Introducing Simulated Stem Cells into a Bio-Inspired Cell-Cell Communication Mechanism for Structure Regeneration

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***Abstract*—The planarian flatworm has been essential for learning about the regeneration process of organisms. One of the reasons for this exceptional capability is the abundance of adult stem cells, denominated neoblasts, distributed throughout planarian's body. Its body contains around 20% to 30% of neoblasts. Moreover, experiments *in vivo* show that irradiated worms with no neoblasts lose their regenerative capabilities. In this paper we add the concept of simulated neoblasts to a previous bio-inspired cell-cell communication mechanism of dynamic structure discovery and regeneration. We simulate a 3D organism structure resembling the planarian's, with the inclusion of two types of cells: the *neoblasts* that are capable of creating new morphological messages (packets) and *differentiated cells* that only relay such messages. After a cut in half, the mechanism uses morphological information created by neoblasts and exchanged across cells to regrow the missing tissue. We vary model parameters such as the frequency of packets created by neoblasts, how many segments a packet might have before backtracking, and the probability of a packet changing direction. After a large number of simulation runs, we confirm the efficacy of the model for distinct proportions of neoblasts, showing that there exist parameter assignments that fully regenerated the worm, even for simulations containing 10% of neoblasts.**

I. Introduction

In planarians, adult stem cells (called neoblasts) are the only cells that are capable of dividing and differentiating into any other cell type [1]. Those cells can be selectively killed by irradiation, which prevents the planarian from regenerating [2]. Moreover, a single transplanted neoblast can restore the restorative capabilities of lethally irradiated worms [3]. Therefore, neoblasts are necessary for the regeneration process in planarians [4]. However, it is still unknown how the various cell types in planaria contribute instructive influences for establishing specific patterning outcomes.

We previously proposed [5] a bio-inspired cell-cell communication mechanism that could dynamically discover morphological information and use it later for regenerating lesioned areas in the morphology. Although the model has not been linked yet to biological mechanisms, it showed various functional properties of regeneration displayed by planaria. Thus, the model is a valuable asset that might work as a proof-of-concept of how morphological information can be discovered and used for regeneration.

We intend to find a mapping between our proposed mechanism to biological processes. In order to find this mapping, if such mapping exists, we have to improve the model complexity to replicate behaviors of cells, and then compare the outcomes of the model to experiments *in vivo*. Thus, in this paper we modified our previous cell-cell communication mechanism to account for the presence of a new cell type responsible for regeneration in planaria (the neoblasts). We hypothesize here that only neoblasts can create new information about the morphology of the organism. Previously, the model contained only one type of cell capable of creating and relaying morphological messages. Here, there are two distinct types of cells: *neoblasts* and *differentiated cells*. While neoblasts act in the exact same way as cells in the original model (i.e., creating and relaying messages), differentiated cells can only relay messages they receive. We aim to verify the necessary proportion of neoblasts to completely regenerate the organism from a large tissue removal, similar to the behavior that real planarians exhibit.

In the remainder of the paper we first present our original cell-cell communication mechanism for structure discovery and regeneration and then we discuss the results that showed the efficacy of the original mechanism. In addition, we examine some developmental models that incorporate regeneration capabilities. Next, we describe the modifications of the model in order to add the two distinct cell types. We also show the experiments we performed aiming to understand the limits of the model when only a subset of cells creates new morphological information. After that, we show the results of our experiments. We then discuss the implications of those results for guiding regeneration research. Finally, we present our conclusions and proposals for future work.

II. Previous Work and Background

Cell-cell communication is determinant in the regeneration process performed by planarians. For example, when there is damage on the shape of the organism, cells must communicate

in order to determine the type, pattern, location, and scaling of the missing structures that need to be recreated. Moreover, the information about the large-scale anatomy to which the organism must regenerate to (called target morphology) is available to every piece of the worm (holographically encoded) and can be reached from any initial configuration (different types of cuts) [6]. Despite considerable progress in the molecular biology of mechanisms necessary for regeneration [7], it is still largely unknown how cell networks process the information in dynamics that are sufficient to regenerate a perfect worm from diverse types of damage [8], [9], [1]. Thus, we are interested in modeling algorithmic process by which cells encode the target morphology and store it for future regenerations.

We proposed a dynamic messaging mechanism in which cells exchange morphological information with their neighbors and then use that information to repair missing parts of the organism. We implemented this mechanism as an agent-based model in which each cell is an agent that sends and receives messages (named "packets") at each discrete time. The packets that exist across cells at a specific time describe the morphology of the organism at that time. At some point in time, those packets return to the location where they were created, through the same path they came from (i.e., "backtrack"). If there are missing cells on packets' paths, these cells are repaired until the packets complete their path. This simple mechanism can dynamically discover and repair new morphologies.

