Modeling Of Stem Cells Differentiation

Massimo Pisu[1], Alessandro Concas[1], Giacomo Cao[1,2,3*]

[1]CRS4 (Center for Advanced Studies, Research and Development in Sardinia), Parco Scientifico e Tecnologico POLARIS, Edificio 1, 09010 Pula, Cagliari, Italy
[2]Dipartimento di Ingegneria Chimica e Materiali, Università degli Studi di Cagliari, Piazza d'Armi, 09123 Cagliari, Italy
[3]Centro Interdipartimentale Metalli in Medicina, c/o Dipartimento di Scienze Chimiche, S.P. Monserrato - Sestu Km 0.700, 09042 Monserrato, Cagliari, Italy

1. Introduction

Stem cells represent a potential source of cells for transplantation since they have the ability to self-renew and differentiate into functional cells of various tissues. Adult stem cells may be obtained from tissues (liver, intestine, retina, skin, muscle, neural, mammary glands and others) of individual patients so that reimplantation of in vitro cultivated cells/tissues would avoid problems of rejection. Mesenchymal stem cells (MSC), also known as marrow stromal cells, which are progenitors of all connective tissue cells, can be on the other hand isolated using standard techniques, expanded in culture, and stimulated to differentiate into connective tissue cells. MSC may differentiate into specialized cells to form bone, cartilage, tendon, dermal, adipose, muscle tissues. When forming connective tissues, cells secrete macromolecules (collagen and proteoglycans mainly represented by glycosaminoglycan, GAG) which constitute the extra-cellular matrix (ECM). A very important role on cell differentiation is played by growth factors (GFs), which are proteins that bind to receptors on the cell surface, with the primary result of activating cellular proliferation and/or differentiation Several works are focused on experimental studies concerning the mesenchymal stem cell differentiation into chondrocytes stimulated by TGF-β superfamily (Barry et al., 2001; Bai et al., 2004). With the aim to facilitate experiments, thus helping to find the optimal operating conditions and at the same time contributing to the understanding of biological mechanisms and stem cell behaviour several contributions on the modeling of these systems have been performed starting from the stochastic model by Till et al. (1964) up to the recent work by Hentschel et al. (2004) which simulated the dynamic mechanisms for skeletal pattern formation in the vertebrate limb when growth factors (FGF, and TGF-βs) are used.

In the present work we propose a novel mathematical model to simulate stem cells differentiation into specialized cells. The model, is based upon material balances for extra cellular matrix compounds, growth factors and nutrients as well as mass-structured population balance to simulate cell growth, differentiation and proliferation in vivo or during in vitro cultivation (Pisu et al., 2007). Literature experimental data concerning the differentiation of mesenchymal stem cells into chondrocytes in terms of total DNA and GAG content are successfully compared with model results, thus demonstrating the

validity of the proposed model as well as its predictive capability. A further test of model capability is performed for the case of in vivo fracture healing during which mesenchymal stem cells differentiate into chondrocytes and osteoblasts.

2. Modeling

The mathematical model proposed in the present work is based on the cell differentiation pathway schematically shown in Figure 1. Stem cells (e.g. mesenchymes) may differentiate into specialized cells of type 1 (e.g. chondrocytes) under the influence of specific growth factors (e.g. BMP-2 and BMP-4). Stem cells may also differentiate into specialized cells of type 2 (e.g. osteoblasts) by means of a different class of growth factors (e.g. TGF-β1). These cells may be also obtained by the differentiation of specialized cells of type 1 under the influence of the same growth factor (e.g. TGF-β1). All the cells involved in the pathway shown in Figure 1 undergo mitosis and may synthesize two extra cellular matrix compounds, i.e. ECM1 (e.g. GAG) and ECM2 (e.g. collagen).

