Carbohydrate Polymers 112 (2014) 277–283

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Preparation of aligned porous chitin nanowhisker foams by directional freeze–casting technique

Yiming Zhou[a,b], Shiyu Fu[a], Yunqiao Pu[b], Shaobo Pan[b], Arthur J. Ragauskas[b,*]

[a] State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China
[b] Institute of Paper Science and Technology, Georgia Institute of Technology, Atlanta, GA 30332, USA

ARTICLE INFO

Article history:
Received 10 October 2013
Received in revised form 23 May 2014
Accepted 24 May 2014
Available online 2 June 2014

Keywords:
Chitin foams
Directional freeze–casting
Aligned porous structures
Slurry formulation

ABSTRACT

Structured biofoams with aligned porous structures were fabricated from nanosized chitin by employing a directional freeze–casting technique. The effects of the freezing conditions and slurry formulation on nanochitin foam morphology were investigated. The morphology of obtained foams was characterized using scanning electron microscopy (SEM). It was found that the pore structure of the obtained foams was a likewise of the ice crystals formed during the directional freezing. The results indicate that directional freeze–casting protocol can significantly influence the morphological features and microstructures of the obtained biofoams which could have numerous applications, including engineered carriers, scaffolds, filters and specifically as a template for potential multi-layered composites after infusion with a second phase.

1. Introduction

During the past decade, considerable interest has been focused on porous foam biomaterials with homogeneous and well defined architectures, which have been found to have numerous benefits when applied in research fields such as tissue engineering, drug delivery, catalyst support, separation, green packaging, and automotive components (Köhnke, Lin, Elder, Theliander, & Ragauskas, 2012; Lee & Deng, 2011). Foams can have a high strength-to-weight ratio and a large porosity to store or carry other materials depending on their architecture. Therefore, it is very important to control their architectural properties such as pore size, pore morphology, and porous space arrangement.

Among the available techniques used to prepare foams, directional freeze–casting has been shown to be a versatile, easily implemented, and a promising technique. The technique yields materials with porous structures where the final porosity is a replica of the solidified solvent structure. Since the directional freeze–casting process is mainly dependent on the physical entrapment of particles or polymers between ice crystals, it does not bring impurities into the samples and further purification of products is therefore not needed. With the directional freeze–casting method, aqueous solutions are usually used to prepare porous materials in which water is an environment-friendly solvent and the use of ice crystals is green and sustainable. In addition, it has been reported that this technique makes it possible to produce materials with a wealth of pore morphologies by tailoring the freezing process (Gutiérrez, Ferrer, & del Monte, 2008; Qian & Zhang, 2011).

Recently, significant attention has been devoted to the fabrication of nanoparticle-based biofoams with oriented structures. The effects of key factors such as suspension concentration, particle size, crystal structure as well as freezing conditions on the biofoams have been studied in detail. For example, various groups have investigated the production of biofoams by using directional freeze–casting technique and most of these studies have employed nanocellulose-based raw materials (i.e., cellulose nanowhiskers or nanofibrils) (Köhnke et al., 2012; Lee & Deng, 2011; Sehaqui, Salajková, Zhou, & Berglund, 2010). To the authors' best knowledge, the potential of using chitin nanowhiskers (ChN) as raw material to design aligned foams with the directional freeze–casting technique has not reported yet.

Chitin abundantly exists in nature, particularly as a structural polysaccharide in exoskeleton of crustacean, cuticle of insects, and cell wall fungi. It is estimated that about 10^{10} to 10^{11} t of this polymer are synthesized each year and its rate of turnover in the biosphere is even greater than that of cellulose (Abdou, Nagy, & Elsabee, 2008). Chitin has a similar chemical structure to cellulose with its 2-hydroxy replaced with an acetamide group, resulting in a polymer of β(1 → 4)-2-acetamido-2-deoxy-D-glucopyranosyl

* Corresponding author. Tel.: +1 404 894 9701.
E-mail addresses: arthur.ragauskas@chemistry.gatech.edu, art.ragauskas@ipst.gatech.edu (A.J. Ragauskas).

