Wound Closure

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Introduction

Healing in mammals is a complex process where a series of biomechanical and biochemical reponses occur to close a wound by cell migration and contraction. The forces created during this process contribute to the closure, but represent a source of high mechanical stress to the skin that conforms the scar, causing pain, disfigurement and loss of tissue funtion. This forces could also lead to an abnormal healing. A greater comprehension of the biological mechanics behind the healing could represent a benefit for the clinical management of normal and abnormal wounds.

For our present work we will use the equations presented by Oster, Murray et al, and we will expand the work done by Maini et al for the two dimensional case with a finite difference approach.

Some definitions

Collagen
Main protein of connective tissue in animals and most abundant protein in mammals.

Extracellular matrix
Is the part of animal tissue that provides structural support to the animal cells.

Fibroblast
Type of cell that synthesizes the extracellular matrix and collagen. Most common

cells of connective tissue in animals.

Growth Factor
Naturally occurring substance capable of stimulating cellular growth, proliferation and cellular differentiation.

The healing process

This process starts seconds after the injury is made and it can last even for years. It is also a susceptible process, as experience can tell, it can be easily interrupted. We can identify three phases: inflammation, proliferation and remodeling. This phases have not clear boundaries between them and overlap in time.

- Inflammatory phase

A cloth is formed to stop the bleeding and growth factors are released to attract inflammatory cells to eliminate debris, bacteria and damaged tissue. Fibrin and fibronectin from a plug that will be the main structural support before collagen is deposited. Migratory cells use this as an temporary matrix to move across the wound. New growth factors are released to speed the rate of proliferation and migration, and to induce angiogenesis. It usually last 2-3 days after the injury, if its last too long it can cause tissue damage leading to a chronic wound. It will last until there is debris in the site.

- Proliferative phase

Fibroblasts start entering the wound site, endothelial cells from non injured blood vessels go through the ECM matrix into the wound site, all these occur under hypoxic and high lactic acid environment, and when these conditions are no longer fulfilled the rate of the migration and proliferation is reduced. Fibroblasts from normal tissue migrate into the wound site from its margins, initially using the plug of fibrin, then they deposit collagen into the wound, creating a provisional ECM matrix. Fibroblasts create growth factors to attract epithelial cells.

Collagen is important because it strengthens the wound, before the only thing holding the wound closed is the cloth of fibrin. Although fibroblast create new collagen, there is degradation of it by collagenases, in early stages synthesis exceeds degradation,

increasing the amount of it, at a later time these will be equal indicating the maturation of the wound. Migration of epithelial cells creates a barrier between the damaged tissue and the environment. These cells can only migrate across living tissue. Before they start to migrate they must destroy the desmosomes, the attachments to other cells and the ECM matrix. Cells climb over one another in the migration stage, the first cells attach to basement membrane, creating the stratum basale. The faster the migration less scar it will be. Epithelial cells have the ability to kill bacteria and dead tissue that could obstruct their path. As the cell front advance new cells are produced at the wound edge, until the cells meet at the center of the wound and then stop their movement, creating attachments to the ECM matrix. Around a week after, the contraction takes place lasting even for several weeks. Its purpose is to reduce the size of the wound.

- Remodeling phase

This stage can take up to a year or more, its purpose is to convert the provisional ECM matrix into something more like the original ECM matrix. Rearranging collagen fibers increasing the tensile strength of the wound.

The model

The model presented by Oster et al, is a model for mesenchymal cell morphogenesis based on the mechanical interaction between the cells and the ECM matrix. This model is based on the following two properties, cells are capable of generating large traction forces which can deform the ECM through which they move, the deformations they produce affect the direction of their movement

It consists of three equations, density of the cells, the density of the ECM matrix, and the mechanical balance of forces.

$$\frac{\partial n}{\partial t} + \frac{\partial}{\partial x}\left[n\frac{\partial u}{\partial t} + \chi(\rho)n\frac{\partial \rho}{\partial x} - D(\rho)\frac{\partial n}{\partial x}\right] = P(n,\rho), \tag{1}$$

$$\frac{\partial \rho}{\partial t} + \frac{\partial}{\partial x}(\rho\frac{\partial u}{\partial t}) = B(n,\rho), \tag{2}$$

$$\frac{\partial}{\partial x}\left[\mu\frac{\partial^2 u}{\partial x \partial t} + E\frac{\partial u}{\partial x} + \tau(n,\rho)\right] \quad = \quad F(n,\rho) \tag{3}$$

Where, $\varrho(x,t)$ is the ECM density ,$n(x,t)$ is the cell density and $u(x,t)$is the displacement.

Body forces

$F = s\rho u$, where $s > 0$ measures the strength of the ECM attachements to underlying tissues. The ECM matrix is generally attached elastically to epithelial layer.

Cell traction forces

The cell traction τ, depend of the adhesion between cell surface and binding sites on collagen fibers, it reasonable to assume that τ is proportional to $n\rho$. Therefore,

$$\tau(n,\rho) = \frac{T_0 n\rho}{R^2 + \rho^2}$$

Assumptions:

- For normal tissue, without a wound, we set the cell density and ECM density to one.
- Fibroelastic cells prolifarate according to a logistic growth law, $P = rn(1-n)$, where r is the linear growth rate and $r > 0$
- Set $D > 0$ a constant.
- The rate of collagen biosynthesis and degradation are assumed to be proportional to n and $-n\rho$. So it will take the form of $B = \epsilon n(1-\rho)$,where ϵ is very small in order to introduce the fact that the ECM remodeling takes more time than the proliferation of cells.

