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Systems biology

Development of a two-dimensional agent-based model for chronic chagasic cardiomyopathy after stem cell transplantation

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ABSTRACT

Motivation: A significant issue in stem cell therapy is to understand the role of this type of cell in the tissue regeneration. To explain this mechanism, an experimental study has quantified that the bone marrow cell transplantation decreases the number of inflammatory cells and reduces the fibrosis area in chagasic mice. Using this experimental data, we have developed an agent-based computational model to investigate the regeneration of the chronic chagasic cardiomyopathy after bone marrow stem cell transplantation.

Results: Our model includes six different types of agents: inflammatory cell, fibrosis area, cardiomyocyte, proinflammatory cytokine tumor necrosis factor-α, *Trypanosoma cruzi* parasite and bone marrow stem cell. This latter promotes apoptosis in inflammatory cells, reduction in the fibrosis area and can differentiate into cardiomyocyte. Proinflammatory cytokine tumor necrosis factor-α can increase the fibrosis area and *T.cruzi* can increase the number of inflammatory cells. Our results for both apoptosis of inflammatory cells and reduction in the fibrosis area were compared with experimental data. They suggest that the concentration pattern is the most important factor to characterize the kinetics of cardiac tissue regeneration after bone marrow stem cell transplantation.

Availability: The source code of our software is available online at www.vivas.ufba.br/bone/bone.zip

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Supplementaty information: Supplementary data are available at *Bioinformatics* online.

1 INTRODUCTION

Chronic chagasic cardiomyopathy is an endemic illness affecting millions of people in the world. The etiological agent of this disease is the hemoflagellate parasite *Trypanosoma cruzi*. This protozoan is transmitted by more than 100 species of insects of the family Ruduviidae, subfamily Triatominae or by blood transfusion. According to the World Health Organization, there are 16–18 millions of people infected only in South America. This disease is still incurable, however most of the contaminated people remain asymptomatic, i.e. they have an indeterminate form and around 30% of them develop a chronic inflammatory disease, with cardiac and/or digestive complications. The clinical symptoms of the heart pathology are cardiomyopathy, heart failure and arrhythmia (Andrade and Andrews, 2005; Coura *et al*., 2002; Higuchi *et al*., 2003; Kelly, 2000; Zacks *et al*., 2005).

Chronic chagasic cardiomyopathy is characterized by a diffuse inflammatory reaction composed mainly of mononuclear cells, and a severe fibrosis (Higuchi *et al*., 2003; Soares *et al*., 2001). The number of *T.cruzi* parasites in the chronic phase of this disease is disproportionately low in relation to the intensity of inflammation. However, recent studies have verified high frequencies of *T.cruzi* antigens in this disease, providing that the occurrence of this parasite is associated with myocardial inflammation (Higuchi *et al*., 2003). The development of fibrosis is caused by an elevate production in the number of proinflammatory cytokines tumor necrosis factor-α (TNF-α) in the heart (Soares *et al*., 2004).

The contribution of the scientific literature in computational models for parasites infection includes interaction between *T.cruzi* parasites and antibodies during the acute phase of Chagas infection (Condat *et al*., 2003; Isasi *et al*., 2001; Sibona and Condat, 2002), competitive parasite–antibody interaction in the intracellular and extracellular phase of the Chagas' disease (Sibona *et al*., 2005), cell-mediated immune response to Chagas' disease (Nelson and Velasco-Hernández, 2002) and dynamics of *Plasmodium falciparum* invasion of the human erythrocyte cells (Ferrer *et al*., 2007; Hoschen *et al*., 2000; McKenzie and Bossert, 2005; Peleg *et al*., 2002).

