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TITLE:

Synthesis Method for Cellulose Nanofiber Biotemplated Palladium Composite Aerogels

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SHORT ABSTRACT:

A synthesis method for cellulose nanofiber biotemplated palladium composite aerogels is presented. The resulting composite aerogel materials offer potential for catalysis, sensing, and hydrogen gas storage applications.

LONG ABSTRACT:

Here, a method to synthesize cellulose nanofiber biotemplated palladium composite aerogels is presented. Noble metal aerogel synthesis methods often result in fragile aerogels with poor shape control. The use of carboxymethylated cellulose nanofibers (CNFs) to form a covalently bonded hydrogel allows for the reduction of metal ions such as palladium on the CNFs with control over both nanostructure and macroscopic aerogel monolith shape after supercritical drying. Crosslinking the carboxymethylated cellulose nanofibers is achieved using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in the presence of ethylenediamine. The CNF hydrogels maintain their shape throughout synthesis steps including covalent crosslinking, equilibration with precursor ions, metal reduction with high concentration reducing agent, rinsing in water, ethanol solvent exchange, and CO₂ supercritical drying. Varying the precursor palladium ion concentration allows for control over the metal content in the final aerogel

composite through a direct ion chemical reduction rather than relying on the relatively slow coalescence of pre-formed nanoparticles used in other sol-gel techniques. With diffusion as the basis to introduce and remove chemical species into and out of the hydrogel, this method is suitable for smaller bulk geometries and thin films. Characterization of the cellulose nanofiber-palladium composite aerogels with scanning electron microscopy, X-ray diffractometry, thermal gravimetric analysis, nitrogen gas adsorption, electrochemical impedance spectroscopy, and cyclic voltammetry indicates a high surface area, metallized palladium porous structure.

INTRODUCTION:

Aerogels, first reported by Kistler, offer porous structures orders of magnitude less dense than their bulk material counterparts¹⁻³. Noble metal aerogels have attracted scientific interest for their potential in power and energy, catalytic, and sensor applications. Noble metal aerogels have recently been synthesized via two basic strategies. One strategy is to induce the coalescence of pre-formed nanoparticles⁴⁻⁷. Sol-gel coalescence of nanoparticles can be driven by linker molecules, changes in solution ionic strength, or simple nanoparticle surface free energy minimization⁷⁻⁹. The other strategy is to form aerogels in a single reduction step from metal precursor solutions⁹⁻¹³. This approach has also been used to form bimetallic and alloy noble metal aerogels. The first strategy is generally slow and may require up to many weeks for nanoparticle coalescence¹⁴. The direct reduction approach, while generally more rapid, suffers from poor shape control over the macroscopic aerogel monolith.

One possible synthesis approach to address challenges with control of noble metal aerogel macroscopic shape and nanostructure is to employ biotemplating¹⁵. Biotemplating uses biological molecules ranging from collagen, gelatin, DNA, viruses, to cellulose to provide a shape-directing template for the synthesis of nanostructures, where the resulting metal-based nanostructures assume the geometry of the biological template molecule^{16,17}. Cellulose nanofibers are appealing as a biotemplate given the high natural abundance of cellulosic materials, their high aspect ratio linear geometry, and ability to chemically functionalize their glucose monomers¹⁸⁻²³. Cellulose nanofibers (CNF) have been used to synthesize three dimensional TiO₂ nanowires for photoanodes²⁴, silver nanowires for transparent paper electronics²⁵, and palladium aerogel composites for catalysis²⁶. Further, TEMPO-oxidized cellulose nanofibers have been used both as a biotemplate and reducing agent in the preparation of palladium decorated CNF aerogels²⁷.

Here, a method to synthesize cellulose nanofiber biotemplated palladium composite aerogels is presented²⁶. Fragile aerogels with poor shape control occurs for a range noble metal aerogel synthesis methods. Carboxymethylated cellulose nanofibers (CNFs) used to form a covalent hydrogel allow for the reduction of metal ions such as palladium on the CNFs providing control over both nanostructure and macroscopic aerogel monolith shape after supercritical drying. Carboxymethylated cellulose nanofiber crosslinking is achieved using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in the presence of ethylendiamine as a linker molecule between CNFs. The CNF hydrogels maintain their shape throughout the synthesis steps including covalent crosslinking, equilibration with precursor ions, metal reduction with high concentration reducing agent, rinsing in water, ethanol solvent exchange, and CO₂ supercritical

drying. Precursor ion concentration variation allows for control over the final aerogel metal content through a direct ion reduction rather than relying on the relatively slow coalescence of pre-formed nanoparticles used in sol-gel methods. With diffusion as the basis to introduce and remove chemical species into and out of the hydrogel, this method is suitable for smaller bulk geometries and thin films. Characterization of the cellulose nanofiber-palladium composite aerogels with scanning electron microscopy, X-ray diffractometry, thermal gravimetric analysis, nitrogen gas adsorption, electrochemical impedance spectroscopy, and cyclic voltammetry indicates a high surface area, metalized palladium porous structure.

PROTOCOL:

CAUTION: Consult all relevant safety data sheets (SDS) before use. Use appropriate safety practices when performing chemical reactions, to include the use of a fume hood and personal protective equipment (PPE). Rapid hydrogen gas evolution can cause high pressure in reaction tubes causing caps to pop and solutions to spray out. Ensure that reaction tubes remain open and pointed away from the experimenter as specified in the protocol.

1. Cellulose nanofiber hydrogel preparation

1.1. Preparation of cellulose nanofiber solution: Prepare 3% (w/w) cellulose nanofiber solution by mixing 1.5 g of carboxymethyl cellulose nanofibers with 50 mL of deionized water. Shake the solution and vortex for 1 min. Sonicate the solution in a bath sonicator at ambient temperature for 24 h to ensure complete mixing.

1.2. Preparation of cross-linking solution: First add 0.959 g of EDC and 0.195 g of 2-(N-morpholino)ethanesulfonic acid (MES) buffer to 2.833 mL of deionized water. Vortex. Add 0.167 mL of ethylenediamine. Vortex for 15 s. Adjust the final volume to 10 mL and pH to 4.5 by adding 1.0 M HCl and deionized water.

NOTE: Final crosslinking solution concentrations are 0.5 M EDC, 0.25 M ethylenediamine, and 0.1 M MES buffer.

1.3. Centrifugation of cellulose nanofiber solution: Pipette 0.25 mL of the 3 % (w/w) cellulose nanofiber solution into each of 6 microfuge tubes (1.7 mL or 2.0 mL). Centrifuge the microfuge tubes for 20 min at 21,000 x g. Remove excess water above the compacted CNFs with a pipette avoiding contact with the top surface.

NOTE: After centrifuging, the cellulose nanofiber solutions present a distinct interface between the concentrated CNF's and the clear supernatant. Based on removal of the excess water, the final CNF concentration will be approximately 3.8%.

1.4. Cross-link the cellulose nanofiber hydrogels. Pipette 1.0 mL of the EDC and diamine crosslinking solution above the compacted cellulose nanofibers in each of the microfuge tubes. Wait at least 24 h for the crosslinking solution to diffuse through the gels and crosslink the CNFs.

1.5. Gel rinsing: Remove the crosslinking solution supernatant in the microfuge tubes with a pipette. With the microfuge tube caps open, immerse the microfuge tubes containing the crosslinked CNF gels in 1 L of deionized water for at least 24 h to remove excess crosslinking solution from within the CNF hydrogels.

1.6. Fourier-transform infrared (FTIR) spectroscopy: Place approximately 0.5 mL of 3% (w/w) CNF solution in deionized water on the sample stage and scan percent transmittance for 650 – 4000 cm^{-1} . Use the same scan conditions and repeat for a CNF crosslinked hydrogel from Step 1.5.

2. Preparation of cellulose nanofiber - palladium composite hydrogels

2.1. Prepare $\text{Pd}(\text{NH}_3)_4\text{Cl}_2$ solution. Prepare 10 mL of 1.0 M $\text{Pd}(\text{NH}_3)_4\text{Cl}_2$ solution. Vortex the solution for 15 s. Dilute 1.0 M $\text{Pd}(\text{NH}_3)_4\text{Cl}_2$ solution to 1 mL volumes at 1, 10, 50, 100, 500, and 1000 mM.

NOTE: 1.0 M NaPdCl_4 solution and respective dilutions may be used and results in similar final aerogel structures.

2.2. Equilibrate cellulose nanofiber hydrogels in palladium solutions. Pipette 1 mL of the 1, 10, 50, 100, 500, and 1000 mM $\text{Pd}(\text{NH}_3)_4\text{Cl}_2$ solutions on the top of the cellulose nanofiber hydrogels in the microfuge tubes. Wait at least 24 h for the palladium solution to equilibrate within the hydrogels.

2.3. Prepare NaBH_4 reducing agent solution. Prepare 60 mL of 2 M NaBH_4 solution. Aliquot 10 mL of NaBH_4 solution into each of six 15 mL conical tubes.

NOTE: The 2 M NaBH_4 solution is a highly concentrated reducing agent solution and should be handled within a chemical fume hood. Spontaneous decomposition and hydrogen gas evolution will be observed. Ensure that the tubes are pointed away from the experimenter and that proper PPE is worn.

2.4. First reduction of palladium salts on cellulose nanofiber hydrogels: Invert the microfuge tubes with the palladium equilibrated CNF hydrogels and gently tap to remove the hydrogels. In a chemical fume hood, with flat tweezers, place each of the palladium equilibrated CNF hydrogels into each of the 15 mL conical tubes with 10 mL of NaBH_4 solution. Allow the reduction to proceed for 24 h.

NOTE: Upon placing the palladium equilibrated CNF gels into the 2 M NaBH_4 solution, violent hydrogen gas evolution will occur. Ensure that reaction tubes remain open and pointed away from the experimenter.

2.5. Prepare second NaBH_4 reducing agent solution. Prepare 60 mL of 0.5 M NaBH_4 solution.

Aliquot 10 mL of NaBH₄ solution into each of six 15 mL conical tubes.

2.6. Second reduction of palladium salts on cellulose nanofiber hydrogels: In a fume hood, using a pair of flat tweezers transfer each of hydrogels from the 2 M NaBH₄ solutions into the 0.5 M NaBH₄ solutions. Allow the reduction to proceed for 24 h.

