BENG 221

Modeling EDTA Diffusion into Bone for Demineralization

10/14/2016

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1. Introduction

1.1 Background

Osseous tissue is known to contain a high mineral content, which includes a high concentration of calcium (Ca^{2+}). For applications involving conventional histology, i.e. microtome sectioning, staining, and visualization via light microscopy, this mineral must be removed prior to bone preparation and sectioning for these applications. For the demineralizing process, two common agents are used: acid demineralizers and chelating agents. Acid demineralizers, such as hydrochloric acid (HCl), are known for their ability to rapidly demineralize bone tissue, but often these chemicals cause undesirable distortion of tissue structures causing loss of nuclear staining and maceration of tissues (figure 1) [1-3].

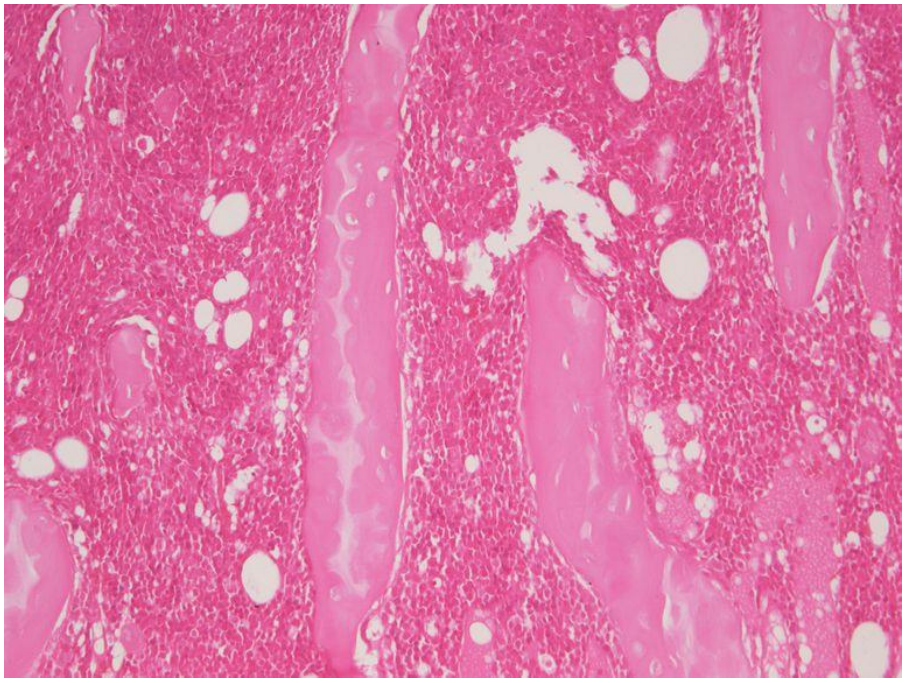

Figure 1: A section of decalcified cancellous bone decalcified with HCl. The staining is poor with an absence of nuclear staining. [1]

Alternatively, chelating agents can be used to demineralize bone tissue while preserving the integrity of intercellular structures. Chelating agents capture polyvalent ions, such as calcium, from the surface of mineral crystals in bone and slowly reducing their size. However, these agents generally remove minerals at a much slower rate than acid demineralizers. One such chelating agent, ethylenediaminetetraacetic acid (EDTA), binds to the calcium ions and effectively removes them from the surrounding tissue when introduced into porous bone (Figure 2) [3]. Salts of EDTA are noncolloidal organic chelating agents which form soluble nonionic chelates with polyvalent ions. Calcium is strongly chelated above pH 6 with activity plateauing above pH 7.5 [3]. Using X-ray, the process of demineralization can be tracked to specified end points (Figure 3).

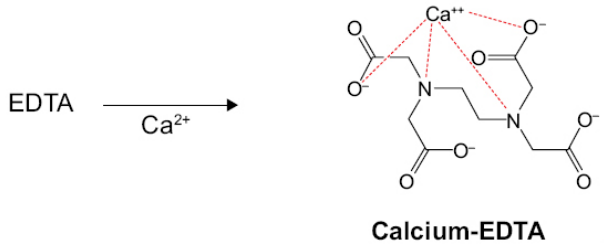

Figure 2: Chelating reaction of EDTA with calcium [4]

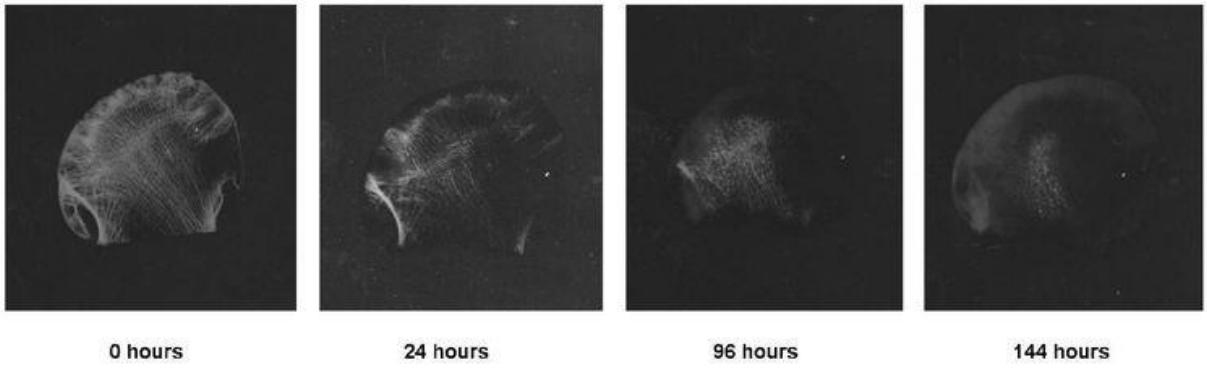

Figure 3: Radiographs following the process of decalcification of a femoral head [1]

