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Development of an Electrospun Nanofibrous Web with Hyaluronic Acid

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Abstract
Textile materials with an electrospun nanofibrous web can be used fo ar wide range of applications, including medicine and health care. In this research, polyamide-6 and hyaluronic acid were used for the development of a nanofibrous web via electrospinning. Hyaluronic acid is one of the most interesting ingredients used in skin care. It is very important that the electrospun polyamide-6 nanofibrous structure binds nanoparticles of hyaluronic acid not covering the surface of these particles. The main goal of this work was to develop an electrospun nanofibrous polyamide-6 web with hyaluronic acid which can be used for health care and/or cosmetology A. polyamide-6 nanofibrous web with hyaluronic acid was successfully developed via electrospinning. The presence of hyaluronic acid in the nanoweb was confirmed after web treatment with hot (95%) water. Hyaluronic acid was transported from the spinning solution to the electrospun web, was not isolated from the environment by polyamide-6, and could interact with human skin.

Key words: *electrospinning, nanofibres, hyaluronic acid, polyamide.*

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Introduction

Nanoparticle-based medicine is a quite recent emerging field, bringing together various disciplines and clinically relevant approaches. This is currently an exciting challenge for academic and industrial developers. Nanotechnology has the potential to transform the specificity and efficacy of existing drugs as well as facilitate natural health tools. The alectrospun nanofibrous structure binds nanoparticles into the web but does not cover the surface of particles. Particles are active as they are only stuck to the nanofibres (in the case of polyamide PA nanofibres) or become active after the nanofibres melt (in the case of poly-vinyl-alcohol PVA nanofibres). The process of electrospinning enables to develop a continuous web with even distribution of functionally active nanoparticles. During electrospinning, it is possible to regulate the density of the nanofibrous structure as well as the equality of the functional particle distribution. Electrospinning is a well-known process used for the formation of nano or/and micro fibres due to electrostatic forces between two electrodes. Nanofibres can be formed from polymer solution or melt, and their diameter is usually from 10 to 1000 nm [1-3].

Textile materials with an electrospun nanofibrous web can be used for the manufacturing of goods for a wide range of applications, including medicine and health care, for example to perform protective and healing functions. The electrospinning technique, owing to its simplicity and high-adaptability nature, is considered an ideal strategy for the manufacturing of nanofibrous dressing, scaffolds and bandages for wound healing and regeneration. Nanofibrous biofunctionalized webs can be considered promising materials for skin tissue-engineering applications [4, 5]. Electrospun webs can be used as substrates for pharmaceuticals. Drugs may be involved directly during the electrospinning process. It is known that due to the different chemical nature of electrospun fibres and because of their possible surface modification, materials are used to deliver various types of drugs: antibiotics, protein, and other antineoplastic drugs [6].

Thus a prospective area of electrospinning method application is the development of new materials for cosmetology and regenerative medicine. As a result of the analysis of the different kinds of active components, we decided to choose hyaluronic acid as an additive to the solution for web electrospinning.

Hyaluronic acid (HA) has many benefits and is one of the most interesting ingredients in skin care. HA is a substance that is naturally present in the human body, found in the highest concentrations in fluids in the eyes and joints. HA is also used as a lip filler in plastic surgery, and can be applied to the skin for healing wounds, burns, skin ulcers, and as a moisturizer. HA plays a critical role in skin health with its unique ability to hold in moisture (1000 ml of water per gram of hyaluronic acid). HA can adjust its moisture absorption rate based on the humidity – relative to the season and climate. Interestingly the average human

has approximately 15 grams of HA in the body, one-third of which is degraded and synthesised every day [7-9].

HA is a lubricating, clear substance that is produced by the body naturally. In the human body, HA is found in the greatest concentrations in the skin, inside joints, within the eye sockets and in other tissues, where it helps retain collagen, increase moisture, and provide elasticity and flexibility. HA is a natural *glycosaminoglycan* (polysaccharides that are an important component of connective tissue) and can be derived from multiple resources, foods, supplements and HA powders. The HA used as medicine is extracted from rooster combs or made by bacteria in the laboratory. The chemical structure of HA is presented in ***Figure 1*** [10].

