Investigations of a Tissue Growth Model: On the Mechanism of Wing Size Determination in Fly Development

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1 Abstract

Understanding why cellular tissues in animals stop growing at a certain point, is somewhat of a conundrum that is not completely understood by modern day science. Many models have been created to better understand this phenomena. One such model was constructed by Hufnagel, Teleman, and their team which explored the imaginal disc in a wing of Drosophila. This group determined that decapentaplegic (Dpp) morphogen gradient in the wing does not adapt to disc size. They proposed that the level of Dpp in the cell, combined with the mechanical stress in the tissue caused by the nonuniformity of Dpp, will eventually cause tissue growth to cease. This paper follows up on the work done by Hufnagel and his team. A model for cell growth and division was created that accounts for the length of the Dpp strip, and pressure (both tension and compression) in the cell. The results obtained from this work show that the number of cells in the tissue will eventually reach a constant value after a long enough time period, due to the tensile pressure and Dpp distribution, much like the work done by Hufnagel and his team. Additional findings include that as the length of the Dpp strip is proportional to tissue size. The data shows that as tensile pressure in the cell reaches large enough values, tissue growth is ceased. Tensile pressure encourages cells to divide, however this model also shows that as the tensile pressure is increased the final tissue size will be decreased. This analysis further continues work on tissue development.

2 Statement of the Problem

Many researchers have searched for the mechanism that controls the growth, and cessation of growth, in cells. Hufnagel, Teleman, and their team attempted to determine and understand how a "growing tissue knows when it has achieved its correct final size" [1] with regards to the wing of Drosophila (a type of fruit fly). In other words, what causes the tissue to cease growing and the cells to stop diving. They also explored how the tissue stops growing evenly, even though the morphogen gradient is not uniform throughout the cell. In this paper, the work others' have done on the subject is explored along with what Hufnagel, Teleman, and their group have researched in order to understand this process. If these mechanisms were understood, researchers may better understand and possibly control cells that grow and divide too much, or cease too early.

3 Background

This mechanism has been studied by many other scientists as well. For example, Stephen Day and Peter Lawrence [2] searched for this mechanism in the Drosophila. Their conclusion was that the steepness of the morphogen gradient determines whether or not a cell will grow and divide. Morphogen is any type of signaling molecule that acts on cells to cause a specific response, working in a concentration-dependent manner. This means that whether the cell responds to the morphogen is based

on the amount of morphogen being supplied to the cell. The specific morphogen in the Drosophila wing causing cell growth is known as decapentaplegic, or Dpp. There is a strip of cells located in the center of the wing from which the Dpp diffuses out of and moves toward the edge of the tissue, creating a gradient, (see Figure 1 A,B). Day and Lawrence believed that as the wing grows this "strip" of Dpp is stretched, reducing the gradient and causing the Dpp to fall below the threshold needed for continued division and growth. This conclusion was found to be false by Hufnagel and his team. His group found that found that the length of the Dpp strip is independent of the disk size [Figure 1 D].

Other models have been proposed that do not depend on the morphogen gradient, but are instead based on the idea of cell growth dependent on positional values. A cell's positional value is it's spatial coordinates, or where it is located in relation to its neighbors. As cells divide, the neighbors change and cells are moved around, lowering the positional value. When the positional value of a cell is too low, below a specific threshold, cell growth stops. However, experiments have been conducted, such as the one in Germany [4], which showed that cells moved from the location governed by it's positional value would still grow based on the concentration of Dpp.

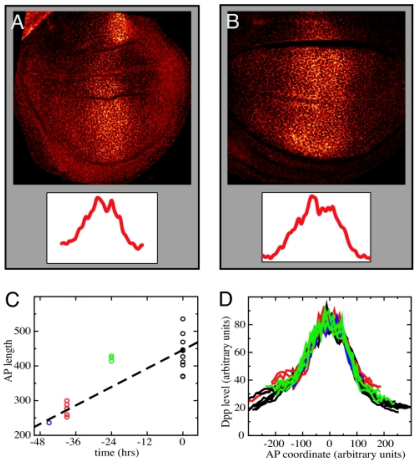


Figure 1. This figure, from the paper by Hufnagel and Teleman [1], shows the "strip" of cells containing Dpp, running vertically down the imaginal disc (A and B). The dpp is represented by the fluorescence in the wing. The brighter the color, the higher the concentration of Dpp. The concentration lessens as the Dpp moves toward the edges of the wing, creating the gradient. C shows the growth of the anterior-posterior length in the imaginal disc. The different colored circle represent different stages of the fly during development. D is a graph of the level of Dpp plotted against the length of the imaginal disc. The colors correspond to various ages of the Drosophila. From the overlaid graphs, Hufnagel's team concluded that the length size of the imaginal disk is independent of the level of Dpp produced.

