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Evaluation report of the PhD thesis of Moein Zarei entitled:

'Biodegradable and biomimetic fibrous composites of hierarchical structure'.

The dissertation was submitted to West Pomeranian University of Technology in Szczecin (Zachodniopomorski Uniwersytet Technologiczny w Szczecinie), Faculty of Chemical Technology and Engineering, Department of Polymer and Biomaterials Science and completed under the supervision of Prof. dr hab. inż. Mirosława El Fray.

The aim of PhD study of Moein Zarei was to design hierarchical structures, mimicking vascular grafts by combining micro and nanometric features. He developed novel biodegradable copolymers, such as poly(butylene succinate-dilinoleic succinate-ethoxy succinate) (PBS-DLS), adjusting segmental compositions and modifying with poly(ethylene glycol) (PEG) to obtain PBS-DLS-PEG. The objective of the work was to replicate the complex architecture of blood vessels, which consists of three distinct layers with different cell types, thicknesses, and functionalities. He combined 3D printing, electrospinning and a layer of gelatine to obtain a composite with tubular structures.

Considering the general structure of this dissertation, it contains 6 chapters and is clearly presented and compact with the useful list of abbreviations before the introduction. The literature review covers the following topics: challenges in cardiovascular disease and their treatments, vessel construction and materials, the historical development of vascular grafts, state of the art in vascular graft tissue engineering. Of course, the Author introduced the anatomy of blood vessels and materials used in vascular prostheses, dividing them into biodegradable polymers with a focus on poly(butylene succinate) (PBS) and its synthesis and modification details. In the first part of the thesis, nanocomposites strategies in materials science are mentioned, and nanocellulose (NC) is pointed out. The following fabrication techniques are included in the thesis: electrospinning and additive manufacturing fused filament fabrication and their combination, as well as endothelialization. The endothelialization relates to the coating of the lumen with biomimetic proteins, such as collagen or gelatin, to provide a favorable microenvironment that mimics the native extracellular matrix, facilitating the adhesion and growth of endothelial cells. Notably, the author summarized the key findings from the literature review as follows to show the research gaps and indicate the importance of his studies. Further, the aim of his PhD study is stated as the creation of a hierarchical tubular structure that closely emulates the complexity and functionality of natural blood vessels using a series of synthesized copolymers with varying segmental ratios of PBS, DLA, and PEG components. The key challenge of this research is in the integration of 3D printing and electrospun layers. To achieve that, a home-made collector was designed for electrospinning, to directly deposit electrospun fibers and facilitate bonding between layers. Additionally, between them, a gelatin coating was added via perfusion to promote future blood compatibility and endothelialization, which is essential for successful graft performance. Further the mechanical and physical characterization was described including the fabricated multiscale structures mimicking vascular grafts undergoing mechanical and physical characterization, and including tensile strength tests, compliance analysis, and burst pressure tests.

In the Chapter 4, we are led to the experimental methods, starting with the materials description and synthesis procedure of polymers. The materials characterization begins with the verification of the chemical structures using proton nuclear magnetic resonance, Fourier transform infrared spectroscopy, gel permeation chromatography, and viscosity of solution measurements. The thermal properties were investigated using a differential scanning calorimeter (DSC), dynamic mechanical and thermal analysis (DMTA), and melt crystallization. Surface properties were verified with water contact angle measurements and mechanical with the tensile testing. The sample and cells morphology were validated by scanning electron microscopy (SEM). The degradation experiments were performed utilizing enzymatic biodegradation, mass loss, and water uptake tests. Moreover, the bioactivity was verified in simulated body fluid (SBF) and the Biocompatibility with fibroblast cells (L292 cell line) using indirect and direct cytotoxicity tests. Additionally, the cell attachments to polymer surfaces were visualized using SEM.

The proposed fabrication technology in this thesis is very interesting as it involves coating the before 3D printed cylinders with gels and electrospun fibers using a self-built-in setup. Similarly, the burst pressure liquid leakage tests are performed in the built-in equipment.

In Chapter 5, the results are described, starting from the synthesis and properties of new copolymers PBS-DLS and PBS-DLS-PEG, then follows with their thermal properties, including DSC phase transitions of the synthesized copolymers, thermomechanical properties done by DMTA. The degradation study was conducted over a period of 20 days, with sampling at 6 different time intervals. This approach allowed for a comprehensive understanding of the degradation behavior of the copolymers, with a PCL polymer included as a control.

