Perfusion-enhanced growth of tissue-engineered cartilage in a bioreactor: A finite element study

Martijn Cox

Supervisors: dr. ir. Cees Oomens
             ir. Bram Sengers
Date: 30-12-2002
Abstract

Experiments on tissue-engineering cartilage in a static bioreactor often show an inhomogeneous matrix-production. The method described in this article is aimed at answering the question if it is possible to enhance matrix-production by perfusion through the cartilage-construct. Goal is to investigate the possibilities of different kinds of flow-histories on the increase and homogenization of extracellular matrix (production) in a bioreactor for tissue-engineered cartilage. The method to be used is a Finite Element Model using biphasic mechanics, advective and diffusive solute transport, uptake, biosynthesis and changing mechanical properties of the tissue-engineered construct. The key idea is that matrix production depends on local oxygen-levels and concentration of (an arbitrary) growth factor. The diffusion speed of oxygen is high compared to the speed of transport by perfusion. Oxygen-transport is therefore thought to be little influenced by perfusion. However, applying a flow to the bio-construct can control the transport of the bigger growth-factor molecules, where diffusion takes place very slow.

Results show the importance of flow in enhancing matrix-production, and the positive effect of alternating flow on creating a more homogeneous matrix. Experiment are proposed for qualitative determination of the importance of oxygen or growth factor in matrix growth. Further experiments are needed to evaluate and validate the numerical method as proposed in this article.
Introduction

The research described in this article forms part of IMBIOTOR, a European Commission project in which several European universities and companies have combined their forces to develop an intelligent bioreactor for the production of tissue-engineered cartilage. One of the major difficulties in tissue-engineering cartilage in static bioreactors is inhomogeneous matrix-production (Martin et al., 1999; Freed and Vunjak-Novakovic, 2000; Martin et al., 2000). Peripheral cartilage growth exceeds that of the inner layers. Peripheral circumstances for matrix-production are ideal, with high concentrations of oxygen and growth factors, which are assumed to be the most important factors for matrix production (Obradovic et al., 2000; Bailón-Plaza et al., 2001; Blunk et al., 2002; Pei et al., 2002). Peripheral uptake and utilization of oxygen and growth factors leads to low concentrations in deeper layers of the bioconstruct. This is likely to result in inhomogeneous matrix-production. Because higher matrix-production leads to lower permeability this process is self-enforcing. The more matrix-growth in outer regions, the less permeability of the bioconstruct, which leads to even lower concentrations in deeper layers.

The question is if it is possible to increase inner concentrations of growth factor by applying a pressure gradient, which will lead to flow through the bioreactor. The relatively slow transport of growth-factor molecules (e.g. IGF-I, TGF-β) will be influenced by this flow. Diffusion of oxygen is relatively fast; therefore oxygen concentrations will differ only slightly from the situation without flow.

This article aims at investigating the possibilities of different kinds of flow-histories to increase and homogenize extracellular matrix (-production) in a bioreactor for tissue-engineered cartilage.
The Finite Element Model used in this article is that proposed by Sengers (submitted). This model provides a numerical framework for tissue engineering, which can be used for modeling many different kinds of aspects of the process of tissue engineering of cartilage. The modeling approach described here relates mechanical loading to solute transport and uptake. This leads to biosynthesis of extra cellular matrix, which leads to changing mechanical properties (stiffness and permeability) of the cartilage construct. Growth factor concentrations in deeper regions are thought to be increased by flow due to mechanical loading. Different kinds of load-histories are compared to the situation without flow.

Possible negative side effect of applying a load to the cartilage tissue is the compression of the pores in the medium, which may lead to a great decrease in permeability of the construct. In the second problem, to better illustrate the possible effects of compression on the permeability an extension is made to the model, using a more realistic permeability law.

Results show that, under modeling assumptions, flow does increase and homogenize matrix production. An experiment is proposed to determine the relative importance of oxygen and growth factor in matrix production. Further experiments are needed to validate and evaluate the results of the method as described here.

