A Reaction-Diffusion Numerical Model to Predict Cardiac Tissues Regeneration Via Stem Cell Therapy

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Abstract

Myocardial infarction is a leading cause of morbidity and mortality in the industrialized world. Extensive loss of cardiomyocytes, substituted by scarred tissue, is the key pathological mechanism leading to post infarction heart failure [1]. The use of exogenous cells to replace lost cardiomyocytes has been demonstrated in animal models and in clinical trials by transplanting mesenchymal stem cells (MSCs) into the infarcted area. To optimize the initial conditions of the stem cell therapy, many experimental studies are focused on determining the number and the localization of stem cells that must be implanted near the necrotic area. In this work we develop a quantitative numerical model able to simulate substrate concentration profile, stem cells distribution and their proliferation near the ischemic area. The model describes the cell growth, the nutrient transport and its consumption through reaction-diffusion equations. The shrinking of the necrotic area leads in fact to a moving boundary problem. Some preliminary results, obtained in a 3D framework, are shown and discussed.

Keywords: convection-diffusion-reaction equations, Free boundary problem, Stem cell therapy, Congestive heart failure.

1. Introduction

Congestive heart failure is a leading cause of morbidity and mortality in the industrialized world. In the United States, more then 5 million people suffer heart failure. Each year about 550,000 new cases are diagnosed. The European Society of Cardiology has evaluated at least 15 million patients with heart failure in 51 countries [2]. Stem cell therapies could have unique potential for the treatment of heart failure through regeneration of cardiac tissue and restoration of cardiac contractility [3]. Many experimental studies are performed in order to determine the number of stem cells which should be implanted near the necrotic area. The density of implantable stem cells seems to be one of the critical parameters, depending on cell metabolism and histological environment (damage, oxygen O_2 and carbon dioxide CO_2 concentration, residual microcirculation, temperature, pH etc.). The localization of the implant is also important. Indeed the stem cells must be reached easily by nutrients as oxygen and glucose. Studies investigating the issues just recalled have been performed "in vitro" and in "vivo" [4, 5, 6]. In recent years, also mathematical modeling has been playing an important role in the analysis of such issues (see, for example, [7, 8, 9]). The main goal of this work is to develop a numerical model enabling one to simulate the substrate concentration profile, the stem cells distribution and their proliferation near the ischemic area, as well as the transport of nutrients.

For the sake of computational simplicity, in this preliminary work we keep an assumption of spherical symmetry. Thus we consider a small spherical shell of inflamed tissue, enclosing a spherical necrotic core. The transport of both stem cells and nutrients is described by a convection-diffusion-reaction system. The model aims at describing the initial development of a first regenerated layer

around the ischemic core. Therefore the onset of angiogenesis in the regenerated portion, which is beyond the scopes of this work. The width of the regenerated layer is therefore limited by the effective reach of passive diffusion of nutrients, and can not be expected to exceed $\approx 75\mu m$.

The amount of implantable stem cells, the oxygen concentration in the treated area and the extension of ischemic area after cell proliferation can be predicted by a number of basic numerical simulations.

2. The biological mechanism

Let us consider a spherical 3D portion of cardiac tissue subjected to an ischemia in the central core. This portion will be represented by a sphere Ω of radius R (fig. 1), and the ischemic (necrotic) core N_0 will consist initially in a concentric sphere of radius $R_0 < R$. A limited amount of stem cells is injected in a controlled manner over the external boundary of Ω: they will migrate and diffuse in Ω through the underlying tissue which constitutes a homogeneous substrate of partially inflamed cells. As an effect of the damage, a gradient of a chemoattractant exists in Ω, attracting the stem cells towards the necrotic zone. Due to extremely adverse conditions, only a small percentage ($\sim 10\%$) of stem cells survive to reach the necrotic core, differentiate and regenerate the necrotic area, forming a new thin layer $D(t)$. Consequently, the necrotic area $N(t)$ shrinks, keeping, in our model, its spherical shape. The set given by difference between Ω and $N(t)$ is the inflamed cell region, denoted by $G(t) = \Omega - N(t)$. We also let $\Gamma(t)$ be the interface between G and N, which coincides with the internal boundary of G and the external boundary of N. This interface moves inwards with velocity V due to the deposition of stem cells causing a progressive shrinking of N. From a mathematical point of view, this leads to a free boundary problem, since the internal boundary changes in time in an a priori unknown way [10, 11].

