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Viscoelastic and mechanical properties of hyaluronan films and hydrogels modified by carbodiimide

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ABSTRACT

This study investigated an effect of different ways of the preparation of insoluble hyaluronan material on its mechanical and viscoelastic properties. Hyaluronan (NaHy) of molecular weight M_w = 500,000 g mol^{-1} was modified with *N*-(3-dimethylaminopropyl-*N*′-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS), to be able absorb liquid without changing its mechanical properties. The modified, water insoluble NaHy materials were prepared in different geometry; as modified films and modified cylinders with exact dimensions. The occurrence of modification was confirmed by FT-IR (Fourier transform infrared spectroscopy) and ^{1}H NMR (proton nuclear magnetic resonance) spectroscopy and swelling test. The determined mechanical and viscoelastic properties of unmodified and modified hyaluronan revealed the high dependency of elasticity changes depending on the gel processing method. Moreover, NaHy gels in the cylindrical form with the sponge-like structure predominant them as a convenient geometry for application in a humid environment.

1. Introduction

Hyaluronic acid (HA) is a linear biopolymer with a high molecular weight, naturally presented in vertebrate organism. The highest amount of hyaluronic acid was discovered in the extracellular matrix of connective tissues. Furthermore, it can be found as a constituent in all body fluids and in the vitreous humour of the eye and the synovial fluid in the joints (Manasa, Sridevi, Chandana Lakshmi, & Dedeepya, 2012; Fraser, Laurent, & Laurent, 1997). Its structural unit consists of *N*-acetyl-D-glucosamine and D-glucuronic acid connecting with alternating β-1,3 and β-1,4 glycosidic bond (Laurent & Fraser, 1992). HA chain may expand to thousand fold of its original volume and form a loose hydrated network because of hydrophilic nature. The molecular weight of NaHy can reach 10^6 to 10^8 g mol^{-1} according to different sources and methods used to its determination (Cowman & Matsuoka, 2005). Due to such high molecular weight and its viscoelastic and rheological properties predestine hyaluronic acid to play important roles in living organisms (Kogen, Šoltés, Stern, & Gemeiner, 2007; Price, Berry, & Navsaria, 2007; Brown & Jones, 2005; Guillaumie et al., 2009). The above mentioned, unmodified hyaluronic acid has many important applications in drug delivery and surgery as well as in the field of ophthalmology, tissue engineering and wound healing (Saettone, Monti, & Torracca, 1994; Moore & Willoughby, 1995; Davidson, Nanney, Broadley, Whitsett, & Aquino, 1991; Vercruysse & Prestwich, 1998; Prestwitch, Marecak, Marecak, Vercruysse, & Ziebell, 1998; Collins & Birkinshaw, 2013). Many authors have reported the important influence of molecular weight and degree of substitution of NaHy on the final film formation (Minařík, Smolka, & Lapčík, 2011; Mlčochová et al., 2006; Li et al., 2007; Obadait et al., 2010). Chemical modification of sodium salt hyaluronan (NaHy) allows modifying its solubility, viscosity, amphiphilicity, rate of degradation and *in vivo* residence time. Schanté, Zuber, Herlin, and Vandamme (2011) reviewed a number of chemical modification methods relevant for NaHy. The most common modification of hyaluronic acid is a chemical cross-linking, which can fix its structure (Lu, Lai, Ma, & Hsiue, 2008). The intensive research

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was devoted to study of NaHy cross-linking mechanism (Collins & Birkinshaw, 2007). The functional groups enabling cross-linking of HA molecules are carboxyl and hydroxyl groups, whereby carboxyl groups can be easy cross-linked generating an ether bond, and hydroxyl groups form ester bond. Moreover, other reactive groups such as amino groups can be also introduced in NaHy. Water-insoluble NaHy films can be prepared by an esterification of hyaluronic acid with mono-functional organic halides whereas highly swollen gels or insoluble plastic materials by cross-linking with divinylsulfone, bisepoxides, formaldehyde, glutaraldehyde, bishalides and carbodiimide (Balazs & Leshchiner, 1986; Balazs & Leshchiner, 1987; De Belder & Mälson, 1986; Malson & Lindqvist, 1986; Valle & Romeo, 1988; Valle & Romeo, 1987; Hu, Sabelman, Tsai, Tan, & Hentz, 2000; Zhang et al., 2011; Perng Ch, Wang, Tsi Ch, & Ma, 2011). Hamilton, Fox, Acharya, and Walts (1970) patented in 1970 cross-linking of HA by carbodiimide. Further experiments concerning modification of NaHy by carbodiimides were performed by many other authors (Xuejun, Netti, Ambrosio, Nicolais, & Sannino, 2004; Tomihata & Ikada, 1997; Kuo, Swann, & Prestwich, 1991; Sannino et al., 2005). Generally, carbodiimide is considered to be a preferable cross-linking agent with low cytotoxicity and water solubility.