Values and descriptions of constants used in this article are summarized in table 1.
Table 1: Values of constants used

<table>
<thead>
<tr>
<th>Constant</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_{O_2}$</td>
<td>0.25</td>
<td>Relative anaerobic matrix production</td>
</tr>
<tr>
<td>$C_f$</td>
<td>$8.1 \times 10^{12}$ N m$^{-4}$</td>
<td>Isotropic friction coefficient</td>
</tr>
<tr>
<td>$c_{GF_{sat}}$</td>
<td>1 M</td>
<td>External concentration of growth factor</td>
</tr>
<tr>
<td>$c_{O_2_{sat}}$</td>
<td>$2 \times 10^{-10}$ mol mm$^{-3}$</td>
<td>External concentration of oxygen</td>
</tr>
<tr>
<td>$Cm_1$</td>
<td>Variable</td>
<td>Constant for growth factor dependent matrix production</td>
</tr>
<tr>
<td>$Cm_2$</td>
<td>Variable</td>
<td>Constant for oxygen dependent matrix production</td>
</tr>
<tr>
<td>$Cm_{O_2}$</td>
<td>$2 \times 10^{-10}$ mol mm$^{-3}$</td>
<td>Michaelis-Menten constant oxygen</td>
</tr>
<tr>
<td>$Cm_{GF}$</td>
<td>0.1 M</td>
<td>Michaelis-Menten constant growth factor</td>
</tr>
<tr>
<td>$D_{GF}$</td>
<td>$5 \times 10^{-8}$ cm$^2$ s$^{-1}$</td>
<td>Growth factor diffusion constant</td>
</tr>
<tr>
<td>$D_{O_2}$</td>
<td>$1.5 \times 10^{-5}$ cm$^2$ s$^{-1}$</td>
<td>Oxygen diffusion constant</td>
</tr>
<tr>
<td>$G_0$</td>
<td>4.5 kPa</td>
<td>Shear modulus at $t = 0$</td>
</tr>
<tr>
<td>$K_{max_{O_2}}$</td>
<td>$10^{12}$ s$^{-1}$</td>
<td>Max. rate of oxygen consumption</td>
</tr>
<tr>
<td>$K_{max_{GF}}$</td>
<td>$10^{-3}$ s$^{-1}$</td>
<td>Max. rate of growth factor consumption</td>
</tr>
<tr>
<td>$M$</td>
<td>8.0</td>
<td>Deformation-dependent permeability constant</td>
</tr>
<tr>
<td>$n_0^f$</td>
<td>0.97</td>
<td>Fluid volume fraction at $t = 0$</td>
</tr>
<tr>
<td>$n_{max}^s$</td>
<td>0.30</td>
<td>Max. solid volume fraction</td>
</tr>
<tr>
<td>$dn_{max}^s/dt$</td>
<td>$4 \times 10^{-7}$ s$^{-1}$</td>
<td>Max. rate of matrix production</td>
</tr>
<tr>
<td>$P$</td>
<td>$1 \times 10^{-3}$ N mm$^{-2}$</td>
<td>External hydrostatic pressure</td>
</tr>
<tr>
<td>$\kappa_0$</td>
<td>4.2 kPa</td>
<td>Bulk modulus at $t = 0$</td>
</tr>
</tbody>
</table>
Methods

Mathematical model

Fig. 1 shows a schematic representation of the model. The fluid saturated scaffold is modeled by using biphasic mixture mechanics. The computed velocity field is used to calculate the local concentration of oxygen and growth factor due to advection and diffusion. The volume fraction of cells and scaffold material is assumed to be constant and equal to zero. Therefore, the solid volume fraction can be assumed to be essentially equal to the volume fraction of the extra cellular matrix. This solid volume fraction is chosen to be 0.03 at the start of the simulation, which corresponds to a porosity of 97% for a PGA scaffold (Freed and Vunjak-Novakovic, 1998).

Matrix production depends only on growth factor and oxygen concentrations and is calculated using operator splitting. More matrix leads to higher stiffness and less permeability, which will be used in the biphasic mechanics part.

