Agent-Based Modelling of Stem Cell Self-organisation in a Niche

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Abstract. It is our belief that modelling the behaviour of stem cells in the adult human body as an agent-based system is the most appropriate way of understanding the process of self-organisation. We have undertaken several case studies where formal and/or computational models of stem cell systems, have been re-developed using an agent-based approach. This paper presents details of one of these case studies where we have used an agent-based approach as opposed to a cellular automata approach. A formalisation of the non-agent and agent-based approach is given, and from the results of this investigation, we aim to demonstrate the advantages of the agent-based approach for developing biologically plausible models with emergent self-organising dynamics. The aim of this paper first to discuss the importance of modelling and simulating stem cells, because of certain experimental limitations, but also to demonstrate that the multi-agent approach to modelling is the most appropriate.

1 Introduction

In recent years there has been a growing debate about how stem cells behave in the human body; whether the fate of stem cells is pre-determined [11] or stochastic [13, 19], and whether the fate of cells relies on their internal state [12], or on extra-cellular micro-environmental factors [21]. There have been several attempts to build formal models of these theories, so that predictions can be made about how and why stem cells behave either individually or collectively. An excellent review of these formal approaches can be found in a recent publication [22].

Recent experimental evidence has suggested that stem cells development may be more complicated than was originally thought. The standard model of stem cell development is that a stem cell becomes increasingly differentiated over time along a well-defined cell lineage and eventually becomes a fully functional cell. This model has been challenged by many researchers including one of our collaborators, Neil Theise [7, 18, 16]. Several years ago, new theories were proposed by our collaborator and others that challenged the prevailing view because new experimental data suggested that stem cell fate is both *reversible*, i.e. cells can become less differentiated or behave more like stem cells, and *plastic*, i.e. cells can migrate from one cell lineage to another.

Whilst working on Cell, with an interdisciplinary team including Theise and the artist Jane Prophet, it became clear to us that the most appropriate way to model stem

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cells in the adult human body was as a dynamic system of self-organising agents. Our work to date has used our existing, well-established techniques for specifying and modelling agent-based systems in general [4, 6, 9, 10] and progressed along two parallel strands. The first strand of our work has been an attempt to develop an agent-based model of Theise's theory of stem cell behaviour and organisation [17, 5]. The second strand has been to use the same agent-based approach to analyse and re-develop existing models to ensure that our framework is sufficiently flexible to model more than one theory and to understand how other work differs from our own. In other words, we have been working on re-implementing agent-based versions of cellular automata and equational models of stem cells in order to support our claim that the agent approach is more suitable than other current modelling approaches.

In this paper we consider one of the latest models of stem cell systems and show what can be gained from evaluating them using our agent framework. The aim of this paper is to show why we need simulations of stem cell behaviour in general, to demonstrate the role of formal modelling in developing these simulations, and to show the benefits of a multi-agent approach over other possible modelling approaches. We also aim to substantiate our belief that stem cell self-organisation and behaviour is an emergent of the individual interactions of individual stem cells with each other and with the environment in which they are situated.

Before we consider this work in detail, we first consider the reasons why we might want to build models of stem cell systems in general.

1.1 Formal Modelling of Stem Cells

The mathematical modelling, conceptualisation and simulation of stem cell behaviour is beginning to receive a substantial amount of interest from a number of researchers [14, 1, 8]. As has been pointed out by others, predictive models of stem cell systems, could provide important new understandings of the self-regulating mechanisms that result in well known global properties of stem cells. These include the following qualities of a healthy human adult.

1. There are always a sufficient number of stem cells.
2. Fully determined cells are sufficiently replenished as they die.
3. The system of stem cells can recover after serious injury or disease.

As has been discussed by a number of authors [22, 14] there are several reasons why formal predictive models of stem cells will receive an increasing amount of attention in the near future. Though the first model we know of was published in 1964 ([20]) there has been surprisingly little work in this field until the last couple of years. Indeed over the last few years, there has been a noticeable climate change in this respect, and there is now a growing awareness of the need to use mathematical modelling and computer simulation to understand the processes and behaviours of stem cells in the body. An excellent review of existing models has been recently published [22].

We summarize what we see are the key reasons for the systematic development of formal models and simulations to consider hypothesis about the nature and behaviour of stem cells.

