CHEMICAL ENGINEERING TRANSACTIONS Volume 24, 2011
Editor Sauro Pierucci
 ISBN 978-88-95608-15-0 ISSN 1974-9791
DOI: 10.3303/CET1124168

A Population Balance Model to Investigate the Effect of Microgravity on the Kinetics of *in Vitro* Cell Proliferation

Sarah Fadda[1], Alberto Cincotti[1*], Giacomo Cao[1,2 *]

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

In this work a mathematical model helpful to investigate the effects of microgravity on the kinetics of *in vitro* proliferation of adherent cells is proposed. The model is based on a Population Balance (PB) approach that allows to describe cell cycle progression through the different phases experienced by any cell of the entire population during its own life. Specifically, the proposed model has been developed as a multi-staged 2-D PB, by considering a different sub-population of cells for any single phase of the cell cycle. These sub-populations composing the entire population of cells of the cultivation system are discriminated through cellular volume and DNA content, that both increase during the mitotic cycle. A series of numerical simulations related to the *in vitro* proliferation kinetics of adherent cells is here reported for illustrating model capabilities. It is found that, the change of one adjustable parameter related to cell volume growth rate in response to a change of gravity is able to mimic cell culture behaviour under microgravity conditions, as reported in the technical literature.

1. Introduction – State of the art of microgravity effects on cell culturing

When the history of space biology started in the fifties, research was characterised by the so-called 'fishing experiment', that is the investigation, without a specific working hypothesis, of the behaviour in space of an organism. The rationale for such studies was the scientific curiosity to expose living systems to an environment never experienced before during the evolution and not reproducible on Earth. More recently the attention has been focused moving towards the *in vitro* study of basic cellular processes as well as the cell culture behaviour. Specifically, research during the last twenty years demonstrated unequivocally that gravity (hyper- or micro-gravity) affects cells in culture. It is now widely recognized that gravity should be regarded as one of the operative conditions (just like temperature or pH) that need to be taken into account when investigating cell cultures, so much so that cellular machinery may be better studied and understood also by changing gravity level (Schmitt, 1999). Even if the analysis of the technical literature may look dispersive at first sight, a conclusive point may be reached anyway: generally speaking, the main effect of microgravity consists of the inhibition of cell cycle kinetics and the decrease of expansion rate, for suspended cells as well as for adherent cells (cf. Hughes-Fulford, 2003; Coinu *et al.* , 2006).

Please cite this article as: Fadda S., Cincotti A. and Cao G., 2011, A population balance model to investigate the effect of microgravity on the kinetics of in-vitro cell proliferation, Chemical Engineering Transactions, 24, 1003-1008
DOI: 10.3303/CET1124168

However, several questions still remain open also because a systematic experimental campaign has never been conducted, for economical reasons. That's why the scientific literature on this specific subject is limited, and frequently the experimental runs are not really conducted during a space flight, but are actually ground-based with "simulated microgravity" attained in special machines (van Loon, 2007). Moreover, the studies on the slowing-down effect of proliferation kinetics as gravity is reduced are conducted by performing experimental runs at different operative conditions (i.e. different cell lineages and seeding densities, different sampling times for kinetic measurements) analysed through different characterization techniques by focusing on different aspects of cellular metabolism and typically from a qualitative perspective, only.
In this picture, a mathematical model able to interpret experimental data from a quantitative point of view may represent a helpful tool to shed some light on the complex mechanisms that rule the biological behaviour of a *in vitro* cell cultivation system under microgravity conditions. In addition, the carrying out of the experimental campaign on such complex (i.e. highly non linear) system could be supported by the effective simulation of its behavior. On the basis of these considerations, in this article a population balance model to investigate the effects of microgravity on the kinetics of *in vitro* proliferation of adherent cells is developed. The proposed model is based on a multi-staged Population Balance (PB) approach that allows to describe cell cycle progression, taking into account both volume and DNA content evolution (2-D) during the life of a population of cycling cells.

