Cellulose nanofiber hydrogels are produced by osmotic dehydration. We analyse the evolution of their hierarchical porosity in wet state depending on solid content (0.7-12 wt%). We measure their water physical characteristics, mechanical properties, ion-conductivity and redispersion. This method is straightforward, reproducible, does not require heat, vacuum and minimizes nanofibrillar aggregation.

Nanocellulose is a class of naturally derived nanomaterials that offers excellent mechanical properties, biocompatibility, application range and versatile possibilities for chemical modification coupled with bio-based and renewable sourcing.\textsuperscript{1-3} This class encompasses cellulose nanocrystals (CNCs) and nanofibres (CNFs); the first are highly crystalline rigid rods, the second are flexible semicrystalline filaments. Although their high water affinity can represent a challenge in composites and other applications targeted at load-bearing, water retention is a significant asset for creating structural hydrogels.\textsuperscript{2-6} Because of their tendency to entangle, CNFs easily form viscoelastic solids (hydrogels) upon dehydration at concentrations as low as 0.25 wt%.\textsuperscript{7} When the water/solids content needs to be tuned, several methods are available to dehydrate hydrogels.\textsuperscript{8} Solvent evaporation poses the difficulty of precisely controlling the final solid content as time, heat, relative humidity, and ventilation all contribute to it. Moreover, it produces highly aggregated materials. Dehydration via filtration, spray, freeze and CO\textsubscript{2} drying are time-consuming, complex and costly, and it can induce irreversible aggregation of the nanofibres, which decreases their redispersion and optical properties (e.g. transparency).\textsuperscript{9} Control over porosity is also difficult with these methods. Such difficulties have most probably led to the to coagulating or cross-linking suspensions with a solids content of <2 wt%.\textsuperscript{3,5} CNFs and nanocellulose in general are particularly appealing compared to polymeric hydrogels in tissue engineering, simulation of extracellular matrix, wound healing, solid state cell factories and biomimicking applications, as the 3D porous network can be tailored over a vast range of mechanical properties without losing its structural integrity.\textsuperscript{2,10,11} Especially for those applications involving living cells, the amount and type of porosity must be tailored for the specific biological proliferation, as gas and fluid transport within the 3D structure define how suitable the material is for the specific application. In this respect, the fabrication technique plays a critical role. New bottom-up methods are needed to control the pore structure in order to increase the performance, to aid commercialization and promote further material exploration of nanocellulose-based materials.\textsuperscript{5,12,13} Previously, Guccini et al.\textsuperscript{14} described the use of osmotic dehydration to precisely control the solids content of CNF suspensions (0.5 to 4.9 wt%), while minimizing the aggregation of the nanofibres. In the present work we demonstrate the untouched potential of osmotic dehydration (OD) as innovative method to prepare hydrogels. For the first time we provide a detailed picture of the porosity evolution in wet state of the CNF hydrogels depending on their solid content (0.7 to 12 wt%), to which we correlate the conductivity, water physical characteristics and mechanical properties. Additionally, we show that OD preserves the rheological properties of the CNFs upon rewetting. The carboxylated CNFs (Fig. S1) used in this study were obtained by TEMPO-mediated oxidation.\textsuperscript{15} Fig. 1A schematically shows the OD set-up used to manufacture the CNF hydrogels. The top compartment containing the CNFs, separated from the magnetically stirred PEG solution (35 kDa) by a semipermeable fabrication of hydrogels by bottom-up approach mostly limited
membrane (cut off 6-8 kDa), which allows only the passage of water. Given the higher concentration of the PEG solution (10 - 25 wt%), water flows from the CNF to the PEG compartment by osmosis, thus dehydrating the CNF suspension. The rate of the diffusion can be tuned by PEG concentration, thus the dehydration can be easily controlled and performed at room temperature and atmospheric pressure. The OD leads to homogenous and smooth hydrogels whose dimensions can be potentially scaled up (Fig. S2). Fig. 1B shows that the solids content increases exponentially from 0.7 ± 0.1 to 12.4 ± 0.8 wt%, depending on the PEG concentration, which corresponds to a decrease in moisture content. Despite the pivotal role that the dehydration plays in nanocellulose assembly, rheological and mechanical properties, this effect is rarely systematically correlated with the physical-chemical properties of the derived hydrogels, such as porosity and water interaction.8,9 This can be achieved using OD. Fig. 1C shows the freeze-dried cross-sections of two of the hydrogels imaged by SEM. At 0.7 wt% the hydrogel appears much more porous, with pores mainly below 100 μm and walls that are a couple of micrometres thick. At 12 wt% the cross-section appears very compact and dense. Their thickness is also different, ≈ 400 μm at 0.7 wt% and ≈ 20 μm at 12 wt%. Despite the big difference in the solids content, both cross-sections show the typical layered structure, suggesting that its formation happens progressively during dehydration. We note that the characterization of the wet (or swollen) porosity from SEM imaging may not be totally accurate because of the artifacts that can be produced during freeze-drying of the sample. Thermoporosimetry measures the porosity directly in wet state,16-18 and has recently been successfully employed to characterize pore size distribution and water characteristics in cellulosic fibres19,20 as well as in CNC systems.21,22 Fig. 2A shows the wet porosity of the CNF hydrogels as a function of the CNF solids content. During the dehydration the distance between the nanofibres decreases, creating an interconnected 3D network that provides the porosity in wet state. The total pore volume stays relatively constant at around 17 ml g⁻¹ between 0.7 and 1.2 wt% solid content. Once the solid content reaches 12 wt%, it decreases to a minimum of 3.9 ± 0.7 ml g⁻¹. Most of the pores are in the mesoporous range, accounting to ≈97 % at the lowest CNF concentration and ≈99 % at the highest. The pore size distribution (PSD) of the hydrogels is shown in Figure 2B. The peak of each distribution is in all cases above 10 nm and decreases from ≈20 to 16 nm as the solids content increases. Thermoporosimetry can only offer an estimation of the macroporosity18 and therefore, the micrometer sized porosity visible from the SEM images is not accounted in the distribution. Nevertheless, a clear trend can be seen. The amount of macropores between 50 and 100 nm sharply decreases with the increase of solid content. These results provide a detailed picture of the porosity evolution in wet state of the hydrogels as a function of their solid content. CNF films prepared by vacuum filtration have shown a pore volume in wet state three orders of magnitude lower, but bigger pore diameters (= 30 nm).18 Most probably these differences originate from nanofiber aggregation induced during drying by filtration, which may also explain why the pore dimensions are constant despite a 200 h swelling in water. By carefully modulating the porosity via OD, it is possible to tune the water interactions as well as the liquid and gas uptakes of the hydrogels. Indeed, the macropores aid the fast water uptake and swelling but allow low water penetration within the solids structure, while the mesopores aid slower water absorption but larger penetration via capillary forces.3 Briefly, the water in nanocellulose matrixes can be defined as free and bound water with respect to its thermodynamic properties, which are influenced by the effect of the confinement within the matrix and strong nanocellulose-water interaction.23 “Free water” has the same chemical-physical properties as bulk water and it is chiefly responsible for swelling of the CNF matrix. “Bound freezing water” is confined and weakly bound in the sub-microscopic space created from the CNF network. Such confinement affects its solid-liquid phase transition. “Non-freezing water” is tightly bound at the cellulose surface and does not freeze due to strong interaction. Fig. 2C shows the percentage of bound and free water respect to the total freezing water. Below 1.2 wt% the ratio is relatively even, after which the amount of free water decreases while the amount of bound water decreases at higher solids contents. At 12 wt% the share of bound water is nearly 90% of the total water. The increase in bound water with the CNF solids content relates to the decrease of porosity in the hydrogels (Fig. 2A and 2B), which ultimately provides more confinement for the water and thus changes in its thermodynamic properties. The fact that below 1.2 wt% the porosity is relatively stable, but the moisture content increases (Fig. 1B), suggests that the free water is capable of swelling the hydrogel structure increasing its

