

PAPER • OPEN ACCESS

A preliminary study on sodium hyaluronate loaded polyvinyl alcohol nanofiber webs obtained via roller electrospinning

To cite this article: S Jegina *et al* 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **500** 012024

View the [article online](#) for updates and enhancements.

A preliminary study on sodium hyaluronate loaded polyvinyl alcohol nanofiber webs obtained via roller electrospinning

Jegina S¹, Salaka L¹, Kukle S¹, Livkisa D², Gravitis J³

¹Institute of Design Technologies, Riga Technical University, Riga, Latvia

²Laboratory of Bioanalytical methods and Biodosimetry, University of Latvia

³Latvian State Institute of Wood Chemistry, Riga, Latvia

E-mail: Sandra.Jegina@rtu.lv

Abstract. The aim of this study was to investigate whether it is possible to integrate cosmetic grade sodium hyaluronate or hyaluronic acid (HA) into polyvinyl alcohol (PVA) nanofiber matrix, as well as to analyse how different concentrations of HA effects the viscosity and conductivity of the spinning solution, fiber diameters, morphology and mechanical properties of the nanowebs.

1. Introduction

In biomedical field hyaluronic acid/sodium hyaluronate (HA) is widely used because of its properties- it is a nontoxic, biodegradable, biocompatible natural linear polysaccharide. HA can be chemically modified due to the presence of multiple acid and hydroxyl groups in the molecule [1]. In medical applications HA plays a significant role in wound healing, tissue engineering, drug delivery and immunomodulation [2].

HA belongs to the extracellular matrix (ECM) molecules. Recently, it is appreciated that ECM molecules that lie between cells, in addition to providing a constructive framework, they exert major effects on cellular function. Although ECM molecules appear amorphous by light microscopy, they form a highly organized structure, comprising mainly of glycosaminoglycan (GAG) with a unique capacity to bind and retain water molecules [3], proteoglycans, growth factors and structural proteins such as collagens. Yet, the predominant component of the skin ECM is HA [4].

In dermatology and cosmetology topical delivery of molecules into the human skin is one of the main issues and as studies have shown- HA with low molecular weight (20-300 kDa) passes through the outermost layer of the epidermis compared to the impermeability of high molecular weight HA (1000-1400 kDa) [5].

Besides effective consumer products with HA and therapeutic injections [6], HA has been studied also in terms of electrospinning to expand its applications using polyamide solution in formic acid as matrix with HA 0.04 %wt to prepare nano fibres by needleless spinning [7].

A number of studies have proven the efficient use of PVA to develop electro spun nano fibrous mats as a novel dressing matrix for controlled release of drugs and biopolymers for regenerative medicine [8-13]. Considering that polyvinyl alcohol (PVA) is a biocompatible and nontoxic polymer



as confirmed by numerous studies including our previous experiments, in this study it is used as matrix to investigate spin ability and obtained web properties with different HA proportions in spinning solutions.

2. Materials and methods

Cosmetic grade low molecular weight (50 kDa) HA was bought in a local biocosmetic store “BB Factory”, while PVA (Mowiol® 18-88, Mw ~130,000, Sigma-Aldrich) bought in Labochemia Latvija” Ltd.

Both HA and PVA were dissolved in distilled water in different temperatures- HA in +20°C and PVA in 120-135°C, respectively. While the applied stirring speed was the same for both ingredients (1000 rpm), stirring time differed- 2 hours for PVA, 30 minutes for HA as HA dissolves relatively fast in water due to its well-known hydrophilic properties.

Five concentrations of HA were prepared and mixed into 10 wt% PVA solution (Table 1). To ensure stability of the bioactive ingredients of HA, PVA was cooled down to +20°C after dissolving process. When both solutions had the same temperature, they were mixed together using magnetic stirrer. Stirring time- 1 hour, speed- 1000 rpm, no heating was applied.

Table 1. Variants of polyvinyl alcohol (PVA) and PVA/hyaluronic acid (HA) spinning solutions

Composition	HA content wt%
PVA (10 wt%)	0
PVA (10 wt%)/HA	0.,04; 0.06; 0.07; 0.08; 0.1;

Electroconductivity and viscosity measured before electrospinning to prognose the success of nanofiber acquisition. It is known that viscosity values should be in the range 100-2000 mPa·s for solution to be spin able [14, 15].

