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FABRICATION OF BIOACTIVE GLASS CONTAINING COMPOSITE FIBERS USING ELECTROSPINNING METHODS

Summary: The aim of the work was to fabricate nanocomposite fibers containing bioglass using electrospinning technique. Two different types of fibers were produced in this study. Quality of the composite fibers was studied using optical microscope. Moreover *in vitro* bioactivity test was performed in Simulated Body Fluid in order to confirm the bioactive character of the obtained fibers.

Słowa kluczowe: Electrospinning, PCL, Bioglass, Bioglass-Zinc, Simulated Body Fluid, Bioactivity

WYTWARZANIE KOMPOZYTOWYCH WŁÓKIEN ZAWIERAJĄCYCH BIOSZKŁO METODĄ ELEKTROPRZĘDZENIA

Streszczenie: Celem pracy było wytworzenie włókien kompozytowych zawierających bioszkło metodą elektroprzędzenia. Wytworzono dwa różne rodzaje włókien, które poddano ocenie przy użyciu mikroskopu optycznego. Ponadto przeprowadzono test bioaktywności *in vitro* w symulowanym płynie ustrojowym (płyn SBF). Przeprowadzone badania wykazały bioaktywny charakter wytworzonych włókien.

Keywords: Elektroprzędzenie, PCL, Bioszkło, Bioszkło z cynkiem, płyn SBF, Bioaktywność

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1. Introduction

Electrospinning (ES) is a technique to obtain microfibers and nanofibers from a wide variety of materials, such as polymers, ceramics, composites. This method allows an easy and reliable way to obtain fibers. The principle of electrospinning consists in applying high voltage to a polymer solution. High voltage is applied to the tip of a syringe that contains the polymer solution [1]. When electrical charges break the surface tension of polymer solution drops, solution jets are ejected to a spinning collector. This process evaporates the solvent, collecting fibers on the spinning surface in woven form. The setup of a typical electrospinning machine is composed of a syringe with the polymer solution, a collector (flat or cylindrical), a high voltage power supply. This equipment can be set up for different parameters. Depending of which set up is used or variations made in solution and equipment parameters, different fibers can be obtained, using these properties for a wide variety of applications such as industrial, medical purposes [2].

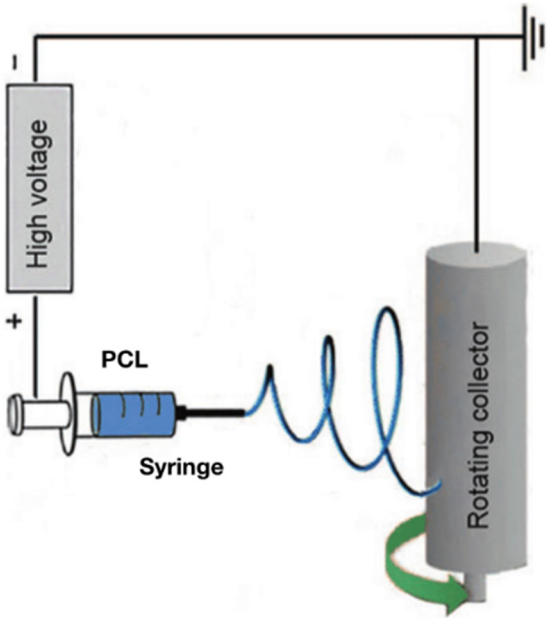

Figure 1. Scheme of electrospinning process.

1.1. Electrospinning parameters

There are several factors that affect the electrospinning process. These factors are classified as process parameters, solution and environmental parameters [3].

Concentration

The concentration of the solution play an important role in the fiber formation during the electrospinning process. When the solution concentration is low, formation of beads or beaded nanofibers appear. If the concentration is high, beyond a critical

value, helix-shaped micro-ribbons will appear. When the concentration is suitable, smooth nanofibers can be obtained.

Molecular Weight

In principle, molecular weight reflects the entanglement of polymer chains in solutions, namely the solution viscosity. Keep the concentration fixed, lowering the molecular weight of the polymer trends to form beads rather than smooth fiber. Increasing the molecular weight, smooth fiber will be obtained, increasing the molecular weight above this point, micro-ribbon will be obtained.

Viscosity

Continuous and smooth fibers cannot be obtained in very low viscosity, as contrary very high viscosity results in the hard ejection of jets from solution. Generally, the solution viscosity can be tuned by adjusting the polymer concentration of the solution.