NOTE: The initially reduced CNF gels in the 2 M NaBH₄ solution will be mechanically stable during the transfer step. However, light pressure should be used with the flat tweezers during solution transfer steps to avoid gel compaction.

2.7. Rinse the cellulose nanofiber-palladium composite gels. Using flat tweezers, transfer each of the reduced palladium-CNF gels into 50 mL deionized water in conical tubes. Exchange deionized water after 12 h and allow the gels to rinse for at least an additional 12 h.

2.8. Perform ethanol solvent exchange in cellulose nanofiber-palladium gels. Use flat tweezers to transfer the rinsed CNF-palladium gels successively into 50 mL of 25%, 50%, 75%, and 100% ethanol solutions with at least 6 h in each solution.

3. Aerogel preparation

3.1. After solvent exchange with ethanol, dry the CNF-palladium gels using CO₂ in a supercritical dryer with a set point of 35 °C and 1200 psi. After supercritical drying is complete, allow the chamber to equilibrate for at least 12 h prior to opening and removal of the aerogels.

NOTE: Occasionally, the 500 mM and 1000 mM samples have been observed to combust when removed from the supercritical dryer which is attributed to the presence of palladium hydride. The 12 h supercritical chamber equilibration is intended to allow for outgassing of hydrogen.

4. Composite aerogel material characterization

4.1. Scanning electron microscopy (SEM): Cut the CNF-palladium aerogel with a razor blade to obtain a thin film approximately 1 – 2 mm thick. Affix the thin film sample with carbon tape on a SEM sample stub. Initially use an accelerating voltage of 15 kV and beam current of 2.7 – 5.4 pA to perform imaging.

4.2. X-ray diffractometry (XRD): Place the CNF-palladium aerogel in a sample holder and align the top of the aerogel with the top of holder. Alternatively, place a thin film sample section, as in Step 4.1, on a glass slide. Perform XRD scans for diffraction angles 2 θ from 5 ° to 90 ° at 45 kV and 40 mA with Cu K α radiation (1.54060 Å), a 2 θ step size of 0.0130 °, and 20 s per step.

4.3. Thermal gravimetric analysis (TGA): Place the aerogel sample in the instrument crucible. Perform analysis by flowing nitrogen gas at 60 mL/min and heating at 10 °/min from ambient temperature to 700 °C.

4.4. Nitrogen gas adsorption-desorption: Degas the samples for 24 h at room temperature. Use nitrogen at -196 °C as the test gas with equilibration times for adsorption and desorption of 60 s and 120 s, respectively.

NOTE: Elevated degas temperatures are not recommended to avoid decomposition of the cellulose nanofibers.

4.5. Electrochemical characterization.

4.5.1. Immerse the aerogel samples in 0.5 M H₂SO₄ electrolyte for 24 h.

4.5.2. Use a 3-electrode cell with a Ag/AgCl (3 M NaCl) reference electrode, a 0.5 mm diameter Pt wire auxiliary/counter electrode, and a lacquer coated 0.5 mm diameter platinum working electrode. Place the lacquer coated wire with a 1 mm exposed tip in contact with the top surface of the aerogel at the bottom of the electrochemical vial¹².

4.5.3. Perform electrochemical impedance spectroscopy (EIS) from 1 MHz to 1 mHz with a 10 mV sine wave.

4.5.4. Perform cyclic voltammetry (CV) using a voltage range of -0.2 to 1.2 V (vs. Ag/AgCl) with scan rates of 10, 25, 50, 75, and 100 mV/s.

REPRESENTATIVE RESULTS:

The scheme to covalently crosslink cellulose nanofibers with EDC in the presence of ethylenediamine is depicted in **Figure 1**. EDC crosslinking results in an amide bond between a carboxyl and primary amine functional group. Given that the carboxymethyl cellulose nanofibers possess only carboxyl groups for crosslinking, the presence of a diamine linker molecule such as ethylenediamine is essential to covalently link two adjacent CNFs via two amide bonds. To confirm crosslinking, **Figure 2** shows FTIR spectra for 3% (w/w) CNF solutions compared to CNF hydrogels after crosslinking with using EDC in the presence of ethylenediamine. Both CNF and crosslinked CNF hydrogels were equilibrated in deionized water prior to FTIR analysis. The 3% (w/w) CNF solution presents a broad peak between approximately 3200 to 3600 cm⁻¹ and is attributed to O-H stretching²⁸. The prominent peak at 1595 cm⁻¹ is likely attributed to the vibration of the -COO⁻Na⁺ groups on the carboxymethyl cellulose nanofibers²⁹. After crosslinking the carboxymethyl cellulose nanofibers with EDC in the presence of ethylenediamine, three bonding possibilities result. The first is an effective crosslink between two CNF's with ethylenediamine forming two amide bonds with carboxylates on the CNF's. The second is ethylenediamine forming a single amide bond with a CNF carboxylate with a primary amine at the other end of the diamine molecule. The third possibility is EDC forming an unstable o-acylisourea intermediate that hydrolyzes to reform the initial carboxyl group³⁰.

After crosslinking, the broad O-H absorption band between 3200 to 3600 cm⁻¹ decreases, with the emergence of prominent peaks at 3284 and 3335 cm⁻¹, attributed to primary amines and amide bonds resulting from both amides of crosslinked CNFs, and single amide bonds between

CNFs and ethylenediamine resulting in a primary amine at the terminal end of the ethylenediamine molecule^{28,31}. The peak at 2903 cm⁻¹ associated with C-H stretching becomes more prominent after crosslinking and is attributed to the increased presence of -NH₃⁺ from the terminal primary amines. The decrease in the carbonyl stretch at a wavenumber of 1595 cm⁻¹ is attributed to the decreased number of -COO⁻Na⁺ groups due to crosslinking with ethylenediamine. The formation of amide bonds due to crosslinking is seen in the amide peaks at 1693 and 1668 cm⁻¹, as well as at 1540 cm⁻¹, and a small peak at 1236 cm⁻¹ ^{28,29,31}.

Figure 3 depicts photographs of each of the synthesis steps to include: the covalently crosslinked CNF hydrogels (**Figure 3a**); CNFs equilibrated across a concentration range of 1, 10, 50, 100, 500, and 1000 mM Pd(NH₃)₄Cl₂ (**Figure 3b**), or Na₂PdCl₄ (**Figure 3c**) solution; reduced CNF-palladium gels (**Figure 3d**); and supercritically dried aerogel composites (**Figure 3e**). The photographs demonstrate the shape control offered by this synthesis method.

The SEM images in **Figure 4a-f** depict composite CNF-palladium aerogels synthesized from 1, 10, 50, 100, 500, and 1000 mM Pd(NH₃)₄Cl₂ solutions, respectively. In general, the aerogels present interconnected fibrillary ligaments with increasing nanoparticle size correlating with increasing palladium solution concentration. The average nanoparticle diameters and pore sizes for lower concentration samples are: 1 mM) 12.6 ± 2.2 nm and 32.4 ± 13.3 nm; and 10 mM) 12.4 ± 2.0 nm and 32.2 ± 10.4 nm. Aerogels synthesized with 50 mM and higher palladium concentrations present more distinctly interconnected nanoparticles. The average nanoparticle diameters resulting from 50, 100, 500, and 1000 mM palladium synthesis concentrations are 19.5 ± 5.0 nm, 41.9 ± 10.0 nm, 45.6 ± 14.6 nm, and 59.0 ± 16.4 nm, respectively.

XRD spectra for 2θ angles from 20 – 70 ° in **Figure 5** indicate peaks for palladium and palladium hydride indexed to Joint Committee on Powder Diffraction Standards (JCPDS) reference numbers 01-087-0643 and 00-018-0951, respectively. The palladium hydride and palladium peaks become more convoluted with increasing palladium synthesis concentration, where they are not distinguishable at 1000 mM. The decrease in peak broadening correlates with the increase in nanoparticle diameters observed in **Figure 4**.

The thermogravimetric spectra shown in **Figure 6** indicate increasing metal content in the CNF-palladium composite aerogels with increasing synthesis palladium solution concentration. The weight% versus palladium synthesis concentration shown in **Figure 6c** demonstrates control of metal content in the aerogel composite between 0 – 75.5%.

Nitrogen adsorption-desorption isotherms, and corresponding cumulative pore volumes with differential pore volume are shown for aerogel composites synthesized from 1, 100, and 1000 mM palladium solutions in **Figure 7a-b**, **Figure 7c-d**, and **Figure 7e-f**, respectively. The physisorption data indicates type IV adsorption-desorption isotherms indicating mesoporous and macroporous structure. The Brunauer-Emmett-Teller (BET) specific surface areas were 582, 456, and 171 m²/g for the 1, 100, and 1000 mM palladium samples, respectively, indicating decreasing specific surface area with increasing metal content³². Barrett-Joyner-Halenda (BJH) pore size analysis also indicates that as the aerogel palladium content increases, there is a decreasing

frequency of mesopores³³. Using BJH analysis of the desorption curves, the cumulative pore volumes (V_{pore}) for the 1, 100 and 1000 mM samples were 7.37 cm³/g, 6.10 cm³/g, and 2.40 cm³/g. Average sample specific volumes (V_{sample}) were determined by measuring the volume and dividing by the sample mass. Aerogel porosities were 97.3%, 95.0%, and 90.4% for the 1, 100, and 1000 mM, respectively using Equation (1),

$$\% \text{ Porosity} = (V_{\text{pore}} / V_{\text{sample}}) \times 100 \% \quad (1)$$

With the same starting CNF covalent hydrogel and pore size distribution, sample porosities decrease with increasing metal content as the reduced metal fills the pore space.

Figure 8a shows the EIS spectra conducted in 0.5 M H₂SO₄ using a 10 mA amplitude sine wave across a frequency range of 140 kHz to 15 mHz. The incomplete semicircle in the high frequency region shown in **Figure 8b** indicates low charge transfer resistance and double layer capacitance for the CNF-palladium composite aerogel. CV scans performed in 0.5 M H₂SO₄ from -0.2 V to 1.2 V (vs Ag/AgCl) at scan rates of 10, 25, 50, and 75 mV/s are shown in **Figure 8c**, with the 10 mV/s scan shown separately in **Figure 8d**. The CV scans indicate hydrogen adsorption and desorption at potentials less than 0 V, as well as characteristic oxidation and reduction peaks for palladium greater than 0.5 V.

