distance, it stimulates the stored TGF-β to be activated. Therefore, a higher portion of latent TGF-β will be activated which then promotes further chondrogenic differentiation, potentially resulting in a more efficient path to differentiate all the seeded MSCs.

A schematic diagram of an idealized version of the strategies used in this research is shown in Figure 3. Here a layer of chondrocytes lies above a layer of MSCs, with or without the application of a strain force.



- Case study 1: layered MSCs (bottom) and chondrocytes (top) cell seeding without mechanical stimulation.
- Case study 2: layered MSCs (bottom) and chondrocytes (top) cell seeding with mechanical stimulation.

2. Methodology

Model of a linearly elastic scaffold

To track the amount of mechanical strain the material undergoes we follow its displacement, defined as the difference between the current and initial positions of a particle. In a Lagrangian coordinate system this is labelled as $u(X,t) = x(X,t) - X$. [4](1). The porous scaffold (typically a hydrogel) is assumed to be a linear elastic material, so that at time $t = 0$, when a strain is applied, its initial position is $u(X,0) = -u_{in}$. Because this material is elastic, it is able to recover to its reference state of $u = 0$ within $t = t_0$, and assume it is a linear response, the relationship of displacement between time and position are $u(X,t) = -u_{in} \times (t/t_0) - 1$ (2), the diagram is shown in Figure 3.

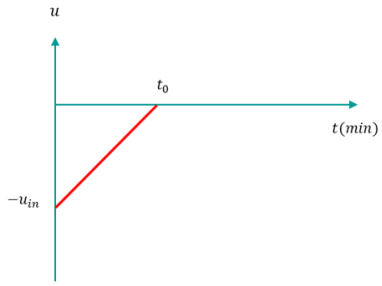


Figure 3: Relationship of displacement and time within a single movement.

A periodic forcing on the scaffold is of practical interest, since this for a single strain allows the material to move backwards and forwards similarly to the forces experienced by natural cartilage in a joint. To this end a forcing based on a cosine is included. The reference differentiation rate of MSCs to chondrocytes is $6 \times 10^{-3}/min$. Therefore, in dimensionless time an oscillatory forcing of one cycle per second corresponds to the following function: (3)

$$(\cos(6.28 \times 10^4 \times t) + 1) \times 0.5$$

Overall, the function becomes

$$u(X,t) = -u_{in} \times (t/t_0 - 1) \times 0.5 \times (\cos(6.28 \times 10^4 \times t) + 1) \quad (4)$$

Model of the cell populations and TGF-β dynamics

Based on the schematic shown in Figure 2, a mechanistic mathematical model is developed to describe the populations of MSCs and chondrocytes and the concentrations of the various forms of TGF-β. We assume that these vary over one spatial dimension x, which is parallel to the direction depth of the scaffold, and that all quantities vary over time in response to diffusion of TGF-β. It is assumed that the chondrocytes neither proliferate nor dedifferentiate, and that the extracellular matrix with latent bound TGF-β is always fixed relative to the cell population when strains are applied.

The variables used to represent the various cell densities and different forms of TGF-β are summarized in Table 1.

Table 1. Description of the model variable along with their units.

Dependent variable	Description	Unit
m	Density of MSCs	Mio/mL
n	Density of chondrocytes	Mio/mL
c	Concentration of inbound, latent TGF-β	ng/mL
b	Concentration of bound, latent TGF-β	ng/mL
a	Concentration of inbound, active TGF-β	ng/mL
f	Fraction of bound TGF-β receptors per MSCs	-
g	Fraction of bound TGF-β receptors per chondrocytes	-

In this model, two cell types are considered, denote by $m(x,t)$ and $n(x,t)$ to represent the density of MSCs and chondrocytes at depth x and time t. It is assumed that the biological differentiation process occurs when the TGF- β is bound to an MSC receptors, , denote by $f(x,t)$, exceeds a certain threshold, denoted by f_d. From the principle of mass balance of these two distinct type cells, we derive the following equations:

$$\text{MSCs}: \quad \frac{\partial m}{\partial t} = \underbrace{-\Lambda_1 H\left(f - f_{\mathrm{d}}\right) m}_{\text{differentiation of MSCs to chondrocytes}} \tag{5}$$

$$\text{chondrocytes}: \quad \frac{\partial n}{\partial t} = \underbrace{\Lambda_1 H\left(f - f_{\mathrm{d}}\right) m}_{\text{differentiation of MSCs to chondrocytes}} \tag{6}$$

From the above equations (5) and (6), $H(\cdot)$ is the Heaviside step function ($H(z) = 1\ if\ z > 0$; $H(z) = 0$ otherwise). So, this process starts to differentiate when $f > f_d$. This is a reasonable approximate to the dynamics of this biological process. The coefficient Λ_1 is the rate at which MSCs differentiate when $f > f_d$, a representative value of this parameter is given in Table 2.

