O6-3	**Encapsulation of animal cells as a tool to obtain high density cell cultures and increased recombinant protein production in bioreactors: A realistic goal or pipe dream**	

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INTRODUCTION

The use of animal cell culture is one of only a few methods which can produce highly glycosylated recombinant proteins for medical applications (Breguet 2007a). However, by comparison with yeast and bacteria, mammalian cells are relatively fragile and as a result high density cell cultures in bioreactors may be limited by mechanical and shear forces, which result from agitation and aeration. This leads to a low specific productivity and maximum cell density. Productivity can be increased by using perfusion cultures with cell retention systems; however this can be very complex and expensive (Breguet 2007a).

Immobilization of animal cells within the confines of a semi-permeable hydrogel membrane has proving to be a promising technique to provide an optimal growth environment over conventional suspension cultures. This is the case as it provides an environment free from destructive hydrodynamic shear stresses and forces, thus effectively enhancing cell growth, viability and recovery of the product (Gugerli 2002).

Several types of microcapsules have been used for growing mammalian cells. However, it has yet to be demonstrated whether such systems offer advantages over classical suspension cultures in terms of specific growth rate and productivity (Breguet 2007a). Numerous cell encapsulation studies have focused on using the classical alginate/poly-L-lysine (PLL) system developed by Lim and Sun in the 1970's.

Our research group is focused on developing and implementing new encapsulation and analytical (PAT) methods to increase the overall production and quality of recombinant proteins in animal cell cultures; the overall aim to transfer this knowledge to an industrial scale with a commercial partner. This short paper will give a brief description of some of the results obtained in our lab, while also discussing limitations, and present work to obtain the overall objective.

EXPERIMENTS AND RESULTS

Encapsulation of animal cells

CHO SSF3 cells, which produce recombinant human secretary component (rhSC), where encapsulated within the classical alginate-PLL system. Microcapsules were prepared using the vibrating nozzle technique performed on an Inotech Encapsulator and encapsulated cells were compared to freely suspended cells when added to a bioreactor. Table 1 show the results of this experiment (Breguet 2007a). In contrast with the suspension cell culture (μ=0.028 h^{-1}), the specific growth rate in the capsules decreased with time (from 0.027 to 0.013 h^{-1}). The microcapsules were completely retained within the bioreactor, becoming colonized with cells to a maximum concentration of 4.4 X 10^6 cell·mL^{-1}reactor (6.6 X 10^7 cell·mL^{-1} caps), which most importantly is over 3-fold higher compared to the suspension culture. This enabled more than a 3-fold increase in the production of the recombinant protein despite a reduction in the specific growth rate. An advantage of this system is that much higher cell densities may be attained in the bioreactor (greater than 10^7 cell·mL^{-1} reactor), simply by increasing the volume of microcapsules. The unexpectedly low colonization values (17%) indicate that cells were not able to grow freely within the core of the microcapsules.

Table 1: Comparison of freely suspended and encapsulated CHO SSF3 cells when cultured in a bioreactor to produce (rhSC)

Value	Units	Suspension cells	Encapsulated cells
1st μ	h^{-1}	0.028	0.027
2nd μ	h^{-1}	-	0.013
Time	h	160	650
X_{max} reactor	cell·mL^{-1} reactor	1.41 X10^6	4.4 X 10^6
$X_{max\ caps}$	cell·mL^{-1} caps	-	6.6 X 10^7
$Cells_{max}$	cell·$caps^{-1}$	-	17,000
б*	%	-	17
q_P	mg·$cell^{-1}h^{-1}$	1.7 X 10^{-9}	5.3 X 10^{-9}

б* - % of capsule occupied by the cells

Limitations of cell encapsulation

As shown in numerous studies the alginate/PLL system for cell encapsulation is subjected to two main limitations which prevent its application at an industrial level. These include (1) the poor mechanical stability of the alginate hydrogel and its sensitivity towards chelating agents such as phosphate and citrate, or other anti-gelling agents like Na^+ and Mg^{2+}. These can reduce the mechanical stability of the hydrogel and in some cases cause complete dissolution of the gel network structure (Breguet 2007b) and (2) the inability of the encapsulated cells to fully colonize the inner core. After dissolution of the core large amounts of the alginate (46-67%), which is termed intracapsular alginate, can remain inside the core and will inhibit the growth of the entrapped cells (Breguet 2007b).

