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Sustained alignment of nanocellulose from magnetic field exposure

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Title (English)

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Abstract

A method to sustain the alignment of nanocellulose, which a magnetic field induces in a solution of cellulose crystals, was tested. The nanocellulose was modified to incorporate metal chelating cysteine groups through oxidations and Schiff base coupling. Water solutions of the modified cellulose was exposed to a magnetic field of one Tesla. To stabilise the alignment iron ions were added to the solutions. The material was topographically studied with scanning electron microscopy. The results were inconclusive due to inadequate field exposure.

Keywords

Nanocellulose, magnetic alignment, metal chelators, cysteine

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Populärvetenskaplig sammanfattning

För att möta tidens utmaningar krävs det nya smarta material och nya smarta sätt att använda sådant som redan finns. Ett material som besitter en oerhörd potential är cellulosa. Den kan framställas ur träd och växter. Cellulosan finns i cellväggar och har fått sitt namn av den anledningen. Ur ett miljö- och energiperspektiv skulle det vara väldigt intressant att kunna använda sig av cellulosa istället för andra mindre hållbara alternativ som oljebaserade plaster eller ovanliga metaller. Det kan även vara bra att hitta på nya sätt att använda cellulosa på. Om det här projektet lyckas kan det kanske bidra till att kunna använda en av algarterna som orsakar algblomningen i Östersjön till något nyttigt.

När en lagom andel cellulosakristaller i vatten utsätts för ett magnetiskt fält lägger sig kristallerna så att alla pekar åt samma håll. I projektet undersöks det om det är möjligt att få kristallerna att stanna på det sättet. För att lyckas med det behöver cellulosan ändras på något sätt. Genom att sätta fast cystein på cellulosan gör det att den kan hålla fast metalljoner. Cystein är en aminosyra som används för att bygga upp olika proteiner. Tanken är att cysteinet på cellulosan och metalljoner i lösningen ska hindra cellulosakristallerna från att flytta på sig när magnetfältet tas bort.

Examensarbete 30 hp
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Contents

Introduction

Cellulose can be used as a latticework on which cells can be grown. To better control the conditions in such growth experiments it is hypothesised that an aligned nanocellulose material could be utilised. Nanofibers of cellulose have been shown to align perpendicular to magnetic fields (Sugiyama *et al*. 1992). To build on these results the aim of the project was to maintain the alignment of nanocellulose fibres achieved through exposure to a magnetic field. The alignment is however lost over time when the magnetic field no longer is present. To make the fibres stay in the aligned configuration they were modified to include metal chelating groups. These metal chelators provide additional fixation of the acquired alignment. The cellulose fibres were prepared in gel suspensions to further increase the effect of the alignment by making it harder for the fibres to shift from the alignment due to steric/spatial hindrance.

Background

Previous work

In 1992 the group of Sugiyama discovered that cellulose crystals can be oriented in a sufficiently strong magnetic field. The reason to achieve orientation of the crystals is to be able to better control the properties of materials in which the cellulose can be incorporated. Crystalline cellulose has some very interesting properties. When characterised mechanically the perfect cellulose crystal is comparable to steel and Kevlar in terms of tensile strength and stiffness. Of major impact is that it also can be deemed as a renewable resource. Therefore it would be of great benefit to be able to develop new and improved materials that utilise some of these aspects. What the Sugiyama group did was to apply magnetic fields of up to 7 T on aqueous suspensions of non-flocculated cellulose microcrystals (Sugiyama *et al*. 1992). The microcrystals used were extracted from tunicate species, a type of sea invertebrate. The preparation was done through repeated bleaching followed by homogenisation and acid hydrolysis. To study the influence of a magnetic field on the ordering of the microcrystals, samples were allowed to dry on glass slides placed inside the homogenous region of the magnetic field. The samples were then analysed by electron diffraction or X-ray diffraction depending on the thickness of the layers. To get a visual image of the samples Transmission Electron Microscope, TEM, was used. An illustration of the different states of aligned and

unaligned fibres are shown in Figure 1.

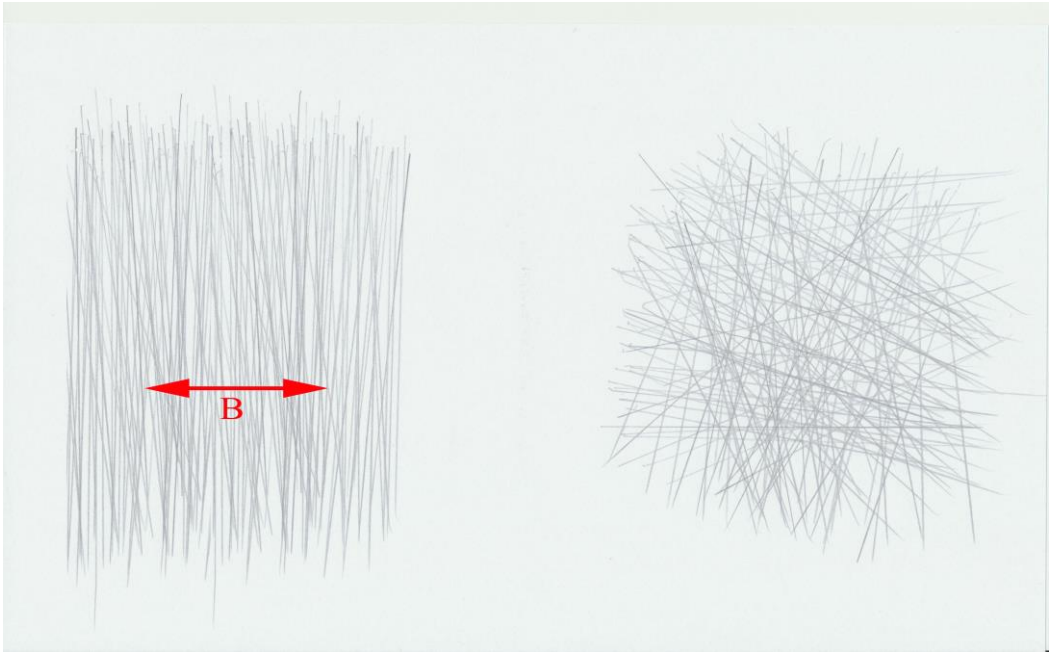

Figure 1. Illustration of aligned (left) and unaligned (right) fibres. The alignment is induced by the magnetic field B and is perpendicular to its direction.

All samples prepared inside the magnetic field showed a high degree of order compared to control samples prepared outside. The ordering was a clear alignment of the microcrystals with their long axis perpendicular to the applied magnetic field (Sugiyama *et al*. 1992).

Chiral nematic ordering is a phenomenon recorded in suspensions of rod-like particles. Dispersions of crystalline nano cellulose display this kind of ordering. To obtain liquid crystal behaviour the cellulose content need to be above a certain concentration, the critical volume fraction. This concentration is dependent on the length of the cellulose crystals and the degree of polydispersity of crystals. Above the critical volume fraction the dispersion will separate into an upper isotropic and a lower anisotropic phase (Schütz, 2015). Due to the rod-like nature of the particles they will align themselves uni-axially, that is with their long axis pointing in the same direction (Kimura *et al*. 2005). The alignment is parallel to what is called the director of the medium. This ordering has been observed in a number of different nanoparticle suspensions, including nanocellulose suspensions (Habibi *et al*. 2010; Kimura *et al*. 2005; Kvien & Oksman 2007; Orts *et al*. 1998; Sugiyama *et al*. 1992). The behaviour is caused by the diamagnetic properties of the cellulose molecules (Kimura *et al*., 2005). Diamagnetism is a characteristic of materials that is observed when they are placed in magnetic fields (Britannica, n.d.). Furthermore it has been shown that the alignment can be directed through the use of external fields, for example magnetic and electric fields as well as

shearing forces (Kimura *et al*. 2005). One of the conclusions on the alignments that have been drawn is that the cellulose crystals will align perpendicular to an applied magnetic field (Sugiyama *et al*. 1992). Having a highly ordered orientation is a property that is sought after in many applications concerning nano- and nanocomposite materials. It could be of great impact to be able to control the ordering of nanoparticles in the current use of nanomaterials and it would also enable new approaches and solutions to a number of different fields. Some examples of applications being researched are multifunctional thin films (Lagerwall *et al*., 2014), scaffolds for tissue engineering (Roychowdhury *et al*., 2009), different bulk materials, aero- and hydrogels (Saito *et al*., 2011).

Cellulose

Cellulose is regarded as the most abundant polymer in the world (Klemm *et al*. 2011). It is present in the cell wall of most plants. Several other types of organisms are also able to synthesise it including algae, bacteria, amoebas, fungi and even some animals like the tunicate. Thanks to this abundance it has been used by humans in one way or another at least since the species started to use tools. During the last centuries it has been recognised and used as a chemical compound and raw material (Klemm *et al*. 2011). In the 1920's the polymeric state of the material was discovered which led to the subsequent emergence of polymer science (Habibi *et al*. 2010). With the development of nanoscale characterisation and visualisation techniques the nano-nature of cellulose has been researched in the last decades. When describing cellulose crystals at the nano-scale a number of different names can be used, including microcrystals, whiskers, nanocrystals, nanoparticles, microcrystallites or nanofibers (Habibi *et al*. 2010). In this report it is referred to as nanocellulose or cellulose crystals.

The building blocks of cellulose are glucose molecules that are connected by a beta (1→4) glucosidic bond. Glucose is a sugar molecule consisting of carbon, oxygen and hydrogen and has the chemical formula $C_6H_{12}O_6$. Five of the six carbon atoms present form a pyranose ring together with one of the oxygen atoms. This configuration is hexagonal and each of the carbon atoms has a hydroxyl group bound to it. It is two of these hydroxyl groups,

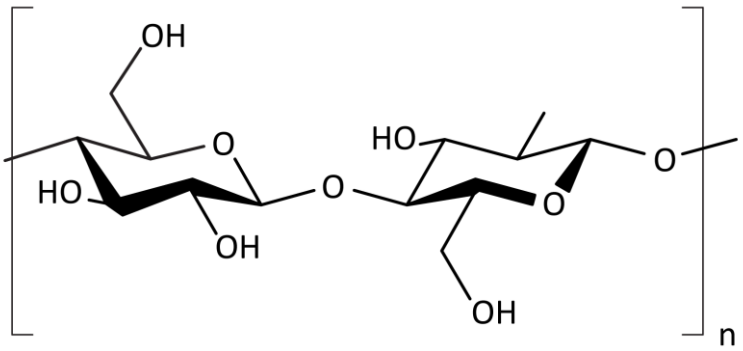

Figure 2. The repetitive unit of cellulose, cellobiose. Two glucose molecules are bound together by a beta(1→4)glucosidic bond.

the ones on the first and fourth carbon, which form the glucosidic bonds and thus the cellobiose molecules. Glucose bound in this fashion makes up the repeating structure of cellulose, as seen in Figure 2. Adjacent cellobiose molecules are flipped 180 degrees compared to each other. This relation enables hydrogen bonds to be formed between hydroxyl groups of glucose. The result of this interaction is the elementary fibrils of cellulose, the fibrils contain both crystalline and amorphous regions. The degree of crystalline cellulose differ depending on where it is originated, different species give different cellulose crystals (Habibi *et al*. 2010). The cellulose in this project is from an algae species called *Cladophora*, and is highly crystalline. The elementary fibrils form microfibrils which are incorporated in the cell wall structure, as seen in Figure 3.

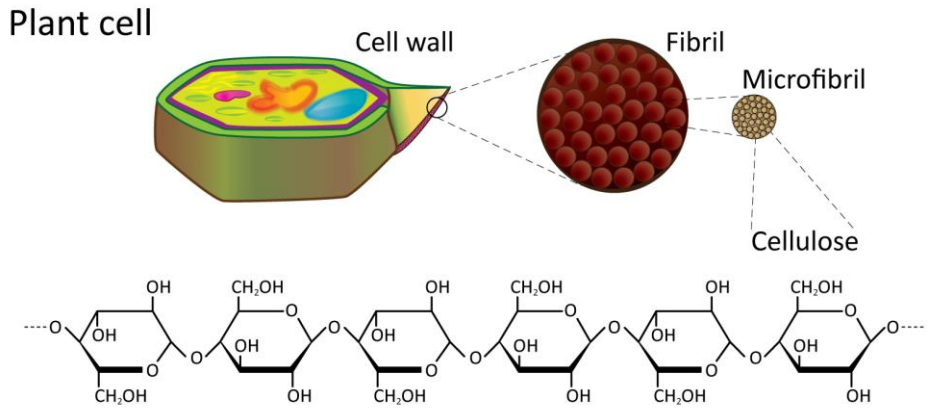

Figure 3. The hierarchy of cellulose structures. The cellulose chains form microfibrils through hydrogen bonding, these are grouped together into fibrils which are incorporated in the cell wall. Modified from Moore *et al.* (1998).

Cladophora cellulose

The *Cladophora* algae is a green algae that grows in lakes and streams, it can be found globally. There are also a few marine species. Most commonly it grows attached to rocks or submerged wood. The filaments can also be found growing freely or forming green carpets, in some distinct cases they can even form free ranging balls. It is quite difficult to tell different species of *Cladophora* apart. The species bloom annually and when they are decomposed by bacteria the oxygen levels can drop to harmful levels for other organisms. Because of this it could be beneficial to find industrial applications where these species can be useful (Mihranyan, 2011).

Liquid crystalline phase behaviour

A liquid crystal is a liquid that exhibits some characteristics found in solid crystals. The most significant similarity is the degree of order of the particles/molecules in the liquid. They display orientations common in crystals but still remain fluid (Singh, 2002). There are a number of different configurations or phases attainable for a liquid crystal based on the degree of order of the molecules. They can be distinguished from each other through their different optic behaviours (Singh, 2002). The simplest phase is the nematic phase, which displays the least amount of order. The molecules in the liquid show only long range orientation. For rod like molecules this means that they align their long-axis in the same direction. They seem to be pointing in the same general direction. This directional vector is called the director. Nematic phases have many anisotropic, directionally dependent, features on the macroscopic scale. The molecules are free to move in relation to each other, however, they retain their long range directional order (Singh, 2002). Due to this ordering the liquid will present some characteristic optic properties, namely birefringence. This means that the liquid exhibits different reflective behaviour in regard to how the light hits the particles. When polarised light is shone along the long axis of the rods the refractive index is different from when the light is perpendicular. When the ordering of the particles also leads to the formation of a helical structure the phase is called chiral nematic instead (Singh, 2002), as seen in Figure 4. This state is often referred to as cholesteric due to its discovery being made on solutions of cholesterol derivatives.

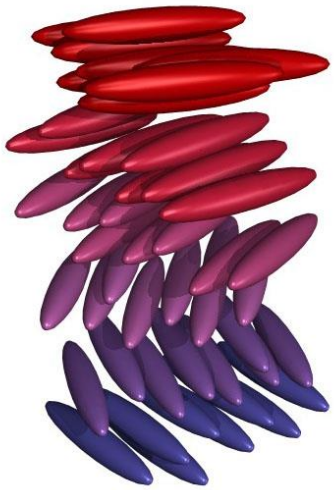

Figure 4. Sketch of chiral nematic, or cholesteric, ordering of rod-shaped molecules. Modified illustration from Wikipedia, *© User:Kebes / Wikimedia Commons / CC-BY-SA-3.0*

The helical structure in liquid crystals can be described as the director of the particles being twisted somewhat throughout the liquid. The result being that the particles instead of pointing in the same general direction in the whole solution form layers of particles pointing in the same direction, which is slightly offset from the direction of the closest layer (Singh, 2002). This behaviour is what is forming the helical structure. The periodicity in which the pattern repeats itself is referred to as the pitch of the helix. Thanks to the helical structure the solution also exhibits new optical properties. When contained in a cell the helical structure will orient itself vertically to the cell walls. Studied between crossed-polarizers the liquid will expose a uniform colour if seen along the helical axis. The liquid will exhibit a fingerprint pattern

instead of the uniform colour if the axis is aligned along the plane of the cell (Kimura *et al*., 2005). Another optic characteristic present in chiral nematic phases but not in nematic phases is the property of a one-dimensional Bragg reflector. If the pitch of the helix matches the wavelength of the light the liquid will exhibit selective reflection. A smectic liquid crystal exhibits further degrees of ordering compared to the nematic phases. The molecules align themselves in layers, in the simplest smectic phase the normal of the layer is parallel to the director of the layer. When the molecules assemble into cylindrical structures the phase is called columnar. It is possible to transition a liquid crystal between different crystal phases through modification of for example concentration or temperature. This is at least theoretically possible for cellulose dispersions too, however the increased viscosity hinders the ordering of the molecules (Schütz, 2015).

