Ewelina Pabjańczyk-Wlazło[1], Izabella Krucińska[1], Michał Chrzanowski[1], Grzegorz Szparaga[1], Agata Chaberska[2], Beata Kolesińska[2], Agnieszka Komisarczyk[1], Maciej Boguń[1*]

[1] Lodz University of Technology, Faculty of Material Technologies and Textile Design, Department of Material and Commodity Sciences and Textile Metrology
Zeromskiego Str. 116, 90-924 Lodz, Poland
* E-mail: maciej.bogun@p.lodz.pl

[2] Lodz University of Technology, Faculty of Chemistry, Institute of Organic Chemistry
Zeromskiego Str. 116, 90-924 Lodz, Poland

Fabrication of Pure Electrospun Materials from Hyaluronic Acid

DOI: 10.5604/01.3001.0010.1688

Abstract
The aim of the research was to develop optimal conditions for manufacturing materials based on hyaluronic acid by the electrospun method. The studies were composed of three stages: the process of selection of the optimal solvent (mixture of solvents), the molecular weight of hyaluronic acid, and the concentration of biopolymer in the spinning solution. The influence of variable parameters on the rheological properties of the spinning solutions and electrospinning trails was tested. Depending on the electrospinning regime applied, the fibers obtained were characterised by a diameter of the order of 20 to 400 nm. As a result of the development works presented, an optimal molecular weight of the polymer, its concentration and system of solvents were determined, together with process parameters, ensuring a stable electrospinning process and relatively homogeneous nanofibers. Additionally studies on the residues of solvents used during electrosun formation were done and parameters of drying of the final materials were examined. This approach (verification of the presence of organic solvent residue in the nanofibrous formed) is important for the suitability of nanofibres as scaffolds for regenerative medicine. This study provides an opportunity for the understanding and identification of process parameters, allowing for predictable manufacturing nanofibers based on natural biopolymers, which makes it tremendously beneficial in terms of customisation.

Key words: *biocompatible polymers, natural polymers, biomimetism, biomimetic scaffolds, regenerative medicine, electrospinning, hyaluronic acid.*

Introduction

Biomimetism is a concept of adapting solutions from nature to science, medicine and technology. The term was proposed by Otto Schmitt in the 1950s [1] and is one of the strongest emerging trends today, setting out guidelines for development in the majority of scientific fields [2] and giving the opportunity to apply a nature-based solution to everyday human practice. Nowadays the most extensive use of biomimetism concerns the broadly defined medical sciences [3]. The concept of biomimetism has also entered into regenerative medicine and tissue engineering for the preparation of scaffolds mimicking the natural architecture of extracellular matrixes able to promote cell growth and proliferation [4]. From the viewpoint of scaffold formation for tissue regeneration, biomaterials (in particular naturally occurring polymers) have gained significant considerations [5], associated with their unique properties, the most important of which is biocompatibility, providing a proper environment for natural tissues. They are also biodegradable, which means that they are not harmful to the environment and are characterized by the other physical and chemical properties allowing their various applications.

One of the most promising polymers for use in medicine and tissue engineering is hyaluronic acid (HA), a glycosaminoglycan occuring in all living organisms. Interestingly hyaluronic acid is the main component of the extracellular matrix (ECM) of skin, joints, the eye and many other tissues and organs [6]. It constitutes a significant factor of the extracellular matrix of connective tissues, with important biological functions including shock absorption, molecular filtering and collagen fibril support. Their natural function of providing appropriate support makes them ideally suited for utilisation as tissue scaffolds [7]. Apart from structural-maintenance properties, it is important to notice that HA plays a significant role in many biological processes such as embryogenesis, inflammation, metastasis and tumour progression, as well as in metabolic activities in the process of healing [8]. Especially here, hyaluronate plays a major role in each phase by stimulating cell migration, differentiation and proliferation, also by participating in the regulation of the metabolism and organisation of the ECM [9]. In the light of these reports, HA is a very promising object of research in the field of fibrous structures useful for regenerative medicine.

Scientific and technical progress has increased in the area of preparation of various types of materials on a nanoscale. Electrospinning is a process in which nanofibers are produced under the influence of an electric field from a stream of polymer solution or polymer melt

List of abbreviations

AW	*ammonia water*
ECM	*extracellular matrix*
HA	*hyaluronic acid*
HA-DTPH	*dithiobis(propanoic dihydrazide)*
NMP	*N-methylpyrrolidone*
PE	*polyethylene*
PEO	*poly(ethylene oxide)*
PEGDA	*poly(ethylene glycol)-diacrylate*
PLA	*poly(lactic acid)*

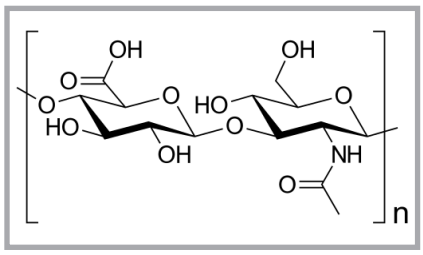


Figure 1. *The structure of hyaluronic acid.*

supplied through nozzle holes of certain geometry and received at collectors with a horizontal or vertical arrangement. This allows to obtain a determined morphology and structure of fibers depending on the process parameters [10]. In this respect, electrospinning stands out compared to other techniques, because there are three groups of parameters that can be optimised: the process parameters, the character of the spinning solution, and environmental conditions. In terms of optimisation, electrospinning is one of the most promising techniques for the preparation of nanofibers; however, unchanging fibers can be achieved only in a narrow range of process parameters which have to be optimised in order to be devoid of beads and to achieve a certain thickness of individual filaments. Depending on the parameters' diameters in the range of 50-2000 nm, a specific morphology and structure can be obtained [11].

