Turning Gels into Cartilage: Modeling Tissue Regeneration in Cell-Seeded Scaffolds

Anh Minh Bui Boi∗ Chen Zhang† Haoyan Chi‡ Kuan Xu§
Luyen Nguyen¶ Mauricio Osorio∥ Nick Benes∗∗ Nicholas Gewecke††

Mentor: Mansoor Haider‡‡

1 Introduction

Articular cartilage is the hydrated biological soft tissue that lines the surfaces of diarthrodial joints such as the knee, shoulder and hip. The most common cause of cartilage degradation is osteoarthritis, which is associated with aging, and can ultimately lead to painful bone-on-bone contact necessitating joint replacement. Tissue engineering strategies for cartilage regeneration or repair can potentially improve the quality of life for millions of people with tissue damage due to osteoarthritis.

One method which shows promise in regenerating or repairing cartilage is the seeding of biocompatible, nutrient-rich hydrogels with cartilage cells. The cell can then absorb and utilize the nutrients to synthesize and release proteins that constitute the tissue’s extracellular matrix, which is the primary structural component of cartilage. For example, such cell seeded scaffolds could be used to repair cartilage by injection or grafting of a cell-seeded hydrogel into a damaged cartilage site. Ideally, over a period of time, the cell-seeded hydrogel will transform into a cartilage-like biomaterial that integrates well with the surrounding native tissue.

In developing a model for evolution of a cell-seeded scaffold, a primary quantity of interest is the regeneration time required to synthesize a specified volume of new cartilage extracellular matrix (ECM), and how this time depends on the key underlying factors in the model. This project focused on one aspect of the tissue regeneration process related to modeling cell-mediated ECM regeneration in the local environment of a single cartilage cell embedded in a hydrogel scaffold.

We formulate a mathematical model of the process based on a system of reaction diffusion equations and associated boundary and interface conditions. Two different approaches for analysis of this model are then considered based on asymptotic analysis and numerical solution techniques.

∗SUNY Buffalo  †SUNY Buffalo  ‡SUNY Buffalo  §New Jersey Institute of Technology  ¶University of Delaware  ∥University of Cincinnati  ∗∗Boston University  ††University of Tennessee, Knoxville  ‡‡North Carolina State University
for the governing partial differential equations. Two numerical approaches are considered: a semi-
discrete model, where only the time variable is discretized, and a finite difference model, where both
time and space are discretized. A brief discussion of results based on model solutions and possible
extensions is also presented.

2 Mathematical Model

Our mathematical model focuses on simulating accumulation of the ECM around a single cell that
is seeded in a hydrogel (Fig. 1). Initially, nutrients diffuse into the cell which, in turn, utilizes these
nutrients to synthesize proteins for ECM formation. As these proteins diffuse out of the cell and
come into contact with the surrounding hydrogel, a reaction occurs and it is assumed that linked
ECM is formed. As linked ECM accumulates, diffusive transport of the nutrients and unlinked
proteins is altered. To simplify the analysis, we focus on a one-dimensional Cartesian system in
which the cell occupies the domain $0 < x < a$ and the ECM, once it forms, occupies the domain
$a < x < b(t)$. We do not explicitly model the hydrogel region. Instead, it is assumed that cell-
synthesized proteins created linked ECM as they come into contact with the hydrogel, and this
phenomenon is modeled via an evolution equation along the boundary $x = b(t)$. It is also assumed
that there is an abundant supply of hydrogel and nutrients in the region external to the domain of
the model.

![Figure 1: Illustration of cartilage regeneration in the local environment of a single cell in a cell-
seeded hydrogel scaffold. (a) At $t = 0^-$, the cell (beige) is surrounded by a nutrient-rich hydrogel
(blue). (b) At $t = 0^+$, the cell utilizes nutrients (red) that it absorbs to synthesize extracellular
matrix (ECM) proteins (green) which diffuse out of the cell. (c) For $t > 0$ the matrix proteins react
with the hydrogel to produce linked ECM (purple) with an advancing ECM-gel interface. For the
purposes of the workshop, the system was idealized to have a one-dimensional Cartesian geometry.

