Enzymatic synthesis of cellulose in space: gravity is a crucial factor for building cellulose II gel structure

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Abstract

We previously reported in vitro synthesis of highly ordered crystalline cellulose II by reverse reaction of cellodextrin phosphorylase from the cellulolytic bacterium Clostridium (Hungateiclostridium) thermocellum (CtCDP), but the formation mechanism of the cellulose crystals and highly ordered structure has long been unclear. Considering the specific density of cellulose versus water, the formation of crystalline and highly ordered structure in an aqueous solution should be affected by gravity. Thus, we synthesized cellulose with CtCDP at the International Space Station, where sedimentation and convection due to gravity are negligible. Optical microscopic observation suggested that cellulose in space has a gel-like appearance without apparent aggregation, in contrast to cellulose synthesized on the ground. Small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) indicated that cellulose synthesized in space has a more uniform particle distribution in the ~100 nm scale region than cellulose synthesized on the ground. Scanning electron microscopy (SEM) showed that both celluloses have a micrometer scale network structure, whereas a fine fiber network was constructed only under microgravity. These results indicate that gravity plays a role in cellulose II crystal sedimentation and the building of network structure, and synthesis in space could play a role in the design of unique materials.

Keywords: cellulose, cellodextrin phosphorylase, synthesis in vitro, microgravity

Declarations

Funding: This work was supported by JSPS KAKENHI Grant Number 19K15884 (grant to NS), by a Grant-in-Aid for Innovative Areas from the Japanese Ministry of Education, Culture, Sports, and Technology (MEXT; grant no. 18H05494 to KI), and the Academy of Finland through research grant SA-FOSSOK [Decision
K.I. thanks the Finnish Funding Agency for Innovation for the support of the Finland Distinguished Professor Program “Advanced approaches for enzymatic biomass utilization and modification (BioAD)”.

Conflicts of interest/Competing interests: Not applicable.

Code availability: Not applicable

Ethics approval: Not applicable

Consent to participate: Not applicable

Consent for publication: Not applicable

Acknowledgments

The authors thank Prof. Motomitsu Kitaoka of Niigata University for providing CtCDP gene and Prof. Tomoya Imai of Kyoto University for helpful discussions. The WAXS and SAXS experiments were conducted at the BL8S3 station of Aichi Synchrotron Radiation Center, Aichi Science & Technology Foundation, Aichi, Japan (Proposal No. 2020D6031). SEM observation was conducted under the supervision of Dr. Satoshi Kimura of the University of Tokyo. We thank Confocal Sciences Inc. and Japan Manned Space Systems Corporation (JAMSS) for sample preparation and launch to the ISS.

Graphical Abstract
**Introduction**

Cellulose is the most abundant carbohydrate on Earth, and has been utilized by humans from ancient times. In nature, cellulose is mostly produced by woody and herbaceous plants as a cell-wall component. It is also synthesized by some microorganisms such as *Komagataeibacter xylinus* (*Acetobacter xylinum*), invertebrate animals (urochordates), or green algae (*Cladophora* species) (VanderHart and Atalla 1984; Belton et al. 1989; Larsson et al. 1997). Cellulose is a linear polymer of exclusively β-1,4-glycosidic-bonded glucose molecules synthesized by cellulose synthase complex on the cell membrane of these species. β-1,4-Glucan chains synthesized by the complex on the cell membrane spontaneously assemble and crystallize to form cellulose microfibrils (CMF; also called cellulose nanofibers, CNF). The shape of CMF depends on the geometry and morphology of the cellulose synthase complex (Brown 1996; Saxena and Brown 2005), but the mechanism of CMF formation is still unknown. Inside CMF, the β-1,4-glucan chains are bound together by hydrogen bonds and hydrophobic interaction to form a specific crystalline structure. Cellulose Iα and Iβ are the smallest crystalline units of natural cellulose, and these two natural crystalline allomorphs are composed of glucan chains in parallel orientation (Atalla and VanderHart 1984; Nishiyama et al. 2002, 2003). In contrast, cellulose II is a non-natural crystalline form originally found in mercerized and regenerated cellulose. The crystalline structure of cellulose II is significantly different from those of natural cellulose Iα and Iβ, having an anti-parallel orientation of cellulose molecules (Kolpak and Blackwell 1976; Langan et al. 1999; Kim et al. 2006). Cellulose II may be thermodynamically more stable, considering that it is formed in preference to metastable cellulose Iα or Iβ when dissolved β-1,4-glucan chains are recrystallized.

