# MODEL-BASED OPTIMIZATION OF THE MEDIUM REFRESHMENT REGIME DURING NEOTISSUE GROWTH IN A PERFUSION BIOREACTOR

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*Abstract*

Computational models are interesting tools to facilitate the translation from the laboratory to the patient. In regenerative medicine, computer models describing bioprocesses taking place in bioreactor environment can assist in designing process conditions leading to robust and economically viable products. In this study we present a low-cost computational model describing the neotissue (cells + extracellular matrix) growth in a perfusion bioreactor set-up. The neotissue growth is influenced by the geometry of the scaffold, the flow-induced shear stress and a number of metabolic factors. After initial model validation, a Genetic Algorithm optimization technique is used to find the best medium refreshment regime (frequency and percentage of medium replaced) resulting in a maximal amount of neotissue being produced in the scaffold in a 28 days of culture period.

*Keywords*

Bone tissue engineering, Perfusion bioreactor, Neotissue growth, Mathematical modeling, Optimization.

## Introduction

Bone tissue engineering (TE) is a field that combines expertise from medical and engineering sciences to come up with solutions for large or non-healing bone defects. Nowadays, the most common technique for producing neotissue (cells and the extracellular matrix they produce) *in vitro*, lies in the combination of different elements such as scaffolds and mesenchymal stem cells (MSCs). In this process, the role of (perfusion) bioreactors is to ensure the supply of nutrients to and removal of metabolic waste from the system (Papantoniou et al., 2013 and Grayson et al., 2011). In addition, they provide mechanical stimulation to the cells to help maintain their viability and stimulate biological activity. Regular 3D porous scaffolds are being used because of their ability to induce reproducible culture conditions that can control specific cell behavior such as proliferation and differentiation. Furthermore, scaffolds can provide fundamental patterns for subsequent cell growth and neotissue (Melchels et al., 2011). MSCs have been shown to be able to differentiate into a variety of cell types, including osteoblasts (bone forming cells) and chondrocytes (cartilage forming cells) (Sonnaert et al., 2014), depending on the specific biochemical and biomechanical environment.

Despite the extensive amount of past and continuing research efforts, the translation of the TE products from bench to bed side remains a challenge. There is a clear need to further elucidate the intricate mechanisms involved in the neotissue formation as well as to develop proper monitoring and control tools to follow-up the bioreactor processes (in real-time). Computational models could play a significant role to help in the clarification of the biological mechanisms taking place during the neotissue growth within the scaffold (Guyot et al., 2015 and Hossain et al., 2015). Moreover, the use of computational models will enable us to optimize the whole process by finding the optimum values for the bioreactor culture settings (e.g. fluid flow rate, nutrients concentration and medium refreshment rate).

In this study, we present a computational model describing the neotissue growth in porous structures during dynamic culture conditions in a perfusion bioreactor. As the purpose of the model is for it to be used in the context of process optimization, the computational cost was a key factor in the model development. A mechanistic model describing the 3D neotissue growth in a mechanistic manner such as the model developed by Guyot et al (2015) is computationally too expensive to be used in rigorous optimization routines, so a cheaper homogenized model was developed. In order to find the best culture strategy (represented in the context of this study by the frequency

and percentage of medium replacement), a Genetic Algorithm (GA) optimization technique was used. The results obtained by GA point towards bioreactor conditions that would lead to an increased volume of neotissue in the same timeframe.

## Methods

*Experimental Set-up*

Calibration and testing of the presented models was performed with the help of experimental data that was, partly, generated for the purpose of model validation by researchers from the Tissue Engineering Unit of the Skeletal Biology and Engineering Research Centre of the KU Leuven (Sonnaert et al., 2014). The set-up used to generate this data consisted of three different compartments which are cells, scaffolds and perfusion bioreactor. Schematic representation of the perfusion bioreactor and the scaffold used in this study are shown in figure 1.

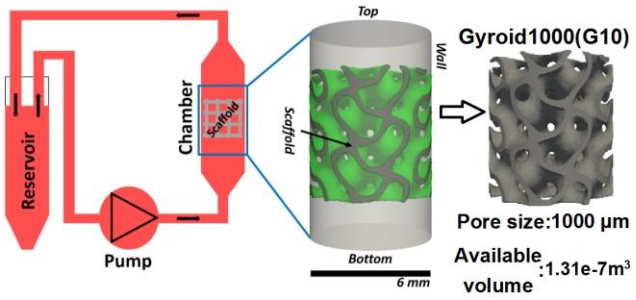


*Figure 1. Schematic representation of the perfusion bioreactor and the scaffold used in this study. The green volume inside the gyroid scaffold (centr) reprents the neotissue.*

The following sections describe theses three compartments in details.