FIGURE AND TABLE LEGENDS:

Figure 1. Aerogel synthesis scheme. (a) Cross linking carboxymethyl cellulose nanofibers (CNF) with EDC and ethylenediamine as a linker molecule. (b, c) Cross-linked carboxymethyl cellulose nanofibers. (d) CNF hydrogel equilibrated with palladium salt solution. (e) CNF biotemplated palladium composite aerogel after reduction with NaBH₄, rinsing, solvent exchange with ethanol, and CO₂ supercritical drying. Reproduced from reference 26 with permission.

Figure 2. FTIR spectra for 3% (w/w) carboxymethyl cellulose nanofiber (CNF) solution in deionized water and CNF hydrogels crosslinked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in the presence of ethylenediamine and subsequently equilibrated in deionized water.

Figure 3. Aerogel synthesis process photos. (a) Cross-linked carboxymethyl cellulose nanofiber hydrogels with EDC and ethylenediamine as a linker molecule. CNF hydrogels equilibrated with palladium salt solutions of 1, 10, 50, 100, 500, and 1000 mM for (b) Pd(NH₃)₄Cl₂, and (c) Na₂PdCl₄. (d) CNF biotemplated palladium aerogel after reduction with NaBH₄. (e) CNF-Pd composite aerogels after rinsing, solvent exchange with ethanol, and CO₂ supercritical drying. Reproduced from reference 26 with permission.

Figure 4. Scanning electron microscopy images of CNF-Pd composite aerogels prepared from Pd(NH₃)₄Cl₂ concentrations of (a) 1 mM; (b) 10 mM; (c) 50 mM; (d) 100 mM; (e) 500 mM; and (f) 1000 mM. Reproduced from reference 26 with permission.

Figure 5. X-ray diffraction spectra for CNF-Pd composite aerogels synthesized from Pd(NH₃)₄Cl₂

salt solution concentrations of 1 mM, 10 mM, 50 mM, 100 mM, 500 mM, and 1000 mM. JCPDS reference 00-018-0951 palladium hydride peak positions are indicated with a light blue dashed line, and dashed gray lines for 01-087-0643 palladium peak positions. Reproduced from reference 26 with permission.

Figure 6. Thermogravimetric analysis (TGA). (a) TGA of aerogels synthesized with $\text{Pd}(\text{NH}_3)_4\text{Cl}_2$ salt solutions. (b) TGA of 50 mM $\text{Pd}(\text{NH}_3)_4\text{Cl}_2$ sample from (a) with differential thermal analysis (DTA). (c) Palladium sample mass at 600 °C from (a) for the varying palladium concentrations. Reproduced from reference 26 with permission.

Figure 7. Brunauer-Emmett-Teller analysis. Nitrogen adsorption–desorption isotherms, and pore size distribution with cumulative pore volume for aerogels synthesized with $\text{Pd}(\text{NH}_3)_4\text{Cl}_2$ salt solutions of (a,b) 0 mM, (c,d) 100 mM and (e,f) 1000 mM. Reproduced from reference 26 with permission.

Figure 8. Electrochemical characterization in 0.5 M H_2SO_4 of CNF-Pd aerogels prepared from 1000 mM $\text{Pd}(\text{NH}_3)_4\text{Cl}_2$. (a) Electrochemical impedance spectroscopy with a 10 mV sine wave was used across frequencies from 140 kHz to 15 mHz. (b) High frequency spectra from 140 kHz to 1.3 kHz from (a). (c) Cyclic voltammetry (CV) at scan rates of 10, 25, 50, and 75 mV/s. (d) CV scan at 10 mV/s from (c). Reproduced from reference 26 with permission.

DISCUSSION:

The noble metal cellulose nanofiber biotemplated aerogel synthesis method presented here results in stable aerogel composites with tunable metal composition. The covalent crosslinking of the compacted cellulose nanofibers after centrifugation results in hydrogels that are mechanically durable during the subsequent synthesis steps of palladium ion equilibration, electrochemical reduction, rinsing, solvent exchange, and supercritical drying. The hydrogel stability is vital during the electrochemical reduction step given the high concentration (2 M NaBH_4) of reducing agent solution and consequent violent hydrogen evolution. The commercially purchased TEMPO-oxidized cellulose nanofibrils used in this study had a nominal $-\text{COO}^-\text{Na}^+$ density of 1.2 mmol/g with approximate length and width of the cellulose nanofibers of 300 and 10 nm, respectively, and the 3% (w/w) solutions had a pH of 5. Likely due to the short fiber length, crosslinking at concentrations 3% (w/w) and less did not result in stable hydrogels. Centrifuging the 3% (w/w) solutions to compact the fibers to an approximate concentration of 3.8% (w/w) resulting in well crosslinked hydrogels that were stable during the electrochemical reduction of palladium step. The high NaBH_4 concentration is necessary to drive the reducing agent diffusion into the hydrogel biotemplate. The preservation of the covalent hydrogel macroscopic shape and biotemplated mesoporous structure is a key advantage of this synthesis method. In the absence of covalent crosslinking using EDC in the presence of a diamine linker, compacted ionic CNF hydrogels disaggregate during the chemical reduction step. Further, no palladium nanoparticles were observed to diffuse away from the CNF-palladium aerogel composites during the reduction step suggesting that all of the reduced palladium is bound within the resulting aerogels.

Critical to synthesizing homogenous aerogel composites is to allow sufficient time for diffusion

in each of the synthesis steps. Using shorter times than indicated in the protocol will result in unstable gels and incomplete metallization throughout the cross-section of the aerogels. This manifests in disaggregation during the reduction, rinsing, solvent exchange, and drying steps, and a ring-like metallization pattern in the aerogel cross-section with metallization near the outer surface and incomplete metallization, or bare cellulose toward the center of the monolith.

The presented synthesis method's primary benefit is the ability to control the aerogel monolith shape, control the composite aerogel metal content, and achieve a high surface area mesoporous structure. Material characterization with SEM, XRD, TGA, nitrogen gas adsorption, EIS, and CV indicate meaningful and reproducible results that correlate well with the nanostructures observed with SEM. Further, other noble metal salts such as $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, K_2PtCl_4 , $\text{Pt}(\text{NH}_3)_4\text{Cl}_2$, and Na_2PtCl_6 may be employed to achieve similar noble metal composite aerogels¹¹.

The protocol may be varied by changing the shape of the cellulose nanofiber covalent hydrogel template. Compacted CNFs may be shaped into flat films through spin coating, or conformally applied to arbitrary geometries and then crosslinked and processed in accordance with the presented method. The primary limitation of the method is the dependence of each synthesis step on the diffusion time of chemical species correlating with the thickness of the biotemplate hydrogel, and consequent diffusion path length. This poses a practical limit on the size and thickness of the resulting aerogels. Future work includes mass transfer modeling to determine the practical limits of the synthesis method based on diffusion, as well as convective flow approaches to overcome these limitations. Another potential issue with extended use of the CNF-palladium aerogel composite for catalytic applications is palladium leaching with detachment of palladium nanoparticles from the CNF template.

The synthesis method presented here offers an advancement in mechanically stable, shape-controlled, high surface area composite noble metal aerogels with tunable metal content. The covalent cellulose nanofiber hydrogels provide a material synthesis approach for a range of metal composites for energy, catalysis, and sensor applications.

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DISCLOSURES:

The authors have nothing to disclose.

