Monodisperse Highly Ordered Chitosan/Cellulose Nanocomposite Foams

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Abstract

In solid foams, most physical properties are determined by the pore size and shape distributions and the organisation of the pores. For this reason, it is important to control the structure of porous materials. We recently tackled this issue with the help of microfluidic-aided foam templating, which allowed us to generate monodisperse and highly ordered chitosan foams. However, the properties of foams also depend on the properties of the pore wall constituents. In case of chitosan-based foams, the foams have poor absolute mechanical properties, simply due to the fact that the solubility of chitosan in water is very low, so that the relative density of the freeze-dried foams becomes very small. Drawing inspiration from the field of nanocomposites, we incorporated cellulose nanofibres into the foamed chitosan solutions, with a view to strengthening the pore walls in the foam and thus the mechanical properties of the final foam. We report here how the cellulose nanofibres affect the structure of both the liquid foam template and the solid foam. The resulting nanocomposite foams have improved mechanical properties, which, however, are not proportional to the amount of cellulose nanofibres in the composites. One reason for this observation is the disturbance of the porous structure of the solid foams by the cellulose nanofibres.

Keywords: Cellulose (A), Foams (A), Nanocomposites (A), Mechanical testing (D)

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1. Introduction

Polymer foams are materials consisting of a gas phase dispersed in a polymer matrix. The gas bubbles within the polymer matrix do not affect the intrinsic properties of the polymer such as its chemical reactivity or wettability. However, the presence of the gas bubbles results in materials which are lightweight and which have a large specific surface area. The extent to which the dispersed gas phase affects the properties of a porous polymer depends on the morphology of the foam, i.e. on its relative density, the average pore size, the interconnectivity between the pores, the thickness of the pore walls etc. Twenty years ago, Gibson and Ashby[1] reported on the effect of the relative density of porous polymers on their response to compression, and on the interdependency between architecture and mechanical properties.

Although the size of the pores does not significantly affect the mechanical strength of the material for a given relative density, it is an important parameter for biological applications where solid foams are used as scaffolds for tissue engineering. Cell colonization calls for monodisperse pores with sizes between 100 μm and 500 μm [2]. The foams also have to be open-cell in order to allow the transportation of nutrients and oxygen throughout the scaffold. Well-defined scaffolds are required for systematic studies as local differences of the structure affect cell growth and result in different biodegradation rates of the scaffolds[3–5]. Moreover, the optimal pore size differs for each type of cell to be cultivated which is why we need methods allowing an easy and straightforward tailoring of the scaffold's pore size.

High Internal Phase Emulsions (HIPEs) have been proposed as templates for porous polymers (thus named polyHIPEs) with an improved control over their structures[6]. However, typical polyHIPEs are materials with still too large pore size distributions and pore sizes below 100 μm . It was only recently that both the control of polydispersity and of the pore size were tackled by using microfluidic techniques, which allowed for the production of pores with diameters in the range of 25 - 250 μm [5]. But a major problem remains, specific to the use of emulsions: the need for an organic solvent. Whether it is used as the dispersed phase itself or to wash the dispersed phase out of the material, traces of solvent in scaffolds are always undesirable. Foam templating, on the contrary, uses gas as the dispersed phase, which solves the problem of the need for an organic solvent[3]. Thus, we use foam templating combined with microfluidics to reach a high structural order and to tune the bubble sizes[4,7–11].

Foam templating, like any other type of colloidal templating, is based on the idea that the liquid template can be solidified in order to produce a solid material that is, ideally, the exact image of its liquid counterpart. The aim of templating is to tailor the pore size, the pore size distribution and the pore morphology of the solid foam by tailoring the structure of the liquid template.

The polymer used in the study at hand is chitosan, which is a polysaccharide derived from chitin. Chitin is extracted from the shells of crustaceans[12]. Chitosan is slightly soluble in acidic solutions, in which its amino groups are protonated. Chitosan is thus a polycation and displays the common properties of polyelectrolytes[13]. Chitosan has already been studied extensively as a potential material for tissue engineering and drug release[14,15]. Indeed, thanks to its amino side groups, chitosan is intrinsically antibacterial. Chitosan also displays little reaction with foreign bodies and can be degraded via enzymatic activity into oligosaccharides[14].

We have shown in our previous work how to generate monodisperse chitosan foams with interesting morphological properties via foam templating[16,17]. However, with an elastic modulus below 100 kPa[18] such foams are too weak to be used as scaffolds for tissue engineering. Following the work of Svagan et al.[19], who designed amylopectin/cellulose nanocomposite foams, Wang et al.[20] showed that by adding cellulose nanofibres (CNF) to a chitosan-based solution, one obtains improved mechanical properties of the resulting solid foams. We thus followed a similar route to improve the mechanical performance of the monodisperse chitosan foams. Indeed, we incorporated different amounts of positively charged quaternised CNF into the 4 wt % chitosan solution used in[17] to produce monodisperse highly ordered nanocomposite foams. A nanocomposite is a material with a matrix reinforced by a filler having at least one dimension in the nanometre range. The matrix in the present study is chitosan and the filler is quaternised cellulose nanofibres (CNF), which are described in detail in[21]. The nanometre range dimension of the fibres is their diameter, which is on average 1.6-2.1 nm.

