A review of nanocellulose as a novel vehicle for drug delivery

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KEYWORDS: Cellulose nanocrystals, Cellulose nanofibrils, Bacterial cellulose, Antimicrobial, Antibiotic, Anti-cancer, Drug delivery

SUMMARY: The current state of research into nanocellulose in drug delivery is reviewed in this article. There are three types of nanocellulose: cellulose nanocrystals (CNC), cellulose nanofibrils (CNF) and bacterial cellulose (BC), all of which may be produced in suitable amounts at reasonable cost. All three have been investigated as drug delivery vehicles with CNC and CNF reported to bind and release some water-soluble drugs via ionic interactions whereas BC has been used to release drugs from flexible membranes. The rationale for using nanocellulose is the high surface area-to-volume ratio of the material that may enable high levels of drug binding at the surface. All forms of nanocellulose can be chemically modified to expand the range of drugs that may bind to the surface. Most studies are academic in nature and have not focused on formulating specific drugs for specific disease applications. Furthermore, there are few studies that have investigated the assumed biocompatibility or fate of nanocellulose *in vivo*. Arguably, a logical first application might be for wound dressings in which a nanocellulose-based antibiotic formulation could provide a controlled drug release aspect uncomplicated by internal biocompatibility or clearance concerns. The prospects for further research into nanocelluloses in drug delivery are discussed.

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Nanocellulosic materials can be divided into three broad categories, depending upon whether the cellulose nanofibers are of bacterial origin or are produced by chemical or mechanical means. These categories are described as bacterial cellulose (BC), cellulose nanocrystals (CNC) and cellulose nanofibrils (CNF), respectively. The material now known as CNF has also been previously described as microfibrillated cellulose (MFC) or nanofibrillated cellulose (NFC). Each of these forms of nanocellulose has been the subject of extensive research covering a very wide range of potential uses and has been reviewed in a number of books and journal articles (Azizi Samir et al. 2005; Dufresne 2012; Gama et al. 2013; Hamad *in press*; Iguchi et al. 2000; Siró, Plackett 2010).

Interest in the production and utilization of nanocellulose in its various forms is reflected in the scientific literature, where there has been a steady increase in publications in recent years (*Fig 1*). In the case of CNC and CNF, development of these materials has generally been viewed positively by the European and North American forest industries because of the potential for new value-added products to complement the existing portfolio of pulp and paper commodities and specialty paper products. The possible applications cited for the various forms of nanocellulose are quite diverse and have included polymer composites, packaging materials, cosmetics, rheology modifiers in foods, and additives for oil-drilling muds, as well as a range of biomedical uses such as tissue culture scaffolds, implants, wound dressings and vehicles for drug delivery.

The interest in application of nanocelluloses in medicine has been stimulated by their perceived non-toxicity, biocompatibility, good mechanical properties, high surface area-to-volume ratio and potential versatility in terms of chemical modification. The latter feature can potentially be used very effectively for the binding and release of therapeutic agents. Such use of nanocellulose falls into the broader field of nanomedicine and is attracting interest because of its potential as a means of achieving targeted and sustained release of drugs (e.g., anti-cancer agents, antibiotics) at safe and clinically appropriate levels. In practice, for improved efficacy, sustained release of drugs over a period of days or weeks is often preferable to immediate release of the total drug content. Furthermore, there is a trend in the pharmaceutical industry towards more hydrophobic, water-insoluble drugs and therefore binding or encapsulation at the nanoscale can provide a means of enhancing the amount of drug available in a given volume of an aqueous formulation. Nanomedicine, of which new forms of drug delivery are just one part, is now the key theme of various scientific journals (e.g., Nanomedicine, Journal of Nanomedicine, International Journal of Nanomedicine, European Journal of Nanomedicine) and has been the subject of a number of recent books (Jain 2008; Mishra 2013; Prasad 2012; Yeo 2013).

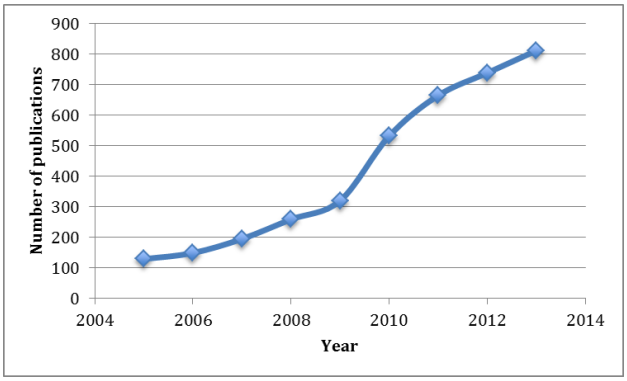

Fig 1 - Publications including journal articles and patents during the period 2005 to 2013 using the SciFinder® database and the keywords "microfibrillated cellulose", "nanofibrillated cellulose", "microfibrillar cellulose", "nanofibrillar cellulose", "bacterial cellulose", "microbial cellulose", "nanocrystalline cellulose", "cellulose nanofibrils", cellulose whiskers" and "cellulose nanocrystals". The search was carried out on 27 January 2014.

This review on nanocellulose and drug delivery is presented in the form of a state-of-the-art summary of research published in the scientific literature and is divided into sections according to each particular type of nanocellulose. As with other applications of these nanomaterials, the number of related scientific publications has become increasingly significant since the year 2000. There has also been an increase in the number of patents covering the preparation and use of nanocelluloses, as discussed in two recent reviews on this topic (Duran et al. 2012; Charreau et al. 2013). In the summary of this review we provide some overall comments concerning the work reported to date, research areas that still require attention and the practical challenges that may be faced before nanocelluloses can be widely adopted in the field of drug delivery.

Cellulose nanocrystals (CNC)

Background

CNC is the term now used for a highly crystalline, needle-like form of nanocellulose, which has been derived from wood pulp, pulp industry wastes, native cellulose in the form of cotton, cellulosic agricultural residues (e.g., sugar beet pulp) or microcrystalline cellulose (MCC) by acid hydrolysis (Aspler et al. 2013). MCC, consisting of highly crystalline regions of cellulose, is produced by acid hydrolysis of the amorphous part of cellulose until a level-off degree of polymerization (LODP) is achieved. By filtering and spray drying, MCC particle agglomerates composed of microcrystals are obtained, the particle size distribution and moisture content of which can be varied via the spray drying process. The LODP of MCC is typically in the order of 200-300 and, in the case of the commercial MCC Avicel® (FMC BioPolymer) as an example, products with nominal particle sizes ranging from 20 to 180 μm are currently available. Since MCC is non-toxic, readily binds to itself without adhesive and can also bind various additives, it has been widely used for tableting of pharmaceuticals (Saigal et al. 2009).

