Modeling Cell Migration in a Simulated Bioelectrical Signaling Network for Anatomical Regeneration

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Abstract

A key question in developmental biology and regenerative medicine concerns the physiological mechanisms by which cells coordinate their behaviors toward the construction and repair of complex anatomical structures. Gap junctional communication among cells enables bioelectrical signaling within a network that enables collections of cells to cooperate during morphogenesis. During regeneration in amputated planarian flatworms, cells capable of dividing must migrate to areas where new tissue is needed. Moreover, these cells must stop proliferating when the needed structures are completed. We previously proposed a cell-cell communication mechanism that enables structure discovery and regeneration by cell networks. In this paper, we further develop the mechanism to address two important simplifications of the previous model: cell division was not limited to adult stem cells (as it is *in vivo*), and adult stem cells did not migrate to injured areas to initiate the regeneration process. Thus, here we limit cell division to a specific cell type (neoblasts) and propose a second message type that guides neoblasts to locations where cell division is needed. Our results show that even after incorporating these two constraints, our cell-cell communication model maintained its regeneration capabilities against a large tissue removal.

Introduction

In animals that can regenerate their bodies in response to damage, cell-cell communication plays an important role in coordinating the large-scale regeneration process. Cellular activity must be coordinated toward the production of precisely the missing organs, in the right location, orientation and size. Cells communicate across long distances to identify areas where tissue is missing and to determine what type of cells needs to be created to repair to the target morphology (Pezzulo and Levin, 2015). Understanding the process of regeneration in organisms, such as planaria (Sheiman and Kreshchenko, 2015), may shed light on diverse research areas, from regenerative medicine to aging and birth defects (Birnbaum and Alvarado, 2008). Recently, progress has been made using models of gene regulation and physiological signaling to explain parts of the regeneration processes (Umesono et al., 2013; Durant et al., 2016). While it is important to identify the genes necessary for the process, functional control of pattern for regenerative medicine and synthetic bioengineering applications require discovery of the algorithms that are sufficient for implementing complex pattern repair.

A key question about the regeneration process in Planaria is how adult stem-cells (neoblasts) detect missing parts in the organism's body and initiate regeneration (Lobo et al., 2012). Part of the challenge lies in the fact that the process of cell migration in Planaria is not completely understood. Saló and Baguñà (1985) proposed that cell migration happens randomly through the organism due to cell proliferation. However, there is evidence that signals coming from the wound guide neoblasts to the area of the injury (Reddien and Alvarado, 2004). For instance, in a partially irradiated worm regeneration does not start immediately following injury. Instead, it takes around 4 weeks to create a blastema (mass of cells) capable of differentiating into the missing parts. This suggests that the wound keeps sending signals to the neoblasts, and that the neoblasts can migrate over long distances until they reach the area of the injury.

In this paper, we propose a signaling mechanism that indicates damage to an organism's morphology. These signals guide neoblasts to areas where a high level of cell proliferation is necessary. Our mechanism is based upon a previous model of cell-cell communication for dynamic morphology discovery and morphological repair (Ferreira et al., 2016). This work introduces two improvements to that model: we restricted cell division to adult stem cells, and we added stem cell migration.

All cells create and transmit *morphological messages* to discover the shape of the worm – one of the first mechanistic models of anatomical surveillance, a critical but poorly understood aspect of plasticity under anatomical change (Bryant et al., 2017). In our model, these messages backtrack until they reach where they started or there is no receiver cell. If a neoblast receives a backtracking message to a location with no receiver, then it divides, placing a new cell at that location. On the other hand, if a somatic cell receives a backtracking *morphological message*, then it cre-

ates a *migration message*, which is relayed until it reaches a neoblast. Information in the *migration message* then guides the neoblast to the target area. To test our modifications to the model, we performed an anterior cut that removed half of the simulated planarian's body. The process of sending and receiving messages corresponds to well-established mechanisms such as secretion and reception of chemical morphogen pulses, or propagation of bioelectric states across tissues (Levin and Martyniuk, 2018; Webb et al., 2005).

