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Review of the pHd thesis submitted by Nehar Celikkin, MSC entitled *3D Printed Gelatin Metacrylate Scaffolds for Bone Tissue Engineering*

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Assessment of the choice of the desideration topic

In the case of bone defects or bone diseases related to the destruction of bone tissue, still allogeneic transplantation remains the gold standard. Unfortunately, this solution is not always accepted by the patient's body. Novel approaches dedicated orthopaedic and traumatology more often used a new materials and technologies tailored to the patient's defect. An increasing number of implant or construct formation techniques take into account the need to monitor the regeneration of damaged tissue. Moreover, it is possible to modify the material in such a way that the self-repair process of the damage can be tracked. However, in this respect, the bone tissue is a demanding research example. This composite porous system with a diverse architecture and sufficient strength is still a challenge for scientists in the field of materials science. Such a conscious approach results in the application of novel solutions, which is a decision taken both by the doctor and the patient.

The topic of scaffolds dedicated to bone tissue engineering would not be novel if it were not for the use of 3D printing technology. Recent decades (from 2000) have seen a rapid development of techniques based on 3D printing and 3D bioprinting. Three-dimensional (3D) printing is a state-of-the-art technology to fabricate biological constructs with hierarchical architecture mimicking their native counterparts. Thanks to these techniques, it is possible to control the scaffold porosity on both macro and micro scales as well as to modify the base material with non-organic and organic factors, including cells (bioprinting). 3D printing allows for the proper distribution and positioning of biomaterials and signaling factors. When employing 3D bioprinting we get heterogeneous cells in high densities to form tissue-engineering constructs. The quality of the final printed scaffold is assessed in terms of biocompatibility, biodegradability, cellular response, and the tissue microenvironment it is exposed to. The 3D printed scaffolds with interconnected pores and large surface areas support the cell attachment, growth, intercellular communication and exchange of gas and nutrients, which is a notable advantage over the traditional techniques of solvent casting, phase separation and melt molding. With this perspective, 3D printing is gaining overwhelming acceptance from doctors and researchers across the globe as a viable option to improve the lives of disease-stricken patients.

Therefore, taking up such intresting topic by Nehar Celikkin, MSc. seems to be a good choice. The issue corespondence with other scientific projects realized by Biomaterials Group in Faculty of Materials Science Warsaw University of Technology

Content-related assessment

The reviewed PhD thesis of Nehar Celikkin, MSc. consists of 173 pages, of which 16 pages are the own thesis including introduction, aim and scope of the work and the summarized final conclusions. The rest of the PhD thesis consists of 92 pages and includes 4 publications that form the background of the PhD. The cited literature includes an impressive set of 357 literature items (the oldest of which dates back to 1993).

In the short introduction, the PhD student introduces the ideas of regenerative medicine and tissue engineering. It is rightly pointed out that it is clinical needs that stimulate scientists to develop mimetic biomaterials. There is a greater chance of increasing the active implant interface and thus a better interaction of the scaffold/implant with the surrounding tissues, using natural materials and their compositions.

The next chapter is the cited review whose first author is Ms Celikkin. The work introduces the reader to a group of materials used as starting materials for scaffolding, cell encapsulation materials and 3D bio-printing techniques. Much space in the manuscript is devoted to the research involving natural materials conducted *in vivo* on animals. Frequently the paper includes the data describing imperfections of the tested materials and technological solutions which pose difficulties in regenerating a specific tissue or the extracellular matrix. I believe this is a good starting point for a PhD student to show how much still needs to be explored and explained in the field of 3D scaffolding and the bench-to-bedside research.

The third chapter is an introduction to the actual topic of the work. It shows the clue of the medical problem of supplementing and regenerating bone defects. Treating bone as a living material, the work refers to the possibilities of tissue engineering. It also indicates that stimulation of regenerative processes cannot be expected based on only synthetic or natural scaffolds. It must be admitted that Ms Celikkin fairly assesses the possibilities of various techniques of obtaining scaffolds, pointing to their advantages and disadvantages. The paper also realistically evaluates practical solutions where clinicians monitor the tissue regeneration process using CT, MRI, SPECT or PET.