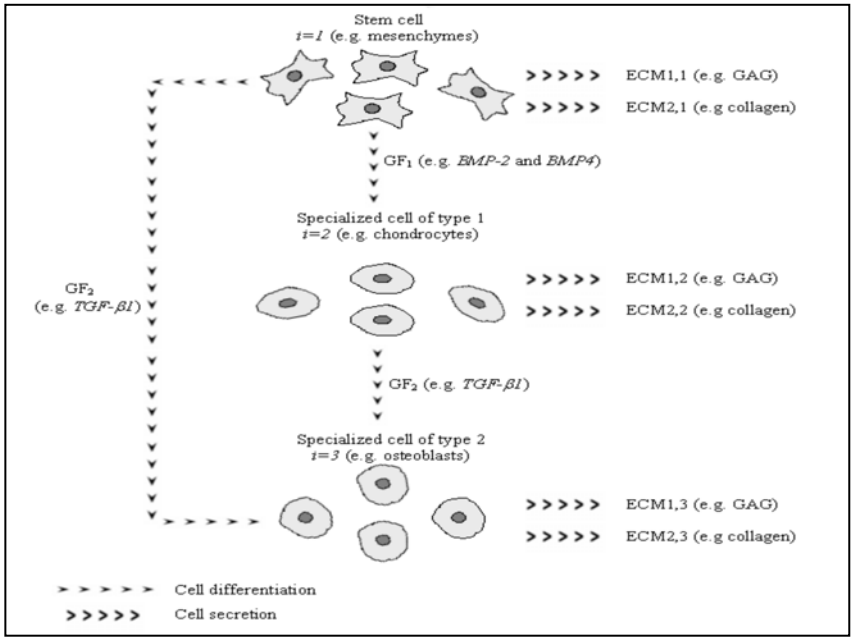

Figure 1. Schematic representation of the mathematical model.

To describe cell growth, proliferation and differentiation into intermediate or specialized cells during cell cultivation in the presence of culture medium and specific growth factors, the following mass structured population balance for the generic cell of i-th type may be written:

$$\frac{\partial \psi_i(m_i,t)}{\partial t}+\frac{\partial\left[v_i\psi_i(m_i,t)\right]}{\partial m_i}=2\int_m^{\infty}\psi_i(m'_i,t)\Gamma_i^F(m'_i,C_{O_2})\,p(m_i,m'_i)\,dm_i' \qquad (1)$$
$$-\psi_i(m_i,t)\Gamma_i^F(m_i,C_{O_2})+\psi_k(m_k,t)\Gamma_{kj}^T(m_k,C_{GF,j})-\psi_i(m_i,t)\Gamma_{ij}^T(m_i,C_{GF,j})$$

for i = 1,…,NC and k≠ i, along with:

$$\psi_i(m,\,t) = \psi_i{}^0(m,\,0) \qquad \text{for} \quad t = 0 \quad \text{and} \quad \forall\, m \tag{2}$$

$$\psi_i(m,t) = 0 \qquad \text{for} \quad t > 0 \quad \text{and} \quad m = 0 \tag{3}$$

where symbol's significance is reported elsewhere (Pisu et al., 2007) for the sake of brevity. Mitosis rate as determined by cell division rate, and cell growth as determined by rate of cell mass, appearing in equation (1), are expressed as follows:

$$\Gamma_i^F(m_i, C_{O_2}) = \frac{\nu_i(m_i, C_{O_2})\, f(m_i)}{1 - \int_0^m f(m'_i)\, dm'_i} \tag{4}$$

$$\nu_i(m_i, C_{O_2}) = \left(\frac{3}{d_{c,i}}\right)^{2/3} (4\,\pi)^{1/3}\, m_i^{\,2/3}\, \frac{\mu_i'\, C_{O_2}}{C_m + C_{O_2}} - \mu_{c,i} m_i \tag{5}$$

where

$$f(m) = \frac{1}{\sqrt{2\pi\sigma_i^{\,2}}} \exp \frac{-(m - \mu_{o,i})^2}{2\sigma_i^{\,2}} \tag{6}$$

$$p(m, m') = \frac{1}{\beta(q_i, q_i)} \frac{1}{m'} \left(\frac{m}{m'}\right)^{q_i - 1} \left(1 - \frac{m}{m'}\right)^{q_i - 1} \tag{7}$$

