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Review of Adam Mirek's M.Sc. Eng. doctoral thesis "Development of new drug delivery systems made with electrostatic and 3D bioprinting techniques."

I had the privilege of reviewing Adam Mirek's M.Sc. Eng. doctoral thesis, "Development of new drug delivery systems made with electrostatic and 3D bioprinting techniques".The thesis was written under the guidance of two esteemed professors: Dorota Lewińska, Ph.D., D.Sc., a Professor at the Nalecz Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, and Mikhael Bechelany, Ph.D., D.Sc., from Institut Européen des Membranes, University of Montpellier. The main objective of the research is to explore cutting-edge drug delivery systems utilizing electrostatic techniques. This study expands on the groundwork of the Laboratory of Electrostatic Methods of Bioencapsulation, which Professor Dorota Lewińska has been spearheading for several years.

The Dissertation adheres to a classic structure, comprising 49 numbered pages (all 145 pages), annexes, 11 Figures, and two tables. The Ph.D. student holds the position of the primary author in a series of five articles that have been published in esteemed journals. Notably, four of these journals have Impact Factors, while the fifth is undergoing evaluation, with expectations of a similar impact. The articles are preceded by a comprehensive introduction that serves as a collection guide. The work is initiated with acknowledgments, followed by a list of publications used to compose the dissertation, summaries in Polish, English, and French, and lists of abbreviations.

The introductory portion of the work comprises a significant portion, approximately one-third, and effectively explains the rationale behind developing and utilizing drug delivery systems. It also provides an overview of the essential prerequisites for creating an efficient drug delivery system and a comprehensive review of the current state of knowledge in the drug delivery systems field.

Despite its brevity of only seven pages, the experimental section is appropriately concise and justified as it was developed through five different works' experiments. The results section,

which constitutes another third of the work, is logically subdivided into three subsections that meticulously describe each presented thesis.

The Dissertation is comprised of five works that provide a thorough summary of the research, complete with conclusive findings and 113 literature references. Alongside the works, the annex provides declarations of co-authors' shares and a detailed account of the Ph.D. student's research activity that extends beyond the scope of the monothematic set of articles. The Dissertation also includes two articles that have been published in peer-reviewed journals, a Polish patent, a list of four domestic and foreign conferences, and a summer school where the Ph.D. student actively participated.

It is worth mentioning that Adam Mirek, M.Sc. Eng.'s works have garnered positive assessments from reviewers prior to their publication as articles. As a reviewer of the doctoral dissertation, my role is supportive rather than investigative, and I am tasked with accentuating the benefits of employing these positively evaluated works as a monothematic series of scientific publications.

Efficient drug delivery systems are crucial in medicine, with significant applications in treating various medical conditions. This topic is intensely researched by materials engineers, biomedical engineers, and pharmacists as it has the potential to be highly effective in treating lifestyle diseases such as osteoporosis and cancer. Creating an effective drug delivery system is a major objective for many pharmaceutical applications.

Adam Mirek, MSc. Eng implemented an ambitious project in two research centers: the Institute of Biocybernetics and Biomedical Engineering of the Polish Academy of Sciences in Warsaw and the Institut Européen des Membranes, University of Montpellier. Despite the challenging pandemic, he achieved positive results with the potential for significant practical applications. His work focused on addressing a critical problem in creating drug delivery systems.

Currently, commercialized drug delivery systems based on microspheres, such as Lupron Depot, are known for their non-linear drug delivery to the body, which has a physical basis. As the drug is released from the microsphere, its subsequent spherical layers become available, and the active volume of the microsphere is dependent on the third power of its radius. This results in exponential drug release, with a significant decrease in the amount of pharmaceuticals delivered over time. Adam Mirek’s MSc.Eng research aimed to reduce this problem, and the results are promising for developing more efficient drug delivery systems.

