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USE OF 3D-BIOPRINTING IN TISSUE ENGINEERING SCAFFOLD PRODUCTION

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Abstract: 3D bioprinting is the hi-tech approach in tissue engineering science. Applying of modern 3D bioprinting systems allows production of tissue-like structures that could be used in regenerative medicine. Such tissues or primitive organ constructs could be used for substituting the parts of damaged organs, or for changing of organs in whole. Biomaterials must fulfill specific requirement to be applied in regenerative medicine: biocompatibility, biodegradability and possessing appropriate mechanical properties. PEG and PCL are widely used today in tissue engineering. We optimized wide range of PCL, PEG solutions alone and in combination. For 3D bioprinting was used Inkredible+ device. Optimization of experimental parameters for creating a scaffold that can be used in tissue engineering is a demanding process. As it is the first stage of developing the blood vessel, there is a high importance of ensuring they are formed well and with the desired properties. This innovative approach may be promising for further fabrication of a blood vessel.

Keywords: 3D bioprinting, Scaffolds, Regenerative medicine

1. INTRODUCTION

Repair, recovery and replacement of parts of tissue in relations of the reappearance of regular physiological functions is the main goal of regenerative medicine and tissue engineering. In that sense, the use of many smart approaches is essential. Bioprinting is in very fast and active progress with a very wide assortment of applications [1]. Bioprinting can be defined as a specific application of the biomaterial layer-by-layer. This procedure is typically followed by specialized software allowing us the printing of 3D constructs in high resolution. Materials of natural origin, such as fibrinogen, collagen, gelatin; then chemically synthesized biocompatible materials such as various polymers and many others are used in this purpose. Depending on the application, these materials are often applied in mixtures with live cells, which accept the new environment, micro and macro structure forming tissue-like constructs. The application of bioprinting is very wide and covers the areas of tissue engineering and regenerative medicine, tissue and cancer investigation, transplantation, drug development and application. Some of the most important examples of the use of bioprinting in tissue engineering are the creation of bone-like structures [2], skin [3], cardiac tissue structures [4].

Tissue engineering mainly combines the use of highly porous biocompatible materials, which serve as a scaffold on which cells are grown for regeneration or replacement of tissue [5]. Scaffolds mimic the natural surroundings of the cell in which they can normally grow, proliferate and manifest their physiological functions. That's why scaffold chemistry is a very popular branch of science. Scaffolds must meet 3 basic requirements in order to be able to apply in regenerative medicine: biocompatibility, biodegradability and possessing appropriate mechanical properties. In other words, if a particular material possesses such traits that cells communicate with the material most naturally, i.e. no rejection observation, possesses the appropriate mechanical properties, and if it is subjected to completely uniform biodegradation and non-toxic absorption / secretion, this material can be considered as a good candidate for use [6].

Synthetic polymers are usually the first choice to many manufactures for fabrication of scaffolds. The reason lies in hydrophobic nature, consistent arrangement of atoms/molecules and firm texture that guarantee optimal hardness and density. However, from biological aspects there are severe limitation: low ability of a polymers to maintain contact with a liquid resulting. Up to 90% of human body consist of water and if synthetic polymers aren't able to form intermolecular connection there will be no cell attachment and furthermore migration, and proliferation. Fortunately, there are ways to overcome low bio-affinity of synthetic polymers. First, washing out scaffold after fabrication makes a possibility for reaching surface with small pores that will enable many biological processes of cells, starting with attachment. Second, instead of crystalline polymers (that have consistent arrangement of atoms/molecules), semi-crystalline polymer (mixture of both crystalline and amorphous polymers) can be used.

PCL and PEG are polymers that fulfill terms needed for tissue engineering: semi-crystalline, biodegradable, biocompatible, compatible with other synthetic polymers and soluble in water (7). Nevertheless, different ratio of PCL and PEG in hybrid scaffold results in distinctive physical and chemical properties that are crucial for potential application of scaffold in many areas of industry. For instance, PCL is widely used for bone tissue regeneration in combination with hydroxyapatite (HA) due to mechanical strength, good porosity and reabsorption of manufactured scaffold (8). Since the PCL has a low degree of surface wetting, entrenched interaction will be weak and cell adhesion will not be possible. Adding HA to PCL contributes to manufacturing of scaffolds with better osteogenic features (9). PCL limitations can be overcome with PEG too. PEG is soluble in water in all the proportions and higher hydrophilicity can increase flexibility and degradation rate of hybrid scaffolds. In addition, PCL has low bioactivity, but PCL/PEG scaffold will better stimulate biological response due to physical and biological improvements (higher hydrophilicity and porosity) (10).

