RANDOM-WALK SIMULATION OF CELL MIGRATION AND PROLIFERATION

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Abstract. *Cell migration and proliferation has been modelled in several works of the literature as a process similar to diffusion. However, diffusion models to simulate the proliferation and migration of cells tend to create a homogeneous distribution in the cell density, but this result is not real. Diffusion is not the mechanism of cell dispersal: cells disperse by crawling or proliferation, or are transported in a moving fluid. The use of stochastic models or other (cellular automata, models particles, etc...) can modify this limitation. Therefore, this paper presents a stochastic model (random-walk) to simulate the proliferation and migration of cells. Both processes are considered as completely stochastic as discrete. The model developed aims to predict the behavior of in vitro cell cultures performed with adult muscle satellite cells. Non homogeneous distribution of cells has been observed inside the culture well. Using previous stochastic model we have been able to predict the non homogeneous cell distribution and accurate quantitative results have been computed. In a future, the model will allow us to incorporate other aspects such as cell differentiation, incorporate several cell populations simultaneously, etc.*

1 INTRODUCTION

Cell migration and proliferation has been modelled in several works of the literature as a process similar to diffusion[1]. Bailon-Plaza and van der Meulen (2003)[2] simulate cell migration as a diffusive process taking account gradients in matrix density (haptotaxis). However, using a diffusion model to simulate the migration and proliferation tends to create a smooth variation in cell density, but this result may not be enough. The use of stochastic models (random-walk) or other (cellular automata, particles models, etc...) [3,4] can modify this limitation. Furthermore, random-walk models can simulate not only a preferred direction of migration (resulting from, for e.g., convection or chemoattractant control of migration) but proliferation can also be explicitly modelled by multiplying cell numbers during dispersal. Moreover, using a random-walk model these aspects could be included for several cell populations simultaneously.

Experiments demonstrating random movement of cells were done many years ago. For example, Ambrose (1961)[5] observed the movement of an isolated fibroblast over the surface of a tissue culture dish as mostly random while Carter (1965)[6] was among the first to demonstrate that cells execute a random walk on surfaces. Gail and Boone (1970)[7] quantified that cell migration differs from the pure random walk in that the angles between successive turns are closer to zero; therefore, cells show persistence in their movements. More recently, Palsson and Bahatia (2004)[8] observed, in an in vivo analysis, that a random spatial distribution could be produced during stem cell proliferation. Zohar et al (1998)[9] observed experimentally that mesenchymal stem cells (MSCs) disperse by crawling and convection in the fluid. The directional nature of movement is most apparent with fibroblasts; during wound healing, they become highly motile and migrate in large numbers towards the wound[10]; diffusion-type models that reproduce this effect have been developed [11,12].

Therefore, this paper presents a stochastic model (random-walk) to simulate the proliferation and migration of cells[13]. Both processes have been considered as completely stochastic. The model developed aims to predict the behavior of in vitro cell cultures performed with adult muscle satellite cells. Non uniform cells distribution has been observed inside the culture well [14]. Satellite cells are stem cells or muscle pre-cells which serve to aid the regeneration of adult skeletal muscle [15]. As result of the proliferation (when the cells are reproduced for satellite) and the latter differentiation (when the nucleus changes into a specific type of cell, in this case, a muscle cell), satellite cells are fused between them or with damaged muscle adjacent fibres, this increases the number of myonuclei in the fibres for its growth and regeneration. The proliferation of satellite cells is necessary for supplying more nuclei to the muscle cells. The differentiation is also necessary for the new nuclei to behave as muscular nuclei. The number of myonuceli directly determines the capacity of the muscle cell to produce proteins, including androgen receptor.

For the validation of the model several experiments have been performed with muscle satellite cells of control mouse Wild Type (WT) and transgenic (TR), moreover the cells come from two types of fibres - Fast (anaerobic) and aerobic – Slow (aerobic). The Fast cell type come from a tissue with a fast muscle contraction, while Slow cells type derive from tissue with postural functions[16,17].

