

Optimization of Seeding Density in Microencapsulated Recombinant CHO Cell Culture

Y. Zhang,^{a,b} J. Zhou,^{a,b} X. Zhang,^{a,b} W. Yu,^a X. Guo,^a W. Wang,^a and X. Ma^{a,*}

^aLaboratory of Biomedical Material Engineering, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, P. R. China

^bGraduate School of the Chinese Academy of Sciences, Chinese Academy of Sciences, 19 Yuquan Road, Beijing 100039, P.R. China

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Microencapsulation technology is an alternative large-scale mammalian cell culture method. The semi-permeable membrane of the microcapsule allows free diffusion of nutrients, oxygen and toxic metabolites to support cell growth, and the microcapsule membrane can protect the cells from the mechanical damage of shear forces associated with agitation and aeration. Many polymers have been used to make microcapsules, such as chitosan, polyacrylates, alginate, polyamino acids, and polyamides. One of these microcapsules is Alginate–polylysine–alginate (APA) microcapsule. Using the electrostatic droplet generator and polyelectrolyte complexation, we developed APA microcapsules with a diameter of 300 μm and assessed the effect of the seeding density on microencapsulated recombinant Chinese hamster ovary (CHO) cell growth, metabolism and endostatin production. The results showed that the cell growth was the best and endostatin production was the highest when the seeding density was $3 \cdot 10^6$ cells mL^{-1} microcapsule, at which the maximal cell density reached $1.82 \cdot 10^7$ cells mL^{-1} microcapsule and endostatin production reached $12.3 \mu\text{g mL}^{-1}$ at day 9. The energy metabolism was more efficient when the seeding density was $3 \cdot 10^6$ cells mL^{-1} microcapsule, at which more glucose and glutamine were utilized for biosynthesis and less lactate and ammonium were produced.

Key words:

Microencapsulation, seeding density, recombinant CHO cell, endostatin

Introduction

Microencapsulation technology is an alternative biotechnology and the special microenvironment provided by microcapsules affects the cellular behaviors greatly. For example, cells always form aggregates and organize into three-dimensional structure to proliferate, differentiate and function.^{1,2} This technology has been applied to large-scale mammalian cell culture by Damon Biotech in the 1980s.³ The microencapsulated cell culture has many advantages over other immobilized cell technology, such as micro-carrier and hollow fiber. The microcapsules provide a mild and comfortable environment for cell growth because the microcapsule membrane allows bi-directional diffusion of nutrients, oxygen and toxic metabolites, and the microcapsule membrane confines cells inside the microcapsule increasing the cell-to-cell and cell-to-matrix interactions through direct contact and/or secreted protein molecules such as growth factor and hormone. Especially, the microcapsule membrane can protect cells from mechanical dam-

age of shear forces associated with agitation and aeration. Therefore, microencapsulated cell culture benefits to cell growth and can obtain higher cell density and protein production. The maximal cell density can reach $2 \cdot 10^8$ cells mL^{-1} microcapsule after 20 days of culture.¹

At present, many polymers, such as chitosan, polyacrylates, alginate, polyamino acids and polyamides, have been used to make microcapsules.^{4,5,6} Alginate–polylysine–alginate (APA) microcapsule is one of the most widely studied microcapsules because it is the first system utilized to fully explore the encapsulation technology and most findings with this system are pertinent to many applications.⁷ The procedure to prepare an APA microcapsule includes formation of calcium alginate beads, reaction with positively charged polylysine to form alginate-polylysine membrane, coating with alginate and liquefaction of the inner alginate matrix.

One of the major parameters in an immobilized cell culture is the initial cell density. As one desired to produce large amounts of recombinant protein, the high seeding density were frequently used. Insufficient inoculum may result in a hindered growth rate and reduced growth extent. Therefore, a mini-