To elucidate the formation mechanism of CMF and to develop new materials applications, synthesis of various forms of artificial cellulose has been attempted (Uryu et al. 1983, 1985; Nakatsubo et al. 1996). Early efforts showed poor regio- and stereo-selectivity, and thus highly substrate-selective enzymatic approaches were adopted (Kobayashi et al. 1991, 2000; Kobayashi and Shoda 1995; Kobayashi 2005; Tanaka et al. 2007). Cellodextrin phosphorylase (CDP) is one of the enzymes utilized for the synthesis of cellulose *in vitro*. Although CDP catalyzes phosphorolysis of cellodextrin (cellooligosaccharide), it is possible to
synthesize cellulose via the reverse reaction by using high concentrations of α-D-glucose-1-phosphate (α-G1P) as a glycosyl donor, with glucose and cellobiose as primary glycosyl acceptors (Alexander 1968; Sheth and Alexander 1969; Krishnareddy et al. 2002). The glycosyl donors form β-1,4-glycoside bonds with the non-reducing ends of glycosyl acceptors. In this manner, platelet lamellae of crystalline cellulose having the degree of polymerization (DP) 9 were formed in vitro (Hiraishi et al. 2009). All these studies aiming to synthesize cellulose in vitro afforded cellulose II. Pylkkänen et al. have found that concentrated cellulose II synthesized by CDP from Clostridium (Hungateiclostridium) thermocellum (CtCDP) formed crystalline platelet lamellae and ribbon-like higher-ordered network structure (Pylkkänen et al. 2020). However, the mechanism of formation of cellulose II’s supermolecular structure is still unknown, as is that of natural cellulose Iα and Iβ.

Protein crystallization in space enhances the quality of protein crystals due to decreased sedimentation and convection under microgravity (Vekilov 1999). This affords more orderly crystals than can be obtained on the ground, enabling researchers to obtain higher-quality X-ray diffraction data (Snell et al. 1995; Inaka et al. 2011; Nakamura et al. 2015; Tachioka et al. 2017; Yamaguchi et al. 2021). A crystal of alloy semiconductor grown on the International Space Station (ISS) also showed better quality than one grown on the ground (Inatomi et al. 2015), and an NaCl crystal grown on the ISS had different morphology from a crystal grown on Earth (Fontana et al. 2011). On the other hand, the synthesis and crystal formation of organic polymers such as cellulose under microgravity in space have not yet been investigated.

In the present study, cellulose II was synthesized in vitro using CtCDP on the ISS. We investigated how gravity affects cellulose II crystalline or higher-order structure formation by comparing the product with material synthesized in the same way on the ground, employing small-angle x-ray scattering (SAXS), wide-angle X-ray scattering (WAXS), and scanning electron microscopy (SEM).
Materials and Methods

Materials.

α-G1P and pET-28b vector were purchased from Sigma-Aldrich Co. LLC (MO, US). Cellulbiose and other chemical reagents were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Overnight Express auto-induction medium and BugBuster reagents were purchased from Merck KGaA (Darmstadt, Germany). CDP from Clostridium (Hungateiclostridium) thermocellum strain YM4 was initially provided by Prof. Momomitsu Kitaoka of Niigata University, Japan. E. coli BL21 (DE3) competent cells were purchased from Nippon Gene (Tokyo, Japan). C-Tube counter-diffusion (Otálora et al. 2009) quartz capillaries were purchased from Confocal Sciences Inc. (Tokyo, Japan).

Enzyme preparation.

