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Numerical model for *in-vitro* ultrasound stimulation of bone cells: a parametric study

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1. Introduction

In the early 1950s, the first clinical observations on the effect of *Ultrasound Stimulation on Bone Regeneration (USBR)* to stimulate osteogenesis, were reported (Buchta 1952). However, due to the large number of different protocols proposed in the literature, and because the effects of this ultrasonic mechanotransduction are still unclear, *USBR* remains controversial (Padilla et al. 2014).

To better understand the interaction between ultrasound and bone cells, the development of *in-vitro* experiments is a key step.

In order to characterize and control the ultrasound dose delivered to the cells inside a Petri dish, an innovative experimental set-up has been proposed. In this set-up, an absorbing lid has been designed to avoid standing waves inside the Petri dish and an acoustic lens is used to enlarge and homogenise the acoustic field generated at the cells.

In this study, a numerical model using finite element (FE) method is developed in order to determine the shape of this acoustic lens, its material composition, the excitation frequency and the distance f_d between the transducer.

2. Methods

A numerical model of the *in-vitro* stimulation experimental set-up is developed in *COMSOL Multiphysics*® v5.5, by using the Pressure Acoustics module (transient) for the propagation of acoustic wave in the fluid parts and the Solid Mechanics module for the dish. The multiphysics coupling is automatically managed at the boundaries by the software.

2.1 Numerical model

Due to cylindrical nature of experimental set-up, the corresponding numerical model is considered to be 2D axis-symmetric, as shown on figure 1.

Everything is considered as fluids, except the Petri dish, in which no cells are present for this study. The acoustic attenuation is not taken into account in this preliminary study.

Petri dish

The Petri dish used in these experiments is a Corning model (ref. 430196) with an external diameter of 55 mm, a depth of $H_{dish} = 15$ mm and a wall and bottom thickness of $pdwTh = pdbTh = 0.8$ mm. The material constituting the Petri dish

is polystyrene. A home-made absorbing lid is mounted above the Petri dish. The absorbing system is a thermoplastic polyoxymethylene (POM) tube containing degassed water and absorbing foam (Aptflex F28, Precision Acoustics Ltd, Dorchester, UK). It has been experimentally validated to avoid wave reflection and standing wave formation inside the Petri dish (Majnooni et al. 2021). The dimensions, assembly and materials have been carefully thought out and chosen to be compatible with cell culture. The culture area is reduced to a diameter of 35 mm with a POM annulus placed at the bottom of the dish (Fig. 1).

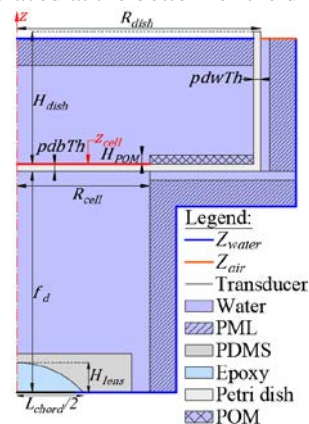


Figure 1 Schematic of the 2D axis-symmetric model

Acoustic signal

The ultrasound stimulation is a $f=1$ MHz-pulsed signal generated with a transducer of 13 mm in diameter:

$$P(t) = P_0 \sin(2\pi ft), \quad (1)$$

$$\text{with } \begin{cases} P_0 = 57 \text{ kPa for } 0 \leq t \leq 200 \mu\text{s}, \\ P_0 = 0 \text{ kPa for } 200 \mu\text{s} < t \leq 1 \text{ ms}. \end{cases}$$

Acoustic dose

In designing and development of US medical applications, acoustic intensity which is defined as the rate of energy transfer per unit time per unit area, is a key parameter.

In order to characterize the acoustic field inside the Petri dish, different parameters are calculated:

- The *Temporal Average Intensity* I_{TA} .
- The *Spatial Average Temporal Average Intensity* I_{SATA} which corresponds to the I_{TA} averaged over the beam cross-sectional area.
- The *Spatial Peak Temporal Average Intensity* I_{SPTA} , which corresponds to the maximum value of I_{TA} .

