Development of 3D Bioprinting Technology Using Modified Natural and Synthetic Hydrogels for Engineering Construction of Organs

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ABSTRACT

The shortage of human organs for transplantation is one of the critical and unresolved problems in clinical medicine. An average of 18 people die every day in the world due to a shortage of donor organs. Regenerative medicine is an alternative to donor organs. The use of digital imaging technologies using CT and 3D bioprinter (bioprinting) is the so-called biomedical application of layered three-dimensional printing in order to solve the problem of obtaining copies of living organs. In the present article, we studied general bioprinting technologies and testing of chitosan-based bioplastics and hydrogels using 3D printing and different fillability (porosity) of the material for biocompatibility using stem cells. Also, the "DoctorCT" software was used for 3D printing of substrates of biocompatible plastics.

Key Words: *hydrogel, bioprinting, 3D-printer, matrix, chitosan.*

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INTRODUCTION

Medical devices demonstrate the advancement of the medical field [1] and conjointly provide a larger contribution on lives. [2] Today, bioprinting and 3D printing is paving the way for key innovations in many areas, such as medicine, engineering, and education. Recent advances in science have made it possible to carry

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out 3D printing of biocompatible materials, cells, and their auxiliary components, with the further creation of fully functional living tissues on their basis. 3D bioprinting can be used in regenerative medicine for the transplantation of necessary tissues and organs. [3-8] The digital information from different scanners can be sent to the 3D printing facility without the risk of losing any accuracy. [9] Compared to 3D printing from inorganic materials, there are factors complicating the process in biopress, such as the choice of materials, cell types, their growth and differentiation factors, as well as technical difficulties associated with cell sensitivity and tissue formation. [10-12] To solve these problems, the interaction of technologies from the field of engineering, science of biomaterials, cell biology, physics, and medicine is necessary. The 3D bioprinting method is already used for growing and transplanting some tissues, including multilayer epithelium, bone, vascular grafts, heart, and cartilage structures. [5-8, 10-16] Other applications of three-dimensional bioprinting include modeling of tissues with high pharmacodynamic parameters for research purposes, as well as for the development of new drugs and toxicological analysis (http://bioprinting.strikingly.com).

3D printing gives doctors, young scientists, and students an affordable opportunity to design, visualize, enfold their ideas and test them in real conditions. The 3D printing method was first described in 1986 by Charles V. Hull. His method, called "stereolithography", thin layers of material, hardening under the action of ultraviolet rays, consistently form layers of a solid three-dimensional structure. [4, 10, 17-19] Later, this method was used to create composite forms for the cultivation of cells on three-dimensional carrier substrates, the so-called "scaffolds", from biological materials. The development of water-based, solvent-free systems made it possible to directly print scaffolds from biomaterials to use in transplantation, with or without stem cells. [3, 20, 21] There are several approaches to 3D bioprinting, including biomimicry, autonomous self-assembly, and buildup from fabric spheroids. [3]

Now scientists are developing the above approaches in order to create three-dimensional structures functioning in the human body that will have the biological and mechanical properties necessary for tissue healing and organ functioning. [22, 23] One of the most important problems is the need to adopt the technologies created for printing molten plastic and metal to print sensitive, living biological materials. [24, 25] At the same time, work is needed to resolve the issue of reproducing the complex microarchitecture of the components of the ECM (extracellular matrix) and numerous cell types in sufficient quantity to perform biological functions. [10]

MATERIAL AND METHODS

Used materials

The materials included Polylactide (PLA), sodium alginate, agarose, chitosan, sodium casein, sodium hydroxide. Due to its biocompatibility, polylactide is widely used in medicine, for the production of surgical yarns and pins, as well as in drug delivery systems. [21, 26] Polylactide corresponds to the concept of sustainable development since annually renewable natural resources are used for its synthesis. Bioprinting technologies from Cellink [27] are the most adopted in this field, allowing printing on a bioprinter with already prepared commercialized hydrogel (allowing to keep the printed form of the organ) with differentiated stem cells. That is why we also used Cellink hydrogel for evaluation and comparison with our hydrogels.

Used equipment

The experiment was carried out with the usage of 3D printer "3Dison Multi", [13] which is able to print as a polymer plastic, and biocompatible hydrogels, and pastes of different structures. “3Dison Multi” is one of the most accurate 3D printers to date (print layer thickness of 0.025 mm). Despite its small size, MULTI can print objects with large enough volumes (the print area is 270×148×180 mm) at fairly high speed (300 mm/s in fast mode). [28]

Creating a 3D reconstruction of organs (blueprints) for bioprinting

Medical image processing (DICOM), the 3D reconstruction of the organ models (blueprints) for bioprinting, and creating multi-layer tissue-engineering scaffolds using hydrogels for bioprinting were carried out. For bioprinting the organ engineering scaffolds, the natural and synthetic hydrogel of our own design has been applied. It consists of chitosan and synthetics and functions as a connecting structure and a base. Spheroids with cells made by a hanging drop technique, which were placed in it. There were also plans to exclude spheroids and put only the cells into the hydrogel to form an organ instead. After that the hydrogel was placed into cartridges (20 ml), it was inserted in the 3D printer. Then the bioprinting of the engineering organ scaffold, tissue or plant parts was carried out. After that, the tissue-engineering organ structure was placed into a bioreactor for the engineering scaffold to grow into the real organ. [27, 29, 30] As a result, a 3D bioprinter is just a part of the organ bioprinting line since it is necessary to supply it with a draft, and various materials. Then, the resulting organ model from the cells needs to be grown.

