Simulating Avascular Tumors with Membrane Systems

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Summary. Tumor growth has received a considerable attention by the scientific community. In the earliest stages of development, tumor growth seems to be regulated by direct diffusion of nutrients and wastes from and to surrounding tissue. In this paper we present an approach of the use of membrane computing techniques to simulate and predict the growth of a tumor in the avascular stage. We present a preliminary version of our software to simulate this growth and some future research lines.

1 Introduction

Tumor growth is a fundamental problem that has received considerable attention by the scientific community. There are many mathematical models that describe the initial avascular stage of solid tumor growth (see [13, 14, 6, 3, 23, 20, 11]), and these models are becoming increasingly sophisticated.

The avascular stage of tumor growth is characterized by small tumors which gain the nutrients and oxygen they need for survival and growth by diffusion from external blood vessels. Since there are no blood vessels within the tumor to supply the mass needed for such volume expansion, this must also enter through the tumor's periphery.

An individual tumor cell has the potential, over successive divisions, to develop into a cluster of tumor cells. Further growth and proliferation lead to the development of an avascular tumor consisting of approximately 10^6 cells which feed on oxygen and other nutrients present in the local environment.

The rapid growth and resilience of tumors make it difficult to believe that they behave as random, disorganized and diffuse cell masses and suggests instead that they are emerging, opportunistic systems. If this hypothesis holds true, the growing tumor and not only the single cell must be investigated and treated as a self-organizing complex dynamic system. This cannot be done with currently available

in vitro/in vivo models or common mathematical approaches. We propose the use of membranes systems as a new tool for the simulation and study of avascular tumors.

In membrane systems a local, modular and topological modelling of biological phenomena is easily achieved. Thus, using this bioinspired model of computation, we can get a detailed representation of each individual tumor cell, of their adaptive behaviors and processes, as well as the interactions among cells and between cells and a heterogeneous environment. All these features are not easily achieved when using other models, such as differential equations or cellular automata.

As we will see, membrane systems own several interesting features which make them suitable for this study. In particular, P systems treat the discrete nature of actual cells realistically. Each cell can be seen as an independent computing unit with its own behavior. In this way, a local modelling of the process can be simulated and then, the evolution of the whole tumor can be studied as the sum of all local performances together with the network of interactions among the cells.

Furthermore, membrane systems are a flexible framework where different approaches, such as discrete, continuous, stochastic, etc. (see [7], [19], [8]), can be considered within the same model to represent different facets of the biological phenomenon which is investigated. This interpretation allows the model to serve as an intuitive complement to the results obtained from a continuum model.

The paper is organized as follows. First, the spheroid model of avascular tumor growing is presented in the next section. In section 3 we recall some important features of membrane computing. The simulation and preliminary results are presented in section 4. Finally, some conclusions and future research lines are given in the last section.

2 The Spheroid Model

The early development of solid tumors has been extensively studied, both experimentally via the multicellular spheroid model, and theoretically using mathematical modelling. The vast majority of models apply specifically to multi-cell spheroids which have a characteristic structure of a proliferating rim and a necrotic core, separated by a band of quiescent cells.

Over the years, researchers have devised several methods for producing tumor cells aggregated or *spheroids* that can be used to study tumor invasion in a 3D model system.

In the earliest stages of development, tumor growth seems to be regulated by direct diffusion of nutrients and wastes from and to surrounding tissue. When a tumor is very small, every cell receives nourishment by simple diffusion and the growth rate is exponential in time. However, this stage cannot be sustained because as a nutrient is consumed its concentration must decrease towards the center of the tumor. The concentration of a vital nutrient at the center will fall below a critical level.

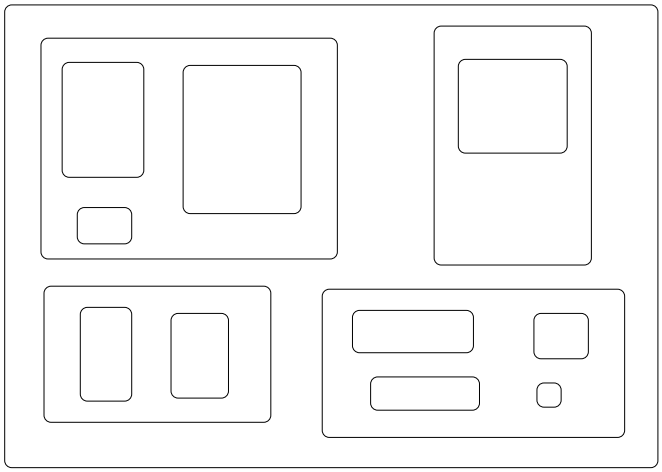

Fig. 1. Membrane structure of a P system.

