Modelisation in fluid dynamics and transport phenomena in porous media.
Bone Tissue Engineering Applications

L.I. Meijer
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Supervisors:
Prof. C. Oddou
Université Paris XII
Dr. Ir. R. van Donkelaar
Technische Universiteit Eindhoven

Eindhoven University of Technology
Department of Tissue-Engineering and Biomechanics
Section Materials Technology

In cooperation with:
Université Paris XII
Laboratoire de Biomechanique et Biomateriaux osteo articulaires-LMP
Abstract

Tissue engineering is a very promising technique for creating bone implants. Bone cells are grown in vitro and then placed inside a scaffold to grow into a bony shape. A limiting factor for the growth of the cells is the transportation through the scaffold. Since trabecular bone has no vascular system, nutrients and waste products have to be distributed through diffusion. Transportation can be improved by applying a medium flow to the scaffold. The influence of this medium flow on the penetration of nutrients, in this case oxygen, was investigated. With the known velocity field in a pore of the scaffold and a cell specific oxygen consumption factor, OCF, oxygen concentration at every point in this pore can be computed. Three different factors that influence the depth of penetration of oxygen are examined. Increasing the inlet velocity of the medium will have a linear increasing effect on the oxygen penetration length, OPL. The initial concentration of oxygen in the medium also has a linear effect on the OPL. The specific consumption factor of the cells in the scaffold, OCF, has an inverse effect on the OPL.
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A Oxygen penetration on different lines
Chapter 1

Introduction

Patients with large bone defects can profit from the use of bone implants in the healing process. Ideally, the (bio)material of the implant has mechanical properties similar to bone. Osteogenic cells will be seeded in a porous scaffold of a geometric shape such that the cells will grow in a structure similar as bone. Because trabecular bone has no vascular supply, it is dependent on diffusion for exchange of nutrients and waste products in the tissue. Growth of cells is often limited to the outer parts of the scaffolds due to lack of nutrients in the inner part [Rose-2004]. The transportation of nutrients can be improved by applying a flow to the scaffold. A bioreactor can provide a circulation of fluid that functions as medium to supply nutrients and remove waste products. Besides, it maintains conditions for cells to grow, such as temperature and pH.

It is known that cell growth of chondrocytes was inhibited due to transport limitations within a polymer scaffold [Galban-1999]. It is assumed, that the possibility of osteocytes to proliferate is restricted to the presence of oxygen. By improving penetration of oxygen through the entire scaffold, cell proliferation can expand to the interior of the scaffold.

Earlier research of Das ([Das-2003]) has led to the development of a perfusion bioreactor to apply a dynamic, continuous fluid flow through the scaffold. In a study of Federici streamlines through the porous medium in this bioreactor were visualized, and it was shown that the hydrodynamic conditions inside the scaffold are homogenous. A model of a single pore by Federici showed the penetration of oxygen by convection and diffusion, with oxygen consumption by osteocytes on the surface of the scaffold represented by a Michaelis-Menten law ([Fede-2003]). This model consists of a simple 2D geometry and assumes a constant velocity at each point inside the scaffold. In the model of Federici a non realistic velocity for the medium was used. In this report, the velocity profile through the scaffold was solved locally, using fluid mechanics equations. This local velocity was then used to determine convection of oxygen by the medium. A gradient of oxygen is caused by consumption of oxygen by the cells present on the scaffold. The oxygen concentration through the entire scaffold was calculated with the aid of simulations. This concentration gives information about cell viability. Choosing a minimum concentration at which cells can survive, determines qualitative at what site in the scaffold cells will grow and proliferate. The aim of this report is to find what parameters are most important in determining the local oxygen concentration and cell viability. Changing this parameters will give insight in their influence quantitatively.

Simulations were performed with a finite element method based program, FEMlab (designed by the Comsol company), that allows to solve coupled physics equations.
For the geometry of the porous medium a special scaffold was used with a known geometry ([Diet-2000]).
Chapter 2

Theory

The oxygen penetration in a scaffold is determined by diffusion and convection. An oxygen gradient is created due to consumption of oxygen by cells in the scaffold. The latter is discussed in more detail in section 2.4 Diffusion is depending on the type of medium inside the scaffold. Convection is mainly influenced by the local velocity field. To determine the velocity field in every point in the scaffold, some assumptions and simplifications must be made. The geometry of the scaffold will be explained in detail in chapter 3. In this paragraph, only a single pore of the porous scaffold is considered. Over the scaffold, a pressure gradient is present. The fluid flow through the pore can be seen as a flow through a tube, associated with a pressure field. For this situation, Navier-Stokes equations can be used to solve the velocity field.

2.1 Fluid Dynamics; Navier-Stokes equations

This paragraph gives the Navier-Stokes equation in a general form and some fluid characteristics. It has to be associated with an equation stipulating that the flow is such that the fluid behaves as an incompressible fluid.

The equation is given by:

\[
\frac{\partial u}{\partial t} + u \nabla u = F - \frac{1}{\rho} \nabla p + \nu \Delta u,
\]  

(2.1)

with \( u = [u_x, u_y, u_z]^T \), the velocity in \( x, y \) and \( z \) direction, \( \rho \) the density of the fluid and \( \nu \) its viscosity. The pressure gradient of the fluid is given by \( \Delta p \).