Background and Previous Work

We proposed a proof-of-concept model for structure regeneration based on cell-cell communication. In our model cells send messages that travel through the organism, hence dynamically discovering its shape. Morphology information exists in a distributed form across the set of existent messages in the organism at a given time. The genome does not encode the entire morphology, instead it encodes the machinery of how to treat those messages. The claim that Planaria contain some kind of distributed morphology information which guides regeneration is grounded by the fact that the target morphology (i.e., the shape to which the worm regenerates) can be stably edited without any modification of the genomic sequence. For example, treatment of planarians with reagents that temporarily block cell-cell communication leads to a permanent modification of the target morphology of the animal: pieces cut from such worms, without further treatment, continue to regenerate as bipolar 2-headed forms (Nogi and Levin, 2005; Oviedo et al., 2010; Durant et al., 2017).

In our agent-based model for structure discovery and repair, each cell is an agent that exchange messages with their neighbors. These messages contain information about the path they traveled through the structure. We denominate these messages as *morphology messages*. Thus, during a "discovery phase", morphology messages travel through the structure discovering the shape of the organism until they start a "backtracking phase". At this second phase, packets return through the same path they moved during the "discovery phase". If during the "backtracking phase" a cell has to send a message to a location where there is no receiver, then the holder of the morphology message divides and the daughter cell is correctly positioned to receive the message.

We showed that this cell-cell communication mechanism can maintain the morphology of a simulated worm even though some cells may randomly die due to, for example, natural aging or irradiation (Ferreira et al., 2016). In a second paper (Ferreira et al., 2017b), we proposed an "activation mechanism" to reduce the detrimental effects of noise that might exist during cell-cell communication. In Ferreira et al. (2017a) we introduced simulated neoblasts to our regeneration mechanism. We hypothesized that neoblasts are the only type of cells capable of creating new morphological messages. The *somatic cells* in the organism (defined there as "differentiated cells") only relay those messages. We showed that ratios as small as 10% of neoblasts were sufficient to fully regenerate a worm from an anterior cut that removed half of the worm's body.

Several researchers in the artificial life community have addressed the regeneration problem (also defined as shape homeostasis). Most of these works use the same mechanism for the development and regeneration processes. For example, Hotz (2003) and Andersen et al. (2009) independently evolved genetic-regulatory networks (GRNs) to develop organisms capable of self-repair. Epigenetic tracking (Fontana and Wróbel, 2013) and cellular automata development models (Basanta et al., 2008; Gerlee et al., 2011) have also been evolved and have shown regeneration capabilities.

Two models especially found results consistent with conclusions we derived from our model. Brodsky (2016) proposed a physics-based model for development of epithelial tissues. Due to the partial redundancy presented in his model, the developed tissue is robust against damage and environmental variation. Partial redundancy has also been important to our model in order for the "activation mechanism" to work against noisy configurations. Recently, Gerlee et al. (2017) evolved network models of cell dynamics for a shape development and maintenance. Each evolution contained one permutation of the model in which three model characteristics might exist: cell-cell communication, different cell actions (i.e., apoptosis and cell migration) and division polarity. The authors found solutions robust against mutations and wounding. Finally, they concluded that a long-range communication was the most important parameter in the evolved solutions. This conclusion is also what we found in our model, where the length of messages has a direct correlation to the performance of the model in regenerating the morphology against a large tissue removal.

Modeling Cell Migration and Proliferation

The previous version of our model rests on the assumption that all cells on the simulated planarian are capable of dividing. Thus, if a cell has to backtrack a message to a location without a receiver, then the cell divides and the newly created cell is rightly positioned to receive the message. However, this is not realistic since, in planarians, neoblasts are the only cell type capable of dividing and differentiating into any cell type (Scimone et al., 2014). In this work, we improve the model to allow only the neoblasts to proliferate. Cells need space to divide, thus it is necessary to create a guiding mechanism for neoblasts to migrate to the area where it is possible for them to proliferate. We also propose a new type of message that guides neoblasts to these areas in order for the regeneration process to complete.