Unfortunately this is not the end of the process. Indeed a majority of tumors exhibit the phenomenon of angiogenesis marking the transition from the relatively harmless and localized avascular state described above to the more dangerous vascular state wherein the tumor develops the ability to proliferate, invade surrounding tissues, and metastasize to distant parts of the body.

After the early stages of growth, the avascular spheroids consist structurally of an inner zone of necrotic cells (dead due to lack of nutrients) and an outer zone of living cells. This outer zone can be further divided into a layer largely composed of quiescent cells and a layer largely composed of proliferating cells, although dead cells are also found adjacent to both quiescent and proliferating cells [21]. At this stage the spheroids tend to reach a finite size of at most a few millimeters in diameter [10]. In this state of dynamic equilibrium there is a balance between mitosis and the death and disintegration of tumor cells into waste products, mainly water.

3 Membrane Computing

Membrane computing is a new non-deterministic framework for devising computing models which starts from the assumption that the processes taking place in the compartmental structure of a living cell can be interpreted as computations.

Roughly speaking, a P system consists of a cell-like membrane structure, in the compartments of which one places multisets of objects which evolve according to given rules in a synchronous non-deterministic maximally parallel manner[1].

[1] A layman-oriented introduction can be found in [18], a comprehensive presentation can be found in [17], and further bibliography at [24].

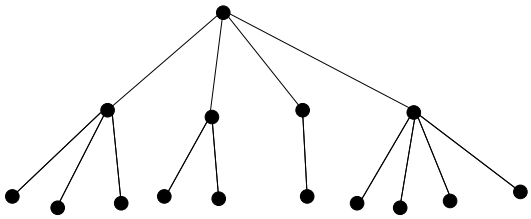

Fig. 2. Membrane structure as a tree.

3.1 The classical view

Basically, a P system consists of a set of membranes, usually organized in a hierarchical structure, as illustrated in Figure 1.

There exists a skin membrane which embraces all the others, separating the system from the external environment. The membranes which do not contain other membranes inside are called elementary membranes. The regions delimited by the membranes (that is, the space bounded by a membrane and the immediately lower membranes, if there are any) can contain certain objects, that are allowed to be repeated. By means of the application of fixed evolution rules associated with the membranes (or regions), these objects can transform themselves into different ones, and can even go from a region to an adjacent one, crossing the membrane that separates them. These P systems offer two levels of parallelism: on the one hand, the rules within a membrane are applied simultaneously; on the other hand, these operations are performed in parallel in all the membranes of the system.

Each region can be seen as a computing unit (a processor), having its own data (biological substances) and its local program (given by biochemical reactions). So, the cell can be seen as an unconventional computing device.

To sum up, P systems have the following properties:

- Each region is a discrete computing unit.
- The evolution of the P system is the result of the evolution of these discrete units together with the interaction among them via their communication channels.
- Some chemical compounds (e.g., nourishment or wastes) can be sent from one membrane to others via the communication channels.
- Depending on the rules and the chemical compounds placed in the different regions, new membranes can appear or existing ones can be dissolved.

These properties together with the new representation shown in the next subsection, lead us to consider P systems as an appropriate model to simulate the spheroid model of avascular tumors.

3.2 A new perspective

In this paper we explore P systems from a new perspective. The spheroid model of avascular tumors can be seen as a hierarchical arrangement of cells rooted in the center of the tumor. The arrangement of cells has a discrete nature, while its growth is regulated by division of existing cells and it depends on the diffusion of nourishment from the perimeter of the tumor.

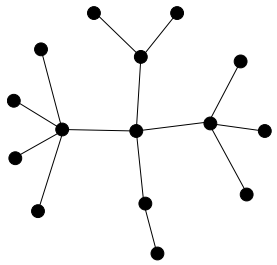

Fig. 3. Radial representation of the membrane structure.

Membrane systems admit a graphical representation which allows considering the whole P system as an avascular tumor. In this way, the membrane structure of the P system of Figure 1 is depicted as a tree in Figure 2. This rooted tree admits a radial representation (Figure 3) and this representation is similar to the usual model of spheroids (Figure 4)[2].

With this new perspective, the tumor is represented by the whole P system; the skin of the P system represents the central cell of the spheroid and each membrane of the P system is a cell of the tumor.

With this new representation it is quite natural to study the main processes related to the growth of an avascular tumor in the framework of P systems. The growth of a tumor is carried out by the apparition of new cells.

Increasing the number of membranes is usually made via division of existing ones or by creating new membranes from objects[3].

Inspired in living cells, P systems abstract the way of obtaining new membranes. These process are basically two: *mitosis* (membrane division) and *autopoiesis* (membrane creation), see [16]. Both ways of generating new membranes have given rise to different variants of P systems: *P systems with active membranes*, where the new workspace is generated by membrane division, and *P systems with membrane creation*, where the new membranes are created from objects.