4 Proposal

The work performed by Hufnagel, Teleman, and their team further confirmed that cell growth is governed by the morphogen gradient. As long as the gradient stayed above a certain level, cell growth would continue. Hufnagel and his team hypothesized that tissues stopped growing uniformly, not due to morphogen or positional value thresholds, but instead due to negative feedback from mechanical stress on the cells. Negative feedback is when the outcome of a process influences the process to change. In this instance, the morphogen that causes cells to grow and divide also causes the cells to become more 'squished', placing stress on the cells and eventually causing the cells to stop growing.

This paper continues their work by examining the qualitative and quantitative representations of their model. A generalized version of a tissue growth model was coded in MATLAB and compared to the results produced by Hufnagel's team.

5 Hufnagel's Model

The model for Hufnagel's project makes several key assumptions about the biology and cell structure of cells in the imaginal disc. Firstly, it is assumed that Dpp is the only morphogen required to promote cell growth. The other morphogen, Wingless (Wg), is not assumed to impact cell development. The cells in this model began in mechanical equilibrium, where the total energy of the system is minimal. This value depends on the location of the cell, the perimeter of the cell, and the stress from its neighboring cells. The cell geometry is structured as polygons, as shown in Figure 2. This is a two dimensional model. Each cell has its own position, shape and height dependent on its mechanical equilibrium.

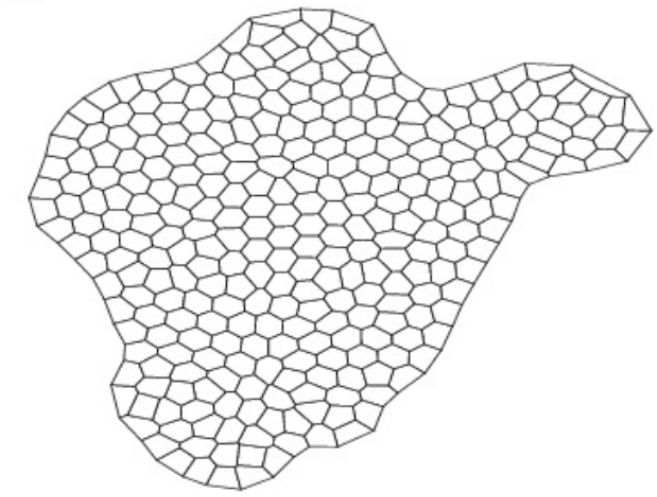


Figure 2. (Hufnagel and Teleman [1]) This figure shows the structure of the cells in this model. Each polygon represents a cell.

When a cell divides, it is assumed that the division is a random Poisson process with a fixed rate. This rate will depend only on the morphogen concentration and the mechanical stress in the cell. It is also assumed that upon cell division, the daughter cells grow to their full size instantaneously, and are in mechanical equilibrium. This process continues until the induced mechanical stress forces the cells to stop growing, completing the wing.

5.1 Mathematical Model

As discussed in the previous section, the first assumption in the tissue growth arrest model concerns the morphogen distribution and gradient. Only the Dpp morphogen was considered in this model with the assumption that is it secreted by a single cell, at a constant rate, in the middle of the tissue. The time necessary to establish the spatial morphogen profile is short compared to the rates needed for cell growth and cell division. The spatial dynamics are governed by diffusion and a constant degradation rate. Thus, the result is a fixed morphogen distribution profile that decays exponentially with distance to

the source. The static axial morphogen gradient takes the form:

$$M(r) = me^{-r/l},$$

Where r is the position, m is the morphogen amplitude, and l is the characteristic length scale. (Recall from section *2 Background*, the characteristic length scale is determined by the length of the strip of cells from which the Dpp diffuses from and moves towards the edge of the tissue, creating the gradient.)

