Nano-modified non-woven filter fabrics capable of holding viruses

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Introduction

The method of producing nanofibres in the electric field, commonly referred to as electrospinning, was already known as early as in 1934, and it was developed and patented by Formhals. He was the first person to describe the process of obtaining fibres from cellulose acetate. Until the early 1990s the electrospinning process was practically unused for industrial purposes. It was due to insufficient knowledge of phenomena occurring during the electrospinning process, low efficiency and the lack of possibility to control the whole process. The early 1990s saw a sudden development in nanotechnology, and the nanofibre electrospinning process was adopted by many research institutes. A few research teams presented the possibility of producing nanofibres by electrospinning from the polymer solutions and molten polymers [1÷5].

Comparing different methods for obtaining fibres using conventional techniques (*wet spinning, dry spinning, melt blown*) [6÷8], the electrospinning from a solution allows for obtaining fibres much lower in diameter, in fact as low as 100 nm. This is a new method, allowing for obtaining nanofibres. By using an appropriate process for collecting nanofibres, it is possible to, depending on actual needs, obtain nanofibres in dry spinning or in a water bath, which provides huge possibilities for industrial applications, i.a. to produce membranes, biomedical structure components (used in tissue engineering, administering drugs, artificial blood vessel organs), special protective fabrics, in separation industry, to reinforce composites, etc. [9].

In addition, nanofibres can be treated with nanoparticles or other biocides providing them with specific properties, e.g. bactericidal effect.

Chlorhexidine used in research is a synthetic antiseptic that exerts a strong effect on Gram-positive and Gram-negative bacteria, moulds, yeasts and some viruses. It is used for disinfecting skin, mucous membranes and surgical instruments. Its antibacterial properties consist in damaging the bacterial cell membrane. Chlorhexidine is used mainly in dentistry, but also as an antibacterial agent in dressings [10÷14].

If we face a risk of pandemic caused by one of influenza viruses, it is essential to enhance the personal protection (protective masks), or collective protection (filters installed in the ventilation systems of public utility buildings) by using antibacterial agents.

Experimental part

Materials and their preparation

The basic material for obtaining nanofibres was acetylcellulose. In the studies, acetylcellulose with a molecular mass of approx. 270 thousand was used.

Acetylcellulose was converted to the form of a solution with chloroform used as a solvent. For producing nanofibres, the solution must be perfectly clear, without any solid contaminations. To obtain such a solution, acetylcellulose after dissolving was centrifuged using a laboratory centrifuge at 2,500 rpm.

Research equipment

Producing nanofibres via electrospinning from a solution requires specialty equipment. A high voltage stabiliser equipped with a stepless output voltage adjustment ranging from 0 to 50 kV, with positive polarity, and stepless adjustment of this current ranging from 0 to 2000 μA was used. Another key equipment component is a feeding electrode, made of high quality glass and a metal capillary, fitted with a thermostat allowing for the constant temperature of the nanofibre production process. Another component is a rotary reception electrode equipped with replaceable reception plates – electrodes (Photo. 1), with a stepless rotational speed adjustment.

Photo. 1. Reception electrode

The whole equipment is located in an air conditioned and ventilated room allowing for keeping constant, proper temperature parameters for the nanofibre electroforming process. Another important component is a low-flow syringe pump used to apply the spinning solution delivered to the feeding electrode. All the equipment subassemblies were located in a chamber specially designed for this purpose, made of a material featuring strong ambience insulating properties, as well as providing safety for persons operating and controlling the whole electrospinning process.

Research methods

In the case of producing nanofibres from acetylcellulose, the method of electrospinning from a solution was adopted.

To obtain nanofibres proper technical parameters have been determined for a specific polymer solution used. The basic parameter is the concentration of solution, at which the nanofibre electroforming process will be most efficient. It should be noted that the electrospinning solution must include a proper solvent, the one that evaporates easily during the nanofibre production process. While optimising the concentration of electrospinning solution, a rotary rheometer Anton Paar MCR 101 fitted with an electrorheology unit was used, allowing for accurate determination of any changes occurring in the electrospinning solution after placing it in the electric field applied during the nanofibre electroforming process.