We made a slight modification on the definition of the parameter $NeoblastRatio$ proposed in Ferreira et al. (2017a). Previously, $NeoblastRatio$ was the ratio of neoblasts uniformly distributed in the worm at the start of the simulation.

Here, $NeoblastRatio$ is also the ratio of neoblasts or *somatic cells* outcomes from division. More specifically, when a neoblast divides, it checks the value of this parameter to decide whether the daughter cell is another neoblast or a *somatic cell*. This way, the model tries to maintain the same ratio of neoblasts during the entire simulation.

We hypothesize that cells incapable of dividing (*somatic cells*) create a second type of message (defined here as *migration messages*) indicating to the neoblasts that there are missing cells in a location and a neoblast needs to migrate to that area. The following algorithms detail how the simulation works. At each cycle, the simulation performs Algorithm 1. First, all cells process messages received at the last cycle (Algorithm 2). When the message being processed is a *morphology message*, then the simulation performs Algorithm 3, otherwise the simulation runs Algorithm 5. After all cells processed all messages, cells create new *morphology messages* and send them along with the processed messages to their neighbors. When a neoblast is migrating, it performs the migration process of Algorithm 6. The conflict resolution we propose for cells acting in the same cycle is based upon the "id" of the cell (i.e., cell 1 acts before cell 2).

Algorithm 1 Process performed by the simulation for each cycle.

newCycle()

1: $cycle++$
2: **for all** $Cell\ i \in CellList$ **do**
3: $\quad processMessages(i, i.MessageList)$
4: **end for**
5: **for all** $Cell\ i \in CellList$ **do**
6: $\quad i.createNewMorphologyMessages()$
7: $\quad i.sendAllMessages()$
8: $\quad$ **if** $i.cellType == Neoblast$ **then**
9: $\quad\quad$ **if** $isMigrating(i)$ **then**
10: $\quad\quad\quad neoblastMigration(i, i.migMsg)$
11: $\quad\quad$ **end if**
12: $\quad$ **end if**
13: **end for**

Algorithm 2 Method performed by cells to decide what to do to each message.

processMessages(i, $MessageList$)

1: **for all** $Message\ \beta \in MessageList$ **do**
2: $\quad$ **if** $\beta_{type} == Morphology$ **then**
3: $\quad\quad processMorphologyMessage(i, \beta)$
4: $\quad$ **else**
5: $\quad\quad processMigrationMessage(i, \beta)$
6: $\quad$ **end if**
7: **end for**

Morphology messages start at *Discovery* mode. The next destination of these messages depends on two model's parameters: $MinSegments$ and $NewSegmentProb$. Algorithm 4 shows the method performed by the simulation to select whether a message must start the *Backtrack* mode, must continue on the same direction, or must change direction. If the morphology message is at the *Backtrack* mode, the cell checks whether there is a neighbor cell to receive this message. When there is no receiver, a neoblast divides and places its outcome from division in the specific location to receive the message. On the other hand, a somatic cell creates a *migration message* and sends it to a random direction.

Algorithm 3 Method performed by cells when they receive a morphology message.

processMorphologyMessage(i, β)

1: $top \Leftarrow \beta_V.top$
2: **if** $\beta_{mode} == Discovery$ **then**
3: $\quad dest \Leftarrow selectMsgDestination(i, \beta)$
4: $\quad i.sendMsg(\beta, dest)$
5: **else**
6: $\quad$ **if** $isAlive(i.Neighbors[reverse(top_{Direction})])$ **then**
7: $\quad\quad i.sendMsg(\beta, reverse(top_{Direction}))$
8: $\quad$ **else**
9: $\quad\quad dest \Leftarrow reverse(top_{Direction})$
10: $\quad\quad$ **if** $i.cellType == Neoblast$ **then**
11: $\quad\quad\quad cellDivision(i, dest)$
12: $\quad\quad\quad i.sendMsg(\beta, dest)$
13: $\quad\quad$ **else**
14: $\quad\quad\quad msgDest \Leftarrow getRandomDirection()$
15: $\quad\quad\quad migMsg \Leftarrow MigrationMsg(\beta, msgDest)$
16: $\quad\quad\quad i.sendMsg(migMsg, msgDest)$
17: $\quad\quad$ **end if**
18: $\quad$ **end if**
19: **end if**

