Bioresorbable polymer matrices impregnated with BMP-2

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Abstract. Bioresorbable matrices from poly(lactic-co-glycolic acid) containing therapeutically significant concentrations of bone morphogenetic protein 2 (BMP-2) was formed by supercritical fluid encapsulation and surface-selective laser sintering. Biocompatibility of these matrices and the kinetics of BMP-2 release from their volume into physiological saline was investigated. It was shown experimentally that the release of BMP-2 was almost uniform for 15 days. The subsequent use of laser sintering contributed to a longer retention of BMP-2. High cytocompatibility of the obtained matrices with cultures of mesenchymal stem cells was shown in vitro.

1. Introduction
Three-dimensional polymeric matrices with encapsulated bioactive substances are widely used today in regenerative medicine. One of the most effective osteogenic inductors in bone tissue engineering is bone morphogenetic protein 2 (BMP-2). However, this protein is easily and rapidly destroyed in culture media (the half-time degradation of BMP-2 in vitro is about 1 hour) [1]. One of the ways to solve this problem is encapsulation BMP-2 in bioresorbable materials and the subsequent development of matrixes for tissue engineering based on resulting composites. Supercritical fluid technologies (SCF) for encapsulating proteins into polymer materials has a number of advantages over traditional technologies. First of all - there is no need to use toxic solvents. All processes can be carried out at temperatures close to physiological (≤ 40°C). Biologically active substances almost completely retain their activity under these conditions [2]. Surface-selective laser sintering (SSLS) [3] is used to form matrix structures of polymer powders with encapsulated biologically active substances by heating and fusing mainly surface layers of sintered particles, also bioactivity inside the main volume material is saved [4]. The purpose of our study was the development of matrices based on bioresorbable poly(lactic-co-glycolic acid) (PLGA) and BMP-2 using the methods of SCF plasticization and SSLS as well as the study of kinetics of BMP-2 release from them into physiological saline and their cytocompatibility.
2. Models and Methods
Sterile BMP-2 solution with concentration of 10 μg / ml (AcronB iotech, USA, cat. AK8356) and copolymer of lactic (75%) and glycolic (25%) acids PLGA (Purasorb PDLG 7507, Corbion Purac, Netherlands) with a characteristic viscosity of 0.7 dl / g. were used in the experiments.

Bioactive matrices based on PLG were manufactured by the SCF plasticization of the initial polymer mixture with BMP-2 (3.57 µg BMP-2 per 1 gram) in cylindrical (5 mm diameter, 5 mm height) molds at a temperature 33 ± 0.1ºC and pressure 10 ± 0.1 MPa and its subsequent foaming at a pressure drop to the atmospheric value [5]. Then, the obtained cylindrical samples were cut into discs with thickness 0.3 mm and 1 mm. Discs with 0.3 mm thickness were used for subsequent laser sintering, 1 mm thick discs were used to study kinetics of BMP-2 release from them into physiological saline and their cytocompatibility.

SSLS using water as the sensitizer of heating was carried out using thulium laser with 1,94 μm wavelength (IPG Photonics), laser power of 3 W, beam size 400 μm, scanning speed 30 mm/s and hatching frequency 1 mm⁻¹.

In order to study the kinetics of BMP-2 release matrices impregnated with BMP-2 (4 samples) were placed in physiological saline. The experiment was carried out under sterile conditions. The solution containing released BMP-2 was taken out from the glass vial every day and frozen to -20 ºC. After that, a new portion of physiological solution was added to the matrices. The experiment was conducted during 14 days. The kinetics of BMP-2 release was determined using enzyme-linked immunosorbent assay (ELISA, R&D, USA).

Cytocompatibility of bioactive matrices was investigated using mesenchymal stem cells (MSC) from subcutaneous fatty tissue, which were previously obtained and characterized according to [6]. MTT-test was used to evaluate cytotoxicity [7].

3. Results and Discussion
The process of sorption of carbon dioxide at high (more than 5.0 MPa) pressures in the volume of the amorphous polymer leads to a decrease in its glass transition temperature and plasticization. Under these conditions solid polymer particles are transformed into a gel-like mass and integrate the BMP-2 microparticles, which are initially distributed on their surface.

After the CO₂ pressure is released to the atmospheric value the polymer composite is cured again, the porous polymer matrix with BMP-2 is formed in the shape of a cylinder with the diameter of 5 mm. The image of cross section of the bioactive PLG matrix was obtained by scanning electronic microscope (SEM) and is shown in Figure 1.

The SSLS using water as the sensitizer of heating allows to melt the surface of the matrix without significant overheating of its volume [8]. It is possible to change the kinetics of BMP-2 release due to this laser processing with maintaining its activity. SEM image of the surface of the matrix after SSLS is shown in Figure 2.

![Figure 1. Cross section of the matrix.](image1)

![Figure 2. Surface of the matrix after SSLS.](image2)
All investigated samples of bioactive matrices showed high level of cytocompatibility. Cell survival on the surface of the matrices was comparable to the control, and was about 100%. Within 7 days of observation the cells on the surface of the matrices were spread, acquiring an elongated or polygonal shape (PKH26-labeled) (Figure 3-4).

The results of experiments of the kinetics of BMP-2 release from PLG matrices in physiological solution are shown in Figure 5.

Matrices formed by supercritical CO₂ provided a smooth release of BMP-2 for 15 days. During this time almost 100% of the encapsulated protein was released from the matrices. Laser sintering contributed to longer retention of BMP-2. The peak release was observed from 13 to 15 days. During this time up to 80% of protein was extracted from sintered samples.

4. Conclusion
Bone morphogenetic protein BMP-2 was encapsulated in the bioresorbable aliphatic polyether, poly(lactic-co-glycolic acid), using method of supercritical fluid plasticization. Porous bioactive matrices were formed from this composition by SCF foaming. Their high cytocompatibility was shown by in vitro tests using cultures mesenchymal stem cells. Matrices without laser sintering provided a gradual BMP-2 release for 15 days. Laser sintering contributed to longer retention of BMP-2 in matrix structure. In our opinion, combination of SCF technologies and PSLS is very promising from the perspective of development and formation of composite osteoplastic materials that require the controlled prolonged release of various growth factors or other drugs.
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