# Opponent's review

## Thesis: Novel Avenues for the Preparation of Antifouling Scaffolds for Tissue Engineering

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Tissue engineering is one of the new and modern methods of treatment of sick or wounded patients via replacement of the damaged tissue on the cell level. Three basic approaches of this process are being used; (i) cell injection method, (ii) close loop method and (iii) scaffolds application. The last of them has in principle been the topic of the thesis and, therefore, the work is focused on a study and development of hydrogel-type materials, capable of serving as scaffolds for controlled cells growing.

The author formulated the following three main parts of the thesis:

i. Choice of the convenient hydrophilic monomers for a preparation of scaffolds using polymer brushes as model systems
ii. Synthesis of antifouling hydrogels via random copolymerization of the selected monomers with zwitterionic comonomers ensuring antifouling and mechanical properties of the products
iii. Modification of nanofibers surface with selected copolymer brushes

Prevailingly, the polymers and copolymers were synthesized via Atom-transfer radical polymerization (ATRP), giving a chance to prepare not only products with predetermined properties but, among others, also to grow polymer brushes from functionalized surface.

In the first part, polymer brushes of hydroxyethylMA (HEMA), oligoethyleneMA (MOEGMA, HOEGMA), hydroxypropyl methacrylamide (HPMA), carboxybetaine(meth) acrylamide (CBMA), sulfobetaineMA (SBMA) and other monomers under study were synthesized on gold surfaces, using anchored ATRP initiator, functionalized with mercapto-group. The prepared polymer brushes were tested for fouling properties using various proteins and, the most convenient monomers for further studies were chosen.

The next part is devoted to a solution of the problem of poor mechanical properties of the polymers on carboxybetaine (meth)acrylamide basis, having otherwise antifouling ability

almost perfect. Actually, this study is tightly connected with the paper of the author, published earlier, and shows the way of choice. Carboxybetaine-containing monomers, (CB) (meth)acrylamide and methacrylate, were copolymerized with HEMA via photopolymerization, initiated with benzoin ethyl ether. Characterization of copolymer products shows that even a low concentration of the CB monomers distinctly improves the swelling behavior and antifouling properties of the copolymer keeping, at the same time, its mechanical properties on the sufficient level.

In the next study, the above mentioned copolymers were prepared by photopolymerization in the presence of Laponite (LN) as a nanofiller. Interesting results were obtained, because the formed products exhibited some mechanical properties like nanocomposites, i.e. good mechanical robustness, self-healing ability and, at the same time, with a high wettability and antifouling character. More, the author prepared macromer from PEG and PolyCL and, consequently used it, in cooperation with foreign laboratories, for copolymerization with CB-type monomer via stereolitography. It can be claimed that the results, mentioned in this paragraph, seem to be good base for further studies.

In the third part of the thesis, the study is presented of the grafting of polycaprolactone (PCL) fibers with the hydrophilic monomers exhibiting desired effect on the protein adhesion that have been selected in the foregoing parts of the research. Due to the fact that PCL alone contains a low concentration of hydroxyl-groups, necessary for functionalization of the fibers with ATRP initiator, the first step of this synthesis was a modification of PCL fibers with poly(dopamine) layer. This compound was consequently reacted with 2-bromoisobutyryl bromide (BIBB) as well as in the foregoing parts of the work. The fibers, functionalized in this way, were then covered with layer of polyMeOEGMA, HOEGMA, HPMA and CBAA, respectively. The tests of protein fouling and cell adhesion documented almost no fouling from complex biological fluids as well as strong depression of the cell adhesion.

In all parts, the thesis contains important and interesting results, representing valuable information for further development and design of tissue engineering as modern method of medicine. Therefore, it can be regarded as a good piece of research in the serious and interdisciplinary area. The work is composed of the copies of author's publications being prevailingly already published in impacted journals and the concomitant text, explaining some of the details. I have not read minutely the publication copies as they had already been evaluated by the appropriate editors and reviewers, but I have paid my attention on the author's text and interpretation of the obtained results, especially from the chemical point of

view. I can state, that I have not found any serious mistakes or errors – nevertheless, I am putting up couple of the notes and queries:

- In chapt 2, p. 48, copolymers of HEMA and CBAA or CBMA were synthesized and tested for mechanical and antifouling properties, however, no information is given about copolymerization parameters, i.e. monomer reactivity ratios. Are these data available (I am afraid not) and, more, are the copolymerization diagrams for these monomer pairs known or not?
- In the same chapter, p. 50, IR spectra of the synthesized copolymers are presented – can the author explain the interpretation of the data in more detail? More, could be verified information on the composition of the copolymers, obtained from vibration spectroscopy using another method?
- Chapt. 3, p. 55: Nanocomposite-type hydrogels (copolymers mentioned above) were prepared by copolymerization of the chosen feed in the presence of the filler (Laponite). In the text no information about morphology of the composites are presented. Thus, was the morphology checked, e.g., using X-ray diffraction or electron microscopy? Perhaps, it can be assumed that amino-group in the CB-type monomer strongly affects intercalation or exfoliation of the filler, nevertheless, the experimental data would be useful.
- Chapt 5, p. 71: PLLA fibers were modified gradually with poly(dopamine) (PD), anchored ATRP initiator and, finely with a layer of the chosen polymer or copolymer. Can the author describe formation of PD layer, bond of the ATRP initiator to PD in more detail? Scheme 6 does not seem to be sufficient and appropriately legible.

At the end of my review, I can state, that the thesis of Ing Kostina presents the results of high-quality interdisciplinary research in the field of high importance. I believe, that most of the data she obtained could be a good base for further development.

I recommend the evaluated thesis as an excellent base for the appropriate defense and for awarding the author with PhD degree.

Prague, Feb 2, 2016

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