Figure 1 shows a functional diagram of our original mechanism. At each cycle, all cells receive packets from neighbor cells and decide upon the next owners of those packets. A cell can hold the packet, send it to the same direction, send the packet to another direction (adding a segment) or backtrack the packet. First, the cell checks whether the packet is discovering new morphologies or backtracking. If the packet is backtracking and there is no cell positioned to receive this packet in the next cycle, the cell holding the packet divides and position itself to receive the packet. Otherwise, the cell checks if the packet has at least $MinSegments$ segments, then this packet backtracks. Otherwise, the cell checks if the distance of the top segment in the packet is greater than $MinTopLengthToHold$, then there is a probability $HoldProb$ that the cell will hold the packet. Otherwise, there is a probability $NewSegmentProb$ that the cell will either send the packet to a different direction or send it to the same direction. After deciding what to do to all packets, all cells create a specific number of new packets defined by the parameter $PacketFreq$ and send them to random directions.

We introduced this cell-cell communication mechanism in a paper which aimed to verify whether the model was capable of repairing the structure of the organism in light of random cell death that can occur through irradiation [5]. In that paper, we varied the probability of random cells dying at each cycle and we verified that even at 4% of cell death rate, there were parameter assignments that maintained more than 90% of alive cells indefinitely. More recently, we modified the model to account for noise that can occur during message transmission [10]. In that paper, we applied noise on both elements of packet segments (i.e., the distance and direction) when they backtracked. Both noises prevented the model to fully regenerate the worm from a cut on its anterior part. We then proposed an activation mechanism by which various packets are necessary to reach a missing cell position in order to regrow a new cell there. Although this activation mechanism reduced the number of parameter assignments that fully regenerated the worm in configurations with no noise, it significantly improved the performance of the model when there was noise on packets.

Several developmental models also show regeneration capabilities. The first model with these characteristics was proposed by Eggenberger Hotz [11]. In his model he evolved regenerative systems with shape resembling the planarians' and the author used a genetic regulatory network (GRN) in order to reduce the number of genetic parameters in the system. In addition, a developmental mechanism which defines cells after all cell division was also defined. Other developmental models with regeneration capabilities were proposed using distinct approaches: GRN [12], Cartesian genetic programming [13] and cellular automata [14], [15].

The model from Fontana and Wróbel [16], though it is still a developmental model, is the most similar to ours. This model uses two types of cells: *normal* cells and *driver* cells. Driver cells are responsible for creating new cells during development and later for receiving chemical signals that are used to detect damages to the morphology thereafter starting the regeneration process. The authors showed a genome that developed into a lizard-like organism capable of completely regenerating its limbs, head and tail.

III. Experiments

The goal of these experiments was to define the minimum number of neoblasts that is necessary to fully regenerate a simulated worm after damage on its anterior part. Thus, the modified model works as follows: first, the simulator creates $NumCells \times NeoblastRatio$ neoblast cells and $NumCells \times (1 - NeoblastRatio)$ differentiated cells. Then it uses these cells to build the fixed structure of the organism. Neoblasts are uniformly placed across the structure (i.e., all locations in the organism have the same probability of having a neoblast). Similar to the original model, while the current cycle is different from $EndCycle$, all cells sense packets received from the last cycle as well as packets held in the last cycle, and cells decide upon the next destination of those packets (hold, send to same direction, send to another direction, start backtracking). If the packet is backtracking and the cell must send it to a direction where there is no cell to receive the packet, the cell divides and creates a new differentiated cell in that location. Therefore, in our model, neoblasts are placed only at the beginning of the simulation since all new cells in the organism are differentiated cells. Finally, after all cells decide on the owners of all packets at the next cycle, differently from the original model, only neoblasts create $PacketFreq$ new packets and send them to random directions. At the end of the simulation, we calculate $RegeneratedRatio$ (equation 1).

