

Mathematical Modeling of Extracellular Matrix Accumulation and Nutrient Diffusion in Hydrogel for a Cartilage Repair Problem

Graduate Student Mathematical Modeling Camp

Rensselaer Polytechnic Institute, June 2006.

- **Mentor :** Dr. Mansoor Haider, North Carolina State University

- **Student Group :**

Aisha Alwehebi , Mississippi State University

Aranya Chakraborty, Rensselaer Polytechnic Institute

Yiming Cheng, New Jersey Institute of Technology

Michael Franklin, Claremont Graduate University

Tom Kiehl, Rensselaer Polytechnic Institute

Noam Zeev, University of Delaware

1 Introduction

In this report we describe a mathematical modeling procedure for the biological phenomenon of extracellular matrix (ECM) accumulation and nutrient diffusion in hydrogel solution for a cartilage repair problem. Articular cartilage is the hydrated biological soft tissue that lines surfaces of bones in joints such as the knee, shoulder and hip. Cartilage contains no blood vessels or nerve endings but is populated with cells (chondrocytes) that maintain the extracellular matrix by regulating their metabolic activity in response to the local extracellular environment. However, depending on circumstances, cartilage may lose its structural integrity and, ultimately, the result may be complete tissue degradation with painful bone-on-bone contact necessitating joint replacement. Osteoarthritis is one such condition in which cartilage can exhibit holes called osteochondral defects that, in theory, could be filled with biocompatible materials that facilitate restoration of the tissue's structural integrity. A conceptual model for this process can be considered as a cell surrounded by an accumulating layer of ECM in a bed of hydrogel. Nutrients diffuse through the hydrogel and ECM layers and enter the cell to enhance production of ECM constituents, thereby enabling the tissue to regain its lost stiffness.

The main objective of this modeling study is to mathematically investigate the physical behavior of cell-seeded hydrogels, and the capacity for this procedure to synthesize materials with similar properties to endogenous cartilage. Examples of targeted material properties include stiffness, porosity and hydraulic permeability. Since the material properties are highly dependent on features of the synthesis procedure such as hydrogel concentration, nutrient supply and initial cell seeding density, mathematical modeling of this integrated complex biological phenomenon is a challenging task.

In this report, we address this complex problem by proposing both a dynamic ODE model and a spatially varying PDE model for an important component of the cartilage repair process. We then apply these models to simulate the effects of the different model parameters on the response of the variables. The report is organized as follows : in section 2 we describe the dynamic model and a possible model bifurcation observed for some values of the initial hydrogel concentration; we present the simulation results and comment on them thereafter. In section 3 we modify the model by incorporating effects of spatial variations of the different concentration variables, and propose a PDE model; we employ this model in simulations and comment on its behavior as compared to the dynamic model. Section 4 concludes the report.

2 Dynamic Model

To develop an ordinary differential equation model for the matrix accumulation and nutrient diffusion phenomena, we first define the dynamic variables as

$$\begin{aligned} M(t) &= \text{Concentration of ECM (mg/mL)} \\ N(t) &= \text{Concentration of Nutrients in the cell (moles/mL)} \\ H(t) &= \text{Concentration of hydrogel (mg/mL)}, \end{aligned}$$

and make the following assumptions :

- Cell seeding density is constant.
- External supply of nutrients is unlimited.
- Cell needs a minimum level of nutrient concentration to survive.
- The hydrogel layer eventually attains a saturation level that inhibits nutrient supply to the cell.
- The hydrogel is more diffusive than the Extracellular Matrix (ECM).
- The hydrogel gets replaced by accumulating ECM via a chemical reaction.

Based on the above assumptions we propose the following state variable model :

$$\dot{M} = c_1 (N - N_c) - d_m \tag{1}$$

$$\dot{N} = c_2 (M^* - M) + c_3 H(H_s - H) \tag{2}$$

$$\dot{H} = -c_4 H M \tag{3}$$

with initial conditions $M(0) = 0$, $N(0) = N_0 > N_c$, $H(0) = H_0$, where $c_1, c_2, c_3, c_4 > 0$ are constants. The new parameters appearing on the RHS of (1), (2) and (3) are defined as follows :

1. d_m is a constant decay rate for ECM due to inherent matrix degradation
2. N_c is the minimum nutrient concentration required for survival of the cell
3. M^* is the target level of ECM
4. H_s is the saturating hydrogel concentration, beyond which the dense hydrogel bed starts impeding the diffusion of nutrients into the cell.