Previous research data indicate that the electrospinning of nanofibrous mats composed only of pure biopolymers has been difficult to achieve. Some HA characteristic features like solubility in water and high surface tension in aqueous solutions determine its processability by electrospinning. Especially the main obstacle is to determine the proper characteristic of the spinning solution that will result in electrospun fibrous material of defined features. These factors include primarily the molar mass of the polymer, concentration, viscosity and surface tension [12, 13]. A successful electrospinning of pure HA fibers at an ambient temperature was reported, where the diameter of fibers formed were between 20-110 nm [14]. HA was dissolved in a mixture of solvents containing deionized water (DW), formic acid (FA) and dimethylformamide (DMF) in different ratios. A three-dimensional hyaluronic acid nanofibrous structure mimicking the architecture of a natural extracellular matrix based on electrospinning was reported. However, for this purpose it was necessary use chemically modified hyaluronic acid derivatives (a thiolated HA derivative: 3,3-dithiobis(propanoic dihydrazide) (HA-DTPH)) in the presence of an auxiliary agent (poly(ethylene oxide) (PEO)) and cross-linking derivative (poly(ethylene glycol)-diacrylate (PEGDA)) [15]. Yet it has been found that this approach is not useful regenerative medicine because of the presence of residual organic solvents and/or toxic additives during the formation of non-woven material. These and many other studies outline the critical role of biopolymer processing with respect to their application in different fields, especially medicine. (***Figure 1***)

However, processing studies on electrospinning biopolymers are seldom the subject of research, which causes that in the literature there is the lack of a technological approach aiming at optimisation of the manufacturing of scaffolds. Additionally the use of pure unmodified hyaluronic acid or its salts is very limited. In this respect, how modifications influence the properties of HA that affect its behavior and adoption in the natural environment should be examined. In view of this, two primary aims of this study were posed: 1. to determine an optimal molecular weight of the polymer, its concentration, the composition of the solvent mixture and process parameters in order to achieve homogeneous nanofibers; 2. to ascertain the amount of NMP residues in hyaluronic acid-based non-woven in order to verify its suitability for application in regenerative medicine.

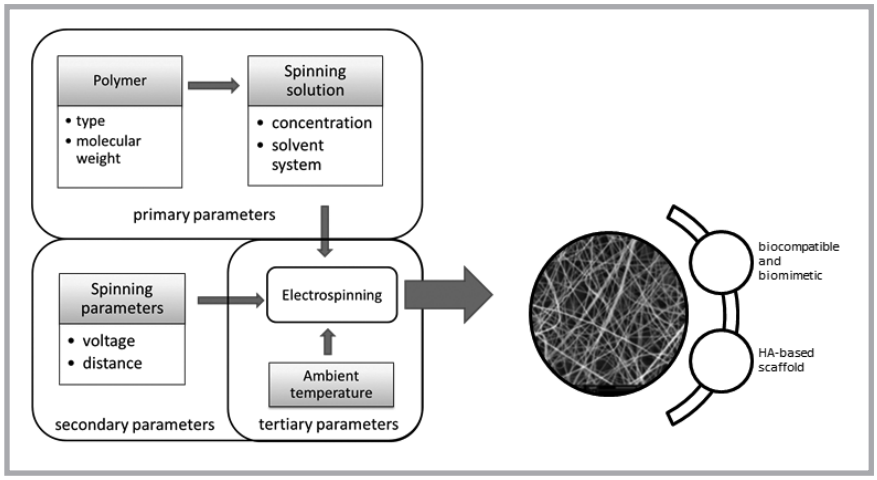


Figure 2. *Schematic presentation of the concept of research. Source: own elaboration.*

Objective of the study and research concept

The general objective of the study was to design and characterise materials useful as scaffolds for tissue engineering that are based on pure hyaluronic acid sodium salts and manufactured by electrospinning. The originality of the approach applied is the application of pure hyaluronic acid, in contrast to several recent studies that have been carried out on polymeric matrices based on other types of polymers, where HA was only a component of the matrix and its role was to enhance the biocompatibility of the entire composite [16]. The step-wise process describing the research concept is schematically presented in ***Figure 2***.

Firstly selection of the biopolymer and its molecular weight was completed. Criteria for selecting the type of polymer included the natural origin, non-toxicity, and occurrence in human tissues. All these parameters ensured the highest degree of biocomptability. In this respect, hyaluronic acid and its sodium salts have the highest potential. The choice of molecular weight from the available scope (starting from 80-130 kDa, to 2,0-2,2 MDa) was preceded by preliminary studies [17]. The result of this step was the selection of 100-150 kDa HA sodium salt, which allowed sufficiently high concentrations of spinning solutions, simultaneously not causing an excessive increase in the viscosity of the solution, which may cause instability of the polymer stream at the exit of the spinneret.

Secondly the preparation step of the spinning solution was carried out, involving the selection of the proper system of solvents and concentration of the polymer. Based on the preliminary studies (conductivity of the system of solvents and its rheological parameters), the concentration