A detailed description of the conservation laws and constitutive equations is found in Sengers (submitted). Summarizing the biphasic mixture is described by the following set of coupled equations:

\begin{align}
\vec{v} \cdot \sigma + \nabla p &= 0, \\
K^{-1} \cdot \varepsilon + \nabla p &= 0,
\end{align}

\textit{figure 1: A schematic representation of the model}
\[ \nabla \cdot \mathbf{v}^f + \nabla \cdot \mathbf{v} = 0, \quad (3) \]
\[ \mathbf{v}^\beta - \mathbf{v}^f = -\frac{K}{n^f} \cdot \nabla p - \frac{D}{c^\beta} \cdot \nabla c^\beta \quad (4) \]

in which \( \sigma^e \) is the effective Cauchy stress tensor for the solid matrix, \( p \) the hydrostatic pore pressure, while \( \mathbf{v}^f \) is the velocity of the solid phase. \( n^f \) represents the fluid volume fraction. We start our simulations with \( n_0^f = 0.97 \) (Freed and Vunjak-Novakovic, 1998). The specific discharge is defined as 
\[ q = n^f (\mathbf{v}^f - \mathbf{v}^s). \]
\( K \) is the permeability tensor.

The relative velocity of a solute \( \beta \) (oxygen or growth factor) with respect to the solid matrix is given by (4), while \( D \) denotes the diffusion tensor, and \( c^\beta \) the concentration of solute \( \beta \). The diffusion constant of oxygen is set to \( D_{O2} = 1.5 \cdot 10^{-5} \text{ cm}^2 \text{ s}^{-1} \) (Obradovic et al., 2000; Haselgrove et al., 1993). The growth factor diffusion constant is equal to \( D_{GF} = 5 \cdot 10^{-8} \text{ cm}^2 \text{ s}^{-1} \), which is in the same order of magnitude as the diffusion constant of growth factor TGF-\( \beta \) as used by Bailón-Plaza et al., 2001.

The right-hand side of equation (4) exists of a perfusion part and a diffusion part. In oxygen transport, the diffusion part is dominant. Growth factor transport is mainly determined by the perfusion part.

The uptake of oxygen by the cells is described by Michaelis-Menten kinetics:

\[ \frac{dc^{O2}}{dt} = -\frac{K_{max,O2} \cdot c^{O2}}{C_{m,O2} + c^{O2}} \quad (5) \]

in which \( \frac{dc^{O2}}{dt} \) represents the oxygen uptake rate of the cells in \( \text{mol mm}^{-3} \text{ s}^{-1} \). \( K_{max,O2} \) is the maximum rate of oxygen consumption and \( C_{m,O2} \) the Michaelis-
Menten constant for O₂. \( K_{max, O_2} \) and \( C_{m, O_2} \) are chosen in such a way that the oxygen concentration profile along the depth of the bioreactor is approximately similar to that of the model by Obradovic et al., 2000. \( K_{max, O_2} \) equals \( 10^{-12} \text{ s}^{-1} \), \( C_{m, O_2} \) equals \( 2 \cdot 10^{-16} \text{ mol mm}^{-3} \). The disappearance of growth factor is a much more difficult process. Growth factor might disappear due to utilization or uptake, but also by biodegradation. In this case focus will not be on the exact process of growth factor disappearance, therefore we assume that this process can be described in a similar way as the uptake of oxygen:

\[
\frac{dc^{GF}}{dt} = -\frac{K_{max, GF} \cdot c^{GF}}{C_{m, GF} + c^{GF}}
\]

\( \frac{dc^{GF}}{dt} \) denotes the uptake, usage or degradation rate of growth factor. \( K_{max, GF} \) and \( C_{m, GF} \) are chosen in such a way that the time constant for uptake of growth factor is in the same order of magnitude as the time constant for transport of growth factor. \( K_{max, GF} \) is \( 10^{-3} \text{ s}^{-1} \), \( C_{m, GF} \) is 0.1 M.

Matrix production is assumed to depend on both oxygen and growth factor local concentrations (Obradovic et al., 2000; Bailón-Plaza et al., 2001; Blunk et al., 2002; Pei et al., 2002). In absence of oxygen, still matrix production (or degradation) takes place (Lee et al., 1997). For large concentrations of oxygen and/or growth factor, matrix production reaches a maximum level. Due to before-mentioned restrictions, matrix production represents an increase in solid volume fraction, \( n^s \). This results in:

\[
\frac{dn^s}{dt} = \frac{dn^s_{max}}{dt} \left[ k_1 \cdot \left( \frac{c^{GF}}{C_{m, GF} + c^{GF}} \cdot \left( \frac{c^{O_2}}{C_{m, O_2} + c^{O_2}} + anO_2 \right) \right) \right] - n^s
\]
in which $\frac{dn^s}{dt}$ is the rate of matrix growth or degradation (s$^{-1}$), $\frac{dn_{max}^s}{dt}$ is the maximum rate of matrix production, set to $4 \cdot 10^{-7}$ s$^{-1}$. $Cm_1$ and $Cm_2$ are constants that determine the growth factor and oxygen dependent part of matrix production, respectively. They are varied during the simulations. The relative contribution of anaerobic matrix production is represented by $anO_2$, it is assumed to be equal to 0.25 (Lee et al., 1997). As can be seen in (eq. 7), a decrease in growth factor and/or oxygen may lead to matrix degradation. $k_1$ finally is a constant that limits the maximum amount of matrix in the tissue-engineered construct. $k_1$ is defined by:

$$k_1 = \frac{n_{max}^s}{Cm_1 + c^{GF_{out}} + \left(1 - anO_2\right) \cdot \frac{c^{O_2_{out}}}{Cm_2 + c^{O_2_{out}} + anO_2}}$$

in which $n_{max}^s$ is the maximum relative amount of matrix, i.e. the maximum solid volume fraction. $c^{GF_{out}}$ and $c^{O_2_{out}}$ denote the concentrations of oxygen and growth factor in the surrounding solution. $c^{GF_{out}}$ is chosen equal to 1M, because it is an arbitrary growth factor. $c^{O_2_{out}}$ is equal to $2 \cdot 10^{-10}$ mol mm$^{-3}$, (Sengers, submitted; Obradovic et al., 2000).

For $c^{GF} = c^{GF_{out}}$ and $c^{O_2} = c^{O_2_{out}}$ equation (7) reduces to:

$$\frac{dn^s}{dt} = \frac{dn_{max}^s}{dt} \cdot \left[n_{max}^s - n^s\right]$$

The maximum solid volume fraction is chosen to be 0.30, which corresponds with a fluid volume fraction of 60% to 85% in natural cartilage (Hasler et al., 1999).

The matrix is assumed to be a compressible hyperelastic neo-Hookean material. The effective stress tensor is given by:

$$\sigma^e = \kappa(J-1)I + \frac{G}{J} \left(F \cdot F^e - J^2 I \right),$$

where $I$ is the identity matrix, $J$ is the determinant of $F$, $F^e$ is the right Cauchy-Green deformation tensor, and $\kappa$ and $G$ are the bulk and shear modulus, respectively.
where $\kappa$ is the bulk modulus and $G$ the shear modulus. $F$ is the deformation tensor and $J = \det(F)$.

The bulk and shear moduli are assumed to be constant for the matrix fraction. For the whole mixture they are assumed to be proportional with the solid volume fraction:

$$\kappa = \frac{n^s}{n^0_s} \cdot \kappa_0$$

(11)

$$G = \frac{n^s}{n^0_s} \cdot G_0$$

(12)

In which $\kappa$ and $G$ are the bulk and shear moduli of the mixture. Based on the order of magnitude of the equilibrium modulus found in confined compression for three days old tissue-engineered cartilage constructs [4], and a Poisson’s ratio of $\nu = 0.1$ (Sengers, submitted; Wong, 2000), the resulting moduli at $t = 0$ are $\kappa_0 = 4.2$ kPa and $G_0 = 4.5$ kPa.
Numerical implementation

Oxygen and growth factor transport and uptake are relatively fast processes, which set to an equilibrium in less than an hour. To be able to simulate these processes a numerical model needs to use small time steps (minutes). Matrix production, on the other hand, is a process of days and weeks. It would take very many small time steps to see any difference in matrix production at all. Therefore we work with two different time scales. First, an equilibrium is reached for the transport of oxygen and growth factor on a small time scale ($\Delta t = 1$ min). Equilibrium is assumed to be reached when the following inequality holds for every $z$:

$$\frac{c^\beta(z,t)}{c^\beta(z,t-\Delta t)} - 1 < 0.01$$  \hfill (13)

in which $c^\beta(z,t)$ is either oxygen or growth factor concentration at position $z$ and time $t$, $\Delta t$ denotes a small time step. From eq. (13) we can see that equilibrium is reached when relative change in solute concentration during one small time step is less then 1\% everywhere in the medium.

