FABRICATION AND PLASMA MODIFICATION OF POLYMER SCAFFOLDS FOR REGENERATIVE AND REPLACEMENT MEDICINE.

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Introduction

Materials that serve as analogues for the native extra-cellular matrix (ECM) can be used in medicine to aid in either the reconstruction or regeneration of damaged tissue and organs. Polymer matrices have been used for regeneration of bone, liver, pancreas, skin and other tissues [1]. Macroporous. biodegradable polymer scaffolds have been prepared by numerous techniques including solvent casting/salt leaching, phase separation, solvent evaporation, and fiber bonding to form a polymer mesh.

The main goal of this research was to apply novel physical processing techniques to fabricate and modify highly porous implantable biodegradable scaffolds. More specifically, this involves fabricating scaffolds using electrospinning, piezoelectric printing, gel sublimation techniques and finally modification of matrixes by plasma treatment in order to control chemical structure and morphology of scaffolds.

Materials and Methods

Poly(oxybutirate-co-valerate), POB (Aldrich, USA) and collagen containing heterogenic biopolymer hydrogel (HBH) (BIOMIR service, Russia) obtained from hydrolyzate of farm animal tissues [2] , were used for preparation of polymer scaffolds and 2D predefined structures by electrospinning, piezoelectric printing and gel sublimation methods.

The homemade apparatus used for electrospinning included a pump driven by stepping motor, a high voltage power supply and a syringe as the reservoir for the polymer solution to which an blunt-end needle was attached. The stainless plate (10.0 cm * 8 cm * 0.5 cm) was used as the collection target. All electrospinning parameters including applied voltage (10 – 25 kV), distance between the needle and grounded plate (10 – 25 cm), solution dispensing rate (0.2 - 2mL/min) and concentration of polymer in methyl chloride (5 -15 %). could be changed to optimize the morphology of scaffolds. Three milliliters of solution was electrospun for each matrix.

Piezoelectric ink jet printing is a thermally constant process that can be carried out at room temperature or in a localized cold environment. The piezoelectric printhead consists of a piezoelectric transducer, nozzles, manifolds, ink pumping chambers, and fluid inlet passages. When a voltage is applied to the lead zirconate titanate (PZT) piezoelectric transducer, the transducer deforms and creates mechanical vibrations. For example, the ink jet cartridge in the Dimatix Materials Printer DMP 2831 used in these experiments is powered by a thin piezoelectric unimorph, which is constructed in the plane of the wafer. This structure consists of patterned PZT bonded to a silicon diaphragm. Actuation of the PZT piezoelectric transducer is in the plane of the wafer (bender mode). A die consists of 16 individually addressable jets

that release drops perpendicular to the wafer from an array of inline nozzles that are spaced 254 μm apart. The effective diameter of the nozzle is 21.5 μm, which provides a drop in the ~10 pL range.

Gel sublimation at low temperature was used for the fabrication of scaffolds from the cooled mixture of POB with two solvents: acetic acid and carbon tetrachloride. Due to the different crystallization parameters of these solvents a two dimensional porous structure was formed in the process of pumping the mixture and removing the solvents at low temperature. The morphology of the matrix could be varied by changing the relative concentration of the solvents and pumping temperature.

After fabrication polymer scaffolds were placed between the electrodes of dielectric barrier discharge (DBD) in atmospheric air (22kV, 5μs pulse duration) in order to modify their surface chemical composition and improve biocompatibility. ATR FTIR spectroscopy was used to analyze surface chemical composition before and after plasma treatment. The morphology of polymer scaffolds was investigated by AFM and SEM techniques

Biocompatibility of polymer scaffolds was estimated by different biological tests: hemolysis, cell toxicity and cell proliferation experiments. Fibroblast cells NIH 3T3 were used for cell toxicity and cell proliferation testing experiments.

Results and discussion

Figure 1 shows SEM pictures of polymer scaffolds fabricated from POB by electrospinning technology. By variation the main parameters of the electrospinning process listed above it was possible to change the fibers diameter in the range 0.1 -10 microns and verify porous size in the range 10 – 50 microns.

