

## Video Article

# Microhoneycomb Monoliths Prepared by the Unidirectional Freeze-drying of Cellulose Nanofiber Based Sols: Method and Extensions

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## Abstract

Monolithic honeycomb structures have been attractive to multidisciplinary fields due to their high strength-to-weight ratio. Particularly, microhoneycomb monoliths (MHMs) with micrometer-scale channels are expected as efficient platforms for reactions and separations because of their large surface areas. Up to now, MHMs have been prepared by a unidirectional freeze-drying (UDF) method only from very limited precursors. Herein, we report a protocol from which a series of MHMs consisting of different components can be obtained. Recently, we found that cellulose nanofibers function as a distinct structure-directing agent towards the formation of MHMs through the UDF process. By mixing the cellulose nanofibers with water soluble substances which do not yield MHMs, a variety of composite MHMs can be prepared. This significantly enriches the chemical constitution of MHMs towards versatile applications.

## Video Link

The video component of this article can be found at <https://www.jove.com/video/57144/>

## Introduction

As a brand-new material, microhoneycomb monolith (denoted MHM) has recently attracted tremendous attention from multidisciplinary fields<sup>1,2,3,4,5,6,7,8</sup>. The MHM was first prepared by S. Mukai *et al.* through a modified unidirectional freeze-drying (UDF) approach as a monolith with an array of straight microchannels with honeycomb-like cross sections<sup>9</sup>. MHM possesses the general advantages of honeycomb structures, *i.e.*, efficient tessellation, high strength-to-weight ratio, and low pressure drop. Moreover, compared with the honeycomb monolith with a larger channel size, the MHM has a much larger specific surface area. The UDF method involves the unidirectional growth of ice crystals and simultaneous phase separation upon freezing. After the removal of the ice crystals, a solid component molded by the ice crystal is obtained. The morphology formed upon the phase separation depends on the intrinsic nature of the precursor (sol or gel), and in most of cases, lamella<sup>10</sup>, fiber<sup>11</sup>, and fishbone<sup>12</sup> structures are likely to form rather than the MHMs. As a result, the formation of MHMs has been reported only in limited precursors, and this has significantly hampered the diversity of their chemical property. We have recently found that cellulose nanofibers have a strong structure-directing function toward forming the MHM structure through the UDF process<sup>13</sup>. Simply by mixing the cellulose nanofibers with other water-dispersible components, it is possible to prepare a variety of MHMs with different chemical properties. Moreover, their exterior shapes and channel sizes are flexibly and easily controlled<sup>13</sup>. Thus, MHMs are expected to be used as filters, catalyst supports, flow-type electrodes, sensors and scaffolds for biomaterials.

In this paper, we first explain the basic preparation technique of MHMs from the aqueous dispersion of cellulose nanofibers through the UDF process in detail. Moreover, we demonstrate the preparation of several different types of composite MHMs.

## Protocol

### 1. Preparation of 1 wt% 2,2,6,6-Tetramethylpiperidin-1-oxyl (TEMPO)-mediated Oxidized Cellulose Nanofiber (TOCN) Sol

NOTE: The sol is defined as a colloidal suspension of very small solid particles in a continuous liquid medium.

