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Strain-Induced Stiffening of Nanocellulose-Reinforced Poly(vinyl alcohol) Hydrogels Mimicking Collagenous Soft Tissues

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Soft tissues possess remarkable mechanical strength for their high water content, which is hard to mimic in synthetic materials. Here, we demonstrate how strain-induced stiffening in hydrogels plays a major role in mimicking the mechanical properties of collagenous soft tissues. In particular, nanocellulose reinforced polyvinyl alcohol (PVA) hydrogels of exceptionally high water content (90-93 % wt.) are shown to exhibit collagen-like mechanical behavior typical for soft tissues. High water content and co-existence of both soft and rigid domains in the gel network are the main factors responsible for strain-induced stiffening. This observed effect due to alignment of rigid components of the hydrogel is simulated through modeling and visualized through strain-induced birefringence experiments. Design parameters such as nanocellulose aspect ratio and solvent composition are also shown important to control the mechanical properties. In addition, owing to their transparency (90-95 % at 550 nm) and hyperelastic properties (250 – 350 % strain), the described hydrogels are promising materials for biomedical applications, especially in ophthalmology.

1. Introduction

Synthetic and natural hydrogels are predominantly hydrophilic, cross-linked polymeric networks of high water content. Soft and strong hydrogels are highly demanded, because on one hand excessive stiffness may induce undesired damage and on the other hand the material should be sufficiently strong to be handled.¹ Such soft yet strong hydrogels are particularly useful in ophthalmology.

The choice of the matrix-forming polymer for an ophthalmic application depends on several factors like optical properties, mechanical strength, biocompatibility, biodegradability, surface properties, and permeability. Poly(vinyl alcohol), PVA, could be one option and has proven to be a flexible, biocompatible matrix capable of addressing many demanding medical needs. Its availability, unique chemical and physical properties have led to its inclusion in many implants and biomaterials that have been used in the fields of keratoprosthesis,² vascular prosthesis³ as well as articular cartilage and meniscus replacement.⁴⁻⁶

PVA has also been used in ophthalmic compositions for a long time: PVA films and gels have been reported as ophthalmic inserts in the lower conjunctival sac when loaded with antibiotics.⁷⁻¹⁰ Due to its great alterability, PVA sponges are used as surgical spears in eye surgery.^{11, 12} These eye sponges present an enhanced tactile feel to the surgeon while remaining

non-abrasive to the eye.¹³ The applicability of PVA for in vivo keratoprosthesis was evaluated by Tsuk et al.² PVA based synthetic cornea was developed and tested on rabbit animal model to show that limbal epithelial cells can migrate on to the synthetic cornea.¹⁴ Chemically modified PVA based soft contact lenses are available today marketed under the trade name Nelfilcon A.^{15, 16}

The scope of hydrogel applications is often limited by poor mechanical behavior at high water content. In order to enhance the mechanical properties of hydrogels, reinforcing materials like carbon nanotubes,¹⁷ nanocellulose,¹⁸ clay¹⁹ and metallic particles²⁰ have been used. In addition, crosslinking by chemical agents or physical methods, e.g. irradiation or cryogelation, is typically used to reinforce the hydrogels.

Cellulose is the most abundant natural polymer produced not only by trees but also by some algae, fungi and bacteria, and has widespread use in biomedical applications.^{21, 22} Cellulose derivatives, e.g., carboxymethyl cellulose, hydroxypropyl methylcellulose, are used in contact lens conditioning solutions to enhance wettability and wear comfort due to their water retention ability and transparency while in solution.²²⁻²⁴ Recently regenerated cellulose (dissolved and reprecipitated) hydrogels were used to develop a bandage lens for ocular