Chapter 6: Conclusions, summarizes this PhD thesis's main outcome and overall conclusions and suggestions for future work. It is important to notice that many of the results have been published in excellent journals and presented at international conferences. In the thesis, the following publications as the first author are listed:

1. Zarei M, Michalkiewicz B, El Fray M. Boosting the biodegradation and bioactivity of PBS-DLS copolymers via incorporating PEG, *ChemRxiv*. Cambridge: Cambridge Open Engage; 2023; 10.26434/chemrxiv-2023-xdj5w-v3
2. Zarei M, Żwir MJ, Wiśniewska E, Michalkiewicz B, El Fray M. Melt and solution processability of poly (butylene succinate-dilinoleic succinate) copolymers modified with poly (ethylene glycol) using 3D printing and electrospinning. *Polym Adv Techn.,* 2023;34:3586–3602, http://doi.org/10.1002/pat.6159.
3. Zarei, M.; El Fray, M. Synthesis of Hydrophilic Poly(butylene succinate-butylene dilinoleate) (PBS-DLS) Copolymers Containing Poly(Ethylene Glycol) (PEG) of Variable Molecular Weights. Polymers 2021, 13, 3177.

with many more as co-authors.

The summary is related to the already published papers. At the end, we have the list of references that reaches 202 positions.

Comments to the discussion:

1. All the characterization of the synthesized copolymers you have done on films, how is that related to later manufactured via 3D printing and electrospinning samples that are known to have different properties due to the processing parameter?
2. On page 75, you wrote, “A higher contact angle suggests a more hydrophobic surface, while a lower angle indicates higher surface wettability.” The wetting

properties should be clearly specify, where are the borders for hydrophobicity? The errors should be provided to the all values.

3. While presenting the mechanical properties, it would be useful to include the toughness values as later you discuss the energy absorption upon deformation.

4. On page 29, you refer to electrospinning fibrous film, but you need to be aware that you produce membranes or mats with almost 90 % porosity.

5. Did you observe via SEM electrospun fibers or 3D printed scaffolds after the degradation, especially to prove the surface erosion mechanism in the degradation of your copolymers in the form you use for vascular grafts? The architecture of mats has a considerable effect on the degradation.
6. Table 8, what is the error on the test?
7. Figure 27, error bars are missing on columns?
8. In the photo of the set-up shown in Figure 13, there is a Plexi box. Are you able to control the temperature and humidity during electrospinning to ensure the reproducibility of your samples?

Editorial comments:

1. Page 1 - (PBS-DLA-PEG) should be (PBS-DLS-PEG), which is the abbreviation you use later in the thesis.
2. In many photos presented in figures, the scale bars are missing, for example, the photograph in Figure 54.
3. Two dots on page 52 in the last sentence.
4. Minutes or seconds could be used as 'min' and 's' in SI format.
5. Adding a) b) in the figures would make it easier to refer to them in the discussion text.
6. The connection lines between the points in Figure 50 are unnecessary; there are separate measurements.

7. Figures 31 and 32 (DSC results) the y axis description is hardly visible.
8. The schematics in Figure 37 could be combined with Figure 36, showing SEM micrographs.

In summary, this PhD thesis aims are clear and logically presented with an up-to-date bibliography. Moein Zarei's thesis is an excellent example of fundamental polymer studies, including synthesis and properties studies with the optimization for biomedical applications. The preceding comments are subject to debate as they contain editorial remarks. However, it's important to note that these remarks do not detract from the overall positive evaluation of the work. Instead, they serve as constructive guidelines to enhance the PhD student's scientific and research development.

Upon thoroughly examining the dissertation and carefully analyzing its outcomes, I am pleased to acknowledge the comprehensive and meticulous description and explanation of the research objectives. This thesis stands as a remarkable example of interdisciplinary research, seamlessly integrating polymer manufacturing techniques with biological responses of the produced vascular grafts. Its significance lies in addressing pivotal topics with direct relevance to practical applications stemming from the obtained results. Therefore, I apply for the distinction of this doctoral thesis.

The dissertation authored by Moein Zarei encapsulates invaluable research findings and their insightful discussion. From my perspective, this scholarly endeavor impeccably aligns with the criteria outlined for doctoral dissertations, as stipulated by the Law on Higher Education and Science. Therefore, I earnestly recommend the esteemed The Council of Materials Engineering Discipline to accept the dissertation and grant Moein Zarei the opportunity to defend his work publicly.

Urszula Stachewicz,