From equation (2.1) a characteristic dimensionless number of the flow can be derived by introducing dimensionless variables. This will be explained in section 3.5adimen).

\[
Re = \frac{u_0 \varepsilon}{\nu}
\]  

(2.2)

\( Re \), the Reynolds number, gives the ratio between inertial and viscous effects defined from a given length scale \( \varepsilon \), of the flow topology and a given velocity scale \( u_0 \).
2.2 Dimensionless equations

To simplify the comparison between the fluid dynamics model with other models, for instance with convection-diffusion equation, all parameters used in the model will be made dimensionless.

To make a dimensionless equation, the dimensionless variables $x'$, $u'$ and $p'$ are introduced according to:

$$x = \varepsilon x' \quad u = U_0 u' \quad p = \rho U_0^2 p'$$

(2.3)

with $\varepsilon$ the thickness of a pore.

For example, solving the $x$-component of the Navier-Stokes equation (2.1) for a stationary flow ($\frac{\partial u}{\partial t} = 0$, $F = 0$) and using the dimensionless variables gives:

$$\frac{U_0^2}{\varepsilon} \frac{\partial u}{\partial t} = -\frac{\rho U_0^2}{\varepsilon} \frac{\partial p'}{\partial x'} + \nu \frac{U_0}{\varepsilon^2} \frac{\partial^2 u}{\partial x^2}$$

(2.4)

This gives for the general form of the adimensionalised Navier-Stokes equation:

$$u' \nabla' u' = -\nabla' p' + \nu \frac{U_0}{\varepsilon} \Delta' u' = -\nabla' p' + \frac{1}{Re} \Delta' u'$$

(2.5)

with $\nabla' = \frac{\partial}{\partial x'}$ and $\Delta' = \frac{\partial^2}{\partial x'^2}$.

2.3 Convection-diffusion equations

Transport of oxygen through a medium can be determined by a contribution of convection and diffusion to the flux. Local flux (2.6) of molecules caused by diffusion is depending on the oxygen concentration gradient and the diffusion constant, $D_{O_2}$, for oxygen through the fluid phase of the porous medium.

$$\vec{J}_D = -D_{O_2} \nabla C_{O_2}$$

(2.6)

The contribution of convection to the local flux, which is depending on the local concentration of oxygen and the local velocity is given by:

$$\vec{J}_C = -C_{O_2} \vec{u}$$

(2.7)

Combining equations (2.6) and (2.7) gives the total local flux determined by diffusion and convection.

$$\vec{J} = -D_{O_2} \nabla C_{O_2} + C_{O_2} \vec{u}$$

(2.8)

When both fluid dynamics and transport equations are considered and rendered a-dimensional, characteristic numbers arise: $Sc = \frac{D}{\nu}$ and $Pe = \frac{U \varepsilon}{D}$. $Sc$, the Schmidt number, is the ratio between kinetic viscosity and molecular diffusivity. $Pe$ gives the ratio between the Reynolds number and the Schmidt number, i.e. the effect of the ratio between the diffusivity of the solute particles in the solvent and the diffusivity of momentum within the solvent medium.
2.4 Oxygen consumption factor

The consumption of oxygen by a single cell is dependent of a cell-specific consumption factor and the local concentration of oxygen. Total oxygen consumption per surface unit is hence given by multiplying with the cells surface density. The effect of cell consumption on the concentration of oxygen can be described by Michalis-Menten kinetics, see equation (2.9).

\[ -N_{O_2}\vec{n} = -\rho_{cell}V_{max} \frac{C_{O_2}}{\kappa_M C_{O_2}} \]  \hspace{1cm} (2.9)

The surface density of the cells is given by \( \rho_{cell} \), whereas \( V_{max} \) is the maximum oxygen consumption by one cell and \( \kappa_M \) the Michaelis-Menten constant.

Considering a balance of inflow and outflow per unity of volume, for cell consumption and convection and diffusion, equations (2.8) and (2.9) can be written in a differential form:

\[ \frac{\partial^2 C_{O_2}}{\partial x^2} - \frac{u}{D_{O_2}} \frac{\partial C_{O_2}}{\partial x} = 2\rho_{cell}V_{max} D_\varepsilon \left( \frac{C_{O_2}}{\kappa_M + C_{O_2}} \right) \]  \hspace{1cm} (2.10)

This equation can be made dimensionless, as already described in section 2.2 for Navier-Stokes equation, if a characteristic length \( L \) is defined (such that \( L = \varepsilon \)), using \( C_{O_2} = \frac{\varepsilon}{\kappa_M} \), and defining an oxygen consumption parameter \( \Omega = \frac{\rho_{cell}V_{max} \varepsilon}{\kappa_M D} \) that represents the ratio between the fluxes by cell consumption and diffusion. The Peclet number is defined by \( Pe = \frac{u \varepsilon}{D_{O_2}} \) and gives the relative importance of convection versus diffusion.