1. It is not possible to investigate how stem cells react by looking at dead tissue, and much stem cell research is based on observation of dead, 2-D slides. Building simulations allows researchers to test possible cell behaviours that can then be related back to observable laboratory results.
2. In the adult body, stem cells cannot be distinguished morphologically from other primitive non-determined cell types. It is therefore hard, if not impossible, to observe their behaviour in the dynamic system of which they are a part.
3. The size and complexity of stem cell systems mean that without simulation, it is not possible to consider the whole system. Simulations provide an important tool for understanding the global behaviour of complex systems reacting agents.
4. Clearly any formal model, and resulting simulation, of stem cells will necessarily incur massive simplifications and abstractions about the machinations of the human body. It is our belief, however, that theoretical simplifications are often key to understanding fundamental properties of natural systems.
5. It is the *potential* of cells to behave in lots of different ways which makes them more or less stem like. It may be that stem cell is a notion rather than an artifact and refers to the wide-ranging set of potential behaviours that it might have that are influenced by internal, environmental, and stochastic processes. Simulations provide a way of determining which behaviours are essential to stem cells and which are incidental in systems that have been studied in the laboratory.
6. When you consider experimental evidence you have seen only one behaviour. This behaviour may have been one of many, and it is the potential for cells to behave in certain ways that might be key to defining them. Modelling and simulation is a much more effective device for understanding "behavioural potential" than looking at completed chains of events in the lab.
7. Though our work has been explicitly concerned with modelling the adult human body, it is clear that simulation does not involve any ethical difficulties such as extracting stem cells from an embryo in such a way that it is sacrificed.
8. And of course, simulation is cheap.

This should give the reader an indication of why we believe this will become a growing field in the next few years. In our approach we have used an agent-based approach to the formal modelling and simulation of stem cells, and we make the following claims which we will attempt to substantiate in this paper.

1. An agent-based approach provides more flexibility than other more limited approaches and so delivers greater potential for modelling more sophisticated, globally emergent, behaviour.
2. An agent-based approach can also provide more biological plausibility than existing approaches such as cellular automata and other mathematical approaches. One of the main reasons that biological plausibility is important is to attract biologists to use and work with any models and simulations that are created.
3. Stem cells are a prime example of a self-organising system where individual cells react to their local physical, chemical and biological environment. The system should therefore be most suitably modelled as a system of interacting reactive

agents, where the reaction at the micro level gives rise to the emergent behaviour at the system level.

4. Even though we are simulating cells and environment, the Brooksian idea of an agent being something which is both situated and embodied ([2]), is a fundamental driving force of our use of agents as the appropriate modelling paradigm. Cells modelled as agents have a physical, chemical and biological presence and are situated in a physical, chemical and biological environment in which they react. The way in which they react will then influence the way other cells react in the future and so on. This then, becomes a complex system, as we have claimed before, that stem cell systems should be modelled as complex adaptive systems.
5. By situating our simulation work in a wider formal framework we can compare and evaluate different models. We believe that this is necessary for this new field to develop in a systematic manner.
6. Moreover, the formal framework allows us to "agentify" existing models, making it very clear what the relationship between the existing version and the agent version is.
7. By building a formal model using a specification language from software engineering, there are techniques to ensure that the simulation correctly implements the model.

In this paper we go some way to justifying our claims above by looking at one case study in detail. We consider the work of Agur et al. who have developed a cellular automata model of stem cells, and show that by re-caging this work in terms of an agent model, we can highlight difficulties of the cellular automata approach in general, but also increase the biological plausibility of the model.

In what follows below we will provide formal specifications of the original model and the agent-based reformulation using the language Z [15]. We have a history of using Z to build specifications of agent and non-agent computational systems that allows us to compare and evaluate different models and approaches [6].

2 A Cellular Automata Approach to Modelling Stem Cells

In recent work, Agur et al. [1] built a cellular automata model to show how the number of stem cells in the bone marrow could be maintained and how they could produce a continuous output of determined cells. The bone marrow is considered to be a stem cell *niche* where most biologists believe that the human body's supply of hematopoietic stem cells are situated and maintained.

This work is important because it is one of the few examples where a mathematical model has been used to show what properties of stem cells might be required to enable the maintenance of the system's homeostasis. The model demonstrates a possible mechanism that allows a niche to maintain a reasonably fixed number of stem cells, produce supply of mature (determined) cells, and to be capable of returning to this state even after very large perturbations that might occur through injury or disease. The behaviour of a cell is determined (equally differentiated) by both internal (intrinsic) factors, e.g. a

local counter, and external (extrinsic) factors, e.g. the prevalence of stem cells nearby, as stated by the authors as follows.

