Metabolic activity of Jurkat cell line after encapsulation in PGA-HSA coated beads

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Introduction

Calcium alginate gel beads represent the most common method to immobilize living cells (Orive, 2003). The beads are usually coated with a polycationic polymer such as poly-L-lysine (PLL), which forms an ionic bonded membrane at the surface. However, the lack of permanent bonds in the membrane results in a limited stability (Thu, 1996).
In our laboratory, we develop a simple method for creating a stable membrane around alginate beads (Lévy, 1996, Edwards-Lévy, 1999). The method is based on a transacylation reaction between ester groups of an alginate derivative (propylene-glycol alginate, PGA) and free amino groups of a protein, which can be human serum albumin (HSA) for therapeutic purposes. Upon alkalization, covalent amide bonds are created between the two polymers. This reaction, applied around alginate gel beads, can be restricted to external layers of the beads, thus forming a membrane. The method has already been applied to bioencapsulation studies, and hepatocytes (Joly, 1997) or genetically modified cells (Shinya, 1999) survived the procedure and remained functional after encapsulation.
The aim of the present work was to assess if the method could preserve for prolonged periods the viability and functionality of the Jurkat cell line (human T cell leukemia), used as a model. This work also explored the possibility of cryopreserving the encapsulated cells in liquid nitrogen, in such conditions that the membrane integrity and cell viability would be respected.

Material and methods

Preparation of PGA-HSA coated beads

The coated beads were prepared as described elsewhere (Shinya, 1999), with minor modifications. The polymer solution was prepared by dissolving 1.25 % (w/v) sodium alginate (Manucol DH®, ISP), 2.5 % PGA (Kelcoloïd S®, ISP) and 6.25 % HSA (Baxter) in saline. After 2 min centrifugation at 5000 rpm to eliminate air bubbles, 4 ml of this solution was carefully mixed with 1 ml of cell suspension (5.10^6 cells/ml in saline). The beads were then prepared by dropwise addition of the mixture into a 1.7 % $CaCl_2$ solution. After a stirring time of 5 min, the supernatant was eliminated. The transacylation reaction was started by adding the gel beads in a 0.01M NaOH solution. After 5 min stirring, the coated beads were neutralized by soaking in a pH7 buffer for 5 min, and were then rinsed three times with saline, and finally with the culture medium (90 % RPMI 1640 + Glutamax + 10 % decomplemented foetal calf serum (FCS) containing 1% penicillin/streptomycin, Gibco). The encapsulated cells were then placed in a flask with culture medium, in an incubator at 37°C and 5 % CO_2.
Control beads without cells were prepared in the same manner, replacing the cell suspension by saline.

Preparation of control Alginate-PLL-alginate beads

Control gel beads coated with an ionic membrane were prepared from a 1.5 % sodium alginate solution in saline, extruded in the same calcium bath than for the first series. The beads were then rinsed with saline, incubated for 5 min in 25 ml of a 0.05 % PLL (Sigma) solution in saline, rinsed again with saline, and incubated for 3 min in 25 ml of a 0.05 % sodium alginate solution in saline. They were rinsed 3 times with saline, and with the culture medium, before being transferred in a culture flask containing culture medium.

Citrate treatment of the beads

Samples of coated beads were gently soaked for 30 min in 20 ml of a 2 % sodium citrate solution, in order to reliquify the internal calcium alginate gel core. The resulting particles were then rinsed with saline and the membranes surrounding liquid drops were observed.

Microscopic observations

After cell encapsulation, the beads were daily observed with a stereomicroscope (SZ-11, Olympus) equipped with a CCD camera (DP50, Olympus) and using the AnalySIS software (Soft Imaging System) for the measurement of the beads and archiving of the photographs.

Cellular activity assay

Cellular activity inside the beads was periodically quantified using the method of H. Uludag and M.V. Sefton (Uludag 1990). 3 beads were placed in a test tube with 1 ml of culture medium and 250 µl of a 3-[4,5-dimethylthiazol-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma) solution (5 mg/ml in phosphate buffered saline). After a 5 h incubation time at 37°C, the supernatant was discarded and replaced by 2 ml of dimethylsulfoxide (DMSO, Fisher Scientific). The formazan produced by the enzymatic reduction of the MTT by the living cells was recovered by disrupting the beads with a metallic tip, vortexing the tube for 2 min, and centrifuging the mixture at 4°C-400 g for 10 min. The absorbance of the supernatant at 630 nm was subtracted from the absorbance at 530 nm. The same assay was performed on empty beads, and on free cells at various concentrations, as controls. Each assay was triplicated.

