

Reviewer's report

Author of Thesis: Zuzana Kočí, MSc.

Title of Thesis: Mesenchymal stromal cells and biological scaffolds for neural tissue regeneration

Study programme: Neurosciences

Reviewer: Eva Filová, MSc, Ph.D.

The thesis has a standard structure, e.g. Abstract, Declaration, Acknowledgements, Content, List of abbreviation, Introduction, etc. The thesis is well arranged into chapters and subchapters including 34 figures. Introduction describes spinal cord tissue regeneration, spinal cord anatomy, injury, endogenous regeneration, and experimental treatment of spinal cord injury. The third chapter focuses on stem cells, their sources, properties and interaction with the host tissue mainly in regard to neural regeneration. The fourth chapter describes synthetic and natural biomaterials used for the treatment of spinal cord injury. The fifth chapter in detail describes different types of extracellular matrix used for tissue regeneration. The introduction contains 8 illustrative pictures and 2 tables. In the Methods section, the author used various methods and techniques e.g. cell isolation, cell culture and characterization, qPCR, ECM hydrogel preparation and characterization, surgery, histology, behavioral testing which allowed complex characterization of samples.

The hypothesis and the study aims section specifies 4 key aims which are processed in Methods, Results, and Conclusion sections separately for each aim and followed by the Discussion of all results.

The thesis aims deal with use of up-to-date topic concerning spinal cord injury treatment using stem cells and biomaterials. The first part compares MSC from bone marrow, adipose tissue and Wharton's jelly in terms of surface marker expression, differentiation potential, migratory capacity, and secretion of cytokines and growth factors. The second part studies intrathecal application of hWJ-MSCs in the spinal cord injury using different dose or repeated application of hWJ-MSCs. In the third part, injectable ECM hydrogels have been prepared from different tissues of the CNS and non-CNS origin, characterized for biochemical composition, viscoelastic properties, and cell proliferation and migration in vitro and in vivo. The fourth part evaluates neuro-regenerative properties of CNS and non-CNS-derived extracellular matrix in a model of spinal cord injury on rats.

All aims have been fulfilled. Other sections of the thesis include Evaluation of hypotheses and study aims, Summary and Bibliography. Bibliography contains more than 250 publications.

The results have been published in three international journals with impact factors 4.92, 3.485 and 3.226; Mrs. Kočí is the first author of one publication. Moreover, she participated in other nine articles which are not included in the thesis.

The thesis significantly contributed to the research of spinal cord injury regeneration using hWJ-MSCs, which are being recently studied by other laboratories. The comparison of hWJ-MSCs with other stem cells often used for spinal cord regeneration showed their appropriate features and positive results from in vitro and in vivo studies. In addition, development of novel natural biomaterials for tissue regeneration is very important as they provide cells with environment and signals similar to normal tissue. The optimal source of decellularized natural tissue has not been found yet. The study explained positive features as well as limitations of this methodology which is valuable for further research.

Comments:

The list of abbreviations is very long. It is not necessary to abbreviate common words e.g. tissue engineering, or chemicals which are mentioned 2 or 3 times, i.e. polypyrrolle, methylprednisolone.

In Part 4, the section 8.3.1 contains evaluation of ECM hydrogel without cells while the section 8.3.2 contains hydrogels seeded with hWJ-MSC. However, the figure 32 describing hWJ-MSC – seeded hydrogel is included in the section 8.3.1. It is not clear whether non-seeded or cell-seeded ECM was used.

Questions:

In part 4.1.3 you have mentioned WST-1 assay which was repeated five times. Do you use 5 replicates or the whole experiment was repeated 5 times?

In section 4.1.6. regarding secretome analysis you have pooled three donors in a cell culture. It is not clear why in the results section 5.3.7 there are figures 14-17 containing samples with or without deviation. In a figure legend, it is not specified what deviation was used.

What time after spinal cord injury is the best for application of stem cells or biomaterials?

In part 3 you have described decreased healing of spinal cord lesion treated by ECM hydrogel and cysts formation after 4 and 8 weeks due to the fast degradation of hydrogel. Would it be better to decrease the degradation of hydrogels, or would you prefer other materials or their combination?

Conclusion: Zuzana Kočí showed the ability of independent and creative scientific work and published her results in international journal with impact factor. I recommend the thesis for the defense and for granting of PhD. title to Zuzana Kočí.

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