The basis for generation of CNC first arose in the work of Nickerson and Habrle (1947) who discovered that the degradation of cellulose fibers in boiling acid solutions reached a limit after a certain length of treatment. These researchers proposed that the acid preferentially attacked the sections of cellulose linking the cellulose crystallites. This work then led to the pioneering efforts of Rånby and coworkers who studied the controlled sulfuric acid-catalyzed hydrolysis of cellulose in various forms (Rånby 1949; Rånby, Ribi 1950; Rånby 1951; Rånby 1952). During the same period, acid-based degradation of cellulose followed by ultra-sonic treatment was investigated and this research eventually led to the commercialization of MCC (Battista, 1950; Battista et al. 1956). In the intervening years, the production, properties and possible applications of CNC have been the subject of extensive international research including most notably at McGill University in Montreal, Québec, FP Innovations in Québec City and Vancouver, BC and at the US Forest Products Laboratory (USFPL) in Madison, Wisconsin, as well as within a number of other substantial research programmes in North America, Europe, Japan and elsewhere. Laboratory processes for manufacturing CNC typically involve acid hydrolysis of a selected cellulosic substrate, followed by purification through repeated centrifugation and suspension in water and finally dialysis and freeze drying. As noted by Peng et al. (2011), depending on source and processing conditions, the diameter of the cellulose nanocrystals is generally in the single digits or tens of nanometers as determined by techniques such as transmission electron microscopy (TEM) or atomic force microscopy (AFM).

The most significant commercial development of CNC to date has been based upon research at FP Innovations in Canada. As a result of this work and with financial support from the Canadian Federal Government and Domtar, a commercial operation under the name CelluForce started manufacturing CNC in bulk (CelluForce NCC™) in early 2012. In parallel with this development, the Canadian research network ArboraNano was established in 2009. The research carried out by this network over the period 2009-2013 has covered both basic science and the potential for practical application of CNC in a wide variety of uses. The CelluForce CNC production plant in Windsor, Québec and the ArboraNano research network are the largest of their kind so far which have been focused specifically on CNC (NRCan 2013). Research conducted at USFPL in Madison resulted in the opening of the first US CNC-manufacturing facility in mid-2012. Production at this factory, owned by the US Forest Service, is to be ramped up in support of what the US National Science Foundation has claimed will be a $600 billion industry by 2020 (Ferguson 2012). Within Europe, Rettenmeier in Germany manufactures CNC as an inert filler for pharmaceuticals.

Examples of CNC and drug delivery from the scientific literature

The potential for use of CNC as a means of binding and releasing drugs has been frequently referred to in the relevant scientific literature. For example, although not focusing specifically on drug delivery, Dong and Roman (2007) developed a method for fluorescently labeling CNC with a view to further studies on cellular uptake and biodistribution of this nanomaterial using bioimaging. Their work involved the introduction of epoxy groups to the surface of CNC, followed by epoxy ring opening with ammonium hydroxide to create primary amine groups, to which fluorescein-5′-isothiocyanate (FITC) could be covalently attached. These researchers cited earlier work in which the potential applications of CNC or other polysaccharide nanocrystals for delivery of therapeutics have been mentioned within several review articles (Azizi Samir et al. 2005; de Souza Lima, Borsali 2004; Fleming et al. 2001). In a more recent study, Lin et al. (2011) reported the use of CNC, as well as chitin nanowhiskers and starch nanocrystals, as additives in calcium cross-linked alginate microspheres. The addition of these nanoadditives stabilised the cross-linked alginate matrix, resulting in a higher encapsulation efficiency and sustained release profile for theophylline, a chosen model drug that is in clinical use for treatment of respiratory

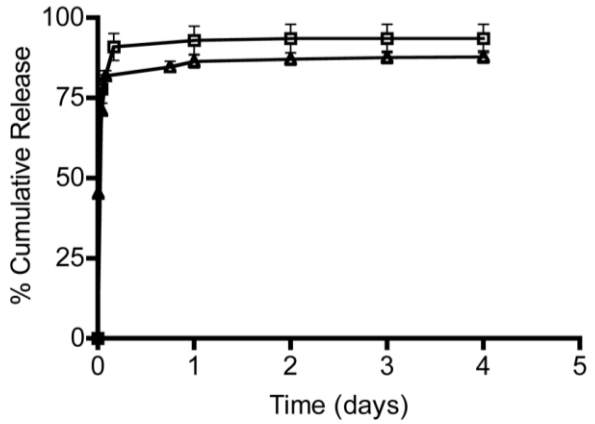

Fig 2 - The *in vitro* release of doxorubicin (Δ) and tetracycline (□) from CNC in 10mM phosphate-buffered saline at 37ºC (Reprinted with permission from Jackson et al. 2011. Copyright 2011 Dove Medical Press).

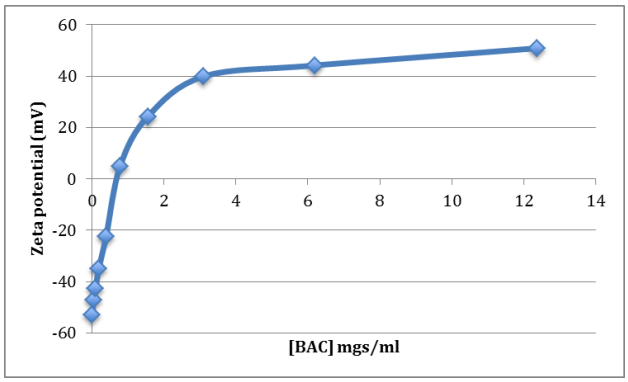

Fig 3 - Change in zeta potential of a 4.1 mgs/ml suspension of CNC upon addition of benzalkonium chloride (BAC) solutions of increasing concentration (1.5 mls BAC solution added to 0.5 mls CNC suspension for each measurement).

diseases. This work followed an earlier study by Zhang et al. (2010) in which the use of polysaccharide nanocrystals in supramolecular hydrogels with cyclodextrin inclusion not only improved mechanical strength but also enhanced the sustained release profile of bovine serum albumin (BSA) as a model drug. Wang and Roman (2011) examined the formation of polyelectrolyte-macroion complexes (PMCs) between chitosan, a cationic polysaccharide, and CNC, with a view to determining the composition, size, shape and net charge of the PMC particles as a function of CNC concentration and mixing sequence, and consequently their suitability for oral drug delivery applications. The authors concluded that PMCs with suitable properties for intestinal absorption (i.e., net positive charge and particle size less than 10 μm) could be obtained at chitosan amine/CNC sulfate group molar ratios (N/S) ≥ 1.33.

