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The Effects of Cellular Seeding Density on Microencapsulated Recombinant Cho Cells Growth, Metabolism and Production of Endostatin

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Abstract: Microencapsulation technology is an alternative large-scale mammalian cells culture method. The semi-permeable membrane of microcapsule allows free diffusion of nutrients, oxygen and toxic metabolites, provides a mild and comfortable environment for cell growth and it can also protect cells from mechanical damage of shear forces associated with agitation and aeration; therefore the microencapsulated cells culture favor to long-term growth of cells and can obtain higher cell density. The cellular seeding density is important control parameter in mammalian cell culture _Cit directly effects cell growth, metabolism and protein production. Optimization of the cellular seeding density is very important for establishing ideal culture conditions. The aim of this study was to assess the effects of different cellular seeding density on microencapsulated recombinant CHO cell growth, metabolism and endostatin production and optimize the cellular seeding density. The results showed that the maximal cell density and yield of endostatin reached 1.91×10 7 cells mL^{-1} microcapsule and 615.1ng mL^{-1} during the culture when the cellular seeding density was 3×10 6 cells mL^{-1} microcapsule. The metabolism behavior of glucose and glutamine shifted as the seeding density was altered. The energy metabolism was more efficient as the cellular seeding density was at a lower level, more glucose and glutamine were utilized for biosynthesis and less lactate and ammonium were produced.

Key word: Microencapsulation, cellular seeding density, CHO cell, endostain

INTRODUCTION

Microencapsulation technology is an alternative biotechnology that has been applied to a wide range of therapeutic treatments including anemia (Koo *et al.*,[1]) dwarfism (Chang *et al.*,[2] ; Al-Hendy *et al.*,[3]), hemophilia B (Liu *et al.*,[4]; Hortelano *et al.*,[5]), kidney (Cieslinski *et al.*,[6]) and liver failure (Chang *et al.*,[7]),, pituitary (Aebischer *et al.*,[8]), Central Nervous System (CNS) insufficiencies (Aebischer *et al.*,[9]; Maysinger *et al.*,[10]) and diabetes (Lim *et al.*,[11]; Soon-Shiong *et al.*,[12]; Soon-Shiong *et al.*,[13]; Grohn *et al.*,[14]) and it is first applied for large-scale mammalian cell culture by Damon Biotech (Posillico *et al.*,[15]) in the early 1980s. Microencapsulated cell culture has many advantages over other methods of cell immobilization in mammalian cell culture. First, liquid core of microcapsule gives a larger free space for cells growth and the microcapsule membrane confines cells inside the microcapsule which increases the cell-to-cell and cell-to-matrix interactions through direct contact and/or secreted protein molecules such as growth factor, hormone, therefore microencapsulated cell culture benefits to cell growth and can obtain higher cell density. The maximal cell density can reach 10^8 cells mL^{-1} microcapsule in microencapsulated hybridoma cell culture (Posillico *et al.*,[15]). Second, there is less mass transfer resistance inside the microcapsule and the microcapsule membrane allows bi-directional diffusion of nutrients, oxygen and toxic metabolites, thus provides a mild and comfortable environment for cell growth. Last, the microcapsule can protect cells from mechanical damage of shear forces associated with agitation and aeration; therefore microencapsulated cell culture can be stirred at higher rate, which can improve nutrients and oxygen exchange.

The cellular seeding density is an important parameter in immobilized cell culture, it is different in different culture conditions. As one desired to produce large amounts of protein, the high cell seeding density are frequently used. Supplying insufficient inoculum could result in a retard of growth rate and a reduction of growth extent. W. S. Hu (Hu *et al.*,[16]; Hu *et al.*,[17]) reported that

