

Kraków, 10.04.2026 r.



prof. dr hab. Jan Zawala
Jerzy Haber Institute of Catalysis and Surface Chemistry,
Polish Academy of Sciences
ul. Niezapominajek 8, 30-239 Krakow
e-mail: jan.zawala@ikifp.edu.pl
tel.: (12) 63 95 103, 507 525 408

Report

on PhD Thesis by **Debjita Mukherjee** entitled:

***"Microfluidics-assisted high-throughput cell encapsulation for microtissue engineering:
from cancer models to artificial pancreatic islets"***

Institute of Physical Chemistry Polish Academy of Sciences, Warsaw, Poland

1. General characteristics of the thesis

To investigate civilization diseases such as cancer progression and to evaluate treatment strategies, the development of appropriate disease models is crucial. This is highly complex and challenging task, mainly due to the heterogeneity of cancer both across and within tumor types, as well as the requirement that an effective model must reproduce the fundamental hallmarks of tumor biology. Furthermore, for clinical relevance, such models should also reflect the complexity of the tumor microenvironment. The assessed thesis clearly demonstrates that droplet microfluidics can serve as a powerful tool for the development of such models in a time-efficient manner. It highlights the significant potential of microfluidic approaches in biomedical sciences for designing robust systems with high physiological relevance, not only for testing anti-cancer drugs, but also for the production of artificial pancreatic islet cells, which may find future applications in diabetes therapy.

2. Assessment of thesis structure and scope

The doctoral thesis is a written work comprising 158 pages and is divided into two main parts – A and B – containing four and three chapters, respectively. The results are supported by 74 figures and 5 tables and are discussed in the context of 245 references. Part A presents the results of studies on optimizing of the systems used including microfluidics chips design and patterning, as well as droplet encapsulation (and re-encapsulation) procedures. These findings provide a foundation for more advanced experiments aimed at the development of 3D cell models in microdroplets for direct biomedical applications particularly cancer treatment and artificial pancreatic islets generation, described in the Part B. The thesis follows a non-typical structure – it is not divided into separate theoretical (literature review) and experimental sections. Instead, each chapter begins with a theoretical background, followed by a materials and methods section, results description, and discussion, in which the findings are interpreted in the context of similar studies conducted by other researchers.

Chapter 1 provides fundamental and general information on the geometries of microfluidic devices and the dynamics of droplet formation. It also includes a description of microfluidic chips fabrication, as well as methods for analysis, testing, and optimization. In the case of a chip designed for high-throughput droplet production, the parameters influencing the monodispersity of bare droplets are discussed. Notably, the Candidate successfully designed and optimized a circular microfluidic chip exhibiting outstanding production efficiency (in terms of production rates – up to 10^5 per minute) that has not been previously reported in the literature for cell encapsulation applications.

Chapter 2 focuses on the optimization of crosslinking conditions and the development of a rapid crosslinking system aimed at stabilizing droplets formed via water-in-oil emulsification using hydrogels. The theoretical section discusses the fundamentals of crosslinking processes, including their types and limitations. Subsequently, the mechanism of methacrylated gelatin (GelMA) crosslinking is described, along with the procedure for mouse fibroblast encapsulation. The results include an analysis of the influence of illumination conditions (light intensity and wavelength range) on crosslinking efficiency, as well as the effect of temperature. Furthermore, the relationship between crosslinking efficiency, cell viability, and size stability is examined. A comparison with similar studies conducted by other researchers indicates that the system optimized by the Candidate demonstrates superior performance.

In **chapter 3** optimization of hydrogels for 3D-cell culture applications are described. The main aim was to investigate different hydrogel compositions to find the hydrogel most suitable for artificial islets models, investigated in details in chapter 7. In addition, chip optimization, especially related to procedures for enhancement of wettability of surfaces of channels, was described. Then bioink testing on the design hydrogel chip was performed and results were analyzed in terms of bioink compatibility. All of these efforts allow for determination of the most suitable bioink and the best hydrogel for the cell growth.