Cells: The type of cells used in this experimental set-up is human Periosteal Derived Cells (hPDCs). This cell type has been chosen for its pluripotency and its ability to form bone tissue engineered construct. All the necessary ethical approval for prelevation and use of these cells are in place.

Scaffolds: 3D additively manufactured open porous Ti6Al4V scaffolds produced using a non-commercial, in-house developed selective laser melting machine were used (Van Bael et al., 2012). The outer dimensions of the cylindrical scaffold were 6mm diameter and 6mm height. The scaffolds used in this study had a triply periodic internal structure (Gyroid, Dcup or Schwarz). For scaffolds with a Gyroid structure, 2 different sizes of internal periodicity were tested (Gyroid10, G10, having a bigger pore size than Gyroid 7, G7)

Perfusion Bioreactor culture: Prior to the start of the experiment, cells were trypsinized with Tryple Express (Invitrogen) and seeded on the scaffolds. A static drop-seeding protocol was used for seeding cells onto pre wetted 3D Ti scaffolds. 0.2 million cells per scaffold were seeded and left to attach overnight (t=24h). Scaffolds were positioned in the bioreactor perfusion chambers with a random orientation (top-bottom) with respect to the direction of drop seeding (Impens et al., 2010). Scaffolds were cultured for 28 days. For dynamic culturing, TE constructs were placed in an in-house developed perfusion bioreactor equipped with parallel perfusion circuits. Each perfusion chamber (Length=26 mm, Diameter=6mm), holding a single scaffold, was connected to an individual medium reservoir containing 10 ml of cell culture medium via a Tygon (Cole Parmer) tubing and a two stop tubing (BPT, Cole Parmer) connected to a peristaltic pump (IPC 24, Ismatec SA). Basic Growth Medium was fully refreshed (100%) every three days for the entire culture period.

*Model Set-up*

Guyot et al (2015) developed a model describing the neotissue growth in great detail, making it computationally very expensive. In this study, we have adapted the model in order to reduce the cost but maintain a similar level of mechanistic detail by essentially removing all references to spatial heterogeneity. The evolution of different model species such as neotissue volume ($V_n$), oxygen concentration ($C_o$), glucose concentration ($C_g$) and lactate concentration ($C_{la}$) are modeled based on Michaelis-Menten kinetics as follows:

$$\frac{dV_n}{dt} = A.f_s(\tau).f_c(\kappa).h_1(C_o).h_2(C_g).h_3(pH).\frac{V_n}{K_{V_n} + \lambda V_n} \quad (1)$$

$$\frac{dC_o}{dt} = -\alpha Vn.\phi_{Cells}.V_O.\frac{C_o}{K_o + \gamma C_o} \quad (2)$$

$$\frac{dC_g}{dt} = -\beta Vn.\phi_{Cells}.V_g.\frac{C_g}{K_g + C_g} \quad (3)$$

$$\frac{dC_{la}}{dt} = 2.Vn.\phi_{Cells}.V_g.\frac{C_g}{K_g + C_g} \quad (4)$$

$$pH = 7.4 - 0.0406.La. \quad (5)$$

In Eq. (1), the neotissue volume ($V_n$) is expressed as a function of oxygen ($C_o$), glucose ($C_g$), pH level (pH), mean curvature ($\kappa$) of the neotissue inside the 3D scaffold and the shear stress ($\tau$) caused by the medium that is perfused through the scaffold. As neither flow-induced shear stress nor mean curvature can be calculated in a spatially homogenized model, relationships between these variables and the neotissue volume (nf%) were derived from the mechanistic heterogeneous model developed by Guyot et al. (2015) shown Eq. (6) and (7) below, with parameters $s_i$

and $c_i$ determined through a fitting procedure for each scaffold geometry.

$$\tau = \begin{cases} s_1 V_n & : nf\% < T_1 \\ s_2 V_n & : nf\% \geq T_1 \end{cases} \quad (6)$$

$$\kappa = \begin{cases} c_1 V_n & : nf\% < T_2 \\ c_2 V_n & : nf\% \geq T_2 \end{cases} \quad (7)$$

The influence of oxygen and glucose concentrations on the produced neotissue in Eq. (1) are taken into account through the functions $h_1$ and $h_2$ where neotissue volume reduces when the level of these species decrease.

$$h_1(C_o) = \frac{C_o}{K_o + C_o} \quad (8)$$

$$h_2(C_g) = \frac{C_g}{K_g + C_g} \quad (9)$$

In Eq. (2) to Eq. (4), the right hand side terms show the production or consumption of the species by the cells which is modeled using the Michaelis-Menten kinetics. Equation (5) is proposed by Hossain et al., (2015), in which they give a linear relation between the lactate concentration and the pH level.

