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Review of the PhD Thesis
of MSc Debjita Mukherjee, entitled:
“Microfluidics-assisted High-throughput Cell
Encapsulation for Microtissue Engineering:
from Cancer Models to Artificial Pancreatic Islets”

Supervisor: dr hab. Jan Guzowski

1. Scope and significance of the thesis

In recent years, increasing attention has been paid to the development of advanced three-dimensional (3D) cell culture systems for biomedical applications. Conventional two-dimensional cell cultures do not accurately reflect the complexity of native tissues, where cells remain surrounded by extracellular matrix and continuously interact with neighboring cells. As a consequence, standard 2D cultures often show limited physiological relevance and

reduced predictive value in studies related to disease progression, drug screening, and regenerative medicine. Therefore, there is a strong need for technologies enabling controlled fabrication of reproducible 3D cellular systems that better mimic the native tissue microenvironment.

One of the rapidly developing approaches in this area is microfluidics. Droplet microfluidics enables generation of highly uniform microdroplets with precisely controlled size, composition, and encapsulation conditions. Compared with conventional bulk emulsification methods, microfluidic systems provide significantly better reproducibility and allow generation of monodisperse hydrogel particles suitable for cell culture applications. The small dimensions of the droplets additionally improve nutrient and oxygen transport, creating favorable conditions for growth of encapsulated cells and formation of multicellular microtissues. Another important advantage of droplet microfluidics is the possibility of fabricating complex multicompartment structures. Native tissues and organs consist of multiple cell populations organized within separate but interacting compartments. Such spatial organization is essential for maintaining proper biological function. Droplet microfluidics enables fabrication of advanced structures such as core-shell droplets, Janus particles, and droplet-in-droplet systems, which allow controlled co-culture and spatial separation of different cell types. These systems make it possible to recreate more physiologically relevant tissue architectures compared with conventional cell culture methods.

Development of 3D cancer models is currently one of the important applications of droplet microfluidics. Cancer remains among the leading causes of mortality worldwide, and there is a continuous need for reliable in vitro tumor models for studies of tumor progression and anticancer therapies. Microfluidic microdroplet systems enable rapid and reproducible formation of tumor spheroids and multicellular cancer models with improved physiological relevance. Moreover, multicompartment droplet systems allow co-culture of cancer cells with stromal or supporting cells, enabling better recreation of tumor heterogeneity.

Microfluidic encapsulation systems are also increasingly investigated for regenerative medicine and cell therapy applications. In particular, encapsulation of pancreatic β -cells and pancreatic islets has emerged as a promising strategy for treatment of Type 1 diabetes mellitus. Hydrogel microcapsules may protect transplanted cells against immune rejection while preserving insulin secretion and cell viability. Microfluidic techniques allow fabrication of highly uniform microcapsules with controlled size and permeability, improving reproducibility and stability of the encapsulation process.

Despite significant progress in the field, several technological limitations remain unresolved. One of the major challenges concerns simultaneous achievement of high production throughput, efficient hydrogel crosslinking, good droplet monodispersity, high-density cell encapsulation in single droplet or multi-cell encapsulation in droplets-in-droplets. Many previously reported systems were designed mainly for single-cell encapsulation or low numbers of cells per droplet. In addition, increasing flow rates frequently results in unstable droplet formation and reduced size uniformity. Therefore, development of scalable

microfluidic systems capable of generating large numbers of highly reproducible, cell-laden hydrogel droplets remains an important research direction in modern biomedical engineering. Considering the above aspects, the subject of the reviewed doctoral thesis should be regarded as highly actual and scientifically important. The presented work addresses significant challenges related to high-throughput production of cell-laden hydrogel microdroplets and development of physiologically relevant 3D culture systems. MSc Debjita Mukherjee developed a microfluidic platform enabling generation of highly monodisperse gelatin-based microdroplets with high cell density and additionally demonstrated complex droplet-in-droplet systems for compartmentalized co-culture applications. The scope of the conducted studies is well aligned with current research trends related to tissue engineering, disease modelling, drug testing, and regenerative medicine.