The formulation of the different expressions in the above model was motivated by a qualitative analysis of the relations between the defined state variables. Equation (1) indicates that an increase in nutrient concentration over the critical nutrient level required for matrix production enhances ECM aggregation while, simultaneously, the matrix slowly degrades at a constant rate. Equation (2) captures the phenomenon that initially, when the matrix concentration is low, nutrients can diffuse rapidly into the cell, but as M approaches a critical value M^* the diffusion becomes slower. Also, in a manner that depends on whether the initial hydrogel concentration is less than or more than a critical saturating level, matrix accumulation is either enhanced or impeded, as modeled by the second term in (2). Equation (3) is a mass action equation for the chemical reaction between ECM and hydrogel by which the latter gets replaced gradually.

2.1 Non-dimesionalisation of the ODE model

We next non-dimensionalise the dynamic model in (1), (2) and (3), by defining the following normalized variables:

$$\bar{M} = \frac{M}{M^*} \quad \bar{N} = \frac{N}{N_c} \quad \bar{H} = \frac{H}{H_s} \quad \bar{t} = \frac{t}{t_w} \quad (4)$$

and the concentration ratios

$$\mu_1 = u_N \frac{N_c}{M^*} \quad \mu_2 = \frac{H_s}{u_N N_c} \quad \mu_3 = \frac{M^*}{H_s} \quad (5)$$

where u_N is the molecular weight of the nutrients and t_w is taken as a week. We also define the time constants associated with the different interactions between the variables in the system as follows : τ_1 is the time constant for matrix accumulation due to nutrient diffusion, τ_2 and τ_3 are time constants corresponding to the slowing down of nutrient diffusion due to increasing matrix density and hydrogel saturation, respectively, τ_4 is a time constant for the mass action reaction, while τ_d is a time scale for ECM decay. Typical values of the concentration ratios and the time constants used for simulation purposes can be found in the Appendix. In terms of the defined normalized variables and time constants, the ODE model in (1), (2) and (3) can be written as

$$\begin{aligned}
\dot{\bar{M}} &= \frac{\mu_1}{\tau_1} (\bar{N} - 1) - \frac{1}{\tau_d} \\
\dot{\bar{N}} &= \frac{1}{\mu_1 \tau_2} (1 - \bar{M}) + \frac{\mu_2^2}{\tau_3} \bar{H} (1 - \bar{H}) \\
\dot{\bar{H}} &= -\frac{\mu_3}{\tau_4} \bar{H} \bar{M}
\end{aligned} \tag{6}$$

with initial conditions $\bar{M}(0) = 0$, $\bar{N}(0) > 1$ and $\bar{H}(0) = \frac{H_0}{H_s}$.

The physical meaning of the constants c_1, c_2, c_3, c_4 in (1), (2) and (3) can now be interpreted in terms of the concentration ratios and time constants from the above normalized model. Moreover, the dependence of the solution of (6) on the parameter $\bar{H}(0)$ is of primary interest since it allows us to analyze how the rates of matrix accumulation and nutrient diffusion change for dense and dilute concentrations of hydrogel at the beginning of the repair process.

In the following sections we describe simulations based on the dynamic model (6) that were performed in Matlab.

2.2 Model Bifurcation due to Initial hydrogel Concentration

Since accurate numerical values for the different parameters in the model (6) are not available, representative values based on qualitative estimates of time scales in the repair process were chosen for the purpose of simulation. For one chosen set of parameter values, the simulations demonstrated an interesting mathematical feature. The dynamic model exhibits a bifurcation as the parameter $\bar{H}(0)$, the initial dimensionless hydrogel concentration is varied, with all the other parameters being fixed to some nominal values. For example, for a fixed set of values of the time constants and the concentration ratios, the phase plane diagrams for the state variables \bar{N} and \bar{M} for two different values of initial hydrogel concentration, are shown in Figures 1(a) and 1(b). The results clearly indicate that there exists some critical value for this bifurcation parameter $\bar{H}(0)$ depending on the other parameters of the system, at which instability sets in for the model (6).

