Development and validation of a novel phonomimetic bioreactor

Claus Gerstenberger*, Andrijana Kirsch, David Hortobagyi, Tanja Grossmann, Markus Gugatschka

Division of Phoniatrics, ENT University Hospital Graz, Medical University of Graz, Graz, Austria

vocal fold fibroblasts, bioreactor; cell culture; vibration

Introduction

Vocal fold fibroblasts (VFF) constitute the main cell type of the vocal fold's lamina propria, produce the extracellular matrix and thereby determine the tissue characteristics. To study VFF behavior under *in vitro* conditions it is important to mimic the dynamic environment of the *in vivo* state [1]. The aim of our study was to develop and validate a novel phonomimetic bioreactor system mainly based on commercially available components. The use of cell culture dishes with flexible silicone bottoms in combination with a suitable loudspeaker made it possible to expose the cells to various kinds of phonatory stimuli.

Methods

The vibration system consisted of a loudspeaker mounted into a custom-made polyoxymethylene (POM) housing with cell culture plate fixation elements (Fig 1A). The loudspeaker (AL 170, Visaton GmbH, Haan, Germany) was connected via an XLR audio cable to an audio power amplifier (XLS 1502, 775W, Crown International, Elkhart, IN, USA). The audio cable was pulled through a sealed hole in the back of the incubator (ICO105, Memmert GmbH, Schwabach, Germany). We used 6-well-plates with a flexible silicone base (BioFlex, Flexcell International Corporation, Burlington, NC, USA) which were directly stimulated via sound waves. Stimulation sound files were generated with the open source audio software Audacity (audacityteam.org) and exported to a mobile audio player (iPod touch 4G, Apple Inc., Cupertino, CA, USA). The voltage at the audio output of the audio power amplifier served as a measure of the vibration intensity and was determined with a digital multimeter (Fluke 179, Fluke, Everett, WA, USA). The laboratory setting of the components is shown in Fig 1B. We were able to couple any stimulation pattern with high amplitudes and frequencies up to 10 kHz. An example of a 24 hours pattern is shown in Fig 1C.

The vibration behavior of the silicone membranes in the culture plates was investigated by laser Doppler vibrometry (Polytec OFV 3001, Waldbronn, Germany) [2].

Immortalized human vocal fold fibroblasts (hVFF) [3] seeded on the flexible culture plates and allowed to attach for 24 hours in static conditions and then transferred to the vibration bioreactor for 48 hours. Non-vibrational control cells were cultivated in parallel in a separate incubator. After a one-hour rest period, cells and supernatants were harvested for subsequent analyses.

Results and Discussion

We present a new type of phonomimetic bioreactor. Compared to previous models, our device is easy to assemble and cost-effective, yet can provide a wide spectrum of phonatory stimuli based on the entire dynamic range of the human voice. Depending on the amplifier's set volume and the selected stimulation frequency displacements up to 1 mm in the center of a well could be achieved. Gene expression data of VFF cultured in our phonomimetic bioreactor show a significant effect of vibration on ECM metabolism, which illustrates the efficacy of our device. Gene expression of hyaluronan synthase 2, collagen III, fibronectin and TGFß-1 was significantly upregulated in VFF exposed to vibration, compared to static control. Vibration also significantly upregulated collagen I gene and protein expression.

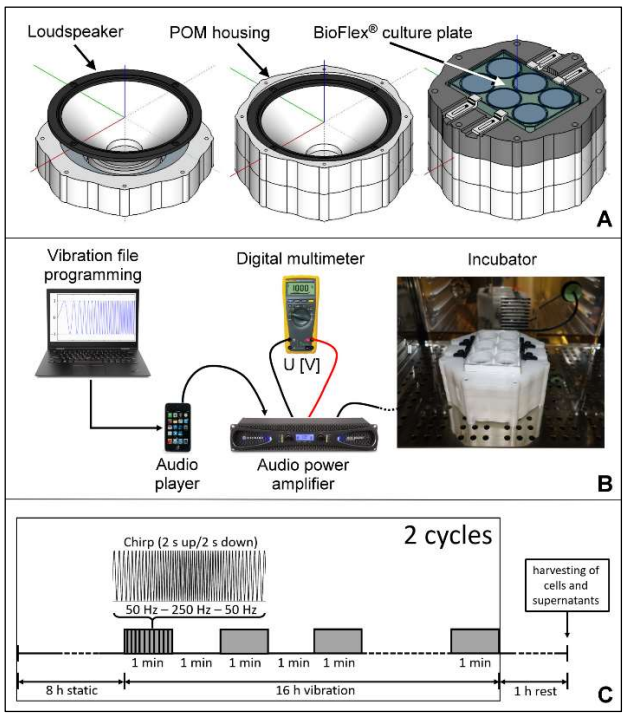

Figure 1: Construction of the bioreactor. A custom-made POM housing, designed using a 3D CAD software, accommodated the BioFlex plate (A). The schematic diagrams depict the assembly of all the components of the bioreactor (B) and the vibration pattern to which the cells were exposed to (C).

References

[1] Catten M *et al*, Otolaryngol Head Neck Surg. 1998 May; 118(5):663–7.
[2] Kirsch *et al*, PLoS One. 2019 Mar 14; 14(3): e0213788
[3] Chen X *et al*, Tissue Eng Part C Methods. 2009 Jun; 15(2):201–12

*corresponding author: claus.gerstenberger@medunigraz.at