- The positive parameters μ and E quantify the viscous and elastic contributions.
- We neglect the haptotactic contribution.

First step

To prove that we have understood the problem and its details the first thing to do is to reproduce the results obteined by Maini et al. We will take the same values of the parameters used in the article, and recall their assumptions.

Boundary conditions:

By defining the initial wound space as, $-1 \leq x \leq 1$, and using symmetry at $x = 0$ (wound center), we restrict ourselves to the semi-infinite domain $0 \leq x < \infty$. The initial half- wound is set to unity by the scaling of x. Therefore, we have:

$$\frac{\partial n}{\partial x}(0,t) = \frac{\partial \rho}{\partial x}(0,t) = u(0,t) = 0 \tag{4}$$

Also we set the limits of the wound, meaning that tissue far away of the damaged site does not have anything to do with it and it is in perfect conditions. We set our infinity to x=4.

$$\begin{aligned} n(\infty,t) = \rho(\infty,t) = 1, \\ u(\infty,t) = 0. \end{aligned} \tag{5}$$

Initial Conditions:

$$\begin{aligned} n(x,0) = H(x-1), \\ \rho(x,0) = \rho_i + (1-\rho_i)H(x-1), \\ u(x,0) = 0. \end{aligned} \tag{6}$$

where the initial ECM density ρ_i inside the wound is due to the early, provisional wound matrix, which is low in collagen and satisfies $0 < \rho_i < 1$, and H is the heaviside step function.

We use the finite difference approximations to solve the above set of partial differential equations.

Discretization in one dimension

We discritise the above system using finite difference approximations in space and Euler explicit for time. For the second derivative in space we use central difference approximations while for first derivative we use forward or backward schemes depending on the direction of the flow. We need to pay attention to the transport term when discretizing, because it depends on the direction of the flow. First, we give the discretization of the transport term when the flow is in the negative direction, where we used forward difference approximation, backward approximation otherwise.

If $\frac{\partial u}{\partial t} < 0$ we have;

$$C = \frac{n_{i+1}^N(u_{i+1}^{N+1} - u_{i+1}^N) - n_i^N(u_i^{N+1} - u_i^N)}{dx},$$

$$A = \frac{\rho_{i+1}^N(u_{i+1}^{N+1} - u_{i+1}^N) - \rho_i^N(u_i^{N+1} - u_i^N)}{dx}. \tag{7}$$

If $\frac{\partial u}{\partial t} > 0$ we have;

$$C = \frac{n_i^N(u_i^{N+1} - u_i^N) - n_{i-1}^N(u_{i-1}^{N+1} - u_{i-1}^N)}{dx},$$

$$A = \frac{\rho_i^N(u_i^{N+1} - u_i^N) - \rho_{i-1}^N(u_{i-1}^{N+1} - u_{i-1}^N)}{dx}. \tag{8}$$

Therefore, we have our discritized equations as;

$$n_i^{N+1} = n_i^N - C - \frac{\chi_i n_i dt(\rho_{i+1}^N - \rho_i^N) - \chi_{i-1} n_{i-1} dt(\rho_i^N - \rho_{i-1}^N)}{dx^2} + \frac{D_i dt(n_{i+1}^N - n_i^N) - D_{i-1} dt(n_i^N - n_{i-1}^N)}{dx^2}$$

$$+ dt P(n, \rho)_{i,}$$

$$\rho_i^{N+1} = \rho_i^N - A + B(n, \rho)_{i,}$$

$$(\frac{\mu}{dt} + E)(\frac{-u_{i+1}^{N+1} + 2u_i^{N+1} - u_{i-1}^{N+1}}{dx^2}) + F_i = \frac{\mu}{dt}(\frac{-u_{i+1}^N + 2u_i^N - u_{i-1}^N}{dx^2}) + \frac{\tau_{i+1}^N - \tau_i^N}{dx} \quad (9)$$

where

$$F_i = s\rho_i^N u_i^{N+1}.$$

From equation (9) we have the following system:

$$(\frac{\mu}{dt} + E)Bu^{N+1} = \frac{\mu}{dt} B_1 u^N + \tau^N. \quad (10)$$

where, B, B_1 are two tridiagonal matrix, which are symmetric and definite positive.

We note that the matrix on the left side is a time evolving matrix because we introduced the F=spu term into the matrix. And because of the fact that rho is always positive, we have will also have a definite positive matrix.

We solve the above system iteratively, together with equations of ρ and n .

Implementation

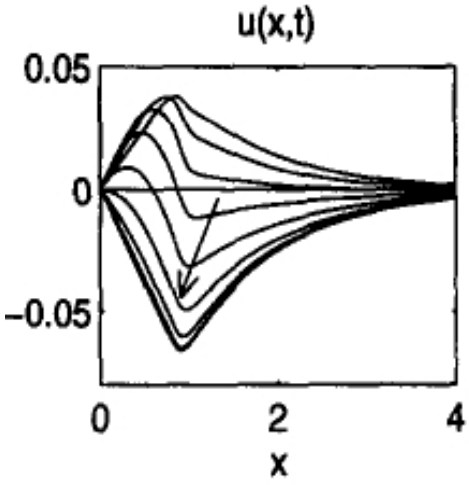
For getting the results we will use the aid of the Scilab software, and make a program that succesfully solves the problem. In this part we first need to construct the matrix for our linear system, that in this case has the shape of a tridiagonal matrix.

