Composite Hydrogel Spheroids Based on Cellulose Nanofibrils and Nanofibrous Chiral Coordination Polymer by Green Synthesis

Emile R. Engel,* Vincenzo Calabrese, Kazi M. Zakir Hossain, Karen J. Edler, and Janet L. Scott

Cellulose-based hydrogels are promising sustainable materials for a variety of applications, including tissue engineering, water treatment, and drug delivery. However, the tailoring of diverse properties by efficient green chemistry methods is an ongoing challenge. Here, composite hydrogels of consistent spheroidal structure, incorporating TEMPO-oxidized cellulose nanofibrils and nanofibrous chiral Cu(II) aspartate coordination polymer, are presented. The hydrogels are prepared by a single-step procedure in aqueous media at ambient temperature and pressure, adhering to the principles of green chemistry. With a view to adapting this method for a variety of alternative coordination polymers (to tailor functional properties), the following critical factors for formation of robust composite hydrogel spheroids are identified: rheological properties of the primary matrix used for spheroidal hydrogel formation and coordination polymer self-assembly rate.

1. Introduction

Cellulose and other biopolymer-based hydrogels have received considerable attention as promising materials for tissue engineering as scaffolds and extracellular growth matrices.[1-3] Beyond tissue engineering, cellulose-based hydrogels have been explored for applications in water treatment,[4,5] drug delivery,[6-8] and personal hygiene products.[9] It is well-established that aqueous ionic solutions induce gelation of cellulose nanocrystal[10,11] and nanofibril[12] dispersions. In the case of nanocrystalline cellulose bearing negatively charged sulfate ester groups, ion-induced gelation has been attributed to a charge screening effect that promotes intermolecular attraction.[11] Similarly, for oxidized cellulose nanofibrils bearing carboxylate groups, gelation has been attributed to specific cation-carboxylate interactions and charge screening[12-15] and this gelation mechanism has been exploited for the fabrication of long-living threads via flow-focusing microfluidics.[16] In the present work, chemical compound (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO)-oxidized cellulose nanofibrils (OCNF) with a degree of oxidation of 23%, average fibril radius of 3.5 nm and average fibril length of 160 nm[18] have been used (surface chemistry of OCNF depicted in Figure 1A).

Cellulose and coordination polymers (CPs), including the subclass of metal-organic frameworks, are attractive complementary materials for the fabrication of composites. Cellulose is a versatile structural material, useful for constructing films and membranes, packaging materials and, of course, hydrogels. However, introducing certain functional properties, such as chiral recognition,[17] magnetism,[18,19] and luminescence,[20,21] to cellulose-based materials is challenging. Incorporating CPs into cellulose-based materials is a strategy for introducing a wide range of properties, given the great diversity of existing and possible CPs, including 1D CPs,[22] and the associated range of functional properties including chiral recognition,[23] porosity,[24] magnetism,[25] optical properties,[26] and catalytic activity.[27] Obvious potential applications for cellulose/CP composite hydrogels include the adsorption of pollutants from waste water, slow release of drugs, nutrients or pesticides, and functionalized (e.g., antibacterial) wound dressings but the full range of potential applications will be much greater if a variety of functional properties can be introduced by incorporation of various CPs. In addition, the selective, anisotropic incorporation of CPs into hydrogels could open new avenues for the production of “Janus-like” composite hydrogels for dual (synergistic) activity or enhancement of mechanical properties. The creation of “anisotropy and directionality within the networks” has been described as an ongoing research challenge in a recent review of structured macroporous hydrogels.[28] Zhu and co-workers prepared alginate hydrogels incorporating various metal-organic frameworks isotropically in a multistep procedure involving metal-organic framework
synthesis at elevated temperatures, in mixtures of methanol and water or ethanol and water. However, cellulose is more naturally abundant and more structurally robust than alginate, making it preferable for large-scale applications. Moreover, a simpler ambient temperature procedure is conceivable based on cellulose nanofibrils and CPs that self-assemble in aqueous solution.

CP nanofibers combining Cu\(^{2+}\) and enantiomerically pure aspartic acid were first reported by Imaz et al. The blue crystalline nanofibers of the Cu(II) \(\text{L-}{\text{aspartate}}\) or Cu(II) \(\text{D-}{\text{aspartate}}\) CP, herein referred to simply as \(\text{Cu-Asp}\), are prepared by combining aqueous Cu(NO\(_3\))\(_2\) and an alkaline solution of L- or D-aspartate (L-Asp or D-Asp) (Figure 1B). We prepared \(\text{Cu-Asp}\) by this known method and confirmed the identity of the phase by powder X-ray diffraction analysis (see Figure S1 in the Supporting Information). Wu et al. have demonstrated that an alkaline solution of the aspartate racemate resolves in the presence of Cu\(^{2+}\), yielding a mixture of the L- and D- versions of \(\text{Cu-Asp}\).\(^{[31]}\)