Despite of considerable research concerning of physico-chemical properties of cross-linked or modified NaHy (Haxaire, Braccini, Milas, Rinaudo, & Pérez, 2000; Hamilton et al., 1970) rather less attention has been paid to the investigation of viscoelastic properties of modified NaHy materials depending on its geometry. We hypothesize that cross-linking and modification of NaHy change not only its solubility but especially viscoelastic properties. Moreover, the geometric form of the final modified NaHy can significantly influence its mechanical and viscoelastic properties. In the literature it is evident, that the most common forms of modified NaHy are hydrogels in the form of films. We have supposed that modified NaHy in the form of cylindrical hydrogel (NaHy_B_cylinder) could promote rather intermolecular than intramolecular modification, contributing to the overall stabilization and probably more superior mechanical properties in comparison with flat hydrogel (NaHy_B_film). In addition to this, cylindrical hydrogel (NaHy_B_cylinder) may find a vacancy in applications such as wound healing, drug delivery substrates, scaffolds for tissue engineering or absorbent medications. Moreover, NaHy_B_cylinder due to its geometry may be applicable to remove heavy metal ions from aqueous solutions. In this work the water-insoluble hyaluronan hydrogels in the form of films and cylinders were prepared and characterized by nuclear magnetic resonance (^{1}H NMR), FT-IR spectroscopy, tensile testing and dynamic mechanical analysis (DMA).

2. Materials and methods

The sodium hyaluronate sample (NaHy) of molecular weight M_w = 500,000 g mol^{-1} was provided by Contipro, (Czech Republic). Cross-linking agents *N*-(3-dimethylaminopropyl-*N*′-ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimid (HMS) were purchased from Sigma-Aldrich. All of the chemicals were of analytical grade and used without further purification.

2.1. Preparation of unmodified hyaluronan films

Stock solutions of NaHy in concentration of 1% (w/w) were prepared by dissolving the polymer in Milli-*Q* water using gentle stirring for 24 h at 50 °C. The solution of hyaluronan was then poured into a polystyrene mould with dimensions of 100 × 80 × 0.05 mm and dried to constant weight at 50 °C.

2.2. Preparation of modified hyaluronan films

Cross-linking agent EDC works as the activating ingredient of the carboxylic groups (100 mM) and HMS as the proton exchanger in a weight ratio 2:1 were added to already prepared 1% (w/w) hyaluronan and stirred for 1 h at 25 °C. This solution was then titrated with 0.1 M HCl to adjusting pH in the range of 4.5–4.75 under continuous stirring 1 h. Resulting solution was then poured into a polystyrene mould with dimensions of 100 × 80 × 0.05 mm and dried to constant weight at 50 °C.

2.3. Preparation of modified hyaluronan cylinders

The NaHy cylindrical hydrogels with a sponge-like solid structure were prepared as follows: 100 mM EDC was added to 1% (w/w) hyaluronan solution and stirred for 1 h at 25 °C. A 0.1 M HCl was then added to adjust pH to 4.5–4.75 under continuous stirring during 1 h. Subsequently, this solution was casted in polyethylene bins and kept at −18 °C for 72 h to prepare hyaluronan hydrogels in the cylindrical form with the diameter of 2.8 cm and the height of 0.7 cm.

2.4. Swelling test

The swelling test was used to determine an extent of modification of hyaluronan materials. Prepared hyaluronan samples were kept in a desiccator for one week. Afterwards were cylinders cut to round shape samples of diameter 11 mm, and dried to constant weight at 50 °C. The modified hyaluronan samples were then swelled in Milli-*Q* water and reweighed immediately at predetermined time intervals. The water content (W_{H_2O}%) in samples was calculated according to the following equation:

$$W_{H_2O}(\%) = \frac{(w_i - w_0)}{w_i} \times 100 \quad (1)$$

where w_i is the weight of the swollen sample and w_0 is the weight of the dry sample before swelling. The presented results are average values of 4 measurements.