* Corresponding Author: Professor Xiaojun Ma
Tel/Fax: +86-411-84379096; E-mail: maxj@dicp.ac.cn

imum seeding density is necessary to initiate a batch culture for immobilized cell culture. Hu W. S. reported that the seeding density obviously affected cell growth on microcarriers.^{8,9} When the seeding density was $4 \cdot 10^5$ cells mL⁻¹, the maximal cell density could attain $1.2 \cdot 10^6$ cells mL⁻¹. Decreasing the seeding density resulted in an obvious reduction of both growth rate and growth extent. Arús L. *et al.* have studied the effect of the seeding density on microencapsulated hybridoma cell growth. The results showed the cells in the APA microcapsules had a good growth when the seeding density was $1 \cdot 10^7$ cells mL⁻¹ microcapsule, and the cells had a poor growth when the seeding densities were decreased to $1 \cdot 10^6$ or $5 \cdot 10^6$ cells mL⁻¹ microcapsule.¹⁰ The monoclonal antibody expressed by microencapsulated cells was the highest when the seeding density was $1 \cdot 10^7$ cells mL⁻¹ microcapsule and the maximal mass concentration was $\gamma = 29.1$ μ g mL⁻¹ at day 17. However, the initial seeding density of $1 \cdot 10^7$ cells mL⁻¹ was considerably high, even exceeded the maximal cell density in suspension, and increased the difficulty of inoculum cell culture and microcapsule preparation. Therefore, it was necessary to optimize the seeding density for acquiring better cell growth and production of recombinant protein in lower seeding density.

In this study, the microcapsules with different seeding density were prepared to determine the effect of the seeding density on microencapsulated recombinant CHO cell growth, metabolism and endostatin production.

Materials and methods

Cell and culture

The recombinant Chinese hamster ovary (CHO) cells transfected with the endostatin gene were kindly donated by Dr. Huaining Teng (Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences). The cells were routinely cultivated in 250 mL T-flasks and were incubated at 37 °C in a humidified 5 % CO₂ atmosphere. The medium was DMEM/F12 (1 : 1) medium (Sigma, U.S.A.) supplemented with 10 % fetal bovine serum (FBS, Sanli Biology, Beijing, China), 100 units mL⁻¹ penicillin and 100 μ g mL⁻¹ streptomycin, 5 μ g mL⁻¹ puromycin (Sigma, U.S.A.). The cells were subcultivated every 2 or 3 days.

Preparation of APA microencapsulated cells

Alginate-Poly-L-lysine-Alginate (APA) microcapsules containing recombinant CHO cells were prepared as described previously with some modification.¹¹ Briefly, exponentially growing CHO cells

were harvested and resuspended in a $w = 1.5$ % filtered sodium alginate solution (Sigma, U.S.A.). Four microcapsules with different seeding density were prepared: $5 \cdot 10^5$, 10^6 , $3 \cdot 10^6$ and 10^7 cells mL⁻¹ microcapsule. Then the cell suspension was extruded through a 0.4-mm needle into a $c = 100$ mmol L⁻¹ CaCl₂ solution using an electrostatic droplet generator (Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China) to form calcium alginate gel beads. The gel beads were incubated with $w = 0.05$ % poly-L-lysine (MW 21 900; Sigma, U.S.A) to form alginate-L-polylysine membrane around the surface. After washing the beads with saline, $w = 0.15$ % alginate was added to counteract the excess charge on the membrane of microcapsule for 5 min. The membrane-enclosed gel beads were further suspended in $c = 55$ mmol L⁻¹ sodium citrate to liquefy the alginate gel core. The microcapsules with recombinant CHO cells were cultured at 37 °C in a humidified 5 % CO₂ atmosphere.

Batch cultures of microencapsulated cells with different seeding density

The microcapsules with different seeding density were suspended in DMEM/F12 (1 : 1) medium. One mL medium with 0.1 mL microcapsules were inoculated into each well of a 24-well plate, and incubated at 37 °C in a humidified $w = 5$ % CO₂ atmosphere. At day 9 of culture, the media were collected and kept frozen at -20 °C for later analysis to determine the concentration of endostatin.

MTT Assay of microencapsulated cells to determine cell number

The viable cell concentration in the microcapsules was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] assay.¹² Briefly, 100 μ L MTT solutions ($\gamma = 5$ mg mL⁻¹; Sigma, U.S.A.) were added into each well of a 24-well plate and incubated at 37 °C for an additional 24 h. The medium containing MTT was removed and microcapsules were washed twice with 0.9 % saline, and then 1 mL DMSO was added to solubilize the MTT tetrazolium crystal. The absorbance (A) was determined at 570 nm and 630 nm as reference using a plate reader (Wellsan MK3, Labsystems, Finland). Triplicate samples were used at each time point, and the results were expressed as mean \pm SD. The cell numbers were calculated from the value of OD₅₇₀ according to a standard curve. Known numbers of recombinant CHO cells were microencapsulated in APA microcapsules and the value of OD₅₇₀ of these microencapsulated CHO cells was measured using the above method in order to obtain the standard curve

of cell numbers vs. value of OD₅₇₀. Finally, the cell number was derived from the value of OD₅₇₀ according to the standard curve.

