Application of cells microbeads for bioartificial liver in fluidized bed bioreactor

A. Gautier, B. Carpentier, M. Dufresne, P. Paullier, C. Legallais*

Université de Technologie de Compiègne, CNRS UMR 6600,
Biomechanics and Biomedical Engineering, Compiègne, France
(cecile.legallais@utc.fr)

Introduction

The study consists in the optimization of a bioartificial liver (BAL) allowing patients suffering from hepatic diseases to wait for a graft. BAL principle is to exploit performances of hepatocytes immobilized into an extracorporeal bioreactor to mimic liver functions. In our system, hepatocytes are entrapped in a tri-dimensional anchorage framework : alginate beads. This immobilization allows preservation of cells in a hospitable environment, the porous structure of alginate ensuring their immuno-protection [1] without preventing any interaction with the external medium. Indeed, alginate porosity allows transfers of nutrients, oxygen and metabolites.

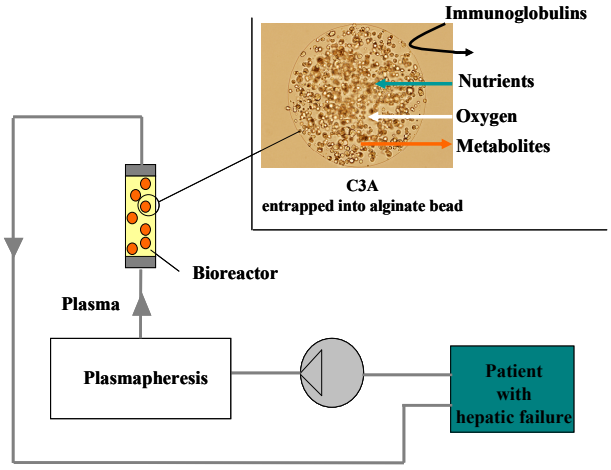

Figure 1 Bioartificial liver system

The alginate beads are then placed into a fluidized bed bioreactor (FBBAL) (Figure 1). This technology issued from chemical engineering achieves a permanent bead motion in a confined volume. As for other tissue engineering application, a key issue in the success of such reactor is the maintenance of high cell viability and functionality through adequate oxygen and nutrients supply. Diffusive mass transfer takes place in the bead itself while additional convection effects occur in the supernatant. To our knowledge, data on the optimum size of beads in the FBBAL have not been established yet.

Bead production should be optimized according to the following parameters: diameter, alginate density, alginate type. For this purpose, both mass transfer effects and cells functions should be considered as output. For liver supply, substances such as albumin, urea, ammonia, glucose are exchanged between the surrounding media (the patient's plasma in clinical application) and the encapsulated hepatocytes.

Material and Methods

Cells encapsulation into alginate bead

C3A cells provided by ATCC (American Type Culture Collection) were cultivated in standard T75 Falcon culture flask (Merk Eurolab, Strasbourg, France) with 20 ml of Minimum Essential Medium Earles salts (MEM), L-glutamine supplemented with penicillin (100 units/ml) and streptomycin (100 µg/ml), 1 % of non-essential Amino Acids, 1 % of Hepes buffer solution 1 M, 1 % of sodium pyruvate 100 mM and 10 % of fetal calf serum (FCS), all purchased from Gibco (Cergy Pontoise, France). The number of cells in confluence ranged from 15 to 20 millions cells per flask.

Low viscosity alginate was purchased from Sigma (Saint Quentin, France). First, cells were detached, counted and gently mixed with sterile alginate solution 2,2%. Ten mL of the final suspension containing 5. 10^6 cells /mL of alginate were extruded as droplets through a nozzle system (external diameter: 0.55 mm, length : 25 mm) designed in our laboratory. Droplets were size controlled (600 µm and 1000 µm diameter) using parallel air flow and gellified in a casting solution $CaCl_2$ (Figure 3).

