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FUNCTIONALITY OF A RHEOMETER-BIOREACTOR TO STRESS AND ENGINEER TISSUE AT SONIC FREQUENCIES

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Introduction

Millions of Americans suffer from hearing loss, voice problems or repetitive motion injuries. When tissues are set into vibration at sonic frequencies, the (complex) shear modulus largely governs their macroscopic vibrational properties. Conditioning engineered tissues at sonic physiologic vibrations is believed to improve their functionality when transplanted into patients. Instruments called bioreactors, uniquely designed for the purpose of growing tissues, exposing the tissue to vibration, and quantifying their mechanical response, are needed to investigate the potential public health benefits of such treatment. Primary functionalities of a bioreactor include (1) establishing uniform cell distributions in 3D constructs, (2) maintaining gas and nutrient concentrations, (3) providing fluid circulation, and (4) exposing developing tissue to physical stimuli. (Freed and Vunjak-Novakovic, 2000) A stress-controlled rheometer was adapted to function as a bioreactor. The degree to which the rheometer-bioreactor could satisfy the four criteria and simulate vocalization forces were evaluated.

Methods

The Gemini rotational shear rheometer (Malvern Instruments, UK) was used. Appliance adaptations were used to accommodate a 3D matrix and a monolayer of cells. For both adaptations, a custom cup, capable of being sterilized and holding cell culture medium, was fitted to the rheometer base. For the 3D configurations, a polyurethane substrate, used in other bioreactor experiments, (Titze and others, 2004) was seeded with fibroblast cells. The cells were incubated in static conditions for two weeks, and then exposed to vibrations in the rheometer bioreactor. Cell viability was quantified and compared to control conditions, applied torque and resulting strains were recorded, along with linear viscoelastic measurements of the 3D construct for three different vibration conditions. A similar experiment was performed using a dermal equivalent 3D matrix cell culture medium. For the monolayer configuration, cells were seeded onto a coverslip. The coverslip was then attached to either the stationary base or the top rotating plate. Vibration was exerted in the bioreactor by immersing the coverslip in cell culture medium augmented with methyl cellulose to increase fluid viscosity. Stresses up to 4000 Pa at 100 Hz were turned on and off every 10 s for 2 h.

Results

Fibroblast-seeded discs maintained comparable cell viability to controls, whether in static or vibrational conditions. Resulting strains was quantified throughout testing, and rheologic data were obtained.(Klemuk, Jaiswal, and Titze, 2008) Highly cellularized 100 μm thick dermal membranes were unaffected by 10 Hz vibration but were morphologically changed after 1 hour exposure to 100 Hz, 60% strain vibration. A monolayer of laryngeal fibroblast cells, subjected to 2 hours of vibraton, remained unchanged for stress exposures up to 3000 Pa and 100 Hz, but with higher forces, the cells were stripped or adversely deformed.

Discussion

These results demonstrate observable tissue response to phonation-like forces in the rheometer-bioreactor at the cellular level and at the macroscopic properties level, even when exposure time is limited. By modifying cup and plate attachments, tissue constructs of varying architectures can be studied. Modifications to the rheometer-bioreactor are ongoing to increase testing duration and extend vibration conditions.

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