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A simulation model for stem cells differentiation into specialized cells of non-connective tissues

Massimo Pisu[a], Alessandro Concas[a], Sarah Fadda[b], Alberto Cincotti[b], Giacomo Cao[a,b,*]

[a] CRS4 (Center for Advanced Studies, Research and Development in Sardinia), Località Piscinamanna, Edificio 1, 09010 Pula, Cagliari, Italy
[b] Dipartimento di Ingegneria Chimica e Materiali, Università degli Studi di Cagliari, Piazza d'Armi, 09123 Cagliari, Italy

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ABSTRACT

A novel mathematical model to simulate stem cells differentiation into specialized cells of non-connective tissues is proposed. The model is based upon material balances for growth factors coupled with a mass-structured population balance describing cell growth, proliferation and differentiation. The proposed model is written in a general form and it may be used to simulate a generic cell differentiation pathway during *in vitro* cultivation when specific growth factors are used. Literature experimental data concerning the differentiation of central nervous stem cells into astrocytes are successfully compared with model results, thus demonstrating the validity of the proposed model as well as its predictive capability. Finally, sensitivity analysis of model parameters is also performed in order to clarify what mechanisms most strongly influence differentiation and cell types distribution.

1. Introduction

Stem cell therapies based on the differentiation of adult or embryonic cells into specialized ones appear to be an effective method in the treatment of human diseases (Henningson et al., 2003; Baksh et al., 2004). Stem cells, the progenitors of all body tissues, have the remarkable and critical abilities to exist *in vivo* in a quiescent, undifferentiated state and to propagate. They are characterized initially as being totipotent, pluripotent, or multipotent (Henningson et al., 2003). These cells may differentiate into functional cells of various tissues (Baksh et al., 2004; Khademhosseini and Zandstra, 2004; Henningson et al., 2003). Since the proliferative capacity of many adult tissue-specific cells is very limited, thus making difficult their *in vitro* expansion, current research is focused on the use of stem cells instead of tissue-specific cells (Kuo and Tuan, 2003). Today, adult stem cell therapies are very promising in medicine, even if the diseases they are used to treat are limited to very specific types of disorders. Autologous stem cells may be obtained from tissues (skin, muscle, retina, neural, liver, intestine, mammary glands and others) of individual patients so that reimplantation of *in vitro* cultivated cells/tissues would avoid rejection problems. A wide range of diseases may be potentially treated by using totipotent embryonic stem cells which are object of ethic debates and discussions (Macklin, 2000; Moore et al., 2006).

A crucial role for cell differentiation is played by growth factors (GFs), which generally are proteins that bind to receptors on the cell surface, with the primary result of activating cellular proliferation and/or differentiation. Several growth factors are quite versatile, stimulating cellular division in numerous different cell types, while others are specific to a particular cell type. In this context the well-known *TGF-β* superfamily plays an important role in the development of cells. This family comprises a variety of growth factors that feature different functions in stem cells biology. These factors regulate both "stemness" and various cell differentiation pathways (Pucéat, 2007). Despite intensive research work, the role of this family of growth factors in stem cells differentiation is still unclear (Pucéat, 2007). Further studies are required since the effect of this type of growth factors are in some cases contradictory and mechanisms concerning cell proliferation/differentiation as well as the interaction with growth factors need to be elucidated.

An important contribution along these lines may be provided by predictive models which should facilitate the experiments, thus helping researchers to find optimal operating conditions and at the same time contributing to the understanding of biological mechanisms and stem cell behavior. For this reason several papers on modeling stem cell proliferation/differentiation are available in the literature starting with the stochastic model proposed by Till et al. (1964). A remarkable attempt to simulate cell differentiation for ex vivo hematopoiesis in the presence of *TGF-β*1 was done by Nielsen et al. (1998). These authors developed a mathematical

* Corresponding author at: Dipartimento di Ingegneria Chimica e Materiali, Università degli Studi di Cagliari, Piazza d'Armi, 09123 Cagliari, Italy.
Tel.: +39 070 675 5058; fax: +39 070 675 5057.
E-mail address: cao@visnu.dicm.unica.it (G. Cao).

