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● *Original Contribution*

THEORETICAL EVALUATION OF THE ACOUSTIC FIELD IN AN ULTRASONIC BIOREACTOR

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Abstract—Ultrasound-assisted bioreactors that provide mechanical conditioning to cells have broad applicability in tissue engineering, but biological experiments with ultrasound are very sensitive to environmental conditions. A mathematical model was developed to complement experimental measurements, as well as to describe ultrasonic fields existing in regions where measurements are impossible, specifically, within microporous tissue engineering scaffolds. The model uniquely combines Biot theory to predict the ultrasonic field in the scaffold with an electromechanical transducer model to couple the mechanical stimulation experienced by cells to the external electrical input. In the specific example examined here, cells immobilized on scaffolds are subjected to different forms of ultrasonic stimulation due to the formation of standing wave fields and vertical high-pressure bands. The model confirms the sensitivity of the supplied acoustic power to the liquid level in sonobioreactors and identifies the input electrical impedance as a method of detecting resonance effects. (E-mail: hviljoen1@unlnotes.unl.edu) **© 2015 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound, Bio-acoustics, Mechanotransduction, Tissue engineering, Finite volume method, Spectral method, Biot theory.**

INTRODUCTION

Ultrasound is frequently used in therapeutic applications such as wound healing, targeted drug delivery and lithotripsy (Dalecki 2004; Naito et al. 2010; Rubin et al. 2001), as well as in *in vitro* cultures (Hsu et al. 2006a, 2006b; Nishikori et al. 2002; Parvizi et al. 1999; Zhang et al. 2003). Ultrasonic bioreactors (also known as sonobioreactors) (Fig. 1) can provide the necessary mechanical stimulation required to reproduce physiologic conditions and promote synthesis of biological tissue substitutes (Hsu et al. 2007; Whitney and Lamb 2012; Zhang et al. 2003). Cells are most often stimulated by pulsed ultrasound, but recent work has indicated that the stimulation of *in vitro* chondrocyte cultures by continuous ultrasound has a positive influence on several factors, including cell proliferation, viability and gene expression of select chondrocytic and load inducible-markers (Hasanova et al. 2011; Louw et al. 2013; Noriega et al. 2013; Whitney and Lamb 2012). Despite the many applications of ultrasound in biology, the exact mechanism whereby ultrasound affects cells is not well understood (Dalecki 2004; Fowlkes 2008; Nelson et al. 2009).

Sonobioreactors and the ability to conduct reproducible *in vitro* experiments are central to tissue engineering and medical ultrasound research in general. The effects of ultrasound are usually investigated by stimulating a group of cells and measuring the average biological response. It is important to ensure that all cells in the treatment group receive similar acoustic simulation to accurately correlate cellular responses to ultrasonic stimulation. Furthermore, acoustic regimens must be controlled very precisely to avoid negative bio-effects induced by cavitation or thermal events (Karande et al. 2005; Kinoshita and Hynynen 2007; Mitragotri 2005).

Ultrasound wave propagation in routinely used *in vitro* setups have been analyzed by using either a combination of simulations and hydrophone measurements (Hensel et al. 2011) or a combination of pulse-echo ultrasound, laser Doppler vibrometry and Schlieren imaging (Leskinen and Hynynen 2012). These studies highlighted the sensitivity of experiments in ultrasonic bioreactors to external disturbances, with frequency effects creating uncertainties of up to 700%, and concluded that detailed analyses are required to control experimental variations (Leskinen and Hynynen 2012).

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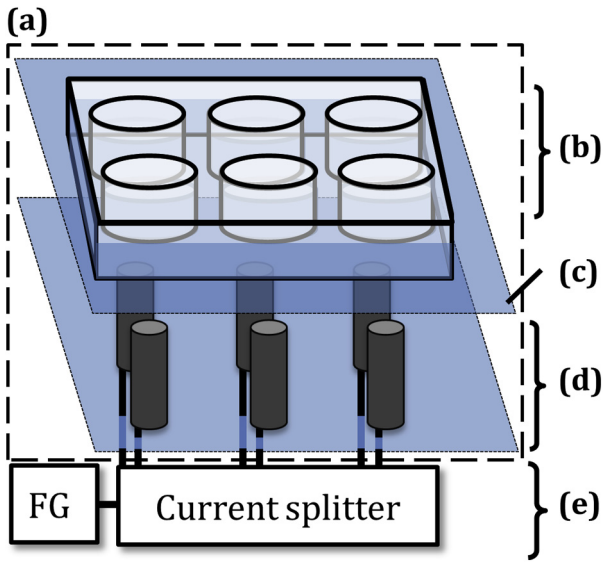