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Figure 1. AFM images and physical appearance of oxidized (a), (c) CNC and (b), (d) CNF

injuries.²² Furthermore, nanocellulose is reported to give better mechanical properties than cellulose macro-sized fibers.²⁵ Moreover, nanocellulose in the form of crystals (CNC) and fibers (CNF) is widely used as a reinforcing material owing to its high aspect ratio, exceptional strength, hydrophilicity, transparency, availability and biocompatibility. These properties led us to choose nanocellulose as our reinforcing material.^{26, 27} We used TEMPO oxidized cellulose due to its high water holding capacity, transparency, ease of production, and scalability. Furthermore, TEMPO oxidized cellulose has been shown to be cytocompatible.²⁸⁻³⁰

Previously, several groups have reported composites made from PVA and nanocellulose using both physical and chemical crosslinking methods.³¹⁻³⁶ However, we were the first to present composite hydrogels with water content as high as 90% and a transparency over 90% in the visible range. This was achieved by a cryotropic gelation method resulting in a composite hydrogel with a macroporous structure and a refractive index very close to that of water. The composite hydrogel has strong absorbing properties in the UV-B region and low absorbing in UV-A region.³⁷ The material also showed cytocompatibility with human corneal epithelial cells and was suturable on a porcine cornea.³⁸ The unique properties of PVA and nanocellulose combined, make the hydrogel a good contender for contact lens and other ocular applications.

A previous study summarized the mechanical properties of different contact lens materials available on the market today. In the present study, transparent composite hydrogels developed for soft contact lens applications in our group were subjected to mechanical characterization and a model was developed to describe strain-induced stiffening behaviour of the composite material. The objective of this study was to enhance the mechanical properties of the PVA hydrogel by

introducing CNC or CNF without altering the water content and the transparency.

2. Results and Discussion

In this study, CNC and CNF were used to reinforce PVA hydrogels to improve the mechanical properties of PVA hydrogel without compromising its transparency. The physico-chemical and optical properties of CNC and CNF-reinforced PVA

Figure 2. Image of transparent hydrogel sheet covering lower half of eye (printed image). The image of the eye was obtained with permission from Thomas Tolkien

hydrogels were summarized in our previous work.³⁷

AFM imaging was used to investigate the morphology of the CNC and CNF samples. **Figures 1a, 1b** shows AFM images of CNC and CNF samples. It is seen in Figures 1a, 1b that CNC consisted of individual whiskers, a few hundred nm long, in contrast to entangled CNF, which are several μm long. CNF gel is self-supporting, while CNC gel resembles a viscous free-flowing dispersion as shown in Figures 1c, 1d. The high aspect ratio of CNF is likely responsible for the high viscosity leading to formation of self-supporting gel as shown in Figure 1d. Accordingly, the free-flowing viscous CNC dispersion is due to the relatively low aspect ratio of cellulose whiskers.³⁹

All hydrogels were highly transparent (80-95% at 550 nm) irrespective of the reinforcing material used. **Figure 2** shows the typical hydrogel sample obtained after solvent exchange with deionized water. The optical characterization of the hydrogels will not be covered here any further, and the reader is directed to our earlier publication.³⁷

Figure 3. Mechanical properties of composite hydrogel materials with different reinforcements and solvent compositions (DMSO:Water). (a) True* tensile strength (b) Elongation at break (c) Modulus in toe region (d) True* Modulus before failure. Clear trend is observed for pure PVA but it is not followed in composite hydrogels due to variance in nanocellulose distribution in the matrix.

Figure 3 summarizes the results of mechanical characterization of hydrogels. Because the hydrogels were highly elastic and deformable, it was necessary to compensate the calculated value of the tensile strength and modulus due to continuous change in the original dimensions of the hydrogel sample. The inclusion of nanocellulose of varying aspect ratio clearly affected the mechanical properties of the hydrogels with respect to their strength, elongation, and modulus values. Furthermore, the composition of the liquid phase also affected the mechanical properties of PVA hydrogels. For clarity, the effect of each of the parameters will be discussed separately.

2.1. Effect of liquid phase composition

The mechanical strength and elasticity of hydrogels is a direct consequence of the hydrogel network structure, which in turn depends on the gelation mechanism. Gelation occurs in pure PVA system by physical cryotropic crosslinking.^{40, 41} The crosslinking density is affected by several factors such as PVA concentration, degree of acetylation, molecular weight, and freezing temperature.^{40, 42} Here, among other factors, the strength and elasticity of the hydrogel were shown to be directly influenced by the composition of the liquid phase during cryotropic gelation, i.e. DMSO:water ratio.

