# Microenvironmental restricted cell growth inside various types of microcarriers

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## Introduction

A cell immobilization within various types of microcarriers has been widely utilized in food technology, biomedicine and tissue engineering (Bugarski et al., 1993, 1999; Nedovic et al., 2001). Owing to the very gentle, simple and rapid procedure, the entrapments of cells in Ca-alginate microbeads, as well as, in alginate-poly-L-lysine (alginate-PLL) microcapsules are still the most frequently used methods for immobilization. Of the basic interest for various applications is to optimize cell growth dynamics including efficient supply of cells by nutrients, suitable microenvironment conditions for cells inside the microcarriers. Bugarski et. al. (1999), Nedovic et al. (2001) optimized the internal nutrients transfer and performance of the alginate-PLL membrane for both microcapsules and Ca-alginate microbeads.

However, after nutrient optimization it was found that only about 20% of the microcapsule volume was filled with cells, indicating the existence of other factors acting restrictive on cells growth. Various microenvironmental restrictive factors, dependently on types of cells and microcarriers, could be a cause of this phenomenon. The mechanism of microenvironmental resistance toward cell growth is complex and includes various multi component and multi-level processes. These processes represent the consequence of the various types of interactions. The interactions arise between cells itself and between cells and microcarrier structure constituents. These processes are interrelated and determine the evolution of the biological systems toward the equilibrium states. However, it is the large number of processes. For further analysis, it is necessary to reduce the number of processes and finding the optimal resolution for the particular modeling considerations. Such type of complexity was modeled by Pajic-Lijakovic et al., (2007a, 2007b, 2007c) for two biological systems. The first system represents the growth of yeast cells within the Ca-alginate microbeads while the second one represents the growth of hybridoma cells within the alginate-PLL microcapsules.

The microenvironmental restrictive action on cell growth is considered on two different biological systems due to elucidate the complex mechanism of microenvironmental resistance. At first it is necessary to identify various restrictive factors which act in various regions of microcarriers.

## Materials and methods

We consider the microenvironmental restricted cell growth and elaborate this problem on two biological systems. The first biological system represents the growth of yeast cells inside the Ca-alginate microbeads, while the second represents the growth of hybridoma cells inside the alginate-PLL microcapsules.

For the first biological system, the consideration is based on experimentally obtained data for the intra-bead yeast cell concentration profile, after reached the equilibrium state (after 150 h), as well as, total yeast cell concentration per microbed and microbead volume as function of time.

Total yeast cell concentration in the beads was estimated by using Thoma counting chamber after dissolution of beads.

The initial yeast concentration ($\rho_0$) in beads was about 6 x $10^6$ cells/ml. Microbeads were also sampled twice per day from the cultivation flask and cell concentration is measured in the same

way. Local cell concentration per microbead layers is calculated from experimentally determined surface fraction of cells for various microbead cross sections. Surface fraction of cells was estimated by ultramicroton cutting the microbead. Alginate microbeads sampled for image analysis were fixed in 2.5 % glutaraldehyde, embedded in araldite, cross-sectioned by LKB III ultramicrotom and stained with hematoxylin and eosin (H&E). The images of microbeads cross-sections (number of sections was six for each bead) where acquired using a solid-state CCD camera (Hitachi) mounted on an inverted microscope (Nikon Diaphot), digitized by a CG-7 frame Grabber (Scion Corp., Frederick, MD) and analyzed using Image Pro Plus software. The detail experimental procedure is given by Pajic-Lijakovic et al. (2007b).

For the second biological system, the consideration is based on experimentally obtained data for the intra-bead hybridoma cell concentration profile, after reached the equilibrium state (after 23 days), as well as, total hybridoma cell concentration per microcapsules.

Total hybridoma cell concentrations in the microcapsules were estimated by counting the cells using hemocytometer from 10 microcapsules. The microcapsules were previously mechanically ruptured, while the cell viability was assessed using Trypan blue exclusion test.

The initial hybridoma concentration ( $\rho_0$ ) in microcapsules was about 1 x $10^6$ cells/ml. The local cell concentrations within the microcapsules were experimentally obtained by examining 20 μm-thin layers stained with hematoxylin and eosin. The microcapsules, removed from bioreactor on day 21 of cultivation, were frozen on dry ice and stored at -70°C untill the series of thin layers were prepared. Twenty five layers of 20μm thickness equaled to the half of the microcapsule volume. The detail experimental procedure is given by Pajic-Lijakovic et al. (2007a).

**Results and discussion**

We made the parallel presentation of the experimental data for both biological systems. For experimental considerations, microcarriers are divided into thin layers with various distances from the center.