Similarly, the equations for the different forms of TGF-β is given by equations (3)-(7), with representative values of the various parameters given in Table 2. These equations describe the process shown in Figure 2, and see Chen et al. for more detail of each term.

$$\frac{\partial c}{\partial t} = \underbrace{D_c \frac{\partial^2 c}{\partial x^2}}_{\text{diffusion}} + \underbrace{\lambda_3 n}_{\substack{\text{constitutive production} \\ \text{by chondrocytes}}} - \underbrace{\lambda_4 c}_{\text{binding to ECM}} - \underbrace{\lambda_5 c}_{\text{natural decay}} \tag{7}$$

$$\frac{\partial b}{\partial t} = \underbrace{\lambda_4 c}_{\text{binding to ECM}} - \underbrace{\lambda_6 b}_{\text{chemical activation}} - \underbrace{\lambda_7 b}_{\text{natural decay}} \tag{8}$$

$$\frac{\partial a}{\partial t} = \underbrace{D_a \frac{\partial^2 a}{\partial x^2}}_{\text{diffusion}} + \underbrace{\lambda_6 b}_{\text{chemical activation}} - \underbrace{\lambda_8 a}_{\text{decay}} \underbrace{-\left(\lambda_{m9} amF_{\mathrm{tot}}\left(1-f\right) + \lambda_{n9} anG_{\mathrm{tot}}\left(1-g\right)\right)}_{\text{binding to cell receptors}} \tag{9}$$

$$\frac{\partial (fm)}{\partial t} = \underbrace{\lambda^*_{m9} am(1-f)}_{\substack{\text{binding to MSC} \\ \text{receptors}}} - \underbrace{\lambda_{m10} fm}_{\substack{\text{internalization} \\ \text{(MSCs)}}} - \underbrace{\Lambda_1 H(f - f_{\mathrm{d}}) fm,}_{\substack{\text{loss of bound TGF-}\beta \\ \text{due to MSC differentiation}}} \tag{10}$$

$$\frac{\partial (gn)}{\partial t} = \underbrace{\lambda^*_{n9} an(1-g)}_{\substack{\text{binding to chondro.} \\ \text{receptors}}} - \underbrace{\lambda_{n10} gn}_{\substack{\text{internalization} \\ \text{(chondrocytes)}}} + \underbrace{\Lambda_1 H(f - f_d) fm.}_{\substack{\text{gain of bound TGF-}\beta \\ \text{due to MSC differentiation}}} \tag{11}$$

If mechanical stimulation is applied on ECM which contains latent bound TGF-β, a new term is introduced to equations for latent bound TGF-β (b) and active TGF-β (a), namely $\lambda_{99} bH(u + u_0)$ (again based on the principle of mass balance). The new equations are listed below:

latent bound: $$\frac{\partial b}{\partial t} = \underbrace{\lambda_4 c}_{\text{transfer from unbound}} - \underbrace{\lambda_6 qb}_{\text{chemical activation}} - \underbrace{\lambda_7 b}_{\text{natural decay}} - \lambda_{99} bH(u+u_0) \quad (12)$$

active: $$\frac{\partial a}{\partial t} = \underbrace{D_a \frac{\partial^2 a}{\partial x^2}}_{\text{diffusion}} + \underbrace{\lambda_6 qb}_{\text{chemical activation}} - \underbrace{\lambda_8 a}_{\text{decay}} - \underbrace{(\lambda_{m9} am(1-f) + \lambda_{n9} an(1-g))}_{\text{binding to cell receptors}} + \lambda_{99} bH(u+u_0) \quad (13)$$

3. Results/discussion

A previous study [2] found that there are two possible outcomes for the culture environment without any mechanical stimulation. If the chondrocytes are seeded with a low density, then MSCs in the bottom layer would not start to differentiation; if the seeded chondrocyte density is above a threshold value of $n_{crit} = 0.27$, then all MSCs would differentiate. Figure 4 shows how the long-term state of the system depends on this initial density n_0. Given the critical value n_{crit} a solid line. The shaded region indicates the time taken to achieve 90% full differentiation. It is observed that a higher initial density of chondrocytes results in a less time to differentiate.