Lactate production in the medium is in direct relation with the pH level of the medium according to Eq. (5) and has a negative influence on the neotissue growth. This behavior is incorporated with the introduction of the local pH and its negative effect on cells when the medium becomes more acidic. In (Wuertz et al., 2009), authors determined a detrimental effect of pH on cells using Eq. (10) where the neotissue growth rate decreases linearly when the pH level of the medium decreases.

$$h_3(pH) = \begin{cases} 0 & : pH < 6.375 \\ \frac{4pH}{3} - 8.5 & : 6.375 \leq pH < 7.1 \\ 1 & : pH \geq 7.1 \end{cases} \quad (10)$$

In Chapman et al., (2014), authors introduce a growth model for cell aggregates in hollow fiber bioreactors where cell population growth varies depending on wall shear stresses experienced by cells. These findings have been incorporated in this study through Eq. (11). Using this function enables us to take into account an optimal shear stress ($\tau$) range enhancing growth as well as a detrimental value of $\tau$ that inhibits growth. This function is defined as follows:

$$f_s(\tau) = \begin{cases} 0.5 + \frac{0.5\tau}{a_1} & : 0 \leq \tau < a_1 \\ 1 & : a_1 \leq \tau < a_2 \\ \frac{\tau - a_3}{a_2 - a_3} & : a_2 \leq \tau < a_3 \\ 0 & : x \geq a_3 \end{cases}. \quad (11)$$

According to this function, the shear stress has a beneficial effect on cells enhancing the growth if it is between $a_1$ and $a_2$ ($f_s = 1$). For values of $\tau$ above the threshold $a_3$, the shear stress has a detrimental effect and inhibits the cellular activity resulting the growth to become zero ($f_s = 0$).

The function describing the effect of curvature on growth is expressed using a linear function as follow:

$$f_c(\kappa) = \kappa \quad (12)$$

An overview of all parameter values used in this study is provided in Table 1. Parameters $A$, $\alpha, \beta, \gamma, \lambda$ are homogenization parameters required to capture a 3D heterogeneous reality by means of a homogenized set of equations, determined through a fitting procedure. A sensitivity analysis of this fitting was performed showing that the fitting is most sensitive to the value of $A$.

*Table 1. Overview of all parameter values used in this study.*

| Parameter | Value | Reference |
|---|---|---|
| $A$ | 1.8e-17 | Estimated |
| $\alpha$ | 251 | Estimated |
| $\beta$ | 28e4 | Estimated |
| $\gamma$ | 0.61 | Estimated |
| $\lambda$ | 0.4817 | Estimated |
| $s_1$ | -3e-5 | (Guyot et al., 2015) |
| $s_2$ | 1.5e-4 | (Guyot et al., 2015) |
| $c_1$ | 20 | (Guyot et al., 2015) |
| $c_2$ | -250 | (Guyot et al., 2015) |
| $T_1$ | 60 | (Guyot et al., 2015) |
| $T_2$ | 70 | (Guyot et al., 2015) |
| $a_1$ | 0.01 | (Chapman et al., 2014) |
| $a_2$ | 0.03 | (Chapman et al., 2014) |
| $a_3$ | 0.05 | (Chapman et al., 2014) |
| $V_O$ | 1.09e-17 mol/cell/s | (Lambrechts et al., 2014) |
| $V_g$ | 9.5e-17 mol/cell/s | (Zhou et al., 2013) |
| $K_o$ | 1.82e-3 mM | (Carlier et al., 2014) |
| $K_g$ | 0.3 mM | (Hossain et al., 2015) |

| | | |
|---|---|---|
| $\phi_{Cells}$ | 2.5e13 cells/m$^3$ | (Guyot et al., 2016) |

*Model Implementation*

The model was implemented in Matlab®. The capacity of predicting the evolution of the neotissue growth using the model developed in this study, is tested on four additional scaffold geometries. These scaffolds all had triply periodic internal surfaces and have been suggested as interesting structures in the context of tissue engineering (Shin et al., 2012).