The aim of the present study was to incorporate CP nanofibers of \(\text{Cu-Asp}\), by in situ self-assembly, into stable OCNF hydrogels by an elegant method, adhering to the principles of green chemistry, exploiting ion-induced gelation of OCNF aqueous dispersions. Such a method could be adapted to incorporate a variety of alternative CPs of different functional properties. The structures of ion-induced OCNF hydrogels have already been thoroughly investigated elsewhere.\(^{[34,33]}\) The intention of the present work was to establish, as represented in Figure 1C, whether introducing a ligand-containing dispersion into a metal–salt solution, where the ligand and metal salt are known to form a CP by spontaneous self-assembly, would result in hydrogels containing: i) an isotropic distribution of CP (either an extended network or discrete particles), ii) an anisotropic distribution of crystallites, or iii) no CP. Furthermore, we sought to identify key parameters for controlling the properties of the resultant hydrogels.

2. Results and Discussion

We set out to prepare composite hydrogels from a 1 wt% OCNF dispersion containing 5 wt% silica nanoparticles (SiNPs) and varying concentrations of aspartic acid and NaOH. SiNPs have been proven to act as a noninteracting filler in an OCNF-based hydrogel, preserving the network architecture while, at this concentration, inducing smooth yielding of the OCNF–SiNP gel.\(^{[34]}\) Specifically, the ability of the SiNP to suppress the onset of stress overshoots upon yielding has been herein exploited to form hydrogels with defined structures, such as spheroids and filaments. Figure 2A shows the formation of filaments (see also Video S1 in the Supporting Information) from a dispersion containing only OCNF and SiNP. These filaments were stable for several months in the metal salt solution and were robust enough to be removed from the ionic solution and exposed to air. OCNF, based on the well-established method of TEMPO-mediated oxidation,\(^{[35]}\) was obtained as an 8 wt% paste of solids in water with a degree of oxidation of 25% by conductometric titration.\(^{[14]}\) A 3 wt% stock dispersion of OCNF was prepared by dispersing the solids in water using a sonication probe (\(\approx 8000 \text{ J}\) over 60 min of processing time per batch of 40 mL). A 10 wt% SiNP stock dispersion was prepared using silica powder (Sigma-Aldrich).
Aldrich 718 483), comprising SiNP of diameter 1570 ± 1.3 nm (Z-average). The silica powder was suspended in water, followed by sonication (=240 J over 2 min of processing time per batch of 20 mL). The OCNF and SiNP dispersions were then combined in appropriate proportions and subjected to a short period of sonication (=120 J over 1 min of processing time per 10 mL sample). Aspartic acid was added to dispersions as an alkaline solution of 1:2 aspartic acid/NaOH. NaOH served to deprotonate the ligand as well as the OCNF carbohydrate group. The integrity of the matrix was preserved despite the addition of NaOH at relatively high concentrations; there were no indications of OCNF degradation. Additional details of sample preparation procedures are provided in the Experimental Section. Further references to “OCNF dispersions” imply dispersions containing 1 wt% OCNF, 5 wt% SiNP, and concentrations of aspartic acid and NaOH as specified, unless otherwise stated.

Droplets of ligand-containing OCNF dispersions were ejected from a syringe needle or micropipette into an aqueous solution of 100 × 10⁻³ M Cu(NO₃)₂ to form hydrogel spheroids. For a series of OCNF dispersions containing l-Asp at different concentrations in the range 0–125 × 10⁻³ M, representative examples of the resultant hydrogel spheroids are shown in Figure 2B. As expected, the Cu(NO₃)₂ solution induced gelation of the OCNF dispersion on a time scale of seconds, as previously described for similar systems, which is attributable to specific cation–carboxylate interactions and counterion charge screening of the OCNF surface. The control sample in the absence of l-Asp formed a flattened structure with a depression in the middle. At 25 × 10⁻³ M l-Asp, the spheroid is partially flattened. At 50 × 10⁻³ M l-Asp, the structure is a tractable hydrogel with an ellipsoidal shape. At 75 × 10⁻³ M l-Asp, a conical shape results, and at higher concentrations, the structures are considerably elongated. The dispersion containing 50 × 10⁻³ M l-Asp was determined to be most favorable for preparing hydrogels of well-defined spheroidal structure and consistent sizes of 2–4 mm in diameter. The spheroids were robust enough to be removed from the ionic solution and exposed to air. They were also stable to repeated washing and storage in pure deionized water over periods of several hours (see Figure S2 in the Supporting Information).