2. Experimental

2.1 Chemicals

Chitosan with a molecular weight of $30\,000\text{ g mol}^{-1}$ and a deacetylation degree DD of 90.56% (data from the supplier) was purchased from Glentham Life Sciences Ltd. The white-light yellow powder was used as received. The cross-linker, genipin, was purchased from Challenge Bioproducts Co., Ltd. and had a purity of 98% (determined by HPLC, data from the supplier). The cross-linking reaction between genipin and chitosan is shown in Scheme 1 a). We dissolved 1 wt % genipin in 1 vol % AcOH. Once the genipin was dissolved, we added the surfactant at a concentration of 0.1 wt % with respect to the solvent. The acetic acid was 100% pure and purchased from VWR. The bidistilled water was demineralised using an ion exchange column. The surfactant (Plantacare 2000 UP, an alkyl polyglycoside) was donated from Cognis Deutschland GmbH & Co (today BASF) and has an active matter between 51% and 55%. The alkyl chain contains between 8 and 16 carbons and the head group has on average 1.5 glycoside groups. Its chemical structure is shown in Scheme 1 b). Perfluorohexane was 98% pure and purchased from Alfa Aesar.

For the preparation of the cellulose-free chitosan solutions, later described as C40_000 and C41_000, the chitosan was dissolved in a 1 vol % acetic acid solution with a magnetic stirrer for at least 2 h. The abbreviations were defined as follows: C40_*** and C41_*** mean that the chitosan concentrations were 4.0 wt % and 4.1 wt %, respectively; and C**_000, C**_025, C**_050, C**_075 and C**_100 mean that the CNF concentrations were 0.000 wt %, 0.050 wt %, 0.075 wt % and 0.100 wt %, respectively. To ensure a good dissolution, we treated the chitosan solution with an ultrasonic homogeniser SONOPLUS HD2200 from Bandelin for 5 min at a power of 40%. To prevent the solution from heating up, it was placed in an ice bath during homogenisation. The surfactant was then added to the solution ($c_{\text{surfactant}} = 0.1\text{ wt \%}$), and the solution was left in an ultrasound bath (SONOREX SK 100H from Bandelin) to remove the bubbles that formed while stirring the solution.

The quaternised cellulose nanofibres (CNF) was prepared as a gel-like suspension in water, and the structure of the nanofibres is shown in Scheme 1 c). The cellulose content was 0.14 wt %. To prepare the mixed chitosan/CNF solutions, later described as C40_050, C40_075 and C40_100², we first added water and acetic acid to the CNF dispersion to obtain a solution with the desired CNF concentration in 1 vol % AcOH. The

a)

Chitosan

Genipin

Cross-linked chitosan

b)

Plantacare 2000 UP

c)

Core: crystalline cellulose

Surface: Quaternised Cellulose Derivatives

$R = H$ or $\text{CH}_2\text{CH}_2\text{N}^+(\text{CH}_3)_2\text{Cl}^-$

2.2 Rotational rheometry

2.3 Microfluidics

The general microfluidic set-up is shown in Figure 1 (top). The gas phase was pressure controlled using a pressure pump (OB1 Mk2 Pressure Controller from Elveflow) connected to a nitrogen tap. The pressure pump was connected to a glass bottle sealed with a GL45 cap from Vaplock containing perfluorohexane C_6F_{14} (which is a volatile liquid) to the microfluidic chip. Due to the high vapour pressure of perfluorohexane, traces of perfluorohexane were carried along with the nitrogen flow. The bubbles formed with microfluidics had thus a gas phase composed of nitrogen with traces of perfluorohexane. We used syringe pumps to push the liquid phases through the channels. Since chitosan and genipin were dissolved in different solutions we used one syringe pump for each solution. The syringe pumps (Pump 11 Elite from Harvard Apparatus) allowed us to apply a constant flow rate, namely $Q_{\text{chitosan}} = 180 \mu\text{L min}^{-1}$ for the chitosan solution and $Q_{\text{genipin}} = 30 \mu\text{L min}^{-1}$ for the genipin solution. We controlled the bubble size by varying the gas pressure p_{gas} , which we controlled using the software Elveflow Smart Interface. The chip used was purchased from Dolomite Microfluidics and had a T-junction geometry (see Figure 1 (bottom)). It was $190 \mu\text{m}$ deep and its constriction was $195 \mu\text{m}$ large. Note that we used in previous works a microfluidic chip with a cross-section instead of a T-junction[16,17]. The reason for the change of geometry was practical: we have never managed to generate a stable flow of monodisperse bubbles with solutions containing CNF using a cross-flow geometry. However, microfluidic bubbling was possible for chitosan solutions containing CNF using a T-junction. Since the addition of CNF does not significantly change the viscosity of the solution at the flow rates involved (see Section 3.1), we postulate that the geometry is responsible for the impossibility to produce monodisperse foams with a cross-flow geometry if the chitosan solution contains CNF. Indeed, we know that the CNFs can be up to $2 \mu\text{m}$ in length[21] and are elongated due to the high shear rates within the microfluidic channels. While the flow has to follow an elbow in the cross-flow geometry (the direction of the incoming flow is perpendicular to the direction of the outgoing flow), the direction of the liquid flow does not change in the T-junction during bubbling (see Figure 1). Thus, in a T-junction, the cellulose nanofibres may remain elongated in the direction of the flow while in a cross-flow geometry the 90° angle can induce a contraction of the fibres and flow instabilities preventing monodisperse bubbling.

