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An Agent-based Computational Approach for Representing Aspects of *In Vitro* Multi-cellular Tumor Spheroid Growth

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Abstract—**There have been many efforts to explain and simulate tumor growth with mathematical and computational models. However, none have systematically examined the behaviors of tumor spheroids during growth. The interactions among tumor cells during growth are also not well understood. We have implemented an agent-based computational approach to study the macro- and micro- behaviors of avascular tumor spheroids during growth. Our simulations of tumor spheroid growth begin with a single tumor cell in optimal environmental conditions. We observe an initial phase of rapid growth, during which the shape of the collective approximates a spheroid. Subsequently a characteristic layered structure develops, consisting of an outermost proliferating cell layer, an intermediate quiescent cell layer, and a central necrotic core. These behaviors of our *in silico* spheroids map well to experimental *in vitro* observations.**

Keywords—**Agent-Based, Computational, Multicellular, Tumor Spheroid, *in vitro*, simulation**

I. Introduction

In vivo, early stage tumor growth is a complicated process, involving interactions among multiple subprocesses, such as proliferation, quiescence, necrosis, apoptosis, and angiogenesis. Moreover, early stage tumor growth *in vivo* is very difficult to observe, particularly in the avascular phase. Multicellular tumor spheroids (MTS) are an *in vitro* model system that has been developed to imitate the early, avascular growth phase of *in vivo* tumors. Computational models of this process that are able to account for the observed behavior of the *in vitro* system and allow *in silico* experimentation could prove valuable.

Both mathematical and computational models have been formulated to simulate MTS growth. Drasdo [1] used a Monte-Carlo approach to model the initial exponential growth phase of avascular tumors. Dormann and Deutsch [2] used a hybrid cellular automaton to simulate avascular tumor growth. Kansal et. al. [3] developed a cellular automaton model of brain tumor growth. Casciari et. al. [4] and Ward and King [5] are examples of differential equation models have also been developed to study these processes. These models, however, are unable to represent certain aspects of this system well. For example, differential equation models cannot easily represent either the individual and spatial heterogeneity of tumor cells or their adaptive behaviors. Cellular automaton models do not provide for detailed representation of individual tumor cell behavior and state, and lack the flexibility to represent the adaptive behavior of individual tumor cells. In addition, none of these approaches allows the detailed study of nutrient and waste transport through a spheroid.

We believe an agent-based approach is sufficiently flexible to model this system. Agents are software objects that have the ability to add, remove, and modify objects and events. Philosophically, they are objects that have their own motivation and can initiate causal chains, as opposed to simply participating in a sequence of events created elsewhere. Agent-based models are discrete in most dimensions, including time, state, and the rules used to govern agent behaviors. Agent-based models were specifically developed to provide more natural descriptions of complex adaptive systems in various contexts [6].

Detailed descriptions of *in vitro* multicellular tumor spheroids are provided in [7,8] Briefly, *in vitro* tumor cells consume oxygen and nutrients and release metabolic byproducts. Under optimal environmental conditions—sufficient oxygen and nutrients and low levels of byproducts—tumor cells undergo mitosis, producing new tumor cells. This cell proliferation leads to an initial exponential growth phase and a multicellular spheroid. As spheroid size radius increases, oxygen and nutrient availability internally is reduced, and metabolic byproducts accumulate within the spheroid. Such conditions are thought to contribute to necrosis of cells near the center of the spheroid. The byproducts of necrosis can be cytotoxic, and their presence can further inhibit tumor cell proliferation. Tumor cells near the surface of the spheroid, on the other hand, can continue to proliferate because of the favorable environmental conditions. Cells located between this outer proliferating layer and the inner necrotic regions become quiescent—environmental conditions are adequate to their survival, but not for proliferation. This dynamic results in the characteristic layered structures of tumor spheroids illustrated in below Fig. 1.

