



POSTER PRESENTATIONS

Obtained results as presented in (Table 2) with the lowest concentration of ZnO on washed viscose fibres were tested for antimicrobial efficiency and they confirmed very good percent of reduction of *Escherichia coli* which was more than 99%.

CONCLUSIONS

Presented material (sample 1, 2, 3, 4, 5, 6, 7, 8) was very well covered with ZnO, and ZnO/silica. The excellent percent of reduction of *Escherichia coli* were confirmed for samples of washed viscose.

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REFERENCES

1. Yudanov TN, Reshetov IV. Modern wound dressings: manufacturing and properties. Pharm. Chem. J. 2006; 40: 24 – 31.
2. BITENC, Marko, PODBRŠČEK, Peter, CRNJAK OREL, Zorica, CLEVELAND, Michael A., PARAMO, Jorge Antonio, PETERS, Raul M., STRZHEMECHNY, Yuri M. Correlation between morphology and defect luminescence in precipitated ZnO nanorod powders. Cryst. growth des. 2009, vol. 9, no. 2, str. 997-1001.
3. BITENC, Marko, DRAŽIĆ, Goran, CRNJAK OREL, Zorica. Characterization of crystalline zinc oxide in the form of hexagonal bipods. Cryst. growth des. 2010, vol. 10, no. 2, str. 830-837
4. Zhang L, Jiang Y, Ding Y, Daskalakis N, Jeuken L, Povey M, O'Neill A, York D. Mechanistic investigation into antibacterial behaviour of suspensions of ZnO nanoparticles against *E.coli*. J. Nanopart. Res. 2010; 12: 1625 – 1636.
5. Sawai J. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. J. Microbiol. Methods. 2003; 54: 177 – 182.

ELECTROSPUN NANOFIBERS FOR TOPICAL DRUG DELIVERY

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INTRODUCTION

In the beginning of the XXIst century, which is the great period of biotechnology, one of the largest challenges in the field of pharmaceutical technology is the formulation of biopharmaceuticals, which are generally very sensitive to the physical and chemical properties of their environment. After the biotechnological process biodrugs are in aqueous medium, where many of them have low stability, what can lead low stability in the product as well (1). Therefore it might be very advantageous to design solid forms what provides good stability of biodrugs and easy administration. However, the available techniques, such as freeze drying, have several drawbacks. Another very challenging field for pharmaceutical technologists is formulation of poorly water soluble drugs (2). Recently the number of drug candidates of low water solubility increases continuously due to the applied screening and test methods and due to the limited patentability of molecules with low molecular weight. Preparation of nanofibers by electrostatic spinning has been introduced recently and used successfully in various scientific fields such as composites, filters, tissue engineering and pharmaceutical technology as well (3,4,5). In this work an antiviral protein, a probiotic bacteria and Carvedilol with poor water solubility were used as model drugs to investigate the capabilities of electrospun materials to improve the stability of biodrugs and the dissolution rate of Carvedilol. Besides using this technology the topical drug delivery (creating an orally dissolving web (ODW) for instance) can be feasible.

MATERIALS AND METHODS

Materials

Polyvinyl-pyrrolidone (PVP) (molecular weight: PVP K30: ~ 50000 Da) was kindly provided by BASF (Ludwigshafen, Germany). Carvedilol was originated from Sigma-Aldrich (Budapest, Hungary).

Electrostatic spinning process

The electrostatic spinner used for the experiments was equipped with NT-35 High Voltage DC Supply. The utilized electrical potential on the spinneret electrode was between 10–35 kV which was adjusted during the experiments. A grounded steel plate covered with teflon was used as collector. Polymer solutions were dosed by SEP-10S Plus syringe pump. The experiments were performed at ambient temperature (25°C).

Dissolution test followed by UV-VIS

Dissolution of the Carvedilol from the electrospun mats were performed by Erweka DT6 dissolution tester. Concentrations of the collected samples were measured by Hewlett Packard 8452A type UV spectrophotometer.

