MEDICAL UNIVERSITY OF VIENNA

Photoresponsive hydrogels for mechanic stimulation in ligament cell-on-chip applications

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Objective

Ligamentopathies and tendinopathies are multifactorial disorders with increasing prevalence among athletes and the general population. They involve inflammation and extracellular matrix degradation triggered by abnormal mechanical loading, yet their underlying cellular mechanisms remain poorly defined. Most existing in vitro models inadequately reproduce the native mechanical microenvironment, which plays a critical role in the mechano-inflammation process in these pathologies.

This project addresses these limitations by developing a cell-on-chip system enabling controlled mechano-stimulation of multiple cell-laden hydrogel constructs (microactuators) and simultaneous real-time readouts with integrated biosensors. Mechanical actuation utilizes a thermoresponsive hydrogel segment within the construct, which contracts upon 532 nm laser-induced plasmonic heating.

Results

Microactuators were fabricated by stop-flow lithography in PDMS microfluidic chips using photocrosslinkable hydrogels. A two-material structure consisting of a thermoresponsive PEGDA/PNIPAM hydrogel and a passive PEGDA hydrogel was produced in a single fabrication step. Gold nanoparticles were incorporated into the active hydrogel to enable photothermal actuation using a 532 nm laser.
Our characterization of the microactuator demonstrated reversible contraction and relaxation under repeated laser on/off stimulation. Furthermore, we showed stable actuation over multiple cycles, with a slow drift in the maximum and minimum values of the thermoresponsive area over an actuation period of one hour. This is attributed to the fact that repeated actuation leads to heat accumulation in the surrounding medium.

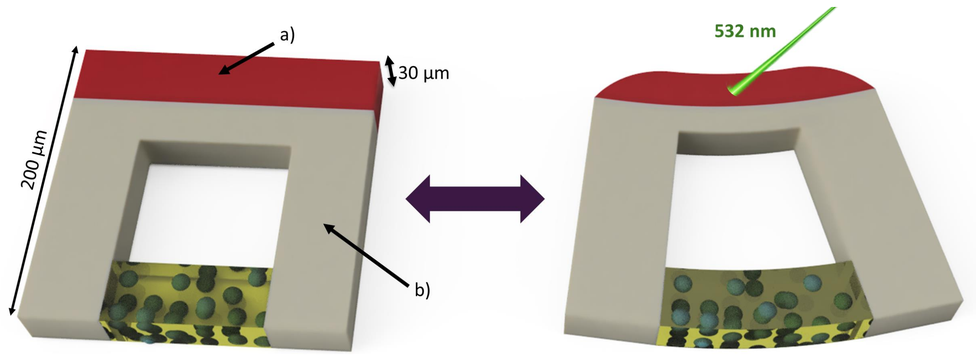


Fig. 1: Schematic images of the microactuator in the relaxed (left) and actuated (right) states. **(a)** Thermoresponsive PEGDA/PNIPAM segment, **(b)** passive PEGDA segment, and (c) biocompatible hydrogel with encapsulated cells.

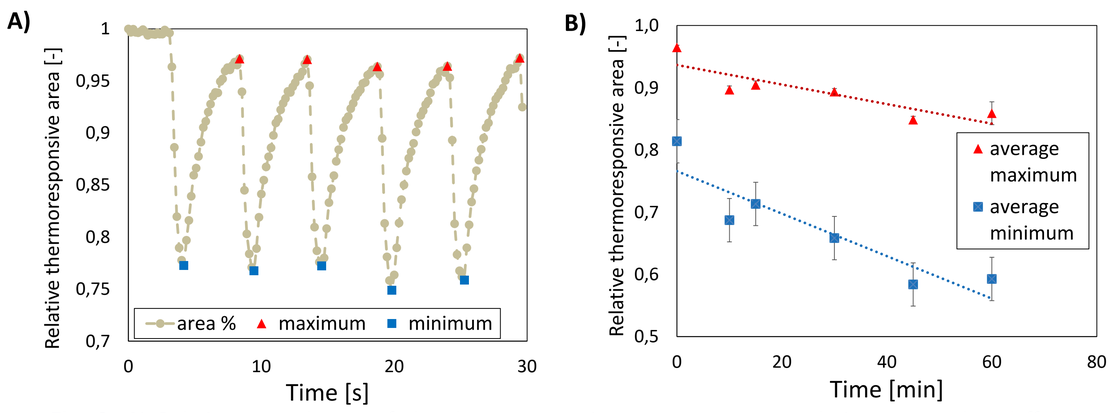
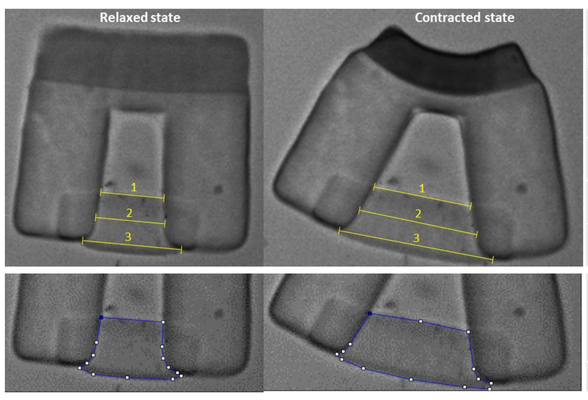


Fig. 2: A) Relative area change of the thermoresponsive actuator segment during repeated on/off laser activation. **B)** Time-dependent shift in average maximum and minimum area values during prolonged cyclic stimulation.



