Influence of Calcium-Phosphate Coating on Wettability of Hybrid Piezoelectric Scaffolds

A Zviagin[1], R Chernozem[1], M Surmeneva[1], K Loza[2], O. Prymak[2], M Ulbricht[3], M Epple[2] and R Surmenev[1, *]

[1] Physical Materials Science and Composite Materials Centre, National Research Tomsk Polytechnic University, 634050, Tomsk, Russia
[2] Inorganic Chemistry and Center for Nanointegration Duisburg-Essen (CeNIDE), University of Duisburg-Essen, 45141 Essen, Germany
[3] Technical Chemistry II, University of Duisburg-Essen, 45141 Essen, Germany

[*]E-mail: rsurmenev@mail.ru

Abstract. Herein, electrospun biodegradable scaffolds based on polycaprolactone (PCL), poly(3-hydroxybutyrate) (PHB) and polyaniline (PANi) polymers were fabricated. A calcium-phosphate (CaP) coating was deposited on the surface of the scaffolds via an improved soaking process. Influence of the deposition cycles and ethanol concentration in the solution on the relative increase of the scaffolds weight and water contact angle (WCA) are determined. The characterization of the molecular and crystal structure confirmed the formation of CaP phase. Importantly, WCA results showed that the pristine scaffolds have the hydrophobic surface, while the deposition of CaP coating onto scaffolds allows to significantly improve the surface wetting behavior, and infiltration of the water droplets into the CaP-coated scaffolds was observed. Thus, the fabricated hybrid biodegradable piezoelectric scaffolds can be utilized for regenerative medicine.

1. Introduction

The porous scaffold development is of significant importance in bone tissue engineering, which allows to provide the functional support of damaged tissues and ensure stimulation of bone regeneration. Numerous studies have reported successful application of biodegradable polymers for the scaffold fabrication [1]. In addition, relatively recently, the possibility of piezoelectric polymers for tissues recovery has been investigated [2]. Piezoelectric polymers are materials that can generate electrical charges in response to applied mechanical stress. Electrically charged surfaces influence on cell behavior, e.g. cell growth, adhesion and morphology of different cell types [3]. Poly(3-hydroxybutyrate) (PHB) is a promising biodegradable polymer for regenerative medicine that possesses piezoelectric properties, but the piezoelectric response of PHB is relatively low in comparison with other piezoelectric polymers as PVDF or piezoceramics [4]. In order to improve the piezoelectric response of PHB electrospun scaffolds, conductive polyaniline (PANi) can be added to PHB solution before the scaffolds fabrication process [5]. Nevertheless, such scaffolds have the hydrophobic surface, thereby limiting their successful application in bone tissue engineering [6]. To improve these properties, calcium phosphate (CaP) coatings are proposed, since CaP is the major component of the inorganic phase of bone tissue and its presence on the surface of the scaffolds can significantly increase the bioactivity of the material, thus, resulting in the repair of damaged bone tissue [7]. For CaP coating deposition, a soaking was used.

To prepare CaP coating onto fiber scaffold surface, a simple and low cost method, such as soaking can be successfully utilized [8]. Since the deposition is carried out in the media, this method allows to form a coating on the entire surface of the porous electrospun scaffold, unlike other deposition methods, such as, for example, physical vapor deposition. Therefore, the aim of this study is to deposit CaP coating on the porous piezoelectric electrospun scaffolds and to study the influence of the coating on the scaffolds wettability.

2. Materials and methods

2.1. Materials

Polycaprolactone (PCL, Mn = 80,000 g/mol), poly(3-hydroxybutyrate) (PHB, Mw = 300,000 g/mol) Polyaniline (PANi, emeraldine salt, Mw > 15,000 g/mol), calcium chloride ($ZnCl_2$), disodium hydrogen phosphate (Na_2PO_4) and ethanol 96 % (C_2H_5OH) were purchased from Sigma-Aldrich, Germany. Chloroform ($CHCl_3$) was purchased from EKOS-1, Russia.