Factors influencing the rate of decalcification include concentration, temperature, agitation, and fluid access [1]. In addition, it has been emphasized that diffusion plays an important role in the progression of decalcification [5]. The appropriate concentration balances the need for rapid decalcification with the degree with tissue damage. As the demineralizing agent combines with calcium, the concentration of active agent will be depleted with time. Similarly, temperature increases the speed of decalcification through enhanced kinetics, but will also increase the rate of tissue damage. Agitation of the fluid as well as fluid access to the bone specimen will also enhance the rate of demineralization. To maximize diffusion and ingress of the demineralizing agent into the specimen, all surfaces of the specimen should be exposed.

1.2 Problem Statement

This purpose of this project was to develop a mathematical model displaying the diffusion of a demineralizing agent into bone given various parameters. These parameters are outlined as follows:

a. Diffusion of EDTA into thin bone slices, with the bone placed in an apparatus that allows for diffusion of steady-state EDTA in a single direction through the bone.
b. Comparison of diffusion with alterations to the demineralizing agent (EDTA vs. HCl).
c. EDTA reacts with calcium as a function of time.
d. EDTA pumped at a controlled rate into the bone slice.

2. Analytical Solution: Case 1

2.1 Assumptions

In the diffusion of EDTA into thin bone slices we will assume that the apparatus is designed such that EDTA diffuses through only one face of the slice and that there is no flux at the bottom face. This assumption reduces diffusion to a single dimension. In addition, EDTA will be introduced in excess at t = 0 s where there is initially no EDTA in the bone slice, and there is a constant concentration of C_0 at the diffusing face. The mathematical assumptions are described below (Table 2).

Table 1: Mathematical assumptions for diffusion of EDTA into thin bone slices

Assumption	Mathematical Relation
EDTA diffuses through only one face of the slice	$\partial C/\partial t = D\,\partial^2 C/\partial x^2$
EDTA (C_o) is introduced in excess at t = 0 s There is initially no EDTA in the bone slice	$C(x,0) = C_o\delta(x)$
There is zero flux at the bottom edge	$\partial C/\partial x\,(L,t) = o$
There is enough EDTA to saturate the bone	$C(x,t\rightarrow\infty) = C_o$

1-D Diffusion

$$\frac{\partial c}{dt} = D\frac{\partial^2 c}{\partial x^2}$$

Initial Conditions

$$c(x,0) = C_0\delta(x)$$

Boundary Conditions

$$(0,t) = C_0$$

$$\frac{\partial c}{dx}(L,t) = 0$$

Solution to an inhomogeneous PDE with no source term and constant spatial boundaries is of the form:

$$c(x,t) = c_p(x,t) + c_h(x,t)$$

<u>Using the steady state condition to find the particular solution:</u>

$$t \rightarrow \infty$$

$$\frac{\partial c_p}{dt} = 0$$

$$\frac{\partial c_p}{dt} = D\frac{\partial^2 c_p}{\partial x^2}$$

$$0 = D\frac{\partial^2 c}{\partial x^2}$$

The equation for the particular solution can be reduced to an ordinary differential equation of the form:

$$c_p(x) = Ax + B$$

Applying boundary conditions to solve for coefficients:

$$\boldsymbol{BC}: \boldsymbol{c(0) = C_0}$$
$$c_p(0) = A(0) + B = C_0$$
$$B = C_0$$
$$c_p(x) = Ax + C_0$$
$$\boldsymbol{BC}: \boldsymbol{\frac{\partial c}{\partial x}(L) = 0}$$
$$\frac{\partial c_p}{dx}(x) = A$$
$$\frac{\partial c_p}{dx}(L) = A = 0$$
$$A = 0$$
$$c_p(x) = C_0$$

Solving for the homogenous solution:

Homogenous Boundary Conditions

$$c_h(0,t) = 0$$
$$\frac{\partial c_h}{dx}(L,t) = 0$$

Deriving spatial boundaries:

$$c_h(0,t) = \emptyset(0) * G(t) = 0$$
$$\emptyset(0) = 0$$
$$\frac{\partial c_h}{dx}(L,t) = \emptyset'(L) * G(t) = 0$$
$$\emptyset'(L) = 0$$

Solving for the homogenous solution using separation of variables:

$$c_h(x,t) = \emptyset(x) * G(t)$$
$$\frac{\partial c_h}{dt} = \emptyset(x) * G'(t)$$
$$\frac{\partial c_h}{dx} = \emptyset'(x) * G(t)$$
$$\frac{\partial^2 c_h}{\partial x^2} = \emptyset''(x) * G(t)$$
$$G'(t) * \emptyset(x) = D * \emptyset''(x) * G(t)$$
$$\frac{1}{D} * \frac{G'(t)}{G(t)} = \frac{\emptyset''(x)}{\emptyset(x)} = -\lambda$$

For the case where λ = 0:

$$\emptyset''(x) = 0$$
$$\emptyset(x) = Ax + B$$

Input BCs:

$$BC: \emptyset(0) = 0$$
$$\emptyset(0) = Ax + B = 0$$
$$\emptyset(0) = A(0) + B = 0$$
$$B = 0$$
$$\emptyset(x) = Ax$$
$$BC: \emptyset(L) = 0$$
$$\emptyset(L) = AL = 0$$
$$A = 0$$

