

Review of the Doctoral Dissertation
Scientific Council of the Institute of Physical Chemistry
Polish Academy of Sciences (IChF PAN)

Candidate's Name: Maria Celeste Tirelli

Title of the Doctoral Dissertation: Microfluidic-Assisted 3D Printing of Functionally Graded Porous Biomaterials

Supervisor: Dr hab. Marco Costantini

Reviewer: Dr hab. Filippo Pierini

1. Dissertation Topic

Microfluidic-assisted 3D printing of porous biomaterials is a highly timely and scientifically significant topic, driven by the increasing demand for fine-tuned, porosity-controlled biomaterials in tissue engineering, biointerfaces, and drug delivery systems fabricated via advanced biomedical manufacturing. Conventional fabrication methods often lack precise control over pore architecture, material distribution, and microscale structural properties, which are essential for achieving optimal biological and mechanical performance, especially at the interface of different tissues.

Microfluidic-assisted 3D printing represents an emerging and innovative approach that enables precise manipulation of material flow and scaffold microstructure during fabrication. This technology offers enhanced control over porosity, interconnectivity, and material composition, thereby improving cell viability, nutrient transport, and integration among different tissue types.

The dissertation submitted by Maria Celeste Tirelli deeply investigated this area, which, due to its high interdisciplinarity, is remarkably challenging. The applicant integrated basic knowledge and advanced skills in microfluidics, additive manufacturing, and biomaterials science, in order to achieve stable 3D printing conditions while maintaining controlled structural porosity and biocompatibility. The performed interdisciplinary research activities have strong relevance and significant potential to advance next-generation fabrication technologies for biomedical applications.

2. Theoretical Knowledge

The doctoral activities performed by the applicant to develop microfluidic-assisted 3D printing for the fabrication of biomaterials with graded porosity require a broad and rigorous theoretical foundation across specific areas within the tackled multidisciplinary field, including biomaterial design, fabrication, characterization, and application testing.

Maria Celeste Tirelli demonstrated a fundamental understanding of fluid rheology and its connection to physical principles, necessary for designing devices that control fluid behavior at the microscale. She demonstrated a theoretical understanding of flow stability, droplet generation, mixing phenomena, and transport mechanisms, all of which are essential for the precise manipulation of multiphase bioinks during microfluidics-based fabrication processes.

The applicant demonstrated knowledge of polymer chemistry, which was important, as the properties of biomaterials strongly depend on polymer composition, crosslinking mechanisms, degradation behavior, and interactions with biological environments. Understanding these aspects was crucial for the applicant to design 3D-printable materials with suitable mechanical, chemical, and biological properties.

The mechanical stability, elasticity, and structural integrity of printed scaffolds are directly influenced by pore size, geometry, and distribution, as well as material architecture, making micromechanical analysis essential for optimizing scaffold functionality. These fundamentals in micromechanics were required to evaluate the relationship between advanced porous microstructures and their mechanical performance, as Maria Celeste Tirelli clearly demonstrated.

Knowledge of additive manufacturing is necessary to design biomaterial fabrication, including process parameters, layer-by-layer fabrication strategies, and the influence of printing conditions on structural fidelity and reproducibility, which are particularly challenging in porous materials development, which requires multiple fabrication steps. Such understanding enables the applicant to develop solid manufacturing protocols for complex biomaterial systems.

Finally, a basic understanding of biology is essential due to the biomedical applications of the developed porous materials. Knowledge of cell behavior, biocompatibility, and cellular interactions with biomaterials was required to properly design structures that support biological functions and promote tissue integration, as the applicant successfully did.

Maria Celeste Tirelli clearly demonstrated a high level of scientific rigor, technical proficiency, and research capability. These accomplishments confirm her ability to conduct interdisciplinary research effectively and to integrate methodologies and theoretical frameworks from multiple scientific domains within a coherent research strategy. The dissertation evidences the candidate's advanced competence in combining concepts from microfluidics, biomaterials science, additive manufacturing, polymer chemistry, micromechanics, and biology.

3. Research Independence

The quality and scientific value of a PhD trajectory are strongly influenced not only by the candidate's individual capabilities but also by the research environment in which the doctoral work is conducted. In particular, the scientific level, experience, and academic guidance provided by the supervisor play a fundamental role in shaping the development of the doctoral research. Indeed, a scientifically stimulating environment, as well as the supervisor's expertise, scientific vision, and international recognition, are essential for a valuable academic exposure of a PhD candidate. On the other hand, a doctoral dissertation is an individual's path, and a few indicators highlight the candidate's originality, critical thinking, and research excellence. From that perspective, Maria Celeste Tirelli achieved several research goals, all deeply characterized by high quality.