Also, a great deal of research has been carried out to investigate the kinetics of stem cell transplantation (Bianco *et al*., 2001; Grove *et al*., 2004; Sell, 2004; Temple, 2001). A stem cell is a singular type of cell that can renew itself and possesses ability to divide in multiple types of specialized cells often for indefinite periods (Sell, 2004). The discovery of the plasticity of adult bone marrow cells has allowed new perspectives for the treatment of incurable chronic disease. Adult bone marrow cells include two types of bone marrow stem cells, i.e. hematopoietic stem cells and mesenchymal stem cells. Hematopoietic stem cells give rise to all blood lineages and mesenchymal stem cells give rise to bone, cartilage and fat. Experimental evidences have shown that adult bone marrow stem cell can differentiate into other types of cells, including skeletal muscle, cardiac muscle, hepatocytes, keratinocytes and neurons (Bianco *et al*., 2001; Grove *et al*., 2004; Sell, 2004).

Diverse experimental models have confirmed the regenerative potential of bone marrow stem cells. The transplant of bone marrow

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stem cells improves the injured tissue because this type of stem cell can promote apoptosis in non-normal cells. Also, bone marrow stem cells can differentiate into a normal cell of the tissue (Sell, 2004). An experimental model to describe the effects of adult bone marrow cell transplantation in dilated cardiomyopathy was developed by Soares *et al*. (2004). In this model, bone marrow cells containing bone marrow stem cells were injected into chronic chagasic mice leading to a considerable reduction in the inflammatory infiltrate and in the fibrosis area. The authors suggest that bone marrow stem cells promote apoptosis of inflammatory cells and reduction in the fibrosis area.

Recently, different theoretical models have been proposed to understand the stem cell differentiation and proliferation. The models developed for describing these processes are based on differential equations (Lemon *et al*., 2007; Tannenbaum *et al*., 2005), co-clustering latent variable models (Joung *et al*., 2006) and Bayesian network (Woolf *et al*., 2005). However, we can also represent stem cells by using autonomous agents to evaluate the influence of this population. For instance, the different types of cells in the chronic chagasic cardiomyopathy can be represented by different types of agents. Thus, we propose in this article a two-dimensional agent-based model for chronic chagasic cardiomyopathy regeneration after bone marrow stem cell transplantation.

2 COMPUTATIONAL MODEL

A two-dimensional lattice is employed to represent the cardiac tissue and the state of the sites is chosen and distributed on a regular lattice. Each site represents the region of the space in which only a type of autonomous agent can occupy in the chagasic tissue. The time evolution is equally run in the entire lattice and each site changes its state according to a local rule, which depends only on the adjacent neighbors. In addition, our model has different types of agents to describe the chagasic cardiac tissue. In this model, each type of agent represents a different type of cell. An agent lives in an environment in which it can identify the types of agents through their attributes and can interact with other agents (Wooldridge and Jennings, 1995). There are also empty spaces to allow the movement of some types of cells. We have developed a software program to simulate the chronic chagasic cardiomyopathy regeneration after bone marrow stem cell transplantation. The source code was developed in C++ language and it is available at http://www.vivas.ufba.br/bone/bone.zip.

2.1 State of the sites

The computational model generates a two-dimensional rectangular lattice consisting of a grid with 500×500 points in size, which represents a block of virtual chagasic cardiac tissue. This lattice possesses 250 000 sites and periodic boundary conditions. Initially, the parameters of this model are the total number of agents, initial fraction of fibrosis area, initial fraction of inflammatory cells, initial fraction of bone marrow stem cells, initial fraction of TNF-α and initial fraction of *T.cruzi* parasites. The fraction of cardiomyocytes is given by the difference between the total number of agents and the total number of the others types of cells. Here, we use the experimental data obtained by Soares *et al*. (2004) to simulate the initial fraction of inflammatory cells and initial fraction of fibrosis area. Thus, in our simulations we employ the same quantity for these two parameters.

The quantity of each type of agent is calculated by multiplying the initial fraction of each type of agent by the total number of agents. The quantity of bone marrow stem cells, *T.cruzi* parasites and TNF-α is constant during the simulation. The initial distribution of the agents is heterogeneous. Initially, some fibrosis areas are randomly placed and the others are placed in empty spaces in the neighborhood of a fibrosis area with a certain probability. This probability depends on the quantity of fibrosis areas around the empty space. The same occurs to the inflammatory cells. The probabilities are given by:

$$P_\beta = \frac{T_\beta}{8} \tag{1}$$

$$P_\gamma = \frac{T_\gamma}{8} \tag{2}$$

where, P_β is the probability of an empty space being occupied by a fibrosis area, T_β is the total number of fibrosis areas surrounding the empty space, P_γ is the probability of an empty space being occupied by an inflammatory cell and T_γ is the total number of inflammatory cells surrounding the empty space.