The differential equation for dimensionless oxygen concentration per unity of volume becomes:

\[ \frac{\partial^2 C'_{O_2}}{\partial x^2} - Pe \cdot u \frac{\partial C'_{O_2}}{\partial x} = 2\Omega \frac{C'_{O_2}}{1 + C'_{O_2}} \]  \hspace{1cm} (2.11)
Chapter 3

Model

First, the geometry of the model will be described. The scaffold used in this report is made of a biodegradable polymer \textit{Poly(\varepsilon-Caprolactone)} (PCL). It is fabricated by fused deposition modelling ([Diet-2000]) and has a known geometry. An example of such a scaffold is shown in 3.1, where layers of fibres are placed in different angles to each other.

![Figure 3.1: Cross-sectional view of freeze-fractured PCL scaffold with lay-down pattern 0/72/144/36/108 [Diet-2000]](image)

The scaffold that is considered in this report has layers of fibres in an angle of 90 degrees to each other. Figure 3.2(a) shows a schematic 3D side view of the scaffold. Along the line indicated in this figure, a 2D section plane was taken. This section plane is shown in figure 3.2(b).
The dashed lines in Figure 3.2(b) indicate symmetry lines. The shaded area gives a simplification of the scaffold geometry and is shown again in Figure 3.3.

The geometry consists now of a single 'pore' with circular solid shapes of a diameter $\varepsilon$ and where the distance between each of the cylinders in the 2D plane is equal to $\varepsilon$ too.

The size of the cross-section area is $200 \varepsilon$ by $200 \varepsilon$. Fluid mechanics equations are valid for the model in macroscale, where the total length of the pore is $200 \varepsilon$. In section 3.5 (micromodel) another method to determine the permeability of oxygen through the pore is explained. This method makes use of a microscale model, with a size of $\varepsilon$ by $\varepsilon$. The parameter found with the microscale model can be applied on the model as a whole.
3.1 Fluid dynamics model: Navier-Stokes

Now the velocity profile can be solved for the model using Navier-Stokes equations. For this, a 2D incompressible Navier-Stokes model is solved using FEMlab. To decrease computation time while evaluating results for this fluid dynamics model, the length of the geometry is decreased to $6\varepsilon$, instead of $200\varepsilon$. The value of $\varepsilon$ is $125 \text{ m}$. This geometry is shown in Figure 3.3 and Figure 3.4.

![Model geometry with mesh distribution and boundary numbers](image)

Figure 3.4: Model geometry with mesh distribution and boundary numbers

On the inlet boundary, 1, a constant velocity of $u_0$ in x-direction is prescribed. The pressure at the outlet, boundary 7, is set to 0. On the borders, boundary 8 to 13, the fluid has no velocity so no slip conditions have been set. All other boundaries are symmetrical. All boundary settings are given in Table 3.1. The fluid has a density $\rho$ and a viscosity $\eta$.

<table>
<thead>
<tr>
<th>Number of Boundaries</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$u = u_0$</td>
</tr>
<tr>
<td>2-6</td>
<td>Symmetry</td>
</tr>
<tr>
<td>7</td>
<td>$p = 0$</td>
</tr>
<tr>
<td>8-13</td>
<td>No Slip: $u, v = 0$</td>
</tr>
</tbody>
</table>

Table 3.1: Boundary conditions in model with dimensions

For the values of the physical properties and geometrical parameters as well as flow boundary conditions those values have been used that match experimental conditions. The medium is assumed to have the properties of water:

- $\varepsilon = 1.25 \cdot 10^{-4}$ m for the diameter of the pores
- $\rho = 1 \cdot 10^3$ kg/m$^3$ and $\eta = 1 \cdot 10^{-3}$ Pa · s are the characteristics of the fluid
- $u_0 = 1.68 \cdot 10^{-4}$ m/s is the velocity of the fluid at the inlet boundary
3.2 Adimensionalisation of the fluid dynamics equations

Recalling equation (2.5) from Section 2.2 gives the general form for the dimensionless Navier-Stokes equation.

\[ u' \nabla' u' = - \nabla' p' + \frac{\nu'}{\varepsilon u_0} \Delta' u', \quad (3.1) \]

recall that \( \nabla' = \nabla \cdot \varepsilon \).

The values listed below have been used in the model of a pore as explained in section 3.1 and can be used here to compute the variables needed in the dimensionless Navier-Stokes equation.

- \( \epsilon = 1.25 \cdot 10^{-4} m \)
- \( \rho = 1 \cdot 10^3 kg/m^3 \)
- \( \eta = 1 \cdot 10^{-3} Pa \cdot s \)
- \( \nu = \frac{\eta}{\rho} = 10^{-6} m^2/s \)
- \( U_0 = 1.68 \cdot 10^{-4} m/s \)

Filling in these values into (2.5) results in:

\[ u' \nabla' u' = - \nabla' p' + 47.619 \Delta' u'. \quad (3.2) \]
FEMlab stipulates the Navier-Stokes equation in the following form to solve the stationary flow problem:

\[-\nabla \eta (\nabla u + \nabla u)^T + \rho (u \nabla)u + \nabla p = F \tag{3.3}\]

and

\[\nabla \cdot u = 0\]

for incompressibility.

Comparing (3.2) with (3.3) gives the values of the constants to be used for the simulations in FEMlab. The values that have been used for the dimensionless model are:

\[\rho = 1; \quad \eta = 47,62; \quad u_0 = 1; \quad \varepsilon = 1; \quad F = 0\]

The geometry and boundary settings are the same as the model with dimensions.