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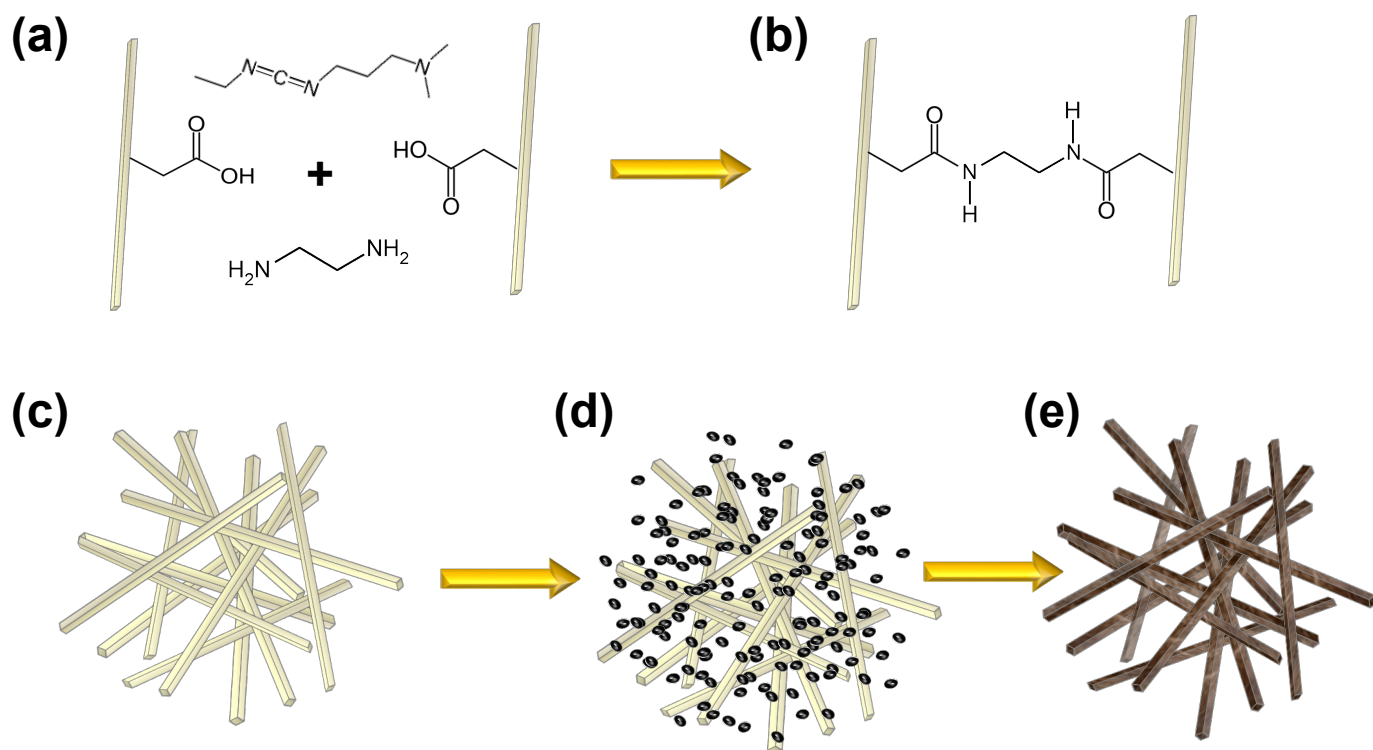
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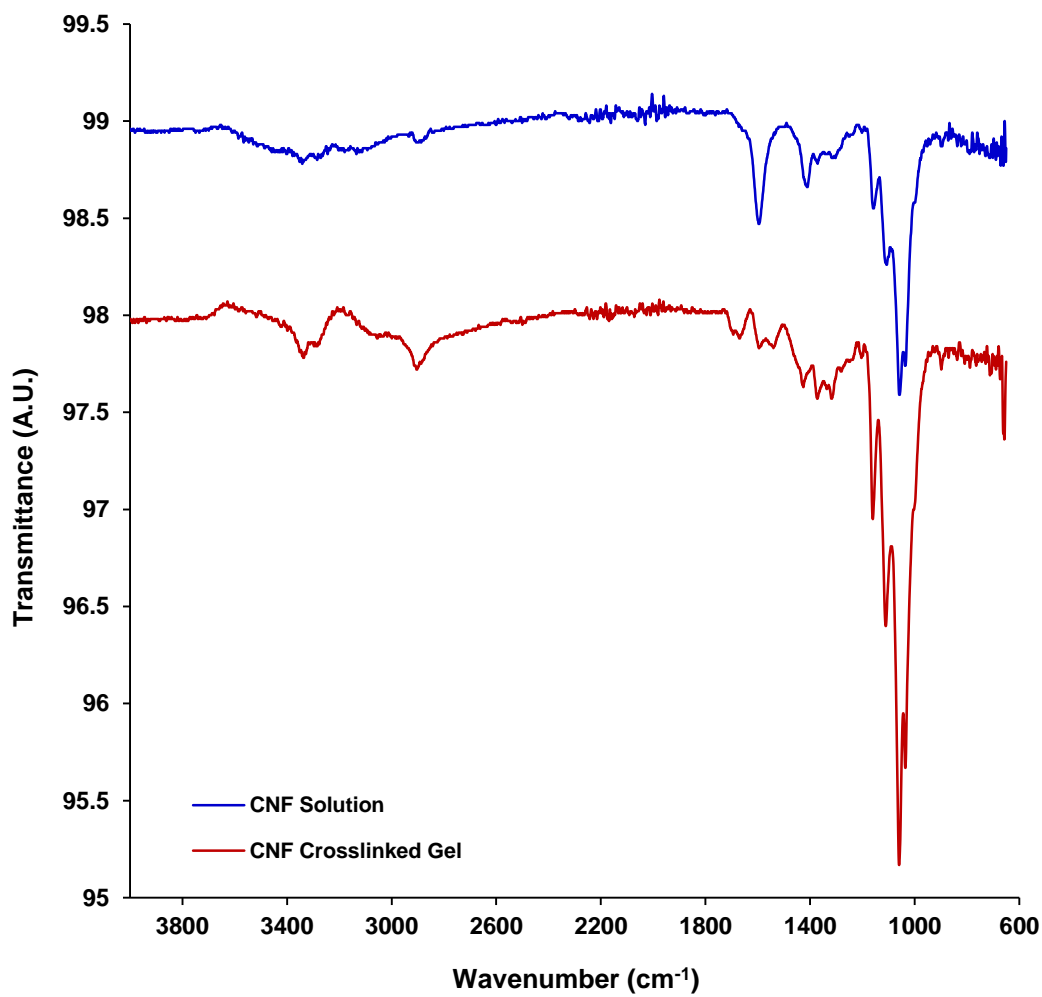
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Figure 1

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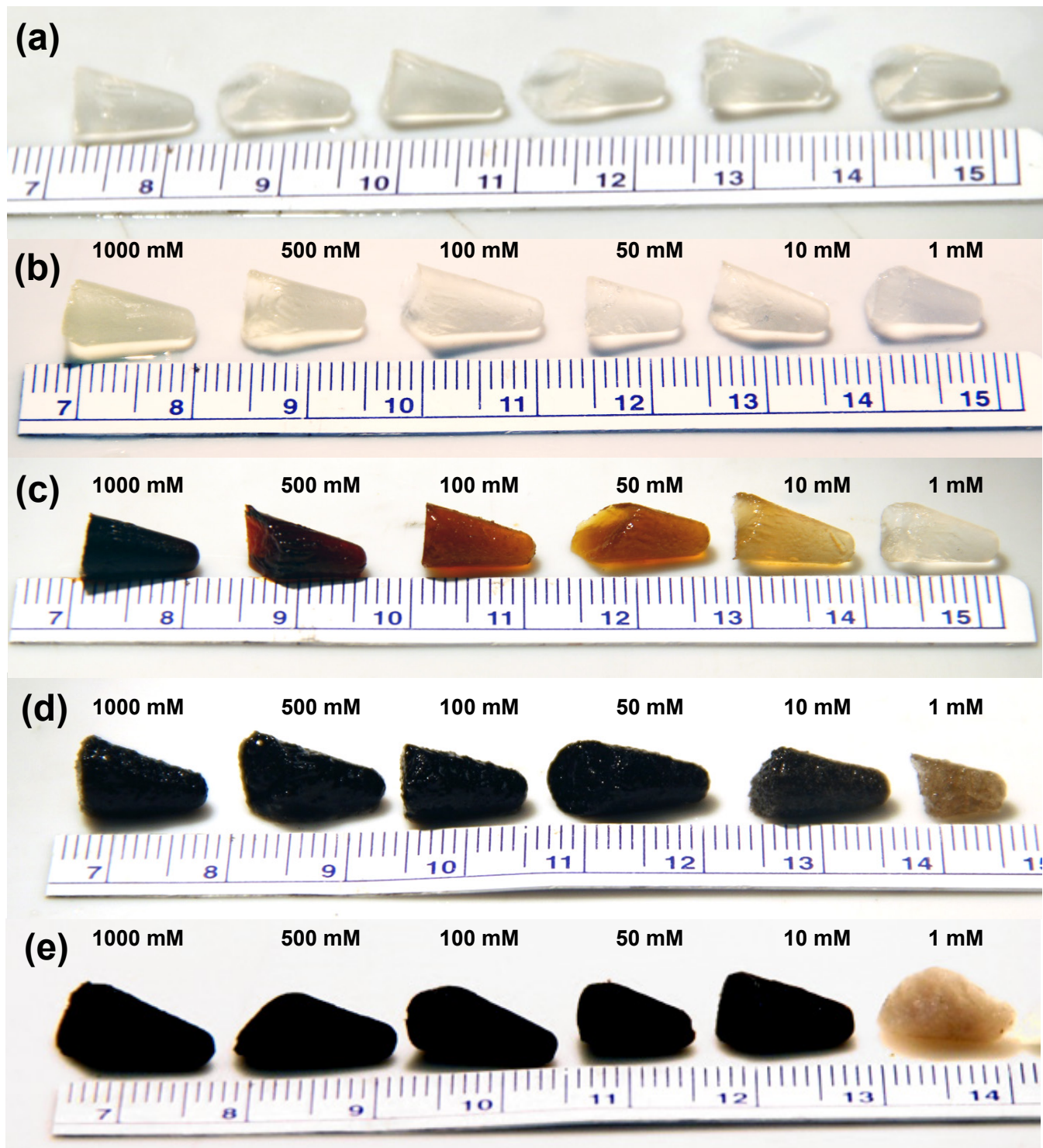
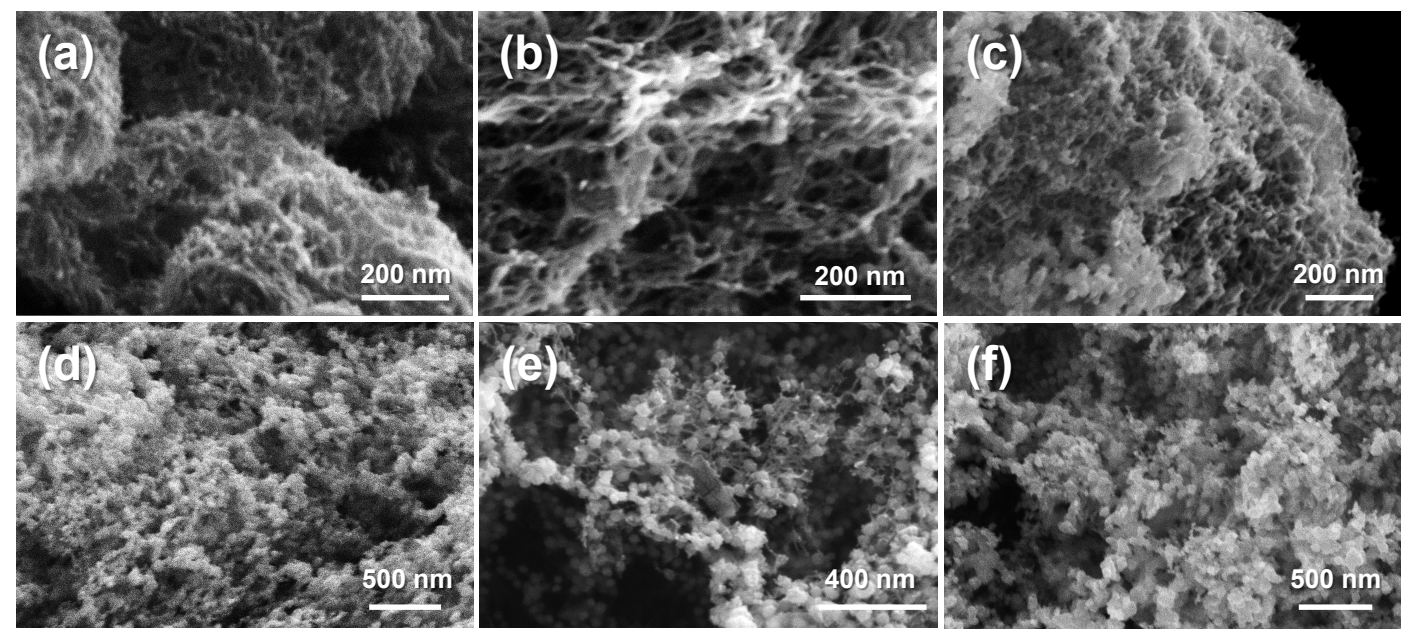