2.5. FT-IR analysis

The FT-IR spectra of dried NaHy film, modified NaHy film and cylinder were recorded with Nicolet 6700 FT-IR spectrometer in ATR arrangement with diamond crystal. All spectra were recorded in the absorbance mode in the wave number range 4000–400 cm^{-1} and 64 accumulations at the resolutions of 4 cm^{-1}.

2.6. ^{1}H NMR

The NaHy samples and EDC were dissolved in D_2O (Sigma-Aldrich, 99.9 atom% D, with no internal standard), and the spectra were calibrated to 4.70 ppm for residual HDO signal. Samples investigated by NMR were modified using deuterized water and 0.1 M deuterium chloride acid solution (prepared from 35 wt% DCl in D_2O (Sigma-Aldrich, 99 atom% D) and D_2O) as described in Section 2.3 and transferred into NMR cuvette under an inert atmosphere. For quantitative analysis was taken 0.5 cm^3 of liquid that was gently compressed from hydrogel, and 0.01 cm^3 of DMSO (used as an internal standard). Concentrations of ingredients were calculated according Eq. (2) where *I* is integral intensity of ^{1}H NMR signal, *N* is the number of H-atoms providing signal under consideration. The volume change related to the mixing of 0.5 cm^3 of sample with 0.01 cm^3 of DMSO was ignored.

$$c_i = \frac{I_i}{N_i} \times \frac{N_{DMSO}}{I_{DMSO}} \times c_{DMSO} \quad (2)$$

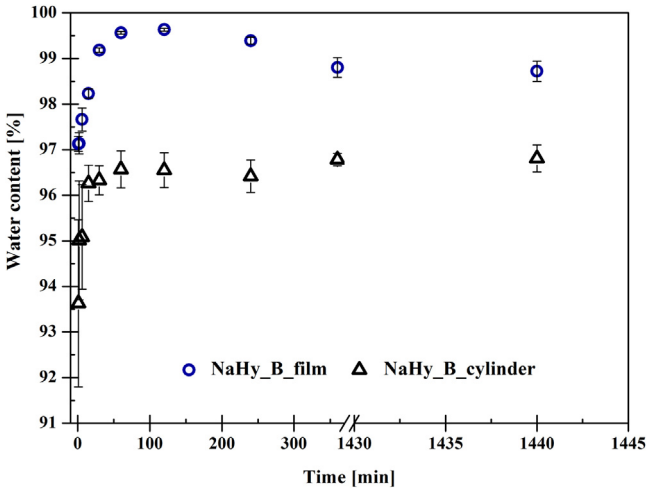

Fig. 1. Dependence of the water content of hyaluronan samples modified with EDC/HMS respective EDC.

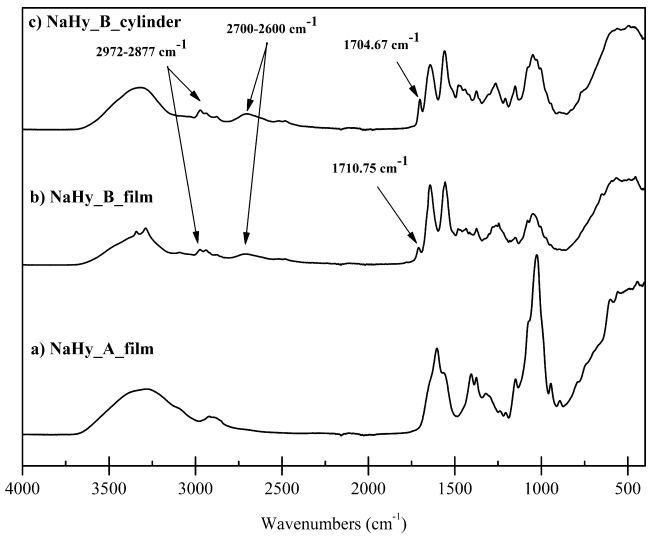

Fig. 2. FT-IR spectra of dried (a) unmodified hyaluronan film (NaHy_A_film), (b) modified hyaluronan film (NaHy_B_film), (c) NaHy hydrogel (NaHy_B_cylinder).

The concentration of carboxylic groups available for modification was calculated according Eq. (3) where FW_{MU} is formula weight of mer unit.

$$c_{COOH} = \frac{c_{NaHy} \times FW_{NaHy}}{FW_{MU}} \quad (3)$$

^{1}H NMR spectra were recorded using a BRUKER AVANCE 300 spectrometer at frequency of 300.15 MHz and processed using ACD/NMR Processor AE v. 12.01.