1. Cell behaviour is determined by the number of its stem cell neighbours. This assumption is aimed at simply describing the fact that cytokines, secreted by cells into the micro-environment are capable of activating quiescent stem cells into proliferation and determination.
2. Each cell has internal counters that determine stem cell proliferation and stem cell transition into determination as well as the transit time of a differentiated cell before migrating to the peripheral blood.

In the cellular automata model, the niche is modelled as a connected, locally finite, undirected graph.

$$[Node]$$

$$[X]$$
$$connected\ \_ : \mathbb{P}(\mathbb{P}(X \times X))$$

This can be represented as a symmetric relation on the set of nodes, such that no node relates to itself. We also assume that a graph is connected.

$$graph : Node \leftrightarrow Node$$
$$neighbours : Node \rightarrow (\mathbb{P}\, Node)$$
$$\forall\, n : Node \bullet (n, n) \notin graph$$
$$graph^{\sim} = graph$$
$$\forall\, n : Node \bullet neighbours\ n = \mathrm{ran}(\{n\} \lhd graph)$$
$$connected\ \ graph$$

Any *Node* is either empty, or it is occupied by either a stem cell or a determined cell. Here we introduce a naming convention that we shall use throughout where we add a two letter suffix to all names specific to a model, in the case of the Agur model we add the suffix "Ag".

$$TypeAg ::= EmptyAg \mid StemAg \mid DeterminedAg$$

The state of any node is given by the node location, the state, and an internal clock.

$$NodeStateAg$$
$$node : Node$$
$$type : TypeAg$$
$$counter : \mathbb{N}$$

The set of all such nodes is then given below, and defines the system state. We also define a function that returns the neighbouring node states for any given node state.

$$
\begin{array}{|l}
\hline
SystemStateAg \\
nodes : \mathbb{P}\, NodeStateAg \\
neighboursAg : NodeStateAg \rightarrow \mathbb{P}\, NodeStateAg \\
\hline
\{n : nodes \bullet n.node\} = Node \\
\#nodes = \#Node \\
\forall\, n, m : NodeStateAg \bullet \\
\quad m \in (neighboursAg\ n) \Leftrightarrow \\
\quad m.node \in (neighbours\ n.node) \\
\hline
\end{array}
$$

There are three constant values, we will call them $LeaveNicheAg$, $CyclingPhaseAg$ and $NeighbourEmptyAg$ in our specification, that are used to reflect experimental observation. $LeaveNicheAg$ represents the time taken for a determined cell to leave the niche. $CyclingPhaseAg$ represents the cycling phase of a stem cell; a certain number of ticks of the counter are needed before the cell is ready to consider dividing. Finally, $NeighbourEmptyAg$ represents the amount of time it takes for an empty space that is continuously neighboured by a stem cell, to be populated by a descendent from the neighbouring stem cell.

$$
LeaveNicheAg, CyclingPhaseAg, NeighbourEmptyAg : \mathbb{N}
$$

We now specify how the system changes over time. Whenever there is a change of state in the system, we identify the node that we are considering as $node$. As a consequence of each change $node$ is removed and replaced with a new node, $newnode$, that represents the updated state. All locations are updated simultaneously.

$$
\begin{array}{|l}
\hline
\Delta SystemStateAg \\
SystemStateAg \\
SystemStateAg' \\
node, newnode : NodeStateAg \\
\hline
nodes' = (nodes \setminus \{node\}) \cup \{newnode\} \\
\hline
\end{array}
$$

The rules of this model, which determine what happens at a node based on internal and external factors are described and specified below.

1. Determined cell nodes
   (a) If the internal counter of a node representing a determined cell has reached $LeaveNicheAg$ then the cell leaves the niche; the internal counter of the node is reset to 0, and the new state at the node becomes empty.

$$
\begin{array}{|l}
\hline
DeterminedLeaveNicheAg \\
\Delta SystemStateAg \\
\hline
node.type = DeterminedAg \\
node.counter = LeaveNicheAg \\
newnode.type = EmptyAg \\
newnode.counter = 0 \\
\hline
\end{array}
$$

(b) If the internal counter has not yet reached $LeaveNicheAg$ then the internal conter is incremented.

$$
\begin{array}{|l}
\hline
DeterminedStayNicheAg \\
\Delta SystemStateAg \\
\hline
node.type = DeterminedAg \\
node.counter < LeaveNicheAg \\
newnode.type = node.type \\
newnode.counter = node.counter + 1 \\
\hline
\end{array}
$$