http://dx.doi.org/10.1016/j.carbpol.2014.05.062
0144-8617/

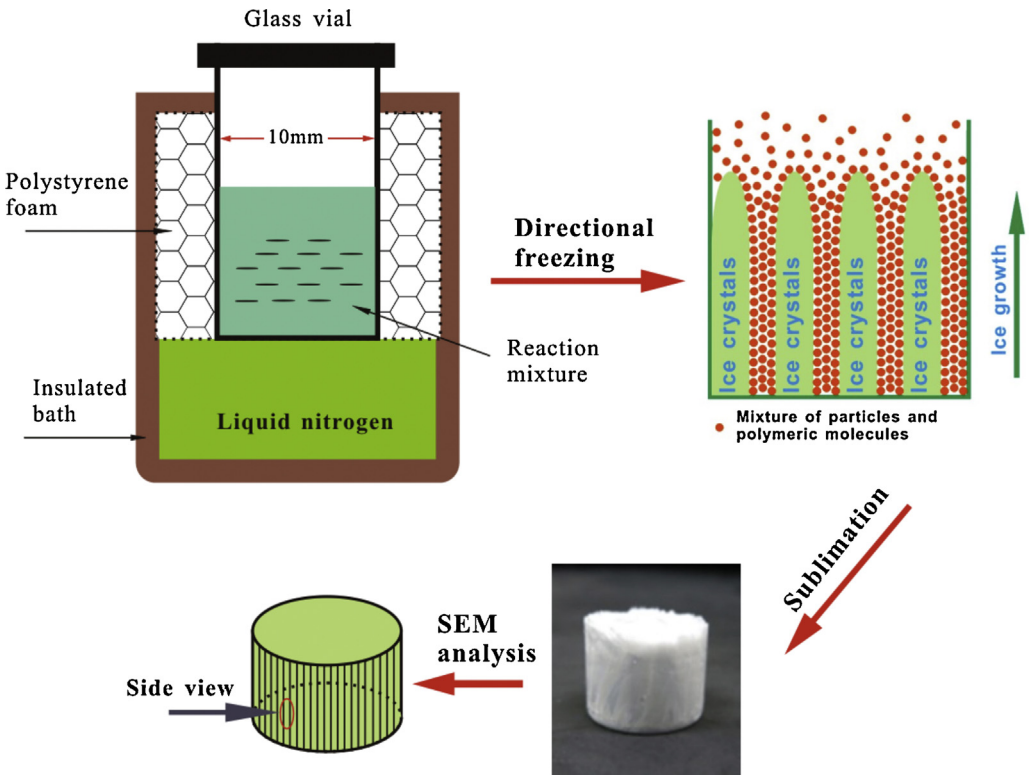

Fig. 1. Schematic representation of the directional freezing method for the preparation of ChN foams.

structural units. Chitin has been known to form microfibrillar structures in living organisms. These fibrils are usually embedded in a protein matrix and have a diameter from around 2.5 to 25 nm depending on their biological origins. Chitin nanowhiskers can be isolated from chitin containing organisms by the similar method toward preparation of cellulose nanowhisker through hydrolysis in strong acid aqueous medium. Recent studies have proposed the use of chitin nanowhiskers as building blocks (Habibi & Lucia, 2012), as catalytically active biofibers (Das et al., 2012), and as nanofillers in nanocomposites (Uddin, Fujie, Sembo, & Gotoh, 2012). Utilizing the directional freeze–casting technique, we attempted to fabricate aligned biofoam using chitin nanowhisker and investigate the effects of the freezing parameters on the obtained foam microstructures, which has not been reported so far. We expect that the use of ChN in foam formulations would be another potential application for these biodegradable, readily available fishery waste resources.

2. Experimental

2.1. Preparation of chitin nanowhiskers

Chitin sourced from shrimp shells by Aldrich was used as a starting material for chitin nanowhiskers (ChN) preparation. The purification of the chitin and preparation of the ChN is similar to previous published procedures (Morin & Dufresne, 2002). In brief, the chitin samples were first boiled and stirred in a 5% KOH solution for 6 h to remove most of the proteins. The suspension was subsequently kept at room temperature overnight under stirring, filtered, and washed several times with distilled water. The chitin powder (10.00 g) was then bleached with 17.00 g of $NaClO_2$ in 1 L of water containing 0.3 M sodium acetate buffer for 6 h at 80 °C. The bleaching solution was changed every 2 h followed by abundant washing with distilled water. After bleaching, the suspension was kept in a 5% KOH solution for 48 h to remove residual proteins. The resulting suspension was centrifuged at 5000 rpm for 15 min to obtain the protein-free chitin.

Chitin nanowhisker suspension was prepared by hydrolyzing the purified chitin sample with HCl (3.0 N, 30 mL g^{-1} of chitin) boiling for 90 min under stirring. After acid hydrolysis, the suspension was diluted with distilled water followed by centrifugation (12,000 rpm for 15 min). This process was repeated three times. The suspension was then transferred to a dialysis bag and dialyzed in running water for 6 h and then overnight in distilled water until a pH > 6 was reached. The dispersion of ChN was completed by a further 5-min ultrasonic treatment for every 30 cm^3 aliquot. The obtained ChN suspension was stored at 6 °C until used.