2.7. Tensile tests

In order to obtain information about mechanical properties of unmodified NaHy and modified NaHy films the Instron tensile machine with 100 N force sensor was utilized, using a crosshead speed of 1 mm min^{-1} at 25 °C. Dimensions of the specimens were following: for modified film with length of 30 mm, width 4 mm and thickness of 0.17 mm, for unmodified film with length of 30 mm, width of 4 mm and thickness of 0.05 mm. At least five specimens were tested in each case. The change of mechanical properties of the samples was studied from the point of statistical significance by the analysis of variance (ANOVA) using Origin (version 8.5). Differences among mean values were processed by Tukey test at a level of significance of $p < 0.05$.

2.8. Dynamic mechanical analysis (DMA)

Dynamic mechanical analysis (DMA) was carried out under (a) tensile and (b) compression geometry in transient mode as creep/creep recovery experiments using instrument DMA Q800 (TA Instruments, USA). Creep testing was performed by applying 0.01 N of static preloaded force, 20 min isotherm at 25 °C, following by applying of constant stress of 1 kPa for 3 min and relaxation without stress for 3 min. Samples used for DMA had following dimensions: (a) strips with a width of 4 mm were cut from films and mounted in the DMA with a gauge length of 10 mm, (b) cylinders with the diameter of 15 mm were punched out from the original hyaluronan cylindrical hydrogels and excess water was squeezed between filter paper to reach final sample height of 6 mm.

The viscoelastic properties of hyaluronan samples obtained by creep/creep recovery measurements were evaluated as creep compliance (J) and creep recovery (R). The creep compliance, J expresses the sample's willingness to yield (or compress in the compression mode) and can be calculated as an inverse of the modulus:

$$J = \frac{d\varepsilon}{d\sigma} \quad (4)$$

where ε is a strain and σ is a stress at a specific time.

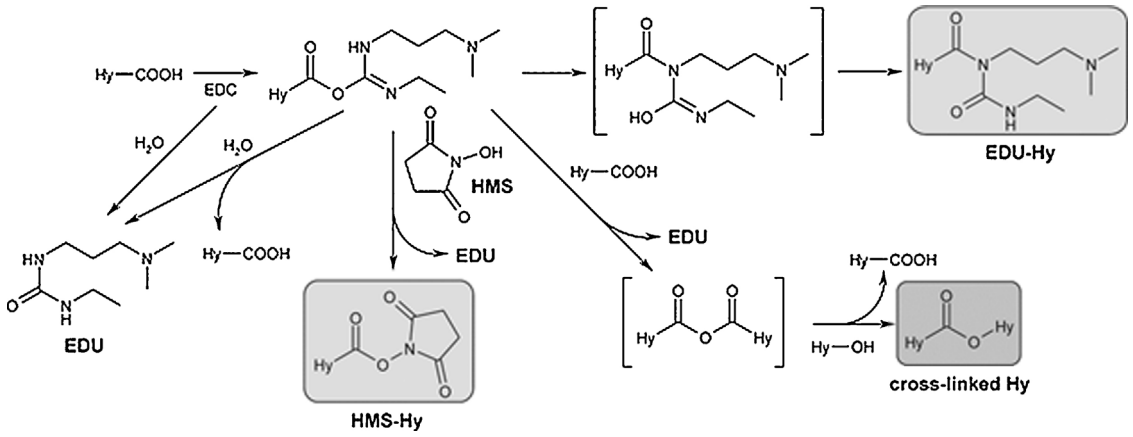

Fig. 3. The possible cross-linking reaction of hyaluronan with EDC and HMS.

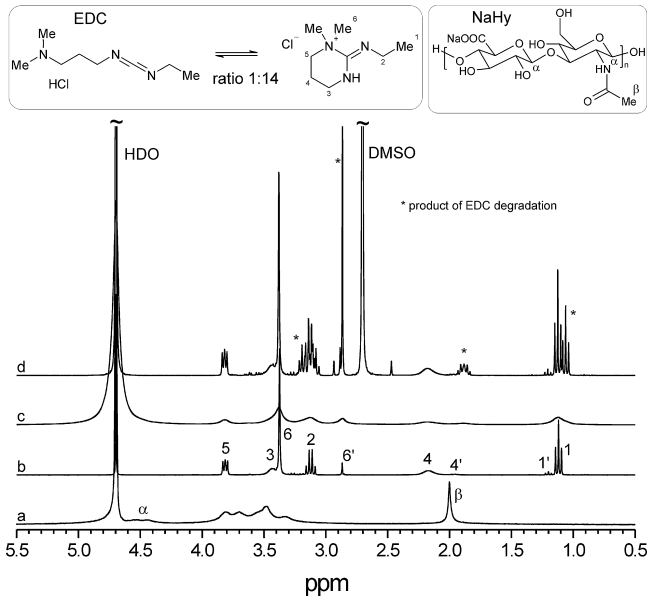

Fig. 4. Stacking plot of partial ^{1}H NMR spectra of (a) sodium salt of hyaluronic acid in D_2O at 30 °C; (b) standard EDC sample in D_2O at 30 °C; (c) sponge product (NaHy hydrogel) in mother liquor; (d) the mother liquor after sponge removing (DMSO was added as an internal standard).