Future applications

Magnetically aligned cellulose has potential to be used in a number of different applications. The most prominent is to use the cellulose as a composite with other materials. The aligned cellulose fibres in the composite materials can influence the properties of the material, for example, making it more resilient and tough. Cellulose fibres in themselves can be very strong in certain configurations, outshining other fibres such as Kevlar on a large scale (Netravali *et al*., 2007). Other applications include cellulose based displays and other electronics and cosmetics or pharmaceuticals. The aim of the nanogroup at Uppsala University is to be able to use aligned cellulose as a matrix for cell growth experiments. It has been shown that the surface on which the cells are grown may have a quite large impact on the proliferation of the cells. By using aligned cellulose fibres the hope is that it will be possible to better control the parameters during cell growth. The experiments may be more easily reproduced if the surface can be adjusted to the same setting from time to time. It is also possible that the aligned structure can have a positive effect on the proliferation of cells. Thus the alignment can be used to improve recovery of damaged tissue.

Experimental setup

In this project we are working with the crystalline regions of the cellulose fibres. In order to isolate the crystalline parts of cellulose it has to be chemically treated. This is most often done through acid hydrolysis and the treatment degrades the amorphous areas and leaves the crystalline parts of the cellulose intact (Klemm *et al*. 2011) (Habibi *et al*. 2010). Once the crystals have been isolated it is possible to form colloidal suspensions of cellulose crystals. These suspensions exhibit liquid crystal behaviour due to the rod-like shape of the crystals.

The crystals align themselves with their long axis oriented in the same direction. In the nineties it was discovered that it was possible to manipulate the ordering of cellulose crystals in suspension through the use of strong magnetic fields (Sugiyama *et al*. 1992). This project aims to expand on this fact. Through incorporation of metal coordinating groups onto the surface of cellulose crystals it is believed that the enhanced ordering from exposing them to a magnetic field can be maintained.

In order to obtain individual fibrils in a reasonably energy efficient manner charged groups can be introduced at the surface of the cellulose. These charges enable the fibrils to be separated from each other via repulsion and hinders aggregation in the suspension. This has been done through oxidation of the hydroxyl groups of the cellulose polymer. Two different oxidation routes have been utilised, both a tetramethylpiperidineoxyl-mediated, or TEMPO-mediated, pathway and a peroxymonosulfate pathway. Both pathways target mainly the primary alcohol available on the cellulose crystal surface. After this first oxidation the cellulose was sonicated to obtain suspensions of dispersed crystals. To be able to attach the coordinating groups onto the cellulose it was required to introduce suitable auxiliaries, which was achieved by another oxidation, this time using a metaperiodate compound to target the secondary alcohols in the polymer. Now the metal coordinating groups will have available positions to attach to. The groups used in the project were cysteine and picolylamine, with a focus on cysteine, which would be preferable in future applications. The attachment utilises Schiff-base reactions, where the aldehyde groups on the cellulose react with the amine groups of the metal coordinators to form covalent bonds. After this attachment the structure was stabilised through reduction.

Once the modifications have been performed on the cellulose material it will be placed in a magnetic field. Metal ions will be added to the samples to determine if the modifications increase the duration of the magnetic alignment of the cellulose material.

Materials and methods

Chemistry

The chemical steps involved in the modification of the cellulose consists of four parts, shown in Figure 5. They are oxidation with TEMPO or Oxone, oxidation with periodate, coupling of amine through Schiff base coupling and finally stabilisation through reduction.

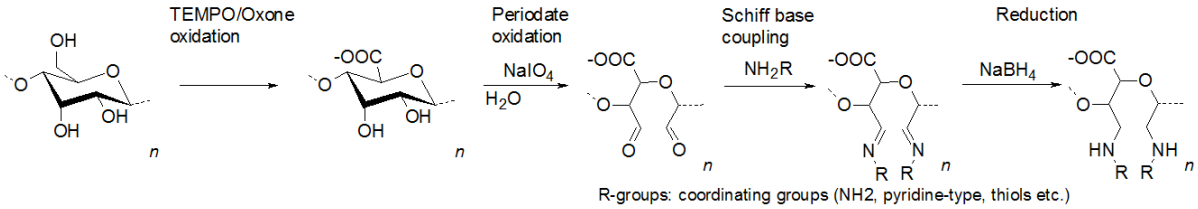

Figure 5. The general reaction scheme for this project. Oxidation of the cellulose with Oxone or TEMPO followed by oxidation with periodate and coupling of cysteine to the cellulose and lastly stabilisation through reduction.

TEMPO oxidation

TEMPO or 2,2,6,6,-tetramethylpiperidine-1-oxyl is a reagent used to catalyse the oxidation of primary alcohols into aldehydes and subsequently carboxyls (Isogai *et al*. 2011). The reactive species is the oxoammonium ion that contains an electron radical. When applied to cellulose it quite specifically attacks the hydroxyl group on the C6 carbons. Higher order of alcohols can be oxidised by this reaction, however, with much lower specificity and rate. To keep the reaction going the oxoammonium ions have to be regenerated. This is accomplished by introducing a pair of secondary oxidants (Isogai *et al*. 2011). Usually sodium hypochlorite and sodium bromide are employed. The reaction scheme can be seen in Figure 6. These substances are quite expensive and environmentally harmful. Because of this, studies have been conducted to see if they can be replaced by regeneration through for example electrolysis (Carlsson *et al*., 2014).

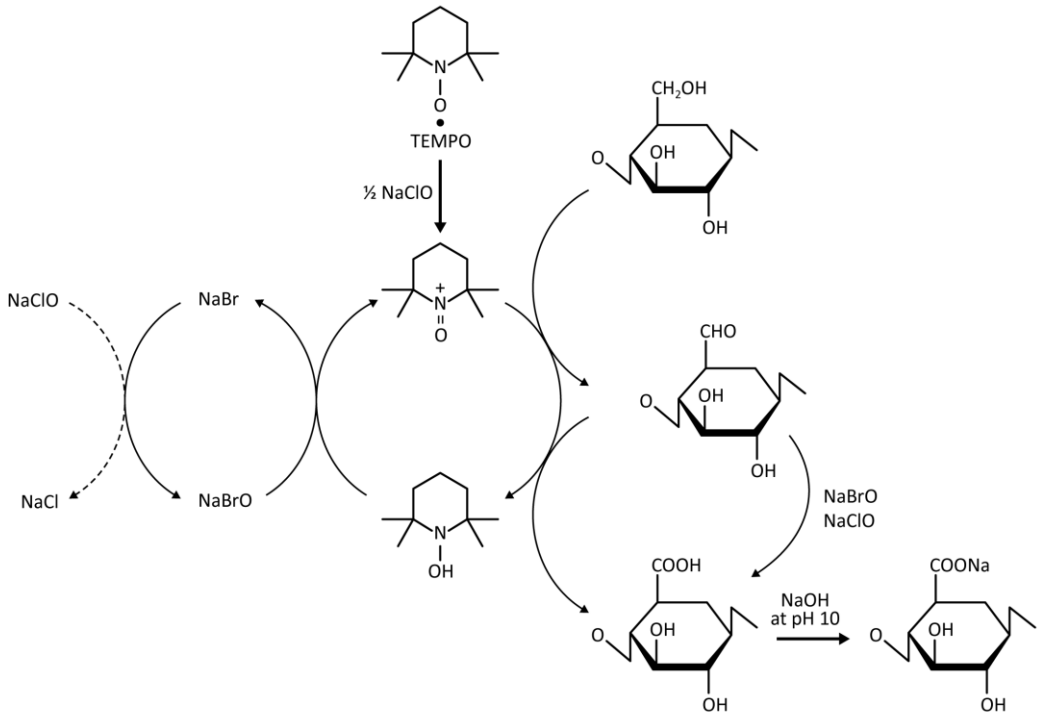

Figure 6. Reaction of TEMPO-mediated oxidation as described by Isogai *et al*. (2011). The TEMPO-molecule targets the primary alcohol on the glucose and oxidises it to an aldehyde. To keep the reaction going the TEMPO need to be regenerated by secondary oxidants NaClO and NaBr.

Oxone oxidation

Oxone is one of the trade names for potassium peroxymonosulfate and it is commonly used as an oxidising agent. As with TEMPO-mediated oxidation the peroxymonosulfate targets primary alcohols and catalyses the oxidation of these into aldehydes and carboxyls.

Periodate oxidation

Periodate is an anion consisting of one iodine atom and four oxygen atoms. The oxidative periodate molecule attacks the C2 and C3 carbons of glucose in the cellulose chain. The attack is highly specific since it requires two accessible hydroxyl groups in close vicinity, which is present only at the C2-C3 carbons in cellulose. This reaction results in a dialdehyde formation at these carbons, breaking the bond between the carbons and turning the cellulose chain into dialdehyde cellulose (DAC) (Kim *et al*. 2000), see Figure 7.

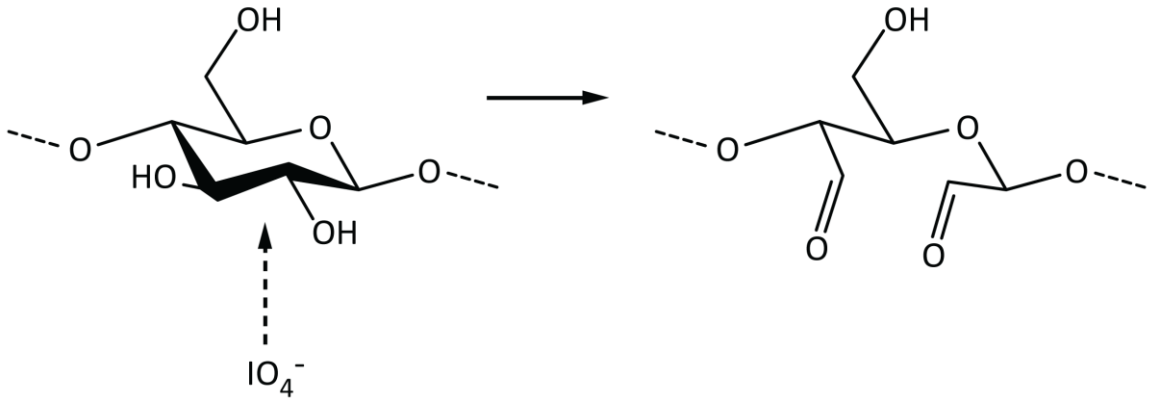

Figure 7. The general principle of periodate oxidation of glucose as described by Kristiansen *et al.*(2010). The periodate ion attacks the hydroxyls at the C2 and C3 carbons on the glucose and turns them into aldehydes and thereby opens the ring structure of the glucose. The resulting cellulose chain is called dialdehyde cellulose.

The resulting aldehydes can be utilised in further functionalisation of the cellulose chain. The reaction takes place in both amorphous and crystalline regions. Due to the crystalline nature of cellulose the site of reaction is more difficult to access, leading to a lower rate of reaction in these regions. The mode of attack is proposed to proceed in a highly heterogeneous fashion according to Kim *et al.* (2000) thereby forming isolated patches of oxidation along the micro/nanofibrils. This is thought to be due to the loss of crystallinity at these positions, making the remaining hydroxyls more susceptible to oxidation (Kim *et al*. 2000). Such mode of oxidation has the effect that the thinning of the micro/nanofibrills is minimised, unlike the results from a previous study (Sassi and Chanzy, 1995).

Experimental work

The following description of the experimental work highlights the procedure for obtaining the final modified cellulose product. Introductions to some of the experimental methods can be found in Appendix 1. Several different batches of oxidised cellulose were made to increase the amount of available material to work with. All were made in the same manner as described below and presented in Appendix 2.

Materials

Nanocellulose from *Cladophora* algae was provided by FMC Biopolymer. Sodium metaperiodate, L-cysteine, TEMPO, Oxone, picolylamine and other chemicals used were of analytical or reagent grade and were used as received. Unless otherwise stated the chemicals

were provided by Sigma Aldrich. Deionized water was used throughout the experimental procedures.

TEMPO-mediated oxidation

The preparation of the cellulose material started with TEMPO-mediated oxidation of *Cladophora* cellulose. To begin with 5.0 g of cellulose was dispersed in 700 ml of water. The dispersion was mechanically stirred at 160 rpm. To the dispersion a solution of 25.4 mg of TEMPO (Alfa Aesar) and 260 mg of sodium bromide (NaBr) in 50 ml sodium hypochlorite solution (NaClO, 10 wt. %) was added. To maintain the optimal pH of around 10.2 for the reaction the pH was monitored and NaOH added when needed. The reaction was allowed to proceed for 2 hours and was then quenched with an addition of 10 ml ethanol. To collect the cellulose the dispersion was centrifuged for 10 minutes at 4690 x *g*. Additional centrifugation was needed in order to obtain pellets of the cellulose. The pellets were then washed with water through dispersion and centrifuged four times, the supernatant was removed.

Oxone oxidation

A preparation of Oxone oxidised cellulose was prepared by dispersing 2 g *Cladophora* cellulose in 400 ml of water to which 7.6 g of Oxone ($KHSO_5$) was added. The dispersion was stirred and kept at 60°C overnight. The reaction was stopped by centrifuging the solution at 4690 x *g* until the cellulose had pelleted to the bottom of the centrifuge vessels. It was then washed repeatedly with water in the same way as the TEMPO-oxidised cellulose.

As with the TEMPO-oxidised cellulose, samples were taken and freeze dried or vacuum centrifuged for analysis. To determine the amount of cellulose in the solution it was dried in a vacuum oven. Preparation of 0.5 wt. % cellulose was made from calculation of dried cellulose content for sonication, presented in Table 5 in Appendix 2. 0.5 g of dry content cellulose was dispersed in 100 ml of water. The samples were sonicated at 20 % amplitude at 30 second pulse for 1 hour (Q500 Sonicator, QSonica, USA). The sonicated cellulose was vacuum centrifuged to thin films for analysis in SEM.

Following, sonications was conducted in a rosette beaker to enhance the effect of the sonication. In addition to this the time for sonication was reduced to ten minutes, as it had been shown to give satisfactory results by other groups (Kvien and Oksman, 2007; Saito *et al*., 2011).

Periodate oxidation

The sonicated cellulose was treated with sodiummetaperiodate. The first TEMPO oxidised cellulose batch yielded 0.08 g of dry weight cellulose, to this 0.3 g of sodiummetaperiodate was added in 75 ml of acetate buffer (10 mM, pH 4.5). The reaction was allowed to go on for one hour and 20 minutes before being quenched with 10 ml ethylene glycol. The material was washed repeatedly with water by dispersion and centrifugation. Samples of unmodified cellulose, oxidised cellulose, both TEMPO and Oxone mediated, and periodate oxidised TEMPO cellulose were dried in a vacuum oven for infra-red spectroscopy analysis. The solvent was changed from water to methanol to facilitate the functionalisation with picolylamine. Metaperiodate oxidation of the Oxone modified cellulose was conducted in the same manner. The first batch was made from sonicated supernatant of cellulose dilutions containing 0.035 g/ml and 0.0248 g/ml dry weight cellulose material. Of these solutions 120 ml was used, equalling a total content of 3.588 g cellulose material. For every gram of dry weight cellulose 3.75 g of sodium metaperiodate is needed to acquire sufficient oxidation, theoretically all available hydroxyls will react. 13.455 g sodium metaperiodate was added to the 120 ml of cellulose solution as well as 100 ml of acetate buffer into a beaker and stirred for two hours, after which 10 ml ethylene glycol was added to quench the reaction. The material was then washed with water in a centrifuge.

Functionalisation

The remaining cellulose material, 0.08 g dry weight, was dissolved in 30 ml of methanol to which 270 µl of picolylamine was added, 5 mole equivalents. The reaction was run over night. The yield from this reaction was much too low to be able to continue with. The Oxone and periodate oxidised cellulose was also functionalised with picolylamine. 5 ml picolylamine was added to 3.588 cellulose material in 60 ml of methanol and run over night while stirred. To stabilise the cellulose 1.5 g of sodium borohydride was added and allowed to react for 30 minutes. To quench the reaction and neutralise any remaining reduction agent, a few drops of water was added and 5 drops of acetic acid was added in intervals of a couple of minutes until the solution was neutral/acidic. The material was then washed with methanol followed by water through centrifugation.

A gelling experiment was conducted for a number of different concentrations of picolylamine functionalised cellulose. Nine different samples were prepared.

Cysteine coupling to oxidised cellulose was performed through mixing of cellulose material and cysteine in a 1:1 mole equivalent ratio. To begin with, the mixing was done in acetate buffer of 10 mM and pH 4.5. The first round consisted of 0.16 grams of cellulose material and 0.12 grams of cysteine in 50 ml acetate buffer, and was kept stirred over night. The next day the oxidised cellulose structure was stabilised with 0.07 g of the reductive agent $NaCNBH_3$, one mole equivalent. To neutralise any remaining $NaCNBH_3$ the solution was quenched with drop wise added acetic acid until the pH reached acidic values. The material was then washed repeatedly with water through centrifugation.

Linker addition

As an alternative to the Schiff-base coupling another method that adds a linker between the cellulose and the functional amines was tested. To add a linker between the cellulose and the metal chelating cysteine a procedure to add DNA to cellulose by utilising oxidised sugars was adapted to add amines to cellulose. The procedure for DNA is described by Larry Moss *et al.* (1981). The first setup used in this project was with 50 ml of cellulose suspension to which 2.5 ml of the epoxide linker, diglycidyl ether, was added and to start the reaction a mixture of 0.6 M NaOH and 4 mg/ml $NaBH_4$ was used. The reaction was carried out in a small round bottom flask over night. Then the mixture was washed with ethanol and water. To couple cysteine to the epoxide linker one mole equivalents of cysteine to the cellulose in the samples were added to saturate the available positions. A lot of the cellulose material was washed away and the procedure was repeated with higher concentrations of cellulose.