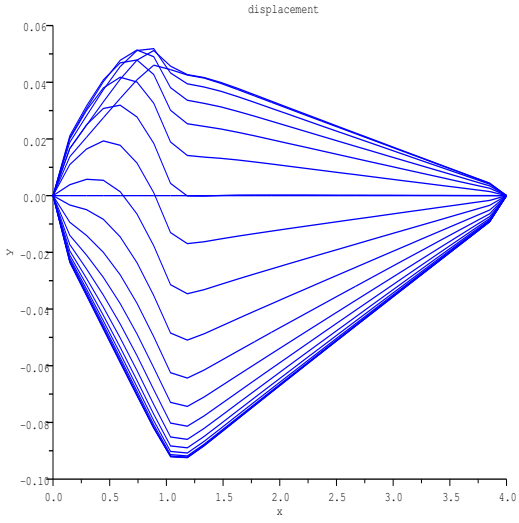
$$\frac{1}{dx^2}\begin{pmatrix} 2 & -1 & 0 & \cdots & 0 \\ -1 & 2 & -1 & \ddots & \vdots \\ 0 & \ddots & \ddots & \ddots & 0 \\ \vdots & \ddots & -1 & 2 & -1 \\ 0 & \cdots & 0 & -1 & 2 \end{pmatrix}$$

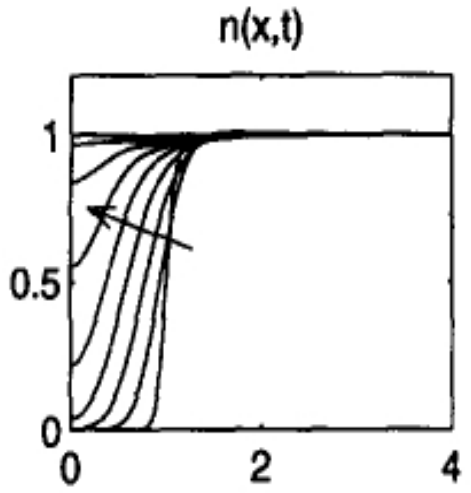
Once we have the values of the displacement for the next time step we proceed with the cell and ECM matrix values.

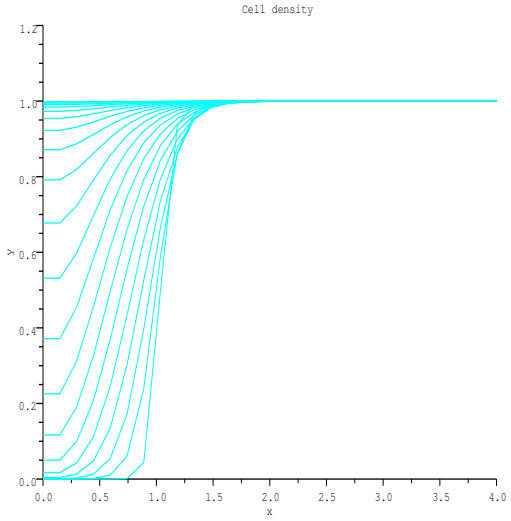
Results on one dimension

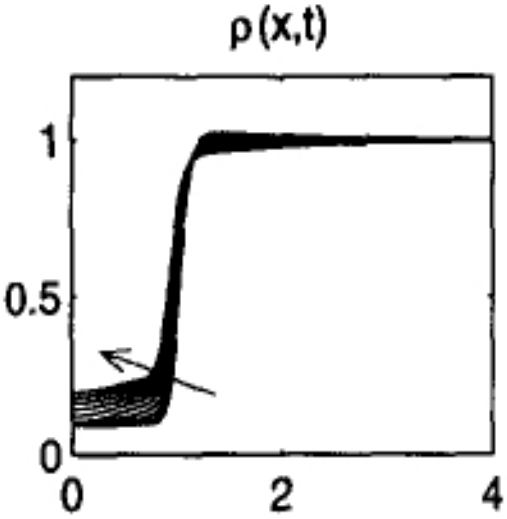
The results we expect is that both the cell and ECM density return to the unity at the end of time, and that u will go to zero, having normal skin again.

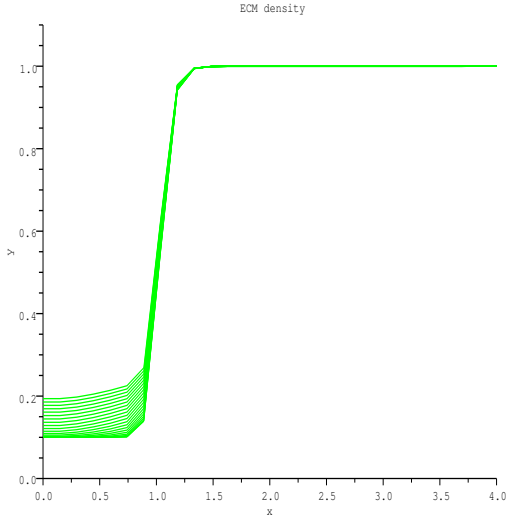












On the left hand side we have plotted the results obteined for the displacement, cell density and ECM matrix density in the article by Maini et al, on the right we have

plotted our results. As it can be seen the graphs are very similar.

