PREPARATION OF ANTIBACTERIAL ELECTROSPUN SCAFFOLD FOR SKIN CELLS CULTURE

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Abstract

In this work the function and application of titanium dioxide as a filler in a composite system polycaprolactone/titanium dioxide (PCL/TiO_2) was examined. Titanium dioxide was applied by ultrasonic bath on already electrospun PCL fibrous scaffold treated and non-treated with NaOH. A procedure of surface modification of the electrospun PCL fibrous scaffold was made to enhance the interaction of the surface with the TiO_2 particles. The surface modification was performed using NaOH for the formation of carboxyl groups on the fibers' surfaces. The water contact angle was measured by goniometer to prove the change from hydrophobic to hydrophilic polymer surface. SEM was used to study the morphology structure of the electrospun PCL fibrous scaffold before and after NaOH treatment and introduction of TiO_2. The content of TiO_2 on the electrospun PCL fibrous scaffold was determined by TGA. After NaOH treatment the surface of the electrospun PCL fibrous scaffolds changed from hydrophobic to hydrophilic. SEM micrographs show that with the sonification of 30 min homogeneous TiO_2 particles distribution was obtained, while after sonification of 60 min, the TiO_2 particles tend to agglomerate. The modification of the scaffold surface with NaOH enhances the adhesion of the TiO_2 filler. TG analysis show that longer treatment of the electrospun PCL fibrous scaffolds in the ultrasonic bath gives lower thermal stability. The time of 30 minutes in the ultrasonic bath is optimal to provide sufficient amount of the TiO_2 particles on the electrospun PCL fibrous scaffold.

Keywords: Electrospinning, NaOH, surface modification, titanium dioxide

1. INTRODUCTION

The tendency of human organism, tissue and organs to get injured and the flaws of human tissue caused by innate defects are everyday problems that physicians encounter. Treatment usually focuses on tissue transplantation from one site to another in the same patient (autograft) or from one person, the donor to the other (alograft or transplant). Although these treatments are revolutionary and save human lives, they face many problems. This is why tissue engineering has been developed with the aim of repairing and regenerating damaged tissue by developing biological replacements that restore, maintain, or improve tissue function. Tissue engineering uses different natural and artificial biomaterials as 3D scaffolds where the cells previously taken from the patient's body are planted. 3D scaffold structure is important for imaging specific microgeometry of tissue. In order to obtain scaffold of the exact 3D microgeometry and topography, electrospinning technique is used in combination with 3D printing. First, 3D printing technique is used to obtain collector of specific topography, and then they are used in the electrospinning procedure for fiber collecting. Electrospinning enables the production of very fine fibers of precisely specified properties. This way, certain micro-topography scaffolds are created which is important for linking the cells and the development of new tissue [1]. Recently, biodegradable fiber polymers are used as matrices for the cultivation and growth of different tissue cells. Specifically, mats obtained by electrospinning of poly (ε-caprolactone) (PCL) exhibit good potential as skin regeneration tissue carriers as they do not produce harmful degradation products such as poly (lactic acid) (PLA) or poly (lacto-co-glycolic acid) (PLGA). In addition, PCL has good mechanical properties and prolonged degradation time [2,3]. Poly (ε-caprolactone) (PCL) is a partially crystalline aliphatic polyester with a melting point at 60 ° C. The low melting point makes it easier to process. PCL is hydrophobic due to the presence of nonpolar methylene groups. PCL is the most widely used polyester in tissue engineering. In this paper, PCL scaffolds were electrospun on the collector of parallel channel geometry [4]. Subsequently, TiO_2 was applied from aqueous solution, to neat PCL scaffold and to scaffold previously treated with NaOH, using ultrasonic bath. The aim of this work is to investigate how pre-treatment of the surface and the time in the ultrasonic bath affects the adhesion of TiO_2 to the electrospun PCL fibrous scaffold in order to use the material for fibroblast cell growth and regeneration of the skin tissue.