With respect to mechanical equilibrium, it is assumed that the cell layer reaches this state, with the cells adhering to each other, on a time scale faster than that of cell growth and division. The surface tension term (depending on the perimeter of the cell), a bulk contribution term (defining the preferred volume of the cell), a term coupling neighboring cells, and a term that regulates the stiffness of the cell's thickness all comprise the mechanical properties of a cell in the tissue layer. The equilibrium positions of a tissue with N_C cells and N_V vertices are found by the following Hamiltonian:

$$H(r_1,\ldots, r_{NV}, \xi_1,\ldots, \xi_{NC}) = \sum_{\alpha=\text{cell}} [\, \rho_\alpha + a(V_\alpha - V_0)^2 + b \sum_{\beta=\nu(\alpha)} (\xi_\alpha - \xi_\beta)^2 + c(\xi_\alpha - 1)^2]$$

where ρ_α is the cell perimeter, V_α is the cell volume, and ξ_α is the cell height. With $V_\alpha = A_\alpha \xi_\alpha$, the cell area A_α is determined by the positions of its vertices, r_i. $\nu(\alpha)$ denotes the collection of all cells adjacent to cell α. Deviations of V_α from V_0 are accounted for by a, b imposes a penalty on the variation of ξ_α between adjacent cells, and c controls deviations of ξ_α from an unstressed value (without loss of generality, Hufnagel and his team take $\xi_\alpha = 1$).

During mechanical equilibrium, the energy of the system is minimal. The above Hamiltonian (which minimizes the energy of the system) suggests that the volume V_α of the cell should be close to its intrinsic volume $V_{0,a}(t)$, while also minimizing the perimeter and differences in thickness to its neighbors. The intrinsic volume of the cell $V_{0,a}(t)$ is defined by the time dependent cell mass. [1]

The cell geometry is altered by cell division (and resultant growth of daughter cells) and by growth related topical rearrangement of the cells. As described in section *3 The Model*, cells divide at random as a Poisson process, but at a fixed rate, once the cell's volume exceeds a certain threshold value. (Again, without loss of generality, this value is set to 1.)

Newly born cells grow with a rate k directly following cell division as:

$$d/dt(V_{0,a}(t)) = k_a \text{ and } V_{0,a}(0) = \underline{V}_{0,a}$$

where $\underline{V}_{0,a}$ is the volume of the cell a directly after division. k_a is the growth rate of cellular volume regulated by both morphogen concentration and mechanical stress. At distances large compared to cell size, the mechanical equilibrium interaction (influenced by cell geometry and proliferation) between cells

is reduced to that of continuum elasticity.

The experiment shows that the dependence on position and time arises from its dependence on the morphogen level and on stress. This parameterization can be expressed as:

$$\partial\,\gamma(r,t)=\Gamma(M(r,t),p(r,t))$$

In this equation, M represents the morphogen concentration and p represents the local uniaxial compression stress within the cell layer.

Elastic strain energy is the energy stored in an elastic material upon deformation, which depends on displacement of all direction and transverse strains. The elastic energy caused by displacement can be represented as $E=\frac{K}{2}x^2$, where E is energy, K is the spring constant coefficient, and x is the displacement. By determining the minimal elastic strain energy, one can find the relation between pressure and layer height. The equation of minimal elastic energy is stated as:

$$H=\int d^2r[\tfrac{\mu}{2}(\Delta u_{ab}-\tfrac{\delta_{ab}}{2}\Delta u_{cc})^2+\tfrac{K}{2}(\Delta u_{cc}-\Delta t\gamma)^2+\tfrac{w^2}{2}(\partial_a\Delta\xi)^2+\tfrac{\beta}{2}\Delta\xi(\Delta u_{cc}-\Delta t\gamma)+\tfrac{K_\xi}{2}\Delta\xi^2]$$

And through calculation, this leads to following equation:

$$-w^2\nabla^2\xi(r,t)+\xi(r,y)=ap(r,t)$$

In this equation, ξ represents the layer height and p is the stress. Two major conclusions are drawn from this equation. First, the layer height $\xi(r,t)$ is proportional to the local stress. Therefore the layer height (ξ) is used in the growth model, rather that stress (p) which yields the following model:

$$\gamma=\Gamma(\xi,M)=\Gamma M(M)[1-q(\xi-\xi_0)^2]$$

Second, the layer height (ξ) cannot vary too rapidly with position and has a characteristic length *w,* below which its variation is suppressed.