It was hypothesized that Cu²⁺ and aspartate anions would self-assemble in situ to form Cu-Asp within the OCNF hydrogel matrix, and that Cu-Asp would interact with the OCNF via electrostatic interactions between the CP and the OCNF carboxylate and hydroxyl groups. In a separate experiment Cu-Asp was prepared at larger scale in the presence of OCNF, without addition of SiNP (details in the Experimental Section). Bright blue Cu-Asp formed in both dispersions and was evenly distributed throughout. However, after centrifugation at 1660 × g for 15 min, the blue Cu-Asp component became separated from the white OCNF component (Figure 3A). This suggests Cu-Asp is not bound to OCNF via strong cation–carbohydrate coordination bonds. Rather, Cu-Asp is present as discrete crystallites and only weak interactions exist between the Cu-Asp and OCNF components.

To further clarify the interactions between the gel matrix and the CP, combined small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) were performed (Figure 3B). This technique accessed a q-range of 0.01–1.5 Å⁻¹, where q is defined as the scattering vector, thus probing structural changes (d-spacing) between 628 and 4 Å, where d-spacing = 2π/q. At first, WAXS patterns for various samples of the composite hydrogels are compared with the reference pattern for pure Cu-Asp, prepared in water, which was further characterized by powder X-ray diffraction to confirm that Cu-Asp is indeed the same CP phase as described by Imaz et al. (Supporting Information shows powder X-ray diffraction patterns expressed as a function of q vector and 2θ). The peak positions in the WAXS pattern for the composite hydrogels correspond with WAXS (Figure 3B) and powder X-ray diffraction (Figure S1c, Supporting Information) patterns for the reference Cu-Asp and the reported powder X-ray diffraction patterns in the literature (Figure S1a,b, Supporting Information).

Considering WAXS length scales, at 50 × 10⁻³ M l-Asp, the scattering pattern is dominated by OCNF and SiNP. However, at concentrations of 75 × 10⁻³ M and greater, peaks corresponding to Cu-Asp are observed at the same q as for the pure Cu-Asp assembled in water (reference sample, see Figure S1 in the Supporting Information), confirming the presence and phase purity of the CP within the hydrogel matrix. Scanning
Figure 3. A) OCNF dispersions (without SiNP) containing Cu-Asp after centrifugation at 1660 × g for 15 min and decanting the excess water: a) dispersion containing 10 wt% Cu-Asp (with respect to OCNF dry content) and b) dispersion containing 20 wt% Cu-Asp, showing separation of OCNF gel from CP particles. B) Combined SAXS and WAXS patterns, according to SAXS and WAXS regimes described above, for pure Cu-Asp as reference (prepared in water) and OCNF dispersions containing l-Asp at the following concentrations: a) 0 M (control sample), b) 50 × 10⁻³ M, c) 75 × 10⁻³ M, d) 100 × 10⁻³ M, and e) 125 × 10⁻³ M. C) SEM image of the cross-section of an air-dried hydrogel showing bundles of Cu-Asp crystallites.

Figure 4. G′ and tan δ for OCNF dispersions containing l-Asp at concentrations in the range 0–150 × 10⁻³ M (in the absence of Cu(NO₃)₂). The dotted line is drawn as a guide, while darkening of the background indicates the liquid (tan δ > 1) to gel-like (tan δ < 1) transition.

electron microscopy (SEM) imaging (Figure 3C) shows the presence of fibrous bundles of CP crystallites, which are ∼50 μm in length and 10–30 μm in width, within the hydrogel matrix. In addition, the occurrence of the CP peaks at the same q as for the Cu-Asp reference sample is consistent with a lack of strong Cu-Asp–matrix interactions, further supporting the presence of CP within the gel matrix as discrete crystallites as previously discussed. In the SAXS region, similar slopes (q≈3 m) are observed for all OCNF containing dispersions, confirming that no substantial structural changes occur at these length scales. A detailed structural investigation of OCNF–SiNP hydrogels (where gelation was induced by NaCl) has been previously reported using contrast matched small angle neutron scattering (SANS), revealing that the presence of SiNP did not perturb the OCNF network architecture within the accessible q range.³⁴