Fig. 1. Typical sonobioreactor setup. (a) The entire setup, excluding electrical components, is kept in an incubator with temperature and oxygen controlled. (b) Cell seeded scaffolds are placed in the wells of a tissue culture plate; each well contains growth medium. Tissue culture plates can contain 6, 12 or 24 wells. (c) The tissue culture plate is partially submerged in an aquarium so that no air bubbles exist below the tissue culture wells, while the culture medium is kept isolated from the aquarium. The aquarium acoustically couples the wells to the ultrasonic transducers. (d) An ultrasonic immersion transducer is placed below each of the tissue culture wells to provide mechanical stimulation (bottom-up sonication). Alternatively, the transducers may be placed directly above the tissue culture well, partially immersed in the culture medium, for top-down sonication. (e) A function generator (FG) and current splitter provide the voltage input to each ultrasound transducer. The input is sinusoidal with variable frequency and voltage amplitude.

A mathematical model is one such analysis method. Such a model would be especially useful in predicting ultrasonic effects within regions inaccessible to measurement, such as the interior of tissue engineering scaffolds. The original theory for the propagation of sound through porous media was developed by M.A. Biot (1962), and is valid whenever the acoustic wavelength is much larger than the characteristic dimensions of the porous structure under investigation, as is the case here. Furthermore, the bioreactor is driven by a real transducer with a complex beam shape that must be accounted for. The power delivered to the bioreactor by the transducer is dependent on the electrical input as well as the transducer impedance. It is necessary to couple the bioreactor model to an electromechanical transducer model to quantitatively predict the ultrasonic field in the bioreactor and scaffold. A unique combination of mathematical models, including the effects of porous media, real transducers and electrical inputs, is necessary to model an ultrasonic bioreactor.

Our goals in this work were to (i) develop a framework for applying an appropriate mathematical model with the ability to predict the propagation of ultrasound through liquids, elastic solids and porous media, to a sonobioreactor; (ii) identify and validate appropriate numerical methods for solving the mathematical model; (iii) couple the model-predicted ultrasonic field to the measureable transducer electrical input; (iv) use the mathematical model to identify possible disturbances that may cause experimental error by affecting the ultrasonic field; and (v) identify potential measurement and control variables as well as design criteria that can be used to ensure repeatability of sonobioreactor experiments. An accurate and efficient mathematical model can be used to design experiments aimed at discovering the biological effects of ultrasound and optimize bioreactors for the eventual production of synthetic tissues and other high-value biological products.

METHODS

Mathematical modeling

An axisymmetric mathematical model suffices for the bioreactor setup illustrated in Figure 2(a). The bioreactor consists of fluids (water in aquarium, culture media), solids (tissue culture well) and poro-elastic media (scaffolds). The ultrasonic transducer acts as the acoustic source and fixes the fluid velocity $v = [v_r, v_z]$ in the z-direction such that $v_z(r, z; t) = v_0(t)$ at the transducer face. The velocity amplitude v_0 depends on the electromechanical coupling occurring between the supplied voltage source and the transducer face by means of a piezoelectric slab, as predicted by the Krimholtz–Leedom–Matthaei (KLM) transducer model (Krimholtz et al. 1970).

Two numerical methods are investigated and compared in terms of accuracy and computational efficiency. The transfer matrix/angular spectrum approach (TM/ASA) is a semi-analytical method acting under the fundamental assumption that acoustic wave reflections in the radial direction can be neglected (Louw 2013). Mathematically, the TM/ASA represents the ultrasonic transducer as being set in an infinite, rigid baffle and approximates every bioreactor component (scaffold, tissue culture well, *etc*.) as infinitely wide, hence no radial reflections (see Fig. 2b). In practice, the assumption is justified when each acoustic component has a diameter greater than that of the ultrasonic beam; this postulate is evaluated by comparing TM/ASA results to the output of a second numerical method: the finite volume method (FVM) (Saenger et al. 2000; Virieux 1986). The FVM is a full numerical method that accounts for the complex geometry of the entire system, but has much higher computational cost than the TM/ASA. By comparing