In vitro Platform for Acoustic and Electrophysiological Investigations of Ultrasound Neuromodulation

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Abstract. With the increasing age of human population, prevalence of neurodegenerative diseases, such as Parkinson’s disease or Alzheimer’s disease, increases. Brain pacemakers are used to treat their symptoms. For its implantation surgery is needed. Risky surgical interventions can be avoided using transcranial stimulation methods such as Transcranial Magnetic Stimulation (TMS). However, the TMS suffers from low spatial resolution. Ultrasound (US) has gained lots of attention recently because of its transcranial and focusing capabilities. Many researchers have investigated ultrasound neuromodulation through in vivo and in vitro studies, thus US has the potential to develop to a promising alternative for neuromodulation. However, results show that US can influence the neural activity but the mechanisms are not yet fully understood. For systematic investigations, an in vitro platform for ultrasound excitation of neuronal cells based on microelectrode arrays is proposed. This setup will allow investigations over a wide range of frequencies and sound pressure, in order to learn more about the effect on neuronal cells. In this work, the acoustic characterization of the platform is presented.

Keywords

neuromodulation, ultrasound, neuron, neural activity, microelectrode array, simulation, finite elements method

1. Introduction

The quality of life has increased dramatically over the past decades. According to the United Nations (UN), approximately one billion individuals worldwide will be aged 65 years or older by 2030 [1]. The demographic change is one of the major growth drivers of the worldwide neuromodulation market. In 2015, it reached ~$3.3B and is forecasted to hit ~$4.8B by 2020 [1]. Brain pacemakers are used for Deep Brain Stimulation (DBS) and are well established devices of the neuromodulation market. For their installation surgery is needed because holes must be drilled through the patient’s skull to set up the electrodes for stimulation. Risky surgical interventions can be avoided using transcranial stimulation methods such as Transcranial Magnetic Stimulation (TMS) and transcranial Direct Current Stimulation (tDCS). However, the TMS suffers from low spatial resolution due to the induced magnetic field by the coils [2].

Ultrasound (US) has gained lots of attention recently because of its transcranial and focusing capabilities. Many researchers have investigated ultrasound neuromodulation through in vivo and in vitro studies. They have processed experiments on different body parts of humans and animals, e.g., human and cat cochlea [3, 4], human brain [5] and intact mouse cortex and hippocampus [2]. Parameters applied in the experiments are frequency, acoustic power, pulsed or continuous excitation and beam form [6]. Results show that US can influence the neural activity but the mechanisms are not yet fully understood. Over 60 years ago, the Hodgkin-Huxley model (H-H) [7] was presented to describe the electrophysiological activity of neurons. It explains the electrical processes during signal generation and propagation in neurons.

However, the model fails to describe how mechanical US waves influence the action potentials occurring in the neuron. Plaksin et al. [8] modified the H-H model by including a so-called bilayer sonophore model [9] to describe the response of the lipid membrane to ultrasound (NICE model). In the H-H model, the membrane capacitance \( C_M \) is assumed to be constant as the membrane geometry is flat (see Fig. 1a). It is well known that US induces intramembrane nanobubbles which lead to oscillations in the local curvature of the lipid membrane (see Fig. 1b). In the NICE model, this membrane deformation is considered and \( C_M \) is not constant anymore. The change in capacitance leads to an AC current flowing across the lipid membrane.
In *vitro* experiments with neurons on a microelectrode array (MEA) chip are a good opportunity to investigate neural activity resulting from ultrasound exposure. Multi-unit recordings from neuronal networks cultured on MEAs are a widely used approach to achieve basic understanding of network’s electrophysiological activity as well as the realization of cell-based biosensors. In contrast to *in vivo* experiments, they allow the examination of individual mechanisms in a controllable environment. For this reason, an *in vitro* platform is set up to investigate the effects of ultrasound on neuronal cells plated on a MEA chip (see Fig. 2).

The setup includes a signal and pulse generator, an amplifier and an US transducer for the generation of pulsed ultrasound waves. In order to achieve good control and low losses, it was decided to couple the generated sound waves directly into the cell culture medium in the container of the MEA chip. To allow for an adjustable distance between transducer and MEA chip, the container ring is extended by a fluid-filled, acoustic wave guide (see Fig. 3, wall extension). A cover closes the wave guide and reduces environmental influences (see Fig. 3, cap). The electrophysiological activity of the neurons is measured with a recording system connected to the MEA chip.

In a first approach and with the goal to quantify the acoustic pressure distribution in the relatively small geometry (compared to the wavelength), simulations based on a simplified model of the MEA chip using the finite elements method (FEM) are performed. With this method, physics based problems are solved numerically with the help of partial differential equations and proper boundary conditions. The object of interest is divided into many small elements (finite elements).

