Ultrasound in Med. & Biol., Vol. ■, No. ■, pp. 1–11, 2015

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2015.03.020

● *Original Contribution*

BIOLOGICALLY AND ACOUSTICALLY COMPATIBLE CHAMBER FOR STUDYING ULTRASOUND-MEDIATED DELIVERY OF THERAPEUTIC COMPOUNDS

Dario Carugo, Joshua Owen, Calum Crake, Jeong Yu Lee, and Eleanor Stride
Department of Engineering Science, Institute of Biomedical Engineering, University of Oxford, Oxford, United Kingdom

(*Received* 19 *December* 2014; *revised* 13 *February* 2015; *in final form* 18 *March* 2015)

Abstract—Ultrasound (US), in combination with microbubbles, has been found to be a potential alternative to viral therapies for transfecting biological cells. The translation of this technique to the clinical environment, however, requires robust and systematic optimization of the acoustic parameters needed to achieve a desired therapeutic effect. Currently, a variety of different devices have been developed to transfect cells *in vitro*, resulting in a lack of standardized experimental conditions and difficulty in comparing results from different laboratories. To overcome this limitation, we propose an easy-to-fabricate and cost-effective device for application in US-mediated delivery of therapeutic compounds. It comprises a commercially available cell culture dish coupled with a silicon-based "lid" developed in-house that enables the device to be immersed in a water bath for US exposure. Described here are the design of the device, characterization of the sound field and fluid dynamics inside the chamber and an example protocol for a therapeutic delivery experiment. (E-mail: eleanor.stride@eng.ox.ac.uk) © **2015 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound-mediated drug delivery, Small interfering RNA, *In vitro* assay, Cell culture device, Sonoporation.**

INTRODUCTION

The transfection of cells has enormous potential for the treatment of cancer and other major diseases. Currently, however, the low permeability of cell membranes to genetic materials represents a major hindrance to its clinical application. Viral vectors have been found to significantly improve intracellular delivery, but their use is limited by the risk of immunogenic responses and/or off-target effects (Edelstein et al. 2007). Non-viral transfection methods have consequently been investigated extensively; but, to date, the majority has yielded relatively low transfection efficiencies and/or unacceptable loss of cell viability (Glover et al. 2005). One method that has shown promise is the use of focused ultrasound (US) in combination with microbubble contrast agents. Under US exposure, the oscillation of the microbubbles induces a temporary permeabilization of nearby cell membranes via a process known as sonoporation (Lentacker et al. 2014). This technique has been used to deliver a variety of therapeutic compounds including small molecule drugs (Escoffre et al. 2011), deoxyribonucleic acid (DNA) (Miller et al. 2002) and small interfering RNA (siRNA) (Kinoshita and Hynynen 2005). However, much better understanding of the governing biophysical mechanisms and optimization of the exposure conditions for particular applications are required for development into a clinically relevant procedure. These, in turn, require the development of robust and reproducible experimental methods for generating and analyzing US-mediated cell transfection *in vitro*. Notably, no device has been designed specifically to fulfill this aim, and in most cases, devices developed for another purpose have been adapted to transfect cells via sonoporation, leading to significant variability between different studies.

On the basis of their geometry/architecture, devices can be classified in three main categories: (i) tubes, (ii) well plates or chambers and (iii) parallel-plate chambers (the most commonly used being OptiCell from Thermo Fisher Scientific, Waltham, MA, USA). Coupling of plastic round-bottom tubes (Fechheimer et al. 1987) and commercially available tissue culture tubes (Ogawa et al. 2002) with US transducers has been reported for

Address correspondence to: Eleanor Stride, Department of Engineering Science, Institute of Biomedical Engineering, University of Oxford, Old Road Campus Research Building, Oxford OX3 7 DQ, UK. E-mail: eleanor.stride@eng.ox.ac.uk
DC and JO contributed equally to this work.

transfection of mammalian cells. Although simple to use, these devices are largely limited to the treatment of cells in suspension, and it is difficult to predict the acoustic field in the target region, particularly if acoustic standing waves are generated as a result of reflection from the tube walls, a problem particularly in low-aspect-ratio tubes. Similar problems may occur in well plates and cylindrical chambers coupled with piezo-electric transducers that have been used to investigate accumulation of drugs such as paclitaxel and doxorubicin in endothelial and cancer cells (Jackson et al. 2011) and to evaluate the biological response of cells undergoing sonoporation (Schlicher et al. 2006; Zhong et al. 2011). Well plates are particularly suitable for multiparametric studies as they provide the advantage of fast, post-sonication readouts, for example, using automatic plate readers. However, they suffer from limited flexibility with respect to the alignment of the US source and the significant mismatch in acoustic impedance between cell medium and the constitutive materials (*i.e.*, polystyrene [Bruus 2012]) and/or the air–water interface when insonified from below. The latter remains a problem even when plates with a flexible surface membrane (*e.g.*, BioFlex by Flexcell International, Burlington, NC, USA) are used.