Two Dimensional case

Now that we have seen that our approach is good, we can proceed to model the two dimensional case. The first thing to do in these part is to transform the equations into 2d equations, thus getting:

$$\frac{\partial n}{\partial t} + div\left[n\frac{\partial \mathbf{u}}{\partial t} + \chi(\rho)n\nabla\rho - D(\rho)\nabla n\right] = P(n,\rho) \tag{11}$$

$$\frac{\partial \rho}{\partial t} + div(\rho\frac{\partial \mathbf{u}}{\partial t}) = B(n,\rho) \tag{12}$$

$$-div\left[\mu\frac{\partial(\nabla\mathbf{u})}{\partial t} + E\nabla\mathbf{u} + \tau(n,\rho)I\right] + F(n,\rho) = 0 \tag{13}$$

since $u = (u_1, u_2)$, the above set of equations can be written explicitly as

$$\frac{\partial n}{\partial t} + \frac{\partial}{\partial x}\left[n\frac{\partial u_1}{\partial t} + \chi(\rho)n\frac{\partial \rho}{\partial x} - D(\rho)\frac{\partial n}{\partial x}\right] + \frac{\partial}{\partial y}\left[n\frac{\partial u_2}{\partial t} + \chi(\rho)n\frac{\partial \rho}{\partial y} - D(\rho)\frac{\partial n}{\partial y}\right]$$
$$= P(n,\rho) \tag{14}$$

,

$$\frac{\partial \rho}{\partial t} + \frac{\partial}{\partial x}(\rho\frac{\partial u_1}{\partial t}) + \frac{\partial}{\partial y}(\rho\frac{\partial u_2}{\partial t}) = B(n,\rho) \tag{15}$$

$$-\left[\mu\begin{pmatrix}\frac{\partial(\Delta u_1)}{\partial t}\\ \frac{\partial(\Delta u_2)}{\partial t}\end{pmatrix} + E\begin{pmatrix}\Delta u_1\\ \Delta u_2\end{pmatrix} + \begin{pmatrix}\frac{\partial \tau}{\partial x}\\ \frac{\partial \tau}{\partial y}\end{pmatrix}\right] + F(n,\rho) = 0 \tag{16}$$

In the displacement equation we have decomposed it, in two parts u_1 that describes the movement of the ECM matrix in the $x-$ direction and u_2 for the $y-$ direction.

Boundary and Initial conditions

By defining the initial wound space as, $0 \leq x \leq L$,$0 \leq y \leq h$. Therefore, we have:

$$n(x,0,t) = n(x,\infty,t) = \rho(x,0,t) = \rho(x,\infty,t) = 1$$

$$\frac{\partial n}{\partial x}(0,y,t) = \frac{\partial n}{\partial x}(\infty,y,t) = \frac{\partial \rho}{\partial x}(0,y,t) = \frac{\partial \rho}{\partial x}(\infty,y,t) \tag{17}$$

$$u(x,0,t) = u(x,\infty,t) = u(0,y,t) = u(\infty,y,t) = 0$$

For the boundary conditions we assume that the walls of our domain are complete tissue, we mean without any damage, so we suppose the density for both the ECM matrix and the cell are equal to one. As initial condition we have no displacement at time zero. Also we neglect any flux coming from the boundary sides $x = 0$ and $x = N_x$.

Discretization

We discritize and make some simplification of the above using central difference for the second derivative, forward difference and backward difference and also explicit Euler scheme in time to get the following equations;

In order to discretise well, we have to pay more attention on $\frac{\partial}{\partial x}(n\frac{\partial u_1}{\partial t})$ which depends on the direction of the velocity. Therefore, we will use the following notations in

order to pay attention in the discretization whether the velocity is in the positive direction or negative direction. If the flow is in the positive x -direction or y-direction we will use backward difference approximation , forward difference otherwise. Therefore, we have:

If $\frac{\partial u_1}{\partial t} < 0$ we have:

$$C_1 = \frac{n^N_{i+1,j}(u^{N+1}_{1(i+1,j)} - u^N_{1(i+1,j)}) - n^N_{i,j}(u^{N+1}_{1(i,j)} - u^N_{1(i,j)})}{dx},$$

$$G_1 = \frac{\rho^N_{i+1,j}(u^{N+1}_{1(i+1,j)} - u^N_{1(i+1,j)}) - \rho^N_{i,j}(u^{N+1}_{1(i,j)} - u^N_{1(i,j)})}{dx}. \tag{18}$$

If $\frac{\partial u_1}{\partial t} > 0$ we have:

$$C_1 = \frac{n^N_{i,j}(u^{N+1}_{1(i,j)} - u^N_{1(i,j)}) - n^N_{i-1,j}(u^{N+1}_{1(i-1,j)} - u^N_{1(i-1,j)})}{dx},$$

$$G_1 = \frac{\rho^N_{i,j}(u^{N+1}_{1(i,j)} - u^N_{1(i,j)}) - \rho^N_{i-1,j}(u^{N+1}_{1(i-1,j)} - u^N_{1(i-1,j)})}{dx}. \tag{19}$$

If $\frac{\partial u_2}{\partial t} < 0$ we have:
,

$$C_2 = \frac{n^N_{i,j+1}(u^{N+1}_{2(i,j+1)} - u^N_{2(i,j+1)}) - n^N_{i,j}(u^{N+1}_{2(i,j)} - u^N_{2(i,j)})}{dy}$$

$$G_2 = \frac{\rho^N_{i,j+1}(u^{N+1}_{2(i,j+1)} - u^N_{2(i,j+1)}) - \rho^N_{i,j}(u^{N+1}_{2(i,j)} - u^N_{2(i,j)})}{dy}. \tag{20}$$

