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REVIEW ARTICLE

The potential of nanofibers and nanobiocides in water purification

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Abstract
Electrospun nanofibers and nanobiocides show potential in the improvement of water filtration membranes. Biofouling of membranes caused by the bacterial load in water reduces the quality of drinking water and has become a major problem. Several studies showed inhibition of these bacteria after exposure to nanofibers with functionalized surfaces. Nanobiocides such as metal nanoparticles and engineered nanomaterials are successfully incorporated into nanofibers showing high antimicrobial activity and stability in water. Research on the applications of nanofibers and nanobiocides in water purification, the fabrication thereof and recently published patents are reviewed in this article.

Keywords: *Nanofibers; Electrospinning; Nanobiocides, Water purification; Water filtration systems; Antibacterial activity*

Introduction

Drinking water contaminated with bacteria and viruses is the main cause of diseases in development countries (Jain and Pradeep, 2004). One-sixth of the world population (1.1 billion people) doesn't have access to safe water (WHO, 2004). Currently, chemical disinfectants such as chlorine and membrane-based water filtration systems are employed to control microbial pathogens. However the reliability of these methods is under question. The formation of harmful disinfection byproducts from chemical disinfectants and the rise in resistant pathogens are problematic (Li et al., 2008). Two significant obstacles in water treatment membrane filtration are biofouling and virus penetration (Barhate and Ramakrishna, 2007). Membrane permeability and membrane lifetime are reduced and energy costs are increased. Nanofiltration and reverse osmosis are effective in removing viruses however both are high energy filtration schemes. Low pressure membranes with anti-biofouling and antiviral properties are therefore in urgent demand (Zodrow et al., 2009).

Filtration systems may be improved by the use of nanofibrous media. The low basis weight, high permeability and small pore size make them appropriate to remove unwanted particles smaller than 0.3 µm (Barhate and Ramakrishna, 2007). Nanofibrous filtering media may differ in thickness, fiber diameter and distribution, representative pore size, porosity, and tortuosity factor (indicating geometry and interconnectivity of pores) depending on the selected polymer(s) (Frenot and Chronakis, 2003). Nanobiocides such as metal nanoparticles, antibiotics and bacterial agents, can be added to the polymer solution and encapsulated in the dry nanofibers. These nanofibers are synthesized by a new and efficient fabrication process, namely electrospinning (Frenot and Chronakis, 2003).

This review will focus on the fabrication and antimicrobial activity of nanofibers and nanoparticles and possible applications in water purification.

Fabrication of nanofibers

Electrospinning is a novel technology for the fabrication of non-woven fibrous articles (nanofibers) with diameters lower than 100 nm (Li and Zia, 2004; Renekar and Chun, 1996). The first patent on the production of nanofibers was published in the USA (Cooley, 1902). Many patents followed from 1934 to 1944, which described the experimental setup using electrostatic force (Formhals, 1934). In Russia, nanofibrous filter media, known as filters of Petryanov, was kept top

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secret and never reached the Western Community (Lushnikov, 1997). These filter materials were used after World War II to protect the environment from nuclear-active aerosol release. Five enterprises were established in Russia by the end of 1960 producing more than twenty types of fiber filter materials (Lushnikov, 1997; Shepelev and Rykunov, 1995; www.krunk.ee/eng/2/21.shtml). Production of nanofibrous materials in America gained momentum in 1980 and only started in the 1990s in Europe (www.krunk.ee/eng/2/21.shtml). Currently more than twenty enterprises are keeping interest in production or use of nanofiber filter media (Barhate and Ramakrishna, 2007).