2. Form and scientific content

The doctoral thesis entitled “Microfluidics-assisted High-throughput Cell Encapsulation for Microtissue Engineering: from Cancer Models to Artificial Pancreatic Islets” addresses a broad and interdisciplinary research area at the interface of microfluidics, tissue engineering, and disease modelling. The scope of the thesis includes development of a high-throughput microfluidic encapsulation platform, its application in fabrication of 3D cancer models, encapsulation of pancreatic β -cells, and engineering of artificial pancreatic islet-like systems. The dissertation consists of 7 main chapters and comprises 156 pages. The thesis is divided into two main parts: PART-A: High-throughput Encapsulation System Development, and PART-B: Application in Cancer Models and Artificial Pancreatic Islets.

The thesis begins with an introductory chapter that briefly presents the historical development of microfluidics in the context of biomedical applications. Although the MSc Debjita Mukherjee correctly emphasizes the importance of microfluidics for disease modelling and regenerative medicine, the chapter remains rather short and general in character. The introduction lacks a more detailed discussion of the current state of the art, existing technological limitations, and the specific scientific gap addressed in the thesis.

Moreover, the chapter does not provide a clearly formulated research motivation, scientific objectives, or working hypothesis, which are typically expected elements of a doctoral dissertation. A more focused discussion of droplet microfluidics, cell encapsulation systems, and their biomedical relevance would strengthen the scientific value of the thesis and better justify the novelty and scope of the presented work. Nevertheless, the Candidate clearly indicates that the thesis aims to apply microfluidic technologies for development of miniaturized models for cancer research and cell-based therapies for Type 1 diabetes. Inclusion of more specific aims and hypotheses at the end of the chapter would further improve the clarity and overall structure of the dissertation.

Chapter 1, entitled *“Droplet Microfluidics and the Process of High-throughput Monodisperse Droplet Generation for Cell Encapsulation”*, presents the technological foundations and initial optimization studies related to the development of a microfluidic platform for generation of hydrogel droplets and then cell-laden hydrogel droplets. The chapter provides a detailed overview of the fabrication and operation of microfluidic chips based on T-junction and step-emulsification geometries used for droplet generation. The chapter contains descriptions of key mechanisms such as Laplace pressure, Rayleigh–Plateau instability, capillary-number-dependent flow regimes, and passive droplet formation strategies. It demonstrates that MSc Debjita Mukherjee possesses substantial technical understanding of droplet formation physics, interfacial phenomena, and hydrogel-based encapsulation systems.

The Candidate describes the fabrication procedures for both polycarbonate and PDMS-based microfluidic chips, including bonding methods, surface treatment, and channel characterization. Moreover, the systematic optimization of flow rates, phase ratios, and chip geometries are presented to achieve stable high-throughput generation of highly monodisperse droplets suitable for cell encapsulation. It is shown that the initially developed parallel T-junction chips were unable to provide stable and reproducible droplet formation at high flow rates, although more experimental evidence supporting this limitation should have been presented. To overcome these problems, MSc Debjita Mukherjee developed a novel circular step-emulsification chip (called DPC), containing 64 radially distributed wedge-shaped droplet generators. The optimized geometry together with properly selected polymer and oil flow rates enabled generation of highly monodisperse droplets with diameters of approximately 200 μm at high throughput. A coefficient of variation below 3% was claimed, however, this result was not sufficiently supported by quantitative analysis or statistical evidence.

Another strength of the work is the investigation of cell distribution inside the droplets at different cell loading concentrations. The Candidate showed that increasing the cell concentration from 10 to 20 million cells/mL reduced the number of empty droplets and improved overall cell distribution. Although ideal Poisson distribution was not fully achieved, the obtained results demonstrated relatively uniform cell encapsulation (about 50 cells/droplet) suitable for microtissue engineering applications. An important part of this chapter is also comparison of the developed system with previously reported high-throughput droplet generation platforms.