A gene coding ΔCys-CtCDP based on CDP from C. thermocellum strain YM4 (GenBank: AB061316.1) was designed, in which all 11 cysteine residues were replaced with serine residues. None of the cysteine residues in CtCDP are thought to form disulfide bonds. This gene was codon-optimized for expression in E. coli and synthesized by GenScript (NJ, US) with a 6x His tag at the C-terminal. It was inserted into the pET-28b vector between the NcoI and XhoI sites with Ligation High (Toyobo, Osaka, Japan). The vector was transformed into E. coli BL21 (DE3). ΔCys-CtCDP was expressed while transformed cells were cultivated in an Erlenmeyer flask filled with 1 L of Overnight Express auto-induction medium at 30ºC. After 18 hours of cultivation, the cells were collected by centrifugation, and the crude enzyme was obtained after cell lysis with BugBuster reagents. The crude enzyme was purified on a TALON his-tag cobalt affinity column (Clontech Takara Bio USA, CA, US). The His-tagged target protein was eluted with a linear gradient of 20 mM Tris-HCl buffer pH 7.5 containing 100 mM NaCl and 500 mM imidazole. The His-tagged protein was then dialyzed against 20 mM Tris-HCl buffer with an Amicon apparatus with 10,000 MWCO Biomax membrane filter (Merck). Anion exchange chromatography with TOYOPEARL DEAE-650S (Tosoh, Tokyo, Japan) was employed for further purification. Highly purified ΔCys-CtCDP was eluted with a linear gradient of 20 mM Tris-HCl buffer pH 7.5 containing 250 mM NaCl and used for cellulose synthesis.
Cellulose synthesis in vitro.

0.10 µg/ml ΔCys-CtCDP and 10 mM cellobiose were introduced into a C-tube counter-diffusion (Otálora et al. 2009) quartz capillary placed in 10 mM cellobiose and 200 mM α-G1P solution three days before launch. The counter-diffusion capillary consists of a 2 mm diameter quartz capillary and silicon tubing containing agarose gel; this arrangement allows the outer solution to diffuse into the capillary. Inside the counter-diffusion capillary, the initial α-G1P concentration was set to 0 mM and this gradually increased as α-G1P diffused from the gel tube (Fig. 1A). The α-G1P concentration was controlled to minimize the influence of gravity during cellulose synthesis before arrival at the ISS. The Kirara service (JAMSS, Tokyo, Japan) was used to launch the experiment to the ISS. The sample was kept in the microgravity environment of the ISS for one month at 20 °C inside a thermostated box (Fig. 1B). The cellulose synthesized on Earth was prepared similarly, except for the presence of gravity, as a control.

WAXS measurements.

WAXS measurements were done at the BL8S3 station of Aichi Synchrotron Radiation Center (Aichi, Japan) with a 205.85 mm camera length. R-Axis IV++ (Rigaku, Tokyo, Japan) was used to record the diffraction, and radial integration of diffraction intensity was performed with the program FIT2D (ESRF, Grenoble, France). Sample capillaries were attached to the cell holder, and measurements were conducted at the upper part (10 mm from the capillary top), middle part (14 mm from the capillary top), and bottom part (18 mm from the capillary top) of the capillary (Fig. 1C).

Igor Pro (Wavemetrics, OR, US) was used to perform WAXS peak fit analysis and to create graphics. FWHM (full width at half maximum) of peaks assigned to the 020 plane of cellulose II and peak areas were determined, assuming that scattering due to water was smooth and would not form any peak.

SAXS measurements.

SAXS experiment was conducted at the BL8S3 station of Aichi Synchrotron Radiation Center under the following conditions: diffraction of 0.92 Å X-rays was recorded on an R-Axis IV++ at a camera length of 3975.85 mm. Radial integration of diffraction intensity was performed with the program FIT2D.
Sample capillaries were attached to the cell holder, and measurements were done at the upper part (10 mm from the capillary top), middle part (14 mm from the capillary top), and bottom part (18 mm from the capillary top) of each sample capillary (Fig. 1C).