Figure 4



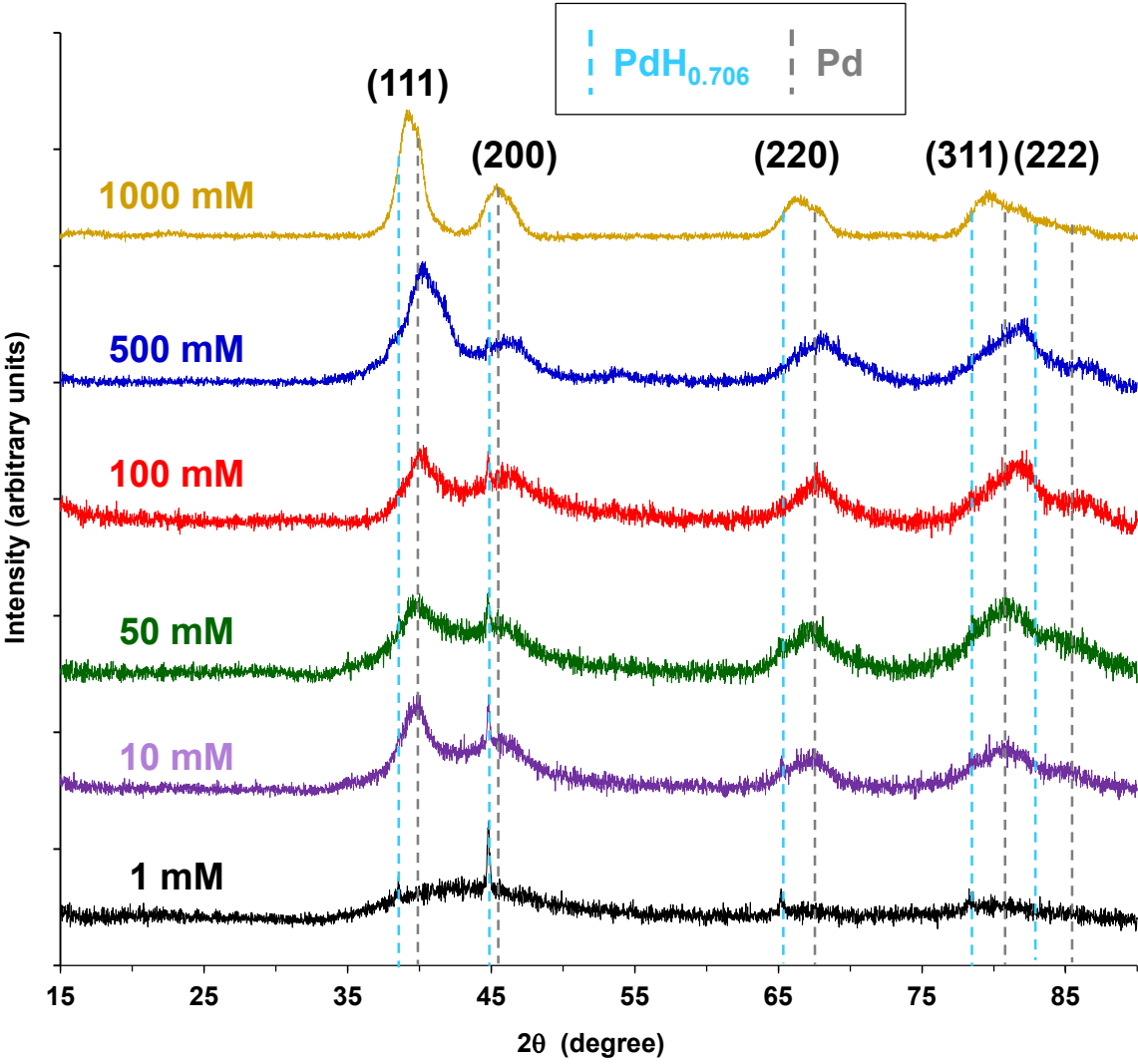


Figure 6

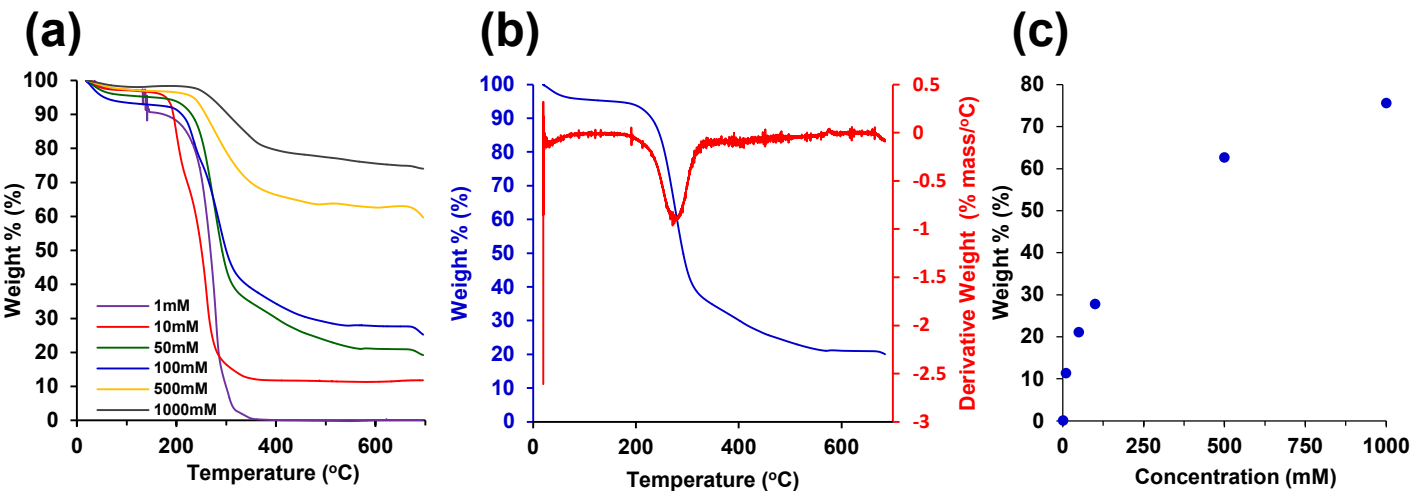


Figure 7

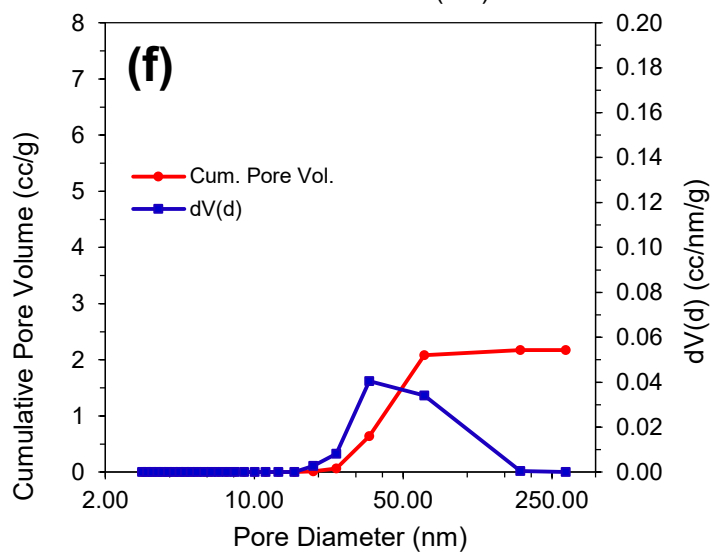
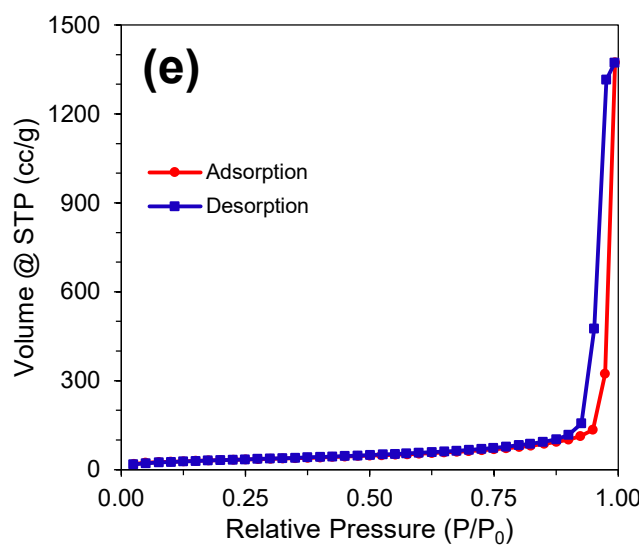
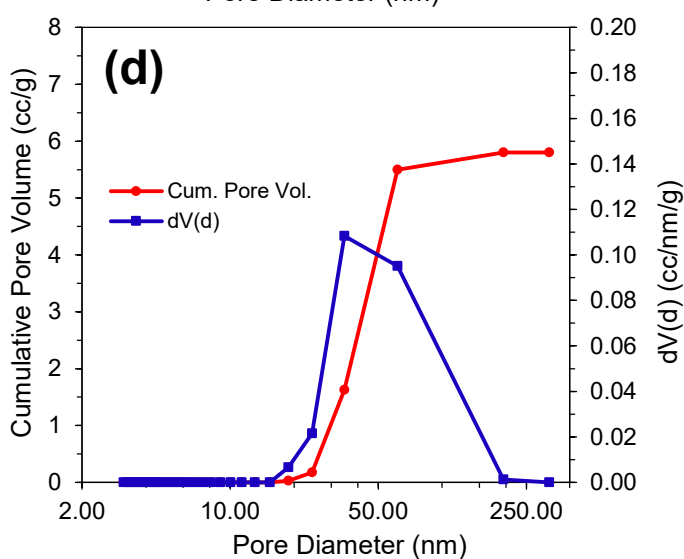
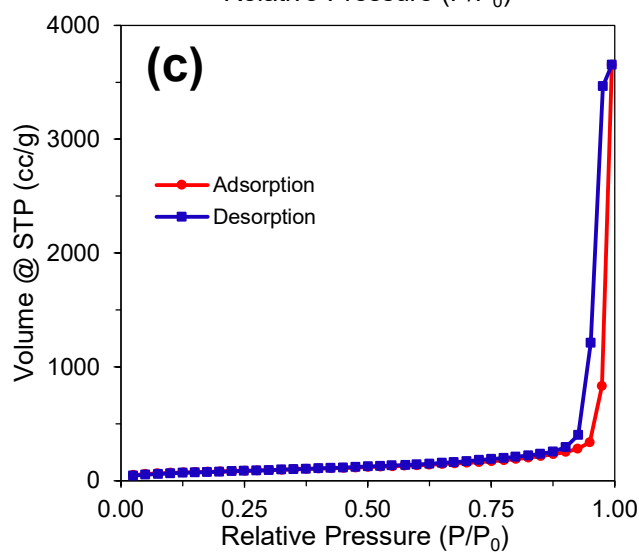
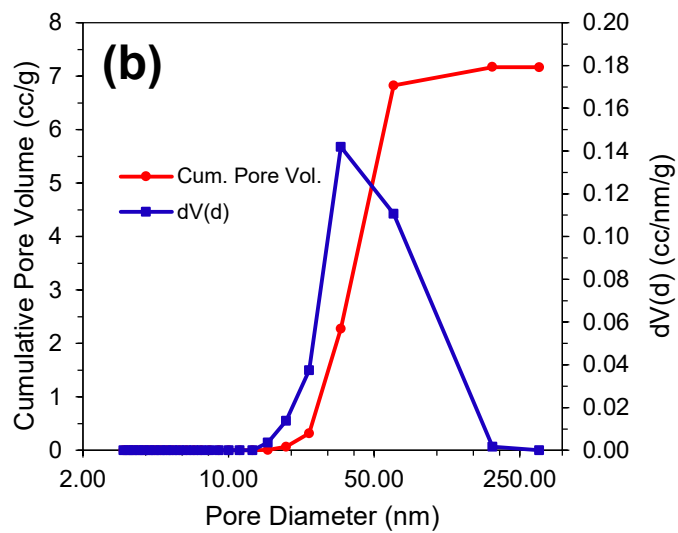
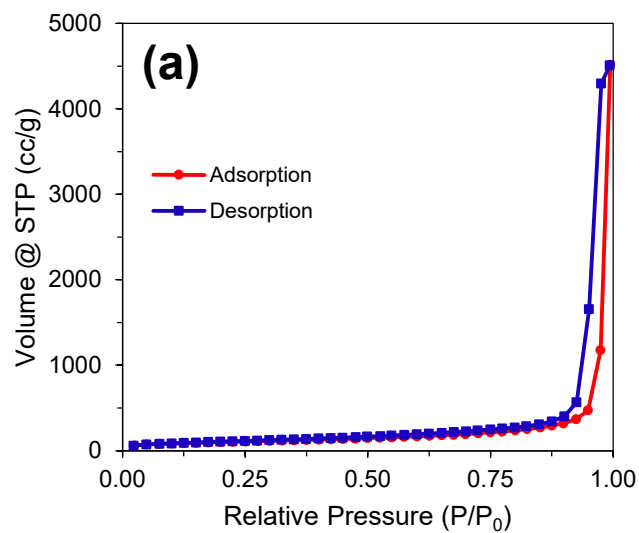
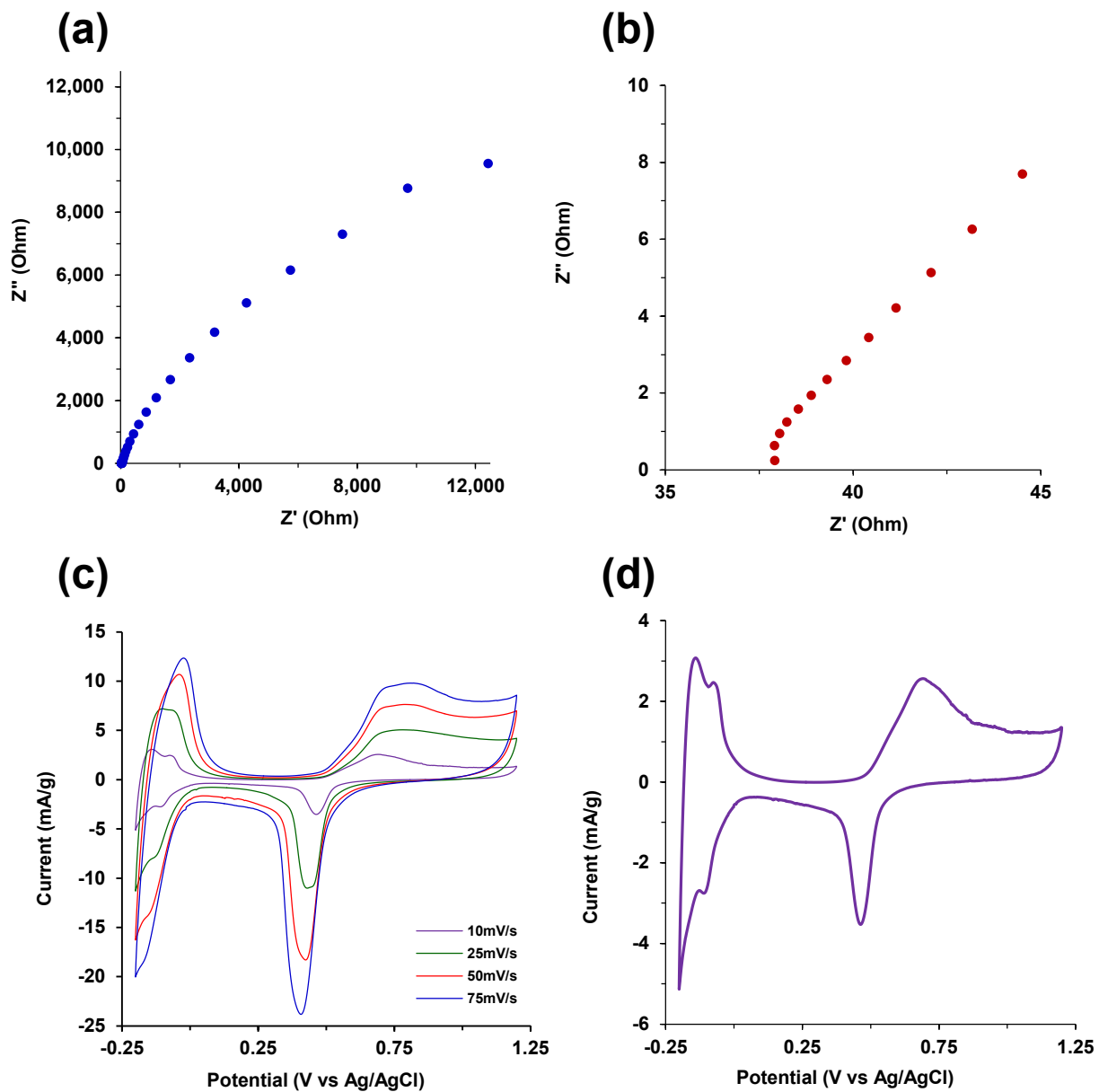


Figure 8



Name of Material/ Equipment	Company	Catalog Number	Comments/Description
0.5 mm platinum wire electrode	BASi	MW-4130	Used for auxillary electrode and separately for lacquer coating and use as a working electrode
1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)	Sigma-Aldrich	1892-57-5	
2-(N-morpholino)ethanesulfonic acid (MES)	Sigma-Aldrich	117961-21-4	
Ag/AgCl (3M NaCl) Reference Electrode	BASi	MF-2052	
	University of Maine Process		
Carboxymethyl cellulose, TEMPO Cellulose Nanofibrils, Dry Powder	Development Center	No 8	
Ethanol, 200 proof	PHARMCO-AAPER	241000200	
Ethylenediamine	Sigma-Aldrich	107-15-3	
Fourier-Transform Infrared (FTIR) Spectrometer, Frontier	Perkin Elmer	L1280044	
Hydrochloric Acid	CORCO	7647-01-0	
Na ₂ PdCl ₄	Sigma-Aldrich	13820-40-1	
NaBH ₄	Sigma-Aldrich	16940-66-2	
Pd(NH ₃) ₄ Cl ₂	Sigma-Aldrich	13933-31-8	
Potentiostat	Biologic-USA	VMP-3	Electrochemical analysis-EIS, CV
Scanning Electron Microscope (SEM) Helios 600 Nanolab	ThermoFisher Scientific		
Supercritical Dryer	Leica	EM CPD300	Aerogel supercritical drying with CO ₂
Surface and Pore Analyzer	Quantachrome	NOVA 4000e	Nitrogen gas adsorption
Thermal Gravimetric Analysis	TA instruments	TGA Q500	
Ultrasonic Cleaner	MTI	EQ-VGT-1860QTD	
XRD	PanAlytical	Empyrean	X-ray diffractometry