### 3.3 Convection-diffusion equations with oxygen consumption term

For the same geometry as given in section 3.1, with a length of 200\(\varepsilon\), a diffusion-convection model has been build in FEMlab. For the calculations, a constant velocity through the pore has been used with a value \(u_0\). On the boundary of the solid phase, 8-13, a layer of zero thickness represents the presence of cells. Here, cell consumption is represented by the oxygen consumption factor (OCF), \(\Omega\), in the boundary settings. For the constants, values are used that match experimental conditions:

- \(\rho_{cell} = 1.7 \cdot 10^9 \text{ cells/m}^2\)
- \(D_{O_2} = 2 \cdot 10^{-9} \text{ m}^2/\text{s}\)
- \(V_{max} = 0.38 \cdot 10^{-15} \text{ mol/cell} \cdot \text{s}\)
- \(\kappa_M = 0.01 \text{ mol/m}^3\)
- \(C_0 = 0.2 \text{ mol/l}\)

With the constants given above, a diffusion characteristic length can be derived by homogeneity consideration of the diffusion equation, that gives a value also found in the literature for diffusion in living tissues.

\[L_D = \sqrt{\frac{DC_0}{\rho V S_v}} \approx 200 \mu \tag{3.4}\]

With \(S_v\) the specific surface (pore envelop area by volume unit) of the porous medium

\[S_v = \frac{1}{\varepsilon} = 10^4 \text{ m}^{-1} \tag{3.5}\]

An approached expression for the characteristic length of convection alone can be derived by solute mass conservation:

\[L_C = \frac{\varepsilon U_0 C_0}{\rho V} \approx 5 \text{ mm} \tag{3.6}\]

For the oxygen consumption, an approximation is made of the Michaelis-Menten curve as shown in Figure 3.5. For concentrations above \(K\), cells are assumed to have a constant consumption, below this value their consumption is linearly decreasing.
CHAPTER 3. MODEL

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Figure 3.5: Michaelis-Menten curve with approximation (dashed line)

3.4 Coupled Model

The Navier-Stokes and diffusion-convection equations are coupled through multi-physics in FEMlab. The velocity profile has been solved from the Navier-Stokes equations and used to calculate the oxygen concentration with the convection-diffusion equation. FEMlab uses the following set of equations on the subdomain:

$$
- \nabla \cdot \left( D_{O_2} \nabla C_{O_2} + 10.5 \cdot \bar{c} \cdot \nabla C_{O_2} \right) = 0
$$

$$
\rho \bar{u} - \nabla \eta (\nabla \bar{u} + (\nabla \bar{u})^T) + \rho (\bar{u} \cdot \nabla) \bar{u} + \nabla p = 0
\quad \nabla \cdot \bar{u} = 0
$$

The upper equation comes from the diffusion-convection balance, the middle represents the Navier-Stokes equation and the lower equation comes from the supposed incompressibility of the fluid.

Oxygen consumption by cells is applied as a boundary condition on the surface of the solid phase, as discussed in section 3.3:

$$
n \cdot (c \nabla C_{O_2} + C_{O_2}) + \frac{\Omega}{\kappa_M + C_{O_2}} = 0 \quad (3.7)
$$

For the dimensionless formulation, values of the parameters have been calculated as explained in section 2.4, leading to:

$$
\Omega = 4; \quad C'_0 = 20; \quad u_0 = 1
$$

By changing each of these parameters, their influence on the oxygen penetration will be tested. It was assumed that cells need enough oxygen to proliferate and to be viable. Under a certain amount of oxygen, cells can not proliferate and will eventually die. To find out how deep cells can grow, oxygen concentration must be known at each point in the pore. By choosing a critical value of oxygen, a length can be determined till which cells will penetrate into the pore. This length is called the Oxygen Penetration Length (OPL).
3.5 Determining a macro-permeability constant with a micro-scale model

3.5.1 Geometry

The Darcy Law gives a relationship for fluid flow through a porous medium, as in our model. The general formulation of this law gives the vectorial flow rate:

$$\overrightarrow{Q} = \frac{K}{\eta} \cdot A \cdot \frac{\Delta P}{L},$$

with:

- $\Delta P_L$ the macroscopic pressure gradient vector,
- $L$ the macroscopic length scale,
- $K$ the permeability tensor,
- $A$ the flow area,
- $\eta$ the dynamic viscosity of the fluid.

The Darcy law can be applied on the macro-scale of the model, whereas in the micro-scale the Navier-Stokes equations are valid. The model geometry can be described in a different macroscopic and a microscopic scale, as shown in Figure 3.6. The geometry of the micro-scale is presented in Figure 3.7.

At the macroscopic scale the Darcy law changes into 3.9, since the flow per area gives the mean velocity of the fluid.

$$\langle u \rangle = -\frac{K}{\eta} \nabla p$$

with $\langle u \rangle = \frac{1}{n} \int_{\Omega} u d\Omega$ as the mean velocity in the area of the microscopic scale.
3.5.2 Boundary conditions

There is symmetry on boundaries 1 and 4, and boundaries 2 and 3. The following periodic boundary conditions have been set in the microscopic model:

\[
\begin{align*}
    u^{(0)}|_{(2)} &= u^{(0)}|_{(2)} \\
    u^{(0)}|_{(1)} &= u^{(0)}|_{(4)} \\
    p^{(1)}|_{(2)} &= p^{(1)}|_{(3)} \\
    p^{(1)}|_{(1)} &= p^{(1)}|_{(4)}
\end{align*}
\]

\[\nabla \eta^{(0)} = 0\] for incompressibility

\[\eta^{(0)}|_{\Gamma} = 0\] assuming there is no slip on the solid-liquid interface.

