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Doctoral Thesis Review

Review of Magdalena Bigaj-Józefowska's Doctoral Thesis submitted to the Faculty of Chemistry, Adam Mickiewicz University.

Title of the Doctoral Thesis:

Application of cancer cell membrane coated nanoparticles in advanced liver cancer treatment

Ph.D. candidate: Magdalena Bigaj-Józefowska

1. Objective

The Thesis mainly focuses on developing biocompatible multifunctional nanoparticles with an extraordinary ability to treat liver cancer efficiently. The entire material fabrication was optimized using a step-by-step approach guided by the pieces of evidence collected during the chemical, structural, and physical characterization, as well as evaluating the interaction of the nanomaterials developed at different project stages with living cells and animal models.

The overall research aim of the presented activity is "*Contributing to the field of nanomedicine by addressing the gap in the literature regarding the synthesis and application of membrane-coated PDA-based nanoparticles for liver cancer therapy and imaging*". In order to reach that goal, different sub-aims have been targeted by Magdalena Bigaj-Józefowska in her Thesis, including: a) synthesis of the nanoparticles (which includes several fabrication steps); b) chemical, structural, and physical characterization; c) testing the drug release features of the system; d) evaluating the material *in vitro* biocompatibility, ability to kill cancer cells and MRI properties of the system; and e) evaluating the applicability of the proposed

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theranostic cancer treatment *in vivo*.

The presented study has been approached considering the urgent request to solve the problem of efficiently diagnosing and curing liver cancer using a single material activable on demand. The entire research activity has been technically designed properly, firstly investigating and enhancing the capability of the fabricated materials using a methodological approach based on material characterization. Moreover, an impressive number of biomedical-oriented *in vitro* and *in vivo* tests have been carried out to prove the final applicability of the developed nanostructures, which undoubtedly improve the influence of Magdalena Bigaj-Józefowska's activities on the nanomedicine field.

It is worth reminding that the doctoral activities of the candidate were not only well-design but even had an incredibly high impact on the application of the proposed nanomaterials, as proved by the results of the *in vivo* tests on mice. To achieve the main target, Magdalena Bigaj-Józefowska was able to fulfill all the subtasks previously set at the beginning of her studies, therefore, it is evident that all goals have been completely reached.

2. Originality

Liver cancer is a severe disease that profoundly impacts human society. This medical condition develops over a period of time, and it is influenced by chronic diseases (e.g., cirrhosis, hepatitis, etc) as well as human misbehaviors (e.g., alcohol consumption and smoking). The typical liver cancer diagnosis includes blood tests, imaging (including MRI), and liver biopsy to confirm the existence of cancerous cells. After the diagnosis, chemotherapy, radiation therapy, and the surgical removal of the tumor are the most applied strategies. Looking at the stat-of-art, the identification and treatment of this disease are very invasive; moreover, an early diagnosis and treatment can significantly improve outcomes. The doctoral studies of Magdalena Bigaj-Józefowska focused on solving the main limitation of the present clinical approach, indeed, a theranostic tactic based on both diagnosis and cure of the condition with a minimally-invasive targeted strategy is at the core of the research efforts in this specific field.

Nanotechnology-based therapeutic approaches play a vital role in revolutionizing

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medicine. Firstly, the physical appearance of nanomaterials gives them an obvious advantage, indeed, they can be used in small quantities and work efficiently thanks to their high surface area, moreover, nanostructured materials with the ability to be used as imaging agents to deliver drugs or other therapeutics can be targeted in a specific body tissue. These features enhance the therapeutic efficacy of treatments while minimizing side effects. The field is uninterruptedly growing, and scientists such as Mrs. Bigaj-Józefowska are designing and investigating new material development strategies to improve the efficacy of targeted teranostic agents.