2.2. Fabrication of electrospun scaffolds

PCL (9 wt.%) and PHB (6 wt.%) electrospun solutions were prepared by dissolving polymers in the $CHCl_3$. The solutions for composite PHB/PANi scaffolds were prepared by doping 1, 2 and 3 wt.% of PANi to PHB powder before being dissolved. Polymers were dissolved in an ultrasonic bath during 3 hours. A syringe pump (Aitecs 2016, Lithuania) was used to feed the solutions into the needle tip (inner diameter of 0.58 mm). Electrospinning was performed at room temperature, 6.5 kV of the applied voltage, 1.5 mL/h injection flowrate and 600 rpm of the cylindrical collector rotation speed.

2.3. CaP deposition process

The CaP formation on the scaffolds was carried out by an alternate soaking process and improved soaking process. The procedure in detail is reported elsewhere [8]. The calcium chloride and disodium hydrogen phosphate were dissolved in deionized water and in mixed solvent of ultrapure water and ethanol (25 v/v%), and with final concentrations of 0.5 M and 0.3 M, respectively. The ethanol was added to reduce the surface tension of the resulting solution. The samples were soaked into calcium solution and then rinsed in ultrapure water, further soaked into phosphate solution, and then rinsed, indicating one cycle for the soaking process. The samples were soaked for 10 minutes during the first cycle and 5 minutes during subsequent cycles. After the deposition process, the samples were dried in oven at the temperature of 50 ºC for 2 hours.

2.4. Characterization of the fibrous scaffolds

The morphology of the scaffolds was characterized by scanning electron microscopy (SEM) (FEI Quanta 250 FEG ESEM, USA) operated at 8 kV. The average fiber diameter of the scaffolds was calculated by measuring 100 different fibers from each type of scaffolds using the software package, ImageJ. To study the phase composition and structure, X-ray diffraction was used (D8 Advance Bruker, Germany) with Cu Kα radiation ($\lambda = 0.154$ nm) in the 2θ range from 5° to 90° with a step size of 0.01° at 40 kV and 40 mA. Before XRD measurements, all samples were fixed on the tesa-film which then affected on the obtained patterns. The molecular bonds of the samples were studied using Fourier transform infrared (FTIR) spectroscopy (ALPHA FTIR Spectrometer, Germany) in the frequency range from 600 to 4000 cm^{-1} with a step size of 4 cm^{-1}. Water contact angle (WCA) in air was measured using the sessile drop method via a contact angle analyzer (OCA 15 Plus Data Physics Instruments GmbH, Germany) at room temperature. A minimum of 5 droplets (5 μL, 1 μL/s) of ultrapure Milli-Q water were examined for each sample.

3. Results and discussions

According to SEM results (Figure 1A-E), the morphology of electrospun scaffolds had a porous fibrous structure. The average fiber diameter of the scaffolds varied from 2.2±0.4 μm to 5.5±1.5 μm for

PHB/PANi2% and PCL scaffold, respectively (Figure 1F). The WCA measurements revealed the hydrophobic surface (WCA>90°) for all scaffold compositions (Figure 1A-E). The addition of PANi to PHB scaffolds (PHB/PANi2% and PHB/PANi3%) resulted in a slight increase in the WCA. This effect has been also observed by Zhou et al. [9].