The reason behind this instability can be easily investigated by analyzing the slope function for $\bar{N}(t)$ in (6) for small values of time t . Since at time $t = 0$, we have $\bar{M}(0) = 0$, the slope function at initial times reduces to a quadratic form in $\bar{H}(0)$ and the solution of this quadratic equation determines the critical value for the bifurcation parameter at which the model becomes unstable. This can be easily confirmed by linearising the model around some close neighborhood of the origin and varying $\bar{H}(0)$ so that the eigenvalues shift from the open left half plane to the unstable regime. Solving the aforementioned quadratic equation with respect to $\bar{H}(0)$, the critical value for initial hydrogel concentration can be derived as,

$$H_c = \frac{1}{2} + \frac{1}{2} \sqrt{1 + \frac{4\tau_3}{\mu_1 \mu_2^2 \tau_2}} \tag{7}$$

It may be noted that the critical bifurcation parameter value is a function of the dimensionless parameters τ_2, τ_3, μ_1 and μ_2 .

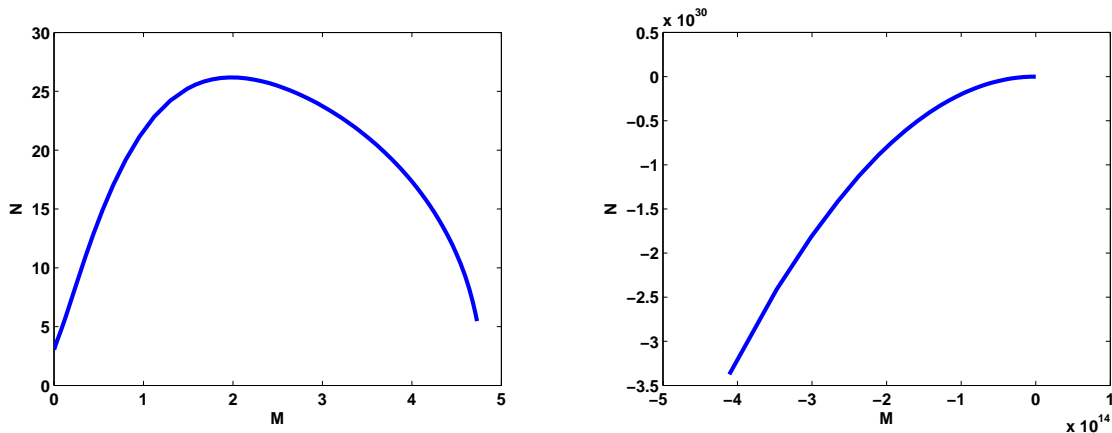


Figure 1: Model bifurcation due to changes in initial hydrogel concentration with respect to a critical concentration

2.3 Simulation Results for the Dynamic Model

Choosing the parameters γ , β and ϵ (the mathematical definitions of these parameters have been stated in the Appendix) to satisfy $\gamma\beta\epsilon = 20$ which fixes \bar{H}_c to a value greater than 1.1, we vary $\bar{H}(0)$ from 0.9 to 1.1 (which indicates that the initial hydrogel concentration is on the stable side of the bifurcation), and simulate the dynamic model (6) for these two values of $\bar{H}(0)$. The simulation results are shown in Figure 2.

The results can be seen to mimic the associated physical process. When the initial hydrogel concentration is less than the saturating value, nutrient diffusion is enhanced and hence the initial rate of increase of nutrient concentration inside the cell is high, as can be seen from the second figure on the left hand column in Figure 2. As nutrient diffusion into the cell increases, ECM accumulates around the cell at a fast rate (first figure on the left column), but after a certain point in time ECM accumulation starts impeding the diffusion of nutrients, as evident from the first figure. As expected, the response for hydrogel concentration is opposite of that for matrix accumulation, and is shown in the third figure of the left hand column. Consistent results are also obtained for the second case where we consider the initial hydrogel concentration to be greater than the saturating value. Since in this case, the process begins on a dense bed of hydrogel which impedes the diffusion of nutrients into the cell, nutrient concentration takes a considerable amount of time to increase. As a result matrix production gets delayed significantly, as can be seen from the first figure on the right hand column. hydrogel replacement, in turn, takes place slowly and eventually the hydrogel concentration settles down to an equilibrium value as the process gets shut down when N reaches N_c , the minimum concentration of nutrients required by the cell to survive.

Thus far, we have considered an ODE model for the repair process by ignoring spatial variations of nutrients in the matrix and hydrogel layers that surround the cells. In the next section, we consider an alternative PDE model to take into account these spatial variations.