Single functions characterize macromaterials, while nanomaterials offer the opportunity to show multiple and even extremely different capabilities at the nanoscale with one single piece of material. Indeed, nanomaterials can be engineered to combine various characteristics, such as electrical, optical, mechanical, and chemical properties, into a single nanostructured material. Allowing such structures to perform multiple tasks or applications simultaneously makes them highly versatile and valuable in various fields. Designing and developing multifunctional nanomaterials are at the forefront of nanoscience research thanks to the opportunity to solve problems and improve existing technologies offered by these materials. In this context, the applicant developed and characterized nanoparticles with therapeutic and diagnostic features where therapeutic (e.g., drug delivery and photothermal therapy) and diagnostic (e.g., imaging) functions are combined to target specific therapies and simultaneously monitor the treatment's progress.

Researchers in the biomedical engineering field are spending time and effort to develop materials that are not only targetable in a specific body tissue but even materials activable by anthropogenic stimuli. Light-activable biomaterials offer a versatile and non-invasive means of controlling and manipulating biological systems and materials, providing precise control over the applied therapeutic processes. Magdalena Bigaj-Józefowska was able to efficiently work on one of the most advanced and difficult topics of material engineering, developing NIR light-activable drug delivery systems.

Magdalena Bigaj-Józefowska fine-tuned an impressive number of chemical, structural, and physical properties of all the intermediated and final nanomaterials; furthermore, she evaluated the investigated features with sophisticated instruments,

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which have been correctly selected to confirm the material properties. Finally, the applicability of the nanostructured materials has been fully proven, which is undoubtedly a great plus for this doctoral study.

Mrs. Bigaj-Józefowska studied nanomaterials that, due to their nature and potential timing, are extremely interesting. This Thesis work shows the successful development of groundbreaking biomaterials for theranostic cure of liver cancer; therefore, it is expected that it will lead to impactful publications in the treated field.

3. Presentation

The Thesis of Mrs. Bigaj-Józefowska is a text that is composed of 178 pages and shows the research activities in an exhaustive narrative story.

The first section is a deeply presented Introduction, which is longer than 20 pages ad includes 6 sub-sections that have been perfectly designed to introduce all the research areas touched in the doctoral work. Images could be improved to help the readers (e.g., Figure 4, which does not include all the therapeutic approaches involved in this Thesis, and Figure 6, in which figure descriptions are missing), moreover, some of them have minor inaccuracies (Figure 3 shows "estimated" values for a period time passed a few years ago) In general, the Introduction is comprehensive, but it is still easy to read and does not include trivial pieces of information.

"*2. Aim of the study"* is well-prepared as the candidate listed all the necessary points concisely, giving the right pieces of information to the reader. Anyway, even this section could be slightly improved as not all the points have been inserted in the right place. Indeed, the reader would expect to follow a temporal path, which cannot be followed for" *Characterization and Analysis"*. Moreover, the last point ("*Contribution to Nanomedicine")* seems to be an overall aim, which should be reported at a higher level than the previously listed objectives.

"3. Materials and Method" section is methodologically prepared following the style of a typical research article in the field. The given information is very detailed and allows any scientist working in the investigated area to repeat the proposed experiment. Anyway, more attention should be given to the chemical formula, which has not been

mentioned in the list of materials close to the name of the compounds. The applicant should keep in mind that readers, who may be specialists in completely different disciplines, could be interested in this work. Therefore, taking care of any aspect of the comprehension of the text is important.

"4. Results and Discussion" section is the core of the Thesis. Due to its nature and importance, this is the longer section. Mrs. Magdalena Bigaj-Józefowska described here every important scientific achievement, discussing the technical data but even evaluating problems and connected solutions, which is vital in the material development process involving different subsequent steps. The text is very technical and direct toward showing the results, which makes reading it very enjoyable.

" 5. Conclusions" section reflects the high quality of the conducted research in terms of content. Anyway, not all the aspects have been taken care of in deep detail. Indeed, I expect to read a section in which the list of achievements is designed by looking at the list of the aims reported in the "*2. Aim of the study*" section, but no direct connection between these two sections is present. Most importantly, reading at least a paragraph describing the future perspective of this research work would be appreciated.

Five technical sections have been listed at the end of the Thesis to help the reader understand the technical information reported in the text (abbreviation, figure, tables, and references), as well as the applicant's scientific achievements (*"Publications and Conferences"*).