Chapter 4 describes methods for the generation of complex droplet structures (re-encapsulation of droplets within droplets) required for cell co-culture. The Candidate developed a two-stage method for producing core-shell particles, enabling the creation of an oil-free system. By employing a single T-junction chip, efficient re-encapsulation of previously generated microdroplets was achieved. It was demonstrated that each droplet can encapsulate one or more core droplets, and that different types of droplets can be combined within a single shell droplet. Interestingly, the number of re-encapsulated droplets was found to depend on the flow rates of both the hydrogel containing droplets and the oil phase. For the most efficient re-encapsulation conditions (defined based on the re-encapsulation parameter, Re), single- and double-droplet encapsulation occurred most frequently. The developed system was subsequently applied to the successful cultivation of encapsulated cells, including fibroblasts (as model cells) and pancreatic islet cells.

Chapters 5, 6, and 7, which constitute Part B of the thesis, focus on application-oriented studies and present highly interesting results regarding the use of the developed high-throughput system and re-encapsulation techniques for cell encapsulation in 3D culture systems for drug testing and artificial cell formation.

Chapter 5, in particular, describes the results obtained for chronic myeloid leukemia and breast cancer models. The results demonstrate that the developed re-encapsulation system is well suited for drug testing applications, offering several important advantages, including high physiological relevance, the ability to culture different types of cancer cells, time efficiency, and the relatively straightforward fabrication of large populations of droplets.

Chapter 6 describes the encapsulation of rat and human pancreatic islet cells, including a detailed analysis of long-term cell growth, proliferation, and function (e.g., morphological assessment, DNA damage, oxidative stress, and insulin secretion). These studies enabled the optimization of both the hydrogel type and the photocrosslinking wavelength to achieve the most efficient insulin production by the cells. It was demonstrated that the low levels of DNA damage and oxidative stress experienced by the cells during the encapsulation process do not significantly affect their structural integrity or functional performance during long-term culture. The techniques developed in Chapter 6 were subsequently applied to successfully generate the core of artificial pancreatic islets, as described in **Chapter 7**. As shown, the developed and well-optimized protocols allowed for the production of artificial islets capable of maintaining both their structure and functionality. Based on the presented results, these artificial islets can be considered promising candidates for future clinical applications.

I have no major objections regarding the overall structure of the thesis, however, in my opinion, it leads to certain information being presented outside the sections where it would typically be expected. For instance, Chapter 1 (mistakenly labeled as Chapter 5) outlines the procedure for hydrogel

preparation (Section 1.3, page 21 – methacrylated gelatin, GelMA) and its application in droplet production, whereas the theoretical background and detailed methodology for this specific hydrogel are only introduced in Chapter 3. This arrangement makes the thesis more difficult to follow and may hinder comprehension. Could the Author clarify whether there was a specific reason for not including this information earlier, for example on page 21? Another example concerns the description of the fundamental properties of hydrogels. In my view, it would be more beneficial for the reader if this information were presented earlier in the thesis, preferably at the beginning of Chapter 2. I highly appreciated the discussion sections presented at the end of each chapter, in which the Candidate summarized the results and provided a critical analysis of the developed systems in relation to the existing literature. The Candidate also offered clear insights into potential future investigations aimed at improving the developed systems. In my opinion, the inclusion of perspectives on further research and future directions is particularly valuable, as it demonstrates the Author's scientific maturity and ability to design complex scientific experiments.

The scope of the thesis is highly multidisciplinary, encompassing several contemporary and rapidly advancing fields, including microfluidics, biomaterials development, 3D cell culture, tissue engineering, organoid development, therapeutics, and personalized medicine. This interdisciplinary nature reflects the Candidate's extensive knowledge and her ability to accurately identify highly relevant and current scientific challenges, particularly in the context of civilization-related diseases.

3. Substantial remarks

In my opinion, the thesis is of very high quality and deserves strong recognition, particularly for the extensive experimental work undertaken. The Candidate has systematically analyzed and optimized a large number of parameters, demonstrating exceptional diligence and a well-structured approach to achieving the intended research objectives. The multidisciplinary nature of the work is especially noteworthy, as it integrates concepts and methodologies from several advanced research fields. Special recognition should be given to the Candidate for independently designing and fabricating the microfluidic systems, resulting in the creation of highly optimized platforms for efficient droplet generation and cell encapsulation. This aspect clearly highlights the Candidate's technical competence, creativity, and ability to translate theoretical concepts into functional experimental systems. Furthermore, the applied character of the research and its strong relevance to current biomedical challenges should be emphasized. The results presented in this thesis have significant potential to contribute to the development of future procedures and technologies aimed at the treatment of serious civilization-related diseases. Among the most important achievements that can be distinguished from the research results presented in the dissertation, I include:

- Development of an experimental system for producing monodisperse crosslinked hydrogel microdroplets with superior generation rates and encapsulation efficiency not previously reported in the literature (over 50,000 droplets per minute, with an average of 50 encapsulated cells per droplet).
- Development of a crosslinking procedure enabling the efficient production of stable, fully crosslinked single droplets and droplet-in-droplet systems, while preserving the viability of encapsulated cells.
- Development of a two-stage method for generating core-shell, oil-free particles (re-encapsulation of droplets within droplets), suitable for 3D cell culture applications.
- Successful cultivation of various cancer cell types in the developed re-encapsulated systems, demonstrating strong potential for applications in drug testing.
- Development and optimization of protocols for the generation of artificial pancreatic islets capable of maintaining their structure and function, with potential readiness for clinical translation.

The thesis is carefully prepared from an editorial standpoint, however, the Author has not avoided a few minor editorial errors, which are listed below in accordance with my duties as a reviewer:

1. Page 15 – Chapter 5 instead of Chapter 1
2. Page 15, 3rd paragraph, line 5 – “*The In particular ...*”, „*The*” should be removed
3. Page 15, 3rd paragraph, line 12 – sentence repetition „*Droplet formation ...*”
4. Page 16, section „*Common Microfluidics Channel Geometries*” – first sentence repetition „*For most applications ...*”
5. Page 19, last line from bottom “*upto*” lacks of a space
6. Page 21, section 1.3 – GelMA synthesis procedure is shown in Chapter 3 not 2
7. Table 2-1 on page 35-37 (as an example) – references numbering issue in most of the tables

Furthermore, during the reading, I noticed some inconsistencies but also particularly interesting issues which should be addressed by the Candidate:

- On page 15 it is stated that: “... *Generally, (...) the continuous phase needs surfactant to minimize interactions (...) and prevent coalescence of the generated droplets ...*”. Could the Author clarify why the surfactant cannot be introduced into the dispersed phase for the same purpose?
- Which method for contact angle determination was used in the context of PDMS surface hydrophilization (tangent, circle fit, etc.)?
- In Fig. 3–5 on page 68, image A.i., it appears that the contact angle is indeed around 104°, however, the tangential lines drawn to the bubble surface suggest a value of approximately 140°. Could the Author clarify this discrepancy?
- The purpose of using bioinks in the experiments described in Chapter 3 is not entirely clear. Could the Author clarify their role? Were these bioinks subsequently used as dyes in the experiments presented in Chapter 4?
- Has the Author tested, or considered testing, alternative methods of chemical crosslinking for the droplets?

The comments presented above arise solely from scientific curiosity and a desire to engage in an inspiring discussion during the public defense, and in no way diminish my high evaluation of the doctoral dissertation. This positive assessment stems primarily from the scientific and practical value of the research problem addressed, the systematic investigation of numerous parameters influencing the properties of highly complex systems, and the accurate interpretation of the results, all of which demonstrate the Candidate’s high level of expertise.

4. Final conclusion

In summary, the doctoral dissertation submitted by Ms. Debjita Mukherjee, M.Sc., entitled “*Microfluidics-assisted high-throughput cell encapsulation for microtissue engineering: from cancer models to artificial pancreatic islets*” constitutes an original solution to a complex scientific problem. The Author of the dissertation has demonstrated extensive theoretical knowledge and the ability to conduct independent scientific research, both in terms of planning and carrying out experiments, as well as interpreting the results of complex experimental studies. My overall assessment is therefore fully positive.

I conclude that the dissertation by Ms. Debjita Mukherjee, M.Sc., fully meets the requirements set out in Article 187 of the Act of 20 July 2018 – Law on Higher Education and Science (Journal of Laws of 2024, item 1571, as amended). Therefore, I submit to the High Council of the Institute of Physical Chemistry Polish Academy of Sciences in Warsaw a motion to admit the Candidate to the further stages of the doctoral proceedings.

Furthermore, in my opinion, considering the aspects of the thesis and achievements mentioned in the Section 3 of this review, the dissertation deserves to be awarded with distinction.