Cardiomyocytes, bone marrow stem cells, TNF-α, and *T.cruzi* have a random distribution. The distribution pattern of all types of agents is based on experimental data of Soares *et al*. (2004). In this case, the fibrosis area and inflammatory cells have a non-uniform density; therefore, we use a similar concentration distribution in these types of agents.

2.2 Neighborhood

Inflammatory cells, cardiomyocytes, TNF-α, *T.cruzi* parasites and bone marrow stem cells can move through the empty spaces. Each type of cell (agent) is chosen randomly making it to jump to empty space. These types of agents move randomly using the Moore neighborhood. In this type of neighborhood, each cell has eight neighbors situated in positions north, south, east, west, northeast, southeast, northwest and southwest (Wolfram, 1986). Only the fibrosis area is fixed.

2.3 Transition rules of states

The time evolution depends on a set of rules that determines the state of all sites. The state of the sites is determined through the simulated stem cell properties. This computational model simulates the properties of apoptosis and differentiation. Bone marrow stem cells can promote apoptosis of inflammatory cells, reduction of the fibrosis area (Soares *et al*., 2004) and can differentiate into cardiomyocytes (Orlic *et al*., 2001). Also, TNF-α can increase the fibrosis area (Soares *et al*., 2004) and *T.cruzi* can increase the number of inflammatory cells (Higuchi *et al*., 2003). Then, the transition rules of this computational model are as follow:

- In the neighborhood of an inflammatory cell, if the number of bone marrow stem cells is different from zero, the apoptosis of this inflammatory cell occurs (Soares *et al*., 2004), i.e. the site of the inflammatory cell changes into empty space.

- In the neighborhood of a fibrosis area, if the number of bone marrow stem cells is different from zero, the reduction in this fibrosis area occurs (Soares *et al*., 2004), i.e. the site of the fibrosis area changes into empty space.
- In the neighborhood of an empty space site, if there is a cardiomyocyte and the number of bone marrow stem cells is greater than a determined value, the differentiation of bone marrow stem cells into cardiomyocyte occurs (Orlic *et al*., 2001), i.e. the empty space changes into cardiomyocyte.
- In the neighborhood of an empty space site, if the number of inflammatory cells and *T. cruzi* parasites is different from zero, the empty space site changes into an inflammatory cell (Higuchi *et al*., 2003).
- In the neighborhood of an empty space site, if the number of TNF-α and fibrosis areas is different from zero, the empty space site changes into a fibrosis area (Soares *et al*., 2004).

3 RESULTS AND DISCUSSION

A total of 20 simulations were performed to estimate the best parameters that describe the kinetics of bone marrow stem cells in the chagasic cardiac tissue. All the parameters were varied and the parameter optimization was done using the best fit with the experimental data. All the steps of the model were equally followed in all simulations. The data of fibrosis area and inflammatory cells were normalized to enable a comparison between computational and experimental results. The normalization of experimental and computational results is done in the same way. All the data are divided by the initial value of the respective dataset. Then, the normalized data correspond to the fraction of cells in relation to the first month. This can be calculated by using the equation:

$$N_i' = \frac{N_i}{N_o} \tag{3}$$

where N_i' represents the normalized data, N_i the original data and N_o the data in the first month. As defined in the model rules, bone marrow stem cells can promote apoptosis of inflammatory cells, reduction in the fibrosis area and generation of cardiomyocytes. TNF-α increases the fibrosis area and *T.cruzi* parasites increase the number of inflammatory cells.