Surface Tension

With the concentration fixed, reducing the surface tension of the solution, beaded fibers can be converted into smooth fibers. Additionally, the surface tension and solution viscosity can be adjusted by changing the mass ratio of solvent mix and fiber morphologies.

Conductivity/Surface Charge Density

Solution conductivity is mainly determined by the polymer type, solvent sort, and the salt.

Voltage

A flow of current through a metallic needle at a critical voltage will cause the drop to deform into a Taylor cone and form ultrafine nanofibers. The critical value varies depending on the polymer. We can find that voltage does influence fiber diameter, but the level of significances varies with the polymer solution concentration and on the distance between the tip and the collector.

Flow Rate

Lower flow rate is more recommended as the polymer solution will get enough time for polarization. If the flow rate is very high, beads fibers with thick diameter will form and leads to increasing the pore size, rather than the smooth fiber with thin diameter owing to the short drying time prior to reaching the collector and low stretching forces.

Collectors

With the need of fibers transferring, diverse collectors have been developed including wire mesh, pin, grids, parallel or gridded bar, rotating rods or wheel, liquid bath, and so on.

Distance between the collector and the tip of the syringe

The nanofiber morphology depends on the deposition time, evaporation rate and whipping. If the distance is too short, the fiber will not have enough time to solidify before reaching the collector, whereas if the distance is too long, bead fiber can be obtained.

Ambient Parameters

Ambient parameters can also affect the fiber diameters and morphologies such as humidity, temperature. Increasing temperature favors the thinner fiber diameter. As for the humidity, low may dry the solvent totally and increase the velocity of the solvent evaporation. On the other hand, high humidity will lead to the thick fiber diameter owing to the charges on the jet can be neutralized and the stretching forces become small [4-6].

1.2. Polycaprolactone (PCL) and Bioglass (BG)

Polycaprolactone (PCL) is a semicrystalline polymer with a glass transition temperature of around 60 ˚C. It is a polymer with low viscosity and easy to process. The PCL presents a high permeability to water, oxygen and CO_2, in addition to mechanical properties comparable to those of low density polyethylene. In this way, PCL can be considered an excellent material to improve the mechanical properties of other polymers. It can also be mixed with ceramic materials in order to fabricate polymer/ceramic composites [7]. The use of synthetic polymers for biological applications is a rapidly growing field of research. However they do not have such excellent bioactivity as ceramic materials (hydroxyapatite or bioglass).

Bioactive glasses from the CaO–P2O5–SiO2-NaO2 system have been proved as a bioactive materials (it means: when implanted bond directly to living bone). The mechanisms of bioactivity and bone bonding of bioactive glass have been widely studied, and they are described in detail elsewhere [8]. Based on those studies, the bonding of glass to bone has been attributed to the formation of a apatite layer on the glass surface in contact with the body fluid. This layer is very similar to the mineral part of bone and it bonds with living tissues [9].

Simulated Body Fluid (SBF) is an artificial fluid and has ion concentration similar to human blood plasma. This fluid is widely used to test bioactivity of materials.

The aim of this study was to fabricate a PCL bioactive fibers. In order to ensure bioactivity of the polymer PCL fibers, the bioglass was added to the polymer solution during the manufacturing process. We have fabricated two types of bioactive fibers

using electrospinning method. Then fibers were incubated for two weeks in SBF to check if the apatite layer is forming on the surface of composite fibers – proving its bioactivity.

2. Materials and Methods

Polycaprolactone with the molecular weight of 80 kDa (PCL, Sigma-Aldrich) was used in the study. Two different types of bioglass were used: BG and BG_Zn (made at University of Science and Technology AGH). Chloroform and methanol (POCH, Poland) were used as solvents. To prepare the solution 5 g of PCL with 25 ml of chloroform and methanol were used. The solution was mixed using a magnetic stirrer until the PCL was fully dissolved. Then the BG and BG_Zn powders were added to each solution. The syringes with the solutions were placed in the electrospinning machine to start the process. The process parameters are presented in the Table 1. All of these parameters directly affect the morphology of fibers.

Three samples were obtained: (1) pure PCL fibers, (2) PCL_BG fibers and (3) PCL_BG_Zn.

Table 1. Parameters during the electrospinning process.