This yields the trivial solution for $\lambda = 0$

For the case where λ < 0:

$$\emptyset''(x) + \lambda * \emptyset(x) = 0$$
$$\emptyset(x) = Ae^{\sqrt{-\lambda}*x} + Be^{-\sqrt{-\lambda}*x}$$

This yields the trivial solution for $\lambda > 0$

For the case where λ > 0:

$$\emptyset''(x) + \lambda * \emptyset(x) = 0$$
$$\emptyset(x) = Acos(\sqrt{\lambda}x) + Bsin(\sqrt{\lambda}x)$$

Input BCs:

$$BC: \emptyset(0) = 0$$
$$\emptyset(0) = Acos(0) + Bsin(0) = 0$$
$$A = 0$$
$$\emptyset(x) = Bsin(\sqrt{\lambda}x)$$
$$BC: \emptyset'(L) = 0$$
$$\emptyset'(x) = -\sqrt{\lambda}Bcos(\sqrt{\lambda}x)$$
$$\emptyset'(L) = -\sqrt{\lambda}Bcos(\sqrt{\lambda}L) = 0$$
$$\sqrt{\lambda}L = \frac{(2n+1)}{2}\pi \quad n = 0,1,2,\ldots$$
$$\lambda = \left(\frac{(2n+1)}{2L}\pi\right)^2$$
$$\emptyset_n(x) = B_n sin\left(\frac{(2n+1)}{2L}\pi x\right)$$

Solving for G(t) where λ > 0:

$$G'(t) + \lambda D * G(t) = 0$$
$$G(t) = e^{-\lambda D t}$$

Putting together the homogenous solution:

$$c_{h_n}(x,t) = B_n sin\left(\frac{(2n+1)}{2L}\pi x\right) e^{-\lambda D t}$$

$$c_{h_n}(x,t) = B_n sin\left(\frac{(2n+1)}{2L}\pi x\right) e^{-D\left(\frac{(2n+1)}{2L}\pi\right)^2 t}$$

$$c_h(x,t) = \sum_{n=0}^{m} B_n sin\left(\frac{(2n+1)}{2L}\pi x\right) e^{-D\left(\frac{(2n+1)}{2L}\pi\right)^2 t}$$

Recall that $C(x,t) = C_p(x,t) + C_h(x,t)$:

$$c(x,t) = C_0 + \sum_{n=0}^{m} B_n sin\left(\frac{(2n+1)}{2L}\pi x\right) e^{-D\left(\frac{(2n+1)}{2L}\pi\right)^2 t}$$

Solving for B_n:

$$B_n = \frac{2}{L}\int_0^L \left[(c(x,0) - c_p(x)) * c_h(x)\right] dx$$

$$B_n = \frac{2}{L}\int_0^L \left[(C_0\delta(x) - C_0) sin\left(\frac{(2n+1)}{2L}\pi x\right)\right] dx$$

$$B_n = \frac{2C_0}{L}\int_0^L (\delta(x) - C_0) sin\left(\frac{(2n+1)}{2L}\pi x\right) dx$$

$$B_n = \frac{-4C_0}{(2n+1)\pi}$$

Final solution:

$$c(x,t) = C_0 + \sum_{n=0}^{m} \frac{-4C_0}{(2n+1)\pi} sin\left(\frac{(2n+1)}{2L}\pi x\right) e^{-D\left(\frac{(2n+1)}{2L}\pi\right)^2 t}$$

2.2 Plot of the Analytical Solution

The analytical solution derived above was plotted using the first 20 terms of the series to generate the following graph (Figure 4). Initially, there is only EDTA at x=0, and no EDTA throughout the domain of x. This illustrates the initial conditions where $C(x,0) = C_o\boldsymbol{\delta}(x)$. However, oscillations can be seen throughout the initial time point. This phenomenon, the Gibb's Phenomenon, occurs when fourier series are used to model jump discontinuities, such as the delta function, resulting in oscillations near the jump. In addition, as t➡∞ the plot saturates at C_0, where C_0=0.7 M EDTA, reflecting the zero flux boundary condition at x=L=2 mm. Thus, the graph supports the initial and boundary conditions assumed. Therefore this model for the diffusion of EDTA through thin bone slices implies that the given bone slice will saturate with EDTA as t➡∞ at a rate dependent on the diffusivity.

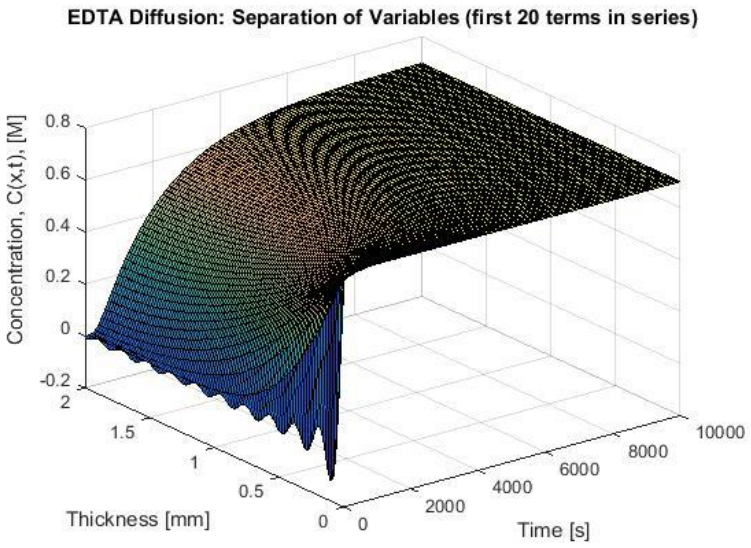

Figure 4: Fourier expansion of the analytical solution using the first 20 terms.