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the cellular seeding density obviously effected cells growth on microcarriers, the maximal cell density could attain 1.2×10^6 cells mL^{-1} when seeding density was 4×10^5 cells mL^{-1}. Decreasing the seeding density resulted in an obvious reduction of both growth rate and growth extent. For microencapsulated cell culture a minimum cellular feeding density is necessary to initiate a batch culture. L. Arús *et al.* (Arús *et al.*,[18]) had studied the effects of cellular.seeding density on microencapsulated hybridoma cells, the results showed that the seeding density was at least 1×10^7 cells mL^{-1} for the microencapsulated cells to keep survival and growth and the lower cell seeding densities (1×10^6 or 5×10^6 cells mL^{-1}) showed poor cell growth. The cellular seeding density affected also production of monoclonal antibody, when seeding density was 1×10^7 cells mL^{-1} microcapsule; the maximal monoclonal antibody concentration was 29.1 μg mL^{-1} at day 17. But the initial seeding density of 1×10^7 cells mL^{-1} was considerably high, even exceeded the maximal cell density in suspension, it increased the difficulty of inoculum cells culture and confined the application of microencapsulation technology in cell culture. Therefore it was necessary to optimize the cellular seeding density for acquiring better cell growth and production of recombinant protein in lower cellular seeding density. In this study, the microcapsules with different cellular seeding density were prepared to determine the effects of the cellular seeding density on microencapsulated recombinant CHO cells growth, metabolism and endostatin production.

MATERIALS AND METHODS

Cell line and culture medium: A recombinant CHO cell line transfected with the endostatin gene (CHO-endo) were kindly donated by Dr. Huaining Teng (Shanghai institutes for biological sciences, Chinese Academy of Sciences) .The cells were routinely cultivated in 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, Beijing, China), 100 units mL^{-1} penicillin and 100 μg 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 (Ma *et al.*,[19]). Briefly, exponentially growing CHO cells were harvested and resuspended in a 1.5% (w/v) filtered sodium alginate solution (Qingdao, China). Four microcapsules with different cell density were.prepared: 5×10^5, 10^6, 3×10^6 and 10^7 cells mL^{-1} microcapsule. The cell suspension was extruded through a 0.4-mm needle into a 100 mmol CaCl2 solution using an electrostatic droplet generator to form calcium alginate gel beads. The gel beads were incubated with 0.05% w/v poly-L-lysine (Mw21, 900; Sigma, U.S.A) to form alginate-poly-L-lysine membrane around the surface. After washing the beads in saline, a further coating of alginate was applied by suspending them in 0.15% (w/v) alginate for a few minutes. The membrane-enclosed gel beads were further suspended in 55 mmol sodium citrate to liquefy the alginate gel core. The APA microcapsules were 300-350 μ m in diameter. The microcapsules with recombinant CHO cells were cultured at 37°C in a humidified 5% CO2 atmosphere.

Batch cultures at different cellular seeding density: The microcapsules with different seeding density were suspended with DMEM/F12 (1:1) medium and 0.1 mL microcapsule with 1mL medium were incubated into each well of 24-well tissue culture plates in triplicate and incubated at 37°C in a humidified 5% CO2 atmosphere. The medium contained 32.5 mmol glucose and 8mmol glutamine. The culture supernatants were collected and kept frozen at -20 every day for later analyses.

Mtt assay of microencapsulated cells: The viable cell concentration in the microcapsules was determined by MTT [3-(4,5-dimethylthiazol-2-vl)-2,5-diphenyltetrazoliumbromide] assay. MTT assay was performed as described previously with modification (Zhang *et al.*,[20]). Briefly, 100 μL MTT solutions (5 mg mL^{-1}; Sigma, U.S.A.) was added into each well of 24-well tissue culture plates and incubated at 37°C for 24 h. The medium containing MTT was removed and microcapsules were washed twice with 0.9% saline and then add 1mL DMSO to solubilize the MTT tetrazolium. The Absorbance (A) was determined at 570 nm and 630 nm as reference using a plate reader (Wellscan MK3, Labsystems, Finland). Triplicate samples were used for each time point and the results were expressed as mean±SD. The cell number was calculated from the value of OD570 according to a standard curve. In certain range, the value of OD570 is proportional to viable cell number. The recombinant CHO cells in serials of number were microencapsulated in APA microcapsule and the value.of OD570 of these microencapsulated CHO cells was measure using above method in order to get the standard curve of cell number versus 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 instruction.

Determination of concentrations of glucose, lactate, glutamine and ammonium: The concentrations of glucose and lactate were measured using SBA-40C bio-sensitive analyzer (Jinan, China). The concentration of glutamine was analyzed as described previously with modification (Chen *et al.*,[21]). First, the culture supernatants were boiled 30 min in boiling water, the glutamine was hydrolyzed to glutamate and then the glutamate was measured using 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,

$$\mu = (\ln X_2 - \ln X_1)/(t_2 - t_1) \quad (1)$$

Where X1, X2 is viable cell density at time ,t ,2t .