In recent years, parallel-plate chambers have attracted increasing interest in the biomedical US community as a means of investigating US-mediated intracellular drug delivery in a physiologically relevant, confined environment. In particular, the OptiCell cell culture device (now discontinued) was previously used to systematically investigate transfection efficiency under varying acoustic conditions (Rahim et al. 2006), to optimize US-mediated targeted gene delivery via microbubbles to cultured primary endothelial cells (Meijering et al. 2007) and to examine the impact of molecular weight on the mechanism of molecular entry into primary endothelial cells (Meijering et al. 2009). Furthermore, acoustic and magnetic fields in combination have been used for intracellular gene delivery with this system (Stride et al. 2009). Despite their widespread usage, parallel-plate chambers may suffer from a range of drawbacks, including the recurrence of obstructions; the potential for generating high wall shear stresses during priming, which could compromise cell viability; difficulty in removing exogenous, trapped air bubbles; and the need for extracting cells post-sonication for culturing or analysis purposes.

Other methods have also been devised for examining US-mediated therapeutic delivery. For example Hu et al. (2013) developed a cell chamber integrated with a waveguide to sonoporate cells while observing membrane perforation and recovery using confocal microscopy. Karshafian et al. (2010) used an exposure chamber with two Mylar windows to investigate if sonoporation is limited by the size of the macromolecule to be delivered. Agar phantoms with a cavity have also been developed to examine the correlation between acoustic cavitation and cell viability (Lai et al. 2006). However, as these systems are application specific and not commercially available, they could be difficult to easily reproduce on a large scale.

With the aim of overcoming the limitations of previous systems, we have developed an easy-to-use, biologically and acoustically compatible device for application in US-mediated delivery of therapeutic compounds. It comprises a commercially available cell culture dish coupled with an acoustically compatible "lid" developed in-house that enables the dish to be immersed in a water bath for US exposure. The ease of fabrication and operation, together with its compatibility with standard cell culturing techniques and analytical instruments, makes it suitable for widespread application in different laboratory settings, potentially opening the way for the adoption of standard experimental protocols in the field of *in vitro* US-mediated delivery of therapeutic compounds. To facilitate the replication of the device by different laboratories, in addition to the description below, the full technical drawings and fabrication instructions are freely available on-line (www.sonofluidics.co.uk).

METHODS

Lid design and fabrication

The lid was fabricated in polydimethylsiloxane (PDMS) via replica moulding. PDMS has been extensively used for fabricating microfluidic devices, mainly because of its biocompatibility (Leclerc et al. 2003; Sia and Whitesides 2003), optical transparency (Whitesides 2006), permeability to gases (Zanzotto et al. 2004) and ease of moulding (Prebiotic 1995).

For this purpose, a manifold was constructed comprising three main components (Fig. 1a): (i) circular base plate (84 mm in diameter, 10 mm thick); (ii) 6.3-mm-thick disk with a step-like variation in diameter (*i.e.*, from 34 to 33 mm); and (iii) 49-mm-inner-diameter ring. Individual components were designed using SolidWorks 2012 (Dassault Systèmes SolidWorks, France) and fabricated in either Teflon or poly(methyl methacrylate) (PMMA) using a lathe machine (Weiler Praktikant VCD, Weiler, Emskirchen/Mausdorf, Germany). The three components were assembled in a single manifold by inserting the ring and the disk into two recesses milled within the base plate. Two segments of 1.6-mm–outer diameter polyether ether ketone (PEEK) rod (IDEX, Lake Forest, IL, USA) were glued to the top surface of the disk. Two circular recesses (0.5 mm deep) were milled on the disk surface to facilitate positioning of the rods.

Degassed, liquid PDMS (curing agent:monomer ratio = 1:10 w/w; Sylgard 184, Dow Corning, Corning,