TENSILE TESTING OF CELL SHEETS: AN EXPERIMENTAL APPROACH

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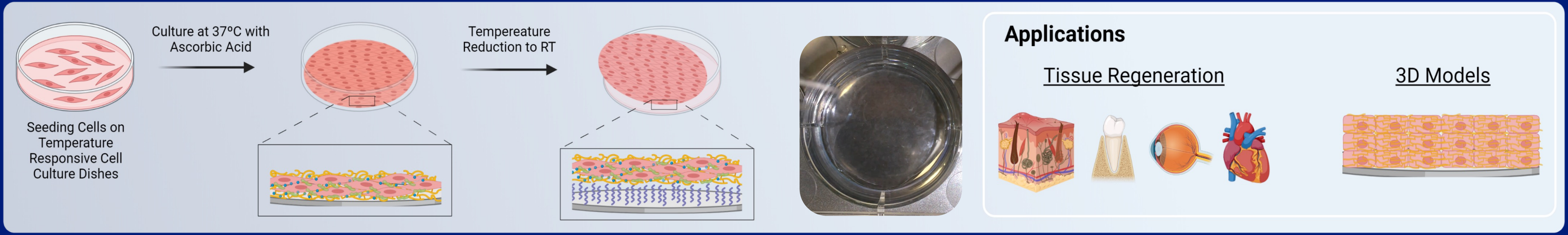
Introduction

Cell Sheet Technology



- This technology relies on the capacity of cells to deposit a robust extracellular matrix (ECM) allowing its harvesting as a sheet, in which cells are embedded in its ECM;

- Cell Sheets are relevant 3D platform that mimic the microenvironment of native tissues, being a powerful tool to study ECM biomechanical and biochemical properties of different tissues

- Applications:
  - Tissue Regeneration Approaches: Cell Sheets are directly transplanted to host tissues;
  - *In vitro* 3D models: stacking individual Cell Sheets to recreate tissue analogues - ECM acts as natural glue.

The biological relevance of Cell Sheets is well established - less is known about their mechanical properties

INTRODUCTION

- There is a growing realization of the importance of study the Cell Sheets mechanical properties;

- Most of the studies measure the local mechanical properties using Atomic Force Microscopy (AFM)
    - measures the mechanical properties at nano/micro scale
    - limited by the surface region of the sample

- Tensile testing is the most common approach to estimate the global mechanical properties
    - measures the mechanical properties at macro scale
    - requires a firm attachment of the sample edges, which is a technical problem for fragile sample like Cell Sheets

PROBLEM

To perform tensile testing in Cell Sheets is necessary to establish a compromise between appropriate grips and clamping force to keep sample edge integrity and reduce slippage.