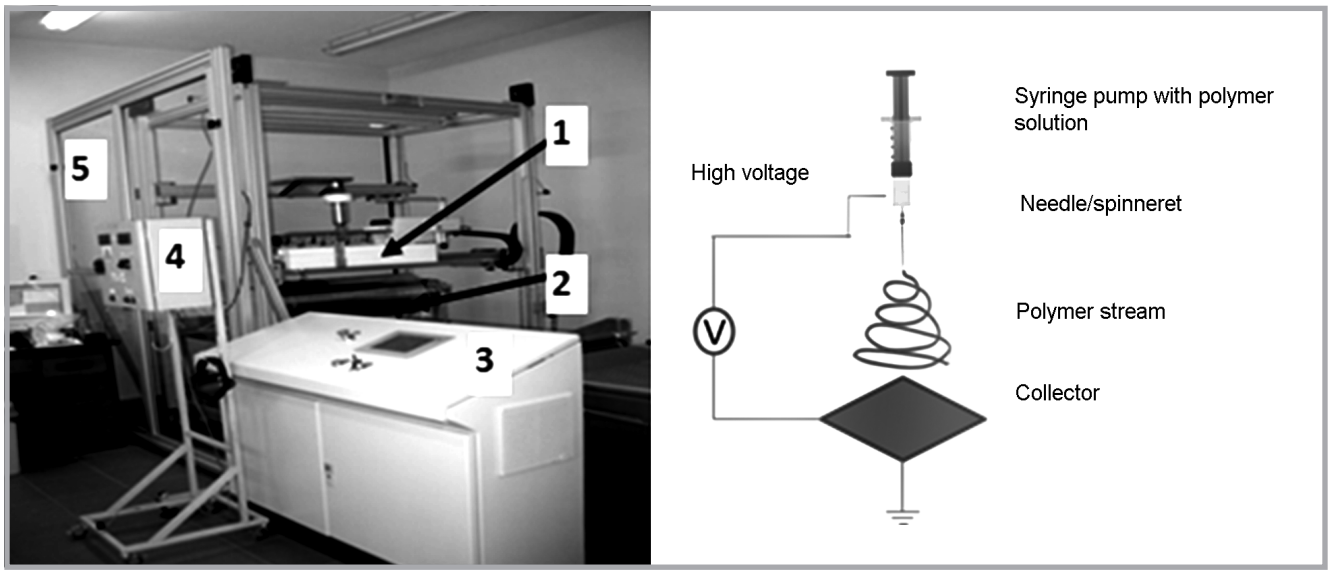


Figure 3. *Schematic view of the electrospinning set and laboratory stand: 1-spinning head with a peristaltic pump, 2-receiver, 3-control panel, 4-high-voltage generator, 5-housing protection against high voltage. Source: own elaboration.*

range for providing a stable polymer flow in the process was set at 12%. From the pool of available organic solvents, including dimethyloformamide (DMF), ethanol (EtOH), 25% ammonia water (AW) and N-methylopirolidone (NMP), based on preliminary data, a mixture of AW and NMP in the ratio of 2:1 was selected. Although water is an excellent solvent of hyaluronic acid sodium salts, it was excluded from the group of potential solvents since it has a very high surface tension of the order of 70 ($mN \cdot m^{-1}$). Very high surface tension results in the formation of beads instead of fibers. Primary inclusion criteria concerned lowering the surface tension of the system of solvents, solubility of the polymer, viscosity of the spinning liquid and the evaporation rate that influences the removal of the solvent's residuals in the final nanofibrous scaffolds.

Finally the process parameters were adjusted. In order to identify the optimal set of parameters, a preliminary test was performed, the results of which are shown in the following sections. The following process scope of parameters was applied: distance from the spinneret and collector = 450-550 mm and voltage applied = 20-30 kV. In order to enhance the evaporation rate of the solvent during the electrospinning process, the ambient temperature was adjusted to 30 °C.

The above-mentioned three groups of parameters selected: molecular weight of the polymer – M=100-150 kDa, its concentration (12%), and system of solvents and process parameters applied proved to provide a stable polymer flow and pure hyaluronic acid-based nanofibers in the range of diameters 20-400 nm.

Experimental part

Materials and methods

Polymer Hyaluronic acid (HA) was purchased from Contipro Biotech (Czech Republic) in the form of sodium salt. DSC analysis was performed with a differential scanning calorimeter – DSC (Perkin-Elmer 7, US) with a cooling device and computer software.

Solvents Solvents of chemical purity were purchased from Labomix (Poland): N-methylopirolidone and ammonia water (25%). Surface tension measurements were performed with a process tensiometer using Wilhelmy's plate method [18]. The conductivity of the solution system selected was measured with a multifunctionmeter – CX-701 (Elmetron, Poland) and conductivity sensor – EC-60 (Elmetron, Poland) at a reference temperature of 25 °C. The result of conductivity measurements was an average value from five measurements.

Spinning solutions Prior to the process of electrospinning, rheological properties of the spinning solutions were tested using a rotational rheometer – Anton Paar RheolabQC (Great Britain). Measurements were carried out in a shear rate range of 0.1-160 s^{-1} using a cylinder – CC27. Rheological parameters were determined for a range of the shear rate of 1-80 s^{-1} using the software Rheoplus/322 V3.21. For determination of the basic rheological parameter, the power model of Ostwald de Waele was applied, as one which approximates the behaviour of rheological polymer fluid. The study was carried at temperatures of 15, 20, 25, 30 and 35 °C. The aging of the solution was measured 7 days after its preparation.

Electrospinning Hyaluronic acid-based fibrous scaffolds were electrospun on a vertical electrospinning laboratory stand, illustrated in ***Figure 3***. The polymer stream was fed through a needle of 0.6 mm diameter and at a flow rate of 0.2 ml/min. The process was carried out at 30 °C and 30% RH.

Microscopic examination Analysis of the microscopic structure of the nanofibers was performed based on scanning electron microscopy images. SEM images were taken with a high-resolution scanning electron microscope – FEI Nova NanoSEM 230 (Jeol, US) equipped with an electron gun with field emission (FEG). Histograms of the nanofibers obtained were prepared on the basis of the SEM images with the use of the dedicated software. Thickness distribution was determined on the basis of the sample size N = 50.

NMP residuals examination Hyaluronic acid samples were dissolved in water. Aqueous solutions thus prepared were filtered through VIVASPIN filters (Sar-

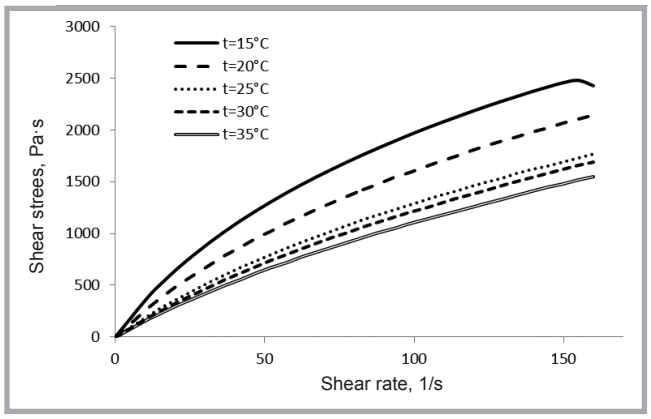


Figure 4. *Rheological properties of 12% hyaluronic acid solution: dependence of the shear stress on the shear rate.*