Chapter 2, *“Optimization of Crosslinking Conditions and Development of an On-the-fly Crosslinking System”*, addresses an important technological aspect of hydrogel-based microfluidics, namely stabilization of generated droplets through efficient photocrosslinking. The chapter combines theoretical background on free radical polymerization with experimental optimization of an integrated on-the-fly crosslinking platform for continuous production of cell-laden GelMA microdroplets.

The chapter introduces the basic principles of hydrogel crosslinking and briefly discusses conventional physical and chemical crosslinking strategies used in biomaterials research. The principles of photo-crosslinking based on free radical polymerization are correctly described. Inclusion of the polymerization rate equation together with discussion of photoinitiator concentration and light intensity demonstrates good understanding of the physicochemical background of photocrosslinkable hydrogel systems. More critical discussion regarding potential limitations of photocrosslinking, such as oxygen inhibition, heterogeneous crosslinking, UV-induced cytotoxicity, and diffusion limitations in larger droplets, would further strengthen this introduction.

A major strength of this chapter is the development of on-the-fly (OTF) photocrosslinking system and systematic optimization of the photocrosslinking process. Particularly valuable is the successful integration of the OTF crosslinking system directly with the droplet generation platform, enabling continuous high-throughput production of stable hydrogel microgels. The development of an additional temperature-controlled enclosure for process stabilization should be also underlined. MSc Debjita Mukherjee interestingly investigated the influence of light wavelength, irradiance, exposure time, channel length, and flow conditions on GelMA crosslinking efficiency and cell viability. The presented work shows that the developed platform enabled generation of crosslinked cell-laden droplets, including core-shell structures, at relatively high throughput. However, it remains unclear what the actual maximum production rate was (16,000 droplets/min or 50,000 droplets/min). At the same time, the system maintained acceptable cell viability at the level of approximately 70%, and in some cases even around 80%.

Although optimization studies are extensive, some aspects of the analysis remain qualitative. In particular, quantitative characterization of crosslinking homogeneity, mechanical stability of the generated microgels, and long-term effects of UV exposure on encapsulated cells could have been explored in greater detail. The rheological characterization also appears somewhat limited and could benefit from more comprehensive mechanical analysis of the final crosslinked structures. The biggest concern are the results of LIVE/DEAD viability assays. Although the LIVE/DEAD staining qualitatively suggests that a substantial proportion of encapsulated cells remained viable after photocrosslinking, the presented fluorescence images do not convincingly support the reported viability values of approximately 70% -80%. The images are relatively low in resolution and do not allow reliable assessment of the ratio between live and dead cells. Furthermore, details regarding quantitative image analysis, number of analyzed droplets, and statistical evaluation are limited.

The next chapter, Chapter 3, provides an overview of hydrogel optimizations for 3D-Cell culture applications. MSc Debjita Mukherjee starts with discussing the basic properties and classifications of hydrogels together with their applications as biomimetic scaffolds for cell culture. Particular attention is given to methacrylated natural hydrogels such as GelMA and HAMA, which were used in the experimental part of the thesis. The section also discusses the potential role of decellularized extracellular matrix (dECM) in cell growth, differentiation, and

maintenance of tissue-specific functions. Then GelMA-, HAMA-, and pancreas-derived dECM-based hydrogels was applied with the aim of developing a suitable bioink for high-throughput droplet encapsulation and artificial islet engineering. Bioinks and their components (GelMA, HAMA, and porcine pancreatic dECM) were provided by Fundacja Badań i Rozwoju Nauki and Polbionica. MSc Debjita Mukherjee evaluated multiple hydrogel formulations, investigated the effect of different dECM digestion protocols, and analyzed the influence of GelMA and HAMA concentrations on droplet stability, cell viability, and proliferation. Particularly valuable is the observation that partially crosslinked droplets resulted in reduced viability and stressed cell phenotypes at the droplet boundaries. The work also demonstrates substantial technical effort related to development of the PDMS-based hydrogel screening platform and optimization of surface hydrophilization methods. The chapter additionally includes a relatively broad range of characterization techniques, including LIVE/DEAD staining, CCK-8 metabolic assays, contact angle measurements, and XPS surface analysis.