Magnetic alignment

For the test in a magnetic field samples of different modifications of the cellulose were prepared. There were four different samples in total, two each of TEMPO and Oxone oxidised cellulose to which cysteine had been added either through periodate oxidation or linker functionalisation. The aim was to prepare samples of about 5 wt. % cellulose content. Due to there not being equal amounts of materials of the different modifications the actual content varied from 2.93 % to 5.70 %.

To test the magnet the cellulose that had been modified with Oxone and periodate oxidation and functionalised with cysteine was used. Samples of 250 µl cellulose suspensions were placed in 3 ml cuvettes. The cuvette was placed between the coils of the magnet to be subjected to the magnetic field of about 1 Tesla. To the suspension a few drops of iron ions dissolved in water was added either before or after being exposed to the magnetic field for 45

minutes. The solution of iron chloride was prepared by dissolving 2.7 g $Cl_3Fe{\cdot}6H_2O$ in 10 ml of water.

Two samples were allowed to dry through evaporation under ordinary room conditions and two samples were snap frozen in liquid nitrogen directly after being subjected to the magnetic field and then freeze-dried in a vacuum centrifuge.

SEM

After the samples had dried they were prepared for SEM analysis. A description of how SEM works can be found in Appendix 1. The samples were attached to aluminium sample stubs with double adhesive tape. Then they were sputter coated with a mixture of palladium and gold (Polaron SC7640, Thermo VG Scientific, England), before being analysed in SEM (Leo 1550 SEM, Zeiss, Germany).

Conductometric titration

Conductometric titration was carried out on samples of about 100 mg cellulose. The cellulose was dried and weighed to determine its mass. The amount of TEMPO and Oxone oxidised cellulose were 118 mg and 115 mg respectively. The cellulose was dried in a vacuum oven. It was then dissolved in 30 ml of 1 mM aqueous NaCl prepared through dilution of 116.88 mg NaCl in two litres of water. The NaCl suspensions were sonicated for 6 minutes with 30/30-pulse and 30% amplitude. The titration was then performed with a titrator (G20 compact titrator, Mettler Toledo, Switzerland), the pH of the samples was lowered to around three by addition of HCl (1 M) to the solutions. By using the LabX titration interface the rate of addition of 0.01 M NaOH was set to 0.05 ml/min, and the titration took about six hours to achieve the wanted plateaus from which the conductivity could be deduced. The theory describing conductometric titration can be read in Appendix 1.

Zeta-potential measurements

Zeta-potential measurements were done on 0.02 % cellulose dispersions made through dissolving 10 mg of dry content cellulose in ~10 mM NaCl solution, 526 mg NaCl in 900 ml of water. A short description of zeta-potential measurements can be read in Appendix 1. The pH was measured and adjusted with the titrator. By addition of NaOH the pH was increased from the stem solutions of 7.74 for Oxone and 6.17 for TEMPO oxidised cellulose in intervals of around one pH unit. The measurements were conducted on 1 ml samples using a dip-cell and the following parameters in a Zetasizer Nano Z (Malvern, UK).

- Measuring type: zeta potential
- Material: Cellulose
- Dispersant: Water
- F(Ka) selection: Smoluchowski
- Temperature: 25°C
- Cell: Dip-cell
- Measurement: Automatic, min 10 runs, max 100 runs
- Number of measurements: 6
- Delay: 2 min
- Automatic attonation selection: yes
- Automatic voltage selection: yes
- Analysis model: Automatic

Interpretation of results and discussion

SEM micrographs

The results were mainly analysed with SEM and thus the study is focused on the morphology of the cellulose fibres. Since no greater alignment could be observed after subjecting the cellulose solution to the magnetic field the first conclusion that can be drawn is that the magnet used could not generate a field strong enough or maintain it long enough to influence the cellulose. The magnet had a capacity of just ~1 T and could be run only briefly, 45 minutes. The run time was limited because the cooling for the magnet was not available.

Cellulose modified with cysteine added to it through Oxone and periodate oxidation was used for the runs in the magnet. This was the modification of which there was most material available, see Table 1.

Table 1. Final product obtained for magnetic alignment.

	Mass of cellulose material (wet) (g)	Dry weight cellulose (g)	Volume water to make solution (ml)	%w/w
TEMPO + periodate + cysteine	1.13	0.07	1.50	4.81
TEMPO + linker + cysteine	0.19	0.01	0.50	2.93
Oxone + periodate + cysteine	3.00	0.20	4.00	4.99
Oxone + linker + cysteine	0.52	0.06	1.00	5.70

Micrographs taken on cellulose oxidised with TEMPO or Oxone show no apparent alignment of the fibres. However, there are some examples of regions where the order seem to be higher. This could be due to shearing forces during drying in the vacuum centrifuge, which have been shown to occur by others (Orts *et al*., 1998). It could also happen as a result of ice crystal formation during freeze-drying (Han *et al*., 2013). In one of the samples taken from freeze-dried TEMPO oxidised cellulose some cellulose fibres align and appear to be rather parallel, as seen in Figure 8.

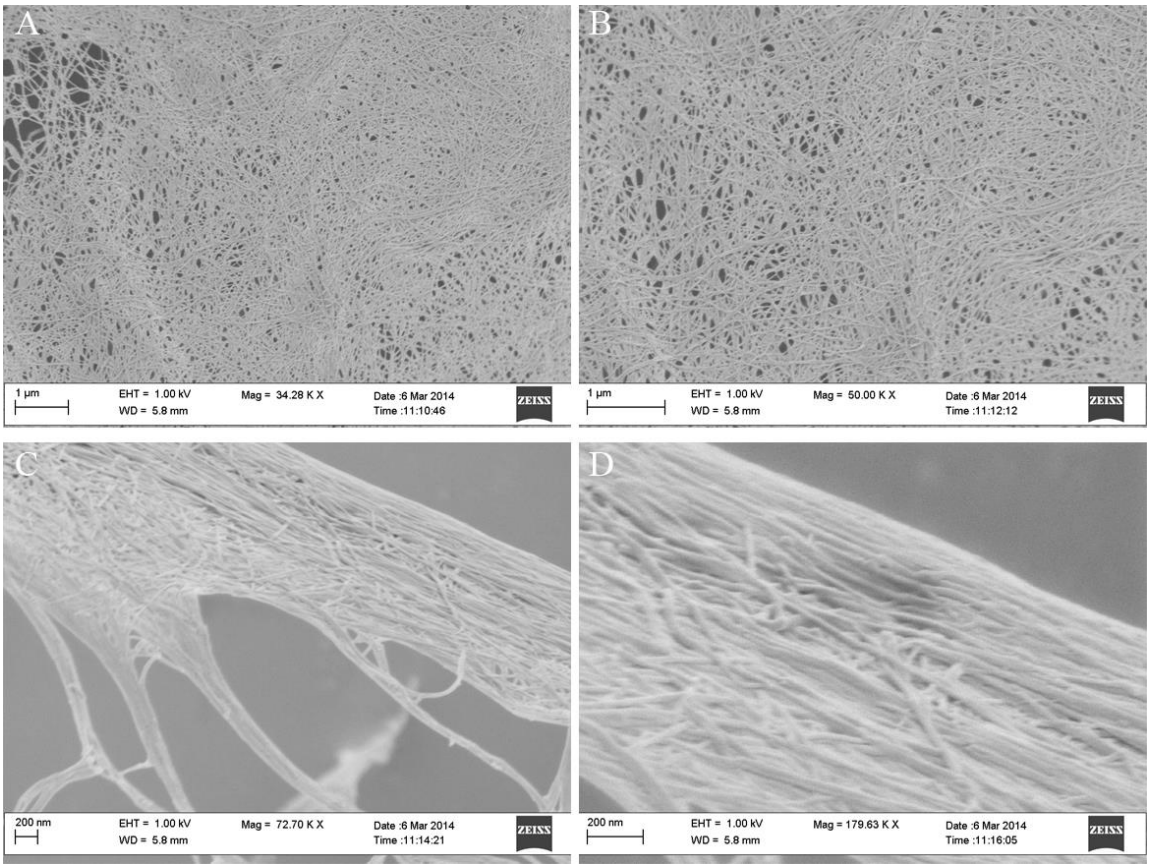

Figure 8. TEMPO oxidised cellulose freeze-dried for 24 h. The cellulose shows regions of higher degree of alignment. Especially in panels C and D. The magnification is 34.28k×, 50.00k×, 72.70k× and 178.63k× for panels A, B, C and D respectively.

This kind of alignment is due to shearing forces when the samples are centrifuged. Although there is alignment from this rather simple method the results are unpredictable and only parts of the material is affected. With magnetic alignment the whole sample would be affected in the same way as long as the magnetic field is homogenous and includes the entire sample. This would lead to better predictability of the final alignment and the results could more easily be reproduced. The other samples look more or less the same as the examples shown in Figure 9 and Figure 10, all micrographs taken can be seen in Appendix 2.

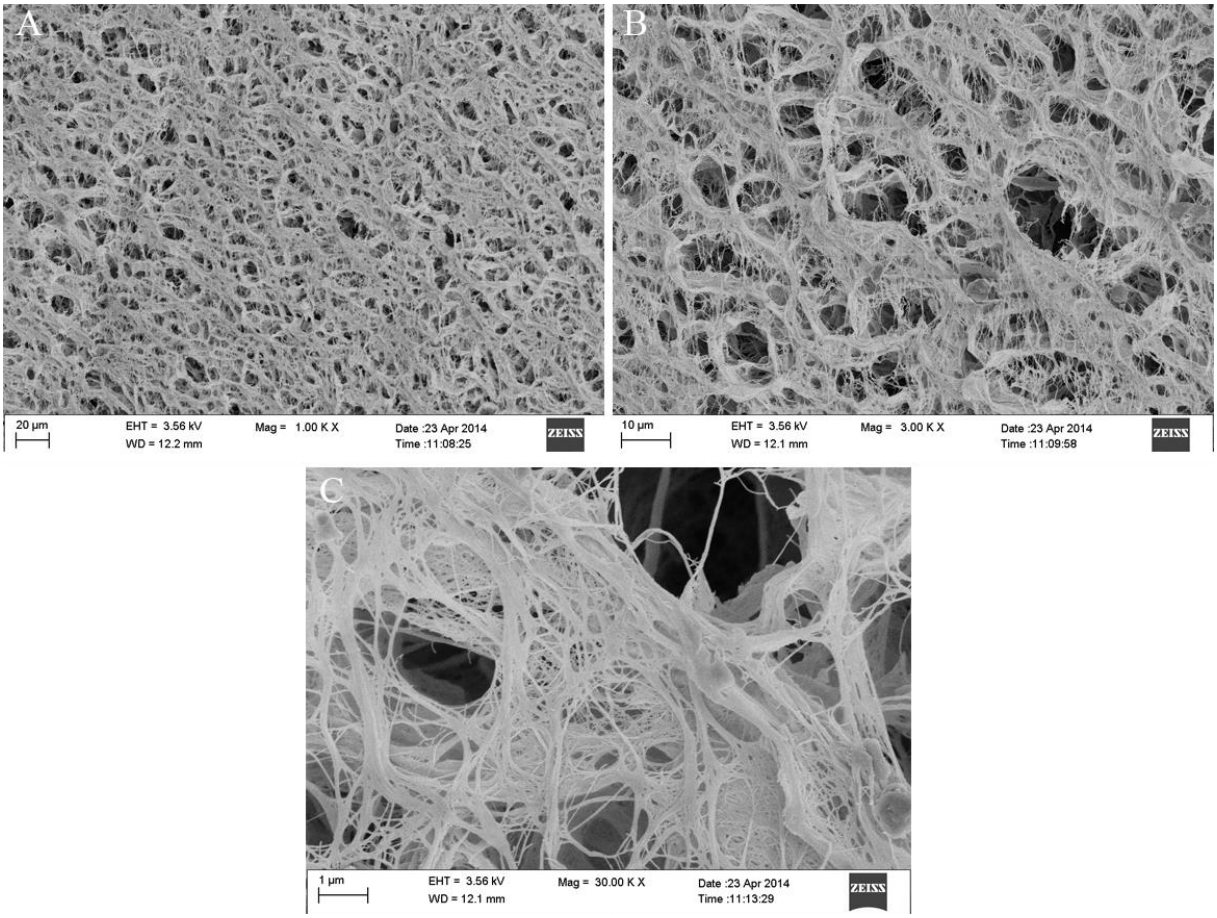

Figure 9. TEMPO oxidised cellulose that has been sonicated and vacuum centrifuged. The fibres show random alignment in a corkscrew like pattern. The magnification is 1.00k×, 3.00k× and 30.00k× for panels A, B and C respectively.

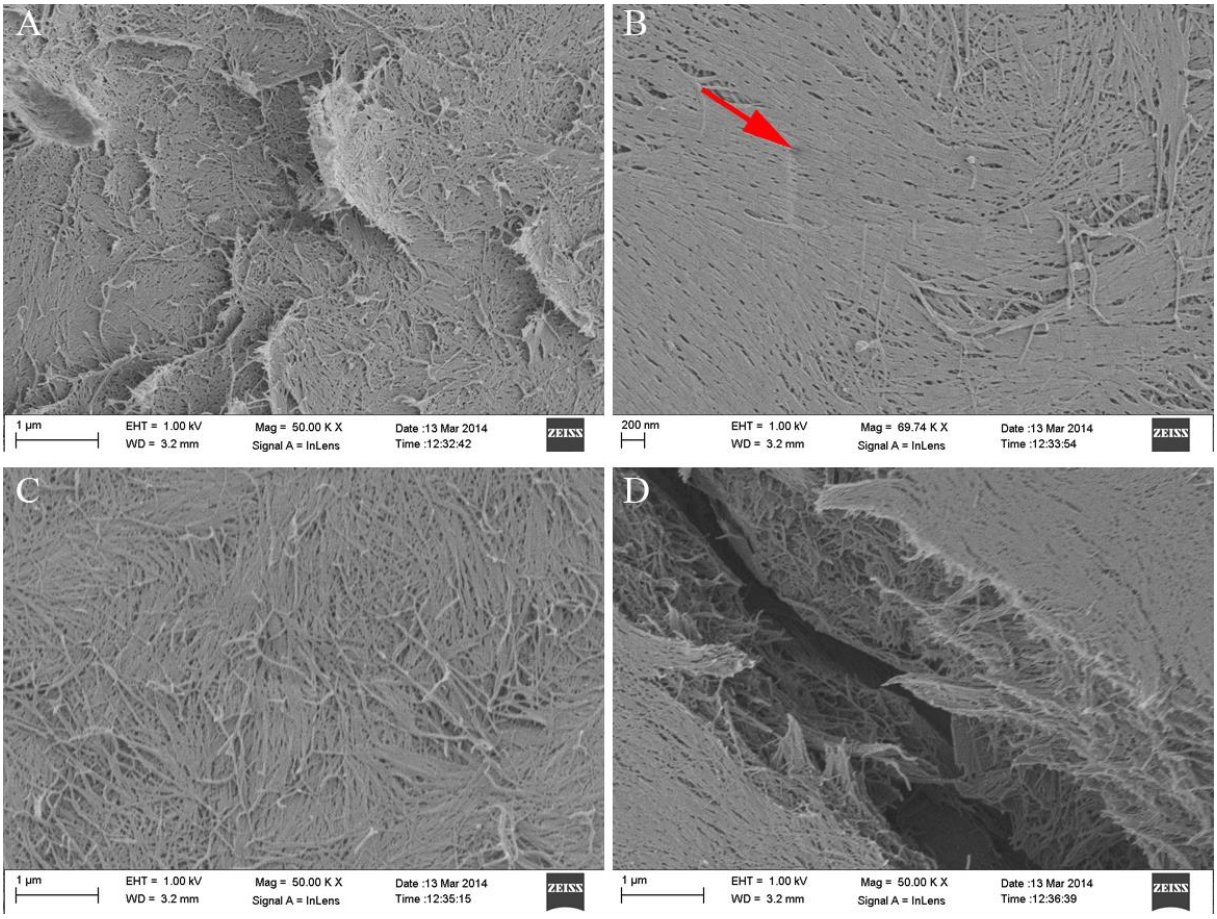

Figure 10. Vacuum centrifuged Oxone oxidised cellulose. Tightly packed fibres with random order alignment. In panel B a rectangle can be seen by the arrow where the electrons of the microscope have melted the material. The magnification is 50.00k×, 69.74k×, 50.00k× and 50.00k× for panels A, B, C and D respectively.

Conductometric titration

When compared to each other the oxidation with TEMPO, Figure 11 and Figure 12, seem to be more efficient than the oxidation with Oxone, Figure 13 and Figure 14. The missing, or at least very small, plateau in Figure 13 is due to inadequate oxidation with Oxone. The material was grainier and had a slight yellow colour compared to previous iterations. The oxidation was probably not allowed ample time to carry out the reactions and it may have been too cold for optimal efficiency. The plateau regions of the charts revealed that oxidation of the cellulose had taken place. The amount of oxidation was calculated from the intercepts of the linearised decrease and increase of conductivity. The mole amount of carboxyls were 0.36 and 0.15 per gram of cellulose for the first measurements of TEMPO and Oxone oxidised cellulose respectively. For the second measurement the results were 0.40 and 0.10 mole carboxyls per gram of cellulose. These results implies that the oxidation has taken place.