Used software for 3D printing substrates of biocompatible plastics

To create biocompatible and bioresorbable carrier matrices or substrates (matrices and scaffolds) were used

for tissue engineering of certain chemical composition, form, and internal (on nano, micro, and macro levels) architectonics using the technology of 3D layered printing (FDM). CreatorK [30] comes with the 3Dison 3D printer. This program edits 3D models and writes specific code for a 3D printer about the finished model, information about plastics, etc.

For the preparation of blueprints and forwarding them to 3D printing, we developed the software package «DoctorCT» allowing the creating three-dimensional models of internal organs (with blood vessels, channels, and neoplasms) on the basis of images of computer and magnetic resonance tomography.

In the course of the work, cells were cultivated on various substrates, such as plastic, hydrogel, and also on the previously listed substrates, only after specific treatment with chemical reagents (glutaraldehyde, polylysine, and collagen). They also immobilized suspension somatic cells on porous cellular structures (scaffolds), in order to preserve their viability in the immobilized state. Cells were cultured in specialized nutrient media (Eagle's medium, DMEM).

Based on DICOM files obtained from computer tomography (CT) of a particular patient or laboratory animal, the three-dimensional models are created (e.g., the model of tibia and its cartilage, thyroid). [20, 26] The resulting multi-layer model is placed into the program DoctorCT designed for bioprinting. Also, in addition to DoctorCT a program named CreatorK is used (15), which provides the option to edit blueprints (3D models) and improve the quality of models for 3D printing. After processing and reconstructing the 3D organ model in the program DoctorCT, the resulting file in STL format is sent to the 3D printer for printing. As a result, a tissue-engineering scaffold of the future organ is obtained, which is to be placed in a nutrient medium to prevent hypoxia. The next step is the placement of the tissue-engineering scaffold into the bioreactor for growing (ripening) in special environments. In the process of creating the software package, a freeware program, 3D Slicer 4.5.0.1 was used. [28, 31] Slicer 4.5.0.1 is a multi-platform free software kit with the open architecture that is used for visualization of medical images and calculations. Also, the program has a modular structure, so it is possible to add your own elements and delete the existing ones.

This method is based on a polymer (natural and synthetic hydrogel with cells), which is rapidly degraded (dissolve) and then, as a result, the cell material remains from the dissolved frame. The function of the frame is taken by the cells of already grown organ themselves. [27, 30]

RESULTS AND DISCUSSION

One of the most important trends in the development of modern tissue engineering and regenerative medicine is associated with the use of biodegradable matrix-carriers with open porosity of a certain scale, capable of supporting the migration, growth, and differentiation of stem cells. Such a matrix with stem cells introduced into it, having the necessary form and internal architectonics, should be gradually resorbed in the body medium, while the rate of biodegradation should not exceed the rate of tissue regeneration processes.

To prepare blueprints and output them to 3D printing, we have developed the software complex "DoctorCT", allowing to create three-dimensional models of internal organs (vessels, channels, and neoplasms) by image-based computer and magnetic resonance tomography. Three-dimensional models are created based on DICOM files obtained by CT of a particular patient or laboratory animal. We made a layered model that is placed in a personally developed program to print DoctorCT (Figure 1). We also used it as a Supplement to the print program CreatorK. [13]

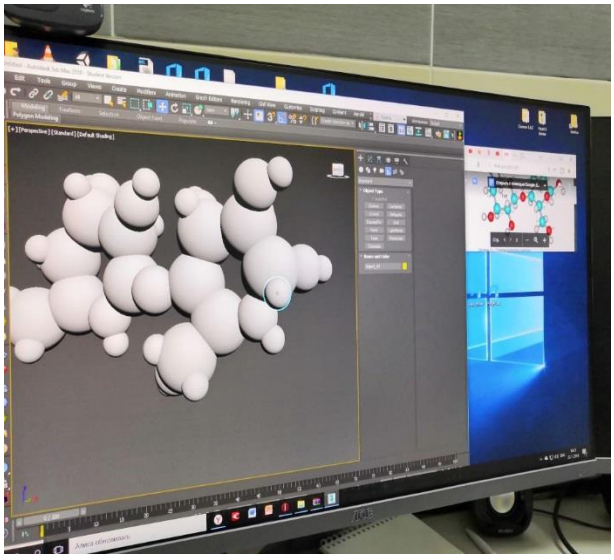


Figure 1: Bioprinting modeling.