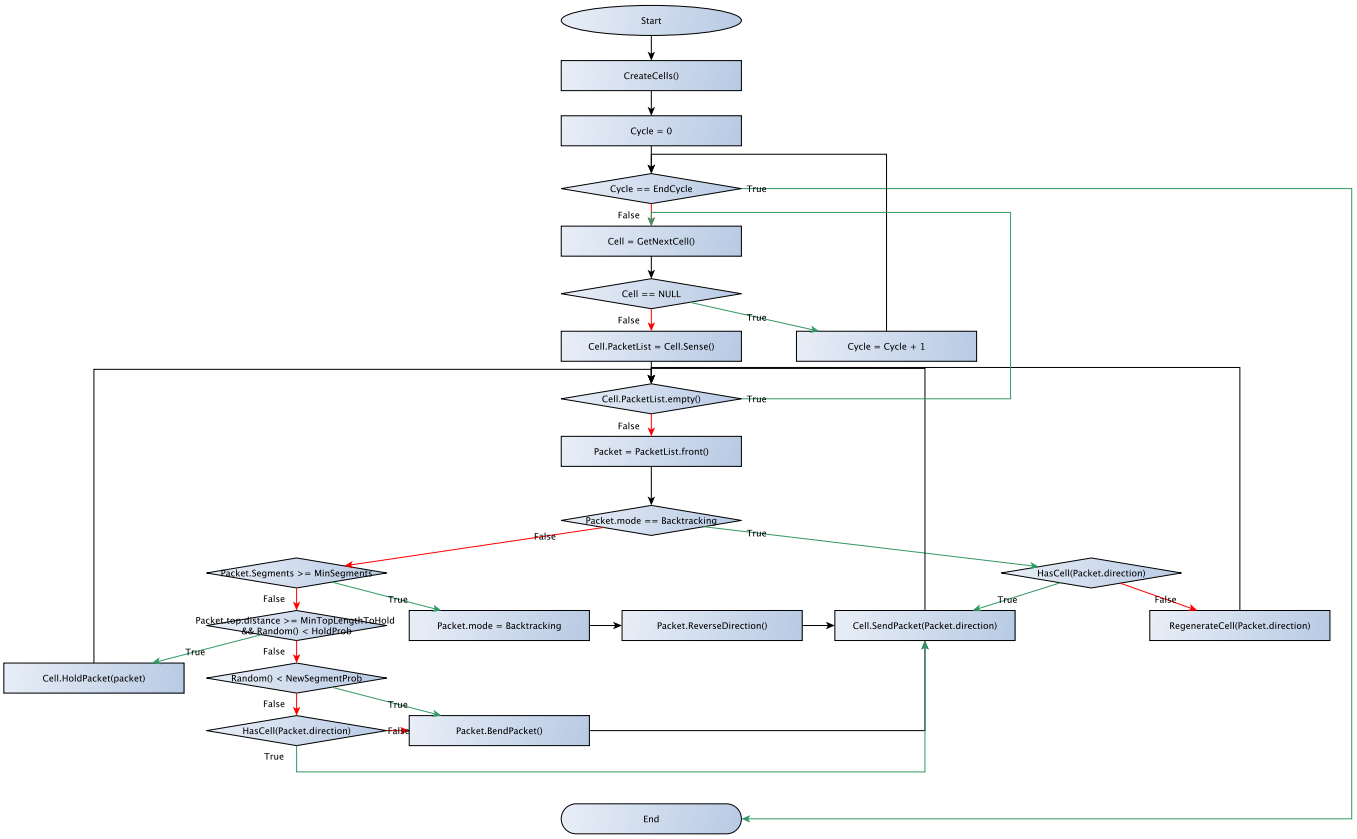


Fig. 1. Functional diagram of the cell-cell communication model.

$$RegenerationRatio = \frac{RegeneratedCells}{RemovedCells} \quad (1)$$

where *RemovedCells* is the number of cells removed by the cut. *RegeneratedCells* is the number of cells regenerated by the end of the simulation. Therefore, a value of $RegenerationRatio = 1$ represents full regeneration of the worm.

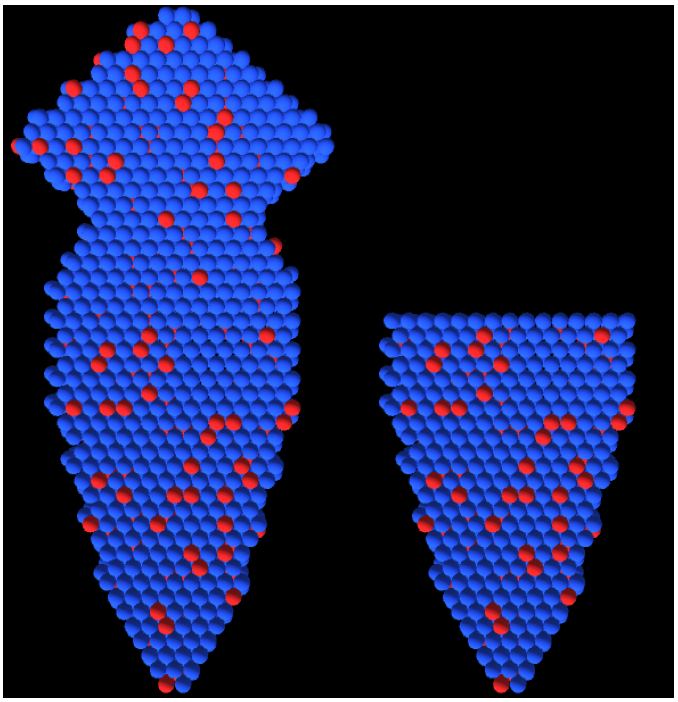
In order to verify the efficacy of our model, we fixed some model parameters and varied others. First, we considered a 3D planarian-like structure containing 4 layers of 525 cells each (2100 cells total). The structure is damaged in $CutTime = 60$ when 50.19% of its anterior tissue area is removed ($RemovedCells = 1054$). Figure 2 shows a dorsal view of the original worm and the same worm immediately after the cut performed in our experiments. After the damage, the model has 40 more cycles to regenerate the missing part of the worm. In this version of the model, all packets are perfectly transmitted between cells (i.e., there is no noise applied to packets).

We fixed the two parameters related to packets being held by cells: $MinTopLengthToHold = 3$ and $HoldProb = 0.5$. With these parameters, cells have a probability of 50% of holding packets that contains the top segment with distance greater than 3.

We varied the ratio of neoblasts in the organism $NeoblastRatio \in \{0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1\}$. A value of $NeoblastRatio = 1$ is equivalent to the original model in which all cells create new packets at each cycle. We expect a positive correlation between $NeoblastRatio$ and $RegeneratedRatio$ because as we increase the number of cells creating new packets we increase the number of existing packets during the simulation, therefore the morphological information describes a larger area of the organism.