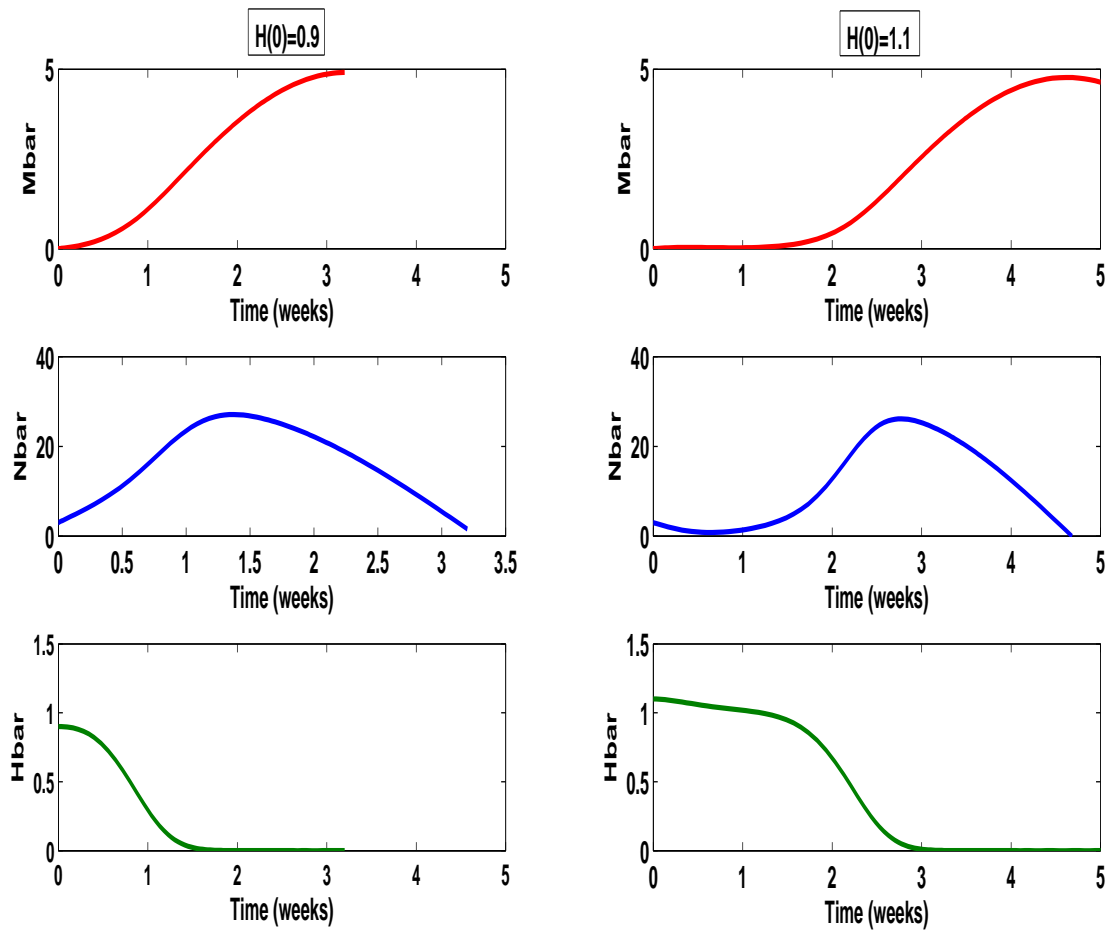


Figure 2: State responses for two different initial hydrogel concentrations

3 Justification of PDE Model

The ODE model of extracellular matrix (ECM) production gives insight into the physical phenomena occurring inside the evolving hydrogel-tissue construct. However, spatial effects are left out of the model, thus potentially limiting its capability in accurately describing the system. For example, the ODE model describes the concentration of the quantities $H(t)$ (hydrogel), $N(t)$ (nutrients) and $M(t)$ (ECM), but neglects any diffusion of nutrients through the ECM. This is a crucial aspect to the ECM production because as the ECM grows around the cell, it limits the amount of nutrients able to nourish the cell. The ODE model also cannot take into account the differences in diffusion coefficients in between the cell and ECM. Because of these features, a PDE model is proposed which can take into account these spatially varying characteristics of the system.