After processing and reconstruction of 3D models on DoctorCT, we received a file in STL format, that was sent to a 3D printer or a professional bio-printer.

Material samples from biocompatible plastic (polymer), PLA, were used for the study. Scaffold matrices (substrates) were produced to immobilize suspension somatic cells on cellular structures in order to ensure optimal conditions for cell growth on the matrices.

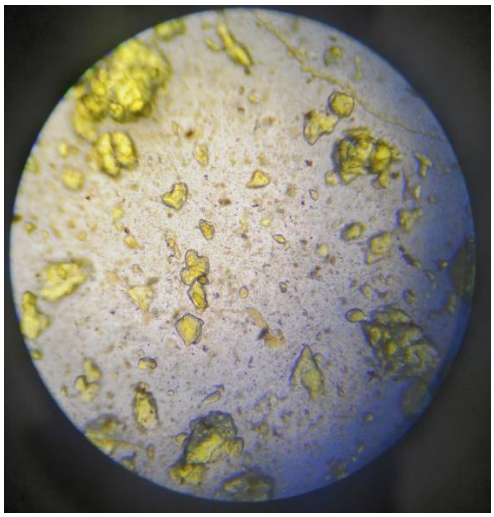
As a result of an experiment using a 3D printer (using 3D layered 3D printing technology (FDM)), PLA biopolymer was used to print and create four square-shaped substrates (scaffolds) with different open porosity (material density)

equal to 40%, 50%, 60% , 80% and a certain block size equal to 1 by 1.5 cm (Figure 2).

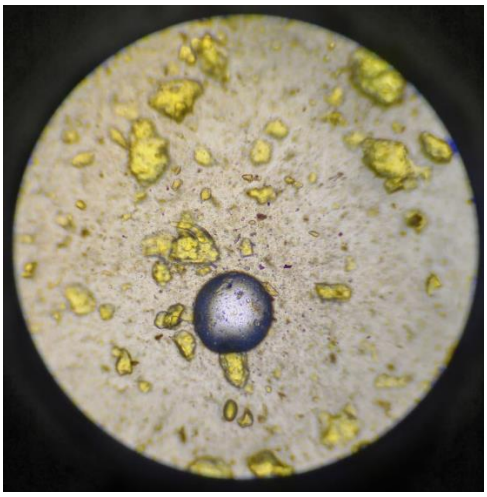
Figure 2: Hydrogel printing.

Models of substrates are capable of supporting the migration, growth, and differentiation of stem cells. Such a matrix with stem cells introduced into it, having the necessary shape and internal architectonics, should gradually sorb organism in a medium, wherein the biodegradation rate must not exceed the rate of tissue regeneration processes.

The prepared hydrogel was compared with the control commercial sample Cellink by microscopy. In General, according to the photos in Figure 3, we obtained a similar hydrogel, but with a strengthened matrix due to the use of chitosan (black inclusion in the photo). Thus, in the course of the experiment, a hydrogel comparable to a commercial analog was obtained.



Cellink hydrogel



Experimental hydrogel

Figure 3: Microphoto of printed models.

The result is a tissue-engineered design of the future organ, which is placed in a special environment to eliminate hypoxia. The next step is to place a tissue-engineered structure in a bioreactor for growing (maturing) under special conditions. Growing progress and the final results will be described later.

CONCLUSION

Bioprinting is an innovative technology that made a revolution in tissue engineering and medicine. Unlike animal cells, bioprinting with the usage of plant tissue cells is still in a poorly developed state. It has not yet been fully studied. One of the main advantages of plant cells is that all the tissues and organs can be obtained from totipotent meristematic cells. An application of bioprinting can help in obtaining plants with predetermined shapes and sizes and also serve for the mass production of the required tissues, rare medicinal plant species or even plant-based production of biomaterial for the industrial use. [5] The printing is possible both with meristematic (totipatent) cells of higher plants as well as with algal or even bacterial cells.

Practice potential

Bioprinting with plant materials has a high potential for its application with ornamental and agricultural purposes as well as for the biotechnological production. The basic concept of bioprinting with plant cells is the exact location of the cells in the proper place of the print area (2D) or volume (3D), which will allow them to differentiate under the influence of the environment composition, including phytohormones, in particular tissues and organs (i.e. the direct organogenesis). With the sufficient elaboration of plant bioprinting techniques with a predetermined shape, a lot of opportunities emerge in the design of decorative plants such as bonsai or flowers

in a test tube. It is also possible to assume that the plant tissue can be printed as a basis for the production of natural materials, for example, grown in the laboratory wooden boards or wooden blocks for construction purposes. This reduces deforestation and enables the creation of unique blocks of rare and valuable wood. Such blocks of wood can be printed with optional shapes and sizes without limitations in trunk thickness. [7]

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