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Bioinspired lubricating films of cellulose nanofibrils and hyaluronic acid

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Graphical Abstract

Highlights

- Tribological properties of thin films of cellulose nanofibrils (CNF) were analyzed
- Hyaluronic acid (HA) was attached to CNF films through an esterification reaction
- The attachment of HA improved remarkably the lubrication properties of CNF films
- HA-CNF films exhibited similar friction coefficients as articular cartilage
- Surface and friction forces were measured using the colloid probe technique

Abstract

The development of materials that combine the excellent mechanical strength of cellulose nanofibrils (CNF) with the lubricating properties of hyaluronic acid (HA) is a new, promising approach to cartilage implants not explored so far. A simple, solvent-free method to produce a very lubricating, strong cellulosic material by covalently attaching HA to the surface of CNF films is described in this work. A detailed analysis of the tribological properties of the CNF films with and without HA is also presented. Surface and friction forces at micro/nanoscale between model hard surfaces (glass microspheres) and the CNF thin films were measured using an atomic force microscope and the colloid probe technique. The effect of HA attachment, the pH and the ionic strength of the aqueous medium on the forces was examined. Excellent lubrication was observed for CNF films with HA attached in conditions where the HA layer was highly hydrated. These results pave the way for the development of new nanocellulose-based materials with good lubrication properties that could be used in biomedical applications.

Keywords: cellulose nanofibrils; hyaluronic acid; thin films; lubrication; colloid probe technique.

1. INTRODUCTION

In line with the growing concern for environment and sustainability, much effort has lately been directed towards new renewable materials with superior properties. In this scenario, cellulose and, especially, nanofibrillated cellulose or cellulose nanofibrils (CNF) can play an extraordinary role. With typical diameters in the range 5-60 nm and lengths up to several micrometers, CNF is produced by the mechanical fibrillation of cellulose pulp, usually assisted by chemical or enzymatic pretreatments according to well-established procedures.¹⁻⁶ Individual fibrils and networks of CNF exhibit elastic moduli in the range of 10-150 GPa with remarkably high wet strength.⁷⁻¹¹ The excellent mechanical properties, together with its natural origin and non-toxicity, have made CNF a very interesting material for new applications beyond the traditional uses of cellulose in paper, cardboard, textiles and construction materials. CNF is an attractive candidate for the reinforcement of composites. It has been combined with different polymers and nanoparticles in order to develop efficient adhesives and cellulose-based electroconductive materials, as well as nanocomposites with superior thermal, mechanical and barrier properties with potential applications in the packaging of food and electronics.¹²⁻¹⁶ Transparency and low weight are usually added values for those materials. CNF and nanocellulose of bacterial origin (bacterial cellulose, BC) are also promising materials in biomedicine. CNF and BC hydrogels have been successfully used as scaffolds for cell culture and differentiation,¹⁷⁻²⁰ and have potential for tissue replacement and regeneration.^{21,22} BC materials for skin repair and wound healing are already available in the market.^{21,22}

The unique characteristics and performance of CNF-based materials depend not only on the structure and mechanical properties of CNF, but also on the intermolecular and surface forces between CNF fibrils and between CNF and other components of the composite material. The atomic force microscope,

in combination with the colloid probe technique, has proved to be very useful for the measurement of forces between surfaces at micro/nanoscale with high sensitivity.^{23,24} In particular, this technique has been applied to analyze the forces between cellulose surfaces such as cellulose microspheres and CNF films, coated or not with different polymers.^{25–30} Friction forces between fibrils also play a very important role in the behavior of cellulose materials. Huang *et al.*³¹ have measured friction forces between two cellulose fibers, whereas Rutland *et al.*^{25,28,32} have studied the effect of different polymers or surfactants on the friction forces between two cellulose microspheres. It has been observed that the adsorption of carboxymethyl cellulose, modified by grafting short chains of polyethylene glycol (CMC-g-PEG), remarkably improves the lubrication of both CNF films and reconstituted cellulose fiber networks.^{29,33} Moderate adhesion together with low friction coefficients between modified fibrils seem to be the basis for the improved mechanical properties of composites made of CNF coated with CMC-g-PEG, which are significantly tougher and stronger than CNF films.³⁰