Since the resistance to flow and elasticity of the OCNF dispersions (which form the primary matrix) strongly dictate the shape of the spheroidal hydrogels generated by ejection from a syringe needle into the metal salt solution, we investigated the rheological properties of the OCNF dispersions at different l-Asp concentrations (Figure 4). Opposing trends are observed for the complex modulus, G′ = (G′Ⅰ + G′Ⅱ) where G′Ⅰ and G′Ⅱ are the storage and loss modulus, respectively, and tan δ, where tan δ is defined as tan δ = G″/G′, as a function of l-Asp concentration, obtained from the linear viscoelastic region of a strain sweep at 1 rad s⁻¹. The increase in G″ as a function of l-Asp concentration indicates toughening of the dispersion. The further decrease of tan δ beyond tan δ < 1 points to enhanced elasticity of the dispersion and the formation of a material, which is dominated by the solid (gel-like) contribution. Noticeably, at [l-Asp] > 25 × 10⁻³ M, the dispersions behave as gel-like materials, which correlates well with the variations in hydrogel structure represented in Figure 2B where a transition occurs from concave edges at 0 M l-Asp to distinctly convex edges at 50 × 10⁻³ M l-Asp. At 50 × 10⁻³ M l-Asp, the change in tan δ approaches a constant value. At l-Asp concentrations beyond 50 × 10⁻³ M, further toughening (i.e., an increase in G″) results in more elongated hydrogel structures.

It had been previously demonstrated that racemic aspartic acid self-assembles with Cu²⁺ at a slower rate than enantiomerically pure D- or L-Asp.³¹ To investigate the effect of this change in reaction rate, spheroids were prepared from a series of OCNF dispersions containing 50 × 10⁻³ M l-Asp and doped with D-Asp at concentrations in the range 0–50 × 10⁻³ M (Figure 5). Rheological analysis of a similar series of dispersions (Figure S5, Supporting Information) revealed no considerable changes in elasticity as captured by tan δ. An anisotropic distribution of Cu-Asp crystallites within the hydrogel matrices is
observed, as is a clear trend in the distribution of crystallites as a function of the ratio of \([\text{l-Asp}]:[\text{d-Asp}]\). In all cases, the crystallites are located near the perimeter of the spheroid. For the sample containing \(\text{l-Asp}\) at \(5 \times 10^{-3}\) and 0 \(\text{m d-Asp}\) (Figure 5a), a dense outer ring of crystallites is visible, along with a sparsely populated secondary inner ring. Additional photographs of spheroids prepared from a dispersion containing \(\text{l-Asp}\) but no \(\text{d-Asp}\) are available in Figures S2 and S3 in the Supporting Information. SEM images of a dried cross-section of a hydrogel spheroid, showing the ring of crystallites, and the absence of crystallites in the center of the spheroid, are provided in Figure S4 in the Supporting Information. For the \(\text{d-Asp}\)-containing samples in Figure 5b–e, only a single ring of crystallites is visible. The density of crystallites declines with increasing \(\text{d-Asp}\) mole fraction. At 1:1 \([\text{l-Asp}]:[\text{d-Asp}]\) (Figure 5f), essentially no crystallites are present. Evidently, the slower reaction rate, induced by increasing the concentration of \(\text{d-Asp}\), allows aspartate ions to diffuse out of the OCNF matrix into the surrounding aqueous solution before crystallization occurs. Thus, a sufficiently high rate of CP self-assembly is a necessary condition for in situ preparation of such composite hydrogels.

The kinetics of \(\text{Cu-Asp}\) self-assembly within the OCNF/SiNP hydrogel matrix were investigated in greater detail by image analysis. The experiment involved adding 200 \(\mu\)L of \(100 \times 10^{-3}\) \(\text{m aqueous Cu(NO}_3\text{)}_2\) to 100 \(\mu\)L of a dispersion containing OCNF, SiNP, and ligand, and collecting photomicrographs at 10 s intervals (Figure 6A). Crystallite propagation and

---

**Figure 5.** Photographs and photomicrographs of spheroids prepared from an OCNF dispersion containing \(5 \times 10^{-3}\) \(\text{m l-Asp}\) doped with \(\text{d-Asp}\) at concentrations of: a) 0 \(\text{m (control), b) 5 \times 10^{-3}\), c) \(10 \times 10^{-3}\), d) \(15 \times 10^{-3}\), e) \(20 \times 10^{-3}\), and f) \(50 \times 10^{-3}\) (racemic mixture).**

---

**Figure 6.** A) Schematic representation of time-lapse optical microscopy experiments. B) Relative image darkness (proportional to crystallite density), \(d\), as a function of time for 100 \(\mu\)L of OCNF dispersions with the following ligand concentrations: a) \(5 \times 10^{-3}\) \(\text{m l-Asp}\) only (measured in triplicate); b) \(50 \times 10^{-3}\) \(\text{m l-Asp} + 15 \times 10^{-3}\) \(\text{m d-Asp}\) (measured in triplicate) and c) \(5 \times 10^{-3}\) \(\text{m l-Asp} + 50 \times 10^{-3}\) \(\text{m d-Asp}\) (measured in duplicate). Arrows indicate the points at which crystallites of \(\text{Cu-Asp}\) were observed.
the increase in crystallite density with time are proportional to the darkening of greyscale photomicrographs. Relative image darkness, $d$, as a function of time, $t$, was determined as an indicator of crystallite density. The value of $d$ is the “mean gray value,” measured by image analysis using the “Measure” tool in ImageJ [37,38] and given in arbitrary units.