Migration messages contain all the information from the *morphology message* that created originated them. Thus, a neoblast using a specific message to migrate will move to the location where the *morphology message* was created. When a somatic cell or a neoblast which is already migrating, receive a *migration message*, the cell relays the message to the same direction. The exception occurs when the message reaches the organism's boundaries. In this case, the cell holding the message sends it to another direction.

When a neoblast receives a *migration message*, it starts the migration procedure. Algorithm 6 details this process. First the neoblast signals the cell located in the position where the neoblast should move to. This signal informs the cell that its location will change and therefore, the cell needs to update any held messages. Then, the neoblast and the cell change locations and update their neighbors. Finally, Algorithm 7 details the process of updating any held message before the cell changes its location.

Algorithm 4 Selects the next destination of a message in the Discovery mode according to some model's parameters.

selectMsgDestination(i, β)

```
1: top ⇐ β_V.top
2: if β.Segments ≥ MinSegments then
3:    β_mode ⇐ Backtracking
4: else
5:    if random() < NewSegmentProb then
6:       β_V.push(getNewDirection(top_Direction))
7:    else
8:       if i.Neighbors[top_Direction] ≠ nil then
9:          top.length++
10:      else
11:         β_V.push(getNewDirection(top_Direction))
12:      end if
13:   end if
14: end if
```

Algorithm 5 Method performed by cells when they receive a migration message.

processMigrationMessage(i, β)

```
1: top ⇐ β_V.top
2: if !isAlive(i.Neighbors[top_Direction]) then
3:    dest ⇐ getRandomDirection()
4:    i.sendMsg(β, dest)
5: else
6:    if i.cellType == Somatic then
7:       i.sendMsg(β, top_Direction)
8:    else
9:       if isMigrating(i) then
10:         i.sendMsg(β, top_Direction)
11:      else
12:         i.startMigration(β)
13:      end if
14:   end if
15: end if
```

Experiments

The purpose of this model is to test whether the worm can recover from an injury that removed half of its tissue. In these experiments, all cells could create new messages, but only *neoblasts* had the ability to proliferate. In addition, *somatic cells* could create *migration messages* to guide *neoblasts* to the area of the injury.

In these experiments, we fixed some model's parameters and varied others. We fixed the shape of the organism as a 2D planarian-like structure containing 525 cells. Figure 4a shows the cell network we used in our experiments. We removed 262 cells on the anterior part of the worm at $CutCycle = 50$. Messages starting at the topmost cells, need 21 cycles (in the best case), to reach the edges of the injury. Thus, 50 cycles are sufficient for messages to discover the entire shape. We then ran the simulation for 100 more cycles ($EndCycle = 150$) and verified the similarity of the regenerated worm to the original worm. Equation 1 shows the similarity metric Sim.

Algorithm 6 Method performed by neoblasts when they are migrating.

neoblastMigration(i, β)

```
1: top ⇐ β_V.top
2: dest ⇐ top_Direction
3: migrationSignal(i.Neighbors[dest], dest)
4: changeLocation(i, i.Neighbors[dest])
```

Algorithm 7 Method performed by a cell when it receives a signal from a neoblast informing that the neoblast will move to the cell location.