5.2 Computational Model

Hufnagel made a few assumptions to simplify the model; he assumed that all cell has same size and same shape, after the cell's splitting daughter cells immediately grow to full size, and cells behave like solid rather than fluid, thus the deformation is very small (0.1%). In order to further simplify the model, all Hufnagel's assumptions were used in our model, and instead assume all cells are hexagonal, all cells were treated as squares.

As Hufnagel pointed out in his paper, local pressure is linearly proportional to its position to the center of the tissue. Therefore, after a cell splits, the daughter cell will grow to full size, and push cells close it to the direction where the pressure is lowest. Thus, the process is equivalent to adding new cells to the lowest pressure position on the edge, where the closest empty spot is near the splitting cells.

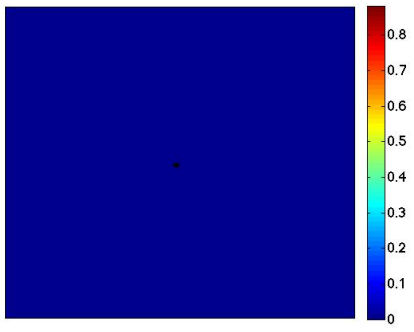
Based on these assumptions, the following algorithms were used to simulate the cell growing of imaginal discs (wings).

First an empty space was gridded and each grid was assigned a value to determine if it was a cell or an empty space. For each turn, all the grids were searched, and checked to find an empty space and/or cells. Then each cell was assigned the value of morphogen concentration and local pressure for this turn, which were calculated based on its position. After that each cell's possibility was calculated based on its morphogen concentration and local pressure. A random number generator was used to determine whether or not the cell split based on previous possibility. If the random number generator decided to split a cell, the daughter cell immediately grew to full size, and pushed a cell to the nearest empty spot. Empty spaces near the edges were checked to find the nearest place, and a new cell was generated in that empty spot. For each turn the total number of cells was calculated to find the tissue size and total growth rate. The edge of the tissue was also calculated to determine the bounding box's perimeter, which is the disc size of the tissue. The above steps were repeated until the maximum time was elapsed.