To date, one of the most detailed investigations on drug binding and release from CNC has been that of Jackson et al. (2011), in which the binding to and release of the antimicrobials doxorubicin hydrochloride (DOX) and tetracycline hydrochloride (TET) from CNC was characterized. These drugs and other therapeutics, which carry a positive charge under physiological pH conditions, probably form strong ionic bonds with the negatively charged sulfate groups resident on the CNC surface as a result of the sulfuric acid hydrolysis process. Approximately every tenth hydroxyl group on CNC produced by sulfuric acid hydrolysis can be sulfated and therefore carry a negative charge. As shown in *Fig 2*, both DOX and TET released more than 80% of the estimated bound drug within four hours. In an additional study, the modification of CNC with cetyl trimethylammonium bromide (CTAB) provided a hydrophobic domain around the cellulose nanofibers, which could be used to entrap hydrophobic anti-cancer drugs such as paclitaxel (PTX), docetaxel (DTX) and etoposide (ETOP). Delayed release of these drugs in phosphate-buffered saline (PBS) was demonstrated and cellular uptake of fluorescein-labeled CNC/CTAB was also confirmed. In subsequent research, the binding and release of other antimicrobials, such as benzalkonium chloride (BAC), has been similarly demonstrated. In each case the interaction with CNC can also be followed using zeta potential measurements, as shown for BAC treatment of sulfated CNC in *Fig 3*. In another recently published study on CNC for drug binding and release, Akhlaghi et al. (2013) modified CNC using TEMPO to introduce carboxyl groups. The modified CNC were then reacted with a chitosan oligosaccharide (Mn = 500Da) by the carbodiimide reaction, involving use of N-hydroxysuccinimide and 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide. After extensive characterization, the binding efficiency of the local anaesthetic drug, procaine hydrochloride, to the chitosan-complexed oxidized CNC was determined by electromotive force measurements (EMF) using a drug membrane electrode prepared from a plasticized, carboxylated PVC-procaine hydrochloride complex. As part of the characterization work, data from isothermal titration calorimetry (ITC) were plotted as kcal/mole of injectant as a function of molar ratio and this indicated that much greater interaction occurred between the model drug and chitosan oligosaccharide-CNC complex at pH 8 (as indicated by a greater heat of reaction) than at either pH 6 or 7. Therefore, pH 8 was chosen for the drug loading experiments (*Fig 4*). Drug binding and loading efficiencies for procaine hydrochloride on chitosan oligosaccharide-oxidized cellulose were 21.5 and 14% w/w respectively. The authors noted that these values were similar to those reported by Jackson et al (2011) for TET bound to sulfated CNC. Release of procaine hydrochloride at pH 8 showed a large initial burst phase over about 10 minutes in which some 80% of the drug was released. This was followed by much slower release of drug over the following one hour. The authors suggested that the rapid release profile might be suitable for wound dressing applications.

The question of safety naturally arises when considering potential applications of nanocellulose in drug delivery but, to date, this issue has only been considered in detail in a few studies. Mahmoud et al. (2010) used methods for fluorescently labeling CNC with either rhodamine B isothiocyanate (RBITC) or FITC to indicate that

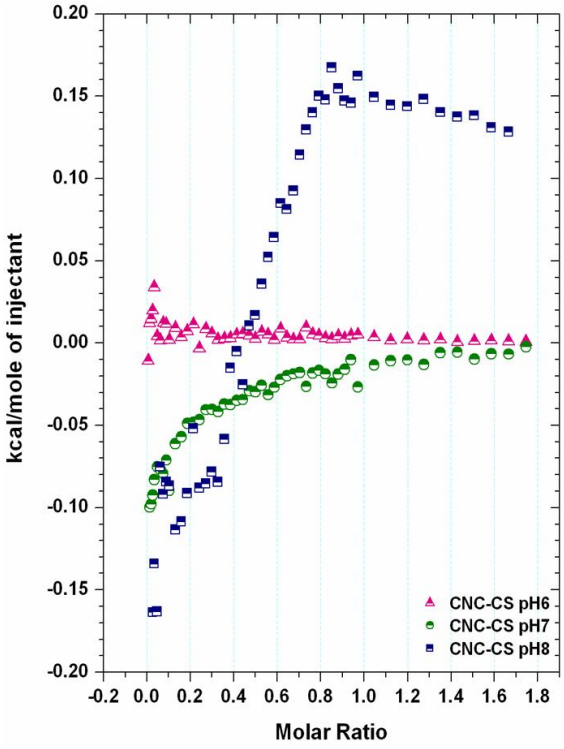

Fig 4 - Data from isothermal titration calorimetry (ITC) showing heats of reaction at pHs 6,7 and 8 when titrating 10mM procaine hydrochloride against 0.05% w/w CNC-chitosan oligosaccharide (Reprinted with permission from Akhlaghi et al. 2013. Copyright 2013 Springer).

functionalized CNC could penetrate cells, with no indication of cytotoxicity. In particular, human embryonic kidney 293 (HEK 293) and *Spodoptera frugiperda* (Sf9) cells took up CNC-RBITC without affecting cell membrane integrity. No cytotoxicity was observed at the concentrations tested. The authors commented that these findings, in combination with the possibility to bind drugs to the cellulose surface, pointed the way towards the development of new systems for drug delivery or optical bioimaging. As part of a large investigation on treatments for cerebrovascular inflammatory diseases, Roman et al. (2009) assessed whether CNC would exhibit any toxicity towards human brain microvascular endothelial cells (HBMECs). This research involved the determination of the cytotoxicity of CNC as measured by MTT assay (Mosmann 1983) as well as cellular uptake studies based on the use of CNC labeled with FITC. The results from this research indicated that CNC were non-toxic to HBMECs over the 0-500 µg/ml concentration range. This finding could be explained by results from the cell uptake studies, which showed that there was limited uptake of CNC after 24 hours' exposure. In a more recently reported study, Dong et al. (2012) assessed the toxicity of CNCs against nine cell lines, including HBMECs, and found no signs of cytotoxicity at the concentration range and exposure time studied (0-50 µg/ml and 48 hours respectively). As outlined by Peng et al. (2011) in their review on the chemistry and applications of nanocellulose, CNC have also been found to be safe in a recent and comprehensive ecotoxicological assessment (Kovacs et al. 2010).

Cellulose nanofibrils (CNF)

Background

The first investigations related to CNF, or MFC as it was then known, date back to the work of Herrick et al. (1983) and Turbak et al. (1983) at the ITT Rayonier labs in the USA. In this initial work, dilute softwood pulp suspensions (1-2% w/w) in water were forced through a laboratory Gaulin-type milk homogenizer at a temperature of 70-90^{o}C. During this process, the pulp suspension was subjected to high shear forces and pumped at high pressure through a spring-loaded assembly. The forces involved in this process led to a high degree of fibrillation and the production of gel-like CNF. The energy requirements for the process were very high and this deterred further progress in this direction for some considerable time. For example, Nakagaito and Yano (2004) noted that values for energy demand in CNF production could exceed 30,000 kWh per ton and even higher values have been reported (Eriksen et al. 2008). In more recent years, and especially as a result of research at the Royal Institute of Technology (KTH) and Innventia in Stockholm, Sweden, significant progress has been made in reducing the energy required to produce CNF. This has been achieved through the use of various enzymatic or chemical pre-treatments to facilitate wood fiber breakdown. As noted in the review by Klemm et al. (2011), carboxymethylation pre-treatment of kraft/sulfite pulp has reduced the energy demand for CNF production to 500 kWh per ton and a combined enzymatic treatment/refining approach resulted in a corresponding figure of 1500 kWh per ton (Pääkkö et al. 2007; Wågberg et al. 1987). In addition, research at KTH and Innventia has led to a series of pretreated types of CNF (e.g., anionic, cationic), which may offer opportunities for a wide range of diversified products based on CNF. Research on CNF and commercial applications has been a particular focus in Europe, with significant activity in recent years in Finland and Norway as well as in Sweden. In addition to pilot facilities for manufacturing CNF at various international research locations, and as noted by Williamson (2012), some major companies in the global pulp and paper sector have also started trial production. For example, UPM Kymmene, usually referred to as UPM, announced in November 2011 that it was starting pre-commercial CNF manufacturing in Finland. In June 2013, the same company announced that it was setting up collaboration with Ashland, Inc. on development of the Biofibrils™ range of micro- and nano-fibrillated cellulose products (http://www.upm.com, 2013). Stora Enso also started up a CNF pilot plant in Eastern Finland in late 2011 and Borregaard in Norway has established a pilot-scale plant for CNF production. Daicel in Japan has until recently offered its NanoCelish™ product, which was described as a cellulose derivative incorporating cellulose fibers that have been reduced to nanoscale. A detailed review of CNF and their surface modification has recently been published (Missoum et al. 2013).