We varied the number of new packets generated by each neoblast at each cycle $PacketFreq \in \{1, 11, 21\}$. Increasing the number of new packets at each cycle culminates in more morphology information existing across the organism. We expect that for a small value of $NeoblastRatio$, more packets would significantly change $RegeneratedRatio$, because more packets are necessary to cover a larger area of the organism. On the other hand, for high values of $NeoblastRatio$, increasing the number of packets would not discover new areas because they would be covered by packets created by other neoblasts.

There are two parameters responsible for controlling the navigation patterns of packets. The first is the number of segments inside the packets before the packet starts backtracking ($MinSegments$); the second is the probability of the packet to change direction, adding a new segment to



(a) (b)

Fig. 2. A dorsal view of the simulated worm containing 2100 cells. (a) shows the structure before the cut and (b) shows the structure immediately after the cut. Blue cells are differentiated cells and red cells are neoblasts.

the packet ($NewSegmentProb$). We varied both parameters $MinSegments \in \{3, 5, 7\}$ and $NewSegmentProb \in \{0.1, 0.2, 0.3\}$. We expect that it is preferable to have more segments and longer packets in order for each packet to describe a longer path. Hence, one packet can regenerate more cells during backtracking. Due to the stochastic behavior of our model, for each parameter assignment we ran 100 distinct random seeds, a total of 27000 simulations.

IV. Results

The average $RegenerationRatio$ of our parameter sweep was 0.895 with a standard deviation of 0.150. Table I shows the mean and standard deviation of $RegenerationRatio$ for all values of $NeoblastRatio$. As expected, a higher value of $NeoblastRatio$ increased the amount of area that was regenerated after the cut because there were more cells creating new packets hence the organism contained more morphology information. However, $RegenerationRatio$ did not increase linearly as $NeoblastRatio$ increased. More specifically, for large values of $NeoblastRatio$, as we increased $NeoblastRatio$ instead of discovering larger areas, there existed more redundant morphological information across the organism.

Figure 3 shows dorsal views of final structures of eight simulation runs. Each picture used different independent parameters. Figure 3a shows the worm that regenerated the fewest number of cells $RegenerationRatio = 0.199$. The parameters assigned to this run were $NeoblastRatio = 0.1$, $PacketFreq = 1$, $MinSegments = 3$ and

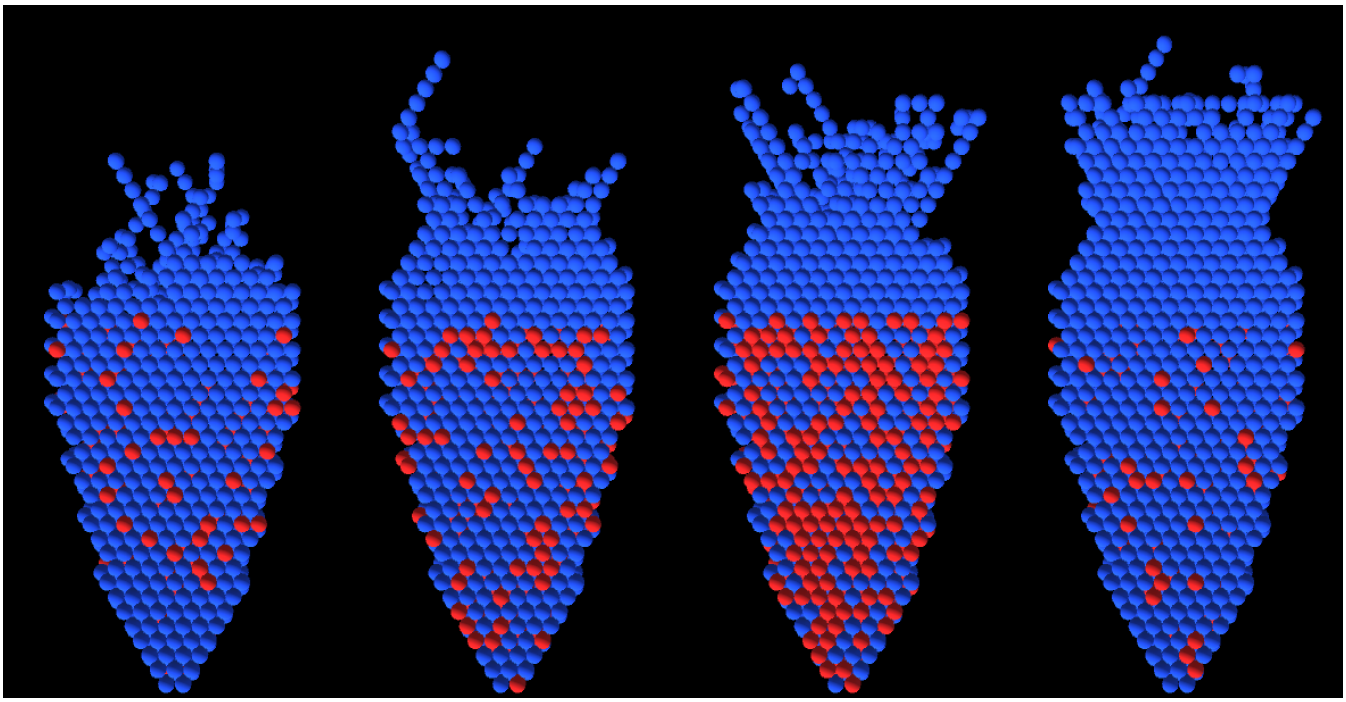
$NeoblastRatio$	$RegeneratedRatio$
0.1	0.772 ± 0.205
0.2	0.841 ± 0.173
0.3	0.874 ± 0.156
0.4	0.895 ± 0.142
0.5	0.909 ± 0.132
0.6	0.919 ± 0.124
0.7	0.927 ± 0.118
0.8	0.933 ± 0.112
0.9	0.939 ± 0.107
1.0	0.943 ± 0.103