The subset (0) indicates that \( u \) is the microscopic velocity field. The microscopic pressure has been indicated by subset (1).

The Dirichlet boundary conditions according to \( h \cdot u = r \) are for boundaries 2 and 3:

\[
\begin{bmatrix}
1 & 0 & 0 \\
0 & 1 & 0 \\
0 & 0 & 1
\end{bmatrix}
\begin{bmatrix}
u \\
p
\end{bmatrix}
\rvert_{(3)}
+ 
\begin{bmatrix}
-1 & 0 & 0 \\
0 & -1 & 0 \\
0 & 0 & -1
\end{bmatrix}
\begin{bmatrix}
u \\
p
\end{bmatrix}
\rvert_{(2)}
= 
\begin{bmatrix}
0 \\
0 \\
0
\end{bmatrix}
\rvert_{(3)}
+ 
\begin{bmatrix}
0 \\
0 \\
0
\end{bmatrix}
\rvert_{(2)}
\]

and have been set the same way as for boundaries 1 and 4.

The permeability tensor \( \mathbf{K} \) is given by:

\[
\mathbf{K} = \begin{pmatrix} K_{xx} & K_{xy} \\ K_{yx} & K_{yy} \end{pmatrix}
\]

(3.10)

And can be computed from the Darcy law, using

\[
\begin{align*}
K_{xx} &= K_{yy} = \langle K_{xx} \rangle = \langle K_{yy} \rangle \\
K_{xy} &= K_{yx} = \langle K_{xy} \rangle = \langle K_{yx} \rangle
\end{align*}
\]

\[
\begin{pmatrix}
\eta^{(0)}_x \\
\eta^{(0)}_y
\end{pmatrix} = -\frac{1}{\eta} \begin{pmatrix} K_{xx} & K_{xy} \\ K_{yx} & K_{yy} \end{pmatrix} \cdot \begin{pmatrix} \nabla p^{(0)}_x \\ \nabla p^{(0)}_y \end{pmatrix}
\]

(3.11)

The macroscopic pressure increment \( \Delta p^{(1)} \) over the length-scale \( L \) is given by \( p_0 - p_i \).
So the macroscopic pressure gradient field is given by:

\[
\begin{pmatrix}
\nabla p_x^{(0)} \\
\nabla p_y^{(0)}
\end{pmatrix} = \left( \frac{p_0 - p_i}{L} \right)
\]

(3.12)

The trick in this homogenization process is to consider that, at the microscopic scale, the flow is generated by a volume density of force \( F \) resulting from the imposed macroscopic pressure gradient.

FEMlab uses again the Navier-Stokes equation (3.3) to solve this problem on the microscopic domain.

\[
-\nabla \eta (\nabla u^{(0)} + \nabla u^{(0)})^T + \rho (u^{(0)} \nabla)u^{(0)} + \nabla p^{(1)} = F
\]

(3.13)

FEMlab needs for the execution of the simulations the values for \( \eta, \rho \) and \( F \). Therefore they must be set in the sub-domain settings. The force \( F \) is imposed by the macroscopic pressure gradient, and therefore only the \( x \)-gradient of the force has a value. \( \eta \) is the characteristic dynamic viscosity of the fluid. The different values are given below.

\[
\begin{align*}
\rho &= 0 \\
\eta &= 1 \cdot 10^{-3} \text{ Pa } \cdot \text{s} \\
F_x &= \Delta p^{(1)} \\
F_y &= 0
\end{align*}
\]
Chapter 4

Results

4.1 Comparing the 2 Navier-Stokes models

In both the dimensional and dimensionless formulation of the same model, effects of increasing flow on $\Delta p$ have been tested. For the model with dimensions, $U_0$ has been defined by $U_0 = n \cdot 1.68 \cdot 10^{-4} \text{ m/s}$ with $n = 1, 2, \ldots, 10$. In the solution, the integral over boundary 1 has been calculated and then divided by the length of the boundary. The pressure on the outlet boundary 4 has been maintained at 0. The mean pressure difference over the total length is hence given by:

$$\langle p \rangle = \frac{1}{\Omega} \int p \, d\Omega \quad (4.1)$$

The length of the model is $6 \varepsilon$. To obtain a pressure difference per unity of length $\varepsilon$, results have been divided by 6. They are presented in Figure 4.1 and assume a linear relation between the flow at inlet and the pressure gradient over the model.

![Graph showing the relation between input velocity and the mean pressure gradient](image)

Figure 4.1: Relation between input velocity and the mean pressure gradient
The relation between flow and $\Delta p$ is depending on the Reynolds number. The dimensionless problem has been solved in three cases:

1. Reynolds number $<< 1$,
2. Reynolds number $\approx 1$,
3. Reynolds number $>> 1$.

In Figure 4.2 these relations are shown. To change the Reynolds number, initial $U_0$ has been set to $10^{-1} \cdot U_0, 10^2 \cdot U_0$ and $10^3 \cdot U_0$ respectively.