The most important indicator, from this point of view, is the publication of a few original research articles as first author in the most prestigious scientific journals in the field, where finding space for a manuscript is very challenging. These achievements demonstrate her capacity to formulate relevant research objectives (under the guidance of her supervisor), design and implement appropriate experimental and methodological strategies, critically analyze and interpret results, discuss complex scientific investigations, as well as, to draft and revise manuscripts to reach publication.

4. Originality of the Dissertation

The integration of microfluidics with 3D printing and the development of porous biomaterials are established research areas rather than entirely new concepts. Indeed, the use of microfluidics to support and improve 3D printing processes has already attracted significant attention in recent years. Microfluidic systems have been explored for precise control of material flow, droplet generation, gradient formation, and multi-material deposition, enabling enhanced print resolution and more sophisticated biofabrication strategies. The combination of microfluidics with 3D bioprinting has been used to fabricate a wide range of materials. Moreover, research on porous biomaterials has been ongoing for decades, particularly in tissue engineering and regenerative medicine. Previous studies on this proposed family of biomaterials have demonstrated their importance as scaffolds and biointerfaces, especially at interfaces between different living tissues, where a porous gradient is needed. Anyway, even though enormous effort has been put in by materials engineers and various fabrication techniques have been developed, the development of effective polymer-based biomaterials with tailor-designed porosity has remained unfulfilled. Keeping the solution of this challenging scientific problem as the main goal, Maria Celeste Tirelli incorporates substantial elements of fundamental scientific advancement in microfluidics into the 3D printing of biomaterials in a completely original way. The

applicant's work is predominantly application-oriented, with a solid theoretical background and a focus on addressing translational and implementation-focused challenges with clear societal relevance.

Before delving into the main innovations proposed by the applicant, I want to stress that publishing articles in well-recognized journals (such as *Advanced Materials Technologies* and *Materials Advances*) is a challenging process, deeply driven by the novelty of the manuscripts. Therefore, when editorial assessments in leading journals in the field (performed by the most experts in the field, such as their editors) are fully positive, it is already a sign of the innovative and original nature of the research activities.

Maria Celeste Tirelli applied a wide range of original solutions to the state of the art in 3D printing of gradient polymer-based porous materials. Anyway, I would like to spotlight that the applicant reported a very original and innovative solution in Chapter 4. In this section, Maria Celeste Tirelli reported on the development of a system that enables the simultaneous generation of compositional and density gradients within a single 3D-printed biomaterial construct. Subsequent evaluation using 3T3 fibroblast cell culture on gradient-engineered substrates demonstrated that such scaffolds facilitate high-throughput screening of multiple experimental conditions within a single specimen, while also supporting spatially resolved cellular responses. Moreover, she showed that the proposed printhead architecture is readily scalable through the integration of additional continuous-phase inlets, thereby further expanding the design space and spatial control achievable in biomaterial patterning, reaching a level of innovation never reported in the literature before.

5. Questions and/or Critical Remarks

The PhD dissertation of Maria Celeste Tirelli is presented in the form of a discursive thesis. The document is 174 pages long, and it includes a series of preface sections (e.i., Funding acknowledgment; List of publications; Abstract; Streszczenie (Abstract in polish); List of figures; List of abbreviations) which help the readers from a technical point of view. Then, five chapters constitute the main core of the document, and a reference list is included at the end. Chapter 1 is an extremely detailed introduction section, which serves to give an overview of all the treated topics (which is very important due to the interdisciplinary nature of the dissertation). Moreover, this chapter includes a subsection focused on the scope and structure of the thesis. Chapters 2, 3, and 4 highlight the three main research activities carried out by the applicant, respectively. Each of their contents is associated with a specific scientific article prepared by the applicant; most importantly, they are technically sound and scientifically rigorous. Chapter 5 is a section intended to summarize Maria Celeste Tirelli's main scientific achievements.