3. Numerical Solution

3.1 Case 1: Modeling Different Acid Demineralizers

Given that the diffusion of EDTA through thin bone slices implies that the given bone slice will saturate with EDTA as $t \rightarrow \infty$ at a rate dependent on the diffusivity, the diffusivity of two concentration of HCl, an acid demineralizing agent, were input into the model according to the following table (Table 3). Although acid demineralizers such as HCl are known to cause undesirable distortion of tissue structures they have been shown to result in more rapid decalcification of bone slices.

Table 2: Diffusion coefficients for various demineralization agents used for histological sections.

	D (m^2/s)
0.7 M EDTA [6]	0.95×10^{-9}
0.5 M HCl [7]	2.31×10^{-9}
2 M HCL [7]	1.33×10^{-9}

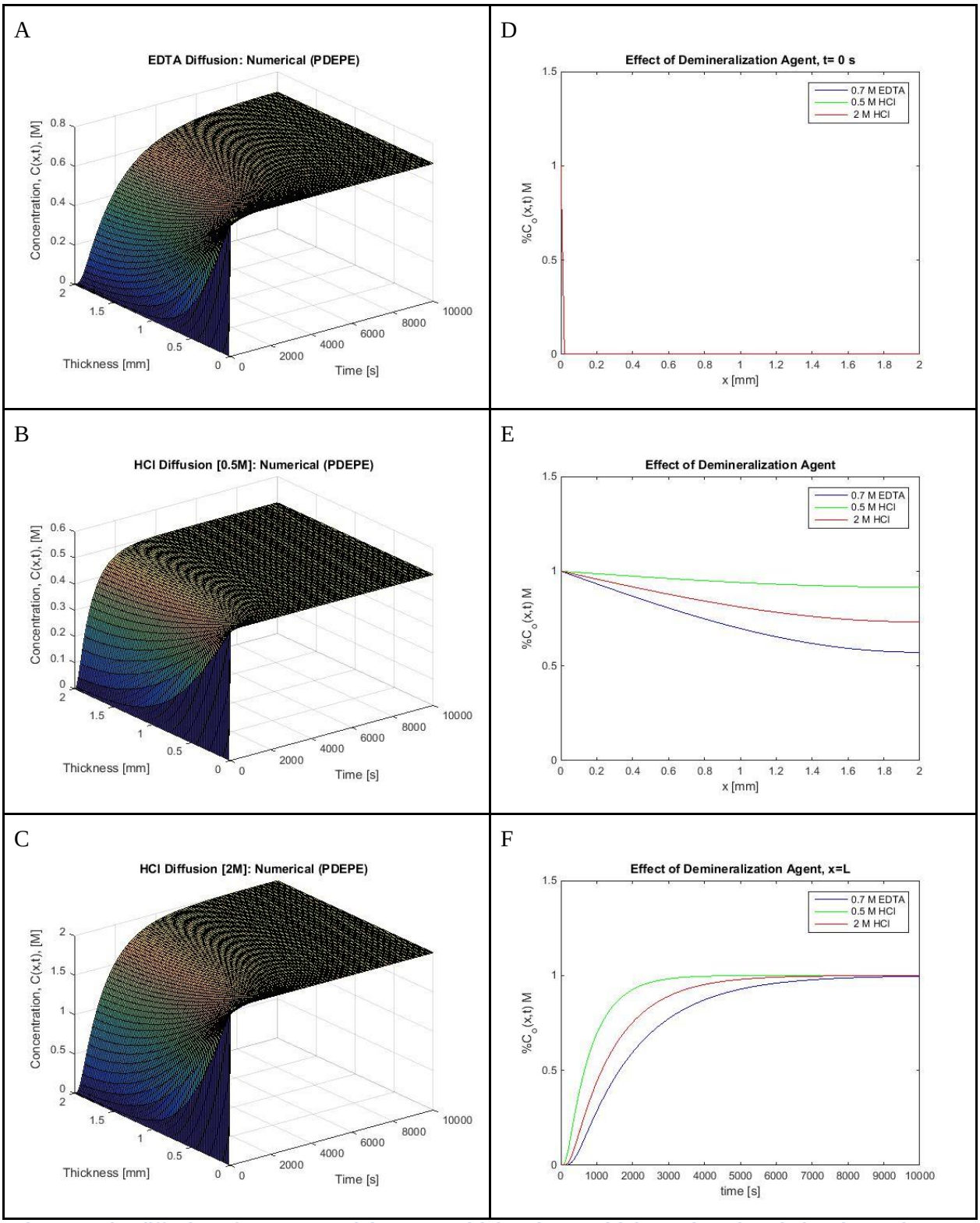

Figure 5: The diffusion of 0.7 M EDTA (A), 0.5 M HCl (B) and 2M HCl (C) are plotted. Isolating time points at t=0 (D) and t=2000 s (E) illustrate how diffusivity affects diffusion of the agent through the substrate. In addition, by plotting the concentration as a function of time at the bottom edge of the slice (F) the time required to reach steady state can be calculated.

The above graphs (Figure 5) illustrate the dependence of diffusion on the diffusion coefficient. Agents that have a higher diffusivity diffuse through the sample at a faster rate and reach steady state the quickest. Diffusivity of a liquid is dependent on temperature and viscosity. Accordingly, the higher concentration of HCL (2M v. 0.5M) has a lower diffusivity in part due to its higher viscosity. Therefore, although it may react faster with the bone than lower concentrations, the ingress of 2M HCl into the bone will be slower. In addition the action of acid decalcifiers compared to other agents (such as EDTA) on the bone must be considered. Thus, when choosing a demineralizing agent to decalcify a histologic sample it is important to consider not only the rate at which the bone can be demineralized but also the effect each agent will have on the bone sample.

