



**HAL**  
open science

# MECHANICAL CHARACTERIZATION OF A ROTATING BIOREACTOR FOR TISSUE ENGINEERING

Romane Lesieur, Marlène Durand, Agnès Drochon

► **To cite this version:**

Romane Lesieur, Marlène Durand, Agnès Drochon. MECHANICAL CHARACTERIZATION OF A ROTATING BIOREACTOR FOR TISSUE ENGINEERING. BIOMAT Congress 2021, Oct 2021, Bourg-Saint-Maurice, France. hal-03358466v2

**HAL Id: hal-03358466**

**<https://hal.utc.fr/hal-03358466v2>**

Submitted on 27 Oct 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



# Mechanical characterization of a rotating bioreactor for tissue engineering

Romane Lesieur<sup>1,2,3</sup>, Marlène Durand<sup>1,2,3</sup>, Agnès Drochon<sup>4</sup>

<sup>1</sup> CHU Bordeaux, CIC1401, 33000 Bordeaux, France

<sup>2</sup> Inserm, CIC1401, 33000 Bordeaux, France

<sup>3</sup> Bordeaux University, Inserm, UMR 1026, BIOTIS, 33000 Bordeaux, France

<sup>4</sup> Bordeaux University, UMR CNRS 5295, Institut de Mécanique et d'Ingénierie, 33000 Bordeaux, France

## 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 deterision 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 Hematein (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).