Besides structural and mechanical characterization, a complete understanding of the tribological properties of CNF is needed. Studies devoted to the analysis of friction and lubrication of CNF films are still scarce, and only occur in some of the works mentioned above. The development of highly lubricating CNF films would represent an important breakthrough towards new commercial applications of CNF. Highly lubricating CNF films could have potential applications in medicine, for instance in cartilage replacement. In that direction, the combination of CNF with natural lubricating polysaccharides like hyaluronic acid (HA) –an important component of cartilage and synovial fluid in joints– represents a promising approach to cartilage implants not explored so far. This work aims at advancing the micro/nano-tribological characterization of CNF and, especially, at developing highly lubricating CNF films. To reach this goal, the surface and friction forces at micro/nanoscale between CNF films and glass microspheres were studied in different aqueous media by using an atomic force microscope and the

colloid probe technique. The covalent attachment of HA to CNF films and the consequent improvement of lubrication properties have been, to the best of our knowledge, studied for the first time in this work.

2. MATERIALS AND METHODS

2.1. Materials

CNF was obtained by mechanical fibrillation of never-dried kraft hardwood birch pulps from Finnish pulp mills using a high-pressure fluidizer (Microfluidics, M-110Y, Microfluidics Int. Co., Newton, MA). No chemical or enzymatic pretreatment was used prior to fibrillation. The CNF obtained by this method showed typical fibril diameters of 8-9 nm and a zeta potential of -3 mV (untreated, low charged nanofibrillated cellulose in reference 34). Hyaluronic acid sodium salt (molecular weight 2000-2400 kDa) from *Streptococcus equi* was supplied by Sigma-Aldrich (St. Louis, MO) and used without further purification. All chemicals used in this work were of analytical grade and purchased from Sigma-Aldrich except those otherwise indicated.

2.2. Preparation of substrates

CNF thin films were prepared by spin-coating a CNF dispersion onto gold-coated quartz crystals (Q-Sense, Biolin Scientific, Stockholm, Sweden). A dispersion of 1.2 g/l CNF was prepared by diluting CNF hydrogel (1.2% dry matter content) in deionized water followed by ultrasonication at 25% amplitude for 5 minutes using a Branson sonifier S-450 D (Branson Corp., Danbury, CT). The CNF dispersion was then centrifuged at 8000 g for 30 minutes at room temperature with an Eppendorf centrifuge 5804R (Eppendorf AG, Hamburg, Germany) to separate CNF fibrils from larger fibril bundles. The supernatant fraction with the finest CNF fibrils (about 0.15% dry matter content) was

collected and spin-coated onto the gold-coated quartz crystals at 4000 rpm for 1 minute using a Laurell spin-coater WS-650SX-6NPP-Lite (Laurell Technologies Corp., North Wales, PA). In order to enhance substrate retention of CNF during the spin-coating process, polyethylene imine (PEI) was previously adsorbed on the surface of the gold-coated quartz crystals. Drops of 2.5 mg/ml PEI solution were deposited on the crystals and polymer adsorption was allowed to take place for 10 minutes, after which the crystals were thoroughly rinsed with deionized water and dried under flowing nitrogen.

HA was covalently bound to the CNF film surface by the esterification reaction between their carboxyl and hydroxyl groups. Drops of 5 mg/ml HA solution in deionized water were deposited on the CNF films formed by spin-coating on quartz crystals as described above. The substrates were dried in an oven at 105 °C for 5-10 minutes and then heated at 155 °C for 3-5 minutes. Finally, the substrates were thoroughly rinsed with deionized water and dried under flowing nitrogen.

2.3. Quartz crystal microbalance with dissipation monitoring (QCM-D)

The adsorption of HA on CNF films was studied by QCM-D using a Q-Sense E4 instrument (Q-sense, Västra Frölunda, Sweden). CNF films, spin-coated on gold-coated quartz crystals as described above, were allowed to swell in deionized water or in different buffers for a few hours in the chambers of the instrument until a stable baseline was obtained. Then 100 µg/ml HA solutions in deionized water or in different buffers were injected in the chambers at 0.1 ml/min rate. Changes in the resonance frequency of the quartz crystal (5 MHz) and its overtones were monitored to quantify HA adsorption. The chambers were finally rinsed with deionized water or the corresponding buffers without HA. The experiments were carried out at room temperature.