Roller or needleless electrospinning was performed on Nanospider™ LAB 200 (Elmarco, Czech Republic) with following parameters- distance between electrodes 14 cm, roller speed- 2 rpm, applied voltage 65 kV, temperature- +20°C.

Fiber diameters were measured and morphology of the nanoweb was evaluated on atomic force microscope (AFM) Dimension Edge Veeco (Bruker, USA) using silicone cantilever OTESPA-R3, $f_0=300\text{kHz}$, $k=26\text{N/m}$ in tapping mode. 100 measurements of each sample were taken for nanofiber diameter analysis.

Fourier Transform Infra-Red (FTIR) spectroscopy was used to determinate HA in obtained nanofiber mats using equipment Thermo Scientific „Nicolet iS50 FT-IR“.

Mechanical properties were tested on Instron Universal Tester Model 2519-107 (deformation speed- 1 mm/min., applied load- 1 N) and the results were collected through program Instron Bluehill. 10 samples (size 10 mm x 30 mm) of each composite were prepared, thickness was measured before placing them on a paper frame (Figure 1) for testing [13]. The paper frame ensure samples tightly fixed and do not damaged by the equipment’s grippers in the testing process.

1.1 %wt HA and additional 5.2 %wt HA nano webs were tested on two cell lines- 3T3 mouse fibroblasts and HaCaT human keratinocytes [16]. Method- Neutral Red Uptake (NRU) assay.

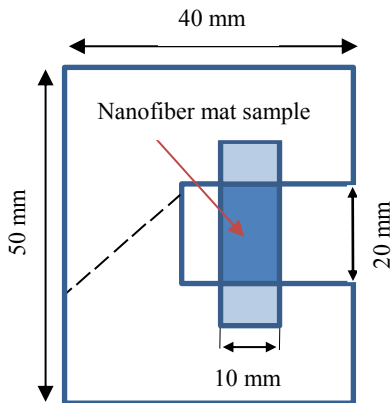


Figure 1. Preparation of nanofiber mat sample in Instron Universal Tester (Model 2519-107) [13]

3. Results and discussion

3.1. Viscosity and conductivity

Graphics of Figure 2 demonstrate that HA does not influence solution electro conductivity, which varies within the standard error, but viscosity proportionally increases with the HA concentration increase (equation (1)) from 796 to 1137 mPa-s, exceeding PVA solution viscosity (597 mPa-s) by 90%.

$$Y_V = 542,9 + 6170 X, \quad R^2 = 0,94, \quad (1)$$

Where: Y_V - viscosity, mPa-s; X - HA wt% of spinning solution.

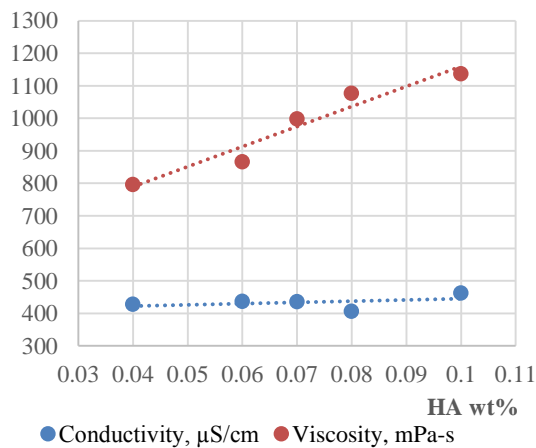


Figure 2. Properties of spinning solution

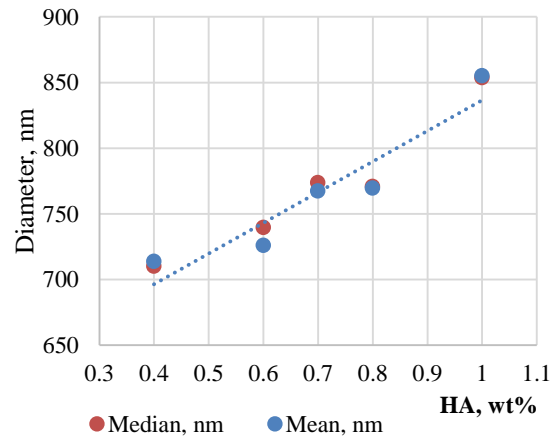


Figure 3. Fibers median and mean diameters

3.2. Diameters of nanofibers

Nanofiber diameter is the most important structural characteristic and its role in drug release process is parameters of electrospinning process [15]. Parameters of electrospinning process (applied voltage, distance between electrodes, relative humidity, temperature, etc.) can influence nanofibers diameters remarkably [16].