All transport of matrix proteins and nutrients is assumed to occur, exclusively, due to diffusion.
Diffusivities in the cell and ECM regions are assumed to be constant, but distinct diffusivities are
considered for the nutrients, which are small solutes, and the matrix proteins, which are larger
solutes based on values in [1-2]. The transformation of unlinked matrix proteins into linked ECM
is idealized as a reaction along the boundary $x = b(t)$ that causes the location of the boundary to
advance in proportion to the concentration of matrix proteins that are, instantaneously, present at
$x = b(t)$. 

2
In our model, \(N(x, t)\) denotes the concentration of nutrients and \(M(x, t)\) denotes the concentration of unlinked matrix proteins present in the system. \(D^N_i\) and \(D^M_i\) denote the diffusivities of the nutrients and unlinked matrix proteins, respectively, with \(i = 1\) referring to the cellular region \((0 < x < a)\) and \(i = 2\) referring to the extracellular region \((a < x < b(t))\). \(k_N\) denotes the rate at which the cell absorbs or utilizes nutrients, and \(k_M\) denotes the rate at which the cell synthesizes unlinked matrix proteins as a result of nutrient absorption. We note that, at \(t = 0\), the ECM domain has not yet formed so that \(b(0^+) = a\). One of the challenges of developing solutions for this model is ensuring accurate computation of the first few time steps, where the ECM domain \((a < x < b(t))\) first forms, and this is the primary focus of this study.

Based on the assumptions described above, the governing equations for our model consist of a set of reaction-diffusion equations inside the cell:

\[
\frac{\partial N}{\partial t} = D^N_1 \Delta N - k_N(N - N^*), \quad 0 < x < a, t > 0
\]

\[
\frac{\partial M}{\partial t} = D^M_1 \Delta M + k_M(N - N^*),
\]

and a set of diffusion equations inside the ECM:

\[
\frac{\partial N}{\partial t} = D^N_2 \Delta N, \quad a < x < b(t), t > 0
\]

\[
\frac{\partial M}{\partial t} = D^M_2 \Delta M
\]

where \(N^*\) is the homeostatic nutrient level in the cell. At \(x = 0\), we assume a no flux condition on \(N\) and \(M\), so that:

\[
\frac{\partial N}{\partial n} = \frac{\partial M}{\partial n} = 0.
\]

Along the interface \(x = a\), continuity of concentrations and diffusive fluxes is enforced:

\[
[M]^+_{a} = [N]^+_{a} = 0
\]

\[
(D^N_2 \nabla N|_{a^+} - D^N_1 \nabla N|_{a^-}) \cdot \mathbf{n} = 0
\]

\[
(D^M_2 \nabla M|_{a^+} - D^M_1 \nabla M|_{a^-}) \cdot \mathbf{n} = 0
\]

Along the interface \(x = b(t)\), the nutrient level is assumed to match the nutrient concentration of the hydrogel \((N_H > N^*)\) and a no-flux condition on unlinked matrix proteins is enforced:

\[
N = N_H
\]

\[
\frac{\partial M}{\partial n} = 0
\]

Linking of the matrix proteins as they diffuse through ECM and come into contact with the hydrogel is modeled via the following interface evolution equation along \(x = b(t)\):

\[
\frac{db}{dt} = k_b M(b(t), t), \quad t > 0
\]

Finally, the following set of initial conditions is also enforced:

\[
b(0) = a, \quad N(x, 0) = N^*, \quad M(x, 0) = 0
\]

In the following three sections, we describe different solution methods to analyze the behavior of this system in the early time regime of its evolution. In particular, \(9\) is a nonlinear equation for the evolution of the position of the ECM-hydrogel boundary, and our focus is on its accurate evaluation for early times.
3 Asymptotic Solution

For convenience in the non-dimensionalization to follow, we re-write the model with the dimensional independent variables taken as \(x'\) and \(t'\). For \(0 < x' < a, t' > 0\), the reaction-diffusion equations in the cell are:

\[
\frac{\partial N}{\partial t'} = D^{(1)}_N \frac{\partial^2 N}{\partial x'^2} - k_N (N - N_*) \tag{11}
\]

\[
\frac{\partial M}{\partial t'} = D^{(1)}_M \frac{\partial^2 M}{\partial x'^2} + k_M (N - N_*) \tag{12}
\]

