O5-3	**Human pancreatic islet encapsulation in microfluidic device**

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INTRODUCTION AND OBJECTIVES

Transplantation of human pancreatic encapsulated islets appears to be a new promising therapy for a long term treatment of the type 1 diabetes (Calafiore 2006). First studies (De Vos 2006) have demonstrated that the success of islet grafting depends on two main issues; first, the biocompatibility of the micro-porous capsule (mainly composed of alginate); second, the encapsulation process which can damage islets before implantation.
Standard encapsulation techniques are using dripping methods combined with an external gelation of alginate (Prüsse 2008). But other methods are in progress. For instance, micro-flow focusing devices which allow the production of monodispersed droplets from an aqueous phase into a continuous organic flow can also be employed for cell encapsulation (Le Vot 2008).
In this paper, we present, to our knowledge for the first time, the encapsulation of human pancreatic islets with a microfluidic device and an external gelation procedure. Viability and insulin secretion of islets were assessed for respectively ten and three days after our encapsulation process. Results showed no toxicity: encapsulated human islets were viable and kept their functionality.

MATERIALS AND METHODS

Microfluidic device fabrication

Microfluidic device was fabricated using standard lithography techniques. Substrate and walls were made of silicon and a glass cover seals the flow channels. The channel depth and width were 500µm. The nozzle had a 2mm long triangular enlargement with a base of 500µm (figure 1). Walls were silanized to achieve hydrophobic coating (water contact angle: 108-110°). Production of aqueous alginate droplets without the use of surfactant was then possible.

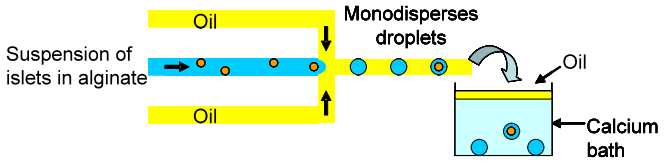

Figure 1: Formation of droplet in our microsystem

Before the experiments, the chip was sterilized by injecting 70% ethanol for 10 minutes, 1% Penicillin for 10 minutes, and finally deionized water for 10 minutes.

Islets culture

Two days after isolation, human pancreatic islets were kindly provided by the Dc Richard's team from the French Blood Establishment (St Ismier, France). The amount of provided islet was 20000 Islet equivalents (IEQ), corresponding to approximately 4500 islets with different size and shape. The purity grade was 90%.
Culture medium used before and after islet encapsulation was composed of CMRL (Gibco 21530), 1% Penicillin, 2.5% Hepes 1M, 2.5% bicarbonate Na, 1% pyruvate Na 100mM, 10% FBS (Biowest-S1810).

Procedure of encapsulation

Super refined soybean oil (CRODA EP-NP-LQ-(MH)), was used as continuous phase. The encapsulation polymer was a 2% (w/w) Pronova SLG 100 alginate from Novamatrix (271108/3). Alginate was prepared in an aqueous solution of 150 mM NaCl, 10mM Hepes. The pH of the alginate solution was adjusted to 7.4. Oil and alginate solutions were motioned by regulated pressures delivered by micropumps (Fluigent® micro-pumps MFCS-8C). Pressure of oil and alginate were fixed respectively to 400 mbars and 450 mbars. External gelation time was fixed to 5 minutes, in a solution composed of 100mM CaCl2, 80mM NaCl, 10mM Hepes. Then, capsules were washed three times with culture medium before being stored into a 37°C incubator supplemented by 5% CO2.

Measurements of islet functionality

The viability of islets was determined by a live and dead fluorescence test. Viable cells were stained with a solution of 0,67 µM fluorescein diacetate (FDA) and dead cells with a solution of 4µM Propidium iodure (PI). Stained cells were visualized using a fluorescent microscope (Axioplan 2 Imaging Zeiss, software used Cell A). Images analysis was performed using Image J software (Open source software). A home-made program calculates the magnitude of PI (green) and FDA (red) fluorescent signals. The viability rate was determined to be the ratio of PI signal over the total (PI+FDA) fluorescence signal. Islets were considered to be viable for a ratio superior to 50%. Viability assessment was performed for 10 days after encapsulation on at least 45 islets each day.

An insulino secretion test (Elisa, Mercodia 10-1250-01) was done to assess the islet functionality for three days after encapsulation. Nonencapsulated and encapsulated islets were sequentially incubated for 2 hours into low (LGS, 0.8g/L) and high (HGS, 8g/L) glucose concentration buffers. Glucose buffers were prepared from a Krebs

Ringer buffer (0.5% of BSA) supplemented with glucose. After incubations, supernatants were sampled. Islets were then stored for one night at 4°C in a 95% Ethanol and 1.5% HCl solution in order to extract the total amount of insulin. The test was done in triplicate and each test was performed on 20 islets. Samples were stored at -20°C before being assayed.