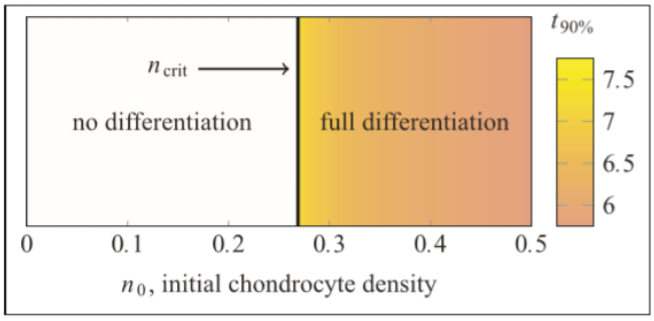


Figure 4: The final yield of chondrocytes in the lower layer for case study 1 with varying n_0, the initial seeded chondrocytes density (from [2])..

As described in [2], in practice since the differentiation process is only related to the initial density of seeded chondrocytes, with a limited supply of the number of chondrocytes in tissue engineering application it is controllable by changing the depth of a the

chondrocytes layer. Therefore, with the same density of seeded chondrocytes, a thin layer would require fewer chondrocytes than a thick layer. It is suggested that such a seeding strategy may be an effective when limited number of chondrocytes are available.

We now consider the new situation of the application of mechanical stimulation, the coefficient of λ_{99} represents the differentiation rate, and u_0 stands for a threshold value of displacement, its representative value is given in Table 2, the simulation invested the relationship of λ_{99} ranging from 0.01 to 0.2, the threshold value n_{crit} found in previous strategy and the time taken by for 90% differentiation. The result is displayed in Figure 5. It is observed that increasing the value of λ_{99} requires a lower initial chondrocytes density to trigger chondrogenesis. For example, when no mechanical stimulation is used a critical value of 0.27 is found, but with the addition of strain the critical initial chondrocyte density is decreased to 0.11 (for a value of the mechanical coefficient λ_{99}=0.2).

Table 2: Summary of the dimensional parameters that appear in equations (1)-(13), together with estimated their values.

Quantity	Description	Representative Value
$\boldsymbol{\Lambda_1}$	MSC to chondrocyte differentiation rate	$6 \times 10^{-3}/min$
$\boldsymbol{f_d}$	MSC to chondrocyte differentiation threshold	0.01[6]
$\boldsymbol{\lambda_3}$	Rate o constitutive TGF-β production by chondrocytes	$1.094 \times 10^{-1} ng/(\min Mio)$[8]
$\boldsymbol{\lambda_4}$	Transfer rate between bound and unbound latent TGF-β	$10^{-1}/min$[12]
$\boldsymbol{\lambda_5}$	Decay rate of unbound latent TGF-β	$6 \times 10^{-2}/min$[8]
$\boldsymbol{\lambda_6}$	Activation rate due to chemical interactions	$6 \times 10^{-3}/min$[13]
$\boldsymbol{\lambda_7}$	Decay rate of bound latent TGF-β	$6 \times 10^{-3}/min$[13]
$\boldsymbol{\lambda_8}$	Decay rate of active TGF- β	$0.258/min$[13]
$\boldsymbol{\lambda_{m9}}$	Binding rate of active TGF- β to MSC receptors	$6 \times 10^{-2}/(\min ng/mL)$[14]
$\boldsymbol{\lambda_{n9}}$	Binding rate of active TGF- β to chondrocyte receptors	$6 \times 10^{-2}/(\min ng/mL)$[14]
$\boldsymbol{\lambda_{m10}}$	Internalization rate of TGF- β bound MSC receptors	$0.25/min$[15]
$\boldsymbol{\lambda_{n10}}$	Internalization rate of TGF- β bound chondrocytes receptors	$0.25/min$[15]
$\boldsymbol{F_{tot}}$	Maximum receptor bound TGF- β per MSC	$6.23 \times 10^{-1} ng/Mio$[16]
$\boldsymbol{G_{tot}}$	Maximum receptor bound TGF- β per chondrocyte	$6.23 \times 10^{-1} ng/Mio$[16]