Measuring point	Length in pixel	Length in pixel	Elongation in %	Mean elongation in %
1	39	64	63	66,7
2	42	75	78	
3	59	94	59	

Area relaxed in pixel	Area contracted in pixel	Area increase in %
1634,0	2.755,00	69

Fig. 3: Analysis of maximum applicable strain with the microactuator. The PNIPAM + AU NP layer of the microactuator was illuminated for 4 seconds with a 300 mW laser at 532 nm at room temperature in a 0,1% Pluronic solution. The resulting elongation of the Dex-HEMA construct was measured relative to the relaxed condition.

To establish SPR-based detection of relevant analytes such as IL-6 or MMP-3 for future cell-on-chip applications, we developed a prism-coupled SPR system with simultaneous fluorescence readout. Sensitivity is enhanced through a sandwich immunoassay format employing a fluorescently labeled secondary detection antibody (dAB), enabling plasmon-enhanced fluorescence (PEF) detection.

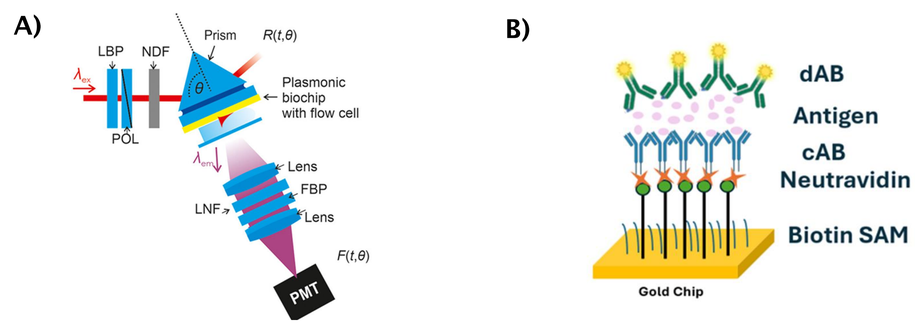


Fig. 4: A) Schematic image of the Angular-resolved prism-based SPR with simultaneous fluorescence readout. **B)** Schematic of our biotin–neutravidin-mediated immobilization strategy on a gold SPR sensor surface, forming a sandwich immunoassay.

