An Agent Oriented Software Engineering Approach for the Adult Stem-Cell Modeling, Simulation and Visualization

Maíra Athanázio Gatti, José Eurico de Vasconcellos Filho and Carlos Lucena

Departamento de Informática – PUC-Rio
Rua Marques de São Vicente 225, 4º andar RDC
Rio de Janeiro – RJ – Brasil
{mgatti,jfilho,lucena}@inf.puc-rio.br

Resumo. Este artigo descreve os primeiros resultados obtidos na modelagem, simulação e visualização do comportamento de células-tronco. Um modelo conceitual da célula foi gerado e, com base nesse modelo e nos processos do ciclo de vida, divisão e diferenciação celular, modelou-se uma ferramenta de simulação baseada em agentes. Em paralelo e considerando o mesmo modelo conceitual e processos, foi desenvolvido um simulador modelado em COOPN de modo a possibilitar a comparação dos resultados obtidos através das duas abordagens. Acredita-se que a simulação do comportamento das células-tronco seja um primeiro passo para facilitar a pesquisa do comportamento de células-tronco.

Abstract. This paper presents the first results achieved towards an agent-based adult stem cell behavior modeling, simulation and visualization. We developed a cell conceptual model on which the model is based and we built the agent-based model and simulation considering the cell life-cycle and the basic processes related to the cell division and differentiation. Based in the same conceptual model, we also modeled and simulated the behavior through the COOPN technique to compare the results. We believe that stem cell simulation behavior is a first step towards the stem cell therapy costs minimization.

1. Introduction

A stem-cell is a primitive cell which can either self renew (reproduce itself) or give rise to more specialized cell types. In recent years there has been a growing debate about how stem cells behave in the human body. In parallel to these debates, there have been several attempts to build formal models (for instance, ordinary differential equations (Lei & Mackey, 2007) and cellular automata (Agur et al., 2002)) so that predictions can be made about how and why stem cells behave either individually or collectively.

With regard to the medicine point of view, if the model is sufficiently detailed and accurate, it serves as a reference, a guide for interpreting experimental results, and a powerful means of suggesting new hypotheses. Moreover, the simulation lets physicians test experimentally unfeasible scenarios and can potentially reduce experimental costs.

It’s our belief that the Agent-Oriented Software Engineering (AOSE) provides clean design in the modeling phase and efficient numerical routines in the simulation phase. Those techniques address the currents drawback of other existents approaches related to the model's semantic (it is more expressive), and to the model and software reuse (Jennings, 2000). Furthermore, the dynamic structures present in biological systems can...
be intuitively represented and efficiently implemented in agent-oriented simulators (d’Inverno and Prophet, 2004), (d’Inverno and Saunders, 2005).

Outline

This paper is organized as follow: section 2 describes our research hypothesis, section 3 highlight the related work, section 4 describes de domain analysis (detailing the processes, scope and the conceptual model developed) and the agent-based solution developed for the modeling and simulation of the stem cell behavior. In section 5 we discussed about the advantages and disadvantages existents in our solution and briefly compare with a COOPN (Biberstein et al., 2001) solution. And finally, section 6 concludes this paper and presents the future work.

2. The Research Hypothesis and the MAS adequacy

![Diagram](image-url)

Figure 1 - The Stem Cell Simulation: A Step before the Stem Cell Therapy
Figure 1 details the medicine point of view motivation for the stem cell simulation. The reconstituting potential of tissue stem cells makes them target cells in different types of clinical settings, particularly with respect to the emerging field of regenerative medicine. In order to use the full functional potential of stem cells, it is necessary to achieve a comprehensive insight into general regulatory principles of cellular differentiation and lineage specification. Only on the basis of such a comprehensive understanding it will be possible to quantitatively describe and predict cellular differentiation and, therefore, to control regenerative processes in vitro and/or in vivo, e.g. by the targeted (re-)programming of cells.