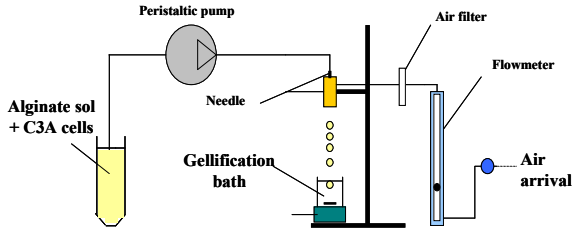

Figure 2 : Airjet method for C3A bioencapsulation

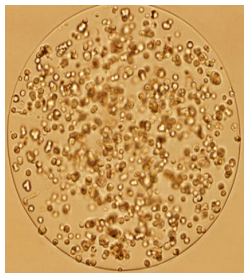

Figure 3 : C3A hepatocytes entrapped into alginate bead (1000 µm diameter)

The alginate beads hosting cells were then placed into the bioreactor (height: 17 cm, internal diameter: 1.35 cm) and fluidized at a flow rate adapted to optimal fluidization. The height of the fluidized bed was monitored. Samples of culture medium were taken at different times : 0h, 18h, 24h, 42h, 48h. Their contents were analyzed with a biochemistry automatus for urea, ammonia, glucose and alpha-foeto-protein (AFP) or by Elisa method for albumin, to assess cell functions. Cell viability was determined by trypan blue exclusion test or by fluorescence staining with acridine orange and propidium iodide.

Mass transfer studies with Vitamin B12 marker

We performed mass transfer studies with empty beads and vitamin B12 as well calibrated molecular marker [2, 3]. Different types of alginate were used : medium viscosity 1,5 % and low viscosity 2 % and 2,2 %. Once the 600 µm or 1000 µm beads were produced, they were placed in the bioreactor under perfusion conditions. The vitamin B12 concentration (initial concentration C_0 = 7,3.10^{-5} mol/L) was monitored on line at different time steps by spectrophotometer at 360 nm, until equilibrium was reached. Mass balance was then performed to quantify the amount of solutes disappearing from the tank and thus penetrating into the beads. A mass transfer model already described in [2] was applied to determine an overall mass transfer coefficient K for the system.

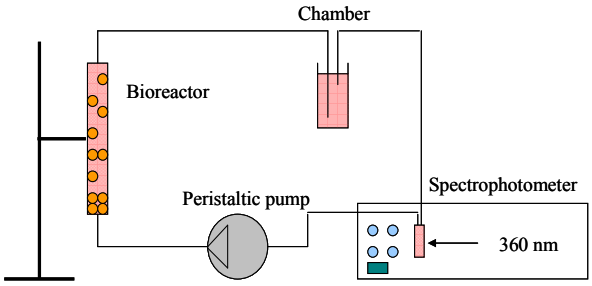

Figure 4 Experimental set up for Vitamin B12 mass transfer study.

Results and Discussion

Effect of bead diameter on cell functions

The functions of C3A cells were assessed in the bioreactor over 48h. Figures 5 and 6 exhibit the time course of urea and AFP concentrations in the supernatant, respectively. These substances covered a broad range of molecular weight, from 60 Da for urea to 69 000 Da for AFP. Both concentrations increased in the supernatant, since these substances synthesized by the cells were then released through the porous structure of alginate. We intuitively expected higher nutrients, oxygen and metabolites transfers with 600 µm bead diameter than 1000 µm, due to a reduced diffusive length in the smaller diameter. Although this trend was respected, the results were not statistically significant, due to large errors bars classical in biological studies.