The chapter is very extensive and partially lacks focus. The hydrogel optimization process appears somewhat empirical, and the rationale behind selection of certain hydrogel compositions or experimental parameters is not always sufficiently justified from the perspective of hydrogel physics or cell–material interactions. More quantitative characterization of hydrogel mechanical properties, swelling behavior, degradation kinetics, and crosslinking density would strengthen the conclusions regarding the suitability of the selected formulations. Similarly, the biological assessment relies mainly on short-term viability assays. The LIVE/DEAD images do not convincingly support the very high reported viability values, particularly considering the dense and heterogeneous 3D cell distribution within the droplets. The presented data do not fully confirm the claimed long-term structural stability and biological superiority of the selected hydrogel formulation.

Chapter 4 presents the development of a droplets-in-droplet microfluidic platform for compartmentalized co-culture of different cell populations. It starts with discussing various classes of complex microparticles, including core-shell, Janus, and ternary structures, together with microfluidic strategies used for their fabrication. Without clear reason, the Candidate applied a modified T-junction chip for encapsulation of one or multiple hydrogel “cores” within larger hydrogel “shells”. Particularly valuable is the attempt to develop an oil-free final core-shell system more suitable for long-term biological culture. The optimization of flow rates and packing fractions for controlling re-encapsulation efficiency is also a strong aspect of the work. MSc Debjita Mukherjee demonstrated how flow conditions influenced shell size, number of encapsulated cores, and probability of successful encapsulation. The use of fluorescently-labelled GelMA and cell-tracking dyes enabled clear visualization of compartmentalization and provided convincing qualitative evidence of successful re-encapsulation. Achieving reported re-encapsulation efficiencies approaching 95% at optimized conditions represents an interesting result.

The main weakness of this chapter is that parameters such as shell thickness uniformity, spatial distribution of cores, hydrogel diffusion properties, and mechanical stability of the final

multicompartment structures were not analyzed in sufficient detail. The biological characterization also remains preliminary and relies mainly on LIVE/DEAD staining and qualitative observations of proliferation. Some conclusions appear somewhat overstated relative to the presented evidence, for example, claims regarding “tissue-like” organization. Moreover, diffusion of nutrients, oxygen, cytokines, and signalling molecules between core and shell compartments were not sufficiently discussed. In addition, the long-term stability of the shell structures and reproducibility of multicore encapsulation remain insufficiently quantified.

The next chapter presents the Candidate’s approach to the development of 3D cancer cell models in microdroplets using the previously developed microfluidic platform for spatially separated co-culture configurations of biologically relevant multicellular cancer models. After briefly introducing the tumour microenvironment, the Candidate investigates co-culture systems composed of cancer cells and fibroblast-like stromal cells to evaluate their role in tumour development. The chapter combines substantial experimental work related to hydrogel optimization, encapsulation conditions, fluorescence-based analysis of cell interactions, and preliminary drug-resistance studies.

For the co-culture model of chronic myeloid leukemia, leukemia cells (K562) and bone marrow fibroblasts (HS-5) were mixed in a 1:1 ratio and encapsulated in GelMA microgels. After culture, an irregular tissue-like morphology was observed. Cell interaction analysis using fluorescently labelled cells demonstrated visible connections between cancer and stromal cells. To investigate whether these interactions affected cancer growth, drug-resistance analysis with Imatinib was successfully demonstrated. However, the interpretation of drug-testing results maybe complicated by hydrogel degradation, diffusion of fluorescent molecules, and migration of cells outside the droplets. To mimic a breast cancer microenvironment, a co-culture model was also developed using MDA-MB-231 cells in the “core” and hDFs in the “shell” The results showed that the cores and shells retained their structure for approximately one week of culture. However, after day 3, migration of cells from the core to the shell was observed. The claims regarding “spheroid formation” were not always convincing. Moreover, the clinical relevance of the developed models was not discussed. Nevertheless, Chapter 5 represents an ambitious attempt to translate the developed microfluidic technology toward biologically relevant cancer modelling applications and drug testing.