2. Stem cells nodes

(a) If the internal counter of a node representing a stem cell has reached the constant $CyclingPhaseAg$, and all of the nodes neighbours are stem cells, then the state of the node becomes a determined cell and the internal counter is reset to 0.

$$
\begin{array}{|l}
\hline
StemToDeterminedNodeAg \\
\Delta SystemStateAg \\
\hline
node.type = StemAg \\
node.counter = CyclingPhaseAg \\
\forall\, n : (neighboursAg\ node) \bullet n.type = StemAg \\
newnode.type = DeterminedAg \\
newnode.counter = 0 \\
\hline
\end{array}
$$

(b) If the internal counter of a node representing a stem cell is equal to $CyclingPhaseAg$ but not all the node's neighbours are stem cells then do nothing; leave the internal counter unchanged.

$$
\begin{array}{|l}
\hline
RemainAsStem1Ag \\
\Delta SystemStateAg \\
\hline
node.type = StemAg \\
node.counter = CyclingPhaseAg \\
\neg\, (\forall\, n : (neighboursAg\ node) \bullet n.type = StemAg) \\
newnode.type = node.type \\
newnode.counter = node.counter \\
\hline
\end{array}
$$

(c) If the counter has not reached $CyclingPhaseAg$ then do nothing except increment counter by 1.

$$
\begin{array}{|l}
\hline
RemainAsStem2Ag \\
\Delta SystemStateAg \\
\hline
node.type = StemAg \\
node.counter < CyclingPhaseAg \\
newnode.type = node.type \\
newnode.counter = node.counter + 1 \\
\hline
\end{array}
$$

3. Empty nodes
   (a) If the internal counter at an empty node has reached $NeighbourEmptyAg$ and there is a stem cell neighbour then introduce, i.e. give birth to, a stem cell in that location. The internal counter of the node is reset to 0.

$$
\begin{array}{|l}
\hline
BecomeStemAg \\
\Delta SystemStateAg \\
\hline
node.type = EmptyAg \\
node.counter = NeighbourEmptyAg \\
\exists\, n : (neighboursAg\ node) \bullet n.type = StemAg \\
newnode.type = StemAg \\
newnode.counter = 0 \\
\hline
\end{array}
$$

   (b) If the counter at an empty grid has not reached $NeighbourEmptyAg$ and there is exists a stem cell neighbour then increment the counter by 1.

$$
\begin{array}{|l}
\hline
RemainEmpty1Ag \\
\Delta SystemStateAg \\
\hline
node.type = EmptyAg \\
node.counter < NeighbourEmptyAg \\
\exists\, n : (neighboursAg\ node) \bullet n.type = StemAg \\
newnode.type = EmptyAg \\
newnode.counter = node.counter + 1 \\
\hline
\end{array}
$$

   (c) If there are no stem cell neighbours at all then reset the internal counter to 0.

$$
\begin{array}{|l}
\hline
RemainEmpty2Ag \\
\Delta SystemStateAg \\
\hline
node.type = EmptyAg \\
\neg\, (\exists\, n : (neighboursAg\ node) \bullet n.type = StemAg) \\
newnode.type = EmptyAg \\
newnode.counter = 0 \\
\hline
\end{array}
$$

2.1 Discussion About the Cellular Automata Approach

We now have provided a specification of this system, and this formal model immediately identifies a number of issues with this cellular automata work.

1. The specification clearly reveals that niche spaces, i.e. empty nodes, must have counters for this model to work. In a sense, empty space is having to do some computational work. Clearly this lacks biological feasibility and is against what the authors state about modelling cells, rather than empty locations, having counters.
2. Stem cell division is not explicitly represented, instead stem cells are brought into being by empty space.

3. More subtly, these stem cells appear when empty nodes have been surrounded by at least one stem cell for a particular period of time. However, the location of the neighbouring stem cell can vary at each step. Even though the model details the fact that if a stem cell is next to an empty space long enough then it will divide so that it's descendent occupies this space. However, the rule does not state that the neighbouring stem cell must be the same stem cell for every tick of the counter. It states something much weaker; that there must be a neighbouring cell, possibly different each time, for each tick of the counter from 1 to $NeighbourEmptyAg$. Biologically, it would seem more intuitive that the same stem cell should be next to an empty niche space for this length of time in order for "division" to occur into the space but the model lacks a "directional component".
4. The state of a stem cell after division is not defined. Let us for a moment assume that the neighbouring stem cell (S) is fixed for all counts from 1 to $NeighbourEmptyAg$ from some specific location (N). Nothing is said about what happens to S after a new stem cell appears in N. For example, should the counter of S be reset after division? Neither does it give any preconditions on S. For example, does S's local counter need to have reached an appropriate point in its cycling phase for this to happen?