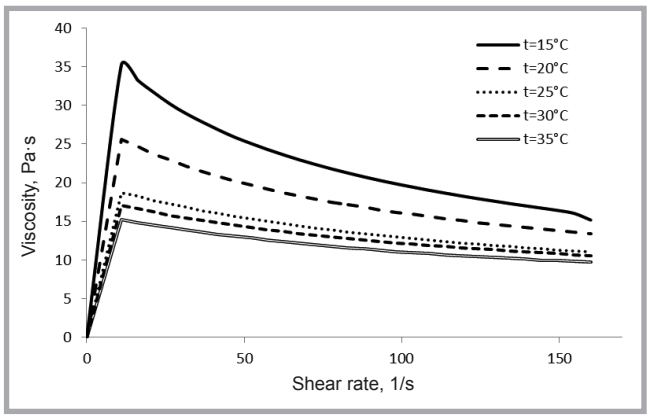


Figure 5. *Rheological properties of 12% hyaluronic acid solution: the dependence of the apparent dynamic viscosity on the shear rate.*

torius Stedim Biotech, Germany) with a membrane of 2,000 MWCO HY and then centrifuged (900 rpm) (Hettich, Universal 230R), thereby obtaining an aqueous solution of compounds of low molecular weight -below 2000 g/mol. The aqueous solutions were analysed by a UV spectrophotometer (Shimadzu UV-1601PC, Japan). The absorbance of the NMP was read at 282.5 nm. For the NMP calibration curve, analytical grade solutions of NMP (Across) were prepared.

Preparation of spinning solutions

The sodium salt of hyaluronic acid was dissolved in the type of solvent system selected taking into account the need to achieve appropriate characteristics of the spinning solution which stemmed from the preliminary studies – viscosity, concentration and surface tension. The high solubility of HA in water excluded its application in the electrospinning process, due to the high heat of evaporation of water and the inability of achieving a continuous stream of the polymer during the electrospinning process (referring to the high surface tension). Therefore a mixture of two available solvents was used – ammonia water (25% aqueous ammonium solution) and N-methylpyrrolidone in the ratio of 2:1 respectively. Spinning solutions were prepared with the use of a mechanical stirrer. The mixing process lasted for 5h constantly and solutions were used after degassing in ambient temperature.

Formation of hyaluronic acid-based nanofibers

Nanofibers were formed using electrospinning. The construction of the laboratory stand utilised for forming nanofibers is protected by the European patent [19]. ***Figure 3*** illustrates the laboratory stand. The operating voltage applied was predetermined based on the preliminary data and amounted to 20, 25 and 30 kV. The distance from the needle to the collector ranged from 450 to 550 mm based on the initial tests.

Result and discussion

Rheological properties

For the concentration (12%), molecular weight of the polymer (100-150 kDa) and composition of the spinning solution (AW:NMP, ratio 2:1) initially selected, rheological analysis was performed in the range of predetermined temperatures. The results are presented in ***Table 1***.

Analysis of the flow curves of the solutions examined are presented in ***Figures 4*** and ***5***.

The flow curve analysis shows that regardless of the temperature, the solutions tested are non-Newtonian shear thinned liquids (which refers to the parameter $n < 1$). In the case of the solutions investigated, the shear stress increases less proportionally with the increasing shear rate and curves pass through the origin of the coordinate system, which means that they are liquids without a yield point [20]. The solutions prepared exhibited a susceptibility to change in the shear stress, therefore we called them non-Newtonian liquids. In addition, a theoretical relation between the increasing temperature and decrease in the apparent dynamic viscosity was also confirmed. Higher temperature of the measurement caused a bigger decrease in the apparent dynamic viscosity. The rise in temperature increases the internal energy of the molecules (by supplying energy in the form of heat) and the vibrations of atoms, causing that their resistance to flow becomes smaller, and thus the viscosity of the solution decreases. Therefore changes in the dynamic apparent viscosity as a function of the shear rate for all the solutions studied exhibit a typical course, like that of the liquid solutions of the polymers. The investigation of the rheological properties brought insights in the behaviour of the polymer solution at different temperatures. Following this analysis, the most favourable ambient temperature was chosen for conducting a stable electrospinning process (30 °C).

Table 1. *Characteristics of spinning solutions.*

Sample description	Temperature, °C					Aging, 20°C	Surface tension, mNm^{-1}
	15	20	25	30	35		
12% HA WA:NMP	k = 39.096	k = 25.035	k = 17.543	k = 16.935	k = 14.906	k = 29.768	60.073
	n = 0.904	n = 0.958	n = 0.981	n = 0.966	n = 0.972	n = 0.950	

Sample description	Conductivity, µS	Standard deviation
WA:NMP 2:1	116.088	3.104

Morphology of the nanofibers

The second group of parameters affecting the electrospinning process and morphology of the fibrous substrates obtained are secondary parameters which relate to the process of electrospinning itself. ***Table 2*** depicts SEM images with histograms of the fiber diameter distribution of fibrous structures in various regimes of the electrospinning process.

Considering the parameters applied, it was possible to obtain fibrous materials with elementary fibers of dimensions in the range of 20 to 400 nm. In the case of the experiment using a 450 mm distance from the spinneret to the collector, the fibers obtained were characterised by dimensions in the range of 60-280 nm, 60-360 nm and 20-400 nm for 450, 500 m and 550 mm, respectively. Noticeable is also the decrease in efficiency of the process at the lowest voltage applied – 20 kV. Interestingly the increase in the distance between the spinneret and collector influenced the distribution of diameters by slightly lowering the uniformity of the fibers collected, and also the distribution of their diameters is slightly shifted toward higher values. With regard to the surface area of the fibers, which is connected to their dimensions and especially important in the case of biomedical applications, the most optimal process parameters determined were 450 mm distance from the collector and 25 kV voltage. It can be concluded that in this case, fibers are characterised by the narrowest distribution of diameters, of which over 45% constitute 80-100 nm.