The Thesis has been presented in English language by the applicant. The style and grammar are correct. Anyway, some details concerning the presentation of the whole story could be improved to enhance the fruition of the content. On the other hand, the text remains reader-friendly, which is a difficult objective to achieve in the case of a long text as the submitted Thesis. Therefore, my opinion on the overall presentation is positive.

4. Methods and Results

The research activities performed by Magdalena Bigaj-Józefowska as part of her doctoral studies are purely experimental. The primary aim of Bigaj-Józefowska's

research activity is to design, synthesize, characterize, and finally prove the applicability in the field of liver cancer of multifunctional, stimuli-responsive nanoparticles.

At the beginning of the Ph.D. pathway, a series of MDPA and MDPA@Fe NPs have been synthesized and characterized to prove structural, morphological, and chemical features. Several conditions have been tested, and looking at the obtained data, only the most perspective system was moved to the next stage. Besides the great quality of the results, I believe that there is still space for material optimization, especially looking at the Zeta Potential values indicating the colloidal sol stability; moreover, the release of a substance in the range of temperature of the PTT application (1 in Figure 11) still remains unclear. Furthermore, these particles showed the ability to be loaded with important bioactive molecules such as doxorubicin and release this drug on demand. The activable features of the developed DDSs have been proved, anyway, testing the actability a few times during the investigated range of time can easily open new gates to the application of the developed particles.

The synthesized nanoparticles have been successfully encapsulated: MPDAFe@Mem. This structure was fully characterized, too; moreover, thermoresponsivity and applicability as MRI agents were proved. As an outlook, I suggest trying to minimize the intensity of the triggering NIR light to be more in line with the clinical need and to uniform it along all the experiments reported in the whole Thesis (*in vitro* and *in vivo* studies).

The capability of killing cancer cells (HepG2 line) *in vitro* upon activation has been proven with detailed studies. The biocompatibility of the developed nanostructures was tested using MRC5 cells, which is undoubtedly correct; anyway, the effect of the whole therapeutic approach to biosafety could be studied more in detail in the future.

At this point, it is worth mentioning that Mrs. Bigaj-Józefowska did not only take care of the material science-oriented details and carried out *in vitro* tests with different cell lines. Indeed, the last section of the *"4. Results and Discussion"* is dedicated to *in vivo* studies using Bulb/c nude mice, aimed at proving the applicability (therapeutic and imaging features) and safety of both MPDAFe@Mem

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NPs and MPDAFe@DOX@Mem NPs, with and without laser irradiation.

Overall, the developed research's scientific quality is undoubtedly high.

5. Conclusion

Evaluating all the mentioned aspects, which span from the presentation to the scientific achievements of the Thesis presented by the applicant, my overall opinion is highly positive. Magdalena Bigaj-Józefowska reached all the set objectives and technical targets; for this reason, I propose allowing her to defend her Thesis in the public dissertation.

The scientific area in which the applicant focused her activities is extraordinarily dynamic and complex due to the multidisciplinary aspects of it. The candidate successfully dealt with multifunctional systems composed of different materials and assembled in different steps, which is not an obvious achievement for a scientist at this stage of her career. Additionally, the ability to develop a light-responsive nanomaterial that can be targeted to a specific site makes the obtained structure even more interesting from the material engineering point of view. Impressively, the candidate showed the ability to successfully work in the field of material science and even in the biomedical application-oriented area. Indeed, her research included an in-depth evaluation of the *in vitro* effect and the *in vivo* therapeutic outcome of the applied strategy.

Magdalena Bigaj-Józefowska successfully tackled an enormous amount of research issues that are extremely important for the scientific community. The final material has unique features such as multifunctionality, targetability, activability by NIR-light, biocompatibility, and the ability to detect and eradicate liver cancer using a combination of therapies. The scientific findings shown in this Thesis are, without any doubt, of enormous value in the investigated field; for this reason, I even suggest considering Magdalena Bigaj-Józefowska for distinction.

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