After equilibrium is reached a large timestep is applied ($\Delta t = 400.000 \text{ s} \approx 4.6$ days) in which matrix is formed. Then again oxygen and growth factor transport takes place until steady state and so on. This way it is possible to simulate the fast transport processes as well as the slow matrix production in a reasonable simulation time.
Geometry

The bioreactor is modeled as a disc with a radius of 10 mm and a height of 3.0 mm. The disc is surrounded by a fluid with high concentrations of oxygen and growth factor. In this article only a 1D-problem is considered for a small bar in the center of the disc, as shown in figure 2. The surrounding fluid is assumed to be well mixed, which leads to constant concentrations of oxygen and growth factor at both ends of the bar. The problem is solved using the SEPRAN finite element package (Segal, 2000). A three-dimensional 27-node brick element with quadratic interpolation functions is used. For a more detailed description see Sengers (submitted).

![Geometry of the modeled bioreactor](image)

For the first problem, the permeability tensor $K$ is defined as (Sengers, submitted; Huyghe and Janssen, 1997; Almeida and Spilker, 1997):

$$K = \left(\frac{n}{f}\right)^2 C_F^{-1},$$  \hspace{1cm} (14)

in which $C_F$ is the friction tensor between the solid and the fluid phase. The isotropic friction coefficient $C_F = 8.1 \cdot 10^{12}$ N s m$^{-4}$, which results from before-mentioned shear and bulk moduli and the order of magnitude of permeability found in confined compression for three days old tissue-engineered cartilage constructs (Martin et al., 2000).

All simulations are done with different combinations of $Cm_1$ and $Cm_2$ (eq. 7). $Cm_1$ and $Cm_2$ are first small compared to the outer concentrations of oxygen and
growth factor ($Cm_1 = 0.01 \cdot c^{GF_{ext}}$ and $Cm_2 = 0.01 \cdot c^{O_{2_{ext}}}$). Small variations in oxygen and growth factor concentration have little influence on matrix production. After that we consider a situation where $Cm_1 = c^{GF_{ext}}$. In this case small decreases in growth factor levels lead to fast decrease of matrix production. After that, the same is done for oxygen. Reality is expected to be somewhere between these extreme situations.

In the first simulation the right side of the bar (figure 2) is fixed in space, while the left side of the bar is loaded with an external hydrostatic pressure of $1 \cdot 10^{-3}$ N mm$^{-2}$. The pressure is chosen to keep deformations small (<10%). There is only flow from left to right.

The second simulation uses alternating flow. In the first large time step, load is applied on the left side of the bar. In the second large time step, load is applied on the right side. For modeling convenience, the right side is still fixed in space. Flow is moving from right to left now, until equilibrium is reached. After that, matrix production is calculated and flow is alternated again.

Experimental results show that permeability decreases rapidly, even when fluid volume fractions decrease only slightly (Martin et al., 2000; Freed et al., 1998). Therefore in the second problem the permeability law (eq 13) is extended with a deformation-dependent part (Lai et al., 1981):

$$K = J^M \cdot \left(\frac{\text{n}^f}{\text{n}^f + 1}\right)^2 \mathbf{C}_F^{-I},$$

in which $J$ is the determinant of the deformation tensor $F$.

Literature values for $M$ vary between 5 and 10 (Chen et al., 2001; Holmes et al., 1985; Holmes and Mow, 1990). In this case $M$ is set to 8. $Cm_1$ and $Cm_2$ are varied in the second problem just like in the first.
The number of elements used varies between 40 and 80. To avoid numerical oscillations in growth factor concentrations at the right edge of the bar, a strong bias is necessary. In case of alternating flow, a double bias is used.
Results

Generally after approximately 30 to 75 minutes, the solute transport reaches an equilibrium. An example of thus obtained oxygen and growth factor concentration profiles along the depth of the bio-construct is shown in figure 3.

In figure 4, matrix volume fractions along the depth of the bioreactor are shown in deformed state for different combinations of flows and $Cm_1$ and $Cm_2$. Each line corresponds with a time step of approximately 4.6 days. Matrix growth is simulated for 46 days.