- The ratio of surface covered by at least 25% I_{SPTA} over the total surface (Preston 1991).
- The mean value and standard deviation of the gradient of the absolute value of normalized I_{TA} (Horne et al 2020).

Acoustic lens

Preliminary study (Majnooni et al. 2021) has revealed that the acoustic field delivered at the cell's location is smaller than the targeted area (35 mm in diameter) and the acoustic intensity distribution is heterogeneous. To fix the problem, an acoustic lens is developed. The inner part of the lens is made in epoxy and the outer part, in polydimethylsiloxane (PDMS) (Yang et al. 2017). Its efficiency is tested in a numerical model.

The properties of the different materials used in the numerical model are given in table 1.

Material	Density [kg/m ³]	Velocity (L) [m/s]	Impedance [MRayls]
Water (17.8°C)	1000	1474	1.47
Epoxy	1080	2640	2.85
Polystyrene	1050	2367	2.49
PDMS Sylgard 160	1580	950	1.5
PDMS Sylgard 164	1570	1049	1.65
PDMS Sylgard 184	970	1200	1.16

Table 1 Characteristics of the different materials used

2.2 Parametric studies

Different parametric sweeps are performed in specified ranges in order to select an optimal set of parameters:

- The lens geometry: the height H_{lens} of the epoxy part varies between 1 mm and 5 mm so its curvature radius R_{lens} varies between 29 mm and 8 mm, in order to keep the width of the epoxy part $L_{chord} = 15$ mm.
- The lens composition: the effect of the PDMS part of the lens was tested for three types of PDMS: Sylgard 160, Sylgard 164 and Sylgard 184 (Dow Corning, Midland MI) (Table 1).
- The distance f_d between the transducer and the Petri dish: two distances had been tested ($f_d = 25$ mm and $f_d = 30$ mm), for four different frequencies (1 MHz, 1.2 MHz, 1.5 MHz and 2 MHz).

These 3 studies are conducted independently of each other.

3. Results and discussion

Figure 2 presents the I_{TA} , inside the Petri dish (in the plane perpendicular to the z -axis), calculated for excitation frequencies of 1 MHz and 2 MHz, and normalized by the I_{SPTA} at 1 MHz. These results were obtained with a chosen lens geometry such that $H_{lens} = 3.36$ mm and $R_{lens} = 10$ mm, PDMS Sylgard 184 and the distance $f_d = 30$ mm. The normalized I_{TA} are given at $z = 30.81$ mm corresponding to the future cell's height inside the Petri dish.

The ratio of the I_{SPTA} at 2 MHz over the I_{SPTA} at 1 MHz is equal to 1.27. The ratio between both I_{SATA} is equal to 1.02.

In both configurations the targeted area is covered and the levels of intensity are close. But, for this configuration, a frequency of 2 MHz provides a more homogeneous distribution of the intensity inside the dish.

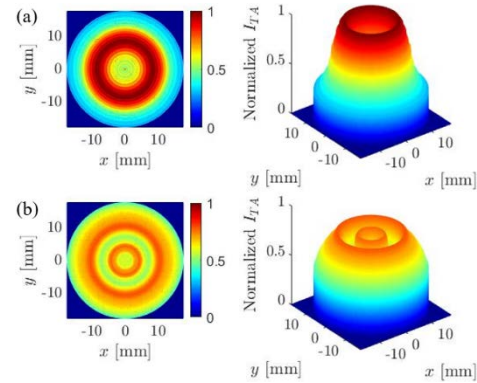


Figure 2 Comparison of normalized I_{TA} for a frequency of (a) 1 MHz and (b) 2 MHz

4. Conclusions

This work proposes a numerical FE-model to guide the design of an acoustic lens, in order to enlarge and homogenise the acoustic intensity delivered inside a Petri dish in an *in-vitro* ultrasound stimulation of cells context. The next step will be to build the acoustic lens and experimentally measure the intensity field inside the Petri dish to compare it with the simulated one, and finally to test this set-up in *in-vitro* conditions with murine osteocytes cells (MLO-Y4 cell line, Kerfast, Boston, USA), in 2D monolayered culture.

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