Figure 4.2: Relation between $u$ and $\Delta P$ for different Reynolds numbers

After this, simulations have been performed with 1 to 8 times $u_0$. Figure 4.2 shows different relations between the input velocity and the pressure gradient for different Reynolds numbers. For a Reynolds number in the order of 1 or higher, a non-linear relationship holds which is attributed to the inertial effects within the flow at the scale of the pore. For a Reynolds number much smaller than one, a linear relationship can be seen. In the dimensional model that satisfies experimental conditions, the Reynolds number is 0.021 ($<< 1$). As can be seen from Figure 4.2, a linear relation has been found between inlet velocity and pressure gradient, as is the case for a Reynolds number $<< 1$ in the dimensionless model.

Figure 4.3(a) and 4.3(b) show the velocity profiles of the dimensionless and the dimensional model, respectively. The scale for the problem with dimensions has been made proportional to that of the dimensionless problem, according to formula $u = u_0 \cdot u'$, given in section 2.2. No differences can be mentioned between the velocity profiles of both models.

To be able to make a better comparison between the dimensionless and the dimensional model, the values of the dimensionless model can be scaled back to the dimensions. Figure 4.4 shows the velocity in $x$-direction of the dimensional model and the scaled velocity of the dimensionless model at the outlet boundary.
The velocities of the two models are not exactly the same but show very slight difference.

![Figure 4.3: Velocity fields for problems with and without dimension](image)

The same comparison can be made for the pressure along boundary 1 per unity of length, $\varepsilon$. See Figure 4.5.

Also the pressures of the two models show slight differences to be attributed to the numerical errors.

### 4.2 Coupled model

Figure 4.6 shows a part of the solution for the coupled model. The colored surface represents the oxygen concentration, the black arrows give the fluid velocity and the white lines are concentration iso-lines. It can be seen that not all cells, present on the solid surface, experience the same oxygen concentration. Also, due to different velocities along the solid surface, cells at different sites will experience different shear stresses. So, there are different living conditions for the cells in different positions on the surface.
Figure 4.5: Comparison of pressure at outlet between dimensional and dimensionless model

Figure 4.7 shows the oxygen concentration through the pore for the dimensionless model, on a line at $y = 0.5$, halfway in the pore along the solid phases. Disturbances in the concentration are caused by the effect of presence of the solid phase on the velocity field. The initial velocity for the situation in Figure 4.11 is set to 1, initial concentration, $C_0$, is $0.2 \text{ mol/m}^3$ and $\Omega = 4$.

As pointed out in section 3.4, parameters $\Omega$ (the oxygen consumption factor), $U_0$ (the inlet velocity) and $C_0$ (the initial oxygen concentration) are changed, to evaluate their influence on the penetration of oxygen. An oxygen penetration length (OPL) is defined as the distance in the pore at which an oxygen concentration of $0.1 \text{ ml/m}^3$ occurs. This penetration length has been plotted as a function of the changing parameters. Figure 4.8 and 4.9 show a linear relation between respectively inlet velocity and initial concentration and the OPL.

Figure 4.10 shows a non-linear relation between the OCF and the OPL. The relation should surely be an inverse one, as can be easily demonstrated by simple considerations on oxygen mass conservation.

The relative importance of the change of the parameters to each other can be shown by representing on one graph (Figure 4.11) their changes in proportion. For this, a reference value is chosen with $C_0 = 0.2 \text{ mol/m}^3$, $U_0 = 1$ and the $OCF = 4$, for which change is set to 0. A line is drawn through all measuring points.

Figure 4.11 shows that a change of initial concentration has in proportion a slightly greater effect on the OPL than a change of initial concentration. Changing the OCF has a large effect on the OPL for values smaller than the reference value of $0.2 \text{ mol/m}^3$, which decreases with rising values of OCF.
4.3 Determining K

With the results of the simulation, the permeability K of the porous substrate can be determined according to equation (3.9) given in section 3.5.1. The mean velocities of the fluid in \( x \) and \( y \) direction can be computed in FEMlab using the sub-domain integration for any given value of \( F_x \). The relation between \( F_x \) and the mean fluid velocity is linear, so equation (4.3) can be computed for any given value of \( F_x \).

\[
\frac{\langle u \rangle}{F_x} = \frac{\langle u \rangle}{\nabla p_x^{(0)}} = C \tag{4.2}
\]

The following constants follow from the simulation in FEMlab:

\[
\frac{\langle u \rangle}{F_x} = 6.5 \cdot 10^{-7} \\
\frac{\langle v \rangle}{F_x} = 7.9 \cdot 10^{-10}
\]

Now, \( K \) can be computed:

\[
K_{xx} = \langle K_{xx} \rangle = -\frac{\eta \cdot \langle u \rangle}{\Delta p_x^{(0)}} = \eta \cdot \frac{\langle u \rangle}{F_x} = 6.5 \cdot 10^{-10},
\]

\[
K_{xy} = \langle K_{xy} \rangle = -\frac{\eta \cdot \langle v \rangle}{\Delta p_x^{(0)}} = \eta \cdot \frac{\langle v \rangle}{F_x} = 7.9 \cdot 10^{-13}.
\]