Determination of endostatin concentration

Endostatin concentration in conditioned medium was determined by ELISA (Accucyte Human Endostatin Kit, Cytimmolune Sciences, College Park, MD, USA) following the manufacturer's instructions.

Determination of concentrations of glucose, lactate, glutamine, and ammonium

The concentrations of glucose and lactate were measured by SBA-40C bio-sensitive analyzer (Jinan, China). The concentration of glutamine was analyzed as described previously with modification.¹³ First, the culture supernatants were boiled 30 min in water, the glutamine was hydrolyzed to glutamate, and then the glutamate was measured by SBA-40C bio-sensitive analyzer (Jinan, China). Ammonium concentration was measured with an ammonium assay kit (Zhongsheng, China).

Calculations and level of significance

The specific growth rate (μ) was calculated by the general formula,¹⁴

$$\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1} \quad (1)$$

Where N_1 , N_2 is cell number at time t_1 , t_2 .

The specific consumption rate of glucose (q_{glc}), the specific consumption rate of glutamine (q_{gln}), the specific production rate of lactate q_{lac} and the specific production rate of ammonium (q_{amm}) for the metabolic parameters were calculated by the general formula,¹⁵

$$q_s = \frac{n_2 - n_1}{t_2 - t_1} \cdot \frac{\ln N_2 - \ln N_1}{N_2 - N_1} \quad (2)$$

Where n_1 , n_2 is the amount of glucose, glutamine or lactate, ammonium at time t_1 , t_2 and N_1 and N_2 is the viable cell number at time t_1 , t_2 .

The yield of lactate from glucose ($Y_{lac/glc}$) and the yield of ammonium from glutamine ($Y_{amm/gln}$) are,¹⁶

$$Y(\text{mol mol}^{-1}) = \frac{\text{mol of product produced}}{\text{mol of product consumed}} \quad (3)$$

The yield of cell from glucose ($Y_{x/glc}$) and the yield of cell from glutamine ($Y_{x/gln}$) are

$$Y(10^6 \text{ cells mmol}^{-1}) = \frac{\text{cell number}}{\text{mol of product consumed}} \quad (4)$$

Results and discussion

Effect of the seeding density on microencapsulated recombinant CHO cells growth

The growth profile of microencapsulated recombinant CHO cells was monitored by a light microscope, which showed that the cells in microcapsules created spherical aggregates during the culture (Fig. 1). The cell spheroids filled up most of the microcapsular interior at day 9 when the cell density was $\rho = 3 \cdot 10^6$ cells mL⁻¹ microcapsule and the maximal viable cell density (ρ_s) of $2.01 \pm 0.18 \cdot 10^7$ cells mL⁻¹ microcapsule was obtained at day 6 (Fig. 1 and Fig. 2). Decreasing the seeding density to $1 \cdot 10^6$ cells mL⁻¹ microcapsule resulted in a decrease of final growth extent, at which the maximal cells density only reached $1.65 \pm 0.09 \cdot 10^7$ cells mL⁻¹ microcapsule. Further decreasing the