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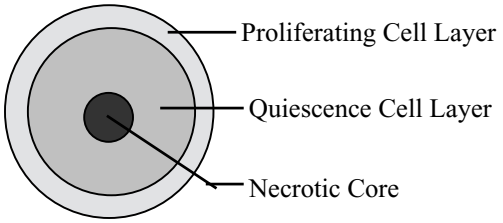

Fig. 1. Tumor Spheroid Schematic Diagram

II. Computational Approach and Methods

Swarm[1] is a software library that was designed to facilitate multi-agent simulation by managing many programming tasks common to agent-based models, thus freeing the user to focus on the specific requirements of the simulation. Core Swarm libraries provide vital functions such as object creation, memory allocation, scheduling of activities, and list management. All agents and objects in Swarm can be probed. Once attached the probe can send a message, change a variable, or retrieve values.

We have implemented an agent-based model of multicellular tumor spheroid growth using the Swarm framework. Swarm enables and facilitates the simulation of collections of concurrently interacting agents.

Our model contains 4 computational spaces (Fig. 2.): *TumorSpace*, *OxygenSpace*, *NutrientSpace*, *InhibitorSpace*. Each space is represented as a (toroidal) 2-D square lattice. They overlap as shown below. All factors other than oxygen that contribute to growth and division are lumped together into a class called *Nutrients*. Similarly, all factors that inhibit growth or that are harmful to tumor cells are grouped into the class *Inhibitor*. Corresponding objects in each class are designated OXYGEN, NUTRIENT, and INHIBITOR to distinguish them from their biological referents[2].

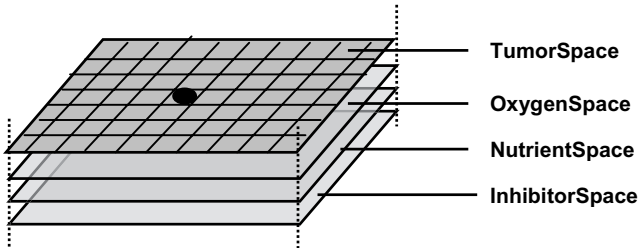

Fig. 2. Schematic representation of overlapping grid spaces

[1] See http://www.swarm.org. for further information on Swarm.
[2] The same word may be used to refer to molecules, cells, system components, or events in the referent system and to the corresponding objects, agents, or events within the *in silico* system. In the latter case the word is written using small caps.

TumorSpace contains tumor cell agents. Each location can by occupied by a single agent. OXYGEN, NUTRIENT, and INHIBITOR are integer values at each location in their respective spaces.

For simulation of oxygen transport in *OxygenSpace*, we use Swarm's built-in diffusion algorithm. Swarm provides a discrete 2nd-order approximation of 2-D diffusion with evaporation. For simulation of NUTRIENT and INHIBITOR transport, we have implemented data structures and algorithms to represent the transport of these quantities along the edges between cells. We call this the *EdgeFlow* function. The *EdgeFlow* algorithm is implemented similar to diffusion, except that edges can be "open" or "closed," and transport occurs only along open edges. This allows us to simulate the heterogeneous transport of these materials through a spheroid in a detailed manner.

Tumor cell agents, designated TUMOR AGENTs, have the following internal attributes that, collectively, control their behavior:

- state: proliferating, quiescent, or dead
- rates of OXYGEN consumption, NUTRIENT consumption and INHIBITOR production
- OXYGEN minimum and maximum requirements
- NUTRIENT minimum and maximum requirements
- INHIBITOR minimum and maximum requirements

The initial conditions are as follows: Initializing *OxygenSpace* sets the value of OXYGEN at each location to O_C. Initializing *NutrientSpace* sets the value of NUTRIENT at each location to N_C. One TUMOR AGENT in the proliferating state is placed at the center of *TumorSpace*. The simulation is started. At each time step thereafter, OXYGEN and NUTRIENT values are "replenished" outside the spheroid: for each location in the *TumorSpace* that neither contains a TUMOR AGENT nor is surrounded by TUMOR AGENTs, the corresponding location in *OxygenSpace* and *NutrientSpace* is reset to O_C or N_C.