The specific consumption rates of glucose, glutamine and the specific production rates lactate, ammonium for the metabolic parameters were calculated by the general formula,

$$q = [(C_2 - C_1)/(t_2 - t_1)]/[(X_2 - X_1)/(\ln X_2 - \ln X_1)] \quad (2)$$

Where C1, C2 is the concentration of glucose, glutamine or lactate, ammonium at time t1, t_2 and X_1, X_2 is viable cell density at time t_1, t_2.

RESULTS AND DISCUSSION

Effects of cellular seeding density on cell growth: The growth profiles for microencapsulated CHO cells at various seeding densities were.showed in Fig. 1. With an initial seeding density of 3×10^6 cells mL^{-1} microcapsule, a maximal viable cell density (Xmax) of $1.91\pm0.18\times10^7$ (average±standard deviation, n = 3) cells mL^{-1} microcapsule were obtained at day 5 and cell number increased 4.57-fold. Though decreasing cellular seeding density to 1×10^6 cells mL^{-1} microcapsule could get a higher cell multiplication ratio, it also resulted in a final growth extent decrease. The cell multiplication ratio could reach 7.53-fold but the maximal cells density could only reach $1.37\pm0.09\times10^7$ cells mL^{-1} microcapsule. Further decreasing seeding density to 5×10^5 cells mL^{-1} microcapsule resulted in an obvious decrease of cell growth rate and growth extent. Maximal cells density only reached $4.46\pm0.68\times10^6$ cells mL^{-1} microcapsule and cell number only increased 5.32-fold. When seeding density increased to 1×10^7 cells mL^{-1} microcapsule, the maximal cell density only reached $1.49\pm0.19\times10^7$ cells mL^{-1} microcapsule and cellular multiplication ratio was only 1.68-fold.

The maximal specific growth rates (μ_{max}) were presented in Table 1. The μ_{max} was largest when seeding density was 1×10^6 cells mL^{-1} microcapsule, attained 0.616 d^{-1} at day 7. The μ_{max} was decreased to 0.325 d^{-1} or 0.214 d^{-1} as cells seeding density was increased to 3×10 6 or 1×10 7 cells mL^{-1} microcapsule. And the μ^{max} was only 0.243 when cells seeding density was 5×10 5 cells mL^{-1} microcapsule.

There was no lag phase in cell growth when seeding density was 1×10^7 cells mL^{-1} microcapsule, the cells rapidly grew and enter exponential growth phase at first day. But the cells growth span was only 4 days, the viable cell density declined after day 4. This growth decline was not due to the exhaustion of any macro nutrient components, but the inhibition of metabolic byproducts resulted in the decline of μ in the early stage of cultivation. The metabolism of glucose and glutamine produced a mass of lactate and ammonium due to cell rapid growth in a quite short time (Fig. 2 and 3); the

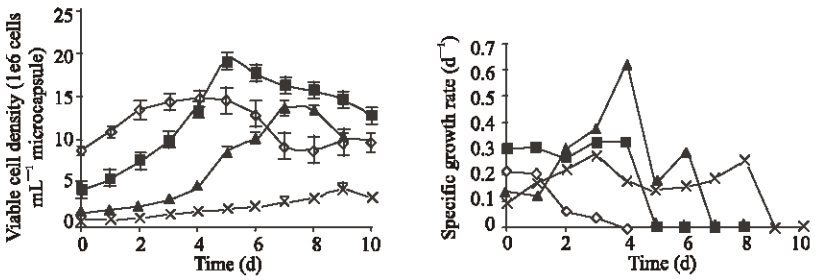

Fig. 1: Effects of different cellular seeding density on growth of CHO-endo cells in batch cultures. The cellular seeding density in the microcapsules were 1×10^7 cells mL^{-1} microcapsule (◇), 3×10^6 cells mL^{-1} microcapsule (■), 1×10^6 cells mL^{-1} microcapsule (▲), 5×10^5 cells mL^{-1} microcapsule (×)

Table 1: Culture data of microencapsulated CHO cells in different cellular feeding density

