3D BIOPRINTING – DETERMINATION OF HYDROGEL BIOINKS PROPERTIES FOR A PRINTER WITH DEDICATED CONSTRUCTION

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Introduction
Biofabrication technology is defined as the production of complex living and nonliving biological microtissues from raw materials such as living cells, molecules, extracellular matrices and biomaterials [1]. Biofabrication is an area of tissue engineering where many solutions can be developed using additive manufacturing (AM), also known as 3D printing (layer-by-layer material deposition). Three different biofabrication approaches can be distinguished – scaffold-based tissue engineering, scaffold-free tissue engineering or bioprinting [2]. Bioprinting is an emerging field that makes a revolutionary impact on medical sciences.
Current medical procedures aim to restore tissue function to patients with diseased or damaged tissues through tissue transplantation and implants. In tissue engineering scaffolds provide an optimum environment or housing for cell attachment and growth, tissue regeneration, fluid movement, and structural integrity. Adaptation of 3D printing into tissue engineering brings unique capabilities in rapid fabrication of tissue scaffolds with controlled porosity and internal architecture, tunable mechanical and structural properties.
Due to its individuation and controllable properties, the ability to print with patient's cells but also from reproducibility, high resolution in microscale production, in the future, bioprinting may develop into a potential tool for organ regeneration and have promising applications in tissue engineering.
Bioprinting allows for the fabrication of 3D tissue constructs with pre-programmed structures and geometries containing biomaterials and/or living cells (together termed the bioink) by synchronizing the bioink deposition/cross-linking with the motorized stage movement [3]. Various 3D printing methods can be used for bioprinting: inkjet (droplet), laser-assisted and extrusion-based [4]. The bioprinting modalities develop significantly, nevertheless their applications are limited by the lack of appropriate bioinks, which both need to meet the requirements for bioprinting and have the proper bioactivity of the different cell types. In order to generate tissue constructs with adequate mechanical strength, retain the tissue-matching mechanics, adjust gelation and stabilization to aid the bioprinting of structures with high shape fidelity, biocompatibility and, if necessary, biodegradability, the bioink should possess the desired physiochemical properties (mechanical, rheological, chemical) and biological characteristics [5].

Materials and Methods
In order to talk about an effective bioprinting, we need to choose the right printing material (bioink), AM technique and parameters. In our laboratory the new construction of 3D printer was designed. Such elements as nozzle length and diameter were firstly numerically simulated and then calibrated trough real prints adjusted the applied bioinks (sodium alginate/gelatin hydrogels). Single line and three dimensional lattice prints were performed in order to configure all movement of the printer and to achieve correctness of prints. Various solvents for hydrogels and different crosslinking method were checked. Rheological properties (rheometer), mechanical properties (Young's modulus and compressive strength) and chemical analysis (FTIR) of hydrogels were conducted. Biological response was evaluated using cells lines in order to check the influence of AM method onto the bioink. The biocompatibility was checked conducting the live/dead and XTT tests according to the ISO norm rules (extracts response and direct reaction to the material).

Results and Discussion
Tests showed that the parameters of custom 3D bioprinter have to be adjusted and tested in real use experiments in order to confirm the numerical simulations. Thanks to the simulation and test confirmation the optimal diameter of the nozzle of 0.32 mm was chosen (adjusted to the peristaltic pump and all the printer configuration). The modified extrusion-based printing method, which were applied in this custom 3D bioprinter, with adjusted temperature and cross-linking method, let obtain the highest possible level of reproduction of the correctness of the final prints. The verified hydrogels composition with various solvents and structure analysis showed that bioinks prepared on the basis of media are better materials for cells proliferation. Porous topography of the hydrogels limits the cells proliferation although they biocompatibility was confirmed.

Conclusions
The simulation allowed to set the output parameters and match them to bioink intended for direct printing usage. Development of the 3D bioprinter must be carried out in strictly controlled parameters for the bioink. The temperature, cross linking agent and method, pressure, composition influence the printability and affect compressive strength of the hydrogels. In the second stage all these factors have an impact on topography and thus on the cells viability.

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