A list of functional TUMOR AGENTs is maintained, (proliferating or quiescent). At each time step, each TUMOR AGENT in the list caries out the following activities. Consume OXYGEN and NUTRIENT only from the corresponding location in *OxygenSpace* and *NutrientSpace*, and release an amount of INHIBITOR to *InhibitorSpace*, following individual internal rate values. Use resulting OXYGEN, NUTRIENT, and INHIBITOR amounts to determine new TUMOR AGENT state for the next time step.

The determination of the TUMOR AGENT state is made as follows: the TUMOR AGENT compares OXYGEN, NUTRIENT, and INHIBITOR values against its internal minimum and maximum requirements. If OXYGEN and NUTRIENT values are greater than the maximum requirements and INHIBITOR value is less than the minimum requirement (representing ideal conditions for the tumor cell), the TUMOR AGENT is proliferating. If any of these values are between the agent's minimum and maximum levels, the TUMOR AGENT may take

on the proliferating or quiescent state, with varying probabilities. If either OXYGEN or NUTRIENT is less than the agent's minimum requirements, or INHIBITOR is greater than the maximum requirement (representing adverse conditions), the agent goes to the dead state with a certain probability.

If the new state of the TUMOR AGENT is proliferating, attempt to divide. First, examine the eight neighboring locations in *TumorSpace*. If any neighboring location is unoccupied, create a new TUMOR AGENT in the proliferating state and place it in the unoccupied location. If no neighboring locations are unoccupied, simply remain in the proliferating state. For every TUMOR AGENT in the necrotic state increment *InhibitorSpace* in the corresponding location to represent accumulation of toxic necrotic material.

During the initial stage of simulation, each TUMOR AGENT represents a single tumor cell. As the spheroid increases in size and fills *TumorSpace*, a "zoom function" is introduced. This allows a TUMOR AGENT to represent multiple tumor cells without distorting the spheroid shape and behaviors. The numbers of tumor cells a TUMOR AGENT represents increases each time the *zoomFunction* is applied. This allows the model to simulate larger spheroids, in which the total number of tumor cells may reach 10^{10}, without representing each cell individually, which would be computationally intractable.

III. RESULTS

Following initiation of a simulation (Fig. 3a), TUMOR AGENTs proliferate, taking advantage of the optimal environmental conditions. This results in a spheroid of proliferating TUMOR AGENTs (Fig. 3b). The size of spheroid increases rapidly, which causes OXYGEN and NUTRIENT availability in the interior to decrease and INHIBITOR levels to increase. This results in decreasing gradients of OXYGEN and NUTRIENT and increasing gradient of INHIBITOR, moving from the outer edges towards the center. As a result, the TUMOR AGENTs toward the center become quiescent, and proliferating TUMOR AGENTs are limited to the outer regions (Fig. 3c). Eventually, three layers are formed (Fig. 3d). Conditions in the interior become increasingly adverse, causing transition of TUMOR AGENTs from quiescent to necrotic state and the formation of a "necrotic core."

For initial results and validation of our model, we demonstrate the effects of varying OXYGEN and NUTRIENT supply on spheroid growth. OXYGEN and NUTRIENT supply are determined by the parameters O_C and N_C (the values to which OXYGEN and NUTRIENT are replenished outside the spheroid). We use Proliferating Fraction as an outcome variable to measure spheroid growth. Proliferating Fraction is defined as the number of proliferating TUMOR AGENTs divided by the total number of TUMOR AGENTs.