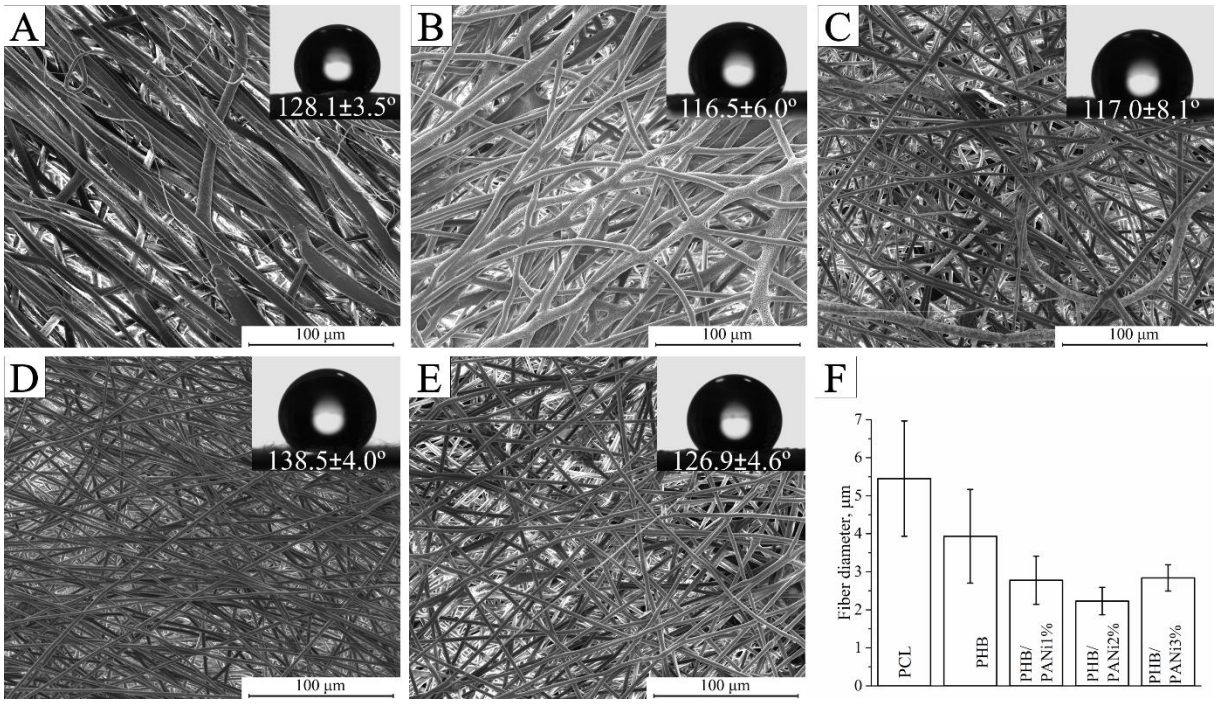

Figure 1. (A-E) SEM images of scaffold surface and insets of their WCA: A – PCL scaffold; B – PHB scaffold; C – PHB/PANi1% scaffold; D – PHB/PANi2% scaffold; E – PHB/PANi3% scaffold. (F) Average fiber diameters of the scaffolds

After investigations of the pristine scaffolds, an influence of CaP deposition parameters, such as the number of deposition cycles and ethanol concentration, on the relative increase in mass and WCA of PHB scaffolds were studied (Table 1).

Table 1. Changes in the relative increase in mass and WCA of PHB scaffolds due to varying the ethanol concentration and number of deposition cycles.

Number of deposition cycles	Ethanol concentration, v/v%	Changes of the relative sample mass, %	WCA, °
3	0	5.1	66.2±6.8
5	0	9.0	50.7±6.6
7	0	9.2	16.8±8.5
3	25	33.6	-
5	25	25.3	-
7	25	48.6	-

The increase of the number of deposition cycles led to increasing of the relative sample mass, probably, due to the increase of the CaP coating thickness/density or coated fibers surface area. CaP deposition from solutions containing 25 v/v% of ethanol also led to significantly increased relative sample mass. This effect can be due to the fact that the surface of the scaffolds with ethanol is better wetted compared to that of water. Besides, the results of WCA measurements revealed that water droplets immediately infiltrated into the scaffolds coated in ethanol, therefore, it was impossible to

correctly determine the initial WCA. However, this effect indicated that the wettability of the scaffolds coated with CaP in ethanol solution was improved in comparison with the scaffolds coated in water solution. Thus, the increase of the number of deposition cycles and the doping of ethanol lead to the decrease of WCA values of electrospun PHB scaffolds. Based on these results, 3 cycles of CaP deposition on the scaffolds from the solutions with 25 v/v% of ethanol was chosen for further studies.

The SEM results and energy-dispersive X-ray spectroscopy (EDS) of the scaffolds with deposited CaP coating presented in the Figure 2. The pores between the fibers were partially filled with the deposited CaP coating. In some places on the surface of the scaffolds, fibers with the finest diameter were completely coated with the CaP coating (Figure 2C-D). In case, when initial fibrous structure of the scaffolds should be preserved, the number of deposition cycles to prevent the formation of such a thick coating on the surface of the scaffolds should be reduced. EDS spectra showed the peaks corresponding to calcium and phosphorus as well as peaks of sodium and chlorine, which are byproducts of the reaction to prepare CaP coating. A high magnification image of the CaP coating (Figure 2F) showed that the coating has a rough surface which is similar with the surface of biphasic CaP bioceramics [10].

