Study of the processes of three-dimensional printing of caprolactone copolymers with methylphosphate groups

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Abstract. Reconstruction of the damaged or missed organs or skeleton fragments is an ambitious target for regenerative medicine. Tissue engineering construction (TEC) comprising three-dimensional bioresorbable scaffold and living cells can become native tissue substitute. Scaffold should correspond to strict requirements. Generally, its physical-mechanical and biochemical characteristics should be comparable with tissue to be replaced. Besides, resorption rate of scaffold material should correlate with the tissue regeneration rate in each case. Thus, the effect of scaffold manufacturing methods on its properties is an important point in TEC design and fabrication. At present study, thermo-extrusion 3D printing of bioresorbable scaffolds based on polycaprolactone and its copolymer with polyethylene glycol and methylphosphate groups, synthesized by novel chemical route, was investigated. Scaffold surface morphology and its internal structures were studied by optical and scanning electron microscopy. The effect of thermal-induced degradation on polymers properties at various 3D printing processing parameters was analysed by gel-permeation chromatography, differential scanning calorimetry and mechanical testing. It was shown that optimizing experimental parameters the developed methods of thermo-extrusion 3D printing enable effective polymer scaffolds fabrication in accordance with their 3D computer model without significant changes of physicochemical properties of initial materials.

1. Introduction

Tissue engineering is a promising methodology that allows to create tissues and organs in vitro as well as to use biocompatible materials and cells to repair damaged or inherently abnormal tissues [1]. The combination of a bioresorbable scaffold, living cells and biologically active substances is called tissue-engineering construction (TEC) [2]. Scaffold is a three-dimensional substrate for cells, which should provide attachment, proliferation, neovascularization, possibility of nutrients penetration, induce tissue formation and even promote cell differentiation [3]. Thus, important aspects are both the choice of material for the scaffold and the methods of its manufacturing.

The scaffold material should be non-toxic and non-immunogenic. Its mechanical properties should be comparable with the characteristics of the native tissue. In addition, an important property of the
scaffold material is biodegradability [4]. Besides, the resorption rate of the scaffold material should correlate with the rate of tissue regeneration in each case. Materials used for the scaffold fabrication can have either artificial or natural origin [5]. Synthetic polymers demonstrate promising features since their physicochemical and biochemical properties, and as a result, their mechanical properties and degradation rate, can be specially optimized for a particular application [6]. Nowadays, aliphatic polyesters are often chosen as the polymer base for TECs. Poly-ε-caprolactones and its copolymers are one of the main representatives of this class [7].

Nowadays, there is a huge variety of methods of polymer scaffolds fabrication for TECs. In particular, additive manufacturing (AM) technologies which based on layer-by-layer fabrication of solid copies of 3D computer models [8]. These methods are widely spread as they allow to produce complex custom-designed constructions of external shape and internal pore geometry. The main types of AM techniques currently used to fabricate polymer or composite structures are: different types of inkjet printing of polymer powder [9], fused deposition modeling (FDM) by molten polymer filament [10], hydrogel extrusion printing followed by chemical or UV solidifying of structures [11], laser stereolithography [12] and selective laser sintering [13]. Presumably, the most promising 3D printing method with thermoplastic polycaprolactones (and composites based on it) is a modified version of FDM. It allows using a wide range of thermoplastic polymer melts with high viscosity. Probability of thermal-induced degradation and initial molecular weight changes of thermolabile biocompatible polymers decreases in comparison with classical FDM [14]. One of the important issues is the effect of the thermo-extrusion printing process on physicochemical and mechanical properties of the initial materials and, accordingly, the spatial resolution and properties of the fabricated scaffolds.

Thus, the aims of the study were investigation of thermo-extrusion printing capability for biodegradable scaffolds made of new copolymers and analysis of the effect of printing process parameters on physical-chemical and mechanical properties of initial polymers and scaffolds made of them.

Materials and methods

1.1. Materials
Poly-ε-caprolactone (Mw = 39.4 kDa) as well as copolymer of caprolactone with polyethylene glycol and methylphosphate groups (Mw = 42.9 kDa) were synthesized by novel chemical route enabling polycaprolactone and its copolymer fabrication avoiding any presence of toxic tin octoate catalyst. Briefly, Poly-ε-caprolactone (PCL) synthesis was carried out with polymerization of ε-caprolactone and BnO-MgBHT as a catalyst (in ratio 200:1). Synthesis of copolymer PEG 2000 1.2% - caprolactone 86.6% - methylphosphate 12.2% (PEG-PCL-McPh) was carried out with sequential polymerization of ε-caprolactone, methylphosphate, PEG and BnO-MgBHT as a catalyst (in ratio 28:12:1) [15].