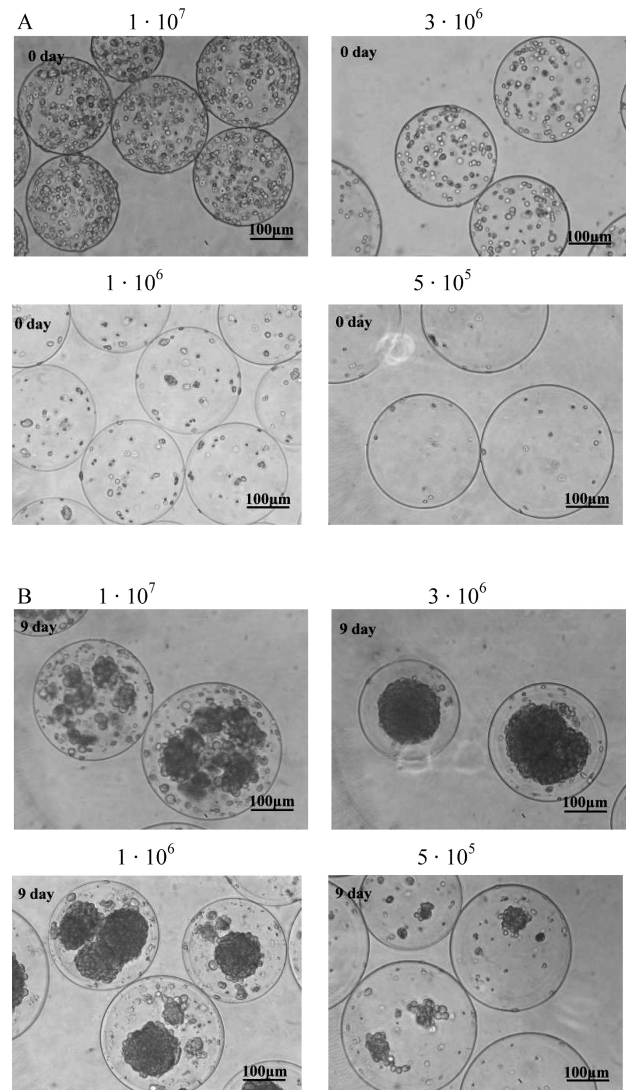


Fig. 1 – Morphology of the growth profile of recombinant CHO cells microencapsulated in APA microcapsules with different seeding density. A: culture 0 day, B: culture 9 day

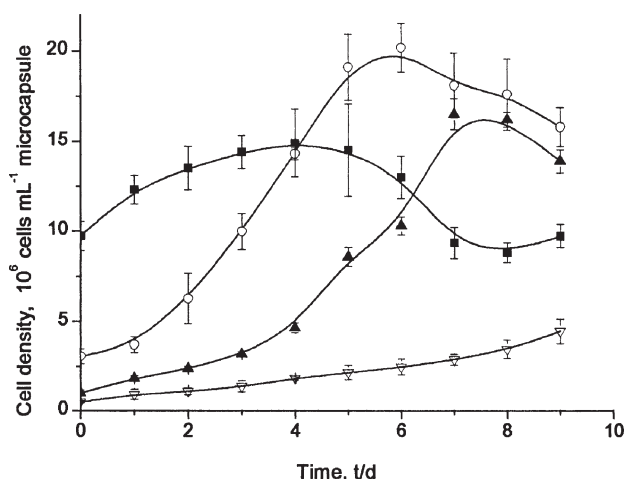


Fig. 2 – Effect of seeding density on growth of microencapsulated recombinant CHO cells. The seeding density was $1 \cdot 10^7$ cells mL^{-1} microcapsule (■), $3 \cdot 10^6$ cells mL^{-1} microcapsule (○), $1 \cdot 10^6$ cells mL^{-1} microcapsule (▲) and $5 \cdot 10^5$ cells mL^{-1} microcapsule (▽)

seeding density to $5 \cdot 10^5$ cells mL^{-1} microcapsule resulted in an obvious decrease of the cell growth rate and growth extent, and the maximal cells density only reached $4.46 \pm 0.68 \cdot 10^6$ cells mL^{-1} microcapsule. The maximal cell density only reached $1.49 \pm 0.19 \cdot 10^7$ cells mL^{-1} microcapsule when the seeding density increased to $1 \cdot 10^7$ cells mL^{-1} microcapsule. The μ_{\max} was larger when the seeding density was $1 \cdot 10^6$ or $3 \cdot 10^6$ cells mL^{-1} microcapsule, attained 0.511 d^{-1} or 0.561 d^{-1} , and decreased to 0.193 d^{-1} when the seeding density increased to

Table 1 – Culture data of microencapsulated CHO cells in different seeding densities

	Seeding densities / mL^{-1}			
	$1 \cdot 10^7$	$3 \cdot 10^6$	$1 \cdot 10^6$	$5 \cdot 10^5$
N_{\max} , 10^6 cells	14.9	20.2	16.5	4.46
μ_{\max} , d^{-1}	0.193	0.561	0.511	0.307
q_{glc} , $\text{mmol} \cdot (1 \cdot 10^6 \text{ cells}) \cdot (10^6 \text{ cells})^{-1} \cdot \text{d}^{-1}$	0.399	0.380	0.399	0.390
q_{lac} , $\text{mmol} \cdot (1 \cdot 10^6 \text{ cells}) \cdot (10^6 \text{ cells})^{-1} \cdot \text{d}^{-1}$	0.707	0.671	0.566	0.557
q_{gln} , $\text{mmol} \cdot (1 \cdot 10^6 \text{ cells}) \cdot (10^6 \text{ cells})^{-1} \cdot \text{d}^{-1}$	0.110	0.105	0.113	0.117
q_{amm} , $\text{mmol} \cdot (1 \cdot 10^6 \text{ cells}) \cdot (10^6 \text{ cells})^{-1} \cdot \text{d}^{-1}$	0.104	0.039	0.053	0.086
$Y_{\text{N/glc}}$, $1 \cdot 10^6 \text{ cells} \text{ mmol}^{-1}$	0.324	0.801	0.637	0.476
$Y_{\text{N/gln}}$, $1 \cdot 10^6 \text{ cells} \text{ mmol}^{-1}$	0.925	2.908	2.237	1.589
$Y_{\text{lac/glc}}$, $\text{mmol} \text{ mmol}^{-1}$	1.895	1.770	1.730	1.357
$Y_{\text{amm/gln}}$, $\text{mmol} \text{ mmol}^{-1}$	0.942	0.351	0.596	0.697

