Affects of surface detection on shape and growth of model tumor spheroids

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Abstract

This report discusses the cross-section shapes and growth curves created by different proliferation algorithms in an in silico model of in vitro multicellular tumor spheroid growth (MTS). The in vitro MTS system is a model of in vivo tumor growth. The in silico model described herein represents aspects of MTS growth, including its layered structure and growth patterns. An exploration of four algorithms designed to mimic different kinds of cellular proliferation demonstrates that different proliferation algorithms will create distinctly dissimilar cross section shapes and growth curves. Algorithms that minimize surface irregularity and area are the most likely to produce simulated MTS that achieve and maintain a constant size.

1. INTRODUCTION

In vitro multicellular tumor spheroid (MTS) are model systems for the growth of tumors in vivo before angiogenesis. The in vitro MTS grows exponentially, then linearly, and eventually stabilizes in size and cell number as cells within the center of the MTS begin to die from lack of oxygen and nutrient. An MTS develops a concentric layered structure with actively proliferating cells on the outside, quiescent cells in the middle, and a core of necrotic cells and debris [1]. There is some debate as to whether growth stabilization requires the presence of an internally generated necrotic inhibitor that slows the growth of the MTS or whether it is purely due to volume loss from dying cells balancing growth on the outside surface of the MTS [2,3]. We previously developed an agent-based in silico model of MTS growth that reproduced key aspects of in vitro MTS growth, including the layered structure and growth curves, but the model did so through the action of a necrotic inhibitor [4]. The current version of the model does not include a necrotic inhibitor and produces stabilization through a balance of cell growth and death. In the process of developing this model, we discovered that different proliferation algorithms lead to different in silico MTS shapes. We anticipate that these algorithms are placeholders for high level, in silico operating principles that will map to in vitro MTS counterparts. Understanding how each algorithm influences in silico phenotype may be critical to subsequent discovery of those operating principles.

This article explores the basis for the differing shapes and their affect on surface area and MTS growth. Four algorithms are considered: the first algorithm allows outside cells to proliferate in a random space-filling manner, the second calculates the "pressure" felt by individual cells to rank order cellular proliferation; the third calculates the "stress" felt by individual cells to rank order proliferation; the fourth calculates the "stress" felt by individual cells to influence the likelihood of proliferation. The space-filling algorithm creates an irregularly circular MTS, the pressure-based algorithm creates an MTS with a hexagonal cross section, the stress-based algorithm creates an MTS with a 12 sided cross-section, and the stress-likelihood-based algorithm creates an MTS that varies between a circle and a 12 sided shape.

2. METHODS

The model consists of agents representing cells that interact on a hexagonal 2D grid, as well as nutrients that exist on a second grid through which they diffuse based on a discrete version of the heat diffusion equation $du/dt = D\nabla^2 u$. In silico cells consume nutrient and follow rules that determine proliferation, movement, and state change. Cells exist in three different states: proliferating, quiescent, and necrotic. Cells in the proliferating and quiescent states consume nutrient and will change from proliferating to quiescent to necrotic if the nutrient value drops below the parameters *proNut* and *quiNut.* Once a cell enters the necrotic state it will disappear from the simulation after a random number of simulation cycles between 0 and *removeDelay.* When a cell disappears, an empty space remains, into which other cells can move. The empty space effectively moves in a random walk toward the outside of the MTS, eventually leaving the MTS when a cell on the surface moves into the space. Cellular proliferation is executed according to one of four possible algorithms: space-filling, pressure-based, stress-based, and a stress-likelihood-based.