Once the composition of the electrospinning solution is determined, proper process technical parameters were defined. The most important one includes voltage applied to the nanofibre production capillary. The voltage should be set based on a photo taken with an electron microscope, allowing for defining a proper structure of nanofibres obtained and their diameter. To reduce the diameter of obtained nanofibre, it is possible to reduce the diameter of the electrode capillary feeding the spinning solution. The quality of nanofibre obtained depends also on the distance from the feeding electrode to the reception electrode for the nanofibres obtained. When changing this parameter, remember to adjust voltage, as a larger distance between the electrodes requires higher voltage to maintain constant electric field properties.

While producing nanofibres from a natural polymer, the following parameters for the spinning solution were assumed (Tab.1).

Table 1

Acetylcellulose solution parameters

Spinning solution concentration	5%
Solvent	chloroform
Spinning solution temperature	20°C
Chamber temperature	20°C
Inner capillary diameter	0.8 mm
Voltage	10.0 – 25.0 kV
Distance between electrodes	150 mm
Nanofibre application time	30 minutes

Nanofibres were applied onto the surface of cellulose filter discs, used as a carrier. Nanofibres obtained by this method were treated with selected antibacterial agents.

Using chlorhexidine in the process of acetylcellulose nanofibre-based filters allows for producing a material whose high mechanical properties will be enriched with bacteriostatic and bactericidal properties. The acetylcellulose nanofibres containing chlorhexidine were obtained by using two methods: a direct method by electrospinning of acetylcellulose fibres with chlorhexidine added and an indirect method, by incubating acetylcellulose fibres in titanium triethanolamine solved in isopropanol at room temperature. The titanium-nanofibre binding was fixed at 110°C, and then gently rinsed in distilled water. Dry acetylcellulose nanofibres with the titanium triethanolamine bound was placed in the solution of chlorhexidine digluconate. The chlorhexidine digluconate – titanium binding was fixed at 90°C, and then it was rinsed with distilled water and dried at room temperature.

To check the biocidal properties of the modifier used, the microbiological tests of: acetylcellulose nanofibres without chlorhexidine added, electrospun acetylcellulose nanofibres with chlorhexidine and the chlorhexidine digluconate modified acetylcellulose nanofibres were performed. Incubation with *Bacillus subtilis* was conducted at 37°C for 18 hrs.

Results

The quality of nanofibres obtained at various electrospinning process parameters was determined using the photos of the surface structure taken with an electron microscope (Photo 2÷10).