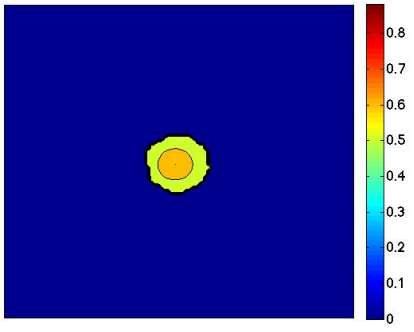
6 Results

6.1 Execution Results of the MATLAB Code for the Tissue Growth Model

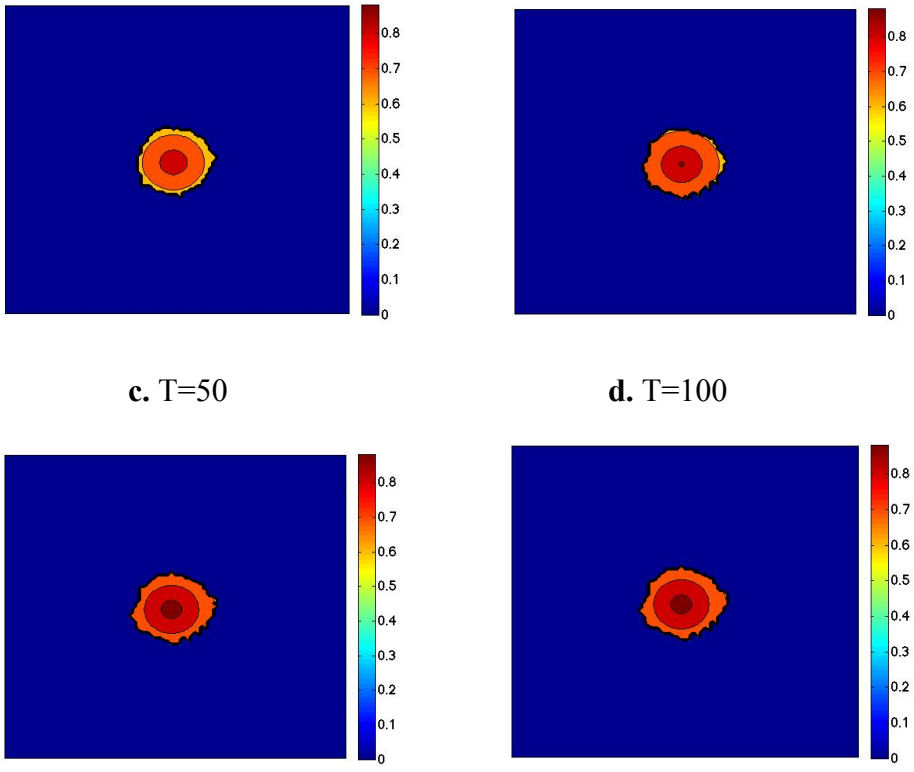
As described in the previous section, *5.2 Computational Model*, cell growth was modeled as a function of morphogen concentration and pressure. The following snapshots of cell growth were generated with a lambda value of 3 and a maximum time interval of 200 time steps. (See appendix for a copy of the MATLAB code used to generate this simulation.)



a. T = 1



b.T=20



c. T=50 **d.** T=100

e. T=150 **f.** T = 200

Figure 3. These figures, a-f, are snap-shots displaying cell growth at various time steps (T).

6.2 Cell Growth and Its Relation to the Morphogen Concentration and Gradient

Section 3, *Background*, describes the behavior of the cell in response to the amount of morphogen being supplied to it. Recall, the morphogen gradient is created by a strip of cells in the center of the wing from which the Dpp diffuse out of and moves toward the edge of the tissue. The following graph, Morphogen vs. Position, describes the spread of the Dpp in relation to distance from the center of the cell.

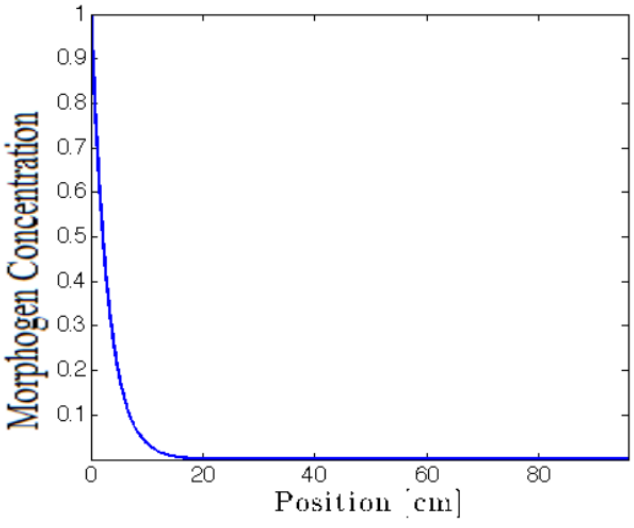


Figure 4. This graph illustrates exponential decay of morphogen concentration with distance from the source.

Recall also, that researchers Day and Lawrence hypothesized that as cell growth occurred, the strip of Dpp experienced tensile force, reducing the gradient, causing the Dpp to fall below a threshold value necessary for cell division and growth. The work performed by Hufnagel and his team proved this hypothesis false. Although the morphogen concentration with respect to distance behaves as the graph illustrated above, they determined the length of the Dpp strip was independent of the imaginal disc size.

From the previous graph, it is shown that the fixed morphogen profile exhibits exponential decay with distance from the source. The distance measurement is attenuated by the characteristic length scale, l (the length of the strip of Dpp). Experimentally, Hufnagel and his team found this value to be approximately constant. The graph below illustrates cell growth as a function of time for three different values of the characteristic length scale, l .