The creep recovery, R presents an ability of sample relax after a removal of stress:

$$R = \left(1 - \frac{J - J_R}{J}\right) \times 100 \quad (5)$$

where J is a maximum reached creep compliance and J_R is a recoverable compliance at the end of relaxation time.

At least three specimens were tested in each case. The change of creep recovery of the samples was studied from the point of statistical significance by the analysis of variance (ANOVA) using Origin (version 8.5). Differences among mean values were processed by Tukey test at a level of significance of $p < 0.05$.

3. Results and discussion

3.1. Swelling tests

The swelling test was applied only for modified samples due to the high solubility of unmodified hyaluronan in water. Dependence of the water content of modified hyaluronan film (NaHy_A_film) and cylindrical hydrogel (NaHy_B_cylinder) is shown in Fig. 1. As can be seen, the modified film reached stabilization following a rapid increase in the water contents. The swelling tendency is similar for both materials. Swelling test showed that, the modified NaHy samples absorb of 99.2% (film) or 97% (cylindrical hydrogel) of water. It can be seen that the applied modification and the geometrical form determine an extent of water uptake. It was already shown that the extent of modification and hydrophobicity of the system determine the rate, at which hydrogels reach their water content equilibrium (Collins & Birkinshaw, 2008). The high ability of modified material to absorb water adverts to low extent of cross-linking. From the previous research it is known (Lai, 2012), that swelling of hyaluronan based materials with chemical cross-linking agent may enlarge the intermolecular distance which results in decrease of the collision frequency of polysaccharide molecules and thus the rate at which the covalent bond are formed between polymeric chains. In addition, the lower swelling ratio indicates higher cross-linking density of NaHy hydrogel (Lai, 2012). Additionally, cross-linking density can be indicated by changes in mechanical properties of NaHy samples.

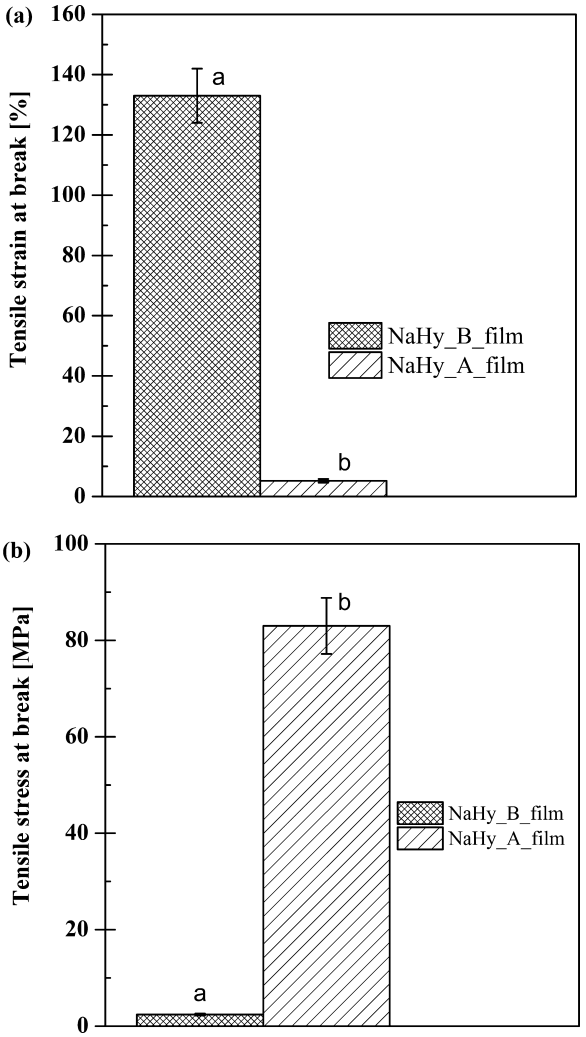

Fig. 5. Mechanical properties dependence of EDC modified NaHy films (a) Tensile strain at maximum of NaHy_A_film and NaHy_B_film, (b) Tensile stress at maximum of NaHy_A_film and NaHy_B_film. The superscript letters indicate the results of multiple comparison tests for the individual mechanical properties, calculated separated for each sample. Group averages with different superscript letters differ significantly ($p < 0.05$). Error bar means standard deviation from average value of five measurements.

