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Doctoral dissertation evaluation of M.Sc. Maria Celeste Tirelli

Entitled: **Microfluidic-Assisted 3D Printing of Functionally Graded Porous
Biomaterials**

This review was prepared at the request of the Deputy Director of Scientific Affairs, IPC PAS, dr hab. Maciej Kubicki, prof. IPC PAS (letter dated Sept 16, 2026). The formal basis for this opinion is the resolution passed by the Scientific Council of IPC PAS on April 15, 2026, appointing me as a reviewer for the aforementioned doctoral dissertation.

Formal Information regarding the Dissertation

The dissertation submitted for review comprises a thematically coherent series of three articles, supplemented by a comprehensive descriptive section, yielding 172 pages. The work is structured into several key parts: an overview of the doctoral candidate's achievements, an introduction, a discussion of the results, and a list of references. The core descriptive section — which includes an introduction, a statement of the research objectives, and a summary of the main findings — focuses primarily on the preparation of porous polymeric materials (hydrogels). This part, along with the summary and conclusions, consists of 95 pages of typescript. The dissertation concludes the main achievement described in the publications and one that is under preparation; however, publications and relevant supporting information are not added to the thesis. The co-author's statements are missing, since the PhD candidate is a first author in one manuscript, one PhD candidate is the second author, and the third one is under preparation/review, so the statements will be useful. The doctoral thesis was prepared under the supervision of Dr. hab. Marco Costantini, Professor at the Institute of Physical Chemistry, Polish Academy of Sciences.

Upon reviewing the dissertation, I have structured my assessment into the following three categories:

a) Originality and importance of the research problem

The proposed subject of the doctoral dissertation is devoted to the preparation of porous biomaterials by the combination of droplet microfluidic templating with the extrusion-based 3D printing. That was a challenging task, which is in line with cutting-edge research in this field. This was evidenced by the publication in the high-impact journals in the field. The main aim is to possess control over the structural and functional properties of the obtained materials by varying the preparation conditions. In turn, those materials will be useful as bone scaffolds, co-culture systems for cells, or to mimic tissue models. However, the main applications are bone scaffolds since the first two manuscripts primarily focus on the porous materials with (Chapter 3) or without hydroxyapatite nanoparticles (Chapter 2) used for bone regeneration.

In Chapter 2, the preparation of Functionally Graded Materials (FGMs) is described by direct deposition of an O/W emulsion formed within a flow-focusing microfluidic chip and extruded into a support medium. By varying the ratio between the oil droplets (serving as pore templates) and the continuous phase (forming the structural matrix) during printing,

the authors can locally control the porosity and mechanical properties of the material, which is a great achievement. Finally, the ultra-lightweight and highly porous materials are created (Figure 2.6) with relatively high hydrolytic stability under physiological conditions. Moreover, it was possible to fabricate complex architectures (a chess knight and a pawn, the sorting hat from Harry Potter, St. Peter's dome, a DNA helix) in agarose fluid-gel as a support bath, including intricate gradient structures with alternating material compositions.

In Chapter 3, the unpublished results are described in the work entitled "Microfluidic-Assisted Digital Manufacturing of Functionally Graded Porous Ceramics with Bone-Like Architecture." This is a continuation of previous research; however, gelatin B serves as the matrix for scaffold preparation, and hydroxyapatite nanoparticles are added. The functionalization of gelatin allows for direct UV-induced crosslinking of emulsion droplets within the agarose bath, facilitating instantaneous solidification upon deposition. The novel, valuable part is the bioactivity and osteoinductive capacity of the obtained scaffolds. The Alamar Blue assay was used to investigate the proliferation of hMSC cells, showing time-resolved cell migration throughout the scaffold's surface and interior pore network. Moreover, immunofluorescence analysis confirmed that, rather than being restricted to localized niches, osteogenic markers were expressed throughout the full volume of the three-dimensional construct. Importantly, the hydroxyapatite-containing scaffold independently drives hMSC osteogenesis, achieving a gene expression signature comparable to that of cells cultured in traditional osteogenic supplements.

In Chapter 4, dextran-methacrylate and gelatin-methacryloyl were used as matrices for the emulsion fiber composition and preparation of various geometries composed of two superimposed layers. This combination of two distinct biopolymers enables their precise programmable gradients, allowing for localized control over mechanical properties and cellular interactions. Finally, the biocompatibility of gradient hydrogels was tested against 3T3 fibroblasts. Interestingly, mechanical characterization via compression testing confirmed a distinct stiffness gradient, with Young's modulus varying significantly as a function of ink composition, and the stiffness can be controlled through increasing GelMA content, causing its decrease. It is especially important for cell adhesion, spreading, and migration.

This is a clearly and concisely written dissertation. The description of material synthesis and formation is straightforward, and the methodology is good; however, the analysis of reaction products is mandatory. The findings are well-discussed and transparently presented, with no notable errors. While the summary provides a strong overview of the

doctoral student's impressive achievements, a more critical discussion comparing these results to broader literature would have been beneficial. Overall, I evaluate the thesis as high-level research.

