

Review report on PhD Thesis

Reviewer: Ing. František Ondreáš, Ph.D.

PhD thesis title: Development and Evaluation of a Multi-Modal Hyaluronic Acid Hydrogel for Anti-Inflammatory Drug Delivery for Multiple Sclerosis Therapy

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The thesis focuses on biopolymer-based hybrid hydrogels combining covalent crosslinking with polyelectrolyte complexes for drugs delivery and controlled release with the specific aim developing advanced platform for multiple sclerosis treatment utilizing repurposed active compounds. The thesis approach nicely combines several drug delivery development principles and benefits from their advantages. Critical aspects for material development, controlled drug delivery, administration pathway, treatment adherence, active compounds development, and patient-centric approach are discussed and taken into consideration in the thesis aims and results interpretation. The thesis topic is very actual as we stand before a challenge to treat chronic diseases such as multiple sclerosis more effectively and advanced platforms such as injectable hydrogels with tailored properties and repurposed drugs is a vital way to achieve it.

The review summarizes current disease-modifying therapies for multiple sclerosis treatment, defining their advantages and generalizing their shortcomings. It mainly focuses on drug administration approaches to patient compliance (patient-centric), which are very important in this kind of treatment, thus defining the space for new systems for treating this disease, mainly as implantable hydrogels with controlled drug release. Based on the review, minocycline and synthetic preimplantation factor were chosen. The review defines current drug delivery approaches well, focusing on polyelectrolyte complexes and hydrogels, and defines their strengths and weaknesses. The thesis aims are well defined thanks to utilizing effectively the current state of the art. The formal structure of the dissertation and its language level are good, and thesis structure is well established.

The problem-solving process and dissertation results are of high quality. The work provides an interesting concept for drug delivery systems that combines cross-linked hydrogels with polyelectrolyte complexes to create implantable systems with improved active compounds release to ensure an optimal therapeutic window for APIs that otherwise fail in this aspect. The advanced derivatives of biopolymers, such as oxidized hyaluronic acid and chondroitin sulfate, were utilized, when the latter was also synthesized during the own work. Substantial viscoelastic parameters for the characterization of hydrogels, such as storage modulus, LVE area, and gelation time, of developed hydrogels were well characterized together with the extrusion force, which is very important for injection application.

Two models based on rubber elasticity and swelling phenomenon were used, for which the necessary data were obtained, and structural parameters such as crosslinking density, M_c , and

mesh size were calculated. The results of these structural parameters were discussed with respect to the composition and determined properties, especially the drug release characteristics. The relationship between compositions, structural parameters, physical properties, and the active compounds release profile was appropriately described and well interpreted, considering current knowledge described in the literature. In addition to the interpretation itself, the limits of the structural models used and calculated values were also discussed regarding the functional behavior of developed systems. Controlled release of selected drugs as the key property relevant to the intended application and contribution against the current state of knowledge was determined by an appropriate method and discussed with respect to composition and structural parameters, and described by the appropriate model, enabling comparison of the application-relevant parameters. The developed systems made it possible to reduce the burst release and prolong the release when the release of a sufficient dose was achieved, thus guaranteeing the extension of the delivery of the drug in the optimal therapeutic window. The results highlight the critical role of physical interactions between the active compounds and hydrogel components able to form polyelectrolyte complexes, which are covalently bound to its structure, for achieving the desired extension of release time over other parameters and approaches such as mesh size, swelling profile, or physical polyelectrolyte complexes without covalent bound to the hydrogel structure.

The thesis aims were fulfilled as the novel implantable hydrogel platform combining the advantages of a covalent network and electrostatic complexes was developed, which showed an advantageous drug release profile with minimized burst release, prolonging therapeutic delivery while maintaining hydrogel structural stability, ensuring appropriate mechanical properties and injectability. Specifically, two optimized systems with beneficial drug release of minocycline and SPIF, respectively, were developed. The bioactivity of developed systems was successfully validated in in-vitro experiments.

Both significance for practice and development of the field of study were achieved. The thesis results provide knowledge about hybrid hydrogel systems, mainly structure-property relationships, and underline the suitability of the chosen concept and developed hydrogel platform applicable not only for treatment of multiple sclerosis but also for development of similar systems for the treatment of various diseases, for which implantable drug delivery systems with an extended drug release could improve current state of treatment.

A dissertation meets the legal requirements when it demonstrates the ability and readiness for independent activity in the field of research or development and contains original and published results.

Comments and questions:

1. Is it possible to create a system combining MN and SPIF?
2. How was the used concentration of HAOX, CSOX, CS, PDHA, and calcium selected?
3. Could HAOX-PECOX have an advantageous release profile without gelatine, with simple or chelated minocycline?
4. Did the injectability parameters change with the changes in the content of CSOX, FTIC-SPIF, and PDHA content for the systems in Chapter 4.2?
5. It is strange that in the range of HAOX17 to HAOX37, with changing CSOX content, the modulus drops significantly (Figure 4.17), but the M_c decreases (Table 4.9, particularly values in the rubber elasticity theory column regarding this data), which are inversely proportional

(this is also evident from equations 3.7 and 3.15). Similarly, for these samples, the crosslinking density decreases (Table 4.6), which is inversely proportional to the M_c (equation 3.13). Based on these data and equations, M_c should instead increase with increasing CSOX content in the range HAOX_17->HACOX_27->HACOX37. Please, can you explain this?

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