2.2. Preparation of porous samples

For the preparation of porous ChN samples, 2.0 wt% ChN suspension was mixed with 10.0 wt% PVA solution (dissolved in water), and the final volumes (100 mL) were made by adding water. The added amounts of ChN were calculated in order to prepare the final mixtures with constant PVA content of 1.2 wt% but containing 0.4, 0.8, and 1.2 wt% ChN, respectively. For the samples with constant ChN content (0.8 wt%), the PVA was added to prepare the final mixtures containing 0, 0.4, 1.2, 1.6 and 2.0 wt% PVA, respectively. The mixture was slowly stirred at room temperature for 4 h in order to avoid the generation of bubbles. A 10.0 mL suspension mixture was transferred into a glass vial and frozen by two methods: (i) plunging in liquid N_2 for 5 min (−196 °C); (ii) directional freezing in which only the bottom of the vial was in contact with liquid N_2, as illustrated in Fig. 1. To prevent vertical heat transfer to the ChN suspension, the vial was insulated with polystyrene foam. Frozen samples were immediately put in a freeze-dryer for 24 h to remove ice crystal templates by sublimation. The average diameter of the cylindrical samples after freeze–drying was 10 mm.

2.3. Density measurements

The foam density was calculated from the sample weight divided by the sample volume. The dimensions of the samples were measured using a digital caliper. The relative density was defined as the ratio between foam density and the density of cell wall, ρ^*/ρ_s, where ρ^* is the foam density and ρ_s is the cell wall density. The porosity was obtained from the formula of $1-[\rho^*/\rho_s]$. The cell wall density was approximated as the theoretical density of the cell-wall, ρ_t, which was calculated from the densities of the cell-wall constituents (ρ_c: ChN; ρ_p: PVA), and their respective weight fractions, W_c and W_p (Svagan, Berglund, & Jensen, 2011):

$$\rho_s \approx \rho_t = \frac{1}{\left(W_c/\rho_c + W_p/\rho_p\right)}$$

The densities, ρ_c, ρ_p, used in the calculation were 1425 and 1200 kg m^{-3} for ChN (Ding et al., 2012) and PVA (Striolo & Prausnitz, 2000), respectively.

2.4. Analytical methods

Transmission electron microscopy (TEM) observations were made with a JEM 100CX-2 electron microscope. A droplet of a dilute suspension of ChN was deposited and allowed to dry on a carbon-coated grid. The accelerating voltage was 80 kV. The morphology of the nanowhiskers was determined and averaged from five representative images that were observed to be typical over 100 TEM images. Scanning electron microscopy (SEM) was performed to investigate the morphology of porous samples with a JEOL-1530 thermally assisted field emission (TFE) instrument operated at 3 kV. Before acquiring images, the surfaces of all the samples were coated with gold in a sputter coater.

3. Results and discussion

3.1. Effect of ChN concentrations under non-directional freezing

The morphology and size of ChN in this study has been analyzed by transmission electron microscopy (TEM) (Fig. 2). The individual ChN possessed an average width of 15–20 nm and a length in the range of 150–400 nm. The ChN suspension also showed birefringence, which provided supporting evidence for the existence of rod-like ChN. In the non-directional freezing experiments, a series of suspensions (ChN concentration: 0.4–1.2 wt%, PVA content: 0.8 wt%) were directly plunged into liquid N_2 for 5 min and the ChN foams were successfully prepared without collapse and shrinkage. Under these freezing conditions, crystals can nucleate at any direction and have no preferred growth direction, which resulted in structures with completely random orientation of the honeycomb-like pores (Fig. 3a–c). For the foams made with various ChN concentrations, the pore diameter can be varied from 8.6 to 2.0 μm with the higher ChN concentration resulting in the finer microstructure. The physical properties of ChN foams are summarized in Table 1. The density of the foams prepared from non-directional freezing varied from 13.7 to 20.5 kg m^{-3}. With an increase in ChN concentration, the resulted

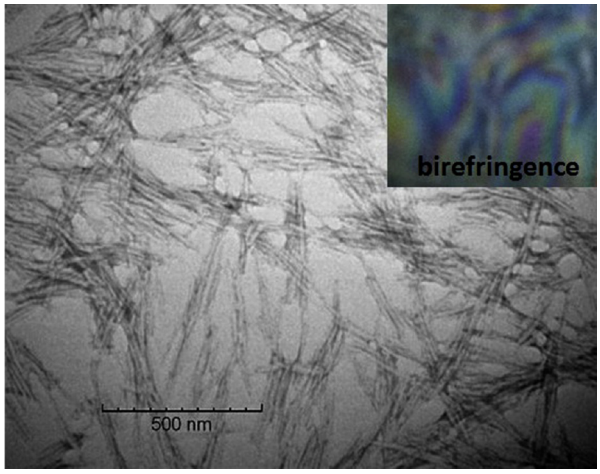

Fig. 2. TEM image of a dilute suspension of chitin nanowhiskers (inset: photograph of the suspension of chitin whiskers observed between cross nicols, showing the formation of birefringent domains.