Figure 6B tracks $d$ for the OCNF dispersions containing l-Asp and varying amounts of d-Asp immediately after being submerged in aqueous Cu(NO$_3$)$_2$. For the control sample containing 50 × 10$^{-3}$ m l-Asp and 0 m d-Asp (a in Figure 6B), the first crystallites are observable at 70 s after submersion in Cu(NO$_3$)$_2$ at $t = 0$ s (see Figure S6 in the Supporting Information). The earliest observation of crystallites coincides with an inflection point in the plot (a in Figure 6B). We propose that during 0–70 s metastable coordination complexes form, an inflection point in the plot. We propose that during 0–70 s metastable coordination complexes form, causing mild darkening of the solution, and nucleation of Cu-Asp occurs at $\approx 70$ s. With respect to the 50 × 10$^{-3}$ m l-Asp and 15 × 10$^{-3}$ m d-Asp sample (b in Figure 6B), the inflection at $\approx 160$ s also corresponds to the observation of microscopic crystallites via an optical microscope (Figure S7, Supporting Information). For the sample containing equimolar amounts of l-Asp and d-Asp (c in Figure 6B), the plot displays an analogous broad inflection at $\approx 1000$–1100 s, where microscopic crystallites appear (Figure S8, Supporting Information). These results confirm retardation of the onset of Cu-Asp crystallization, which allows aspartate to diffuse from the hydrogel matrix into the surrounding aqueous medium during the preparation of spheroids.

### 3. Conclusion

A green chemistry method has been designed for the preparation of OCNF hydrogel spheroids incorporating a nanofibrous crystalline chiral CP. The present procedure is carried out at ambient temperature in water, with the CP self-assembly occurring in situ. In principle, this method could be used to prepare analogous hydrogel structures incorporating a variety of CPs provided a number of conditions are met, some of which have been established via the present investigation. First, rheological parameters including elasticity and toughness of the dispersion used to prepare the composite hydrogels must be carefully controlled to ensure the desired structures (e.g., concave/convex spheroids or elongated shapes) can be achieved. The ligand and a deprotonating agent (NaOH) were found to have a strong influence on the mechanical properties of the dispersion. Therefore, the final dispersion, composed of the OCNF/SiNP primary matrix, ligand, and deprotonating agents should be adjusted to achieve specific mechanical properties via, for instance, fine tuning the concentration of an additive such as SiNP. Importantly, the rate of CP self-assembly is key to achieving a high yield of entrapped crystallites. A high rate of self-assembly prevents diffusion of the free ligand out of the OCNF matrix. The understanding provided in this study could be rapidly exploited in other systems involving a divalent metal salt and organic ligand, where the CP or metal–organic framework self-assembles spontaneously. Provided specific conditions are met, the preparation of composite hydrogels of well-defined structure could be extended to different combinations of matrices, metal cations, and ligands to tailor specific functional properties in the composites. Earlier work on OCNF hydrogels demonstrates that these gels are stable, and in fact, increase in strength up to at least 80 °C, so we expect that these constructs will be similarly stable, enabling extension to a wider range of synthesis conditions and also facilitating applications as catalyst supports. Combining the vast array of existing CP materials with a stable OCNF hydrogel matrix foreshadows applications in fields ranging from controlled release to chiral separations as well as selective sorption and molecular storage.

### 4. Experimental Section

**Materials:** L-Asp, d-Asp, Cu(NO$_3$)$_2$, 3H$_2$O, and NaOH were purchased from Sigma-Aldrich and used as received. SiNP of diameter 157 ± 1 nm (Z-average) and dynamic light scattering (DLS) polydispersity index (intensity distribution) of 0.14 (DLS instrument details provided below) were purchased as silica powder from Sigma-Aldrich (product number 718483). The OCNF [39,42] was previously described [46] and was an 8 wt% paste of solids in water.

**OCNF:** The degree of oxidation, with respect to number of anhydroglucose units, was 25% by conductometric titration, average fibril radius 3.5 nm and average fibril length 160 nm. The OCNF was acidified to pH 3 using 1 m HCl (aq) and purified by dialysis against deionized water using Sigma-Aldrich cellulose dialysis tubing with a molecular weight cut-off of 12 400 Da. Dialysis was carried out for 3 days, with daily replacement of deionized water, after which the pH of the OCNF suspension was adjusted to 7 using 0.1 m NaOH (aq). A stock dispersion of 3.01 ± 0.06 wt% OCNF was prepared by mechanical shearing for 30 min at 10 000 rpm using an IKA T18 digital Ultra-Turrax, followed by sonication. Batches of 40 mL 3.01 ± 0.06 wt% OCNF were dispersed by sonication (instrument details and method provided below) for a total processing time of 60 min (~7500 J) per batch.