So,

\[
K = \begin{pmatrix}
6.5 \cdot 10^{-10} & 7.9 \cdot 10^{-13} \\
7.9 \cdot 10^{-13} & 6.5 \cdot 10^{-10}
\end{pmatrix} m^2.
\]
Figure 4.7: Oxygen concentration through the pore

Figure 4.8: OPL as a function of the inlet velocity
Figure 4.9: OPL as a function of the initial oxygen concentration

Figure 4.10: OPL as a function of OCF
Figure 4.11: Change of parameters represented in percentages
Chapter 5

Experimental Results

5.1 Compression test

To find the Young’s modulus of the samples supplied by a team of the University of Singapore [Diet-2000], a pressure test has been performed on one of the samples. A schematic view of the geometry is shown in Figure 5.1(a). Layers have been placed on each-other in an angle of 0, 60 and 120. Initial size of the sample is $10 \times 10.5 \times 7.2 \text{ mm}$, as shown in Figure 5.1(b). Notice that this sample has a different geometry than the one use for the model described in Chapter 3.

![Schematic figure of scaffold](Zein-2002)

![Initial conditions](b) Initial conditions

Figure 5.1: Schematic overview and initial conditions

A ramp-relaxation test has been done under unconfined compression loading with a fixed ramp velocity of the displacement frame of 1 mm/min for 10.8 sec., so the final deformation is 2.5%. After this, displacement is stopped and the sample was let free to relax for 103 minutes. This is shown in Figure 5.2.

During the experiments the force on the scaffold has been recorded and the results are presented in Figure 5.3. On the x-axis, the time is represented and on the y-axis, the force.
5.2 Young’s Modulus

Now the Young’s modulus of the sample can be calculated from equation (5.1):

\[ E = \frac{F}{\delta} \]  \hspace{1cm} (5.1)

With \( A \) the surface of the sample on which the force \( F \) is applied, so that

\[ \frac{F}{A} = \sigma \]  \hspace{1cm} (5.2)

is the compression stress.

\( \delta \) is the displacement of the sample in y-direction, and \( L \) the original height of the sample, so that:

\[ \frac{\delta}{L} = \varepsilon \]  \hspace{1cm} (5.3)

is the compression strain.
CHAPTER 5. EXPERIMENTAL RESULTS

Values for these parameters have been determined to be:

\[ A = 10.5 \text{ mm} \times 10 \text{ mm} = 105 \text{ mm}^2 \]
\[ L_{//} = 7.2 \text{ mm} \]
\[ \delta = 0.18 \text{ mm} \]
\[ F = 10.4 \text{ DaN} \text{ (measured from Figure 5.3)} \]

Substituting these values into equation (5.2) and (5.3) the Young’s Modulus can be calculated using equation (5.1).

\[
E = \frac{9.9 \times 10^5}{0.025} = 3.96 \times 10^7 \text{ N m}^{-2} = 39.6 \text{ MPa}.
\]

Remark; Earlier research on these scaffolds showed a stiffness of 47.1 ± 1.9 MPa ([Zein-2002]).

5.3 Permeability constant

In Figure 5.3, the crossing point between the tangent to the beginning of the graph and the asymptote, gives an estimation of the relaxation time \( \tau \). If one assumes that the relaxation process is essentially due to the poroelasticity of the sample, the permeability constant, \( K \), can be calculated from this \( \tau \), the dynamic viscosity of air, \( \eta \), the Young’s modulus, \( E \), and the area of the sample, \( A \), according to equation (5.4). ([Fede-2003])

\[
K = \frac{\eta A}{\tau E} = \frac{18.5 \times 10^{-6} \times 105 \times 10^{-6}}{10.8 \times 3.96 \times 10^7} = 4.5 \times 10^{-18} \text{ m}^2
\]

In fact, many relaxation processes can be put forward in order to interpret the observed phenomena. They are related to friction processes taking place at different length scales:

- Polymeric macromolecules visco-elasticity (10-100 Å)
- Flow within pores of the structure (10-100 \( \mu \text{m} \))
- Plastic flow
- Friction at the scale of the fibres (≥ 1 mm)

What has been investigated over here is the hypothesis of dominating poroelasticity phenomena, with the characteristic time involved being:

\[
\tau = \frac{\eta L_{\perp}^2}{K E}
\]

It appears that the result in terms of permeability constant is in full disagreement with what is known about the microstructure of the porous medium (pores of the order of 100 \( \mu \text{m} \) leading to a value of \( K = 10^{-8} \text{m}^2 \))
Chapter 6

Discussion and recommendations

For a good growth of cells inside the scaffold, it is important that oxygen can go as far as possible into the pores. Cells can only proliferate in the presence of nutrients, in this case oxygen. The described model can give a good insight in the penetration of oxygen through a pore. Three parameters are most important in determining the length of penetration, the OPL. The initial concentration of oxygen and the inlet velocity are found to have a linear effect on the OPL. The consumption of oxygen by the cells in the scaffold, the OCF, has an inverse effect on the OPL. This oxygen consumption factor is mainly determined by the type of cells present in the scaffold. Therefore, this value can not be changed in experiments. Other factors that determine the OCF per surface unit are the local concentration of oxygen and the density of cells on the surface of the scaffold. For the OCF as present in the boundary equations, a value is chosen that is known for hepatocytes. It is likely that the OCF for osteocytes is smaller than that for hepatocytes. Analysis of the relative importance of each of the parameters has shown that OCF’s smaller than 4 have a large effect on the OPL. Therefore it is important to find a better estimation for the OCF of osteocytes. It is possible to have a quantitative idea of the influence of different parameters on the OPL. For future work it is necessary to find a better estimation for this OCF, for example from experiments. With this it would be possible to give a more quantitative value for the OPL.