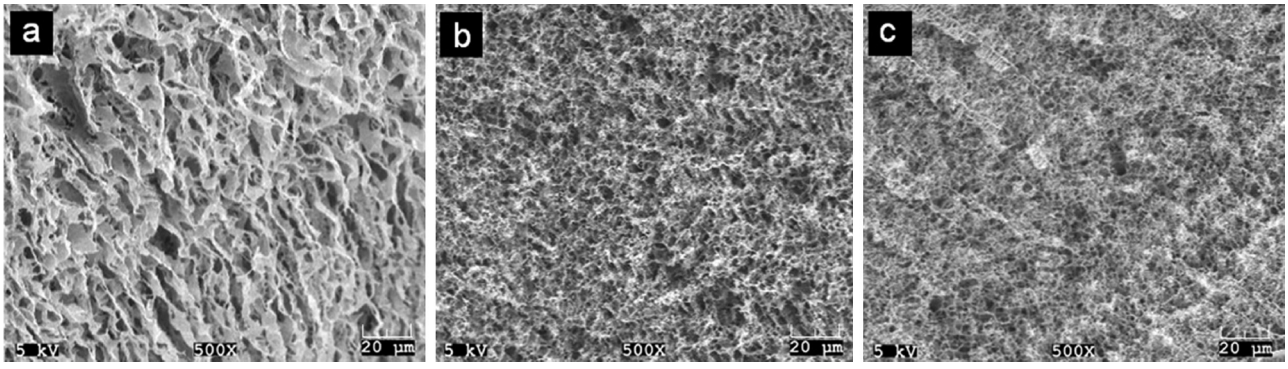

Fig. 3. SEM images of ChN foams made with various ChN suspensions under non-directional freezing. The suspension concentrations are (a) 0.4 wt%, (b) 0.8 wt%, (c) 1.2 wt%.

Table 1
Physical properties of ChN foams with varying ChN and PVA contents.

Freezing methods	Non-directional freezing			Directional freezing						
ChN content (wt%)	0.4	0.8	1.2	0.4	0.8	1.2	0.8	0.8	0.8	0.8
PVA content (wt%)	1.2	1.2	1.2	1.2	1.2	1.2	0	0.4	1.6	2.0
Density (kg m^{-3})	13.7 ± 2.3	17.1 ± 3.2	20.5 ± 2.9	14.5 ± 1.7	18.2 ± 3.4	22.1 ± 2.8	6.8 ± 1.0	10.3 ± 2.1	22.4 ± 4.3	23.9 ± 3.9
Relative density[a] (%)	1.10	1.33	1.57	1.16	1.42	17.0	0.48	0.77	1.77	1.90
Porosity (%)	98.9	98.7	98.4	98.8	98.6	98.3	99.5	99.2	98.2	98.1

[a] Calculated using 1425 kg m^{-3} for chitin and 1200 kg m^{-3} for PVA as references.

