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● *Original Contribution*

SONOPORATION OF ADHERENT CELLS UNDER REGULATED ULTRASOUND CAVITATION CONDITIONS

Pauline Muleki Seya, Manuela Fouqueray, Jacqueline Ngo, Adrien Poizat, Claude Inserra, and Jean-Christophe Béra

Inserm, U1032, LabTau, Lyon, F-69003, France; Université de Lyon, Lyon, F-69003, France

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Abstract—A sonoporation device dedicated to the adherent cell monolayer has been implemented with a regulation process allowing the real-time monitoring and control of inertial cavitation activity. Use of the cavitation-regulated device revealed first that adherent cell sonoporation efficiency is related to inertial cavitation activity, without inducing additional cell mortality. Reproducibility is enhanced for the highest sonoporation rates (up to 17%); sonoporation efficiency can reach 26% when advantage is taken of the standing wave acoustic configuration by applying a frequency sweep with ultrasound frequency tuned to the modal acoustic modes of the cavity. This device allows sonoporation of adherent and suspended cells, and the use of regulation allows some environmental parameters such as the temperature of the medium to be overcome, resulting in the possibility of cell sonoporation even at ambient temperature. (E-mail: pauline.muleki@gmail.com) **© 2015 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Sonoporation, Adherent cells, Inertial cavitation, Cavitation regulation.**

INTRODUCTION

Sonoporation, the enhancement of cell membrane permeability and creation of transient pores with ultrasound (Miller et al. 1996; Qiu et al. 2010), is a promising alternative technique for transferring molecules or genetic material into cells (Ng and Liu 2002). Although the physical mechanisms underlying sonoporation are not fully understood, it is commonly thought that acoustic cavitation plays a main role in pore formation. There are two kinds of acoustic cavitation activity (Leighton 1994): non-inertial cavitation corresponding to bubble oscillations, and inertial cavitation corresponding to bubble collapse. These can be differentiated from each other by analysis of the acoustic waves radiated by the bubbles. Non-inertial cavitation is characterized by the appearance of subharmonics of the fundamental frequency, whereas inertial cavitation results in broadband noise on the acoustic spectrum. The contribution of each state of cavitation to the sonoporation process is still unclear. Nevertheless, several works have linked the role of inertial cavitation activity to sonoporation efficiency (Sundaram et al. 2003; Zhou et al. 2008). To obtain insight into the physical mechanisms underlying sonoporation, some devices have been designed to visualize, during sonication and observation, the interactions between bubbles, cells and medium.

A single cell in the adherent (Marmottant and Hilgenfeldt 2003; Sankin et al. 2010; Zhou et al. 2012) or suspension (Le Gac et al. 2007) configuration has been studied with a single bubble created and trapped by a laser (Le Gac et al. 2007; Sankin et al. 2010; Zhou et al. 2012), created by air injection and attached to a wall (Marmottant and Hilgenfeldt 2003) or nucleated in a microfluidic system (Nejad et al. 2011). Adherent cell monolayers have been submitted to multiple bubbles (ultrasound contrast agent in most cases) near cells (Okada et al. 2005; van Wamel et al. 2004, 2006; Wolfrum et al. 2002) or attached to cells (Kudo et al. 2009). These devices allow the cell membrane deformations that result from bubble oscillations (van Wamel et al. 2004, 2006), linked to contraction/expansion bubble phases (Okada et al. 2005), and appear where bubbles are attached (Kudo et al. 2009) or are caused by microstreaming (Marmottant and Hilgenfeldt 2003; Sankin et al. 2010), microjetting (Okada et al. 2005; Sankin et al. 2010) or

Address correspondence to: Pauline Muleki Seya, Inserm, U1032, LabTau, Lyon, F-69003, France; Université de Lyon, Lyon, F-69003, France. E-mail: pauline.muleki@gmail.com