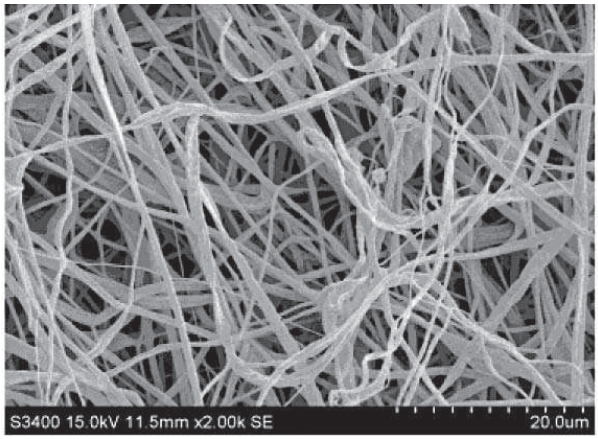

Photo. 2. SEM photo of acetylcellulose nanofibre surface

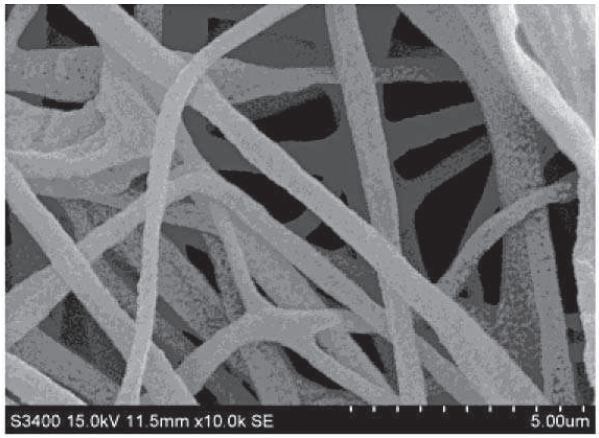

Photo. 3. SEM photo of acetylcellulose nanofibre surface

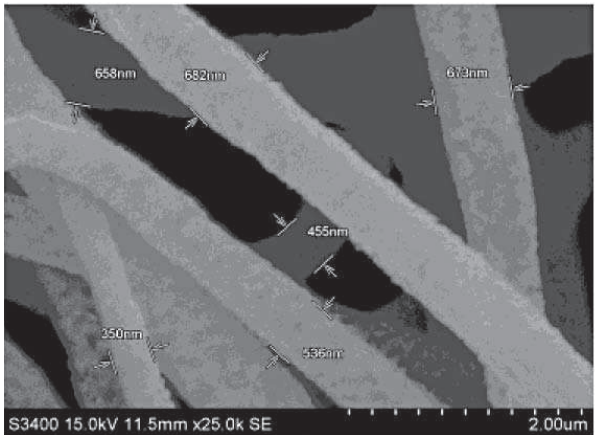

Photo. 4. SEM photo of acetylcellulose nanofibre surface

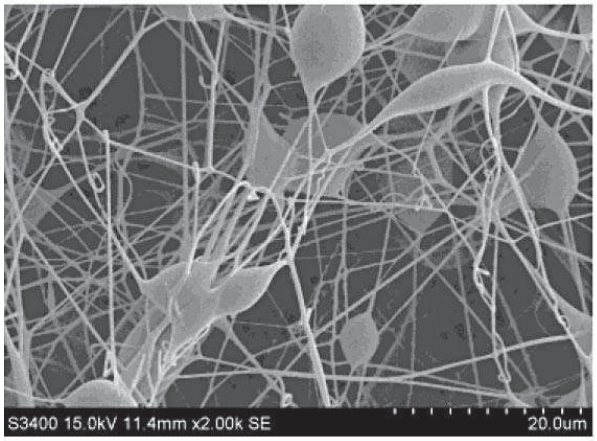

Photo. 5. SEM photo of acetylcellulose nanofibre surface

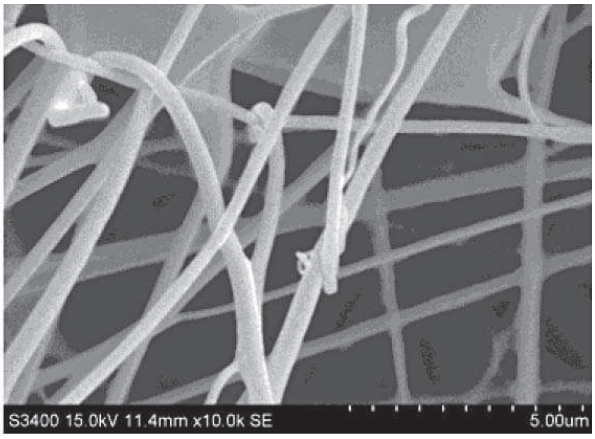

Photo. 6. SEM photo of acetylcellulose nanofibre surface

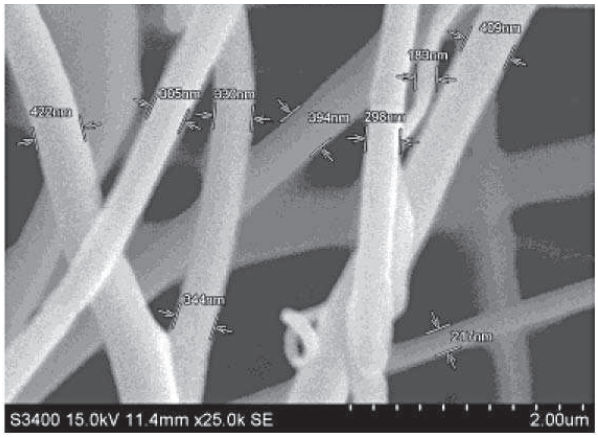

Photo. 7. SEM photo of acetylcellulose nanofibre surface

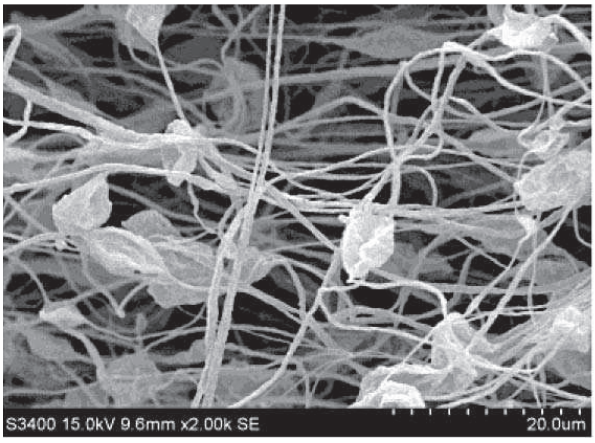

Photo. 8. SEM photo of acetylcellulose nanofibre surface

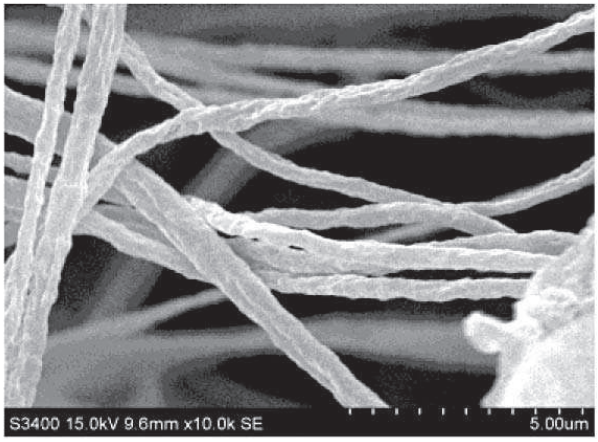