2. EXPERIMENTAL

2.1. Materials

In this work PCL (Polycaprolactone 440744-500G) average molecular mass *Mn* of 70,000-90,000 g/mol by GPC, Mw/Mn<2, dencity 1.145 g/ml at 25°C) was supplied by Sigma-Aldrich, Germany. As solvents glacial acetic acid and acetone from Sigma-Aldrich, Germany were used.

2.2. Preparation of polymer solutions

Polymer solution of 18 % PCL with 8:2 ratio of glacial acetic acid and acetone was made. It was mixed by magnetic stirer at the temperature of 50 °C.

2.3. Electrospinning procedure

Electrospinning of PCL solutions was made on electrospinning machine, NT-ESS-300, NTSEE Co. Ltd. South Korea. The conditions for electrospinning were: electric voltage: 15 kV, distance from the needle end to collector 18 cm, speed of solution flow 1 mL/h and total time of 4 hours. Syringe is filled with 4 mL of PCL solution. It is then connected to the pump where flow conditions are regulated. One end of the source is connected to the needle and the other to the collector for the dispersion of the solution. 3D printed collector is set to distance of 18 cm from the syringe with the needle and the electrospinning is performed for 4 hours. After that time the source is unplugged and electrospun scaffold is used for further analysis.

2.4. NaOH treatment

Suface of samples is treated before applying TiO_2 with NaOH to change the surface energy and lower down the contact angle with water to make hydrophillic suface of electrospun PCL fiber from originally hydrophobic surface. If the surface is hydrophillic it is considered that it will react better with applied polar particles of TiO_2.The solution of NaOH was 1 N and the samples were treated in it for 1 hour then washed with distilled water until pH neutral.

2.5. Application of TiO_2

TiO_2 was applied on the electrospun PCL fibrous scaffold from 1 % water solution afterwards by ultrasonic bath device. In total 4 (2 without and 2 treated with NaOH) samples were held in the ultrasonic bath for 30 and 60 minutes to make comparison and decide the best technique of applying the highest amount of equally dispersed TiO_2 particles on the electrospun PCL fibrous scaffold without agglomerations.

2.6. Experimental part

Water contact angle were determined by measuring on a contact angle goniometer DataPhysics OCA 20 Instruments GmbH by *Sessile drop* method. The water contact angle was measured for non-modified and PCL modified by NaOH. The volume of the drop was from 1.000 µL to 5.000 µL. On every sample 3 drops were ejected and after 20 seconds the contact angle was measured.

For determination of the fiber's morphology samples are scanned by electron microscope SEM QUANTA 250, FEI. The magnification was 50, 1000, 2000 or 3000 times on different parts of the samples. The thermal stability was measured by TGA analyzer Q500 from TA Instruments. Samples of about 10 mg were analyzed in a nitrogen atmosphere with heat speed of 10°C/min, in a temperature range from 25 °C to 600 °C.

3. RESULTS AND DISCUSION

3.1. Water contact angle measurement

The water contact angle was measured before and after NaOH treatment. The interaction of water and the surface of the sample is detected from the contact angle data. Contact angle measurement plays an important role in a characterization of the surface of materials. It is a measurement of moisturizing the solid with the liquid. A drop is spilled on a solid surface and the balanced shape is awaited. Contact angle is formed from the forces of the periphery of a drop and a solid surface. Surface condition is determined concerning it's polarity, homogeneity and roughness. Hidrophobicity of the electrospun PCL fibrous scaffold could be changed by NaOH treatment. To confirm the change from hydrophobic to hydrophilic water contact angle measurement for treated and non-treated electrospun PCL fibrous scaffold is made. It is well-known that the electrospun PCL fibrous scaffold has a water contact angle of around 80°. The water contact angle measurements are shown in **Figure 1**. For non-treated electrospun PCL fibrous scaffold contact angles on two different position of the scaffold were obtained around 90° and 125.8 ± 5.6°, which clearly show hydrophobicity (**Figure 1a and 1b**). On the other hand the electrospun PCL fibrous scaffold treated with NaOH shows extreme hydrophilicity with water contact angle of about 0° which proves that the surface of the PCL became polar. Unfortunately, it was not possible to take contact angle images of a drop for the electrospun PCL fibrous scaffold treated with NaOH because the material instantly soaked the drop of water. Generally, NaOH treatment is made to lower the polymer surface hydrophobicity and to enhance the interaction between the polymer and TiO_2.