**SiNP:** A 10.04 ± 1 wt% stock dispersion was prepared by suspending SiNP in water and dispersing by sonication, using the method below, in 20 mL batches for a total processing time of 2 min (240 J) per batch.

**Preparation of the Primary Matrix:** The 3.01 ± 0.06 wt% OCNF and 10.04 ± 0.01 wt% SiNP stock dispersions were combined in appropriate proportions with water to prepare the primary (ligand-free) matrix comprising 1.00 ± 0.06 wt% OCNF and 5.02 ± 0.01 wt% SiNP. This dispersion was homogenized in batches of 10 mL by the sonication method below for a total processing time of 60 s (~120 J) per batch.

**Preparation of the Ligand-Containing Matrix:** L-Asp or d-Asp was combined with NaOH in water at 1:2 molar ratio of Asp:NaOH to deprotonate and solubilize the ligand. The 3.01 ± 0.06 wt% OCNF and 10.04 ± 0.01 wt% SiNP stock dispersions were combined in appropriate proportions with water, L-Asp and/or d-Asp, and NaOH to prepare the ligand-containing matrix comprising 1.00 ± 0.06 wt% OCNF, 5.02 ± 0.01 wt% SiNP, and specific concentrations of L-Asp and d-Asp. These dispersions were homogenized in batches of 10 mL by the sonication method below for a total processing time of 60 s (~120 J) per batch.

**Sonication:** All methods involving sonication employed an Ultrasonic Processor FB-505, Fisher, 200 W cm$^{-2}$, equipped with a 6 mm probe. The instrument was operated at 30% amplitude in the “1 s on 1 s off” pulse mode.

**DLS:** DLS measurements were carried out on dilute samples of SiNP (0.01 wt%) using a Malvern Zetasizer Nano ZSP. Samples were measured as an average of 4 measurements from 100 scans each. All DLS data were collected within 48 h of sample preparation.

**Preparation of Composite Hydrogel Spheres:** Droplets of 20 μL of the primary matrix or ligand-containing matrix were ejected via micropipette or syringe needle, at 10 mm above the air/water interface, into a bath of 100 × 10$^{-3}$ m Cu(NO$_3$)$_2$ to produce hydrogel spheroids.
Centrifugation: Two 20 mL dispersions of 1 wt% OCNF (dispersions A and B) were prepared. 1 mL of 100 × 10⁻³ M Cu(NO₃)₂ was added to dispersion A and 2 mL of 100 × 10⁻³ M Cu(NO₃)₂ solution was added to dispersion B and these dispersions were stirred for 1 h. Thereafter, 1 mL of 100 × 10⁻³ M l-Asp (200 × 10⁻¹ M NaOH) was added to dispersion A to produce 10 wt% Cu-Asp (with respect to OCNF dry content) and 2 mL of 100 × 10⁻³ M l-Asp (200 × 10⁻¹ M NaOH) was added to dispersion B to generate 20 wt% Cu-Asp. Bright blue Cu-Asp formed in both dispersions and was evenly distributed. These dispersions were subjected to centrifugation at 1660 × g (3000 rpm) for 15 min using a Thermo Scientific Heraeus Megafuge 16 equipped with TX-200 rotor with round buckets.

Powder X-Ray Diffraction: Data were collected on pure Cu-Asp crystallites (prepared by a previously reported[30] procedure) at ambient temperature using a STOE STADI P diffractometer, equipped with germanium monochromator and STOE IP-PSD image plate. The instrument was operated in transmission mode with Cu Kα (λ = 1.5406 Å) radiation. Data were collected in the 2θ range 2°–75° (4893 data points) and processed using WinXPOW.

SAXS and WAXS: SAXS and WAXS data were collected on a SANSpoint 2.0 (Anton Paar) equipped with Primux 100 micro Cu and Mo X-ray sources and a 2D EIGER R 1M hybrid photon counting detector (Dectris). Cu Kα radiation (λ = 1.542 Å) was used. Samples were loaded in PasteCell (Anton Paar) sample holders. Background data were collected using an empty sample cell and this background was subtracted from each sample dataset. Data reduction as (Iq) versus q was conducted using SAXSanalysis (Anton Paar) while background subtraction and data processing were performed using the Irena Package[40] within IGOR Pro (Wavemetrics, Inc.).