$1 \cdot 10^7$ cells mL^{-1} microcapsule. The μ_{\max} was only 0.307 d^{-1} at the seeding density of $5 \cdot 10^5$ cells mL^{-1} microcapsule (Table 1).

The cell growth was fast in the first 2 days of culture when the seeding density was $1 \cdot 10^7$ cells mL^{-1} microcapsule, but the span was only 4 days and the viable cell density declined after 4 days of culture. The cells formed multiple aggregates and only filled up half space of the microcapsules at day 9 (Fig. 1). This growth decline was not due to the exhaustion of any macro-nutrient components, but the rapid production of metabolic byproducts resulting in a decline of the specific growth rate in the culture. The rapid metabolism of glucose and glutamine produced a mass of lactate and ammonium when the seeding density was $1 \cdot 10^7$ cells mL^{-1} microcapsule (Fig. 2 and Fig. 3), at which the concentration of lactate reached $23.67 \text{ mmol L}^{-1}$ at day 2 and it was notably higher than the concentration reported in the literature as being inhibitory (18 mmol L^{-1}).¹⁷ Although the concentration of metabolic byproducts was rather high at the end of the

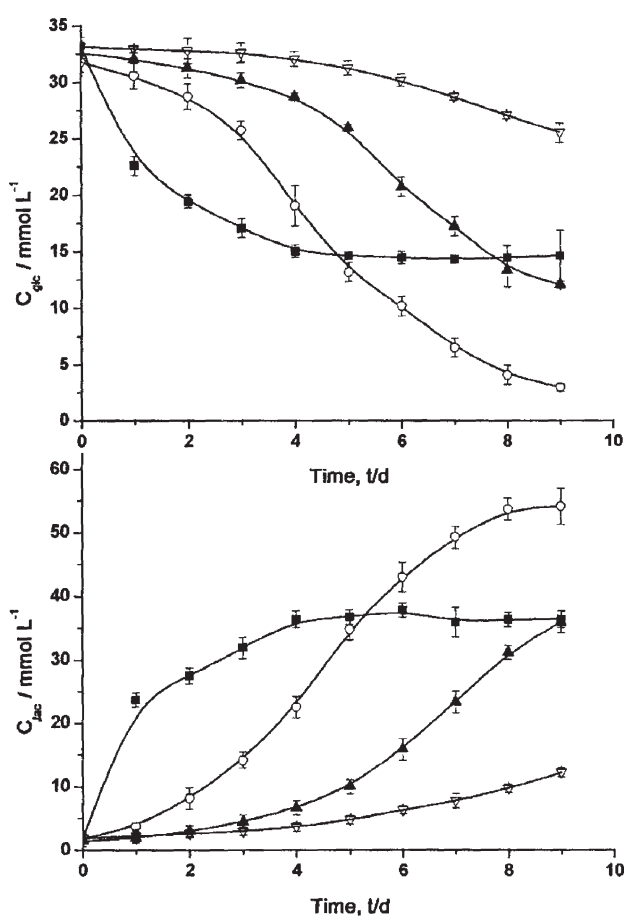


Fig. 3 – Effect of different seeding densities on glucose metabolism and lactate production of microencapsulated recombinant CHO cells. Seeding densities were: $1 \cdot 10^7$ cells mL^{-1} microcapsule (■), $3 \cdot 10^6$ cells mL^{-1} microcapsule (○), $1 \cdot 10^6$ cells mL^{-1} microcapsule (▲), and $5 \cdot 10^5$ cells mL^{-1} microcapsule (▽)