In our lab we are looking to overcome these problems by examining the use of different polymer systems to make stronger and more robust microcapsules, as well as directly preparing aqueous-core microcapsules surrounded by a hydrogel membrane.

Polymer choice (mechanical stability)

While a vast amount of polymers can be used to make microcapsules, most work has focused on using alginate. In our research group we have obtained a polymer (termed polymer X) which has shown the capability to make the desired microcapsules using a simple, gentle, rapid and reproducible process. The main advantages of this system over the conventional alginate-PLL system is its ability to produce stronger and more resistant capsules (Table 2), whilst also being able to fully control the molecular weight cut off of the membrane, and also its thickness.

Table 2: Comparison of the mechanical resistance of capsules made from different polymers

Polymer System	Force to Break Capsule (g)
Alginate-PLL (30-70 kDa)	2.09
Polymer X (15 kDa)	80.2
" (22.5 kDa)	70.43
" (25 kDa)	65.34
" (35 kDa)	34.34

Cell growth within microcapsules (colonizing of the capsule core)

Mammalian cell growth and viability within alginate/PLL microcapsules is limited, and is believed to be caused by the presence of intracapsular alginate within the core (Breguet 2007b). In our lab we are developing methods to avoid the use of polymers such as alginate in the core of the capsules by directly preparing fully aqueous-core microcapsules surrounded by a hydrogel membrane (Figure 1). In this case the cells can freely proliferate to completely colonize the core and achieve very high cell densities. At present this work is at a preliminary stage but to date has shown promising results to help prevent growth limitation and achieve high levels of cell colonization.

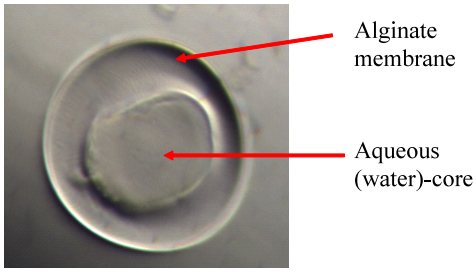

Figure 1: (a) Light microscope image displaying a microcapsules core, which is completely aqueous, produced directly using the co-extrusion jet break up technique.

PAT and cell encapsulation

At present the main goal of our research group is to integrate animal cell encapsulation with PAT tools involved in the monitoring and control of bioprocessing operations (Figure 2), in order to help develop further a better understanding of the behaviour of the encapsulated culture within the microcapsules; the overall aim being to further increase the specific growth and productivity of the desired product.

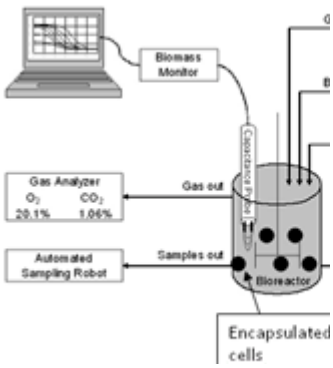

Figure 2: Schematic of the PAT setup used in our lab to help monitor encapsulated cells and other essential conditions involved in obtaining high density cell cultures and increased product concentrations

CONCLUSION

While a lot of work (and breakthroughs) is still required in order for cell encapsulation to be used as a tool for increasing recombinant protein production at a large scale industrial level; recent advances make this goal quite obtainable. These developments include the ability to produce stronger microcapsules under very simple conditions, whereby the capsules produced bear a core which is completely liquid, and this should enable high levels of cell colonizing to be reached. The recent application of PAT tools to help monitor and control key conditions for cell growth and product production should fast-track the application of animal cell encapsulation as a viable methodology to produce large quantities of recombinant glycosylated proteins at an industrial level – turning this pipe dream of the past into a very obtainable and realistic goal of the future.

REFERENCES

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