3.2. FT-IR analysis

FT-IR experiments were carried out to obtain deeper insight into the modifying mechanism of NaHy by carbodiimide. Fig. 2, line (a) shows typical spectrum of hyaluronan (NaHy_A_film) with absorption bands at 3307 and 943 cm^{-1} (O—H group), 2927 and 2875 cm^{-1} (asym. and sym. stretching vibrations of CH_2 group), 1608, 1411 and 605 cm^{-1} (N—H deformation and C—N stretching vibrations of amide), 1608 and 1377 cm^{-1} (carboxyl vibrations), 1143, 1079, 1040 and 946 cm^{-1} (C—O—C vibrations) (Gilli, Kacuráková, Mathlouthi, Navarini, & Paoletti, 1994). Spectra of modified NaHy samples (NaHy_B_film and NaHy_B_cylinder, see Fig. 2 lines (b) and (c)) approved the accomplished modification. An occurrence of the ester bond can be suggested by additional bands belonging to ν(CH) and ν(CH_2) vibrations of alkyl chains located in the range of 2850–2930 cm^{-1} (Mlčochová et al., 2006). Another new peak appears in the range of 2700–2600 cm^{-1} and may be assigned to —CH_2 stretching vibrations of protonated urea. The vibration

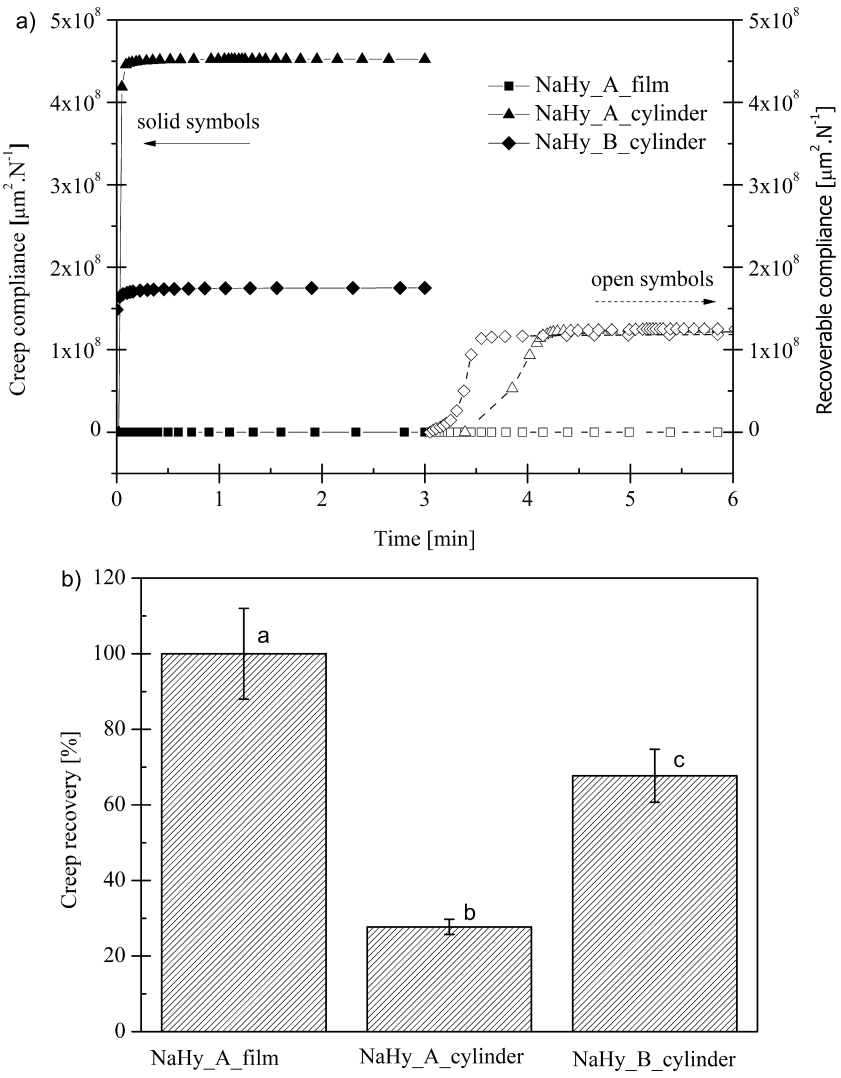

Fig. 6. Creep properties of hyaluronan samples: (a) creep compliance and recoverable compliance (b) creep recovery. The superscript letters indicate the results of multiple comparison tests for the individual creep recovery data, calculated separated for each sample. Group averages with different superscript letters differ significantly ($p < 0.05$). Error bar means standard deviation from average value of three measurements.