	Feeding density			
	1×10^7 cells mL^{-1}	3×10^6 cells mL^{-1}	1×10^6 cells mL^{-1}	5×10^5 cells mL^{-1}
X_{max} ($\times 10^6$ cells)	14.9	19.1	13.7	4.46
μ_{max} (d^{-1})	0.214	0.325	0.616	0.243
Multiplication ratio	1.68	4.57	7.53	5.32
q_{glc} (μmol/1 $\times 10^6$ cells*d)	3.99	3.80	3.99	3.90
q_{lac} (μmol/1 $\times 10^6$ cells*d)	7.07	6.71	5.66	5.57
q_{gln} (μmol/1 $\times 10^6$ cells*d)	1.10	1.05	1.13	1.17
q_{amm} (μmol/1 $\times 10^6$ cells*d)	1.04	0.39	0.53	0.86
$Y_{x/glc}$ (1 $\times 10^9$ cells/mmol)	3.24	8.01	6.37	4.76
$Y_{x/gln}$ (1 $\times 10^9$ cells/mmol)	9.25	29.08	22.37	15.89
$Y_{lac/glc}$ (mmol/mmol)	1.895	1.770	1.730	1.357
$Y_{amm/gln}$ (mmol/mmol)	0.942	0.351	0.596	0.697

concentration of lactate reached 23.67 mmol at day 2, notably higher than the concentration reported in the literature as being inhibitory (18 mmol; Kurano *et al.*,[22]). Although the concentration of metabolic byproducts were rather high at the end of the cultures when the seeding density was 3×10^6 or 1×10^6 cells mL^{-1} microcapsule, the cell growth was not inhibited. It was because the metabolic byproducts were gradually produced during the cultures and the cells could gradually adapt the environment of high lactate and ammonium concentration, therefore the inhibition of the lactate and ammonium on cells growth would decrease. The lactate concentration exceeded by inhibitory concentration at day 4 or day 7.when seeding density were 3×10^6 or 1×10^6 cells mL^{-1} microcapsule, prolonged the cell growth span which benefited to acquire higher cell density and larger multiplication ratio. There were only few cells even no cells in each microcapsule when seeding density was 5×10^5 cells mL^{-1} microcapsule, it effected cell-to-cell and cell-to-matrix interactions through direct contact and/or secreted protein molecules such as growth factor, hormone. So the cells growth was retarded and only attained a lower cell density. In conclusion, the suited cellular seeding density was between 1×10^6 cells mL^{-1} microcapsule and 3×10^6 cells mL^{-1} microcapsule and the higher seeding density (3×10^6 cells mL^{-1} microcapsule) benefited to acquire higher cells concentration and lower seeding density (1×10^6 cells mL^{-1} microcapsule) benefit to acquire larger multiplication ratio.

Effects of cellular seeding density on glucose metabolism: The effects of cellular seeding density on glucose consumption and lactate production were showed in Fig. 2. When cellular seeding density was higher, catabolism rate of glucose was faster to provide more energy for cell growth and cell survival and thus generated more lactate which could inhibit cell growth and cell viability of metabolism. The consumption of glucose and production of lactate was very rapid at the first day when cellular seeding density was 1×10^7 cells mL^{-1} microcapsule and the lactate concentration reached 23.67 mmol. Subsequently _Cthe consumption of glucose and production of lactate rapidly decreased and almost ceased after day 5, the glucose was not exhausted at the end of culture. The rates of glucose consumption and lactate production slowed when cellular seeding density was 3×10^6 or 1×10^6 cells mL^{-1} microcapsule, it prolonged the span of culture. Although the lactate concentration was high at the end of culture, the cell growth and glucose metabolism were not apparently inhibited.