2. Modeling section

The mathematical model proposed in this work describes cellular growth and proliferation (expansion by mitosis) during *in vitro* cultivation in a batch system (i.e. mono-layer adherent cells on Petri dish) by addressing the dynamic behaviour of three subpopulation of cells, i.e. the cells belonging to the three stages of the classic cell cycle life ($G1 \Rightarrow S \Rightarrow G2/M$). During the expansion of a cell population two main phenomena take place: proliferation (i.e. increase in cell number) and maturation (i.e. increase in cell size and DNA content). Specifically, in the proposed model cell size growth is assumed to occur throughout the entire cycle life, i.e. in all the three stages considered in this work (G1, S, G2/M). On the other hand, DNA increases only during the S phase, which separates the "young" cells (daughters) with a minimum DNA level and belonging to the G1 phase, from the "old" cells (mothers) with a doubled DNA content and belonging to the G2/M phase, ready to split at mitosis into two new G1 cells. In this work, only relatively short cultivation times are taken into account, thus avoiding to describe cell culture behavior when reaching full confluence, i.e. when the limited surface area of the Petri dish is completely occupied, while proliferation stops because of contact inhibition. The Population Balance (PB) Equations for the three different stages (G1, S, G2/M) are reported in Table 1 along with the corresponding boundary conditions. $n^P(v,t)$ represents the number concentration density distribution of cells in the generic phase P (= G1, G2/M) with volume v at time t, which is assumed to be distributed spatially uniform on the Petri dish. On the other hand, the cells of

phase S are represented by a 2D distribution, $\bar{n}^{S}(v,x,t)$, thus depending not only on volume v, but also on DNA content x (i.e. 2 internal coordinates), as well as on time t.

Table 1: Model Equations

Population Balances

$$\frac{\partial n^{G1}(v,t)}{\partial t}+\frac{\partial\left(r_v^{G1}(v)\cdot n^{G1}(v,t)\right)}{\partial v}= \quad (1)$$
$$2\cdot\int_{v}^{v_{\max}} p^{G2/M}(v,v')\cdot\Gamma^{G2/M}(v')\cdot n^{G2/M}(v',t)\,dv'-\Gamma^{G1}(v)\cdot n^{G1}(v,t)$$

$$n^{G1}(v_{\min},t)=0 \quad for \quad v=v_{\min} \quad and \quad t>0 \quad (2)$$

$$\frac{\partial\bar{n}^{S}(v,x,t)}{\partial t}+\frac{\partial\left(r_v^{S}(v)\cdot\bar{n}^{S}(v,x,t)\right)}{\partial v}+\frac{\partial\left(r_x^{S}(v)\cdot\bar{n}^{S}(v,x,t)\right)}{\partial x}=0 \quad (3)$$

$$\bar{n}^{S}(v_{\min},x,t)=0 \quad for \quad v=v_{\min} \quad and \quad \forall x;t>0 \quad (4)$$

$$r_x^{S}(v)\cdot\bar{n}^{S}(v,1,t)=\Gamma^{G1}(v)\cdot n^{G1}(v,t) \quad for \quad x=1 \quad and \quad \forall v;t>0 \quad (5)$$

$$\frac{\partial n^{G2/M}(v,t)}{\partial t}+\frac{\partial\left(r_v^{G2/M}(v)\cdot n^{G2/M}(v,t)\right)}{\partial v}= \quad (6)$$
$$-\Gamma^{G2/M}(v)\cdot n^{G2/M}(v,t)+r_x^{S}(v)\cdot n^{S}(v,2,t)$$

$$n^{G2/M}(v_{\min},t)=0 \quad for \quad v=v_{\min} \quad and \quad t>0 \quad (7)$$

Partitioning function, Transition rates and growth rates

$$p^{G2/;M}(v,v')=\frac{1}{\beta(q,q)}\frac{1}{v'}\left(\frac{v}{v'}\right)^{q-1}\left(1-\frac{v}{v'}\right)^{q-1} \quad (8)$$

with symmetrical beta function for $\beta(q,q)$ and gamma function for $\Gamma(q)$

$$\Gamma^{P}(v)=r_v^{P}(v)\cdot\gamma^{P\to P+1}(v) \quad for \quad P=G1,G2/M \quad and \quad P+1=S,G1 \quad (9)$$

with $\gamma^{P\to P+1}(v)=\dfrac{f^{P\to P+1}(v)}{1-\int_{0}^{v} f^{P\to P+1}(v')dv'}$ and $f^{P\to P+1}(v)=\dfrac{k}{\lambda^{k}}\cdot v^{k-1}\exp\left(-\left(\dfrac{v}{\lambda}\right)^{k}\right)$

$$r_v^{P}=k_v\cdot v \qquad for \quad P=G1,S,G2/M \quad (10)$$

$$r_x^{S}=k_S\cdot v \quad (11)$$

Evidently, cells in G1 and G2/M do not show a distribution of DNA relative content, which is equal to 1 and 2, correspondingly. In Table 1, the partitioning function, the transition rates and the growth rates adopted are also reported. Specifically, a Weibull distribution function (characterized by two adjustable parameters k an λ, the shape and scale factors, respectively) is used to describe the cell volume condition that rules the transitions of cells G1→S and G2/M→G1. This choice is related to the assumption of statistical transitions, i.e. these transitions do not occur only at a precise critical