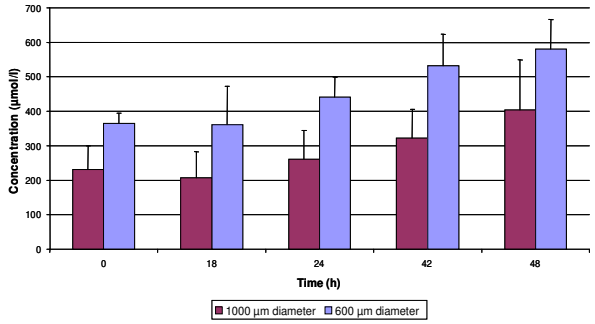

Figure 5 Urea concentration time course in fluidized bed bioreactor

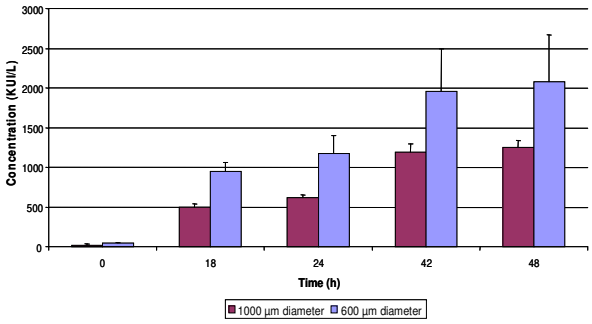

Figure 6 AFP concentration time course in fluidized bed bioreactor

Cell viability was also evaluated with different markers showing alive (Figure 7) and dead cells (Figure 8). Trypan blue test shows that 88 to 92 % of cells were viable after 48h.

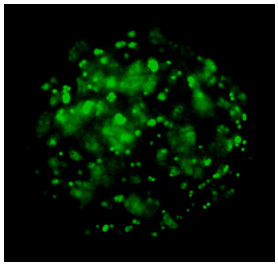

Figure 7 Orange acridine test showing viable cells (green)

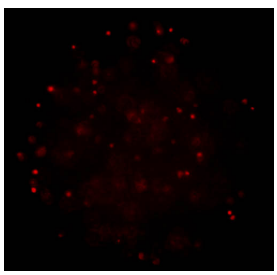

Figure 8 Propidium iodide test showing dead cells (red)

Mass transfer with Vitamin B12

To optimize alginate concentration with alleviated experiments, and to analyze the previous results obtained with cells, a model based on vitamin B12 mass transfer was applied [2]. Figure 9 summarizes the mass transfer coefficients K calculated from the experiments performed with 3 types of alginate and two bead diameters.

With the concentration employed, the type of alginate did not seem to significantly influence the mass transfer efficiency. Surprisingly, mass transfer coefficients were higher for beads of 1000 µm diameter. This trend was attenuated when the bead surface area (A) was taken into account : the area to volume ratio was higher for the 600 µm bead, leading to equivalent mass transfer K*A. Nevertheless, these unexpected results could be due to inadequate gellification time for the small diameter beads, or to different convection effects in the bioreactor.

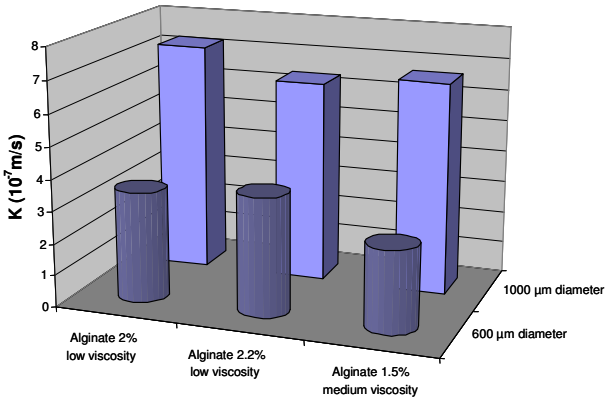

Figure 9 Mass transfer coefficients in the bioreactor for beads 600 µm and 1000 µm

Conclusions

Culture of hepatocytes in the fluidized bed bioreactor was successfully maintained over 48h under sterile conditions. After 48h, cells were viable and demonstrated maintained basic functions such as urea synthesis or AFP production.

According to these preliminary results, there was no significant difference in term of mass transfer or cell viability and functions, related to bead diameters or alginate type. These results however need to be confirmed by further biological assays and mass transfer experiments with a broad range of molecular weight markers.

References

[1] B. David et al. (2004) In vitro assessment of encaspulated C3A hepatocytes functions in a fluidized bed bioreactor. Biotech. Prog. 20 1204-1212

[2] B. David et al. (2004) Mass transfers in a fluidized bed bioreactor using alginate beads for a future bioartificial liver. The International Journal of Artificial Organs 27 (4) 284-293

[3] D. Lewinska et al. (2002) Mass transfer coefficient in characterization of gel beads and microcapsules. Journal of Membrane Science 209 533-540.