2.1 Space-filling proliferation

At every simulation cycle (SC) each cell that is in the proliferating state will decrement the variable *prolifCounter,* which is initially equal to *prolifDelay.* When this variable reaches zero the cell will randomly choose an empty neighboring location and create a new cell in that location and then resets *prolifCounter* to *prolifDelay.*

2.2 Pressure-based proliferation

The pressure-based algorithm also decrements *prolifCounter* at each turn, but when this variable reaches zero (and if the cell is on the outside of the MTS) the cell does not proliferate, but instead increments the global variable *n* and resets *prolifCounter.* At the end of each SC the algorithm will create a number of new cells equal to *n.* To calculate where these new cells are placed, the algorithm queries each cell on the outside of the MTS for the number of empty spaces in its neighborhood. A list of cells is created ranked increasingly by the number of empty neighbors, so the cells with the fewest neighbors will be first on the list. The first *n* cells on the list are chosen to proliferate, which involves creating a new cell in one of their empty neighboring locations.

2.3 Stress-based proliferation

This algorithm is similar to the pressure-based algorithm, except that instead of being ranked by the number of empty neighbors, the list is ranked by the stress felt by each cell. The stress is calculated as follows. Each outside cell calculates its initial stress by counting the empty locations in its neighborhood and subtracting by 2. These cells then count the number of outside cells in their neighborhood. If a cell has 1, 3, or 4 outside neighbors its final stress equals its initial stress plus 1. If it has 2 outside neighbors it will sum the initial stress of the two outside neighbors and calculate its final stress based on this logic: initial stress minus 1 if the sum is less than 0; initial stress plus 1 if the sum is greater than 1; initial stress otherwise. Cells that are in pits and channels will have low stress values while cells on peninsulas will have high stress values.

2.4 Stress-likelihood-based proliferation

The stress-likelihood-based algorithm calculates the stress of cells similar to the stress-based algorithm, but no ranked list is created. Instead, every cell on the outside of the MTS has the possibility of proliferating when its *prolifCounter* reaches zero. The probability of a cell proliferating is biased based on its stress. The cells with the highest stress have the lowest probability of proliferation, while the cells with the lowest stress have the highest probability of proliferation. This algorithm generates less frequent proliferation because the likelihoods of proliferation for individual cells are typically low, so it is necessary to lower the value of *prolifDelay* to compensate.

2.5 Data analysis

MTS shape is assessed through empirical image analysis, while cross sectional area is measured by averaging MTS extents to calculate a radius and assuming the MTS has a roughly circular area. This similarity measure [5] mimics the method used for calculating the volume of in vitro MTS [6].

3. RESULTS

Simulations were run to 70,000 SCs. Cross sectional images were captured every 100 SCs. Cross sectional area is calculated in mm^2, assuming that individual cells have a diameter of 10 μm.

3.1 MTS Shape

MTS simulated with the space-filling algorithm exhibit a roughly circular shape with irregular borders, as seen in Figure 1a. New cells are added at random to the MTS and thus the circularity is not perfect. The diffusion algorithm, which acts over distance rather than steps causes the quiescent area of the MTS to be almost perfectly circular. When the MTS is grown with a higher *consumptionRate* (Figure 1b) quiescent and necrotic cells appear earlier in the simulation and increase the raggedness of the MTS border.

MTS simulated with the pressure-based algorithm maintain a consistent hexagonal shape throughout growth. In the initial stages the spheroid grows outward in a hexagonal conformation, adding new layers randomly so that the overall shape is maintained. Once cell death begins and spaces start to move outward, channels form at the edges of the spheroid. These channels may be filled in by the proliferation algorithm, but only under certain parameter settings. Although the MTS in Figure 1c and 1d appear similar, the MTS simulated with a higher *consumptionRate* will stabilize in growth, as shown in Figure 2. The shape remains consistent for different parameter settings.

The stress-based algorithm creates MTS with a 12 sided shape. This pattern of growth is readily visible at early stages but becomes less so later as empty spaces move outward from the center of the spheroid. The 12 sided shape also is conserved between different parameter settings.

MTS simulated with the stress-likelihood-based algorithm vary between a circular shape and a 12 sided shape. It is necessary to use a lower value of *prolifVal* (75 instead of 1500) to approximate the growth speed of the other algorithms because the biased probability of proliferation is significantly lower for individual cells and a *prolifVal* of 1500 creates a spheroid that grows very slowly and cannot be accurately compared to other runs. Again, the shape is roughly conserved at different parameter settings.