Membranes are created in living cells, for instance, in the process of vesicle mediated transport and in order to keep molecules close to each other to facilitate

[2] The classical schematic representation of the spheroid can be seen in [4] or [15].

[3] Recently, new operations to change the membrane structure have been explored, such as *merging membranes*, or the operations of *endocytosis*, *exocytosis* or *gemmation*.

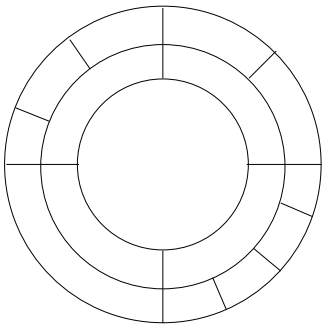

Fig. 4. Membrane structure as an avascular tumor.

their reactions. Membranes can also be created in a laboratory – see [16]. Here we abstract the operation of creation of new membranes under the influence of existing chemical substances to define P systems with membrane creation.

Real tumors grow due to mitosis of cells in the proliferating rim, but the suitable abstract model to add new cells is still a matter of study.

The decrease of the number of membranes is made by applying a so-called *dissolution rule* $[a]_e \rightarrow b$ in which the object a inside a membrane with label e produces the dissolution of the membrane, a disappears and a new element b and the rest of the multiset in the membrane go to its father (more precisely, they go to the closest non-dissolved ancestor in the membrane hierarchy, since several membranes can dissolve in the same step).

Finally, the diffusion of nourishment from the perimeter of the tumor can be easily simulated by using send-in communication rules. If these rules are combined with evolution rules, which represent the consumption of nourishment per cell, the amount of nourishment sent to the innermost layers decreases.

4 The Simulation

Based on membrane computing principles, we have developed a prototype of a simulator[4]. It has been written in SWI-prolog (see [25]), together with its graphical extension XPCE. For that we have considered ten parameters. Seven of them are related to the tumor whose growth we want to simulate and three of them are related to the graphical representation. The parameters are the following:

- **Factor growing:** This parameter represents the number of cells obtained from a single proliferating cell in a unit of time.
- **Initial nourishment:** Every cell of the perimeter is in touch with the surrounding tissue. These outermost cells obtain from the environment this initial amount of nourishment.

[4] The software can be obtained from the authors via e-mail.

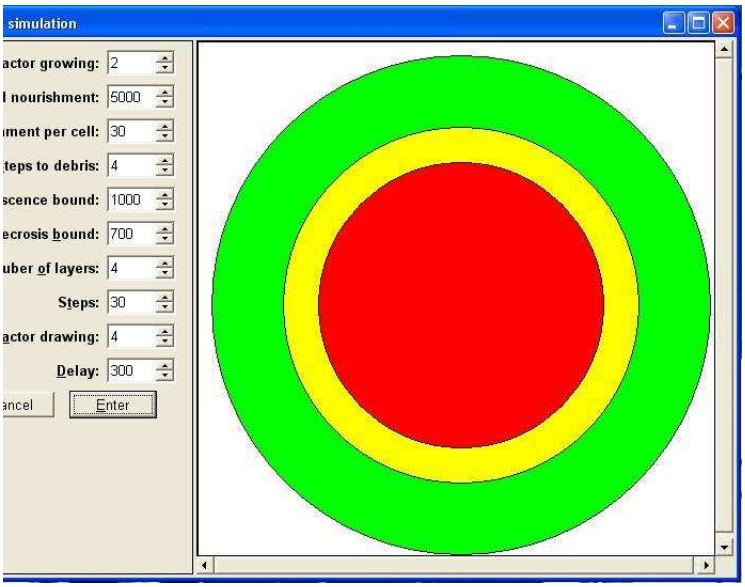

Fig. 5. Interface of the simulator.

- **Nourishment per cell:** Every cell obtain the nourishment from the surrounding tissue (if it is in the outermost layer) or by diffusion from the external adjacent layer. Part of this nourishment is burnt by the cell for its vital processes and the rest is sent to the internal adjacent layer.
- **Quiescence threshold:** When the amount of nourishment which reaches a cell goes down under this threshold, the cell remains alive, but it is not able to divide. It reaches a *quiescent* mode.
- **Necrosis threshold:** The cells in quiescent mode also need nourishment, so the amount of nourishment going on decreasing towards the center of the tumor. If the amount of nourishment goes down under this parameter, the cell die.
- **Steps to debris:** When a cell dies, it preserves its volume for a few more steps. After these time, the compounds of the cell become debris and they are not considered in the volume of the tumor.
- **Initial layers:** We start the simulation from an initial spheroid with a few layers where all the cells are considered in the proliferating mode.