After a deep revision of the whole PhD thesis presented by Maria Celeste Tirelli, a few technical and specific points deserve to be elucidated in greater detail:

- Section 1.6.1. Creating a point-by-point list of the hypotheses and objectives could help figure out the scope of the PhD path.
- Chapters 2, 3, and 4. UV light intensity applied for the polymer crosslinking in combination with photoinitiators should be mentioned.
- Chapter 2. The applicant claimed, "The two-layer scaffold ($\phi = 0.3/0.7$) showed three regions of varying slope (Figure 2.5b)." Is the discussion an assignment of these different regions assumed by the applicant or taken from literature? Moreover, why is the Young's modulus calculated from the very first region? Finally, given the complexity of such an analysis and its interpretation, I would define Young's Modulus as "apparent".
- Chapter 3. The microfluidic-based manufacturing reported here is very interesting; however, it is time-, effort-, and cost-consuming compared with conventional techniques (e.g., molding). Did the applicant analyze the advantages of applying the developed fabrication technique?
- Chapter 3. Alamar Blue assay was performed in triplicate, but no statistical information is reported in the results (e.g., STDs). Moreover, I was wondering why no data had been collected before day 7 (at least to show the data considered as 100%). Finally, did the applicant perform this test on TCP or any other reference material?
- Chapter 3. Cell colonization is well-proven. Anyway, looking at the pore dimension, I would ask to discuss the cell penetration into the scaffold in more detail, too.
- Chapter 4. The applicant stated that "Progressive variations in peak intensity were observed along the scaffold height, consistent with the programmed DexMA-to-GelMA gradient". I would point out that peak intensity itself is not a reliable piece of information for this purpose. It is necessary to quantify the intensity ratio between the investigated fingerprint signal and a reference peak in each specific evaluated spectrum.
- Chapter 4. Data connected to day 1 in Figure 4.7 suggest a direct beneficial impact of lowering the substrate Young's modulus (e.g., higher GelMa content) during the seeding stage. On the other hand, its impact on the cell spreading stage (investigated at day 7) cannot be quantified solely by cell numbers, as those values are strongly influenced by the number of living cells seeded (day 1 data). Therefore, I suggest further elaborating the data, calculating the growth rate at day 7, and using day 1 data as the reference. The hypothesis that the substrate stiffness impacts differently at the very early stage than in the second part of the investigated culture, in terms of time, is confirmed by the fact that the trend reported in Figure 4.8b differs substantially from that in Figure 4.7 (especially on day 1). It would be interesting to analyze the obtained data to determine the impact of the modified properties (polymer content and, in turn, Young's modulus) over time during the cell culture process.

- Chapter 5. To assess the achievement of all planned scientific targets, I suggest creating a list of completed results/goals, linking them to the specific PhD objectives outlined in Chapter 1.

6. Final Evaluation with Justification

During her doctoral studies at the Institute of Physical Chemistry Polish Academy of Sciences, Maria Celeste Tirelli has faced a range of scientifically complex and technically demanding research problems. The dissertation is underpinned by a robust methodological framework, coherent scientific strategy, and sustained critical evaluation of results, collectively reflecting a high degree of scholarly development and scientific competence. The work consistently evidences autonomy of thought, originality in problem formulation, and the ability to operate effectively within complex interdisciplinary research environments. The thesis further demonstrates the candidate's capacity to connect theoretical background with experimental execution in a logically structured and methodologically sound manner. The research design is well-justified and appropriately implemented, indicating strong methodological competencies and substantial independence in scientific decision-making. The candidate's strong publication record further corroborates the research's impact and relevance. These outputs represent meaningful contributions to advancing microfluidic-based 3D printing processes for fabricating porous materials and are likely to serve as building blocks in the future development of effective porous biomaterials with a clinical translation perspective.

In conclusion, my overall evaluation of Maria Celeste Tirelli's doctoral dissertation is very positive, as it achieved the highest level of quality across all evaluated points and it meets the conditions specified in attached Article 187 of the Act of July 20, 2018 Law on Higher Education and Science (Journal of Laws of 2024, item 1571, as amended). Considering the aforementioned evidence, I support allowing the candidate to proceed with the public defense of her doctoral dissertation. Furthermore, I would like to recommend the candidate for distinction in recognition of the high scientific quality, originality, and overall academic merit of the work, especially due to the outstanding impactful nature of the scientific activities presented in Chapter 4 and the associated article: Tirelli M.C., Nalin F., Celikkin N., Górecka Ż., Posabella P., Świążkowski W., Costantini M. "Microfluidic-mixer assisted 3D printing of functionally graded multimaterial hydrogels for engineering complex tissue interfaces" *Mater. Adv.* 7, 2641–2651, 2026.

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Signature of the Reviewer