3.1 The PDE Model

The primary governing equation of diffusion comes from Fick's equation, which states

$$\frac{\partial U}{\partial t} = D(t)\nabla^2 U,$$

where the variable $U = U(x, t)$ is a concentration and $D(t)$ is a time-dependent diffusion coefficient. In this case, we use spherical coordinates for the quantities of interest (ie $N = N(r, t)$) where we assume no angular dependence. Figure 3 shows the spatial model of the cell with ECM growing radially around it. In the PDE model, the most important quantity governing the ECM production is the amount of nutrients reaching the cell, given as $N_1(r, t)$. More specifically we are only concerned about the amount of nutrients reaching the boundary of the cell, $N_1(a, t)$ since this quantity regulates the ECM production. In order to capture this mathematically in the model, we describe the time rate of change of the outer ECM radius $b(t)$ as having a direct dependence on $N_1(a, t)$.

The amount of nutrients within the ECM is given similarly as $N_2(r, t)$, which has a value of N_H (the hydrogel nutrient concentration) at the outer radius $b(t)$. The diffusion coefficients of the cell and ECM are given by D_1 and $D_2(t)$, respectively. Note that D_1 is independent of time, which indicates that the cell's ability to transport nutrients is assumed constant, whereas the diffusion coefficient in the ECM is taken as a decreasing function of time due to matrix accumulation. The mathematical model describing the system is given by

$$\frac{\partial N_1}{\partial t} = D_1 \left(\frac{\partial^2 N_1}{\partial r^2} + \frac{2}{r} \frac{\partial N_1}{\partial r} \right), \quad (8)$$

$$\frac{\partial N_2}{\partial t} = D_2(t) \left(\frac{\partial^2 N_2}{\partial r^2} + \frac{2}{r} \frac{\partial N_2}{\partial r} \right), \quad (9)$$

$$\frac{db}{dt} = \alpha(N_1(a, t) - N_c) \quad (10)$$

with boundary conditions given by

$$N_1(a, t) = N_2(a, t), \quad (11)$$

$$D_1 \frac{\partial}{\partial t} N_1(a, t) = D_2(t) \frac{\partial}{\partial t} N_2(a, t), \quad (12)$$

$$N_1(0, t) > 0, \quad (13)$$

$$N_2(b(t), t) = N_H, \quad (14)$$

AND initial conditions given by

$$N_1(r, 0) = N_0 > N_c, \quad (15)$$

$$N_2(r, 0) = N_H, \quad (16)$$

$$b(0) = a. \quad (17)$$

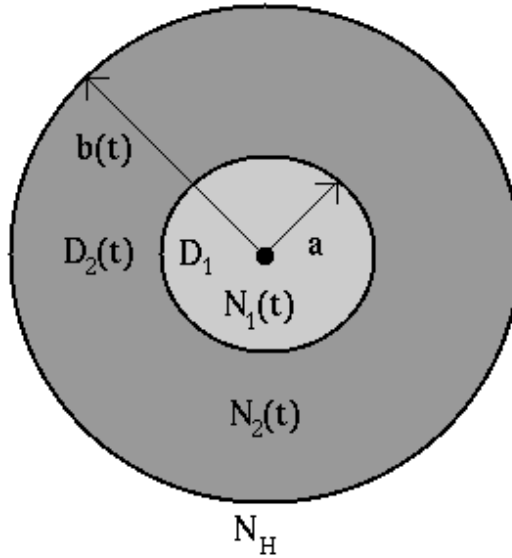


Figure 3: Illustration of the cell (lighter region) with ECM growth around it (darker region). Outside the ECM is the hydrogel.

The first two boundary conditions enforce continuity of nutrient concentration and continuity of nutrient flux across the cell/ECM boundary, respectively. It is possible to quantitatively describe the ECM diffusion coefficient $D_2(t)$ as any time-varying, monotone decreasing function, since we expect the diffusion coefficient to decrease as ECM thickness increases. For example, a linear relationship as in Figure 4 can be given by

$$D_2(t) = \frac{D_\star - D_H}{R - a}(b(t) - a) + D_H,$$

where $b(t) = R$ is some expected value of the outer radius with respective expected diffusion D_\star , and D_H is the maximum diffusion rate corresponding to ECM with zero thickness (ie $D_2(0) = D_H$ when $b(0) = a$).

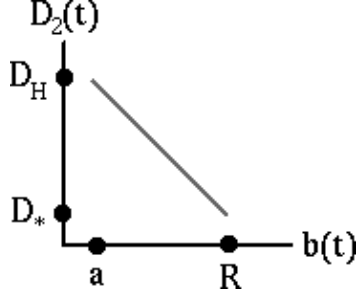


Figure 4: Linear relationship for decreasing ECM diffusion coefficient, $D_2(t)$.