To compare the predictions of the agent-based model, we carried out a fit of the experimental data (Soares *et al*., 2004). In this experiment, 8-week-old female or male BALB/c mice were used for *T.cruzi* infection. These mice were infected by the inoculation of *T.cruzi* trypomastigotes and treated 6 month later with adult bone marrow cells obtained from normal BALB/c mice by the intravenous route. These bone marrow cells contained several different types of cells, including bone marrow stem cells. Bone marrow cells were used to treat chagasic mice. These mice were sacrificed 1, 2, 3, 5 and 6 months after treatment for measurement of inflammatory cells per square millimeter and percentage of fibrosis area in the heart. In this experiment, the authors measured the fibrosis area and counted the number of inflammatory cells only 1 month after bone marrow cell transplantation.

Several studies have emphasized that the quantity of parasite in the chronic phase of Chagas' disease is disproportionately low (Higuchi *et al*., 2003). However, in the experiment of Soares *et al*. (2004) parasites could not be observed either in the blood or in heart sections 1, 2 and 4 weeks and 2 to 6 months after bone marrow cell transplantation, by using optical microscopy. Also, in this experiment the migration of transplanted bone marrow cells was demonstrated, but the number of bone marrow stem cell was not quantified. Also, the number of TNF-α in the chagasic cardiac tissue was not quantified.

Figure 1a shows heart sections of chronic chagasic mice treated with saline and bone marrow cells obtained by Soares *et al*. (2004). In these heart sections, the regions of fibrosis and inflammatory cells are greater and more concentrated in the non-transplanted mice. Figure 1b–d shows a graphic visualization of our computational model. In this representation, for clarity, the states of the sites have different patterns (see Supplementary Material for color figure). The distribution of the different types of agents in our model describes the concentration pattern of fibrosis area and inflammatory cells in the chagasic cardiac tissue. The model evolution simulates the dissolution and reduction of fibrosis area and inflammatory cells. Therefore, initially the regions of fibrosis and inflammatory cells are more concentrated, but they do not possess a uniform density. The concentration pattern of the different types of agents has a significant importance because it is responsible for the chagasic tissue regeneration.

In this computational model, 500 time steps correspond to the experimental data of 1 month; therefore, each time step corresponds to ~1.44 h of 'real time'. Hence, we have performed 2500 time steps. In all the simulations, we use the initial fraction of inflammatory cells equaling 0.08, and the initial fraction of fibrosis area equaling 0.22. We have used these values because these are the fractions obtained by Soares *et al*. (2004), 1 month after bone marrow cell transplantation. We have compared our computational data with the experimental data 1, 2, 3, 5 and 6 months after treatment.

In Figure 2, we show the kinetics of apoptosis of inflammatory cells and reduction in the fibrosis area for different fractions of lattice-site occupation. Our results indicate that the smaller the number of empty spaces, the smaller the final fraction of inflammatory cells and fibrosis area. This happens because it is more difficult for a *T.cruzi* parasite to meet an inflammatory cell when there are few empty spaces. Therefore, when the number of empty spaces is small, the increase in the number of inflammatory cells occurs more slowly. This is related to the random movement of inflammatory cells and *T.cruzi*. The same occurs for TNF-α.

In Figure 2a, the experimental curve of inflammatory cells Soares *et al*. (2004) shows an increase that probably occurs since the mice are infected with *T.cruzi* and, consequently, continue generating inflammatory cells. In Figure 2b, the experimental curve of fibrosis area (Soares *et al*., 2004) first decreases, then increases and in the sixth month decreases again. The experimental curve of fibrosis area increases in the third month because there is TNF-α in the cardiac tissue of mice; therefore, the development of fibrosis area continues. However, the reduction of fibrosis in the sixth month can perhaps be attributed to the effect of decreases in the number of inflammatory cells, TNF-α-producing cells in the heart.