So the basic problem is that this model relies on allowing both unfilled niche locations as well as stem and determined cells to have counters. Moreover, it does not investigate or model the nature of a stem cell before and after division. We now attempt to re-interpret these rules using an agent-based approach that still retains the overall qualities of the model.

2.2 Re-formulation Using an Agent-Based Approach

One of the biggest differences between the original cellular automata model and our re-formulation is the change in the role of graph nodes. In the cellular automata model each node represents either a cell or an empty space. In our re-formulation, each node represents a space that may or may not contain an agent that represents a cell. This difference in the two models is illustrated in Figure 1.

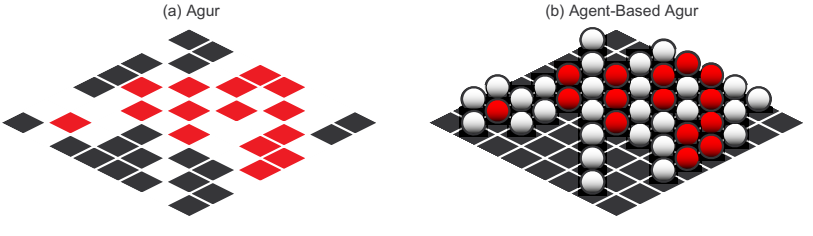

Fig. 1. A comparison of the original Agur cellular automata model and our reformulation as a grid-based agent model. In the original model the nodes maintain the state of the cells, whereas in our re-formulation the nodes contain agents and it is the agents that maintain the state of the cells

With the agent approach we also provide each cell with a unique identifier. We model all cells as having one internal counter as before. In addition there is a counter associated with each of the neighbouring nodes. The counters associated with neighbouring nodes record how long the neighbouring location has been empty. Moreover, cells can sense the type of cell at each of its neighbours, although this perception ability is only used by stem cells. If an agent represents a stem cell then it can potentially divide into any location where the counter has reached $NeighbourEmptyAg$.

$[AgentId]$

AgentCellAg

$$
\begin{array}{l}
id : AgentId \\
type : TypeAg \\
counter : \mathbb{N} \\
nscounter : Node \pfun \mathbb{N} \\
nstype : Node \pfun TypeAg \\
\hline
type = StemAg \lor type = DeterminedAg \\
\mathrm{dom}\ nscounter = \mathrm{dom}\ nstype \\
\forall n : Node \mid nstype\ n \neq EmptyAg \bullet nscounter\ n = 0
\end{array}
$$

A stem cell agent is defined as follows.

AgentStemCellAg

$$
\begin{array}{l}
AgentCellAg \\
\hline
type = StemAg
\end{array}
$$

A determined cell agent is defined as follows.

AgentDeterminedCellAg

$$
\begin{array}{l}
AgentCellAg \\
\hline
type = DeterminedAg
\end{array}
$$

The initial state of an stem cell agent is defined as follows.

InitAgentStemCellAg

$$
\begin{array}{l}
AgentCellAg \\
\hline
counter = 0 \\
\mathrm{ran}\ nscounter = \{0\}
\end{array}
$$

The initial state of a determined cell agent is defined as follows.

InitAgentDeterminedCellAg

$$
\begin{array}{l}
AgentDeterminedCellAg \\
\hline
counter = 0
\end{array}
$$

We define a mature stem cell as one which is ready to divide.

$$
\begin{array}{|l}
\hline
\textit{MatureAgentStemCellAg} \\
\textit{AgentStemCellAg} \\
\hline
\textit{counter} = \textit{CyclingPhaseAg} \\
\hline
\end{array}
$$

The system state consists of the niche where some nodes are filled with cells. The first predicate simply states that the empty nodes are those nodes which do not contain a cell. The second predicate states that the neighbours are defined by the graph to which the cells are attached.

$$
\begin{array}{|l}
\hline
\textit{AgentSystemStateAg} \\
\textit{cells} : \textit{Node} \rightarrow \textit{AgentCellAg} \\
\textit{emptynodes} : \mathbb{P}\, \textit{Node} \\
\hline
\textit{emptynodes} = \textit{Node} \setminus (\mathrm{dom}\ \textit{cells}) \\
\forall\, n : \textit{Node};\ c : \textit{AgentCellAg} \mid (n, c) \in \textit{cells} \land c.\textit{type} = \textit{StemAg} \bullet \\
\qquad \mathrm{dom}\ c.\textit{nscounter} = \mathrm{ran}(\{n\} \lhd \textit{graph}) \\
\hline
\end{array}
$$

2.3 Operation

Space does not permit us giving a full treatment, and of course many of the operations would be identical to that which we have specified before, but we outline the basic operations here.

