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Opinion on a Thesis

Development of new drug delivery systems made with electrostatic and bioprinting techniques

submitted

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This opinion was prepared based on a letter No. SN/003/3.2/2022 from 14 June 2023 sent to my attention by Prof. Dorota Pijanowska, Ph.D., D.Sc., the Vice-Director of the Institute for Science.

In his thesis, **Adam Mirek** designed, manufactured, and characterised novel controlled drug delivery systems using electrospinning and 3D bioprinting techniques with better biological performance, particularly related to reduced burst release and improved active substance loading. Moreover, the author developed an original method for the production of drug-loaded microspheres by suspension electrospinning combined with pulsed voltage, which were embedded in printed scaffolds or nanofibrous mats. The development of new drug delivery systems allowing the controlled release of biologically active molecules with less adverse

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effects is of key importance in a variety of branches of medicine, because the available treatment strategies are not always successful or sufficient. Thus, the research conducted by Adam Mirek is original, timely, and well addressed.

The thesis contains five chapters: *1. Introduction*, *2. Main goal and research theses*, *3. Experimental part, 4. Overview of the results, 5. Final conclusions,* followed by a list of *References.* This part has 48 pages. At the beginning there is also *Summary* in English, Polish, and French, so formal statutory requirements have been met as well. In the second part of the thesis, the author included five publications and two Appendices containing *Declarations of authors' contributions* and *Other research not included in the thesis.*

In *the Introduction,* the author addressed the need to use controlled drug delivery systems and the major factors to be considered when designing them, including biocompatibility, precision, stability, and sustainability. Then, he focused on the historical overview and evolution of drug delivery systems including nanoparticles and microspheres, electrospun fibrous mats, 3D bioprinted constructs, and hybrid systems. This part is very well written and is based on an appropriate recent bibliography, so it can be recommended as the state of the art in the design of drug delivery systems. I found only one mistake on page 14: instead of "granulate colony-stimulating factor (G-CSF)" it should be "granulocyte colony-stimulating factor (G-CSF)".

In *Chapter 2* main goal and three research theses are shown in a consistent and informative way.

In *Chapter 3* the experimental part is presented including the electrospinning process with pulsed voltage, production of electrospun fibres modified with microspheres, 3D bioprinting of hydrogel matrices and 3D bioprinting of microsphere-loaded hydrogel matrices. In the last part of this chapter, the electrostatic formation of microspheres is described. The author used two polymer types to produce drug loaded microparticles – degradable polycaprolactone and non-degradable polyetherosulphone. I really appreciate

that it is possible to obtain micropatrticles from both degradable and nondegradable polymers, but what is the rationale for producing drug carriers from non-degradable polyetherosulphone? Does the author expect controlled release of drugs from non-degradable particles? What will be the fate of such drug carriers? These are questions that I would like to ask during the public defence of the thesis, and I would be happy to obtain some comments from the author on this matter.

In *Chapter 4* the author provided an overview of the most important results to address the main aim of the doctoral dissertation and three main theses.

Thesis (1) was formulated as follows: *The use of pulsed voltage (PV) with additional controllable electrical parameters, such as the electrical pulse duration and frequency, stabilizes the process of electrospinning and electrostatic droplet formation, enabling the production of synthetic polymer fibers or microspheres of the desired diameter.* To prove this thesis stated in the dissertation, the author presented and discussed the most important results shown in Figures 4 and 5, which were published in his articles (1) and (2). The results are convincing and show that it is feasible to optimise the manufacturing process by applying PV particular parameters to obtain either nanofibrous mats or microparticles with high efficiency.

Thesis (2) was formulated as follows: *Appropriate selection of the cross-linking method and agents used to treat the electrospun or 3D printed constructs leads to obtaining stable, water-insoluble structures of polyvinylpyrrolidone, gelatin and sodium alginate, and in the case of hydrogels, eliminates the burst effect in a 3D bioprinted drug delivery system.* In this case, the author extracted the most important results from his articles (3), (4) and (5) and depicted them in Figures 6, 7 and 8. It was confirmed that prolonged exposure of poly(vinyl pirolidone) nanofibrous mats to UV irradiation resulted in better cross-linking, which improves their degradation in aqueous conditions. In the case of gelatin-alginate matrices, the degradation time, which is correlated with the release of the active encapsulated substance, was shown to be controlled by the concentration of

cross-linking agents (calcium ions or glutaraldehyde) and exposition time on the cross-linking agents. In the case of matrices based on methacrylated gelatin, it can additionally be modulated with the use of microparticles (non-degradable or degradable). Therefore, Thesis 2 was also positively verified. However, I have one comment related to using rhodamine as a model of an active substance to assess its release kinetics: Do the author plan to test release of active pharmaceutical ingredients, such as antibiotics, with the use of other direct methods, e.g. HPLC? What are the limitations of using rhodamine as a drug model?

Thesis (3) was formulated as follows: *Non-aggregated, drug-loaded synthetic polymer microspheres can be produced and used as an additive to electrospinning suspension or bioink for 3D bioprinting, resulting in an increased drug capacity of the electrospun or 3D bioprinted drug delivery system, and in the case of electrospun fibers, an elimination of the burst effect.* To prove this thesis the author showed and discussed the most important results displayed in Figures 9, 10 and 11, which were published in his articles (2), (3) and (4). This thesis was also verified positively: microparticle powders can be produced from non-degradable and degradable polymers by pulsed voltage electrospray and they can be embedded into electrospun nanofibers or 3D printed matrices. I have only one question about the results of the released rhodamine from PVP fibrous mats displayed in Fig. 10 F. Can the Ph.D. candidate explain why after ca. 30 min one can observe the maximal concentration of rhodamine, which is decreasing afterwards and stabilises at much lower level after 3 h?

In *Chapter 5* the author presented the final conclusions of his work and in Tables 1 and 2 compared strengths and weaknesses of developed materials as drug delivery systems. This part shows that the Ph.D. candidate can analyse his results and extract key pieces of information.

The results presented in the dissertation have already been published in very good journals: *Materials and Design*. 183, 108106; *Colloids and Surfaces A: Physicochemical and Engineering Aspects.* 648, 129246 (2022); *Biomaterials Advances.* 147, 213330 (2023) and 150, 213436 (2023); and

Journal of Materials Chemistry B. 10, 8862–8874 (2022). In every article Adam Mirek is the first author, and in 3 of them he is also the corresponding author.

According to the letters of author's contribution provided by other co-authors of the articles introduced in this thesis, the role of the Ph.D. candidate was significant and included: conceptualization, methodology, formal analysis, investigation, data curation, visualization and writing – original draft, as well as writing – review & editing.

In my opinion, the Ph.D. thesis of Adam Mirek is a comprehensive study of the effect of manufacturing conditions on the properties of advanced drug delivery systems, which is particularly important in view of new emerging technologies in biomedical engineering.

To sum up, I state that the thesis submitted by Adam Mirek entitled *Development of new drug delivery systems made with electrostatic and bioprinting techniques* fulfils all the requirements for the Ph.D. degree according to relevant law.

Taking into account the scientific quality of the thesis, the fact that the research was published in highly ranked journals, and that the Ph.D. candidate has exceptional scientific achievements as reflected by the number of publications (7 papers in JCR journals), as well as oral and poster presentations at international conferences, my recommendation is that the Ph.D. title is awarded to Adam Mirek with distinction.