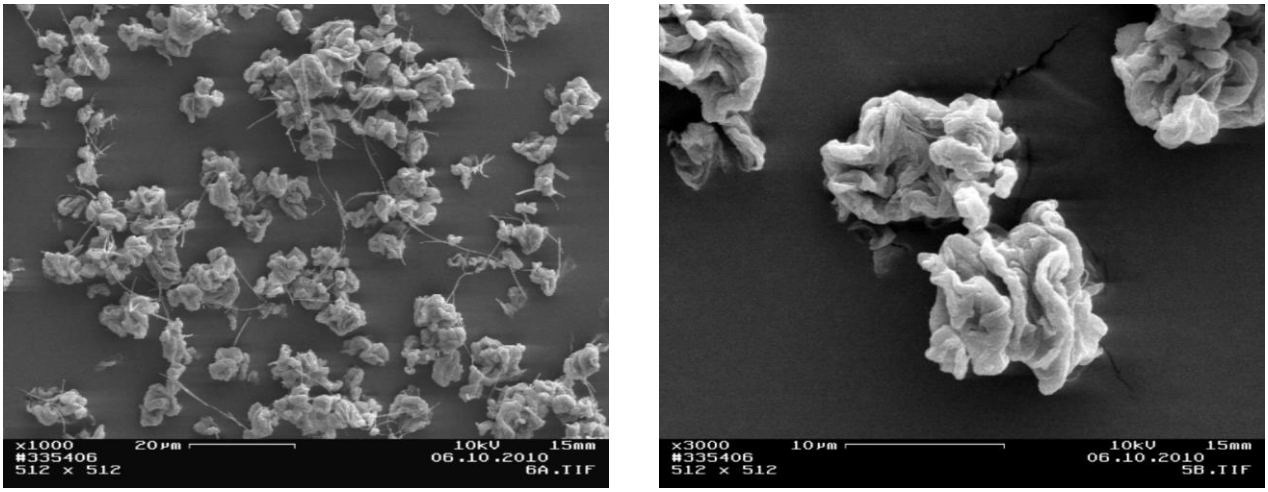

Fig 5 - SEM images of spray-dried CNF microparticles containing 20% indomethacin at lower (left) and higher (right) magnifiication (Reprinted with permission from Kolakovic et al. 2012a. Copyright 2012 Royal Society of Chemistry).

Examples of CNF and drug delivery from the scientific literature

Detailed investigations on the use of CNF for drug delivery applications have been carried out at the Faculty of Pharmacy of the University of Helsinki in cooperation with UPM. These studies have included comparison of CNF with MCC as a pharmaceutical excipient, drug release from spray-dried CNF microparticles, drug binding to and release from CNF films and the investigation of drug binding to CNF using ITC (Kolakovic et al. 2011;. 2012a, 2012b, 2013; Kolakovic 2013). In the investigation of spray-dried CNF microparticles, six model drugs were selected (indo-methacin, metoprolol tartrate, verapamil hydrochloride, nadolol, ibuprofen and atenolol). The drugs were dissolved in either water or 50 mM ammonium hydroxide, mixed in specific ratios with CNF suspensions and then sonicated and mechanically stirred before spray drying. SEM images of CNF microparticles loaded with indomethacin were obtained (*Fig 5*). Drug concentrations were determined by dissolving the CNF microparticles in 1-N-allyl-3-methylimidazolium chloride (AMIMCl), an ionic liquid, which is reported to be a good solvent for native cellulose (Zhang et al. 2005). Solutions in AMIMCl were then diluted with dimethyl sulfoxide (DMSO) and analyzed for drug content using an HPLC method. With the exception of indomethacin, all the drugs were encapsulated predominantly in the amorphous form. Indomethacin on the other hand showed evidence of a polymorphic change from the γ to the α form. After removing unbound drug fractions, release studies were carried out in deionized water or phosphate buffer at pH 7.4 and release kinetics were studied using a mathematical model previously developed for spherical particles (Baker, Lonsdale 1974). The drug release curves showed a rapid release phase in the first 10-14 days, followed by a much slower release rate over a period up to 60 days. Release kinetics varied according to drug type, which the authors attributed to differences in solubility and binding affinity to CNF (Kolakovic et al. 2012a). In the study on CNF films for controlled drug delivery, mixtures of the drugs indomethacin, itraconazole or beclamethasone dipropionate with CNF suspensions were prepared, sonicated and then filtered through 0.2 µm polyvinylidene fluoride (PVDF) membranes to obtain a cellulose nanofiber network containing within it particles of the largely water-insoluble drugs. The resulting filtrate was analyzed in each case, using HPLC to determine the amount of unbound drug. Entrapment efficiencies were found to be in the range of 86.5–99.9%. Thermal analysis and x-ray diffraction studies confirmed that in all cases the loaded drugs remained in their original crystalline state. The drug release studies showed that indomethacin was released in a sustainable way from the CNF films over a period of 15-30 days. Indomethacin release could also be modeled using a simple Higuchi equation (Higuchi 1963). In contrast, beclamethasone dipropionate and itraconazole were released more slowly from the CNF films over a period of three months, leading the authors to conclude that a simple diffusion process did not apply in the case of these other two drugs. Instead, zero-order kinetics provided the closest fit to the release data. The authors suggested that the difference in release profiles could be attributed to different drug solubilities, differences in drug binding to CNF and variations in drug particle size in the cellulose nanofiber network, leading to differences in the lamellar structure and tortuosity in these matrices. It was concluded that the drug-loaded

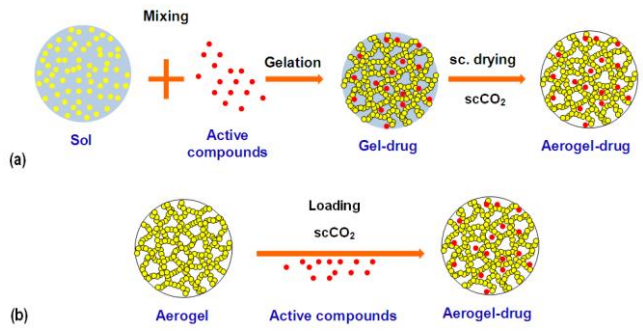

Fig 6 - Drug loading of aerogels: a) during the sol-gel process, and b) in the aerogel matrix by the supercritical CO_2 ($scCO_2$) impregnation post-treatment method (Reprinted with permission from Garćia-González et al. 2011. Copyright 2011 Elsevier).