culture, the cell growth was not inhibited when the seeding density was $3 \cdot 10^6$ or $1 \cdot 10^6$ cells mL^{-1} microcapsule. It was because the cells could gradually adapt to the environment of high lactate and ammonium concentration when the metabolic by-products were gradually produced during the culture. It was necessary to inoculate with an adequate cell density for the quick growth of the cells and insufficient inoculum could result in hindered cell growth and reduced cell proliferation. Hu W. S. reported that the cell growth ceased when the seeding density was less than 7 cells per microcarrier. When the seeding density was $5 \cdot 10^5$ cells mL^{-1} microcapsule, the cells did not grow in many microcapsules because the seed cell numbers in them were less than 7 cells per microcapsule. (The mean diameter of the microcapsule was $300 \mu\text{m}$. There were about $7 \cdot 10^4$ microcapsules per mL and so the average cell number per microcapsule was 7. As the seed cells randomly distribute in the microcapsules during the preparation of the microcapsule, in some of the microcapsules there were more than 7 cells and in some there were less than 7 cells. This influenced cell-to-cell and cell-to-matrix interactions through direct contact and/or secreted protein molecules such as growth factor, hormone. Therefore, the cell growth was good only in a few of the microcapsules with the seeding density over 7 cells per microcapsule and the maximal cell density only reached $1.65 \pm 0.09 \cdot 10^7$ cells mL^{-1} microcapsule (Fig. 1 and Fig. 2).

Effect of the seeding density on glucose metabolism of microencapsulated cells

The effect of the seeding density on glucose consumption and lactate production is shown in Fig. 3. When the seeding density was higher, the catabolism rate of glucose was faster to provide more energy for the cell growth and survival. The consumption of glucose and production of lactate were very rapid in the first day when the seeding density was $1 \cdot 10^7$ cells mL^{-1} microcapsule, and the lactate concentration reached $c = 23.67 \text{ mmol L}^{-1}$ in the first day. Subsequently, the rate of glucose consumption and lactate production rapidly decreased. The glucose metabolism almost ceased after 5 days of culture and the glucose was not exhausted at the end of culture. The rate of glucose consumption and lactate production slowed down with the decrease of the seeding density. Although the lactate concentration was high at the end of culture when the seeding density was $3 \cdot 10^6$ or $1 \cdot 10^6$ cells mL^{-1} microcapsule, the cell growth and the glucose metabolism apparently were not inhibited. The shift of the seeding density did not affect the specific glucose consumption rate (Table 1). The q_{glc} was from 3.90 mmol d^{-1} per 10^6 cells at the seeding density of

$5 \cdot 10^5$ cells mL^{-1} microcapsule to 3.99 mmol d^{-1} per 10^6 cells at the seeding density of $1 \cdot 10^7$ cells mL^{-1} microcapsule. However, the efficiency of glucose utilization was different when the seeding density was changed. The yield of cell from glucose ($Y_{x/\text{glc}}$) was the highest when the seeding density was $3 \cdot 10^6$ cells mL^{-1} microcapsule, attained $8.01 \cdot 10^6$ cells mmol^{-1} and the $Y_{x/\text{glc}}$ decreased when the seeding density decreased or increased. The specific lactate production rate increased with the increase of the seeding density, and increased from 5.57 mmol d^{-1} per 10^6 cells to 7.07 mmol d^{-1} per 10^6 cells when the seeding density increased from $5 \cdot 10^5$ cells mL^{-1} microcapsule to $1 \cdot 10^7$ cells mL^{-1} microcapsule. The yield of lactate from glucose ($Y_{\text{lac}/\text{glc}}$) decreased from 1.895 to 1.357 mmol mmol^{-1} when the seeding density decreasing from $1 \cdot 10^7$ cells mL^{-1} microcapsule to $5 \cdot 10^5$ cells mL^{-1} microcapsule.