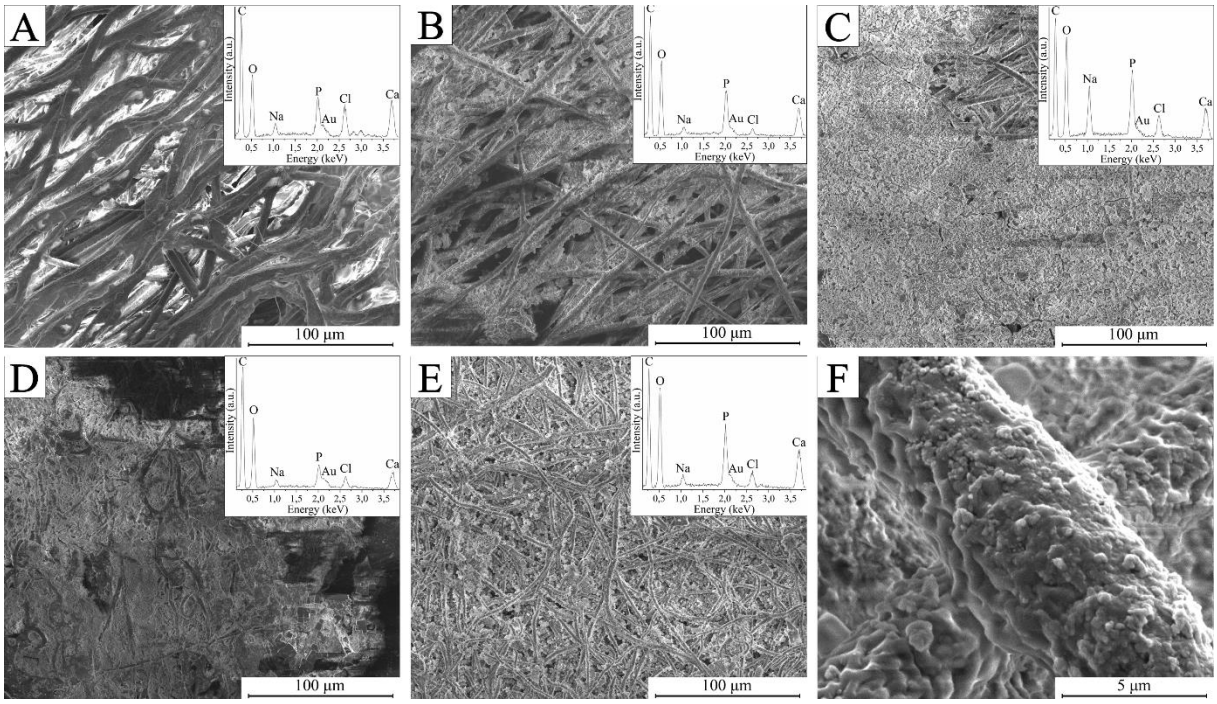

Figure 2. SEM images and EDS spectra of the scaffolds with CaP coating: A – PCL scaffold; B – PHB scaffold; C – PHB/PANi1% scaffold; D – PHB/PANi2% scaffold; E – PHB/PANi3% scaffold; F – high magnification of CaP coating on the PHB scaffold

To characterize the molecular and phase composition of the prepared coating, FTIR and XRD analyses were carried out. FTIR spectra exhibited all the characteristic peaks of PCL and PHB polymers for pristine scaffolds [11]. After the addition of PANi in PHB, new peak at 743 cm^{-1}, which attributes to the C-H out-of-plane bending on 1,2- aromatic rings in PANi structure, was observed [12]. In turn, after CaP deposition, FTIR spectra revealed the presence of typical phosphate peaks (PO_4^{3-}) at 559 (ν4), 959 (ν3) and 1019 (ν3) cm^{-1}, as well as the OH^- peak at 600 cm^{-1} from hydroxyapatite (HA) or amorphous CaP [8].