Median and average diameters of the nanofiber webs shown in Figure 3. Diameters tend to increase by added HA concentration and can be briefly described by equation (2).

$$Y_D = 602.87 + 233.64 x, \quad R^2 = 0.89 \quad (2)$$

3.3. Morphology of nanofiber mats

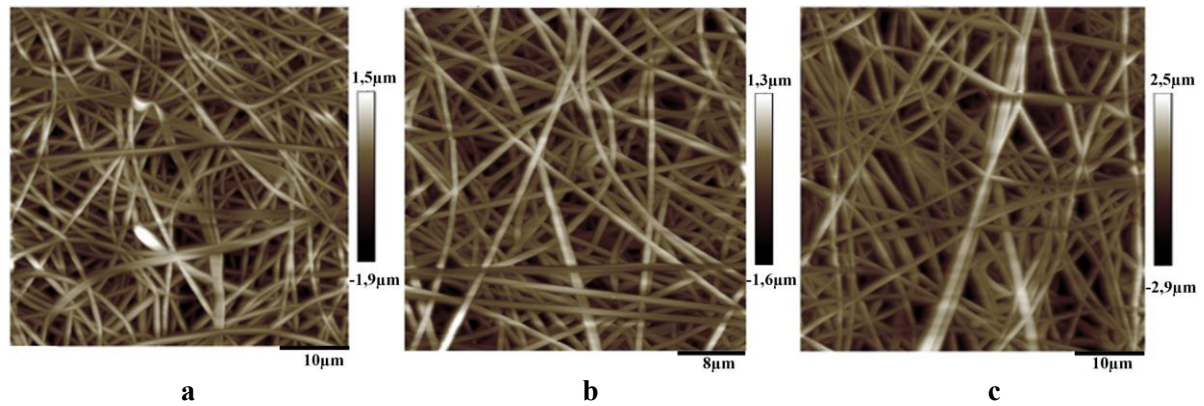


Figure 4. AFM images of nanofiber mats- **a** (PVA/0.4 wt% HA), **b** (PVA/0.8 wt% HA), **c** (PVA/1.1 wt% HA)

As shown in AFM images (Figure 4), 0.8 wt% HA nanofiber mat has even and dense surface compared to 0.4 and 1 wt% HA samples. This could explain why 0.8 wt% sample demonstrated the best mechanical properties compared to other concentrations.

3.4. Mechanical properties of nanofiber mats

Table 2. Characteristics of nanofiber webs

	Web thickness, μm		Web surface roughness, μm	Agglutinated fibres
	Mean	-/+		
PVA 10 wt%	91.8	14.0	3.5	+
HA 0.4 wt%	147.7	6.3	3.4	+++
HA 0.6 wt%	113.5	8.9	4.1	++
HA 0.7 wt%	75.1	8.8	2.3	-
HA 0.8 wt%	91.1	13.2	2.9	+
HA 1.1 wt%	102.3	10.8	5.4	++++

Although all samples were electro spun within the same period (5 minutes), thickness and surface roughness of the nanofiber webs varied, as well as the amount of agglutinated nanofibers (Table 2). As seen from graphs of Figure 5, nonlinear variation nature of variants thickness and surface roughness with a HA in a range from 0.6 – 1.1 wt % are quite similar. Though surface roughness of the HA 0.4 wt percentage web is close to the relevant indicator of net PVA, the web thickness differ substantially. The high share of agglutinated fibres in the web cross-section could be considered as a reason of difference (Table 2).