Nowadays, the stem cells are cultivated in the lab in order to differentiate in a specific mature cell. Today it is hard to predict the stem cell behavior under some substances. Moreover, all the infrastructure necessary to maintain the stem cell culture is very expensive and many stem cells are waste if the inject substance didn’t lead the culture to the desired mature cell. Thus, the stem cell simulation is a powerful tool for reducing all those costs and accelerating the process for the stem cell therapy.

Basically, stem cells are: a potentially heterogeneous population of functionally undifferentiated cells; composed of multi-cellular organisms; capable of homing to an appropriate growth heterogeneous environment, proliferation, production of a large number of differentiated progeny, self-renewing or self-maintaining their population, regenerating the functional tissue after injury with flexibility and reversibility in the use of these options (Loeffler & Roeder, 2002). All of those characteristics lead us to a Multi-Agent Systems-based solution if we analyze the basic agent’s concepts and capabilities (Jennings, 2000):

- Agents are autonomous and interactive entities: an agent is capable of acting without direct external intervention and communicates with the environment and other agents;
- Agents and multi-agents systems have the capacity for adaptation: an agent is capable of responding to other agents and/or its environment to some degree, and a multi-agent system might adapt itself to a specific state through the learning processes;
- Multi-agent systems provide abstractions that allow decomposing a biological system to a set of agents;
- Multi-agent systems provide flexibility for modeling more sophisticated, globally emergent behavior: the global effect resulting from the interaction of the individuals is often unpredictable and non-deterministic;
- Multi-agent systems by their nature are powerful tool for modeling biological systems (Gatti & Lucena, 2007). Biological systems are complex systems and their modeling implies a deep understanding of the system both in terms of its structure and its behavior and multi-agent systems allows this specification.
- Software agents embody distribution and heterogeneity and, thus, they are indicated as the new abstraction for the engineering of complex distributed systems;
- Multi-agent systems are capable of being open systems: agents may enter and leave the environment at their will, and the system has no single point of control.
Multi-agent systems are capable of being self-organized: agents could be organized in a structure that might evolve to a different structure according to the agent’s behavior, performance, and others.

That said, considering that the underlying mechanisms and the regulatory principles of stem cells organization are still widely unknown and that they are self-organizing system, MAS is an effective way to understand how stem cells organize themselves, and to deal with it emergent global behavior.

The agent-based simulation suggests how tiny changes in individual stem cell behavior might lead to disease at the global through the emergent behavior, allows temporal analysis, reduce costs and risks, and could avoid some ethical issues.

3. Related Work and the MAS advantages

In order to support the claim that the agent approach is more suitable than other modeling approaches, existing approaches have been taken and recast in the agent-based modeling and simulation framework, which has demonstrated a number of clear advantages of the agent approach over existing approaches (d’Inverno & Saunders, 2005).

To start with, mathematical models (Agur et al., 2002, Lei & Mackey, 2007) don’t allow expressing partial information about a system, i.e. to formally describe open systems, as an open MAS does. Moreover, depending on the system complexity, there would be an explosion of differential equations to model it with more than 50 equations to model a subsystem, for example. Another drawback is the absence of an abstraction for the models. Physicians have to know deeply mathematical methods in order to model the system, while MAS can provide the right level of abstraction for that.

Concerning the conceptual comparison, MAS are not just probabilistic dependent as the Monte Carlo methods (Metropolis & Ulam, 1949). More than reproducing the emergent behavior, agent-based simulation can provide advanced mechanisms as learning and adaptation that, as far as we know, is not possible to implement through Monte Carlo simulation. Those mechanisms not only make the model more complete but allow the optimization of self-organization, for instance.

To date (Theise & d’Inverno, d’Inverno & Prophet, d’Inverno & Saunders, 2003, 2004, 2005) have produced formal and mutually consistent specifications of the leading of some predictive models of stem cell behavior within their agent framework. They have also produced simulations and visualizations of these models. In their approach, each stem cell is implemented as an agent. They modeled and simulate the stem cells in a dynamic environment with the capabilities of division and determined (stem cells which have reached their cycle phase and which are surrounded by stem cells become determined). However, the stem cell behavior modeled was too simple, feasible and not adequate or evolvable to an adequate model for the physicians’ experiments.