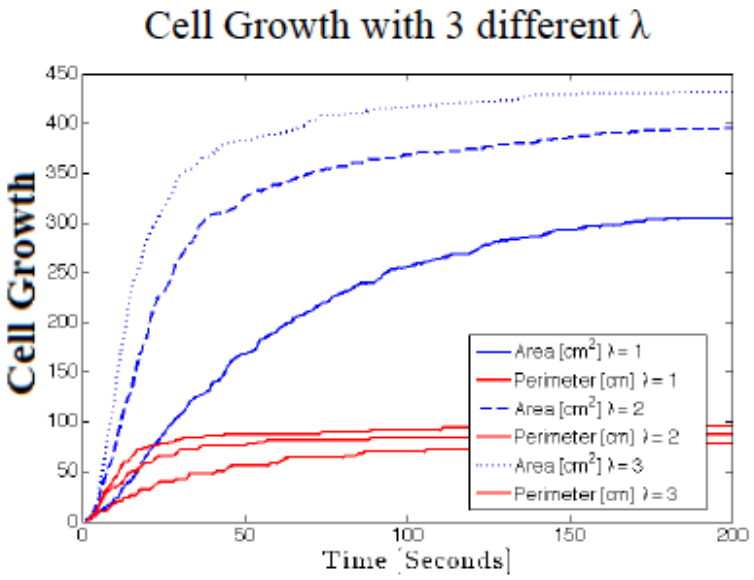


Figure 5. This graph plots cell growth with respect to time for 3 different Dpp strip lengths.

Although Hufnagel and his team concluded that l was independent of the imaginal disc size, it is clear from the above graph that as l increases, cell growth increases. This is an intuitive result, for when the strip of Dpp is larger, more morphogen can be distributed throughout the cell. This is on par with the results of Hufnagel's team as they determined that cell growth is dependent on the parameters of morphogen production and spreading.

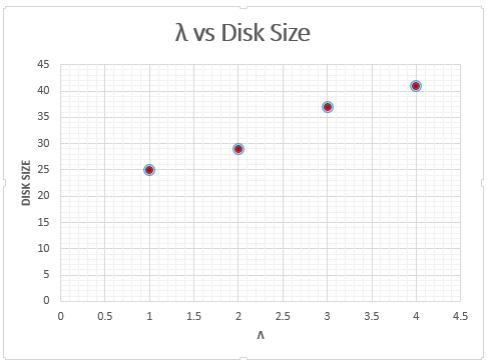


Figure 6. Graph a presents the findings from the computational model.

Graph b is the results of Hufnagel.

a. Computational model results **b.**Hufnagel's Results**.**

6.3 Cell Growth and Its Relation to Mechanical Stress

Hufnagel's team also investigated how mechanics regulate growth in imaginal discs. Thier model utilizes a cellular cycle beginning with a state of mechanical equilibrium, followed by cell growth and division, involving parameters such as cell perimeter, area, height, volume, rearrangement of cells, (T1 and T2 transitions), and elastic strain (both tension and compression), and concluding the cycle by returning to a state of equilibrium. The model proposed in this paper presents a more generalized version utilizing a more simplified cell geometry and tensile pressure as the sole source of mechanical stress.
Cell growth rate has a functional dependence on stress (pressure) within the cell layer for a fixed morphogen level. In the model discussed in this paper, the growth rate is presented as a function of pressure and modeled by the following quadratic equation:

$$\text{growth rate} = -1.053 \times (\text{pressure})^2 - (\text{pressure}) \times 0.527 + 0.987$$
with pressure values ranging [-1.25,0.75]

This equation was derived from a system of equations inferred graphically in [1]. The following figure, describing the relationship between growth rate and pressure, resulted from the computational model discussed in section 7.