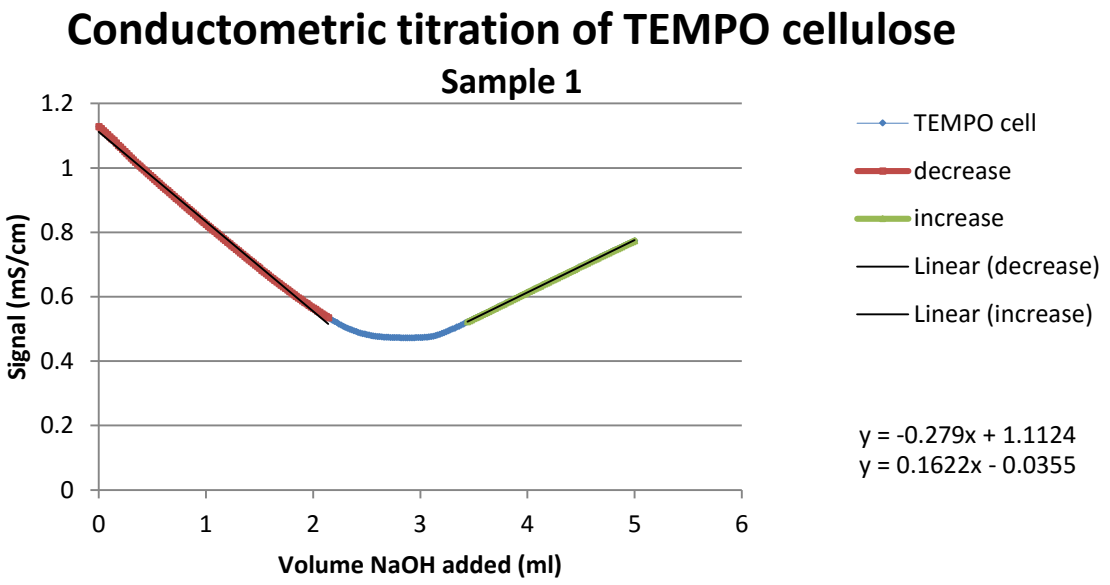

Figure 11. Conductometric titration of TEMPO oxidised cellulose. The linear phase to the left is decrease of conductivity due to H+ ions being oxidised, and the linear phase to the right corresponds to increased conductivity with added free OH- groups in the solution. The plateau in the middle is oxidation of hydroxyl groups on the cellulose material.

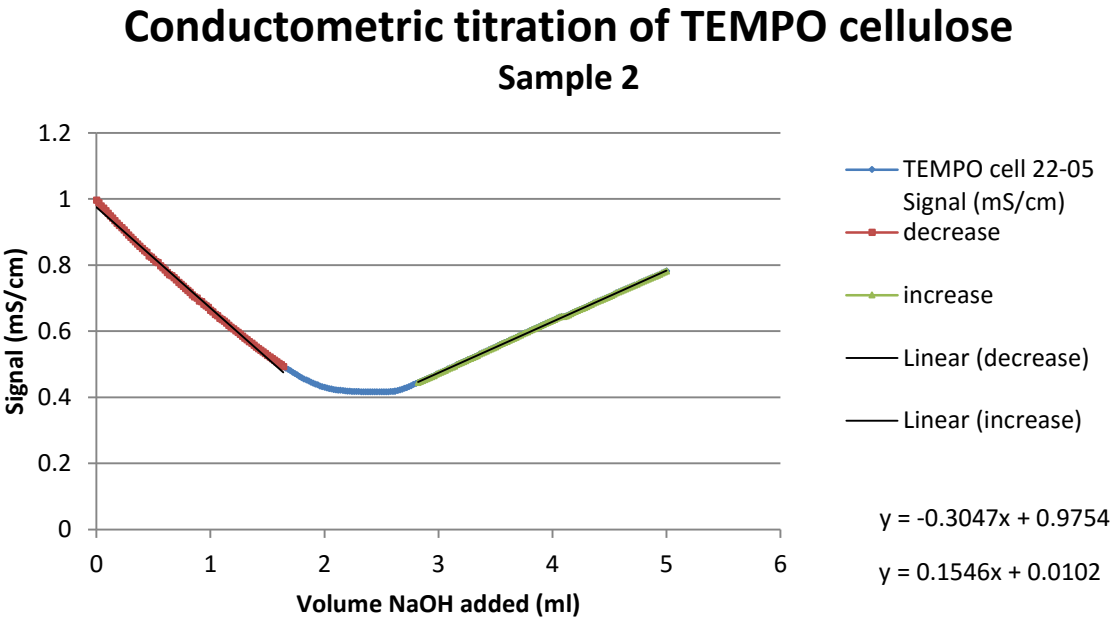

Figure 12. Conductometric titration of TEMPO oxidised cellulose, taken on 2014-05-22.

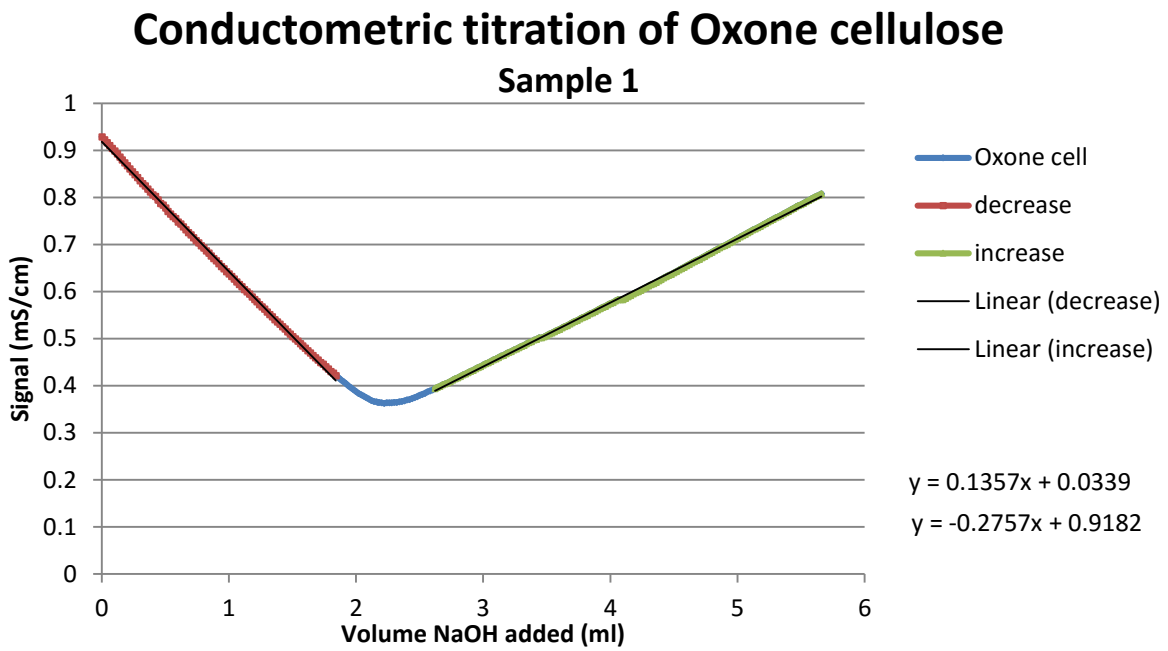

Figure 13. Conductometric titration of Oxone oxidised cellulose.

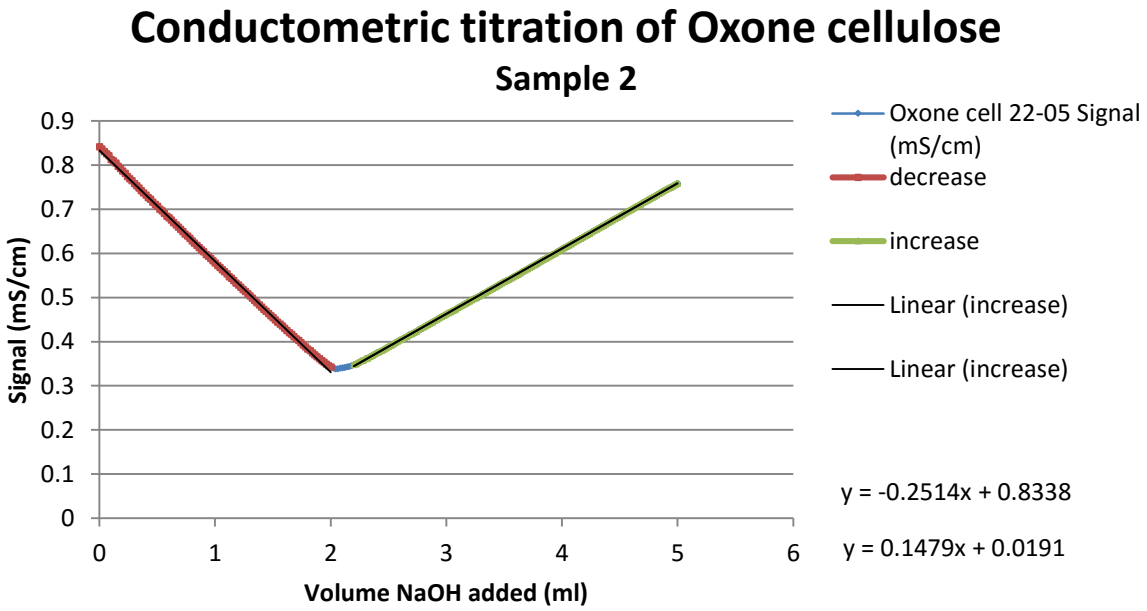

Figure 14. Conductometric titration of Oxone oxidised cellulose, taken on 2014-05-22.

From the data in Table 2 it can be said that the yield of cellulose material after sonication is not very high, 70 % at best. Given that all steps after the sonication contain a number of centrifugations it is quite certain that the total yield of cellulose material is quite low. The amount of final product was highest for Oxone and periodate oxidised cellulose with cysteine coupled to it at 0.19 g dry weight, and considerably lower for all the other samples.

Table 2. Yield of cellulose material after sonication and centrifugation.

	TEMPO	Oxone #1	Oxone #2
Cellulose mass/10 ml (g)	0.03	0.03	0.02
Total cellulose mass in solution (g)	0.35	0.34	0.25
Cellulose before sonication (g)	0.50	0.50	0.50
Yield	0.69	0.68	0.50
Yield %	69.40	68.00	49.60

Zeta-potential measurements

The measured zeta potentials of the oxidised cellulose samples, with both TEMPO and Oxone, are in line with expectations. Unmodified crystalline cellulose would have a zeta potential of around -10 mV due to the hydroxyls present (Zhou, 2012). When oxidising the cellulose additional surface charges are added and thus the zeta potential drops. In this case to between -30 mV to -40 mV, for TEMPO and Oxone oxidised cellulose respectively, see Table 3 and Table 4. It would have been good to make measurements on the lower range of pH values as well in order to make more assertive conclusions. The results obtained are still in line with expectations for the surface charges of the modified cellulose.

Table 3. Zeta potential measurements on TEMPO oxidised cellulose.

pH	6.10	8.60	9.10
Zeta potential (mV)	-37.63	-41.20	-41.97

Table 4. Zeta potential measurements on Oxone oxidised cellulose.

pH	7.70	8.80	9.10
Zeta potential (mV)	-32.67	-30.20	-32.67

Future work

If the results had been more conclusive further analysis of the cellulose material could have been conducted. The first step though before any further studies is to try with a stronger magnet and run it for a longer time to see if the alignment is possible to achieve. Then the results for the modified cellulose should be compared to unmodified cellulose to determine whether the modification in some way improves the sustainability of the induced order.

Dynamic light scattering

Dynamic light scattering is a method that can be used to determine the size distribution of particles in a suspension. When shone on by a laser the particles in the suspension will scatter the light. Depending on their size the particles will scatter the light differently. Small particles, compared to the wavelength of the incoming light, will scatter it in all directions. Due to the diffusion of the particles in the suspension the recorded intensity of scattered light will fluctuate with time. Larger particles move slower and thus the fluctuation will be less than for smaller ones. The intensity profiles can be examined and conclusions on the particles sizes can be drawn. To draw confident conclusions the suspension has to be monodisperse, containing one particle population of the same size. Otherwise a more intricate setup is needed and the data has to be analysed further. This is to be able to cope with parameters such as polydispersity and multiple scattering.

Thermogravimetric analysis

This analysis method is employed to study changes in physical and chemical properties of materials as they are subjected to changes in temperature. The measurements are usually done either as a function of increasing temperature or as a function of time. Depending on which properties are to be studied and what is expected from the material the approach can be varied. It is especially useful to employ when studying materials that exhibit mass changes due to decomposition, oxidation or loss of volatiles. There are a few very common applications; characterisation of materials through analysis of decomposition patterns, studies on mechanisms and reaction kinetics of degradation and organic and inorganic content determination. In order to get useful results it is important to have high precision measurements of three properties: mass change, temperature and temperature change. To conduct a thermogravimetric analysis a precision balance and a programmable furnace is needed. The method relies on detecting mass changes in the sample as the temperature increases. The changes in mass are indicators that some reaction is taking place in the sample

such as decomposition. It can detect phase transitions, physical phenomena, and also give insight into chemical phenomena such as chemisorption and solid-gas reactions. Different reactions occur at different temperatures and are dictated by the composition of the sample and the available pathways.

N_2 H_2O adsorption isotherms (BET)

Adsorption is the process when molecules or species in a liquid or gas adsorbs to a solid material. It can also describe the interface of liquid-liquid or liquid-gas attractions. On the surface of any given phase there are attractive forces that can bind to molecules in adjacent phases.

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Appendix 1: Methods for analysis

A few different methods have been used in order to analyse and characterise the cellulose material produced during the laboratory work.

Fourier transform infra-red spectroscopy FTIR

FTIR is an example of absorption spectroscopy and the aim is to measure a samples absorption of light of certain wavelengths (Griffiths and De Haseth, 2007). It is based on ordinary IR-spectroscopy where light in the infra-red spectrum is shone on the sample one wavelength at a time and the absorption is measured consecutively. FTIR is used to obtain the same information the major difference being that instead of shining monochromatic light (light of a single wavelength/frequency) on the sample a beam of light containing several frequencies are used instead (Griffiths and De Haseth, 2007). The frequencies used are then shifted to a new set of frequencies and a new measurement is taken, this is repeated a number of times and generates a data point for each beam used. The data is then analysed by a computer and the absorption for each wavelength is inferred through calculations of Fourier transforms of the data.

Zeta-potential measurements

To determine the surface properties of particles in a colloidal (liquid suspension) system a method called zeta-potential can be utilized. What is done is a measurement of the difference in electric potential between the medium and the stationary layer attached to the particles (Hunter *et al*., 2013). In any suspension the fluid medium will form a layer of stationary molecules surrounding the particles. This stationary layer will have somewhat different properties than the medium in general. In particular it will display a different electric potential, due in part to the higher order of orientation. The benefit of doing a zeta-potential measurement is that it can be translated into a value of stability for the suspension. In other words it can determine how likely the particles in the solution are to aggregate together. For sufficiently small particles a high zeta-potential indicates that the solution will be stable. If the potential is low this means that the attractive forces between the particles are higher than the repulsive ones. This leads to aggregation and flocculation of the particles (Hunter *et al*., 2013).

Scanning Electron Microscope - SEM

The scanning electron microscope is used to get topographic information of samples at very high degrees of magnification (Thornton, 1968). The basic principle of microscopes is that the

resolution is determined to great extent by the wavelength used in the setup. Thus a scanning electron microscope has a much greater theoretic potential in terms of resolution than ordinary light microscopes (Thornton, 1968). On top of that the magnification possible is also much greater, up to 300 000 times. An electron microscope differ from an optical microscope in mainly one way, it utilizes a beam of electrons instead of a beam of light (Becker William T., 2002). This in turn leads to a few necessary modifications. Instead of lenses to gather and direct the light it uses magnets. However, the major difference is in terms of how the sample is analysed. Since the human eye is only able to detect electromagnetic waves within the visible spectrum some other means of recording is needed. To this end a number of different detectors can be used. These detectors analyse the way that the electron beam interacts with the specimen. To view the surface of the specimen the detector records the action of secondary electrons (Thornton, 1968). Secondary electrons being electrons that are dislodged from the specimen as the beam hits it. Other detectors can record backscattered electrons or X-rays to give information about the composition of the specimen (Thornton, 1968). Because of the scale on which the objects are studied it is also necessary to minimize the amounts of vibrations inside the sample chamber. As well as having the means to adjust the position of the specimen in a very precise way. Another thing that is very important is that the sample is studied under a vacuum. Otherwise all the molecules in the air would interfere with the electron beam and distort the recorded signal. Only limited sample preparation is needed for SEM readings (Becker William T., 2002). The surface of the specimen need to be conductive, or else the incoming electrons will have a degenerating effect. If the sample is not inherently conductive it needs to be covered with a conductive layer (Becker William T., 2002). This is done through sputter coating. A thin film of metal, often gold or palladium or a mixture of both, is deposited onto the surface through an argon gas flux. This treatment protects the sample and makes it possible to do numerous analyses on the same specimen. Also if the specimen contains water, the moisture needs to be removed through drying in some way. Otherwise, the water will vaporize when the vacuum is established (Becker William T., 2002). There are special microscopes available that can handle other environments, apart from vacuum. Those are called environmental SEM and they separate the beam from the specimen. This allows for the chamber with the specimen to be filled with gas, while still containing the high vacuum necessary for the electron beam.