The variation of the initial fraction of bone marrow stem cells for apoptosis of inflammatory cells and reduction in the fibrosis area is shown in the Figure 3. The fit for experimental data are given in Figure 3a and b. In addition, the data of our computational model also increase because it possesses *T.cruzi* and TNF-α. The presence

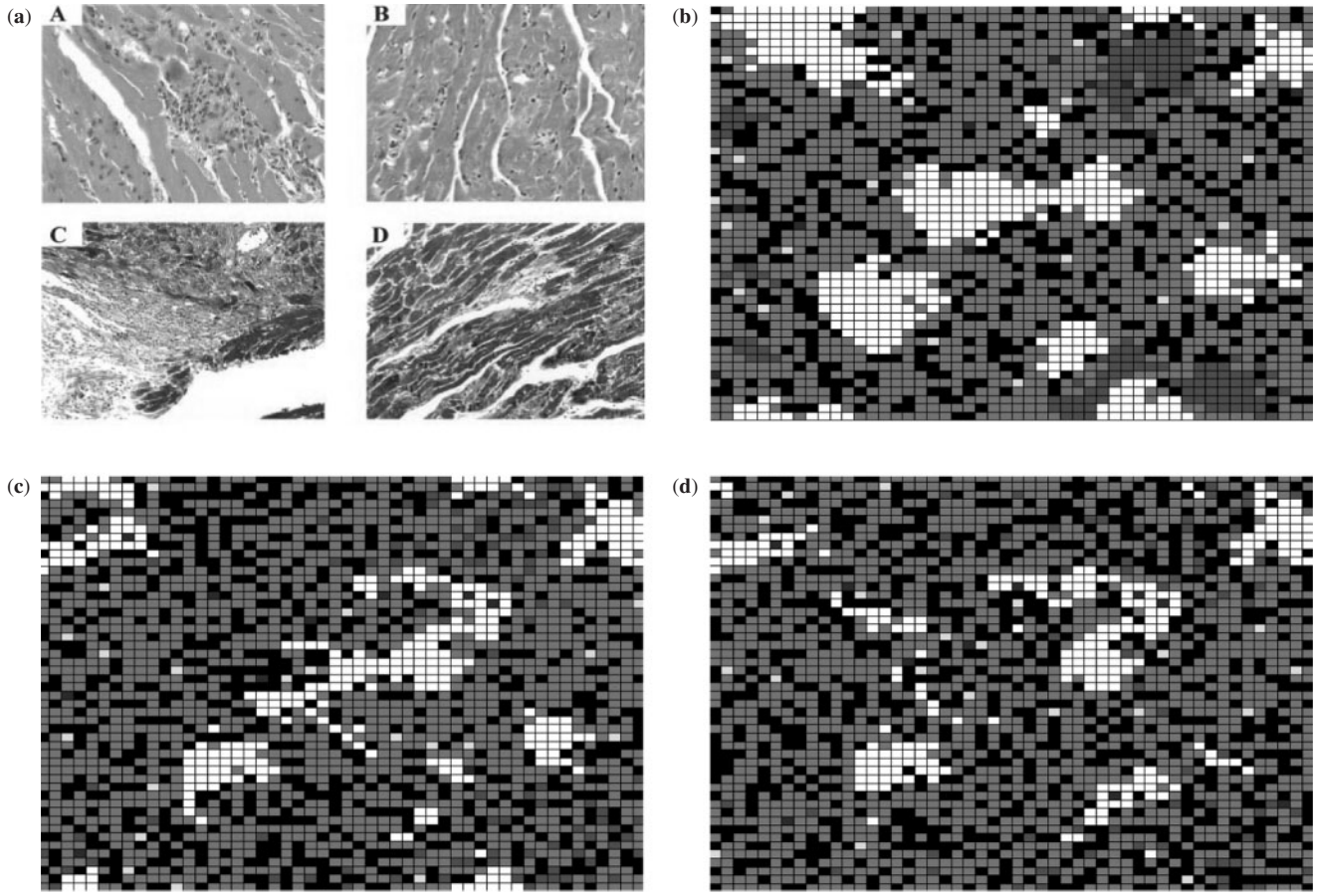

Fig. 1. Comparison of the chagasic cardiac tissue section from Soares *et al*. (2004) with our computational model at different time steps. (**a**) Heart sections of the chronic chagasic BALB/c mice treated with saline (control) and bone marrow cells. Two month later control mice (A and C) or bone marrow cells treated mice (B and D) were sacrificed. In these sections, fibrosis area appears in white, inflammatory cells in dark gray and cardiomyocytes in light gray. In these heart sections, the regions of fibrosis area and inflammatory cells are greater and more concentrated in the control mice (A and C). (Reprinted from Am. J. Pathol. 2004 164: 441–447 with permission from the American Society for Investigative Pathology). (**b**) Graphic representation of our computational model at different time steps. This two-dimensional rectangular lattice consists, for clarity, of 50×50 points. The initial fraction of the lattice-sites occupied (So) is 0.8, the initial fraction of inflammatory cells is 0.08, the initial fraction of fibrosis area is 0.22, the initial fraction of *T.cruzi* (T_c) is 0.00055, the initial fraction of TNF-α is 0.0045, the initial fraction of bone marrow stem cells (Bmst) is 0.01 and the number of bone marrow stem cells required for the differentiation of a bone marrow stem cell into a cardiomyocyte (Dcm) to occur is greater than 2. Empty spaces are represented by black patterns and fibrosis areas by white. The other types of cells are represented by different tones of gray: TNF-α and *T.cruzi* by darkest gray, inflammatory cells by dark gray, cardiomyocytes by intermediate gray and bone marrow stem cells by light gray. Time step $t = 0$. (**c**) Time step $t = 50$. (**d**) Time step $t = 100$. (See Supplementary Material for color figure).

