

Warsaw, ...30.01.2026.....

Author of the doctoral dissertation:**Debjita Mukherjee**.....

Supervisor:**Dr. hab. Jan Guzowski**....., Ph.D., D. Sc.

Auxiliary supervisor or Supervisor:, Ph.D. or, Ph.D., D. Sc.
(* optional only for the new proceedings for the award of a doctoral degree)

Title of the doctoral dissertation: **Microfluidics-assisted High-throughput Cell Encapsulation for Microtissue Engineering: from Cancer Model to Artificial Pancreatic Islets**

Abstract

Droplet microfluidics-based technologies for 3D-cell culture and microtissue engineering are receiving increasing interest in the fields of biomedical engineering, biotechnology, and even preclinical drug development. However, balancing throughput of production, droplet monodispersity, and crosslinking efficiency is quite challenging. In the Thesis, I demonstrate an experimental system suitable to produce more than 50000 crosslinked hydrogel (gelatin-based) droplets of ~200 μm diameter within a minute, with 50 cells per droplet on average. Such high droplet production rates have only been used for encapsulating low cell numbers (<10 cells/droplet) or single cells previously, with much smaller droplet diameters. Owing to the high cell number in each droplet, the hydrogel microspheres were suitable for microtissue engineering. Additionally, I also report complex droplet-in-droplet hydrogel structures to allow compartmentalized co-culturing of different cell types. Process parameters such as flow rates, crosslinking light wavelength, irradiance, exposure time, distance from the crosslinking lamp, and temperature during the crosslinking process were optimized.

A two-chip microfluidic system was developed. First, a chip with parallel channels produced the cell-laden microdroplets, which subsequently flowed to the next chip, where they were crosslinked in flow and finally collected. I showed how different chip designs could affect the droplet production and derived the final design to produce highly monodisperse droplets irrespective of high flow rates, with good distribution of cells in each droplet. Next, crosslinking efficiency was demonstrated at different crosslinking light wavelengths and irradiances at a fixed time of UV exposure for on-chip crosslinking. An interesting phenomenon of a well crosslinked droplet core with a partially crosslinked shell was observed in poorly crosslinked droplets at low irradiances. A “sweet spot” was identified in terms of crosslinking conditions to allow production of completely crosslinked droplets without significantly affecting the viability of the encapsulated cells within. For the complex droplet-in-droplet system, the microdroplet “cores” were produced with the optimized conditions and were encapsulated in a second hydrogel layer (“shell”). Similar optimizations of flow rate and crosslinking light intensity, in a stationary condition, were performed to ensure efficient re-encapsulation of droplets.

Different gelatin-based hydrogels were then tested for use in different applications using the two abovementioned systems. I have used the system for disease modelling and cell therapy

applications. Firstly, I performed encapsulation and co-encapsulation of leukaemia and bone marrow stroma cells to evaluate drug resistance in the leukaemia microenvironment model. I also demonstrated encapsulation of cancer cells in the “cores” and fibroblasts in the “shell” to use the droplet-in-droplet systems for disease modelling and drug testing. Secondly, pancreatic β -cells were encapsulated in the microdroplets with >85% viability as cell therapy for Type 1 diabetes. Additionally, mesenchymal stem cells (MSCs) were reprogrammed within porcine pancreatic ECM (ppECM) containing droplets to function as pancreatic islet-like cells. Attempts were made with the droplets-in-droplet system to enable vasculature development in the artificial pancreatic islets. The thesis shows the immense potential of microfluidics in the field of biomedical sciences to design robust systems with high physiological relevance.