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A microfluidic biochip for the nanoporation of living cells

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ABSTRACT

This paper deals with the development of a microfluidic biochip for the exposure of living cells to nanosecond pulsed electric fields (nsPEF). When exposed to ultra short electric pulses (typical duration of 3–10 ns), disturbances on the plasma membrane and on the intra cellular components occur, modifying the behavioral response of cells exposed to drugs or transgene vectors. This phenomenon permits to envision promising therapies. The presented biochip is composed of thick gold electrodes that are designed to deliver a maximum of energy to the biological medium containing cells. The temporal and spectral distributions of the nsPEF are considered for the design of the chip. In order to validate the fabricated biochip ability to orient the pulse towards the cells flowing within the exposition channels, a frequency analysis is provided. High voltage measurements in the time domain are performed to characterize the amplitude and the shape of the nsPEF within the exposition channels and compared to numerical simulations achieved with a 3D Finite-Difference Time-Domain code. We demonstrate that the biochip is adapted for 3 ns and 10 ns pulses and that the nsPEF are homogenously applied to the biological cells regardless their position along the microfluidic channel. Furthermore, biological tests performed on the developed microfluidic biochip permit to prove its capability to permeabilize living cells with nanopulses. To the best of our knowledge, we report here the first successful use of a microfluidic device optimized for the achievement and real time observation of the nanoporation of living cells.

1. Introduction

The effects of short and intense electric fields on biological cells have been studied for a long time as they are known to induce disturbances on the plasma membrane which can become permeable to various molecules (Tsong, 1991). This temporary permeabilization of the plasma membrane allows genes or drugs entering into the cell cytosol. This technique is very attractive in various applications such as electrochemotherapy (Marty et al., 2006; Mir et al., 2006), cutaneous and subcutaneous tumour nodule treatments (Mir et al., 1998), evaluation of the cytotoxicity of nonpermeant or poorly permeant anticancer drugs (Labanauskiene et al., 2007), gene electrotransfer to various animal tissues (Andre and Mir, 2004; Andre et al., 2008) and latterly with in vivo tests (Gothelf and Gehl, 2010; Heller and Heller, 2006).

More recently, the development of generators allowing to reach very short and high voltage pulses, in the range of 3–10 ns with intensities between 20 and 150 kV/cm, has opened a new important investigation field (Buescher et al., 2004; Vernier et al., 2004). Different works have been reported about the effects on animal cells of these kinds of pulses, commonly named as nanosecond pulsed electric fields (nsPEF). More precisely, it has been shown that the gene electrotransfection efficiency can be improved by nsPEF exposure, also called as nanoporation, (Beebe et al., 2003) and that disturbances on the cell membrane could be sufficient to render it permeable to small molecules, such as propidium iodide (Ibey et al., 2009; Vernier et al., 2006). Beyond their potential effects on the cell membrane, these nsPEF show a great interest because they also offer the possibility to disturb the intra cellular structures and functions (Beebe and Schoenbach, 2005).

However, the mechanisms implied in the effects of nsPEF on living cells remain still misunderstood. This is mainly due to the lack of real-time visualization and monitoring systems during nsPEF application on biological cells. Commercial electroporation chambers or cuvettes are commonly used to apply nanosecond pulses on living cells (Kenaan et al., 2010; Vernier et al., 2006), but are applied without any possibility to visualize cells in real time during the pulse applications which renders difficulty in the study of their effect.

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In parallel, recent developments of microtechnologies and microfluidics techniques permit consideration of the design and fabrication of new innovative tools for biology. The main benefits of these technologies consist in their miniaturization and parallelization capabilities, as well as real-time observation in the case where transparent materials are used for the device fabrication. In the case of electroporation, miniaturized electrodes permit to expose cells on a chip to microsecond duration pulses (typically 100 μs) and intense electric fields (typically 1 kV/cm) (Huang and Rubinsky, 2001; Krishnaswamy et al., 2007; Le Pioufle et al., 2000; Lee et al., 2009; Wang et al., 2009). However, the delivery of the nsPEF (typically 3–10 ns, 20–45 kV/cm) to the cells without deformation of spectral and temporal contents requires a specific design.

In this context, the work presented in this paper describes the design, fabrication and characterization of a miniaturized device specifically optimized for nsPEF exposure of living cells. The proposed device permits the real-time visualization of the nanoporation of cells, under fluorescent microscopy, thanks to the transparency of exposure channels. An electrical characterization of the biochip supported by frequency and time domain measurements and simulations is proposed. Biological characterizations of the cells exposed on the chip to 10 ns pulsed electric fields using a florescent dye are carried out.

The development of such microfluidic devices optimized for the real-time visualization of the effect of nsPEF on living cells is of prime importance as potentially, their use might provide (a) new knowledge on the nanoporation effects (b) high throughput production of permeabilized cells for further therapeutic uses as drug or gene insertion.