RESULTS AND DISCUSSION

Capsule production

The average capsule diameter is 460µm (CV<5%) with a characteristic form of tears (figure 2). This form is characteristic to the external gelation (Capretto 2008).

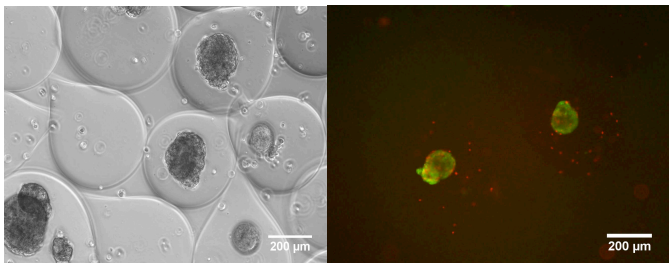

Figure 2: Humans islets encapsulated with a MFFD

Microfluidic device does not overcome empty capsule problem. For an initial concentration of 8000 islets/ml of alginate, 55% of the capsules are empty. In the other 45% of capsules, 80% contain one islet and 20% contain more than one (mainly 2, but up to 4). Less than 1% of these capsules lead to islet protrusion.

Viability

Viability was assessed for a 10-day period after encapsulation (figure 3). 10% (500 islets) of the pancreatic islets batch were kept as reference (nonencapsulated islets).

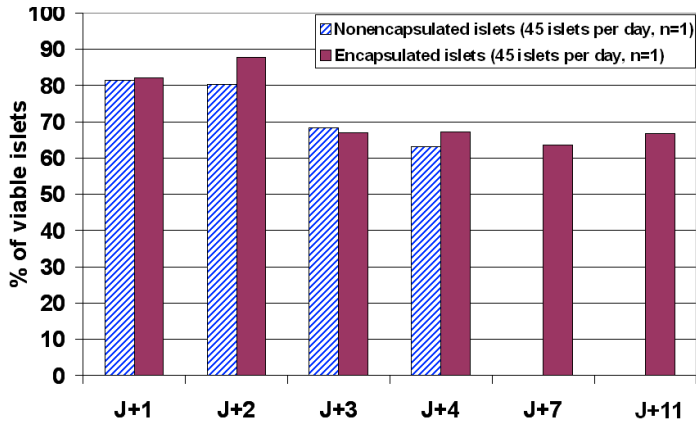

Figure 3: Viability of islets after encapsulation

After 6 days no more nonencapsulated islets are alive whereas 60% of encapsulated islets present more than 50% of viable cells.

Insulino secretion

Figure 4 shows the insulin secretion ratio for nonencapsulated and encapsulated islets. This ratio is defined as the amount of secreted insulin for low and high glucose stimulation over the total amount of extracted insulin. This normalization overcomes islets size distribution.

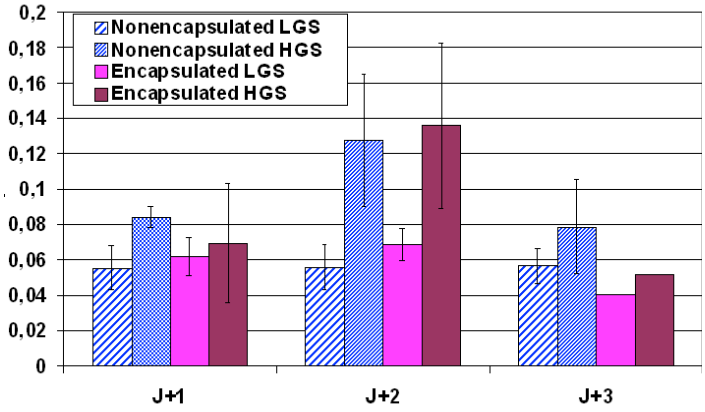

Figure 4: Islet functionality after encapsulation

Results show that this ratio is quite the same for nonencapsulated or encapsulated islets, meaning that capsules produced by our device have the same porosity properties as capsules coming from standard techniques. However, an important variability is observed in our measurements. Lack of islet does not allow us to carry on other experiments.

CONCLUSIONS

We demonstrate that viability and functionality of human pancreatic islets are not affected by an encapsulation into a microfluidic device. Microfluidic systems are promising for islet and cell encapsulation since all the process can be automated (size sorting of islets, gelation process) on the same chip at a reduced scale.

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