Glucose was the main carbon source and important energy source in the mammalian cell culture. The high rate of glucose consumption and lactate production were characteristic of cell *in vitro* culture and the requirement for rapid cell proliferation.¹⁸ The glucose was predominantly metabolized by the glycolytic pathway and pyruvate was produced as the end product, and then pyruvate was transformed to lactate to recover the oxidation of cofactor nicotinamide adenine dinucleotide (NAD). Only a small amount of glucose was completely oxidized to carbon dioxide, even in the presence of saturating oxygen *in vitro* culture.¹⁹ Some glucose was metabolized by the pentose phosphate pathway to provide ribose-5 phosphate for nucleic acid synthesis.¹⁹ Due to a large amount of glucose producing lactate, the accumulation of lactate always occurred in cell *in vitro* culture. The cell growth, metabolism and recombinant protein production would be inhibited because the accumulation of lactate would lead to the acidification of the culture environment and the change in medium osmolarity.¹⁴ The results in this study indicate that more glucose was utilized to cell proliferation and endostatin production because less lactate was produced when the seeding density was $3 \cdot 10^6$ cells mL^{-1} microcapsule or $1 \cdot 10^6$ cells mL^{-1} microcapsule. Therefore, it could acquire higher cell density due to the prolongation of cell growth span benefited from the decrease of lactate production when the seeding density was $3 \cdot 10^6$ cells mL^{-1} microcapsule.

Effect of the seeding density on glutamine metabolism

The effect of the seeding density on glutamine consumption and ammonium production is shown in Fig. 4. The rates of glutamine consumption and ammonium production increased with the increase

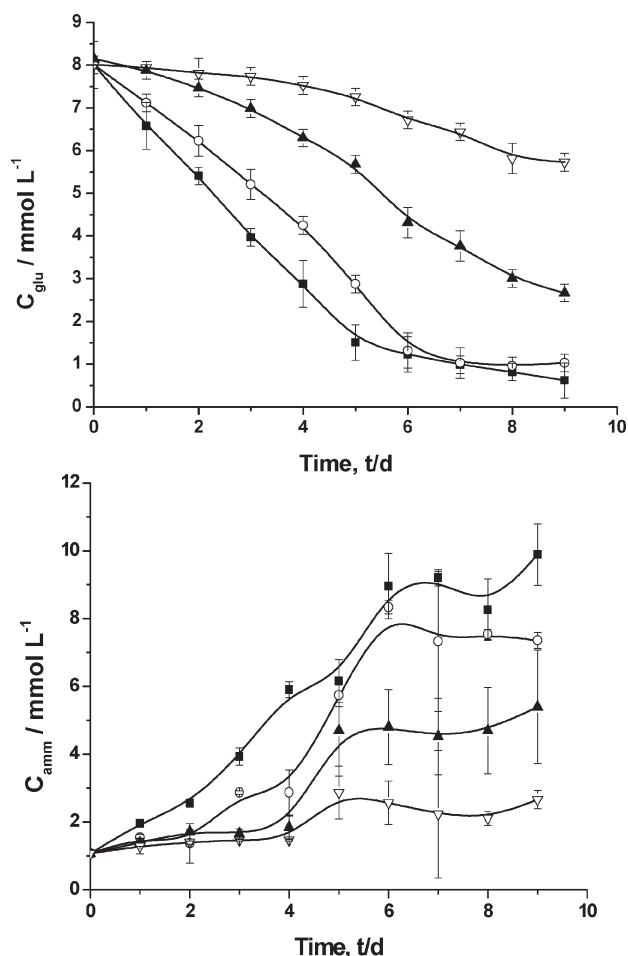


Fig. 4 – Effect of seeding density on glutamine metabolism and ammonium production of microencapsulated recombinant CHO cells. Seeding densities were: $1 \cdot 10^7$ cells mL^{-1} microcapsule (■), $3 \cdot 10^6$ cells mL^{-1} microcapsule (○), $1 \cdot 10^6$ cells mL^{-1} microcapsule (▲), and $5 \cdot 10^5$ cells mL^{-1} microcapsule (▽)

of the seeding density. Although the specific glutamine consumption rates were similar at different seeding density, the specific ammonium production rates were significantly different (Table 1). The specific ammonium production rate was the lowest and the yield of cells from glutamine ($Y_{x/gln}$) was the highest when the seeding density was $3 \cdot 10^6$ cells mL^{-1} microcapsule, which were $0.039 \text{ mmol d}^{-1}$ per 10^6 cells and $29.08 \cdot 10^6$ cells mmol^{-1} , respectively. The $Y_{x/gln}$ decreased with the decrease of the seeding density. It was the lowest and only reached $9.25 \cdot 10^6$ cells mmol^{-1} at the seeding density of $1 \cdot 10^7$ cells mL^{-1} microcapsule. The yield of ammonium from glutamine ($Y_{amm/gln}$) was only $0.39 \text{ mmol mmol}^{-1}$ when the seeding density was $3 \cdot 10^6$ cells mL^{-1} microcapsule, which was lower than the yields at other seeding densities.