migrationSignal($neighbor$, $dest$)

```
1: for all Message β ∈ neighbor.MessageList do
2:    top ⇐ β_V.top
3:    if β_mode ⇐ Backtracking then
4:       if top_Direction == dest then
5:          top.length−−
6:       else
7:          β_V.push(dest)
8:       end if
9:    else
10:      if top_Direction == reverse(dest) then
11:         top.length++
12:      else
13:         β_V.push(reverse(dest))
14:      end if
15:   end if
16: end for
```

$$Sim = \frac{AliveCells - MissingCells}{TotalCells} \quad (1)$$

where $TotalCells$ is the number of cells in the original worm, $AliveCells$ is the number of alive cells in locations where there was a cell in the original worm and $MissingCells$ is the number of locations where there was a cell in the original worm but the model did not regrow a cell. Therefore, $Sim = 1$ constitutes a complete regeneration.

We varied the number of messages all cells create at each cycle $PacketFreq \in \{15, 18, 21, 24, 27\}$. We previously showed that a higher value of this parameter increased the information spatially distributed in the organism, hence increasing the performance of the model. The same behavior was expected here, due to the fact that more messages are necessary to travel through the organism for migration messages to reach neoblasts. We varied the number of segments before backtracking a message $MinSegments \in \{4, 5, 6, 7\}$. We expected a positive correlation with Sim since a higher value of this pa-

rameter also represents more morphological information existing in the organism. We varied the probability of creating a new segment (i.e., change the direction of a message) $NewSegmentProb \in \{0.05, 0.1, 0.15, 0.2\}$. We showed that in experiments with large tissue removal, it was preferable to have longer messages (i.e., lower values of $NewSegmentProb$) because messages that started at the farthest cells needed to reach the edge of the injury. Finally, we varied the proportion of neoblast outcomes $NeoblastRatio \in \{0.1, 0.15, 0.2, 0.25, 0.3\}$. We expected that a higher value of this parameter would improve the performance of the model due to more neoblasts existing in areas close to the injury, hence having to migrate for shorter distances. For each point in this parameter space, we ran 20 distinct simulations, totaling 8000 runs.

Results

Our results showed that the inclusion of *migration messages* was sufficient to maintain the ability of regenerating a simulated worm from a cut that removed half of the worm's body. In 1565 out of 8000 simulation runs (19.56% of the parameter space), the model regenerated the entire shape of the worm. The average value of Sim in our runs was 0.944 and standard deviation equals to 0.078. Therefore, most simulation runs that did not fully regenerate the worm had a good performance, regrowing almost all cells.

Increasing the value of $NeoblastRatio$ increases the chance of fully regenerating the worm ($Sim = 0.890$ for $NeoblastRatio = 0.1$ and $Sim = 0.979$ for $NeoblastRatio = 0.3$). This happens because a higher value of $NeoblastRatio$ implicates more neoblasts close to the area of the injury. Thus, it is easier for *migration messages* to reach neoblasts, and neoblasts have to migrate for shorter distances.

We were interested in the interactions between the other parameters of the model and the $NeoblastRatio$. Figure 1 shows the interaction of $PacketFreq$ and Sim. In general, increasing the number of messages created at each cycle increases the capabilities of regenerating the worm. This happens because more messages existing in the worm at a specific time lead to more morphological information distributed across the cells. Therefore, there is a higher probability of existing messages that regenerates cells in all missing positions.

However, there are specific cases in which parameter assignments with a higher value of $PacketFreq$ perform worse than the same parameter assignments with a lower value of $PacketFreq$. For example, looking at the box-plots of the assignments $NeoblastRatio = 0.1$ and $PacketFreq = 21$ and $PacketFreq = 24$ in Figure 1, one can see that there are at least two runs in the first assignment that have a value of Sim smaller than any other value on the second. The reason for these results is that as neoblasts start the migration process after they receive the first *migration message*, there are no guarantees that this packet is one that contains a lot of morphological information. Thus, there is a chance that the *migration message* that would regrow a specific area of the morphology never reaches a neoblast, hence this area is not regenerated.