We used Teflon tubings with an outer diameter of 1.6 mm and an inner diameter of 0.5 mm from Techlab. We monitored microfluidic bubbling by means of a Nikon SMZ- 800 N optical microscope coupled with an Optronis CL600X2 high-speed camera. The software used to record the images was GenICam. The foams

were collected in polystyrene Petri dishes with a diameter of 3.5 cm and a height of 1 cm. The time required to collect a foam depended strongly on the liquid pressure and ranged from 1 to 30 min.

Figure 1: (top) Generation of monodisperse chitosan/CNF foams using a microfluidic device with a T-junction. (bottom) Picture of the T-junction used with its relevant dimensions given in mm. The channel depth is constant over the whole chip and equal to 0.190 mm.

2.4 Liquid foam characterisation and solidification

The bubble size distributions were determined using the image analysis software ImageJ from pictures taken with the Nikon SMZ-800 N optical microscope. Note that the bubble/pores sizes discussed throughout this work are diameters and not radii. For monodisperse closed-packed foams, the bubble size was determined by measuring the distance between the centres of two bubbles in contact, which we call the centre-to-centre

distance d_{cc} . The bubble centre was calculated by ImageJ and is the average position of all pixels belonging to the bubble. This allowed for a more precise determination of the bubble size if the contours of the bubble are out of focus. For polydisperse foams, the bubble size was calculated from the area of the bubble measured with ImageJ. In the absence of a close-packing the centre-to-centre distance this area is not equal to twice the radius. Note that the visible black rings (see Figure 4) are not the contours of the liquid bubbles, but are an optical effect[23]. At least 40 bubbles were measured for each sample to determine the polydispersity index (*PDI*). The *PDI* was used to assess the monodispersity of the liquid and solid foams.

The liquid fractions of the foams were determined by collecting the foams in a 10 mL graduated cylinder for a given duration Δt . The liquid fraction ϕ of the foam in the cylinder differs from the liquid fraction set by microfluidic bubbling due to drainage. To measure ϕ , we determined the total volume of liquid V_l in the cylinder by first summing the flow rates of the syringe pumps ($Q_{total} = Q_{chitosan} + Q_{genipin}$), which yields $V_l = \Delta t \cdot Q_{total}$. The volume of the drained liquid $V_{l,drain}$ and the foam volume V_{foam} were measured with the graduation of the cylinder. The volume of the liquid contained in the foam $V_{l,foam}$ was the difference between the total volume and the volume of the drained phase, i.e. $V_{l,foam} = V_l - V_{l,drain}$. The liquid fraction ϕ was the ratio of the volume of liquid in the foam and the volume of the foam, i.e. $\phi = V_{l,foam} / V_{foam}$.

The cross-linking procedure was the same for all samples. The Petri dishes containing the foams were sealed with Parafilm and left to cross-link at room temperature for 18 hours. Once cross-linked, the foams were frozen in liquid nitrogen and freeze-dried in an Alpha 1–4 LSC freeze-dryer from CHRIST.

2.5 Solid foam characterisation

Morphology

We investigated the structure of the solid foams with scanning electron microscopy (SEM) using a CamScan CS 44 microscope. The solid foams were frozen with liquid nitrogen before being cut with a scalpel. Without this freezing step, the foams were not stiff enough to be cut and were shredded or were compressed upon cutting. Once the samples were cut, we glued them on a sample holder and sputtered them with gold to coat the samples with a conductive layer. The voltage applied was 5 kV. The software used to record the images

was Edax Genesis. The pore size distributions were measured from the SEM images using ImageJ using at least 40 pores.

Density and mechanical properties

The foams were first conditioned in a room with controlled environment ($50 \pm 2\%$ relative humidity, 23 ± 1 °C) for at least 40 hours prior to testing. The height, diameter (precision caliper 0.01 mm, Mitutoyo) and weight (precision balance 0.1 mg, Mettler-Toledo) were measured for each sample. The mechanical properties of the solid foams were then determined using a single column Universal Testing Machine 5944 (Instron, USA) in compressive mode. Specimens were carefully mounted on the instrument and tested using a strain rate of 0.1 min^{-1} until 90% strain. The modulus was determined as the slope from the initial linear elastic region, and the yield strength (or plateau stress) was calculated as the intersect of tangential lines to the elastic and plateau regions. The density was calculated by dividing the mass of the sample by their calculated volume assuming a cylindrical shape. For each sample, the reported values are average of 4-7 specimens.

The cell wall properties were calculated using Gibson's and Ashby's scaling laws and rule of mixtures for the density of a composite[1], assuming 1400 kg m^{-3} for the density of chitosan and 1500 kg m^{-3} for the density of CNF. It holds

$$\rho_{rel} = \rho^* / \rho_s \quad (1)$$

$$E_s = E^* \cdot \rho_{rel}^{-2} \quad (2)$$

$$\sigma_s = 0.3 \cdot \sigma^* \cdot \rho_{rel}^{-3/2}, \quad (3)$$

with ρ_{rel} the foam's relative density, ρ^* the foam's measured density, ρ_s the density of the cell wall, E_s the elastic modulus of the cell wall, E^* the foam's elastic modulus, σ^* the foam's yield strength and σ_s the yield strength of the cell wall. Finally, the foam porosity is defined as $(1 - \rho_{rel})$.