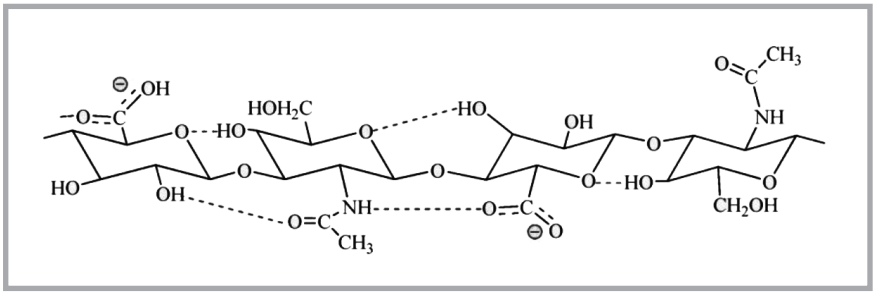

Figure 1. *Chemical structure of HA [10].*

Depending on the number of segments in the molecule of HA, it can have a different weight and length. Based on the weight of the molecules, it is possible to distinguish two types of HA: low molecular weight and high molecular weight. The effectiveness of the product depends on what type of HA is included in its composition. A feature of high molecular acid is the big size of its molecules, which are somewhat larger than 3-20 thousand nanometres. Such a size does not allow it to penetrate into the deeper layers of the skin. Low molecular weight HA has a smaller molecular size (about 5 nanometres), which makes it easy to penetrate into the skin, and hence it works inside at the cellular level. The application of low molecular weight HA increases the internal volume of tissue, significantly activates the activity of fibroblasts, has a stimulating effect on cell division, increases their migration, accelerates the active substance penetration and helps to reduce wrinkles [11-13].

HA is proven to be beneficial in the wound healing process, because hyaluronic acid contributes significantly to cell proliferation and migration. Electrospun HA nanofibres are important for skin regeneration, should mimic the natural architecture of tissue, and thus should provide a wound dressing similar to natural tissue [14, 15]. Moreover the air permeability of the HA nanofibrous web is much higher than that of the solid HA, leading to a better healing result. The drug release profile may be changed by the structure of the nanofibrous material which is used as the supporting structure. The best result can be obtained by combining a biodegradable layer which directly contacts with the damage and has sorption, homeostatic and healing properties, as well as a safety (non-biodegradable) layer to ensure sterile conditions [16-18]. For afore-mentioned layer, PA6 (polyamide-6) nanofibres is the optimal decision. It is known that the thinnest nanofibres can be obtained from PA6 [1]. Functional nanoparticles stick onto the surface of the thin PA6 nanofibres and give the highest functionality as they are not wrapped by the polymer. However, HA is hydrophilic and naturally biodegradable, while PA6 is insoluble in water, and therefore there is a problem to develop a PA6 nanofibrous web with water soluble HA.

The main goal of this work was to develop an electrospun nanofibrous PA6 web with HA for the manufacturing of bandages and/or plasters that can be used for healthcare and/or cosmetology.

Materials and methods

In the initial stage, two types of polyamide-6 (PA6) granules were used as the main polymer for preparation of electrospinning solution: low viscosity PA6 (relative viscosity at 96% H_2SO_4 ((sulphuric acid)) is 2.4–2.8); and high PA6 (relative viscosity at 96% H_2SO_4 is 3.2–3.5). A formic acid was used as a solvent because it is highly volatile and the most preferred for PA6. Hyaluronic acid (HA) HATLM 20-40 of low-molecular weight (Bloomage Freda Biopharm Co Ltd, China) was used in our investigation as this type of HA is preferred by manufacturers for use in healthcare and cosmetology. Small molecules of HA can easily penetrate into the skin. Measurements of viscosity of solutions were made with a rotational viscometer – Rheotec Reometer RC 01/02 (Rheotec, Germany) at a temperature of 20 °C.