SAXS data was processed with ATSAS (Manalastas-Cantos et al. 2021) and SasView (http://www.sasview.org/). The SAXS data were analyzed after subtracting the scattering curve of the negative control solution containing 0.10 µg/ml CtCDP and 10 mM cellobiose in a C-tube capillary. Earlier electron microscopy and atomic microscopy observations showed that complex structural features can co-exist in one reaction system (Hiraishi et al. 2009; Pylkkänen et al. 2020), and therefore we used a unified power law equation for fitting the data (Beaucage 1995; Tajima et al. 2019).

\[
I(Q) = \text{background} + \sum_{i=1}^{2} \left[ G_i \cdot \exp \left( -\frac{Q^2 R g_i^2}{3} \right) + B_i \cdot \exp \left( -\frac{Q^2 R g_i^2 + 1}{3} \right) \right]. \\
\left( \frac{1}{Q_{i}} \right)^{p_i} \right] \\
(1)
\]

\[
Q_i^* = Q \left[ \text{erf} \left( \frac{Q \cdot R g_i}{\sqrt{6}} \right) \right]^{-3} \\
(2)
\]

Q, I(Q), R_g, G, B in equations (1) and (2) are scattering vector, intensity, radius of gyration for a particular scattering body, Guinier function, and Porod-type function, respectively. The scattering vector was defined as \( Q = 4\pi/\lambda \sin \theta \), where \( \theta \) is the scattering angle, and \( \lambda \) is the wavelength.

**Observation with scanning electron microscopy (SEM).**

The solvent in capillaries containing synthesized cellulose was replaced gradually with tert-butyl alcohol and then the cellulose samples were freeze-dried in a lyophilizer (FDU-1200, Eyela, Tokyo, Japan) and collected by breaking the capillaries with a cutting stone (Hampton Research, CA, US). Samples were coated with Pt-Pd, and SEM images were captured with an FE-SEM S-4800 (Hitachi, Tokyo, Japan) at 1 kV.
Results and discussion

Enzyme preparation

CrCDP was found to be unstable and lost its activity over several weeks. Since synthesis of cellulose on the ISS was planned for one month, improving the stability of CrCDP was the first challenge for this study. Alexander et al. suggested that the oxidation state of cysteine residues negatively affects the CrCDP activity, and therefore, we designed ΔCys-CrCDP in which all 11 cysteine residues are replaced with serine residues (Fig. 2). This ΔCys-CrCDP did not lose activity for at least two months. Characterization of the mutated CrCDP will be reported elsewhere.

Optical observation of cellulose synthesized in counter-diffusion capillaries

In the CrCDP reaction using the counter-diffusion reaction vessel, the reaction proceeds as the donor substrate, α-G1P, is supplied from the gel tube by diffusion (Fig. 1A). In the sample capillaries, there was an unreacted region, where no product exists, on the opposite side from the gel tube. This result suggests that the enzymatic reaction proceeded sequentially from the site of the gel tube, regardless of whether the reaction takes place in space or on the ground (Fig 3A and B). However, the appearance of the cellulose synthesized under the two conditions differed significantly.

The cellulose synthesized on the ISS had an overall homogeneous gel-like appearance, and no aggregates could be seen (Fig 3A). However, on the ground, the formation of larger aggregates was observed, and they were more abundant near the base of the gel tube, i.e., in the direction of gravity (Fig 3B). The density of cellulose crystals is approximately 1.5 g/cm³, and under typical aqueous reaction conditions, the synthesized cellulose particles be expected to settle under gravity. This settling would not occur in the microgravity environment in space, suggesting that cellulose synthesis under microgravity prevents the formation of visible highly ordered structures and aggregates, affording more homogeneous cellulose crystals. In addition, the highly ordered structure of cellulose
synthesized under microgravity was sufficiently strong to withstand its weight because no aggregation was observed after the return to the Earth.