If $\frac{\partial u_2}{\partial t} > 0$ we have:

$$\frac{n^N_{i,j}(u^{N+1}_{2(i,j)} - u^N_{2(i,j)}) - n^N_{i,j-1}(u^{N+1}_{2(i,j-1)} - u^N_{2(i,j-1)})}{dy},$$

$$G_2 = \frac{\rho^N_{i,j}(u^{N+1}_{2(i,j)} - u^N_{2(i,j)}) - \rho^N_{i,j-1}(u^{N+1}_{2(i,j-1)} - u^N_{2(i,j-1)}).}{dy} \tag{21}$$

Therefore, we have our discritized equations as:

$$n_{i,j}^{N+1} = n_{i,j}^{N} - C_1 - \frac{dt(\chi_{i,j}^{N} n_{i,j}(\rho_{i+1,j}^{N} - \rho_{i,j}^{N}) - \chi_{i-1,j}^{N} n_{i-1,j}(\rho_{i,j}^{N} - \rho_{i-1,j}^{N}))}{dx^2} +$$

$$\frac{dt(D_{i,j}^{N}(n_{i+1,j}^{N} - n_{i,j}^{N}) - D_{i-1,j}^{N}(n_{i,j}^{N} - n_{i-1,j}^{N}))}{dx^2}$$

$$-C_2 - \frac{dt(\chi_{i.j}^{N} n_{i,j}^{N}(\rho_{i,j+1}^{N} - \rho_{i,j}^{N}) - \chi_{i.j-1}^{N} n_{i,j-1}^{N}(\rho_{i,j}^{N} - \rho_{i,j-1}^{N}))}{dy^2} +$$

$$\frac{dt(D_{i,j}^{N}(n_{i,j+1}^{N} - n_{i,j}^{N}) - D_{i,j-1}^{N}(n_{i,j}^{N} - n_{i,j-1}^{N}))}{dy^2} + dtP_{i,j}^{N}$$

$$\rho_{i,j}^{N+1} = \rho_{i,j}^{N} - G_1 - G_2 + dtB_{i,j}^{N}$$

From the viscolastic equation we get the following equations:

$$(\frac{\mu}{dt} + E)(\frac{u_{1i+1,j}^{N+1} - 2u_{1i,j}^{N+1} + u_{1i-1,j}^{N+1}}{dx^2} + \frac{u_{1i,j+1}^{N+1} - 2u_{1i,j}^{N+1} + u_{1i,j-1}^{N+1}}{dy^2}) + F_{1i,j}^{N+1} =$$

$$\frac{-\mu}{dt}(\frac{u_{1i+1,j}^{N} - 2u_{1i,j}^{N} + u_{1i-1,j}^{N}}{dx^2} + \frac{u_{1i,j+1}^{N} - 2u_{1i,j}^{N} + u_{1i,j-1}^{N}}{dy^2}) + \frac{\tau_{i+1,j}^{N} - \tau_{i,j}^{N}}{dx}$$

$$-(\frac{\mu}{dt} + E)(\frac{u_{2i+1,j}^{N+1} - 2u_{2i,j}^{N+1} + u_{2i-1,j}^{N+1}}{dx^2} + \frac{u_{2i,j+1}^{N+1} - 2u_{2i,j}^{N+1} + u_{2i,j-1}^{N+1}}{dy^2}) + F_{2i,j}^{N+1} =$$

$$-\frac{\mu}{dt}(\frac{u_{2i+1,j}^{N} - 2u_{2i,j}^{N} + u_{2i-1,j}^{N}}{dx^2} + \frac{u_{2i,j+1}^{N} - 2u_{2i,j}^{N} + u_{2i,j-1}^{N}}{dy^2}) + \frac{\tau_{i,j+1}^{N} - \tau_{i,j}^{N}}{dy}$$

where

$$F_{1i,j}^{N+1} = s\rho^N u_{1(i,j)}^{N+1}$$
$$F_{1i,j}^{N+1} = s\rho^N u_{2(i,j)}^{N+1}$$

When we combine the above equation we come up with the following systems:

$$(\frac{\mu}{dt} + E)A_1 u_1^{N+1} = \frac{\mu}{dt} A_2 u_1^N + \tau_1^N$$

$$(\frac{\mu}{dt} + E)A_1 u_2^{N+1} = \frac{\mu}{dt} A_2 u_2^N + \tau_2^N \tag{22}$$

where A_1 and A_2 are symmetric tridiagonal band matrix and definite positive.

When implimenting this problem we considered our domain as :

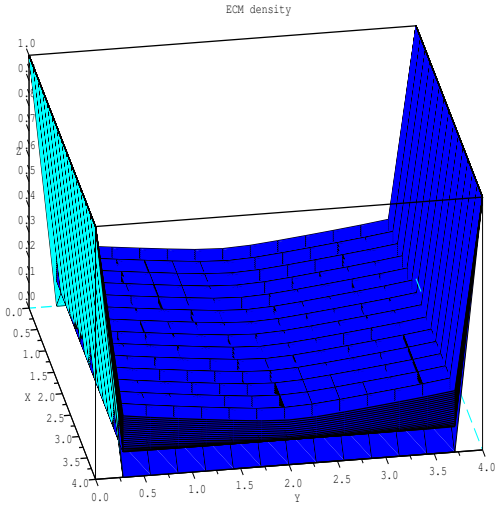
$0 \leq x \leq L$,$0 \leq y \leq h$, with the given boundary conditions on the walls of our domain.