CNF films might be valuable in implants, transdermal patches or ocular applications (Kolakovic et al. 2012b). Drug interactions with CNF were evaluated using permeation and binding studies as well as ITC to determine the thermodynamics of binding (Kolakovic et al. 2013). The permeation tests indicated that diffusion of the drugs through CNF films could be size-dependent while the binding and ITC studies suggested that binding was pH-dependent and involved electrostatic forces as the main mechanism. The latter is feasible because, as noted by the authors, the CNF in this case contained as much as 23% hemicellulose in the form of xylan, which can provide a negative charge to cellulose nanofiber surfaces due to the presence of carboxylic acid groups. Ionization of these carboxylic acid groups may lead to repulsion between fiber or film layers, resulting in increased water permeation and faster drug release.

Aerogels are porous, ultra-lightweight materials derived from gels in which the liquid component has been replaced with a gas. Starting with investigations on silica aerogels in the 1960s, there has been continuing interest in the use of aerogels as host matrices for pharmaceuticals and other therapeutically active compounds. The loading process may either be incorporated in a sol-gel process before or during solvent exchange or by incorporation of the active ingredient in the dry aerogel matrix (*Fig 6*). In this context, polysaccharide-based aerogels have been considered potentially interesting as biodegradable drug delivery vehicles (Garćia-González et al. 2011). Porosities in the 90-99% range have been achieved, with corresponding densities of 0.07–0.46 g/cm^3. Garćia-González et al. (2011) suggested that aerogel-drug combinations, because of their physical structure, might offer enhanced drug loading capacity and bioavailability as well as the potential for controlled release.

In a typical process, a hydrogel is formed from aqueous solution by changes in pH or temperature or by chemical cross-linking. In the next step, the water content is replaced by an alcohol to form an alcogel and the alcohol may then be extracted from the gel (e.g., by supercritical CO_2-assisted drying). Cellulose aerogels can be prepared by two main methods: first, by dissolving cellulose in a suitable solvent (e.g., N-methylmorpholine-N-oxide (NMMO) or ionic liquids) and then introducing water or alcohol or, second, by using various forms of nanocellulose in water suspension, which are then subjected to multi-step solvent exchange using ethanol or acetone. Significant shrinkage typically occurs when preparing cellulose aerogels. Reports of both cellulose aerogel preparation methods are available in the literature (Gavillon, Budtova 2008; Liebner et al. 2010). As noted by Sehaqui et al. (2011), it is possible to form high-porosity, high-specific area aerogels by solvent exchange of CNF gels from water into ethanol and then from ethanol into tert-butanol. The result was aerogels with densities as low as 14 and 29 kg/m^3, which were characterized using field-emission scanning electron microscopy (FE-SEM), nitrogen adsorption to determine specific surface area and pore size, and compression testing. When compared with cellular foams prepared from CNF, the nanofibrous aerogels had lower moduli and lower stress in compression for a given strain and it was suggested that the tert-butanol freeze-drying method could be used to create "soft"aerogels. Valo et al. (2013) reported a study on drug release from nanoparticles embedded in four different nanofibrillar cellulose aerogels. Beclamethasone dipropionate, a glucocorticoid steroid, was used as the model drug and the cellulose matrices were MCC, nanocellulose extracted from quince or pimiento fruit, bacterial cellulose and a TEMPO-oxidized nanocellulose obtained from UPM and prepared as described previously in the literature (Saito et al. 2006a; 2006b; 2007). The release profile of the chosen model drug varied according to the type of cellulose used to create the aerogels. In an earlier investigation, Valo et al. (2011) coupled a genetically engineered hydrophobin fusion protein with cellulose binding domains (CBDs) and coated itraconazole drug nanoparticles for subsequent binding to CNF. Hydrophobins are a group of cysteine-rich proteins produced in nature by various fungi and CBDs, known also as cellulose binding modules (CBMs), are non-catalytic portions of cellulases that allow enzyme binding to cellulose. In this work, a double CBD (DCBD) was selected as it was thought this would exhibit stronger binding to cellulose than a single CBD (Linder et al. 1996). The enclosure of hydrophobin-coated or hydrophobin-DCBD-coated itraconazole nanoparticles in an external CNF matrix notably increased formulation storage stability. Interestingly, the authors noted that the dissolution rate of itraconazole from freeze-dried nanoparticle formulations was significantly faster than that of the pure crystalline itraconazole. The reason for this finding was not completely clear, but it was suggested that the porous cellulose network provided a large surface area for access of the dissolution medium, which would facilitate the dissolution of the drug directly from the cellulose fiber-bound particles. As a consequence, *in vivo* performance of the drug in an animal model was enhanced.

There have been various investigations in which biomedical applications of CNF have been explored and which may also be relevant in respect to parallel therapeutic drug incorporation and release. For example, Eyholzer and co-workers (2011) and Borges et al. (2011) studied hydrogel composite materials prepared by UV polymerization of N-vinyl-2-pyrrolidone in combination with CNF and with Tween® 20 trimethacrylate as a cross-linking agent. Mechanical testing of the material suggested that these biocomposite hydrogels could be suitable implants for nucleus pulposus, the jelly-like material in the middle of the spinal disc. This suggestion was based on the adequate swelling ratio and increased mechanical properties under compression of the CNF hydrogel composites.

Layer-by-layer assembly has been explored as a technique for improved drug delivery (Ariga et al. 2011) and Utsel et al. (2010) developed a thermoresponsive system based on multilayers of CNF and N-isopropyl acrylamide. These researchers noted that this approach could provide a way to control permeability and hence drug release by means of temperature modulation. Although targeted at food packaging films, the research conducted by Liu et al (2013) on CNF/chitosan in

combination with the antibacterial agent BAC may have relevance for new drug delivery systems. The concept behind this work was the use of a novel chitosan-BAC complex absorbed on to CNF and then incorporated into sodium alginate films. A disc diffusion Petri dish method was used to show good activity against *Staphylococcus aureus* (*S.aureus*) and some activity against *Escherichia coli* (*E.coli).* This difference in activity was attributed to the generally greater susceptibility of Gram-positive bacteria to BAC. The strength properties of the alginate films were also very significantly enhanced by addition of the CNF/chitosan-BAC complex.

