Virial stress-A key control parameter for immobilized hybridoma cells

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INTRODUCTION

The prediction of internal mechanical stress generated within immobilized cell population has the major importance for many biotechnological and biomedical applications. The considered system, i.e. micro-bead consists of two subsystems: polymer hydrogel matrix and dispersed cell population. The cell population is entrapped within the matrix in the form of clusters. The motion of cells is restricted to the motion of cells within the clusters. The interactions between clusters can be neglected based on our experimental observations (Pajic-Lijakovic I., 2007b). The number of clusters is approximately the same during the cell growth. In the absence of external forces, composite system of cells and hydrogel could be treated as isotopic (and homogeneous on scale much larger then the cell size). Generated stress influences both cell growth and product formation within hydrogel polymer matrix in the form of micro-bead. It represents the consequence of loading conditions within polymer matrix as compression induced by cell local mechanical pressing to the surrounding during their movement and growth. For modeling the generation of the internal stress, it is necessary to connect the local contributions of cells with the total internal stress (volume averaged stress). Local contributions of cells represent the result of complex behaviors of cell population. Cell movement has been already described as Brownian motion and expressed statistically using Langevin equation (Stokes C.L. et al., 1991; Ochab-Marcinek A. et al., 2004). To our opinion the procedure proposed by Irving J.H. and Kirkwood J.G. (1950) would be suitable for describing the local internal stress within the cell population. On that way, we use micro-rheology approach starting with basic assumptions of Theoretical rheology developed by Kirkwood for considering the problem. To our opinion virial stress (Hill T.L., 1986) can be also suitable for modeling the internal mechanical stress of cell population within the microcarreres. We expanded our previous consideration (Pajic-Lijakovic I. et al., 2007a,b) and formulate the new framework to connect the cell growth dynamic and mAb production with the mechanically induced internal stress within the cell population.

MATHERIAL AND METHODS

Experimental: The cell line used in these experiments was a mouse/mouse-hybridoma for production of monoclonal IgG (kindly provided by INEP, Zemun, Serbia and Montenegro). The cells were grown in Dulbecco's modified Eagle's medium (DMEM, Sigma, St. Louis, MO, USA) supplemented with 10% fetal calf serum (FCS, Sigma, St. Louis, MO, USA) in 75 ml tissue culture flasks (Fisher Scientific) and incubated at 37^{0}C in a humidified atmosphere with 5% CO_2 in air. The optimal density was about $0.3x10^5$ cells/ml, while the doubling time was about 40 h. Cell counts were enumerated using hemocytometer as an average from 10 microbead that were previously mechanically ruptured, while the cell viability was assessed using Trypan blue exclusion test. To determine the concentration of mAb produced an enzyme-linked immune-adsorbent assay (ELISA)

was used. The content of a 75 ml of the medium with cells was collected by centrifugation (10 min at 1000 rpm). The cell suspension was further mixed with sterile Na-alginate solution (1.5 %, Kelco Gel LV). Spherical droplets were formed by extrusion of Na-alginate/hybridoma cell suspension using high voltage generator. After gelling the microbeads were placed in double distillated water to remove un-reacted material and low molecular weight byproducts.

Theoretical: The generation of the internal stress is caused by both, by cell growth and movement on one side and by matrix resistance on the other. The complex changes within both, cell population and extra cellular matrix are induced by particular behavior of cell population. Cell movement within the matrix corresponds to over-damped Brownian motion (Ochab-Marcinek A. et al., 2004). Accordingly, it can be described statistically by Smoluchowski equation. Smoluchowski equation describes the time evolution of cell population toward equilibrium state in phase space in the form of distribution function $P\left(r^N,\tau,t\right)$ (where $r^N \equiv \{r_1,\ r_2,...r_N\}$, $N = N(t)$ is current number of cell within microcarrier, τ is loading time, t is growing time). The previously mentioned current equilibrium state, obtained at every time sets $\left(t,\tau_{eq}\right)$ will be described by the equilibrium distribution $P\left(r^N,\tau_{eq},t\right)$. Then, the local internal stress within cell population $\sigma\left(r,\tau_{eq},t\right)$ will be formulated by modified Irving-Kirkwood procedure (1950). The total internal stress for homogeneously distributed cell population within polymer matrix will be determined by volume averaging the local one as $\Pi_m(t)=\frac{1}{V_T(t)}\int_{V_T(t)} dr\,\sigma\left(r,\tau_{eq},t\right)$. For the homogeneous distribution of cells within the matrix, averaging is possible over the whole microbead volume. Total internal stress for every current equilibrium state will be equilibrated with the total external stress within the microcarrier matrix. The total internal stress obtained from local ones, in the virial form (11, 14), will be expressed as $\Pi_m(t)=aC(t)+bC(t)^2$ (where $\Pi_m(t)$ is virial stress, $C(t)$ is cell concentration in microcarrier, a and b are apparent first and second virial coefficient). The first apparent virial coefficient a represents the measure of the averaged cell kinetic energy, while the second apparent virial coefficient b depends on the pair interaction potential. The pair interaction potential depends on both, cell type and extra cellular medium. For further modeling consideration, it is suitable to transform the eq. into dimensionless form of stress by dividing the left and the right side with the equilibrium stress, $\Pi_m\left(t_{eq}\right)=\Pi_{mC}$ acted on cells which causes the cells to stop their growth. The transformed eq. into dimensionless form was expressed as:

$$Y(t)=a^*X(t)+b^*X(t)^2 \qquad (1)$$

where $Y(t)=\Pi_m(t)/\Pi_{mC}$ is the dimensionless internal mechanical stress, $X(t)=C(t)/C_{eq}$ is the dimensionless cell concentration in microcarrier, C_{eq} is equilibrium cell concentration, $a^*=aC_{eq}/\Pi_{mC}$ and $b^*=bC_{eq}^2/\Pi_{mC}$ are the dimensionless parameters. At equilibrium state for the cells for growing time $t\to t_{eq}$ the value of dimensionless matrix resistance stress is $Y\left(t_{eq}\right)=1$ and the value of dimensionless cell concentration is $X\left(t_{eq}\right)=1$. The parameters a^* and b^* from eq. 1 related as: $b^*=1-a^*$. Further model development includes formulation of the model equation for restricted cell growth. For this purpose, we modified the deterministic part of the model described by Pajic-Lijakovic I. et al. (2007a,b) and neglected the stochastic term at the level of the whole microcarrier. The model equation in the dimensionless form is:

$$\frac{dX(t)}{dt}=\mu\,X(t)-\eta\,Y(t)X(t)-\delta\,X(t)^2 \qquad (2)$$

where μ is the mean kinetic constant of cell growth which is equal to $\mu = \ln 2 / t_d$, while t_d is cell doubling time in our experiments $t_d \approx 40\ h$; η is the model parameter which represents the measure of the restriction of cells growth due to stress action on the cells and δ is the model parameter which represents the magnitude of interactions between cells themselves. Finally, we proposed the suitable dimensionless concentration balance for the product mAb per bioreactor volume:

$$\frac{dM(t)}{dt} = \varphi\ (1 + Y(t))\frac{dX(t)}{dt} - \frac{dY(t)}{dt} \qquad (3)$$

where $M(t) = C_p(t) / C_{peq}$ represents dimensionless concentration of mAb, while $C_p(t)$ is the mAb concentration per bioreactor volume and $C_{p\,eq}$ is the equilibrium concentration, φ is the kinetic constant of mAb production. The number of model parameters was reduced using the initial and boundary conditions: (a) at $t = 0$ the initial cell concentration is equal C_0 and the dimensionless cell concentration is $X_0 = C_0 / C_{eq}$, the initial dimensionless stress is $Y(0) = a^* X_0 + b^* X_0^2$, the initial dimensionless mAb concentration is $M(0) = 0$; (b) at equilibrium state for the cells, the value of dimensionless internal stress is $Y(t_{eq}) = 1$, the value of dimensionless cell concentration is $X(t_{eq}) = 1$, the value of dimensionless mAb concentration is $M(t_{eq}) = 1$, while the rates $dX(t)/dt/_{t=t_{eq}} = dY(t)/dt/_{t=t_{eq}} = dM(t)/dt/_{t=t_{eq}} = 0$. The value of the dimensionless parameter b^* from the eq. 1, as described above, is $b^* = 1 - a^*$. The value of the parameter δ from the eq. 2 is $\delta = \mu - \eta$. Three model parameters: b^*, φ, η from the eq. 3 could be determined by comparing experimental data with the model predictions.

RESULTS AND DISCUSSION

Model presented here describes the impact of mechanically induced internal stress within cell population on the dynamics of hybridoma cell growth and monoclonal antibodies (mAb) production.

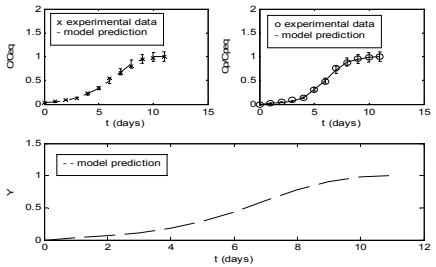

Figure 1. Experimental data and model predictions for cell concentration within microbead (C/C_{eq} vs. t); for mAb concentration in solution (C_p/C_{peq} vs. t) and dimensionless internal stress for cell population within microbead (Y vs. t)

The optimal model parameter from eq. 3 obtained by this fitting procedure that enable the best agreement with the experimental data was: $\tau_\eta = \eta^{-1} = 3.5 \pm 0.3$ *days*. The values of dimensionless parameters from eq. 1 were $a^* = 0.1 \pm 0.02$ and $b^* = 0.9 \pm 0.02$. As shown in Figure 1, model predicted values for the dimensionless cell concentration profile (C/C_{eq} vs. t) correlated well with the experimental data with relative error of 10 %. The restriction time for cell growth was shorter relative to the equilibrium growing time, i.e. $\tau_\eta = 0.32\ t_{eq}$ (where equilibrium growing time is $t_{eq} = 11$ *days*). The model predicted values for the dimensionless internal stress acted on cells within the microcarrier matrixes were also obtained from eqs. 1-2 by fitting procedure (Figure 1-Y vs. t). Using the proposed model the predicted values of dimensionless mAb concentrations were also calculated from eq. 3 by introduced the already calculated values for dimensionless internal stress. Experimental dimensionless mAb concentrations represent the ratios between the experimental mAb concentrations and the equilibrium mAb concentration. The obtained optimal values for kinetic constant for mAb production are $\varphi = 2.2 \pm 0.1$ for microbeads. As shown in Figure 1 (C_p/C_{peq} vs. t), model prediction values for the dimensionless mAb concentrations correlated rather well with the experimental data, i.e. with relative error of 10 %.

CONCLUSIONS

In summary, the results of this study pointed to some important cause-consequence relationships between the mechanically induced internal stress within the immobilized cell population and biomedical application. The generation of internal stress is caused by cell movement, inter-cell interactions and growth. The internal mechanical stress generated within the cell population is equilibrated with the external mechanical stress generated within variously structured alginate matrixes during the many relaxation cycles. We offer the formulation of internal stress in the form of virial stress the local contributions of cells with the total internal stress (volume averaged stress). Local contributions of cells represent the result of complex behaviors of cell population, presented as the sum of kinetic and potential contributions. The first represents the result of cell movement while the second represents the result of inter-cell interactions.

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