**2. Materials and Methods**

**2.1 Model geometry**

To reduce the complexity of the model, it was simplified and only the acoustically relevant parts like the MEA chip reservoir and the piezo element of the US transducer were considered. Neurons are not implemented in the model and the square base plate is not fully covered (only the area underneath the ring).

At first, the cylinder geometry was set up including a wall extension and a base plate. It has a diameter of 19 mm and a height of 50 mm which defines the distance between the base plate of the MEA chip and the US transducer. The main goal is to ensure that the exposure of the neurons is done in the so-called far field of the US transducer where sound decreases inversely with the distance from the source and propagation is unidirectional, whereas the near field provides no simple mathematical rule.

For the calculation of the near field length $N$, the diameter of the aperture (piezo element) $D$, the US frequency $f$ and the speed of sound $c$ are required (see Eq. 1). In this scenario, the near field length is 28.47 mm.
\[ N = \frac{D^2 f}{4c} \]  

(1)

The material used for the domain of the cylinder is water because cell culture medium has similar acoustical characteristics compared to water. For the margins of the reservoir, a glass ring is assumed. Furthermore, for the boundaries of the reservoir, a spherical wave radiation boundary condition is used to minimize the reflections of the sound waves. Above the reservoir, a second smaller cylinder was placed which describes the used piezoelectric transducer. The transducer has a diameter of 13 mm and a thickness of 0.5 mm, whereas the material is defined as lead zirconate titanate (PZT-5H). For the oscillation of the piezo element, the voltage was set to 100 V.

A mesh with 2nd order finite elements was used for spatial discretization of the model. The mesh size is 0.1484 mm which equals 10 elements per wavelength. For the calculation of the wavelength, the speed of sound in water (1,484 m/s) and the frequency of 1 MHz were used. This results in a wavelength of 1.484 mm which must be divided through 10 to realize 10 elements per wavelength. The application of these settings resulted in 61,228 elements in the model.

2.2 Computation

With the Pressure Acoustics, Frequency Domain the distribution of the acoustic pressure for the selected frequency of 1 MHz was investigated. For the computation, a workstation equipped with two CPUs (20 cores and 40 threads) combined with 256 GB RAM was used. The modelling software was COMSOL Multiphysics® 5.2a (Comsol AG, Stockholm, Sweden) with the Acoustics and the Structural Mechanics Module.

3. Results and Discussion

The convergence of the model was determined by processing a parametric sweep over a specific range of elements per wavelength describing the mesh size. In every sequence, the acoustic pressure maximum in the reservoir domain was determined and compared with the reference value. For this model, an appropriate convergence was found at 10 elements per wavelength which equals a mesh size of 0.1484 mm or 137,754 degrees of freedom (DOF).

For visualization of the results, the sound pressure distribution is displayed in Figure 4 in a 3D view for a frequency of 1 MHz. The distance between the US transducer and the MEA bottom, where the neurons will be positioned, is 50 mm. In the near field domain of the US transducer, propagation is not unidirectional. In the far field domain, positive (red sections) and negative (blue sections) sound pressure maxima are located along the centre axis. As expected, the sound pressure decreases here with increasing distance to the source.

The sound pressure distribution on the bottom of the MEA chip is of relevance because the neurons are located on the bottom in the in vitro experiments. For this reason, a slice plot was created describing the sound pressure distribution on the bottom of the chip (see Fig. 5). The slice shows a high sound pressure distribution (red section) around the centre of the reservoir except for a small area near the centre axis (white section). With increasing distance to it, the sound pressure decreases (blue section). In the centre of the MEA chip bottom, the sound pressure is around 100.953 kPa.
When interpreting the results, it is important to take into account that the simulation model is simplified in order to reduce the complexity. Boundary conditions were used to replace physical borders, e.g., base plate and ring wall. In the model, boundaries are assumed to be non-reflective. Due to small geometries, reflections at the interfaces between medium and glass would lead to strong interferences of sound waves. How far real conditions might introduce deviations from the ideal situation needs to be examined in further measurements.

Summary

In this paper, the characterization of an in vitro platform for ultrasound stimulation of neuronal cells on a MEA chip was presented. The platform will allow the variation of different parameters, such as the frequency and sound pressure in a wide range. In this FEM simulation of a simplified MEA chip geometry, the sound pressure distribution on the chip surface was investigated by applying US waves with a frequency of 1 MHz generated by a piezoelectric transducer. The results of the simulation show a near field and a far field behaviour. In the far field, the sound pressure propagates along the centre axis with minima and maxima and it decreases with growing distance from the ultrasound source. In the next step, acoustical measurements and in vitro experiments will be processed to investigate the real behaviour in the setup and to compare the results with those of the simulation afterwards.

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References


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Nico-Wei KÜCK was born in Changchun, People’s Republic of China (PRC), and earned his bachelor’s degree as industrial engineer in February 2016 at the University of Applied Sciences Aschaffenburg. Currently he is enrolled in the scientific master’s program of industrial engineering at the same university.