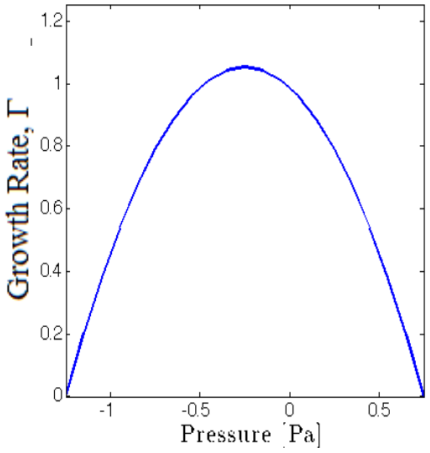
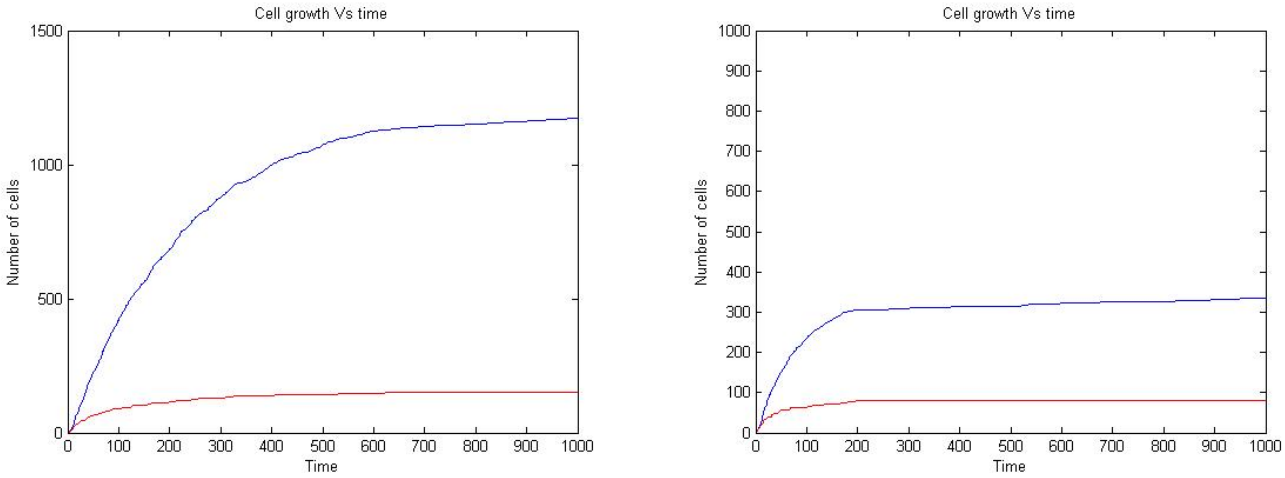


Figure 7. Growth rate as a function of pressure. Note that maximum growth occurs for p<0

One notable feature of the graph illustrates that at sufficiently high pressure values, cell growth is arrested completely. Negative pressure values correspond to cells under tensile stress. The computational model discussed in this paper

illustrates primarily this behavior due to coding design of cells ‘taking’ or ‘occupying’ the nearest empty space in the grid. Hufnagel’s work [1] accounts for positive pressure values corresponding to cells undergoing compression from neighboring cells. In both models, the maximum growth rate occurs for pressure values less than 0. It is concluded that tension promotes growth in epithelial cell cultures [1].

To better understand the effect of tensile pressure on growth rate, two graphs were generated. One illustrates cell growth rate as a function of time without a dependence on pressure. The second graph considers tensile pressure with respect to cellular growth as described above. In both graphs, the characteristic length scale, *l*, was set to 1.



a. Pressure Insensitive Group **b.** Pressure Sensitive Group

Figure 8. **The left graph, a, shows the growth of the tissue for the insensitive pressure group. The graph to the right, b, shows the growth of tissue cells for the pressure sensitive group.**

7 Conclusion

Hufnagel and his team found that morphogen distribution is independent of disc size but dependent on the parameters involving morphogen production and spreading. From their mathematical model and subsequent simulation, it was discovered that when the disc boundary reaches the stress threshold, the arrest of cell proliferation throughout the disc is induced by mechanical stress in the tissue.

The model and subsequent simulation presented in this paper is quantitatively similar to the results produced by Hufnagel’s team. Although the tissue growth model presented here generalizes cell geometry and simplifies the influences of mechanical stress, similar results were obtained, illustrating cellular growth dependence on morphogen concentration and tensile pressure.

Understanding cell and tissue growth mechanisms is beneficial towards further understanding of diseases caused by inadequacies in or excesses of cell growth and division.

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[2] Day SJ, Lawrence PA. Development (Cambridge, UK) 2000;127:2977–2987

[3] Tabata, T.. "Morphogens, their identification and regulation." *Development* 131.4 (2004): 703-712. Print.

