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Fund “Nauka” Project № 19027 Resume – Competition-Based Session 2019:
“Bioprinting and morphological analysis of a 3D scaffold for biosynthetic implants”
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The generation of biosynthetic implants via 3d printing (bioprinting) is a method, in which controlled by a computer robot builds physical objects with accurate dimensional coordinates layer by layer with a composite hydrogel. The resulting object can be created with biopolymers, which are native to the extracellular matrix of the human tissues (collagen, elastin, hyaluronic acid etc.), acquired from animals and plants (alginate, cellulose, cytosine et cetera) or are synthesized artificially (polylactic acid, polycaprolactone, polyethylene, polyglycolic acid et cetera.) Those biopolymers can be combined with additives, such as growth factors (VEGF, IGF-1, bFGF, TGFbeta1 etc.) or high-performance materials (nano-silicates, graphene, titanium, carbon amongst others) into composite hydrogels, which changes the initial properties of the material substantially. The resulting hydrogel can be 3d printed into a complex three-dimensional structure with the characteristics of the extracellular matrix of the human tissue.

This study aims to develop a hydrogel, which consists of alginate, methylcellulose and laponite with increased osteogenic potential and optimal physio-chemical qualities for extrusion-based bioprinting or direct usage as bone-replacement substance. The hydrogel will be developed in two samples – a clean hydrogel and a hydrogel with additives (thrombocyte lysate, FGF). Both samples will be examined as a bioprinted model or as a media for cell culture, after inoculation with mesenchymal progenitor cells. The model will be incubated for 28 days, and then histological and cytological slides will be examined for newly synthesized elements of the extracellular matrix, the cell viability, quantity and morphology of the remaining living cells in the sample.

Achieved results:

A composite polysaccharide-nanosilicate hydrogel was developed and tested, based on alginate, methylcellulose and laponite as a bio ink for extrusion-based 3D printing.

In the 3D printing tests, the bio ink was capable of producing complex porous three dimensional scaffolds with excellent dimensional accuracy that did not deform further during solidification.

The 3D printed scaffolds were cut and mounted as histological slides (to simulate the processing of living tissues) and stained with standard histological protocols. After gel solidification, the polysaccharide fibers formed a porous mesh surrounding interconnected spaces with diameters in the range of less than 100 μm and up to 500 μm. The laponite was visualized as a flaky substance that was stained by the basic dyes and produced a background staining. The partial extraction of the laponite via fixation and washing decreased the background staining. This would allow the development of a protocol for histological and cytological analysis of bioprinted matrices from the proposed hydrogel composite inoculated with cells.

The 3D printed scaffolds were scanned with clinical CT scanner and dental CBCT scanner and the resulting images were compared with a CT scan of a dry human calcaneus. The mean density in HUs of the 3D printed scaffolds was close to the natural bone and can be further tuned by the concentration of the laponite and the porosity of the model, which makes the bio ink suitable as a tissue-equivalent material for imaging phantoms.

All those qualities are needed for the generation of complex multimodal phantoms that can simulate at the same time the biological and radiological properties of the trabecular bone tissue (as well as other tissues) under physiological and pathological conditions. Such devices could be an invaluable tool in the in vitro research of bone pathology and the dependence of the imaging properties on different pathological processes.

The 3D printed composite polysaccharide/ nanosilicate hydrogel matrices can be further supplemented with different growth factors and bioactive substances. Based on the conducted experiments we developed a hypothetical model for the bioprinting of osteogenic scaffold. The model includes enrichment of the composite bio ink with platelet lysate and FGF-2, inoculation of the scaffold with bone marrow derived MSCs, incubation in a bioreactor system with cell culturing media and analysis via histological and cytochemical methods. With this combination of materials and additives, we aim to promote osteogenic differentiation of the MSCs, which will open the possibility for the integration of this method in the clinical practice. The in vitro verification of this hypothetical model is pending.