3. Results and Discussion

3.1 Chitosan/CNF Solutions and Microfluidic Bubbling

Rheological behaviour

In Figure 2 the viscosity η is plotted as a function of the shear rate $\dot{\gamma}$ for the different solutions used in this study. The composition and denomination of each solution are given in Table 1. Looking at Figure 2, one sees that the two solutions without CNF, i.e. C40_000 with $c_{\text{chitosan}} = 4.0$ wt % and C41_000 with $c_{\text{chitosan}} = 4.1$ wt %, have a Newtonian plateau from which one can extract the zero-shear-rate viscosity η_0 . One obtains $\eta_0 = 0.04$ Pa s for the C40_000 solution and $\eta_0 = 0.06$ Pa s for the C41_000 solution. As expected, the solution containing a slightly larger amount of chitosan is more viscous. Moreover, both chitosan solutions display a shear thinning behaviour at high shear rates, i.e. from $\dot{\gamma} > 1000$ s⁻¹. Such a shear thinning behaviour is in accordance with previous studies on the rheology of chitosan solutions[9,24,25].

Looking at the flow curves of the solutions containing CNF, one sees that all solutions have a strong shear thinning behaviour over the whole range of shear rates studied. The absence of a plateau at low shear rates is characteristic of yield fluids. For each curve one can distinguish three different regions with three different slopes. The three-region viscosity model has already been observed and described for CNF suspensions[26,27], but, to the best of our knowledge, it was neither studied in presence of chitosan in particular nor of any other polysaccharide in general. Note that a thorough rheological study requires oscillatory rheometry with the measurement of the storage and loss moduli of the solutions[26]. We chose not to carry out these measurements since such a deep understanding of the interactions between chitosan and CNF is not within the scope of this paper. We are, however, interested in the viscosity of the solutions at the shear rates applied in the chip to fit the microfluidic parameters with the flow properties. For the chip used in this work, the shear rate at the constriction of the T-junction is $\dot{\gamma} \sim 850$ s⁻¹ (see the calculations in[18]). We list the corresponding viscosities at the T-junction η_T in Table 1.

Table 1: Different solutions used to generate monodisperse foams with the viscosity at the T-junction η_T , the gas pressure p_{gas} at which the foam templates were produced and the average bubble size of the foam templates $\langle d_{\text{bubble}} \rangle$.

sample	$c_{\text{chitosan}} / \text{wt } \%$	$c_{\text{CNF}} / \text{wt } \%$	$\eta_T / \text{mPa s}$	$p_{\text{gas}} / \text{mbar}$	$\langle d_{\text{bubble}} \rangle / \mu\text{m}$
C40_100	4.0	0.100	38 ± 3	1700	310 ± 4
C40_075	4.0	0.075	42 ± 3	1750	299 ± 4
C40_050	4.0	0.050	38 ± 2	1500	306 ± 3
C40_000	4.0	0.000	39 ± 3	950	303 ± 4
C41_000	4.1	0.000	56 ± 3	1300	301 ± 6

Figure 2: Viscosity η as a function of the shear rate $\dot{\gamma}$ for the different solutions used for the generation of the liquid foam templates. The solutions are C400_100 with $c_{\text{chitosan}} = 4.0 \text{ wt } \%$ and

$c_{\text{CNF}} = 0.1 \text{ wt } \%$, C40_075 with $c_{\text{chitosan}} = 4.0 \text{ wt } \%$ and $c_{\text{CNF}} = 0.075 \text{ wt } \%$, C40_050 with $c_{\text{chitosan}} = 4.0 \text{ wt } \%$ and $c_{\text{CNF}} = 0.05 \text{ wt } \%$, C40_000 with $c_{\text{chitosan}} = 4 \text{ wt } \%$ and no CNF and C41_000 with $c_{\text{chitosan}} = 4.1 \text{ wt } \%$ and no CNF. The straight line shows the shear rate in the T-junction, namely $\dot{\gamma} \sim 850 \text{ s}^{-1}$.