**WAXS measurements**

The cellulose synthesized in space appeared homogeneous and gel-like. On the other hand, it is known that the crystalline form of cellulose is affected by drying and other factors, so it was necessary to leave the cellulose in the reaction capillary to perform X-ray diffraction measurements. To identify the allomorphs of cellulose synthesized under microgravity and on the ground, WAXS diffraction measurements were conducted. The WAXS diagram is shown in Fig. 4. The scattering intensity increased monotonically in the range of 5 nm\(^{-1}\) < Q < 16 nm\(^{-1}\) due to the presence of an excess amount of water. All measurements showed similar trends (Fig. 4). However, the scattering intensities of cellulose synthesized in space were similar along the height direction of the capillary, in contrast to the scattering intensities of cellulose synthesized on the ground, where the upper part showed higher scattering intensity in all ranges (5 nm\(^{-1}\) < Q < 16 nm\(^{-1}\)). This suggests that cellulose synthesized in space has a more uniform crystal size or more uniform crystal orientation in the height direction of the capillary than cellulose synthesized on the ground.

As shown in Fig. 4, three peaks were detectable in the range of 5 nm\(^{-1}\) < Q < 16 nm\(^{-1}\). There were weak peaks in the WAXS diagram of cellulose synthesized in space, whereas cellulose synthesized on the ground showed sharp peaks. According to the formula \(d = 2\pi/Q\), which describes the relationship between scattering vector (Q) and real space (d), peaks of Q = 8.69, 14.1, and 15.6 nm\(^{-1}\) correspond to real space d = 7.23, 4.45 and 4.03 Å, respectively. A combination of those d-values was matched with lattice spaces in the 110, 110, and 020 planes of cellulose II, respectively (Kobayashi et al. 2011; French 2014). Therefore, celluloses synthesized on the ground and under microgravity were both assigned as crystalline cellulose II. Thus, gravity did not appear to influence the polymorphic form of the product.

The areas and FWHMs of peaks attributed to the 020 plane in Fig. 4 were determined and are summarized in Table 1. The 020 plane areas of ground-synthesized cellulose II were larger than those of space-synthesized cellulose. The
average peak area of cellulose on the ground was twice as large as that of cellulose synthesized in space, and the average FWHM was 10% smaller. Those data suggest that cellulose synthesized in space has a smaller crystal size or reduced degree of crystal orientation.

**SAXS measurements**

WAXS measurements confirmed that the cellulose synthesized in space was not an amorphous gel, but consisted of particles of crystalline cellulose. Therefore, SAXS measurements were carried out to obtain information on this particulate cellulose.

Fig. 5 shows Log-Absolute SAXS plots and residual plots after subtraction of scattering from the bottom part of each capillary. The residual plots indicate that cellulose synthesized in space showed a more uniform density of particles with various radii of gyration throughout the capillary than cellulose synthesized on the ground (Fig. 5B and D). Specifically, there were more components in the region of $Q < 1.0 \text{ nm}^{-1}$ in the upper and middle parts compared to the bottom part, though there was no significant difference between the plots of the upper and middle parts. Thus, the SAXS profile of cellulose synthesized on the ground differed more depending on the position in the capillary, and the difference was particularly pronounced in the region of $Q < 1.5 \text{ nm}^{-1}$, which means the particle region with a radius of gyration $R_g$ greater than 4.18 nm (Fig. 5C). As for cellulose synthesized on the ground, scattering from the middle part of the capillary was higher than scattering from other parts (Fig. 5C and D). Considering that gravitational settling is the main cause of the variation in particle distribution with capillary position, the observation of a higher density in the middle part of the capillary seems strange. However, it might be explained by adsorption of cellulose II lamellar crystals on the quartz glass during sedimentation and aggregation. C/CDP-cellulose II lamellar crystals have a large hydrophilic area with abundant hydroxyl groups on the surface (Hiraishi et al. 2009; Wada et al. 2021), and might therefore bind readily with SiO$_2$ at the surface of the capillary.

To further highlight the differences, a Kratky plot was performed (Fig. 6). The scattering from the center of the ground-synthesized cellulose showed a clear peak at $Q \approx 0.90 \text{ nm}^{-1}$, which is distinctly different from that of space-synthesized
cellulose. While the scattering intensity of cellulose synthesized on the ground varied with the height in the capillary, the scattering intensity of cellulose synthesized under microgravity was relatively homogeneous. These data qualitatively suggest that there was no significant difference in the number and volume of cellulose particle scatterers in the upper or bottom part of the capillary between the ground and space conditions. Nevertheless, there was a significant difference in the number and volume of scatterers in the middle part.