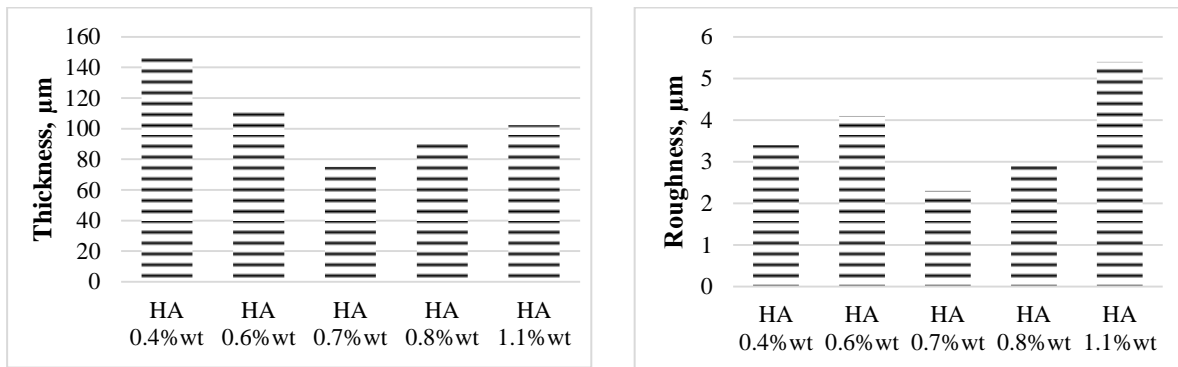


Figure 5. Variations of web variants thickness (left) and surface roughness (right)

Tensile stress and strain at break graphs (Figure 6) show non-linear dynamics depending on HA concentration in fibers, reaching the maximum value at 0,8 wt% HA (1,76 MPa, 17,84%), which is almost equal to the pure PVA sample (1,76 MPa, 18,65%) corresponding values. There is a linear relationship between tensile strain and stress where HA share in fibres fall in a range 0.6 – 0.8 %wt (Figure 6, right).

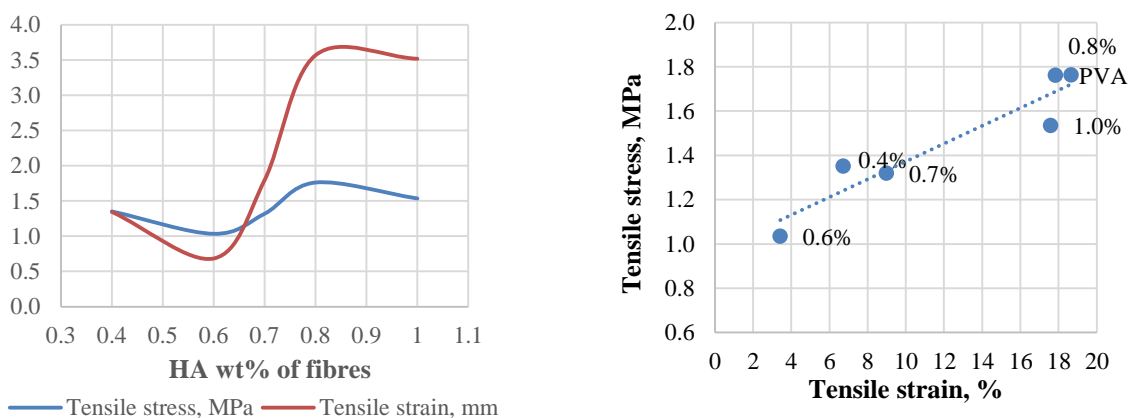


Figure 6. Mechanical properties of nanofiber webs

3.5. Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy of composite PVA and HA nanofiber webs revealed different from net PVA absorption intensity in a frequency range 1557 – 2956 cm⁻¹ and 3176-3462 cm⁻¹(Figure 7).

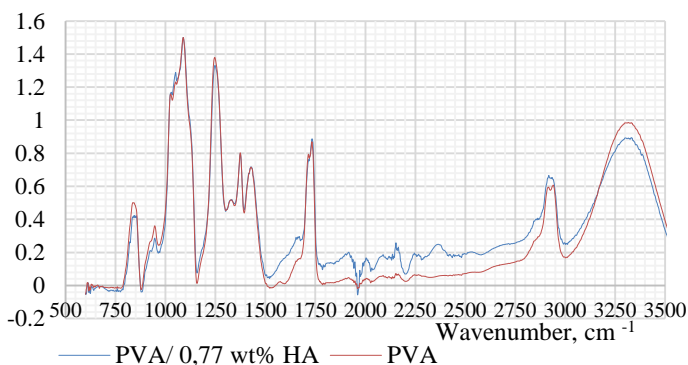


Figure 7. FTIR spectra of net PVA and PVA/HA 0.8 wt% webs

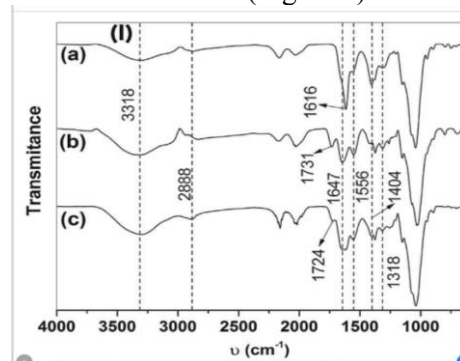


Figure 8. FTIR spectra of HA (a) and modified HA hydrogels[17]

