INCORPORATING ANTIBACTERIAL CLAY MINERALS INTO NANOFIBROUS LAYERS BY ELECTROSPINNING

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Abstract

The aim of this study was to describe electrospinning method for preparation of self-supporting homogenous nanofibrous layers with a presence of pristine clay minerals and clay minerals containing antibacterial agent chlorhexidine acetate in their interlayer space. One clay and two polymers were used. Vermiculite was used as obtained with size fraction < 40 μm. Chlorhexidine/vermiculite was prepared through the intercalation process. Nanofibers were made of hydrophobic polymers, polyurethane (PU) and polycaprolactone (PCL), to gain water stable and durable layers. Polymer solutions for electrospinning contained 2, 5 and 8 wt. % (according to the total weight of the solution) of clay or chlorhexidine/clay. These suspensions were homogenized and immediately spun using 4SPIN LAB. Self-supporting homogenous fibrous layers were prepared under the same electrospinning conditions as neat polymers and were further analysed. Morphology was characterized using scanning electron microscopy (SEM) and presence of clay minerals in the layer was confirmed by digital microscopy and EDX mapping. From SEM images, diameter of the fibres was evaluated. Fibre diameter decreased after adding the clay and was ranging from 600 nm to 1200 nm. Clay particles were present both in fibres and on the surface. Antibacterial chlorhexidine was found in the vermiculite matrix as well as separately in the fibres (result of imperfect intercalation).

Keywords: Clay, nanofibers, electrospinning, chlorhexidine, antibacterial

1. INTRODUCTION

Electrospinning is a method using an electrostatic field/high voltage to fabricate micro- and nanoscaled fibres. This method is constantly evolving and gains attention in both scientific community and industry. It is versatile method which can find novel applications in diverse fields - bioengineering, biomedicine, sensors, filtration or electronics. Electrospun fibres have unique properties such as high specific surface area and large number of smaller pores. There is a large variety of polymer materials which could be used to prepare nanofibers, from natural and to synthetic ones. There is a possibility to prepare simple layers made of a single polymer as well as a composite, which is composed of e.g. several polymers with some organic or inorganic components [1,2]. In this study two polymers were used - polyurethane (PU) and polycaprolactone (PCL). Both are hydrophobic, water stable and biodegradable and are often used for bone tissue engineering, cardiac grafts, wound dressing or for engineering of blood vessel substitutes [1]. Clay minerals are hydrated aluminium phyllosilicates with layered structure. They may undergo process called intercalation, which allows ion exchange with functional molecules or particles. Vermiculite belongs to 2:1 group, its basic structure consists of a magnesium octahedral sheet between two silica tetrahedral sheets. Interlayer space is filled with hydrated cations or water [3]. Since the interlayer space can be easily modified, vermiculite has a great potential in the fields of drug carriers [4-6], tissue engineering and regenerative medicine [7]. In several studies, nanocomposite chlorhexidine acetate/vermiculite was prepared through intercalation process and characterized. Chlorhexidine (CH) was

stable in clay matrix (outflow <5 %) in aqueous solution with conditions close to those in human body [6]. Composite had very good antibacterial effect against *Escherichia faecalis*, *Escherichia coli* and *Staphylococcus aureus*, and showed poor results against *Pseudomonas aeruginosa* [4-6]. Vermiculite was also tested for toxicity on mammal model organism (mice). Neither local nor systemic reaction was observed, therefore toxicity can be excluded [5]. Studies focused on electrospinning of polymers with added clay often investigate the effect of clay content on morphology and processability of prepared nanofibers [8, 9, 10]. Nanofibers with added clay had enhanced filtrating ability [8], increased thermal stability [9,11], improved tensile strength [10, 11] or chemical stability [12]. Wide range of polymers was used with clay such as montmorillonite and hydroxyapatite. After intercalating drugs into the interlayer space nanofiber mats obtained antibacterial properties [13,14] with drug sustained release activity [13]. Prepared materials have a potential in wound healing applications [12], drug delivery systems [13,14], as an antioxidant product in food and pharmaceutical industries [15] or filtration [16,17].

The aim of this study was to find optimal conditions for preparation of self-supporting homogenous nanofibrous layers with a presence of pristine vermiculite and composite chlorhexidine acetate/vermiculite since there is no similar research. Further to characterize morphology of prepared samples using different methods (digital microscopy, scanning electron microscopy, EDX mapping). Prepared materials might have a great potential as inexpensive drug delivery system.