1. Suspend 66.7 g of Nadelholz Bleached Kraft Pulp (NBKP, containing 12 g of cellulose) in 700 mL of deionized (DI) water with a mechanical agitator at 300 rpm for 20 min.
2. Add 20 mL of aqueous TEMPO solution (containing 0.15 g of TEMPO) and 20 mL of aqueous NaBr solution (containing 1.5 g of NaBr) slowly to the above NBKP suspension<sup>14,15</sup>.
3. Adjust the pH of the above suspension to approximately 10.5 (measured with a pH meter) with slowly adding 3.0 M NaOH solution.
4. Slowly add approximately 63.8 g of aqueous NaClO solution (with 6-14 wt% active chlorine) with a pipette to the above mixture to start TEMPO-mediated oxidation.
5. While adding NaClO, continue adding the NaOH solution to keep the pH of the system within the range of 10.0 to ~10.5. This process takes about 2.5 h.
6. Rinse the TEMPO-mediated oxidized cellulose fibers with DI water 3 times (1,200 mL of DI water each time) to remove the residual NaClO, NaOH and other chemicals.
7. Treat the paste with a powerful mechanical blender to disintegrate the oxidized cellulose fibers into nanofibers. Carry out the mechanical treatment carefully several times accompanied with an addition of equal amount of water. Finally, a 1 wt% TEMPO-mediated oxidized cellulose nanofiber (TOCN) sol is obtained. The TOCNs have a diameter of 4 to ~6 nm, and a length of 0.5 to ~2  $\mu\text{m}$ .
8. Store the 1 wt% TOCN sol at 4 °C (cellulose nanofibers tend to rot at an ambient temperature).

### 2. Preparation of TOCN-styrene Butadiene Rubber (SBR) Mixed Sol

1. Add 0.21 g of SBR colloid (48.5 wt%) into 10 g of 1 wt% TOCN sol (step 11.7.) in a 20 mL glass vessel.
2. Agitate the above mixture for 3 min with a vortex mixer at the power level of 6 to achieve an evenly dispersed sol. Store the above mixture sol at 4 °C before use.

### 3. Preparation of TOCN-TiO<sub>2</sub> Mixed Sol

1. Add 0.1 g of TiO<sub>2</sub> nanoparticles (with an average particle size of 20 nm) into 10 g of 1 wt% TOCN sol in a 20 mL glass vessel.
2. Agitate the above mixture with a homogenizer for 10 min to achieve an evenly mixed sol. Perform this 10 min-process intermittently, since a significant amount of heat is generated in the process and results in the rise of temperature, which may degrade TOCNs. Store the mixture sol at 4 °C before use.

### 4. Preparation of TOCN-surface Oxidized Carbon Fiber (SOCF) Mixed Sols

1. Reflux 1.7 g of carbon fiber (300 mesh, with a diameter of 5.5 to ~6.0  $\mu\text{m}$  and a length of approximately 50  $\mu\text{m}$ ) in 150 mL of concentrated nitric acid at 60 °C for 6 h to achieve SOCF<sup>16</sup>. Add 0.01 g of the above SOCF into 10 g of 1 wt% TOCN sol inside a 20 mL glass vessel.
2. Shake-mix the above mixture, and ultra-sonicate the mixture for 5 min to achieve an evenly mixed sol. Store the mixture sol at 4 °C before use.

### 5. Preparation of Microhoneycomb Monolith from the 1 wt% TOCN Sol (Denoted MHM-TOCN)

1. Load a polypropylene (PP) tube (with an inner diameter of 13 mm, an outer diameter of 15 mm, and a length of 150 mm) with glass beads and fill the bottom 5 cm part of the tube<sup>13</sup>.
2. Load a certain amount (the amount is not controlled for each time, but it is normally larger than 2.7 mL to ensure the subsequent cutting process) of the 1 wt% TOCN sol into the above PP tube containing glass beads.  
NOTE: TOCN sol was directly filled into the PP tube without pouring in glass beads for the study of the distance effect that has been involved in the unidirectional freezing process. In this case, the amount of TOCN sol was 11 mL.
3. Carefully remove the bubbles that may have been generated during the sol loading. Keep the PP tube containing the TOCN sol at 4 °C overnight before use.
4. Attach the above PP tube containing the TOCN sol to the dipping machine that is used for unidirectional freezing. Set the relevant parameters and start dipping the PP tube into a thermo jug containing liquid nitrogen (-196 °C) at a constant speed of 50 cm h<sup>-1</sup> (Figure 1).
5. Cut the PP tube part with a saw, and crack the frozen TOCN sol part into several sections. Freeze dry these sections with a freeze-drying machine at -10 °C for 1 day, then at -5 °C for 1 day, and finally at 0 °C for 1 day. The MHM-TOCN was obtained as white-colored monoliths (Figure 1).

