Mechanical and biocompatible properties of the poly(lactide-co-glycolide) matrices produced by antisolvent 3D printing

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Abstract. Three-dimensional scaffolds were made from a solution of poly(lactide-co-glycolide) mixed with tetraglycol using antisolvent 3D printing. The elastic properties and the structure of the obtained matrices were studied. MTT-test and staining with PKH-26, Calcein-AM, DAPI with subsequent fluorescence microscopy were used to study biological properties. The three-dimensional scaffolds had good mechanical properties. Young's modulus value was 18±2 MPa, tensile strength was 0.43±0.05 MPa. The relative survival rate of cells after the first day was 99.58±2.28%, on the 14th day – 98.14±2.22%. The structure of the scaffold promoted cell adhesion and spreading on its surface. The poly(lactide-co-glycolide) matrices produced by antisolvent printing have high porosity, biocompatibility and good mechanical properties. It is allowed to use them in the future as a basis for personalized constructions for the replacement of extensive bone defects.

1 Introduction

The number of conditions associated with the formation of extensive bone defects, particularly of the skull and jaw, is increasing [1,2]. In this context, the development of personalised biocompatible constructions for the restoration of bone defects and the growth of own bone tissue becomes relevant. Reconstructive implants made of artificial materials (titanium, steel, polymers) are widely used to replace extensive defects. However, they have disadvantages, the most important being their lack of osteoinductive properties and their inability to be replaced by bone tissue [3]. In this context, the development of new personalised constructs that can be replaced by bone tissue and act as carriers for bioactive substances is relevant.

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Synthetic bioresorbable polymers serve as a promising basis for such structures. Poly(lactide-co-glycolide) (PLG) is one of the most popular and studied lactic acid copolymers. Variations in volume structure, isomer species and polymer chain length allow the mechanical properties and biodegradation rate of the polymer to be varied [4]. PLG is approved for medical applications and is used to make sutures, screws and plates. There are scaffolds based on it for tissue engineering structures and delivery of bioactive molecules [5].
We have previously improved an antisolvent 3D printing method from a solution of aliphatic poly(lactide-co-glycolide) copolimers in the non-toxic solvent tetraglycol [6]. It allows the creation of scaffolds with a radially oriented interpenetrating pore microstructure and high porosity. Such matrices have some advantages over traditional bone graft materials, which have low porosity, long biodegradation period and prevention of replacement by newly formed bone tissue.
The aim of this article is to study the physical, mechanical and biocompatible properties of matrices produced by antisolvent 3D printing from solutions of poly(lactide-co-glycolide) in tetraglycol.

2 Materials and methods

2.1 Polymer solution

The material used for printing individual fiber or scaffold was poly(lactic-co-glycolic) acid (Corbion, Neteherlands) was used to prepare 10 wt.% solution in the glycofurol (Tetraglycol BioXtra, Sigma-Aldrich, USA). 1g of the PDLG7507 were mixed with the 9g of glucofurol in 20ml glass during 48h at 45ºC using magnetic stirrer (FlatSpin, PCR).

2.2 3D printing

In order to produce a PDLG7507 fiber and scaffolds a specially designed 3D-printer (RFRC “Crystallography and Phonics RAS”, Russia) with syringe dosing system was used. To print the scaffold the PLGA solution was injected according the digital 3D model through the 160 μm nozzle in the water precipitation bath. The temperature condition was maintenance at 25ºC with the 3D-printer built-in thermal controller.
Each scaffold was stored in the 37ºC distilled water during 24h, then washed with the 95% ethanol and dried in room temperature.

2.3 Mechanical tests

The study of the mechanical properties of the used material was carried out on the printed PLG film samples with the size of 20 × 5 × 0.2 mm. The analysis of the mechanical properties of the samples was carried out on the EZ-Test EZ-SX testing machine (Shimadzu, Japan) under the control of the TRAPEZIUM software. The tensile test was performed at a speed of 1 mm/min. The values of the Young's modulus of the samples were calculated from the linear sections of the gained stress-strain curves.

2.4 Microscopy

The macrostructure of the scaffolds was examined with a Bresser Advance stereomicroscope (Bresser, USA) in incident light using ToUp camera software (Touptech, Taiwan, PCR).