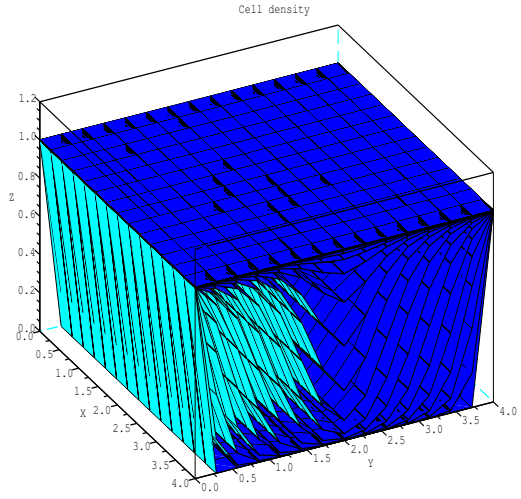
Implementation.

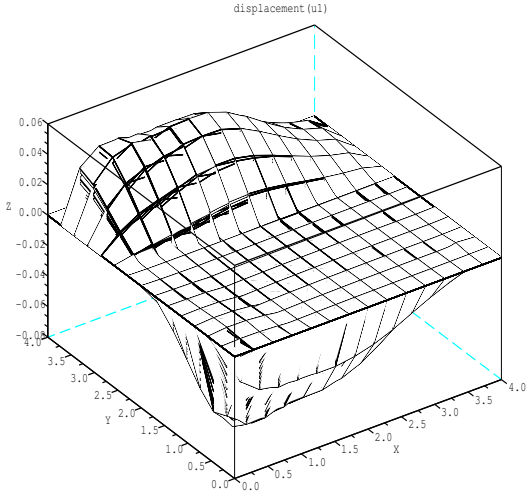
We add many things to our code for having the program of the two dimensional case. As before we will have a linear system to solve, but the shape of our matrix has changed, now we have a tridiagonal matrix with bands.

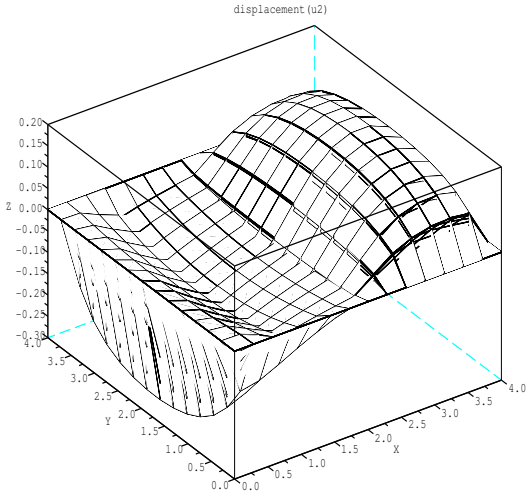
$$\begin{pmatrix}
\frac{2}{dx}+\frac{2}{dy} & \frac{-1}{dx} & 0 & \frac{-1}{dy} & 0 & \cdots & & & 0 \\
\frac{-1}{dx} & \frac{2}{dx}+\frac{2}{dy} & \frac{-1}{dx} & \ddots & & & & & \vdots \\
0 & \frac{-1}{dx} & \ddots & 0 & & & & & \\
\frac{-1}{dy} & \ddots & 0 & & \frac{-1}{dx} & & & & \\
0 & & & \frac{-1}{dx} & & \frac{-1}{dx} & & & 0 \\
\vdots & & & & \frac{-1}{dx} & & 0 & & \frac{-1}{dy} \\
 & & & & & 0 & & \frac{-1}{dx} & 0 \\
 & & & & & & \frac{-1}{dx} & & \frac{-1}{dx} \\
0 & \cdots & & & 0 & \frac{-1}{dy} & 0 & \frac{-1}{dx} & \frac{2}{dx}+\frac{2}{dy}
\end{pmatrix}$$

