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A novel simulation model for stem cells differentiation

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Abstract

A novel mathematical model to simulate mesenchymal stem cells differentiation into specialized cells is proposed. The model is based upon material balances for extracellular matrix compounds, growth factors and nutrients 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 occurring *in vivo* or during *in vitro* cultivation when specific growth factors are used. Literature experimental data concerning the differentiation of mesenchymal stem cells into chondrocytes in terms of total DNA and glycosaminoglycan 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 the model capability is performed for the case of *in vivo* fracture healing during which mesenchymal stem cells differentiate into chondrocytes and osteoblasts. Considerations about the extension of the proposed model to different pathologies beside fracture healing are reported. 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.

Keywords: Simulation model; Stem cell differentiation; Population balance; Growth factor; Extracellular matrix

1. Introduction

Stem cells are currently receiving a great attention since they represent a potential source of cells for transplantation. In fact these cells have the ability to self-renew and differentiate into functional cells of various tissues (Baksh et al., 2004; Khademhosseini and Zandstra, 2004). Proliferative capacity of many adult tissue-specific cells is very limited, making their expansion *in vitro* difficult, even during long-term cultivation which in addition reduces their functional quality. Thus, attention is currently devoted to the use of stem cells or progenitor ones instead of tissue-specific cells (Kuo and Tuan, 2003). 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. Despite this very attractive opportunity, the use of adult stem cells is limited by the ability to identify these rare cells from the heterogeneous tissue population and to expand them *in vitro* (Khademhosseini and Zandstra, 2004). 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 (Caplan and Bruder, 2001; Barry et al., 2001). MSC may differentiate into specialized cells to form bone, cartilage, tendon, dermal, adipose and muscle tissues. In addition, MSC may differentiate into hepatic, renal, cardiac and neural stem cells (Alhadlaq and Mao, 2004). These properties open up therapeutic opportunities for the treatment of lesions in mesenchymal tissues and protocols have been devised for the treatment of defects in articular cartilage, bone, tendon, meniscous and for bone marrow stromal recovery and osteogenesis imperfecta (Barry et al., 2001). When forming connective tissues, cells secrete macromolecules (collagen and proteoglycans mainly represented by glycosaminoglycan, GAG) which constitute the extracellular matrix (ECM). The latter provides an

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Nomenclature

a	parameter appearing in Eqs. (8) and (11) (h^{-1})
b	parameter appearing in Eqs. (8) and (11) (ng/mm^3)
C_{ECM1}	concentration of GAG (dw%)
C_{ECM2}	concentration of collagen (dw%)
C_{GF}	concentration of growth factor (ng/mm^3)
C_m	oxygen concentration at half-maximal consumption ($mmol/mm^3$)
C_{O_2}	concentration of O_2 at saturation condition ($mmol/mm^3$)
C^L	maximum collagen or GAG concentration (dw%)
d	mass density (ng/mm^3)
$f(m)$	division probability density function
k_{ECM1}	rate constant for GAG synthesis (dw% mm^6/(ng mmol h))
k_{ECM2}	rate constant for collagen synthesis (dw% mm^6/(ng mmol h))
k_{GF}^B	rate constant for *in vivo* GF synthesis (ng of GF/(ng of cells h))
m	single cell mass (ng)
N_m	number of grid points for the mass domain
N_C	number of cell type
N_{GF}	number of growth factors
p	partitioning function
q	coefficient appearing in symmetric beta function
t	cultivation time (h)

Greek letters

$\beta(q, q)$	symmetric beta function
Γ^F	division rate function (h^{-1})
Γ^T	differentiation rate function (h^{-1})
μ_c	catabolic rate (h^{-1})
μ_0	average mass of dividing cells (ng)
μ'	maximum rate of cell growth ($ng/(mm^2\,h)$)
ν	time rate of change of cell mass m (ng/h)
ρ	cell number per unit volume ($cells/mm^3$)
ρ^0	initial cell number per unit volume ($cells/mm^3$ or cells/pellets)
σ	standard deviation of the Gaussian distribution (ng)
χ	yield appearing in Eq. (11) (ng of GF/ng of cells)
ψ	cell distribution function ($cells/(ng\,mm^3)$)

Subscripts

c	cells
ECM1	component of ECM, glycosaminoglycan (GAG)
ECM2	component of ECM, collagen
GF	growth factor
i	ith cell type
j	jth growth factor
k	kth cell type
O_2	oxygen
w	water

Superscripts

0	initial conditions
$'$	mother cell

organized environment within which migratory cells can move and interact with one another and stationary cells are anchored (Kuo and Tuan, 2003). Recent studies suggested that the use of three-dimensional scaffold may improve, quantitatively, the differentiation of stem cells (Taqvi and Roy, 2006; Willerth et al., 2006). These investigations are focused on the identification of the more suitable cultivation scaffolds and the optimization of the mechanical and physical properties of such materials. 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. Many growth factors are quite versatile, stimulating cellular division in numerous different cell types, while others are specific to a particular cell-type. Among the various growth factors, bone morphogenetic protein (BMP) which belongs to the transforming growth factor-β (TGF-β) superfamily, plays an important role in the regulation of the differentiation pathway of MSC (Yamaguchi, 1995). In particular, these growth factors may induce the differentiation of MSC into connective cells such as osteoblasts, chondrocytes and adipocytes (Yamaguchi, 1995). Many papers are focused on experimental studies concerning the mesenchymal stem cell differentiation into chondrocytes stimulated by TGF-β superfamily (Johnstone et al., 1998; Barry et al., 2001; Worster et al., 2001; Bai et al., 2004; Bosnakovski et al., 2004; Tsuchiya et al., 2004; Chen et al., 2005; Li et al., 2005; Mauch et al., 2006). Further studies are required since the effect of this type of growth factors are in some cases contradictory and mechanisms concerning cell proliferation/differentiation and 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 to find the optimal operating conditions and at the same time contributing to the understanding of biological mechanisms and stem cell behavior. For this reason several contributions on the modeling of these systems are available starting with the stochastic model of stem cell proliferation proposed by Till et al. (1964). More recently, a remarkable attempt to simulate cell differentiation for *ex vivo* hematopoiesis in the presence of TGF-β1 is done by Nielsen et al. (1998). These authors developed a mathematical model based on population balance which describes the hematopoiesis starting from a colony of hematopoietic stem cell. The model uses a tank and tubular reactor metaphor to describe the (pseudo)-stochastic and deterministic elements of hematopoiesis. Bailon-Plaza and van der Meulen (2001) proposed a two-dimensional mathematical model for simulating the effect of growth factor on fracture healing. The model describes the differentiation of mesenchymal stem cells into chondrocytes and osteoblasts during a fracture healing by accounting for material balance of