Microfluidic bubbling

Figure 3 shows the variation of the bubble size d_{bubble} with the gas pressure p_{gas} for the different solutions. The aim of the chip calibration was to determine for each solution the gas pressure needed to produce a monodisperse liquid foam with the same average bubble size. The bubble size aimed for was ca. $300 \mu\text{m}$ and was arbitrarily chosen. One sees that, for all solutions, the bubble size d_{bubble} increases with increasing gas pressure p_{gas} . As already observed for the cross-flow geometry in [17], the bubble size increases steeply at the lowest bubbling pressures (here for the solutions C40_000 and C40_100), before reaching a second linear regime in which the bubble size increases slower with increasing gas pressure p_{gas} . One clearly sees that the gas pressures p_{gas} needed to reach bubble sizes between $250 \mu\text{m}$ and $300 \mu\text{m}$ are much lower for the solution C40_000 (no CNF, 4 wt % chitosan) than for all other solutions. The difference between the two solutions without CNF (C40_000 and C41_000) can be simply explained by the higher viscosity of the solution containing more chitosan (for $\dot{\gamma} \sim 850 \text{ s}^{-1}$ it holds $\eta_{\text{T}} = 39 \pm 3 \text{ mPa s}$ for C40_000 and $\eta_{\text{T}} = 56 \pm 3 \text{ mPa s}$ for C41_000 (see Table 1)). For the solutions containing CNF, the gas pressures are also shifted to higher values with increasing CNF concentration c_{CNF} , while their viscosities η are all comparable to the viscosity of the C40_000 solution. The viscosity can thus not explain the different gas pressures and one needs to look into the viscoelastic properties of the different solutions to explain this observation. However, a deep investigation of the viscoelasticity of chitosan-CNF solutions was not within the scope of this study as the chip calibration provides sufficient information to produce liquid foam templates with comparable bubble sizes. Figure 4 show pictures of the different liquid foam templates. The gas pressures p_{gas} applied for template production are listed in Table 1.

Figure 3: Bubble diameter d_{bubble} as a function of the gas pressure p_{gas} for the various chitosan solutions. The chitosan flow rate was set to $Q_{\text{chitosan}} = 180 \mu\text{L min}^{-1}$. The error bars correspond to the standard deviations.

3.2 Monodisperse Liquid Chitosan/CNF Foams

Once the appropriate parameters for foam production were determined, we characterised the liquid foam templates. Figure 4 shows pictures of a typical monodisperse liquid foam template for each solution.

Obviously, the presence of CNF in the chitosan solution does not affect ordering.

Figure 4: Pictures of the liquid foam templates for each foaming solution: a) C40_100, b) C40_075, c) C40_050, d) C40_000 and e) C41_000. The scales bars are 500 μm .

Figure 5 shows the evolution of the liquid fraction ϕ of each liquid foam template. One sees that drainage stops and the liquid fraction reaches a plateau for all solutions, but at different times. The values of the final liquid fractions are listed in Table 2. The liquid fractions of the solutions without CNF and the solution C40_050 with $c_{\text{CNF}} = 0.05$ wt % decrease sharply with time at short times. However, at long times, the liquid fractions of the solutions without CNF (C40_000 and C41_000) reach the same value of $\phi = 0.04$. For C40_050, the final value of the liquid fraction is much higher with $\phi = 0.12$. For the solutions C40_100 and C40_075, containing 0.1 wt % CNF and 0.075 wt % CNF, respectively, one observes two linear regions. The first region is a linear decay of the liquid fraction followed by a plateau once drainage has stopped. The final liquid fraction increases with increasing c_{CNF} ($\phi = 0.20$ for $c_{\text{CNF}} = 0.075$ wt % (C40_075) and $\phi = 0.29$ for $c_{\text{CNF}} = 0.1$ wt % (C40_100)).

Figure 5: Liquid fraction ϕ as a function of time t for the different foam templates. The cross-linker genipin was present in the foams and cross-linking occurred during the experiment. Not to bias the foam volume, we conducted the liquid fraction measurements without perfluorohexane.

However, the final liquid fraction is not influenced by the viscosity of the liquid. Indeed, the viscosity only affects the time required for the foams to reach the equilibrium liquid fraction[28]. However, looking at the flow curves in Figure 1, one can split the different solutions into two categories: the CNF-free solutions are shear-thinning with a Newtonian plateau and a zero-shear-rate viscosity, while the solutions containing CNF are yield fluids. Yield fluids have no zero-shear-rate viscosity and do not flow below a given shear stress called yield stress[29]. One sees that the solutions with a zero-shear-rate viscosity have the same plateau liquid fraction of $\phi = 0.04$, while the yield fluids have an increasing plateau liquid fraction with increasing CNF concentration. One sees from Figure 2 that the viscosity at low shear rates also increases with increasing

CNF concentration, indicating a higher yield stress with increasing CNF concentration[29]: drainage stops at higher liquid fractions for liquids with higher yield stresses. Another reason for the increase of the liquid fraction with increasing CNF concentration may be cross-linking. Although we did not measure the gel point for each solution, one can assume that drainage stops upon sufficient cross-linking and the liquid fraction reaches a plateau. The higher viscosities of the solutions with higher CNF concentrations induce a slowing down of drainage. The liquid fraction φ is thus higher when cross-linking arrests drainage. Further studies could determine (a) the influence of c_{CNF} on the yield stress and (b) whether the gel point is shifted to longer times upon addition of CNF. Whichever the reason for the large differences of the liquid fractions, it is clear that the CNF concentration c_{CNF} strongly influences the liquid fraction of the liquid template. One thus can expect a strong dependency of the density of the solid foams on the CNF concentration.

3.3 Monodisperse Solid Chitosan/CNF Foams

We cross-linked and solidified the liquid foam templates following the same procedure as described in[17], namely cross-linking at room temperature for 18 h followed by freeze-drying.