2. MATERIALS AND METHODS

2.1. Preparing solutions for electrospinning

Polycaprolactone (PCL) and chloroform were purchased from Sigma Aldrich. Polyurethane (PU), *N,N*-dimethylformamide (DMF) and tetrahydrofuran (THF) were purchased from BASF Polyurathanes GmbH, Lachner and Honeywell, respectively. Vermiculite from Brazil was used with particle size fraction < 40 μm. Composite chlorhexidine acetate/vermiculite (1:1) was prepared through intercalation process. Both clay samples were provided by Nanotechnology Centre (VSB) and used as received. Two basic polymer solutions were prepared. First one was the 10 wt. % PU dissolved in DMF and THF (1:1). PU was added under constant stirring and solution was stirred until homogenous. The second one was the 10 wt. % PCL dissolved in DMF and chloroform (2:8). PU was added under constant stirring and solution was stirred until homogenous. Suspensions for electrospinning contained 2, 5 and 8 wt. % of vermiculite (Ver) or chlorhexidine/vermiculite (CH/Ver). The weight percent of added clay was with respect to the weight of the final suspension. Each suspension was stirred first in hand and then placed into stand with coolant (water and ice) and homogenized for 5 min by homogenizer IKA® T25 digital Ultra Turrax (5600 rpm, 15 s on, 5 s pause). Prepared suspension was immediately spun.

2.2. Electrospinning conditions

Electrospinning was performed using the 4SPIN LAB device (Contipro, Czech Republic). Two moving needles were used as an emitter and rotating cylinder of width 10 cm covered by substrate (aluminium foil) was used as a collector. The collector rotating speed was 1000 rpm. A distance between the emitter and the collector was set to 18 cm. Each sample have been spun for 120 min, but after 60 min the suspension was changed for newly homogenized one. Feed rate was 20 μl/min for all suspensions except 8 wt. % of CH/Ver in PCL where feed rate had to be increased to 30 μl/min. Applied voltage varied depending on polymer - 25 kV was used for the PU suspensions and 20 kV for the PCL suspension. Ambient conditions were approximately same for all spinning processes - relative humidity was between 30-43 % and temperature was between 23-28 °C.

2.3. Characterization of materials

A digital microscopy was used to confirm the presence of vermiculite in prepared nanofibrous layers, as vermiculite has yellow-brown to green-brown colour and thus was easily distinguishable from polymer. Images

were taken in transmission using the digital microscope VHX Multi Scan Keyence with Keyence ZS-260 lens (magnitude 200x-2000x). All samples were removed from the substrate and placed onto a glass microscope slide. No other treatment of samples was performed. Morphology was characterized by scanning electron microscopy (Zeiss Ultra Plus, Carl ZEISS, applied voltage 3,5 kV). Prior analysis, all samples were coated with a thin layer of chrome. From SEM images (1000 x), diameter of fibres was evaluated using ImageJ 1.48 (average of 30 fibres). Presence of vermiculite and chlorhexidine in the layer was also confirmed by energy-dispersive X-ray spectroscopy mapping (EDX mapping, Zeiss Ultra Plus, Oxford X-MAXN 80). The measurement was conducted at a magnification of 1800, the accelerating voltage was set to 10 kV and the energy range was between 0-10 keV. The intensity of the characteristic X-ray peaks was imaged - for vermiculite (aluminium K-series - 1.557, 1.487, 1.486, magnesium K-series - 1.302, 1.253, silica K-series - 1.836, 1.740, 1.739) and chlorhexidine (chlorine K-series 2.816, 2.622, 2.620).

3. RESULTS AND DISCUSSION

Self-supporting homogenous nanofibrous layers were successfully prepared from above-mentioned suspensions via electrospinning. For clarity, the notation *wt.%Clay_polymer* will be used in the following text, e.g. 2Ver_PU refers to 2 wt.% of vermiculite in polyurethane or 8CH/Ver_PCL refers to 8 wt.% of chlorhexidine/vermiculite in polycaprolactone. Control samples were also prepared (further marked as PU and PCL). Both SEM imaging and EDX mapping were used strictly for qualitative analysis of the composite, to characterize the morphology and whether the clay or CH/clay additive was incorporated into the polymer matrix.

3.1. Digital microscopy

The **Figure 1** shows the differences between 2, 5 and 8 wt.% Ver in PCL (a-c) - the increasing clay content in the nanofibrous layers is evident. All other samples followed the same trend. Some agglomerates in **Figure 1c** exceed the size of 100 μm even though vermiculite size fraction was under 40 μm. This trend was visible especially in samples with PCL. Suspensions with PCL were more viscous, so the viscosity probably affects creation of agglomerates or aggregates.

Figure 1 Digital microscopy images of PCL nanofibers with vermiculite (magnification 200x): a) 2Ver_PCL, b) 5Ver_PCL, c) 8Ver_PCL.