Results




Cell density
Z
X
Y

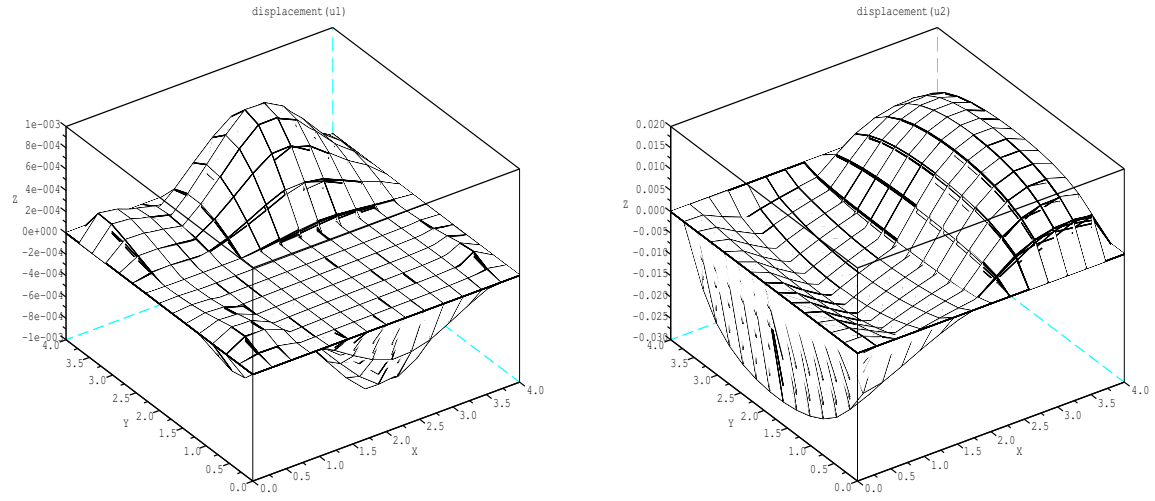

displacement(u1)
Z
Y
X



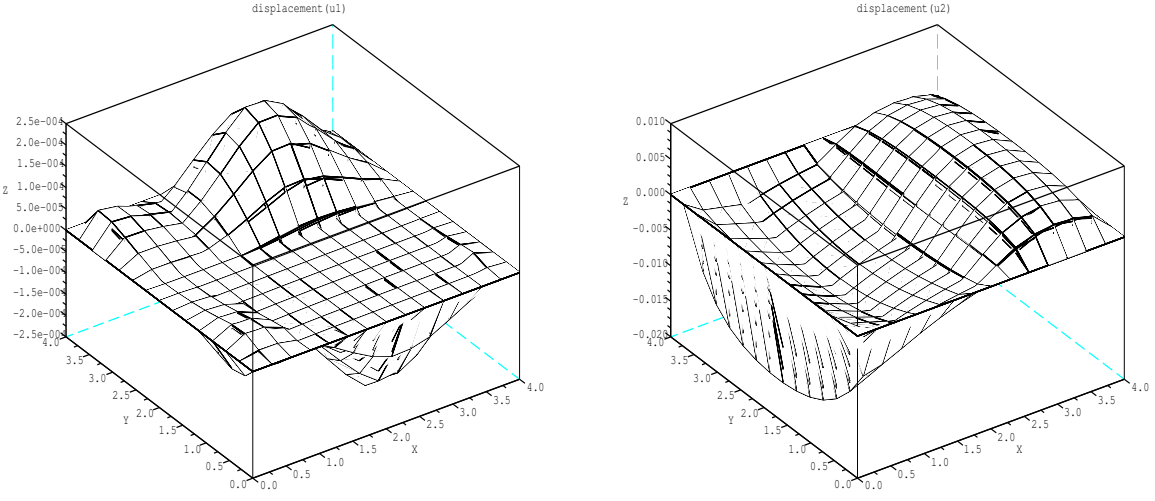
As we can see in the graphs for the ECM matrix and the cell density, the behaviour observed in the 1d case is clearly followed in the 2d case. The cells migrate from the wound edges towards the center, since we do not have flow in the x direction at the boundaries the main contribution comes from the y direction. As expected the cells repopulate the wound area achieving a density of 1. Since the ECM matrix remodelling takes a long time compared with the cell proliferation, we only observe a small change in its original profile.

In the case of the displacement we can see that the cells move from the walls and later they accelerate until they reach the center of the wound. Some cells start to move from the wall in the negative $x-$direction while other cells move from the wall in the positive x-direction and the same thing happens in the $y-$direction. This is true because we do not expect the cells to move out of the wound but into the wound.

Now we will study the effects of the parameters on the solution, in particular we will vary value the viscocity and elasticity to see their impact on the model.

