High throughput electroporation microsystem using sine wave bursts to deliver biomolecules into cell spheroids

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HIGH THROUGHPUT ELECTROPORATION MICROSYSTEM USING SINE WAVE BURSTS TO DELIVER BIOMOLECULES INTO CELL SPHEROIDS

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SUMMARY

Reversible electroporation (EPN) is a method for introducing molecules into cells without permanent damage based on the application of pulsed electric fields. The development of innovative *in vitro* assays exploiting 3D cell models such as spheroids can be of great help to assess the potential of cancer treatments based on EPN. The parallel treatment of tens of spheroids of similar characteristics (size, shape) is required to produce statistical data. To address this challenge, we designed a microfluidic platform enabling culture and electroporation of a large number of spheroids sharing similar characteristics without requiring any manipulation. Here, we demonstrate the delivery of an anti-cancer agent in spheroids using sine wave bursts.

INTRODUCTION

The clinical interest for electroporation-based therapies, including electrochemotherapy (ECT), has drastically increased in the last few years, and spheroids have been identified as a relevant model to study EPN in vitro, as they can mimic various normal and pathological situations. In comparison with cells grown in 2D on flat surfaces, multicellular spheroids reproduce more accurately the structure of a microtissue in which each cell interacts with its neighbors via the formation of junctions ensuring cellular cohesion and communication[1].

In the literature, the proposed approaches usually consist in first fabricating the spheroids using droplet-based microfluidics[2] or hanging drop methods[3], and then introducing them in an electroporation cuvette connected to a pulse generator. Thus, it involves several handling steps, which can potentially damage spheroids. Moreover, it leads to their random distribution in the cuvette, which may induce differences in the electric field perceived from one spheroid to the other. There is therefore a strong need for the development of new tools providing easiness of use, high throughput and results reproducibility.

Our approach intends to overcome those drawbacks as, to the best of our knowledge, there is no existing solution enabling culture of spheroids of controlled size and shape, easy introduction of fresh medium and subsequent electroporation inside a unique device.

EXPERIMENTAL

A reusable polydimethylsiloxane (PDMS) mold is first made from a micro-milled metal master mold with a replica molding process. A thin layer of 2% agarose hydrogel pre-heated at 80°C is poured onto it and an ITO coated glass slide functionnalized with (3-Aminopropyl) triethoxysilane (APTS) is placed over it before demolding (Figure 1a). Human colorectal cancer cells (HT29) are seeded in this porous micro-structured hydrogel (320 wells of 200 µm diameter)[4], and let grown for 3 days until they fill the microwells (Figure 1b). Another ITO coated glass electrode is then placed over the hydrogel to form the EPN microfluidic chamber (Figure 1c), with tubing allowing for the injection of EPN buffer (10 mM Hepes, 1 mM MgCl2(6H2O), 250 mM sucrose, pH=7,1, σ=300 µS/cm) supplemented with an anticancer drug (bleomycin, 20 µg/mL). The distance between both electrodes is 1 mm. Medium exchange in the hydrogel is monitored by impedance measurements.

EPN is performed by applying 2 sine wave bursts (10 kHz, 5 ms)[3,5]. The relevance of such waveform for small molecule delivery into cells has recently been discussed[5]. The electric field effectively perceived by the spheroids (around 800 V/cm) is determined by estimating the electrical losses, from impedance measurements fitted to a theoretical model (data not shown). Electric field distribution in the microfluidic chamber is also determined, thanks to COMSOL modelling.

To evaluate cellular viability 3 days after EPN, cells are marked with two fluorescent agents: fluorescein diacetate (FDA), a marker of living cells, and propidium iodide (PI), indicator of loss of membrane integrity due to cell death.

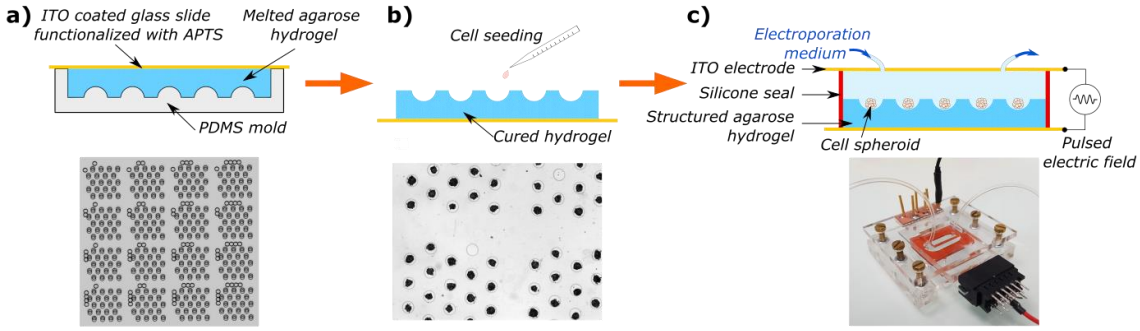

Figure 1: (a) Formation of microwells of 200 µm diameter each in an agarose gel molded on a PDMS master. (b) Cell seeding in this scaffold illustrated with a X2.5 bright field image of the grown spheroids. (c) Integration into a microfluidic device composed of two facing electrodes with apertures for fluid inlet and outlet. The device is transparent, enabling in-situ confocal monitoring.

