Construction and validation of a phonomimetic bioreactor

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Introduction: To date there is no ultimate recommendation on how long patients should rest their voice after an acute injury. Orientating on other medical fields, where a rapid mobilization after surgery is aimed at in order to achieve an earliest possible recovery, there has been a tendency in decreasing the interval of voice rest after phono-microsurgery in the past years. The impact of mechanical forces on the lamina propria has been addressed by several papers, knowing that it plays an important role in phonation.
This in vitro study aims to reveal the impact of mechanical stress by means of vibrational forces on human vocal fold fibroblasts (hVFF), the most abundant cells in the lamina propria, during an acute inflammatory reaction by putting an emphasis on the molecular and cellular level.

Methods: Cells were seeded at a density of 144000 cells per well on two flexible-bottomed BioFlex culture plates. 24 hours later, the medium was changed to a serum-free medium for starvation. After another 24 hours, cells were divided into four groups exposed to different conditions (static or dynamic – with or without cytokines).
An acute inflammatory reaction was simulated by adding 5ng/mL IL1b as well as the same concentration of TGFb1.
By using a phonomimetic bioreactor we were able to apply predefined vibrational stress patterns on hVFF cultured under inflammatory or normal conditions.
Mechanical stimulation was applied four hours daily, over a period of 72 hours.
Subsequently the differences in mRNA proteins, expression as well as the concentrations of being responsible for the extracellular matrix (ECM) composition of the lamina propria, and of proteins, which are known to play an important role in inflammatory reactions, were investigated and compared amongst the different groups.
All experiments were repeated four times.

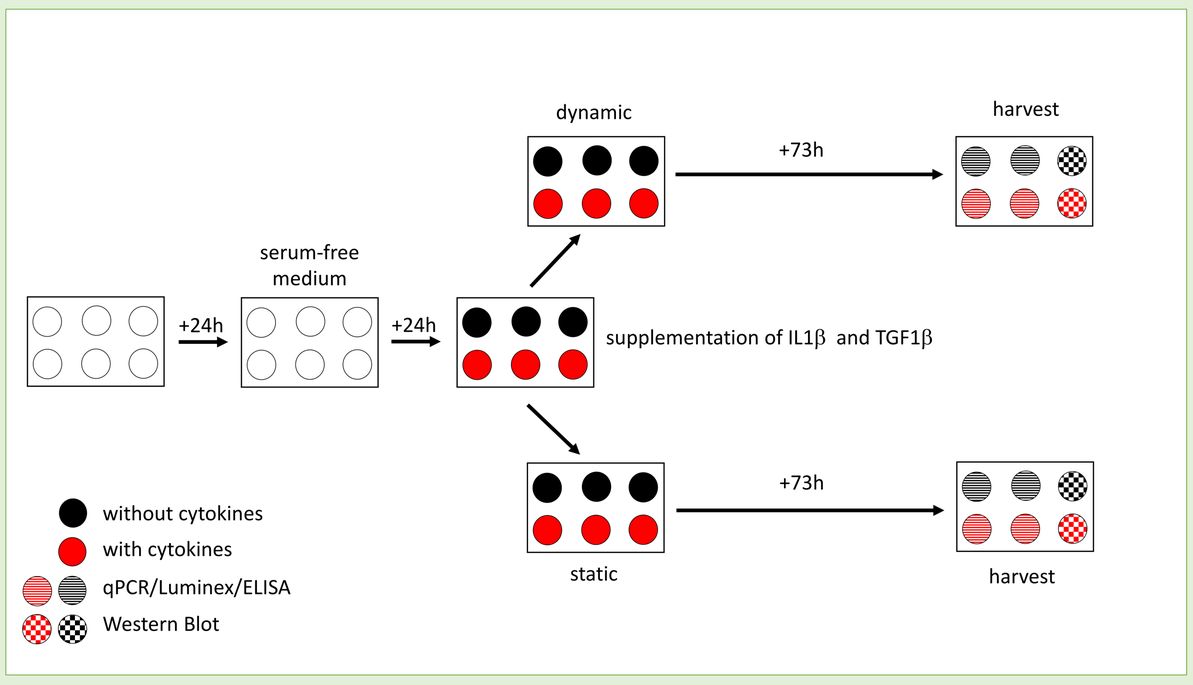

Fig.1: Experimental Design

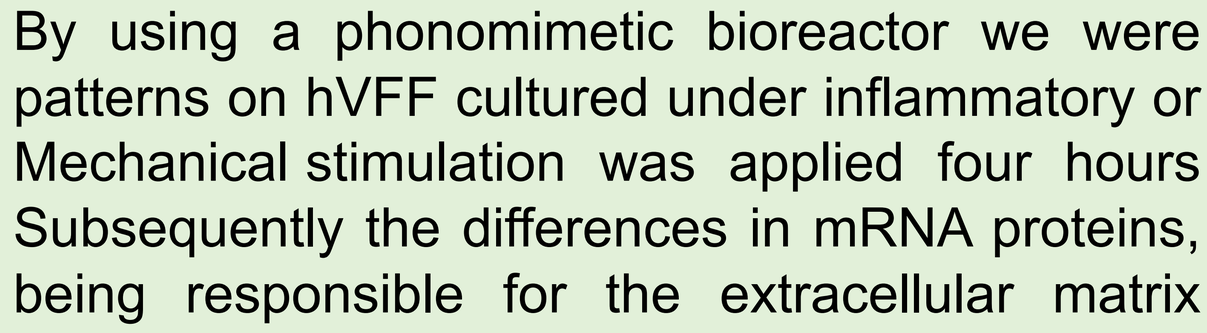

Fig.2: Changes in protein concentration

Results: Cytokine treatment induced a change in the expression of ECM- as well as inflammation-related genes.
The pro-inflammatory cytokine IL11, as well as the myofibroblast marker alpha smooth muscle actin (α-SMA) were significantly reduced when additional vibration was applied. Hyaluronic acid (HA) concentration was significantly increased due to the cytokines, however mechanical stimuli did not show any effect on HA metabolism.

Discussion: The present study aimed to examine the impact of mechanical stimulation on hVFF under inflammatory and pro-fibrotic conditions for the first time. The upregulation of certain genes and proteins by the cytokine treatment was in line with other in vivo experiments. It therefore reflects the applicability of our in vitro inflammatory model. IL11 belongs to the IL6-family and appears to be an essential part in the down-streaming pathway of TGFb1, therefore playing an important role in pro-fibrotic reactions.
During fibrogenesis VFF transform to myofibroblasts, leading to a wound contraction in order to facilitate healing. However, simultaneously, they may impair oscillatory properties of the vocal folds and consequently cause hoarseness. α-SMA is a well-established myofibroblast marker. Reduced concentrations of these two proteins as well as the absence of changes in the desirable anti-fibrotic ECM component HA due to vibration, suggest a beneficial effect of mechanical stimulation following inflammation. These findings corroborate clinical studies which recommend early voice activation following an acute event.

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