2 MATERIAL Y METHODS

2.1 Cell proliferation

The approach for modelling the proliferation of cells is based on the random-walk theory. It is a stochastic process. Initially a cell is presumed (in two dimensions) to be surrounded by four locations that a daughter cell could occupy (Figure 1). Daughter cells are also allowed to remain in the position of the parent cell but opposite "poles" are excluded (as shown in Figure 1) because adjacent positions are far more likely to occur during mitosis. The cells can occupy neighbouring positions with equal probability p (see Figure 1). Although Figure 1 shows four free positions around the cell, this will not, in general, be the case because some positions may already be occupied. Therefore, the model incorporates "contact inhibition" by checking for vacant positions while cells proliferate and depending on the available states (n), the value of the probability p is computed in order to fulfil the condition $\sum_{i=1}^{n} p_i = 1$. If all the surrounded positions are free, the probability p given in Figure 1 will be equal to 1/4. If there is only one vacant position, the probability that it will be filled is equal to one. If all neighbouring positions are occupied, mitosis will not occur.

2.2. Cell migration (n_s, t_s)

Cell migration was also based on the stochastic random-walk approach. Recognising that migration is a more rapid process, a new location for a migrating cell is chosen several times during one iteration of the proliferation process. In the stochastic model proposed, migration is controlled by two parameters: n_s the number of jumps that a cell performed during each proliferation iteration; and t_s the jump size, ie, the distance that the cell moves in each jump. In the simulations presented here, five random jumps are performed for each cell during each iteration of the simulation (n_s=5 and t_s =1). The possible states that cell can occupy after migration, are defined by the nearest wall of the culture wells. At the end of the migration if that position is free is checked. In the event that the location has already been occupied by another cell, a neighbouring location is chosen again randomly, except if the cell population is large enough to prevent the migration of cells. In that case, cells remain in their initial position without migration ("contact inhibition").

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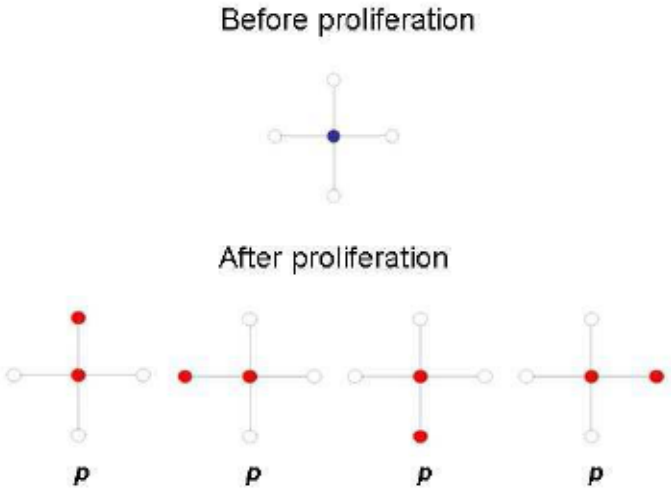

Figure 1: Different possible states for each cell that can occupy after the proliferation. The distance between the sites is only schematic; adjacent sites in the algorithm are considered to be exactly the diameter of a cell. [18].

2.4 Experimental data

In vitro cell cultures with adult muscle satellite cells have been used to validate the model. In vitro cultures have been done in wells of 6.34 mm of diameter (plates of 96 wells) where 1000 cells were initially seeded in culture medium, which was changed daily. The experiment lasted for 5 days during which markers indicating proliferation or mitosis, and differentiation were measured. There were mainly 4 types of experiments depending on the mouse type and the muscle type where that the cells were extracted. There were two mouse types: wild type (WT) and transgenic mouse (TR) which is used as a neurodegenerative model. Cells were extracted from two different muscle tissues: Fast (Anaerobic fibres) when the cells came from a tissue with a fast muscle contraction and Slow (Aerobic fibres) when they came from a tissue with postural functions. For each type of experiment, cells were daily counted and 8 repetitions of each test were done. In each well, cells were counted in 4 areas that correspond to the lateral sides. Therefore, from each well there were 4 data, and a total of 32 values. Mean values were computed and from them, the proliferation rate in each case was obtained. A summary of the experimental data (rate of proliferation) is shown in the Table 1.

Table 1: Summary of the proliferation rate in cell culture in vitro.

Mouse	Fibre type	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
WT	Fast	0.00	2.52	2.14	3.99	2.15	1.94
TR	Fast	0.00	2.38	2.56	2.35	2.07	1.80
WT	Slow	0.00	1.29	1.88	3.54	2.14	1.26
TR	Slow	0.00	2.02	1.47	2.58	2.18	1.72

It was observed that the proliferation behavior was different depending on the mouse type and the muscle fibres from which the cells were extracted. Fast cells proliferated more than from Slow ones. There were also differences between the mouse types (WT vs. TR), the proliferation rate was lower in transgenic mice (TR) and its proliferation rates were also more uniform during the whole experiment.