Figure 4a shows the typical effect of the liquid phase composition on mechanical properties of PVA hydrogels. For clarity, the results are presented for pure PVA samples, and the values for tensile strength, elongation and modulus for all samples are summarized in Figure 3. It is seen from Figure 4a that DMSO:water 60:40 PVA hydrogel was mechanically stronger than PVA samples prepared with 70:30 and 80:20 ratio. Furthermore, the elongation values for 60:40 samples of pure PVA were also higher as compared to 70:30 and 80:20 ratio samples. However, it should be noted that transparency of 60:40 samples was inferior to that of 70:30 and 80:20 samples as shown previously.³⁷

The crosslinking points in the PVA hydrogel network are fine crystallites formed during the cryotropic gelling process.⁴⁰ The strength of the material is proportional to the size and number of these crystallites, which in turn depends on liquid phase composition. Because DMSO is a better solvent for PVA than water,⁴² PVA chains will be more extended in DMSO and more coiled in water. Upon cooling of PVA in a mixed solvent, the solubility of PVA is reduced, which results in gradual phase separation of PVA chains and eventually local crosslinking, resulting in PVA crystallites. With increasing water content and

Figure 4. Typical graphs showing (a) Effect of solvent composition on tensile stress - strain relationship of the pure PVA hydrogel, (b) Effect of nanocellulose reinforcement on tensile stress - strain relationship of the composite hydrogel with solvent composition of DMSO:Water 80:20

decreasing DMSO content the probability of making large crystallites increases. The latter is confirmed by the results of optical and mechanical characterization of pure PVA hydrogels. The above-described mechanism of PVA gelation is slightly altered in the presence of another insoluble component with lot of hydroxyl groups, such as nanocellulose. The results in Figure 4b show that while the mechanical strength of the hydrogels was generally improved upon inclusion of nanocellulose, the effect of the liquid phase composition was less obvious. In particular, for CNF reinforced sample the mechanical strength and modulus values of the 80:20 sample were higher than those of 60:40 sample. It is plausible that in the presence nanocellulose the rigidity, concentration, and local distribution of the nanocrystals/nanofibers is dominating.

2.2. Effect of nanocellulose aspect ratio

Figure 4b illustrates the effect of nanocellulose aspect ratio on PVA hydrogel mechanical properties. For clarity and moreover due to high transparency, the results are presented for 80:20

samples, while values for tensile strength, elongation, and modulus for all samples are summarized in Figure 3. It is seen from Figure 4b that the hydrogels exhibit stress-strain curves characteristic for hyperelastic materials, i.e., high elongation at low stresses and gradual stiffening upon further applied stress. Such behavior is characteristic for collagen-based soft tissues, and normally difficult to mimic in synthetic materials of high water content. It is seen from Figure 4b that inclusion of CNC and CNF strongly affects all mechanical properties. Almost a three-fold increase in tensile strength of CNF-PVA 80:20 sample was recorded as compared to pure PVA 80:20 hydrogel.

The reinforcing effects of CNF and CNC were different. With CNF reinforcement the samples become much stronger than pure PVA but also less elastic. When loaded with CNC, both the maximum stress and elongation increased as compared to pure PVA hydrogel. This difference in behavior could be attributed to the aspect ratio of the CNC and CNF. The results also suggest substantial degree of entanglement between nanocellulose and PVA, probably due to hydrogen bonding. The reinforcing effect of nanocellulose was confirmed for all composition of the liquid phase as summarized in Figure 3. It is interesting to note, that the tensile strength of PVA-CNC 70:30 and 60:40 samples are comparable to those of PVA-CNF, while their elongation is relatively higher. It is plausible that short CNC whiskers are better distributed throughout the hydrogel than corresponding CNFs.