TABLE I
Mean and standard deviation of $RegenerationRatio$ for all tested values of $NeoblastRatio$

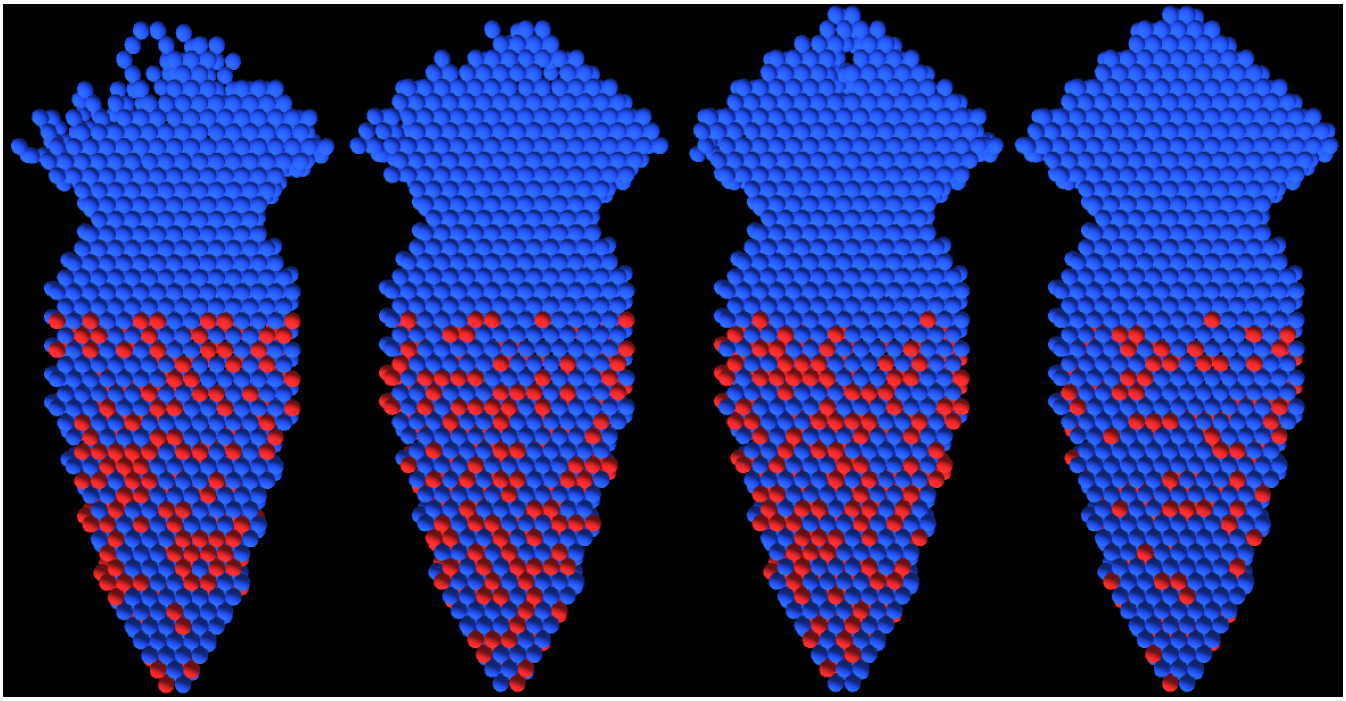
$NewSegmentProb = 0.3$. One can see that this worm completely lost its head and does not present a valid planarian shape anymore. Figure 3b shows one example of worm that regenerated 369 cells ($RegenerationRatio = 0.35$). It worth noting that this figure depicts only one simulation run that regenerated 369 cells. Thus there exist other parameter assignments that display different final shapes. Figures 3c, 3d, 3e and 3g show runs that had $RegenerationRatio$ equals 0.5, 0.65, 0.8 and 0.95 respectively. We can see that the regeneration process happens from the area of the cut to the topmost cells of the worm. Figure 3h confirms that there exist parameter assignments that fully regenerated the worm when there is only a ratio of neoblasts in the organism ($NeoblastRatio = 0.2$ in this example). Finally, we also wanted to verify the shape of the "average" worm. Our results showed a mean $RegenerationRatio = 0.895$, which is equivalent to 943 regenerated cells. Figure 3f shows one simulation run that regrew exactly 943 cells. One can see that, although there are cells missing on the topmost area of the worm, the head structure is still present in the worm.