1.2. 3D-printing
The finding of optimal parameters of thermo-extrusion printing was carried out using our custom-designed and specially developed 3D printer (figure 1). It should be mentioned that in comparison with classical FDM printers the melting zone of our system extruder is limited to a small (ca. 10 mm3) volume located just before the nozzle. Thus, the negative effect of prolonged thermal stress on polymers can be significantly reduced. The process of thermo-extrusion 3D printing was carried out using following procedure. The grounded polymer powder is pressed and subsequently displaced into the heater zone (8) by the piston of the extruder (10). There powder turns into a melt which then is pushed through the nozzle (i.d. ca. 400 µm) of the extruder (7), forming a polymer filament. The working table (6) is move by high precision stepping motors (3, 5), similar to the extruder which is moved by motor (9). That allows to lay down the extruded polymer fiber in accordance with 3D computer model. Optimal parameters of 3D printing by PCL and its copolymer melts were determined using this technique. In addition, testing scaffolds were fabricated to study the thermal-induced degradation of polymers caused by printing process, which in turn effect on the spatial resolution of obtained structures.
1.3. Gel-permeation chromatography (GPC)
The analysis of the molecular weight distribution of PCL and PEG-PCL-MePh (initial polymers and polymers subjected to thermal degradation during 3D printing) was conducted through gel-permeation chromatography on GPC/SEC "Stayer" (AO Akvilon, Russia) with the refractive index detector and column Phenogel 5 mm 10^4 A (300 7.80 mm 5 mm). Tetrahydrofuran was used as an eluent at 40°C and a flow rate of 1 mL/min. The recorded data was automatically processed by the program “MultiChrom 1.6”. The chromatograph was calibrated with polystyrene standards.

1.4. Optical and Scanning Electron Microscopy (SEM)
Surface morphology, external shape and internal geometry of fabricated polymer scaffolds were observed by optical microscopy (Bresser Advance ICD, Germany) and scanning electron microscopy (LEO 1450, Carl Zeiss, Germany). For SEM characterization specimens were coated with a thin (~ 0.05 - 0.1 µm) gold film by sputtering at room temperature.

1.5. Differential scanning calorimetry (DSC)
Thermal effects of phase transitions during the heating of polymers were investigated using a differential scanning calorimetry (Lineis, Germany). The polymer specimens (30 mg) were placed in alumina crucibles with a heating range of 20 to 300°C at a rate of 10°C/min in nitrogen atmosphere. The melting temperature and melting enthalpy of the PCL and its copolymer were determined from the first heating scan.

1.6. Mechanical tests
Mechanical properties of polymer specimens (made of PCL and PEG-PCL-MePh) were analyzed by using mechanical tester Shimadzu EZTest (Japan). 3-point bend test and tension test were carried out. Solid bar specimens (20*5*1 mm3) were fabricated by 3D printing for 3-point bend test. The rate of printing and of the polymer melt feeding were chosen to obtain homogeneous sample with good spatial resolution. For bend testing, a rectangular cross-section sample was put on steel supports. Then it was bent in the center between the supports at a constant speed. The experiment was carried out until sample destruction or a predetermined value of relative deformation/deflection consummation. The stress-strain curves were recorded at a bending speed of 1 mm/min, which is optimal for elastic modulus determination. Dogbone specimens (20*5*1 mm3) were fabricated by 3D printing for tension test. For tension testing, specimen was stretched along its main axis at a constant rate. The stress-strain curves
were recorded at a bending rate of 15 mm/min. Load, deflection and extension were measured constantly.

Results and discussion
Numerous experiments were carried out to determine optimal parameters (temperature of the polymer melt in the extruder nozzle zone, the distance between the nozzle and the working table surface, rate of the polymer melt extrusion, extruder scanning rate, etc.) of 3D printing with melts of PCL and its copolymer with PEG and methylphosphonate groups.

Extruder motion rates (X and Y-axis), rate of the polymer melt extrusion and temperatures of 3D printing were chosen taking the following observations into account:

- the working temperature should be higher than the melting temperature of the polymer in order to ensure the formation of homogeneous polymer fiber, homogeneous in structure and diameter;
- the temperature of the polymer melt should be high enough so that the polymer fiber has steady adhesion to both the working table or the previously printed layer;
- the working temperature of printing should be minimal in order to avoid uncontrollable molecular weight distribution changes and in turn changes of physicochemical properties;
- polymer fiber should cool down as fast as possible in order to avoid polymer melt overspreading and subsequent spatial resolution decrease.

In order to fulfill the last requirement, polymer fiber was cooled straight after extrusion by ventilator airflow. However, as it was demonstrated in our previous study [16], the complete solidification of the printed layer usually takes about 30 seconds, which corresponded to a cooling rate of about 20°C/min.

Scaffolds (multilayer lattice discs) with diameter of 5 mm (figure 2) and 25 mm were fabricated by using our thermo-extrusion 3D printer.