2.3. Effect of applied strain

Figure 5 shows deformation behavior of hydrogels of 80:20 solvent compositions under applied cyclic load at varying strain. Even though several groups reported the mechanical strength of PVA hydrogels produced by freeze-thawing,⁴³ cyclic loading data is scarce. As it has been discussed above the hydrogel exhibits strain-stress curves characteristic to hyperelastic materials. For practical applications, it is important to know the strain threshold above which the material undergoes transition from elastic to plastic deformation. The loop between the loading and unloading curves of a hydrogel arises due to the dissipated energy during test.⁴⁴ For an ideal elastic material the loading and unloading curves follow the same line and no hysteresis loop is expected. The applied energy is dissipated during unloading, when the polymer chains relax and regain their position.

As it is seen in Figure 5, although there is no permanent deformation at low strain values, there is a transient change in the overall structure since the loading and unloading curves do not follow the same path and a hysteresis is clearly observed. The high water content and viscoelastic nature of the material enable these readjustments. It should be noted that the size of the hysteresis varied with the value of the applied strain, and its magnitude was larger, when the applied strain was increased. In particular, for strain values below 25% the loading and unloading paths were more or less identical, whereas at higher loads the magnitude of the hysteresis was progressively increasing. Furthermore, when we compare the hysteresis among the three hydrogels, CNF reinforced sample showed

Figure 5. Effect of loading and unloading cycles on a composite hydrogels of 80:20 solvent compositions. The data points shown are only indicative. Actual data has large number of points.

larger hysteresis, whereas CNC reinforced sample doesn't show much variation when compared to that of pure PVA sample. This is also evident from the stress values of the curves. PVA-CNF samples absorb more energy for a given strain than other two hydrogels and have to dissipate more energy during unloading. The plastic deformation observed in all the three

samples is slightly different at higher strains. When the hydrogel is pulled, the stretching occurs mainly by realignment of soft PVA coils, while the cross-linked domains of the solid matrix are relatively intact. During unloading, the PVA chains relax again, although their conformation is slightly different from the original state. The latter explains why there was nearly 10% elongation of the sample compared to the original size following the loading and unloading experiments as seen in Figure 5. In particular, the loading curve for each successive strain would follow the unloading curve of its predecessor.

PVA-CNF sample showed higher plastic deformation than PVA-CNC sample, which in turn showed higher plastic deformation than pure PVA sample, judging by the size of hysteresis. In PVA-CNF sample the number of interacting points per nanofibril is higher, and they are distributed over several PVA chains in a 3D network. This makes the readjustment of PVA coils during

Figure 6. Illustration of PVA-CNF hydrogel.

unloading more difficult than pure PVA sample. This effect is less prominent in PVA-CNC sample due to low aspect ratio of CNC, leading to lower number of interaction points per cellulose nanocrystal.

2.4. Mechanism of strain-induced stiffening

Hydrogel materials of high water content are generally weak, and there is an inherent conflict between hydrogel softness and strength.⁴⁵⁻⁴⁷ Here we discuss a general mechanism of resolving the softness-strength conflict in hydrogels with high water content, which can potentially be extended to other biomaterials. Other popular strategies include semi interpenetrating networks (semi-IPNs) based on natural polymers.⁴⁸

The stress-strain curves of the hydrogels showed behavior typically observed in collagenous fibrous tissues such as tendons, ligaments, and cornea.⁴⁹⁻⁵² The equilibrium water content of the samples was 90 – 93 wt%. In general, free water filling the macropores has a softening effect on the hydrogel and imparts flexibility. However, considering that all hydrogels investigated in the present study had similar water content, it is obvious that the properties of the solid phase are also important since significant changes were observed upon introduction of nanocellulose. In fact, there was an increase in water content by 3% after reinforcement, whereas the tensile strength of the hydrogels was improved upon inclusion of nanocellulose. In our model, we postulate that it is important to have a solid phase that combines both soft and rigid regions. While it can be argued that pure PVA hydrogel possesses both rigid crystallites and soft randomly coiled chains, the effect of the reinforcement is much clearer when another component of higher rigidity is introduced. The stress-strain curve begins with a toe region where the soft PVA chain network begins to unfold and align in the direction of loading. Since it is easier to stretch out a soft, coiled PVA chain network, this part of the stress-strain curve shows relatively low stiffness. As the PVA chains straighten and rigid components realign, the material gradually stiffens under applied tensile load. Eventually, as individual polymer chains within the network begin to fail, damage accumulates, and the sample finally ruptures. Thus, the overall behavior of the hydrogel depends on the network structure, which in turn depends on the cross linking density, the stiffness of the reinforcing component, and its aspect ratio. The latter is illustrated in **Figure 6**.