Then, the differentiation rate may be expressed as follows:

$$\Gamma_{ij}^T = \frac{a_{ij}\; C_{GF_j}}{b_{ij} + C_{GF_j}} \tag{8}$$

By assuming negligible mass transfer, population balance (1), along with initial (2) and boundary conditions (3) and equations (4)-(8) are coupled with the following material balances for GAG (ECM1) and collagen (ECM2):

$$\frac{\partial C_{ECM1,i}}{\partial t} = k_{ECM1,i} \left(\int_0^\infty m_i\; \psi_i(m_i, t)\, dm_i\right) \left(1 - \frac{C_{ECM1,i}}{C^L{}_{ECM1,i}}\right) C_{O_2} \quad \text{for } i = 1,\ldots,N_C \tag{9}$$

$$\frac{\partial C_{ECM2,i}}{\partial t} = k_{ECM2,i} \left(\int_0^\infty m_i\; \psi_i(m_i, t)\, dm_i\right) \left(1 - \frac{C_{ECM2,i}}{C^L{}_{ECM2,i}}\right) C_{O_2} \text{ for } i = 1,\ldots,N_C \tag{10}$$

Extracellular matrix, quantitatively described by equations (9)-(10), is secreted by the cell of the i-th type, whose differentiation is promoted by growth factor j. It should be noted that the reactive terms of equations (9)-(10), which describe ECM1 and ECM2 synthesis, respectively, are written in form of a product-inhibited kinetics, as discussed in previous works (Pisu et al., 2003; Pisu et al., 2004; Pisu et al., 2006). Growth factor concentration is simulated by the present model either in the case of in vitro (eq. 11a) or in the case of in vivo cultivation (eq. 11b) where an additional term should be introduced to account for the local growth factor production which is assumed to be proportional to the mass of cell of k-th type:

$$\frac{\partial C_{GF_j}}{\partial t} = -\sum_i \chi_{ij} \left(\int_0^\infty m_i \ \psi_i(m_i,t) dm_i \right) \frac{a_{ij} \ C_{GF_j}}{b_{ij} + C_{GF_j}} \qquad \text{for } j = 1,\ldots,N_{GF} \qquad (11a)$$

$$\frac{\partial C_{GF_j}}{\partial t} = -\sum_i \chi_{ij} \left(\int_0^\infty m_i \ \psi_i(m_i,t) dm_i \right) \frac{a_{ij} \ C_{GF_j}}{b_{ij} + C_{GF_j}} \quad \text{for } j=1,\ldots,N_{GF} \qquad (11b)$$
$$+ k^B_{GF_j} \sum_k \left(\int_0^\infty m_k \ \psi_k(m_k,t) dm_k \right)$$

Equation (11) holds the following initial conditions

$$C_{ECM1,i} = C^o_{ECM1,i}; \ C_{ECM2,i} = C^o_{ECM2,i} \ \text{ and } \ C_{GF_j} = C^o_{GF_j} \qquad \text{at } t = 0 \qquad (12)$$

Population balance (1)-(8), coupled with equations (9)-(12), represent a system of partial differential equations which is numerically solved as reported elsewhere (Pisu, et al., 2007). It should be noted that in the simulations we typically use a number of grid points in the mass domain equal to Nm = 30, since finer grids do not provide significant changes in the numerical solution.