The specific glucose consumption rate (q_{glc}) and lactate production rate (q_{lac}) during cell growth were presented in Table 1. The shift of the cellular seeding density didn't affect the specific glucose consumption rate. The qglc was from 3.90 mmol/1 $\times10^6$ cells*d at a seeding density of 5×10^5 cells mL^{-1} microcapsule to 3.99 mmol/1 $\times10^6$ cells*d at 1×10^7 cells mL^{-1} microcapsule. But the efficiency of glucose utilization was different when the cellular seeding density was changed. The yield of cell from glucose ($Y_{x/glc}$) was highest as the cellular seeding.density was 3×10^6 cells mL^{-1} microcapsule, attained 8.01 $\times10^6$ cells/mmol. With the decrease of the cellular seeding density to 1×10^6 or 5×10^5 cells mL^{-1} microcapsule, the Yx/glc decreased to 6.37 $\times10^6$ cells/mmol or 4.76 $\times10^6$ cells/mmol, the decrease was 20.5% or 40.6% compared with the seeding density of 3×10^6 cells mL^{-1} microcapsule. The $Y_{x/glc}$ decreased to 3.24 $\times10^6$ cells/mmol when the cellular seeding density increased to 1×10^7 cells mL^{-1} microcapsule, decreased by 59.6%. As the cellular seeding density increased the specific lactate production rate increased, the qglc was increased from 5.57 mmol/1 $\times10^6$ cells*d at the seeding density of 5×10^5 cells mL^{-1} microcapsule to 7.07 mmol/1 $\times10^6$ cells*d at the seeding density of 1×10^7 cells mL^{-1} microcapsule, an increase of 26.9% was observed. The yield of lactate from glucose ($Y_{lac/glc}$) decreased with the decrease of the cellular seeding density. The $Y_{lac/glc}$ decreased from 1.895 to 1.357 with the cellular seeding density decreasing from 1×10 7 cells mL^{-1} microcapsule to 5×10^5 cells mL^{-1} microcapsule, decreased 28.4%.

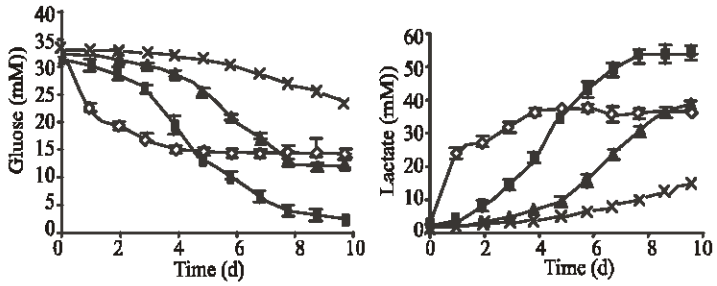

Fig. 2: Effects of different cellular seeding density on metabolism of glucose and production of lactate in batch cultures. The cellular seeding density in the microcapsules were 1×10^7 cells mL^{-1} microcapsule (◊), 3×10^6 cells mL^{-1} microcapsule (■), 1×10^6 cells mL^{-1} microcapsule (▲), 5×10^5 cells mL^{-1} microcapsule (×)

Glucose was main carbon sources and important energy sources in mammalian cell culture. High rate of glucose consumption and lactate production were the characteristic of cell culture in vitro and the requirement of rapid cell proliferation. (Baggetto *et al.*,[23]) The glucose was predominantly metabolized by the glycolytic pathway and produced pyruvate as the end product, the pyruvate was transformed to lactate to recover the oxidation of cofactor Nicotinamide Adenine Dinucieotide (NAD). Only a very small amount of glucose was completely oxidized to carbon dioxide, even in the presence of saturating oxygen (Sharfstein *et al.*,[24]). And a few of glucose metabolized by the pentose phosphate pathway to provide ribose-5phosphate for nucleic acid synthesis (Sharfstein *et al.*,[24]). Due to large amount of glucose produced lactate, the accumulation of lactate always occurred in cell cultures. The accumulation of lactate would lead to the acidification of the culture environment and the change in medium osmolarity, therefore the growth and metabolism of cells and production of recombinant protein would be inhibited (Kurano *et al.*,[22]). The results of this study indicated a significant metabolic shift in different cellular seeding density. Although the specific glucose consumption rate was similar in all cultures, the utilization of glucose was significantly different. The $Y_{x/glc}$ was highest as cellular seeding density was 3×10^6 cells mL^{-1} microcapsule. It demonstrated that more glucose enter into the pentose phosphate pathway to synthesize ribose-5phosphate for nucleic acid synthesis. The $Y_{lac/glc}$ decreased with the decrease of the.cellular seeding density. The high lactate production rates at a cellular seeding density of 1×10^7 cells mL^{-1} microcapsule indicated the cells metabolism was more wasteful with respect to carbon utilization. The more glucose entered into TCA and was completely oxidized to carbon dioxide when the cellular seeding density was lower (3×10^6 cells mL^{-1} microcapsule or 1×10^6 cells mL^{-1} microcapsule). The decrease of lactate production benefited to cell proliferation and the prolongation of cell growth span, therefore it could acquire higher cell concentration.