On the basis of the ***Figures 6*** and *7*, it can be concluded that the application of a higher distance from the spinneret to the collector resulted in slightly bigger distribution of the monofilaments' diameters and bigger average diameters. This general trend can be observed in all cases, apart from one sample (1). In addition to the above, the relation between the distance and morphology of fibers was confirmed. According to previous research, the corresponding distance between the nozzle and collector allows for complete solvent evaporation, which also affects the morphology of the fibers (when the distance is too small fibers stick together) [21, 22].

In the case of dependencies between the average diameter of fibers and the voltage applied, a clear relationship was not defined. Prior studies have noted the importance of the voltage applied in the electrospinning process as it is connected to the creation of the Taylor's cone at the tip of the spinneret which starts the formation of fibers [23]. The voltage applied is the driving force for charge transfer in the stream of the polymer and the density of the loads applied, as the amount of charge per unit of the drop surface is described by the voltage applied, the distance from the nozzle to the collector and the conductivity of the solution. These

Table 2. *Thickness distribution of hyaluronic acid-based nanofibers according to different process parameters.*

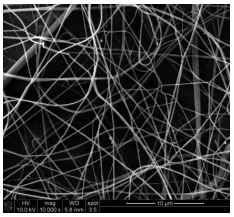
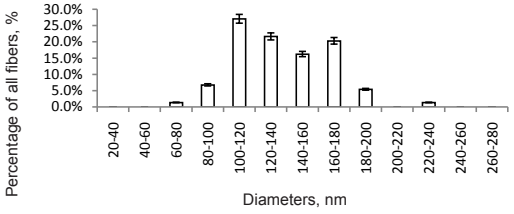
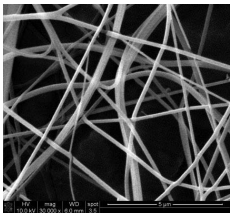
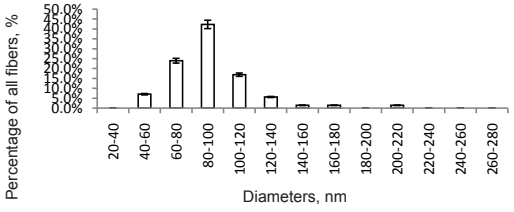
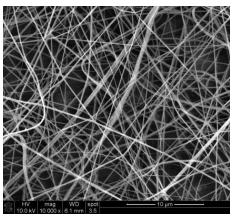
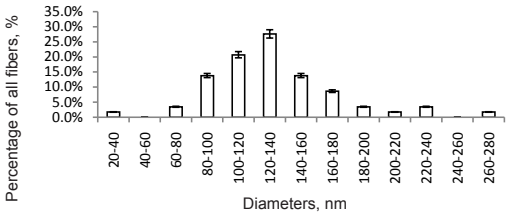
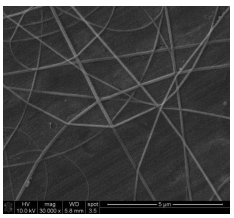
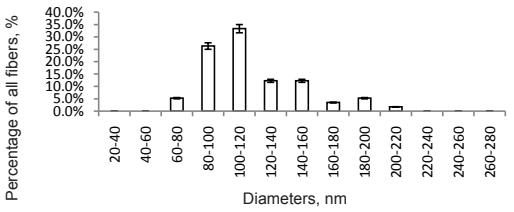
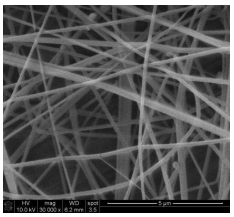
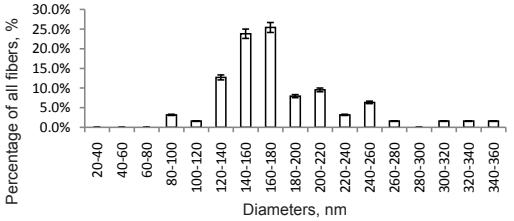
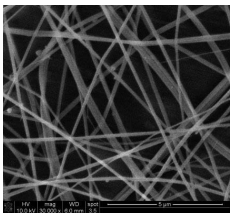
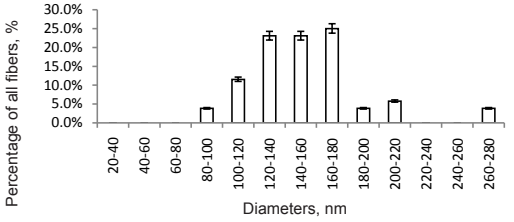
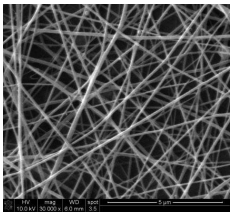
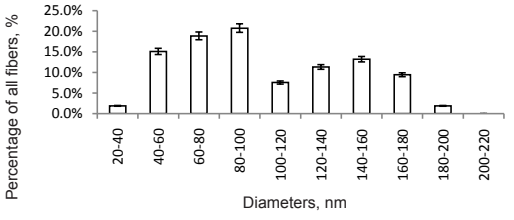
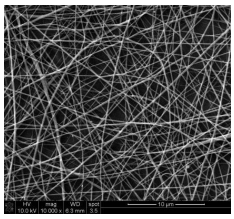
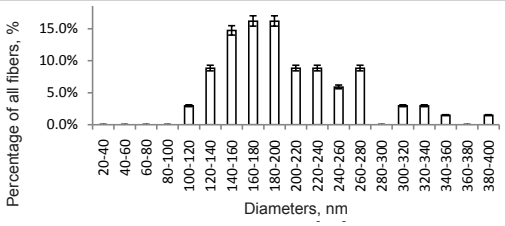
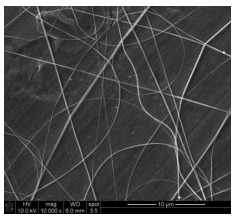
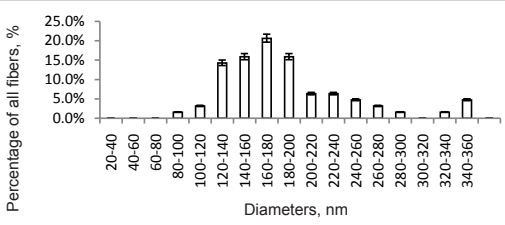
1		
Distance from collector = 450 mm, Voltage = 30 kV, Magnification = 10.000 x		
2		
Distance from collector = 450 mm, Voltage = 25 kV, Magnification = 30.000 x		
3		
Distance from collector = 450 mm, Voltage = 20 kV, Magnification = 10.000 x		
4		
Distance from collector = 500 mm, Voltage = 20 kV, Magnification = 30.000 x		
5		
Distance from collector = 500 mm, Voltage = 25 kV, Magnification = 30.000 x		
6		
Distance from collector = 500 mm, Voltage = 30 kV, Magnification = 30.000 x		
7		
Distance from collector = 550 mm, Voltage = 30 kV, Magnification = 30.000 x		