3.2 Numerical Results for the PDE model

This PDE model for ECM production is solved numerically using finite differences in space and time. The independent variables are discretized as $r_m = m \cdot \Delta r$ for $m = 0, \dots, M$ and $t_n = n \cdot \Delta t$ for $n = 0, \dots, N$, and the dependent variables are approximated as, $N_i(r_m, t_n) \equiv N_{i,m}^n$ for $i = 1, 2$. The scheme is *implicit* and uses $\mathcal{O}(\Delta r^2)$ central differences in space, and $\mathcal{O}(\Delta t)$ backward differences in time.

The update schemes for N_1 and N_2 using the central space, backwards time difference approximations become

$$\begin{aligned} -(\lambda_1 - \lambda_{2,m})N_{1,m-1}^n + (1 + 2\lambda_1)N_{1,m}^n - (\lambda_1 + \lambda_{2,m})N_{1,m+1}^n &= N_{1,m}^{n-1}, \\ -(\gamma_1^n - \gamma_{2,m}^n)N_{2,m-1}^n + (1 + 2\gamma_1^n)N_{2,m}^n - (\gamma_1^n + \gamma_{2,m}^n)N_{2,m+1}^n &= N_{2,m}^{n-1}, \end{aligned}$$

where

$$\lambda_1 = \frac{D_1 \Delta t}{\Delta r^2} \quad , \quad \lambda_{2,m} = \frac{D_1 \Delta t}{r_m \Delta r} \quad , \quad \gamma_1^n = \frac{D_2^n \Delta t}{\Delta r^2} \quad , \quad \gamma_{2,m}^n = \frac{D_2^n \Delta t}{r_m \Delta r}.$$

This scheme yields a tridiagonal system to be solved at each iteration, and is unconditionally stable since it is implicit. The update scheme for the ECM radius $b(t)$ is given by

$$b_m^n = b_m^{n-1} + \alpha \Delta t (N_{1,M}^n - N_c),$$

where $N_{1,M}^n$ is the value of the nutrient concentration at the cell/ECM boundary at time step n .

The results of the numerical scheme are shown in Figure 5. These results show the behavior of the system when the diffusion coefficient of the ECM, $D_2(t)$ is assumed to be constant. The expected behavior of the system is captured in the plots. As time increases, we expect the nutrient concentration reaching the cell to decrease. The top plot shows that the cell's nutrient uptake is largest at $t = 0$ and decreases rapidly toward the critical value of the cell, N_c (shown as a dotted line in the plot). When N_1 is highest, the rate at which the ECM is produced is highest, as captured in the bottom plot. As the cellular nutrients decrease and approach N_c , the ECM continues to be produced, thus the outer radius $b(t)$ continues to grow, but at a slower rate.