*Model Optimization*

In the above described bioreactor experiments performed for studying the neotissue growth in the scaffold, the entire medium supply (100%) flowing through the perfusion bioreactor in a closed loop is refreshed every 3 days based on historic data (which might be suboptimal). As the frequency of the refreshment along with the fraction of medium being refreshed have great impact on the produced neotissue, we can perform the optimization procedure for finding the best time and fraction for refreshing the medium, yielding the maximum amount of neotissue in 21 days. In this study we have used a Genetic Algorithm (GA) for this purpose. The ability of the GA method in solving parameter estimation was demonstrated by Chatterjee et al. (1996). Based on natural selection and genetics (Malhotra et al. 2011), GA searches for the global maximum or minimum of a function without having any knowledge of the problem. Starting from multiple guess points (chromosomes/ individuals) forming the initial population, GA fits (evaluates) each individual directly to the objective function. Then it selects parents, by an appropriate method, and reproduces through natural operators (crossover, mutation) evolving to a new better fitting population. To apply the GA into our problem, there are two parameters that we varied, time between medium refreshments (between 1 and 96 hours) and the fraction of medium refreshed (between 0 and 100%).

**Results**

*Simulation Results*

The simulation results for different model species during 21 days of culture using Gyroid7 scaffold are depicted in Fig. 2 and Fig. 3. The culture medium in this simulation is refreshed every 3 days. As it is shown in the following figures, the neotissue volume filling percentage in the scaffold over time (Fig. 3 bottom) increases resulting in a drop in the oxygen (Fig. 2. top) and glucose (Fig. 3. top) concentrations as the increasing amount of cell will increasingly consume oxygen and glucose. On the other hand, the concentration of lactate, a waste product (Fig. 2. bottom) increases over time with increasing neotissue volume filling.

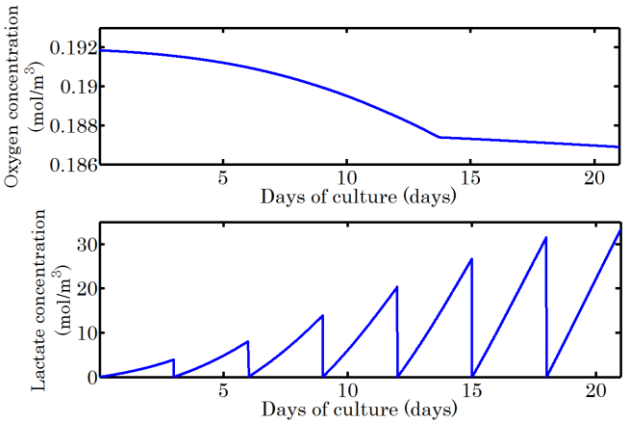


*Figure 2. Oxygen (top) and Lactate (bottom) concentrations over 21 days of culture with medium refreshment every 3 days for gyroid scaffold(G7).*

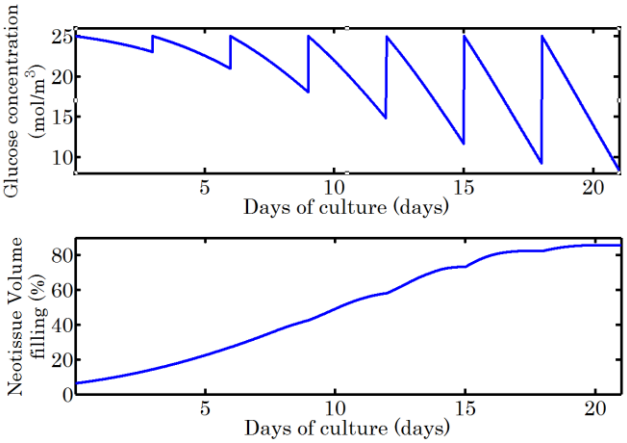


*Figure 3. Glucose concentration (top) and neotissue volume filling percentage (bottom) over 21 days of culture with medium refreshment every 3 days for gyroid scaffold(G7).*

*Experimental Validation of the Model*

Figure 4 shows the comparison between experimental data and computational results for neotissue filling of the Gyroid scaffold (G10) over 28 days of culture. As depicted, a good qualitative and quantitative correlation is obtained for the G10 scaffold for neotissue volume (around 22% at day 28). The computational cost of the model developed in this study is very low (around 1 minute to calculate the neotissue growth in 21 days), allowing for a rigorous optimization procedure to be executed without running into computational problems.

