E010.M

PHOTOTHERMAL SOFT MICROACTUATORS FOR MECHANOSTIMULATION OF ANTERIOR CRUCIATE LIGAMENT

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INTRODUCTION

Cells are highly sensitive to mechanical cues such as matrix stiffness and external forces, which play a key role in regulating their behavior. Understanding these mechanobiological responses requires *in vitro* models that combine **3D cell encapsulation** with **dynamic mechanical stimulation**. Hydrogels are ideal for this purpose thanks to their high water content and tunable mechanical properties.

We aim to identify a **biocompatible, UV-crosslinkable hydrogel** suitable for encapsulating ACL cells within a **soft microactuator**. The actuator, fabricated using stop-flow lithography, employs a thermoresponsive PNIPAM hydrogel activated by focused laser light to generate **reversible cyclic tension**, mimicking physiological strain. Our goal is to establish a cell-supportive hydrogel system that enables reliable and reproducible **mechanostimulation** at the microscale.

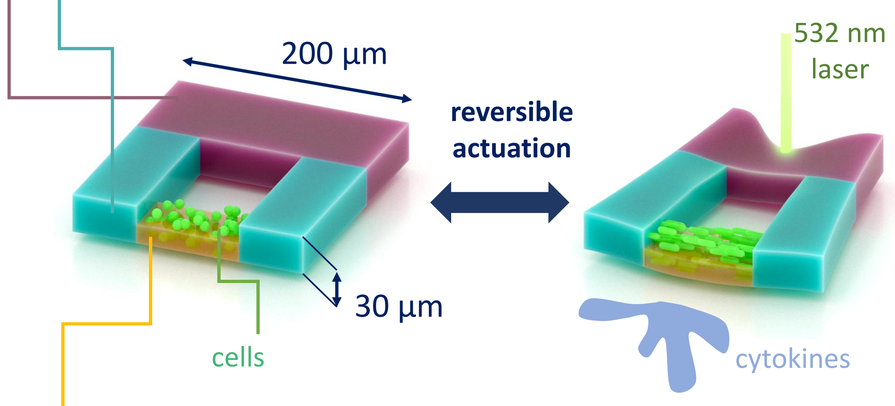
MICROACTUATOR DESIGN

Thermoresponsive hydrogel

- Poly(N-isopropylacrylamide) (**PNIPAM**)
- Lower critical solution temperature (LCST) ≈ 32 °C
- Gold nanoparticles (10 nm) + laser (532 nm)
  → plasmonic heating → collapse of PNIPAM

Non responsive hydrogel

- Polyethylene glycol diacrylate (**PEGDA**)
- Bendable but does not react to temperature shifts



Biocompatible hydrogel

- Supports high viability and cell spreading
- UV-crosslinkable for photolithography

HYDROGEL ACTUATION

25 % hPLMA linkage

- Fabrication via photolithography
- Maximal elongation **28.55** ± 17.43 %
- Estimated microactuators's force from **44.30** ± 16.54 µN to **85.10** ± 10.36 µN depending on laser intensity

Images of a microactuator with attached hPLMA part: a) at rest state
b) at maximal elongation, scale bar 100 µm

Mechanostimulation

- Microactuator allows to stimulate cells at **37 °C**
- Elongation range covers **hypo-** and **hyper-physiological** conditions
- Continuous and consistent stimulation over **at least 1 hour** period

FUTURE WORK

- Simultaneously actuate multiple microactuators
- Encapsulation of cells into the microactuator linkage part
- Long-term mechanostimulation and analysis of cellular responses:
  - **Cytoskeleton remodeling** observation
  - **Cytokine production** monitoring through biosensing

BIOCOMPATIBILITY TESTING

Four hydrogels have been tested for cell encapsulation:

1. Dex-HEMA

- Dextran hydroxyethyl methacrylate
- Biodegradable and biocompatible polysaccharide of bacterial origin
- Synthetized in our laboratory
- Concentration: 15 % (w/w)

2. hPLMA

- Methacrylated human platelet lysate
- Mixture of proteins, growth factors and other bioactive components
- Purchased from Metatissue
- Concentrations: 12 and 25 % (w/w)

3. ColMA

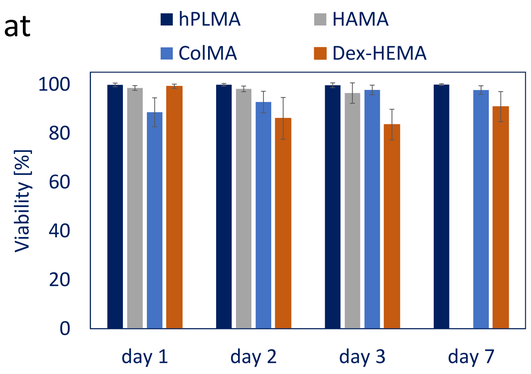
- Methacrylated bovine collagen type I
- Protein with the ability to polymerize by heat and UV
- Purchased from Advanced BioMatrix
- Concentration: 0.3 % (w/w)