To quantitatively evaluate the size of the scatterers, we focused on the small-angle results in the SAXS measurements. We found that a unified power law equation (Beaucage 1995) gave a good fit, with sufficiently small values of chi²/point for all parts of the capillaries (Fig. 7). Especially in the region of 0.07 nm⁻¹ < Q < 0.5 nm⁻¹, all SAXS scatterings were proportional to Q⁻².28 - Q⁻².35, indicating that the particles have a thin plate shape, whether the cellulose is synthesized in space or on the ground (Kratky and Porod 1949; Pedersen 1997). This conclusion is consistent with previous studies showing that Ctcp-cellulose single crystals have a platelet shape (Hiraishi et al. 2009; Pylkkänen et al. 2020; Wada et al. 2021).

The small-angle region of the SAXS results did not show a good fit in Guinier plot analysis for all the samples. This suggests that all the samples obtained consist of a set of aggregates with multiple radii of gyration. Therefore, in this fitting analysis, we focused only on the radius of gyration Rg², which corresponds to the peak at Q ≈ 0.9 nm⁻¹. Table 2 shows all the parameters of the fitting analysis; the average Rg² values for cellulose in space and on the ground were calculated to be 6.61±0.09 nm and 4.57±0.84 nm, respectively. It has been shown that cellulose synthesized in vitro by Ctcp under batch conditions on the ground has a degree of polymerization of 9 and forms plate-like crystals with a thickness of about 5 nm (Hiraishi et al. 2009). This value is similar to the Rg² values of cellulose in space and on the ground. Thus, Ctcp-cellulose’s crystalline lamellar structure existed in cellulosics synthesized both in space and on the ground.

The parameter B₂ in Table 2 represents the number or density of particles having a radius of gyration Rg². Cellulose synthesized in space had uniform B₂ values at all measured points (1.32, 1.34, and 1.27 for the capillary’s upper, middle, and bottom parts, respectively). In contrast, cellulose on the ground had...
different values (1.42, 4.18, and 1.94 for the upper, middle, and bottom parts of the capillary). This difference suggested that cellulose synthesized in space has a quantitatively more uniform density of particles with a radius of gyration $R_{g2}$ in the height direction of capillary, as compared with cellulose on the ground.

**Observation with SEM**

Typical SEM images are shown in Fig. 8. Cellulose synthesized in space (Fig. 8A and B) had a finer network structure than cellulose synthesized on the ground (Fig. 8C and D). The network consisted of ribbon-like structures, which were estimated to be 100-200 nm wide in space-synthesized cellulose (Fig. 8B). In contrast, cellulose synthesized on the ground contained thicker aggregates with micrometer scale width (Fig. 8D), i.e., several times larger. The width of the thin ribbon-like structures synthesized in space was consistent with previous TEM and AFM observations of CDP-synthesized crystalline cellulose II (Hiraishi et al. 2009; Pylkkänen et al. 2020). This indicates that space-cellulose’s ribbon-like structure was comprised of single to several cellulose crystals, while ribbons of cellulose synthesized on the ground contained more crystals. These results and the WAXS peak intensities suggest that the thick aggregated form might assemble through orientation or crystallization. These partial features of the network structure are consistent with the sparse (Fig. 8A and B) and dense (Fig. 8C and D) micrometer-scale appearance of cellulose network structure. The scale of these ribbon widths is similar to the scale of the wavelength of the visible light; therefore, these features would affect the optical appearance (Fig. 3) as well.