3.2 Case 2: Reaction with Calcium

To create a more realistic model of EDTA diffusion and the resultant demineralization of bone, the reaction of EDTA reacts with calcium as a function of time was modeled (Figure 6, 7). As the concentration of EDTA increases throughout the bone segment with time, so does the sequestering of calcium by EDTA. This action was modeled as a time-dependent sink through which the calcium-EDTA complex is removed from the system using a rate constant of 2×10^{-8}, which is ~2 orders of magnitude smaller than the diffusivity for 0.7 M EDTA. Due to the reaction of calcium with EDTA, the concentration of EDTA decreases as t $\rightarrow \infty$.

$$\frac{\partial c}{dt} = D\frac{\partial^2 c}{\partial x^2} - (2 \times 10^{-8})t$$

Initial Conditions

$$c(x, 0) = C_0\delta(x)$$

Boundary Conditions

$$(0, t) = C_0$$

$$\frac{\partial c}{dx}(L, t) = 0$$

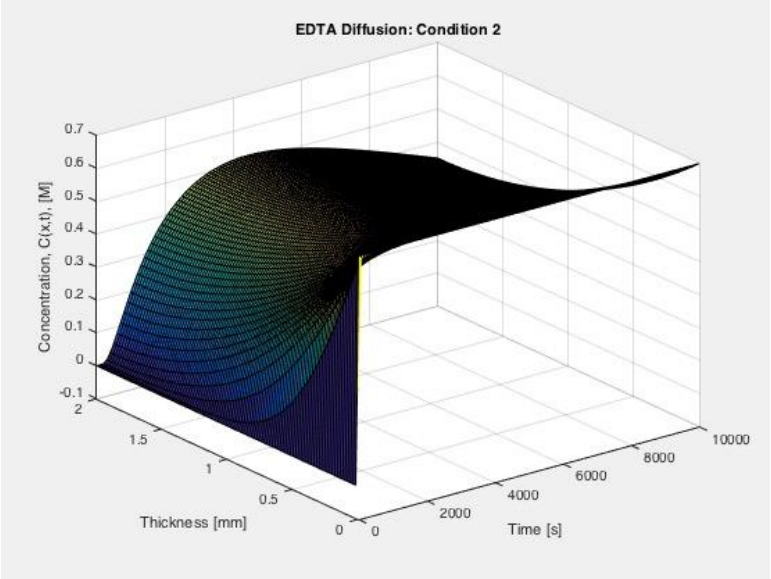

Figure 6: Plot of numerical solution where reaction with calcium is modeled as a constant sink

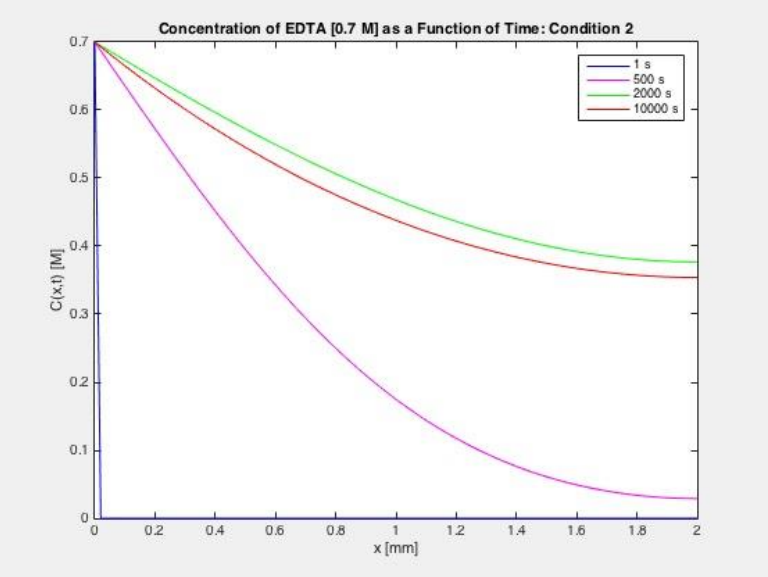

Figure 7: Plot of numerical solution where reaction with calcium is modeled as a constant sink at t= 1s, 500s, 2,000s, and 10,000s

3.3 Case 3: Flow Conditions

Thorough agitation and flow of the demineralizing agent through the bone slice is necessary to enhance the rate of decalcification. Thus, the implementation of a pump which introduces EDTA at a controlled rate through the diffusing face of the slice was modeled as a function of time (Figure 8, 9).

$$\frac{\partial c}{dt} = D\frac{\partial^2 c}{\partial x^2} - (2 \times 10^{-8})t$$