Cryopreservation

Bead samples produced 22 days before or 6 days before were placed in cryovials and suspended in three different media, i.e. the culture medium used for the encapsulated cells, or a medium with a high amount of cryoprotective agents (80 % classical culture medium + 20 % DMSO containing 0.25M sucrose (Heng 2004)), or the solution usually used for the cryopreservation of free Jurkat cells (10 % DMSO, 20 % FCS, 70 % RPMI 1640). Prior to freezing in liquid nitrogen, cryovials were cooled at a rate of 1°C/min until the temperature inside the vials reached -80°C. After one week in liquid nitrogen, the morphology of the beads was observed, and the viability of the encapsulated cells was assessed by a MTT assay, 3 days after rapid thawing of the cryovials in a 37°C bath.

Results and Discussion

Morphology and stability of the empty beads

The preparation procedure led to slightly ovoid particles, with an average diameter of about 3 mm, and a smooth surface. The control alginate-PLL-alginate beads presented a similar morphology, but with a brighter surface. The reliquification of internal gel caused by the citrate treatment revealed the presence of a membrane, thick and strong for HSA-PGA membrane, thin and fragile for Alg-PLL membrane. Placed in culture medium at 37°C, the HSA-PGA coated beads survived for more than 1 month without disruption of the membrane, whereas in the same conditions, Alg-PLL membranes appeared burst open after only 2 days.
It has been previously shown that the covalent bonds involved in the membrane network of PGA-HSA coated beads bring to the particles a remarkable stability in different media (Edwards-Lévy, 1999). This experiment confirms that the coated beads could be used for prolonged periods in a classical cell culture medium without any risk of membrane disruption, ensuring then a “cell-proof” coating around the beads.

Intracapsular cell growth

The photographs of figure 1 show the multiplication of cells inside HSA-PGA coated beads, with time. From day 6 after encapsulation, the first cell clusters became visible as little dots. Then, the clusters grew, became bigger spots, and finally filled the entire submembranar space. The experiment was no further followed after day 37, but it would be interesting to observe the

behaviour of encapsulated cells after the complete filling of the internal volume, and also to determine if the cells of the center of the beads were still alive.

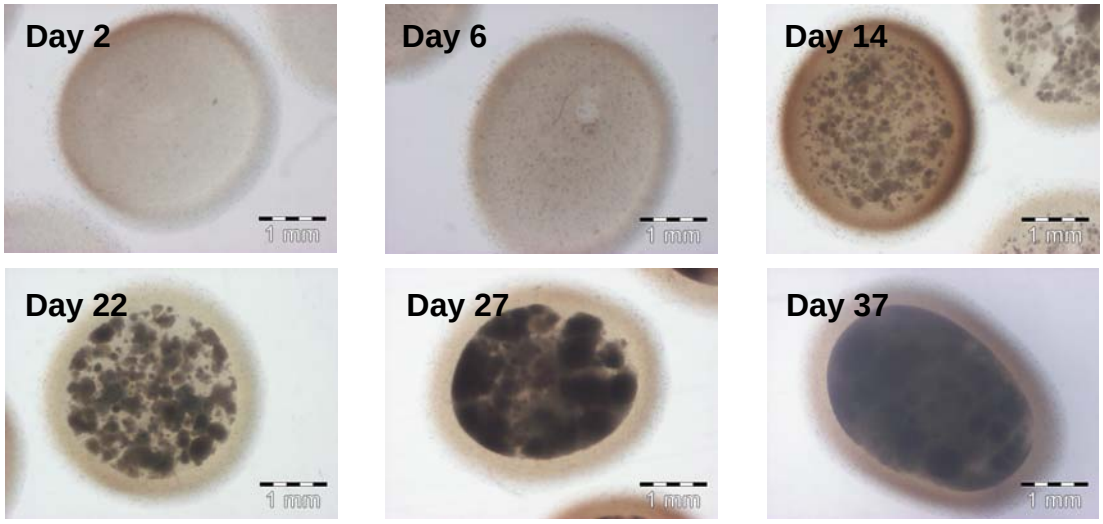

Figure 1. Microphotographs of Jurkat cells encapsulated in PGA-HSA coated beads, different times after encapsulation.