of *T.cruzi* and TNF-α is essential for the simulation of chagasic cardiac regeneration. This fact is evidenced because in the absence of *T.cruzi*, the number of inflammatory cells tends to zero and in the absence of TNF-α, the fibrosis area tends to zero. This vanishing is due to the presence of the bone marrow stem cell.

In Figure 4a, we vary the initial fraction of *T.cruzi* and in Figure 4b the initial fraction of TNF-α. We can observe that a little difference in the initial fraction of the *T.cruzi* changes the behavior of the apoptosis of inflammatory cells. The behavior of the reduction in the fibrosis area is less affected by the variation of the fraction of TNF-α. This happens because it is more difficult for a bone marrow stem cell to penetrate in a place with high density than with low density.

Figure 5 shows the differentiation of the bone marrow stem cells into cardiomyocyte. In all the simulations, the increase is linear. However, in the neighborhood of an empty space site, if there is a cardiomyocyte and the number of bone marrow stem cells is greater than 2, the total number of cardiomyocytes grows around 4.5% and if the number of bone marrow stem cells is greater than 3, the total number of cardiomyocytes grows around 0.6%. It is well known that bone marrow stem cells can differentiate into a normal cell of the tissue (Sell, 2004). As the experimental data of Soares *et al*. (2004) do not quantify the number of new cardiomyocytes, we can only suggest that the increase of cardiomyocytes is less realistic when the number of bone marrow stem cells is greater than 3 because it is very small.