Electrospinning is a simple, rapid, efficient and inexpensive method, run by only three components, namely a high voltage supplier, a capillary tube with a pipette or needle of small diameter (spinneret) and a grounded collecting plate, usually a metal screen, plate, or rotating mandrel (Liang et al., 2007). The nanofibers are produced by applying a high voltage to electrically charge liquid or melt out of pipette. Taylor cones are formed when the electrical field is increased leading to the elongation of the hemispherical surface at the tip of the fluid. The electric force overcomes surface tension of the polymer solution leading to evaporation of the solvent and ejection of a thin polymer drawn toward the collector plate. Ultra fine fibers have been successfully spun from more than fifty different polymers. A polymer solution is prepared by dissolving the polymer in a specific solvent and introducing the fluid into the capillary tube for electrospinning (Huang et al., 2003). Electrospun polymers with wettability and absorbency properties, applied for different types of filters are summarized in Table 1.

Laboratory scale electrospinning yields low quantities of nanofibers. Therefore a multi-jet electro-spinning and blowing-assisted electrospinning technology were recently developed, demonstrating the production capability for fabricating nanofibrous articles on an industrially relevant scale (Chu et al., 2003; Ding et al., 2004a; Um et al., 2004).

Researchers at Elmarco have designed a high-yield industrial application namely Nanospider. This technology produces Taylor cones in close proximity to each other on a cylinder, partly immersed in a polymer solution. A thin film on the cylinder's surface is created from a controlled amount of the polymer solution. Nanofibers weighing between 0.1 and 10 g/m^2 are synthesized and the machine produces 0.1 to 1 g material in less than one minute, sixty times faster than using traditional electrospinning techniques. A variety of polymers are used by Elmarco's Nanospider™ technology to produce a wide range of organic, inorganic and biodegradable nanofibers. Nanofibers may be produced by a water based electrospinning process using poly(vinyl alcohol) (PVA), PEO and biopolymers with a mean fiber diameter of 200, 250, or 300 nm and a fiber diameter distribution of ±30%. Solvent based electrospinning of nylon 6, polyaramid polyurethane (PA6/12), poly(acrylic acid) (PAA), polyurethane (PUR), fluoropolymers, and biopolymers (Collagen, Chitosan) produce fibers with a mean diameter of 80, 100, 150, or 250 nm, and diameter distribution of ±30%. One of Elmarco's core principles is the production of high quality nanofibers. Elmarco's filtration material product, Nanospider AntimicrobeWeb™, has numerous applications and is currently used in the manufacture of surgical masks with almost one hundred percent effectively in the filtering of viruses and bacteria. Elmarco is also in partnership with various industries such as Cummins Filtration in USA to develop innovative solutions in motor vehicle and related filtration systems (www.elmarco.com/ retrieved 24/02/2009).

Nanofibers possess an extremely high surface-to-volume ratio and a small pore size (in microns) (Chen and Hsieh, 2004; Gupta et al., 2004; Han et al., 2004; Huang et al., 2003; Lin et al., 2004; Xu et al., 2003; Yang et al., 2004; Zhang et al., 2005). The composition of the nanofiber mat can be controlled to achieve desired properties and functionality. Parameters influencing the thickness and morphology of nanofibers include (a) molecular weight, molecular-weight distribution, and architecture (branched, linear, etc.) of the polymer (b) solution properties (viscosity, conductivity, and surface tension); (c) process parameters such as electric potential, flow rate, distance between the syringe needle tip

Table 1. Polymers and solvents applied for electrospun nanofibrous material.

Polymer	Solvent	Concentration	Reference
Polyurethane (PU)	Dimethyl formamide	10 wt %	Tsaia et al. (2002)
Polycarboate (PC)	Dichloro-methane	15 wt %	Bognitzki et al. (2000)
	Dimethylformamide: tetrahydrofuran (1:1)	20 wt %	Tsaia et al. (2002)
Polylactic acid (PLA)	Dichloromethane	5 wt %	Bognitzki et al. (2001)
Polyethylene oxide (PEO)	Isopropyle alcohol and water	10 wt %	Morozov et al. (1998)
Polyvinylcarbazole	Dichlormethane	7.5 wt %	Bognitzki et al. (2000)
Polystyrene	Tetrahydrofuran	15 wt %	Torres (2001)
Polyamide (PA)	Dimethylacctamide	Not available	Hajra et al. (2003)
Cellulose acetate (CA)	Acetone, acetic acid	17 %	Han et al. (2002)