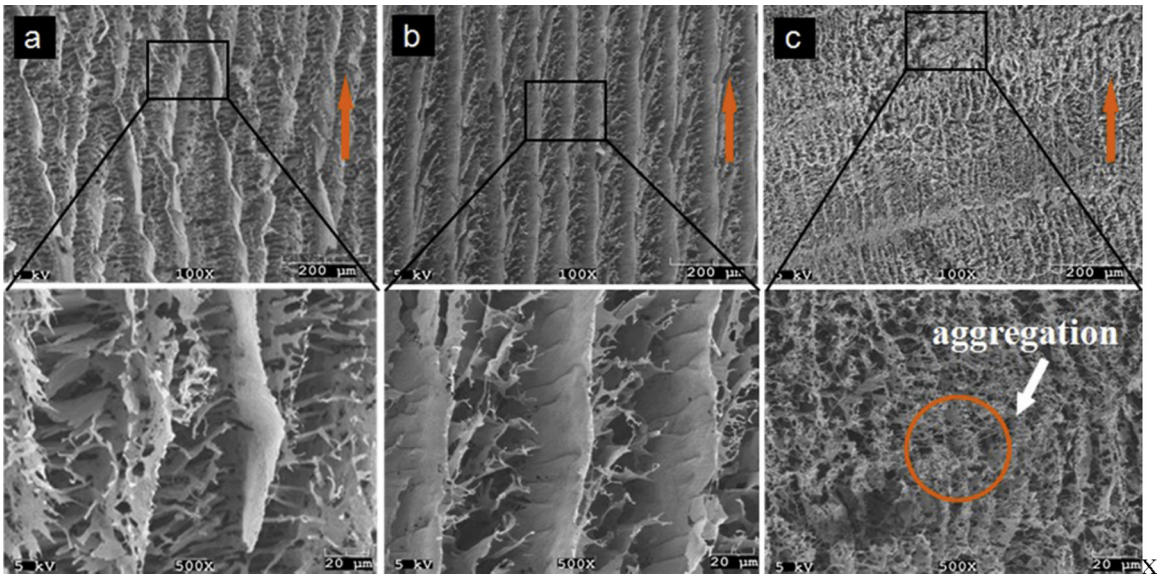

Fig. 4. SEM images of ChN foams made with the various ChN suspensions under directional freezing. The suspension concentrations are (a) 0.4 wt%, (b) 0.8 wt%, (c) 1.2 wt%. Red color arrows indicate the freezing direction. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ChN foam had an increased density. The porosity of non-directional freezing ChN forms appeared comparable in the range of 98.9 to 98.4%.

3.2. Effect of ChN concentrations under directional freezing

Hexagonal water ice crystals exhibit strong anisotropic growth kinetics, varying over about two orders of magnitude with crystallographic orientation (Petrenko & Whitworth, 2002). The freezing process is easier for crystals whose preferred growth directions are parallel to the temperature gradient, and consequently the pores can be oriented depending on the solidification conditions used (Li, Lu, & Walz, 2012). If only the bottom of the vial containing the solution is in contact with liquid N_2 (as illustrated in Fig. 1), the solvent crystals are induced to grow vertically, along the direction of the imposed thermal gradient. Under these conditions, more homogeneous crystal nucleation can be realized, leading to an oriented and continuous channel architecture as clearly shown in Fig. 4. The lamellae with a thickness of a couple of micrometers were observed with a separating space of several dozens of micrometers. As can be seen in Table 1, the directional freezing results in a slight increase in the density of the ChN foam with the increased ChN concentration, while the porosity remains similar.

The change in physical properties was also clearly reflected in the pore morphology of the ChN foams, as shown in Fig. 4 (ChN concentration: 0.4–1.2 wt%, PVA content: 0.8 wt%). For the ChN foams prepared from the suspension at a concentration of 0.4 wt%, the lamellar structured foam was aligned thin membrane layers with inter-spaces about 60–80 μm (Fig. 4a). The lamellar channel showed a tortuous path along the freezing direction, and significant structural defects, rough surface and large size cracks were found on the lamellar wall, as shown in the images at higher magnification. For foams made with 0.8 wt% ChN suspensions (Fig. 4b), the membrane layers were created in parallel to each other and presented a well oriented lamellar architectures and homogeneous character throughout the entire layers. No apparent defects, such as pores, are observed on the lamellar walls. This is a typical phenomenon observed under steady-state freezing conditions where water is being used as a solvent (Dash, Li, & Ragauskas, 2012). However, at the high concentration level (i.e., 1.2 wt%), a more dense structure started to emerge instead of sheet-like structure by sublimating ice molecules, and the aggregations of nanowhiskers were formed in the foams (Fig. 4c).

The mechanism of structure morphology formation can be well understood through the basic physics of water freezing which has been already explained in literature (Butler, 2002; Deville, Saiz, Nalla, & Tomsia, 2006). The primary ice-template structure is dependent on the destabilizing solute interfacial concentration gradient and the surface energy that opposes cell formation. Thus the solute concentration of the slurry is a particularly important processing parameter for controlling foam morphology and must be carefully optimized in the directional freeze–casting process.

3.3. Effect of PVA content under directional freezing

Slurry properties can also be modified by adding additives to achieve needed effects. Additives can be desirable to modify the morphology (through the shape of the solvent crystals) or the interaction between the particles and the solidification front (Deville, 2008). In this study, polyvinyl alcohol (PVA) was used as an organic additives and the ChN concentration of the initial suspension was 0.8 wt%. The effect of PVA content (0–2.0 wt%) on the pore morphology was investigated as shown in Fig. 5. All five samples showed highly ordered lamellar channel structures. The PVA content in the suspension was observed to affect the morphology of ChN foams, as the inter-lamellae spaces varying from 180 μm to 30 μm decreased with an increasing content of the PVA additives. In general, the higher the PVA content in the starting slurry, the denser the resulting microstructure. This can also be seen in Table 1, the directional freezing ChN foams have an increase in the density from 6.84 to 23.9 kg m^{-3} when PVA content increases from 0 to 2.0%.