Conductometric titration

Often referred to as only conductometry is an analytical chemistry method that is used to monitor the progress of chemical reactions (Khopkar, 2012). It is based on the measurements of the conductive properties of a solution, specifically the change in conductivity when an acid or base is titrated. The ions monitored are H+ and Na+, drastic changes in conductivity is the result of these ions being replaced by each other. The hydrogen ion is highly mobile compared to the sodium ion, thus the conductivity is decreased as the H+ ions are replaced. When the conductivity hits a minimum all hydrogen ions have been replaced by sodium. Adding more Na after the equivalence point will lead to an increase in conductivity again. This method is often used on coloured or homogenous solutions where other titration methods are less reliable.

Appendix 2: Supplementary data

Determination of cellulose content

Table 5. Calculated dry/wet weight ratio for samples and how much is needed to make 0.5 wt.% solutions.

Drying of cellulose material	2014-03-06	2014-03-06	2014-03-06	2014-03-18	2014-03-18	2014-03-27
	TEMPO #1	Oxone #1_1	Oxone #1_2	Oxone #2_1	Oxone #2_2	Oxone + picolylamine
Weighing boat (g)	1.03	1.03	1.03	1.03	1.03	1.03
Total weight (g)	1.96	2.50	1.98	1.59	1.81	1.15
Cellulose material (g)	0.92	1.47	0.95	0.56	0.78	0.12
Total weight after drying (g)	1.07	1.10	1.07	1.05	1.06	1.03
Cellulose material after drying (g)	0.04	0.07	0.04	0.02	0.03	0.00
Weight content wet/dry (g)	22.00	19.88	26.31	29.26	31.28	39.67
Mass needed for 0.5% weight solution in 100 ml water (g)	11.00	9.94	13.15	14.63	15.64	19.83
Weight content dry/wet (g)	0.05	0.05	0.04	0.03	0.03	0.03

Drying of cellulose material	2014-04-11	2014-05-14	2014-05-19	2014-06-09	2014-06-09	2014-06-11
	TEMPO #2	TEMPO #3	Oxone #3	Oxone #4_1	Oxone #4_2	Linker + cysteine
Weighing boat (g)	1.03	1.02	1.25	0.85	1.02	0.78
Total weight (g)	1.48	1.52	1.72	1.18	1.60	0.85
Cellulose material (g)	0.45	0.50	0.46	0.34	0.57	0.07

Total weight after drying (g)	1.05	1.06	1.29	0.87	1.06	0.78
Cellulose material after drying (g)	0.02	0.03	0.03	0.02	0.04	0.00
Weight content wet/dry (g)	21.48	14.17	14.00	18.61	15.08	24.00
Mass needed for 0.5% weight solution in 100 ml water (g)	10.74	7.09	7.00	9.31	7.54	12.00
Weight content dry/wet (g)	0.05	0.07	0.07	0.05	0.07	0.04

Drying of cellulose material	2014-06-24	2014-06-26	2014-06-27	2014-06-27	2014-06-30
	TEMPO #4	Oxone #5	Oxone + cysteine	Tempo + cysteine	Oxone + linker + cysteine
Weighing boat (g)	0.22	0.92	0.78	0.14	0.14
Total weight (g)	0.41	1.67	0.85	0.16	0.17
Cellulose material (g)	0.19	0.75	0.07	0.02	0.03
Total weight after drying (g)	0.23	0.98	0.78	0.14	0.14
Cellulose material after drying (g)	0.01	0.07	0.00	0.00	0.00
Weight content wet/dry (g)	20.48	11.19	25.52	25.50	21.20
Mass needed for 0.5% weight solution in 100 ml water (g)	10.24	5.60	12.76	12.75	10.60
Weight content dry/wet (g)	0.05	0.09	0.04	0.04	0.05

Drying of cellulose material	2014-06-30	2014-06-30	2014-06-30	2014-07-02
	Tempo + linker + cysteine	Oxone #6	TEMPO #5	Oxone + periodate + cysteine

Weighing boat (g)	0.14	0.11	0.13	1.82
Total weight (g)	0.15	0.22	0.19	1.86
Cellulose material (g)	0.01	0.11	0.06	0.04
Total weight after drying (g)	0.14	0.12	0.13	1.82
Cellulose material after drying (g)	0.00	0.01	0.00	0.00
Weight content wet/dry (g)	13.50	16.79	18.62	16.71
Mass needed for 0.5% weight solution in 100 ml water (g)	6.75	8.40	9.31	8.35
Weight content dry/wet (g)	0.07	0.06	0.05	0.06

Drying of cellulose material	2014-08-04	2014-08-04	2014-08-04	2014-08-04
	TEMPO + periodate + cysteine	TEMPO + linker + cysteine	Oxone + periodate + cysteine	Oxone + linker + cysteine
Weighing boat (g)	1.84	1.84	1.83	1.83
Total weight (g)	1.91	1.85	1.86	1.85
Cellulose material (g)	0.07	0.01	0.03	0.02
Total weight after drying (g)	1.84	1.84	1.83	1.83
Cellulose material after drying (g)	0.00	0.00	0.00	0.00
Weight content wet/dry (g)	15.68	13.14	15.05	9.12
Mass needed for 0.5% weight solution in 100 ml water (g)	7.84	6.57	7.53	4.56
Weight content dry/wet (g)	0.06	0.08	0.07	0.11

SEM micrographs

Below are micrographs taken on a number of cellulose samples to study morphology and alignment of the fibres. Figures 15-22 were taken on 2014-03-06 and shows TEMPO or Oxone oxidised cellulose that have been either vacuum centrifuged or freeze-dried. Figures 16-23 shows sonicated TEMPO or Oxone oxidised cellulose that have been dried in a vacuum centrifuge. The micrographs were taken on 2014-03-13. Figures 30-32 shows vacuum centrifuged sonicated TEMPO oxidised cellulose, micrographs from 2014-04-28.

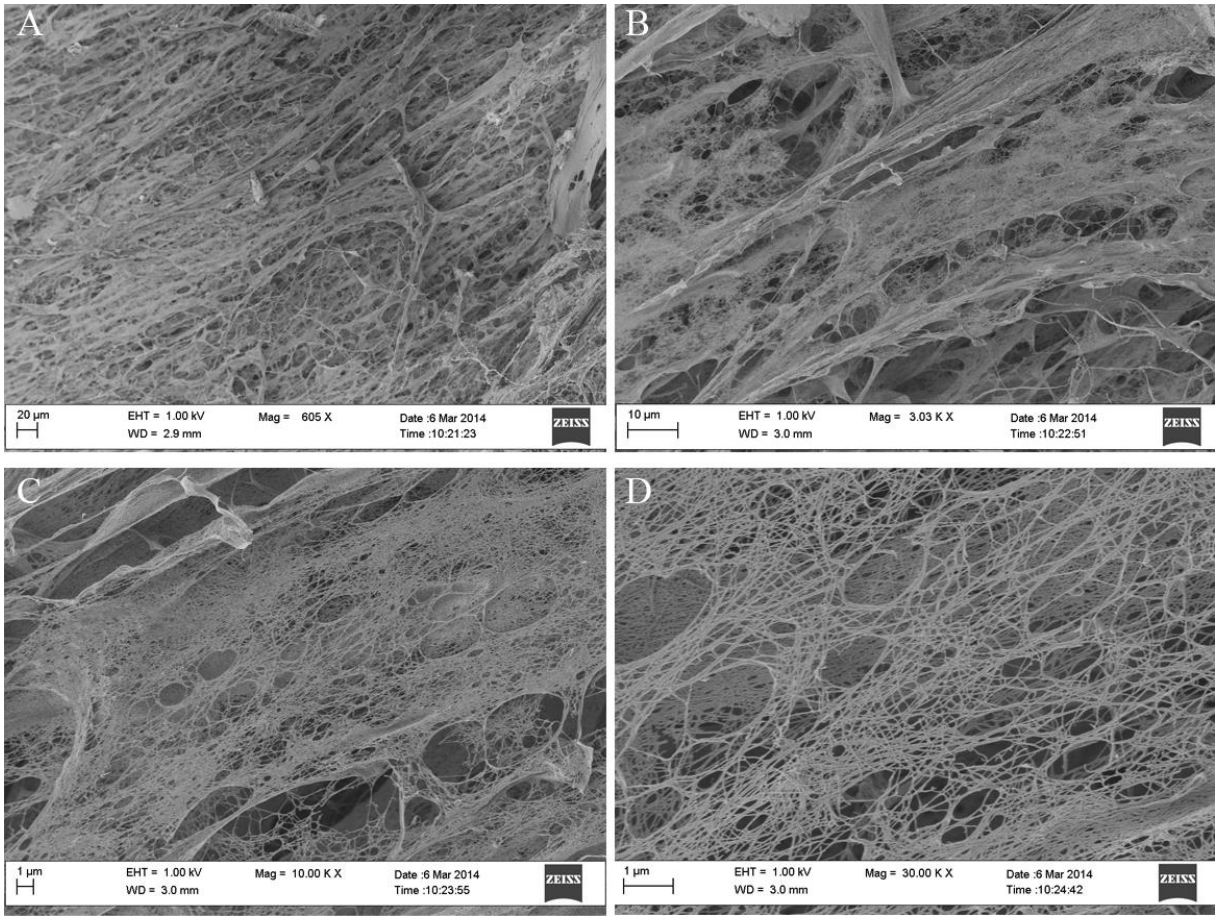

Figure 15. Oxone oxidised cellulose, vacuum centrifuged for 24 hours at 2000 rpm. Porous material with web-like structure and no apparent order. The magnification is 650×, 3.03k×, 10.00k× and 30.00k× for panels A, B, C and D respectively.

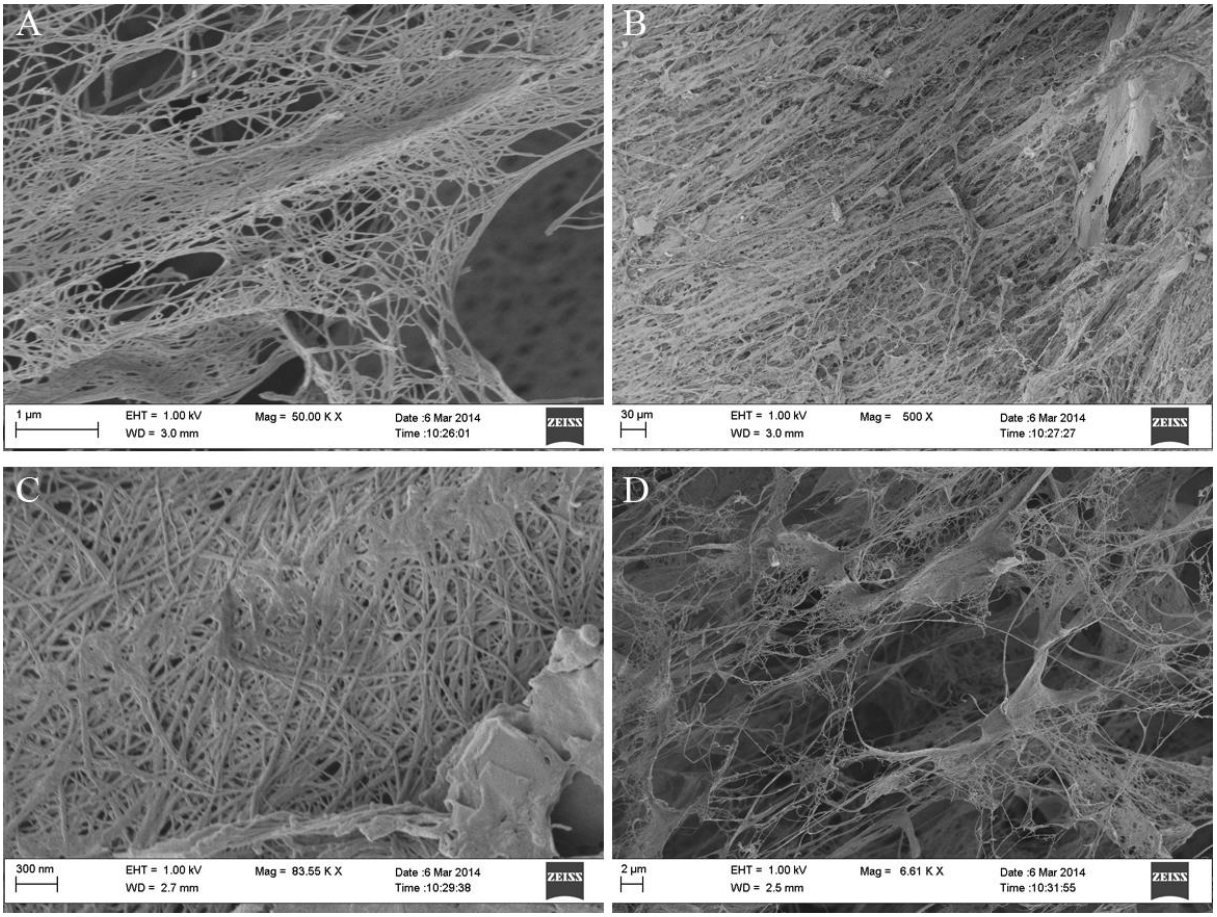

Figure 16. Oxone oxidised cellulose, vacuum centrifuged for 24 hours at 2000 rpm, same sample as previous figure. The magnification is 50.00k×, 500×, 83.55k× and 6.61k× for panels A, B, C and D respectively.

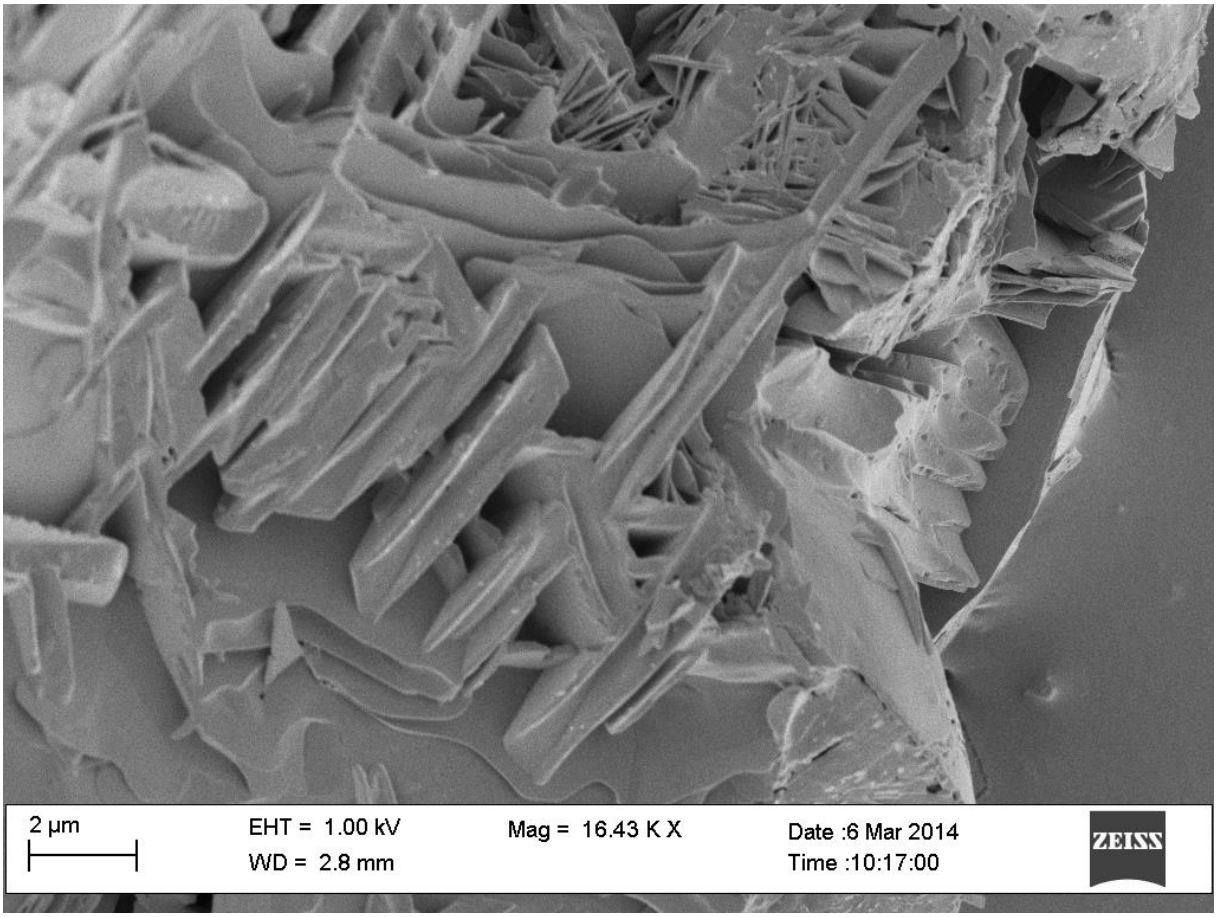

Figure 17. Supernatant from unwashed and first wash of Oxone oxidised cellulose that have been dried in vacuum centrifuge, 2000 rpm for 24 h. No cellulose fibres visible, cluttered with salt crystal complexes. The magnification is 16.43k×.