The glutamine was another essential nutrient in the mammalian cell culture. It was not only the amino group donor in some biosynthetic pathways

such as purine and pyrimidine synthesis, but also the major energy source.¹⁹ Glutamine catabolism could yield α -ketoglutarate and release ammonium molecule and the amido-group could be utilized to synthesize some biomolecules, such as purine and pyrimidine by some biosynthetic pathways.²⁰ Rapid glutamine metabolism was the characteristic of cell growth and the rapid metabolism of glutamine would result in the accumulation of ammonium in media.²¹ The results in this study showed that the yield of ammonium from glutamine ($Y_{amm/gln}$) and the specific ammonium production rate (q_{amm}) were the lowest at the seeding density of $3 \cdot 10^6$ cells mL^{-1} microcapsule. It indicated that more ammonium was utilized to synthesize biomolecules for cell proliferation and protein production. Although the rate of glutamine metabolism was quite rapid when the seeding density was $1 \cdot 10^7$ cells mL^{-1} microcapsule, the efficiency of glutamine was the lowest due to the high ammonium concentrations in media leading to a futile cycle of glutamine metabolism and an increase of the maintenance energy. A majority of energy produced by glucose and glutamine was not utilized for cell growth and recombinant protein production, but was dissipated as heat.

Effect of the seeding density on endostatin production

The production of endostatin is shown in Fig. 5. The maximum yield of $12.3 \mu\text{g mL}^{-1}$ was obtained after 9 days of culture when the seeding density was $3 \cdot 10^6$ cells mL^{-1} microcapsule. The endostatin production reduced with the decrease of the seeding density and only reached 9.65 and $3.53 \mu\text{g mL}^{-1}$ when the seeding density decreased to $1 \cdot 10^6$ cells mL^{-1} microcapsule and $5 \cdot 10^5$ cells mL^{-1} microcapsule. Endostatin production de-

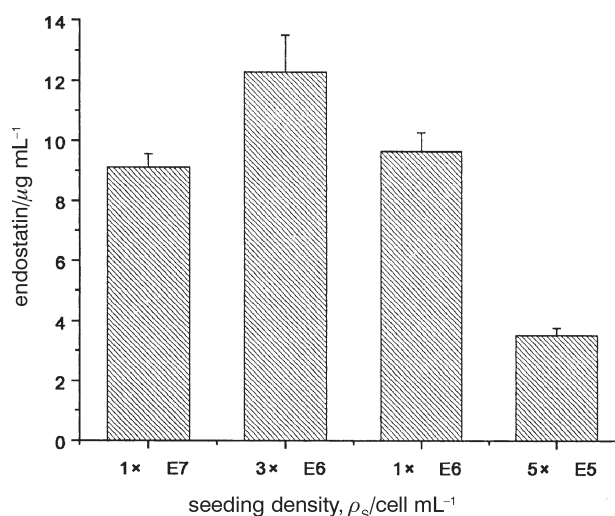


Fig. 5 – Effect of seeding density on endostatin production of microencapsulated recombinant CHO cells

creased to $9.11 \mu\text{g mL}^{-1}$ when the seeding density increased to $1 \cdot 10^7$ cells mL^{-1} microcapsule. The result of endostatin production was associated with the cell growth, and higher cell viability benefited to higher production of the recombinant endostatin.

Conclusion

The seeding density was one of the important control parameters in mammalian cell culture and it directly affected cell growth, metabolism and protein production. Optimization of the seeding density was necessary for establishing ideal conditions in microencapsulated cell culture. In this work, the effects of the seeding density on microencapsulated cell growth, metabolism and endostatin production were studied. The optimized seeding density was $3 \cdot 10^6$ cells mL^{-1} microcapsule in this culture. It provides an important guideline for microencapsulated recombinant cell culture.

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List of symbols

c	– concentration, mmol L^{-1}
C	– viable cells concentration, cells mL^{-1}
n	– amount of substance, mmol
N	– cells number, 10^6 cells
q	– specific consumption rate, $\text{mmol} \cdot (10^6 \text{ cells}) \cdot (10^6 \text{ cells})^{-1} \cdot \text{d}^{-1}$
t	– time, d
T	– temperature, $^{\circ}\text{C}$
w	– mass fraction, %

γ	– mass concentration, $\mu\text{g mL}^{-1}$
μ	– specific growth rate, d^{-1}
ρ_s	– seeding density, cells mL^{-1}

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