volume threshold (λ), but around it. Thus, it is recognized that, not only volume, but also other unspecified (i.e. uncertain and not easily measurable) aspects of cell metabolism are actually involved in these transitions. On the other hand, the transition S→G2/M is assumed to be fully deterministic, as represented by the second term on right hand side of Equation (6). Specifically, all cells (i.e. whatever volumic size they have reached) entering S phase, as coming from G1 phase, possess a relative DNA content equal to 1, which necessarily has to reach a doubled value before transiting to G2/M phase (i.e. whatever volumic size they reach in the meanwhile). Indeed, during S phase a volumic growth takes place simultaneously to DNA content increase, but only DNA relative content rules the progression inside S phase and the transition to G2/M phase, thus following a deterministic path.

3. Results and discussion

The partial integro-differential-equations (1), (3) and (6) in Table 1 have been numerically treated by means of the method of lines. For some simulations performed in this work the selected initial cell distribution is a normal (Gaussian) distribution for G1 phase cells, $n^{G1}(v,0) = \left[A/\left(\sqrt{0.5\cdot\pi}\cdot\sigma\right)\right]\cdot\exp\left\{-0.5\cdot\left[(v-\mu)/\sigma\right]^2\right\}$, characterized by a mean value (μ) and a standard deviation (σ^2), while the other phases are assumed to be empty (i.e. $n^S(v,x,0) = n^{G2/M}(v,0) = 0 \quad \forall v \quad ; \quad \forall x$). This choice allows one to better describe model capabilities and features, since it enhances the dynamical behavior of the sub-populations considered in this work. The values of the model parameters used during the simulations are reported in Table 2, while model results are reported in Figure 1. Specifically, in the left column plots the temporal evolution (up to 120 hours of cultivation time) of the number percentages of cells falling into the different phases is reported in Figure 1a, while the corresponding dynamic behaviour of the normalized volumic distribution of the entire cell population, is given in Figure 1b. It is clearly shown that, the cell number percentages in different phases change (basically, G1 cells decrease while S and G2/M cells increase, being these latter ones formed later than S cells), till constant values are finally reached at about 48 hours of cultivation times. During this time, the total count of cells (also reported in Figure 1a) increases, at least after the very first 8 hours, when G2/M cells mature enough may eventually divide. On the other hand, the evolution of the normalized volumic distribution of the entire cell population reported in Figure 1b for different cultivation times shows that, after an initial transient period of about 48 hours, during which the size distribution grows towards larger volumes, a stationary condition (i.e. the so-called “balanced growth”) is finally reached. In the right column plots of Figure 1, the corresponding model results in terms of cell distributions in the different phases are given at 8 hours of cultivation times, when cells are present in any phase. The volumic distributions for G1 (cf. Figure 1c) and G2/M phases (cf. Figure 1e) are shown along with the corresponding transition rate towards the subsequent phases (dashed lines). The volumic and DNA relative content distributions of cells in S phase are reported as 2-D contour-plots in Figure 1d. The initial condition consisting of a population of cells synchronized in G1 phase is also reported as a red line in Figure 1c. Since the PBE approach is characterized by a

significant number of model parameters, the effect of every single adjustable parameter has been evaluated by performing a sensitivity analysis, whose results are not shown in this work for sake of brevity.

Table 2: Model parameters

Parameter	Value	Unit	Parameter	Value	Unit
A	1.6×10^{4}	-	k_S	8.51×10^{-5}	$\mu m^{-3}\cdot h^{-1}$
μ	15.54	μm	$k_{G1\to S}$	7.8	μm^3
σ	2.13	μm	$k_{G2/M\to G1}$	3.2	μm^3
q	40	-	$\lambda_{G1\to S}$	1.78×10^{3}	μm^3
k_v	0.035	h^{-1}	$\lambda_{G2/M\to G1}$	2.74×10^{3}	μm^3

A significant sensitivity of the model output is found for the proportionality constant (k_v) of the cell volume growth rate. Specifically, by decreasing k_v the apparent doubling time increases (i.e. proliferation kinetics slows down), while proportions at balanced growth among cell numbers in different phases change (i.e. increase for G1 and G2/M phases and decrease for S phase, as shown in Table 3 for two different initial distributions of cells in the phases).