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December 2, 2018

Dear Sir/Ma'am,

We have addressed the editorial and reviewer comments below for our manuscript entitled "Synthesis Method for Cellulose Nanofiber Biotemplated Palladium Composite Aerogels" by Fred J. Burpo, Jesse L. Palmer, Alexander N. Mitropoulos, Enoch A. Nagelli, Lauren A. Morris, Madeline Y. Ryu, and J. Kenneth Wickiser. Our responses to the comments are indicated in red font below.

We appreciate the consideration of our work and the opportunity to address the concerns identified during the review, as well as your patience as members of our research team dealt with medical and other issues. Please let us know if there is anything else needed.

Respectfully,

A handwritten signature in black ink, appearing to read "F. John Burpo", is written over a light gray rectangular background.

F. John Burpo, Sc.D.
Colonel, U.S. Army
Professor and Head, Department of
Chemistry & Life Science

Editorial comments:

Changes to be made by the author(s) regarding the manuscript:

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3. Please expand the Short Abstract to briefly describe the applications of this protocol.

The short abstract was expanded and now reads:

“A synthesis method for cellulose nanofiber biotemplated palladium composite aerogels is presented. The resulting composite aerogel materials offer potential for catalysis, sensing, and hydrogen gas storage applications.”

4. In the protocol, please describe how the products are characterized with different analytical techniques such as SEM, XRD, TGA, and BET analysis and electrochemical characterization because such data are presented in the Representative Results.