For \(a < x' < b(t'), t' > 0\), the diffusion equations in the ECM are:

\[
\frac{\partial N}{\partial t'} = D^{(2)}_N \frac{\partial^2 N}{\partial x'^2} \tag{13}
\]

\[
\frac{\partial M}{\partial t'} = D^{(2)}_M \frac{\partial^2 M}{\partial x'^2} \tag{14}
\]

The set consisting of all boundary and interface conditions can be summarized as:

\[
\frac{\partial N}{\partial x'}(0, t') = 0 \tag{15}
\]

\[
\frac{\partial M}{\partial x'}(0, t') = 0 \tag{16}
\]

\[
\frac{\partial M}{\partial x'}(b(t'), t') = 0 \tag{17}
\]

\[
N(b(t'), t') = N_H \tag{18}
\]

\[
M(a^+, t') = M(a^-, t') \tag{19}
\]

\[
N(a^+, t') = N(a^-, t') \tag{20}
\]

\[
D^{(2)}_N \frac{\partial N}{\partial x'}(a^+, t') = D^{(1)}_N \frac{\partial N}{\partial x'}(a^-, t') \tag{21}
\]

\[
D^{(2)}_M \frac{\partial M}{\partial x'}(a^+, t') = D^{(1)}_M \frac{\partial M}{\partial x'}(a^-, t') \tag{22}
\]

and the interface evolution equation is:

\[
\frac{db}{dt'} = k_b M(b(t'), t') \tag{23}
\]

The initial conditions are:

\[
N(x', 0) = N_* \tag{24}
\]

\[
M(x', 0) = 0 \tag{25}
\]

\[
b(0) = a \tag{26}
\]

We now non-dimensionalize (11-26) via the transformations:

\[
u = \frac{N - N_*}{N_H - N_*}, \quad v = \frac{M}{N_H - N_*}, \quad t = \frac{t'}{a^2/D^{(1)}_N}, \quad x = \frac{x'}{a}, \quad s = \frac{b}{a} \tag{27}
\]
The governing equations (11-12) then become, for $0 < x < 1, t > 0:\n\frac{\partial u}{\partial t} = \frac{\partial^2 u}{\partial x^2} - \frac{a^2 k_N}{D_N^{(1)}} u \tag{28}\\n\frac{\partial v}{\partial t} = \frac{D_M^{(1)} \partial^2 v}{D_N^{(1)} \partial x^2} + \frac{a^2 k_N}{D_N^{(1)}} u \tag{29}\\n
Similarly, the governing equations (13-14) become, for $1 < x < s(t), t > 0:\n\frac{\partial u}{\partial t} = \frac{D_N^{(2)} \partial^2 u}{D_N^{(1)} \partial x^2} \tag{30}\\n\frac{\partial v}{\partial t} = \frac{D_M^{(2)} \partial^2 v}{D_N^{(1)} \partial x^2} \tag{31}\\n
The boundary and interface conditions (15-22) transform to:
\n\frac{\partial u}{\partial x}(0, t) = 0 \tag{32}\\n\frac{\partial v}{\partial x}(0, t) = 0 \tag{33}\\n\frac{\partial v}{\partial x}(s(t), t) = 0 \tag{34}\\nu(s(t), t) = 1 \tag{35}\\nv(1^+, t) = v(1^-, t) \tag{36}\\nu(1^+, t) = u(1^-, t) \tag{37}\\n\frac{\partial v}{\partial x}(1^+, t) = \frac{D_M^{(1)}}{D_M^{(2)}} \frac{\partial v}{\partial x}(1^-, t) \tag{38}\\n\frac{\partial u}{\partial x}(1^+, t) = \frac{D_N^{(1)}}{D_N^{(2)}} \frac{\partial u}{\partial x}(1^-, t) \tag{39}\\n
and the interface evolution equation becomes:
\n\frac{ds}{dt} = \frac{k_b a}{D_N^{(1)}} (N_H - N^*) v(s(t), t) \tag{40}\\n
The initial conditions (24-26) transform to:
\nu(x, 0) = 1 \tag{41}\\nv(x, 0) = 0 \tag{42}\\ns(0) = 1 \tag{43}\\n
Introducing a new set of constants:
\gamma = \frac{a^2 k_N}{D_N^{(1)}}, \ \delta = \frac{k_b a (N_H - N^*)}{D_N^{(1)}}, \ \epsilon \beta = \frac{D_N^{(2)}}{D_N^{(1)}}, \ \epsilon = \frac{D_M^{(2)}}{D_M^{(1)}}, \ \alpha = \frac{D_M^{(1)}}{D_N^{(1)}} \tag{44}
equations (28-29) can be concisely written as, for $0 < x < 1, t > 0$:

\[
\frac{\partial u}{\partial t} = \frac{\partial^2 u}{\partial x^2} - \gamma u \quad (45)
\]
\[
\frac{\partial v}{\partial t} = \alpha \frac{\partial^2 v}{\partial x^2} + \gamma u \quad (46)
\]

Similarly, equations (30-31) can be concisely written as, for $1 < x < s(t), t > 0$:

\[
\frac{\partial u}{\partial t} = \epsilon \beta \frac{\partial^2 u}{\partial x^2} \quad (47)
\]
\[
\frac{\partial v}{\partial t} = \epsilon \frac{\partial^2 v}{\partial x^2} \quad (48)
\]

The boundary and interface conditions (32-39) become:

\[
\frac{\partial u}{\partial x}(0, t) = 0 \quad (49)
\]
\[
\frac{\partial v}{\partial x}(0, t) = 0 \quad (50)
\]
\[
\frac{\partial v}{\partial x}(s(t), t) = 0 \quad (51)
\]
\[
u(s(t), t) = 1 \quad (52)
\]
\[
u(1^+, t) = v(1^-, t) \quad (53)
\]
\[
u(1^+, t) = u(1^-, t) \quad (54)
\]
\[
\epsilon \frac{\partial v}{\partial x}(1^+, t) = \frac{\partial v}{\partial x}(1^-, t) \quad (55)
\]
\[
\epsilon \beta \frac{\partial u}{\partial x}(1^+, t) = \frac{\partial u}{\partial x}(1^-, t) \quad (56)
\]

and the interface evolution equation (40) can be written as:

\[
\frac{ds}{dt} = \delta v(s(t), t) \quad (57)
\]

For completeness, the initial conditions (41-43) are re-written below:

\[
\begin{align*}
u(x, 0) &= 1 \quad (58) \\
v(x, 0) &= 0 \quad (59) \\
s(0) &= 1 \quad (60)
\end{align*}
\]

Our asymptotic solution is based on the assumption that diffusion of matrix proteins in the ECM region will be much slower than diffusion of matrix proteins in the cell. Consequently, the non-dimensional parameter $\epsilon$ in (44) is taken as a small parameter, i.e. $\epsilon << 1$, and an asymptotic solution is developed in the limit $\epsilon \to 0$. Representing the interface location $s(t)$ via an asymptotic expansion of the form:

\[
s(t) \sim 1 + \epsilon s_0(t) + ..., \quad \text{as } \epsilon \to 0 \quad (61)
\]

the following derivation provides an asymptotic solution for $s(t)$. 

6
First, substitute (61) into (57) and use (60) to obtain:

\[
\frac{ds}{dt} = \delta v(s(t), t) \Rightarrow \epsilon s_0'(t) = \delta v(1 + \epsilon s_0(t), t) \Rightarrow \epsilon s_0'(t) = \delta v(1, t) + \delta \epsilon s_0(t)v_x(1, t) + \ldots
\]  

(62)

Since it is expected that \( \delta << 1 \), we can balance terms to obtain the relation:

\[
s_0'(t) = \frac{\delta}{\epsilon} v(1, t)
\]

(63)

For \( t << 1 \), we can differentiate (58-59) with respect to \( x \) and substitute into (45-46) to obtain the approximate solutions on \( 0 < x < 1 \):

\[
\frac{\partial u}{\partial t} = -\gamma u \Rightarrow u(t) \sim e^{-\gamma t}
\]

(64)

\[
\frac{\partial v}{\partial t} = \gamma u \Rightarrow v(t) \sim 1 - e^{\gamma t}
\]

(65)

Substituting (65) into (63), we obtain:

\[
s_0'(t) = \frac{\delta}{\epsilon} (1 - e^{-\gamma t})
\]

