MECHANICAL CHARACTERIZATION OF A ROTATING BIOREACTOR FOR TISSUE ENGINEERING

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Mechanical characterization of a rotating bioreactor for tissue engineering

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1. INTRODUCTION

Biological scaffolds composed of an extracellular matrix (ECM) derived from decellularized tissues are increasingly used in regenerative medicine [1]. **Esophageal tissue engineering** is a promising approach to create an esophageal substitute and improve clinical outcomes in the treatment and surgery of the esophagus. In this study, decellularized scaffolds are prepared from porcine esophagus using mild detergents, acids and enzymes to remove animal cells. Our ultimate goal is to provide scaffolds recellularized with human stem cells, thereby producing a new human esophagus [2]. For this purpose, a flow-through perfusion bioreactor is used: the Rotary Cell Culture System (RCCS), commercially provided by Synthecon (Houston, TX) [3]. The objective of this work is to demonstrate the usability of the RCCS for decellularization and to characterize the flow through the device for recellularization.

2. MATERIALS AND METHODS

BIOLOGICAL CHARACTERIZATION

Decontamination of the sample

The esophagus is incubated under constant agitation at 200 rotation per minute (RPM) for 24 hours at room temperature in a solution with 320mg/L Gentamycin (Sigma-Aldrich), 600mg/L Clindamycin (Sigma-Aldrich), 500mg/L Vancomycin (Sigma-Aldrich), 100mg/L Amphotericine B (Eurobio Scientific). [4]

Decellularization of the sample

3 phases of detersion with chemical solutions [2]:

- Sodium Azide (Sigma-Aldrich)
- Sodium Deoxycholate (Sigma-Aldrich)
- DNase I from bovine pancreas (Sigma-Aldrich)

The process is amplified by mechanical action of the RCCS system. [4]
Histological analysis by paraffin inclusion and sections staining with Saffron Eosin Hemathein (HES).

Detoxification of the sample

Incubation of the matrix in Amberlite XAD16N resin (Sigma-Aldrich) in potassium phosphate buffer (VWR) at pH 6.5, for 72 hours under constant agitation at 150 RPM at 30°C. [4]
Evaluation of cytotoxicity by direct contact with BALB/c 3T3 cells (ISO 10993-5).

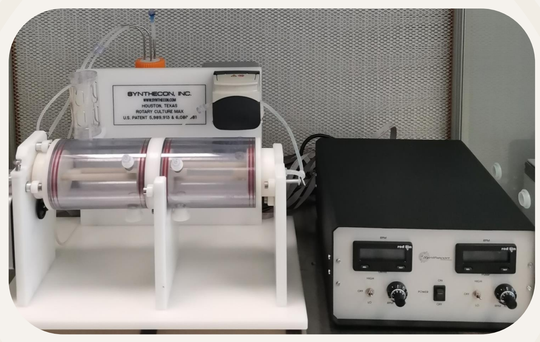

Fig. 1: Rotary Cell Culture System (RCCS)

MECHANICAL CHARACTERIZATION

The rotary cell culture system (RCCS, from Synthecon) allows liquid flow within the tubular esophagus, as well as a mechanical rotation in and around the tissue.

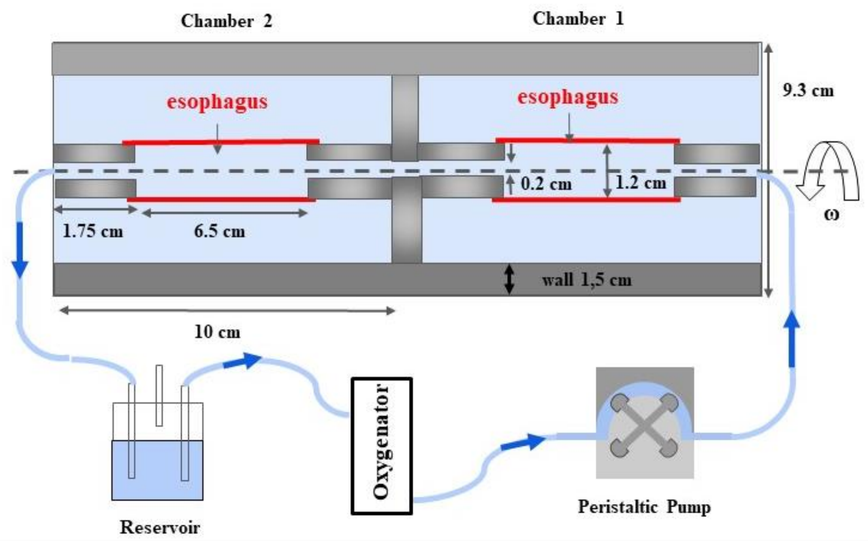

Fig. 2: Schematic representation of the experimental set-up.