Protein analysis

Protein analysis was carried out by Lab-on-a-chip (Laboratory on a chip (LOC)) technique (Agilent bioanalyzer 2100) what performs micro system capillary electrophoresis.

RESULTS AND DISCUSSION

Carvedilol dissolution results

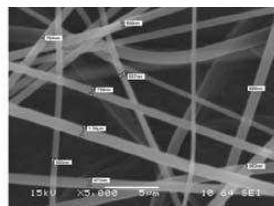


Fig. 1: SEM image of the Carvedilol (12%) loaded PVP nanofibers

SEM image (Fig. 1) confirms that nanofibers were formed with huge surface area which is very promising from the dissolution point of view.

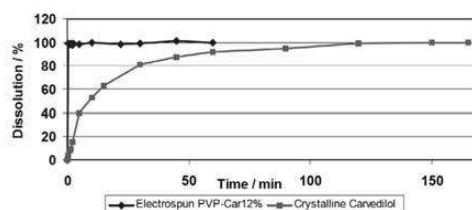


Fig. 2: Dissolution of the Carvedilol samples, Blue: electrospun PVP-Carvedilol (12%), Violet: crystalline Carvedilol

The electrospun mats were dissolved in water immediately as they were immersed in the dissolution media. It means that the drug release was instant (~2 sec.) and the dissolution of Carvedilol was decreased from 2 hours down to few seconds by electrospun nanofibers (Fig. 2).

Protein encapsulation

Generally the biggest challenge during the formulation of proteins is the avoidance of the aggregation. According to our measurements the protein did not aggregate during the electrospinning process. It can be ascribed probably to the gentle treatment and to the protecting action of the PVP



macromolecules. This result means that the formulation of sensitive proteins can be solved this way with avoidance of the aggregation.

Probiotic bacteria encapsulation

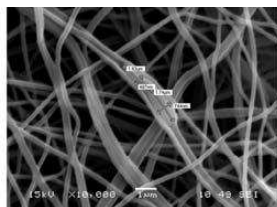


Fig. 3: SEM image of probiotic bacteria embedded in polymer nanofibers

SEM image (Fig. 3) enabled to see how the polymer fiber gets thicker and encompasses two rod-shaped bacterium adhered to each other. It made clear that polymer coating was formed around bacteria during the process. The size of bacteria is similar to their original form (width: $\sim 0.5\text{--}0.9\text{ }\mu\text{m}$ length: $\sim 1.3\text{--}2\text{ }\mu\text{m}$). Viable testing showed the nanofibers contain a huge amount of living bacteria. These results demonstrate that electrospinning is a capable technique to encapsulate living probiotic bacteria into polymer nanofibers forming a biohybrid nanoweb.

REFERENCES

1. Dave A. Parkins, Ulla T. Lashmar, The formulation of biopharmaceutical products. *Pharmaceutical Science & Technology Today* 2000, 129-137
2. Patyi G., Bódis A., Antal I., Vajna B., Nagy ZK., Marosi G. 2010. Thermal and spectroscopic analysis of inclusion complex of spironolactone prepared by evaporation and hot melt methods. *Journal of Thermal Analysis and Calorimetry* 102: 349-355.
3. Kostakova E., Meszaros L., Gregi J. 2009. Composite nanofibers produced by modified needleless electrospinning. *Materials Letters* 63: 2419-2422.
4. Verreck G., Chun I., Peeters J., Rosenblatt J., Brewster M.E., 2003. Preparation and characterization of nanofibers containing amorphous drug dispersions generated by electrostatic spinning. *Pharmaceutical research* 20: 810-817.
5. Nagy, ZK., Nyúl K., Wagner I., Molnár K., Marosi G., 2010. Electrospun water soluble polymer mat for ultra-fast release of Donepezil HCl. *Express Polymer Letters* 4: 763-772.