Photo. 9. SEM photo of acetylcellulose nanofibre surface

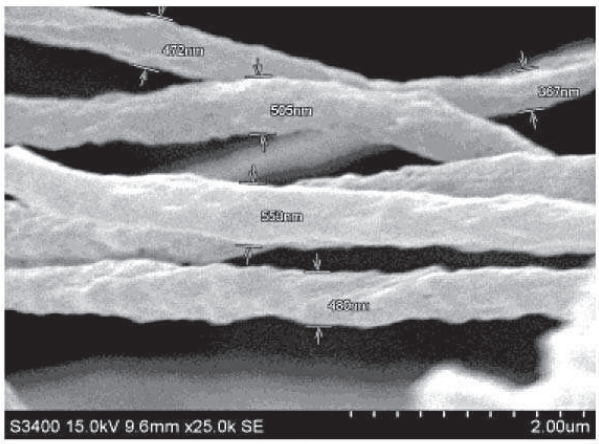

Photo. 10. SEM photo of acetylcellulose nanofibre surface

Photos 11÷13 present the results of microbiological tests of chlorhexidine modified acetylcellulose nanofibres versus a non-modified nanofibre sample.

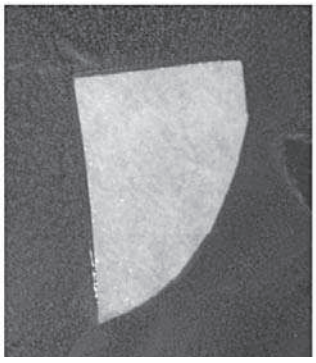

Photo. 11. No zone of inhibition of bacterial growth after control acetylcellulose nanofibre incubation with *Bacillus subtilis* bacteria

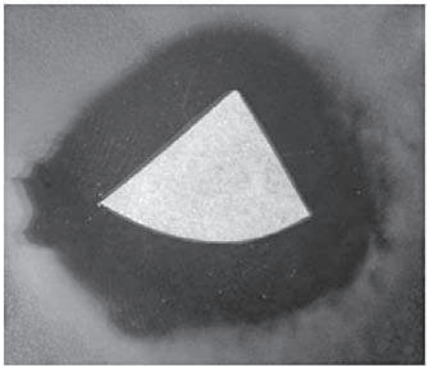

Photo. 12. Zone of inhibition of bacterial growth after the incubation of acetylcellulose nanofibres electrospun with chlorhexidine with *Bacillus subtilis* bacteria