In Chapter 6, Type 1 diabetes is firstly introduced to provide background on the disease and the current challenges associated with its treatment. MSc Debjita Mukherjee shows how microfluidic technologies may offer a potential solution through encapsulation of pancreatic β -cells in hydrogel microdroplets. Rat pancreatic β -cells (RIN-m) and human pancreatic islet cells (hPICs) were encapsulated, and their growth, proliferation, and functionality were investigated.

Initially, RIN-m cells were used as a model system to evaluate droplet formation, cell behaviour, and cellular physiology in different hydrogel compositions optimized in the previous chapters. The cells were cultured within the hydrogel droplets for up to 2 weeks, and their proliferation and insulin secretion were analysed over time. RIN-m or human pancreatic cells were encapsulated at a concentration of 20 million cells/mL using the circular step-emulsification chip combined with either on-the-fly (OTF) or stationary photocrosslinking under 385 nm UV exposure for 15 seconds (QH = 25 $\mu\text{L}/\text{min}$, QO = 125 $\mu\text{L}/\text{min}$). Three hydrogel formulations were tested for long-term cell culture. Among them, the GelMA-HAMA-ECM formulation showed the best overall performance, including high cell viability, improved droplet stability, and limited migration of cells outside the droplets during culture. In addition, this hydrogel composition demonstrated the highest insulin secretion after 14 days of culture, which correlated with increased cell proliferation and clustering within the droplets. Based on these observations, GelMA-HAMA-ECM was selected as the most suitable hydrogel system for subsequent β -cell and pancreatic islet encapsulation studies.

The optimized GelMA-HAMA-ECM hydrogel system was subsequently used for encapsulation of human pancreatic islet cells in order to evaluate the biological performance of the developed microfluidic platform under more clinically relevant conditions. The encapsulated cells were analysed with respect to oxidative stress, DNA damage using the γ -H2AX assay, long-term viability, and structural stability of the hydrogel droplets during extended culture. The obtained results indicated that the on-the-fly (OTF) crosslinking method was less harmful to the encapsulated cells than stationary crosslinking. Cells encapsulated using the OTF system exhibited lower levels of oxidative stress and reduced DNA damage. The encapsulated islet cells remained metabolically active and proliferative throughout the culture period. A large population of insulin-positive β -cells together with smaller populations of glucagon-positive α -cells was identified, demonstrating maintenance of the heterogeneous cellular composition characteristic of native pancreatic islets. These findings suggest that the developed hydrogel microenvironment supported survival and partial functional preservation of encapsulated pancreatic cells during long-term culture.

At the same time, several conclusions appear stronger than what is directly supported by the presented evidence. For example, the claim that the hydrogel system “protects” cells from UV-induced DNA damage cannot be conclusively confirmed, since factors other than hydrogel shielding may also contribute to the observed differences. Furthermore, although insulin secretion and expression of endocrine markers were demonstrated, the long-term functional quality of the encapsulated β -cells was not comprehensively evaluated. More advanced functional analyses, such as glucose-stimulated insulin secretion assays, would significantly strengthen the translational relevance of the study.