It is well known that cellulose II synthesized by CtCDP self-assembles into a network structure (Pylkkänen et al. 2020). Such cellulose II synthesized by CtCDP on the ground was observed as white precipitates or aggregates in the earlier studies, in contrast to the gel-like appearance of space-synthesized cellulose (Fig. 3A). In previous attempts to create gel-like products with supramolecular network architecture on the ground, researchers have added nanocrystals of polymers such as polyethylene glycol and cellulose I$_\beta$ to the reaction mixture for cellulose synthesis by CtCDP (Hata et al. 2017, 2018) to serve as scaffolds. We believe the present report is the first to describe the production of pure cellulose II crystalline gel.
Our observations indicate that once the cellulose II network structure is formed in space, the supermolecular structure is stable after return to Earth. A relatively light polymer (PMMA, 1.2 g/cm³) was reported to form a network structure of crystalline polymer through viscoelastic phase separation on the ground, and gravity appeared to have a negligible influence for at least 12 hours (Tsurusawa et al. 2017). Thus, it is possible that the effect of gravity on cellulose arises because of the high specific gravity of cellulose compared with water. Therefore, the microgravity environment in space may be essential for the production of cellulose II crystalline gel.

**Conclusion**

In the present study, we investigated the possibility that gravity influences the crystallization and formation of highly ordered structure of cellulose II. We found that cellulose synthesized in space did not form aggregates like those of cellulose synthesized on the ground. WAXS demonstrated that similar nano-scale crystalline cellulose II packing occurred on the ground and in space. However, the SAXS experiment showed that cellulose particles in a capillary had higher homogeneity when synthesized in space. SEM observation showed that space-synthesized cellulose had a fine supramolecular network structure on the micrometer scale, and this was strong enough to survive after return to Earth. These findings suggest that gravity influences aggregate formation during self-assembly to form the network. In this work, a bottom-up synthesis of pure cellulose II crystal gel was achieved for the first time. The physical properties of this newly created cellulose II crystalline gel remain to be investigated.
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**Fig. 1.** Experimental settings. (a) The counter-diffusion capillary. (b) Thermostated box containing a counter-diffusion capillary for cellulose synthesis under a microgravity environment (©NAS/ESA, Photo was from https://www.esa.int/ESA_Multimedia/Images/2021/01/ICE_Cube_commercial_COVID-19_experiment). (c) Schematic illustration of wide-angle and small-angle X-ray scattering experiment on the counter-diffusion capillary.
Fig. 2. Locations of mutations on ΔCys-CtCDP are highlighted in yellow in (a). Images of CtCDP tertiary structure are based on CtCDP monomeric structure (PDB ID: 5NZ7, chain A). The distribution of cysteine-to-serine substitutions in the CtCDP protein sequence (984 amino acids) is shown in (b). A graphic of the tertiary structure of CtCDP was created with PyMOL (Schrödinger, NY, US).
Fig. 3. Optical observation of capillaries containing cellulose synthesized in a microgravity environment (a) and on the ground (b). Cellulose synthesized in a microgravity environment showed no apparent aggregation, unlike cellulose synthesized on the ground.
Fig. 4. WAXS diagram of cellulose synthesized in a microgravity environment (a) and on the ground (b). Scattering from the upper, middle, and bottom parts are depicted by dashed, dotted, and solid lines, respectively. Cellulose synthesized in a microgravity environment had more uniform and weaker diffraction peaks of cellulose II than cellulose synthesized on the ground. Each arrow shows the location of a peak corresponding to a lattice space of cellulose II.
Fig. 5. Experimental SAXS curves for CtCDP-cellulose and scattering differences in the height direction of the capillaries. SAXS profiles of cellulose synthesized in space and on the ground are shown in (a) and (b), respectively. The insets show residual scattering after subtraction of the scattering from the bottom part of capillaries. Scattering from the upper, middle, and bottom parts are depicted by dashed, dotted, and solid lines, respectively.
Fig. 6. A Kratky plot demonstrating the difference in CrCDP-cellulose particle distribution in the height direction of the capillaries. Kratky plot of cellulose synthesized on the ground (black lines) and in a microgravity environment (gray lines). Scattering from the upper, middle, and bottom parts is depicted by dashed, dotted, and solid lines, respectively.
Fig. 7. Experimental SAXS profile of CtCDP-cellulose and fitting analysis with equation (1).
Scattering of cellulose synthesized in space from the upper part (a), middle part (b), and the bottom part (c) and scattering of cellulose on the ground from the upper part (d), middle part (e), and the bottom part (f) are depicted in log-log plots. Fitted curves are shown as solid lines, and measured values are shown as gray circles in each figure. All SAXS data in the region $0.07 \text{ nm}^{-1} < Q < 0.5 \text{ nm}^{-1}$ are proportional to approximately $Q^{-2.3}$, indicating that the cellulose particles have a platelet shape.
Fig. 8. Typical SEM images of cellulose synthesized in space and on the ground. Images of cellulose synthesized in space were captured at x500 (a) and x2000 (b) magnification. (c) and (d) show images of cellulose synthesized on the ground at x500 and x2000 magnification, respectively. Cellulose synthesized under a microgravity environment generated a network consisting of thinner ribbons, while cellulose synthesized on the ground had a network structure with matrix-like thick ribbons.
Table 1. Comparison of cellulose synthesized in space and on the ground in terms of peak areas and FWHMs derived from the 020 plane. Peak fit was performed against WAXS profiles of the upper part, middle part, and bottom part of each capillary. Peak areas derived from the 020 plane of cellulose synthesized in space were relatively small, and FWHMs were rather large compared to those of cellulose synthesized on the ground. The unit of area is arbitrary.