Our results showed that 6210 simulations, out of 27000 (23% of the parameter space), completely regenerated the worm. Figure 4 depicts the number of simulations that fully regenerated the worm for each ratio of neoblasts. As expected, more cells creating new packets increased the existing morphological information across the organism, hence increasing the probability of the remaining information regenerating the entire worm. More interestingly was that even when the worm had a small percentage of neoblasts there were parameter assignments that regenerated the worm completely. However, we can also see that the difference in the number of simulations that fully regenerated the worm increased drastically at a ratio between 10% and 30% while after 30% of neoblasts the gains were less significant.

An ANOVA for $RegenerationRatio$ as dependent variable showed significant main effects ($p < .001$) for all independent variables and interactions among them. Figure 5 shows the interaction between $PacketFreq$ on $RegenerationRatio$ for all values of $NeoblastRatio$. We can see that for all values of $NeoblastRatio$, as we increased the number of packets generated at each cycle it also increased the regenerated area of



(a) (b) (c) (d)



(e) (f) (g) (h)

Fig. 3. A dorsal view of eight simulated worms with different parameters at the end of the regeneration process. (a) shows the worst found result in which the model regenerated 210 cells ($RegenerationRatio = 0.199$), (b) shows a run that regenerated 369 cells ($RegenerationRatio = 0.35$), (c) shows a run that regenerated 527 cells ($RegenerationRatio = 0.5$), (d) shows a run that regenerated 685 cells ($RegenerationRatio = 0.65$), (e) shows a run that regenerated 843 cells ($RegenerationRatio = 0.8$), (f) shows the "average" performance of the model in which the model regenerated 943 cells ($RegenerationRatio = 0.895$), (g) shows a run that regenerated 1001 cells ($RegenerationRatio = 0.95$) and (h) shows a full regeneration ($RegenerationRatio = 1$).

the worm. This increment was smaller in the range of $[11, 21]$ than $[1, 11]$. The reason was that as we increased the number of packets created at each cycle, it also increased the number of redundant packets across the organism. These packets did

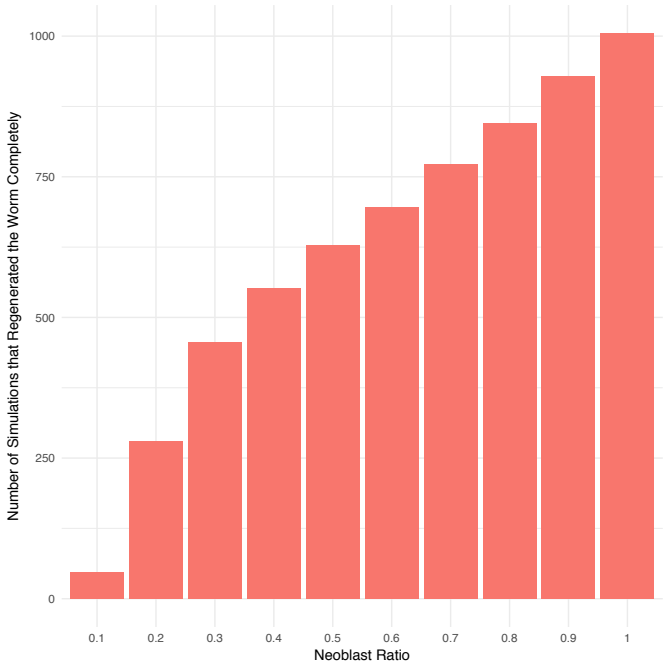


Fig. 4. Histogram of simulations that fully regenerated the worm for each $NeoblastRatio$.

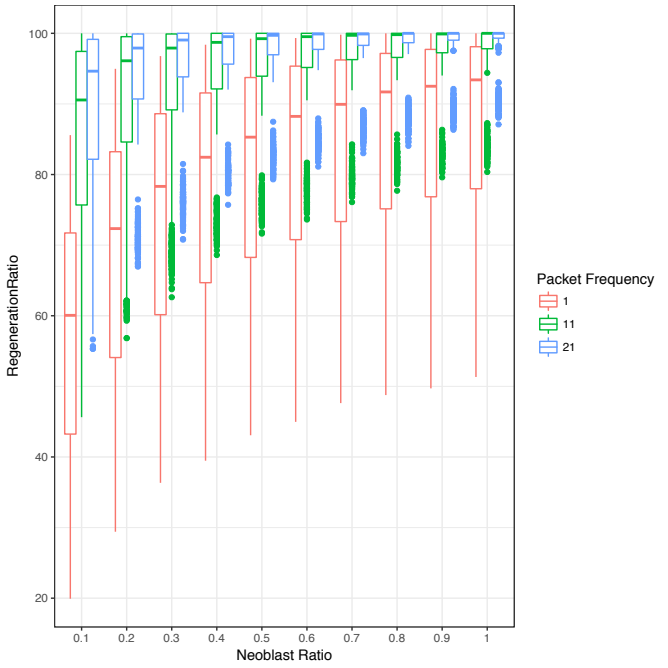


Fig. 5. Box plot of $NeoblastRatio$ and $RegenerationRatio$ for all values of $PacketFreq$.

not help in regeneration because they described areas already represented by other packets.