(66)

subject to the initial condition (from (60-61)):

\[
s_0(0) = 0
\]

(67)

The solution of (66-67) is given by:

\[
s_0(t) = \frac{\delta}{\epsilon} \left( t + \frac{1}{\gamma} (e^{-\gamma t} - 1) \right)
\]

(68)

Substituting (68) into (61) gives an asymptotic solution for \( s(t) \) as:

\[
s(t) \sim 1 + \delta t + \frac{\delta}{\gamma} (e^{-\gamma t} - 1)
\]

(69)

which for, \( t << 1 \), can be further approximated as:

\[
s(t) \sim 1 + \frac{\delta \gamma t^2}{2}
\]

(70)

Returning to the original variables, (70) yields an asymptotic solution for the early-time evolution of the ECM-hydrogel interface location \( b(t) \), using (27) and (44), as:

\[
\Rightarrow b(t) \sim a + \frac{k_b k_N (N_H - N*) t^2}{2}
\]

(71)
4 Semi-Discrete Solution

In one numerical implementation of our 1-dimensional reaction-diffusion model, we considered a semi-discrete approach in which time was discretized but the spatial variable remained continuous. We noted that by replacing

\[ \frac{\partial X}{\partial t} \text{ with } \frac{X^n - X^{n-1}}{\Delta t}, \]

where \( X \in \{M, N\} \), and the superscript denotes the discrete time step, we could reduce the problem to an iterative sequence of ODE systems which could be solved exactly at each iteration. Specifically, we obtained the following discrete versions of the governing equations (1-2) and the equation (9) for evolution of the ECM-gel interface:

\[
\begin{align*}
\frac{d^2 N^n}{dx^2} - \alpha_{N}^1 N^n &= -\beta_{N}^1 N^{n-1} - \gamma_{N}^1 N, & 0 < x < a \\
\frac{d^2 M^n}{dx^2} - \alpha_{M}^1 M^n &= -\beta_{M}^1 M^{n-1} - \gamma_{M}^1 (N^n - N), & 0 < x < a \\
\frac{d^2 N^n}{dx^2} - \alpha_{N}^2 N^n &= -\beta_{N}^2 N^{n-1}, & a < x < b^n \\
\frac{d^2 M^n}{dx^2} - \alpha_{M}^2 M^n &= -\beta_{M}^2 M^{n-1}, & a < x < b^n \\
b^n &= b^{n-1} + (k_b \Delta t) M^n(b^{n-1}),
\end{align*}
\]

where:

\[
\begin{align*}
\alpha_{N}^1 &= \frac{1+k_1^N \Delta t}{D_N^1 \Delta t}, & \beta_{N}^1 &= \frac{1}{D_N^1 \Delta t}, & \gamma_{N}^1 &= \frac{k_1^N}{D_N^1}, \\
\alpha_{M}^1 &= \frac{1}{D_M^1 \Delta t}, & \beta_{M}^1 &= \frac{1}{D_M^1 \Delta t}, & \gamma_{M}^1 &= \frac{k_1^M}{D_M^1}, \\
\alpha_{N}^2 &= \frac{1}{D_N^2 \Delta t}, & \beta_{N}^2 &= \frac{1}{D_N^2 \Delta t}, \\
\alpha_{M}^2 &= \frac{1}{D_M^2 \Delta t}, & \beta_{M}^2 &= \frac{1}{D_M^2 \Delta t},
\end{align*}
\]

The initial conditions (10) are:

\[
\begin{align*}
N^0 &= N, & 0 < x < a = b^0 \\
M^0 &= 0, & 0 < x < a = b^0,
\end{align*}
\]

and the boundary conditions (4-8) become:

\[
\begin{align*}
\frac{dN^n}{dx} &= \frac{dM^n}{dx} = 0 & \text{at } x = 0 \\
[[N^n]] &= [[M^n]] = 0 & \text{at } x = a \\
[[D_N^n \frac{dN^n}{dx}]] &= [[D_M^n \frac{dM^n}{dx}]] = 0 & \text{at } x = a \\
N^n &= N_H & \text{at } x = b^n \\
\frac{dM^n}{dx} &= 0 & \text{at } x = b^n,
\end{align*}
\]

where

\[
[[f(x)]] = \left( \lim_{\xi \to x^-} f(\xi) \right) - \left( \lim_{\xi \to x^+} f(\xi) \right).
\]