2.4. X-ray photoelectron spectroscopy (XPS)

The surface chemical composition of CNF films with and without HA was analyzed by XPS using an Axis ULTRA electron spectrometer (Kratos Analytical) with monochromatic Al K α irradiation at 100W under charge neutralisation. CNF films for XPS analysis were spin-coated on silica substrates following the same procedure described above. In order to avoid signals from the underlying silica substrate in the XPS spectra, CNF was spin-coated 30 times on each silica substrate. As a reference, a sample of pure HA was also prepared by depositing a drop of 5 mg/ml HA solution in deionized water on a bare silica substrate and letting it dry at room temperature.

XPS spectra were recorded after an overnight pre-evacuation, and a fresh in-situ reference sample of 100% cellulose was measured with each analysis batch.³⁵ Both survey and high resolution regional data were obtained from several locations (area of analysis 400 x 800 μ m) for each sample. CasaXPS software was utilized for data analysis. In the case of high resolution data, carbon C 1s and oxygen O 1s regional fits were performed using symmetric Gaussian components. The binding energies were calibrated using the high resolution C 1s aliphatic component at 285.0 eV.³⁶ No sample deterioration during the measurements was observed.

2.5. Atomic force microscopy (AFM)

High-resolution images of CNF films before and after HA attachment were obtained with a MultiMode 8 atomic force microscope equipped with a NanoScope V controller (Bruker Corporation, Billerica, MA), operating in tapping mode. The images were obtained in air using silicon NSC15/AlBS probes (MicroMasch, Tallinn, Estonia) with a tip radius below 10 nm. Research NanoScope 8.15 software (Bruker) was used for image analysis. The only image correction applied was flattening. Average RMS

roughness values were calculated from images of $25\ \mu\text{m}^2$ area at different spots on the same or similarly prepared substrates.

2.6. Measurement of surface and friction forces

Surface and friction forces at micro/nanoscale between CNF films and glass microspheres were measured in aqueous media at different pH and ionic strength using the colloid probe technique and a MultiMode 8 atomic force microscope equipped with a closed-loop PicoForce scanner (Bruker). The colloid probes were prepared by gluing glass spheres with diameters in the range of 15-30 μm (Polysciences Inc., Warrington, PA) at the end of tipless CSC38 cantilevers (MikroMasch) with the help of a micromanipulator (Narishige, Japan) and an optical adhesive (Norland Products Inc., Cranbury, NJ) cured under UV light. In surface force measurements, the colloid probes and the CNF substrates were approached at a speed of 2 $\mu\text{m/s}$ until contact, and then separated. The surface forces, calculated from the vertical deflection and the normal spring constant of the cantilevers, were normalized by the radii of the glass microspheres used as colloid probes. In friction force measurements, the colloid probes slid over the CNF films at a speed of 10 $\mu\text{m/s}$ at different applied (compressive) loads. The friction forces were obtained from the lateral twist and the torsional spring constant of the cantilevers. The normal and torsional spring constants of the cantilevers were determined from the analysis of the thermal vibration spectra of the cantilevers before gluing the glass microspheres and the application of Sader's equations.^{37,38} Values of about 0.05 N/m and 1.2×10^{-9} Nm/rad were obtained for the normal and torsional spring constants, respectively. The experiments were carried out in the following media: *i*) phosphate buffered saline (PBS), pH 7.4 and high ionic strength (10 mM Na_2HPO_4 , 1.8 mM KH_2PO_4 , 137 mM NaCl, 2.7 mM KCl); *ii*) phosphate buffer (PB), pH 7.4 and low ionic strength (10 mM Na_2HPO_4 , 1.8 mM KH_2PO_4); *iii*) PBS, pH 3; and *iv*) PB, pH 3. The last two media were obtained by

adjusting the pH of PBS and PB buffers to 3 with 1 M HCl solution (Merck KGaA, Darmstadt, Germany).