An additional step, Step 4, was added to the protocol and reads as:

4. **Composite aerogel material characterization.**

4.1. **Scanning electron microscopy (SEM).** Cut the CNF-palladium aerogel with a razor blade to obtain a thin film approximately 1 – 2 mm thick. Affix the thin film sample with carbon tape on a SEM sample stub. Initially use an accelerating voltage of 15 kV and beam current of 2.7 – 5.4 pA to perform imaging.

4.2. **X-ray diffractometry (XRD).** Place the CNF-palladium aerogel in a sample holder and align the top of the aerogel with the top of holder. Alternatively, place a thin film sample section, as in Step 4.1, on a glass slide. Perform XRD scans for diffraction angles 2θ from 5° to 90° at 45 kV and 40 mA with Cu K_α radiation (1.54060 \AA), a 2θ step size of 0.0130° , and 20 s per step.

4.3. **Thermal gravimetric analysis (TGA).** Place the aerogel sample in the instrument crucible. Perform analysis by flowing nitrogen gas at 60 mL/min and heating at 10 °/min from ambient temperature to 700 °C.

4.4. **Nitrogen gas adsorption-desorption.** Degas samples for 24 h at room temperature. Use nitrogen at -196 °C as the test gas with equilibration times for adsorption and desorption of 60 s and 120 s, respectively.

Note: Elevated degas temperatures are not recommended to avoid decomposition of the cellulose nanofibers.

4.5. **Electrochemical characterization.**

4.5.1. Immerse aerogel samples in 0.5 M H₂SO₄ electrolyte for 24 h.

4.5.2. Use a 3-electrode cell with an Ag/AgCl (3 M NaCl) reference electrode, a 0.5 mm diameter Pt wire auxiliary/counter electrode, and a lacquer coated 0.5 mm diameter platinum working electrode. Place the lacquer coated wire with a 1 mm exposed tip in contact with the top surface of the aerogel at the bottom of the electrochemical vial.¹²

4.5.3. Perform electrochemical impedance spectroscopy (EIS) from 1 MHz to 1 mHz with a 10 mV sine wave.

4.5.4. Perform cyclic voltammetry (CV) using a voltage range of -0.2 to 1.2 V (vs. Ag/AgCl) with scan rates of 10, 25, 50, 75, and 100 mV/s.

5. Table of Equipment and Materials: Please sort the items in alphabetical order according to the Name of Material/ Equipment.

The Table of Equipment and Materials was updated, re-sorted, and is now alphabetically listed.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

Self-supporting CNF-palladium aerogels were prepared and characterized.

FTIR should be done to prove the crosslinking.

FTIR spectroscopy was performed on 3% (w/w) carboxymethyl cellulose (CNF) solutions and compared to CNF hydrogels after crosslinking with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in the presence of ethylenediamine. Both CNF solutions and crosslinked CNF hydrogels were equilibrated in deionized water prior to FTIR analysis. The FTIR spectra are shown in the figure below and was added as Figure 3 in the manuscript with the subsequent figures renumbered. The Protocol section was updated to include FTIR characterization. The text that follows was added to the Protocol and Representative Results, with references added as indicated:

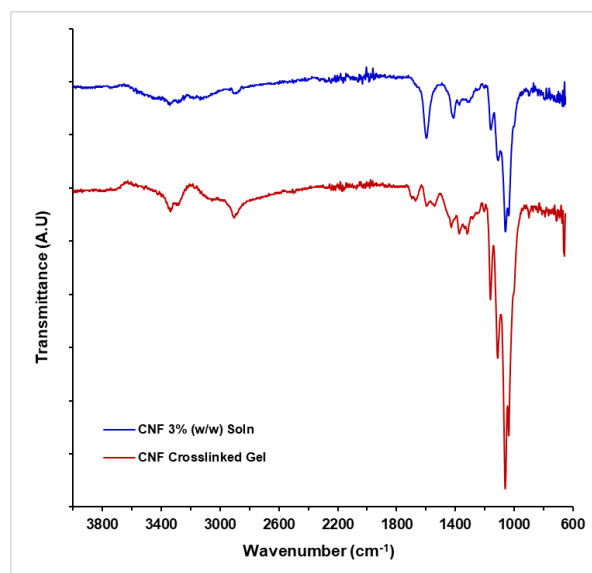


Figure 3. FTIR spectra for 3 % (w/w) carboxymethyl cellulose nanofiber (CNF) solution in deionized water and CNF hydrogels crosslinked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in the presence of ethylenediamine and subsequently equilibrated in deionized water.

Protocol

1.6. Fourier Transform Infrared (FTIR) spectroscopy. Place approximately 0.5 mL of 3 % (w/w) CNF solution in deionized water on the sample stage and scan percent transmittance from 650 – 4000 cm^{-1} . Use the same scan conditions and repeat for a CNF crosslinked hydrogel from Step 1.5.

Representative Results:

“To confirm crosslinking, **Figure 2** shows FTIR spectra for 3% (w/w) carboxymethyl cellulose (CNF) solutions compared to CNF hydrogels after crosslinking with using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in the presence of ethylenediamine. Both CNF and crosslinked CNF hydrogels were equilibrated in deionized water prior to FTIR analysis. The 3% (w/w) CNF solution presents a broad peak between approximately 3200 to 3600 cm^{-1} and is attributed to O-H stretching²⁸. The prominent peak at 1595 cm^{-1} is likely attributed to the vibration of the $-\text{COO}^-\text{Na}^+$ groups on the carboxymethyl cellulose nanofibers²⁹. After crosslinking the carboxymethyl cellulose nanofibers with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in the presence of ethylenediamine, three bonding possibilities result. The first is an effective crosslink between two CNF’s with ethylenediamine forming two amide bonds with carboxylates on the CNF’s. The second is ethylenediamine forming a single amide bond with a CNF carboxylate with a primary amine at the other end of the diamine molecule. The third possibility is EDC forming an unstable o-acylisourea intermediate that hydrolyzes to reform the initial carboxyl group³⁰.

After crosslinking, the broad O-H absorption band between 3200 to 3600 cm^{-1} decreases, with the emergence of prominent peaks at 3284 and 3335 cm^{-1} , attributed to primary amines and amide bonds resulting from both amides of crosslinked CNFs, and single amide bonds between CNFs and ethylenediamine resulting in a primary amine at the terminal end of the ethylenediamine molecule^{28,31}. The peak at 2903 cm^{-1} associated with C-H stretching becomes more prominent after crosslinking and is attributed to the increased presence of $-\text{NH}_3^+$ from the terminal primary amines. The decrease in the carbonyl stretch at a wavenumber of 1595 cm^{-1} is attributed to the decreased number of $-\text{COO}^-\text{Na}^+$ groups due to crosslinking with ethylenediamine. The formation of amide bonds due to crosslinking is

seen in the amide peaks at 1693 and 1668 cm^{-1} , as well as at 1540 cm^{-1} , and a small peak at 1236 cm^{-1} 28,29,31 »

References:

28. Coates, J. in *A Practical Approach. In Encyclopedia of Analytical Chemistry* doi:10.1002/9780470027318.a5606 (ed R. A. Meyers and M. L. McKelvy) (2006).
29. Wang, S. *et al.* Cellulose nanofiber-assisted dispersion of cellulose nanocrystals@polyaniline in water and its conductive films. *RSC Advances*. **6** (12), 10168-10174, (2016).
30. Grabarek, Z. & Gergely, J. Zero-length crosslinking procedure with the use of active esters. *Analytical Biochemistry*. **185** (1), 131-135, (1990).
31. Shabanpour, B., Kazemi, M., Ojagh, S. M. & Pourashouri, P. Bacterial cellulose nanofibers as reinforce in edible fish myofibrillar protein nanocomposite films. *International Journal of Biological Macromolecules*. **117** 742-751, (2018).

One possible application like catalytic experiments can also be done.

The CNF-Pd aerogels were tested using cyclic voltammetry for ethanol oxidation in a 1 M KOH and 1 M ethanol solution with the resulting CV curve shown in the figure below where current is normalized to the palladium mass of the CNF-Pd composite aerogel. The scan rate was 50 mV/s and the second cycle is shown. The forward and reverse current peaks occur at -0.18 V and -0.29 V (vs Ag/AgCl), respectively, with a forward to reverse current ratio of 3.4. While the current peaks voltages are similar to previous reports of palladium aerogels (Fu, *et al.* *ACS Appl. Mater. Interfaces* 2015, 7, 13842–13848; Douk, *et al.* *Electrochim Acta* 275 (2018) 182e191), the peak magnitudes are approximately an order of magnitude lower on a per mass basis. This suggests that despite achieving metal composition as high as 75 % in the CNF-palladium aerogel composites, the electrical conductivity is not sufficient for complete current distribution throughout the aerogel. As a continuation of composite materials development, our group has been pursuing means of pyrolyzing the CNF framework of the aerogels to increase conductivity, as well as integrating carbon nanotubes within the CNF hydrogel templates.

The authors fully agree that a catalytic application would be a worthwhile addition to the manuscript; however, given that the composite material does not currently achieve similar catalytic current densities for ethanol oxidation as pure palladium aerogels on a per mass basis, we recommend that the manuscript remain focused on the synthesis method, retaining the cyclic voltammetry results in H_2SO_4 in Figure 8 (previously Figure 7) to demonstrate the metallization of the aerogel composite.

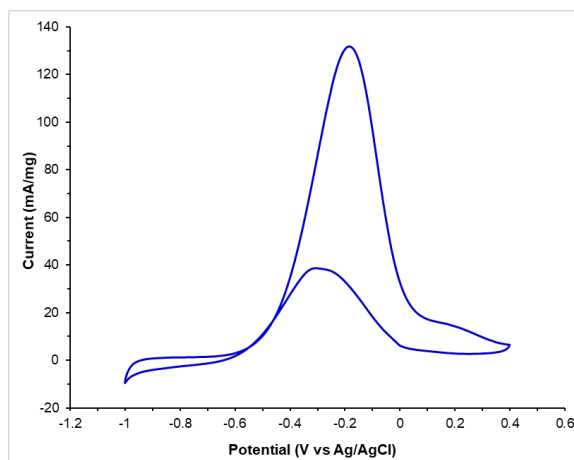


Figure. Cyclic voltammetry of CNF-palladium composite in 1 M KOH and 1 M ethanol at a 50 mV/s scan rate. Current (mA/mg) is normalized to the mass of palladium in the aerogel composite.

Reviewer #2:

Manuscript Summary:

The manuscript is well-written and interesting to a broad audience. It deserves publication in JOVE after the following comments are addressed.