1. Cells set/update counters.
2. Mature stem cells that are surrounded by empty neighbours and have neighbour counters have reached $\textit{NeighbourEmptyAg}$ will make a request to the environment to divide into two daughter stem cells.
3. The environment resolves any conflicts where several cells wish to divide into the same node and informs those mature stem cells that can divide and those that are not able to.
4. Mature stem cells that are able to divide do so. Mature stem cells that are surrounded by stem cells become new determined cells. Mature determined cells which are ready to leave the niche do so.

We consider each of these four stages in turn.

Updating Counters. We use the auxiliary function which increments all the counters of a cell up to the maximum value.

$$
\begin{array}{|l}
\hline
\textit{incrementcounters} : (\textit{Node} \pfun \mathbb{N}) \rightarrow (\textit{Node} \pfun \mathbb{N}) \\
\hline
\forall\, f : \textit{Node} \rightarrow \mathbb{N};\ \textit{max} : \mathbb{N} \bullet \textit{incrementcounters}\ f = \\
\qquad \{\textit{node} : \textit{Node};\ n : \mathbb{N} \mid (\textit{node}, n) \in f \bullet \\
\qquad\qquad (\textit{node}, \mathbf{min}\{n + 1, \textit{NeighbourEmptyAg}\})\} \\
\hline
\end{array}
$$

The reset for all determined cells is straightforward.

$$
\begin{array}{|l}
\hline
\textit{UpdateCounterDeterminedAg} \\
\Delta \textit{AgentCellAg} \\
\hline
\textit{type} = \textit{DeterminedAg} \\
\textit{counter} = \textit{counter}' + 1 \\
\hline
\end{array}
$$

The reset for stem cells depends on whether the cell is mature. In all cases the counters for the empty niche are updated.

$$
\begin{array}{|l}
\hline
\textit{UpdateCounterStemAg} \\
\Delta \textit{AgentCellAg} \\
\hline
\textit{type} = \textit{StemAg} \\
\textit{counter} < \textit{CyclingPhaseAg} \Rightarrow \textit{counter} = \textit{counter}' + 1 \\
\textit{counter} = \textit{CyclingPhaseAg} \Rightarrow \textit{counter}' = \textit{CyclingPhaseAg} \\
\textit{nscounter}' = \\
\quad (\textit{incrementcounters nscounter}) \oplus \\
\quad\quad (\{n : \textit{Node} \mid \textit{nstype } n \neq \textit{EmptyAg} \bullet (n, 0)\}) \\
\hline
\end{array}
$$

Request Division. Our agent-based approach to modelling forces us to consider what happens when two stem cells attempt to divide into the same location. In our model, we specify that when the internal counter reaches $\textit{CyclingPhaseAg}$, it signals to the environment the niche spaces that it is prepared to divide into.

Notice, that this approach is also agent-based in nature. Namely, the agent attempts to do something but the environment is a dynamic and uncertain one. From the perspective of a single cell with its limited sensory abilities the world is no longer deterministic like, it was in the cellular automata model, and not all attempts at action will be successful.

The agent-based model not only considers the nature of acting in a dynamic environment but also addresses issues such as the basic physical limitations of the stem cell niche in general. Once again, it's difficult to see how such issues can be considered, at least explicitly, with the cellular automata approach.

A stem cell agent that is ready to divide, signals to the environment those neighbours that have been empty for long enough, and so are able to receive the new cell. Of course the output may be empty.

$$
\begin{array}{|l}
\hline
\textit{RequestDivision} \\
\textit{AgentCellAg} \\
\textit{possnodes}! : (\textit{AgentId} \times \mathbb{P}\, \textit{Node}) \\
\hline
\textit{counter} = \textit{CyclingPhaseAg} \\
\textit{possnodes}! = (id, \{n : \textit{Node} \mid \textit{nscounter } n = \textit{NeighbourEmptyAg} \bullet n\}) \\
\hline
\end{array}
$$

The Environment Allocates Nodes for Division. The environment receives requests from cells to divide, and non-deterministically assigns those cells that can divide and

those that have insufficient space around them. There are several safety properties that we can specify here:

1. all agents get a reply (first predicate)
2. no agent can be told to divide and not divide (second predicate)
3. no node ever has more than one agent dividing into it (third predicate)
4. cells only get to divide into a node they have requested (fourth predicate)
5. there is no remaining empty node that has been requested by any of the agents not-granted division (fifth predicate).