Rheology: Rheological measurements were performed on the OCNF-based primary and ligand-containing matrices using a stress-controlled rheometer (Discovery HR3, TA Instruments) equipped with sandblasted plate–plate geometry (40 mm). To avoid evaporation, the edges of samples were covered with low viscosity mineral oil and further sandblasted plate–plate geometry (40 mm). To ensure consistent sample history, followed by i) a strain sweep at constant ω = 1 rad s⁻¹ to ensure consistent sample history, followed by ii) a preshearing at 300 s⁻¹ for 30 s and optical microscopy was used to analyze the rate of gelation, TEMPO-oxidized cellulose nanofibrils, coordination polymers, hydrogels, ion-induced gelation, TEMPO-oxidized cellulose.

SEM: The SEM sample was prepared by cutting a cross-section of a hydrogel sphere prepared from a dispersion containing 1.00 ± 0.06 wt% OCNF, 5.02 ± 0.01 wt% SiNP, and 50 × 10⁻³ M l-Asp and allowing this cross-section to dry in air at room temperature. Images were collected using a JEOL JSM-6480LV scanning electron microscope.

Time-Lapse Optical Microscopy: An optical microscope, equipped with digital camera, was used to analyze the rate of Cu-Asp self-assembly in the OCNF dispersions. Dispersions were prepared containing 1.00 ± 0.06 wt% OCNF, 5.02 ± 0.01 wt% SiNP, 50 × 10⁻³ M l-Asp and:

1. 0 M d-Asp (measured in triplicate)
2. 15 × 10⁻³ M d-Asp (measured in triplicate)
3. 50 × 10⁻³ M d-Asp (measured in duplicate)

NaOH was incorporated at 2 molar equivalents per 1 molar equivalent of aspartic acid. For each of the ligand concentrations, 100 mL of the dispersion was place in a small cylindrical vessel. 200 μL of aqueous 100 × 10⁻³ M Cu(NO₃)₂ solution was added at t = 0 s and optical micrographs were collected at a rate of 1 image per 10 s. Image analysis was carried out using ImageJ.[37,38] For each set of images, “mean gray values,” as an indicator of Cu-Asp crystallite density, were determined, using the “Measure” tool, for a rectangular subregion of the image with consistent background and even crystallite distribution. Relative image darkness, d, is the “mean gray value” given in arbitrary units.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements
E.R.E. and V.C. contributed equally to this work. The authors thank Diana Lednitzky of the Material and Chemical Characterisation Facility (MC2) at the University of Bath (UoB) for the collection of SEM images. E.R.E. thanks the Commonwealth Rutherford Fellowships Programme for funding his postdoctoral fellowship and the UoB for institutional support. V.C thanks the UoB for funding his Ph.D. studentship. K.M.Z.H. thanks the EPSRC (EP/N033310/1) for funding his postdoctoral position. Data supporting this work is freely accessible in the UoB research data archive system at https://doi.org/10.15125/BATH-00905.

Conflict of Interest
The authors declare no conflict of interest.

Keywords
cellulose nanofibrils, coordination polymers, hydrogels, ion-induced gelation, TEMPO-oxidized cellulose

Received: March 18, 2020
Revised: July 31, 2020
Published online: September 30, 2020