The initial focus of the Ph.D. student's research was on drug delivery, which included two significant issues. The first concern was the problem of drug burst, which arises when the drug is not evenly dispersed within the drug delivery system, leading to a considerable amount of it settling on the system's surface. This critical concern affects most drug delivery formulations, typically arising from drug and matrix incompatibility. To address this issue, optimizing the drug used in the standard matrix or creating a separate system for each pharmaceutical is necessary; however, this is a technologically challenging task. Additionally, drug release can exceed toxic values, and leading to measurable losses in the active substance, with the burst of a drug covering up to half of the drug delivery system's contents. The Ph.D. student proposed using a hydrogel as a matrix to address these problems, aiming to delay drug delivery and provide a smoother release profile.

The source review analysis was competently executed. While some may raise concerns about the number of references, 113, it is plausible that the Ph.D. student purposefully narrowed their research to encompass only the five articles concisely outlined in the Dissertation, each encompassing 45-55 references. The source review's conclusions were formulated clearly and convincingly.

The author presented three research theses, all of which were confirmed through the conducted research.

The first thesis put forward is that pulsating voltage has the potential to produce fibers of varying sizes. However, the relationship between the pulsed voltage parameters and the fibers' diameter is not clearly defined. It is worth noting that using pulsating voltage can help avoid the difficulties encountered when using alternating voltage. When electrospinning with alternate current, a much higher voltage (such as 60 kV AC) is required compared to 20 kV for DC. The results of the study are both theoretically interesting, as they provide insight into electrostatic methods that incorporate pulsed voltage, and practically significant, as they offer a platform for drug release systems. The research demonstrates that it is possible to prevent the formation of macrospheres when using a PCL solution as a polymer, although this effect was not observed when testing other polymers in different solvents.

The second thesis explored the potential of eliminating the drug burst effect in 3D-printed drug delivery systems. The Ph.D. student discovered that using typical cross-linking agents like calcium ions in alginate resulted in a material that was not water-resistant. Instead, photoinitiators or chemically cross-linking glutaraldehyde had to be used under specific conditions sufficient for photocrosslinking. He also examined the use of appropriate substances

to cross-link the hydrogel matrix. The study found that when these substances were used, there was no observed burst when the standard marker rhodamine was released from microspheres embedded in the hydrogel. Rhodamine was released according to zero-order kinetics.

The third thesis focused on creating drug-containing microspheres for use in electrospinning microfibers or bio-ink for three-dimensional printing. The aim was to increase drug load and prevent drug bursts. By synthesizing drug-releasing microspheres separately, he created a platform for incorporating various therapeutic substances in the microspheres without disrupting the electrospinning process or obtaining 3D bioprinted scaffolds. Encapsulating therapeutics is a proven method for creating effective drug delivery systems.

The study's findings indicated that microspheres with an average size of 14 micrometers released the marker much slower than those with an average size of 6 micrometers. The electrostatic method utilized in creating microspheres facilitated obtaining them in a non-aggregated form, as similarly charged microspheres repel each other and reduce sticking. However, the Ph.D. student surprised me with the results presented in Figure 10F, which showed a decrease in rhodamine concentration during release and requires further explanation. The release of rhodamine from the cross-linked PVP gel also requires explanation.

Throughout the course of experiments, it was possible to effectively showcase the capability of integrating a greater quantity of rhodamine into electrospun fiber systems containing microspheres. The findings revealed the achievement twice the equilibrium concentration during the marker's release. Furthermore, marker-containing microspheres in hydrogels created through three-dimensional printing were successfully incorporated. This led to a remarkable fourfold increase in the equilibrium amount of marker released.

The Ph.D. student has created a groundbreaking platform for controlled drug release, utilizing advanced electrostatic techniques and 3D printing. Through the strategic application of pulsating voltage, he has successfully stabilized the electrostatic processes involved and achieved control over the final product's structure. By cross-linking hydrogel drug release systems, he has effectively influenced release times and potentially reduced drug ejection. These hybrid drug release systems are based on either cross-linked electrospun mats with drug-embedded microspheres or ultraviolet-cross-linked hydrogels that contain drug-embedded microspheres and represent a significant achievement with both theoretical and practical implications.