3.2. Morphology of the fibres

Neat polymer nanofibers were smooth and continuous with inhomogeneous diameter which was (1416 ± 522) nm for PCL and (1629 ± 311) for PU. The **Figure 2** shows selected SEM images (magnification 5000x) of 5Ver_PCL (a), 5CH/Ver_PCL (b), 5Ver_PU (c), and 5CH/Ver_PU (d). Clay particles were probably incorporated into the PCL fibres as there are not many visible particles on their surface. PU fibres are thinner than PCL fibres and there are more clay particles visible on the surface of the fibres. Diameter of clay/PU fibres is also more homogeneous compared to clay/PCL fibres. The **Figure 3** shows a mean diameter of the fibres with a standard deviation. Diameter of the fibres decreased after adding the clay. The highest diameter was

observed at samples with 2 wt. % of clay, then diameter decreased at samples with 8 wt. %, and samples with 5 wt. % of clay had the lowest diameter. But samples with Ver in PCL did not meet this correlation, the highest diameter was at 5Ver_PCL and lowest at 8Ver_PCL. It was observed that electrospun fibres were thicker the more clay they contained [8]. This thickening was caused by higher viscosity of electrospun solution - fibres haven't stretched so much. There is also another factor that affects the final diameter of the fibres. Vermiculite is a clay with nonzero layer charge, so by adding the clay suspension had higher conductivity and therefore was more stretched in the electric field [10]. In this case both principles seemed to combine, suspension with 2 and 5 wt.% stretched more thanks to higher conductivity but suspension with 8 wt.% of clay were too viscose to stretch.

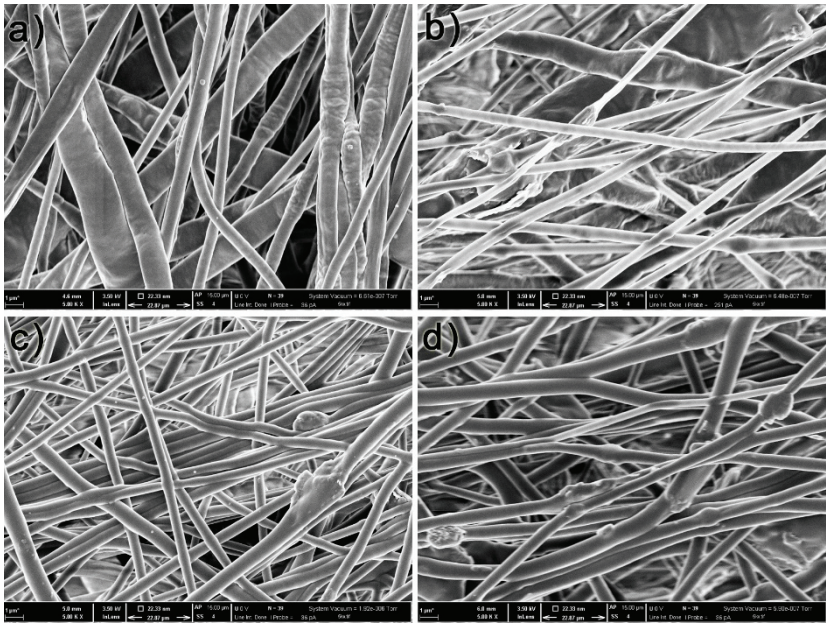

Figure 2 SEM images of PCL or PU nanofibers with vermiculite or chlorhexidine/vermiculite (magnification 5000x): a) 5Ver_PCL, b) 5CH/Ver_PCL, c) 5Ver_PU, d) 5CH/Ver_PU.

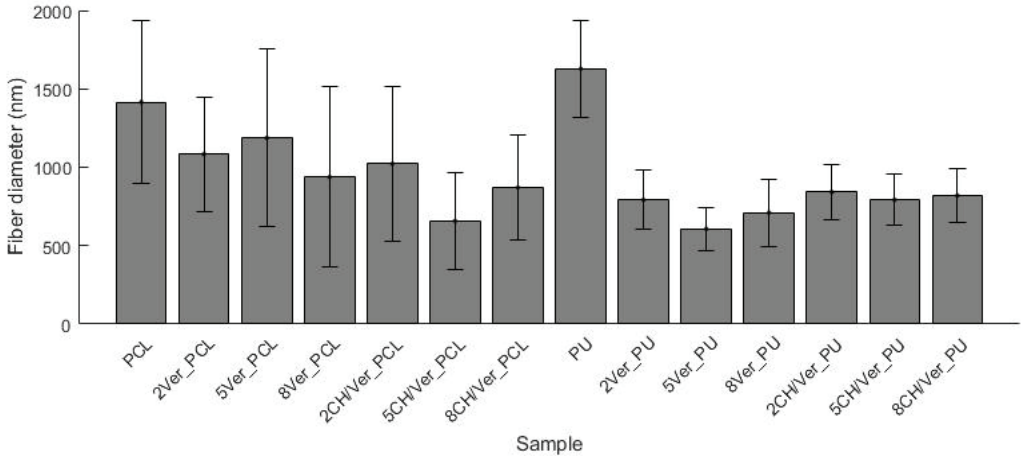

Figure 3 Mean fiber diameter (30 fibres) with standard deviation.