The modelling has been made of a 2D model, from a cross-section of the 3D structure. Not all cross-sections will have the same geometry and therefore not give the same results. Expanding the model to a 3D geometry can have a large effect on the outcome of the simulations.

In the modelling, the concentration of cells on the surface is assumed to be homogenous. In experiments however, it will be impossible to seed the cells equally distributed on the surface of the scaffold. Proliferation and migration can change this concentration into a more homogenous.

Another assumption that has been made, is that the consumption of oxygen by the cells is only dependant of the local concentration of oxygen. In fact, cells change oxygen consumption also with changing conditions. From the solution of the coupled model, shown in Figure 4.4, it is clear that not all cells experience the same conditions. Along the surface of the solid phase, the velocity is changing. This will lead to a different shear stress on the cells and can effect their behavior. In vitro experiments are often done with a pulsatile flow instead of a continuous one. The model is now not suitable.
for a time-dependant flow. In the future it might be interesting to see the effects of changing flow. Although the Young’s modulus found by experiments is of the same order as found in [Zein-2002], there is still a difference of 15%. It must be noted that the experiment was only executed with one scaffold, and additional data are necessary. Results obtained by this experiment are not necessary for the modelling in this report, but can be useful for future work on the scaffolds.

The permeability constant, determined using a micro scale model, was calculated to describe the fluid flow through the porous scaffold in macro-scale. Because the pore size is in the order of 100 $\mu m$, the value of the permeability constant should be around $10^{-8}$ $m^2$. The value found here is in the order of $10^{-10}$ $m^2$. The value for the permeability constant found from experimental data is in the order of $10^{-18}$ $m^2$. This value is much too low. It appears that the right value for the permeability constant cannot be determined with the experimental data.
Chapter 7

Conclusion

Some advice can be given for the design of an experimental setup. In this report it has been shown that changing $U_0$ will have a larger effect on OPL than changing $C_0$. The oxygen consumption factor, $\Omega$, is a characteristic value for the type of cells used in experiments. Therefore this parameter can not be changed. However, it is necessary to know it’s exact value, because it’s influence on the OPL is large, especially for values lower than 4, which has been used as reference value in this report.

Influence of inlet velocity, initial concentration of oxygen and oxygen consumption factor on the penetration of oxygen have been tested. Results show a linear effect for initial oxygen concentration and inlet velocity on the oxygen penetration length. The oxygen consumption factor has an inverse effect on the Oxygen Penetration Length, OPL.

The results come from a model that shows the influence of the parameters described above on the penetration of oxygen through a pore in a porous medium. From the model with dimensions; a dimensionless formulation has been derived, making it possible to combine all equations together in one model. Although results for the dimensionless model and the model with dimensions are not exactly the same, they are in the same range of magnitude and show exactly the same tendencies. The model can be used for future work on the subject.

With a pressure test on the scaffold, a Young’s modulus of 39.6 MPa has been found. This value is in the order of values found in earlier research. ([Zein-2002]) However, due to limited amount of experimental data, no significance can be given to the value found in this report. Besides, it can be concluded that the permeability constant, $K$, can not be calculated from experimental data in this report.
Bibliography


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Appendix A

Oxygen penetration on different lines

For the determination of the oxygen concentration through the pore, was made use of the value along 1 line as shown in the Figure A.1 below. The results in section 4.2 show values along line 1, and a critical concentration of 0.1 $mol/m^3$ in the dimensionless model. In this appendix, the effect of choosing another line or another critical concentration is evaluated. Therefore line 2 is defined as shown in the Figure A.1. A second critical concentration is chosen at 1 $mol/m^3$.

![Figure A.1: Definition of "line 1" and "line 2"](image)

Figure A.2 shows the results for a changing initial oxygen concentration. Of course, for a higher critical concentration the OPL will be smaller. Along line 2, concentrations are higher as along line 1. The same effects appear in Figures A.3 and A.4, for respectively a changing inlet velocity and OCF.

For both the graphs of initial concentration and initial velocity a linear trend-line is drawn between the data-points.

It is obvious that both the choice of a critical concentration and a line on which to determine concentration have their effect on the oxygen penetration length. Oxygen concentration will be the highest on line two, because there the maximum value of the velocity appears, as can be seen in Figure 4.10 in section 4.2. As a cause of convection, the highest value for oxygen concentration will appear at the highest velocity. Therefore, OPL will be larger on line two than on line one. OPL is defined as the length at which a critical concentration of oxygen occurs. Changing this concentration will directly affect the value for the OPL. Choosing a higher critical concentration will therefore decrease the value for OPL.
APPENDIX A. OXYGEN PENETRATION ON DIFFERENT LINES

Figure A.2: Relation between initial oxygen concentration and OPL

Figure A.3: Relation between inlet velocity and OPL
Figure A.4: Relation between OCF and OPL