Let us denote by w the concentration (volume fraction) of underlying cells and by u the concentration of stem cells. For our purposes, it will be assumed that, within a satisfactory approximation, w is spatially uniform and stationary, and that $u + w = 1$.

3. Stem cell dynamics

The dynamics of stem cells in G is governed by the following convection-diffusion-reaction equation:

$$\frac{\partial u}{\partial t} - div\,(D_u \nabla u + \chi u \overrightarrow{r}) = -R_d u \qquad \text{in} \quad G \tag{1}$$

where R_d denotes the stem cell death rate, D_u the diffusion coefficient accounting for stem cell motility, $\chi = \chi(\nabla w)$ a chemoattractant coefficient and $\overrightarrow{r}$ is the unit vector pointing inwards. Indeed chemoattraction (and therefore convection) should play a prominent role in the dynamics of stem cells, in comparison

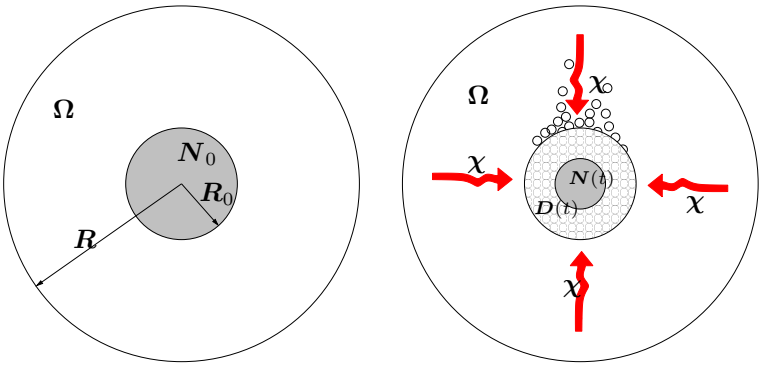

Figure 1: A schematic 2D representation of the damaged area in a simplified symmetric circular geometry. N_0 represents the initial necrotic area (grey), surrounded by an inflamed cell region (white) Ω (figure on the left). The stem cells implanted at the outer boundary are chemoattracted by substances χ released by the inflamed cells towards the necrotic area and build up a regenerated area of differentiated stem cells $D(t)$ (circled). The repaired tissue gradually replaces the necrotic region that shrinks to $N(t)$: its boundary moves at a speed depending on the flux of stem cells at that interface (figure on the right).

with diffusion. The relative size of the two concurrent processes is measured by the Péclet number $Pe = \dfrac{R\chi}{D_u}$. Equation (1) is supplemented by an external boundary condition

$$u = u_0 \exp(-\beta t) \qquad \text{on} \quad \partial G \tag{2}$$

(where β represents the stem cell decay rate), by an internal boundary condition at the interface with the necrotic core

$$u = 0 \qquad \text{on} \quad \Gamma \tag{3}$$

and by an initial condition

$$u = 0 \qquad \text{at} \quad t = 0\ . \tag{4}$$

Equation (3) is prescribed since at the interface Γ stem cells are assumed to either differentiate or die, and thus they exit anyway the population represented by density u. In addition we require

$$V = -\lambda (D_u \nabla u) \cdot \nu \qquad \text{on} \quad \Gamma \tag{5}$$

where V is the normal velocity of the free boundary Γ and ν the outer normal to it. In equation (5), $\lambda \leq 1$ is a parameter expressing the fraction of incoming stem cells that actually are implanted in D. In general λ is an increasing function of the nutrient: $\lambda = \lambda(c)$.