Morphology

Figures 6 (a)-(e) show SEM pictures of the resulting solid foams. The pore size distributions measured from the SEM pictures are also shown along with the bubble size distributions of the corresponding liquid templates. The samples with $c_{\text{CNF}} \leq 0.050$ wt % (C41_000, C40_050 and C40_000) clearly show ordering and retain their monodispersity during solidification, with *PDIs* of 3.5%, 2.5%, and 3.3% respectively. Note that the solid foam with 4 wt % chitosan and no CNF has defects in its ordering and the sample was partly shredded (lower right part in Figure 6 d)), indicating that the cut of the sample was not perfect (this demonstrates the importance of the sample preparation for SEM).

The foams with 0.075 wt % and 0.1 wt % CNF have a broader pore size distribution (see Figures 6 a) and b)), pores with irregular shapes, thicker pore walls, and a lower number of windows between neighbouring pores compared to the other samples. We quantify the number of windows by defining $N_{\text{window}}/N_{\text{pore}}$ which is the average number of windows counted per pore. The values of $N_{\text{window}}/N_{\text{pore}}$ are given in Table 2. One sees that foams with CNF concentrations up to $c_{\text{CNF}} = 0.05$ wt % have in average three windows per pore, which is

coherent with rhombic dodecahedra [17]. This number, however, decreases with increasing c_{CNF} . One can attribute this decrease to the higher densities of the samples (see Table 2), which, in turn, comes from the higher liquid fractions of the templating foams. Since a higher liquid fraction is connected with thicker films; breaking these films during solidification is less likely. Looking closer at the shape of the pore windows (see insets in Figure 6), one sees that the windows of CNF-free foams have smooth oval shapes, whereas the windows of foams containing 0.075 wt % CNF or 0.1 wt % CNF are roughly shaped and frayed. Such irregular shapes do not speak for a slow pore opening mechanism, but rather for a sudden breakage of the film. We have also mentioned defects in pore ordering, which can result in thicker pore walls thus also influencing the opening of the pores.

We propose two possible mechanisms to explain film breakage in the foams with the highest CNF concentrations: (i) The rupture of the film does not occur during solidification due to the enhanced mechanical strength brought about by the addition of CNF, which yields closed-cell foams. The windows observed in Figures 6 (a) and (b) are thus formed due to the high vacuum applied in the sample chamber for taking SEM pictures. The foams are thus per se closed-cell, and the opening of the pores is a consequence of the “post-treatment”. Micro-computed tomography, which is carried out in atmospheric conditions, would allow us to verify this hypothesis provided that the pore walls are thicker than the resolution of the technique. (ii) The cellulose nanofibres create internal stresses within the film that induce a folding back of the material once the film breaks. However, the mechanism of film rupture in absence of CNF being itself not well understood, it would be presumptuous to push the discussion further. Indeed, the literature lacks convincing explanations for a general film rupture mechanism during the solidification of liquid foams. Pore connectivity is an interesting topic that we have not thoroughly investigated and which remains an important gap to fill within our community.

Another peculiarity of the foams containing large amounts of CNF is the porosity of the struts (see e.g. sample C40_100 with $c_{\text{CNF}} = 0.1$ wt %). One can see struts which themselves are porous, some of which are pointed out by the red arrows in Figure 6 b). We attribute this additional porosity to the higher liquid fraction of the corresponding foam. Indeed, a foam with a high liquid fraction has larger Plateau borders. When freezing such a foam, ice crystals form in the Plateau borders—which become struts in the solid state—and leave pores when the ice crystals sublime during freeze-drying.

Figure 6: (left) SEM pictures of different solid foam samples, and (right) the pore size distribution and bubble size distributions of the corresponding templates generated from the following foaming solutions: a) C40_100, b) C40_075, c) C40_050, d) C40_000 and e) C41_000. The scale bars in the insets are 20 μm .

Table 2: Comparison of liquid and solid foam properties for foams with different CNF concentrations.

$\langle d_{\text{bubble}} \rangle$ is the average bubble size of the liquid foam and φ its liquid fraction. $\langle d_{\text{pore}} \rangle$ is the average pore size of the solid foam and ρ^* its density. $N_{\text{window}}/N_{\text{pore}}$ is the average number of windows (or interconnects) per pore in the solid foam.

sample	$\langle d_{\text{bubble}} \rangle / \mu\text{m}$	φ	$\langle d_{\text{pore}} \rangle / \mu\text{m}$	$\rho^* / \text{kg m}^{-3}$	$N_{\text{window}}/N_{\text{pore}}$
C40_100	310 ± 4	0.29	343 ± 21	16.2 ± 1.3	1.7
C40_075	299 ± 4	0.20	268 ± 28	13.0 ± 1.2	2.0
C40_050	306 ± 3	0.12	309 ± 8	13.2 ± 1.0	3.0
C40_000	303 ± 4	0.04	251 ± 25	11.9 ± 0.6	3.0
C41_000	301 ± 6	0.04	282 ± 10	11.5 ± 0.5	3.0

Foam density

The densities of the solid foams ρ^* for the different CNF concentration c_{CNF} are listed in Table 2 together with other morphological properties of the solid foams and the corresponding liquid templates. As expected, the densities of the solid foams ρ^* follow the same trend as the liquid fractions φ of their liquid counterparts: the CNF-free foams have comparable liquid fractions in the liquid state and comparable densities in the solid state. In addition, both the liquid fractions and densities increase with increasing amount of CNF. This nicely illustrates the relation between liquid and solid foams in case of foam templating: the flow properties of the liquid solution, which are highly dependent on the CNF concentration, affect the liquid fraction of the liquid template and, in turn, the density of the solid foam.