Furthermore, the surface of the formed lamellae exhibited a particular topography with dendritic-like features running perpendicular to the ice front. It has been proposed that the dendritic-like features are homogeneous in size and distribution, but their relative morphology varies with the nature of the solvent, the freezing conditions, and the characteristics of the starting nanoparticles (Deville, 2008; Deville, Saiz, & Tomsia, 2007). In the particular case

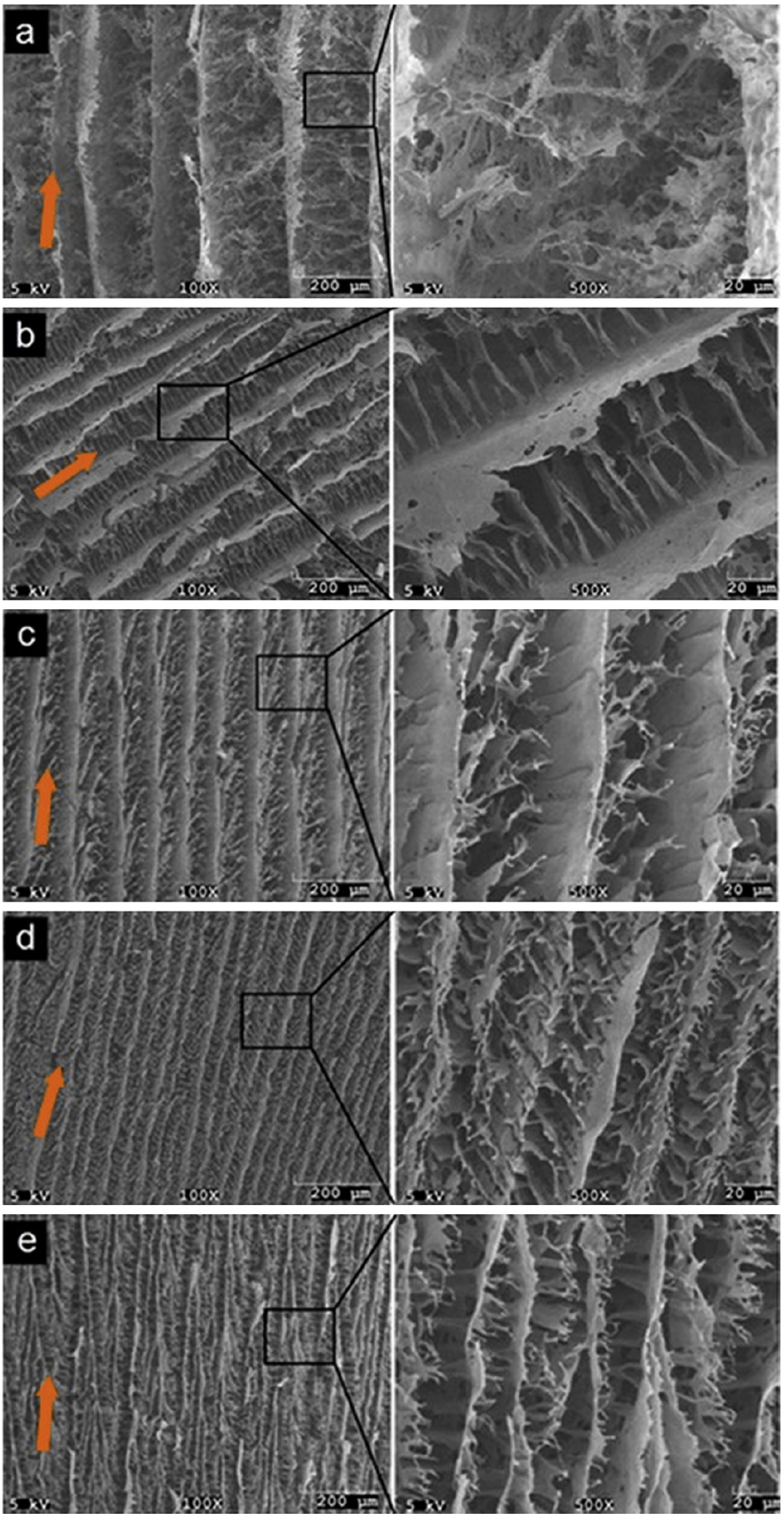

Fig. 5. SEM images of ChN foams made with various PVA contents under directional freezing. The ChN concentration of the initial suspension is 0.8 wt%. The PVA contents are (a) 0 wt%, (b) 0.4 wt%, (c) 1.2 wt%, (d) 1.6 wt%, (e) 2.0 wt%. Red color arrows indicate the freezing direction. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of ChN suspension without PVA, the microstructure between two adjacent lamellae is completely random with irregular morphology fine features protruding from the walls (Fig. 5a). The addition of PVA to the ChN suspension results in quite distinct microstructure from the former. As shown in Fig. 5b–e, a transition from overgrown dendrites that eventually bridge the inter-lamellae gap to a 'fish-bone' dendrite structures occurs gradually as the content of PVA in the suspensions is increased up to 2.0 wt%. This result suggests that the higher the PVA content, the shorter the dendrite structures. The 'fish-bone' morphology in the PVA-added foams results from the side branches that form when PVA freeze-concentrates around the primary ice cells, causing secondary instability formation perpendicular to the freezing direction. The fact that 'fish-bone' structures can be prepared from pure PVA solutions supports this observation (Zhang et al., 2005). The detailed formation mechanism of nanoparticle suspension dendrites is not yet clear. A possible mechanism is based on the splitting and subsequent healing of the ice crystal tip. In the case that the ice front pushes and transports the nanoparticles, a liquid film of sufficient thickness is needed between the ice front and the nanoparticles. If the ice front velocity is too high or the movement speed of the particles is too slow, the liquid film disappears and the nanoparticles are embedded inside the ice crystals (tip splitting) instead of being entrapped between ice crystals. After that, subsequent tip healing occurs to form the bridge structure (Korber, Rau, Cosman, & Cravalho, 1985). In this work, the added PVA likely has a major influence on the properties of the ChN suspension, such as viscosity and surface tension, and modify the supercooling effects, thus producing changes in the critical velocity for particles entrapment during directional freezing. Considering these facts, it is plausible to assume that the addition of PVA in the suspension can be desirable to modify the pore morphology of the ChN foams.