|                  | cellulose synthesized in space | cellulose synthesized on the ground |
|------------------|-------------------------------|-------------------------------------|
|                  | upper part | middle part | bottom part | upper part | middle part | bottom part |
| Area             | 125±34     | 88.8±11.7   | 93.9±13.9   | 188±8      | 234±14      | 246±20      |
| FWHM (°)         | 0.379±0.055 | 0.282±0.025 | 0.296±0.029 | 0.250±0.008 | 0.292±0.011 | 0.316±0.015 |
| Average Area     | 103         |             |             | 222        |             |             |
| Average FWHM (°) | 0.319       |             |             | 0.286      |             |             |
Table 2. Parameters for fitting SAXS profiles to equation (1). Fitting analysis was performed for SAXS profiles of the upper part, middle part, and bottom part of each capillary.

|                  | Cellulose synthesized in space | Cellulose synthesized on the ground |
|------------------|--------------------------------|-------------------------------------|
|                  | upper part  | middle part | bottom part | upper part  | middle part | bottom part |
| Chi²/points      | 0.0849      | 0.0559      | 0.0525      | 0.0492      | 0.195       | 0.140       |
| background       | 0.791±0.066 | 0.746±0.065 | 0.735±0.066 | 0.681±0.068 | 0.763±0.070 | 0.688±0.062 |
| R₉₀ (nm)         | 34.4±0.7    | 35.5±0.7    | 34.4±0.8    | 33.5±0.5    | 32.7±0.2    | 32.4±0.2    |
| P₁               | 2.78±0.032  | 2.75±0.03   | 2.80±0.04   | 2.76±0.03   | 2.66±0.01   | 2.68±0.01   |
| B₁               | 0.619±0.051 | 0.67±0.05   | 0.526±0.056 | 0.721±0.058 | 1.23±0.02   | 0.943±0.021 |
| G₁               | 3360±125    | 3670±151    | 2980±134    | 3500±100    | 4700±53     | 3500±55     |
| R₉₀ (nm)         | 6.48±0.41   | 6.56±0.41   | 6.78±0.45   | 6.22±0.44   | 3.50±0.00   | 4.00±0.00   |
| P₂               | 2.71±0.16   | 2.72±0.15   | 2.61±0.14   | 2.81±0.18   | 5.80±0.64   | 6.39±0.73   |
| B₂               | 1.32±0.10   | 1.34±0.10   | 1.27±0.10   | 1.42±0.10   | 4.18±0.32   | 1.94±0.09   |
| G₂               | 56.4±9.1    | 58.8±9.3    | 58.2±9.9    | 55.9±9.8    | 20.7±0.3    | 23.9±0.4    |