Cellular activity inside the beads

Formazan production by the encapsulated cells as a function of time is illustrated on figure 2. On the whole, the cell activity greatly increased with time, in different steps. A first 14-days phase, where metabolic activity only slowly increased, was attributed to an adaptation phase for the cells, during which nutriments of the culture medium impregnated the beads. Then, a rapid increase was observed, in parallel with an intense cell multiplication inside the beads. The slowing down observed about 28 days after encapsulation could be related to the progressive filling of all the available space inside the membrane, leading to less cell divisions.
The MTT assay could not be applied to control Alg-PLL beads, because these particles did not survive the 5-hour incubation step with MTT.

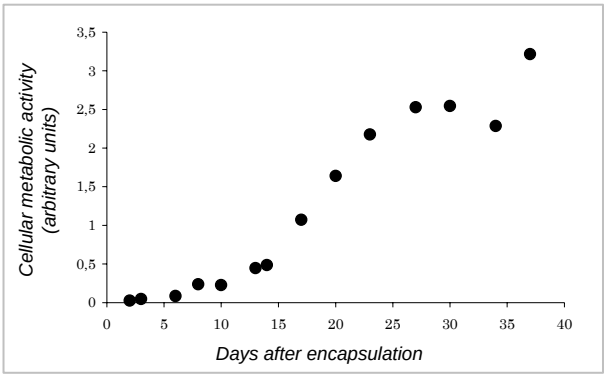

Figure 2. Metabolic activity of encapsulated cells as a function of time after encapsulation.

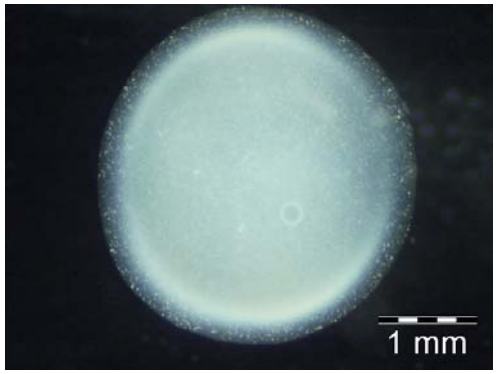

Figure 3 : Morphology of a PGA-HSA coated bead frozen in classical cryopreservation medium at day 6, and rapidly thawed.

Cryopreservation tests

After thawing, an observation of the beads congealed in different media indicated that the better cryoprotective medium for the integrity of the beads was the solution usually used for the

cryopreservation of free Jurkat cells. These tests showed that freezing in liquid nitrogen should take place early after encapsulation (6 days vs. 22 days), otherwise the beads became too weak to resist the treatment. Figure 3 reveals the morphology of the resisting beads, after thawing.
A MTT test performed on freeze-thawed encapsulated cells revealed a cryoprotective effect of the PGA-HSA coated beads on cells, the encapsulated cells keeping 85 % of their activity, whereas free cells kept only 50% of their metabolic activity in the same conditions.
The major challenge in developing cryopreservation protocols for encapsulated cells is that the large size of the capsules makes them particularly prone to cryodamage (Heng, 2004). In this work, we combined the use of a slow-cooling and rapid-thawing protocol, with a medium-concentrated cryopreservative concentration. When applied to PGA-HSA coated beads early after bioencapsulation, this protocol led to 100 % intact beads with a high post-thaw viability of the encapsulated cells. The good mechanical properties of the PGA-HSA covalent membrane, added to the presence of high amounts of protective protein and polysaccharides inside the gel core, might account for this promising result.

Conclusions

The Jurkat cell line, used in this work as a biological model, could be encapsulated in PGA-HSA coated beads. After encapsulation, the cells progressively colonized the whole volume of the beads, and this proliferation was accompanied by a great increase in metabolic activity, as revealed by the MTT assay. During the whole experiment, which lasted 37 days, the coated beads exhibited a remarkable stability, whereas alginate-PLL beads appeared burst open after only 2 days in the same conditions.
The encapsulated cells could be cryopreserved in liquid nitrogen in a convenient protective medium, and the experiments revealed a cryoprotective effect of the PGA-HSA coated beads, leading to the remaining of 85% of the initial metabolic activity of the cells.
This work confirms the interest of the method, based on a simple pH increase, and producing covalent bonds between biocompatible natural polymers, around a gel core protecting the biological encapsulated material. This new type of stable cell containers, allowing rapid exchanges between the culture medium and the intramembranar space, whereas the membrane hinders any cell escape from the beads, brings decisive and promising advantages in the field of bioencapsulation.

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