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Nomenclature

a	parameter appearing in Eqs. (13) and (14) (1/h)
b	parameter appearing in Eqs. (13) and (14) (ng/mm^3)
C_{GF}	concentration of growth factor (ng/mm^3)
C_{O_2}	concentration of O_2 at saturation condition (mmol/mm^3)
C_m	oxygen concentration at half-maximal consumption (mmol/mm^3)
d	mass density (ng/mm^3)
$f(m)$	division probability density function (1/ng)
m	single cell mass (ng)
N_C	number of cell type
N_{GF}	number of growth factors
N_m	number of grid points for the mass domain
n	cell number
n_t	total cell number
p	partitioning function
q	coefficient appearing in symmetric beta function
t	cultivation time (h)
V	total cultivation volume (mm^3)

Greek letters

$\beta(q,q)$	symmetric beta function
$\Gamma(q)$	gamma function
Γ^F	division rate function (1/h)
Γ^T	differentiation rate function (1/h)
μ_0	average mass of dividing cells (ng)
μ_c	catabolic rate (1/h)
μ'	maximum rate of cell growth (ng/(mm^2 h))
ν	time rate of change of cell mass m (ng/h)
σ	standard deviation of the Gaussian distribution (ng)
χ	yield appearing in Eq. (14) (ng of GF/ng of cells)
ψ	cell distribution function (cells/(ng mm^3))

Superscripts

0	initial conditions
$'$	mother cell

Subscripts

c	cells
GF	growth factor
i	ith cell type
j	jth growth factor
k	kth cell type
O_2	oxygen

model based on population balance which simulates hematopoiesis starting from a colony of hematopoietic stem cell. Nielsen et al. (1998) use a tank and tubular reactor metaphor to describe the (pseudo)-stochastic and deterministic elements of hematopoiesis. More recently, Bailon-Plaza and van der Meulen (2001) proposed a two-dimensional mathematical model to describe the effect of growth factor on fracture healing. The model simulates the differentiation of mesenchymal stem cells into chondrocytes and osteoblasts during a fracture healing by accounting for material balance of the involved cells (mesenchymal stem cells, chondrocyte and osteoblast cells) coupled with a material balance on the extracellular matrix (ECM) components and growth factors. Hentschel et al. (2004) proposed a mathematical model which simulates the dynamic mechanisms for skeletal pattern formation in the vertebrate limb when fibroblast growth factor, *FGF*, and *TGF-βs* are present. Beside the works considered above, other authors have addressed the simulation of cell differentiation. For the sake of brevity, a list of the most interesting models available in the literature on this subject is summarized in Table 1. The main limitation of these models is represented by the absence of the description of the cell size distribution and its influence on cellular metabolism and differentiation. With the aim to improve these models a novel mathematic approach to simulate *in vivo* or *in vitro* stem cell differentiation into specialized cells of connective tissues where cells may grow, differentiate and synthesize components of the extracellular matrix (ECM) has been developed by Pisu et al. (2007). The model, which may also describe cell mass distribution, was validated by comparison with available literature experimental data concerning the differentiation of mesenchymal stem cells into chondrocytes.

Table 1
Models concerning the simulation of cell differentiation processes

Model	Reference
Stochastic model of stem cell proliferation for spleen colony-forming cells	Till et al. (1964)
Model of differentiation processes in the thymus	Mehr et al. (1995)
Stochastic model of brain cell differentiation	Yu et al. (1998)
Pseudo-stochastic and deterministic model for ex vivo hematopoiesis	Nielsen et al. (1998)
Stochastic model of proliferation and differentiation of O_2-A progenitor cells	Zorin et al. (2000)
Deterministic two-dimensional model of fracture healing	Bailon-Plaza and van der Meulen (2001)
Dynamic mechanisms for skeletal pattern formation in the vertebrate limb when the growth factors are present	Hentschel et al. (2004)
Dynamic model for cellular differentiation and co-expression properties of switch networks	Cinquin and Demongeot (2005)
Mathematical model for the interaction of transcription factors	Roeder and Glauche (2006)

In the present work we propose a mathematical model to simulate stem cells differentiation into specialized cells of non-connective tissues. The model, along the lines of our previous contributions (Pisu et al., 2003, 2004, 2006, 2007), is based upon material balances for growth factors coupled with a mass-structured population balance to simulate cell growth, differentiation and proliferation *in vivo* or during *in vitro* cultivation. The proposed model is written in a general form and may be used to describe a generic cell differentiation pathway occurring during *in vitro* cultivation of non-connective tissues. Literature experimental data concerning the differentiation of murine central nervous stem cells into astrocytes are successfully compared with model results, thus demonstrating the validity of the proposed model as well as its predictive capability. Finally, sensitivity analysis of model parameters is also performed in order to clarify what mechanisms most strongly influence differentiation and the distribution of cell types.

This approach may be followed to guide the investigation on cell cultivation, growth and differentiation for stem cell therapies or to describe different pathologies involving cell growth and differentiation (i.e., tumors development, infections, etc.).

2. Mathematical modeling

The mathematical model proposed in the present work accounts for the cell generic differentiation pathway schematically shown in Fig. 1. Stem cells (e.g. central nervous system stem cells) may differentiate into specialized cells of type 1 (e.g. astrocytes, $i=2$) under the influence of one or more specific growth factors, GF_1 and GF_2. Stem cells may also differentiate into specialized cells of