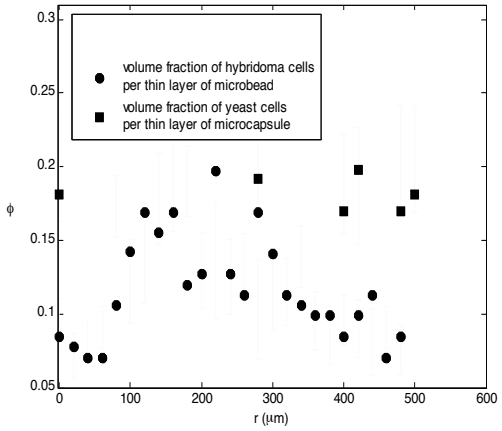


Figure 1. The volume fractions of cells inside the thin layer as function of distance from microcarrier center, for the hybridoma cells inside the alginate-PLL microcapsule and for yeast cells inside the Ca-alginate microbead.

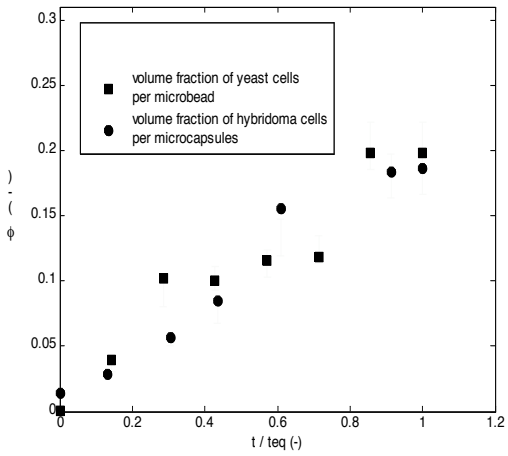


Figure 2. The total volume fractions of cells inside the microcarriers as function of time relative to equilibrium time, for the hybridoma cells inside the alginate-PLL microcapsule and for yeast cells inside the Ca-alginate microbead.

However, cell concentration per layer is not the suitable variable for comparison of the both dynamical systems. The averaged diameter of hybridoma cells is much higher than of yeast cells. Hybridoma cells have the averaged diameter about 20 μm, while yeast cells are smaller with the averaged diameter about 6 μm. For further comparison of the experimental data and model predictions, we used volume fraction of cells per thin layers instead the cell number concentration. The intra-carrier volume fraction profiles of cells are shown in Figure 1.
Relatively uniform yeast cell volume fraction profile is observed inside the Ca-alginate microbead indicated that no internal nutrient diffusion limitations, but microenvironmental restriction, affected dominantly the dynamics of cell growth.
At the same time, the relatively uniform volume fraction profile of hybridoma cells is also observed for the thin layers in the middle part of microcapsule, except for the centre and the region near the membrane. The lower cell volume fraction inside the thin layers near the alginate-PLL membrane could be the consequence of membrane restriction effects. Abrupt decrease of the cell volume fraction inside the thin layers near the centre indicates the presence of another microenvironment influencing the cells growth. It could be the presence the parts of non-dissolved Ca-alginate core region, as indicated in previous reports (Bugarski et al., 1993, 1999, 2004).
The total volume fraction of cells as functions of time relative to the equilibrium time (23 days for hybridoma cells and 150 h for yeast cells) are shown in Figure 2. In the regime I (early stage) for $t/t_{eq}$ up to 0.3, microenvironmental resistance could be neglected for both dynamical systems due to low volume fraction of cells, up to 0.1. Yeast cells growth faster then hybridoma cells. Further, in the regime II (middle stage) for $t/t_{eq}$ from 0.3 to 0.7, microenvironmental resistance increase. In this regime, hybridoma cells growth faster than yeast cells. Finally, in the regime III, for $t/t_{eq}$ from 0.7 to 0.1, microenvironmental resistance becomes high enough to direct both systems into the equilibrium state.

## Conclusions

In summary, the results pointed out that cell volume fraction profiles within the microcarriers could be used for examination the microcarrier matrix behavior identification as well as such structural changes during the cell growth. The cell volume fraction profile within the microbead is relatively uniform indicates matrix homogeneity. However, considering the volume fraction of cells within the microcapsules, two regions are established (the small amount of Ca-alginate core region and dissolved Na-alginate annular region enclosed with alginate-PLL membrane).
The microenvironmental resistance increase faster within the microbeads than within the microcapsules and suppressed the cell growth. Deformation and disintegration of hydrogel has the feedback restrictive action on cell growth within the whole microbeads as well as within the core region of microcapsules. At the same time, the rise of osmotic pressure restricted the cell growth within the liquefied annular region.

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