INVESTIGATION OF PHYSICAL METHODS FOR EFFECTIVE STABILIZATION OF PVA NANOFIBERS

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INTRODUCTION

Electrospun nanofibers are one of the most modern forms of nanocomposite systems and have been widely studied in recent years due to their ease of formulation and unique characteristics, among which their ability to mimic the fundamental elements of the natural extracellular matrix outstands (1). Therefore, nanofibers are promising candidates for many biomedical applications, such as wound dressings, drug delivery and scaffolds for tissue engineering (2). A promising functional material for nanofiber preparation has been polyvinyl alcohol (PVA) due to its good chemical stability, hydrophilicity, biocompatibility, nontoxicity and excellent electrospinnability. However, its water solubility limits its extended application in biomedical field (3).

The aim of this study was to design biomimetic PVA nanofibers with low water solubility or improved water resistance. To achieve that, nanofibers were prepared by electrospinning and further stabilized by different physical methods. Our goal was to increase stability and avoid use of any toxic cross-linking agent.

MATERIALS AND METHODS

Preparation of the solution

PVA solution (8 % w/w) was prepared from PVA powder (Mowiol 20-98, M_w 125 000 g/mol, Sigma-Aldrich) by dissolving the weighted amount of the polymer in distilled water at 90°C for 2 hours.

Electrospinning

The solution was placed in a 20 ml syringe with a 0.8 mm needle on which the high voltage supply of 15 kV was connected. The grounded planar collector covered with aluminium foil was located at a distance of 15 cm from the needle tip and flow rate was set to 0.707 ml/h.

Characterization of nanofibers

Morphology of electrospun nanofibers was examined using a scanning electron microscope (SEM, 235 Supra 35VP-24-13, Carl Zeiss, Germany) operated at an accelerating voltage of 1 kV, with a secondary detector without coating with any conductive layer before imaging.

Stabilization of nanofibers

Different approaches for physical cross linking of obtained nanofibers were used. Nanofibers were soaked in two non-solvents, ethanol and methanol respectively, for 24 hours. Additionally, nanofibers were heat treated at 160°C for 10 min. The hydration of the stabilized nanofibers in physiological environment was studied by immersion in phosphate buffer (pH 7.4) (PBS) and weighting the samples at regular time interval.

RESULTS AND DISCUSSION

Figure 1a shows SEM image of electrospun PVA nanofibers at 5000 x magnification. It can be seen that the matrix is consisted of a very uniform fibers with diameters of $150 \pm 29\text{ nm}$ and smooth surface. However, when the PBS was poured onto white PVA network, it instantly became transparent and has completely dissolved. Thus the unique nanofibrous structure was lost. Therefore, the stabilization of PVA nanofibers is essential in order to successfully use them in physiological environment.

The stabilization of PVA fibers against water can be accomplished using various physical and chemical cross linking methods. Chemical cross linking demands usage of reactive species that can remain in the sample and can compromise biocompatibility. Therefore, the physical stabilization by soaking in non-solvent (methanol and ethanol, respectively) and heat treatment were chosen.

Fig. 1b-c shows SEM images of nanofibers after stabilization. It can be clearly seen that there are no visible changes in nanofiber morphology after heat treatment, whereas nanofiber network stabilized in alcohol has changed, but the fibrous structure was still preserved.

As mentioned before, un-stabilized PVA nanofibers dissolve in PBS instantly. On the other hand all physically stabilized nanofibers retain their integrity for more than 96 h (Fig. 2). Moreover, our results show that stabilization method does not have a significant impact on the hydration.

The main reason for prolonged water resistance of stabilized PVA is increased degree of crystallinity, which acts as physical cross links and stabilize the nano-fibers. This may occur by removal of residual water within the nanofibers by the alcohol or increased temperature, allowing PVA-water hydrogen bonding to be replaced by intermolecular polymer hydrogen bonding resulting in additional crystallization (4). Since such mechanism is present in all three stabilization methods, this is probably the reason why there is no observed major difference in behaviour of nanofibers in physiological environment.