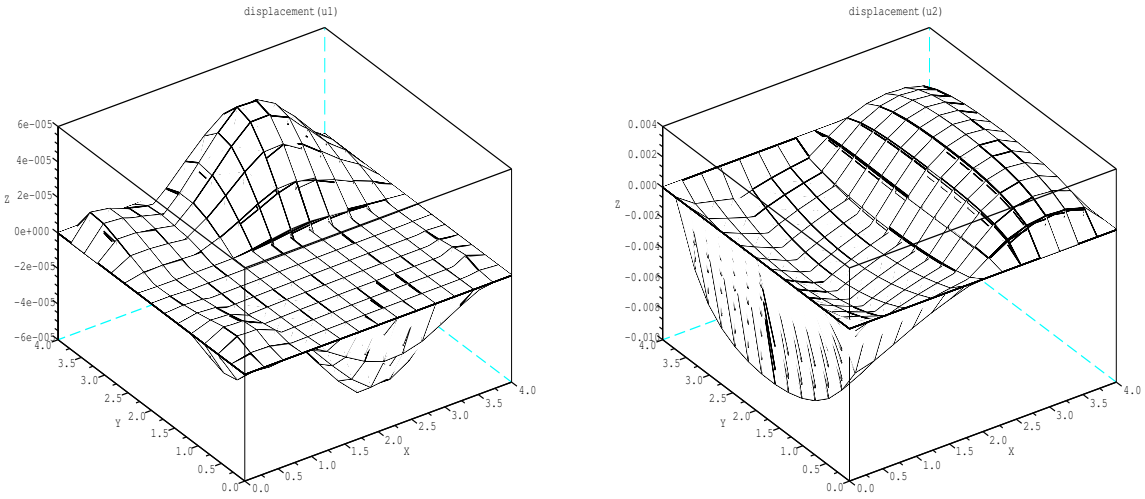


(a) $\mu = 10$

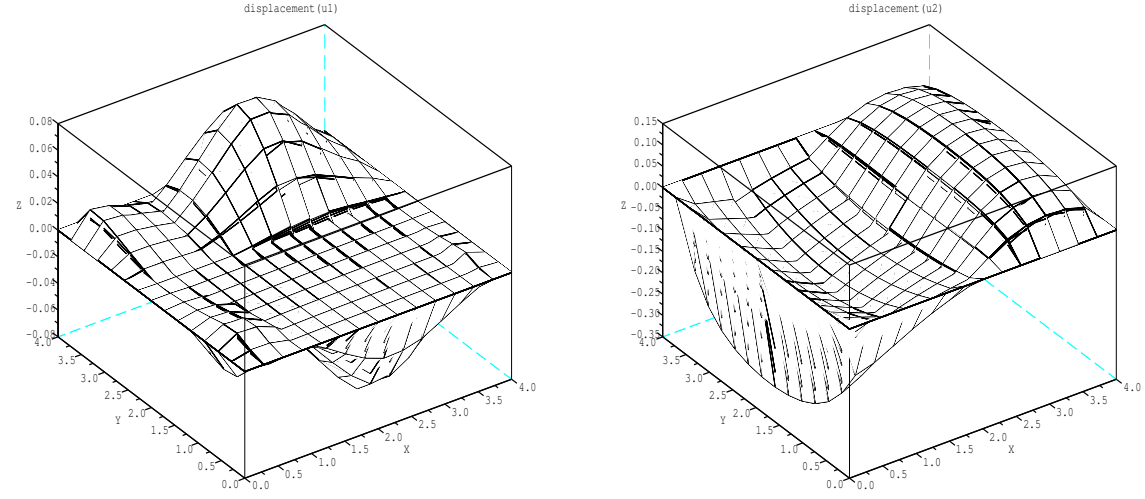


(b) $\mu = 20$

Figure 1: Left hand graphs are the desplacement in x direction. The right ones are for y direction

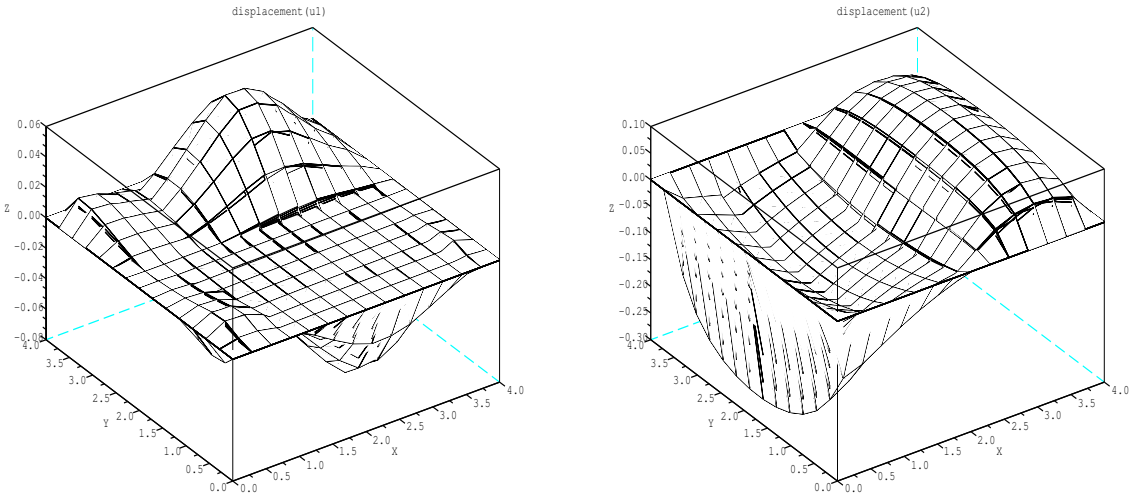


(a) $\mu = 40$

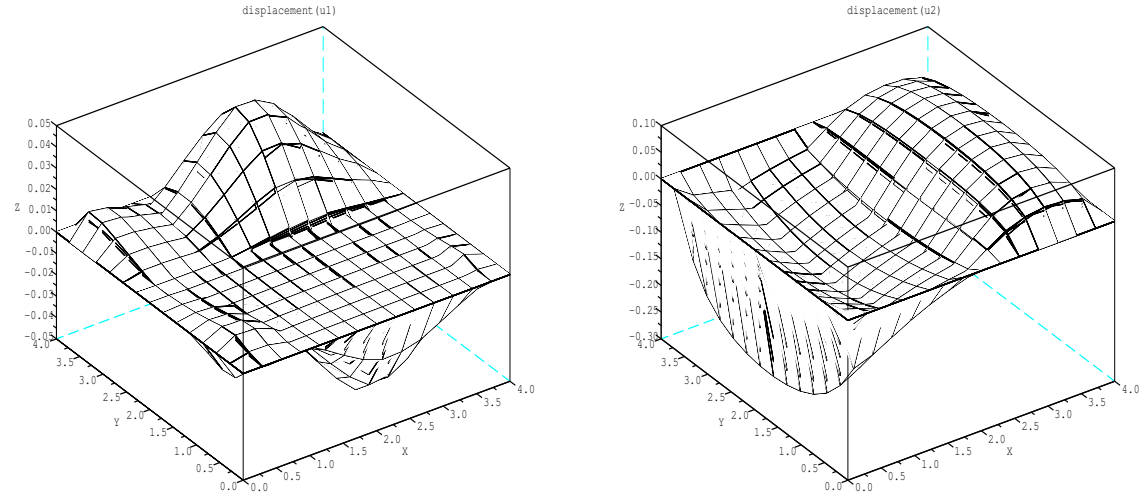


(b) $E = 10$

Figure 2: Left hand graphs are the displacement in x direction. The right ones are for y direction



(a) $E = 20$



(b) $E = 40$

Figure 3: Left hand graphs are the displacement in x direction. The right ones are for y direction

We can see from the above graphs that as we increase the value of viscosity coefficient the displacement become smaller meaning that the cells do not move faster inside the media of ECM.

When we make the viscosity coefficient smaller the displacement become become bigger which implies that the cells move fast through the ECM. This is what is physically expected because the higher the viscosity of the medium the more resistance to the movement of cells. But as we vary the elastic coefficient we see a very small change, which means it does not make a impact on the movement of cells. Also as we take a large value of viscosity coefficient the ECM take a longer time to develop, the same is true for the cell density.