The fiber reorientation behavior at high strains was verified experimentally using strain-induced birefringence experiments. When the hydrogel with anisotropic distribution of crystalline domains, i.e. either nanocellulose or PVA crystallites, is illuminated by polarized light in the relaxed state it appears dark. As the sample is pulled and the rigid components realign, the sample appears progressively bright, a phenomenon known as birefringence. **Figure 7** shows a typical strain-induced birefringence sequence for PVA-CNF hydrogel. Full videos of the sequence for all samples are available in Supporting Information. A similar birefringence behavior was observed in polyamide hydrogels reinforced with clay nanoparticles.^{53, 54} The implications of the nanofiber reorientation are further explored through modeling.

2.5. Modeling the fiber reorientation behavior

The main aim of this section is to predict and understand how the reorientation of the reinforcing elements during a tensile test affects the elastic behaviour of the composite. It should first be noted that the model presented here is different from the model generally used for hyper-elastic tissues⁵⁵ since the change in hyper-elasticity cannot be attributed to the intrinsic properties of the reinforcing elements: CNF or CNC are at least 100 times stiffer than the matrix material (PVA)^{56, 57} and thus are not likely to undergo any significant deformation

Figure 7. Strain induced birefringence images for PVA-CNF hydrogel. Time-lapse images of PVA-CNF hydrogel (a-f) illuminated by a polarized light source. The pulling was set at 50

during the tensile experiment. The change in apparent stiffness is more likely to be the result of the reorientation of the fibrils towards the loading direction resulting from the large deformation of the PVA chains: as the composite is stretched, the particles are reorienting toward the tensile direction and their contribution to the apparent stiffness of the composite is increasing throughout the loading process. The composite is going from a random orientation distribution to a highly aligned structure.

A micromechanics model is developed to describe the mechanical behaviour of the reinforced hydrogels. Before any mechanical loading, the reinforced hydrogel is considered a composite with a random in-plane fibre orientation. During the tensile test, the matrix will expand (up to more than 300%) which will result in a reorientation of the reinforcing particles. The surroundings of the reinforcing element are regarded as a homogenous material, and its properties are assumed unaffected by the presence of the nanoparticles. Moreover the reinforcing elements are not considered to interact with each other. Both of these assumptions might be fair here since the weight fraction of the reinforcing element was kept at 1% but are likely to be invalid for higher weight fractions of cellulose.

Figure 8. (a) Effect of a strain ϵ_x on the reorientation of a particle having an angle ϕ with the loading direction., (b) Predicted fibre orientation distribution for a composite subjected to a given strain, with $\nu_{xy}=0.3$

In this analysis, one nanoparticle oriented at an angle ϕ from the x-axis, which corresponds to the tensile axis, is considered. The reorientation of such a particle to an angle ϕ' due to an applied strain ϵ_x , is illustrated in **Figure 8a**.

The relation between the new nanofibril angle and the applied strain can be expressed from geometrical considerations by⁵⁸

$$\tan \phi' = \frac{l(1 - \nu_{xy}\epsilon_x)}{L(1 + \epsilon_x)} \quad (1)$$

Where ν_{xy} is the in-plane Poisson's ratio, which was obtained here using DIC, see Supporting Information.