Note that for \( n = 0 \), there is ambiguity in the boundary conditions since \( a = b^0 \); in that case, we use the boundary conditions for \( x = b^n \) rather than for \( x = a \).
We employed the following scheme (which we describe below) to solve the system (72-78) at each time step \( t = n \Delta t \):

\[
\begin{align*}
(72) & \quad (74) , \\
\downarrow & \quad \downarrow \\
(73) & \quad (75) \\
\downarrow & \quad \downarrow \\
(76)
\end{align*}
\]

where the numbers refer to the equations from the system of governing ODEs (72-76).

We will now describe this diagram in more detail. Suppose that \( b^{n-1} \) is known, as are \( N^{n-1} \) and \( M^{n-1} \) on the interval \([0, b^{n-1}]\). Then (72) is a non-homogeneous second-order ODE. The corresponding homogeneous equation, \((N^n)'' - \alpha_N N^n = 0\), has solutions \(e^{\pm \sqrt{\alpha_N} x}\). Using variation of parameters, we get

\[
N^n(x) = \frac{\gamma_N}{\alpha_N} N_* + e^{\sqrt{\alpha_N} x} \left( C_1 - \frac{\beta_N}{2\sqrt{\alpha_N}} \int e^{-\sqrt{\alpha_N} \xi} N^{n-1}(\xi) \, d\xi \right) + e^{-\sqrt{\alpha_N} x} \left( C_2 + \frac{\beta_N}{2\sqrt{\alpha_N}} \int e^{\sqrt{\alpha_N} \xi} N^{n-1}(\xi) \, d\xi \right),
\]

(80)

where \( C_1 \) and \( C_2 \) are constants of integration into which the constants from the indefinite integrals above may be absorbed. Similarly, (74) has the solution

\[
N^n(x) = e^{\sqrt{\alpha_N} x} \left( C_3 - \frac{\beta_N}{2\sqrt{\alpha_N}} \int e^{-\sqrt{\alpha_N} \xi} N^{n-1}(\xi) \, d\xi \right) + e^{-\sqrt{\alpha_N} x} \left( C_4 + \frac{\beta_N}{2\sqrt{\alpha_N}} \int e^{\sqrt{\alpha_N} \xi} N^{n-1}(\xi) \, d\xi \right).
\]

(81)

The boundary conditions (78) impose four constraints on solutions (80) and (81) and uniquely determine the coefficients. This gives us unique solutions for \( N^n(x) \) on the intervals \([0, a]\) and \([a, b^{n-1}]\), respectively.

Now that \( N^n \) is known, we can use variation of parameters to solve for \( M^n \). Equation (73) gives us the solution

\[
M^n(x) = -\frac{\gamma_M}{\alpha_M} N_* + e^{\sqrt{\alpha_M} x} \left( C_1 - \frac{1}{2\sqrt{\alpha_M}} \int e^{-\sqrt{\alpha_M} \xi} (\beta_M M^{n-1}(\xi) + \gamma_M N^{n}(\xi)) \, d\xi \right) + e^{-\sqrt{\alpha_M} x} \left( C_2 + \frac{1}{2\sqrt{\alpha_M}} \int e^{\sqrt{\alpha_M} \xi} (\beta_M M^{n-1}(\xi) + \gamma_M N^{n}(\xi)) \, d\xi \right),
\]

(82)

and (75) has the solution

\[
M^n(x) = e^{\sqrt{\alpha_M} x} \left( C_3 - \frac{\beta_M}{2\sqrt{\alpha_M}} \int e^{-\sqrt{\alpha_M} \xi} M^{n-1}(\xi) \, d\xi \right) + e^{-\sqrt{\alpha_M} x} \left( C_4 + \frac{\beta_M}{2\sqrt{\alpha_M}} \int e^{\sqrt{\alpha_M} \xi} M^{n-1}(\xi) \, d\xi \right).
\]

(83)
Again, the boundary conditions determine the constants \( C_1 - C_4 \), and we have unique solutions for \( M^n \) on the intervals \([0, a]\) and \([a, b^{n-1}]\). We conclude by evaluating \( M^n \) at \( x = b^{n-1} \) and using this value in (76) to find \( b^n \).