4. Nutrient dynamics

For simplicity we consider one nutrient only, that is oxygen. The dynamics of the nutrient c is given by a convection-diffusion-reaction equation:

$$\frac{\partial c}{\partial t} - div\,(D_c \nabla c - Uc) = -S(c)(u+w) = -S(c) \qquad \text{in} \quad \Omega \tag{6}$$

where U is a preassigned flow field, D_c is a diffusion coefficient, and

$$S(c) = \frac{R_m}{K_m + c} \tag{7}$$

is a nutrient dependent consumption rate, in the form of a Michaelis-Menten or Monod term, where R_m is the maximum metabolic rate and K_m the saturation constant [12]. The coefficient D_c has a piecewise constant form, i.e.,

$$D_c(x) = \begin{cases} D_c^{max} & \text{if} \quad x \in G(t) \quad \text{(inflamed region)} \\ D_c^{med} & \text{if} \quad x \in D(t) \quad \text{(regenerated region)} \\ D_c^{min} \approx 0 & \text{if} \quad x \in N(t) \quad \text{(necrotic region)} \end{cases} \tag{8}$$

that accounts for different values of oxygen diffusion in different tissues. We prescribe finally the nutrient flux at the external boundary

$$(D_c \nabla c) \cdot \nu = Q \qquad \text{at} \quad \partial\Omega \tag{9}$$

and the initial value

$$c = c_0 \qquad \text{at} \quad t = 0\ . \tag{10}$$

The model depends on many (physical, geometrical, biological) interconnected parameters that should be correctly identified, or, at least, limited in a proper range: some of them come from literature [7, 8, 13, 14], others can be, in the future, directly obtained experimentally or estimated by fitting experimental data with our simulations. In this paper we choose arbitrary but compatible values to the unknown parameters, in order to capture the qualitative behavior of the system (see Table (1)).

5. Numerical simulations and results

As already mentioned, as a first attempt we have assumed radial symmetry. Bearing in mind that we are tackling a free boundary problem, we have integrated numerically (1) and (6) with finite differences and a Backward Differentiation Formulas (BDF) method. Starting at time $t = 0$ with the domain $[R_0, R]$, at each time step we have found via (5) the (new) domain $[r(t),\ R]$, with $r(t) \leq R_0$. Then we have implemented the numerical integration on such

Parameter	Nomenclature	Numerical value
R_m	Maximum reaction rate	$2.88 \cdot 10^{-2}$ g/(cm^3·h)
K_m	Saturation coefficient	$6.3 \cdot 10^{-5}$ g/cm^3
R_d	Cell death parameter	$1.18 \cdot 10^{-3}$ h^{-1}
D_u	Stem cell diffusion coefficient	$6.12 \cdot 10^{-5}$ cm^3/h
D_c^{max}	Nutrient diffusion coefficient	0.5
Q	Nutrient flux	1
R	Radius of Ω	1.0 cm
R_0	Radius of N_0	0.4 cm
λ	Implantation factor	1
β	Stem cell decay rate	$4.18 \cdot 10^{-2}$ h^{-1}

Table 1: The parameters used in the model, their definition and their numerical value used in the simulations.

domain; to avoid complications due to a not-equispaced grid we have rediscretized our domain at each time step, using interpolated values of the previous step. Even if in this way the spatial step, in principle, can change at any time step, we have managed to keep it small.

We stopped the iteration when the internal boundary Γ_T **has moved a distance that does not exceed** $\approx 75\mu m$ **from its initial location towards the necrotic area.** Notice that all the parameters that we used (see Table (1)) were taken from the literature, except Q (nutrient flux) and D_c^{max} (nutrient diffusion coefficient), that were chosen as reasonable and consistent values. Instead, the Péclet number Pe and the initial stem cells concentration u_0 are the control parameters of our simulations. Moreover, we have chosen a stem cell diffusion coefficient two orders of magnitude higher than the corresponding cells diffusion coefficient due to the well known stem cells motility and to the particular nature of the cardiac tissue. The results of our simulations (see Fig. (2)) show the expected qualitative behavior: when the initial stem cells concentration (on the external boundary) is below a certain threshold, the treatment is not successful: all the stem cells die during the migration from the external boundary to the necrotic area (Fig. (2), right panel). Notice that some stem cells eventually reach the necrotic core, but then they do not have enough "strength" to make the internal boundary Γ move via (5) and are subsequently