The antibiotic properties of silver have been known since ancient times and, with the increasing recent concerns associated with drug-resistant bacteria, the use of silver as an antimicrobial (e.g., in wound dressings) has again attracted attention. Current commercial examples include Acticoat® (Smith & Nephew) and Aquacel Ag® (Convatec). The antimicrobial properties of silver in nanoparticulate form (AgNPs) have been the subject of recent reviews (e.g., Marambio-Jones, Hoek 2010; Rai et al. 2009; Sharma et al. 2009). The mechanism of antibiotic action of AgNPs has been much debated but a recent paper strongly suggests that efficacy is linked to the gradual release of silver ions from AgNPs rather than due to some intrinsic activity of the nanoparticles *per se* (Xiu et al. 2012). In this context, Diez et al. (2011) studied the attachment of silver nanoclusters (AgNC) to CNF. In this case the nanoclusters consisted of only a few atoms of silver and were therefore smaller than AgNPs. Fluorescent solutions containing silver nanoclusters were synthesized from silver nitrate and poly(methacrylic acid) (PMAA) using a procedure described in an earlier publication (Diez et al. 2009). CNF films cast on glass were dried and then immersed in silver nanocluster solutions with shaking for several hours. The attachment of AgNC to CNF films was evaluated using the quartz crystal microbalance with dissipation technique (QCM-D) and AFM. The rinsed CNF/AgNC films were then tested for antimicrobial activity by exposure to *E.coli* in Petri dishes. The released silver was found to prevent bacterial growth in an area five times larger than that of the applied CNF/AgNC film (*Fig 7*). The authors suggested that this material could find application in wound healing. The binding of silver in nanoparticulate form to cellulose has been previously reported by Cai et al. (2009), who used aqueous alkali hydroxide-urea solutions to create cellulose gels and then reduced silver *in situ* to form well-dispersed AgNP-cellulose gels. Similar gels were formed using gold or platinum and supercritical drying generated aerogels, which the authors indicated might find use in electro-optical, catalytic or antibacterial applications.

Even though there has been an increase in the commercial use of silver-based dressings in recent years and continuing research on the combination of silver with nanocarriers such as nanocellulose, it is important to note that in some studies the clinical evidence for the efficacy of silver in preventing wound infection and promoting wound healing can be surprisingly lacking. For example, Storm-Versloot et al. (2010) examined 26 randomized

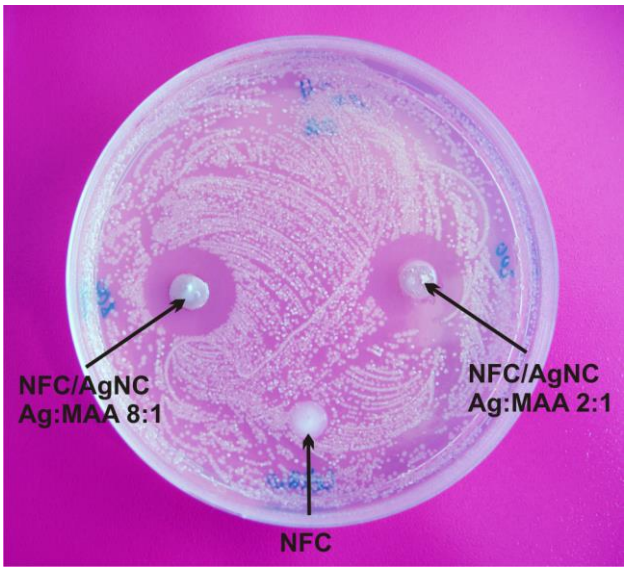

Fig 7 - Antibacterial properties of CNF films functionalized with silver nanoclusters and exposed to *E.coli* in a Petri dish test. Films were dipped for 60 hours in silver nanocluster solutions with molar ratios Ag:MAA 8:1 (left), Ag:MAA 2:1 (right) and unmodified (bottom center) where MAA = methacrylic acid (Reprinted with permission from Diez et al. 2011. Copyright 2011 Wiley).

control trials (RCTs), involving 2066 participants, which compared the efficacy of silver-containing dressings or creams against dressings or creams that did not contain silver. Twenty of the trials were on burn wounds, while the other trials were on a mixture of wound types. As noted by the authors, most trials were small and of poor quality. The conclusion from this particular study was that there is not enough evidence to support the use of silver-containing dressings or creams, as generally these treatments were not found to promote wound healing or prevent wound infections. In contrast, other recent publications, based on systematic reviews or meta-analyses, appear to confirm the effectiveness of silver dressings when used appropriately (Lo et al. 2008, 2009, Carter et al. 2010).

Cellulose fibers have been used as a template for *in situ* synthesis of AgNPs and, by modifying the procedure, it has proven possible to form porous or non-porous silver nanostructures (He et al. 2005). In this case, cellulose sheets were immersed in silver nitrate solution and then, after rinsing with ethanol, were exposed to an aqueous solution of sodium borohydride in order to reduce the silver. The resulting composite sheet was subject to calcination at high temperature to burn off the cellulose and generate a film composed of metallic silver nanofibers with structure inherited from the cellulose template. The type of nanofiber structure was silver concentration-dependent and at low concentrations the population of AgNPs was insufficient to create nanofibrous morphology. Although the authors did not elaborate on specific applications, it is possible to imagine that the silver nanofiber sheet formed on a sacrificial cellulose template might provide another route to antimicrobial materials with biomedical applications. The same authors had earlier published a report in which

cellulose fibers were employed as a nanoreactor (He et al. 2003). In the simple procedure outlined by these researchers, the nanoporous structure of cellulose provided a suitable environment for synthesis of AgNPs, the details of which could be controlled by silver ion concentration.

Bacterial cellulose (BC)

Background

Bacterial cellulose (BC), otherwise known as microbial cellulose, has been the subject of recent reviews in which its use in biomedical applications, particularly in wound healing products, has been discussed (Alvarez et al. 2004; Czaja et al. 2007, 2006; Fontana et al. 1990). BC has joined a list of other microbially derived polysaccharides, such as hyaluronic acid, dextran and alginate, which have attracted medical attention. The review by Czaja et al. (2007) includes a description of BC, its production and properties and contains sections on BC in wound healing systems as well as *in vitro* and *in vivo* tissue engineering. The review notes that research carried out by Fontana et al. (1990) and Mayall et al. (1990) showed that Biofill®, a commercial product, was a very successful wound healing material. Biofill® provided pain relief, protected wounds against infection, accelerated healing processes and reduced the cost of wound treatment. Biofill® is now available as Dermafill™ (Cellulose Solutions LLC, USA). Another BC product, XCell® manufactured by Xylos Corporation was studied by Alvarez et al. (2004) and found to be more effective than conventional dressings when used to treat patients suffering from chronic venous ulcers. Klemm et al. (2001) referred to the use of a tube-like BC product called BASYC® (BActerial SYnthesized Cellulose), which was produced *in situ* as a tube during BC cultivation and considered suitable for use in experimental microsurgery. In the same report, animals treated with a drug-infused BASYC® tube showed improved walking ability post-surgery. Other researchers have found that BC may be suitable as a vehicle for drug delivery. For example, Sokolnicki et al. (2006) concluded that BC can be used to immobilize harmful compounds while also allowing beneficial compounds to pass from a membrane to a wound or diseased area. Suprasorb® X + PHMB (Lohmann & Rauscher, Germany) is a wound dressing product based on BC which has been well tolerated and given good wound healing outcomes (Elzinga et al. 2001; Kingsley et al. 2009). Gengiflex® (Biofill Productos Biotecnologicos, Brazil) is another BC membrane product which has been explored for use in surgery and in dental implants.

