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Review of the Manuscript of the Thesis presented by

Zhanna Kozyreva to get the PhD degree in Skoltech (Skolkovo, Moscow)

Title : FLUORESCENT PHOTOCONVERTIBLE POLYMER MICROCAPSULES FOR CELL LABELING AND TRACKING

The Thesis deals with a development of novel fluorescent photoconvertible microcapsules for cell labelling and tracking. As well known, genetic alterations are unacceptable for some cell types, for instance genetic modification of stem cells could lead to irreversible changing their properties and, as a result, could arise safety questions for personal therapy. On the other hand, the use of lipophilic membrane dyes is limited, since long-term tracking is not possible because of dye leakage from the cells. Therefore the capsules-based technique developed by the author of the Thesis could be considered as an interesting and rather promising alternative approach to both genetic modification and the use of lipophilic drugs. That is why the Thesis is timely and of great interest for sure.

The aim of this study was to design the trackable microparticles, to evaluate them in terms of their physico-chemical properties as well as to test them for possible biomedical applications, namely for cell labeling and tracking.

The manuscript is written in a classical form and consists of Introduction *(Chapter 1),* Review of the literature (*Chapter 2*), Materials and Methods (*Chapter 3*), Results and Discussion (*Chapters 4-6),* General conclusions *(Chapter 7)* and Bibliography (328 references).

In Chapter 1 the author considered background and formulated the aim and objectives of the current study. *Chapter 2* is a Review of Literature. *In Section 2.1* the author described in detail fabrication of polymer microcapsules, their some physicochemical properties as well as encapsulation methods and further promising biomedical applications of these capsules. *The Section 2.2* deals with carbon nanodotes (production, properties, applications), while in *Section 2.3.* there is some interesting information about photoconversion, in particular photoconversion mechanists in fluorescent proteins and organic dyes. Finally, in *Section 2.3* the author focused her attention on cell labelling techniques, namely the methods, where fluorescent proteins or organic fluorophores are used.

It is my pleasure to mention that *Chapter 3* (Materials and Methods) is written very carefully. The author precisely described all used reagents, solutions, instruments, manipulations and procedures as well as all techniques used in the current study. This carefulness really helps reading and understanding of the manuscript.

The obtained Results are described by the author in *Chapters 4-6.* Chapter 4 deals with thermal treatment in PVA gel to facilitate photoconvertible capsule fabrication and capsule characterization. *In Chapter 5.* the author described and discussed in detail the obtained results on non-thermal layer-by-layer synthesis and study of photoconvertible capsules, which have been

prepared without thermal treatment using dye-labeled polyallylamine hydrochloride as well as their characterization.

Finally, *Chapter 6* dealt with very interesting *in vitro* experiments, where photoconvertible polymer capsules were used to evaluate cell labeling and tracking. The author demonstrated that the capsule-based approach could provide observations of movements of several individual cells for several days, as well as the destiny of daughter cells after doubling, without any additional marking for 4 days.

In *Chapter 7* the author concluded that the developed capsules could provide a unique coding system for cell labeling and tracking via a combination of various labels. Moreover, this capsule-based technique could be considered as rather promising alternative to both methods, where lipophilic dyes or green fluorescent proteins are presently used. It should be also mentioned that the proposed approach eliminates a risk of genetic mutations, which is of great importance in personalized medicine.

In general, the manuscript is very well written and easily readable. All parts are well linked to each other, and the manuscript itself represents a nice completed publication. Moreover, the manuscript is very nicely illustrated with 29 Figures and 2 Tables. The author obviously demonstrated really deep knowledges of the subject of her thesis, as well as very good knowledge of the literature related to the field. The discussion of the obtained results as well as the Thesis itself obviously confirm the high competence level of the author in the field of materials science and engineering.

The results of this study have been published in four papers relevant to the Thesis, namely in well known journals, such as International Journal of Molecular Sciences, Heliyon, Nanomaterials, Optical Materials. Additionally, in the publication list of the author there are two papers published in Colloids and Surfaces B: Biointerfaces (2021) and Colloids and Surfaces A: Physicochemical and Engineering Aspects (2024).

In conclusion, the Thesis of Miss **Zhanna Kozyreva** obviously contains novelty. This study is rather important and promising contribution into development of new approaches, which could be proposed for various biomedical applications, namely for cell labeling and tracking. It is my pleasure to support this work and the author of this thesis **Zhanna Kozyreva** in her efforts to get a PhD degree.

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