Characteristic saccharide peaks identified in the sodium hyaluronate sample [17, 18]: at 3318 cm^{-1} ($\nu(\text{O-H})$, stretching band), 2888 cm^{-1} ($\nu(\text{aliphatic C-H})$, stretching band), 1616 cm^{-1} (Amide II), 1556 cm^{-1} ($\delta(-\text{NH}_2)$, bending) fall in observed ranges of experimental web graphs. Peak at frequency 1054 cm^{-1} which refers to skeletal vibrations of C-O and C-C stretching, as well at 1404 cm^{-1} ($\delta(\text{C-H})$ bending) partially overlapping with the bands of PVA (C-C; C-H₂; C-H; C-OH) and therefore not easily identifiable

Further in-depth studies of cosmetic grade HA are necessary to compare IR values to medical grade HA which has been studied extensively. Pharmaceutical grade HA has to be used when the final product developed for tissue engineering, injections and treatment of open wounds. Cosmetic grade HA is suitable for beauty products such as facial mask, skin care cream, lotion, gel, etc.

3.6. Microbiological test results

Biological activity of the composites were tested on two types of cells- 3T3 mouse fibroblast cells and HaCaT human keratinocytes (Figure 9). As 1 wt% concentration showed no positive activity, additional concentration of HA (5 wt%) was prepared and tested. Results show that 5 wt% HA has stronger positive effects on 3T3 cells compared to HaCaT.

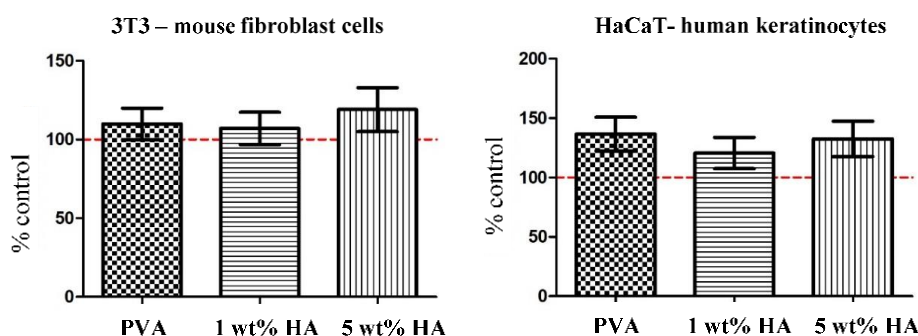


Figure 9. Microbiological test results of nanofiber mats

4. Conclusion

This study shows that spinning solutions based on PVA 10 wt% with the HA additive in a concentration range (0,04 to 0,5) wt% can be successfully electrospun. Viscosity of the spinning solution tends to increase linearly following HA concentration, but conductivity does not have the same dynamics.

Test results of mechanical properties revealed non-linear dynamics depending on HA concentration in fibers. FTIR analysis displayed new absorption peaks in all composites. Nanofiber diameters increased by added HA concentration.

5 wt% HA nanofiber mat sample showed better cell activity compared to 1 wt% HA. In further studies PVA concentration under 10 % wt should be evaluated and combined with a higher concentration of HA to keep appropriate viscosity and ensure better biological activity on cells.

References

- [1] Larrañeta E, Henry M, Irwin N J et al, 2018, Synthesis and characterization of hyaluronic acid hydrogels crosslinked using a solvent-free process for potential biomedical applications, Carbohydr. Polym. 2018, 181, 1194–1205, doi:10.1016/j.carbpol.2017.12.015
- [2] Zamboni F, Vieira S, Reis R L, Miguel Oliveira J, Collins M N, 2018, The potential of hyaluronic acid in immunoprotection and immunomodulation: Chemistry, processing and function. Prog. Mater. Sci. 2018, 97, 97–122, doi:10.1016/j.pmatsci.2018.04.003.
- [3] Baumann L. Skin ageing and its treatment. J Pathol. 2007;211:241–51. doi: 10.1002/path.2098
- [4] Eleni Papakonstantinou, Michael Roth, and George Karakiulakis. Hyaluronic acid: A key molecule in skin aging. Dermatoendocrinol. 2012 Jul 1; 4(3): 253–258.