Table 1. Summary results from adherent sonoporation studies

	Acoustic conditions			Cell medium		
Study	Techno	Excitation	P_a/I_a	Cell line	UCA	T (°C)
Kinoshita and Hynynen (2007)	Plane transducer	CW/1.7 MHz/12–120 s	0–4 W/cm^2	Rat C166	Optison	36.5
Lu and Zhong (2005)	Plane transducer	CW/1 MHz/0–20 s	0.36 MPa	HeLa	None	37
Ohl and Wolfrum (2003)	Lithotripter	Shock wave/1 μs	28 MPa	HeLa	None	25
Kim et al. (1996)	Plane transducer	CW/1 MHz/5–120 s	0.2–0.45 MPa	Rat fibroblast Rat chondrocytes	None	37
Miller and Quddus (2001)	Diagnostic device Doppler mode	PW/3.5 MHz/5 s PRF 4.4 kHz	0.83 MPa	Epidermoid A431 Phagocytic RAW-264.7	Optison	37
Forbes et al. (2008)	Focused transducer	PW/3.15 MHz/30 s/ PRF 10 Hz	0.12–3.5 MPa	Chinese Hamster Ovary	Optison	Room temperature

CW = continuous wave; PW = pulsed wave; PRF = pulse repetition frequency; P_a = acoustic pressure; I_a = acoustic intensity; UCA = ultrasound contrast agent.

bubble collapse (Zhou et al. 2012). For a bubble near a cell, these deformations rise 2.85 μm (Nejad et al. 2011) and can lead to pore creation in cells (Kudo et al. 2009; Sankin et al. 2010; Zhou et al. 2012). These studies provide information on possible mechanisms underlying sonoporation, but not on statistical and parametric results, for example, sonoporation rate.

Only a few devices and studies have dealt with adherent cell sonoporation (Table 1). These studies have tested various biological (Kim et al. 1996; Kinoshita and Hynynen 2007; Lu and Zhong 2005), environmental (Kim et al. 1996) and acoustical (Forbes et al. 2008; Kinoshita and Hynynen 2007; Lu and Zhong 2005; Miller and Quddus 2001; Pan et al. 2005) parameters. Despite the wide disparity in experimental conditions, some key items appear relevant for further analysis. First, in three studies, cells were sonoporated when naturally attached to the bottom of the well or when resuspended before sonication, and sonoporation and transfection rates varied: results for the adherent configuration were lower than (Lu and Zhong 2005), on the same order of (Kim et al. 1996) or higher than (Kinoshita and Hynynen 2007) results for the resuspended configuration. Second, transfection rate appeared to be negligible at room temperature compared with physiologic temperature (Kim et al. 1996). Third, with increasing duration of exposure, the acoustic pressure level and duty cycle induced enhancement of both sonoporation and transfection (Forbes et al. 2008; Kinoshita and Hynynen 2007; Lu and Zhong 2005; Miller and Quddus 2001; Pan et al. 2005) and mortality (Kinoshita and Hynynen 2007; Lu and Zhong, 2005; Miller and Quddus 2001; Pan et al. 2005). And fourth, the acoustic field played an important role, and the sonoporation rate was notably higher in the presence of standing waves (Kinoshita and Hynynen 2007).

It is well known that acoustic cavitation exhibits unstationary behavior, and consequently, in most sonoporation experiments, ultrasound contrast agents (UCAs) are added to the medium to ensure rapid initiation of acoustic cavitation and better reproducibility (Forbes et al. 2008; Kinoshita and Hynynen 2007; Miller and Quddus 2001). To further overcome the limitation induced by cavitation stochasticity, recent research has focused on real-time monitoring and control of cavitation activity (Desjouy et al. 2013; Hockham et al. 2010). As ultrasound transfection and pore formation are correlated with the energy of the broadband noise on the spectrum (Sundaram et al. 2003; Zhou et al. 2008) and, thus, with inertial cavitation, regulation has been implemented on the broadband noise level in the context of sonoporation or transfection applications (Desjouy et al. 2013). For sonoporation of suspended cells, the control of inertial cavitation activity has been performed within the whole volume of the medium, resulting in better reproducibility of biological effects (Lo et al. 2014).

The aim of the work described here was to determine if regulation of cavitation activity is still efficient in the case of adherent cell sonoporation. For this purpose, we developed a sonoporation device dedicated to adherent cells that made possible the real-time monitoring and control of inertial cavitation activity. After testing of environmental and acoustic parameters (temperature, pulse duration, adherent or resuspended condition), regulated-cavitation conditions were compared with fixed acoustic intensity conditions in terms of sonoporation, detachment and mortality rates. Finally, experiments were performed at different frequencies, corresponding to neighboring modes of the standing wave acoustic field, to test the possible spatial character of sonoporation.

METHODS

Ultrasound apparatus and setup

Sonoporation system. The ultrasound setup used in these experiments is illustrated in Figure 1. Two plane