peak at 1700–1710 cm^{-1} can be allocated to ester bond as well as to amid bond. Additionally, the peak at 1700 cm^{-1} can be also assigned to the carbonyl group of amide. The cross-linking agent EDC does not chemically bind to polysaccharides, or more precisely to hyaluronic acid, but activates its carboxyl groups. Furthermore, EDC seems to mediate acid anhydride formation between two carboxyl groups belonging to the same polysaccharide molecules. The resulted acid anhydride may readily react with a hydroxyl group of polysaccharides to yield an ester bond, which functions as a crosslink of polysaccharide molecule (Fig. 3). Therefore, IR spectra of EDC modified NaHy suggest that carboxyl groups of NaHy reacted with hydroxyl groups of NaHy resulting in the formation of ester linkages (Lu et al., 2008; Park, Park, Kim, Song, & Suh, 2002). Carbodiimide induces cross-linking of NaHy and other polysaccharides without taking part in the linkage. EDC reacts with carboxylic acid groups and forms an active *O*-acylisourea intermediate, which is unstable and undergoes rearrangements to a stable *N*-acylurea. In the presence of primary amine, the *O*-acylisourea forming an amide linkage between the primary amine and the carboxyl group (Kuo et al., 1991). An EDC by-product is released as a soluble urea derivate (EDU). In general, carbodiimide as cross-linking agent is preferable because it can induce cross-linking of biomaterials simply by changing then into water-soluble urea derivates that have very low cytotoxicity. In order to prevent the irreversible *N*-acylurea by-product formation, *N*-hydroxy succinimide (HMS) was added to the modified NaHy film (Tomihata & Ikada, 1997; Bulpitt & Aeschlimann, 1999). The chemical reaction between this anhydride and hydroxyl is then responsible for the formation of the ester bond between two NaHy molecules. The reaction cannot occur if any hydroxyl groups do not directly encounter the acid anhydride due to the high instability of the acid anhydride in water solutions at room temperature (Schanté et al., 2011; Sannino et al., 2005; Nakajima & Ikada, 1995). The absorption peaks at 1643 and 1556 cm^{-1} are characteristics for amide bonds.

3.3. ^1H NMR

We attempted to determine the degree of modification of NaHy employing the ^1H NMR spectroscopy. The resulted mixture consisted of sponge-like solid material (cylindrical hydrogel) and clear colourless mother liquor. As it can be seen in Fig. 4, line c, only broadened signals of EDC and of its degradation product (DP) can be observed in the NMR spectrum of sample containing sponge and mother liquor (removing of the sponge led solely to the sharpening of signals as line d depicts). In another words, no signals related to the free or modified NaHy were observed in the

sample after reaction (see Fig. 4 lines a and c/d). Therefore, we decided to determine the portion of non-reacted EDC and of its DP in mother liquor. For this purpose, the 0.5 cm^3 of mother liquor was separated from sponge solid using micropipette and 0.01 cm^3 of DMSO was added as an internal standard. The NMR spectrum of this sample is depicted in Fig. 4, line d. The molar concentrations of EDC and of DP were calculated considering the respective signals of terminal methyl group according to Eq. (1) to reveal that concentration of remaining EDC and DP was 34.6 mM and 23.0 mM, respectively. Considering the initial concentration of EDC of 0.1 M, it is obvious that 42.4 mmol of EDC per dm^3 was consumed. As it is well known that the EDC reacts with carboxylic group of the HA, we were able to calculate the degree of modification. The average formula weight of used NaHy was M_w = 500,000 g mol^{-1}. If we take into account the initial concentration of NaHy of 3.2×10^{-5} M, the formula weight (FW) of the mer unit of 379.32 g mol^{-1} and ignore the slightly higher FW of terminal units, we can employ the Eq. (2) to calculate the concentration of carboxylic groups of 42.1 mM. Upon these calculations we can conclude that essentially all carboxylic groups were modified with EDC under our procedure.