2.5 Implementation algorithm

The algorithm implementation has been represented in the Figure 2. The simulation starts with an initial population of 1000 cells (as in the experiments) randomly distributed in the cell culture. The cells begin to proliferate following the process indicated in section 2.1. The proliferation rate indicates the percentage of population that will proliferate. If the ratio is 100%, all cells proliferate, but if the ratio is lower, the cells that proliferate are selected randomly between the population. It have been defined two parameters that control the proliferation rate: p_1 controls the mouse type (Wild Type WT or transgenic TR); p_2, defines the proliferation rate depending on the fibres type (Fast or Slow). The combination of both parameters results in the proliferation rate p_r ($p_r = p_1.p_2$). The values considered for these parameters have been shown in Table 2. The algorithm also allows to consider preferential directions of the proliferation. The cells prefer to take location near the wells edges.

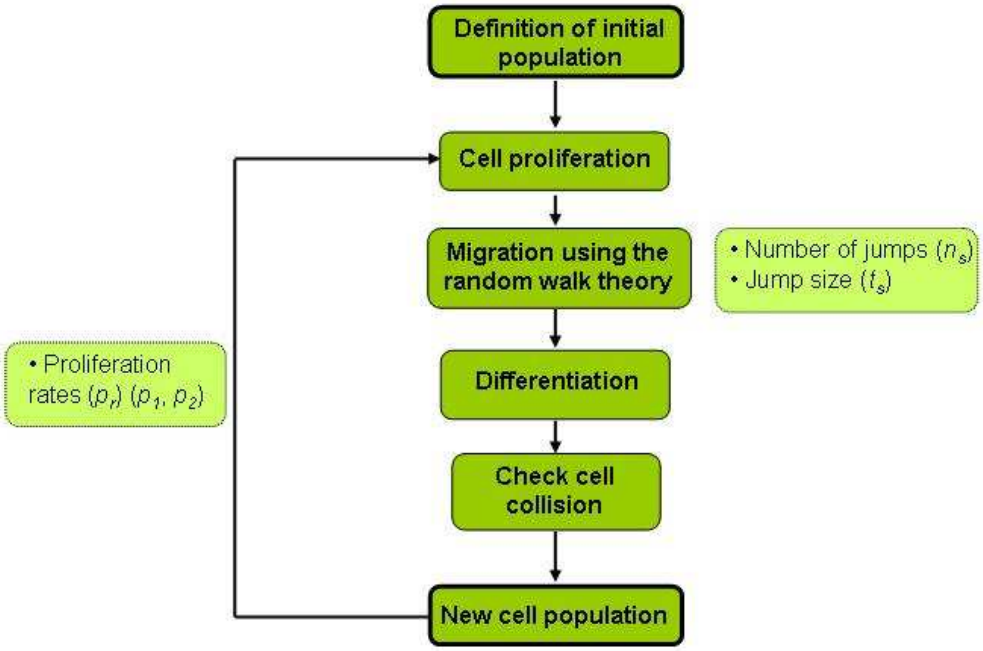

Figure 2: Algorithm implementation.

Once the cells have proliferated, migration is simulated using the random-walk theory described in Section 2.2. The migration process is controlled by two parameters: the number of jumps (n_s) and the jump size (t_s). The values used in the simulation have been shown in Table 2.

Table 2: Parameters of the model used in this simulation.

	p1 (%)		p2 (%)	Migration	
WT	100	Fast	100	ns	ts
TR	80	Slow	65	5	1

After migration, if the new random position is occupied is checked (check the collision - Figure 2). If the position is occupied, one of the cells is removed (apoptosis) and the number of collisions equals the number of cells removed. This process is known in biology as contact inhibition. Finally, the new population initiates a new cycle (Figure 2).

3. RESULT

The proliferation rate obtained from the simulation for the different cases considered and their comparison with the experimental results obtained during 5 days of cell culturing have been represented in Table 3. To obtain the computational results, 5 simulations of each case have been performed. As the simulation is based on a stochastic model, it provides a different result each time. Therefore, the average values of the results have been calculated. The results have been shown in Table 3.