3. Results and discussion

The mathematical model proposed in this work is compared with literature experimental by Barry et al. (2001) who investigated the in vitro chondrogenic differentiation of human mesenchymal stem cells (MSC) into chondrocyte cells (CC) by means of different growth factors (i.e., TGF-β1, TGF-β2 and TGF-β3). Model parameters used in these simulation run are reported elsewhere (Pisu et al., 2007). Figure 2a and 2b show a good agreement between model results and experimental data in terms of DNA and GAG content as a function of cultivation time. It is worth noting that the unknown model parameters, i.e. kinetic constants for GAG synthesis, appearing in equation (9) and parameters a_{11}, b_{11} and χ_{11} of equation (3) are estimated by means of a nonlinear least-square procedure against the experimental data. Analogous results were obtained when simulating experimental results performed with TGF-β2 or TGF-β3 as growth factor. A further test of the model capabilities is performed when simulating experimental data by Bai et al. (2004). These data refer to in vitro differentiation of human mesenchymal stem cell from bone marrow into chondrocyte cells in presence of TGF-β1 and/or CDMP-1 (cartilage-derived morphogenic protein-1). We simulate experimental data concerning GAG content after 21 days of cultivation as a function of different concentration of growth factor CDMP-1 (from 50 to 500 ng/ml) with a constant quantity of TGF-β1 (10 ng/ml). It should be noted that the unknown model parameters (i.e. kinetic constants for GAG synthesis, and parameters a_{11}, b_{11} and χ_{11}) were tuned to fit the GAG contents after 21 days of cultivation when a concentration of CDMP-1 equal to 100 ng/ml was used. Thus, by employing these parameters, the remaining experimental data were predicted by the model as shown in Figure 3. The agreement between model results and experimental data is satisfactory and confirms the predictive capability of the proposed model.

An interesting case to be investigated is represented by the in vivo fracture healing where multipotential stem cells may differentiate into specialized ones responsible for

producing the different tissue involved in the bone regeneration process (Bailon-Plaza and van der Meulen, 2001). By using the model parameters reported elsewhere (Pisu et al., 2007) a typical fracture healing is simulated in terms of cell content (MSC, CC, OC) as a function of time as illustrated in Figure 4. It should be noted that in order to account for the growth factors produced in vivo, equation (11b) has been used instead of equation (11a). Parameters a_{ij}, b_{ij} and χ_{ij} were tuned to simulate, qualitatively, the typical time evolution of the fracture healing in terms of MSC, CC, OC number per unit volume as a function of time. As properly simulated in Figure 4, mesenchymal stem cell content reduces to zero after about 6 days of fracture healing, while chondrocyte content displays a maximum after about 3-4 days of healing time. Osteoblast cells increase during the healing time and become predominant after to about 7-8 days. The main contribution of the proposed model with respect to the present state of the art is constituted by the application of mass-structured population balances which allows one to describe the cell distribution size of all cell type involved during the differentiation process. Cell growth, division, differentiation and extracellular matrix synthesis are strongly connected with the cell size and its distribution during the cultivation so that the quantitative description of these processes is related to cell size and distribution calculated through the proposed mass-structured population balance. Another important feature of the proposed model is its potential ability to simulate a variety of differentiation processes and transformation pathways (not necessarily related to connective tissues) for in vitro/in vivo cultivation. Nevertheless, in some case, as for example when simulating fracture healing, a spatial model would be more appropriate (two-dimensional or three-dimensional), since characteristic local processes (as for example the cell migration and callus replacement) may take place. A possible future direction of our work may concern the introduction of spatial coordinates in the model together with the description of mass transfer processes and migration which may involve cellular matrix compounds, growth factors and nutrients.