Initial Conditions

$$c(x,0) = C_0\delta(x)$$

Boundary Conditions

$$\frac{\partial c}{dx}(0,t) = 0.005\,(\sin t + C_0)$$

$$\frac{\partial c}{dx}(L,t) = 0$$

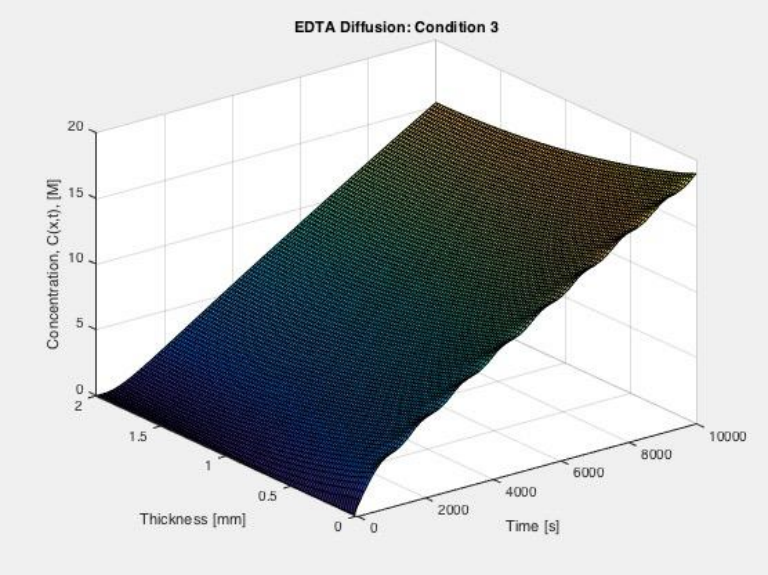

Figure 8: Plot of numerical solution where there is a inhomogeneous flux condition

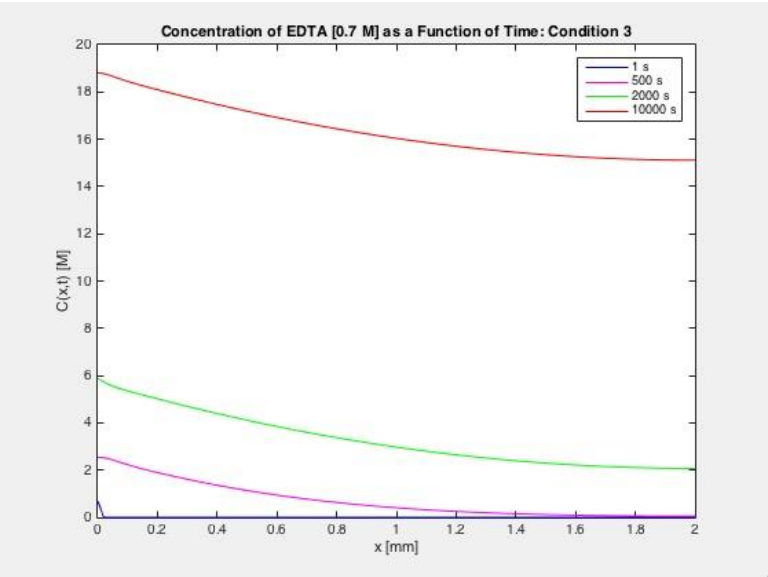

Figure 9: Plot of numerical solution where there is a inhomogeneous flux condition, modeled as a constant sink at t= 1s, 500s, 2,000s, and 10,000s

4. Conclusion

In preparation for histology, it is common to demineralize bone tissue prior to sample sectioning and imaging. For this demineralizing process, the focus is placed largely on removing calcium from the tissue. For our model, we sought to use mathematical methods to examine various components of the demineralizing process and were able to show that the simple diffusion of a demineralizing agent into bone can be affected by several different elements of the process, namely the type of demineralizing agent and how the agent is presented to the bone sample. Through the development of a model, along with several modifications to the model, taking into account various modifications to the experiment, we were able to determine the diffusion rate of a given demineralizing agent into bone.

Moving forward, there are many areas for improvement of the model. One such area falls in taking account for the complex vasculature of the bone tissue; the model could be altered to include not only diffusion through the pores in the tissue but also the vascular channels. Similarly, though our model assumed a flat, rectangular shape for our tissue sample, a future model could encompass different shapes for the tissue section and even diffusion through multiple faces. Though there are also other areas for improvement, taking any of these concepts into consideration would create a more accurate and complex model for our problem.

This model could prove to be useful in many real life applications, namely those pertaining to histology. In lab and experimental applications the largest issue encountered, and the initial reason for the development of this model, is predicting the amount of time for the demineralization to occur. Our model addresses this issue and accounts for many variations of the process. Because of this, the model could prove useful in increasing efficiency of this type of experimentation by allowing for better estimation of osseous demineralization.

5. Matlab Code

5.1 Case 1

5.1.1 Analytical: Modeling EDTA Diffusion

```
close all
clear all
clc

ns = 20;% Number of Terms

global D L c0
D = 0.00095; %diffusion coefficient (mm2/s)
L = 2; %thickness of bone slice (mm)
c0 = .7; %initial concentration (M)

% domain
dx = 0.02; % step size in x dimension
dt = 100; % step size in t dimension
xmesh = 0:dx:L; % domain in x; L/2 = 1
tmesh = 0:dt:10000; % domain in t (s)
nx = length(xmesh); % number of points in x dimension
nt = length(tmesh); % number of points in t dimension

% solution on bounded domain using separation of variables
sol_sep = zeros(nt, nx);
sol_sep=sol_sep+c0;

for n = 0:1:ns
    k = (2*n+1)*pi/2/L;
    for i = 1:length(tmesh)
        for j = 1:length(xmesh)
```

```
            sol_sep(i,j) = sol_sep(i,j) - (4*c0/(2*n+1)/pi) * exp(-
D*(k^2)*tmesh(i)) * sin(k*xmesh(j));
        end
    end
end

% Plot analytical solution
figure(1)
surf(tmesh,xmesh,sol_sep')
title(['EDTA Diffusion: Separation of Variables (first ', num2str(ns), '
terms in series)'])
xlabel('Time [s]')
ylabel('Thickness [mm]')
zlabel('Concentration, C(x,t), [M]')
```