The last chapter 7, *Artificial Pancreatic Islets Engineering and Its Potential in Personalized Therapy*, focuses on the development of artificial pancreatic islet-like systems using the previously optimized microfluidic encapsulation platform. MSc Debjita Mukherjee investigated pancreatic cell encapsulation, stem-cell differentiation, multicompartments

hydrogel systems, endothelial co-culture, and preliminary vasculature engineering. The developed platform combined pancreatic islet cells, fibroblasts, endothelial cells, dECM-containing hydrogels, fibrin matrices, and VEGF-mediated vascular stimulation into a single artificial tissue-like system. Different hydrogel compositions, differentiation conditions, shell formulations, thrombin concentrations, and co-culture strategies were evaluated. The observation that VEGF and fibrin improved HUVEC proliferation and promoted partial vasculature-like organization is scientifically interesting. Similarly, the incorporation of pancreatic dECM into the hydrogel system appears to positively influence differentiation and insulin production.

The most problematic aspect of this study concerns the stem-cell differentiation experiments. The evidence supporting differentiation of BM-MSCs into functional pancreatic islet-like cells is relatively weak and largely indirect. Moreover, the reported insulin secretion levels remain relatively low and were not normalized to cell number or viability. Critically important analyses such as expression of pancreatic transcription factors (PDX1, NKX6.1, MAFA), qPCR, or dynamic glucose-stimulated insulin secretion (GSIS) were not performed. Although HUVEC proliferation and partial network-like structures were observed, no lumen formation, perfusability, endothelial marker quantification (e.g. CD31), or functional vascular assays were shown. The conclusion that “vascularized artificial islets” were generated could be premature. Despite these limitations, Chapter 7 demonstrates an interesting exploratory attempt toward development of complex multicellular microfluidic tissue models for diabetes applications.

The final chapter appropriately highlights the interdisciplinary nature and broad potential applicability of the developed microfluidic platform. MSc Debjita Mukherjee identifies several relevant future directions, including further automation of the encapsulation process, integration of re-encapsulation steps, implementation of iPSC-based approaches, and additional biological characterization of the engineered tissues.

The reference list is extensive (245 positions), comprehensive, and well aligned with the scope of the dissertation. It includes a broad range of relevant literature related to microfluidics, biomaterials, tissue engineering, cancer modelling, and pancreatic islet engineering.

Taking into account the editorial side of the dissertation, the overall quality of the thesis is acceptable. However, the thesis contains some repetition, resembling a textbook-style or technical report rather than a focused doctoral dissertation. The scientific narrative would benefit from clearer presentation of the research gap, novelty, and motivation. The thesis also requires some small editorial errors, and formatting inconsistencies. The quality of several figures is also insufficient for a doctoral dissertation. Some microscopy images have low resolution, limited contrast, or unclear labeling, making interpretation of the results difficult.

3. Main scientific achievements and substantive aspects

The main achievement of this PhD dissertation is the development of an integrated high-throughput droplet microfluidic platform for encapsulation of large numbers of cells within highly monodisperse hydrogel microdroplets for tissue Engineering applications. The developed experimental system enabled production of more than 50,000 photocrosslinked hydrogel droplets per minute with an average diameter of approximately 200 μm and an average encapsulation efficiency of around 50 cells per droplet. Such a combination of high-throughput droplet generation, relatively large droplet size, and high cell occupancy represents a significant advancement compared with previously reported droplet microfluidic systems.

A particularly important accomplishment was the design and optimization of a circular step-emulsification microfluidic chip containing 64 radial wedge-shaped droplet generators together with an integrated on-the-fly photocrosslinking system. MSc Debjita Mukherjee optimized key process parameters, including flow rates, UV wavelength, irradiance, exposure time, temperature, and crosslinking distance, to achieve stable droplet production, efficient crosslinking, and high cell viability.

Another major contribution of the dissertation was the development of complex droplets-in-droplet hydrogel structures for compartmentalized co-culture of multiple cell populations. The proposed multicompartment systems enabled fabrication of core-shell microstructures suitable for modelling cell–cell interactions and more physiologically relevant tissue organization.