The motion of the particle in the rotating frame is governed by the following differential equations:

$$m_P\,\ddot{x} = -k\dot{x} + m_b\omega^2 x + 2m_p\omega\dot{y} - m_b\,g\,\sin(\omega t) \quad \textbf{(1)}$$

$$m_P\,\ddot{y} = -k\dot{y} + m_b\omega^2 y - 2m_p\omega\dot{x} - m_b\,g\,\cos(\omega t) \quad \textbf{(2)}$$

$m_P\,\ddot{x}$ / $m_P\,\ddot{y}$: Acceleration; Particle mass
$-k\dot{x}$ / $-k\dot{y}$: Viscous drag
$m_b\omega^2 x$ / $m_b\omega^2 y$: Centrifugal force
$2m_p\omega\dot{y}$ / $2m_p\omega\dot{x}$: Coriolis force
$m_b\,g\,\sin(\omega t)$ / $m_b\,g\,\cos(\omega t)$: Particle weight compensated by Archimedes

3. RESULTS

BIOLOGICAL

Decellularization

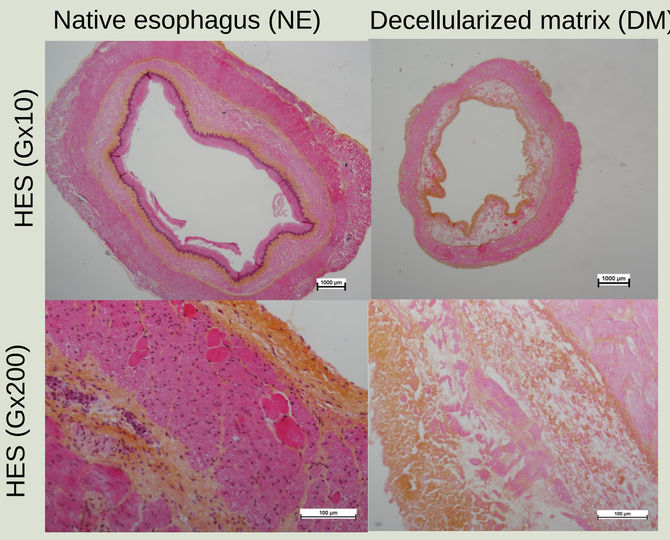

Fig. 3: Histological observations of NE and MD with HES staining.

Cytotoxicity assessment

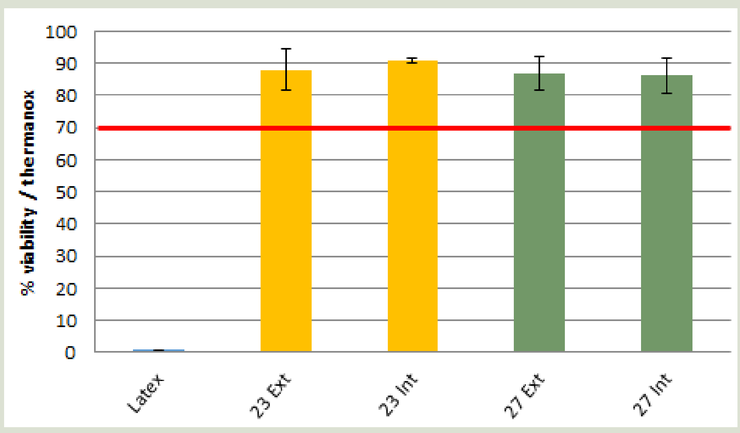

Fig. 4: Cytotoxicity assay by direct contact for 2 samples (n=3)

- Absence of nuclei in the different tissue layers of the MD
- Preservation of the structural framework of the esophagus
- Cell viability is over the 70% threshold required

MECHANICAL

In the rotating frame, the particle follows a periodic nearly circular path in the clockwise direction, associated with a very slow centrifugal drift towards the esophagus wall. In the ground-based frame, the particle appears to have an increasing circle of rotation in the counter-clockwise direction with a stationary center.

4. DISCUSSION

Biological

The methods used confirm the results obtained by G. Luc et al. [2]: obtaining a non-cytotoxic decellularized matrix with good tissue cohesion. This DM will be tested mechanically and analyzed its components mass spectrometry. Its biocompatibility will allow its recellularization with smooth muscle cells, mesenchymal stem cells or epithelial cells.

Mechanical

The use of RCCS for tissue engineering requires that the perfusion rate and rotation speed remain moderate (ω < 20 rpm and Q < 30 ml/min). Equations (1) and (2) indicate that particle motion may be affected by: density difference between fluid and particle, vessel rotation rate, fluid viscosity and particle radius.

5. CONCLUSION

This RCCS device allows to decellularize an esophagus and to obtain a MD. It is also intended to cellularize the scaffold with this system because the setting of the flow and rotation parameters are compatible with this application.

Paper under review: "Mechanical characterization of a rotating bioreactor for tissue engineering"

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