To add HA to the spinning solution, initially 1 g of HA powder was dissolved in 40 ml of warm water (35 °C) by continuous mixing (30-40 min) until a homogeneous gel-like substance was obtained (HA concentration in the gel was 2.4%). In the next step, this substance was added to 200 ml of PA6 spinning solution in formic acid. The quantity of HA gel in the solution was 17% (quantity of pure HA in the solution was 0.4%). To obtain the most homogeneous substance, the solution was heated and agitated periodically (35 °C, 1 hour).

The electrospinning process was carried out using laboratory equipment Nanospider NS LAB (Elmarco, Czech Republic) at an environmental temperature of 20±2 °C and air humidity of 40±2%. Polypropylene nonwoven fabric with 21.5 g/m^2 surface density was used as the supporting material for coating with the PA6-HA nanofibrous web. The supporting material was fixed, and the duration of the electrospinning process was 10 minutes, the distance between electrodes – 13 cm, and the applied voltage – 70 kV. The structure of the newly developed electrospun nanowebs was analysed by Scanning Electron Microscopy (SEM) – Quanta -200 FEG (FEI, Netherlands), using "ImageJ" software.

Results and discussion

Investigation of spinning solution properties using different viscosity PA6

At the first stage of investigations, the influence of polyamide-6 (PA6) concentration on the dynamic viscosity, surface tension and electrical conductivity of the spinning solution was carried out. During investigations, the concentration of low viscosity PA6 in the solution varied from 5% to 15% and that of high viscosity PA6 – from 2.5% to 12%. It was found that increasing the PA6 concentration leads to a insignificant rise in surface tension

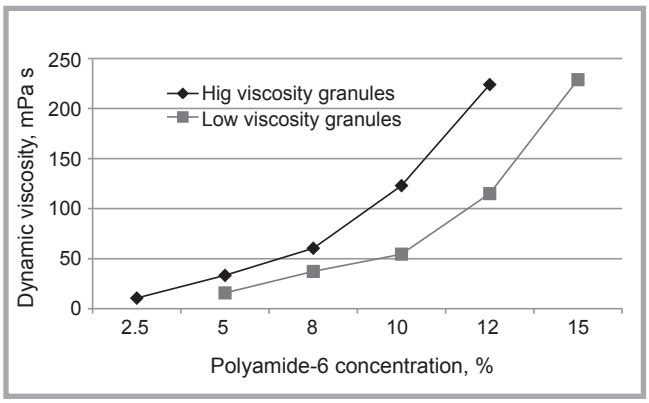

Figure 2. *Influence of polyamide-6 concentration on dynamic viscosity of spinning solutions.*

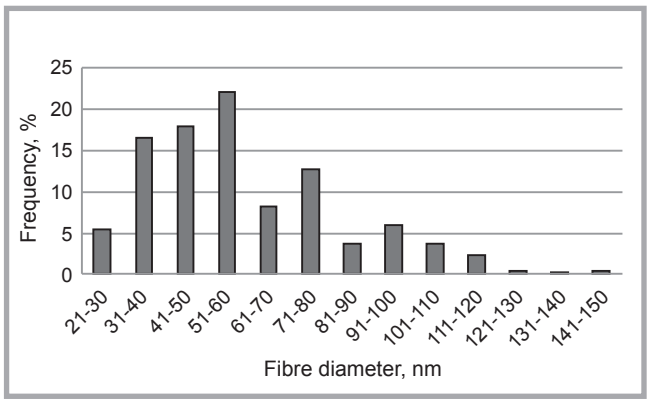

Figure 3. *Distribution of fibre diameter in nanoweb electrospun from high viscosity PA6 and HA solution.*

of the spinning solutions, but did not exceed the recommended limit value for the electrospinning process (5×10^{-2} Pa/m).

Figure 2 shows the dependence of the dynamic viscosity of the spinning solution on various PA6 concentrations. Both types of PA6 granules (low viscosity and high viscosity) allow to obtain s solution with the same viscosity. But in the case of high viscosity PA6, its concentration in the spinning solution can be lower. We can recommend to prepare a spinning solution with at least 12% of low viscosity PA6 or with at least 10% of high viscosity PA6, considering that the dynamic viscosity limit of the spinning solution is 100 mPa·s. It is known that the viscosity of polymer does not influence the electrical conductivity of the spinning solution significantly. All variants of the spinning solution prepared meet requirements for conductivity (from 0.1 till 10 mS/cm).