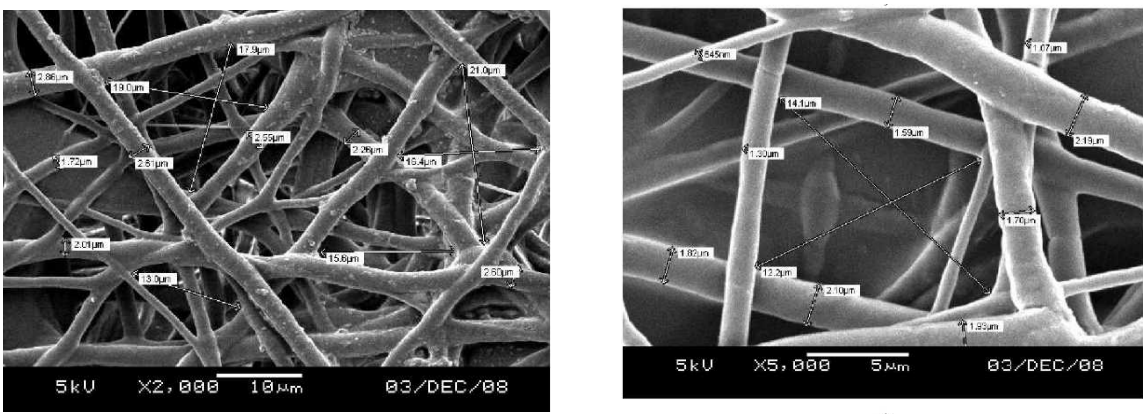

Figure 1. SEM pictures of polymer scaffolds fabricated by electrospinning technology from POB

The matrix presented at Figure 1 was fabricated after optimization of electrospinning parameters to obtain homogeneous and free of defects structure for the POB concentration 15%wt. The optimized structure for this concentration was obtained at the distance between the needle and grounded plate 10 cm and applied voltage 25 kV. The diameter of the fibers in this matrix was in the range 2 – 3 microns and the distance between fibers was 15 – 20 microns approximately. Estimated porosity of this scaffold was in the range 95 – 97%.

The optimum viscosity for jettable fluids in piezo drop-on-demand printheads is ~8–14 mPas (8–14 cps). However, most biological materials including collagens exhibit very low viscosities (0.1–1 cps) and very high surface tension values (58–60 cps dynes/cm). As a result, it is important to be able to adjust the operating parameters of the ink jet printhead to successfully jet low viscosity fluids. In our experiments it was possible to adjust the frequency of the waveform, the voltage to individual nozzles, and the structure of the waveform that drives the movement of the PZT piezoelectric transducer for Dimatix Materials printing system. The collagen containing solution of HBH (in 1.5% acetic acid) was dissolved in phosphate buffer saline (PBS) to the concentration 1mg/ml. Tween 80 surfactant (0.1%; Fisher Scientific, Fair Lawn, NJ, USA) was added to prevent aggregation of collagen. The protein solution was maintained at 28°C, purged through the printhead for uniform droplet formation and then calibrated at a constant velocity of 0.58 m/s for all nozzles prior to deposition. The solution of HBH was printed directly on poly(ethyleneterephthalate) (PET) substrates to develop microscale 2D patterns of materials for possible medical and biological applications. The patterned materials have been examined using several characterization techniques, including optical microscopy, atomic force microscopy, and electron microscopy. Figure 2 shows the optical pictures of the predefined porous structure printed from HBH on PET substrate..

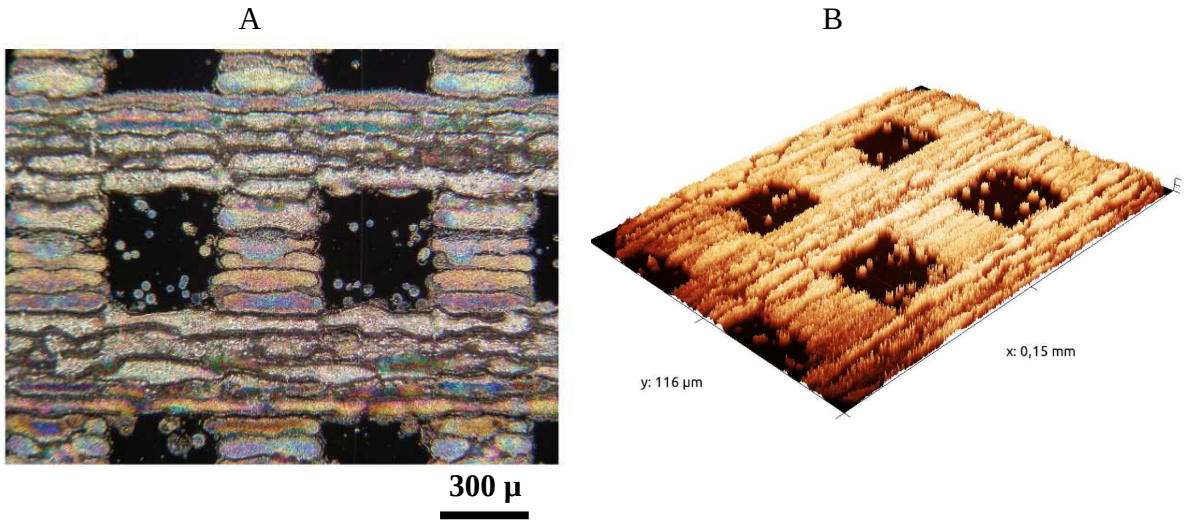

Figure 2. Optical pictures of predefined structures printed from hydrolyzed collagen solution on PET film (A), 3D reconstruction of optical images