3. RESULTS AND DISCUSSION

3.1. Characterization of substrates

CNF thin films were prepared by spin-coating a CNF dispersion onto gold-coated quartz crystals previously covered with PEI. A simple strategy to incorporate HA to the CNF films by adsorption was initially tested. However, QCM-D experiments revealed that the physical adsorption of HA on CNF films was negligible (Figure S1 in Supplementary Material). Therefore, a different method to attach HA on CNF films via the esterification reaction between their carboxyl and hydroxyl groups was applied. The esterification method followed in this work is based on other similar procedures published in the literature for the covalent linking between cellulose and other polycarboxylic acids.^{39–41} However, no literature on attaching HA onto CNF via esterification was found.

The attachment of HA to CNF films was confirmed by XPS after thorough rinsing of substrates (Figure 1a and Table 1). Spectra of the HA-modified CNF films showed the characteristic features of both cellulose and HA. The characteristic components of cellulose (in the carbon and oxygen regions) dominated the XPS spectra of CNF films, but features seen in the HA reference sample (namely nitrogen N 1s and sodium Na 1s in surveys, as well as the non-cellulosic components in high resolution regions of carbon C 1s and oxygen O 1s) were clearly seen in the CNF films subjected to HA binding. By comparing the nitrogen content or the O-C=O component of the carbon peak for HA-modified CNF films with the corresponding values for the pure CNF or HA samples, the HA content at the film surface was estimated to be ca. 30 mol% for CNF+HA. The attachment of HA was also observed on CNF free-

standing films subjected to the same simple esterification procedure followed in this work (results not shown), suggesting that the approach described here can be used for a broader variety of cellulose samples.

Figures 1b and 1c show high-resolution images of some CNF films used in friction experiments. The spin-coating process yielded substrates fully covered by CNF fibrils, with an average RMS roughness of 4.4 ± 0.2 nm. The attachment of HA to CNF did not change the appearance and roughness of the films significantly (RMS roughness 4.0 ± 0.4 nm).

3.2. Surface and friction forces

In order to evaluate the tribological properties of CNF films at micro/nanoscale, surface and friction forces between CNF films and glass microspheres were measured in aqueous media at different pH and ionic strength using an atomic force microscope and the colloid probe technique. Direct surface force measurements give valuable understanding on interactions not available with other methods but, to be meaningful, the technique requires substrates with well-defined geometry and roughness. Because of that, well-defined glass microspheres were chosen in this work as a first-approach model for hard surfaces like bone. Nevertheless, it has to be taken into account that glass and bone have different stiffness, which is a limitation of this approach.

3.2.1. Effect of hyaluronic acid on the tribological properties of CNF in PBS

Figure 2 presents surface and friction forces obtained in PBS pH 7.4 for CNF films with and without HA attached. For reference, surface and friction forces between bare substrates without CNF films are also shown. A characteristic hard contact behavior was observed when the bare substrates approached one another: the lack of interaction between the surfaces was followed by a very steep repulsive force when

contact was reached (Figure 2a). The less steep repulsive force starting at separations around 30 nm observed with a CNF spin-coated substrate corresponds to steric forces due to the compression of the swollen CNF film (Figure 2a). The attachment of HA on the CNF films generated a considerably more swollen and compressible HA-CNF system, with an onset of repulsive forces at separations over 160 nm (Figure 2a). The range of these forces is considerably longer than the expected range of the electrical double-layer repulsion between negatively charged HA-CNF and glass at this ionic strength. At pH 7.4 most of the carboxyl groups on HA (and CNF) are deprotonated. This introduces intermolecular electrostatic repulsions within the film, inducing swelling. Thus, the long-ranged repulsion observed is due to a combination of electrostatic and steric repulsion (electrosteric repulsion)⁴² arising from the compression of the CNF film with HA attached.