Investigation of spinning solution stability

The viscosity of the spinning polymer solution can be unstable over time due to various reasons. Since solution viscosity is an important parameter in the electrospinning process, investigation was carried out to determine changes in the viscosity of PA6 solution with HA over time. It was found that the spinning solutions investigated could segregate over time, which indicates the incomplete compatibility of components in the solution. It is not a positive result. For precise evaluation of the segregation time, solutions of high viscosity and low viscosity PA6 and HA were analysed during different time intervals, using visual control of their homogeneity and measurement of their viscosity.

In the case of low viscosity PA6 and HA solutions, the formation of macromolecular compounds of HA was observed after a few hours, and this is the fact that complicates the process of electrospinning from such a solution, especially its usage in industrial manufacturing. In low viscosity PA6 solution, heterogeneous macromolecules of PA6 and HA have lower energy of intermolecular interaction than homogeneous molecules of concentrated HA solution. Solution with high viscosity PA6 and HA was the most stable, which did not segregate even after three days, with such a period being absolutely sufficient for the electrospinning process of all variants of electrospun webs and is much more useful for industrial manufacturing. After analysis of the results obtained, the spinning solution containing 0.4% HA and 9% high viscosity PA6 with formic acid was used for the next investigations.

Investigation of the polyamide-6 and hyaluronic acid web structure

Solution of 9% high viscosity PA6 and 0.4% HA was used in electrospinning to develop a nanofibrous web (parameters of the electrospinning process are presented in the chapter Materials and methods). After analysis of SEM images of the electrospun nanoweb (see ***Figure 4***) and measurements of the nanofibre diameter (diameter of more than 400 nanofibres was measured), the distribution of all measurements was calculated. That of the nanofibre diameter is presented in ***Figure 3***.

After measurements of the nanofibres diameter, it was found that the average is 59.5 nm (with relative error $\delta = 3.97\%$) and the coefficient of skewness (statistical parameter which characterises the correspondence of the distribution to the Gaussian normal distribution) $A = 0.863$). The results obtained confirm that the technique investigated enables the manufacture of nanoscale fibres because their average diameter does not exceed 100 nm, with a distribution close to the Gaussian normal distribution, and are very close to the results obtained for the electrospun PA6 nanofibrous web without HA [1]. It means that HA does not have a significant influence on PA6 nanofibre diameter. The existence of hyaluronic acid on the electrospun web needed to be confirmed with additional experiments, which was achieved by treatment of the electrospun nanoweb with hot water.

A significant number of web defects, such as spherical solidified droplets (beads) of solution, whose size varied from 150 nm till 1.7 μm, is seen in the SEM image presented in ***Figure 4***. The number of such droplets noticeably exceeds the quantity of similar droplets found in the structure of a web obtained from PA6 solution without HA by this papers' co-authors and published earlier [1].

For explanation of the reasons for the defects in the electrospun nanofibrous web obtained, the following hypotheses have been proposed:

- HA powder was dissolved in the solution with the formation of a gel-like substance. We can state that water molecules react on the surface of this substance with PA6, and this interaction leads to the obtaining of the defects described. It is likely that the solidified droplets consist of the HA and PA6 mixture. In such a case the

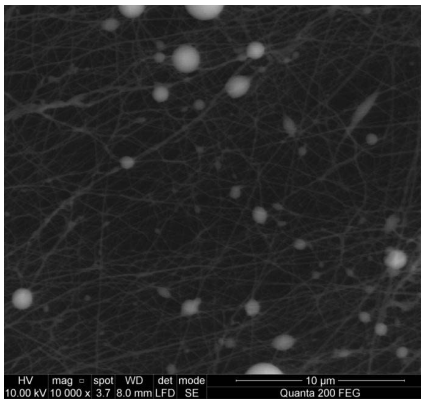

Figure 4. *Electrospun PA6 nanoweb with HA.*

droplets could prevent the active influence of HA on human skin (HA could possibly be locked by PA6) when the materials developed are used, for example, in cosmetology. In the case that HA is not locked in the droplets by PA6, the droplets could give a positive result because they can be active in contact with human skin.