[1] S. Van Vlierberge, P. Dubruel, E. Schacht, Biomacromolecules 2011, 12, 1387.
[2] R. M. A. Domingues, M. E. Gomes, R. L. Reis, Biomacromolecules 2014, 15, 2327.
[3] J. C. Courtenay, M. A. Johns, F. Galembeck, C. Deneke, E. M. Lanzoni, C. A. Costa, J. L. Scott, R. I. Sharma, Cellulose 2017, 24, 253.
[4] N. Mohammed, N. Grishkewich, K. C. Tam, Environ. Sci.: Nano 2018, 5, 623.
[5] K. Bello, B. K. Sarojini, B. Narayana, J. Polym. Res. 2019, 26, 62.
[6] H. Ullah, H. A. Santos, T. Khan, Cellulose 2016, 23, 2291.
[7] M. M. Abeer, M. C. I. Mohd Amin, C. Martin, J. Pharm. Pharmacol. 2014, 66, 1047.
[8] P. Bertsch, L. Schneider, G. Bovone, M. W. Tobbitt, P. Fischer, S. J. Gatzahl, ACS Appl. Mater. Interfaces 2019, 11, 38578.
[9] M. O. Haque, M. I. H. Mondal, in Cellulose-Based Superabsorbent Hydrogels (Ed: M. I. H. Mondal), Springer, Chem 2019, Ch. 45.
[10] M. Chau, S. E. Sriskandha, D. Pichugin, H. Thérien-Aubin, D. Nykypanchuk, G. Chauve, M. Méthot, J. Bouchard, O. Gang, E. Kumacheva, Biomacromolecules 2015, 16, 2455.
[11] P. Bertsch, S. Isabettini, P. Fischer, Biomacromolecules 2017, 18, 4060.
[12] H. Dong, J. F. Snyder, K. S. Williams, J. W. Andzelm, Biomacromolecules 2013, 14, 3338.
[13] L. Mendoza, W. Batchelor, R. F. Tabor, G. Garner, J. Colloid Interface Sci. 2018, 509, 39.
[14] J. Schmitt, V. Calabrese, M. A. Da Silva, S. Lindhoud, V. Alfredsson, J. L. Scott, K. J. Edler, Phys. Chem. Chem. Phys. 2018, 20, 16012.
[15] A. B. Fall, S. B. Lindström, O. Sundman, L. Ödberg, L. Wågberg, *Langmuir* **2011**, *27*, 11332.
[16] K. M. O. Håkansson, A. B. Fall, F. Lundell, S. Yu, C. Krywka, S. V. Roth, G. Santoro, M. Kwick, L. Prahl Wittberg, L. Wågberg, L. D. Söderberg, *Nat. Commun.* **2014**, *5*, 4018.
[17] G. K. E. Scriba, *TrAC, Trends Anal. Chem.* **2019**, *120*, 115639.
[18] X. Sun, L. Yang, Q. Li, J. Zhao, X. Wang, H. Liu, *Chem. Eng. J.* **2014**, *241*, 175.
[19] S. Peng, H. Meng, Y. Ouyang, J. Chang, *Ind. Eng. Chem. Res.* **2014**, *53*, 2106.
[20] K. Junka, J. Guo, I. Filpponen, J. Laine, O. J. Rojas, *Biomacromolecules* **2014**, *15*, 876.
[21] P. Kulpinski, A. Erdman, T. Grzyb, S. Lis, *Polym. Compos.* **2016**, *37*, 153.
[22] C. T. Chen, K. S. Suslick, *Coord. Chem. Rev.* **1993**, *128*, 293.
[23] R. Bruno, N. Marino, L. Bartella, L. Di Donna, G. De Munno, E. Pardo, D. Armentano, *Chem. Commun.* **2018**, *54*, 6356.
[24] S. Horike, S. Shimomura, S. Kitagawa, *Nat. Chem.* **2009**, *1*, 695.
[25] D. Maspoch, D. Ruiz-Molina, J. Veciana, *J. Mater. Chem.* **2004**, *14*, 2713.
[26] X. Zhang, W. Wang, Z. Hu, G. Wang, K. Uvdal, *Coord. Chem. Rev.* **2015**, *284*, 206.
[27] B. Xing, M. F. Choi, B. Xu, *Chem. Eur. J.* **2002**, *8*, 5028.
[28] K. J. De France, F. Xu, T. Hoare, *Adv. Healthcare Mater.* **2018**, *7*, 1700927.
[29] H. Zhu, Q. Zhang, S. Zhu, *ACS Appl. Mater. Interfaces* **2016**, *8*, 17395.
[30] I. Imaz, M. Rubio-Martinez, W. J. Saletra, D. B. Amabilino, D. Maspoch, *J. Am. Chem. Soc.* **2009**, *131*, 18222.
[31] H. Wu, C. Tian, Y. Zhang, C. Yang, S. Zhang, Z. Jiang, *Chem. Commun.* **2015**, *51*, 6329.
[32] P. T. Anastas, J. C. Warner, *Green Chemistry: Theory and Practice*, Oxford University Press, New York **1998**, p. 30.
[33] L. Geng, X. Peng, C. Zhan, A. Naderi, P. R. Sharma, Y. Mao, B. S. Hsiao, *Cellulose* **2017**, *24*, 5417.
[34] V. Calabrese, M. A. da Silva, L. Porcar, S. Bryant, K. M. Z. Hossain, J. L. Scott, K. J. Edler, *Soft Matter* **2020**, *16*, 3303.
[35] T. Saito, S. Kimura, Y. Nishiyama, A. Isogai, *Biomacromolecules* **2007**, *8*, 2485.
[36] A. Fiorati, N. Contessi Negrini, E. Baschenis, L. Altomare, S. Faré, A. Giacometti Schieroni, D. Piovani, R. Mendichi, M. Ferro, F. Castiglione, A. Mele, C. Punta, L. Melone, *Materials* **2020**, *13*, 183.
[37] C. A. Schneider, W. S. Rasband, K. W. Eliceiri, *Nat. Methods* **2012**, *9*, 671.
[38] J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J. Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak, A. Cardona, *Nat. Methods* **2012**, *9*, 676.
[39] V. Calabrese, J. C. Muñoz-García, J. Schmitt, M. A. da Silva, J. L. Scott, J. Angulo, Y. Z. Khimyak, K. J. Edler, *J. Colloid Interface Sci.* **2019**, *535*, 205.
[40] J. Ilavsky, P. R. Jemian, *J. Appl. Crystallogr.* **2009**, *42*, 347.