2. Materials and methods

2.1. Design methodology

The biochip is composed of a 50 μm thick SU8 microfluidic channel including thick gold electrodes with a typical thickness of 25 μm, in which cells suspended in a biological medium are injected. Gold is chosen as material for the electrodes because of its excellent electrical properties and biocompatibility (Dalmay et al., 2010).

The biochip is designed in such a way that the pulsed electric field is absorbed and dissipated mainly in the biological medium placed between the electrodes within which cells to be treated are flowed. To do so, impedance matching is necessary between the generator, the transmission line and the nanoporation biochip.

Considering the frequency spectrum of the applied pulses (typically 0–200 MHz), the electrical biochip behavior is mainly determined by the properties of the medium flowing within the microfluidic channel. It can be described by a parallel CG model, where C and G represent respectively the conductance and the capacitance of the biological medium (1).

$$C = \varepsilon_0 \varepsilon_r \frac{h \cdot L}{d} \quad \text{and} \quad G = \sigma \frac{h \cdot L}{d} \tag{1}$$

where σ and ε_r represent respectively the conductivity and the relative permittivity of the biological solution, d corresponds to the gap between the electrodes containing the medium, h is the thickness of the microchannel and L the length of electrode in contact with the medium into the channel.

The impedance Z_e of this model (2) has to be matched to the impedance of nsPEF generator ($Z_g = 50\,\Omega$) in order to (a) deliver a maximum of energy to the biological medium, (b) avoid reflections of the nsPEF backwards to the generator, (c) avoid temporal distortions of the applied nsPEF.

$$Z_e = \frac{1}{G + jC\omega} = \frac{d}{A(\sigma + j\varepsilon_0 \varepsilon_r \omega)} \tag{2}$$

where $A = L \times h$ represent the area of the electrical current density. Considering the conductivity ($\sigma = 1$ S/m) and the relative permittivity ($\varepsilon_r = 78$) of the used biological media (see Section 2.4), a transition frequency appears:

$$f_t = \frac{\sigma}{2\pi\varepsilon_0\varepsilon_r} = 231 \quad \text{MHz} \tag{3}$$

Regarding that the impedance has to be matched to $Z_g = 50\,\Omega$ for most of the spectrum of the applied nsPEF, we chose to optimize the impedance mostly from continuous up to this transition frequency f_t where most of the energy of the applied nsPEF is concentrated.

We have considered a distance gap (d) between the electrodes set to 150 μm, a height (h) for the SU8 microfluidic channels set to 50 μm and a thickness (t) for the electrodes fixed to 25 μm. The chosen value for the thickness of gold electrodes ($t = 25$ μm) represents a good compromise between technical constraints and homogeneity of the field across the whole microfluidic channel. Then, the length (L) of the electrodes has to be 60 mm. As the biochip electrodes are based on a coplanar line model with two parallel microchannels, their length has thus been set to 30 mm.

2.2. Fabrication process of the microfluidic biochip

The fabrication process starts with a commercial quartz substrate metalized with a 10 nm chromium and a 150 nm gold layer (ACM, France) and is used as the main support for transparent and planar surfaces at the end of the process. From this seed layer, electroplating of 25 μm gold electrodes is achieved using an electrolytic bath based on potassium aurocyanure ($KAu(CN)_2$). Then a standard UV photolithography using a commercial photoresist (S1805, MicroChem) is used to define the electrode shapes. Removal of the excess gold is made on the different parts of the device by wet etching with a solution based on potassium iodide. Then the Cr seed layer is chemically etched. After the removal of the photoresist and the cleaning of the wafer by successive baths of acetone, ethanol and water combined with ultrasonic steps, the microfluidic channels are defined by a UV photolithography of a transparent commercial photoresist, which allows reaching a high aspect ratio (SU8-2025, MicroChem). The SU8 layer is 25 μm thick to reach the total thickness of the microfluidic channel set to 50 μm. To finish, the biochip is packaged using a PDMS (Sylgard® 184 Silicone Elastomer Kit, Dow Corning) membrane. This layer is bonded to the SU8 resist using a silanization process based on (3-aminopropyl)trimethoxysilane (Aldrich) and methanol mixture.

Fig. 1(a) presents the designed microfluidic biochip with actual measured dimensions and Fig. 2 shows a profilometer measurement of the microfluidic biochip. A photograph of the packaged microfluidic biochip is shown in Fig. 1(b). The measured gap between electrodes was $d = 130$ μm, the thickness obtained for the channel is $h = 47$ μm (Fig. 2) and the length of electroplating electrodes is $L = 33$ mm. Considering Eq. (2), the equivalent impedance at low frequencies of the two parallel microchannels is 42 Ω.

2.3. Electrical characterization

2.3.1. Numerical modelling

Assuming the parameters given in the previous section, the biochip is realistically modeled. A FDTD code (Finite Difference Time Domain) (Leveque et al., 1992, 2004; Taflove and Hagness, 2005; Yee, 1966) is used to perform the numerical analysis. The analyzed structure is composed of the biochip whose microfluidic