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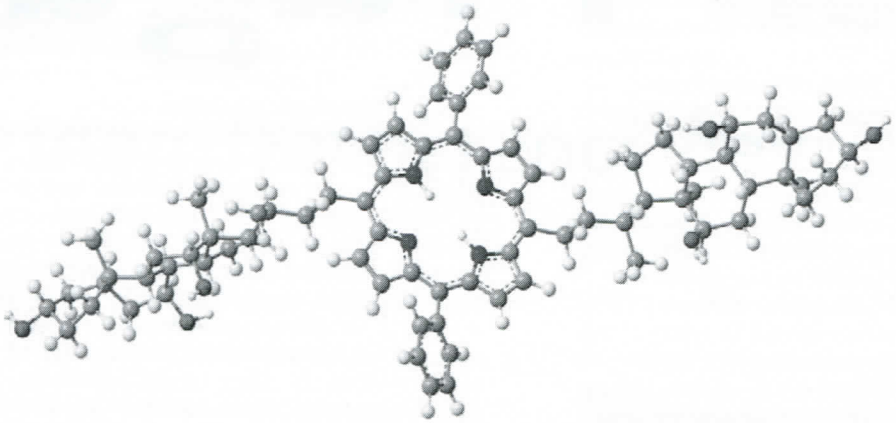

XXIst INTERDISCIPLINARY MEETING OF YOUNG RESEARCHERS AND STUDENTS IN THE FIELD OF CHEMISTRY, BIOCHEMISTRY, MOLECULAR BIOLOGY, AND BIOMATERIALS

May 16 – May 19, 2022

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GLYCOPROFILING OF FIXED LUNG TISSUE

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The human lungs weigh around 1 kg and their role are change of gases between airways and blood through alveoli. Within the lungs, there are about 300 million of alveoli with 90 m^2 of surface exchange area[1]. Formalin-fixed paraffin-embedded (FFPE) sample is a way for preservation of tissues for later research. FFPE tissues are often used in immunohistochemistry – tissues are mounted onto microscopic slide and incubated with specific antibodies[2]. Another type of fixation is alcohol fixation. Ethanol can denature proteins due to its ability to replace water in tissue[3]. Our aim was to develop protocols for protein isolation from fixed tissues into "mild" extraction buffers suitable for mass spectrometry (MS) and lectin-based microarray, that are used in glycomics[4], and apply them for glycoprofiling of proteins extracted from lung tissue samples. We tested commercially available kits for FFPE tissues, optimized our own protocols for protein isolation from ethanol fixed tissues, and evaluated their efficiency for subsequent glycomic/proteomic analyses. Our next step will be the glycoprofiling of fixed tissue samples of patients succumbed to the disease COVID-19. In case of lung tissue, there are some reports about histopathological features in which was mentioned diffuse alveolar damage as main histopathological finding[5]. We want to know if there are some changes in glycans of COVID-19 lungs.

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HARD TISSUES ON A CHIP – DEVELOPMENT OF A PLATFORM FOR GENERATING 3D BONE AND CARTILAGE CULTURES

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Animal models have been used for decades in biomedical research and drug development, representing an indispensable step in pre-clinical studies. Such models are a key for bringing substantial knowledge of the mechanisms initiating diseases as well as for testing their potential therapy. Nevertheless, recently extensive effort is undertaken to develop alternatives aiming to the reduction, refinement, and replacement (3Rs) of animal usage for research or for pre-clinical studies.

Novel methods of three-dimensional (3D) cell cultures became frequently utilised in many research fields due to their enhanced biological functions as compared to conventional two-dimensional (2D) cultures. 3D cell spheroids or organoids can replicate tissue functions, which enables their use both as *in vitro* models or as necessary intermediate step in tissue biofabrication approaches. The most promising tool for generating and analysis of 3D cell structure is a recent application of microfluidic chips. Microfluidic technology allows controlled conditions, automatization, reduced amount of reagents and cells, and mainly dynamic conditions for continuous perfusion of nutrients and removal of the metabolic waste of the cells.

In this study, we developed a polydimethylsiloxane (PDMS) microfluidic platform suitable for 3D bone and cartilaginous cells cultivation. The proper fabrication and preparation led to the development of homogenous 3D cell cultures of MC3T3-E1 osteoblast cells. Moreover, the system was used to study primary chondrocyte differentiation while using WNT inhibitors. These protocols were compared to standard cultivation methods such as hanging drops to reflect the advantages of microfluidic platforms. In addition, the PDMS microfluidic can be connected with the syringe pump to mimic *in vivo* perfusion conditions and increase the value of 3D cell cultivation.