3.4. Comparison between chitin nanowhisker foams vs. cellulose nanowhisker foams

It is well known from the literature that cellulose nanowhiskers (CNWs) represent another source of nano-dimensional sized polysaccharide structures (Dash, Foston, & Ragauskas, 2013). CNWs are typically of 5–20 nm in width, 100–300 nm in length and reported to have a modulus of about 140 GPa (Köhnke et al., 2012; Zeng, He, Li, & Wang, 2013). In this study, CNW foams were also prepared in order to investigate the differences between the two nanowhisker systems (nanowhisker concentration: 0.8 wt%, PVA content: 1.2 wt%).

Under non-directional freezing, the resulting orientation over the side parallel to the ice front was completely random for both foams (ChN foams: Fig. 3b; CNW foams: Electronic Supplementary Information (ESI), Fig. S1). However, ChN foams present a more dense structure than CNW foams. The pore size of ChN foams was much smaller than CNW foams (pore diameter: 2–3 μm vs. 4–8 μm). For the foams prepared with directional freezing, SEM analysis indicated that honeycomb-like pore morphologies were found on the lamellar channel wall of the CNW foams (ESI, Fig. S2). While the ChN foams (Fig. 4b) apparently exhibited a lamellar wall that was much thinner and had more fine tortuous dendrites as compared to CNW foams. The difference in morphological structures may be attributed to the inherent properties of each whiskers and the interaction between the whiskers and the solvent, which can have an influence on the mechanical properties of the final material. Further exploration of these two systems and how they behave during directional freezing is a field of research needing further study.

4. Conclusions

In summary, novel biofoams have been prepared from the readily available biopolymer chitin nanowhiskers by employing a directional freeze–casting technique. The orientation of pore channels in the biofoams can be controlled by modifying the freezing conditions and the slurry formulation (nanoparticle concentration and additive content), which has potential applications especially as a template for multi-layered composites, liquid or gas filters, delivery carriers, and tissue engineering scaffold.

Acknowledgments

Y.M. Zhou is grateful to China Scholarship Council for awarding a scholarship under the State Scholarship Fund to pursue her study. This work will be used by Y.M. Zhou for partial fulfillment of the degree requirement for her doctoral research in the State Key Laboratory of Pulp and Paper Engineering at South China University of Technology, China. This work was partially supported by USDA Forest Service R&D special funding on Cellulose Nano-Materials (2012).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.05.062.