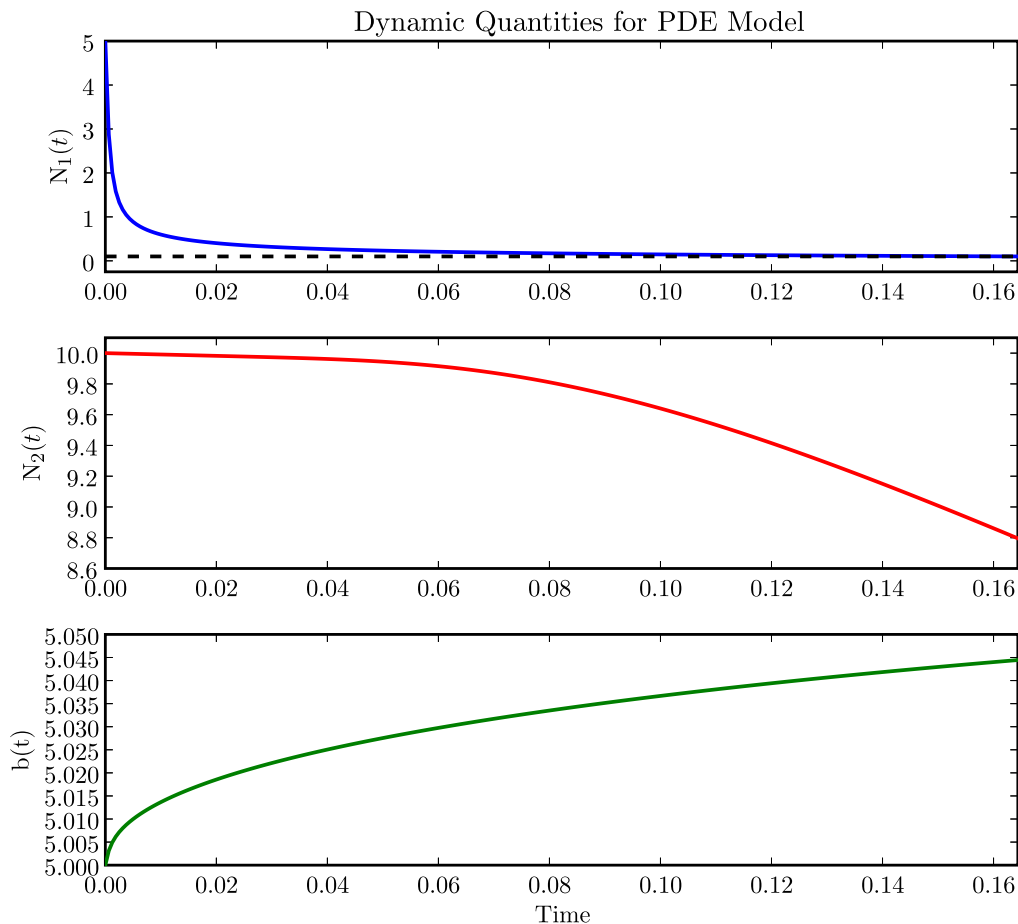


Figure 5: Results of numerical scheme for $N_1(t)$, $N_2(t)$ and $b(t)$.

4 Conclusion

This report documents mathematical modeling of the accumulation of extracellular matrix, diffusion of nutrients into the cell and replacement of hydrogel in a cell-gel construct which serves as a model system for the cartilage repair problem. We have developed ODE and PDE models incorporating spatial effects of moving interface on nutrient diffusion due to accumulating ECM, to model the complex coupled relationships between the primary parameters and variables of interest in experimental applications. The simulation results demonstrate the inhibitory effects of high hydrogel concentration on nutrient diffusion and ECM synthesis and are, hence, consistent with the experimentally observed physical phenomena. Some possible future research directions in this context would be to add more biologically relevant models of hydrogel depletion, improve the numerical structures of the PDE methods, further non-dimensionalization of PDE to avoid scaling issues, and to take into account a reaction-diffusion PDE model to represent the physical processes with greater physical accuracy.

5 References

1. McHale MK, Setton LA and Chilkoti A, Synthesis and in vitro evaluation of enzymatically cross-linked elastin-like polypeptide gels for cartilaginous tissue repair, *Tissue Engineering*, 11(11-12), 1768-1779, Nov 2005.
2. Nettles DL, Vail TP, Morgan MT, Grinstaff MW and Setton LA, Photocrosslinkable hyaluronan as a scaffold for articular cartilage repair, *Annals of Biomedical Engineering*, 32(3): 391-397, Mar 2005.

6 Appendix

6.1 Parameter values chosen for simulation

The different parameters for the dynamic model were chosen as follows :

Time constants :

$$\begin{aligned}\tau_1 &= \tau_3 = \tau_4 = 1 \\ \tau_2 &= \gamma \tau_1 \\ \tau_d &= \delta \tau_1\end{aligned}$$

Concentration ratios :

$$\mu_1 = \epsilon \quad \mu_2^2 = \beta \tau_3 \quad \mu_3 = \alpha \tau_4$$

where $\epsilon = 0.1$, $\gamma = 2$, $\delta = \beta = 100$, $\alpha = 3$.

6.2 Code for solving the PDE model

The following script was written in Python 2.4.