It should be noted that due to the large deformation that the composite undergoes, the Poisson's ratio has a strong influence on the reorientation of the fibrils. The length l and L are related according to

$$l = L \tan(\phi) \quad (2)$$

Hence, introducing equation 1 in equation 2, the relation between ϕ and ϕ' is obtained

$$\phi' = \tan^{-1} \left(\frac{1 - \nu_{xy}\epsilon_x}{1 + \epsilon_x} \tan(\phi) \right) \quad (3)$$

Equation 3 establishes the relation between the initial orientation distribution function $\rho(\phi)$ and the one in the deform configuration $\rho(\phi')$. Examples of fibre orientation distribution resulting from different strains are provided in **Figure 8b**.

After the orientation distribution function was established for each strain, a composite laminate analogy was used to predict the mechanical properties of the composite hydrogel. The main idea of the laminate analogy is to regard the composite with an in plane fibre orientation distribution as a hypothetical laminate of thin unidirectional plate, where the thickness of each plate is replaced by the value of the probability density function.^{59, 60}

$$Q^C = \int_{-\pi/2}^{\pi/2} Q(\theta)\rho(\theta)d\theta \quad (4)$$

Here the value of the density function $\rho(\theta)$ is calculated for every strain increment using equation 1. Some arrangements and simplifications had to be made to evaluate the stiffness of an oriented ply, $Q(\theta)$ since several parameters are lacking to really understand the interactions between the matrix and the reinforcing particles. Indeed, the reinforcing particles here have a limited length, are not likely to be perfectly dispersed in the composite and are not perfectly bounded to the matrix. As a result it is hard to estimate their effective contribution to the stiffness of the composite. Hence, the initial effective contribution of the nanoparticle to the composite stiffness was back calculated from the experimental data for small deformations (5-10% of strain). A ratio of 10 between the longitudinal and transverse properties was assumed, since there are no studies reporting the transversal properties of CNC or CNF. Lastly, Poisson's ratio was here taken directly from the experimental data of the modelled sample. The equations used to calculate the stiffness of a ply, $Q(\theta)$ for a fibre reinforced composite are available in literature.⁶⁰ Note that integral in equation 4 has no simple analytical solution due to the orientation distribution function used in this study. The integral was thus evaluated numerically.

Figure 9 shows the results of the modeling with respective fits to the experimental data. As it is seen from the graph, the fits follow the strength evolution curves very well ($R^2=0.98$). The results of the modeling confirm that the observed stiffening is indeed related to reorientation of the nanocellulose reinforcing fragments. It was also found that the effective contribution to the stiffness of the CNF is 2.5 times higher than the one of the CNC. Even though the CNC has a higher Young's modulus than CNF, the CNF has a much higher aspect ratio. As a result, they are better entangled to the PVA chains and more efficient in sharing load. In order to achieve a high reinforcement, the aspect ratio of the nanoparticle seems a more important parameter than its actual stiffness. In either case the hydrogel is self-standing and can be handled with ease. Future studies will be directed towards thorough evaluation of the biocompatibility of the composite.

Figure 9. Model depicting the behavior of reinforced hydrogels

3. Experimental Methods

3.1. Materials

Microcrystalline cellulose, Avicel PH 101 (MCC), 2,2,6,6-Tetramethylpiperidine-1-oxyl radical (TEMPO), 4-Acetamido-TEMPO, NaBr, NaClO, NaClO₂, CH₃COONa•3H₂O, NaOH, PVA (Average Mw 146 -186 kDa and degree of saponification 99.9%), dimethyl sulfoxide (DMSO), ethanol, HCl were purchased from Sigma Aldrich. All the chemicals used were of reagent or analytical grade. Deionized water was used in all the experiments.