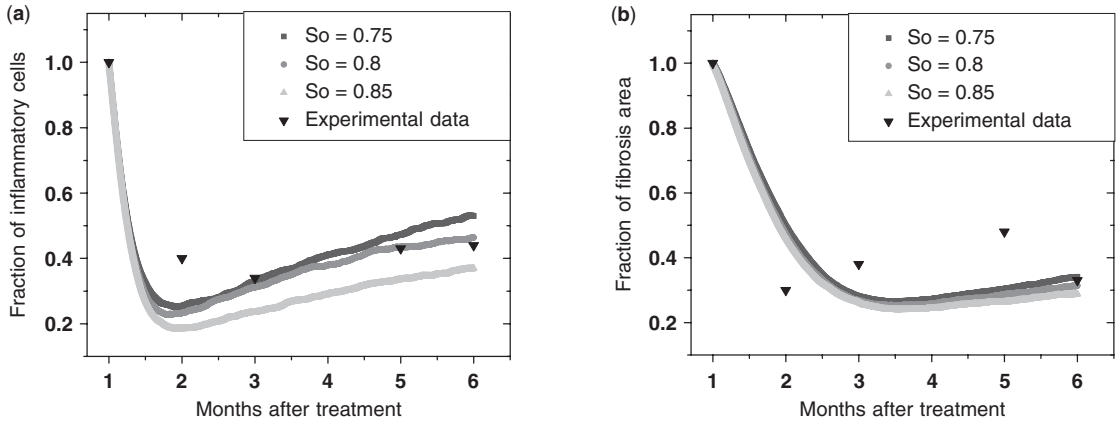

Fig. 2. Kinetics of chagasic tissue regeneration for different fractions of lattice-site occupation. The other parameters used were $T_c = 0.00055$, TNF-$\alpha = 0.0045$, Bmst = 0.01, Dcm > 2. The experimental data were obtained by Soares *et al*. (2004). (**a**) Apoptosis of inflammatory cells. (**b**) Reduction in the fibrosis area. (See Fig. 1 for the meaning of the labels).

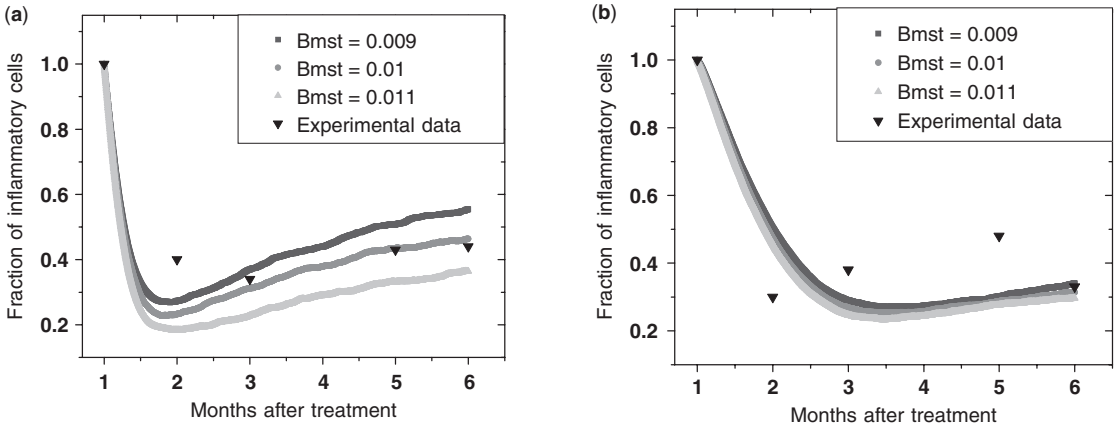

Fig. 3. Kinetics of chagasic tissue regeneration for different fractions of bone marrow stem cells. The other parameters used were $S_o = 0.8$, $T_c = 0.00055$, TNF-$\alpha = 0.0045$ and Dcm > 2. The experimental data were obtained by Soares *et al*. (2004). (**a**) Apoptosis of inflammatory cells. (**b**) Reduction in the fibrosis area. (See Fig. 1 for the meaning of the labels).