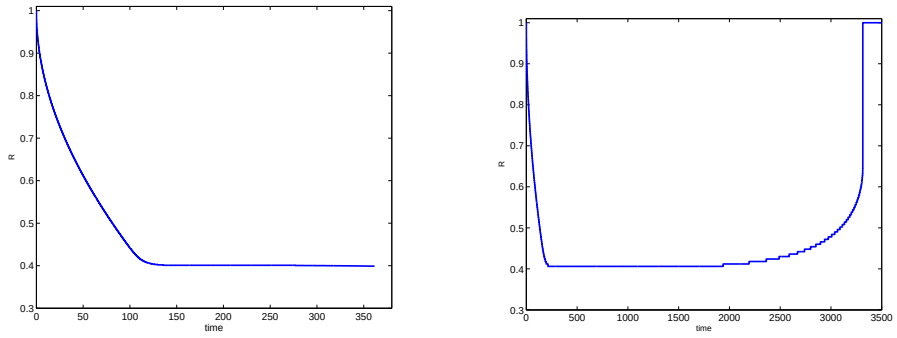

Figure 2: Dynamics of the stem cells front, obtained integrating numerically (1) and (6) with parameters as in Table (1), Péclet number $Pe = 0.1$ and initial stem cells concentration $u_0 = 0.8$ (left panel) and $u_0 = 0.01$ (right panel). Two different behaviors are shown: when $u_0 = 0.8$ (left panel) the front reaches the necrotic area and after some time one new cell layer is formed and the simulation is stopped; when $u_0 = 0.01$ (right panel) the front reaches the necrotic area but does not succeed in forming a new layer and is then cleared up.

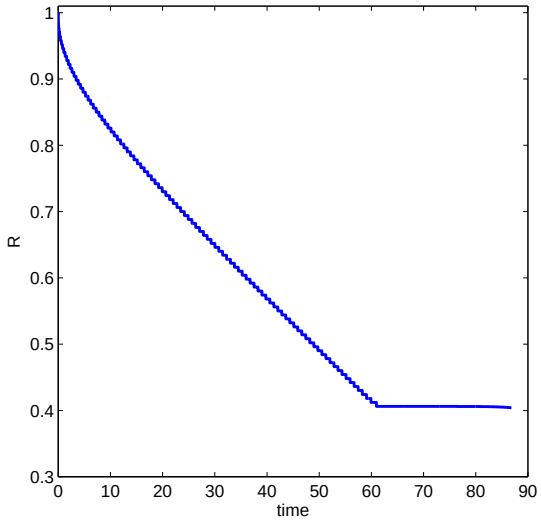

Figure 3: Dynamics of the stem cells front, obtained integrating numerically (1) and (6) with parameters as in Table (1), Péclet number $Pe = 20$ and initial stem cells concentration $u_0 = 0.1$. The stem cells front reaches the necrotic area and builds a new layer in 3/4 days.

cleared up by inflammatory mechanisms.
When instead we place a sufficient amount of stem cells (i. e., when u_0 is over a certain threshold) on the external boundary, one layer of new cells is formed. In the left panel of Fig. (2) this is clearly shown: the stem cells front reaches the necrotic area in approximately the same time of the case previously discussed, and after some time succeed in forming a new cell layer. Then the simulation is terminated.
Fig. (3) shows another simulation where we have chosen different values of the Péclet number Pe and of the initial condition $u0$ such that the stem cells front reaches the necrotic area and builds a new layer in 3/4 days.

6. Conclusions and perspectives

Mathematical models as the one considered in the present paper can highly help physicians to improve stem cell therapies for post infarction heart failure, in order to determine the number of stem cells which should be implanted close to the necrotic area and the position in which the cells should be injected. The proposed model can be considered a first step towards a more realistic representation of the action of stem cells on infarcted cardiac tissues. The numerical simulations show that the model, though simplified, is able to reproduce realistic behaviors, at least by a qualitative point of view. Let us remark that, in absence of experimental data, we have set arbitrary, but physiologically reasonable, values to some fundamental parameters of the model. More information on the experimental values of the unknown parameters will allow us to describe more realistic scenarios.
Our main goal in the forthcoming investigations will be to consider more research models, which could take into account more general domains, without any a priori symmetry hypothesis, different nutrient species, the effects of temperature, pH and other chemical or biological factors etc.