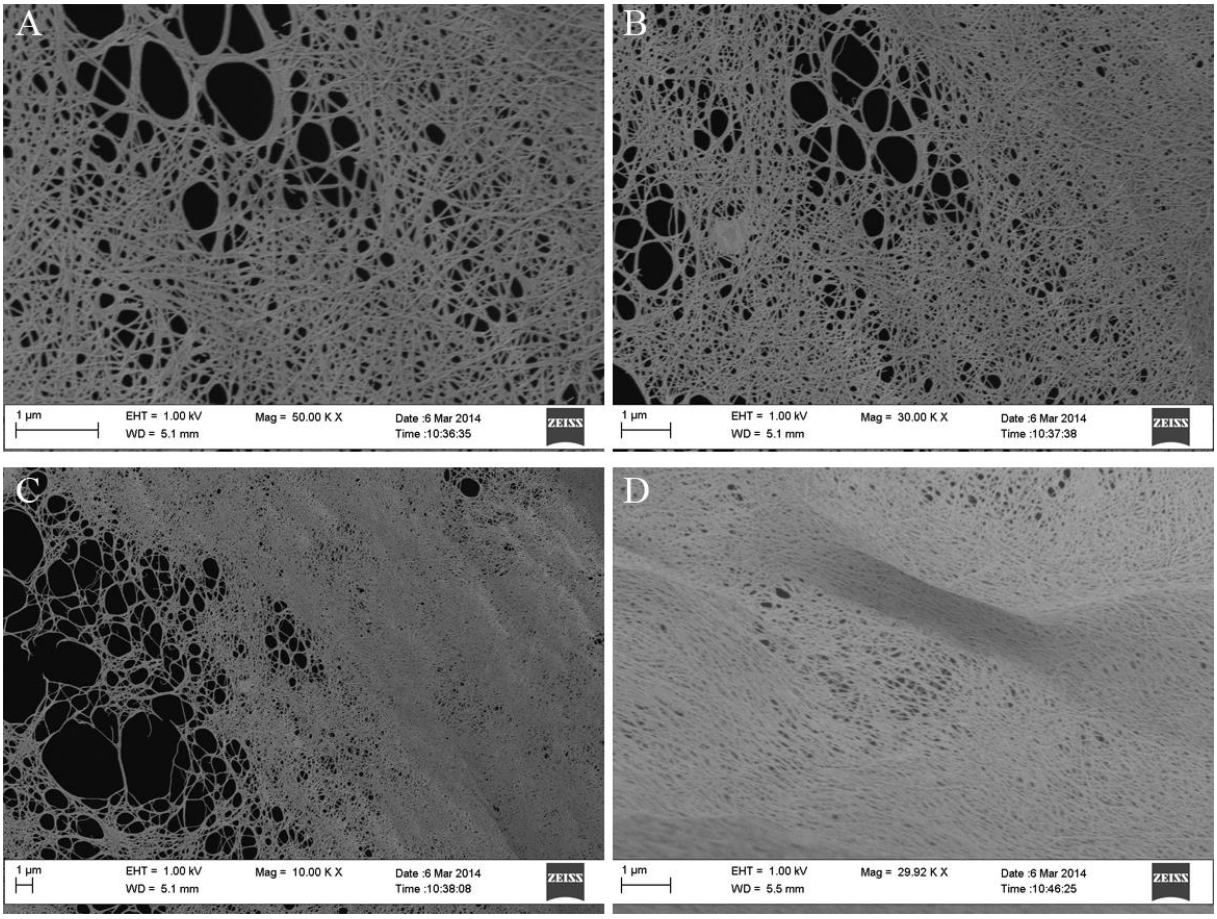

Figure 18. TEMPO oxidised cellulose dried in vacuum centrifuge, 2000 rpm for 24 h. Web-like structure, no apparent order or alignment. The cellulose in the lower right picture looks more tightly packed than the others. The magnification is 50.00k×, 30.00k×, 10.00k× and 29.92k× for panels A, B, C and D respectively.

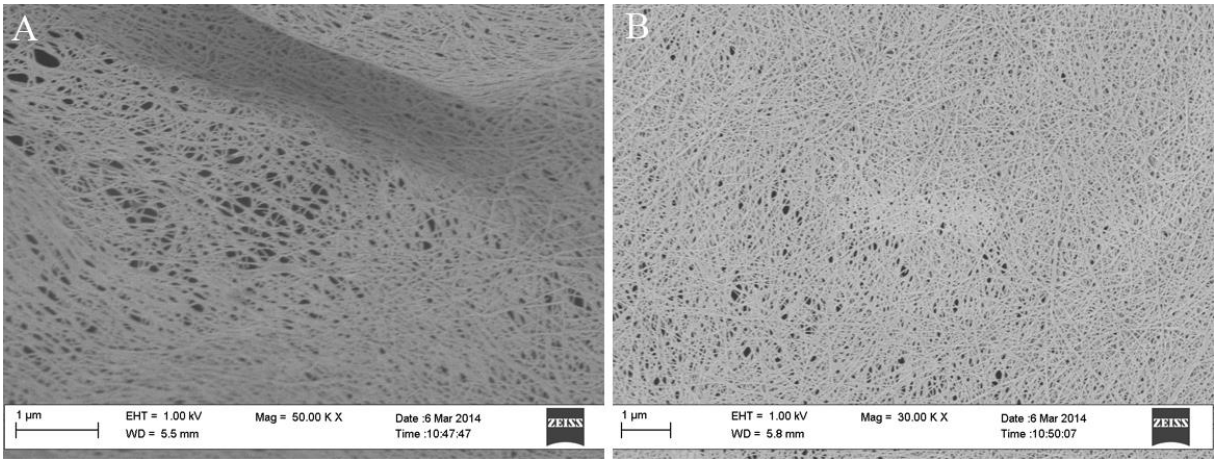

Figure 19. TEMPO oxidised cellulose dried in vacuum centrifuge, 2000 rpm for 24 h. The cellulose fibres looks to be tightly packed. The magnification is 50.00k× and 30.00k× for panels A and B respectively.

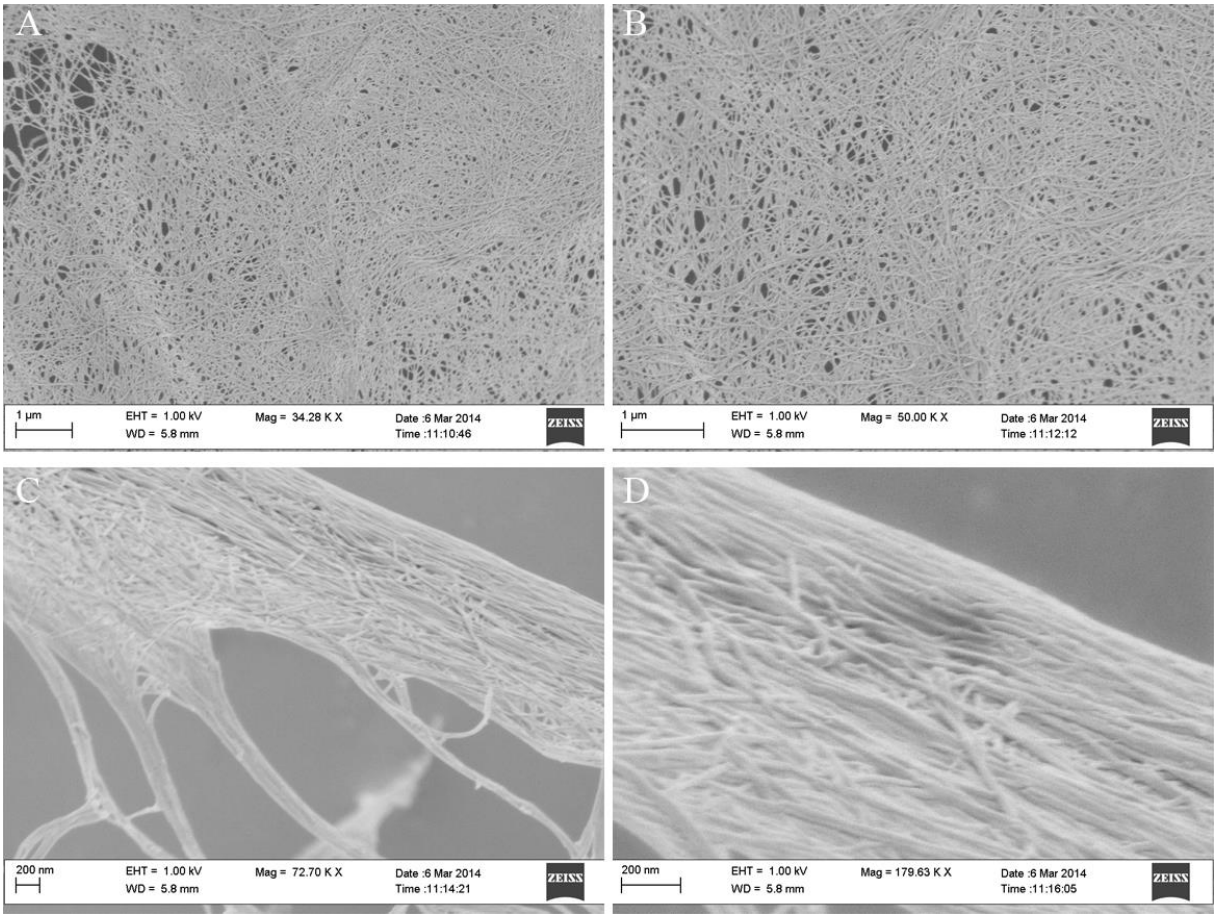

Figure 20. TEMPO oxidised cellulose freeze-dried for 24 h. Shows regions of higher degree of alignment. Especially in panels C and D. The magnification is 34.28k×, 50.00k×, 72.70k× and 178.63k× for panels A, B, C and D respectively.

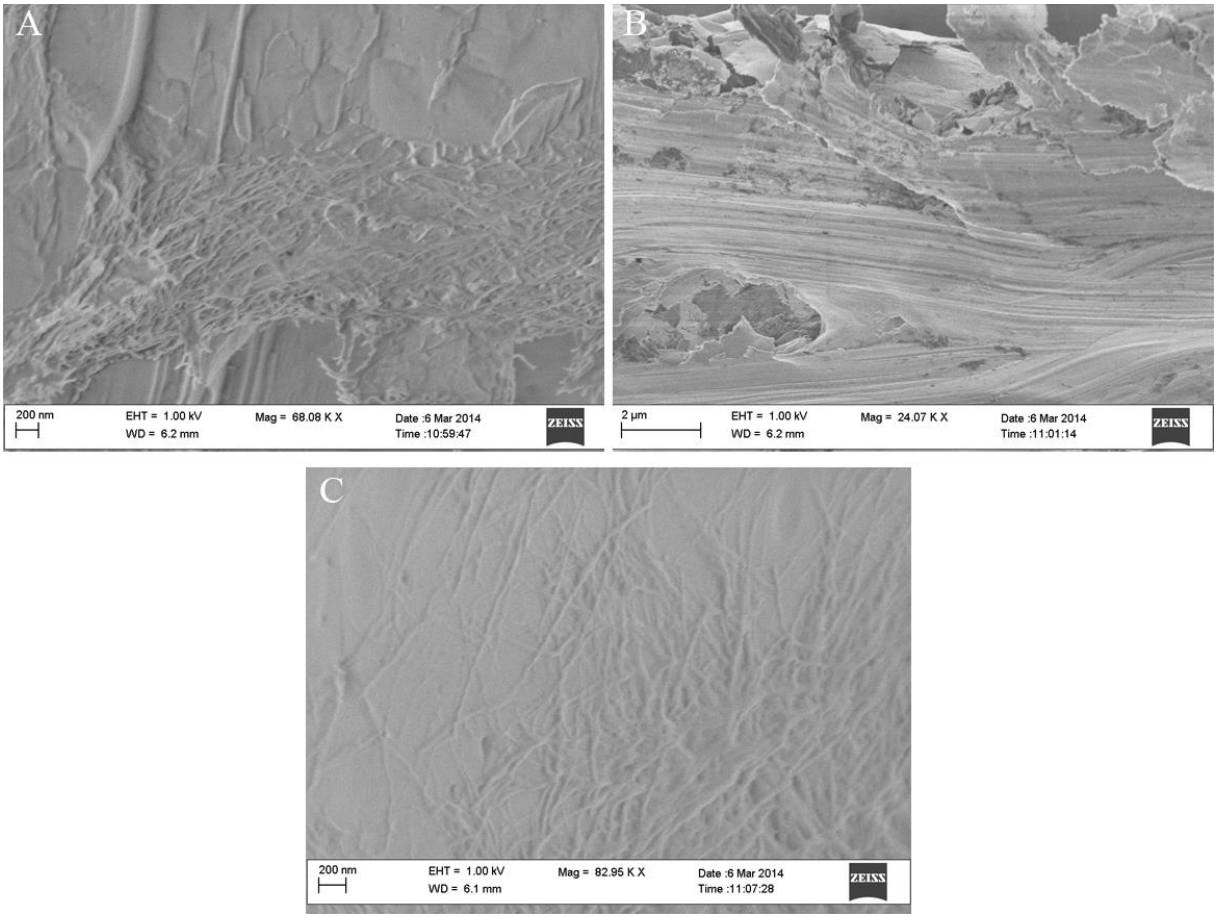

Figure 21. Supernatant from TEMPO oxidised cellulose, vacuum centrifuged, hard to discern cellulose fibres. There are crystals with apparent alignment, these are probably salt crystals. The magnification is 68.08k×, 24.07k× and 82.95k× for panels A, B and C respectively.

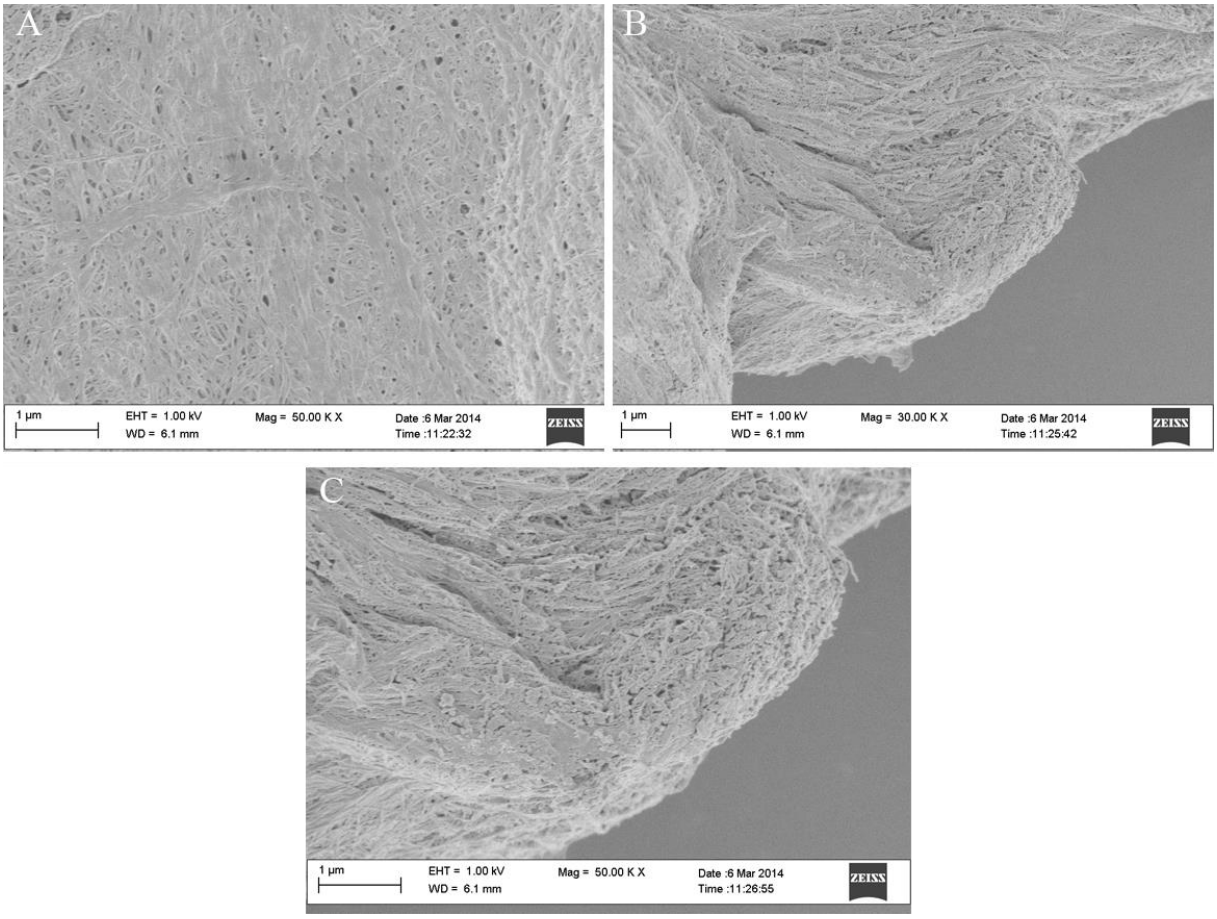

Figure 22. Supernatant of TEMPO oxidised cellulose that has been freeze-dried. The cellulose fibres looks agglomerated and have no higher degree of order. The magnification is 50.00k×, 30.00k× and 50.00k× for panels A, B and C respectively.