Fig. 1. (A) Schematic representation of the Osmotic Dehydration set-up; (B) CNF solid and moisture content of the hydrogels as a function of the PEG concentration; (C) SEM images of the cross-section of the freeze-dried hydrogels prepared using 10 and 25 wt% PEG.
Porosity, only to a certain limit (up to 1.2 wt%) and the excess water is likely phase separated and loosely retained within the CNF network. The variation in mechanical properties with the hydrogels was analysed by a compression test. Fig. 2D shows that the stress increases with the CNF solid content, meaning that the hydrogels become harder to deform. The rate at which the stress increases is higher below 1.2 wt%. This is related with the fact that the deformations due to water loss and densification are more pronounced because of the higher moisture content and porosity (Fig. 1B and 2A). At higher solid content, the larger amount of bound water is clearly less prone to be lost compared to the free water due to its strong association with the CNFs (Fig. 2C). This factor and the lower porosity make the hydrogel stronger. Moreover, the behaviour of CNFs at the nanoscale also contributes. According to our previous study, above 1.0 wt% the nanofiber entangled network goes through an isotropic-anisotropic transition (formation of nematic order), leading to stronger yet stiffer materials. The reliable control of the solid content offered by OD, can be used to gain insight on the fundamental properties of water within the CNF 3D network. Fig. 2E shows the proton conductivity of the hydrogels compared to the freezable water. The conductivity drops nearly 10 times from 0.7 to 1.0 wt% and it is ~100 times lower above 2.4 wt%. Since very little research has been conducted on the relationship between proton conductivity and the amount and state of the water molecules in nanocellulose systems, our well-controllable system is ideal to elucidate this question. Here, we found a strong correlation with the amount of water and likely with the porosity. As the porosity and the free water sharply decreases above 2.4 wt% (Fig. 2A and 2C) the conductivity drops another order of magnitude compared to 0.7 wt%. This shows the predominant role of free water over the bound water in the conductivity, which occurs via water-mediated hydrogen bond (Grötthuss mechanism). These results highlight that bound water cannot sufficiently conduct protons. Interestingly, electrochemical conditions within for example a fuel cell may affect the conduction properties of bound water, as it is suggested by the fact that carboxylate CNF membranes have shown constant conductivity above 65% relative humidity.24

