

Slovak Society of Chemical Engineering Institute of Chemical and Environmental Engineering Slovak University of Technology in Bratislava

## PROCEEDINGS

 $51^{\rm st}$  International Conference of the Slovak Society of Chemical Engineering SSCHE 2025

Hotel DRUŽBA Jasná, Demänovská Dolina, Slovakia May 27 - 30, 2025

Editors: Assoc. Prof. Mário Mihaľ

## ISBN: 978-80-8208-158-2, EAN: 9788082081582

Published by the Faculty of Chemical and Food Technology Slovak Technical University in Bratislava in Slovak Chemistry Library for the Institute of Chemical and Environmental Engineering; Radlinského 9, 812 37 Bratislava, 2024

Mamedova, A., Zlatník, J., Řehoř, I., Rothbauer, M., Heidenberger, J., Tokárová, V.: Development of Microrobots Using Stop-flow Lithography for Mechanostimulation of Encapsulated Cells , Editors: Mihaľ, M., In 51st International Conference of the Slovak Society of Chemical Engineering SSCHE 2025, Jasná, Demänovská Dolina, Slovakia, 2025.

## Development of Microrobots Using Stop-flow Lithography for Mechanostimulation of Encapsulated Cells

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Key words: cell encapsulation, stop-flow lithography, microactuators, mechanostimulation

The encapsulation of tissue cells within a three-dimensional (3D) hydrogel structure presents a valuable alternative to traditional two-dimensional (2D) cultivation methods, such as those utilized in Petri dishes or culture flasks. This 3D setup more accurately replicates the function of the extracellular matrix (ECM) and resembles the tissues found in live organisms. In tendon, ligament, and muscle tissues, the mechanical properties also play a crucial role, significantly influencing cell physiology. Both excessive and insufficient tension can lead to pathological cell behavior. The impact of mechanostimulation remains largely uncharted, and suitable hydrogels containing encapsulated cells can serve as a tool for establishing a controlled biomechanical environment, relevant for research into regenerative medicine and disease models.

This project aims to develop hydrogel microrobots capable of exerting force on encapsulated cells from the anterior cruciate ligament (ACL) to stimulate the production of cytokines—critical signaling and regulatory molecules. The encapsulation of ACL cells within hydrogels and the fabrication of microrobots for cell manipulation utilize the stop-flow lithography (SFL) technique, which involves a microfluidic chip, a photomask, and UV radiation. The microrobot is designed in the shape of a closed horseshoe, consisting of three components made from distinct materials: a rigid polyethylene glycol diacrylate (PEGDA) hydrogel, a thermoresponsive poly N-isopropylacrylamide (pNIPAM) hydrogel embedded with gold nanoparticles, and a septum composed of a biocompatible hydrogel containing encapsulated cells. Actuation is triggered by a 532 nm laser, which heats the gold nanoparticles through surface plasmon resonance, resulting in the contraction of the thermoresponsive hydrogel and the expansion of the septum housing the cells. In this study, four different methacrylated hydrogels were evaluated and compared: methacrylated hydroxyethyl methacrylate collagen (COLMA), methacrylated human platelet lysate (hPLMA), and hydroxyethyl methacrylate-modified dextran (DexHEMA).