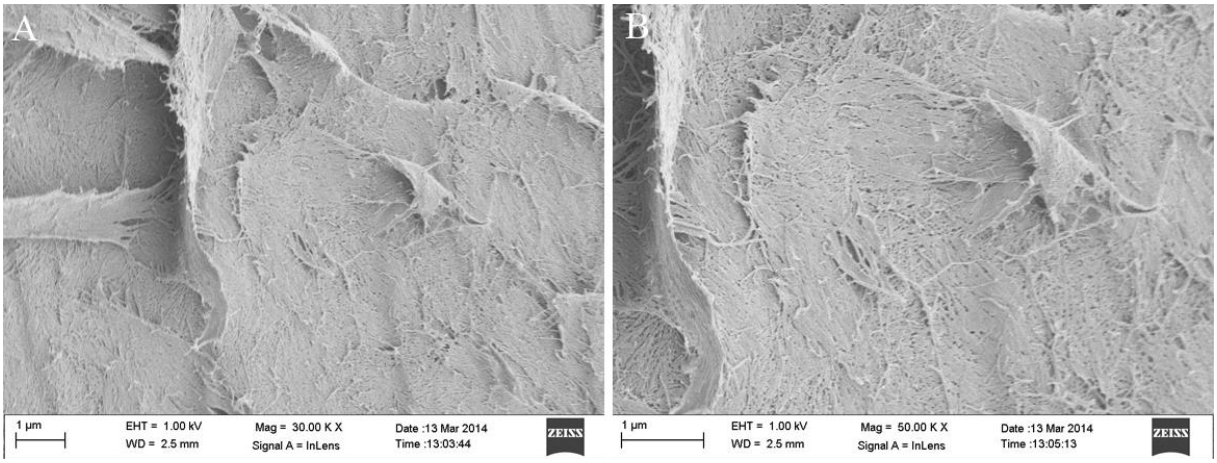

Figure 23. Vacuum centrifuged Oxone oxidised cellulose from the second batch of oxidations. Very tightly packed cellulose fibres form large sheets in multiple layers, no apparent ordering. The magnification is 30.00k× and 50.00k× for panels A and B respectively.

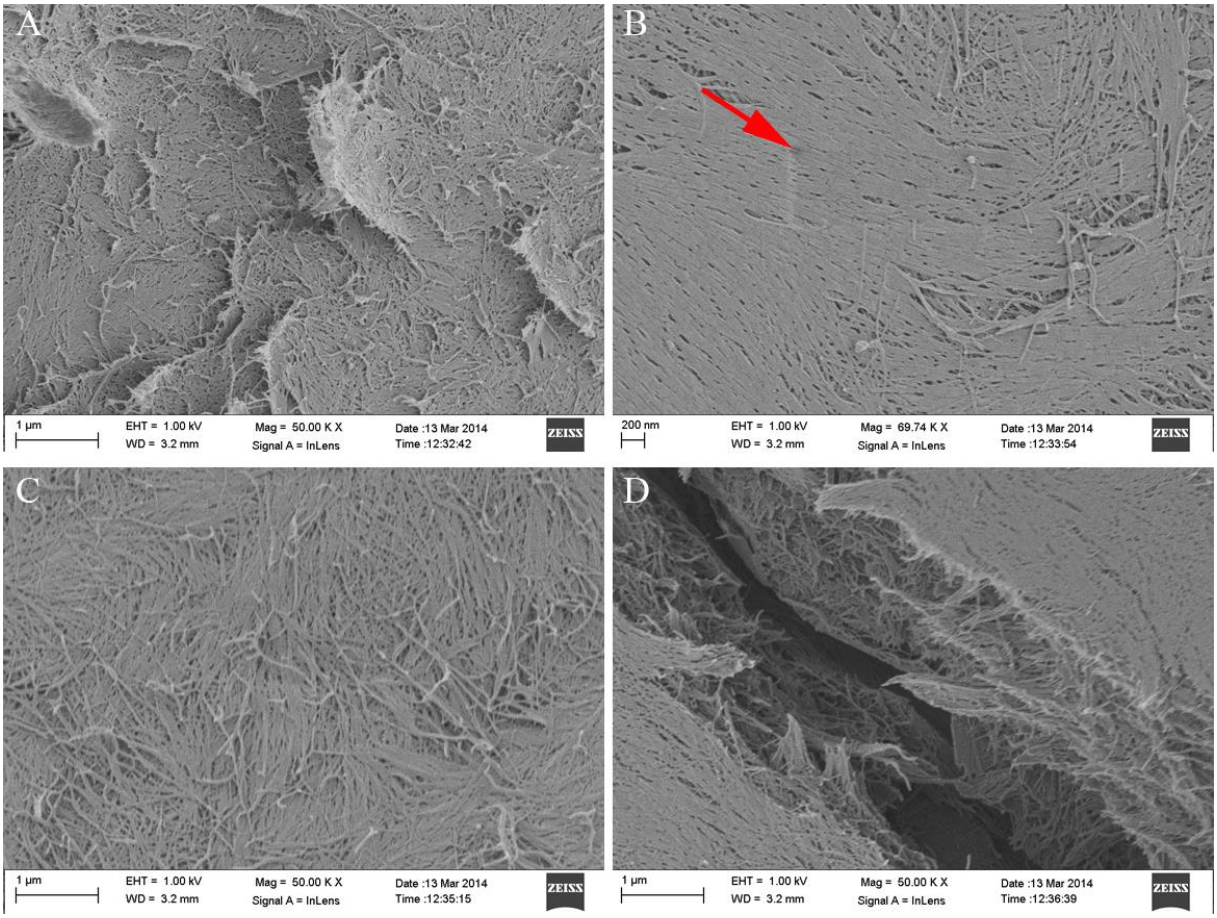

Figure 24. Vacuum centrifuged Oxone oxidised cellulose. Tightly packed fibres with random order alignment. In panel B a rectangle can be seen by the arrow where the electrons of the microscope have melted the material. The magnification is 50.00k×, 69.74k×, 50.00k× and 50.00k× for panels A, B, C and D respectively.

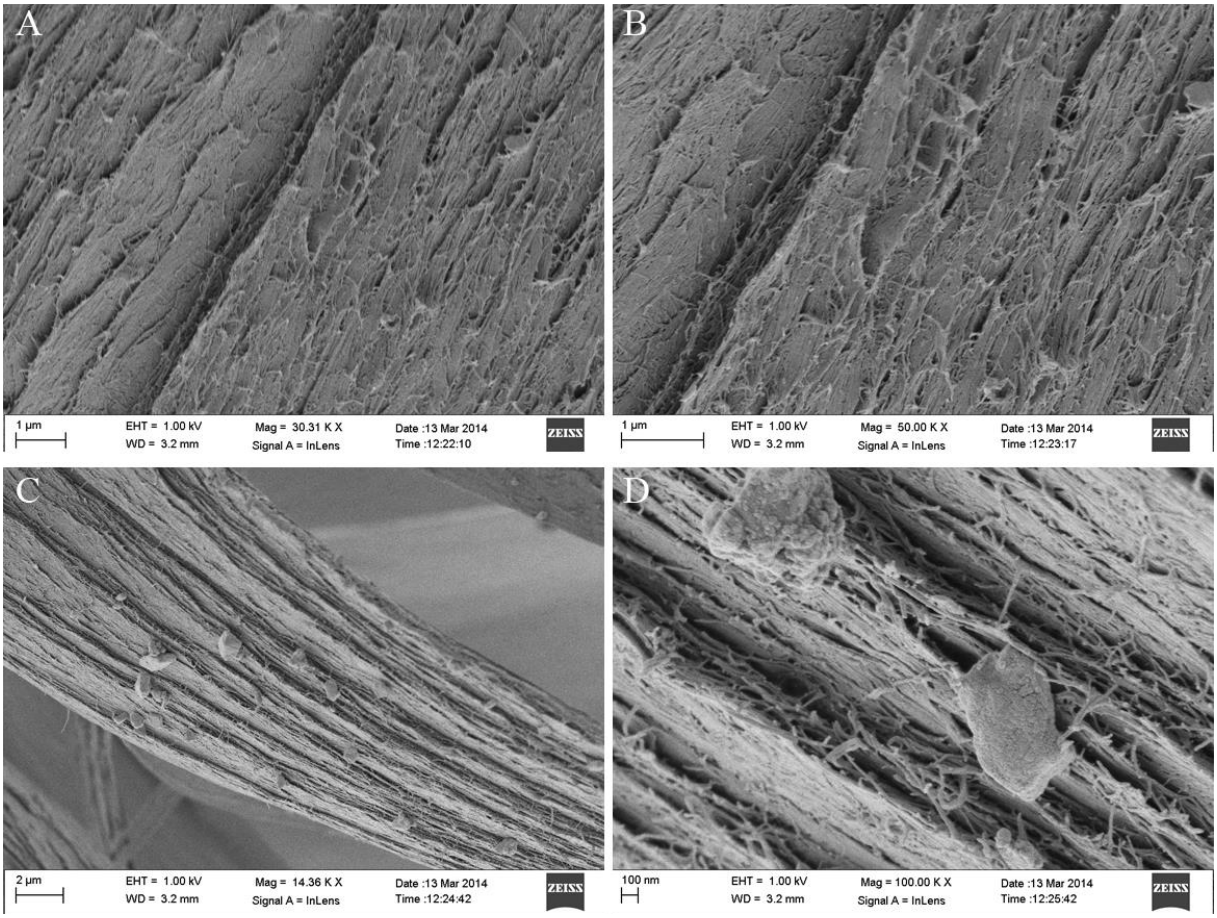

Figure 25. Oxone oxidised cellulose, vacuum centrifuged. This sample seem to have regions of higher order of alignment. The fibres look quite sticky and it is hard to discern them as truly separate. The magnification is 30.31k×, 50.00k×, 14.36k× and 100.00k× for panels A, B, C and D respectively.

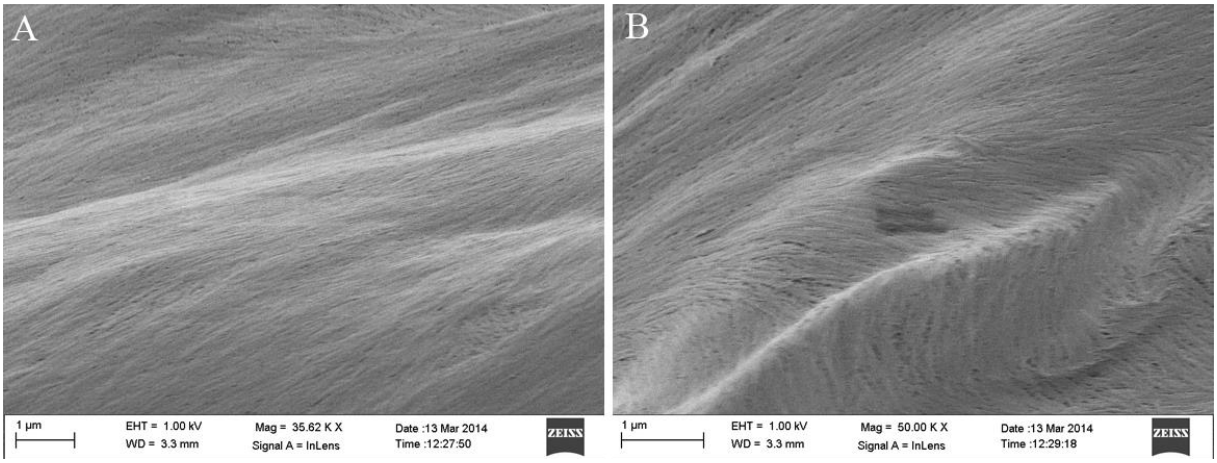

Figure 26. Vacuum centrifuged Oxone oxidised cellulose with a wave-like higher order of alignment. The fibres have adhered tightly to each other. The magnification is 35.62k× and 50.00k× for panels A and B respectively.

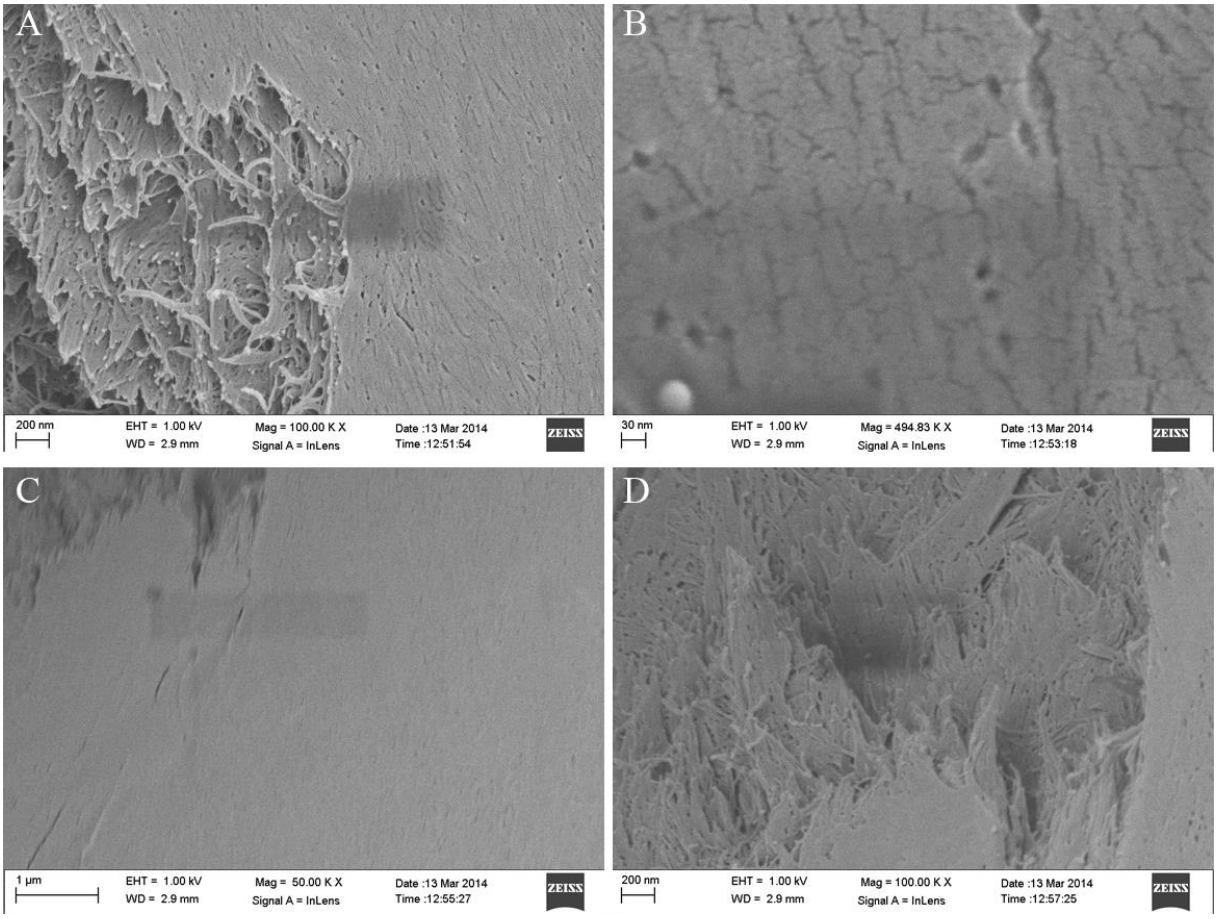

Figure 27. Vacuum centrifuged TEMPO oxidised cellulose. The material heated up very quickly, rectangles of burnt material are visible in all pictures. Hard to get good resolutions at higher magnifications without damaging the surface. The magnification is 100.00k×, 494.83k×, 50.00k× and 100.00k× for panels A, B, C and D respectively.