The next chapter presents the hypothesis and the work scope performed to prove it. The gradually solved research problems concern the characteristics of the photochemically cross-linked and dedicated BTE hydrogel scaffolds. At the first stage, the author focused on determining the optimal concentration of the GelMA hydrogel and its influence on the mechanical and microstructural properties of the scaffolds (including porosity, pore size, and swelling), resulting in a different biological response from MSCs of rat bone marrow.

The next work shows the practical use of the biodegradable GelMA scaffold in the study conducted on an animal model. While carrying out this stage, the author optimized the bioprinting process, with regard to the MSC cells encapsulation and their placement in the scaffolding. The implantation of the scaffold and the *in vivo* monitoring of its behaviour proved its osteoconductive potential, facilitating the overgrowth of bone tissue according to the secondary bone healing mechanism (confirmed by histological tests). Here again, the doctoral

student's honest approach to the process visualization issue should be emphasized, regarding the CT technique as a certain standard. Unfortunately, in the case of polymer scaffolds (materials with a low electron density) CT does not always prove to be right, especially in *in vivo* conditions where the background for the implant is bone tissue which is a high-density material.

The solution to this problem can be found in the chapter on the modification of the GelMA scaffolds with gold nanoparticles (AuNPc). The study presents a sequential approach to the scaffold visualization employing nanoparticles: from selecting such a nanoparticle concentration to ensure the sufficient contrast, up to assessing the scaffold biological properties in contact with MSC cells and the CT tests verifying the solution *in vitro*.

The main achievement of the PhD student is a comprehensive approach to the BTE problem. It includes a decent literature review, followed by the *in vitro* and *in vivo* tests accompanied by the osteogenesis process monitoring. This approach is not only an example of systematic scientific work, but it also forwards the developed material towards clinical practice (bench-to-bedside translation of the engineered bone tissue substitute). It must be admitted that biomaterials engineering is a field of study facing an extremely difficult path before the materials concept can be put into practice.

The PhD student's systematic approach to the problem confirms the readiness of Nahar Celikkin to be an independent scientist. The properly thought out process included the following stages: the gealtine methacrylate synthesis, selecting the optimal concentration of GelMA to ensure high biocompatibility of stem cells, the MSC encapsulation and the use of bioprinting techniques, the implantation and, finally, the monitoring of the *in vivo* behaviour of the scaffold. In the course of the work, the PhD student managed:

- to obtain and characterize the underlying hydrogel (GelMA) and to confirm its biocompatibility in contact with MSC cells,
- to carry out the osteogenic characterization of bulk GelMA hydrogels, followed by the MSC encapsulation during bioprinting,
- to optimize the 3D printing and 3D bioprinting process parameters,
- to characterize *in vivo* the GelMA hydrogels using a rat model and to visualize the implant behavior via MRI and CT techniques,
- to modify GelMA suits with AuNPs to improve the visualization via CT and μCT techniques.

Formal, critical and discussion notes

The presented work layout raises mixed feelings. In my opinion, the introduction should cover all the topics - not only very general assumptions regarding biomaterials/scaffolds for TE/BTE, but also specific problems that currently disqualify the tissue engineering concepts from medical applications. I would expect here Ms Celikkin to point out that the 3D printing technique used in the further part of the work guarantees the best repeatability of the obtained scaffoldings so far. The technique makes it possible to personalize the scaffolding in terms of the shape and composition and to obtain materials with the stiffness/strength or bioactivity tailored to particular cells.

The reception of the PhD dissertation would be easier if the author had initially included a key concept linking all her works published in highly respected journals. The Introduction section could by extended and play a role of a guide through the work by joining general information from the Chapter II with more specific problems/solutions presented in Chapters IV - VI. The current layout suggests that the review manuscript (Chapter I) addresses a slightly different topic (natural carriers of GAGs and proteins) and is included in the presented series only as an additional value as the PhD student is the lead author of the review. This could be a starting point where Ms Celikkin presented the limitations of the materials in terms of forming techniques, properties and the possibility of verifying the in vitro behaviour of scaffolds. Next coming to a specific issue of the tissue engineering which is the bone tissue engineering. In my opinion, there is no clear link between Chapter I and the rest of the work.

The situation is saved in Chapter II with a lot of information explaining the PhD student's approach and the topic importance in the context of statistics, current research and clinical possibilities. Can you, please, explain where this original approach to the work layout comes from?