This paper focuses on the optimization of physical and chemical parameters of the use of these two polymers and their combinations in order to obtain a scaffold for use in tissue engineering, primarily in the production of human blood vessel structure. Optimal physical and chemical properties are crucial for scaffold use because they ensure adequate structural support for cell adhesion and furthermore, development of tissue. Additionally, degradation rate of scaffold can be controlled by changing molecular weight, respectively by changing the ratio of substances.

2. EXPERIMENTAL

Layer-by-layer 3D bioprinting method is used for creation of scaffolds. The parameters of a wide range of PCL, PEG solutions alone and in combination are optimized. For 3D bioprinting we used Inkredible+ device (CellInk, Gothenburg, Sweden). The Inkredible+ 3D bioprinter is equipped with two printheads and UV LED system for crosslinking of bioprints (Fig. 1). The device is connected with PC and supported with appropriate software (printing program Slicer) for controlling of units and process of bioprinting. Three-dimensional CAD models was translated into coordinates and G-code was transferred to the computer. Prepared hydrogels were filled into a 2mL syringes and set on a 3D printer. After 3-axis centering of the syringe, the printing process was started. The XY position precision is 10 μm, while Z is at 2.5 μm. Layer resolution is 100 μm and all samples were printed in petri dish P100. Printer uses pressure to push hydrogel from the syringe and perform the printing process.

Using a variety of PCL and PEG ratio combinations in chloroform/DMF solutions (Table 1), we obtained a series of scaffolds that will be further examined for cell seeding, influence on cell viability

and biodegradability. By changing different parameters, such as the applied temperature, pressure, the solution ratio and concentrations, we obtained many scaffolds, ready to use for cell seeding.

Table 1. *Investigated ratios of polymers and solvents*

POLYMERS RATIO		SOLVENT RATIO	
PCL	PEG	CHLORO-FORM	DMF
60	40	1	1
65	35	1	2
70	30	2	1
75	25	1.5	1
80	20	1	1.5

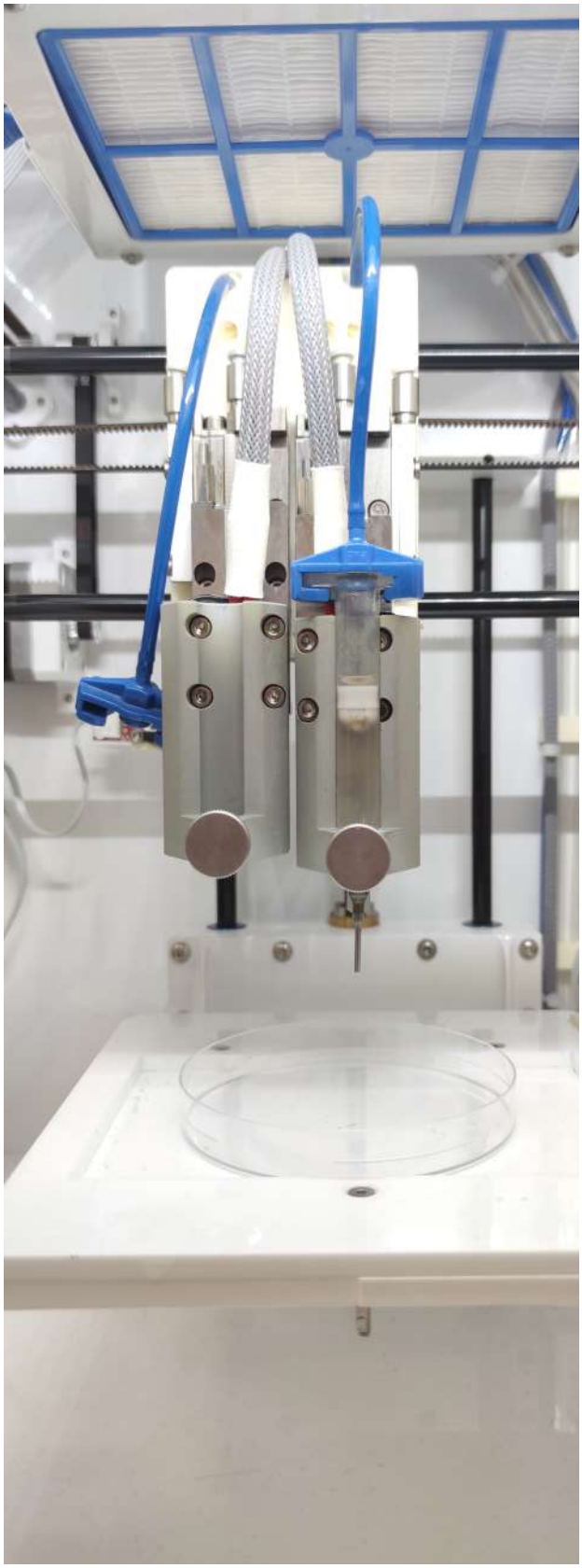

Figure. 1. *3D Printer CellInk Inkredible+.*

Constructs with shape of network are shown in Figure 2. Hydrogels with different concentration of PEG/PCL were used to fabricate two constructs. One construct of each hydrogel was exposed to a 365-nm UV light. After crosslinking, polymer networks showed more homogeneous surface without new defects and higher mechanical qualities.