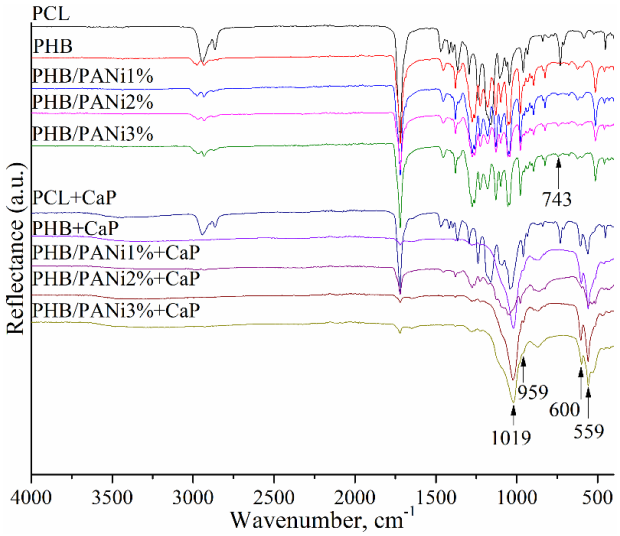

Figure 3. FTIR spectra of electrospun scaffolds before and after CaP coating deposition

The recorded XRD patterns are presented in Figure 4. The main peaks assigned to PCL [13] and PHB (#49-2212 PDF 4+) were observed. After the coating deposition, the peaks around 12°, 21° and 32° assigned to amorphous CaP and HA were observed [8]. Besides, XRD patterns revealed the presence of sodium chloride (NaCl) peak at 32° (JCPDS 5-0628) for the samples after CaP coating deposition. NaCl is a byproduct of the CaP production reaction, which can be removed via additional soaking.

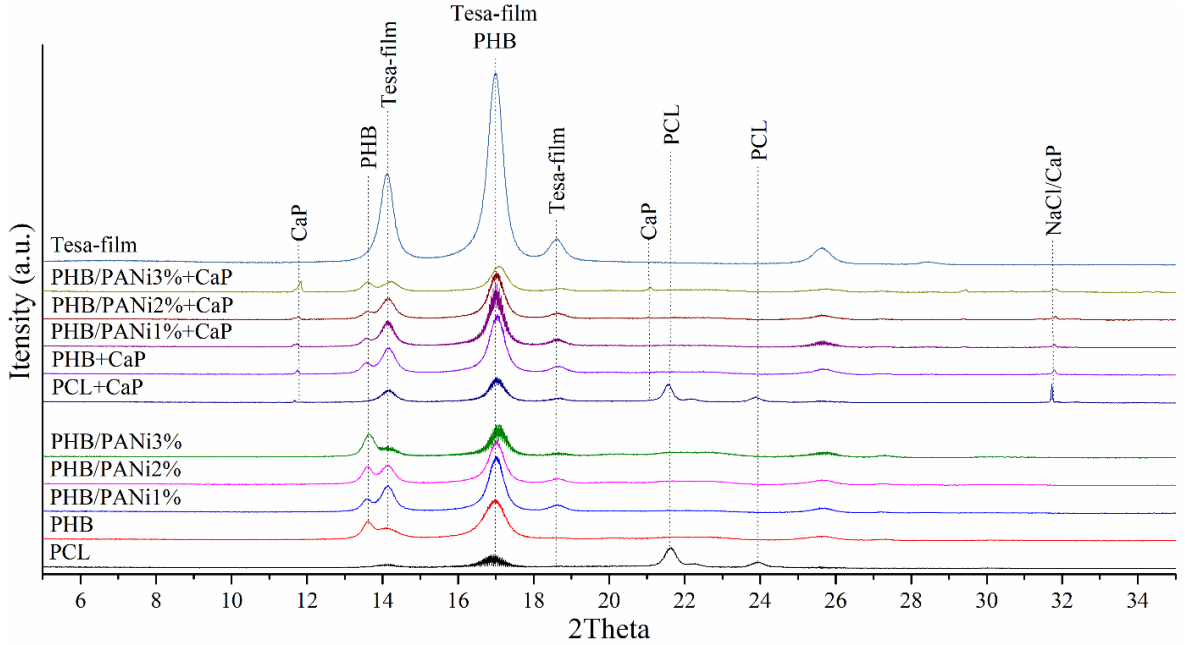

Figure 4. XRD patterns of the electrospun scaffolds before and after CaP coating deposition

Thus, the analysis of FTIR spectra and XRD patterns confirmed the fabrication of a coating corresponding to biphasic CaP bioceramics. Furthermore, this coating successfully improved the wettability of the biodegradable piezoelectric scaffolds.