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References

[1] D. Lloyd–Jones, R.J. Adams, T.M. Brown, M. Carnethon, S. Dai, G. De Simone, T.B. Ferguson, E. Ford, K. Furie, C. Gillespie, A. Go, K. Greenlund, N. Haase, S. Hailpern, P. M. Ho, V. Howard, B. Kissela, S. Kittner, D. Lackland, L. Lisabeth, A. Marelli, M. M. McDermott, J. Meigs, D. Mozaffarian, M. Mussolino, G. Nichol, V. L. Roger, W. Rosamond, R. Sacco, P. Sorlie, R. Stafford, T. Thom, S. Wasserthiel-Smoller, N. D. Wong, & J. Wylie-Rosett, Heart disease and stroke statistics–2010 Update : A report from

the American Heart Association, *Circulation* **121** (2010), e46–e215.

[2] K. Dickstein, A. Cohen-Solal, G. Filippatos, J.J.V. McMurray, P. Ponikowski, P. A. Poole-Wilson, A. Strömberg, D. J. van Veldhuisen, D. Atar, A. W. Hoes, A. Keren, A. Mebazaa, M. Nieminen, S. G. Priori, & K. Swedberg, ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008. The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM), *European Heart Journal* **29** (2008), 2388-2442.

[3] N. Tran, P.R. Franken, F. Maskali, J. Nloga, P. Maureira, S. Poussier, F. Groubatch, C. Vanhove, J.P. Villemot, & P.Y. Marie, Intramyocardial implantation of bone marrow-derived stem cells enhances perfusion in chronic myocardial infarction: dependency on initial perfusion depth and follow-up assessed by gated pinhole SPECT, *J. Nucl. Med.* **48** (2007), 405-412.

[4] S. Zhou, Z. Cui & J.P.G. Urban, Nutrient gradients in engineered cartilage: metabolic kinetics measurement and mass transfer modeling, *Biotechnol. Bioeng.* **101** (2008), 408-421.

[5] R. Kumar, N. Panoskaltsis, F. Stepanek & A. Mantalaris, Coupled oxygen-carbon dioxide transport model for the human bone marrow, *Food and Bioproducts Processing* **86** (2008), 211-219.

[6] B.E. Strauer & R. Kornowski, Stem cell therapy in perspective, *Circulation* **107** (2003), 929-934.

[7] C.A. Chung, C.W. Yang & C.W. Chen, Analysis of cell growth and diffusion in a scaffold for cartilage tissue engineering, *Biotechnol. Bioeng.* **94** (2006), 1138-1146.

[8] C.A. Chung, C.W. Chen, C.P. Chen & C.S. Tseng, Enhancement of cell growth in tissue-engineering constructs under direct perfusion: modeling and simulation, *Biotechnol. Bioeng.* **97** (2007), 1603-1616.

[9] C.J. Galban & B.R. Locke, Analysis of cell growth kinetics and substrate diffusion in a polymer scaffold, *Biotechnol. Bioeng.* **65** (1999), 121-132.

[10] A. Visintin, *Models of Phase Transition*, volume 28 of *Progress in Nonlinear Differential Equations and Their Applications*, Birkhaüser, Boston, MA U.S.A., 1996.

[11] A. M. Meirmanov, *The Stefan Problem*, volume 3 of *de Gruyter Expositions in Mathematics*, Walter de Gruyter, Berlin, Germany, 1992.

[12] J. Monod, The growth of bacterial cultures, *Annu. Rev. Microbiol.* **3** (1949), 371-394.

[13] B. Obradovich, R. L. Carrier, G. Vunjak-Novakovic, & L. E. Freed, Gas exchange is essential for bioreactor cultivation of tissue engineered cartilage, *Biotechnol. Bioeng.* **63** (1999), 197-205.

[14] R. A. I. Windhaber, R. J. Wilkins, & D. Meredith, Functional characterisation of glucose transport in bovine articular chondrocytes, *Pfluerers Arch.* **446** (2003), 572-577.