4. HAMA

- Methacrylated hyaluronic acid
- 100-150 kDa polysaccharide
- Purchased from Advanced BioMatrix
- Concentration: 2 % (w/w)

Methodology

- Anterior cruciate ligament (ACL) cells encapsulated at 1000 cell/µL into 20 µL hydrogel droplets
- Crosslinked under UV for 65 – 90 s
- Incubated in culture media at 37 °C
- Live/dead staining (Calcein-AM/Propidium Iodide)
- Confocal imaging

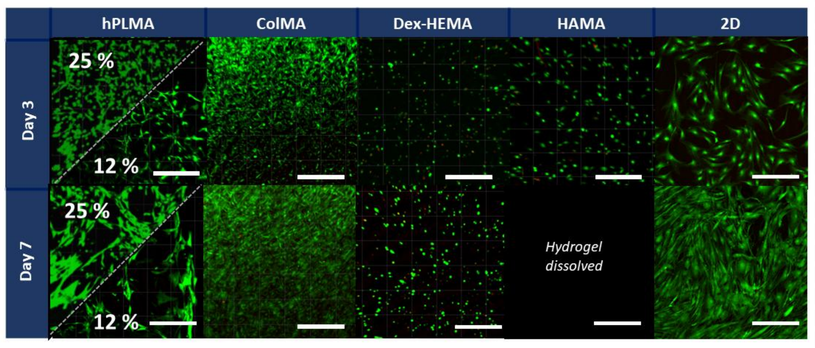


Viability results

- Bulk **viability above 80 %** for all tested hydrogels
- Evaluation after 1, 2, 3 and 7 days
- HAMA dissolved prematurely
- Only **Dex-HEMA** showed **position-dependent viability** due to **diffusion limitations** of the polymeric network

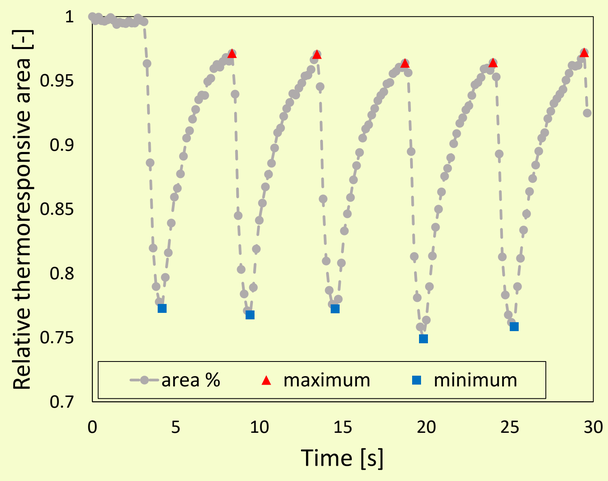
Morphology results

- Only **protein-based** hydrogels hPLMA and ColMA allowed for cell **spreading**
- ColMA proved problematic for photolithography
- Biocompatibility testing identified **hPLMA** as the most suitable candidate



Confocal images of the morphology of encapsulated ACL cells in different hydrogels, stained by Calcein-AM and Propidium Iodide on day 3 and day 7 of incubation, 2D cultivated ACL cells for a comparison, scale bar 300 µm

CYCLIC ACTUATION

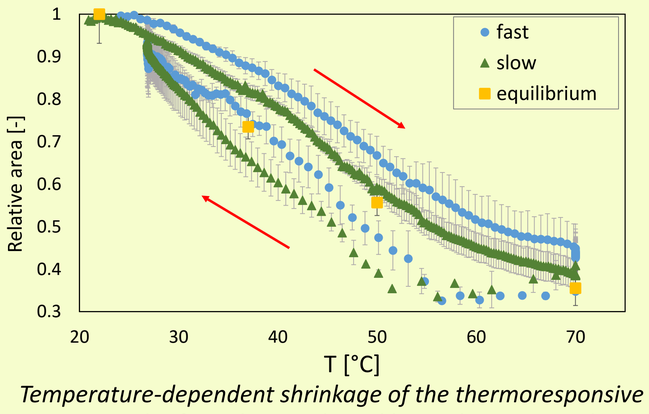


Relative area change of the thermoresponsive actuator segment during repeated on/off laser activation

- Repeated on/off laser stimulation
- Monitoring of the **thermoresponsive segment deformation**
- **Reversible contraction and relaxation** of the active region after each pulse

Thermal shrinkage

- Validation of the thermoresponsive behavior of the actuator's active segment
- Heating protocols: *fast heating* (10 °C/min, blue circles), *slow heating* (1 °C/min, green triangles), and *equilibrium values* (yellow squares)
- Temperature curves exhibit clear hysteresis between the heating and cooling phases



Temperature-dependent shrinkage of the thermoresponsive segment under fast, slow, and equilibrium heating conditions

Laboratory of Biomimetic Engineering

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