Looking specifically at results of $PacketFreq = 1$, we can verify that the model needed 80% of neoblasts in the organism in order to fully regenerate the worm. Moreover, out of 900 simulations with $PacketFreq = 1$ and $NeoblastRatio = 0.8$, only one simulation completely regenerated the worm. On the other hand, looking at the results of $PacketFreq = 21$, in 44 out of 900 simulations with $NeoblastRatio = 0.1$, the model fully regenerated the worm.

Figure 6 shows the interaction between $MinSegments$ on $RegenerationRatio$ for different values of $NeoblastRatio$. As expected, the mean of regenerated area increased as the number of neoblasts in the organism increased. The number of segments before backtracking was important because a higher quantity of segments led to more packets reaching differentiated cells. Hence, those packets discovered paths to regenerate these differentiated cells after damage. For $MinSegments = 3$, it was necessary to have 40% of neoblasts in the organism in order to fully regenerate the worm (2 out of 900 simulations), for $MinSegments = 7$, 39 out of 900 simulations with $NeoblastRatio = 0.1$ completely regenerated the worm.

Finally, Figure 7 shows the interaction between the probability of changing packet direction ($NewSegmentProb$) and $RegenerationRatio$ for all values of $NeoblastRatio$. The results showed that the model performed better with longer packets (small $NewSegmentProb$) than shorter packets. The reason was that the packets that discovered cells on the top area of the worm need to reach cells that were not removed after the damage on the structure. Looking at the simulations with $NewSegmentProb = 0.3$, it was necessary to have 50% of neoblasts in the organism in order to fully regenerate the worm (1 simulation out of 900). For $NewSegmentProb = 0.1$, it was necessary to have only 10% of neoblasts in the organism as 47 simulations completely regenerated the worm.

V. Discussion

Our results showed that our model was capable of regenerating a worm even though only part of the cells created new packets at each cycle. Cells generating a high rate of long packets, with these packets containing a high number of segments before backtracking performed best on a tissue removal experiment. These results show that in order to regrow a large tissue the most important parameter is the $NewSegmentProb$, which can overcome a small number of cells creating new packets at each time.

In all previous versions of the model, every cell generates packets at each cycle which is not what happen in the modification presented here. When all cells create new packets, it is sufficient (but not necessary) for regeneration that one packet created by a missing cell exists in one alive cell. However, in this paper we proposed a modification for the model that contains cells which do not create packets. Thus, in order to regrow differentiated cells, it is necessary for packets created by neoblasts to discover the locations of these differentiated cells. For this reason, it is better for the model's performance that neoblasts exist farther from the edge of the cut. Otherwise, packets need to discover those cells on the top of the worm and then move back to the area of alive cells before the damage

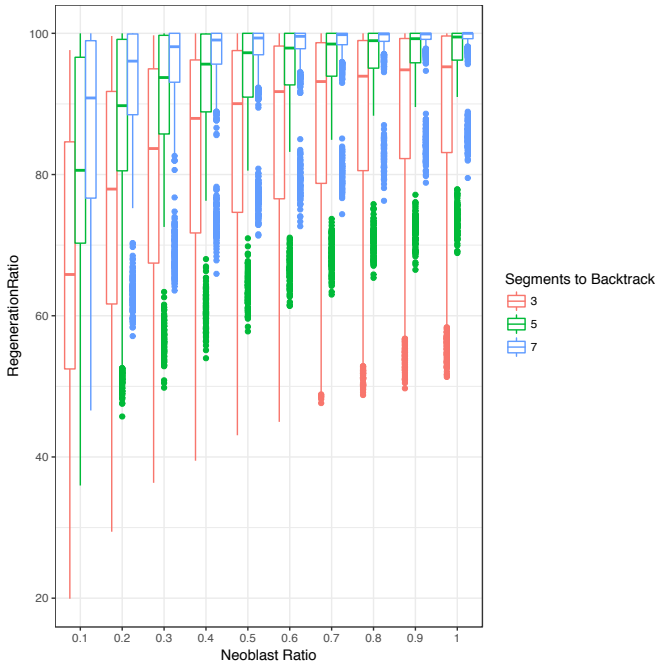


Fig. 6. Box plot of $NeoblastRatio$ and $RegenerationRatio$ for all values of $MinSegments$.