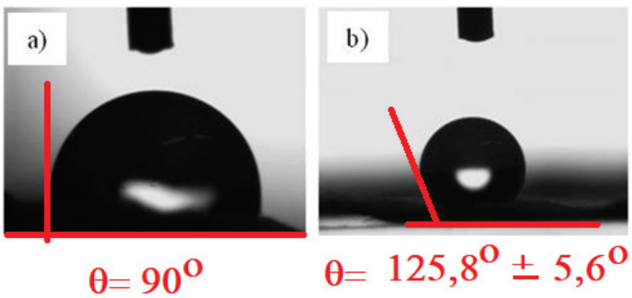

Figure 1 Water contact angle measurements for the electrospun neat PCL scaffold on the different position of the scaffold before treatment with NaOH (Figures 1a and 1b)

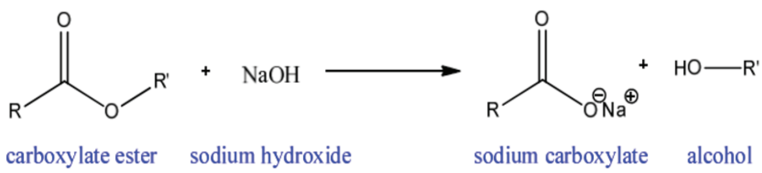

Figure 2 Sodium hydroxide treatment of PCL structure

NaOH hydrolyzes ester bonds of the PCL which leads to carboxyl functional group formation on the electrospun PCL. Carboxyl groups make the surface less hydrophobic as they lower the contact angle value.

Because of the NaOH surface treatment the polymer becomes nano-rough which is enlarging the specific surface that can react with the particles of TiO_2 (**Figure 2**) [5].

3.2. Determination of morphology by scanning electron microscopy (SEM)

Figure 3 shows the structure of the electrospun PCL fibrous scaffold before NaOH treatment and application of TiO_2. Porous, fiber-like structure (**Figure 3a**) that follows geometry of the electrospinning collector (**Figure 3b**) can be seen. Except for fibers, there are also beads formations of the PCL.

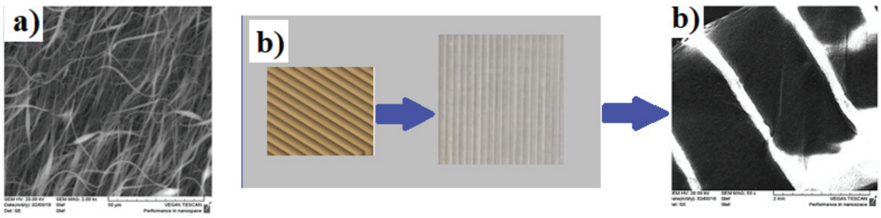

Figure 3 SEM images of the electrospun: a) PCL neat fibrous scaffolds, b) PCL neat fibrous scaffolds on the 3D printed collector, before NaOH treatment and application of TiO_2

The electrospun PCL scaffolds consist of random oriented and intertwined fibers with specific inter pore structure. The surface of the fibers doesn't have any irregularities. The fibers diameters were about 1 µm. The electrospun PCL fibrous scaffold will be used to grow skin cells so it is necessary to provide double porosity of the electrospun fiber carrier by micro pores big enough for skin cells penetration and nano pores small enough to enhance adhesion of the skin cell receptors, transfer of nutrients and oxygen needed for cells growth [6]. For antibacterial properties of the carrier, the TiO_2 is added. **Figure 4a** shows homogeneous TiO_2 particle distribution inside of the electrospun PCL fibrous scaffold after 30 min in the ultrasonic bath.