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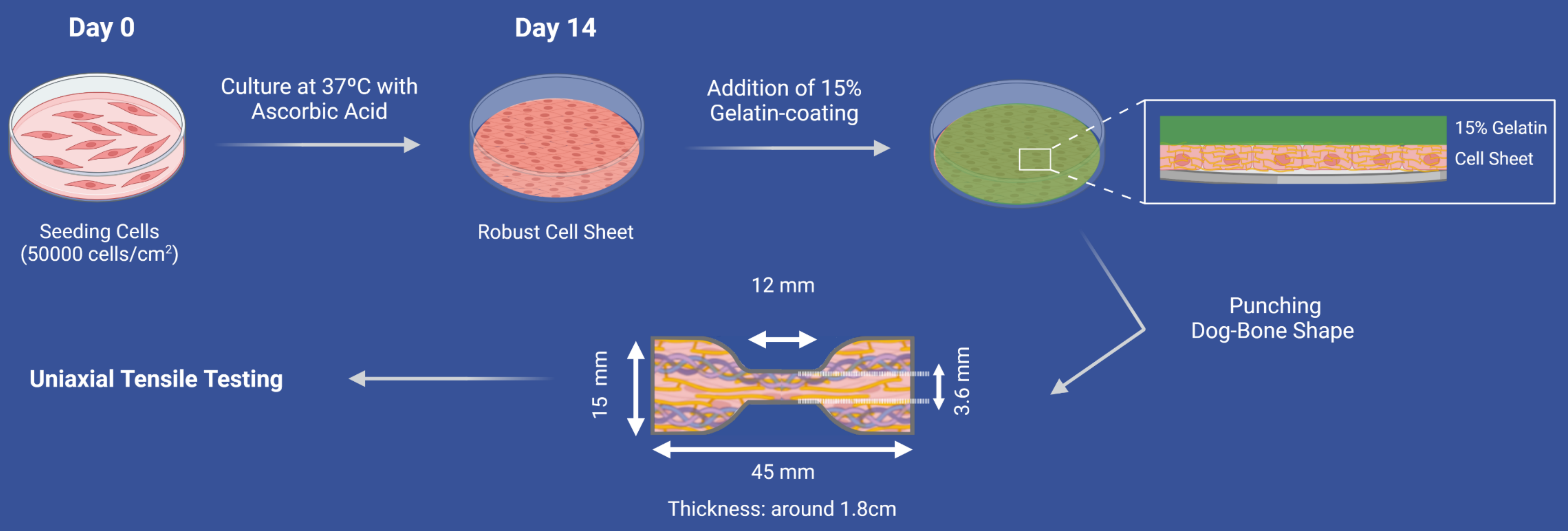
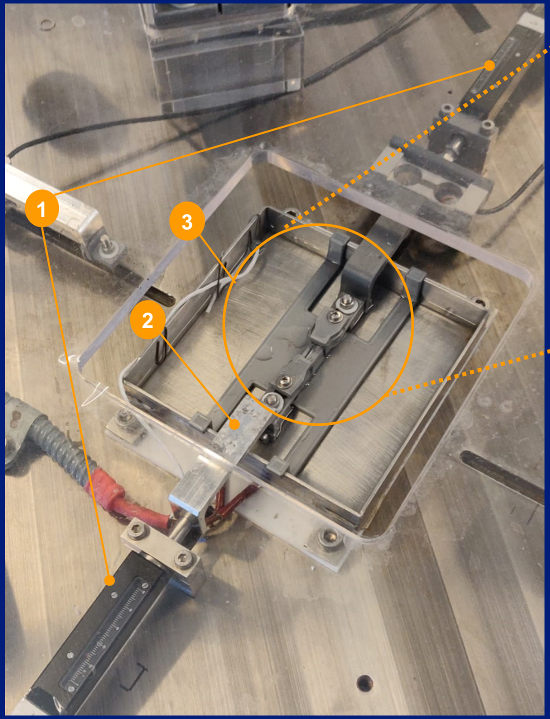
AIM

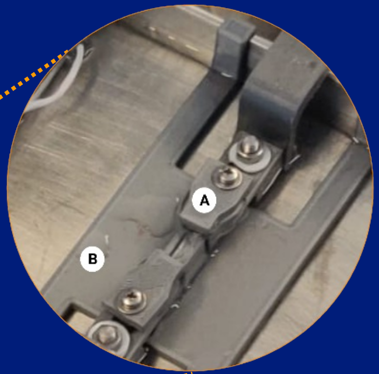
Develop an experimental setup with a mechanical testing prototype and customized 3D printed poly lactic acid(PLA) grips and racks to test mechanical properties of fragile tissues, such as Cell Sheets.