This procedure was implemented in Mathematica and works well for approximately six time steps before the complexity of the integrals in (80)-(83) causes the solutions to blow up. This blow up phenomenon holds for a wide range of parameters and even for \( b^0 > a \), so it appears to be more a problem with the technique than with the size of the time step or the boundary conditions. Nevertheless, we were able to use this method to develop intuition about the early behavior of the system (see Fig. 2). We also used it to estimate the size of \( \Delta t \) needed to make \( b \) advance by one mesh point in the Crank-Nicolson implementation of the system, and this estimate was in good agreement with the estimates obtained via the other methods described in this report. Finally, choosing a set of parameters we fit the power function \( 5 + 1.4784x^{1.6527} \) to the values of \( b \) with \( R^2 = .99932 \) (see Fig. 2(c)); this closely approximates the quadratic expansion predicted by asymptotic methods.

![Figure 2: Early development of (a) nutrient concentration \( N \), (b) matrix protein concentration \( M \), and (c) ECM-gel interface location \( b(t) \) with a power function fit.](image)

### 5 Finite Difference Solution

In developing a finite difference solution for our model (1-10), we employed the following finite difference schemes:

- Crank-Nicolson scheme for the reaction-diffusion equations (1-2)
- First-order Euler for the interface evolution equation (9)
- First-order finite differences for all no flux conditions in (3-9)

Denoting \( N^n_i = N(x_i, t_n) \), we employed a non-uniform discretization \( t_0, t_1, ..., t_n, ... \) in time, and a uniform discretization \( x_0, x_1, ..., x_i, ... \) in space with width \( \Delta x \).

The discrete version of (1) can be written as:

\[
\frac{N_i^{n+1} - N_i^n}{\Delta t} = D_N^{(1)} \left[ \frac{(N_{i+1}^{n+1} - 2N_i^{n+1} + N_{i-1}^{n+1}) + (N_{i+1}^n - 2N_i^n + N_{i-1}^n)}{2\Delta x^2} \right] \\
- k_N \left[ \frac{(N_i^{n+1} - N_s) + (N_i^n - N_s)}{2} \right]
\]
If we let \( r = \frac{D_N^{(1)} \Delta t}{2(\Delta x)^2} \) then the above equation can be more concisely written as:

\[
-rN_i^{n+1} + (1 + 2r + \frac{k_N}{2} \Delta t) N_i^{n+1} - rN_{i-1}^{n+1} = rN_{i+1}^{n+1} + (1 - 2r - \frac{k_N}{2} \Delta t) N_i^{n} - rN_{i-1}^{n} + k_N N_i \Delta t
\]

Similarly, the discrete versions of (2-4) are written as:

\[
-sM_i^{n+1} + (1 + 2s)M_i^{n+1} - sM_{i+1}^{n+1} = sM_{i+1}^{n} + (1 - 2s)M_i^{n} + sN_{i-1}^{n} + k_M \left[ \frac{N_i^{n+1} + N_i^{n}}{2} - N_i \right]
\]  \hspace{1cm} (84)

which can be re-written as:

\[
-sM_i^{n+1} + (1 + 2s)M_i^{n+1} - sM_{i+1}^{n+1} = sM_{i+1}^{n} + (1 - 2s)M_i^{n} + sN_{i-1}^{n} + k_M \left[ \frac{N_i^{n+1} + N_i^{n}}{2} - N_i \right]
\]

Similarly, the discrete versions of (2-4) are written as:

\[
-sM_i^{n+1} + (1 + 2s)M_i^{n+1} - sM_{i+1}^{n+1} = sM_{i+1}^{n} + (1 - 2s)M_i^{n} + sN_{i-1}^{n} + k_M \left[ \frac{N_i^{n+1} + N_i^{n}}{2} - N_i \right]
\]

\[
-sM_i^{n+1} + (1 + 2s)M_i^{n+1} - sM_{i+1}^{n+1} = sM_{i+1}^{n} + (1 - 2s)M_i^{n} + sN_{i-1}^{n} + k_M \left[ \frac{N_i^{n+1} + N_i^{n}}{2} - N_i \right]
\]

where \( s = \frac{D_N^{(1)} \Delta t}{2(\Delta x)^2} \), \( \tilde{r} = \frac{D_N^{(2)} \Delta t}{2(\Delta x)^2} \) and \( \tilde{s} = \frac{D_M^{(2)} \Delta t}{2(\Delta x)^2} \).