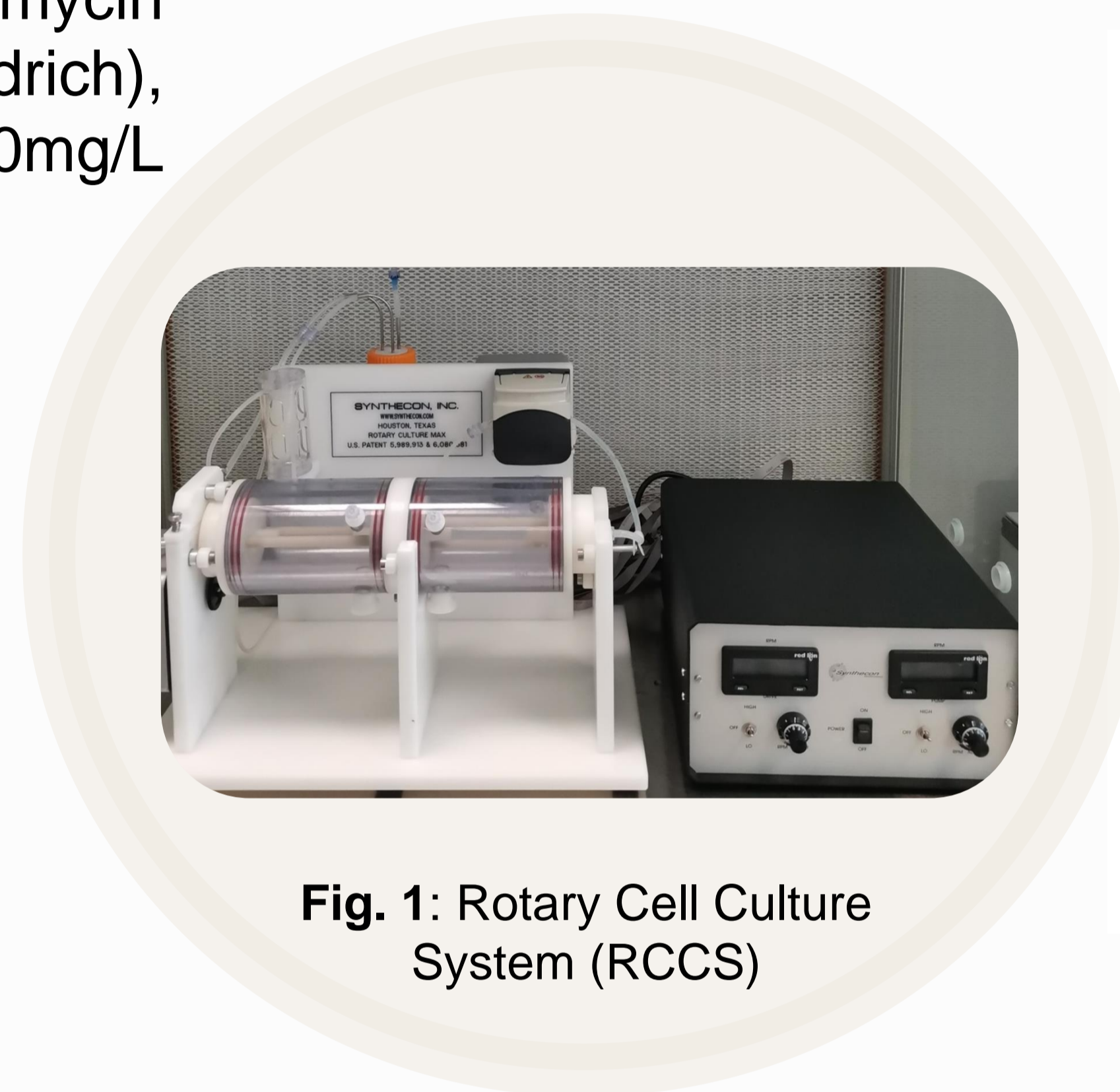


Fig. 1: Rotary Cell Culture System (RCCS)

### 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.

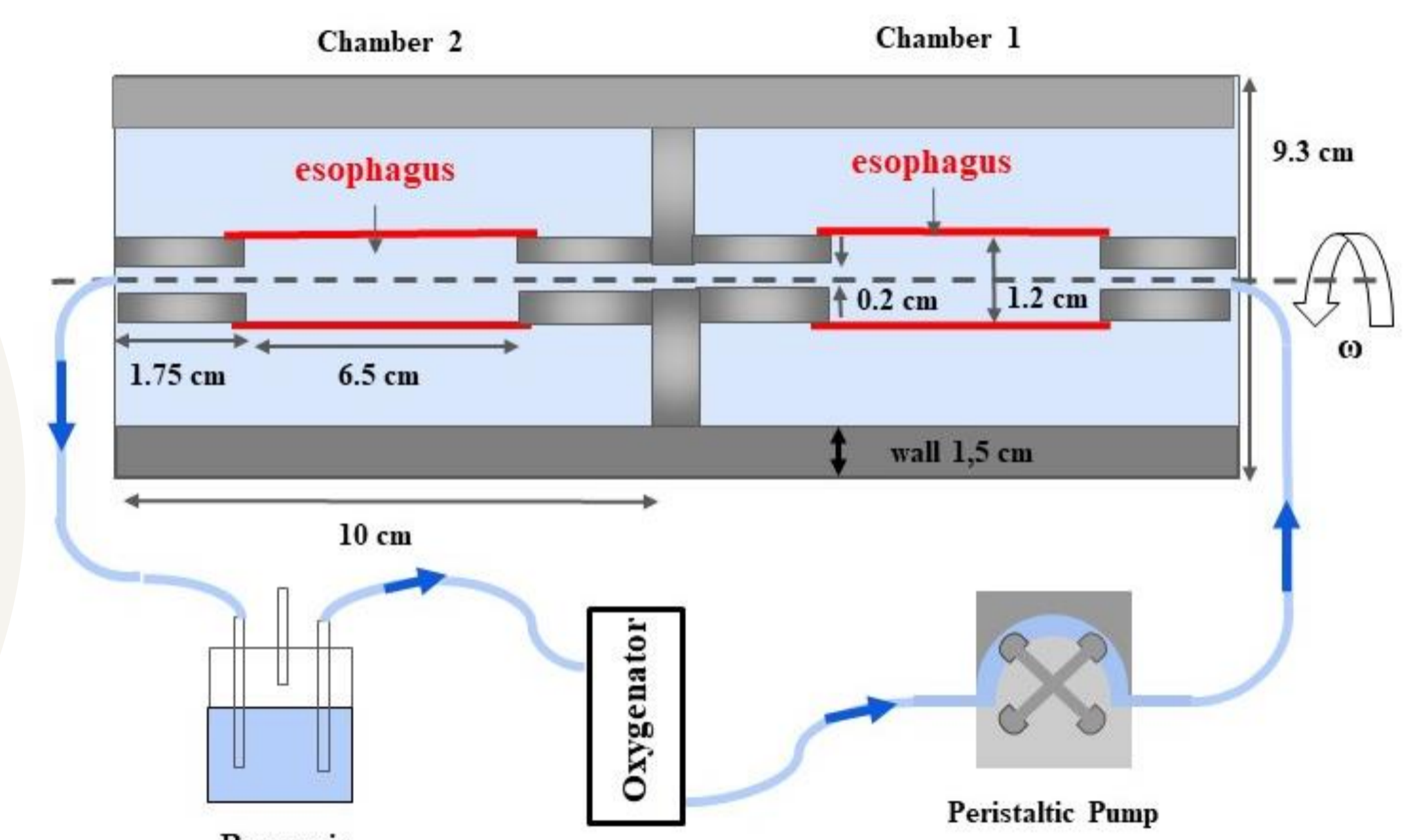


Fig. 2: Schematic representation of the experimental set-up.