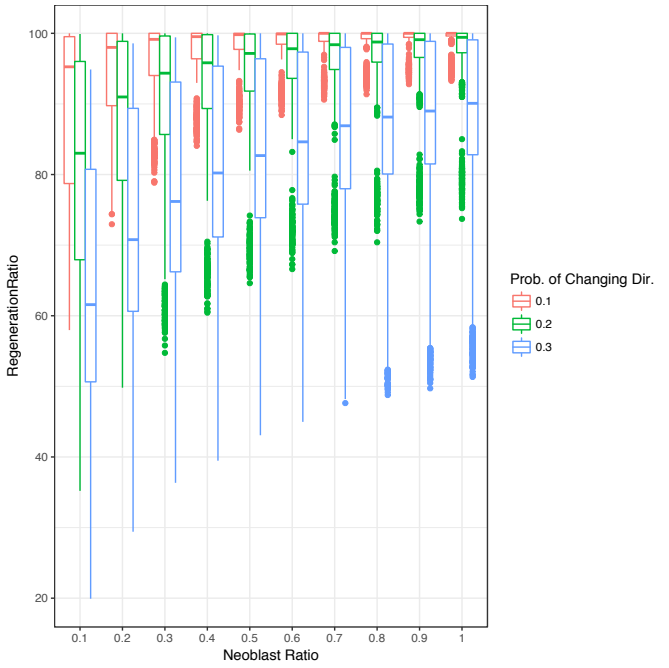


Fig. 7. Box plot of $NeoblastRatio$ and $RegenerationRatio$ for all values of $NewSegmentProb$.

on the structure happens. Therefore, worms that contain more neoblasts on the top of the head have a higher probability of perfectly regenerating their shapes from an anterior area injury.

Although we are not claiming that our model is an explanation of the regeneration process that happens in organisms, the model shows some interesting characteristics that might help understanding regeneration. For instance, cell-cell communication *in-vivo* happens through different forms such as physical forces, bioelectric signaling and chemical signals [17]. The latter is important for showing positional information, which is represented in our model by the distance the packets traverse through the organism.

The regeneration process in planaria does not work exactly as in our model. Worms *in vivo* show an increasing rate of cell division after an injury and then neoblasts migrate to the area of the injury in order to create a mass of new cells called blastema. Thus, a key question yet to be answered is how neoblasts know that there is an injury which allows them to migrate in order to start blastema formation [1]. Our model simplifies this process in two ways: first our model allows both neoblasts and differentiated cells to divide and second our model does not include mechanisms for cell migration. Even though there are simplifications, a similar biological process to what we define here as packets might help neoblasts in the process of migration and formation of blastema. Knowing when to stop growing (when the correct target morphology has been achieved) is a major unsolved problem in this field and will be dealt with in future modeling efforts.

If one removes all neoblasts from our model, the model loses its regeneration capabilities similar to *in vivo* worms. However, a limitation of our model is that all divisions generate differentiated cells. Therefore, after several injuries on the organism, the model will lose its regenerative capabilities. *In vivo*, neoblasts are distributed throughout the entire planarian with the exception of the areas that do not completely regenerate when detached (e.g., pharynx) [18]. These cells represent between 20% and 30% of the entire cell population of the planarian [19]. In our model, fewer neoblasts need more communication (i.e., neoblasts creating more packets) in order for the model to perform a perfect regeneration. Looking at the results of our model for rates of 20% to 30% of neoblasts, one can see that some simulation runs fully regenerated the worm. More specifically, the assignments $PacketFreq = 21$, $MinSegments = 7$ and $NewSegmentProb = 0.1$ showed several distinct runs with $RegenerationRatio = 1$. Thus, the mechanism proposed here might also exist *in vivo* and might be used for guiding biological research in regeneration.

VI. Conclusion

We modified our cell-cell communication mechanism for regeneration to show two types of cells: adult stem cells denominated *neoblasts* and *differentiated cells*. In our model, neoblasts are the only cells capable of creating messages to discover the morphology of the organism. Differentiated cells only relay these messages. We tested this modification in a large set of simulation runs in a 3D shape resembling a planarian's. After damaging the anterior part of the worm, which removed approximately 50% of the worm's tissue, we verified whether the mechanism regenerated the worm for different ratios of neoblasts. Our results showed that even

for small ratios of neoblasts (10% for instance), the cell-cell communication mechanism dynamically discovered the morphology of the worm and regenerated it.

We are interested in understanding the limits of regeneration of organisms. Proposing the use of neoblasts was one of the approaches to understand that. Another hypothesis to explain the outstanding regeneration capabilities of planarians is based upon the simplicity of the organism shape. We believe that the model would fail to regenerate a more complex morphology (i.e., with more corners and turns). Thus, we want to identify whether morphology might be too complex for regeneration and how many segments are necessary to regenerate this complex shape.

In this paper we just started to model the dynamics of those astonishing adult stem cells. In the future, we want to address several simplifications of our model regarding neoblasts. For example, in our model all cells are capable of dividing, which is not true in the planarian, where only neoblasts divide and differentiate. A reasonable next step is to modify the model to account for a more biological plausible cell division. However, in order to implement this new cell division it is inevitable to add a cell migration mechanism to the model. Since in our model only cells on the edge of the cut divide, a guidance mechanism is needed that leads neoblasts to the area of the injury. Saló [20] suggested that neoblasts evenly move to all directions due to cell proliferation instead of "migrating" to the location of the injury. We also plan on testing this hypothesis in our model.

Our main goal is to find a mapping from our model mechanism and a biological equivalent (if such mapping exists). Thus, we want to verify whether our model can replicate behaviors that *in vivo* worms display. For instance, blocking gap junctional communication (GJC) by pharmacological blockers of these electrical synapses leads to a new head emerging at the posterior area of the worm after an injury [21]. We want to verify if reversing packets during backtracking would replicate this finding or if a more complex mechanism must exist.

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