[4] Lecuit T, Brook WJ, Ng M, Calleja M, Sun H, Cohen SM. Nature. 1996;381:387–393

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Appendix

MATLAB code generated for the Tissue Growth Model utilized in this paper:

```
%close all;
clf;
clear;

maxtime = 200;
lambda = 3;
m_threshold = 0.01;
p_min = -1.25;
p_max = 0.75;
```

```
[x,y] = meshgrid (0:0.01:1) % Establishing the Grid

TotalV = zeros(maxtime,1);
perimeter = zeros(maxtime,1);
[n,m] = size(x); % Determining the Size
u = -1*ones(n,m);
u(50,50) = 1;
pressure = 0*ones(n,m);

% Determining the Perimeter (Bounding Box)
title = 'test_';
cnt = 0;

for dt = 1:maxtime

   % Creating the Bounding Box

   iMin = n*10;
   jMin = m*10;
   iMax = 0;
   jMax = 0;

   for i = 1:n
      for j = 1:m
         if u(i,j) > 0
            if i < iMin; iMin = i; end
            if j < jMin; jMin = j; end
            if i > iMax; iMax = i; end
            if j > jMax; jMax = j; end
         end
      end
   end

   perimeter(dt) = 2*(iMax - iMin) + 2*(jMax - jMin);



   % Accounting for Growth
   for i = 2:n-1
      for j = 2:m-1

         if u(i,j) > 0



            d = sqrt((i-50)^2 + ( j-50)^2);

            % Cell Growth Caused by Morphogen

            p_m = exp(-d/lambda);
            if p_m < m_threshold
               p_m = 0;
            end

            % Cell Growth Caused by Pressure

            pressure(i,j) = - (d - perimeter(dt)/2)/50;


            p_p = -1.053*pressure(i,j)^2 - 0.527*pressure(i,j)+0.987;


            rn = rand();

            if p_m *p_p > rn
```

```
            % Finding the Closest Empty Spot

            d_old = 10000;
            for k = iMin - 10:iMax + 10
               for l = jMin - 10:jMax + 10
                  if u(k,l) < 0
                     d_new = sqrt((k-i)^2 + (l-j)^2);
                     if d_new < d_old
                        d_old = d_new;
                        kMin = k;
                        lMin = l;
                     end
                  end
               end
            end

            u(kMin,lMin) = 1;
         end
      end
   end
end

TotalV(dt) = sum(u(:) == 1);

    clf();
    h = figure('visible','off');

    contourf(x,y,pressure);
    colorbar();

    set(gca,'YTick',[])
    set(gca,'YTickLabel',{})

    set(gca,'XTick',[])
    set(gca,'XTickLabel',{})
    set(gca,'FontSize',16)
    set(gca, 'CLim', [0 0.88]);


    number = num2str(cnt);
    out = strcat(title,number);

    print(h,'-r80','-djpeg',out);

    cnt = cnt + 1;


end




figure(2); hold on;
plot(TotalV,'Linewidth',2.0);
plot(perimeter,'r','Linewidth',2.0); hold off;

set(gca,'FontSize',16)

ylabel('Cell Size','FontSize',22,'Interpreter','Latex')
xlabel('Time [Seconds]','FontSize',22,'Interpreter','Latex')
legend('Area [cm^2]','Perimeter [cm]','Location','Best');


%%
```

```
figure(3); % Plotting Growth Rate vs Pressure
r = [-1.25 : 0.1 : 0.75];
gr = -1.053*r.^2 - r.*0.527+0.987;

plot(r, gr,'Linewidth',2.0)

axis([min(r) max(r) min(gr) max(gr) + 0.2 ])

set(gca,'FontSize',16)

ylabel('Growth Rates $\gamma$ [cm$^2$/s]' ,'FontSize',22,'Interpreter','Latex')
xlabel('Pressure [Pa]','FontSize',22,'Interpreter','Latex')



%%

figure(4); % Plotting Morphogen vs Position
position = [0 : 0.1 : perimeter(maxtime)];
morphogen = exp(-position./lambda);

plot(position,morphogen,'Linewidth',2.0)


axis([min(position) max(position) min(morphogen) max(morphogen)])

set(gca,'FontSize',16)

ylabel('Morphogen [-]','FontSize',22,'Interpreter','Latex')
xlabel('Position [cm]','FontSize',22,'Interpreter','Latex')
```