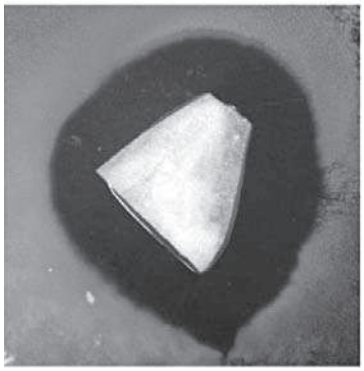

Photo. 13. Zone of inhibition of bacterial growth after the incubation of chlorhexidine digluconate modified acetylcellulose nanofibres with *Bacillus subtilis* bacteria

In addition, research was performed to assess the virus barrier properties of filter discs prepared. First, gene-based structures were prepared (expression vector – pCMV Fut plasmid) containing characteristic marker sequences. The tests were aimed at assessing the efficiency of binding the genetic material by acetylcellulose nanofibres. To do this, 2ml samples of aqueous solutions containing 20ng plasmids in 1μl solution were prepared. The samples were filtered through modified and non-modified filter discs, at a solution flow rate of 100μl per minute. Once the filtering process was completed, the filter discs were cut and incubated in 200μl TE (tris (hydroxymethyl) aminomethane with EDTA) buffer at 37°C for one hour. The concentration of plasmids was measured using a NanoDrop® spectrophotometer. The tests showed a demonstrably higher DNA binding efficiency by the chlorhexidine containing discs.

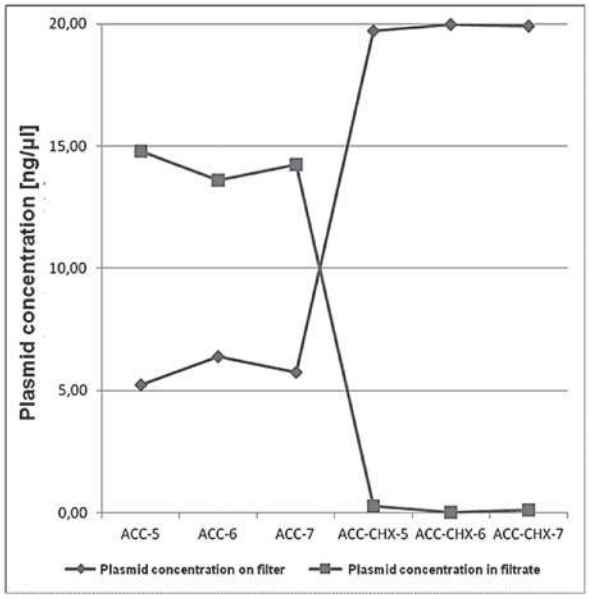

Fig. 1. Result of measuring plasmid on filter and in filtrate. ACC – acetylcellulose filter discs without chlorhexidine, ACC-CHX – chlorhexidine modified acetylcellulose filter discs

Discussion of results

The SEM pictures of nano non-woven fabric surface show that the diameter of nanofibres ranges from 200 to 600 nm. The distribution of nanofibres on the cellulose filter paper is uniform on the whole surface.

The microbiological tests of chlorhexidine modified nanofibres performed show a high inhibition of bacterial growth for all modification methods. No diffusion zone around the discs made only of acetylcellulose nanofibres constituting a control was found.

Initial tests for assessing the barrier properties of nanofibres as antiviral filters showed that they considerably inhibit plasmid penetration into the filtrate at the designed testing unit.

Summary and conclusions

Modifying filters by using nanofibres reduces the diameter of pores, thus making the filtration process more efficient.

Applying active compounds such as: chlorhexidine onto the surface of nanofibres, or incorporating the compound during the electrospinning process, induces antibacterial effect.

Chlorhexidine modified acetylcellulose nanofibres effectively capture plasmids on their surfaces.

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