- The increase in the number of droplets can also be explained by the assumption that droplets of HA are formed in addition to the those of PA6 previously obtained, i.e. these droplets consist of PA6 or HA but not of their blend. In this case, the occurrence of such droplets could give a positive result because it can contribute to higher functional efficiency of the material developed.

Thus it was decided to conduct additional experiments to prove or disprove the hypotheses mentioned above.

Investigation of the influence of treatment by water on the structure of the electrospun web

For more detailed investigation of the electrospun web structure, extra investigations were made. During these, we took into account the different nature of PA6 fibres and HA interaction with water. It was supposed that by soaking samples of the electrospun web in hot water, PA6 nanofibre and PA6 solidified droplets will retain their shape, whereas similar elements of HA, under the influence of hot water, will significantly change their dimensions or fully dissolve.

In order to confirm the presence of HA in the electrospun PA6 nanoweb, samples of the newly developed nanoweb were kept in hot water (temperature 95 °C) for different time intervals: 1 minute, 20 minutes and 30 minutes. After each treatment, SEM images of the treated webs were made. Analysis of these (see ***Figure 5***) show that even after 1 minute treatment by hot water, places can be found on the web where HA transforms from a solid to gel-like state. After treatments of 20 and 30 minutes, these changes in the structure of the web became highly obvious, which can be explained by the interaction between the gel-like substance and water on the surface of PA6 nanofibres and somewhere inside them. Water cannot have any influence on the PA6 nanofibres. By increasing the interval of hot water treatment time, the area unoccupied by the material's nanoweb decreases. This

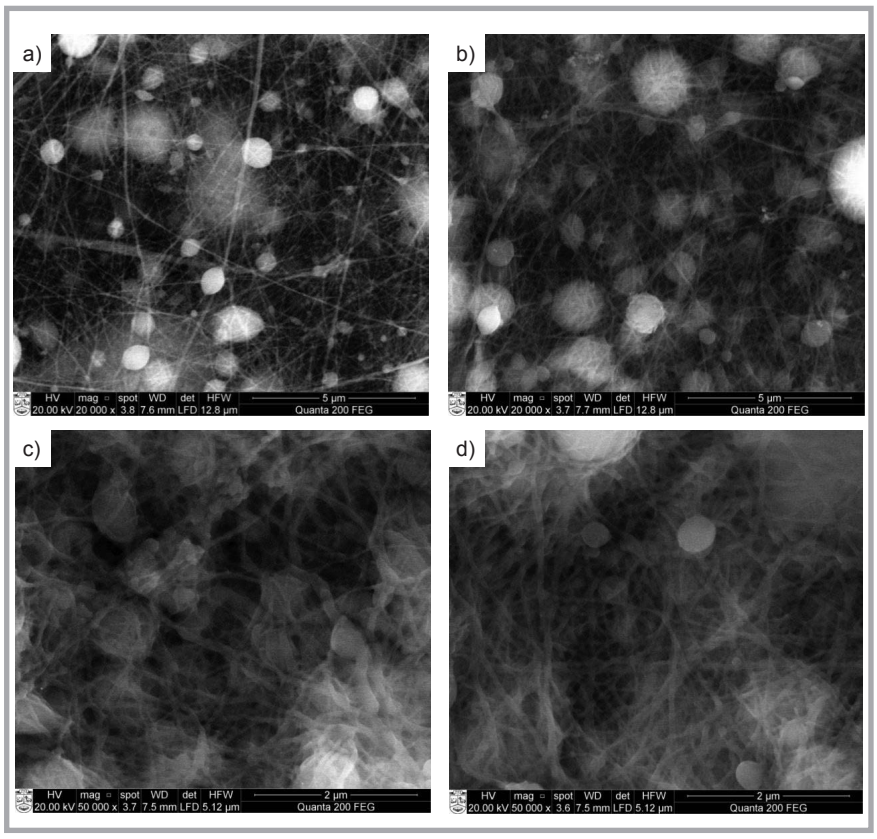

Figure 5. *SEM images of electrospun PA6 and HA nanofibrous web before treatment a) and after treatment with water at 1 min b), 20 min c) and 30 min d).*