Major Concerns:

- Authors must provide more detailed information about the aqueous dispersion of carboxymethyl cellulose nanofibers (fiber morphology, charge density, solution pH...). Also, how is the sonication temperature controlled (was the sonication conducted in an ice bath)?

Carboxymethyl cellulose nanofibers with Na⁺ counterions were purchased from the University of Maine Process Development Center. The freeze dried TEMPO oxidized nanofibers have a nominal length of approximately 300 nm with a width of 10-15 nm confirmed with atomic force microscopy. The concentration of -COO⁻Na⁺ is 1.2 mmol/g of dry powder and the pH of the indicated 3% (w/w) solution was approximately 5. To provide this additional information and context for the CNF solutions, the following text was added to the first paragraph of the Discussion section:

“The TEMPO-oxidized cellulose nanofibrils used in this study had a nominal -COO⁻Na⁺ density of 1.2 mmol/g with approximate length and width of the cellulose nanofibers of 300 and 10 nm, respectively, and the 3 % (w/w) solutions had a pH of 5. Likely due to the short fiber length, crosslinking at concentrations 3 % (w/w) and less did not result in stable hydrogels. Centrifuging the 3 % (w/w) solutions to compact the fibers to an approximate concentration of 3.8 % (w/w) resulting in well crosslinked hydrogels that were stable during the electrochemical reduction of palladium step.”

Sonication was performed in a temperature controlled ultrasonic cleaner purchased from MTI (product number EQ-VGT-1860QTD). The materials list was updated with this information.

- Degassing conditions for the nitrogen sorption analysis are missing.

The samples were vacuum degassed for 24 h at room temperature prior to measurement. The following Step and Note was added in conjunction with the addition of the Step 4 material characterization steps:

4.4. Nitrogen gas adsorption-desorption. Degas samples for 24 h at room temperature. Use nitrogen at -196 °C as the test gas with equilibration times for adsorption and desorption of 60 s and 120 s, respectively.

Note: Elevated degas temperatures are not recommended to avoid decomposition of the cellulose nanofibers.

- Heating rate for the thermogravimetric analysis is missing.

The heating rate for the presented thermogravimetric analysis is 10 °/min. This heating rate is now specified in Step 4 and reads as,

“4.3. Thermal gravimetric analysis (TGA). Place the aerogel sample in the instrument crucible. Perform analysis by flowing nitrogen gas at 60 mL/min and heating at 10 °/min from ambient temperature to 700 °C.”

Minor Concerns:

- Please ensure a consistent space between numerical values and units (e.g. line 114).

The value and unit spacing was corrected on Line 114 and spacing was verified throughout the document.

- In the introduction, please note that TEMPO oxidized cellulose nanofibers were recently used as both templating and reducing agent to prepare Pd@cellulose aerogels (Gu et al. Applied Catalysis B 2018. 237, 482-490).

The introduction has been modified to address the multiple uses of TEMPO-oxidized cellulose nanofibers and added the indicated reference added to References. The second paragraph of the introduction now includes,

“Further, TEMPO-oxidized cellulose nanofibers have been used both as a biotemplate and reducing agent in the preparation of palladium decorated CNF aerogels.²⁷”

- Authors should discussed the possibility of Pd leaching.

The authors acknowledge the reviewers concern and agree that palladium leaching is a significant concern for practical catalytic applications and extended material use. The Discussion section was expanded to include,

“Another potential issue with extended use of the CNF-palladium aerogel composite for catalytic applications is palladium leaching with detachment of palladium nanoparticles from the CNF template.”

Reviewer #3:

Manuscript Summary:

This manuscript provides a method for the synthesis of chemically crosslinked cellulose nanofiber hydrogels that are then metallized by reduction of palladium salts within the hydrogel. The composite material then undergoes supercritical drying to yield palladium aerogels. The diffusion times recommended for infiltration of all reagents in this process has been optimized to ensure uniformity throughout the material. Further, the authors show tunable metal content of the composite corresponding to the starting concentration of the metal salts. Overall, the authors show multiple advantages of this method including the resulting robust structural integrity and shape control of the aerogels.

Major Concerns:

None

Minor Concerns:

- What is meant by the term "electrically addressable," mentioned both in the abstract and introduction?

The phrase “electrically addressable” was intended to connote “conductivity.” To avoid confusion this phrase was deleted from the Abstract and last paragraph in the Introduction.

- Can the authors comment in the introduction about the interaction between palladium and the cellulose nanofibers during the reduction process? Additionally, is there any insight into how this process yields robust aerogels, ie is this attributed to the chemical crosslinking or to the strength of cellulose nanofibers?

The mechanical robustness of the CNF hydrogels and resulting composite aerogels is attributed to the chemical/covalent crosslinking mediated by EDC. Physically entangled CNF hydrogels concentrated using the same method as detailed in Step 1.3, but not covalently cross-linked, do not remain fully intact during and after the chemical reduction step with sodium borohydride. To reflect these observations, the Discussion section was modified to now include,

“CNF hydrogels not covalently crosslinked with EDC did not remain fully intact during the electrochemical reduction step.”

- In the Hydrogel Preparation procedure, it may be helpful to comment on the expected observations during the step of concentrating the 3 % cellulose solution. Is there a noticeable change in viscosity by centrifugation? Is there a clear interface between clarified water and concentrated cellulose or a density gradient? Is the final concentration calculated based on the remaining volume after decanting the supernatant?

Centrifugation results in a concentrated layer of cellulose nanofibers at the bottom of the microfuge tube that is translucent white in color and distinct from the supernatant above with a clearly observable interface. The approximately 3.8 % (w/w) concentration of the concentrated solution was calculated based on the volume of supernatant removed. The resulting concentrated CNF solution is noticeably more viscous than the 3 % (w/w) CNF solution.

Based on the discussion above, the Note after Step 1.3. was modified to read,

“Note: After centrifuging, the cellulose nanofiber solutions present a distinct interface between the concentrated CNF’s and the clear supernatant. Based on removal of the excess water, the final CNF concentration will be approximately 3.8 %.”

- In the Palladium Reduction procedure, is it expected that observable diffusion of palladium particles will occur during the rinsing step, or is the majority of the palladium solution cellulose bound?

No diffusion of palladium particles were observed to diffuse away from the CNF hydrogels during the reduction step and no palladium particles were observed at the bottom of the reaction tubes after reduction was complete. The following line was added to the first paragraph of the Discussion,

“Further, no palladium nanoparticles were observed to diffuse away from the CNF-palladium aerogel composites during the reduction step suggesting that all of the reduced palladium is bound within the resulting aerogels.”

- Overall, the procedure would benefit from providing recommended ratios between reagents in addition to the specific volumes used. For example, the ratio of cellulose, palladium salt solution, and

sodium borohydride volumes appears to be 0.25X, 1X, and 10X. Are these same ratios scalable if the reader wishes to work with different sample sizes?

The author team agrees that generalizing the solution concentrations to ratios would be helpful for scaling up the material synthesis. Given the dependence on chemical species diffusion, and the practical limitations on aerogel monolith size, we recommend maintaining the specific volumes indicated in the manuscript protocol. Our group is currently exploring methods to extend this synthesis approach to larger scaled materials to overcome the long diffusion times required.

- While the analytical methods are not part of the procedure described here, it would be helpful for the authors to comment on the purpose of each technique for evaluating the aerogel properties, especially to educate readers who have less expertise in this field.

To add to the descriptions in the Representative Results sections, Step 4 was added to include details on the material characterization. The new Step 4 now reads,

“4. **Composite aerogel material characterization.**

4.1. **Scanning electron microscopy (SEM).** Cut the CNF-palladium aerogel with a razor blade to obtain a thin film approximately 1 – 2 mm thick. Affix the thin film sample with carbon tape on a SEM sample stub. Initially use an accelerating voltage of 15 kV and beam current of 2.7 – 5.4 pA to perform imaging.

4.2. **X-ray diffractometry (XRD).** Place the CNF-palladium aerogel in a sample holder and align the top of the aerogel with the top of holder. Alternatively, place a thin film sample section, as in Step 4.1, on a glass slide. Perform XRD scans for diffraction angles 2Θ from 5° to 90° at 45 kV and 40 mA with Cu K_α radiation (1.54060 \AA), a 2Θ step size of 0.0130° , and 20 s per step.

4.3. **Thermal gravimetric analysis (TGA).** Place the aerogel sample in the instrument crucible. Perform analysis by flowing nitrogen gas at 60 mL/min and heating at $10^\circ/\text{min}$ from ambient temperature to 700°C .

4.4. **Nitrogen gas adsorption-desorption.** Degas samples for 24 h at room temperature. Use nitrogen at -196°C as the test gas with equilibration times for adsorption and desorption of 60 s and 120 s, respectively.

Note: Elevated degas temperatures are not recommended to avoid decomposition of the cellulose nanofibers.

4.5. **Electrochemical characterization.**

4.5.1. Immerse aerogel samples in 0.5 M H_2SO_4 electrolyte for 24 h.

4.5.2. Use a 3-electrode cell set-up with an Ag/AgCl (3 M saturated) reference electrode, a 0.5 mm diameter Pt wire auxiliary/counter electrode, and the lacquer coated working electrode. Place the lacquer coated wire in contact with the top surface of the aerogel at the bottom of the electrochemical vial.¹²

4.5.3. Perform electrochemical impedance spectroscopy (EIS) from 1 MHz to 1 mHz with a 10 mV sine wave.

4.5.4. Perform cyclic voltammetry (CV) using a voltage range of -0.2 to 1.2 V (vs. Ag/AgCl) with scan rates of 10, 25, 50, 75, and 100 mV/s.”

“Synthesis Method for Cellulose Nanofiber Biotemplated Palladium Composite Aerogels”

Fred J. Burpo, Jesse L. Palmer, Alexander N. Mitropoulos, Enoch A. Nagelli, Lauren A. Morris, Madeline Y. Ryu, and J. Kenneth Wickiser

Figures were used from Reference 26 (Burpo, F. *et al.* Cellulose Nanofiber Biotemplated Palladium Composite Aerogels. *Molecules*. **23** (6), 1405, (2018)) in the journal *Molecules* which is an open access journal allowing authors permission for reuse. The journal re-use permissions are copied here from the weblink <https://www.mdpi.com/about/openaccess> :

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