## 6. Preparation of Microhoneycomb Monolith from the TOCN-SBR Mixed Sol (Denoted MHM-TOCN/SBR) and TOCN-TiO<sub>2</sub> Mixed Sol (Denoted MHM-TOCN/TiO<sub>2</sub>)

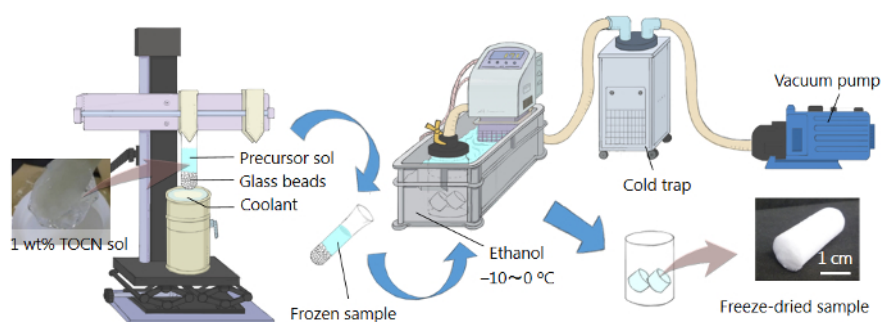
1. Load polypropylene (PP) tubes (with an inner diameter of 13 mm, an outer diameter of 15 mm, and a length of 150 mm) with glass beads, filling the bottom 5 cm part of the tubes.  
NOTE: The glass beads are used to cover the area where unsteady ice crystal growth occurs, to achieve uniform morphology of the resulting sample. Both the size and surface property of the glass beads do not affect the morphology of the resulting sample.
2. Load certain amount (the amount is not controlled for each time, but it is normally larger than 2.7 mL to ensure the subsequent cutting process) of the TOCN-SBR mixed sol or TOCN-TiO<sub>2</sub> mixed sol into the PP tubes containing glass beads.
3. Carefully remove the bubbles that may have been generated during the sol loading. Keep the PP tubes containing the above mixed sol at 4 °C overnight before use.
4. Attach the PP tubes containing the above mixed sols to the dipping machine that is used for unidirectional freezing. Set the relevant parameters and start dipping the PP tube into a tank containing liquid nitrogen (-196 °C) at a constant speed of 20 cm h<sup>-1</sup>.
5. Cut the PP tube part with a saw, and crack the frozen TOCN-SBR mixed sol part into several sections.
6. Freeze dry these sections with a freeze-drying machine at -10 °C for 1 day, then at -5 °C for 1 day, and finally at 0 °C for 1 day. The MHM-TOCN/SBR and MHM-TOCN/TiO<sub>2</sub> were obtained as white monoliths.