$\boldsymbol{D_c}$	Diffusivity of unbound latent TGF-β	$1.278 \times 10^{-9} m^2/min$[17]
$\boldsymbol{D_a}$	Diffusivity of unbound active TGF-β	$1.278 \times 10^{-9} m^2/min$[17]
$\boldsymbol{\lambda_{99}}$	Mechanical differentiation rate	$0.01 - 0.2\ /min$[13]
$\boldsymbol{u_0}$	Threshold displacement value	$0.05m$

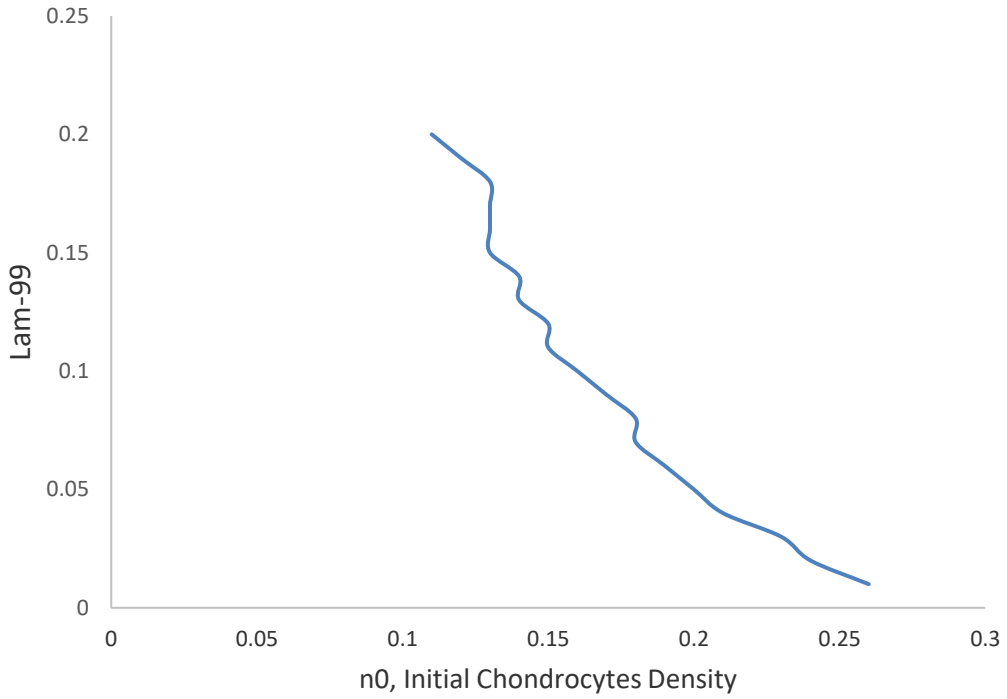


Figure 5: Initial chondrocytes density required for chondrogenesis with an increasing mechanical differentiation rate λ_{99}.

We also consider the time taken for this process, the following graphs in Figure 6 show the trend of the time required for 90% differentiation with different initial chondrocytes density under the conditions of coefficient values of λ_{99} =0.01, 0.05, 0.1 and 0.2, respectively.

