CellEnc™ : New microfluidic platform for automated cell encapsulation

Forvi E., Dalle P., Boizot F., Alessio M., Bellemin-Comte A., Costa G., Caillat P., Benhamou P.Y., Rivera F.*
LETI-CEATech, Grenoble, France (elena.forvi@cea.fr; florence.rivera@cea.fr)

INTRODUCTION

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.

Up to now conventional systems are mainly manual which induces batch disparities and do not provide repeatable and reliable clinical results. Microfluidics devices seems to be promising systems to tackle these drawbacks. They have the ability to couple different functions for the integration of a whole chemical process demonstrating higly reproducible results.

Here we introduce a new automated microfluidic system, called CellEnc™, for on-line cell encapsulation. The purpose of this project is to develop a plug-and-play tool providing clinical grade encapsulated cells.

MATERIAL AND METHODS

CellEnc™ Cartridge

The CellEnc™ microfluidic cartridge is an home-made hybrid system made of three <100> silicon chips sealed with a polymer card.

In the silicon chips, microfluidic channels are dry etched with standard microelectronic technologies to obtain 200µm large and deep microfluidic channels. Each silicon chip is used for a peculiar function as described on Figure 1. The first one is composed of a Micro Flow Focusing Device, MFFD, (Le Vot 2008) for the formation of alginate droplets in oil, and so the encapsulation of cells. The second silicon chip is used to transfer (Dalle 2012) the alginate droplets from the oil phase to a gelling fluid. Once the alginate capsules are completely gelled, the function of the last silicon chip is to transfer them towards a cell media fluid or physiological serum.

The polymer cards are made of PMMA and milled in order to define larger microfluidic channels ranging from 400µm to 2mm. The dimensions of these channels are defined to precisely control the time of "pregelation" and gelation steps.

Finally, walls of both silicon chips and polymer cards are coated with a hydrophobic coating (water contact angle: 108-110°), to produce aqueous alginate droplets without using additional surfactants to avoid toxic effects on cells.

Preparation of solutions

The alginate solution used for these experiments is a 3% (w/w) sterile alginate (Pronova SLG100, Novamatrix), diluted in an aqueous solution of 150mM NaCl, 10mM Hepes. The pH is adjusted to 7.4. Viscosity of alginate solution is 5300mPa.s at zero shear. Hyper-refined soybean oil (CRODA EP-NP-LQ-(MH)) is used as continuous phase and calcium acetate organic salt (Macco Organiques INC) as pre-gelling agent. The gelling phase is a solution of 100mM CaCl2, 80mM NaCl et 10mM Hepes adjusted to pH 7,4. Encapsulated cells are collected in either cell culture media or physiological serum.

Experimental Set-up

Solutions are motioned in a regulated pressure-driven mode, between 0 to 1bar, delivered by micropumps (Fluigent® micro-pumps MFCS-8C). Experiments are visualized under a x5 objective microscope and image sequences acquired with a high speed camera (Mikrotron GmbH, MotionBlitz Eosens) up to 5000fps. All the experiments are performed under sterile and controlled environment.

Parameters for circularity

Capsule sphericity is assessed for 50 capsules via image analysis with ImageJ software through two parameters : the circularity and the aspect ratio of the capsule's fitted ellipse. They are calculated as :

$$c = 4\pi \frac{Area}{Perimeter^2} \quad (1)$$

$$r = \frac{MajorAxis}{MinorAxis} \quad (2)$$

For a perfect circle the values of these both parameters is 1.

RESULTS

Card performances

The whole process for cell encapsulation is integrated in CellEnc™ cartridges, meaning that the input of the cartridge is a suspension of cells in alginate and the output is encapsulated cells in gelled alginate collected in serum. Figure 1 represents the two

geometries of silicon chips: the first one represents the MFFD module in which cells are encapsulated inside liquid alginate droplets. Once the droplets are formed, they start to gel thanks to a gelling agent suspended in oil. The second and third silicon chips use the same geometry which function is to respectively transfer the pregelled alginate droplets from oil towards Ca-based gelling phase (Figure 2) and gelled capsules from the gelling phase to the collecting media (Figure 2).