## 7. Preparation of Microhoneycomb Monolith from the TOCN-SOCF Mixed Sol (Denoted MHM-TOCN/SOCF)

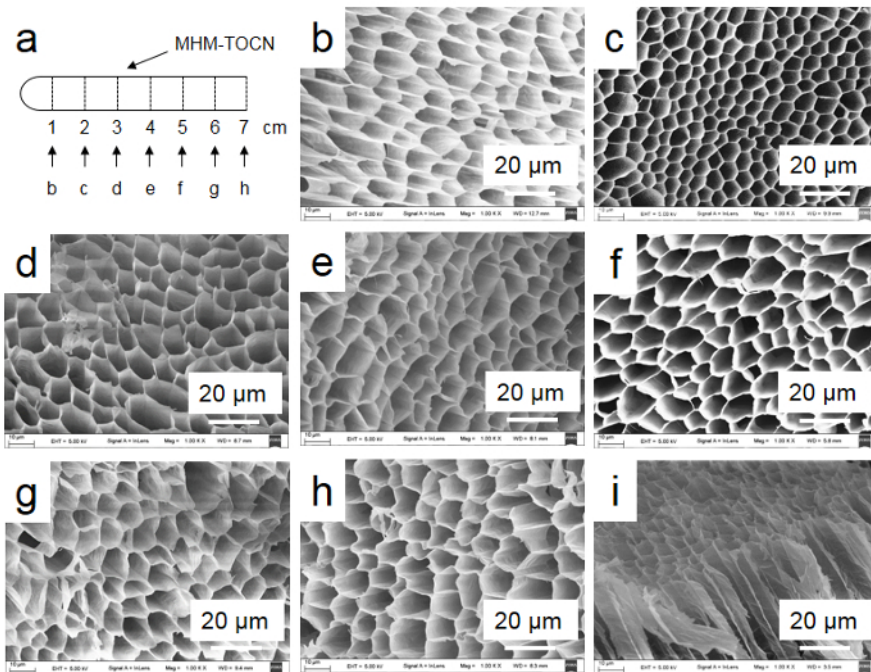
1. Load a polypropylene (PP) tube (with an inner diameter of 13 mm and an outer diameter of 15 mm, and a length of 150 mm) with glass beads, filling the bottom 5 cm part of the tube.
2. Load certain amount (the amount is not controlled for each time, but it is normally larger than 2.7 mL to ensure the subsequent cutting process) of the TOCN-SOCF mixed sol into the above PP tube containing glass beads.
3. Carefully remove the bubbles that may have been generated during the sol loading. Keep the PP tube containing the above mixed sol at 4 °C overnight before use.
4. Attach the PP tube containing the above mixed sol to the dipping machine that is used for unidirectional freezing. Set the relevant parameters and start dipping the PP tube into a tank containing liquid nitrogen (-196 °C) at a constant speed of 20 cm h<sup>-1</sup>.
5. Cut the PP tube part with a saw, and crack the frozen TOCN-SOCF sol part into several sections. Freeze dry these sections with a freeze-drying machine at -10 °C for 1 day, then at -5 °C for 1 day, and finally at 0 °C for 1 day. The MHM-TOCN/SOCF was obtained as a white-grey monolith.

## Representative Results

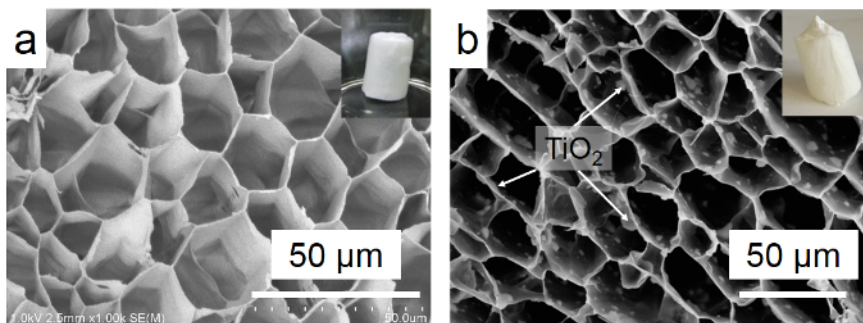
The morphologies for different positions of the MHM-TOCN along the direction of unidirectional freezing are investigated and shown in **Figure 2**. With the position being further away from the bottom part of the MHM-TOCN, a gradual morphology change was revealed (**Figure 2, Discussion**). By introducing a second component into the TOCN sol to form a homogeneous mixture sol, it is possible to prepare various kinds of composite MHMs. For example, composite MHMs including SBR (**Figure 3a**), TiO<sub>2</sub> (**Figure 3b**), or even carbon fiber (**Figure 4**) are prepared.