4. An Agent-based Approach to Model and Simulate Stem-Cell

As stated before, the autonomy, heterogeneity, and temporal dynamics are the main required characteristics for building cells. And Multi-Agent Based Simulation (MABS) models can be implemented through several frameworks, e.g., SWARM (Minar et al., 1996), JADE (Bellifemine et al., 2001 ) e REPASt (Emonet et al., 2005 ).
We developed an agent-based solution to model and simulate the stem cell processes and the internal cell life-cycle. The first step was the domain analysis. At the beginning, many requirements were taken out from a set of biology bibliography, for instance from (Scadden, 2006) and (Alberts et al., 2003). We had to understand a bit more from the domain to avoid basic questions with the physicians. Then we developed the cell conceptual model. The physicians evaluated it and also described the differentiation process. Then we refined the cell conceptual model (presented in sub-section 4.1.1) and finished the domain analysis.

The second step was the modeling phase. We modeled the entities from the conceptual model using the MAS-ML modeling language (Multi-Agent System Modeling Language) (Silva & Lucena, 2004) which was developed by extending the UML based on the TAO (Taming Agents and Objects) conceptual framework (metamodel) (Silva et al., 2003).

4.1. The Stem-Cell Self-Organization Description

An important entity with an active influence in the process is the Niche. The Niche is a specialized cellular environment, which provides stem cells with the support needed for self-renewal, and contains the cells and proteins that constitute the extracellular environment. The Niche has regulatory mechanisms in order to save stem cells from depletion and to protect the host from over-exuberant stem-cell proliferation (Scadden 2006).

4.1.1 The Cell Conceptual Model

In order to simplify the model we had to consider only the more active components during the cell life-cycle and the mitosis division (which is the stem cell division during
the self renew process). By actives components we mean components which contribute and influence more directly the cellular differentiation during the cycle (Figure 3).

The Centrosome is a Microtubule Organizing Center (MTOC) which has a pair of Centriole, and three kinds of Microtubules (Astral, Polar and Kinetochore). The microtubules are nucleated structures capable of growing and shrinking in order to generate force, segregate the chromosomes correctly during cell division and move organelles and cells structures to new locations.

Before the Mitosis phase start, the genetic material in the nucleus is in a loosely bundled coil called Chromatin. The Chromatin condenses together into the Chromosome. There are 46 Chromosomes which condense into two Chromatids during the Mitosis phase.

Each Chromatid has a Centromere, by its turn, has the Kinetochore (which is the point where the kinetochore microtubules are attached to the chromosome.

Besides those elements, the cell modeled is composed of: cell membrane, nuclear membrane, nuclei, substances, protein, and organelles. During the cell life-cycle, many proteins are synthesized, which determines if the cell may continue the division, or not.
### 4.1.2 The Cell Life Cycle Scope

During the cell life-cycle, there is a mechanism called Checkpoint which monitors the cell. For each phase, Gap 0, Gap 1, Synthesis, Gap 2, and so on, the Checkpoint checks if some events have been started or ended in order to prevent the cell to enter in a undesirable state of error. In the table below we describe for each phase, the events associated to it and its checkpoint monitoring:

<table>
<thead>
<tr>
<th>Phase</th>
<th>Events</th>
<th>Checkpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gap 0</td>
<td>Start Cell Life-Cycle</td>
<td>Check if the Chromatins are ok.</td>
</tr>
<tr>
<td>Gap 1</td>
<td>Synthesize Cyclin D</td>
<td>Check if Cyclin D and E were synthesized into the Nuclei.</td>
</tr>
<tr>
<td></td>
<td>Synthesize Cyclin E</td>
<td>Check if the cell is prepared to: divide or differentiate.</td>
</tr>
<tr>
<td></td>
<td>Synthesize Substances</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increase Cell Metabolic Rate</td>
<td></td>
</tr>
<tr>
<td>Synthesis</td>
<td>Synthesize Cyclin A</td>
<td>Check if there are Chromatins replicated, if the Centrosome was</td>
</tr>
<tr>
<td></td>
<td></td>
<td>replicated and if the MPF is deactivated.</td>
</tr>
<tr>
<td></td>
<td>Start Chromatin Replication</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Replicate Centrosome</td>
<td></td>
</tr>
<tr>
<td>Gap 2</td>
<td>Finish Chromatin Replication</td>
<td>Check if all the Chromatins were replicated, if Cyclin M was synthesized</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Synthesize Cyclin M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MPF is activated</td>
<td></td>
</tr>
<tr>
<td>Prophase</td>
<td>Condense Chromatin into Chromosomes</td>
<td>Check if all the Chromatins and their replicas were condensate into</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chromosomes.</td>
</tr>
<tr>
<td></td>
<td>Create Repulsive Forces (for Astral and Polar Microtubules)</td>
<td></td>
</tr>
<tr>
<td>Prometaphase</td>
<td>Create Repulsive Forces (for Kinetochore Microtubules)</td>
<td>Check if all the Kinetochore Microtubules were attached to each Chromosome,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and if the Nuclear Membrane was dissolved.</td>
</tr>
<tr>
<td></td>
<td>Dissolve Nuclear Membrane</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Attach Kinetochore To Microtubule</td>
<td></td>
</tr>
<tr>
<td>Metaphase</td>
<td>Move Chromosome To Equatorial Plane</td>
<td>Check for each Chromosome its position in the cell.</td>
</tr>
<tr>
<td>Anaphase</td>
<td>Separate Chromatids</td>
<td>Check if all the Chromatids were separated.</td>
</tr>
<tr>
<td>Telophase</td>
<td>Demount Mitotic Spindle</td>
<td>Check if the Nuclear Membranes were created, and if all the Chromosomes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>were unfold back to Chromatin</td>
</tr>
<tr>
<td></td>
<td>Shrinking Microtubules</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Create Nuclear Membranes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unfold Chromosomes Back To Chromatin</td>
<td></td>
</tr>
<tr>
<td>Cytokinesis</td>
<td>Constrict Cell Membrane</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Create Abscission</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reorganize and Disappear Non-Kinetochore Microtubules</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1 - Cell Life-Cycle Phases, Events and Checkpoints**

Considering the cell life-cycle, the cell may stay in the G0 phase while it does not receive any signal for starting the cycle. The signal may be a protein, or any other substance. At
the end of the cycle considering the mitosis division, there are two new cells in the G0 phase.

It is possible to change this course depending on which signals the cell receives. Basically, during the G1 phase, the cell receives a signal to: start division, start specialization in the case of being a stem cell, or start differentiation in the case of being a specialized cell.

Furthermore, any cell (stem cell, specialized or differentiated) may do apoptosis. Basically, if any internal process leads the cell to a bad state, or if the niche is overpopulated, the cell kills itself.

4.2. The Agent-based Model

The MAS language provides a set of static and dynamics diagrams. The first step consists in define the environment, active and passive organizations, agents, roles and objects. We defined the Niche the main organization. Each cell (stem cell, progenitor cell and differentiated cell) is an active organization and it plays its respective role in the Niche.

The Cell organization has the sub-organization Cytoplasm, which contains all the organelles, the Centriole, the nuclei with the chromosomes and so on. Every signal in the cell is transmitted to the Cytoplasm. The Cell also contains the cell membrane.

Figure 4 – The Agent-Based Model: Partial View of the Organization Diagrams

Figure 4 illustrates the main-organization Niche and the active sub-organization Cell. The environment, modeled as a passive element, is shown as a package that brings together all the entities that inhabit it.

A Sequence diagram models the interactions between (i) agents playing roles, (ii) organizations playing roles, (iii) environments and (iv) objects while either playing roles or not.

The goal Cell called “metabolize” means to follow the cell life-cycle. To achieve this goal the Cell has to achieve ten sub-goals, one for each phase (G0, S, G1, Prophase, etc). And for each phase there is a plan with a set of actions that allows the agent to achieve the specific plan. Figure 5 illustrates the sequence diagram for the Prometaphase with the goals, sub-goals, plans and actions.
4.3. The Agent-based Simulation

The agent-based simulation was developed with Java. We didn’t use any agent-based framework, middleware or platform because we didn’t want to adapt the modeling to the platform for this first approach. But we intend to reuse a robust platform to provide distribution, performance and scalability. However, we developed a small framework with a set of functionalities as send and receive messages, the instantiation of the environment, and the hierarch structure between agent, role, organization, goal, plan and action.