The structure with the thickness about 50 microns was obtained after 20 layers printing of collagen containing HBH. According to the computer template used for printing, the structure contains rectangular holes with the size 300 *300 microns. These images presented on Figure 2 (A,B) demonstrate that high resolution microscale features may be obtained for collagen containing structures using piezoelectric ink jet digital fabrication technology.

The porous structure with bi-modal controllable distribution function was fabricated from POB by gel sublimation method. Depending on the relative concentrations of acetic acid and carbon tetrachloride solvents different porous structure was obtained after pumping the binary solution of POB in vacuum chamber. The process of gel sublimation technique consists of the

few stages. At first the mixture of POB with acetic acid and carbon tetrachloride was cooled to the frozen temperature -25 - -30C and stored in the refrigerator for 24 hours. After that the frozen sample was placed in vacuum chamber and pumped at the pressure about 100 Torr for 2-3 hours to remove the solvents and fabricate a porous structure.

As shown in Figure 3 the bi-modal porous structure is fabricated by this gel sublimation method.

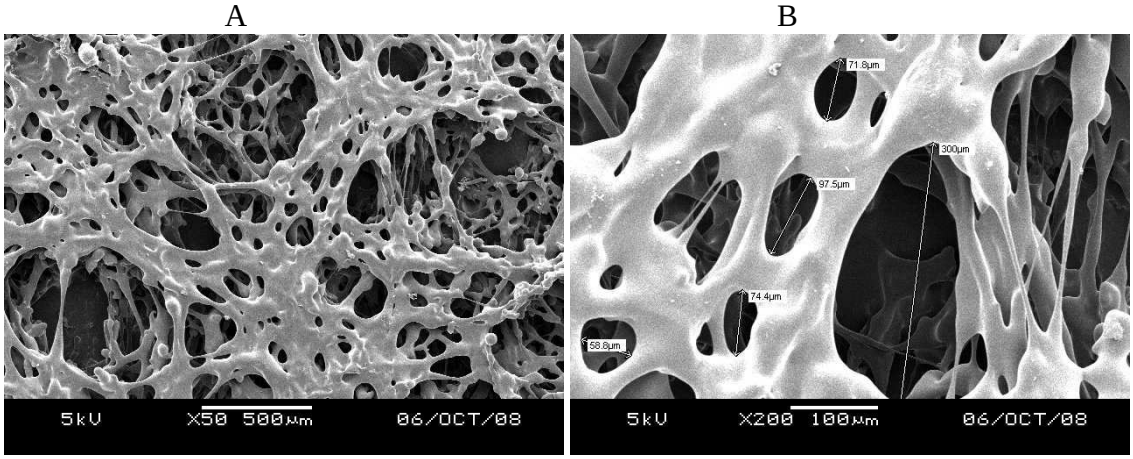

Figure 3 SEM pictures porous structure obtained by gel sublimation method at different magnifications x50 (A) x200 (B)/

The two types of pores are formed as a result of elimination of two differently crystallized solvents. The size of small pores is varied in the range of 5 – 30 microns, while the size of large pores is ranged in 100 – 300 microns.

In order to improve hydrophilicity and biocompatibility of scaffolds obtained by electrospinning and gel sublimation techniques the POB porous structures were treated by atmospheric pressure DBD discharge at different dosages. Plasma treatment leads to the formation of carboxylic groups and provides the increase of polymer surface energy and hydrophilicity as it was proved by ATR FTIR and contact angle measurements [3]. According to various biological testing procedures polymer scaffolds obtained by new fabrication technologies and treated by DBD plasma at optimal conditions have shown no toxic reactions and improve cell proliferation .behavior.

Conclusions

Polymer scaffolds obtained by electrospinning, piezoelectric printing or gel sublimation technologies and treated by atmospheric pressure DBD discharge could provide the attachment and proliferation of various cells and may be used as pre-defined scaffolds in various tissue engineering applications.

References

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