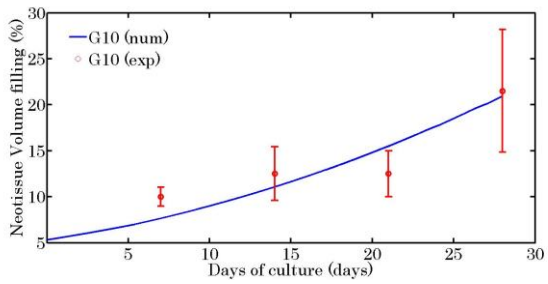


*Figure 4. Percentage of predicted scaffold filling over time (continuous line) compared to experimental data (dots) for gyroid scaffolds (G10).*

*Model Optimization Results*

Figure 5 represents the results obtained by GA. As it is depicted, brighter spots in Fig. 5 correspond to higher neotissue fillings (above 85%). Therefore, if we select the time of refreshment below 50 hours and also choose the refreshment amount above 70% of the present medium, more neotissue will be produced in the scaffold during the same culture time (encircled area in Fig. 5). In other words, decreasing the medium refreshment time or increasing the amount of medium being refreshed will yield in higher neotissue volumes.

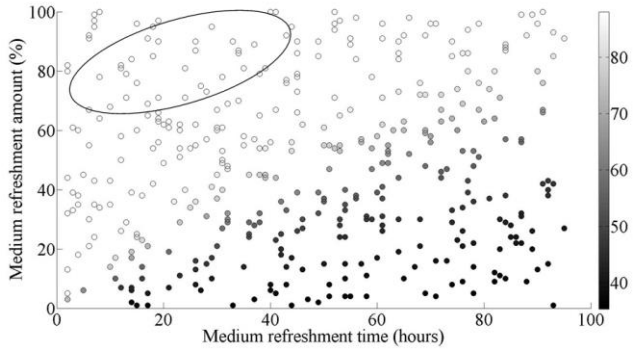


*Figure 5. Representation of neotissue filling for different combinations of medium refreshment time and medium refreshment amount where the grayscale color shows the scaffold filling in % with the brighter spots corresponding to a higher degree scaffold filling.*

**Discussion**

In this work, a computational model describing the neotissue growth in regular scaffolds in a perfusion bioreactor has been developed and the first results of a computational study for optimizing *in vitro* neotissue growth have been presented. In this model all the parameters are represented only as a function of time as, in order to speed up the calculation process, the spatial heterogeneity of different model variables has been homogenized. However, this means that the optimization of the neotissue volume is performed for the scaffold as whole rather than for the individual cells/neotissue areas inside the scaffold.

Nowadays, most of *in vitro* bioreactor cultures settings have been (and are being) determined based on trial and error, which results in wasting time and products. As mentioned earlier, the presented model could tackle this issue by providing to an *in-silico* tool for culture preprocessing to the operator. In this study, we proposed a GA technique for finding the best medium refreshment regime. As GA has its advantages and disadvantages, other general optimizations techniques such as particle swarm optimization (PSO) and Bayesian optimization were also applied (results not shown), yielding similar results. The results obtained by these optimization procedures – although quite obvious with the simplicity of the optimization cost function used at this point- are very promising and show that we can do the medium refreshment more efficiently to get the maximum amount of neotissue in the scaffold. Nevertheless, whereas some of the proposed medium refreshment regimes yield in higher percentage of final neotissue, they could dramatically increase the total cost of the culture as they require overall more medium to be used during a given culture time. Therefore, future objective functions for optimizing the bioreactor set-up will also consider (economic) cost of the culture. In Guyot et al. (2016b), the authors propose an intelligent method for medium refreshment based on the pH level of the medium. It starts by refreshing once a week at the beginning of the culture and gradually increases to (more than) once a day towards the end due to the increased neotissue growth. This way of medium refreshment could be applied in the future and the results, along with their costs of the culture, can be compared.

One of the limitations of this study is the absence of an equation for the representation of cells and extracellular matrix evolution separately. In this study we assumed that the produced neotissue is composed of cell and their extracellular matrix. In Sonnaert et al. (2014), the authors studied the human periosteal-derived cell expansion in a perfusion bioreactor leading to proliferation, differentiation and extracellular matrix formation based on DNA measurements of the neotissue formed inside the scaffold during 28 days of culture. Using these data, the model presented in this study can be extended to include a description of the cells and their matrix in separate variables. Future work includes the incorporation of other objectives in the cost function as well as the investigation of different optimization techniques such as Bayesian optimization which are suitable for models which are computationally expensive. In a future step, also a parameters sensitivity analysis will be conducted in order

to investigate the effect of different model parameters on the final neotissue volume.

In summary, this study proposes a model of neotissue growth in perfusion bioreactors systems that is specifically designed to allow for rigorous optimization of cell culture processes through the design of appropriate bioreactor settings.

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