Table 2 continued. *Thickness distribution of hyaluronic acid-based nanofibers according to different process parameters.*

8		
	Distance from collector = 550 mm, Voltage = 25 kV, Magnification = 30.000 x	
9		
	Distance from collector = 550 mm, Voltage = 20 kV, Magnification = 30.000 x	

values must be adjusted to the type of solvent and polymer concentration; however, this dependency was not confirmed. The increasing voltage should therefore cause an increase in the diameter of fibers, while the value of the critical voltage depends strongly on the concentration of the solution (the threshold increases with the concentration) [24].

NMP residual examination

The final stage of the study included verification of NMP residues in the hyaluronic acid non-woven. Two drying temperatures: 90-95 °C and 130-140 °C were tested, and as a control a polymer which had not been subjected to drying at elevated temperature was used. Drying (heating) was carried out at a pressure of 0.1-0.009 MPa. Different drying times of 6 and 24 hours were used, respectively. In order to remove the hyaluronic acid, after dissolving samples in water, the solutions were subjected to filtration with the use of a VIVASPIN membrane of 2,000 MWCO HY and then centrifuged (900 rpm), resulting in an aqueous solution containing only small molecule compounds. Given that the initial concentration of NMP in ammonia-water amounted to 33.33%, it was expected that NMP residues must be less than this level. Determination of NMP was performed spectrophotometrically by reading the absorbance value at a wavelength of 282.5 nm. The absorbance at this wavelength is also used for the calibration curve. Absorbance was performed for concentrations of 1%, 3%, 5%, 7% and 10% NMP in water. ***Figures 8*** and ***9*** present the UV spectra of aqueous solutions of NMP at different concentrations and the dependency of the absorbance on the NMP concentration in water, respectively.

It was found that drying the samples significantly reduces the amount of NMP residues, which makes it available for medical applications. However, the results obtained indicate that in conditions without further drying, the residue of NMP in hyaluronic acid is 3.81% (***Table 3***).

Heating (drying) of the samples significantly reduces the content of NMP. It is surprising that the effectiveness of the removal of NMP residues in two 24-hour experiments at different temperatures is the same, which is because the minimum content of NMP in both cases is 0.31%.

DSC analysis

From the point of view of solvent residual removal and the processing method – electrospinning at elevated temperature, it was important to check whether the polymer will undergo changes during these processes. According to the results obtained (***Figure 4*** – rheological results), it was confirmed that DSC curves, regardless of the molecular weight of the hyaluronic acid, are characterised by a broad peak with an endothermic peak in the range of 110-130 °C associated with the loss of chemically adsorbed water. This phenomenon has been confirmed in prior works [25]. In contrast, at temperatures above 230 °C polymer degradation occurred which is associated with the occurrence of a narrow exothermic peak. In the case of the drying process (post-heating) of fibrous structures, it is preferred to use temperatures below 120 °C, which in the case of electrospinning was set at 30 °C (***Figure 10***)

Summary

One of the key aspects of electrospinning is the proper selection of the polymer, its molecular weight and concentration and

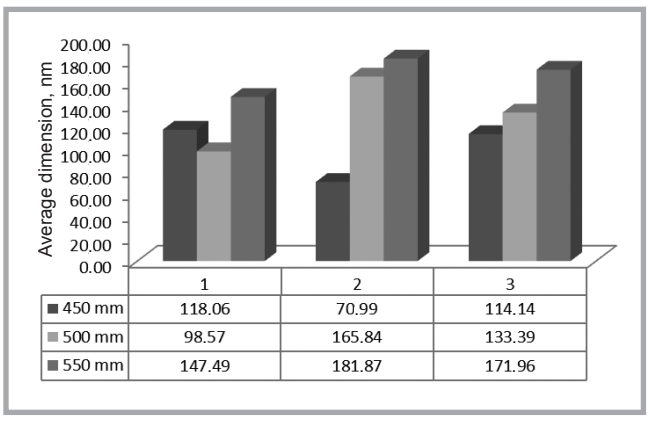


Figure 6. *Dependence of the distance from the collector on the average dimension of nanofibers.*

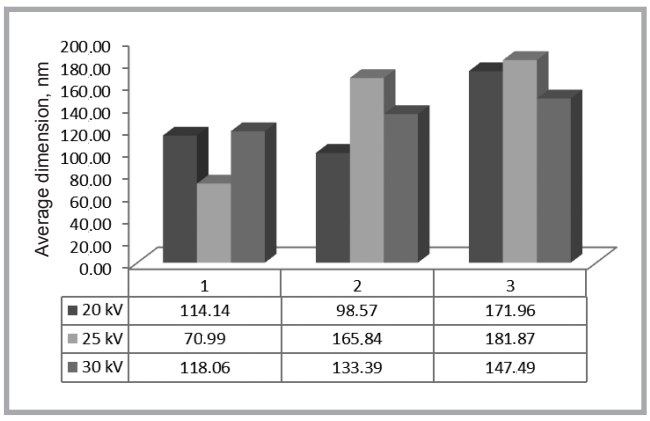


Figure 7. *Dependence of the voltage applied on the average dimension of nanofibers.*