Foam mechanics

The foams were investigated in compression at 23 °C and 50% relative humidity. Table 3 summarizes the elastic moduli and yield strengths of the different samples. The modulus of the foams increases from ~118

kPa to ~175 kPa in the presence of CNF, independent of the CNF concentration. The yield strength follows a similar trend, i.e. it increases from ~7.8 kPa to ~10.5 kPa once CNF is added. For foams without CNF, the C41_000 sample has a slightly higher modulus and strength than C40_000, which is not surprising considering the higher chitosan content in the foaming solution for C41_000. It is important to note that we can discuss general trends only. Differences between the CNF-containing samples or between C41_000 and CNF-reinforced foams cannot be discussed since the values are the same if one considers the standard deviations.

Since the porosity of the present foams (> 98.8%) is much higher than that of typical biological scaffolds we will not compare them. Although a higher porosity is desirable for cell growth it also means less material available for load bearing. One possibility to assess the reinforcement effect of the CNF is to use the Gibson and Ashby scaling laws. One possibility to assess the reinforcement effect of the CNF is to use the Gibson and Ashby scaling laws. This allows calculating cell wall properties based on the relative density and on the foam structure, and removes effects due to density differences (see Eqn 1-3, we refer the reader to [1] for details). The foams in the study at hand have well-defined open-cell structures, so that the calculated values can help in the analysis of the effects of cell wall reinforcement.

Table 3: Physical properties of the solid foams: Modulus (E) and yield strength (σ) for the foam (*) and calculated for the cell wall (s).

sample	foam modulus E^* / kPa	foam yield strength σ^* / kPa	cell wall modulus E_s / GPa	cell wall yield strength σ_s / MPa	relative density ρ^*/ρ_s	porosity %
C40_100	174 ± 32	10.6 ± 0.6	1.30	28	$11.6 \cdot 10^{-3}$	98.8
C40_075	171 ± 25	10.5 ± 2.2	1.98	39	$9.3 \cdot 10^{-3}$	99.1
C40_050	174 ± 7	10.3 ± 0.7	1.96	37	$9.4 \cdot 10^{-3}$	99.1
C40_000	118 ± 11	7.8 ± 0.7	1.63	33	$8.5 \cdot 10^{-3}$	99.2
C41_000	145 ± 26	9.5 ± 0.6	2.15	42	$8.2 \cdot 10^{-3}$	99.2

The calculated cell wall modulus increases from 1.63 GPa for the C40_000 foams to 1.98 GPa for the C40_075 foams. The cell wall modulus then decreases strongly to 1.30 GPa for the foams with the highest CNF content. One can relate this effect to the foam structure. Indeed, when the CNF content becomes larger, the well-defined cellular structure of the foam is damaged, as seen in the SEM images (Figure 6 (a)). The cells have a broader size distribution, some cells are closed, and the cell walls become porous. These structural differences - i.e the loss of order, monodispersity, and connectivity - obviously lead to a weakening of the mechanical properties as is reflected in the calculations of the cell wall moduli. A reasonable speculation is that the cell wall modulus of C40_100 would be significantly higher if the ordered, monodisperse, open-cell structure could be maintained.

Surprisingly, the C41_000 foam has the highest cell wall modulus of 2.15 GPa. Following our line of thoughts, one would expect that ordering, monodispersity, and connectivity of C41_000 foams are better than those of C40_000 foams since their composition is very similar. Indeed, looking at Figure 6 one can clearly see the mentioned structural differences which support our argument. The calculated yield strength of the cell walls follows a similar trend, namely an increase from 33 to 37 and 39 MPa for the low and intermediate CNF concentration, respectively, and a decrease to 28 MPa at the highest CNF content. Here again, C41_000 exhibits the highest yield strength of 42 MPa. The calculated yield strengths of the cell walls are consistent

with those of neat chitosan films cross-linked with genipin[30,31], while our cell walls moduli are slightly greater: Nunes et al. report Young's moduli below 480 MPa, highlighting once again the well-defined structure of the foams.

In conclusion, the effect of CNF addition on the compressive mechanical properties of the cell walls is weaker than expected from theory. We speculate that this observation can be attributed to the fact that CNF addition adversely affects the cellular structure of the foams. In other words, the positive cell wall reinforcement effects are reduced by the negative effects caused by the destruction of the cellular structure. However, one has to keep in mind that adding (not too much) CNF improves the absolute foam properties, i.e the modulus and strength values are higher than those of the CNF-free systems. From an engineering perspective, the most relevant CNF-content for the set chitosan content is 0.05%: at this concentration the ordered, monodisperse, open-cell structure is preserved, while moduli and yield strengths are improved substantially.