The development of a stem cell behavior simulator prototype besides being an instance of the proposed model, has as objectives: offer to the users a visual environment by means of which is possible to follow the Macro and Micro levels of the simulation of stem cell’s cellular life cycle in the niche; perceive the difficulties of implementation of the proposed model; validate initially the model, verifying if the simulated behavior in the prototype has similarities with the behavior of the real entities (stem cell’s); and compare the semantics of a tool built based on MAS approach to this domain with tools built in other approaches.

We understand as Macro level the emergent behavior proceeding of the interactions between the simulated entities. This level is presented by the simulator to the users by means of a visualization area (2D) that represents the niche where the cells evolve in its life-cycles. Each phase of cell life-cycle has a graphical representation, presenting the state of the main components involved in process. These graphical representations, besides presenting a phase of the life-cycle differentiate by means of colors the capacity of differentiation of the cell: red for multipotent, i.e, the stem cell; orange for progenitor; and yellow for differentiated (Figure 6).

As Micro level we understand the state and behavior of each entity simulated individually. In the prototype is possible to obtain the micro level clicking over the cell images presented in the visualization area of the tool. The Cell Data Interface presents the internal state of the cell selected and the Internal Process Interface presents chronologically each action made by the selected cell.

Figure 7 shows a snapshot of the interface of the prototype during a simulation. The Macro level, emergent behavior can be seen in the visualization area and the Micro Level is presented in the Cell Data and Internal Process Interfaces.
5. Discussion

In contrast to the classical mathematical descriptions mainly based on ordinary differential equations, the specification of stem cell behavior is based on behavioral modeling. And Petri Net tools may be used with this purpose. Furthermore, if we want to give richness to the model we might use COOPN (Concurrent Object-Oriented Petri Nets) (Biberstein et al., 2001) COOPN is an object-oriented specification language based on synchronized algebraic Petri nets, COOPN defines Petri nets and coordination between Petri nets using object-oriented approach.

COOPN is based on Algebraic Data Types (ADT), Petri nets, and IWIM coordination models (Arbab, 1996). Hence, COOPN specifications are collections of ADT, class and context (i.e. coordination) modules. Syntactically, each module has the same overall structure; it includes an interface section defining all elements accessible from the outside, and a body section including the local aspects private to the module.

We took the same cell conceptual model and the same processes used for the agent-based modeling and simulation, and we modeled and simulated the stem cell behavior using COOPN. It is also important to highlight that we tried to use Petri Nets but it was unfeasible.

Comparing the results, building the COOPN model and simulation was much easier and fast than building the agent-based solution. However, it was not possible to provide a solution for the experiments that the physicians’ desire, i.e., a mechanism on which the physicians might input some substances and proteins and the system raises an emergent phenomena.
Analyzing separately our agent-based model solution, we realized that the agent-based model solution overloaded the CPU even with few numbers of entities. The visualization was freeze in many times because of the overloading.

Furthermore the agent-based model solution was not developed considering the visualization process, and the process control. Hence it was a hard task to incorporate the interface to the framework.

6. Conclusions and Future Works

The stem cell researchers’ collaborators were very excited with the first results. Basically they observed in the visualization tool the first emergent phenomenon which is similar to the emergent phenomenon in vitro: the differentiated cells are located at the colony’s extremity while the specialized and stem cells are located at the colony’s centre.

The next steps are a review and a set of tests on the proposed model, intending to adjust it for the demand of the biomedical domain. Other activities are to produce reports about Macro and Micro levels to support the comprehension of the information visualized during the simulation process, and identify with the specialists of the domain the trustworthiness of the results gotten from this first prototype in order to allow an adaptation and bias adequate to the reality of the research of the domain.

Acknowledgements

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