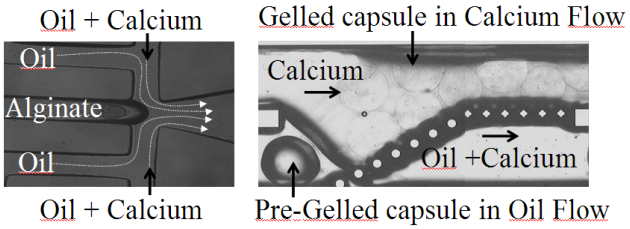

Figure 1: (left) MFFD module; (right) first transfer module

The CellEnc cartridge, as depicted in Figure 2, shows the main 5 integrated steps: droplet formation for cell encapsulation, pregelation, first transfer phase of alginate droplets, gelation and finally second transfer phase. Pregelation and gelation time are precisely controlled thanks to the well-defined lengths of microchannels. The whole encapsulation procedure is performed in a continuous flow meaning that all the alginate capsules should have the same degree of gelation.

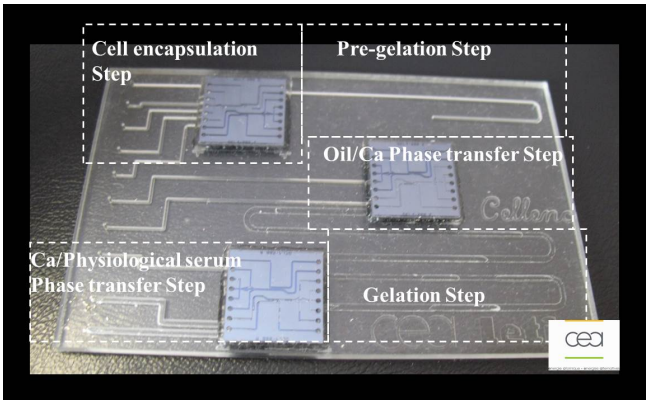

Figure 2: CellEnc™ cards, 5 steps process

Current home-made cartridges demonstrate the ability to supply 80 capsules per minutes with highly viscous alginate solution (5300mPa.s) for an average working time of 1 hour.

The collected capsules are spherical in shape: the measured ratio and circularity parameters were respectively r=1,030 (CV=2,0%) and c=0,87 (CV=1,7%). Capsules are also monodispersed in size (CV 3%), and depending on the applied pressure with an average diameter ranging from 130µm to 160µm.

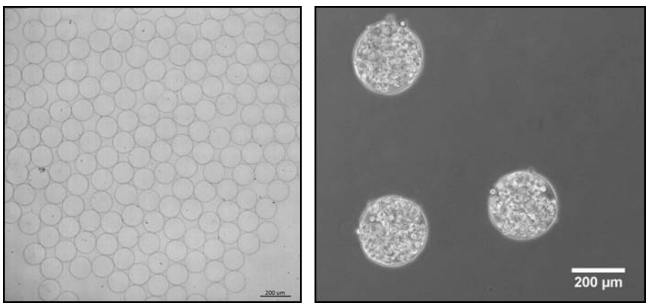

Figure 3: (left) 3% ultrapure alginate capsules; (right) encapsulated Jurkat cells collected in serum

Preliminary results on Jurkat cells encapsulation

Preliminary experiments have been performed to encapsulate floating Jurkat cells in alginate droplets. Figure 3 shows the encapsulated cells collected in serum, demonstrating the ability of our system to perform such a complex task in an automatic and reproducible way.

Cells viability was controlled with standard staining procedures, showing 85% viability**.**

CONCLUSION

To our knowledge, it is the first time that a microfluidic device can safely automate the complex procedure for cells encapsulation. Although current CellEnc™ cards are home-made and can be still optimized, there are good prospects that this new technology can be upgraded to manufacture sterile and disposable devices.

CellEnc™ cards are promising new tools for clinicians, potentially providing them clinical grade encapsulated cells.

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