**Rapporteur's (Prof. habil. dr. Arunas Ramanavicius)**

**Assessment of the doctoral thesis**

**"*Development of new drug delivery systems made with electrostatic and 3D bioprinting techniques*"**

**by Adam MIREK**

The PhD thesis by Adam Mirek summarise the research work, which was performed by Adam Mirek, under guidance of her supervisors and advisers (**Dr. Mikhael Bechelany and Dorota Levinska**) and many other her colleagues. It is very positive that the PhD thesis by Adam Mirek is a result of advanced international collaboration between University of Montpelier and Nalecz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, (Poland).

This work is very important for the future development of drug delivery systems, because above mentioned drug delivery systems are suitable for biomedical applications.

The manuscript of thesis is written in English and in generally it is of very good quality. The investigations, which are described in thesis, are aiming to develop a foundation for new controlled drug delivery systems manufactured using electrostatic and 3D bioprinting techniques, which would be characterized by an increased drug capacity and an elimination of the drug burst release effect (occurring in the first stage of action of systems) based on electrospun polymer fibrous mats or 3D bioprinted hydrogel constructs. Three main research well defined and logical aims have been formulated in this research. All these aims were well achieved.

Methodological aspects are also well defined and are logical. The first part of this work concerned electrostatic techniques. Author adopted a promising approach to the electrospinning process by applying electrical voltage in a pulsed manner. It should be mentioned that significant attention has been dedicated to the application of pulsed voltage with controllable electrical parameters. The control of electrical pulse duration and frequency enabled stabilization of electrospinning process and electrostatic droplet formation, enabling the production of synthetic polymer fibers or microspheres of the desired diameter.

Author prepared and analyzed fibrous mats from polyvinylpyrrolidone (PVP) and polylactide (PLA).

It was determined that the use of pulsed voltage not only stabilizes the process but also provides several additional parameters such as frequency of electric pulses and their duration, which increases the possibilities for the modification of formed structure.

Author developed an interesting and promising method for the formation of drug-loaded polymer microspheres based on biodegradable polycaprolactone (PCL) or non-biodegradable polyethersulfone (PES). Combination of polymer solution treated by pulsed voltage based on electrospraying with solidification of the solution droplets by wet phase inversion technique. It is important that single microspheres formed in this way are characterized by a narrow range of diameters and do not form aggregates. These structures can be stored in the form of a dried powder.

Author combined the results of the above studies, proposing a new method of obtaining polymer fibrous mats modified with microspheres. Drug-loaded microspheres (of PCL or PES) in the form of powder were dispersed evenly in the polymer solution (PVP) and as a result of electrospinning of such a suspension, author obtained mats with increased drug capacity and eliminated burst effect. In order to make the mats insoluble in water, author subjected them to a cross-linking process using a photoinitiator and ultraviolet light, which, as the tests showed, also affected their transport properties. The selection of appropriate cross-linking method and cross-linking agents used to treat the electrospun or 3D bioprinted constructs leaded to the formation stable, water-insoluble drug delivery structures based on polyvinylpyrrolidone, gelatin and sodium alginate. It is important that in the case of hydrogels, the ‘burst effect’ was eliminated, which author reported as advantage for the design of 3D bioprinted drug delivery system. In the second part of research, ‘3D bioprinting’ was applied. Author successfully developed several novel bioinks suitable for 3D bioprinting of hydrogel. One of them consisted of gelatin and sodium alginate. Cross-linking by calcium and glutaraldehyde vapors was applied. In one bioink, author used a suspension of polymer microspheres with immobilized active substance – ampicillin, or marker – rhodamine 640 in gelatin methacrylate and gelatin. Ultraviolet light induced cross-linking was also applied. Non-aggregating, drug-loaded synthetic polymer microspheres were designed and used as additives to the suspension used in electrospinning based 3D bioprinting, resulting in an increased drug load capacity of drug delivery system. Drug transport properties are influenced by factors such as cross-linking time and the type and quantity of microspheres, which enables accurate control over the drug delivery properties of the final product. These systems differ in terms of properties and potential applications, and their manufacturing methods require a distinct approach.

In the final chapter of thesis, author provided a comprehensive evaluation of the strengths and weaknesses of electrospinning and 3D bioprinting techniques for producing hybrid drug delivery systems, as well as the benefits and drawbacks of systems based on electrospun fiber mats or 3D bioprinted hydrogel matrices modified with microspheres.

Author tested the developed constructs using numerous techniques, such as: scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), determination of the specific surface using the BET method, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), spectrophotometric tests of substance marker release, degradation and swelling analysis, mechanical properties tests, antibacterial activity and cytotoxicity tests.

In summary, this work yielded several structures namely microspheres, polymer fibrous mats, 3D bioprinted constructs that have potential to be applied for the controlled drug (ampicillin) delivery systems.

The PhD thesis consists of a thorough and detailed literature review with a very nice and clear description of drug delivery principles and a detailed description of applied methods and achieved results, which are well discussed. The conclusions made by the author indicate that major sated aims and goals were achievement. The overall quality of the manuscript of thesis is very good.

This work is based on five thematically related scientific papers that were published between 2019 and 2023 in peer-reviewed high-ranked international journals reputable journals listed in the JCR database. Adam Mirek is also the author of several conference theses.

There are some very minor shortcomings, which are not decreasing the significance and quality of this PhD thesis, and there are some interesting points for scientific discussion, which will be performed during the defence procedure. Some attention during the research discussion.

**Several questions**:

What are advantages of ‘electrospun fibrous mats’ as drug-delivery systems in comparison to free-drugs.

Why so called ‘burst effect’ should be eliminated or at least reduced in order to improve efficiency of drug delivery system.

Please provide principle and major methodological details on 3D bioprinted constructs.

How cross-linking improves the performance of drug-delivery systems. Which cross-linking agents were found as the most efficient for the design of drug-delivery systems.

Please discuss/comment biodegradation and biocompatibility of in thesis reported drug delivery systems.

**According to my opinion, the Adam Mirek contribution to the study and to research is sufficient and the PhD thesis meets all requirements, which are set for PhD thesis at Montpellier University. Therefore, I am recommending to award Adam Mirek by PhD degree.**

24/08/2023 Prof. habi. Dr. Arunas Ramanavicius
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