RESULTS AND DISCUSSION

With the COMSOL module "Electric Currents in Layered Shells", the device can be modelled as two ITO conductive layers of measured conductivity 7.10^5 S/m and thickness 134 nm, sandwiching a block representing the microfluidic chamber filled with EPN medium. When voltage is applied between both electrodes (Figure 2a), the resulting electric field in the chamber containing hydrogel and spheroids is homogeneous (Figure 2b), with less than 1% variation in intensity over the whole chamber, ensuring that all the spheroids are submitted to the same electric field.

Prior to electroporation, the conductive culture medium is replaced by low conductivity EPN buffer solution which is slowly injected in the microfluidic chamber. Effective medium exchange is confirmed using impedance measurements. Results show that a 2.5 mL injection in 10 min is necessary to reach a plateau (Figure 2c), meaning that the conductivity in the chamber has stabilized and that the culture medium has been fully replaced by the EPN medium. This monitoring of impedance changes guarantees that all the experiments are made in a medium of appropriate conductivity.

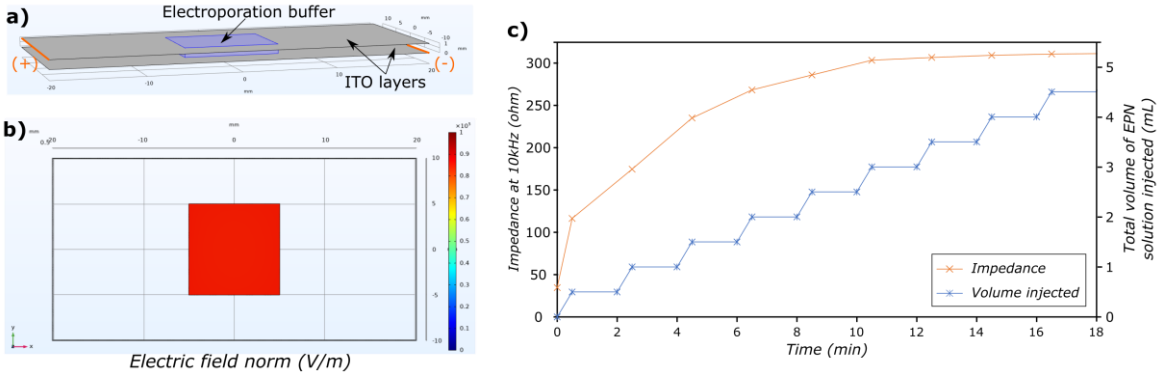

Figure 2: (a) COMSOL model of the device enabling the electric field characterization. (b) Distribution of the electric field intensity in the device. (c) Study of medium diffusion in the micro-structured hydrogel by monitoring impedance when medium is injected.

Figure 3a represents the size evolution of spheroids, measured on the ImageJ software, and calculated with the ratio of size after and before the experiment at several time points (2h, 1 day and 3 days after EPN), for groups submitted to EPN with or without bleomycin, and a control group without EPN. It shows that spheroid growth is inhibited in presence of the anti-cancer drug bleomycin, contrary to the control and EPN without bleomycin groups. This is also visible on figure 3b, along with the cell viability results 3 days after the experiment, suggesting mortality is higher in presence of bleomycin, as the red fluorescence is more present. Thus, the parameters of EPN used here, determined with previous experiment (data not shown), enable a reversible EPN as there are only few dead cells when spheroids are submitted to EPN only. Moreover, delivery of bleomycin in spheroids by EPN is efficient and has the expected impact on cell growth and viability.

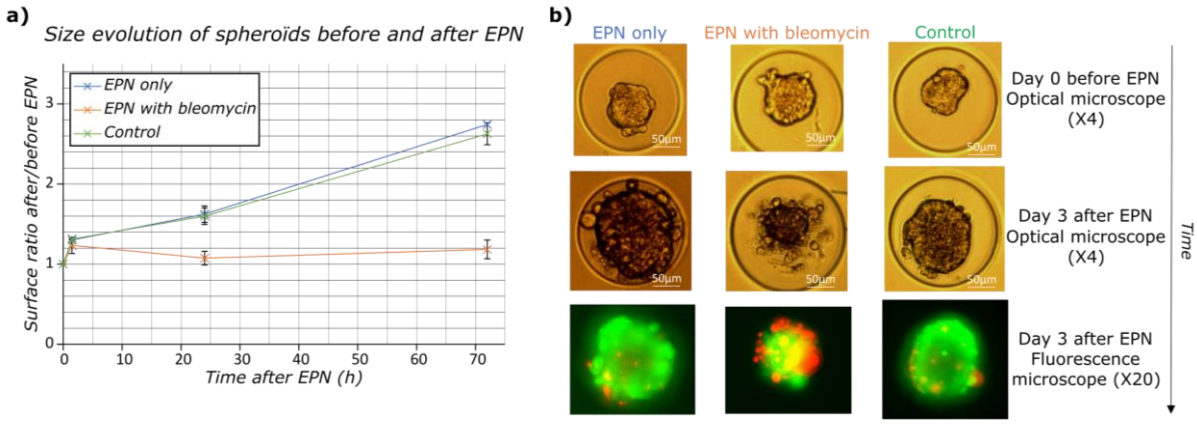

Figure 3 : (a) Evolution of the spheroids size after EPN with or without anti-cancer drug. (b) Representative images obtained by optical microscopy before EPN (first row), 3 days after EPN (2nd row), and viability analysis with epifluorescence (3rd row): Green= FDA staining (living cells); red=IP staining (dead cells).

OUTLOOK

After this proof of concept and first application, we intend to apply EPN of bleomycin to other types of cells, associated to cancerous cells in co-culture spheroids, and to optimize electrode and hydrogel designs in order to be able to monitor spheroid growth with bio-impedance measurements.

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