Examples of BC and drug delivery from the scientific literature

In a recent review on BC-based materials in medical devices the authors suggested that covalent attachment of biologically active ligands such as pharmaceuticals, anticoagulants, growth factors and angiogenic factors could all have great potential (Petersen, Gatenholm 2011). The use of BC membranes for transdermal drug delivery has attracted interest and has been explored by Stoica-Guzun et al. (2007). In their research, BC membrane surfaces were modified using electron beam irradiation and TET was used as a model drug. The permeation of this antibiotic through irradiated and non-irradiated BC membranes was determined using diffusion cells with donor and receptor compartments and quantification of TET permeating through to the receptor compartment by means of UV-visible spectroscopy. The rate of permeation of the drug through the membranes was said to be faster in the case of non-irradiated membranes. Huang et al. (2013) used BC membranes for delivery of berberine hydrochloride and berberine sulfate. Berberine, an isoquinoline alkaloid derived from a number of plant sources, is reported to have anti-fungal activity. The researchers claimed that use of BC as a carrier significantly extended the release time when compared with commercial products and may have application in oral or transdermal drug delivery systems. Release kinetics were analyzed using the Ritger-Peppas equation (Ritger, Peppas 1987) and it was concluded that drug release from BC membranes was a diffusion-controlled process. In addition, the release rate was found to vary according to the pH of the release media, due to both pH-dependent swelling of the fibers and decreased aqueous solubility of the drug under acidic conditions.

In a recent report, Müller et al. (2013) investigated BC as a hydrogel carrier for albumin as a model protein.

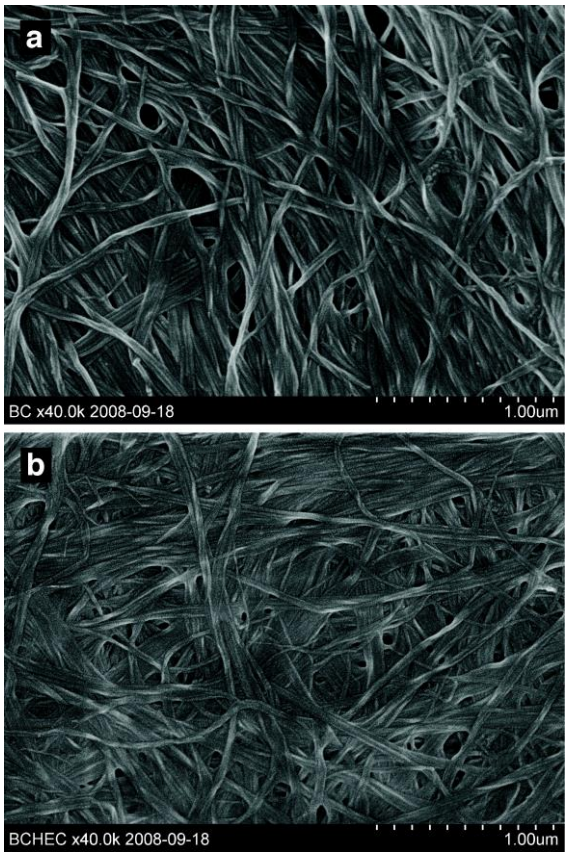

Fig 8 - FE-SEM micrographs of the surface of cellulose nanocomposite films prepared from water suspensions of BC microfibrils: a) control BC, b) BC produced in the presence of 2% w/v hydroxyethyl cellulose (HEC) (Reprinted with permission from Zhou et al. 2009. Copyright 2009 Royal Society of Chemistry).

Loading of the protein was achieved by immersing samples of never-dried or freeze-dried BC hydrogels in phosphate-buffered solutions of bovine serum albumin (BSA), shaking for up to 48 hours at predetermined temperatures and then analysing supernatant solutions using UV spectroscopy. Subsequently, the release of BSA from pretreated BC samples was evaluated by exposure to set quantities of PBS and measuring the amount of BSA released at predetermined intervals. These researchers noted that freeze-dried BC samples exhibited lower uptake of albumin than native, never-dried BC and that release of the model drug was a result of both diffusion- and swelling-controlled processes. Using luciferase as a model, biologically active enzyme that is similar in molecular weight and size to albumin, it was shown that the three-dimensional structure and hence activity of this protein was retained after binding and release from the BC hydrogels. The authors concluded that the highly hydrophilic nature, excellent biocompatibility and ability to control drug loading and release kinetics make BC an attractive biopolymer for controlled drug delivery. As demonstrated by Zhou et al. (2009), BC can be grown in the presence of a water-compatible polymer matrix such as hydroxyethylcellulose (HEC), leading to biocomposite membranes with higher tensile strength than pure BC sheets, cellulose nanopapers or conventional BC/HEC blends (*Fig 8*). Developments in this direction may also open up possibilities for a variety of new drug-loaded biocomposite membranes with potential pharmaceutical applications.

The deposition of AgNPs on BC fibers has been reported (Ifuku et al. 2009). In this study, BC fibers were functionalized using TEMPO, resulting in the formation of carboxylate groups on the nanofiber surfaces. Using exposure to silver nitrate in a hydrothermal process the researchers produced stable AgNPs bound to cellulose (*Fig 9*). An advantage of this method was the narrow size distribution of the AgNPs and therefore the opportunity to modify antimicrobial activity based on nanoparticle size. The use of sheet-shaped BC templates was also mentioned as an advantage because it provides an easy way of handling nano-sized metallic NPs. Berndt et al. (2013) created antimicrobial porous hybrids based on AgNPs in combination with BC. Their approach involved a three-step BC modification procedure in which sterilized BC samples were first activated by treatment with a DMSO solution of N,N'-carbonyldiimidazole (CDI). This step was followed by a second treatment involving sample immersion in 1,4-diaminobutane in anhydrous DMSO and subsequent rinsing in ethanol and deionized water. In a third step, the BC samples were exposed to an aqueous solution of sodium acetate and silver nitrate for one hour at room temperature and then thoroughly washed with deionized water. As a result of this approach, AgNPs grew selectively on the activated BC surfaces and were chemically linked to the cellulose fibers via grafted amine groups on the previously modified BC. As shown by SEM micrographs, there was a clear correlation between the membrane surface area covered by AgNPs and the time of exposure to silver nitrate solution (*Fig 10*). Incubation tests in Petri dishes indicated that the cellulose-based hybrids were active against *E.coli* and could be of interest as a component in antimicrobial wound dressings. In an alternative method, AgNPs were produced *in situ* with BC through hydrolytic decomposition of silver-triethanolamine (TEA) complexes (Barud et al. 2008). Klechkovskaya et al. (2008) also explored the use of BC membranes for the purpose of drug delivery by forming BC membrane templates which could hold zero-valent metallic nanoparticles (i.e., Se and Ag) absorbed from solutions of commercial products stabilized by the addition of poly(vinylpyrrolidone).

There are many reports in the literature citing the biocompatibility of cellulose-based materials; however, there are relatively few studies that have targeted compatibility related to specific applications. One example, related to the use of BC for wound dressings, is the work of Almeida et al. (2013) who ran an *in vivo* skin compatibility investigation and claimed that it was the first such study to evaluate possible human skin irritation when in contact with BC membranes. Using a visual evaluation procedure, these researchers found good skin tolerance after 24-hour patch tests. Membranes containing glycerin were more malleable and also provided a skin moisturizing effect, which could be clinically relevant in certain cases.