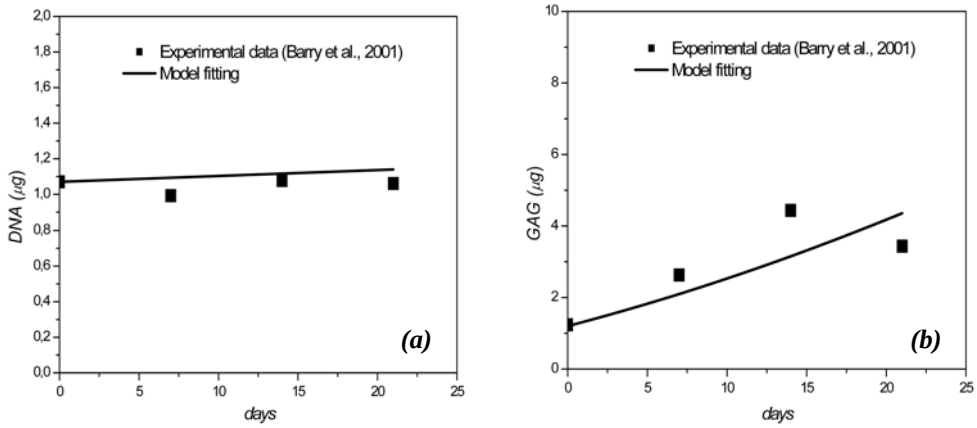

Figure 2. Comparison between model results and experimental data in terms of DNA (a) and GAG content (b) as a function of culture time for experiments carried out with TGF-β1 (Experimental data from Barry et al., 2001).

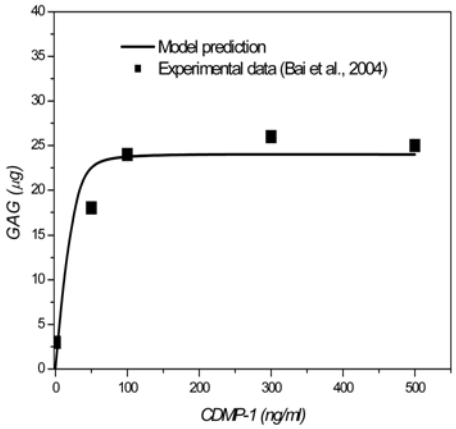

Figure 3. Model prediction in terms of GAG content after 21 days of cultivation as a function of different concentration levels of CDMP-1.

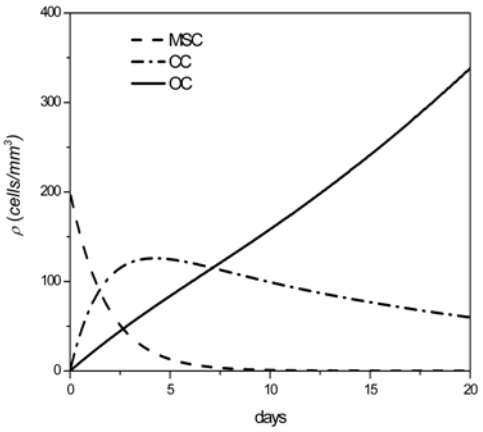

Figure 4. MSC, CC and OC number per unit volume as a function of fracture healing time.

4. Acknowledgements

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5. References

Bai, X., Xiao, Z., Pan, Y., Hu, J., Pohl, J., Wen, J. and L. Li, 2004, Biochemical and Biophysical Research Communications, 325, 453.

Bailon-Plaza, A. and M.C.H. van der Meulen, 2001, Journal of Theoretical Biology, 212, 191.

Barry, F., Boynton, R.E., Liu, B. and J.M. Murphy, 2001, Experimental Cell Research, 268, 189.

Hentschel, H.G.E., Glimm, T., Glazier, J.A. and S.A. Newman, 2004, Proc. R. Soc. Lond. B. 271, 1713.

Pisu, M., Lai, N., Cincotti, A., Delogu, F. and G. Cao, 2003, International Journal of Chemical Reactor Engineering, http://www.bepress.com/ijcre/vol1/A38.

Pisu, M., Lai, N., Cincotti, A., Concas, A. and G. Cao, 2004, Chemical Engineering Science, 59, 5035.

Pisu, M., A., Concas, A., Lai, N. and G. Cao, 2006, Tissue Engineering, 12, 2311.

Pisu, M., A., Concas, A. and G. Cao, 2007, Journal of Biotechnology, submitted.

Till, J.E., McCulloch, E.A. and L. Siminovitch, 1964, Proceedings of the National Academy of Sciences, USA, 51, 29.