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

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

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

Particle mass      Viscous drag      Centrifugal force      Coriolis force      Particle weight compensated by Archimedes

### BIOLOGICAL

## 3. RESULTS

#### Decellularization

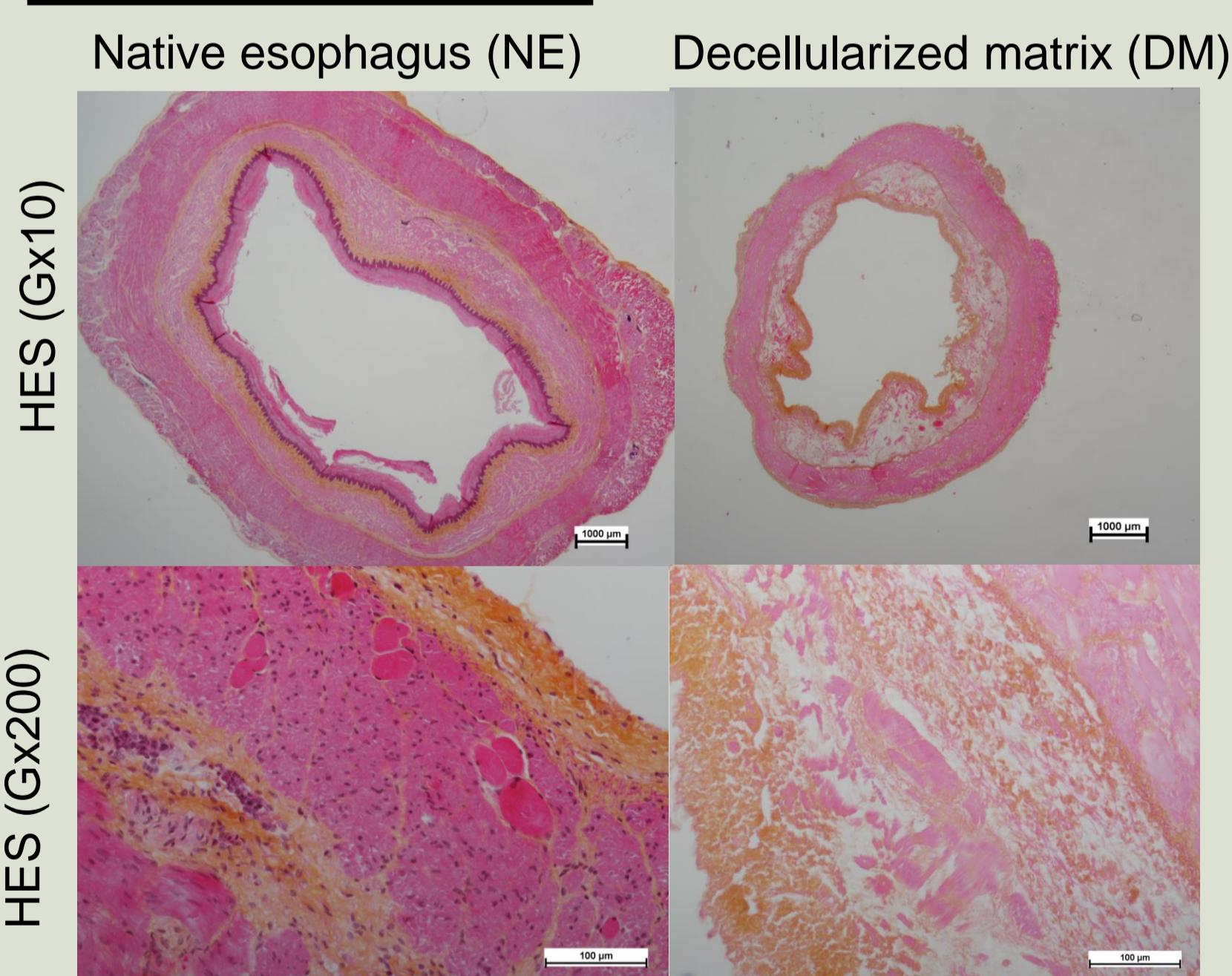


Fig. 3: Histological observations of NE and MD with HES staining.