DetermineDivision

$$
\begin{array}{l}
\Xi AgentSystemStateAg \\
requests? : AgentId \rightarrow (\mathbb{P}\, Node) \\
divide! : AgentId \rightarrowtail (Node \times AgentId) \\
nodivide! : \mathbb{P}\, AgentId \\
\hline
\mathsf{Let}\ cellsdividing == \mathrm{dom}\ divide! \bullet \\
\mathsf{Let}\ cellsnotdividing == nodivide! \bullet \\
\mathsf{Let}\ cellsrequesting == \mathrm{dom}\ requests? \bullet \\
\mathsf{Let}\ nodesreceiving == \\
\quad \{n : Node;\ id : AgentId \mid (n, id) \in (\mathrm{ran}\ divide!) \bullet n\} \bullet \\
cellsdividing \cup cellsnotdividing = cellsrequesting \wedge \\
cellsdividing \cap cellsnotdividing = \{\} \wedge \\
\#cellsdividing = \#nodesreceiving
\end{array}
$$

2.4 Division and Determination

Cells that divide get told where they should divide into. We have two alternatives with the assignment of identifiers to the daughter cells. We can either give both daughters new identifiers, which is useful for tracking where they cells from, or the daughter cell which remains in the node of the previous cell keeps the id of its parent. We specify the first of these alternatives here.

AgentDivideAg

$$
\begin{array}{l}
\Delta AgentSystemStateAg \\
parent? : AgentId \\
to? : Node \\
daughter1Id?, daughter2Id? : AgentId \\
\hline
\mathsf{Let}\ cell == (\mu\, a : AgentCellAg \mid a.id = parent?) \bullet \\
\mathsf{Let}\ currentnode == cells^{\sim} cell \bullet \\
\mathsf{Let}\ daughter1 == \\
\qquad (\mu\, ag : InitAgentStemCellAg \mid ag.id = daughter1Id?) \bullet \\
\mathsf{Let}\ daughter2 == \\
\qquad (\mu\, ag : InitAgentStemCellAg \mid ag.id = daughter2Id?) \bullet \\
cells' = cells \oplus \{(currentnode, daughter1)\} \cup \{(to?, daughter2)\}
\end{array}
$$

If the cell is not allowed to divide then id does nothing.

$$
\begin{array}{|l}
\hline
AgenNoDivideAg \\
\Xi AgentSystemStateAg \\
id? : AgentId \\
\hline
\end{array}
$$

Stem cells which have reached their cycle phase and which are surrounded by stem cells become determined.

$$
\begin{array}{|l}
\hline
AgentDeterminationAg \\
\Delta AgentSystemStateAg \\
cell, newcell : AgentCellAg \\
node : Node \\
\hline
node = cells^{\sim} cell \\
cell.type = StemAg \\
cell.counter = CyclingPhaseAg \\
\text{ran } cell.nstype = \{StemAg\} \\
newcell.type = DeterminedAg \\
newcell.counter = 0 \\
cells' = cells \oplus \{(node, newcell)\} \\
\hline
\end{array}
$$

3 Discussion

We have run hundreds of simulations of both the original CA model and of our agent recapitulation to check that the behaviours of our agent model has the same properties of the CA model. As we explained above, the agent model has allowed us to do is address the issues of biological implausibility.

It is interesting to note that allowing cells to split into all available spaces, i.e. up to four daughters, gives us the closest possible agent-based simulation match to the original CA models, however, any biologically plausibility we may have introduced would be negated by this. By limiting cell division to result in a maximum of at most two daughter cells we still maintain the integrity of the original cellular automata version.

In the next section we now explore how we have used and agent-based approach to extend one of the most sophisticated models of the stem cell niche that we have seen in the literature that proposes an innovative way of understanding how stem cell properties are maintained by the niche.

From a biological viewpoint the model of Agur et al. does not allow any reversibility or plasticity in the basic properties of cells. For example, once a cell has differentiated it cannot become a stem cell again. Moreover, once a cell has left the niche, it cannot return.