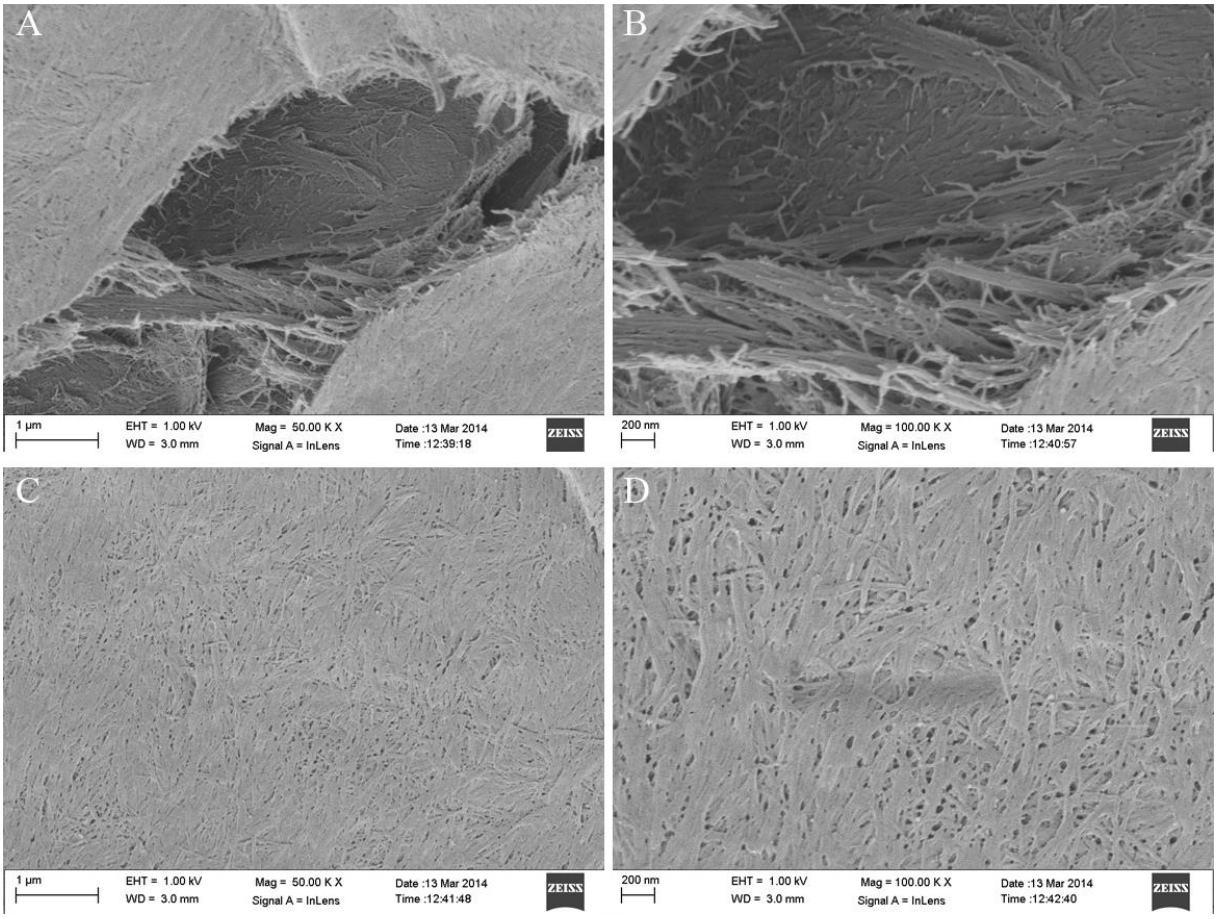

Figure 28. TEMPO oxidised cellulose, vacuum centrifuged. Rather tightly packed fibres, random alignment throughout the sample. The magnification is 50.00k×, 100.00k×, 50.00k× and 100.00k× for panels A, B, C and D respectively.

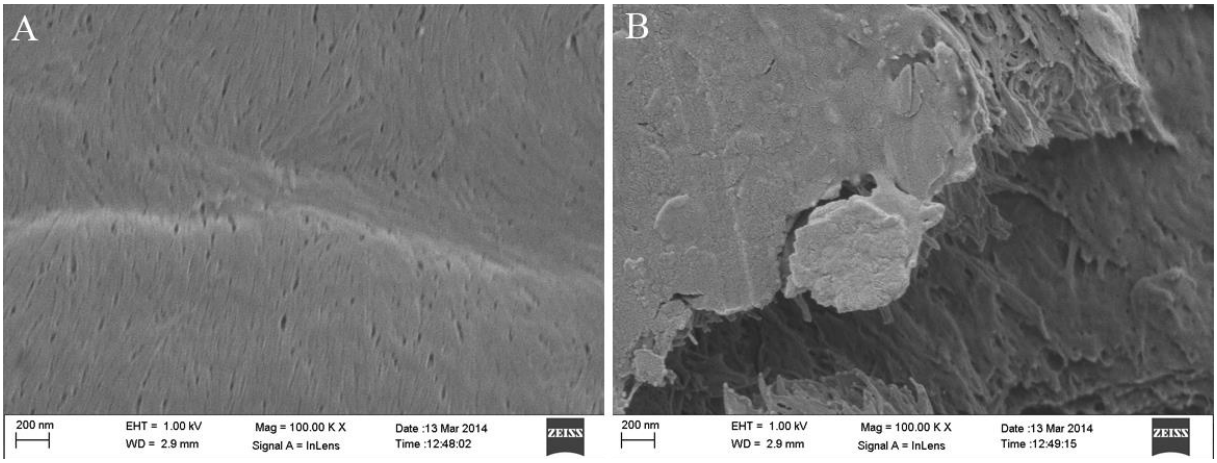

Figure 29. TEMPO oxidised cellulose, vacuum centrifuged. Rather tightly packed fibres, random alignment throughout the sample. The magnification is 100.00k× in both panels A and B.

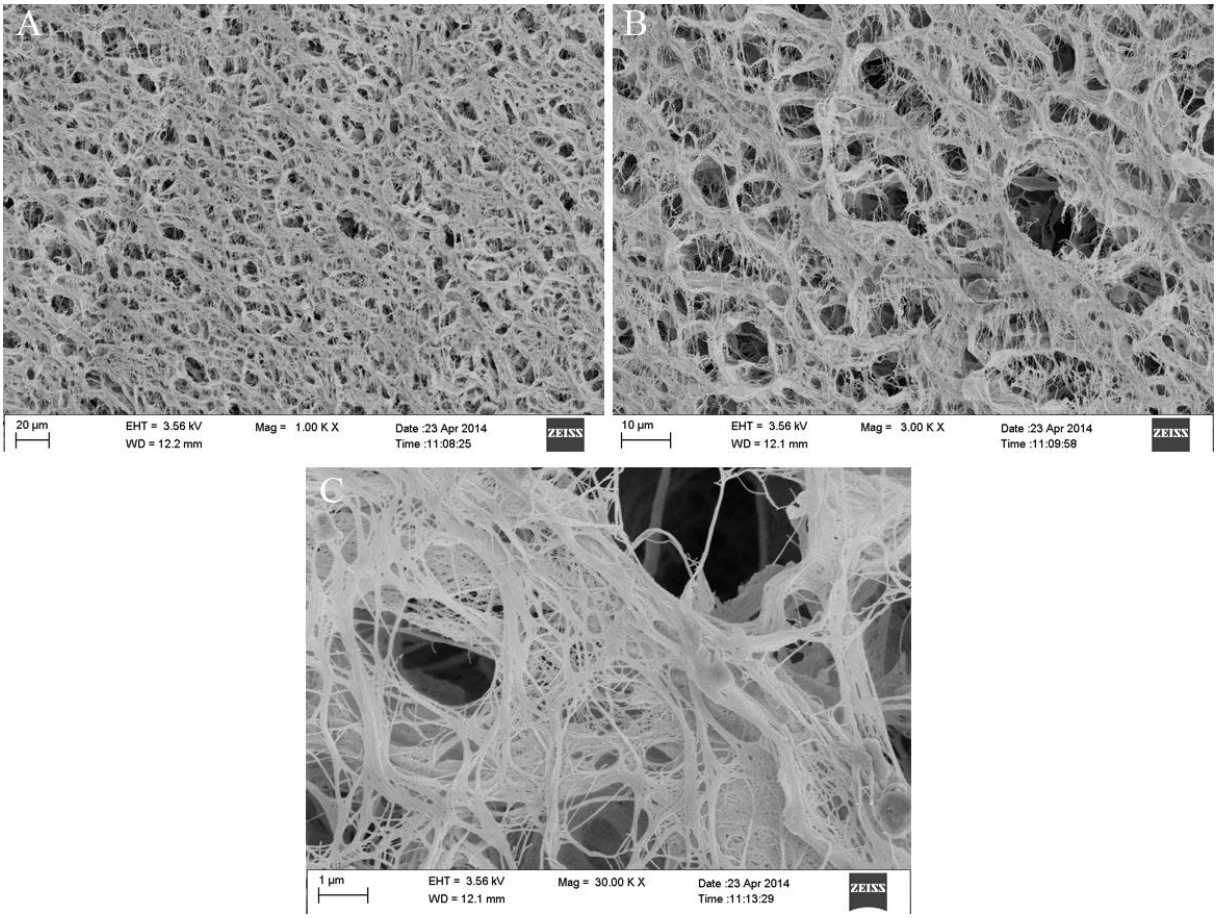

Figure 30. TEMPO oxidised cellulose that has been sonicated and vacuum centrifuged. It shows random alignment in a corkscrew like pattern. The magnification is 1.00k×, 3.00k× and 3.00k× for panels A, B and C respectively.

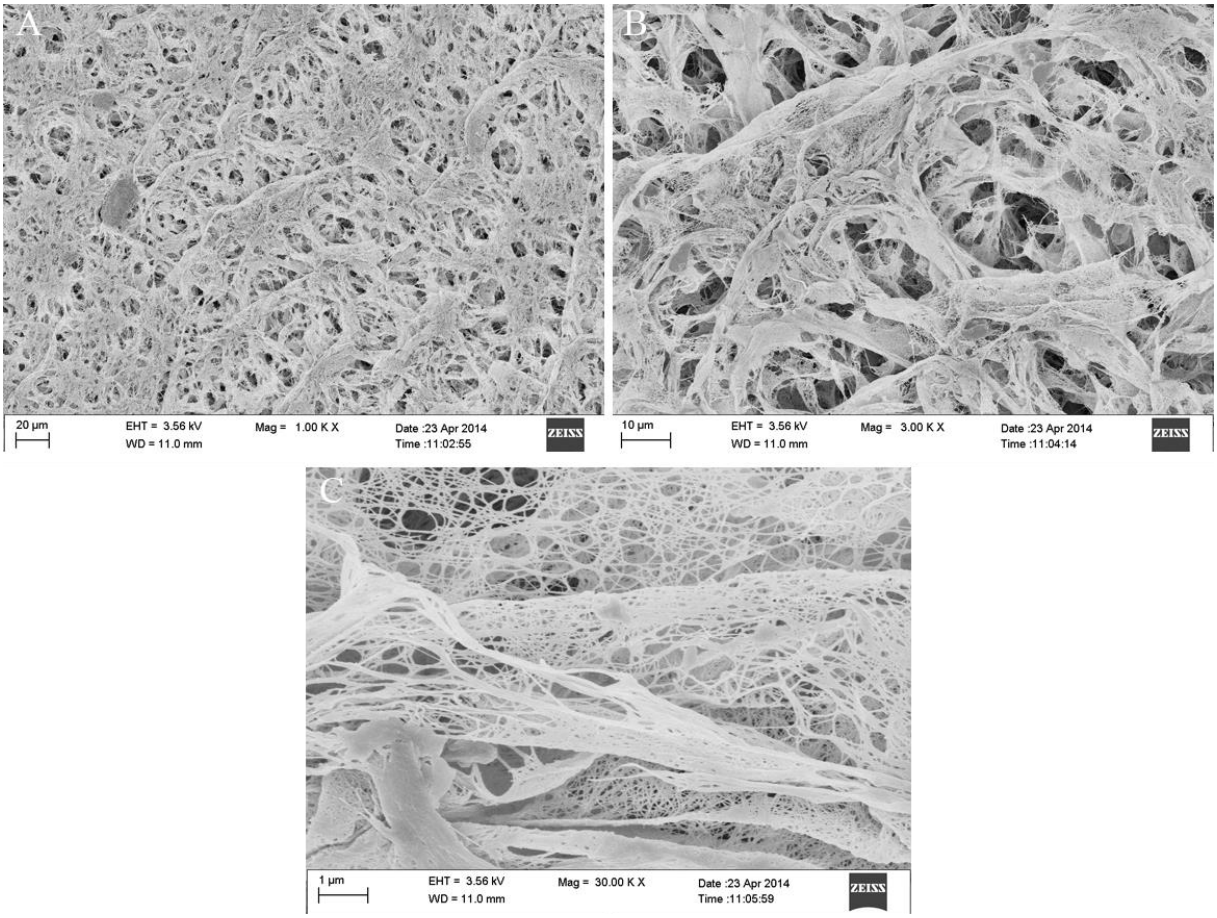

Figure 31. TEMPO oxidised cellulose that has been sonicated and vacuum centrifuged. Also shows random alignment in a corkscrew like pattern. The magnification is 1.00k×, 3.00k× and 30.00k× for panels A, B and C respectively.

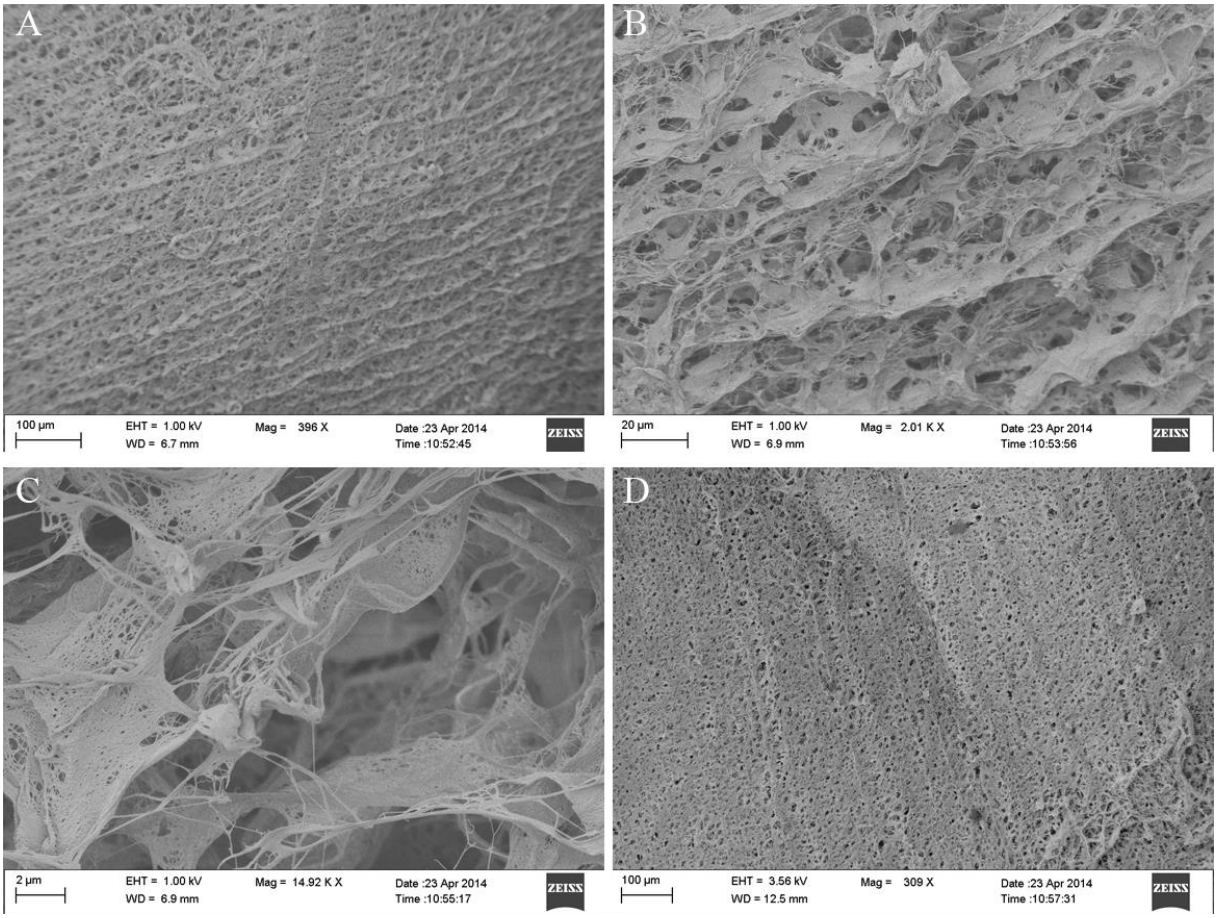

Figure 32. TEMPO oxidised cellulose that has been sonicated and vacuum centrifuged. Repetitive wave-like patterns of unaligned fibres. The magnification is 396×, 2.01k×, 14.92k× and 309× for panels A, B, C and D respectively.

Birefringence

When viewed under polarised light the cellulose suspension shows the characteristics of a chiral nematic phase, Figure 33. These pictures were taken after I had concluded my experimental work, on a trip to test a more powerful magnet.

Figure 33. Oxone and periodate oxidised cellulose with cysteine added to the crystals viewed under polarised light. The anisotropic property of the solution is visible, to the left the polarised light hits the cellulose fibres perpendicular to their general direction and to the right the light is shone parallel to the fibres.