3.3. EDX mapping

In EDX maps (**Figure 4**), major elements contained in vermiculite are blue-coloured (silicon, aluminium and magnesium) and chlorine (recognizable element for chlorhexidine) is red-coloured. Clay particles were mostly

concentrated along the fibres and some bigger particles (size exceeding fibre diameter multiple times) were between the fibres. Chlorhexidine was not always situated with vermiculite, there were many spots, where only chlorhexidine or only vermiculite could be found. This means that the intercalation process was not perfect and not all the chlorhexidine was intercalated into interlayer space of vermiculite. These findings fit with results of Holešová et. al. [6] that even the highest used chlorhexidine concentration above CEC value (cation exchange capacity) did not lead to full chlorhexidine intercalation into vermiculite matrix and chlorhexidine is also anchored on clay surface.

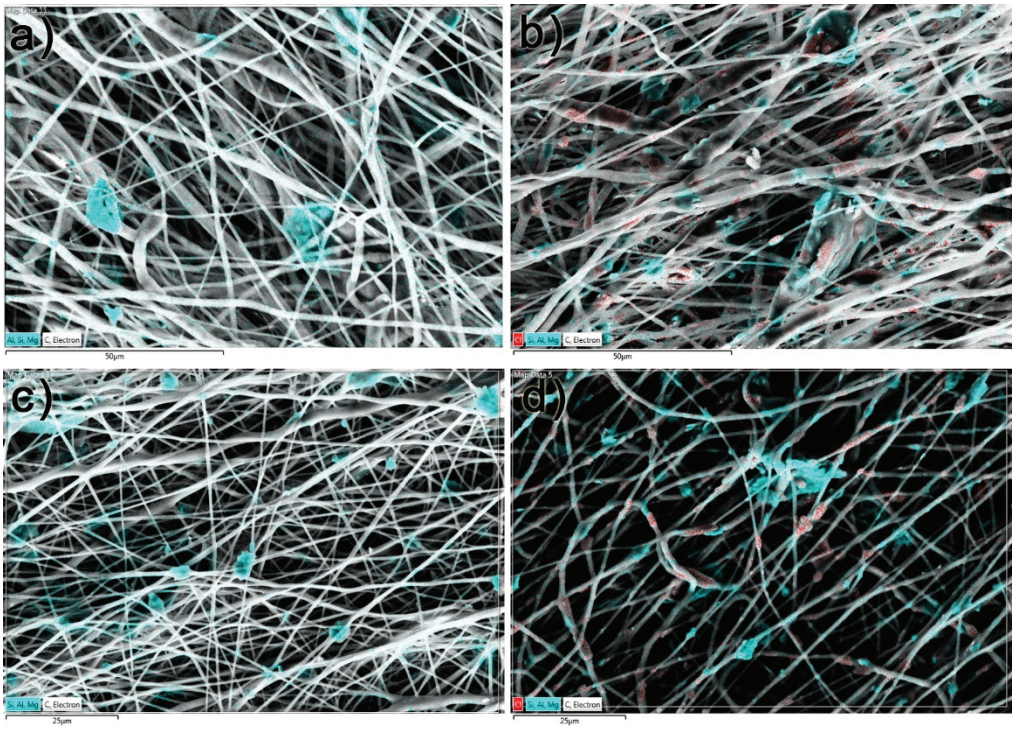

Figure 4 EDX maps, blue coloured: Si, Al, Mg, red coloured: Cl: a) 5Ver_PCL, b) 5CH/Ver_PCL, c) 5Ver_PU, d) 5CH/Ver_PU

4. CONCLUSION

Nanofibrous layers with different weight concentration of vermiculite or chlorhexidine/vermiculite were successfully prepared under the same electrospinning conditions as neat polymers. Fibre diameter decreased after adding the clay and was 600-1200 nm. Clay content in the layer increased with concentration and clay particles were incorporated into the polymer fibres. Antibacterial chlorhexidine was found in the vermiculite matrix as well as separately in the fibres (as a result of imperfect intercalation). Prepared CH/Ver/polymer layers seem to be promising, relatively inexpensive, and easy-to-produce material in the field of antibacterial applications.

ACKNOWLEDGEMENTS

This paper was created in cooperation with Contipro a.s. and in the frame of the SP2018/71 project while financially supported by the Ministry of Education, Youth and Sports of the Czech Republic.

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