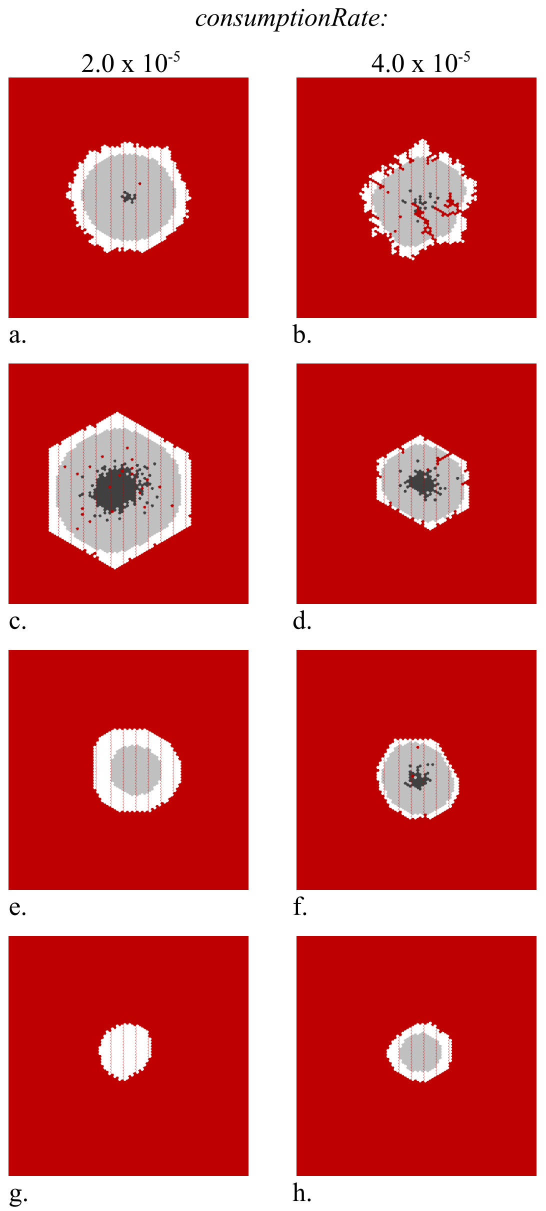

Figure 1. Simulated MTS cross section. **left** | *consumptionRate* of 2.0 x10^{-5}. **right** | *consumptionRate* of 4.0 x10^{-5}. All cross sections taken at 25,000 SCs. **a,b** | Space-filling-based algorithm. **c,d** | Pressure-based algorithm. **e,f** | Stress-based algorithm. **f,g** | Stress-likelihood-based algorithm.

Notice that although the pressure-based and stress-based algorithm allow every cell on the outside of the MTS to contribute to proliferation, there is an obvious difference in the size of the MTS created by the algorithms, as shown in Figure 1c and 1e.

3.2 MTS Growth

There is a striking difference in the overall growth curves of the various simulations. When *consumptionRate* is set to 2.0x10^{-5}, MTS simulated with the stress-based algorithm achieve a stable size after a short period of growth, while MTS simulated with the pressure-based, space-filling-based and stress-likelihood- based algorithms grow exponentially and do not stabilize. When *consumptionRate* is raised to 4.0x10$^{-5,}$ creating conditions less favorable to growth and more favorable to stabilization, the pressure-based algorithm will also stabilize, while the space-filling and stress-likelihood will not (Figure 2b). Simulations at varied parameter settings demonstrate that the stress-based algorithm produces MTS that are more stable than those produced using the pressure-based algorithm and will achieve a stable size at conditions more favorable to growth. The stress-likelihood-based algorithm creates more stable MTS than does the space-filling algorithm, but does not achieve a stable size under the current parameter settings.