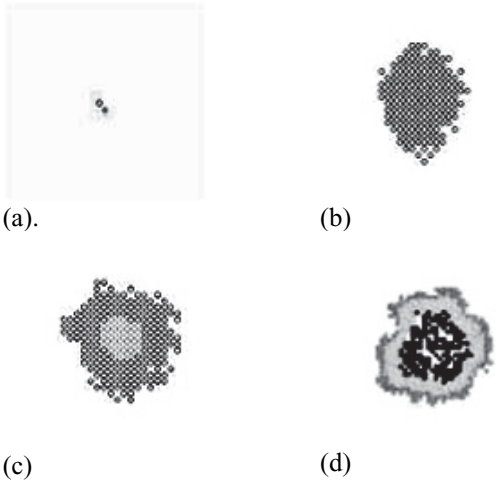

Fig. 3. Tumor growth over time
(a) TUMOR AGENTs at initial simulation (scale 1:1)
(b) TUMOR AGENTs proliferate and form a spheroid (scale 1:1)
(c) quiescent layer forms (scale 1:1)
(d) spheroid increases in size, forms layered structure (scale 1:5)

Three sets of values for these parameters were used in experiments: baseline OXYGEN and NUTRIENT supply (PF1), doubled OXYGEN and NUTRIENT supply (PF2), and halved OXYGEN and NUTRIENT supply (PF3). For each set of values, we averaged the results of five simulations. The results are shown in Fig. 4.

Fig. 4. shows that the Proliferating Fraction declines over time for each of the three set of parameter values. There is a sharp initial decrease, followed by a stabilization. The results also show that Proliferating Fraction varies with OXYGEN and NUTRIENT supply; greater supply of OXYGEN and NUTRIENT result in higher Proliferating Fractions.

We use total number of TUMOR AGENTs to represent spheroid size. Spheroid size rapidly increases in the initial stage of simulation. The rate of increase slows and eventually reaches saturation. Similar to proliferating Fraction, doubling the OXYGEN and NUTRIENT supplies increases total number of TUMOR AGENTs and reducing the supplies to half decreases the total number of TUMOR AGENTs.

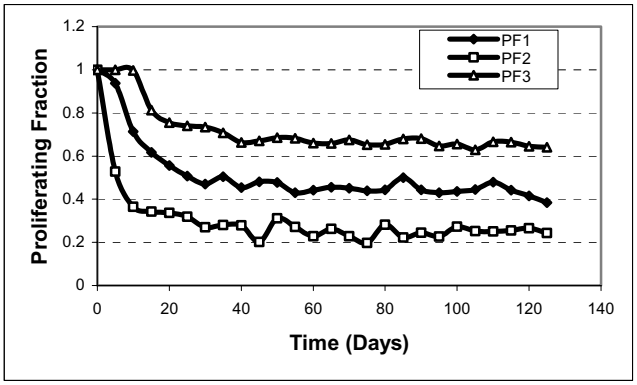

Fig. 4. Proliferating Fraction vs. Time for three different sets of parameter values

III. Discussion

Our model is an initial step towards the goal of providing a flexible model of multicellular tumor spheroids which will allow for *in silico* experimentation. At this stage our model dynamically simulates formation and growth of multicellular tumor spheroids based on a small set of simple rules. The observed development of a spatial layered structure simulates *in vitro* observations, and results concerning major growth indicators, such as those presented above for Proliferating Fraction, are very close to those of *in vitro* data and results of other computational models [9]. Furthermore, a strength of the agent-based approach is that it enables us to observe the microbehaviors of individual agents and their interactions throughout the entire growth process.

Our model at this stage captures a small subset of the biological complexity involved in tumor spheroid growth. However, our approach provides an easy and effective way to add, modify and remove the various parts of the model: agents, rules, etc. As such, it can serve as a foundation for more detailed models of the various biological processes involved.

IV. Conclusion

Substantial progress has been made in various specialized areas of cancer research. The complexity of the disease on both the single cell level as well as the multicellular tumor level has led to the first attempts to describe tumors as complex, dynamic, self-organizing biosystems. To begin to understand the complexity of the system, novel models and simulation methods must be developed, incorporating concepts from many areas such as cancer biology, computer programming techniques, and mathematical and computational applications. Our work is intended to demonstrate that agent-based modeling can be used to represent certain essential aspects of spheroid growth, and thus can serve as the basis for future *in silico* models of this complex system.

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