Table 3: Summary of the proliferation rates from experimental test and computational simulations.

Mouse	Fibres type		Day 1	Day 2	Day 3	Day 4	Day 5
WT	Fast	Experimental	2.52	2.14	3.99	2.15	1.94
		Computational	2.60	2.31	4.53	2.61	1.78
TR	Fast	Experimental	2.38	2.56	2.35	2.07	1.80
		Computational	2.22	2.02	3.57	2.38	1.83
WT	Slow	Experimental	1.29	1.88	3.54	2.14	1.26
		Computational	1.97	1.82	3.07	2.27	1.88
TR	Slow	Experimental	2.02	1.47	2.58	2.18	1.72
		Computational	1.69	1.61	2.42	1.98	1.75

It can be observed in Table 3, as the proliferation rates are very similar between computational and experimental results. The differences may be due to that the differentiation process has not been incorporated in the simulation yet. Special markers were added in the in vitro cultures to indicate if there is cell differentiation. In fact their values indicated that cells experienced differentiation, therefore these differentiated cells stop proliferating. This fact could explain the small differences in the proliferation rates.

During the fist days of the experiments, it was observed that cells were not homogeneously distributed across the surface of the well, but it had a greater cell density in the lateral than in the center (Figure 3a). In the simulation, something similar was predicted, especially during the first three days. There is more concentration of satellite cells in the lateral region of the well than in its center (Figure 3b). By continuing the simulation it can be appreciate that as the number of cells increase, they are distributed uniformly in the culture well.

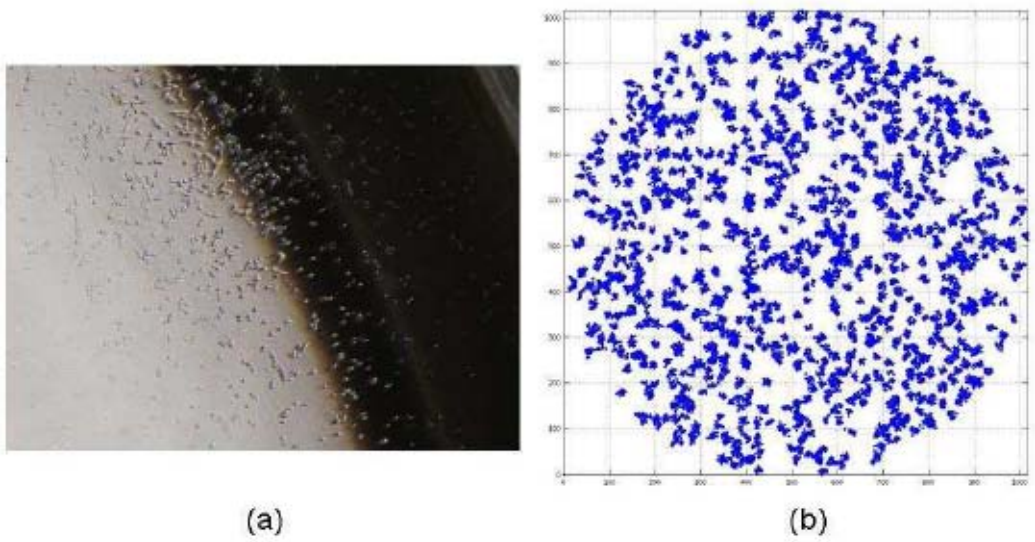

Figure 3: (a) Details of the cell distribution in the lateral of culture well after three days of culture obtained experimentally, (b) cell distribution in the well after the third day of simulation.

The results shown in Figure 3b, were obtained using 5 jumps (ns) during migration and the jump size is 1 (ts). We performed a sensitivity study of these parameters (see Figure 4). It can be observed as increasing the number of jumps (ns) (Figure 4a and b), the cellular distribution is more uniform and there are more cells quantitatively distributed than with a lower number of jumps (Figure 3b). Something similar happens with the size (ts), ie, the distance traveled by the cell at each jump. By increasing the size (Figure 4c and d) a more homogeneous distribution of cells in the culture well is predicted.