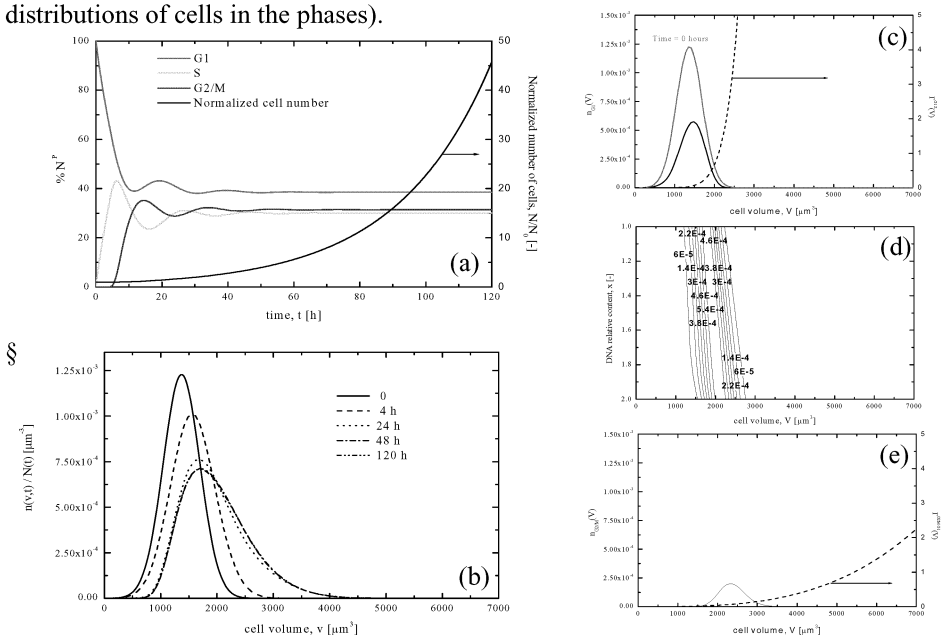

Figure 1: Model results

This system behavior is due to maturation in G1 and G2/M phases that is controlled by volume, while DNA relative content rules the maturation in S phase. Thus, if k_v is reduced basically only the growth in G1 and G2/M phases decrease, leaving that one of the S phase unchanged. The final result is that the cells tend to accumulate in G1 and

G2/M phases, which become the limiting steps in the maturation process along the cell cycle depicted in this work.

Table 3: Model results when varying k_v

	Initial condition	Final condition ("balanced growth")	
		$k_v = 0.0035\ h^{-1}$	$k_v = 0.00175\ h^{-1}$
G1 %	100	40.983	88.467
S %	0	30.162	3.043
G2/M	0	28.855	8.490
G1 %	38.476	38.476	40.437
S %	30.071	30.071	1.987
G2/M	31.473	31.473	57.576

These theoretical predictions perfectly matches (even if only from a qualitative viewpoint) the experimental measurements reported in the literature by Coinu *et al.* (2006) who showed that adherent cells like human breast cancer cells may grow up to one or the other of the two arrests during their life cycle depending on the operative conditions adopted. Specifically, under microgravity conditions after synchronization in G1/G0 through starvation, human breast cancer cells do not grow beyond G1 phase, while cells accumulate in G2/M phase if g is reduced without synchronization.

4. Conclusions

The model proposed and developed in this work is suitable to interpret/simulate the experimental data corresponding to the effect of microgravity on the kinetics of *in-vitro* cell proliferation. The effect of gravity, and, more specifically, the slowing down of cultivation kinetics at microgravity conditions, may be investigated by analyzing the change of the more influencing adjustable parameters of the proposed model (k_v) when gravity changes. As a consequence, these model parameters should be evaluated at different g levels through fitting procedures (i.e. by comparisons between model results and experimental data), in order to identify a phenomenological equation able to track the change of model parameters as a function of gravity level.

References

Coinu R., Chiaviello A., Galleri G., Franconi F., Crescenzi E., Palumbo G., 2006, Exposure to modeled microgravity induces metabolic idleness in malignant human MCF-7 and normal murine VSMC cells, FEBS Letters, 580, 2465-2470.

Hughes-Fulford M., 2003, Function of the cytoskeleton in gravisensing during spaceflight, Adv. Space Res., 32/8, 1585-1593.

Schmitt D., 1999, Summary – Workshop conclusions & recommendations, The Faseb Journal, 13, 175-177.

van Loon J.J.W.A., 2007, Some history and use of the random positioning machine, RPM, in gravity related research, Adv. Space Res., 39, 1161-1165.