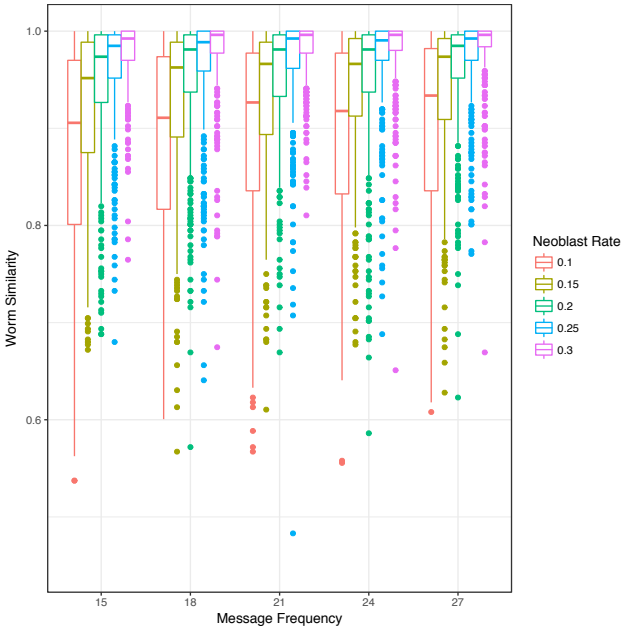


Figure 1: A Tukey Box plot of $PacketFreq$ on Sim for all values of $NeoblastRatio$. For each value of $PacketFreq$, it shows the median, quartiles, range and data outliers of each $NeoblastRatio$.

We can see that a higher value of $MinSegments$ increases the value of Sim (Figure 2). This can be explained by the fact that $MinSegments$ grows the existent morphological information at a specific time. Therefore, there is a higher probability of *morphological messages* to exist in alive cells. But again, rare outliers can happen with higher values of $MinSegments$ when the "best messages" travel through the worm but do not reach neoblasts. Consequently, the worm remains incomplete.

Longer messages (i.e., small value of $NewSegmentProb$) are better for the regeneration process. Figure 3 shows that a small value of $NewSegmentProb$ reduces the detrimental effects of a small ratio of neoblasts. Longer messages are important for two reasons: first, they remain in the structure for a longer period, discovering more of the morphology; second, longer messages are more likely to exist in alive cells after the injury.

Figure 4 depicts temporal changes of a worm configuration. At $cycle = 50$, an anterior cut is made and the first cells (neighbors of neoblasts) start to regrow. The regeneration process continues, with more neoblasts migrating to the area of the injury. At $cycle = 100$, the entire worm is

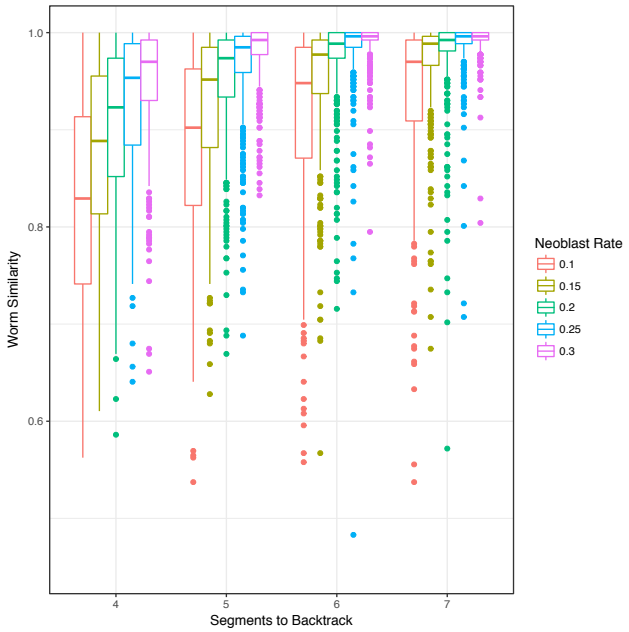


Figure 2: A Tukey Box plot of $MinSegments$ on Sim for all values of $NeoblastRatio$. For each value of $MinSegments$, it shows the median, quartiles, range and data outliers of each $NeoblastRatio$.