Suppose the interface is located at \( x = x_m = m \Delta x \). Then, the interface conditions in (5-6) can be discretized as:

\[
\frac{D_N^{(2)}}{D_N^{(1)}} (N_m^{n+1} - N_m^{n}) = (N_m^{n} - N_{m-1}^{n})
\]

\[
\frac{D_M^{(2)}}{D_M^{(1)}} (M_m^{n+1} - M_m^{n}) = (M_m^{n} - M_{m-1}^{n})
\]

which can be re-written as:

\[
\frac{D_N^{(2)}}{D_N^{(1)}} N_m^{n+1} - \left( 1 + \frac{D_N^{(2)}}{D_N^{(1)}} \right) N_m^{n} + N_{m-1}^{n} = 0
\]

\[
\frac{D_M^{(2)}}{D_M^{(1)}} M_m^{n+1} - \left( 1 + \frac{D_M^{(2)}}{D_M^{(1)}} \right) M_m^{n} + M_{m-1}^{n} = 0
\]

Lastly, the interface evolution equation (9) is discretized as:

\[
\frac{b^{n+1} - b^n}{\Delta t} = k_b M^{n+1}(b(t^n))
\]

The numerical procedure to approximate the solution of the system of differential equations in discrete form was carried out by the following steps:

- **Step 1 (t = 0)**
  Take a single time-step \( \Delta t \). Solve the \( N \) equation (84) in the cell region and use this to solve the \( M \) equation (85) in the cell region \( 0 < x < a \). Use the computed value of \( M \) at \( x = a \) to solve the \( b \) equation (90) and obtain \( b(\Delta t) \). If \( b(\Delta t) = a + 2\Delta x \) (to within a specified tolerance) go to step 2, otherwise adjust \( \Delta t \) and repeat step 1.

- **Step 2 (t = \Delta t)**
  Interpolate to find \( N \) and \( M \) at \( x = a + \Delta x \), using the boundary conditions.
• Step 3 \((t > \Delta t)\)
  Repeat the procedure in Steps 1-2 with the most current value of \(\Delta t\) and find the next value of \(b(t)\). Iterate on \(\Delta t\) until \(b(i) = a + i\Delta x\) (to within a specified tolerance). Continue in this manner for the full time domain of interest.

The procedure described above involves an iterative process to find the value of \(\Delta t\) in each time step in order to advance the boundary (in the ECM region) to the next (fixed width) grid point on the spatial mesh. Note that our Crank-Nicolson scheme requires an uniform mesh size \(\Delta x\) in space. An alternative to the previous procedure, that does not require the iterative procedure, could be to use interpolation and spatial re-meshing to align each successive spatial mesh to the new value of \(b(t)\) obtained with a fixed time step \(\Delta t\). To achieve this, an accurate interpolation method (e.g. cubic splines) would be required to preserve accuracy of our approximations. Finite difference results obtained using the method described in this section were in excellent agreement with the semi-discrete solution (Fig. 2) and consistent with the quadratic growth in time obtained using the asymptotic solution described earlier.

6 Discussion

We have developed three different solution techniques for determination of the early-time behavior of a reaction-diffusion cartilage regeneration model with an advancing interface. Results for three methods agree quite well and demonstrate that, for early time, the newly synthesized cartilage ECM accumulates fairly rapidly and on the order of \(t^2\). While we were unable to get results for larger times, intuitively, the growth rate should decrease over time, as a larger ECM layer would inhibit diffusion of nutrients to the cell and newly synthesized matrix proteins out from the cell (Fig. 1). Refined solution schemes that are capable of simulating the full time scale of the application are required to quantify detailed relationships between tissue regeneration times and the underlying physiological and biophysical mechanisms included in our model. Geometric extension of the model should consider a more realistic geometry such a one-dimensional spherical model, or a two-dimensional axisymmetric model. Extended descriptions could also include a third region modeling evolution of the hydrogel as well as further extensions accounting for material inhomogeneities in the cell and the ECM.

7 References