In addition, I have a few questions that a PhD candidate should answer:

- 1) Can you propose the mechanism of the dextran reaction with GMA?
- 2) Is the analysis of the obtained products of the reaction between dextran and GMA missing (as well as methacrylated gelatin)? It would be good to show the substrate and product by means of NMR spectroscopy.
- 3) Moreover, the polymer characterization by GPC (SEC) is missing. What about the repeatability of the production of porous materials, since the molecular weight and dispersity of polymers are unknown?
Such analysis should also be performed after modification of macromolecules.
- 4) You have used SnCl_2 for the treatment of the channels. Can you determine the amount of Sn allowed by the FDA? Is it possible that your biomaterials contain any amount of Sn? Please point out the method you would use to determine how much tin is present in the materials obtained.
- 5) Material composition was checked by using fluorescently labeled DexMA (rhodamine) and GelMA (FITC)? How was the labelling performed?
- 6) What kind of synthetic polymers can a PhD candidate propose/imagine for the replacement of dextran or gelatin?
- 7) What kind of in vivo testing will a PhD candidate propose to enhance the possibility of the proposed materials' application in real life (as future plans)?
- 8) How can you imagine the scaling up of the production of porous biomaterials? What are the obstacles?

b) Evaluation of the candidate's contribution, including the choice of methodology and documented scientific output

The high quality of PhD candidate research was evidenced by the publication in the high-impact journals in the field, such as Advanced Materials Technologies (IF 6.2) and Materials Advances (IF 4.6); nevertheless, the points from the Ministry of Science and Higher Education are not high. In addition to the three publications included in the dissertation, the PhD candidate's academic record comprises five additional papers in journals, including Lab on a Chip or ACS Appl. Mater. Interfaces. Furthermore, M.Sc. Tirelli's research has been presented at both international and domestic conferences through two oral presentations

and one poster. Other activities, such as applications for grants, research stays abroad, or popularization of science, are not described.

The doctoral candidate chose various techniques for the investigation of the obtained materials, including microscopy, rheology, mechanical testing, and a variety of biocompatibility tests. The combination of microfluidics and 3D printing is impressive; however, it is a main research approach used by the group leader. Therefore, the main drawback is the lack of contribution in every work described in the thesis since those manuscripts have a minimum of seven authors. It should be pointed out who made the microfluidic chips. I can only suppose that the chips or various analyses were done by a PhD candidate without any certainty. The author's contributions are provided in one manuscript (Mater. Adv., 2026, 7, 2641–2651); however, they are very general.

In conclusion, the doctoral candidate's scientific output and accomplishments are highly commendable. Her interdisciplinary research approach reflects a strong proficiency and high level of preparation in the field of materials engineering.

c) Structural and editorial quality of the submitted work

The thesis is well-presented, with appropriate proportions maintained across the individual chapters, notwithstanding a relatively brief introduction in which there is a lack of information about the polymers used for biomedical applications. The errors noted below are primarily editorial, along with a few minor substantive observations. Nevertheless, the main drawback is the information on page 52, which states that chapter 4 is based on the review articles; however, it is a manuscript describing original research results. This was very confusing.

Other comments are as follows:

Page 9: Typu flow-focusing, it should be: o geometrii skupiania przepływu (z *ang.* flow-focusing)

Page 10: zintegrowaną mikrofluidycznie strategię; it should be: Wszystkie te rozwiązania technologiczne pozwalają na sterowanie porowatością, składem i architekturą w ramach jednego procesu technologicznego.

Page 20: CNC is bold

Page 48: The objective of this doctoral thesis is to develop; I would use: The objective of the research presented in this doctoral thesis is to develop.

Page 56: ¹H-NMR should be ¹H-NMR

Page 65: its printability would; therefore, significantly expand

Page 87: SnCl_2 , it should be SnCl_2

Page 108: The arrow in the SEM pictures could be a good indication that it is magnification

Page 120: hours or h???

Page 140: Figure 4.6 is not discussed in detail

Chapter 1.4, 1.5, and 2.3 space is missing in the title

These observations are offered as constructive feedback and do not affect my highly positive evaluation of the thesis. I intend to encourage the doctoral candidate to maintain a more critical eye during the final editorial and proofreading stages.

Summary

In summary, the thesis under review fully complies with the requirements for doctoral dissertations as specified in the Act of 20 July 2018 – The Law on Higher Education and Science (Journal of Laws 2023, item 742). I am pleased to recommend to the Scientific Council of the Institute of Physical Chemistry, Polish Academy of Sciences, that this dissertation be accepted and that M.Sc. Maria Celeste Tirelli is allowed to proceed to the final stages of her doctoral degree process.

Moreover, given her extensive research skills and valuable scientific achievements in the field of porous biomaterials, I highly recommend that Ms. Maria Celeste Tirelli's doctoral dissertation be distinguished.

Marek

Brzeziński

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