Figure 2b shows friction force data at different applied loads. Table 2 presents the corresponding friction coefficients μ , obtained from the slope of the linear relations between friction forces and applied loads according to Amontons' law, $F_{Friction} = \mu F_{Load}$. As can be seen, the friction forces and, consequently, the friction coefficient between the bare substrates increased when a layer of CNF was spin-coated on one of them. Therefore, CNF films did not provide good lubrication between the model hard surfaces studied. The roughness of the CNF films and the considerable adhesion between the glass colloid probes and the CNF films observed in the retraction force curves (Figure S2 in Supplementary Material) are factors that provoke high dissipation of energy when the glass colloid probes slide over the CNF films, which results in high friction forces and friction coefficients. However, a significant reduction in friction force and friction coefficient was observed when HA was attached to CNF films. Two linear regions with two different slopes could be distinguished in this case (Figure 2c). The inflection point was at about 2.5 nN applied load, equivalent to a normalized force F/R of 0.24 mN/m, which corresponds to almost total compression of the HA layer in Figure 2a. Therefore, a correlation between the different

linear regions observed in the friction curve shown in Figure 2c and the compression state of the HA layer attached on the CNF film can be established: at applied loads below 2.5 nN (F/R below 0.24 mN/m) a slightly compressed HA layer provided good lubrication between the substrates with a friction coefficient of 0.18 ± 0.05 ; the friction coefficient increased to 0.33 ± 0.04 when the HA layer was highly compressed at applied loads above 2.5 nN (F/R above 0.24 mN/m). It must be noted that the friction coefficient in the latter case is still considerably lower than in the case of CNF film without HA (0.88 ± 0.03). The improvement in lubrication observed with HA has its origin in the electrosteric repulsion provided by the hydrated HA layer attached to the CNF films, which prevented adhesion to the glass colloid probes (Figure S2 in Supplementary Material) and, consequently, considerably reduced the energy dissipation in friction experiments.

3.2.2. Effect of the ionic strength on the surface and friction forces

Friction experiments were also carried out in PB pH 7.4 to analyze the effect of the ionic strength on the lubrication properties of CNF films. Figures 3a and 3b show surface forces in PB and PBS at pH 7.4. Compared to PBS, the lower ionic strength of PB provoked a larger swelling of the CNF films, especially those with HA attached (higher electrostatic repulsion between charged groups and higher counterion osmotic pressure), which led to longer-range electrosteric repulsions. The onset of the repulsion between pure CNF and glass started at distances of about 80 nm in PB, compared to around 30 nm in PBS (Figure 3a). Similarly, a repulsion larger in magnitude and longer in range was observed for a HA-modified CNF film in PB, starting at separations beyond 400 nm (Figure 3b). A comparison of surface forces between the different systems studied in PB pH 7.4 is presented in Figure 3c, where a similar trend to that in PBS pH 7.4 (Figure 2a) can be observed, with HA-CNF showing the largest repulsion.

Besides surface forces, friction forces were also affected by the ionic strength as shown in Figures 3d and 3e and Table 2. A slight reduction in friction forces and coefficients was observed between CNF film and glass upon decreasing the ionic strength of the medium (Figure 3d and Table 2). The friction reduction was much more significant in the case of CNF with HA attached against glass (Figure 3e and Table 2), in line with the more pronounced electrosteric repulsion measured for that system in PB pH 7.4. As similarly observed in PBS pH 7.4, higher friction forces and higher friction coefficients were measured after spin-coating CNF onto one of the bare surfaces (Figure 3f and Table 2). Nevertheless, excellent lubrication was obtained after attaching HA to CNF films, with friction coefficients as low as 0.007 ± 0.002 (Figure 3f and Table 2).

3.2.3. Effect of the pH on the surface and friction forces

The influence of the pH on the tribological properties of CNF films was also studied. Figure 4 shows surface and friction forces for the different systems measured in PB pH 3. Since most of the carboxyl groups of CNF and HA are protonated at pH 3, a reduction in the swelling of the layers would be expected. That was confirmed by the reduction in the onset of the repulsion upon decreasing the pH. In the case of CNF films, onsets at about 25 nm were observed in PB pH 3, in contrast to the about 80 nm in PB pH 7.4 (Figure 4a). Similarly, onsets at about 300 nm were observed for HA-modified CNF films in PB pH 3, significantly lower than in PB pH 7.4 (Figure 4b). A comparison of surface forces between the different systems studied in PB pH 3 is presented in Figure 4c, which shows qualitatively similar trends to those observed earlier. Higher friction forces and coefficients were obtained between CNF film and glass when decreasing the pH from 7.4 to 3 (Figure 4d and Table 2). That increase in friction correlated with stronger adhesion forces observed in retraction force curves (Figure S3 in Supplementary Material), confirming the importance of adhesion forces in the energy dissipation associated to friction. Friction forces and coefficients were also higher between HA-modified CNF film and glass at pH 3 than