Finally, we also consider three parameters related to some aspects of the simulation:

- **Steps:** Number of time units to carry out the simulation. If the number of steps is large enough, the avascular tumor reach an equilibrium between the necrotic cells with disappear at the center of the tumor and the new cells created in the proliferating rim.

- **Factor drawing** and **Delay:** They controls the scale and speed in which the simulation is depicted.

Figure 5 represents the graphical interface of the simulator.

4.1 Results

The spheroid model that we consider in this paper are mainly due to Burton [2] and Greenspan [13, 14] and, as pointed out by Byrne in [4], it can be considered as an one-dimensional spatial model of avascular tumor growth. When this model was developed, the stress was on the effect of the medium surrounding the tumor on the growth of the tumor [10, 22]. The study focused on the evolution of the radii of the (approximately) radially-symmetric tumors over time.

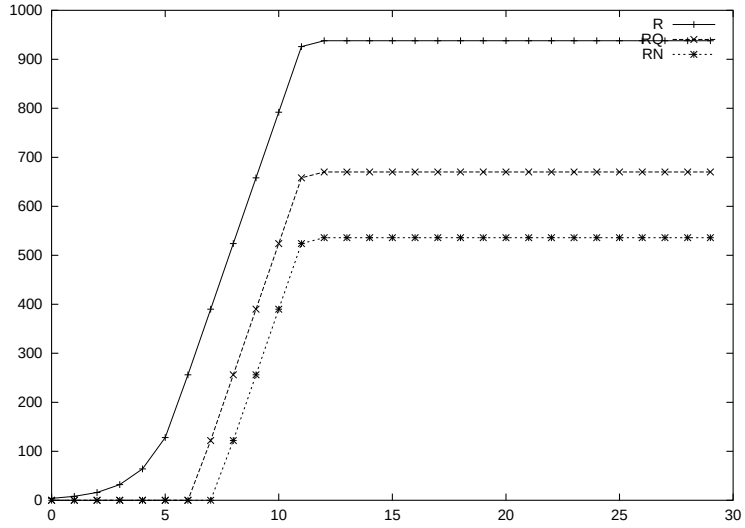

Fig. 6. Evolution of R, R_Q and R_N.

The key variables are $R(t)$, the radius of the tumor, $R_Q(t)$, the outer radius of the quiescence rim, and $R_N(t)$, the radius of the necrotic core, all of them depending on time t. Most of the simulations are based on differential equations[5] and all they need to consider several assumptions, balanced between the necessity of simplification and experimental evidence.

These simulations represent the evolution over time of the radii R, R_Q and R_N. In the first stage, $R_Q = R_N = 0$, i.e., all the cells are proliferating, there are no quiescent or necrotic cells. At this stage, the growth or R is exponential.

[5] An introduction to these simulations can be found in [15] and [4].

In the next stage, R_Q becomes different from zero and later the necrotic rim appears ($R_N \neq 0$). If the considered time is large enough, R, R_Q and R_N become constant. At this point a balanced equilibrium is reached. Figure 6 represents the results obtained with our simulator with the following parameters:

Factor growing = 2
Initial nourishment = 4000
Quiescence threshold = 1000
Initial layers = 4
Steps to debris = 4
Nourishment per cell = 30
Necrosis threshold = 700

5 Conclusions and Future Work

In this paper we have presented the first steps in the use of membrane computing techniques to simulate and study the growth of avascular tumors. As pointed in the previous sections, membrane systems have several features that lead us to consider them as a promising tool for this target. For example, the birth and dead of cells, their discrete nature, and the possibility of transfer objects among membranes are some of these features.

We also have presented the first version of a new software based on membrane computing principles and the preliminary results are encouraging, since the obtained results are quite similar to experimental result as well as the results obtained with classical simulation devices based on differential equations.

Several lines are open for future research. From a theoretical point of view, a deeper study of the different models of membrane systems is necessary (e.g., P systems with active membranes, with creation, tissue P systems, ...) to choose the best one in order to develop the syntax and semantics underlying the modelling of tumor growing.

Another open research line is related to the possibility to consider new parameters in our study in order to make it more and more realistic. Although avascular tumors have been widely investigated, the interaction among cells, which lead to the characteristic three layered structure, are not well understood. This self-organizing structure is the product of the interactions between multiple subprocesses: proliferation, quiescent, necrosis ([12]), apoptosis ([1]), nutrient consumption, cytotoxic byproducts production, diffusion ([5]), cell migration([9]), etc. The next steps in this line can be, for example, to distinguish among different kinds of nutrients or the possibility of considering inhibitors of growing (drugs) and compare the combination of values of the parameters.

Finally, other open line is to consider ideas from [19] and try to incorporate them into our simulation device. In this way, the behavior of each cell would be determined by its chemical signalling network and the growth of the tumor would depend on the evolution of each cell together the interaction among them as a complex system.

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