During the experiments, the Ph.D. student showcased impressive skills in addressing the issue of glutaraldehyde toxicity on cells and proteins. Although initial tests utilizing

glutaraldehyde as a cross-linking agent yielded positive outcomes, he recognized that they may not be practical for real-world applications. Instead, he explored using photoinitiators for a chemically modified hydrogel, with benzophenone being the initial choice due to its historical significance and lower toxicity than glutaraldehyde. However, he discovered even better photoinitiators and selected a more sophisticated one that produced superior results. His work demonstrates a clear progression and an unwavering commitment to learning at every stage, deserving recognition and commendation.

The literature review sheds light on the Ph.D. student's approach to "cell bioprinting" and its associated challenges. While the topic may be impressive for public relations, it requires careful consideration. It is essential to note that cells not nourished with sufficient nutrients and gas exchange will die within a few minutes if they are not vascularized or kept inside a bioreactor. The mentioned adipocyte stem cells (ADSCs) perform optimally and maintain appropriate gene expression in hypoxic conditions. If left in hypoxic conditions, ADSCs will differentiate only into cells that function in these conditions, such as chondrocytes.

It can be challenging to pinpoint weaknesses in a dissertation crafted from articles composed over an extended period, scrutinized by multiple reviewers, and presented with brevity, lucidity, and impartiality. Nevertheless, it falls to the reviewer to detect any potential flaws. From my perspective, apart from previously pointed difficulties, it is crucial to consider the incompatibility of hydrophobic drugs - which form a minor fraction of pharmaceuticals. This lack of compatibility can impede or severely restrict the conveyance of the drug through the hydrogel. Consequently, these drugs are prone to ensnared in the polymer matrix instead of released through the hydrogel, which functions as a barrier.

This dissertation has made a substantial contribution to the field of drug delivery systems. It not only introduced innovative ideas to tackle the prevalent issues faced by these formulations but also conducted hands-on research on both real drugs and model drug-like substances. This commendable feat by the Ph.D. student illustrates the fundamental research element of the work. As such, the dissertation offers both fundamental and practical knowledge of the existing state of technology and knowledge.

This dissertation showcases remarkable innovation in the development of composite drug release systems. The author employed electrostatic methods, utilizing pulsed voltage and 3D printing technology to create microspheres and micro-nanofibers, or microspheres and hydrogels, as composite drug delivery systems. The author's independent and original contributions include successfully creating and testing these systems, using drug release

markers and actual antibiotics. Additionally, the author thoroughly examined the formation and properties of the materials, as well as their effectiveness in fighting real pathogens like *E. coli* and *S. aureus*. The author correctly solved the given issues using appropriate methods and justified the assumptions made.

Despite uncovering some minor inaccuracies, they do not detract from the exceptional quality of the Dissertation as a whole. To conclude, I would like to applaud the remarkable determination and diligent efforts of the Ph.D. student who surmounted obstacles to create an outstanding dissertation.

Under the Act on Scientific Degrees and Scientific Titles, the reviewer is responsible for assessing the doctoral thesis's originality in addressing scientific challenges and the Ph.D. student's theoretical and practical expertise in the Biomedical Engineering field. After a meticulous evaluation of this Dissertation, I am convinced that it fulfills all the criteria set by the Act and has been composed with utmost care. Therefore, I respectfully recommend that the doctoral Dissertation be approved and advanced to subsequent stages of the doctoral process.

Based on the solution of fundamental problems and thorough engagement with applied research, the work performed by M.Sc. Eng. Adam Mirek deserves the highest rating of outstanding and deserves the distinction. Moreover, his significant scientific achievements, including 101 citations across 97 works and a Hirsch index of h=4, further demonstrate his exceptional proficiency in the field.

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