**Figure 1: Schematic of the preparation of MHM-TOCN by unidirectional freeze-drying approach.** The unidirectional freezing is performed with a dipping machine shown in the left. After unidirectional freezing, freeze-drying was carried out with a freeze-drier to yield the MHM-TOCN. This figure has been modified from Pan, Z.-Z. *et al.*<sup>13</sup>. [Please click here to view a larger version of this figure.](#)

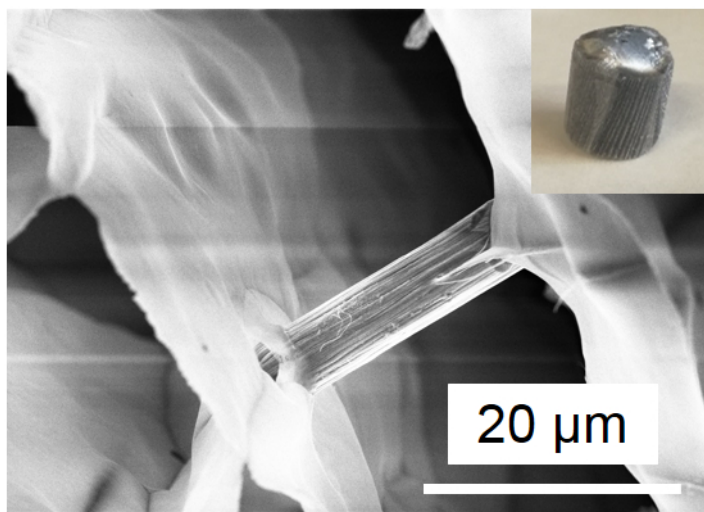


**Figure 2: Morphological characterizations of the different positions of MHM-TOCN.** (a) Schematic with marks labeling the different positions of MHM-TOCN. (b-h) SEM images of the cross section of the MHM-TOCN with a distance to the bottom (the tip) of the MHM-TOCN of 1, 2, 3, 4, 5, 6, 7 cm, respectively. (i) SEM image of the longitudinal section of the MHM-TOCN. Note that in a typical UDF experiment, glass beads are always used to fill the bottom 5 cm part of the PP tube before loading the sol for the unidirectional freezing, so that pseudo-steady growing of ice crystals can be achieved. However, here, TOCN sol was directly filled into the PP tube without pouring in glass beads first to study the distance effect that has involved in the unidirectional freezing process. [Please click here to view a larger version of this figure.](#)



**Figure 3: Morphological characterizations on two MHM composites.** (a and b) show the cross sectional SEM images of (a) MHM-TOCN/SBR and (b) MHM-TOCN/TiO<sub>2</sub>, respectively. The upper-right insets within (a) and (b) are optical images of MHM-TOCN/SBR and MHM-TOCN/TiO<sub>2</sub>, respectively. [Please click here to view a larger version of this figure.](#)





**Figure 4:** SEM image of MHM-TOCN/SOCF. The image shows the novel structure with SOCF connecting the neighboring microhoneycomb walls, and the inset within the image is an optical image of MHM-TOCN/SOCF.