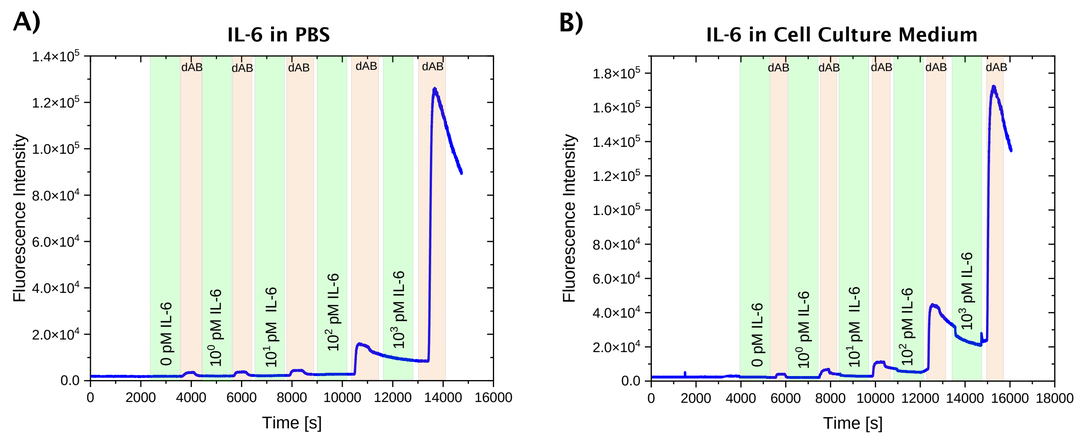
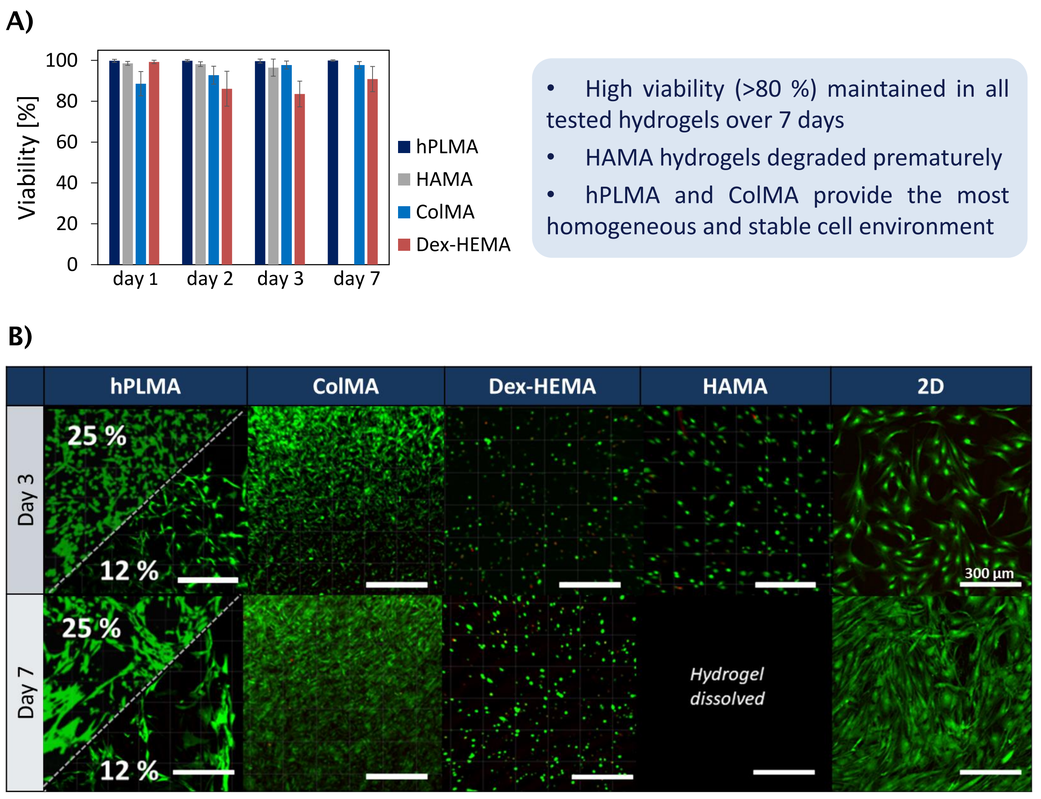


Fig. 5: Fluorescence response traces for IL-6 detection using a prism-coupled SPR/PEF sandwich immunoassay measured in **(A)** PBS and **(B)** cell culture medium (MEM Alpha (1X) Gibco™, 10% ELAREM™ Perform-FD PLUS, 1% Antibiotic-Antimycotic Gibco™). Sequential injections of IL-6 at increasing concentrations (0, 1, 10, 100, and 1000 pM; green phases) were each followed by addition of detection antibody (dAB; orange phases). Signal increments occur predominantly after dAB introduction, consistent with sandwich complex formation and plasmon-enhanced fluorescence readout. The magnitude of the response scales with IL-6 concentration, demonstrating dose-dependent binding. Differences in baseline stability and signal amplitude between PBS and cell culture medium indicate matrix effects.

Ligamentocyte viability and morphology in different hydrogels



- High viability (>80 %) maintained in all tested hydrogels over 7 days
- HAMA hydrogels degraded prematurely
- hPLMA and ColMA provide the most homogeneous and stable cell environment

Fig. 6: Viability of ligamentocytes encapsulated in different hydrogel matrices over time
Á) Quantitative analysis of ligamentocyte viability (%) across hPLMA, HAMA, ColMA, and Dex-HEMA hydrogels at days 1, 2, 3, and 7 post-encapsulation. Viability was assessed via live/dead staining (Calcein AM / Ethidium Homodimer-1) and calculated as the ratio of live to total cells counted. Two-dimensional (2D) monolayer culture served as a reference control. Data are presented as mean ± SD from n = 3 technical replicates. **B)** Representative confocal fluorescence micrographs of ligamentocytes within the indicated hydrogels at days 3 and 7. Viable cells appear green (Calcein AM; cytoplasmic esterase activity) and non-viable cells red (Ethidium Homodimer-1; compromised membrane integrity).

Conclusion

Stop-flow lithography enabled reproducible fabrication of U-shaped microactuators with defined geometry for on-chip mechanical stimulation. Laser-based actuation elicited robust thermoresponsive contraction, achieving 67–69% maximum strain/elongation in the cell-laden segment. Cell viability assays demonstrated over 90% ligamentocyte survival in methacrylate collagen type I, hyaluronic acid methacrylate, and human platelet lysate methacrylate, confirming material biocompatibility. Preliminary SPR confirmed successful detection of IL6 in cell culture medium, supporting integration of label-free molecular readouts with mechanical actuation.

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