Effects of cellularseeding density on glutamine metabolism: The effects of cellular seeding density on glutamine consumption and ammonium production were showed in Fig. 3. As the cellular seeding density increased the rates of glutamine consumption and ammonia production increased, similar to the results of glucose metabolism. The glutamine wasn't exhausted as the maximum viable cell concentration was reached in all cultures, it indicated that the glutamine wasn't limiting nutrient. The ammonium concentration was increased when the seeding density was 1×10^7 cells mL^{-1} microcapsule, 1×10^6 cells mL^{-1} microcapsule or 5×10^5 cells mL^{-1} microcapsule. But when the seeding density was 3×10^6 cells mL^{-1} microcapsule, the ammonium concentration was increased at first 6 days and then gradual decreased, the ammonium was almost exhausted at the end of culture. It would be that the demand of amino acid was large due to cell concentration was high, the cells utilized ammonium for macromolecule synthesis after glutamine was exhausted. The specific glutamine consumption rate (qgln) and ammonium production rate (qamm) during cell growth were presented in Table 1.

The specific glutamine consumption rate wasn't significantly different when the seeding density was changed. The yield of cell from glutamine ($Y_{x/gln}$) was highest as the cellular seeding density was 3×10 6 cells mL^{-1} microcapsule, attained 29.08 $\times10$ 6 cells/mmol. With the decrease of the cellular seeding density, the Yx/gln decreased, the Yx/gln was decreased to 22.37 $\times10^6$ cells/mmol or 15.89 $\times10^6$ cells/mmol when seeding density decreased to 1×10 6 cells mL^{-1} microcapsule or 5×10 5 cells mL^{-1} microcapsule, decreased 23.1% or 45.4%. And the Yx/gln was lowest when the cellular seeding density was 1×10^7 cells mL^{-1} microcapsule, only reached 9.25 $\times10^6$ cells/mmol and decreased 68.2%. The specific ammonium production rate (q_{amm}) was only 0.39 mmol/1 $\times10^6$

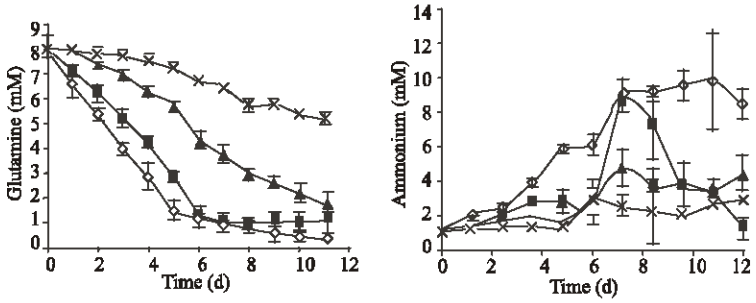

Fig. 3: Effects of different cellular seeding density on metabolism of glutamine and production of ammonium in batch cultures. The cellular seeding density in the microcapsules were 1×10^7 cells mL^{-1} microcapsule(◇), 3×10^6 cells mL^{-1} microcapsule(■), 1×10^6 cells mL^{-1} microcapsule (▲), 5×10 5 cells mL^{-1} microcapsule (×)

cells*d when the cellular seeding density was 3×10^6 cells mL^{-1} microcapsule, lower than other cellular seeding density.