The microstructure of the samples was examined using a Phenom ProX scanning electron microscope (Phenom, Netherlands). The samples were fixed on the conductive carbon tape and tested under 15 kV acceleration voltage.

2.5 *In vitro* study

To evaluate the biological properties of the matrices characterised cultures of rat adipose tissue multipotential mesenchymal stromal cells (MSC) were used. Cells were precipitated by centrifugation for 10 min at 1200 rpm at 15°C and seeded in Petri dishes in DMEM/F12 growth medium ("Paneco", Russia) containing 10% fetal bovine serum (FBS; "PAA Laboratories", USA), 0.584 mg/ml L-glutamine ("Paneco", Russia), 5000 units/ml penicillin ("Paneco", Russia) and 5000 μg/ml streptomycin ("Paneco", Russia) at 37°C and 5% CO2. Cytocompatibility was assessed by the MTT 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. Cells were stained with fluorescent dyes on days 1 and 14 with vital dye PKH-26 (red fluorescent cell linker kit, Sigma, USA). Calcein AM dye (Biotium, USA) was used to detect live cells and DAPI dye (4,6-diamidino-2-phenylindole, Biotium, USA) to detect apoptotic cells. Fluorescence microscopy was performed using a Lionheart FX automated microscope (Agilent BioTek, USA). Cytocompatibility was evaluated after 1 and 14 days.

2.6 Statistical analysis

Graphing and statistical processing were performed using SigmaPlot 12.0 (USA). Data are presented as mean and standard deviation (M±SD). Depending on the Shapiro-Wilk normality test, intergroup comparisons were performed using Student's t-test or Mann-Whitney U-test. Differences were considered statistically significant when the probability was less than 5% ($p < 0.05$). Values that differed from the control values were marked with an asterisk (*) according to the recommendations of the American Physiological Association (APA).

3 Results and discussion

3.1 Mechanical properties of PLG films

Yong's modulus of the tested film samples was in the range of 18±2 MPa, tensile strength - 0.43±0.05 MPa and elongation – 8±2%. The obtained data show that the strength of films printed by the 3D printing method is suitable for implantation purposes. The overall view of the 3D printed scaffold and regions of interest are shown in Fig.1. The mechanical properties of poly(lactide-co-glycolide) matrices were comparable with the previously obtained and investigated matrices from polylactide solutions: their elastic modulus depending on porosity (from 92 to 96%) and molecular weight varied from 1 to 23 MPa [7].

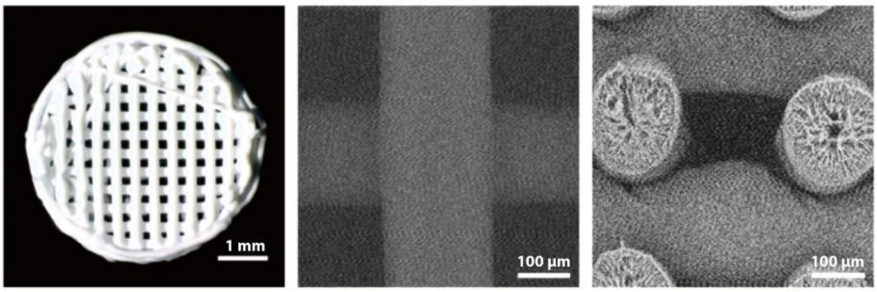

Fig. 1. PLG scaffold manufactured by using the antisolvent 3D-printing method. Overall view, fiber overcrossing and fiber cut section.