5.1.2 Numerical: Modeling Different Acid Demineralizers

```
function ProjectDeCal_PDEPE
close all
clear all
clc

global L
L = 2; %thickness of bone slice (mm)

xmesh = 0:0.02:L; %x domain
tmesh = 0:100:10000; %time domain

%Solution using MATLAB PDE Solver
sol_pdepe = pdepe(0,@pdefun,@ic,@bc,xmesh,tmesh);
sol_pdepeHCl = pdepe(0,@pdefunHCL,@icHCl,@bcHCl,xmesh,tmesh);
sol_pdepeHCl2 = pdepe(0,@pdefunHCL2,@icHCl2,@bcHCl2,xmesh,tmesh);

%3D solution mesh [EDTA]
figure(1)
surf(tmesh,xmesh,sol_pdepe')
title('EDTA Diffusion [0.7M]: Numerical (PDEPE)')
xlabel('Time [s]')
ylabel('Thickness [mm]')
zlabel('Concentration, C(x,t), [M]')

figure(2)
A = sol_pdepe(1,:);
B = sol_pdepe(20,:);
C = sol_pdepe(100,:);
plot(xmesh,A,'b',xmesh,B,'g',xmesh,C,'r');
xlabel('x [mm]');
ylabel('C(x,t) M');
axis([0 2 0 1])
legend('1 s', '2000 s', '10000 s');
title('Concentration in EDTA [0.7M] as a Function of Time')

%3D solution mesh - HCL
figure(3)
```

```
surf(tmesh,xmesh,sol_pdepeHCl')
title('HCl Diffusion [0.5M]: Numerical (PDEPE)')
xlabel('Time [s]')
ylabel('Thickness [mm]')
zlabel('Concentration, C(x,t), [M]')

figure(4)
A = sol_pdepeHCl(1,:);
B = sol_pdepeHCl(20,:);
C = sol_pdepeHCl(100,:);
plot(xmesh,A,'b',xmesh,B,'g',xmesh,C,'r');
xlabel('x [mm]');
ylabel('C(x,t) M');
axis([0 2 0 1])
legend('1 s', '2000 s', '10000 s');
title('Concentration in HCl [0.5M] as a Function of Time')

%3D solution mesh - HCL 2M
figure(5)
surf(tmesh,xmesh,sol_pdepeHCl2')
title('HCl Diffusion [2M]: Numerical (PDEPE)')
xlabel('Time [s]')
ylabel('Thickness [mm]')
zlabel('Concentration, C(x,t), [M]')

figure(6)
A = sol_pdepeHCl2(1,:);
B = sol_pdepeHCl2(20,:);
C = sol_pdepeHCl2(100,:);
plot(xmesh,A,'b',xmesh,B,'g',xmesh,C,'r');
xlabel('x [mm]');
ylabel('C(x,t) M');
axis([0 2 0 2])
legend('0 s', '2000 s', '10000 s');
title('Concentration in HCl [2M] as a Function of Time')
% ------------------------------------------------------------
figure(7)
A = sol_pdepe(20,:);
B = sol_pdepeHCl(20,:);
C = sol_pdepeHCl2(20,:);
plot(xmesh,A,'b',xmesh,B,'g',xmesh,C,'r');
xlabel('x [mm]');
ylabel('%C_o(x,t) M');
axis([0 2 0 1.5])
legend('0.7 M EDTA', '0.5 M HCl', ' 2 M HCl');
title('Effect of Demineralization Agent, t= 2000s')

figure(8)
A0 = sol_pdepe(1,:);
B0 = sol_pdepeHCl(1,:);
C0 = sol_pdepeHCl2(1,:);
plot(xmesh,A0,'b',xmesh,B0,'g',xmesh,C0,'r');
xlabel('x [mm]');
ylabel('%C_o(x,t) M');
axis([0 2 0 1.5])
legend('0.7 M EDTA', '0.5 M HCl', ' 2 M HCl');
```

```
title('Effect of Demineralization Agent, t= 0 s')

figure(9)
A1 = sol_pdepe(:,101);
B1 = sol_pdepeHCl(:,101);
C1 = sol_pdepeHCl2(:,101);
plot(tmesh,A1,'b',tmesh,B1,'g',tmesh,C1,'r');
xlabel('time [s]');
ylabel('%C_o(x,t) M');
axis([0 10000 0 1.5])
legend('0.7 M EDTA', '0.5 M HCl', ' 2 M HCl');
title('Effect of Demineralization Agent, x=L')

% function definitions for pdepe:
% --------------------------------------------------------------

function [c, f, s] = pdefun(x, t, u, DuDx)
% PDE coefficients functions
D=0.00095;
c = 1;
f = D * DuDx; % diffusion
s = 0; % homogeneous, no driving

function [c, f, s] = pdefunHCL(x, t, u, DuDx)
% PDE coefficients functions
D=0.00231; %Diffusion constant HCl 0.5M
c = 1;
f = D * DuDx; % diffusion
s = 0; % homogeneous, no driving term

function [c, f, s] = pdefunHCL2(x, t, u, DuDx)
% PDE coefficients functions
D=0.00133; %Diffusion constant HCl 2M
c = 1;
f = D * DuDx; % diffusion
s = 0; % homogeneous, no driving term

% --------------------------------------------------------------
function u0 = ic(x)
% Initial conditions function
c0=1; %0.7 M (Normalized to 1); %EDTA [M]
u0 = c0 * (x==0); % delta impulse at left boundary condition

function u0 = icHCl(x)
% Initial conditions function
c0=1; %0.5 M (Normalized to 1); %HCl [M]
u0 = c0 * (x==0); % delta impulse at left boundary condition

function u0 = icHCl2(x)
% Initial conditions function
c0=1; %2 M (Normalized to 1); %EDTA [M]
u0 = c0 * (x==0); % delta impulse at left boundary condition
% --------------------------------------------------------------
function [pl, ql, pr, qr] = bc(xl, ul, xr, ur, t)
% Boundary conditions function
```

```
c0=1; %0.7 M (Normalized to 1); %EDTA [M]
pl = c0-ul; % left boundary condition
ql = 0; % no flux left boundary condition
pr = 0; % zero value right boundary condition
qr = 1; % no flux right boundary condition

function [pl, ql, pr, qr] = bcHCl(xl, ul, xr, ur, t)
% Boundary conditions function
c0=1; %0.5 M (Normalized to 1); %EDTA [M]
pl = c0-ul; % left boundary condition
ql = 0; % no flux left boundary condition
pr = 0; % zero value right boundary condition
qr = 1; % no flux right boundary condition

function [pl, ql, pr, qr] = bcHCl2(xl, ul, xr, ur, t)
% Boundary conditions function
c0=1; %2 M (Normalized to 1); %EDTA [M]
pl = c0-ul; % left boundary condition
ql = 0; % no flux left boundary condition
pr = 0; % zero value right boundary condition
qr = 1; % no flux right boundary condition
```