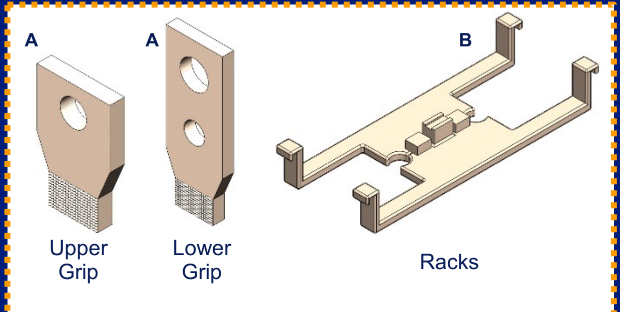
CELL SHEET PRODUCTION



Tensile Testing Experimental Setup







CAD Drawing

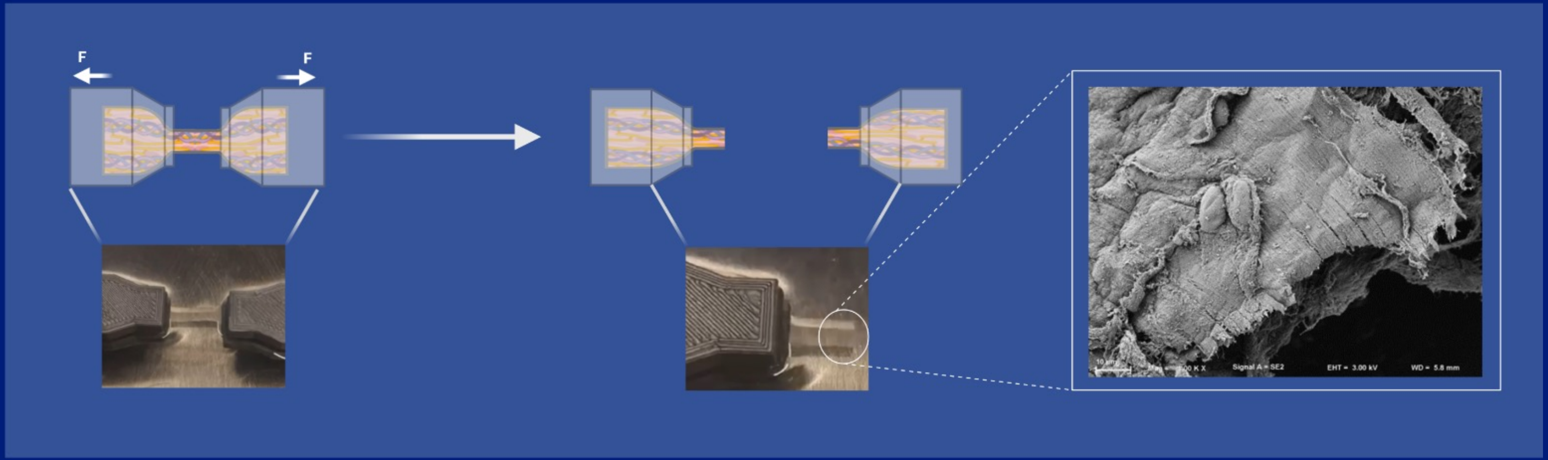
(1) Actuators
(2) Aluminium Arms
(3) 3D Printed PLA Grips and Racks

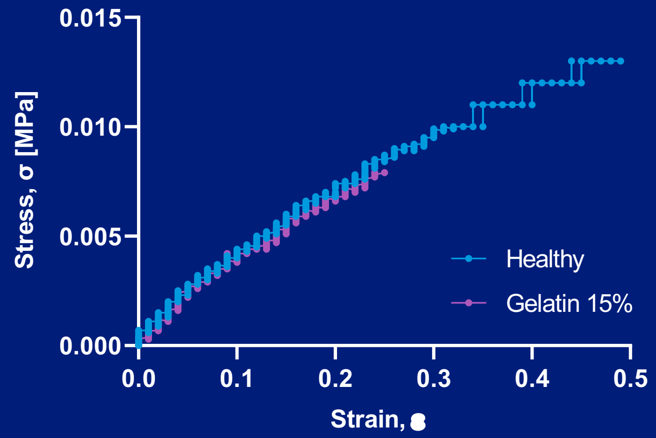
Tensile Test Parameters

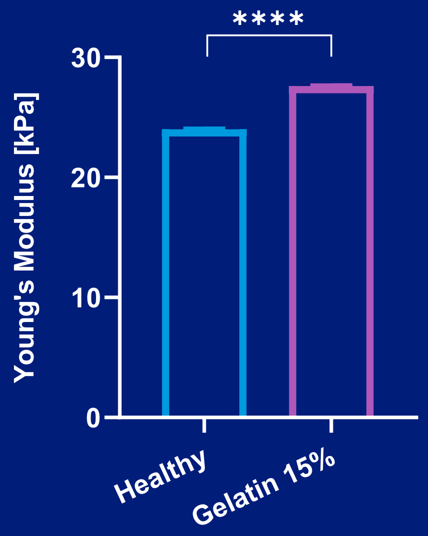
V = 2 mm/min
Load cell = 2.5 N
Temp. RT (PBS or medium)

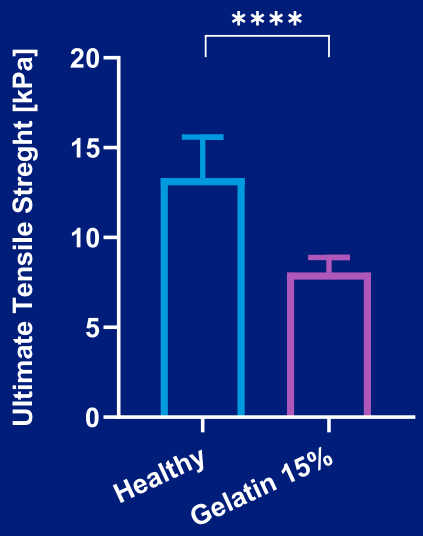
- The prototype consisted of two aluminum alloy arms, connecting two actuators that can be programmed to move independently under deformation control;
- The 3D printed PLA grips and racks were designed with suitable geometry and roughness to avoid structural damage caused by clamping or sample slippage during stretching;
- Samples were attached to actuators by grips and stretched along the longitudinal axis at a constant elongation rate of **2mm/min** until failure;
- D-PBS bath at RT was used to maintain hydration;
- The load was measured using a **2.5 N** load cell throughout the testing period.