The fibre sample has a unisotropic structure with smooth outer surfaces, one of which is no thicker than 1 µm. The inner structure of the fibre was formed by radial finger-like pores with an average length of about 30 µm. It was found that there is a tendency to form an axial void in the center of the antisolvent process casted fiber. The total porosity of the fibre calculated from the cross-section images was higher than 80%.
The use of 3D printing makes it possible to produce poly(lactide-co-glycolide) scaffolds with a complex structure. Their strength properties, including Young's modulus, can match those of mandibular trabecular bone (Young's modulus - from 6.9 to 199.5 MPa) and exceed those of cartilage tissue (Young's modulus - from 0.3 to 20 MPa) [8,9]. This suggests that our 3D printed scaffolds will have mechanical properties that are optimal for the restoration of extensive bone defects. Poly(lactide-co-glycolide) has a significantly lower modulus of elasticity than titanium and its alloys, which are traditionally used for prostheses [10]. However, this avoids the complications experienced by patients with titanium substitutes: the elements cutting through the skin or oral mucosa, deformation of the prosthesis and resorption of native bone tissue in the area of the fixation screws due to the difference in strength properties between the titanium substitutes and the surrounding tissues [11].

3.2 Biocompatibility

The 3D poly(lactide-co-glycolide) scaffold had high biocompatibility and no cytotoxic effect on rat MSC. The relative cell survival rate was 99 ± 2% at day 1 and 98 ± 2% at day 14. There were no statistically significant differences in the relative cell viability values at day 1 and day 14 compared to the control (Fig. 2).

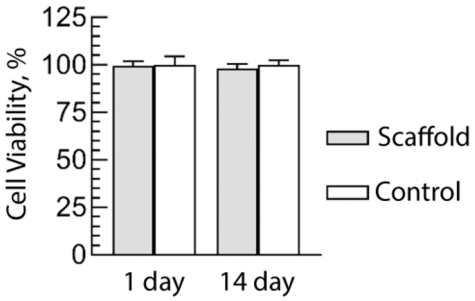

Fig. 2. Cytocompatibility of 3D poly(lactide-co-glycolide) matrices, MTT test

Fluorescence microscopy has also showed that the scaffold has highly cytocompatibility. After 1 day, cells stained with DAPI were not observed. After 14 days there were a significant increase in cell density. Most of cells were stained with Calcein AM. The number of cells stained with DAPI corresponded to the number of dead cells in the control cultures (Fig. 3). The structure of the scaffold favoured the adhesion and spreading of cells on its surface.

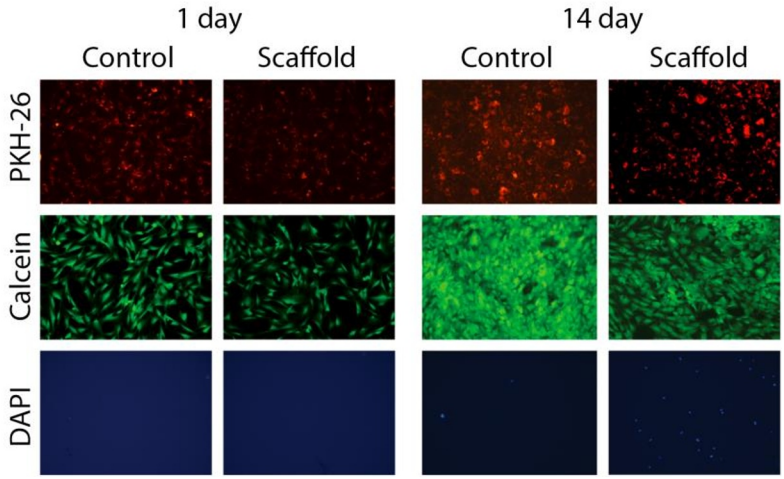

Fig. 3. Cytocompatibility evaluation of 3D poly(lactide-co-glycolide) matrices on rat AT MSC. Cells stained with PKH-26 (red), Calcein AM (green, live cells), DAPI (blue, dead cells). Fluorescence microscopy. Magnification 10x.

Cytocompatibility and cell adhesion of obtained scaffolds were comparable with the matrices made from L-isomers of lactide [7]. But compared to the studied matrices, the 3D scaffolds are produced using antisolvent printing with no excessive heating at the edges of the material. In our opinion, this will allow to impregnate an optimal amount of growth factors into the scaffold [12].

4 Conclusions

The method of antisolvent 3D printing from a solution of aliphatic copolimers of poly(lactide-co-glycolide) in a non-toxic tetraglycol solvent allows the fabrication of biocompatible 3D matrices with high porosity and complex structure. The proposed method can be used to obtain personalised constructs to replace extensive bone defects.

5 Acknowledgements

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