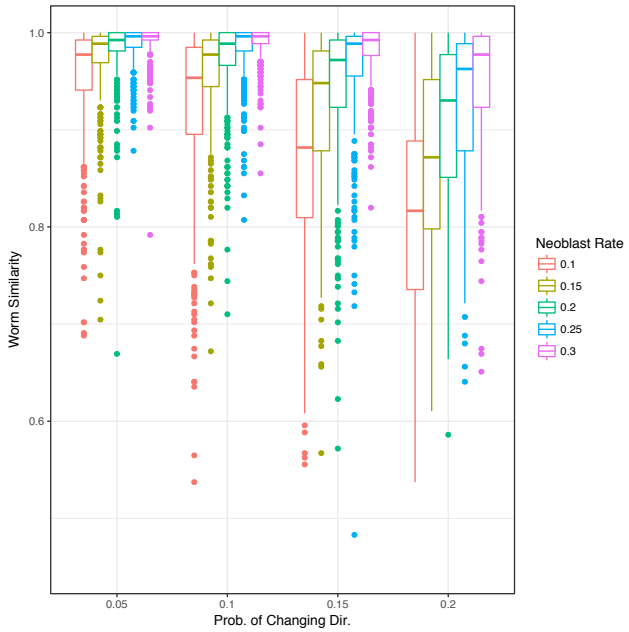


Figure 3: A Tukey Box plot of $NewSegmentProb$ on Sim for all values of $NeoblastRatio$. For each value of $NewSegmentProb$, it shows the median, quartiles, range and data outliers of each $NeoblastRatio$.

regenerated.

Discussion

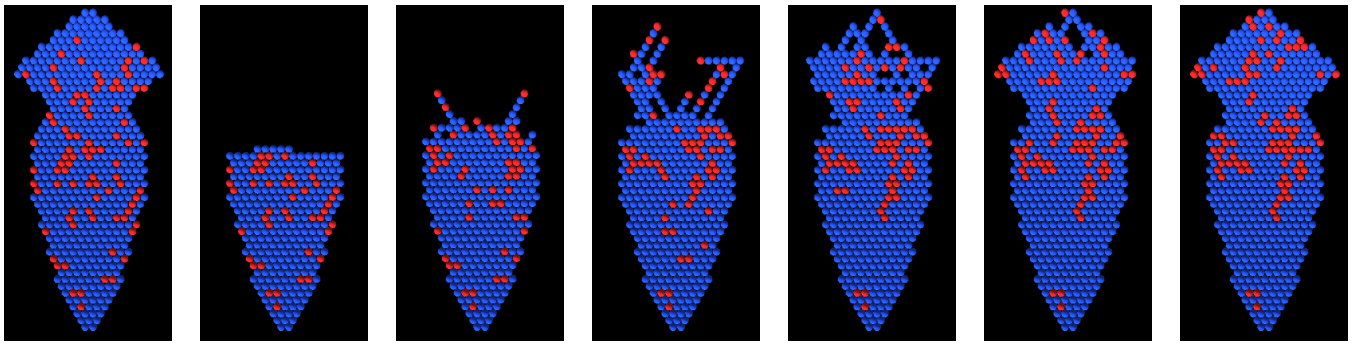
There are two types of regeneration processes *in vivo*: *epimorphosis* and *morphallaxis*. In epimorphosis, the undamaged tissue remains intact after injury, and a blastema forms in the area of the injury. Afterwards, the missing tissues are generated. An example of epimorphic regeneration is the regrowth of limbs, tails and jaws in newts (Morrison et al., 2006). In *morphallaxis*, on the other hand, there is no blastema formation. Instead the remaining tissue is remodeled to create a smaller version of the entire organism (Agata et al., 2007). Planaria regeneration is a mixture of these two processes. There is blastema formation, but when a large tissue is removed the remaining tissue produces missing structures (Morgan, 1898; Saló et al., 2009). Our model only simulates an epimorphic process. A next step is to account for morphallaxis as well.