3.4. Tensile tests

Mechanical properties of NaHy film and modified NaHy film are depicted in Fig. 5. The test specimens prepared from the unmodified NaHy film showed the tensile stress at maximum (83 ± 15) MPa, which is significantly higher than modified NaHy film (2.4 ± 0.2) MPa. In addition, it was found that the tensile strain at maximum, of the unmodified NaHy film was markedly lower (5.2 ± 0.6) % than modified NaHy film (133 ± 9) %. These results could indicate that modified NaHy film is composed of pendant modification too, because the modification does not indispensably cross-link NaHy molecule with other. Additionally, the poorer tensile strength and enhanced elasticity of modified films are characteristic for a lower modified material (Kablik, Monheit, YU, Chang, & Gershkovich, 2009). Another approach to these tensile results mentioned Lai (2012), in his study. They referred to the presence of absorbed water, which may affect the mechanical strength and stability of hydrophilic NaHy networks.

3.5. DMA

Viscoelastic properties of unmodified and modified hyaluronan samples, in the form of films and cylinders were determined by measuring the creep compliance (J) and creep recovery (R). Fig. 6 shows creep behaviour of hyaluronan samples depending on stage and sample form, at a temperature of 25 °C, and an applied stress of 1 kPa. As observed, the lowest creep compliance of 1×10^3 μm^2 N^{-1} was detected for unmodified hyaluronan film. The low willingness to yield of NaHy_A_film is in accordance with determined low tensile strain at maximum (5.2%). On the base of mechanical properties determined by tensile testing, it could be expected that modified hyaluronan film (NaHy_B_film) will show higher creep compliance (25—fold higher tensile strain as unmodified hyaluronan film). However, due to the very low mechanical strength of the modified NaHy film (tensile stress at maximum of 2.4 MPa) no data of creep compliance of this sample were detected. The modified NaHy film was ruptured immediately after gripping of sample in the DMA tensile clamp and the application of preloaded force of 0.01 N (used for the initial stretching of sample). The unmodified and modified NaHy gels in the form of cylinders reached creep compliance of 4.5×10^8 μm^2 N^{-1} and 1.7×10^8 μm^2 N^{-1}, respectively. The determined compression creep response of unmodified cylindrical NaHy hydrogel was different than its tensile response of film sample. By the swelling test no differences depending on sample geometry were detected however, it can be seen that the willingness to change the shape of a material significantly depends on the sample geometry as well as processing method. The creep recovery, based on the detected values of relaxation compliance, can be ordered as follows: NaHy_A_cylinder < NaHy_B_cylinder < NaHy_A_film. Fig. 6b displays creep recovery data (expressed in percentage) in dependence on sample form and processing method. The unmodified film reached the highest relaxation ability of about 100% but with the lowest reached compression of 0.3% in comparison to unmodified cylinder gel with the decreased relaxation ability about 72% but with reached compression of 90%. The modified cylinder gel displayed 88% compression with 68% strain recovery.

4. Conclusions

Water soluble and water insoluble hyaluronan materials in the form of films and cylinders were prepared. Water solubility of hyaluronan gel materials was limited by modification with *N*-(3-dimethylaminopropyl-*N*′-ethylcarbodiimide hydrochloride). The accomplished modification of hyaluronan was confirmed by IR and ^{1}H NMR spectroscopy. Both modified NaHy film and cylinder were in water insoluble in comparison to those unmodified, which were soluble in water after a few minutes. Furthermore, modified film and cylinder can absorb about 99.9% and 97% of water, respectively. The applied modification decreased the tensile strength of NaHy film 24.5 times and increased the tensile strain at break about 80%. The viscoelastic properties of hyaluronan samples in film and cylindrical form were determined by creep/creep recovery testing. It was shown that elasticity of hyaluronan samples highly depend on the sample form as well as applied processing method. It can be concluded that modified hyaluronan material in cylindrical form possess high willingness to change shape after an applied stress as well as high relaxation ability after stress recovery. Moreover, its sponge like solid structure enables to absorb high amounts of liquid. The reported results evidence that modified NaHy in form of cylinder proved improved viscoelastic properties in comparison with hydrogel in the form of film. On the base of our results, we can suggest that modified hyaluronan material with sponge like solid structure in the combination with metal scavengers, for example with lignin (Liu, Zhu, Qin Ch Zhou, Zhao, & Wang, 2013; Albadarin et al., 2011; Mohan, Pittman, & Steele, 2006; Guo, Zhang, & Shan, 2008), could be used as biodegradable composites for water treatment. This will be a subject of our further research.

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