Listing 1: Script written to solve PDE model of ECM production.

```
import math as m
import Numeric as N
import pylab as p

def Npde():
    """Michael Franklin RPI GSMCM 2006
    Cartilage repair problem"""

    # parameters in model.
    NH=10.0
```

```

NC=NH/100.0

# diffusion paramters.
D1=5          # [m**2/sec.]
#D2=3*D1     # static diffusion.
Dstar=1.0    # dynamic diffusion.
Dhydr=5.0
alph=1       # value in b(t) equation.

# inner and outer radius of cell.
Nca=0
Ncb=5

# inner and outer radius of ECM.
Nma=Ncb
Nmb=10

# spatial and time steps.
hr=(0.01)*Ncb
ht=(1.0/4)*(hr**2)
tFinal=5
tnodes=int(tFinal/ht)+1

# number of nodes in each region.
L=int((Ncb-Nca)/hr)

# spatial nodes in each region.
Rc=N.arange(Nca,Ncb+hr,hr)
Rm=N.arange(Nma,Nmb+hr,hr)

# time dependent radius b(t) in ECM.
bm=N.zeros(tnodes,typecode=N.Float64)
bm[0]=Nma

# initializing concentration fields Nc(r,t), Nm(r,t).
Nc=N.zeros((tnodes,L),typecode=N.Float64)
Nm=N.zeros((tnodes,L),typecode=N.Float64)

# initial concentration fields Nc(r,t), Nm(r,t) in each region.
frac=0.5
Nc[0,:]=N.array([frac*NH for k in range(L)],typecode=N.Float64)
Nm[0,:]=N.array([NH for k in range(L)],typecode=N.Float64)

# boundary conditions.
Nm[:,0:L]=N.array([NH for k in range(L)],typecode=N.Float64)

# initial time.
t0=0.0
time=N.zeros(tnodes,typecode=N.Float64)
time[0]=t0
num=int(tnodes)
n=1

while n<num:
    time[n]=n*ht

```

```

# lambda values in FD scheme for Nc.
lamb1c=(D1*ht)/(hr**2)
lamb2c=N.array([(ht*D1)/(Rc[k]*hr) for k in range(1,L-1)],
               typecode=N.Float64)

# constructing tridiagonal matrix for Nc update.
Ac=p.diag(N.array([(1+2.0*lamb1c) for k in range(1,L-1)],
                 typecode=N.Float64))
Ac=Ac+p.diag(N.array([-lamb1c-lamb2c[k] for k in range(1,L-2)],
                    typecode=N.Float64),-1)
Ac=Ac+p.diag(N.array([-lamb1c+lamb2c[k] for k in range(1,L-2)],
                    typecode=N.Float64),1)

# inverting Nc update matrix.
Acinv=p.inverse(Ac)
# FTCS update scheme.
Nc[n,1:L-1]=N.dot(Acinv,Nc[n-1,1:L-1])
# smoothness condition at r=0.
Nc[n,0]=Nc[n,1]

# update scheme for time-varying ECM radius.
bm[n]=bm[n-1]+alph*ht*Nc[n-1,L-1]

# dynamic diffusion in ECM.
D2=((Dstar-Dhydr)/(Nmb-Nma))*(bm[n]-Nma)+Dhydr
Nc[n,L-1]=Nm[n,0]=Nc[n,L-2]+(D2/D1)*(Nm[n,L-1]-Nm[n,L-2])

# lambda values in FD scheme for Nm.
lamb1m=(D2*ht)/(hr**2)
lamb2m=N.array([(ht*D2)/(Rm[k]*hr) for k in range(1,L-1)],
               typecode=N.Float64)

# constructing tridiagonal matrix for Nm update.
Am=p.diag(N.array([(1+2.0*lamb1m) for k in range(1,L-1)],
                 typecode=N.Float64))
Am=Am+p.diag(N.array([-lamb1m-lamb2m[k] for k in range(1,L-2)],
                    typecode=N.Float64),-1)
Am=Am+p.diag(N.array([-lamb1m+lamb2m[k] for k in range(1,L-2)],
                    typecode=N.Float64),1)

# inverting Nm update matrix.
Aminv=p.inverse(Am)
# FTCS update scheme.
Nm[n,1:L-1]=N.dot(Aminv,Nm[n-1,1:L-1])

if Nc[n,L-1]<NC:
    Nmax=n
    n=num
    # plotting of quantities.
    p.figure()
    p.subplot(311),p.plot(time[0:Nmax],Nc[0:Nmax,L-1],'b-')
    p.plot(time[0:Nmax],N.array([NC for k in range(Nmax)]),'k--')
    p.ylabel(r' $N_{1}(t)$ ')
    p.axis([0, time[Nmax-1],-0.25,5])
    p.title(r'Dynamic Quantities for PDE Model')

```

```
p.subplot(312),p.plot(time[0:Nmax],Nm[0:Nmax,int(L/2.0)],'r-')
p.ylabel(r' $N_{2}(t)$ ')
p.axis([0, time[Nmax-1],8.6,10.1])
p.subplot(313),p.plot(time[0:Nmax],bm[0:Nmax],'g-')
p.xlabel('Time'), p.ylabel(r' $b(t)$ ')
p.axis([0, time[Nmax-1],5,5.05])
p.show()
return
Nmax=n
n=n+1
return
```