References

Abdou, E. S., Nagy, K. S. A., & Elsabee, M. Z. (2008). Extraction and characterization of chitin and chitosan from local sources. *Bioresource Technology*, *99*, 1359–1367.
Butler, M. F. (2002). Growth of solutal ice dendrites studied by optical interferometry. *Crystal Growth and Design*, *2*, 59–66.
Dash, R., Li, Y., & Ragauskas, A. J. (2012). Cellulose nanowhisker foams by freeze casting. *Carbohydrate Polymers*, *88*, 789–792.
Das, P., Heuser, T., Wolf, A., Zhu, B. L., Demco, D. E., Ifuku, S., & Walther, A. (2012). Tough and catalytically active hybrid biofibers wet-spun from nanochitin hydrogels. *Biomacromolecules*, *13*, 4205–4212.
Dash, R., Foston, M., & Ragauskas, A. J. (2013). Improving the mechanical and thermal properties of gelatin hydrogels cross-linked by cellulose nanowhiskers. *Carbohydrate Polymers*, *91*, 638–645.
Deville, S. (2008). Freeze–casting of porous ceramics: A review of current achievements and issues. *Advanced Engineering Materials*, *10*, 155–169.
Deville, S., Saiz, E., Nalla, R. K., & Tomsia, A. P. (2006). Freezing as a path to build complex composites. *Science*, *311*, 515–518.
Deville, S., Saiz, E., & Tomsia, A. P. (2007). Ice-templated porous alumina structures. *Acta Materialia*, *55*, 1965–1974.
Ding, B. B., Cai, J., Huang, J. C., Zhang, L. N., Chen, Y., Shi, X. W., Du, Y. M., & Kuga, A. (2012). Facile preparation of robust and biocompatible chitin aerogels. *Journal of Materials Chemistry*, *22*, 5801–5809.
Gutiérrez, M. C., Ferrer, M. L., & del Monte, F. (2008). Ice-templated materials: Sophisticated structures exhibiting enhanced functionalities obtained after unidirectional freezing and ice-segregation-induced self-assembly. *Chemistry of Materials*, *20*, 634–648.
Habibi, Y., & Lucia, L. A. (2012). *Chitin nanofibers as building blocks for advanced materials*. Hoboken, NJ: John Wiley & Sons Press.
Köhnke, T., Lin, A., Elder, T., Theliander, H., & Ragauskas, A. J. (2012). Nanoreinforced xylan–cellulose composite foams by freeze–casting. *Green Chemistry*, *14*, 1864–1869.
Korber, C., Rau, G., Cosman, M. D., & Cravalho, E. G. (1985). Interaction of particles and a moving ice–liquid interface. *Journal of Crystal Growth*, *72*, 649–662.
Lee, J., & Deng, Y. L. (2011). The morphology and mechanical properties of layer structured cellulose microfibril foams from ice-templating methods. *Soft Matter*, *7*, 6034–6040.
Li, W. L., Lu, K., & Walz, J. Y. (2012). Freeze casting of porous materials: Review of critical factors in microstructure evolution. *International Materials Reviews*, *57*, 37–60.
Morin, A., & Dufresne, A. (2002). Nanocomposites of chitin whiskers from riftia tubes and poly(caprolactone). *Macromolecules*, *35*, 2190–2199.
Petrenko, V. F., & Whitworth, R. W. (2002). *Physics of ice*. New York, NY: Oxford University Press.
Qian, L., & Zhang, H. F. (2011). Controlled freezing and freeze drying: A versatile route for porous and micro-/nano-structured materials. *Journal of Chemical Technology and Biotechnology*, *86*, 172–184.

Sehaqui, H., Salajková, M., Zhou, Q., & Berglund, L. A. (2010). Mechanical performance tailoring of tough ultra-high porosity foams prepared from cellulose I nanofiber suspensions. *Soft Matter*, *6*, 1824–1832.

Striolo, A., & Prausnitz, J. M. (2000). Vapor–liquid equilibria for some concentrated aqueous polymer solutions. *Polymer*, *41*, 1109–1117.

Svagan, A. J., Berglund, L. A., & Jensen, P. (2011). Cellulose nanocomposite biopolymer foam—Hierarchical structure effects on energy absorption. *ACS Applied Materials and Interfaces*, *3*, 1411–1417.

Uddin, A. J., Fujie, M., Sembo, S., & Gotoh, Y. (2012). Outstanding reinforcing effect of highly oriented chitin whiskers in PVA nanocomposites. *Carbohydrate Polymers*, *87*, 799–805.

Zeng, J. B., He, Y. S., Li, S. L., & Wang, Y. Z. (2013). Chitin whiskers: An overview. *Biomacromolecules*, *13*, 1–11.

Zhang, H. F., Hussain, I., Brust, M., Butler, M. F., Rannard, S. P., & Cooper, A. I. (2005). Aligned two- and three-dimensional structures by directional freezing of polymers and nanoparticles. *Nature Materials*, *4*, 787–793.