at pH 7.4 (Figure 4e and Table 2). Two linear regions with different slopes were observed at pH 3, with the inflection point at about 3 nN applied load (equivalent to a normalized force F/R of 0.29 mN/m that corresponds to almost total compression of the HA layer in Figure 4b). Good lubrication ($\mu = 0.067 \pm 0.004$) was provided by a slightly compressed HA layer at applied loads below 3 nN (F/R below 0.29 mN/m), but the friction coefficient increased to 0.30 ± 0.03 when the HA layer was highly compressed at applied loads above 3 nN (F/R above 0.29 mN/m). The friction coefficient was, nevertheless, substantially smaller than for CNF films without HA ($\mu = 1.21 \pm 0.08$). Figure 4f presents a comparison of friction curves for the different systems studied in PB pH 3. The considerably high friction forces and friction coefficients obtained with CNF films were again significantly reduced after binding HA (Figure 4f and Table 2).

3.3. Practical implications of the lubricating properties of HA-modified CNF films

Figure 5 compares the surface and friction forces between glass microspheres and CNF films with HA attached in different aqueous media. A clear relation between the swelling of the HA layer and the lubrication of the film is observed: the larger the swelling, the better the lubrication. The strongest electrosteric repulsion and largest swelling of the HA layer occurred in PB pH 7.4, when the ionic strength was low and the carboxyl groups of HA were deprotonated (Figure 5a). In these conditions, remarkably low friction forces and friction coefficient were measured (Figure 5b and Table 2). The swelling of the HA layer decreased either by reducing the effective charge of HA (protonation of carboxyl groups at pH 3) or by increasing the ionic strength of the media (PBS). The combination of both factors in PBS pH 3 provoked the collapse of the HA layer, expressed by the drastic decrease in range and magnitude of repulsion in Figure 5a. Accordingly, the worst lubrication performance of CNF films with HA was observed in PBS pH 3 (Figure 5b and Table 2). Figure 6 schematically illustrates

different conformations adopted by the HA layer depending on the pH and the ionic strength of the medium. Low swelling of HA-CNF films and the resulting higher friction was associated with the higher adhesion observed in the retraction force curves (Figure S4 in Supplementary Material).

The results presented in this work show that spin-coated CNF films do not provide good lubrication between model hard surfaces, independently of the pH and the ionic strength of the medium. High friction coefficients in the range 0.67-1.21 were obtained between glass microspheres and spin-coated CNF films. Similar values of 1.00 ± 0.05 and 0.84 ± 0.08 were reported by Olszewska *et al.*²⁹ for the friction coefficients between cellulose microspheres and CNF films in buffers of low ionic strength and pH 4.5 and 7.3, respectively. Friction coefficients between 0.9 and 1.3 were also obtained by Rutland *et al.*^{25,28,32} between two cellulose microspheres and between a cellulose microsphere and a solvent cast cellulose film in different aqueous solutions.

The lubrication of CNF films was significantly improved by the attachment of HA. HA is a natural polysaccharide with well-known lubricating properties. HA is one of the main components of articular cartilage and synovial fluid, contributing to the excellent lubrication in joints. In this work a reduction between 60% and 90% (depending on pH and ionic strength) in the friction coefficient was observed after attaching HA to CNF films. The friction coefficients in the presence of HA shown in Table 2 are similar to the values obtained by Benz *et al.*⁴³ for the friction between a mica substrate and HA attached to mica or to lipid bilayer-coated mica in PBS pH 7.4 (μ in the range 0.15-0.30). This shows that HA preserves its lubricating properties after being attached to CNF (a substrate that could be more relevant for biomedical applications than mica).