Flow Rate [mL/h]	1,5
Voltage [kV]	25
Volumen of solution [mL]	10
Temperature [ºC]	21,2
Humidity [%]	52
Distance between tip and collector [cm]	20

2.1. SBF preparation

Simulated Body Fluid Solution was prepared according prof T. Kokubo. All reagent are presented in Table 2. Samples were incubated during 2 weeks in 1,5 x concentration of SBF at 37 ºC. After 14 days the samples were taken off , washed with distillated water, weight and observed with microscope. Optical microscope was used for preliminary examinations of nanofibrous materials during manufacturing process. Light microscope (Opta-Tech) equipped with camera and OptaView programm was used to capture all the pictures of the fibers obtained during the electrospinning process, and also before and after incubation of samples in SBF. Magnification between 10x and 40x was used to evaluate the samples.

Table 2. Reagents for SBF solution.

Reagent	Amount [g]
NaCl	12.054
$NaHCO_3$	0.528
KCl	0.3375
$K_2HPO_4 \cdot 3H_2O$	0.345
$MgCl_2 \cdot 6H_2O$	0.4665
HCl(1M)	60ml
$CaCl_2$	0.4395
Na_2SO_4	0.108
Tris	9.0945

3. Results and Discussion

Macroscopic images of the PCL electrospun membranes containing Bioglass and Zn_Bioglass are presented in Fig. 2. The fibers in the form of nonwoven were successfully obtained using an electrospinning technology. Uniform beadless morphology was observed in the case of pure PCL nonwovens. However the macroscopic view of modified samples shows many defects.

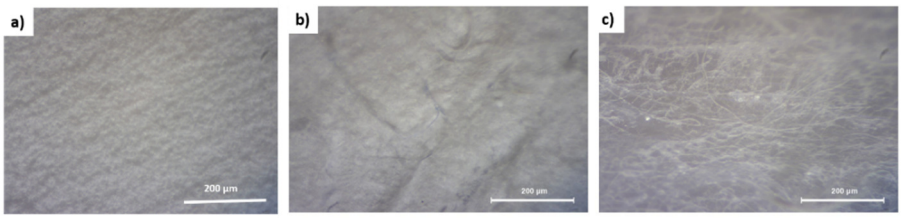

Figure 2. Macroscopic images of PCL samples (a) pure PCL, (b) PCL with bioglass, (c) PCL with Zn_bioglass

The microstructure of obtained samples is presented in Figure 3. Smooth and bead-free electrospun fibers can be observed in the case of pure PCL samples. Unfortunately in the case of bioglass modified fibers (Fig. 3b) and Zn-bioglass modified fibers (Fig. 3c) the formation of beads occurred, which is mainly attributed to the viscosity of the solution and nonevaporation of the solvent. The nonwovens

possess a wide range of fiber diameter and the bioglass particles were successfully incorporated into the fibers structure.

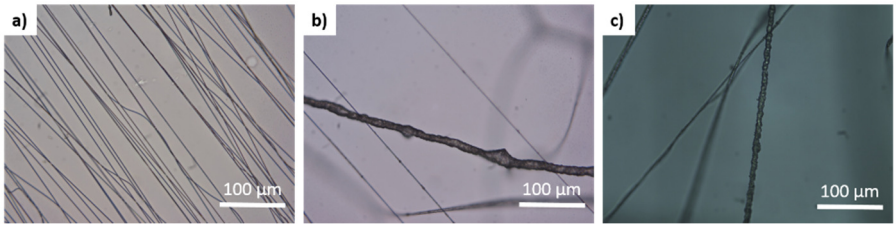

Figure 3. Microscopic images of PCL samples (a) pure PCL, (b) PCL with bioglass, (c) PCL with Zn_bioglass

The changes in the weight of the samples after incubation in Simulated Body Fluid are shown in Figure 4. The weight changes were calculated according to the formula:

$$\Delta m = \frac{mf - mi}{mi} \cdot 100[\%] \tag{1}$$

Where: *mi* - weight of the sample before incubation (initial);
mf - sample weight after incubation period (final).