In Chapter IV related to the influence of the polymer concentration on the properties of GelMA scaffolds, some information requires explanation or addition data. Why only two concentrations of GelMA (5 and 10%) were used in the research (it's too low concentration to 3D printing or bioprinting)? Why such choice of metacrylation degree (57%)? Do you observe microstructure of scaffolds in *in vitro* conditions or just gel insight the medium? It is a pity that the manuscript does not include microscopic pictures showing the morphology of cells. Did one examine the stiffness of the materials for which the MSC cells are extremely sensitive to? What is the effectiveness of GelMA cross-linking? Was the degree of GelMA cross-linking tested or was this assessment based on the swelling ratio and gel fraction studies?

In Chapter V lacks information regarding how the scaffolds were sterilized for the *in vitro* and *in vivo* tests. This information is of high importance when discussing porous polymeric and hydrogel materials. Taking into account the scope of preclinical studies involving animals, it seems crucial to know what the lifetime/degradation degree of the developed materials was. Were there any *in vitro* degradation studies of the obtained scaffolds? What was the degree of the *in vitro* GelMA degradation, and did it correlate with the *in vivo* observations?

In Chapter VI, there is no description of the homogenization method, which is important with regard to nanocomposites (and the GelMA-AuNPs composition is such). Does the method of nanoparticle-prepolymer homogenization change the molecular weight? How was the distribution of AuNPs nanoparticles in the matrix studied? Whether repeatable results of nanoparticle distribution in GelMA matrix were obtained? Does the content of gold nanoparticles affect the degree of crosslinking of GelMA as is the case with carbon nanomaterials (CNTs, GO) added to the GelMA matrix? Was the degradation of the AuNPs-modified scaffolds studied *in vitro*?

The indicated shortcomings seem to result from the young age of the PhD student and some issues which are obvious to the author. However, they require some order so as to provide a clearer message to a reader of the work. The indicated comments are rather debatable. It is known that not all the data obtained during the research stages or even individual

experiments can be included in the publication, but they often constitute a significant supplement which I am asking for.

Dissertation Evaluation

The work presented for the review is an original and professional approach to the subject of BTE scaffolds obtained via 3D printing and bioprinting. The work comprehensive approach is worth emphasizing as it presents the subject from the synthesis, through the physicochemical and biological characterization of the scaffolds up to the preclinical research. It is commendable that the author managed to develop the research material during the PhD studies and publish three very good papers in high-rated journals (three works published in the JCR list journals and one sent to such a journal).

The presented manuscripts are works from the JCR list: two of them are qualified in the fields of materials science and engineering (C, IF 5.091) and polymer science (Polymers MDPI, IF 4.319) in the Q1 area. The other two manuscripts were published (Journal of Biomedical Materials Research Part A, IF 4.396) or are in the process of being published in the Q2 area (Biomedical Materials IF 3.715). In all the works, Ms Celikkin is the first author - thus the lead author. Considering the works scope and their interdisciplinary nature, it is understandable that the publication is composed of the work of multiple authors. In conclusion, I say that the PhD thesis presented for my review is valuable and has a practical aspect. The set goals, both scientific and application-focused, were achieved. The doctoral student has proven that she possesses advanced scientific skills, can use various research methods and, above all, deal with problems that arise during the implementation of the planned experiments.

Assessment of achievements

Her academic achievements should be recognized as outstanding. Ms N. Celikkin is a co-author of 10 publications from the JCR list, a chapter in a book and 4 conference abstracts. She participated in 3 scientific research projects carried out in 4 different research centres. She has participated in 14 conferences and trainings. Her achievements also include 2 scholarships program (master and PhD studies) and her own research project PRELUDIUM.

Final conclusion

I believe that all the objectives of the Ph. D. thesis have been achieved and the Ms. Nehar Celikkin has shown good theoretical and practical skills in the design, implementation of experiments and the correct analysis of the obtained results. The research results obtained by Ph.D student are valuable both in scientific and application terms

A paper submitted for review by Nehar Celikkin, M.Sc. entitled: *3D prointed gelation methacrylate scaffolds for bone tissue engineering* as well as the attached publication file meets the conditions specified in the Law on Higher Education and Science (Journal of Laws of 2008, item 1669 and of 2009, item 39 and item 534) article 20, paragraph 5 of the Act of 14 March 2013 on Scientific Degrees and Academic Title. I hereby request that Ms Celikkin, MSc be admitted to the next stages of her doctoral dissertation procedure.