4. Conclusion

Electrospun hybrid piezoelectric polymer scaffolds were coated with the CaP layer via an improved soaking process. It was shown that the addition of ethanol to the solutions before the deposition as well as increase of the number of deposition cycles from 3 to 7 allow to significantly increase the relative mass of the scaffold from 5 to 49 %, respectively. In addition, all the scaffolds before CaP coating deposition have the hydrophobic surface with WCA of 116.5-138.5°, while CaP coating allows to significantly improve the wettability of all studied scaffolds which allowed infiltration of water droplets into the scaffolds.

Acknowledgements
Andrei Zviagin acknowledges personal financial support from the Leonhard-Euler-Program of DAAD. The financial support from Russian Science Foundation 18-73-10050 is also acknowledged.

References

[1] Rogina A 2014 Electrospinning process: Versatile preparation method for biodegradable and natural polymers and biocomposite systems applied in tissue engineering and drug delivery *Appl. Surf. Sci.* **296** 221-30
[2] Vijaya M 2016 *Piezoelectric materials and devices: applications in engineering and medical sciences* (Boca Raton: CRC Press) p 186
[3] Tandon B, Blaker J J and Cartmell S H 2018 Piezoelectric materials as stimulatory biomedical materials and scaffolds for bone repair *Acta Biomater.* **73** 1-20
[4] Ramier J, Bouderlique T, Stoilova O, Manolova N, Rashkov I, Langlois V, Renard E, Albanese P and Grande D 2014 Biocomposite scaffolds based on electrospun poly (3-hydroxybutyrate) nanofibers and electrosprayed hydroxyapatite nanoparticles for bone tissue engineering applications *Mater. Sci. Eng. C* **38** 161-9
[5] Chernozem R V, Surmeneva M A and Surmenev R A 2018 Hybrid biodegradable scaffolds of piezoelectric polyhydroxybutyrate and conductive polyaniline: Piezocharge constants and electric potential study *Mater. Lett.* **220** 257-60
[6] Timin A S, Muslimov A R, Zyuzin M V, Peltek O O, Karpov T E, Sergeev I S, Dotsenko A I, Goncharenko A A, Yolshin N D and Sinelnik A 2018 Multifunctional scaffolds with improved antimicrobial properties and osteogenicity based on piezoelectric electrospun fibers decorated with bioactive composite microcapsules *ACS Appl. Mater. Interfaces* **10** 34849-68
[7] Bose S and Tarafder S 2012 Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: a review *Acta Biomater.* **8** 1401-21
[8] Watanabe J and Akashi M 2007 Formation of hydroxyapatite provides a tunable protein reservoir within porous polyester membranes by an improved soaking process *Biomacromolecules* **8** 2288-93
[9] Zhou X, Zhang Z, Men X, Yang J, Xu X, Zhu X and Xue Q 2011 Fabrication of superhydrophobic polyaniline films with rapidly switchable wettability *Appl. Surf. Sci.* **258** 285-9
[10] Lobo S E, Glickman R, Da Silva W N, Arinzeh T L and Kerkis I 2015 Response of stem cells from different origins to biphasic calcium phosphate bioceramics *Cell Tissue Res.* **361** 477-95
[11] Liau C P, Bin Ahmad M, Shameli K, Yunus W M Z W, Ibrahim N A, Zainuddin N and Then Y Y 2014 Preparation and characterization of polyhydroxybutyrate/polycaprolactone nanocomposites *Sci. World J.* **2014** 9
[12] Tang J, Jing X, Wang B and Wang F 1988 Infrared spectra of soluble polyaniline *Synth. Met.* **24** 231-8
[13] Balu R, Kumar T, Ramalingam M and Ramakrishna S 2011 Electrospun Polycaprolactone/Poly (1, 4-butylene adipate-co-polycaprolactam) blends: Potential biodegradable scaffold for bone tissue regeneration *J. Biomater. Tissue Eng.* **1** 30-9