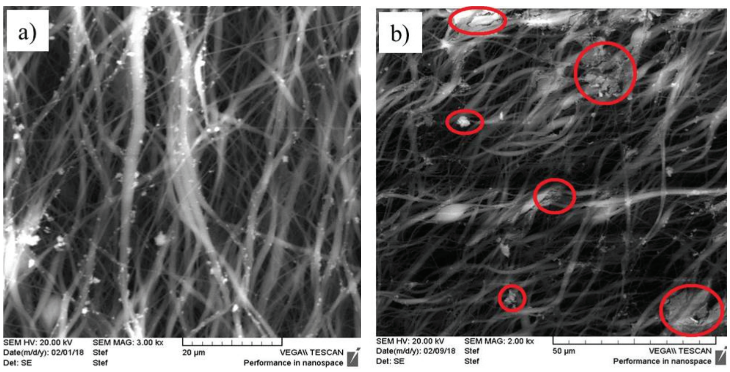

Figure 4 SEM micrographs of the electrospun PCL fibrous scaffold with TiO_2 after a) 30 min and b) 60 min of sonification

After 60 minutes of sonification large quantity of TiO_2 inside of the electrospun PCL fibrous scaffold was obtained, but the particles distribution was not uniform and the particles tend to agglomerate as seen in **Figure 4b**. The modification with NaOH changed the smoothness of the surface. The SEM micrograph in **Figure 5** shows that the geometry of the material changed. In spite of small changes of the surface morphology it is evident in **Figure 5a** that the TiO_2 particles after 30 minutes in an ultrasonic bath got integrated in large agglomerates. After 60 minutes, **Figure 5b**, agglomeration is not so noticeable as without NaOH treatment and distribution of the filler particles is even along the whole surface and between the pores. There is an indication of possible penetration of the filler particles inside of the pores of fiber.

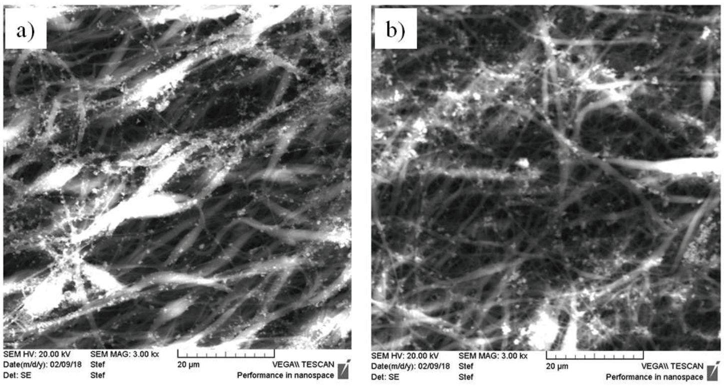

Figure 5 SEM micrographs of the electrospun PCL fibrous scaffold treated with NaOH and with TiO_2 after a) 30 min and b) 60 min of sonification

The mapping micrographs clearly show where the TiO_2 is placed inside the electrospun PCL fibrous scaffolds. The red color marks indicate the titan dioxide particles, thus it is proven that NaOH treatment of the polymer surface enhances the interactions between the polymer and the titanium dioxide (**Figure 6**).