## Discussion

The most critical step for achieving the MHMs is the unidirectional freezing step, during which water solidifies to form columnar ice crystals and push the dispersoid aside to form the framework. The unidirectional freezing process basically involves thermal transfer between the precursor sol and the coolant. In our setup, a dipping machine was used to insert a PP tube containing a precursor sol into the coolant (liquid nitrogen) with a constant velocity. Since liquid nitrogen keeps evaporating all the time, a fluctuant temperature gradient is generated above the nitrogen liquid level. Before touching the nitrogen liquid level, the PP tube has inevitably experienced heat exchange with the cold air above the nitrogen liquid level, which has caused the temperature fluctuation of the bottom part of the PP tube. Further, upon touching the nitrogen liquid level, the temperature of the bottom part of the PP tube immediately dropped to a temperature close to that of the liquid nitrogen ( $-196\text{ }^{\circ}\text{C}$ ), and the adjacent part also quickly cooled down to the temperature of liquid nitrogen. It was not until a certain position that the pseudo-steady heat transfer that is related to the unidirectional freezing started taking place. After freezing, the PP tube was cracked off into sections for freeze-drying. The sections were immediately transferred to a cold well as ice tends to thaw, which would cause morphology deterioration of the resulting samples. In addition, the freeze-drying process was carefully carried out at a temperature that is below  $0\text{ }^{\circ}\text{C}$  to avoid the thawing of ice. We observed different positions of a MHM-TOCN from above its bottom, as are depicted in **Figure 2a**. Positions (b-h) that are 1-7 cm away from the bottom were observed with SEM, as are shown in **Figure 2b-h**. The position (b) that is 1 cm above from the bottom has an oriented morphology toward the center of the bulk (**Figure 2b**). This is similar to that of the monolith prepared from plunge-freezing<sup>13</sup>, which involves the dominant heat exchange along the basal plane. From the position that is 2 cm away from the bottom, a well aligned honeycomb-like morphology was obtained (**Figure 2c-h**), showing the unidirectional growth of ice crystals along the length direction of the PP tube. It should be mentioned that the size of the microhoneycomb experienced an obvious increase from position (c) to (d), and kept steady thereafter. This is ascribed to the distance effect, which in such a lower position as position (c), a higher temperature gradient and a higher growing velocity of the ice crystals<sup>17</sup> were involved, thus leading to smaller ice crystals. However, at higher positions such as position (d), the distance effect no longer applied, and the temperature gradient became relatively stable, thus leading to a steady channel size of  $10\text{ }\mu\text{m}$ . The channel size of the MHM-TOCN would alter in accordance with the dipping speed of the PP tube, but the microhoneycomb morphology retained<sup>13</sup>. The channel size could be tuned within a range of 10 to  $\sim 200\text{ }\mu\text{m}$ <sup>13</sup>, and either a larger or a smaller channel size could only be achieved with special design. **Figure 2i** gives the morphology of the MHM-TOCN along the longitudinal section, showing the unidirectionally penetrating nature of the MHM-TOCN. This differs significantly from the 3-dimensional porous structures that were obtained from the refrigerator-freezing<sup>18</sup> or liquid nitrogen quenching<sup>19</sup>.

The greatest advantage of our methodology is its versatility in controlling the composition of the resulting monolith. We found that TOCNs have a strong tendency toward forming the MHM structure via the UDF process. Simply by preparing a variety of mixture sol, a series of composite MHMs can be achieved. We have shown many examples in our previous report<sup>13</sup>. A typical example is the combination with a water-soluble polymer, and we present another example-SBR here, as shown in **Figure 3a**. These types of composite MHMs have a smooth microhoneycomb wall, showing a homogeneous distribution of the included components. Further, we have confirmed that the MHM-TOCN could be used as a supporter for nanoparticles, as shown in **Figure 3b**. A mixture precursor sol of TOCNs and  $\text{TiO}_2$  nanoparticles yielded a well-ordered MHM with  $\text{TiO}_2$  nanoparticles adhering to the surface of the microhoneycomb walls. This can be further extended to prepare functional MHMs including a variety of nanoparticles.

Finally, our methodology could be further extended for novel constructions with substructure inside the microchannels. We found that by introducing a surface oxidized carbon fiber (SOCF) in the precursor sol, a composite MHM with SOCFs bridging the neighboring microhoneycomb walls was eventually obtained through the UDF process (**Figure 4**). Although further increase of the amount of SOCF interferes with the pseudosteady growth of ice crystals that leads to the MHM, the present result has demonstrated the feasibility of this methodology to be used to explore novel structures. Once a denser structure with certain toughness is achieved, a variety of applications such as energy storage can be imagined for these materials.

## Disclosures

The authors have nothing to disclose.

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