- 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

#### Cytotoxicity assessment

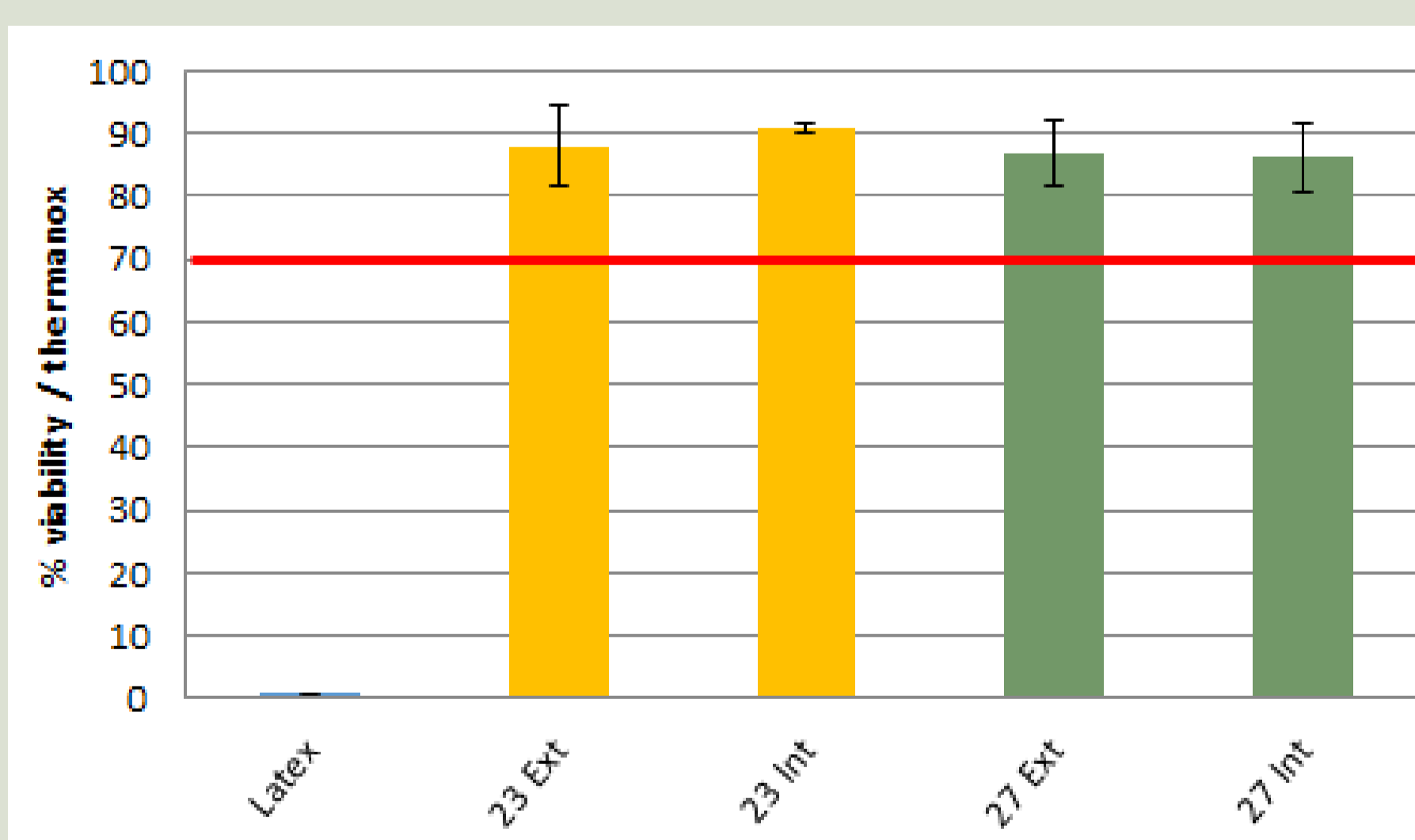


Fig. 4: Cytotoxicity assay by direct contact for 2 samples (n=3)

### 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 ( $\omega < 20$  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"

## ACKNOWLEDGMENTS

## REFERENCES

- [1] Choudhury D., Yee M., Sheng Z. et al. (2020) « Decellularization systems and devices: state of the art » Acta Biomaterialia, Part A, Vol. 115, p 51-59.
- [2] Luc G., Charles G., Gronnier C., et al. (2018) « Decellularized and matured esophageal scaffold for circumferential esophagus replacement: proof of concept in a pig model » Biomaterials, Vol. 175, p1-18.
- [3] https://synthecon.com/pages/perfusion\_bioreactor\_system\_rcmax\_dual\_synthecon\_40.asp
- [4] L. Arakelian, C. Caille, L. Favre et al (2019) « A clinical-grade acellular matrix for esophageal replacement ». J Tissue Eng Regen Med.