4 DISCUSSION

The methodology presented in this paper proposes the use of a stochastic model of random walk to simulate the behaviour of muscle satellite cells, both proliferation and migration [13]. The computational results are similar to those obtained in in-vitro cultures [14] both qualitatively and quantitatively. The proliferation rates between the two results are similar, with slight differences that come from the fact that the simulations do not incorporate differentiation, and experimentally it was demonstrated that there was differentiation by the presence of markers since the third day (Table 3). The proposed methodology has been qualitatively validated by observing the cell population distribution that the different studies of the literature had described, which confirm the random mobility of muscle satellite cells, although no quantitative results have been described. Hence a sensitivity analysis has been done to determine the influence of the migration parameters. It has been reported that they fundamentally affect the homogeneity in the distribution of cells in the culture well. Another limitation of the model is that cell differentiation has not been incorporated, but it will be incorporate in a future work.

In conclusion, the stochastic model of random walk presented in this paper is an approach that simulates cell proliferation and migration, with a methodology that can be easily implemented to simulate problems related to biologic mechanisms or tissue engineering.

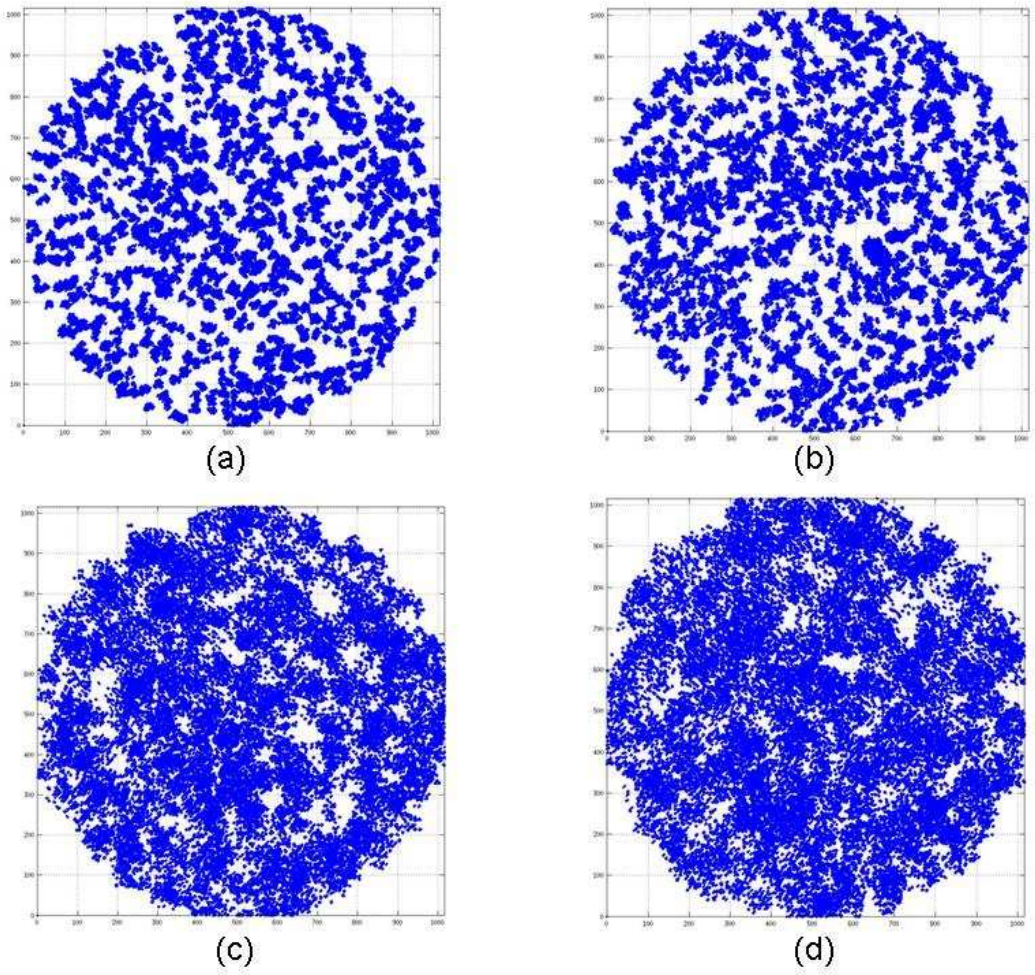

Figure 4: Cell distribution in the well after 5 day simulation compared with parameters different of migration (n_s, t_s): (a) n_s=7, t_s=1; (b) n_s=10, t_s=1; (c) n_s=5, t_s=3; (d) n_s=7, t_s=3

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