4. Conclusion

We describe how one can use microfluidics as a tool to generate highly ordered monodisperse nanocomposite foams, using a foam templating approach developed in earlier studies. The foamed chitosan solutions contain various amounts of cellulose nanofibres which were added with the view of strengthening the resulting solid foams compared to the CNF-free counterparts. As the presence of CNF strongly affects the rheological properties of the chitosan solution, one has to adjust the microfluidic foaming procedure for each concentration of CNF. The monodisperse chitosan/CNF foams are cross-linked with genipin, yielding a foamed hydrogel which is subsequently freeze-dried to obtain monodisperse nanocomposite solid foams. Although the presence of CNF in the chitosan matrix does strengthen the pore walls to a certain degree, this improvement is not necessarily translated into the macroscopic mechanical properties of the solid foams. One may explain this phenomenon by the observation that the ordering, monodispersity, and connectivity of the CNF-containing foams decrease with increasing CNF concentration. In other words, there is a competition between two effects: the improvement of the mechanical properties of the cell walls induced by the presence of CNF, and the decrease of the mechanical properties of the foam as a result of an irregular morphology, also caused by the presence of CNF. Future work should aim at resolving this problem by optimizing the foam templating route. The solidification step is particularly critical, since the disturbed morphologies are observed

in the solid foams only and not in their liquid counterparts. The ability to produce foams with varying CNF concentrations without altering the structure of the resulting solid foam would thus help to determine the optimal CNF content for favorable reinforcement of chitosan-based materials.

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Figure Captions

Figure 1: (top) Generation of monodisperse chitosan/CNF foams using a microfluidic device with a T-junction. (bottom) Picture of the T-junction used with its relevant dimensions given in mm. The channel depth is constant over the whole chip and equal to 0.190 mm.

Figure 2: Viscosity η as a function of the shear rate $\dot{\gamma}$ for the different solutions used for the generation of the liquid foam templates. The solutions are C400_100 with $c_{\text{chitosan}} = 4.0$ wt % and $c_{\text{CNF}} = 0.1$ wt %, C40_075 with $c_{\text{chitosan}} = 4.0$ wt % and $c_{\text{CNF}} = 0.075$ wt %, C40_050 with $c_{\text{chitosan}} = 4.0$ wt % and $c_{\text{CNF}} = 0.05$ wt %, C40_000 with $c_{\text{chitosan}} = 4$ wt % and no CNF and C41_000 with $c_{\text{chitosan}} = 4.1$ wt % and no CNF. The straight line shows the shear rate in the T-junction, namely $\dot{\gamma} \sim 850 \text{ s}^{-1}$.

Figure 3: Bubble diameter d_{bubble} as a function of the gas pressure p_{gas} for the various chitosan solutions. The chitosan flow rate was set to $Q_{\text{chitosan}} = 180 \mu\text{L min}^{-1}$. The error bars correspond to the standard deviations.

Figure 4: Pictures of the liquid foam templates for each foaming solution: a) C40_100, b) C40_075, c) C40_050, d) C40_000 and e) C41_000. The scales bars are 500 μm .

Figure 5: Liquid fraction ϕ as a function of time t for the different foam templates. The cross-linker genipin was present in the foams and cross-linking occurred during the experiment. Not to bias the foam volume, we conducted the liquid fraction measurements without perfluorohexane.

Figure 6: (left) SEM pictures of different solid foam samples, and (right) the pore size distribution and bubble size distributions of the corresponding templates generated from the following foaming solutions: a) C40_100, b) C40_075, c) C40_050, d) C40_000 and e) C41_000. The scale bars in the insets are 20 μm .

Tables

Table 1: Different solutions used to generate monodisperse foams with the viscosity at the T-junction η_T , the gas pressure p_{gas} at which the foam templates were produced and the average bubble size of the foam templates $\langle d_{\text{bubble}} \rangle$.

sample	$c_{\text{chitosan}} / \text{wt } \%$	$c_{\text{CNF}} / \text{wt } \%$	$\eta_T / \text{mPa s}$	$p_{\text{gas}} / \text{mbar}$	$\langle d_{\text{bubble}} \rangle / \mu\text{m}$
C40_100	4.0	0.100	38 ± 3	1700	310 ± 4
C40_075	4.0	0.075	42 ± 3	1750	299 ± 4
C40_050	4.0	0.050	38 ± 2	1500	306 ± 3
C40_000	4.0	0.000	39 ± 3	950	303 ± 4
C41_000	4.1	0.000	56 ± 3	1300	301 ± 6

Table 2: Comparison of liquid and solid foam properties for foams with different CNF concentrations.

$\langle d_{\text{bubble}} \rangle$ is the average bubble size of the liquid foam and φ its liquid fraction. $\langle d_{\text{pore}} \rangle$ is the average pore size of the solid foam and ρ^* its density. $N_{\text{window}}/N_{\text{pore}}$ is the average number of windows (or interconnects) per pore in the solid foam.

sample	$\langle d_{\text{bubble}} \rangle / \mu\text{m}$	φ	$\langle d_{\text{pore}} \rangle / \mu\text{m}$	$\rho^* / \text{kg m}^{-3}$	$N_{\text{window}}/N_{\text{pore}}$
C40_100	310 ± 4	0.29	343 ± 21	16.2 ± 1.3	1.7
C40_075	299 ± 4	0.20	268 ± 28	13.0 ± 1.2	2.0
C40_050	306 ± 3	0.12	309 ± 8	13.2 ± 1.0	3.0
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