![Figure 2. Optical image of PEG-PCL-MePh scaffold (5 mm diameter) fabricated by using thermo-extrusion 3D printer.](image)

The scaffolds surface morphologies and their cross-section structures are shown on SEM images below (figure 3).

The melting temperatures of PCL (69°C) and its copolymer (63°C) were determined by DSC analysis. These temperatures are noticeably lower than the working temperatures of the developed 3D printing process (97°C and 120°C, respectively). However, in consequence of preliminary experiments, necessity of using heightened temperatures was determined. Optimal printing rate (6.5 mm/s for PCL and 4.8 mm/s for PEG-PCL-MePh) also was determined during experiments.
Fabricated polymer scaffolds were designed as a lattice discs with filling of about 40% (figure 2). They had 5 layers of polymer fibers with thickness of ca. 250-300 µm. 3D printing spatial resolution changed from 250 µm to 350-400 µm with PCL and PEG-PCL-MePh, respectively. Probably, this result relates to more apparent effect of the polymer macromolecules orientational relaxation. This effect arises during the process of polymer flow formation, when polymer melt is extruded through nozzle. Generally, scaffolds made of PCL and PEG-PCL-MePh both showed adequate spatial resolution (figure 3 - A1, A2). Overall PCL fiber had smooth and homogenous surface (figure 3 - D1). In some places faint spherulites with a diameter of 15 µm could be observed. Similarly spherulites with a diameter of 10 µm could be observed on the surface of PEG-PCL-MePh fiber (figure 3 – D2). Obtained specific microstructure related to fast heating of polymer powder and rapid extrusion of polymer melt. In this case, polymer powder particles only underwent partly melting in the area of nozzle.

Results of GPC analysis of an average molecular weight (M_w) and polydispersity index (D_m) of investigated polymers before and after thermo-extrusion 3D printing are shown at Table 1.

|                  | M_w, kDa | D_m | T_m, °C | ΔH, J·g⁻¹ |
|------------------|----------|-----|---------|------------|
| Initial PCL      | 73.3     | 1.62| 69.3    | -73.82     |
| PCL after 3D printing | 72.9     | 1.68| 68.9    | -51.36     |
| Initial PEG-PCL-MePh | 31.1     | 1.3 | 63.1    | -47.46     |
| PEG-PCL-MePh after 3D printing | 30.1     | 1.35| 65.9    | -32.51     |
GPC analysis indicated that the average molecular weight loss percent for PCL specimens caused by thermal degradation (printing working temperature 97 °C) was only 0.6%. The average molecular weight loss percent for specimens made of PEG-PCL-MePh was 3.45% after thermo-extrusion printing (printing working temperature 120°C). We suppose that in the second case the weight loss was bigger due to higher working temperature.

To measure melting temperature ($T_m$) and melting enthalpy ($\Delta H$) (see Table 1) of investigated polymers DSC analysis was performed. Thermograms of polymer specimens before and after thermo-extrusion 3D printing were shown in figure 4. Since melting enthalpy is directly proportional to polymer crystallinity, then it can be concluded that for both polymers crystallinity decreases for about 30% after one-time heating.

The results of mechanical (tension) test (see figure 5) and 3-point bend test of polymer specimens are shown in Table 2.

Table 2. Mechanical characteristics of polymer specimens.

|          | Tensile strength, N/mm² | Break strain, % | Elastic modulus, MPa |
|----------|--------------------------|-----------------|----------------------|
| PCL      | 26                       | 842.5           | 300                  |
| PEG-PCL-MePh | 18                     | 455.4           | 195                  |

Figure 4. DSC thermograms of: PCL specimens before (a) and after (b) thermo-extrusion 3D printing process; PEG-PCL-MePh specimens before (c) and after (d) 3D printing process.
Conclusions
A new thermo-extrusion 3D printing process of bioresorbable scaffolds based on polycaprolactone and its copolymer with polyethylene glycol and methylphosphate groups, synthesized by novel chemical route, was developed and studied.
Ultimately, the GPC analysis demonstrated that the average molecular weight loss of polymers used for 3D thermo-extrusion printing is insignificant (less than 3.5%). DSC analysis showed the polymer crystallinity decreased for 30% after one-time heating at working temperatures. The results of mechanical tests analysis indicated that PCL specimen are more elastic, but less mechanically strong than specimen made of caprolactone copolymer with polyethylene glycol and methylphosphate groups.
Based on characteristic evaluation of manufactured scaffolds, we showed that both polymers are promising for further application in TEC scaffold manufacturing. In summary, the developed methods of 3D printing enable effective fabrication of bioresorbable scaffolds in accordance with their 3D computer model without significant change of physicochemical properties of initial polymers.

Acknowledgments
This work was supported by the Ministry of Science and Higher Education within the State assignment FSRC «Crystallography and Photonics» RAS.

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