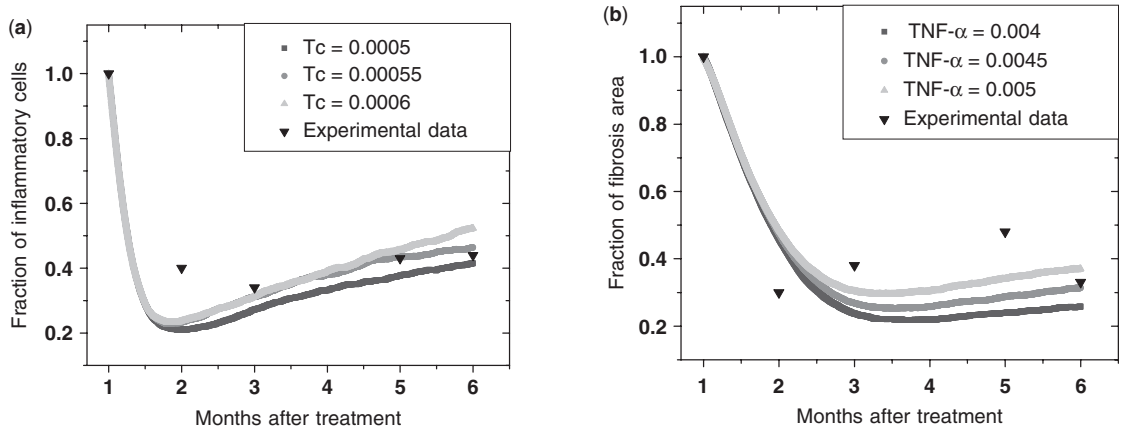

Fig. 4. Kinetics of chagasic tissue regeneration for different fractions of *T.cruzi* and TNF-α. The other parameters used were $S_o = 0.8$, Bmst = 0.01 and Dcm > 2. The experimental data were obtained by Soares *et al*. (2004). (**a**) Apoptosis of inflammatory cells. In this simulation we use TNF-$\alpha = 0.0045$. (**b**) Reduction in the fibrosis area. In this simulation we use $T_c = 0.00055$. (See Fig. 1 for the meaning of the labels).

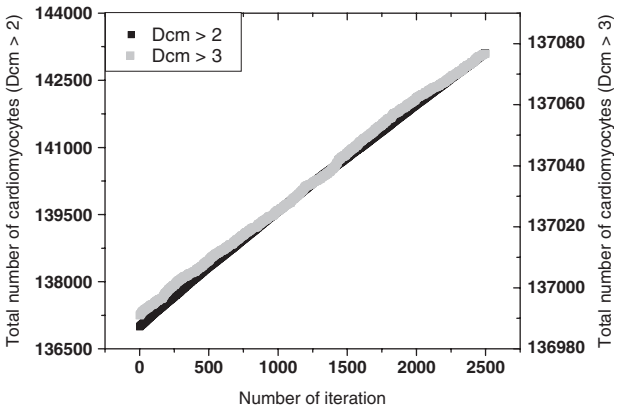

Fig. 5. Kinetics of chagasic tissue regeneration for different number of bone marrow stem cells required for differentiation of a bone marrow stem cell into a cardiomyocyte. (See Fig. 1 for the meaning of the labels).

The best parameters of our computational model to describe the chagasic tissue regeneration were chosen using the best fit with the experimental data. Thus, the parameters selected are the fraction of the number of sites occupied equaling 0.8, fraction of *T.cruzi* parasites equaling 0.00055, fraction of TNF-α equaling 0.0045, fraction of bone marrow stem cells equaling 0.01 and the number of bone marrow stem cells required for the differentiation of a bone marrow stem cell into a cardiomyocyte to occur is greater than 2.

4 CONCLUSIONS

In this article, we have presented a two-dimensional agent-based model to investigate the regeneration of the chronic chagasic cardiomyopathy after bone marrow stem cell transplantation. Our computational model can simulate some stem cells properties including apoptosis and differentiation. Apoptosis occurs in inflammatory cells and the bone marrow stem cells can differentiate in cardiomyocytes. Also, there is reduction in the fibrosis area. The fundamental hypothesis is that the kinetics of chagasic tissue regeneration has a concentration pattern and this can be modeled and represented by using an appropriate computational model. The main aim of our model is to understand the participation of different types of cells in the cardiac tissue regeneration.

Our results were compared with experimental data, excepting for the differentiation of bone marrow stem cells in cardiomyocytes. The results also suggest that the concentration pattern of fibrosis area and inflammatory cells is the most important factor in the kinetics of chronic chagasic cardiomyopathy regeneration after bone marrow stem cell transplantation. In addition, the initial fraction of bone marrow stem cells and *T.cruzi* parasites affects the rate of apoptosis of inflammatory cells. Finally, the initial fraction of bone marrow stem cells and TNF-α affects the rate of reduction in the fibrosis area.

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Conflict of Interest: none declared.

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