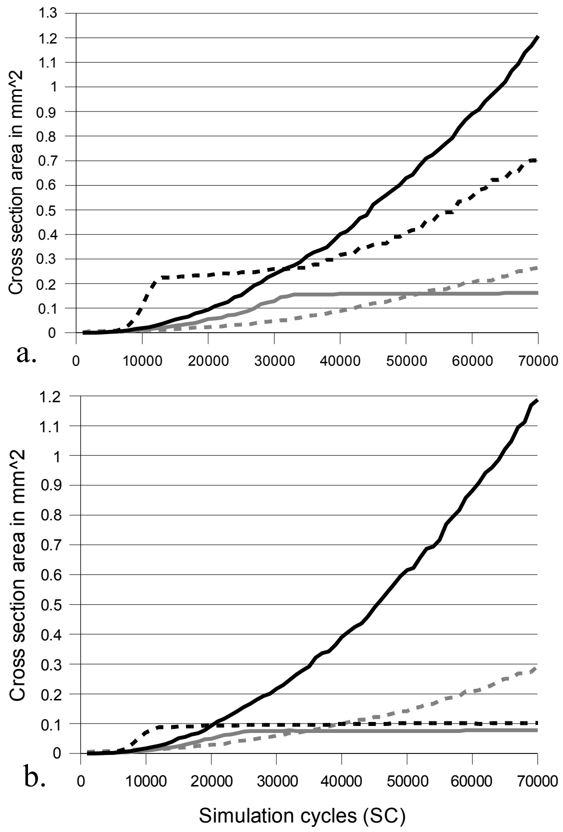

Figure 2. Simulated MTS growth. **black line** | Space-filling-based algorithm. **black dotted line** | Pressure-based algorithm. **gray line** | Stress-based algorithm. **gray dotted line** | Stress-likelihood-based algorithm. **a** | *consumptionRate* of 2.0 x 10^{-5}. **b** | *consumptionRate* of 4.0 x 10^{-5}.

4. CONCLUSION

The MTS shapes produced by the various algorithms were not initially anticipated but do follow logically from the action of the algorithms. The space-filling algorithm creates new cells at random, and thus the shape generated is irregular in nature. This algorithm does not generate a hexagonal MTS because there are more possible combinations of proliferative events that will create a new cell toward the middle of one of the hexagonal faces than toward the corner, thus a hexagonal shape, if formed, would break down over time.

A hexagonal conformation produces the lowest overall pressure, and this is preferred by the pressure-based algorithm. This algorithm treats the MTS like an agent itself, as opposed to allowing all the individual agents to reproduce autonomously, and thus the control of shape is possible. When the MTS takes the shape of a hexagon, with corners in the North/South orientation, every cell in the MTS will have two empty neighbor spaces, except the corners, which will have three. As a result, new growth will generally begin at the corners and progress along an edge, except when more than six new cells are generated during a single SC. The results from the stress-based algorithm are similar, except in this case a 12 side shape minimizes the stress, as the overall stress felt by each cell in this arrangement ends up being equal to 0. Once the ideal conformation is reached, new cells are placed at random on the outside edge of the MTS, creating an imbalance of stress that is corrected by adding new cells on that face.

It was observed that the overall shapes of the MTS generated by the pressure-based and stress-based algorithms were generally balanced, with edge lengths being roughly equal over time. This effect appears to be due to the random selection of new locations for growth when pressure and stress values are similar and shape is regular. The largest face will have a higher likelihood of being the location of newly created cells, but as cells are added to a face its width relative to the other faces will be reduced. As a result a balance is achieved between the size of the faces.

The stress-likelihood-based algorithm is designed to combine the stress algorithm with the local effects of the space-filling algorithm. Locality is a desirable phenotype because cells in vitro are only aware of their immediate physical and chemical environment. This algorithm is a work in progress. Nevertheless, results to date indicate that it can produce an MTS that maintains a roughly circular shape and achieves a stable size after a period of initial exponential growth.

The results of this report indicate that the ability to detect and manage surface irregularity is important for the growth of simulated MTS. MTS simulated using proliferation algorithms that control shape are more likely to stabilize and reach a balance of cellular growth and death.

5. ACKNOWLEDGEMENTS

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