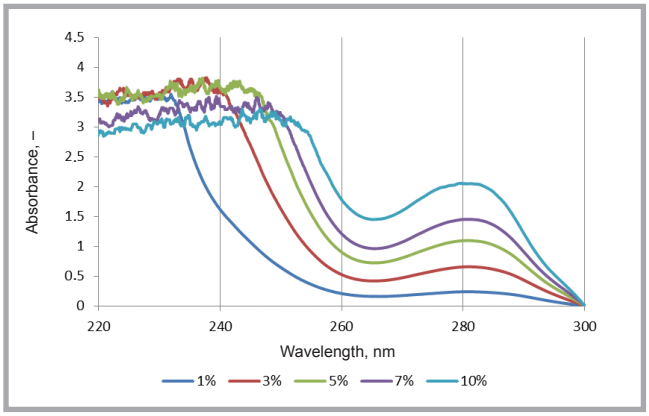


Figure 8. *UV spectra of aqueous solutions of NMP at different concentrations.*

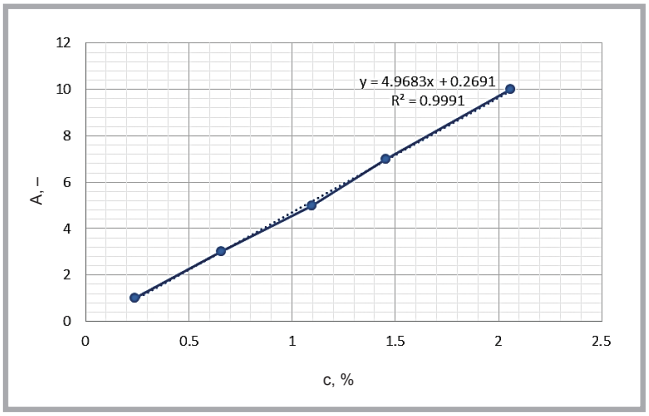


Figure 9. *Dependency of the absorbance on the NMP concentration in water.*

the properties of solvents used. All these parameters affect the spinning solution's viscosity and the surface tension, which decide upon the success of the process. The molecular weight of the polymer and solvent system were selected in such a way that they ensured sufficient surface tension and viscosity. Both these parameters allowed for manufacturing fibers of diameters between 20 to 400 nm. Examination of the influence of the molecular weight on the homogeneity of the final fibers revealed that the most uniform and smooth nanofibers were obtained in the process where HA with a molecular weight of 100-150kDa was used. The solvent system, which ensures the stability of the process of electrospinning, was determined as a mixture of ammonia water and NMP at a ratio of 2:1, respectively, with the most optimal polymer concentration being 12%.

Studies on electrospinning process parameters led to the selection of optimum conditions according to the diameters of the nanofibers obtained. It was found that a distance from the collector (voltage 25 kV) equalling 450 mm is optimal for the process. The optimal fibrous structures obtained are characterised by a size of the diameter of filaments between 80-100 nm, which constitutes 45%.

Studies concerning the presence of NMP in fibrous structures have shown that it is desirable to perform an additional process of heating enabling a significant reduction in the content of the solvent. At the same time the application temperature in the range of 90-95 °C remains efficient and safe to carry out this process. The lack of solvent residues is extremely important from the point of view of the medical use of HA-based non-woven.

The objective of the study was achieved, which included the design and characterisation of promising electrospun scaffolds for prospective use in tissue engineering purely based on hyaluronic acid sodium salt. This research will serve as a basis for future studies on customised biopolymeric scaffolds. It provides a framework for exploration of the influence of different process parameters on the morphology of HA-based fibrous structures. It also confirms previous findings and contributes additional evidence that it is possible to achieve electrospun pure HA scaffolds in the range of predetermined parameters, which gives a great basis for enriching scaffolds with bioactive additives. The studies will be continued to establish a biological response with respect to different morphologies of the fibers obtained and used as a scaffolds.

Acknowledgements

The project was financed by the National Science Center in Poland, awarded on the basis of decision number DEC-2012/07/B/ST8/03378 and the statutory research carried out in the Department of Materials and Commodity Sciences and Textile Metrology.

References

1. Vincent JFV, Bogatyreva OA, Bogatyrev NR, Bowyer A, Pahl AK., Biomimetics: its practice and theory. *Interface Journal of Royal Society* 2006; 3(9), DOI: 10.1098/rsif.2006.0127

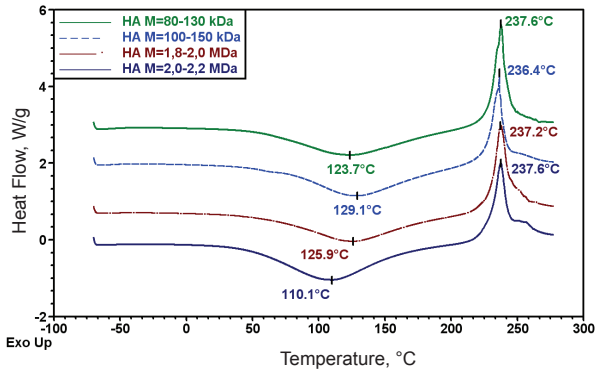


Figure 10. *Comparison of the thermal properties of hyaluronic acid salts of different molecular weights.*

Table 3. *Characteristics of spinning solutions.*

Sample	Heating temp., °C	Heating time, h	Absorbance	Concentration, %
1a	90-95	6	0.0317	0.43
1b		24	0.0088	0.31
2a	130-140	6	0.0201	0.37
2b		24	0.0076	0.31
3 (control)	–	–	0.8202	3.81

