УДК 577.2 EXPLORING THE OSTEOGENIC EFFECT OF AMELOGENIN WITHIN A SPIDER SILK-BASED PROTEIN SCAFFOLD Mouhammad E. (ITMO), Deeb N. (ITMO), Mohamed A. (ITMO) Scientific Supervisor – PhD, Dr. Koshel E.I. (ITMO)

Introduction. A wide range of pathological conditions can be directly or indirectly responsible for skeletal tissue loss like osteoporosis. Traditional treatments such as bone grafts have limitations [1]. Thus, the development of biomimetic scaffolds for bone tissue regeneration is needed as an alternative treatment for these conditions. Spider silk has been suggested due to its mechanical properties, biocompatibility, low density, and biodegradation [2]. Amelogenin, an enamel matrix protein, exhibits osteogenic properties, promoting cell differentiation, and mineralization [1]. And some studies suggested its role as a signaling molecule promoting cell proliferation [3]. However, its application in biomaterials remains poorly studied. The purpose of this study is to study the osteogenic effect of amelogenin within the spider silk scaffold and evaluate its potential for bone regeneration.

Main part. Natural spider silk was collected from ITMO's insectarium and is processed to form scaffolds using the salt leaching method, while optimizing parameters such as solvents and temperature. The genetic construct of pET15b [*AMELX*] was kindly provided by Anastasia Egorova and then transformed into *E. coli* BL21, where protein expression was induced using 0.5 mM IPTG at 37° and its presence confirmed by the SDS-PAGE method. Further confirmation will be carried out using Western blotting. In parallel, work is underway to produce recombinant spider silk: *eADF4* (8x) has been successfully assembled, and the next step will be the expression and purification of the protein for its further processing into a scaffold. The use of the recombinant protein offers tunability and provides consistent quality and scalability.

Conclusion. In this study, methods for processing natural spider silk and forming scaffolds by salt leaching were developed, and recombinant amelogenin in *E. coli* was successfully obtained. The production of recombinant spider silk is also under development, which is an important step towards creating a fully synthetic biomaterial for bone regeneration. Future research will focus on incorporating amelogenin into the scaffold and conducting *in vitro* experiments to evaluate its biological activity.

List of References:

1. Branković, M., Zivic, F., Grujovic, N., Stojadinovic, I., Milenkovic, S., & Kotorcevic, N. (2024). Review of spider silk applications in Biomedical and tissue Engineering. Biomimetics, 9(3), 169.

2. Fiorino, A., Marturano, A., Placella, G., Staderini, E., Domingo, L. I., Cerulli, G. G., Tiribuzi, R., & Blasi, P. (2021). Amelogenin-Derived Peptides in bone Regeneration: A Systematic review. International Journal of Molecular Sciences, 22(17), 9224.

3. Huang, Y., Tanimoto, K., Tanne, Y., Kamiya, T., Kunimatsu, R., Michida, M., Yoshioka, M., Yoshimi, Y., Kato, Y., & Tanne, K. (2010). Effects of human full-length amelogenin on the proliferation of human mesenchymal stem cells derived from bone marrow. Cell and Tissue Research, 342(2), 205–212.