As previously demonstrated, water removal with OD reduces the aggregation of CNF.14 Thus we evaluate OD as a method to improve the redispersibility of concentrated CNFs. After OD, the hydrogels were redispersed to initial concentration (0.2 wt%) simply by magnetic stirring overnight. Fig. 2F shows their storage (G’) and loss (G”) moduli in the linear viscoelastic region, compared with the pristine and oven dried samples. After the OD, the solid-like character (G’ > G”) of the suspensions is preserved, although the elastic behaviour and ability of the CNF network to dissipate stress without irreversible deformation decreases, as can be seen from the lower G’ values and early yield behaviour (onset of nonlinear behaviour and critical stress), respectively (Fig. 5A). This is most probably connected to the formation of small aggregates that disrupt the entanglement network within the suspension. Nevertheless, OD clearly minimizes CNF aggregation, as can also be seen in the inset of Fig. 2F from the unchanged appearance of the redispersed hydrogels (OD) compare to the pristine suspension (Ref). The absence of big aggregates was also confirmed by thermal analysis (Fig. 5B). In contrast, G’ of the oven dried samples decreases more than 10 times, with values
lower than $G''$, indicating irreversible aggregation during drying that is also evident from the sample turbidity (Fig. 2F inset). While several strategies have been implemented for CNCs, CNFs are significantly more difficult to redisperse due to their inherent tendency to entangle\textsuperscript{25,26} Although it is difficult to compare rheological data in general due to the many factors affecting the measurements,\textsuperscript{7} our findings show very good redispersion with very little energy input. This could be further improved, for example, by introducing additives during the water removal or even by simply increasing the shear force of the mixing during redispersion.\textsuperscript{26} Ultimately, our study offers a new platform to regulate the fluid and gas transport in nanocellulose hydrogels. Because of the reliable control of the solid content offered by OD, we were able to elucidate the relationship between porosity, water physical characteristics and mechanical properties, also critical for other nanocellulose-based materials such as membranes, films and foams. The analysed hydrogels (0.7–12 wt%) have a hierarchical porosity in the meso and macroporous range. The total pore volume stays relatively constant up to 1.2 wt%, above which it sharply decreases. At the same time the free water decreases and bound water increases. We found out that the free water has a crucial role in the proton conduction mechanism. Finally, OD minimized the aggregation of the nanofibres which allowed the redispersion of the hydrogels in water without losing their elastic character (G' > G'\textsuperscript{*}).

VG and EK would like to acknowledge the Academy of Finland (AoF) for the Financial support offered by the Project AlgaLEAF, VR and TT the European Union via the project FuturoLEAF (European Union's Horizon 2020 research and innovation programme under grant agreement No 899576), JP and TM the Foundation for Research of Natural Resources in Finland programme under grant agreement No 899576 (and TT (AoF)), VG elastic and bound water increases. The solid content offered by OD, free water decreases, redispersion.

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Notes and references