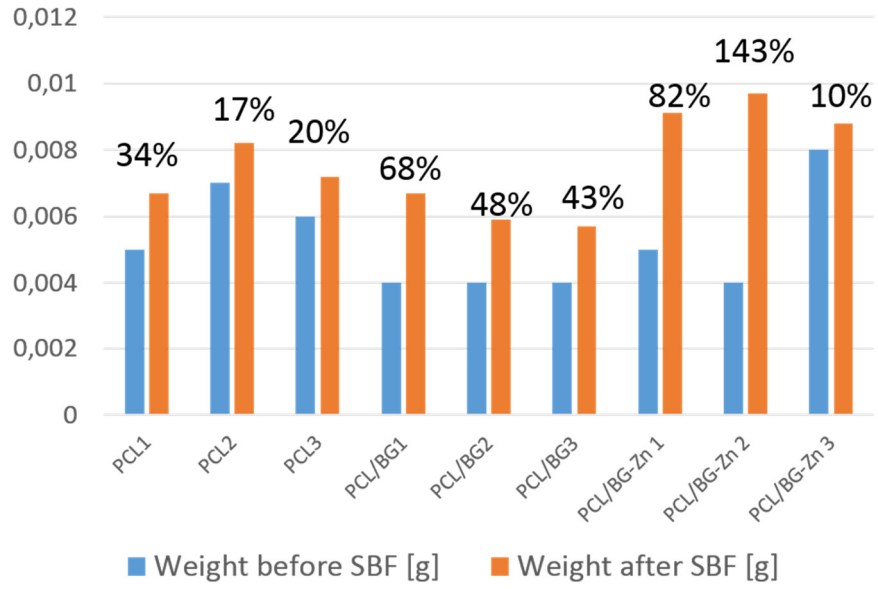

Figure 4. Weight change of samples after incubation in SBF fluid.

Modification of the scaffolds by introducing bioglass to the electrospinning solution of PCL resulted in a significant increase in weight. In the case of a PCL sample modified with a bioglass with zinc and unmodified bioglass, the significant increase of the mass occurred. For pure PCL, there was a slight increase in mass, which is the result of the moisture absorption effect of the polymers. The increase in the mass of

modified samples during the 2-week incubation period is associated with the formation of an apatite layer on the surface of porous scaffolds modified with bioactive additives. In order to confirm the obtained test results, macroscopic and microscopic observations were made of samples after the incubation process.

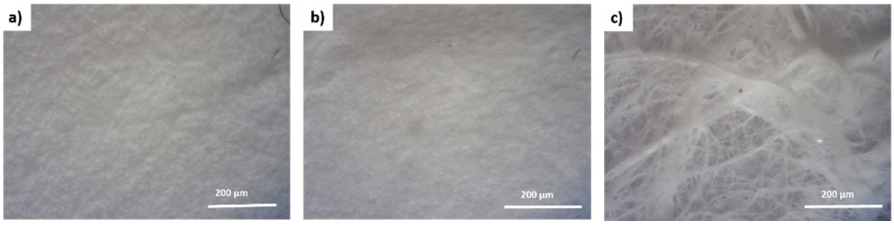

Figure 5. Macroscopic images of PCL samples after immersion in SBF (a) pure PCL, (b) PCL with bioglass, (c) PCL with Zn_bioglass

Figure 5 shows macroscopic images of a nonwovens after incubation in SBF fluid. The surface of the modified materials glittered under the microscope, indicating the precipitation of apatite crystals on the surface of the sample. Also microscopic observations confirmed apatite growth on the fibers surface (Fig. 6).

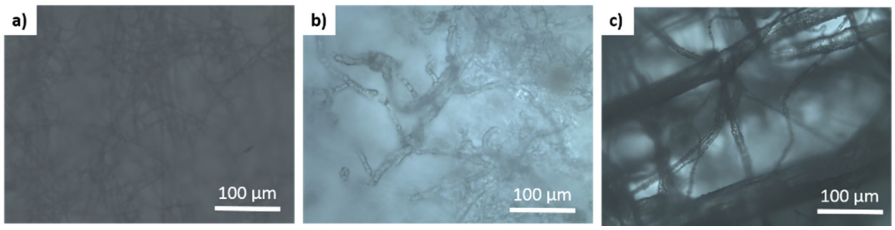

Figure 6. Microscopic images of PCL samples after immersion in SBF (a) pure PCL, (b) PCL with bioglass, (c) PCL with Zn_bioglass

4. Conclusion

Preliminary results obtained on the basis of bioactivity test carried out in SBF fluid confirmed the bioactive nature of modified electrospun fibers. The assessment of bioactivity based on the 14-day SBF test confirmed the possibility of forming the apatite layer on the surface of bioglass and Zn_bioglass modified polycaprolaktone materials. The change in the weight is associated with the formation of an apatite layer on the surface of porous scaffolds modified with bioactive additives. The results of the tests were confirmed by microscopic observations. Unfortunately, limiting resolution of optical microscopy precludes this technique from characterization of electrospun fibers in detail. Optical microscope is used for preliminary examinations of nanofibrous materials during manufacturing process, therefore further evaluations should be done.

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