3.2. TEMPO mediated oxidation of cellulose

To produce CNC, MCC was oxidized according to a previously verified method¹⁹. In short, 5 g MCC was dispersed in 400 mL of deionized water and 90 mg of TEMPO and 1 g of NaBr stirred in 100 mL deionized water were added to it. The mixture was kept stirred at 500 RPM using a magnetic stirrer. To the mixture, 10 mL of 10% (w/w) NaClO was added at intervals of 30 min for 3.5 hours. The pH of NaClO was adjusted to 11 prior to the addition using 1M HCl. 1 M NaOH was used to maintain the system pH at 10.5 for the entire period of reaction. The reaction was quenched after 3.5 hours by adding 10 mL of ethanol. Cellulose was washed 3 times with deionized water by centrifugation and then dialyzed for 3 days. Cellulose was then collected by centrifuging and was dispersed in deionized water by ultrasonication (Vibracell 700 W, 20 KHz, USA) for 5 min to obtain a transparent gel.

Cellulose nanofibrils, CNF, were prepared from never-dried spruce sulfite pulp (provided by Nordic Pulp in Säfte, Sweden) using 4-Acetamido-TEMPO as catalyst based on the method described by Tanaka et. al.⁶¹ The pulp (10 g) was suspended in 0.1 M acetate buffer (1000 mL, pH 4.8) and stirred using an overhead stirrer at 150 RPM until fully dispersed. Thereafter, 4-Acetamido-TEMPO (1 mmol) and sodium chlorite (0.1 mol) were added until dissolution. A buffered (pH 4.5); 30 mL of a 2 M NaClO solution (10.0 mmol) was added to the reaction flask in three steps, 10 mL each/2 hours. The reaction flask was maintained under nitrogen atmosphere over a period of 48

hours at 60 °C with continuous stirring. The oxidation was followed by washing thoroughly with acetate buffer and distilled water under vacuum filtration conditions. Afterwards the pulp fibers were mechanically dispersed in deionized water at 1% wt and passed through a microfluidizer M-110EH (Microfluidics Ind., USA) for a total of four passes, the first two through a set of “large pore” chambers (400 µm followed by 200 µm) and the second set of passes through “small pore” chambers (200 µm followed by 100 µm) with dilution in between. The obtained nanofibril suspension had a solid content of 0.6% wt.

3.3. Atomic force microscopy

The atomic force microscopy images were acquired in air using a Dimension Icon (Bruker, Germany) instrument. The samples were mounted on mica slides by first pre-coating the surface with 0.1% wt poly(L-lysine) solution and then fixing a dilute (0.2% wt) dispersion of nanocellulose onto the mica surface by gentle drying. The images were acquired in the peak-force tapping mode, using the manufacturer’s ScanAsyst Air cantilever and ScanAsyst automatic optimisation algorithm.

3.4. Preparation of hydrogel

The hydrogels were produced as reported earlier.^{37, 38} To produce the hydrogels, 3 g of PVA were added to a mixed solvent system of DMSO and deionized water to a final concentration of 10% (w/w). The composition of solvent was varied by varying the mass ratios of DMSO and water to obtain DMSO:water 80:20, 70:30 and 60:40 mixtures. If the DMSO proportion is further decreased, the processability of the PVA solution becomes problematic due to very high viscosities and buildup of air bubbles, which are difficult to remove. Furthermore, the transparency of the hydrogels is too poor to consider for ophthalmic applications. PVA solutions were obtained by heating the mixture at 100 °C under agitation in an oil bath for about 2 hours. For the preparation of a composite hydrogel, nanocellulose gel equivalent to 30 mg dry weight was added to the PVA solution and further mixed for an hour at 100 °C. The homogenous and transparent solutions obtained by this method were cast in to dumbbell shaped molds and allowed to gel at -20 °C for 24 hours. The dimensions of the mold were 25mm X 6.5mm X 0.5mm. The formed gels were collected from the molds, and DMSO was exchanged with deionized water by dialyzing the gels in excess water for at least 48 hours. The obtained products were stored in deionized water after washing prior to testing.