Perfusion from left to right results in a relatively homogeneous matrix volume fraction profile when $Cm_1$ and $Cm_2$ are low, except for a sharp dip around a depth of approximately 2 mm. When growth factor concentration limits matrix production ($Cm_1$ is high) this profile is much more skew, which resembles the growth factor concentration profile in figure 3. With oxygen as limiting factor the matrix concentration profile is almost symmetric, just like the oxygen concentration in figure 3. With alternating flow, profiles become much more symmetric and homogeneous. When there is no flow, results show only peripheral matrix production.
As shown in figures 5 and 6, the second problem, using deformation-dependent permeability leads to less matrix production. Difference in matrix volume fraction is best seen at the right edge of the bioconstruct. When $Cm_1 = c^{GFe}$ the differences between simple permeability and deformation-dependent permeability are most obvious. Matrix volume fraction profiles are shown in figure 5. Difference in matrix growth is caused by a difference in relative fluid velocity $v^f - v^s$, as shown in figure 6. The relative fluid velocity is much lower for deformation-dependent permeability when $Cm_1 = c^{GFe}$. 

**Figure 4:** Solid volume fraction profiles along the depth of the bioreactor for different combinations of $Cm_1$ and $Cm_2$ and flow histories. Each line corresponds with a time step of 4.6 days.
**Figure 5:** Solid volume fractions after 46 days with different combinations of $Cm_1$ and $Cm_2$ for simple permeability (solid line), and deformation-dependent permeability (dotted line).

**Figure 6:** Relative fluid velocity profiles after 46 days with different combinations of $Cm_1$ and $Cm_2$ for simple permeability (solid line), and deformation-dependent permeability (dotted line).
Discussion

The objective of this finite element study was to investigate the possibilities of perfusion in enhancing matrix growth in tissue-engineered cartilage.

We hypothesized that perfusion would lead to higher concentrations of growth factor molecules in deeper regions of the cartilage construct, which would result in more matrix growth.

Results indicate that, under modeling assumptions, perfusion indeed helps to stimulate matrix growth. Oxygen concentration profiles are almost symmetric, which indicates that oxygen transport mainly takes place by diffusion. Growth factor concentration profiles are more oblique; growth factor transport is mainly caused by the pressure gradient in the bioreactor.

Without perfusion, only peripheral matrix growth takes place, which is in accordance to data found in literature (Martin et al., 1999; Freed and Vunjak-Novakovic, 1998, Martin et al., 2000). Perfusion increases matrix growth in deeper layers. When matrix production is limited by oxygen concentration the profile is nearly symmetrical; growth factor as limiting factor leads to a more oblique profile.

With this information, a perfusion experiment will show the relative importance of oxygen and growth factor in matrix growth. A symmetric matrix profile indicates oxygen as most important factor, an oblique profile is a result of growth factor as main limiter of matrix growth.

When flow is alternated, resulting matrix volume fraction profiles are much more homogeneous, especially when matrix growth is limited by growth factor. In this case flow is alternated with a frequency of 4.6 days. It might be interesting to simulate matrix growth as a function of the alternating flow frequency, in order to find an optimum.
In the second problem focus has been on the permeability of the cartilage construct. Results show that growth factor concentration significantly decreases when a deformation-dependent permeability law is used. Relative fluid velocity is lower, while disappearance rates of growth factor stay the same. When matrix growth is limited by growth factor, the difference in relative fluid velocity is most obvious. This is caused by the asymmetric matrix profile, which leads to differences in local strain along the depth of the bioreactor. High local strains lead to low local permeability and therefore lower relative fluid velocity.

Experimental data show a large decrease in permeability for relatively small changes in fluid volume fractions, even without deformation, which can not be explained by the permeability laws as proposed in this study (Martin et al., 2000; Freed et al., 1998). More experimental data are needed on local permeability in a bioreactor to be able to implement a more realistic permeability law.

Virtually no quantitative experimental data can be found on matrix and solute concentrations along the depth of a bioreactor, except for Obradovic et al. (2000), in which experimental data on oxygen concentration profiles are obtained. Therefore in this study only some extreme situations are shown. Experiments should be done to obtain local matrix volume fractions at different stages in the tissue-engineering process to validate the model proposed here.

In future, in vitro measurement of oxygen and growth factor concentration profiles should be possible. When these results are combined with the model this will provide more insight in the complex processes of tissue-engineering cartilage.
References


Hasler, E.M., Herzog, W., Wu, J.Z., Muller, W. and Wyss, U., 1999. Articular cartilage biomechanics: Theoretical models, material properties, and
biosynthetic response. Critical reviews in Biomedical Engineering 27(6), 415-488.