5.2 Case 2: Numerical: Reaction with Calcium

```
function Condition2
close all
clear all
clc

global D L c0
D = 0.95E-3; %diffusion coefficient (mm2/s)
L = 2; %thickness of bone slice (mm)
c0 = .7; %initial concentration (M)

xmesh = 0:0.02:L; %x domain
tmesh = 0:100:10000; %time domain

%Solution using MATLAB PDE Solver
sol_pdepe = pdepe(0,@pdefun,@ic,@bc,xmesh,tmesh);

%3D solution mesh
figure(1)
surf(tmesh,xmesh,sol_pdepe')
title('EDTA Diffusion: Condition 2')
xlabel('Time [s]')
ylabel('Thickness [mm]')
zlabel('Concentration, C(x,t), [M]')

figure(2)
A = sol_pdepe(1,:);
E = sol_pdepe(5,:);
B = sol_pdepe(20,:);
C = sol_pdepe(100,:);
plot(xmesh,A,'b',xmesh,E,'m',xmesh,B,'g',xmesh,C,'r');
xlabel('x [mm]');
```

```
ylabel('C(x,t) [M]');
legend('1 s', '500 s', '2000 s', '10000 s');
title('Concentration of EDTA [0.7 M] as a Function of Time: Condition 2')

% function definitions for pdepe:
% ------------------------------------------------------------

function [c, f, s] = pdefun(x, t, u, DuDx)
% PDE coefficients functions
global D
c = 1;
f = D * DuDx; % diffusion
s = -0.00000002*t; % sink dependent on time

% ------------------------------------------------------------
function u0 = ic(x)
% Initial conditions function
global c0
u0 = c0 * (x==0); % delta impulse at left boundary condition

% ------------------------------------------------------------
function [pl, ql, pr, qr] = bc(xl, ul, xr, ur, t)
% Boundary conditions function
global c0 L
pl = ul-c0; % constant value left boundary condition
ql = 0; % no flux left boundary condition
pr = 0; % no value right boundary condition
qr = 1; % zero flux right boundary condition
```

5.3 Case 3: Numerical: Flow Conditions

```
function Condition2
close all
clear all
clc

global D L c0
D = 0.95E-3; %diffusion coefficient (mm2/s)
L = 2; %thickness of bone slice (mm)
c0 = .7; %initial concentration (M)

xmesh = 0:0.02:L; %x domain
tmesh = 0:100:10000; %time domain

%Solution using MATLAB PDE Solver
sol_pdepe = pdepe(0,@pdefun,@ic,@bc,xmesh,tmesh);

%3D solution mesh
figure(1)
surf(tmesh,xmesh,sol_pdepe')
title('EDTA Diffusion: Condition 2')
xlabel('Time [s]')
ylabel('Thickness [mm]')
zlabel('Concentration, C(x,t), [M]')

figure(2)
```

```
A = sol_pdepe(1,:);
E = sol_pdepe(5,:);
B = sol_pdepe(20,:);
C = sol_pdepe(100,:);
plot(xmesh,A,'b',xmesh,E,'m',xmesh,B,'g',xmesh,C,'r');
xlabel('x [mm]');
ylabel('C(x,t) [M]');
legend('1 s', '500 s', '2000 s', '10000 s');
title('Concentration of EDTA [0.7 M] as a Function of Time: Condition 2')

% function definitions for pdepe:
% ------------------------------------------------------------

function [c, f, s] = pdefun(x, t, u, DuDx)
% PDE coefficients functions
global D
c = 1;
f = D * DuDx; % diffusion
s = -0.00000002*t; % sink dependent on time

% ------------------------------------------------------------
function u0 = ic(x)
% Initial conditions function
global c0
u0 = c0 * (x==0); % delta impulse at left boundary condition

% ------------------------------------------------------------
function [pl, ql, pr, qr] = bc(xl, ul, xr, ur, t)
% Boundary conditions function
global c0 L
pl = 0.005*(sin(t)+c0); % left flux boundary condition a function of time
ql = 1; % flux left boundary condition exists
pr = 0; % no value right boundary condition
qr = 1; % zero flux right boundary condition
```

6. References

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