- doi: 10.4161/derm.21923
- [5] Essendoubi M, Gobinet C, Reynaud R, Angiboust J F, Manfait M and Piot O, 2016, Human skin penetration of hyaluronic acid of different molecular weights as probed by Raman spectroscopy. *Skin Res Technol*, 22: 55-62. doi:10.1111/srt.12228
 - [6] Nobile V, Buonocore D, Michelotti A, Marzatico F, 2014, Anti-aging and filling efficacy of six types hyaluronic acid based dermo-cosmetic treatment: double blind, randomized clinical trial of efficacy and safety. *Journal of Cosmetic Dermatology*. 2014;13(4):277-287. doi:10.1111/jocd.12120.
 - [7] Milašius, R., Ryklin, D., Yasinskaya, N., Yeutushenka, A., Ragaišienė, A., Rukuižienė, Ž., Mikučionienė, D. Development of an Electrospun Nanofibrous Web with Hyaluronic Acid. *Fibres & Textiles in Eastern Europe*. 2017 | Nr 5 (125) | 8—12.
 - [8] Marcolin C, 2017, Electrospinning of biopolymers for regenerative medicine, PhD thesis, Biomaterial group of the Department of Chemistry, Materials and Chemical Engineering of Politecnico di Milano, <http://hdl.handle.net/10589/132688>.
 - [9] Jannesari M, Varshosaz J, Morshed M, Zamani M, 2011, Composite poly(vinyl alcohol)/poly(vinyl acetate) electrospun nanofibrous mats as a novel wound dressing matrix for controlled release of drugs. *International Journal of Nanomedicine*, 6:993-1003. doi:10.2147/IJN.S17595.
 - [10] Seham Abdelhady, Khaled M. Honsy, Mallesh Kurakula. Electro Spun- Nanofibrous Mats: A Modern Wound Dressing Matrix with a Potential of Drug Delivery and Therapeutics. *Journal of Engineered Fibers and Fabrics*. Volume 10, Issue 4 – 2015, 179-193.
 - [11] Enayati MS, Behzad T, Sajkiewicz P, Rafienia M, Bagheri R, Ghasemi-Mobarakeh L, Kolbuk D, Pahlevanneshan Z, Bonakdar SH. 2018, Development of electrospun poly (vinyl alcohol)-based bionanocomposite scaffolds for bone tissue engineering. *J Biomed Mater Res Part A* 2018;106A:1111–1120.
 - [12] Jegina S, Sutka A, Kukle S and Zelca Z, 2016, The effect of sodium chlorophyllin on polyvinyl alcohol electrospun nanofiber diameters (CYSENI 2016) (Kaunas: Lithuanian Energy Institute), pp 234 – 240.
 - [13] Sutka A, 2015, Technologies, Structure and Properties of Lignocellulosic Nanofibre Bicomponent Filaments. PhD Thesis. Rīga: [RTU], pp 80.
 - [14] Hrib J, Sirc J, Hobzova R et al, 2015, Nanofibers for drug delivery – incorporation and release of model molecules, influence of molecular weight and polymer structure, *Beilstein Journal of Nanotechnology*, vol. 6, pp 1939-1945.
 - [15] Yener F, 2014, New methods in the study of roller electrospinning mechanism. Summary of the thesis. Liberec: Technical University of Liberec, <http://www.ft.tul.cz/document/240>.
 - [16] Olschläger V, Schrader A, Hockertz S. Comparison of primary human fibroblasts and keratinocytes with immortalized cell lines regarding their sensitivity to sodium dodecyl sulfate in a neutral red uptake cytotoxicity assay. *Arzneimittelforschung*. 2009;59(3):146-52. doi: 10.1055/s-0031-1296378.
 - [17] L. Grundelova, A. Gregorova, A. Mracek, R. Vicha, P. Smolka, A. Minarik, Viscoelastic and mechanical properties of hyaluronan films and hydrogels modified by carbodiimide, *Carbohydr. Polym.* 119 (2015) 142–148
 - [18] Javier Perez Quinones, Johanna Jokinen, Salli Keinänen, Carlos Peniche Covas, Oliver Brüggemann, Dmitri Ossipov. Self-assembled hyaluronic acid-testosterone nanocarriers for delivery of anticancer drugs. *Macromolecular Nanotechnology*. *European Polymer Journal* · December 2017 DOI: 10.1016/j.eurpolymj.2017.12.043