3.5. Tensile Testing

The samples were tested instantly after being removed from water to limit dehydration of the hydrogel. The room temperature was 23 °C (±2 °C). The tensile tests were performed using a laboratory tensile test machine (AGX-series, Shimadzu, Kyoto, Japan). The machine was equipped with a 10 N load cell. The specimens were tested at a cross-head speed of 10 mm/min as described in a previous study.⁶² The modulus at the toe region was defined as the slope of the stress-strain curve between 12 % and 25% strain. The composite gels were

also subjected to cyclic loading and unloading with a variable stretch, i.e. 25, 50, 75, 100 % in order to evaluate any hysteresis effects.

3.6. Digital Image Correlation

Digital image correlation (DIC) was used to estimate full-field displacements during the tensile tests. Images were acquired at a sampling frequency of 1 Hz using the commercial system GOM Aramis stereo 5M. The distance from the camera to the sample was 45 cm. Each specimen surface was spray painted in order to create speckles that could be tracked throughout the deformation. Only a few speckles were sprayed on the sample in order to avoid any stiffness variation due to painting. For a few samples, the image acquisition was pursued until failure in order to estimate the true stress of the sample, defined by

$$\sigma_T = \frac{F}{A(t)} \quad (5)$$

Where F is the force measured during the test, whereas $A(t)$ is the cross-sectional area of the sample at the instant t . To calculate the cross sectional area, it was assumed that the shrinkage of the sample was the same in plane and out of plane. The in-plane shrinkage was calculated from the images, by measuring the variation of width during the stretching of the sample.

Every frame recorded by the DIC system was compared with the undeformed state in order to estimate the displacement and strain fields. The acquired strain fields were smoothed by averaging each data pixel with 3×3 surrounding pixels. From the calculated strain field, Poisson's ratio was then determined, in the center of the specimen, according to

$$\nu_{xy} = -\frac{\varepsilon_y}{\varepsilon_x} \quad (6)$$

Where, ε_y is the transverse strain (negative for axial tension) and ε_x is the axial strain (positive for axial tension).

3.7. Strain-induced birefringence

A polarized light source of hexagonally arrayed LEDs was used to detect strain-induced birefringence due to the reorientation of CNC and CNF in the hydrogel during stretching. The experimental setup was as described in our previous work. Briefly, the sample was subjected to tensile test between the cross-polarizers which were set parallel and perpendicular to the direction of strain. Cross-head speed of 50mm/min was used for polarized light photography experiments.

3.8. Equilibrium Water Content

Water content of all samples was determined by drying the samples to constant mass in a laboratory oven at 150 °C. Three measurements were made for each sample.

4. Conclusions

In this article, we describe the strain-induced stiffening of nanocellulose-reinforced PVA hydrogels of high water content. Introducing CNC or CNF in PVA hydrogels allows producing a set of soft yet mechanically strong hydrogels with collagen-like properties. A micromechanics model was developed to understand the mechanism of strain-dependent behaviour of the hydrogels, which stems from re-orientation of the reinforcing elements from anisotropic to highly isotropic state under applied stress. The reorientation of the reinforcing elements is further visualized in strain-induced birefringence experiments. The results have shown that the mechanical properties were controlled by parameters such as composition of the gelling solvent and nanocellulose aspect ratio. The results of this study suggest that soft-tissue-like mechanical properties require high water content and specific type of solid matrix simultaneously composed of soft and rigid domains.

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Supplementary Information

Strain-induced Stiffening of Nanocellulose-Reinforced Poly(vinyl alcohol) Hydrogels Mimicking Collagenous Soft Tissues

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Axial strain measurement using Digital Image Correlation (DIC)

Poisson's ratio used in the modelling was obtained from DIC using the commercial system GOM Aramis stereo 5M. Each sample was spray painted to create speckles that could be tracked during deformation. Images were acquired at a frequency of 1 Hz. Each image of the acquired sample was compared with the initial image to estimate the displacement and strain fields. After some time the system was unable to track the points due to relatively large displacements between the points. Nonetheless, the data obtained at low strains could be used to calculate the Poisson's ratio.

Figure S1. Time lapse images of the pure PVA sample under test conditions. (a) $t=0$ sec, (b) $t= 50$ sec, (c) $t= 100$ sec. Scale shows strain along axial direction, as obtained by DIC.