RESULTS









- Stress-strain data show that the proposed apparatus allows retrieving reproducible and accurate results;
- All samples fractured in the gage region and no slippage was observed during the test.;
- SEM image of fractured region confirmed the rupture and the alignment of matrix components in the direction of applied force;

Other Applications

- Objetive: compare cellsheets of healthy hDFbs (Human dermal fibroblastos) with dystrophic epidermolysis bullosa (DEB) variants with different aggressiveness.

- Objetive: compare cellsheets of healthy hDFbs (Human dermal fibroblastos) with CAFs (Cancer-associated fibroblasts).

- Objective: compare human adipose stromal/stem cells (hASC) with hypothermic preservation, 4ºC (hASC-hypo) and cryopreserved hASCs, -80ºC (hASC-cry).

- (…)

Take Home Messages

- The proposed experimental setup proves the ability to hold soft and fragile samples in place during mechanical testing;

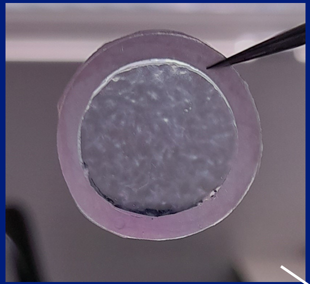
- The proposed experimental setup proved to be suitable:
  - to measure the tensile proprieties of Cell Sheets, overcoming the limitations of current devices
  - opening new perspectives in the mechanical characterization of soft tissue models.

The proposed experimental setup and gelatin-coating can be used as a new strategy to compare the mechanical properties of cell sheets.

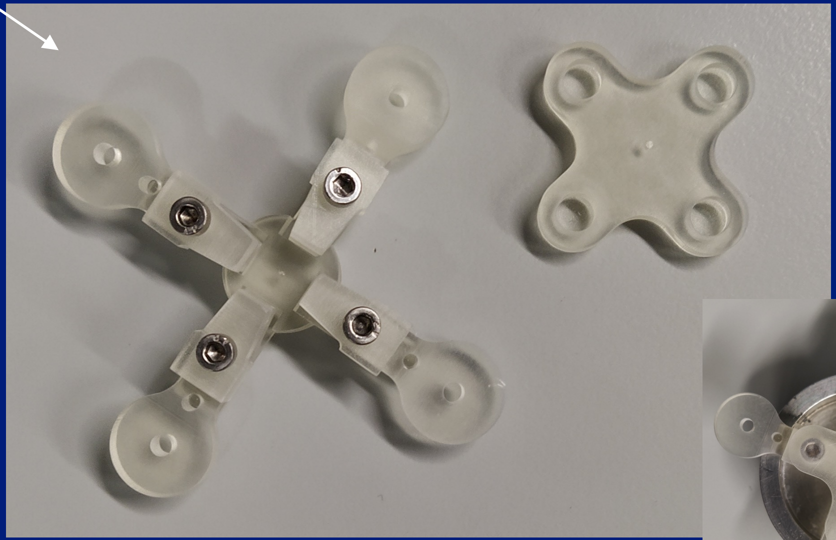
The Shape of Things to Come

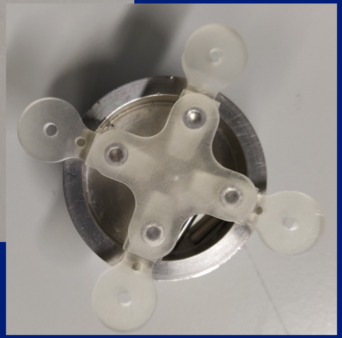
- Remove the gelatin layer ????
- Characterization of 3D models
- Multi-axial testing
- Consider the nonlinear behavior of the cell sheet and the 3D models

3D model



Biaxial testing rig





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Thank you all for your attention!