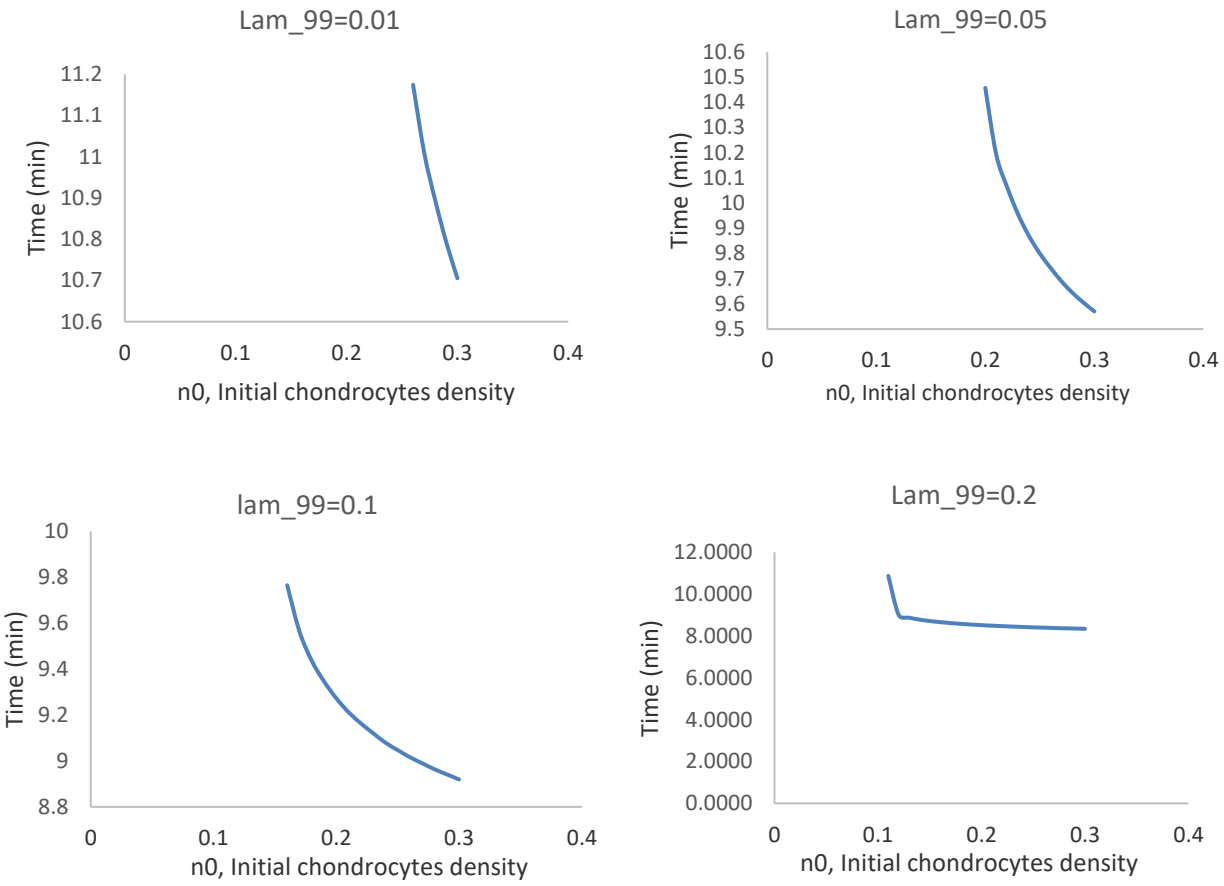


Figure 6: Time required for 90% differentiation with initial chondrocytes density ranging from 0.1-0.3 when $\lambda_{99} = 0.01, 0.05, 0.1, 0.2$.

It is observed that the time required for 90% differentiation descrease as increased value of initial chondrocytes density. However, the value of the coefficient (λ_{99}) also changes the time needed under the same condition, for example:

$$t(n_0, \lambda_{99}) = t(0.3, 0.01) \approx 10.71min,$$
$$t(0.3, 0.05) = 9.57min,$$
$$t(0.3,0.10) = 8.92min$$
$$and\ t(0.3,0.2) = 8.34min$$

Moreover, increasing λ_{99} also lowers the gradient of the decreasing trend and until at a value of 0.2, the time taken stabilized to around 8.3 minutes with an increasing initial chondrocytes density.

In addition to this, the relationship between the time required for 90% differentiation and the value of the coefficient (λ_{99}) with a fixed initial chondrocytes density is shown in Figure 7. It is a observed here that increasing λ_{99} results in a decreasing time required for 90% of the seeded MSCs to differentiate. However, when $\lambda_{99} = 0.15$, the curve of the time required drops suddenly (with an increment 0.01).