The glutamine was another essential nutrient in mammalian cell culture; it was not only an.amino group donor in some biosynthetic pathways such as purine and pyrimidine synthesis, but also a major energy source (Sharfstein *et al.*,[29]). Glutamine catabolism would yield α-ketoglutarate and release ammonium molecule, the amido group would be utilized to synthesize some biomolecules, mainly of the purine and pyrimidine by some biosynthetic pathways (Swierczynski *et al.*,[25]). Rapid glutamine metabolism was the characteristic of cells rapid growth and the rapid metabolism of glutamine would result in the accumulation of ammonium in media (Zhou *et al.*,[26]). High ammonium concentration could inhibit cell growth and reduce metabolism efficiency. The results of this study showed that the yield of ammonium from glutamine ($Y_{amm/gln}$) and the specific ammonium production rate (q_{amm}) was lowest when the cellular seeding density of 3×10^6 cells mL^{-1} microcapsule. It indicated that more ammonium was utilized to synthesize biomolecules for cell proliferation and protein production, the efficiency of glutamine metabolism was highest. Although the rate of glutamine metabolism was quite rapid when the cellular seeding density was 1×10^7 cells mL^{-1} microcapsule, the higher ammonium concentrations leaded to a futile cycle of glutamine metabolism and an increase of maintenance energy. A majority of energy produced by glucose and glutamine was not utilized for cell growth or recombinant protein production, but was dissipated as heat. Therefore the metabolism efficiency of glucose and glutamine was reduced.

Effects of cellular seeding density on endostatin production: The final yield of endostatin was showed in Fig. 4. The maximum yield of 615.1ng mL^{-1} was obtained when cellular seeding density was 3×10^6 cells mL^{-1}

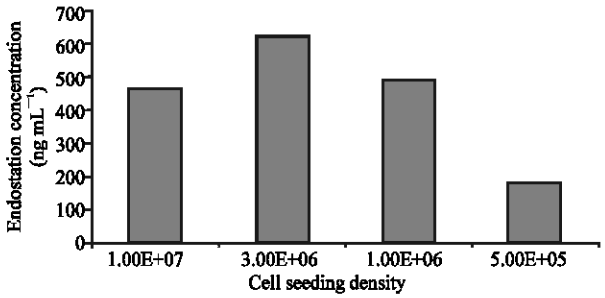

Fig. 4: Effects of different cellular seeding density on production of endostatin in batch cultures

microcapsule. With the decrease of seeding density, the final concentration of endostatin in the media reduced. As cellular seeding density decreased to 1×10^6 cells mL^{-1} microcapsule or 5×10^5 cells mL^{-1} microcapsule, the final concentration of when cellular seeding density increased to 1×10^7 cells mL^{-1} microcapsule, the final concentration of endostatin was only 455.7ng mL^{-1}, decreased by 25.9% compared to the seeding density at 3×10^6 cells mL^{-1} microcapsule.

The production of recombinant protein was associated with the viable cell concentration; higher cell density could be benefit to the higher expression of recombinant protein. The maximum viable cell density was highest when cellular seeding density was 3×10^6 cells mL^{-1} microcapsule, so the final yield of endostatin was highest. This result corresponded with the results of nutrients metabolism, when the cellular seeding density was 3×10^6 cells mL^{-1} microcapsule, the efficiency of glucose and glutamine metabolism were higher, more nutrients were utilized to synthesize endostatin.

CONCLUSION

The cellular seeding density was important control parameter in mammalian cell culture; it directly effected

cell growth, metabolism and protein production. Optimization of the cellular seeding density was important for establishing ideal conditions in microencapsulated cell culture. In this study, the effects of cellular seeding density on microencapsulated cells growth, metabolism and production of recombinant protein was studied. The cell growth was strongly effectd by the cellular seeding density; the cell growth was rapidest as the cellular seeding density was 3×10^6 cells mL^{-1} microcapsule and the maximum cell density reached $1.91\pm0.18\times10^7$ cells mL^{-1} microcapsule. The metabolism of cells was notably altered when the cellular seeding density was changed from 1×10^7 cells mL^{-1} microcapsule to 5×10^5 cells mL^{-1} microcapsule. Although the uptake rate of glucose wasn't markedly different at various seeding density, the efficiency of glucose utilization was significantly different. With the decrease of the cellular seeding density, the qlac and the $Y_{lac/glc}$ decreased. The qamm and the $Y_{amm/gln}$ was lowest when the cellular seeding density was 3×10^6 cells mL^{-1} microcapsule, it indicated that the efficiency of glutamine utilization was highest. The maximal yield of endostatin was observed at a cellular seeding density of 3×10^6 cells mL^{-1} microcapsule, the final concentration was 615.1ng mL^{-1}. So, optimized seeding density was 3×10^6 cells mL^{-1} microcapsule in this microencapsulated recombinant CHO cells culture. Not only the higher cell density and yield of endostatin were observed at this seeding density, but also the higher efficiency of nutrient utilization was acquired.

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