2. Shimomura M. The New Trends in Next Generation Biomimetics Material Technology: Learning from Biodiversity. *Science & Technology Trends Quarterly Review* 2010; 037: 53-75.
3. Sanchez C, Arribart H, Guille MMG. Biomimetism and bioinspiration as tools for the design of innovative materials and systems. *Nature Materials* 2005; 4(4) 12: 277-288.
4. Dhandayuthapani B, Yoshida Y, Maekawa T, Kumar D S. Polymeric Scaffolds in Tissue Engineering Application: A Review. *International Journal of Polymer Science* 2011, Article ID 290602, http://dx.doi.org/10.1155/2011/290602
5. Fa-Ming C and Xiaohua L. Advancing biomaterials of human origin for tissue engineering, *Progress in Polymer Scienc*, 2016; 53(86-168): 86-168. http://dx.doi.org/10.1016/j.progpolymsci.2015.02.004
6. Kogan G, Soltés L, Stern R and Gemeiner P. Hyaluronic acid: a natural biopolymer with a broad range of biomedical and industrial applications. *Biotechnology Letters* 2007; 29: 17-25 DOI: 10.1007/s10529-006-9219-z
7. Ivanova EP, Bazaka K, Crawford RJ. New Functional Biomaterials for Medicine and Healthcare. *Woodhead Publishing* 2013; p. 52-53, ISBN: 9781782422662 DOI: https://doi.org/10.1557/mrs.2014.209
8. Papakonstantinou E, Roth M and Karakiulakis G. Hyaluronic acid: A key molecule in skin aging. *Dermatoendocrinology* 2012; 4(3): 253-258, DOI: 10.4161/derm.21923
9. Olczyk P, Komosińska-Vassev K, Winsz-Szczotka K, Kuźnik-Trocha KK, Olczyk K. Hyaluronan – structure metabolism, function and role in wound healing processes (in Polish). *Postępy Higieny i Medycyny Doświadczalnej* 2008; 62: 651-659, e-ISSN 1732-2693.
10. Tucker N, Stanger JJ, Staiger MP, Razzaq H and Hofman K. The history of the science and technology of electrospinning from 1600 to 1995. *Journal of Engineered Fibers and Fabrics* 2012; 7(3): 50-131.
11. Ramakrishna S. *An introduction to electrospinning and nanofibres*. World Scientific Publishing Company, 2005.
12. Eric K. Brenner, Jessica D. Schiffman, Ebony A. Thompson, Laura J. Toth, Caroline L. Schauer, Electrospinning of hyaluronic acid nanofibers from aqueous ammonium solutions. *Carbohydrate* 2011; Polymers 87: 926-929 http://dx.doi.org/10.1016/j.carbpol.2011.07.033.
13. Ji Y, Ghosh K, Shu X Z, Li B, Sokolov J C, Prestwich GD, Clark RAF, Rafailovich M H. Electrospun three-dimensional hyaluronic acid nanofibrous scaffolds. *Biomaterials* 2006; 27: 3782-3792 DOI: 10.1016/j.biomaterials.2006.02.037
14. Liu Y, Ma G, Fang D, Xu J, Zhang H and Nie J. Effects of solution properties and electric field on the electrospinning of hyaluronic acid. *Carbohydrate Polymers* 2010; 83: 1011-1015 http://dx.doi.org/10.1016/j.carbpol.2010.08.061
15. Wu J and Hong Y. Enhancing cell infiltration of electrospun fibrous scaffolds in tissue regeneration. *Bioactive Materials* 2016; 1(1): 56-64 http://dx.doi.org/10.1016/j.bioactmat.2016.07.001
16. Dhandayuthapani B, Yoshida Y, Maekawa T and Sakthi Kumar D. Polymeric Scaffolds in Tissue Engineering Application: A Review. *International Journal of Polymer Science*, Article ID 290602, 2011; p. 19 http://dx.doi.org/10.1155/2011/290602
17. Pabjańczyk-Wlazło E, Król P, Chrzanowski M, Puchalski M, Krucińska I, Szparaga G and Boguń M. The selection of the polymer-solvent system and process conditions for electrospinning of hyaluronic acid. In: *Proceedings of BioNanoMed 2015, 6th International Congress of Nanotechnology in Medicine and Biology*, 8-10 April 2015, Graz, Austria
18. Biswas SC, Dubreil L and Marion D. Interfacial Behavior of Wheat Puroindolines: Study of Adsorption at the Air–Water Interface from Surface Tension Measurement Using Wilhelmy Plate Method. *Journal of Colloid and Interface Science* 2001; 244(2): 245-253.
19. Krucińska I, Gliścińska EM and Chrzanowski M. System for electrose pinning fibers. Patent Number EP 2325 355 B1, Poland, 2012 http://dx.doi.org/10.1016/S0032-3861(00)00250-0
20. Vinogradov G V. Structure and rheological properties of polymers. *Polymer Mechanics* 1975; 11(1): 139-149, DOI: 10.1007/BF00855434.
21. Deitzel JM, Kleinmeyer J, Harris D and Tan NCB. The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer* 2001; 42: 261-272.
22. Bhardwaj N, Kundu SC. Electrospinning: a fascinating fiber fabrication technique, Biotechnology Advances, 2010; 28(3), 325-347 http://dx.doi.org/10.1016/j.biotechadv.2010.01.004
23. Larrondo L and Manley R.. Electrostatic fiber spinning from polymer melts. II. Examination of the flow field in an electrically driven jet. *Journal of Polymer Science Part B – Polymer Physics* 1981; 19: 921-932 DOI 10.1002/pol.1981.180190602
24. Larrondo L and Manley R. Electrostatic fiber spinning from polymer melts. III. Electrostatic deformation of a pendant drop of polymer melt. *Journal of Polymer Science Part B – Polymer Physics* 1981; 19: 933-940 DOI 10.1002/pol.1981.180190603
25. Shanmugasundaram N, Ravichandran P, Neelakanta RP, Ramamurty N, Pal S and Panduranga R. Collagen-Chitosan polymeric scaffolds for the in vitro culture of human epidermoid carcinoma cells. *Biomaterials* 2001; 22, 1943-1951 http://dx.doi.org/10.1016/S0142-9612(00)00220-9

❑ ***Received 20.02.2017 Reviewed 18.04.2017***