Figure 2. *Printed scaffolds with different PEG and PCL concentrations 1-4 (A- constructs without cross-linking; B-constructs crosslinked with UV).*

3. DISCUSSION

Different polymer composition was prepared and wide range of different scaffolds was manufactured by adjusting 3D printing parameters (temperature, pressure). A large number of preliminary experiments was performed in order to make relevant observations. Final structure of scaffolds was evaluated in order to find what chemical ratio and procedure parameters provide scaffold with the best properties that can be further include in tissue regeneration. Different ratio of compounds and solvents contributed to changes in density that required adjustment of process parameters. Different manufacturing steps changed chemical and physical properties of scaffolds.

Ratio of substances had huge effect on molecular weight, density and porosity of the final scaffolds. By adjusting the printing speed, consistent structure can be obtained. Increasing the speed often caused clogged print heads or scaffolds with incoherent surface. Mixtures with higher density demanded increased temperature and pressure for better scaffold properties.

Park et al. (11) conducted similar study and observed that PCL/PEG scaffolds with small pores in surface can promote bone tissue regeneration by increasing migration and proliferation. In addition, they observed decreasing of mechanical strength with higher PEG concentration, but mechanical properties of construct were adequate for bone tissue regeneration (PCL/PEG that was investigated was 70/30). Study conducted by *Salehi et al.* (12) noticed that higher hydrophilicity improves cellular processes, including proliferation and adhesion. The results observed in our study are consistent with mentioned similar studies, but the further experiments that include *in vitro* analysis (stem cells could be most appropriate) are necessary for higher involvement of this promising scaffolds in regenerative medicine.

4. CONCLUSIONS

Optimization of many experimental parameters for creating a scaffold that can be used in tissue engineering is a demanding process. Scaffolds made with desired properties will be used for seeding of endothelial and smooth muscle cells to investigate their suitability for the creation of a blood vessel *in vitro*.

With a suitable scaffold, cell seeding can take place with aims towards developing an artificial blood vessel. Provided that the cells attach to the scaffold, the new model has aims to be used as a blood vessel within the body. This can only be done once animal and clinical trials have successfully been completed. *In vivo* testing stages are important to determine

the rate at which the scaffold biodegrades and the type of immune response the implantation has. The degradation rate and inflammatory response essentially comes down to the type of materials used in the scaffolds. As it is the first stage of developing the vessel, there is a high importance of ensuring they are formed well and with the desired properties. Our scaffolds show potential for fabricating blood vessels for biomedical application. With this results we have a foundation for the optimization of 3D printed vascular scaffolds in the future.

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УПОТРЕБА 3Д-БИОШТАМПЕ У ТКИВНОМ ИНЖЕЊЕРИНГУ ПРОДУКЦИЈЕ СКАФОЛДА

Сажетак: 3Д штампа је високотехнолошки приступ у науци о ткивном инжењерингу. Примена савремених сисстема 3Д штампе омогућава производњу структура налик ткиву које би се могле користити у регенеративној медицини. Овако добијена ткива или примитивни конструкти органа се могу користити за замену делова оштећених органа или за промену органа у целини. Биоматеријали морају испунити одређење захтеве да би били примењени у регенеративној медицини: биокомпатибилност, биодеградабилност и поседовање одговарајућих механичких особина. ПЕГ и ПЦЛ се широко примењују у ткивном инжењерингу. Оптимизовали смо широк ранг раствора ПЦЛ и ПЕГ појединачно и у комбинацији. За 3Д штампу је коришћен уређај Inkredible+. Оптимизација експерименталних параметара за креирање скафолда који се може применити у ткивном инжењерингу је захтеван процес. С обзиром да је ово прва фаза развоја крвног суда, изузетно је важно осигурати се да су произведени добро и са жељеним карактеристикама. Овај иновативни приступ може бити обећавајући за будућу производну крвног суда.

Кључне ријечи: 3Д штампа, скафолди, регенеративна медицина

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