The developed microfluidic platforms were further successfully applied in several biomedical applications, including leukemia microenvironment modelling, cancer–stromal co-culture systems, pancreatic β -cell encapsulation for Type 1 diabetes therapy, and mesenchymal stem-cell differentiation within pancreatic dECM-containing microgels. The thesis also explored preliminary vascularization approaches using endothelial cell-containing hydrogel shells for development of artificial pancreatic islet-like structures.

Overall, the dissertation combines microfluidics, biomaterials engineering, hydrogel optimization, and cell biology into a versatile and innovative experimental platform for generation of complex multicellular 3D microtissues with potential applications in disease modelling, drug screening, and regenerative medicine.

When reading the PhD thesis, the following comments and questions arise:

1. What was the main reason for selecting step-emulsification instead of flow-focusing geometries for high-density cell encapsulation?
2. How reproducible was the production of >50,000 droplets/min between independent experiments and different chip batches?
3. The thesis reports highly monodisperse droplets with $\text{CV} < 3\%$. How exactly was monodispersity quantified, and how many droplets were analysed statistically?

4. Why were GelMA, HAMA, and pancreatic dECM selected as the main hydrogel components instead of other commonly used biomaterials such as alginate, collagen, or PEG-based hydrogels? Which hydrogel property was the most critical for successful high-throughput cell encapsulation: viscosity, photocrosslinking kinetics, stiffness, or degradation rate?
5. How did the Candidate distinguish between true spheroid formation and simple multicellular aggregation inside the droplets?
6. How stable was the compartmentalization in the droplets-in-droplet systems during long-term culture, and was diffusion between core and shell regions quantified?
7. What were the major limitations preventing full integration of the droplet generation and re-encapsulation processes into a single automated microfluidic platform?
8. How does the developed system compare with existing state-of-the-art encapsulation systems in terms of scalability, reproducibility, and translational potential?
9. What are the main challenges that would need to be solved before the developed artificial islet systems could be considered for in vivo studies or clinical translation?
10. In the Candidate's opinion, which part of the developed platform has the greatest future application potential: high-throughput encapsulation, multicompartment co-culture systems, or artificial pancreatic islets?

4. Final conclusions

Taking into account the presented research problem, the applied experimental approaches, and the obtained results, I conclude that the doctoral dissertation of MSc Debjita Mukherjee, entitled *"Microfluidics-assisted high-throughput cell encapsulation for microtissue engineering"* constitutes a valuable and original scientific contribution in the field of microfluidics and biomedical engineering. The dissertation addresses a highly relevant and contemporary research topic related to the development of advanced microfluidic systems for high-throughput cell encapsulation and engineering of biologically relevant 3D tissue models. The interdisciplinary character of the work, combining microfluidics, biomaterials engineering, hydrogel chemistry, cell biology, and tissue engineering, demonstrates the Candidate's broad scientific knowledge and strong technical competence.

Particular recognition should be given to the development and optimization of a high-throughput step-emulsification microfluidic platform capable of generating highly monodisperse hydrogel droplets with relatively high cell loading densities. The work also includes ambitious attempts toward the fabrication of multicompartment core-shell structures and artificial pancreatic islet-like systems with endothelial and stromal components. The obtained results expand current knowledge regarding the use of droplet microfluidics for multicellular encapsulation, compartmentalized co-culture, and biofabrication of complex hydrogel microstructures with potential biomedical applications.



The dissertation confirms that MSc Debjita Mukherjee possesses extensive theoretical knowledge and practical expertise in the field of microfluidic-based biofabrication.

In summary, I conclude that the doctoral dissertation fulfills the requirements specified in Article 187 of the Act of July 20, 2018, *Law on Higher Education and Science*, and represents a valuable scientific contribution that constitutes a sufficient basis for awarding the doctoral degree. Therefore, I recommend acceptance of the dissertation and admission of MSc Debjita Mukherjee to the public defense.

Sincerely yours,

Wojciech Świąszkowski