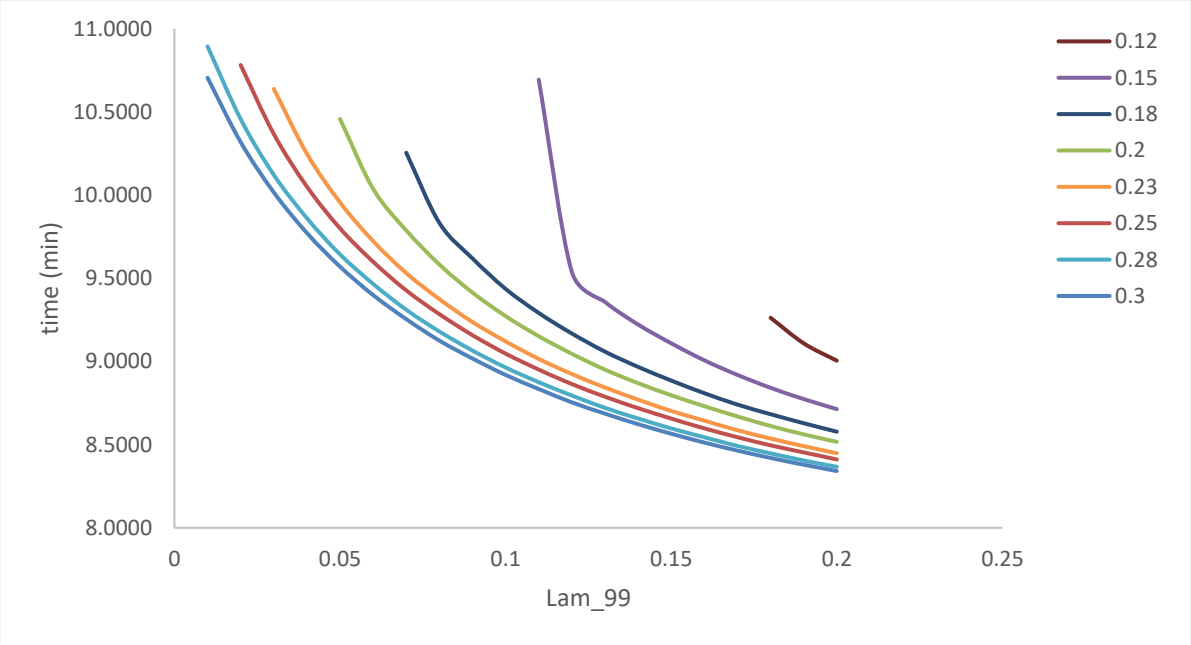


Figure 7: The time require for 90% differentiation with varying mechanical coefficient rate λ_{99} ranging from 0.01-0.2 when seeded initial chondrocytes density equals to 0.12, 0.15, 0.18, 0.2, 0.23, 0.25, 0.28 and 0.3.

4. Conclusion

A mathematical model has been developed to simulate TGF-β driven to differentiation of MSCs into chondrocytes in tissue engineering scaffolds under two assumptions. Firstly, we have assumed this material was linear elastic, so that we could discard all the nonlinear terms in the relation between strain and displacement. However, nonlinear elasticity concerns large deformations that are not negligible in a real applications, and including this extra detail may be an interesting direction for future work.

The second assumption is that the equations we have developed were based on one-dimensional deformation for this scaffold. However, in further development, a three-dimensional mathematic model including this material's thickness and width should be considered to achieve a more accurate representation of this biological simulation.

Here we investigated two seeding strategies of equal layer MSCs and chondrocytes in a scaffold differed from the presence of the mechanical stimulation. In case study 1, from previous research [2], the differentiation of the MSCs in the lower layer was driven by the endogenous production of TGF-β by chondrocytes seeded in the upper layer. Key findings were that

- The initial seeded chondrocytes density must be above a threshold value $n_{crit} = 0.27\ Mio/ml$ to trigger chondrogenesis
- If a fixed number of chondrocytes is available, the depth of the layer can be adjusted to exceed the value of $n_{crit.}$

In case study 2, mechanical stimulation is applied under the same conditions. Chondrogenesis is also driven by endogenous TGF-β here, but is promoted by the inclusion of the mechanical activation of stored TGF- β. Our key findings were that

- A higher mechanical differentiation rate λ_{99} lowers the critical initial chondrocytes density, and also decreases the time required for full differentiation of the seeded cells.
- Where a fixed number of chondrocytes is available, an efficient strategy is to seed them under a high value of mechanical differentiation rate λ_{99}.

Further experiments are required to investigate how this value related to the other factors in the tissue engineering experiment. This value might be dependent on the material properties of the scaffold used, for example it might change for hydrogels with different permeabilities and/or elastic properties.

5. References

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