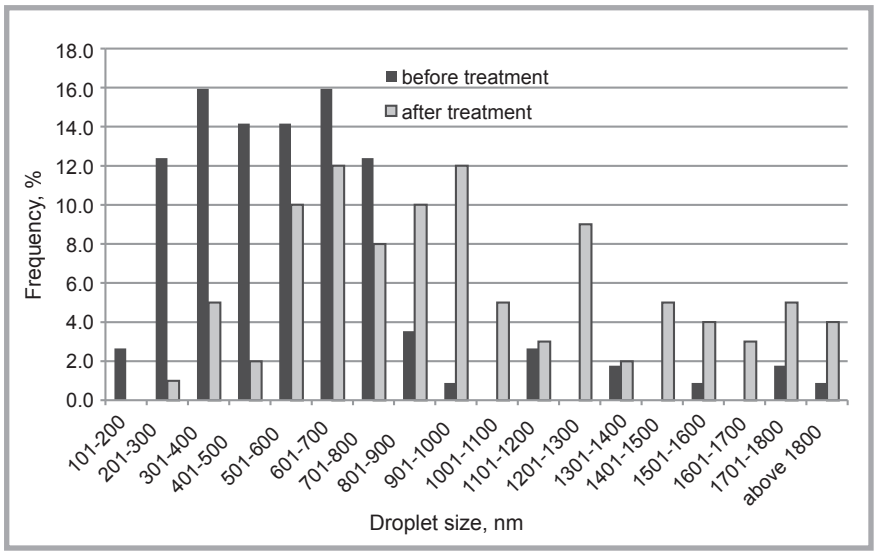

Figure 6. *Distribution of droplet size in web before and after 20 minutes treatment.*

fact can be explained by the swelling of gel droplets formed by reaction of HA with water. It can be noted that the size of these structures varies in a wide range and can reach even 10 μm. Herewith the images obtained after treatment of 20 or 30 minutes (see ***Figures 5.c*** and ***5.d***) showed that HA present in the web not only as droplets (separate and/or mixed with polyamide), but they also cover PA6 nanofibres, which is especially visible after 30 minutes' treatment.

The distribution of the droplet size (see ***Figure 6***) shows that the size (diameter) significantly increases due to the swelling of hyaluronic acid. We found that before hot water treatment, the average value of the droplet size is 583.4 nm ($\delta = 10.21\%$), while after 20 minutes of treatment it increases till 1007.7 nm ($\delta = 8.93\%$).

Hence it was experimentally proven that an increase in the number of droplets in the web occurred due to the fact that the majority of these droplets are made of the gelled HA substance, which was obtained by dissolving HA in water. This fact confirms that HA was transported from the spinning solution to the electrospun web. Furthermore we found that HA is not isolated from the environment by PA6, and the droplets and nanofibres can interact with human skin during usage.

Conslusions

A polyamide-6 (PA6) nanofibrous web with hyaluronic acid (HA) was successfully developed via electrospinning. For electrospinning, low viscosity PA6 (2.4-2.8 relative viscosity at 96% H_2SO_4), formic acid as PA6 solvent, and HA powder (of low-molecular weight) dissolved in warm water till a homogeneous gel-like substance were used. Analysis of the nanofibrous web obtained showed that nanoscale fibres with an average diameter of 59.5 nm were successfully electrospun. The results obtained confirmed that the technique investigated enables to manufacture nanoscale fibres with an average diameter lower than 100 nm and with a diameter distribution close to the Gaussian normal distribution. The presence of HA in the nanoweb was confirmed after web treatment with hot (95%) water. SEM analysis of the nanoweb treated showed that even after 1 minute tratmentsome places on the web transform from a solid to gel-like state, which can only be HA. After 20 and 30 minute treatments, these changes in the structure of the web became highly obvious. Thus it was experimentally proven that an increase in the number of droplets in the web occurred due to the gelled HA substance. This fact confirms that HA was transported from the spinning solution to the electrospun web and that HA is not isolated from the environment by PA6, being able to interact with human skin during nanoweb usage.

Acknowledgements

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