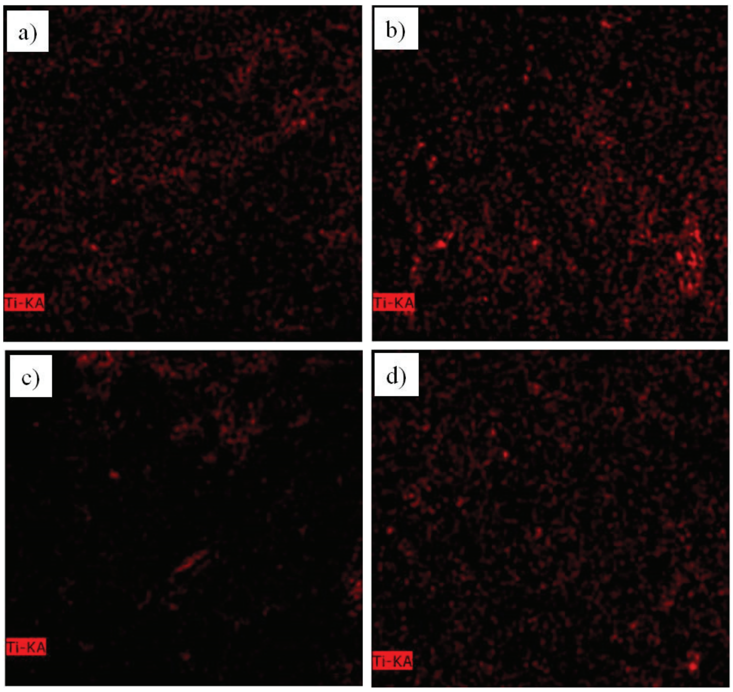

Figure 6 Mapping TiO_2: a) 30 min without NaOH, b) 30 min with NaOH; c) 60 min without NaOH, d) 60 min with NaOH

3.3. Results of thermogravimetric analysis

The effect of the TiO_2 as well as the surface treatment with NaOH on the thermal stability of the electrospun PCL fibrous scaffold was examined by TGA. From the **Table 1** we can see that the addition of TiO_2 in the electrospun PCL fibrous scaffold enhances thermal stability of the scaffold because the initial temperature of degradation increased. Opposite from that, with longer time of ultrasound treatment the degradation starts at lower temperature which means that the time of 60 min is too long for keeping the electrospun PCL fibrous scaffold in the ultrasonic bath. The initial temperature of degradation is also lower for the samples treated with NaOH which affects negatively on the thermal stability of the fibrous scaffold. Another important parameter is the percentage of the residue which is related to the weight of TiO_2 implemented in the electrospun PCL fibrous scaffold. By surface modification with NaOH the weight of the TiO_2 in the PCL fibrous scaffold increases significantly as it is shown in **Table 1**. Also the longer time of treatment enhances the percentage of TiO_2.

Table 1 TGA weight residue and start and end degradation temperatures of the PCL/TiO_2 scaffolds

Sample	Residue at 600°C (mg)	T_i (°C)	T_f (°C)
PCL	0	365.5	426.9
PCL/TiO_2 30 min.	0.67	384.0	452.6
PCL/TiO_2 60 min.	0.79	367.6	426.5
PCL(NaOH)/TiO_2 30 min.	0.80	372.6	432.1
PCL(NaOH)/TiO_2 60 min.	0.88	363.0	427.2

4. CONCLUSION

In this work a possibility to introduce TiO_2 to electrospun PCL fibrous scaffolds by ultrasonic bath method was examined. Further NaOH surface modification of the electrospun PCL fibrous scaffold was made to enhance the interaction with the TiO_2 filler particles. After NaOH treatment the surface of the electrospun PCL fibrous scaffolds changed from hydrophobic to hydrophilic. SEM micrographs show that with the sonification of 30 min homogeneous TiO_2 particles distribution was obtained, while after sonification of 60 min, the TiO_2 particles tend to agglomerate. The modification of the scaffold surface with NaOH enhances the adhesion of the TiO_2 filler. TG analysis show that longer treatment of the electrospun PCL fibrous scaffolds in the ultrasonic bath gives lower thermal stability. The time of 30 minutes in the ultrasonic bath is optimal to provide sufficient amount of the TiO_2 particles on the electrospun PCL fibrous scaffold.

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