A recent example of an approach that uses a more sophisticated model and addresses these issues, is that of Markus Loeffer and Ingo Roeder at the University of Leipzig, who model hematopoietic stem cells using various, but limited, parameters including representing both the growth environment within the marrow, one particular stem cell

niche, and the cycling status of the cell [8]. The ability of cells to both escape and re-enter the niche and to move between high and low niche affinities, referred to as within-tissue plasticity, is stochastically determined.

The validity of their model is demonstrated by the fact that it produces results in global behaviour of the system that match experimental laboratory observations. The point is that the larger patterns of system organization emerge from these few simple rules governing variations in niche-affinity and coordinated changes in cell cycle.

There is no doubt that Roeder's model is one of the most sophisticated ones that we have seen in the literature; it is formal, there is a simulation, it addresses key issues of self-organisation and much of the modelling has an agent-like quality to it. There are, however, a number of issues regarding this model that we have addressed by extending it using our agent framework. Most significantly, they use of a probability function to control the movement of cells between environments, and in the agent-view this is problematic. This probability is calculated from global information relating to the numbers of various cells in the system. Although it useful to assume access to this global information when developing the model of stem cell behaviour, no mechanism is known for how stem cells could have access to this information in real biological systems.

Space presents us to show our work here, but to summarise we have extended the Roeder model to produce an agent-based simulation that increases the biological intuition and plausibility of the model, and allows us to investigate emergence due to the subtle changes in micro-environmental effects for each cell. Modelling cells as agents responding autonomously to their local environment is much more fine grained than the previous model using equations to model cell transitions and allows for a much greater degree of sophistication in the possibilities of understanding how self-organisation actually takes place in the adult human body.

The main point is that an agent does not rely on getting information about the system state, in keeping with the reactive multi-agent systems approach, and we believe that this gives a more biologically plausible handle on how things might be working at the micro-environmental level.

We have extended the Roeder model to incorporate a model of space, albeit only in 2 dimensions so far, so that we can consider cell movement in more detail. We are particularly interested in experimenting with different shapes of niche to discover how these might affect the production or maintenance of stem cells and determined cells.

4 Concluding Remarks

It is perhaps worth noting that Roeder's model is similar in notion to Carriani's view of thermodynamic emergence [3]. It assumes that simple rules, i.e. the transition probability functions, can model complex behaviours of stem cells as they make their transition between niche and non-niche. The assumption is that complex behaviour can be understood by building models with simple behavioural rules that hide the complexities of the underlying interactions between many components, i.e. a top down approach to modelling.

By contrast, our model is more akin to Carriani's ideas of computational emergence. In this view, a series of simple rules gives rise to complex global behaviour, a bottom

up approach if you like, we build simple models of agents and chemical diffusion the lead to the emergence complex system-wide behaviours.

We are currently extending our work by analysing other models and simulations using our formal methods and developing new implementations of these models using agents. We are also continuing to work with Theise to specify new models of his theories using our experiences of analysing and implementing other models of stem cell systems.

We are investigating ways of comparing outputs from our simulation runs, and looking at metrics for determining when one simulation can said to be similar or share the same emergent properties as another simulations. Formal methods have been very useful in that they are re-usable, directly relate to the implementation, and enable us to readily extend and agentify existing work.

From these case studies we can start to produce a kind of generic agent-based framework and simulation environment for modelling and simulating natural biological systems in 2 or 3 dimensions using an agent-based perspective. We believe modelling complex biological systems using an agent-based framework helps to ensure that models have biological plausibility and we also believe they are the most appropriate way of beginning to understand how complex self-organising behaviours occur in natural systems.

In this paper we have had several aims. First, we believe that recent medical evidence suggests that the way to understand how stem cells organise themselves in the body is as a self-organising system, whose global behaviour is an emergent quality of the massive number of interactions of cells with each other and of the environment of which they are a part. We claim. therefore, that the multi-agent system approach to modelling is the most suitable one for exploring means to simulate the behaviour of stem cells and from resulting simulations, suggest how tiny changes in individual stem cell behaviour might lead to disease at the global, and hence observable from an experimental perspective, system level. We have outlined the benefits of this approach by comparing it to a cellular automata approach in detail. Furthermore, we have aimed to demonstrate the pivotal role of formality not only in precision and clarity with modelling and in developing correct and consistent simulations, but as the foundation for a common conceptual framework in a multi-disciplinary project.

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