Friction coefficients between 0.0025 and 0.14 have been obtained in *ex vivo* friction experiments between articular cartilage and other surfaces.⁴⁴⁻⁴⁶ Bonnevie *et al.*⁴⁵ observed that the friction coefficient

for the system articular cartilage/stainless steel probes in PBS pH 7.4 decreased as the sliding velocity increased. Interestingly, at sliding velocities of the same order of magnitude as the one used in our experiments, they obtained friction coefficients similar to the values presented in this work for CNF films with HA attached. The introduction of lubricin –a lubricating protein naturally present in articular cartilage– in the HA-CNF system to further improve the lubrication would be an interesting option to be explored.

Assuming elastic contact between the spherical colloid probe and the CNF film, the maximum contact pressure achieved in our experiments was estimated to be about 1 MPa, a value similar in order of magnitude, but slightly smaller than the peak pressures of 2-3 MPa measured in knee joints by Fukubayashi and Kurosawa at 1000 N applied load.⁴⁷

The binding of HA to CNF films could open the door to the development of nanocellulose-based materials for new applications in the biomedical sector, for instance, in cartilage implants. Attempts to use cellulose materials for cartilage replacement were already started by Greene *et al.*,³³ who combined reconstituted cellulose fiber network (structurally and mechanically different than CNF), carboxymethyl cellulose and CMC-g-PEG to create a cartilage-inspired lubrication system that, however, was not mechanically tough nor robust enough for most practical applications. Different materials, including hydrogels of natural polysaccharides like HA, are currently under study for cartilage replacement.⁴⁸ In spite of their excellent lubricating properties, hydrogels of pure HA lack the mechanical strength to withstand physiological loads, which is a serious disadvantage for their use as weight-bearing implants. However, many of the challenges of current solutions could be avoided by combining the good lubricating properties of HA with the excellent mechanical strength of CNF. A Young's modulus of 11 GPa and tensile strength up to 230 MPa were reported for free-standing films of the CNF used in this work at 50% relative humidity,¹⁰ whereas a Young's modulus of about 0.3 GPa and tensile strength of

about 20 MPa were obtained in wet conditions.¹¹ Considering that CNF has been demonstrated to be nontoxic and to show good biocompatibility,¹⁷ the HA-CNF system studied in this work could be a promising material for the treatment of cartilage damage.

3. CONCLUSIONS

The colloid probe technique was used in this work to analyze the surface and friction forces at micro/nanoscale between spin-coated CNF films and glass microspheres in buffers at different pH and ionic strength. The attachment of HA to CNF via the esterification reaction between carboxyl and hydroxyl groups considerably improves the poor lubrication properties of CNF films. The reduction in friction forces and friction coefficients was especially remarkable at low ionic strengths and high pH, where the repulsion between negatively charged carboxyl groups and the counterion osmotic pressure favors the swelling of the attached HA layer. The fact that the low friction coefficients obtained for CNF films with HA attached are of the same order of magnitude as those for articular cartilage published in the literature suggests that those films could be a promising material for cartilage implants, where the exceptional lubricating properties of HA complements the excellent mechanical properties of CNF.

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Figure Captions

Figure 1

Figure 1. Characterization of CNF films with and without HA attached. a) XPS wide spectra and high resolution spectra of oxygen, nitrogen and carbon of pure CNF, pure HA, and HA attached to CNF. b) AFM height image of a CNF film. c) AFM height image of a CNF film with HA attached.

Figure 2

Figure 2. Surface and friction forces between a glass microsphere and a gold substrate (squares), a CNF film (circles), and a CNF film with HA attached (triangles) in PBS pH 7.4. a) Force curves obtained when approaching the surfaces. b) Friction forces at different applied loads: closed and open symbols correspond to friction force values obtained when increasing and decreasing the applied load, respectively. The friction curve between a glass microsphere and a CNF film with HA attached is shown in more detail in c).

Figure 3

Figure 3. Surface and friction forces for different systems in PBS and PB pH 7.4. a,b) Approach force curves between a glass microsphere and a CNF film (a) and a CNF film with HA attached (b) in PBS pH 7.4 (open symbols) and PB pH 7.4 (closed symbols). c) Approach force curves between a glass microsphere and a gold substrate (squares), a CNF film (circles), and a CNF film with HA attached (triangles) in PB pH 7.4. d,e) Friction forces at different applied loads between a glass microsphere and a CNF film (d) and a CNF film with HA attached (e) in PBS pH 7.4 (circles) and PB pH 7.4 (squares). f) Friction forces between a glass microsphere and a gold substrate (squares), a CNF film (circles), and a CNF film with HA attached (triangles) in PB pH 7.4. Closed and open symbols in d), e) and f) correspond to friction force values obtained when increasing and decreasing the applied load, respectively.