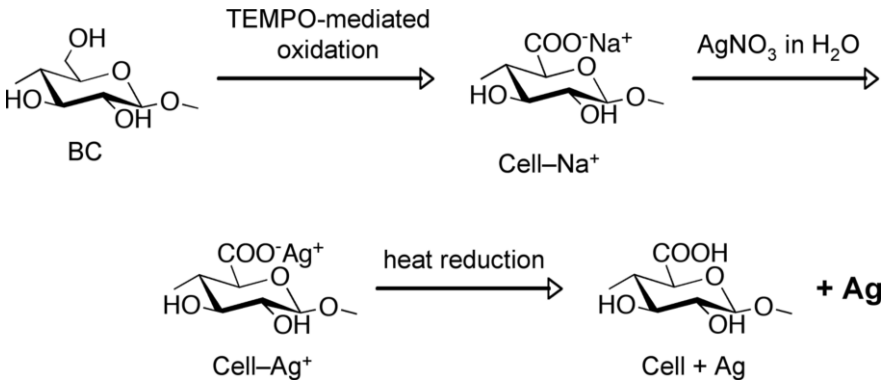

Fig 9 - Synthesis of silver nanoparticles from carboxylate-modified BC nanofibers (Reprinted with permission from Ifuku et al. 2009. Copyright 2009 American Chemical Society).

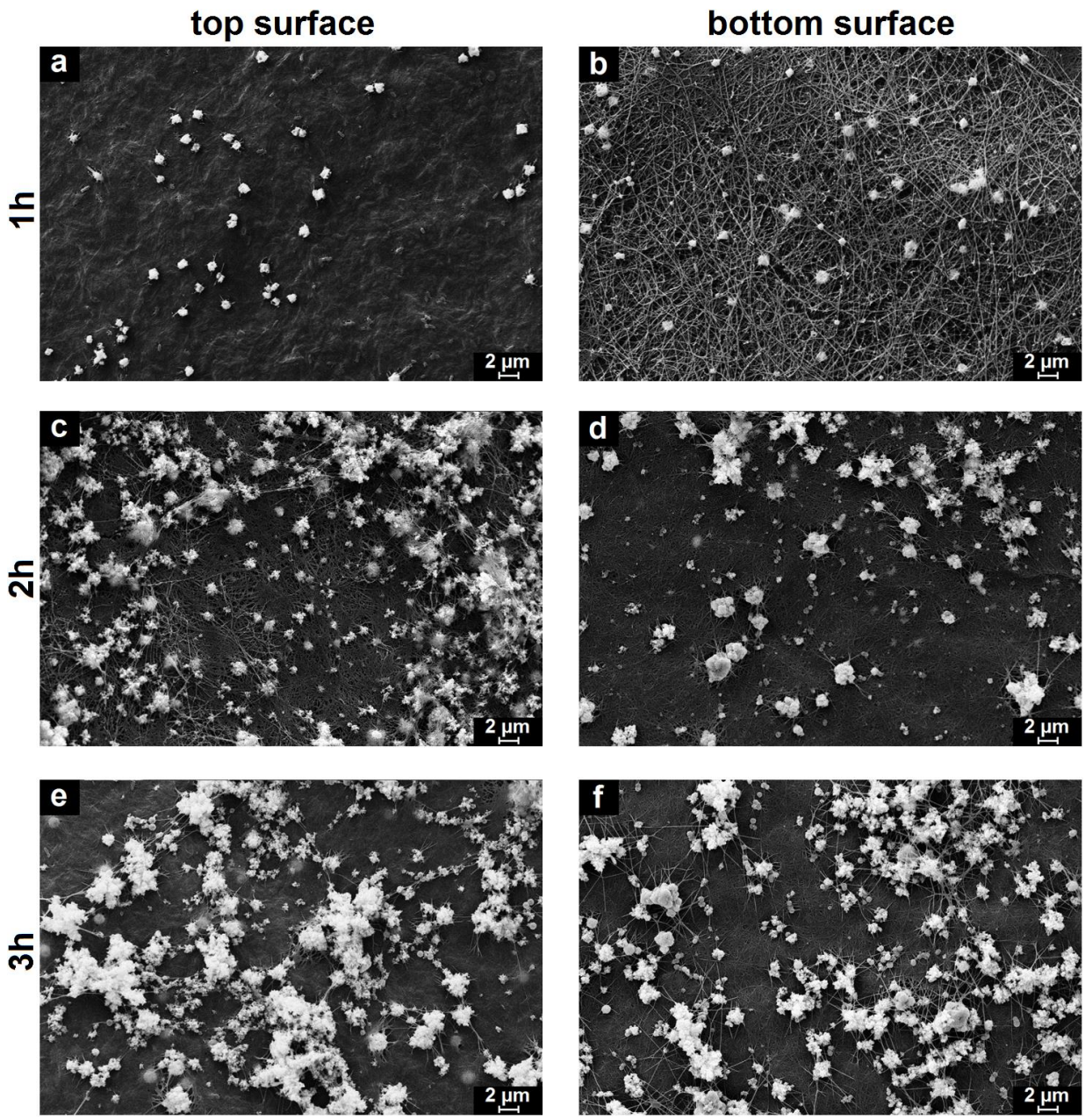

Fig 10 - SEM micrographs of the top and bottom surfaces of BC membranes after treatment with 5 x 10^{-3} M silver nitrate solution for 1-3 hours (Reprinted with permission from Berndt et al. 2013. Copyright 2013 Springer).

Conclusions

Interest in the development of products based on nanocelluloses has increased dramatically over the past several years due to the potential of these materials to provide value-added products that will complement existing pulp and paper commodities and other specialty products. Due to their reported lack of toxicity there is great interest in the use of these nanomaterials for biomedical applications and indeed there are already products on the market for use in wound healing. However, when compared with the overall scientific literature on nanocelluloses, there are relatively few publications to date focused specifically on their use as a vehicle for drug delivery. The high surface area-to-volume ratio and their ease of chemical modification lends these materials to potential use as excipients or matrices for the binding and controlled release of active pharmaceutical ingredients, particularly for topical or transdermal administration. To date, systems investigated include films, fibers and aerogels, which show great promise for delivery of a variety of agents such as anti-inflammatories, anti-infectives (including silver nanoparticles), proteins and steroids. Of particular interest is the possibility to use nanocelluoses to modulate the release of both hydrophobic and hydrophilic compounds, thus providing truly versatile materials with respect to drug delivery. However, most studies to date have explored basic binding and release properties and offered few suggestions or guidance as to how such properties might be developed into a relevant formulation for a specific disease.

This review of the literature highlights the many applications of CNC, CNF or BC, which may be feasible in the field of drug delivery. In addition to building

further on this knowledge base, future research might arguably be aimed increasingly at specific medical applications (e.g., wound healing, transdermal drug delivery) with a view to optimizing formulations and obtaining all the data necessary for clinical approval, including testing in *in vivo* disease state models, which to the authors' knowledge, has yet to be done for these drug delivery systems. There are frequent literature references to the safety and lack of toxicity of the various forms of nanocellulose and, although there have been relatively few reported studies to date, the results so far do support the safety and lack of cytotoxicity of nanocelluloses in unmodified form. However, further development of these materials as drug delivery systems will no doubt require more extensive investigation into their toxicity characteristics.

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