Neoblasts are recognizable because they can divide and also because they have a distinct morphology and transcriptional signature. However, recent findings show that the population of neoblasts within a planarian are heterogeneous (reviewed in Zhu and Pearson (2016)). One of the most important discoveries regarding neoblasts in individual level was made by Wagner et al. (2011). The authors applied varying doses of radiation to planarians and found that at a specific radiation dose (1750 rad), some neoblasts did not die. Moreover, within a week, the surviving neoblasts formed cell colonies. These survivors were denominated "clonogenic neoblasts" (*cNeoblasts*). Later, the authors demonstrated that transplanting of a single *cNeoblast* is sufficient to restore the regeneration capabilities of a planaria without neoblasts.

Our cell-cell communication model uses global parameters that cause all cells to behave identically. However, previous results of our model showed that it was not necessary that all cells constantly create *morphological messages* (Ferreira et al., 2017a). We want to consider configurations where cells are individually parametrized (e.g., each cell has a different gene expression). Thus, each cell might behave differently, with, for example, some neoblasts having different migration or division rates than others.

Although we cannot yet map our model's *migration messages* to a unique biological process, there is evidence that a similar phenomenon occurs *in vivo*. Cells can transmit small molecules (e.g., calcium ions) through gap junctions. This signaling process creates a network of cells electrically connected which transmit information among these cells (McLaughlin and Levin, 2018). There is evidence that this electric field can guide cells to wound areas in the epithelial tissue, and when bioelectrical communication competes with chemical signaling, cells tend to follow the directions of the electric fields (Zhao, 2009).

We previously presented an "activation mechanism" to re-



(a) Cycle = 0 (b) Cycle = 50 (c) Cycle = 60 (d) Cycle = 70 (e) Cycle = 80 (f) Cycle = 90 (g) Cycle = 100

Figure 4: A dorsal view of the simulated worm structure. Blue spheres are somatic cells and red spheres are neoblasts. (a) shows a configuration of the worm at the beginning of the simulation. At $Cycle = 50$ an anterior cut was performed. At $Cycle = 100$ the worm was fully regenerated.

duce the detrimental effects of noise on our cell-cell communication model (Ferreira et al., 2017b). Our assumption of perfect communication in this work is unrealistic: we expect real-world communication to involve noise, signal degradation, etc. Thus, we want to add the activation mechanism to the current version of the model and investigate whether its regeneration capabilities remain.

Planaria are examples of organisms very robust against mutations: despite hundreds of millions of years of somatic mutations (accumulating genomic disorder, due to planarians' asexual mode of reproduction through fission), they exhibit regeneration of their specific morphology with 100% fidelity (Neuhof et al., 2016). It has been hypothesized that they are not even individuals, but a form of organism that did not reach multicellularity yet. Instead, each planaria is a collective of individuals (neoblasts) that cooperate to maintain a specific shape (Fields and Levin, 2018). Thus, a model that explains the regeneration process in planaria also has to be robust against mutations that might happen in each cell. Therefore, testing our model in configurations where cells mutate is also an idea that needs further investigation.

Conclusion

The cell-cell communication mechanism we previously proposed was an attempt to find an algorithmic explanation of how bioelectric signals coordinate cell proliferation to restore the organism's anatomy. In this paper, we expanded the capabilities of the model in two ways. We restricted cell division to adult stem cells (neoblasts) and we added stem cell migration as a possible cell behavior. As part of these additions, we created a new message type that might correspond to *in vivo* phenomena. When somatic cells detect an injury, they create *migration messages* that travel through the organism until they reach a neoblast. These messages guide neoblasts to injury locations where the neoblasts proliferate. We tested these modifications with different ratios of neoblasts in experiments in which half of the planarian's body was removed. We verified that the model was capable of fully regenerating the planarian-like morphology even with a ratio of 10% of neoblasts.

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