Figure 4

Figure 4. Surface and friction forces for different systems in PB pH 7.4 and PB pH 3. a,b) Approach force curves between a glass microsphere and a CNF film (a) and a CNF film with HA attached (b) in PB pH 3 (open symbols) and PB pH 7.4 (closed symbols). c) Approach force curves between a glass microsphere and a gold substrate (squares), a CNF film (circles), and a CNF film with HA attached (triangles) in PB pH 3. d,e) Friction forces at different applied loads between a glass microsphere and a CNF film (d) and a CNF film with HA attached (e) in PB pH 3 (circles) and PB pH 7.4 (squares). f) Friction forces between a glass microsphere and a gold substrate (squares), a CNF film (circles), and a CNF film with HA attached (triangles) in PB pH 3. Closed and open symbols in d), e) and f) correspond to friction force values obtained when increasing and decreasing the applied load, respectively.

Figure 5

Figure 5. Surface and friction forces between a glass microsphere and a CNF film with HA attached in PBS pH 3 (circles), PBS pH 7.4 (squares), PB pH 3 (diamonds), and PB pH 7.4 (triangles). a) Force curves obtained when approaching the surfaces. b) Friction forces at different applied loads: closed and open symbols correspond to friction force values obtained when increasing and decreasing the applied load, respectively.

Figure 6

Figure 6. Schematic representation of the experimental system (drawing not to scale). Different conformations of the HA layer attached to a spin-coated CNF film are shown. At high pH and low ionic strength the repulsion between the charged carboxyl groups of HA and the counterion osmotic pressure give rise to the swelling of the HA layer (left). In contrast, the HA layer collapses at low pH and high ionic strength (right) when most of the carboxyl groups of HA are protonated and the high concentration of ions in solution screens any electrostatic repulsion and reduces the counterion osmotic pressure.

Tables

Table 1. Elemental surface concentrations, relative abundance of carbon bonds, and estimation of HA surface content in different films (CNF, HA, and HA attached to CNF) obtained by XPS analysis. (b.d.l.= below detection limit)

	Elemental concentration (%)				Relative abundance of carbon bonds (%)				HA content (mol%)	
	O 1s	N 1s	Na 1s	C 1s	C-C	C-O	O-C-O	O-C=O	estimated from N 1s	estimated from O-C=O
CNF	39 ± 1	b.d.l.	b.d.l.	61 ± 1	5.8 ± 1.2	72 ± 1	21 ± 1	1.6 ± 0.1	0	0
HA	32 ± 1	3.9 ± 0.1	2.5 ± 0.1	62 ± 1	21 ± 1	51 ± 1	22 ± 1	6.5 ± 0.1	100	100
CNF + HA	36 ± 1	1.2 ± 0.1	1.6 ± 0.2	61 ± 1	13 ± 1	64 ± 1	20 ± 1	2.8 ± 0.1	31	26

Table 2. Friction coefficients of the studied systems in different buffer solutions. Mean values and standard deviations of at least three independent measurements are presented. When two values are shown, the first value corresponds to the friction coefficient at low applied loads, and the second one to high applied loads.

Medium \ System	Friction coefficients		
	Glass colloid probe vs. gold substrate	Glass colloid probe vs. CNF film	Glass colloid probe vs. CNF film with HA attached
PBS pH 7.4	0.67 ± 0.17	0.88 ± 0.03	0.18 ± 0.05 ; 0.33 ± 0.04
PB pH 7.4	0.27 ± 0.01	0.67 ± 0.09	0.007 ± 0.002
PBS pH 3	0.32 ± 0.14	1.14 ± 0.10	0.45 ± 0.03
PB pH 3	0.32 ± 0.04	1.21 ± 0.08	0.067 ± 0.004 ; 0.30 ± 0.03