to ground collection plate; (d) ambient parameters (solution temperature, humidity, and air velocity in the electrospinning chamber), and finally motion of target screen (Reneker et al., 2000; Wang and Hsieh, 2004). In the application of water filtration membranes, nanofibers are not sufficient to withstand impacts during filtration and should be spun directly over a stronger, more rigid support e.g. melt blown supportive fibrous media (Grafe and Graham, 2003) or should be 'sandwiched' between various layers (Wang et al., 2005). To fabricate a self-supporting membrane, post-heat treatment is conducted to fuse the overlapped fibers together attaining a crystalline structure. This improves the structural integrity and mechanical strength of the fibers (Gopal et al., 2006). Nanofibers with mechanical strength close to continuous film and porosity are fabricated using a 10 to 20 wt% polymer suspended in a low boiling solvent with boiling point above 100°C. Fibers adhere at large contact points without deformation (Shutov and Astakhov, 2006).

Despite the remarkable properties mentioned, nanofibers may be further functionalized by spinning blends of specific polymers, coaxial spinning of two polymer solutions, coating of fibers, or inclusion of functional components such as antimicrobial nanoparticles (Kriegel et al., 2008).

Electrospun nanofibrous scaffolds have been widely investigated in the past several years with materials of different compositions applications of varying end uses, such as filtration (Gibson et al., 2001; Schreuder-Gibson et al., 2002; 2004) optical and chemical sensors (Ding et al., 2004b; Ding et al., 2004c; Liu and Kameoka et al., 2004; Wang et al., 2002; Wang and Kim et al., 2004; Wannatong and Sirivat 2004), electrode materials (Kim and Park et al., 2004; Kim and Yang 2003), and biological scaffolds (Khil et al., 2005; Ma et al., 2005; Riboldi et al., 2005; Yang and Murugan et al., 2005).

Antimicrobial nanofibers

The successful preparation of antimicrobial nanofibers depends primarily on the selected surfactants and polymers. Polymer-surfactant interactions influence parameters of the electrospinning process and may alter the three critical factors of electrospun nanofibers, namely rheology, surface tension, and conductivity (Lin et al., 2004; Yao et al., 2003). The hydrophilic and hydrophobic moieties of surfactants enable them to form association colloids and to absorb to various interfaces when dispersed in a solvent (Goodwin 2004; Lange, 1999). Microemulsions are examples of association colloids with lipophilic compounds solubilized within their hydrophobic interior resulting in swollen micelles (Stauffer et al., 1994; Vandamme, 2002; Weiss et al., 1996, 1997, 1999, 2000; Weiss and McClements, 2000). The concentration of the lipophilic compound increases when solubilized in surfactant micelles leading to improved functional properties such as bioavailability, antimicrobial, or antioxidant activities (Weiss et al., 2007). Gaysinksy and co-workers encapsulated lipophilic antimicrobials in microemulsions which increased their interaction with bacterial surfaces, resulting in enhanced activity against Gram-positive and Gram-negative foodborne pathogens (Gaysinsky et al., 2005a,b; 2008).

Conventional membrane materials are mostly hydrophobic and should be hydrophilic for water-based filtration/purification applications. The hydrophobic polymer surface can be rendered into hydrophilic by surface modification and can hereby obtain other functionalities (Yoon et al., 2009). Clean and environmentally friendly ways for surface modification of nanofibers include plasma treatment (Ramakrishana et al., 2005) and surface graft polymerization (Zuwei et al., 2006). Plasma treatment involves changing the surface chemical composition of fibers by treatment with oxygen, ammonia or air that generate free radicals and electrons on the surface. These functional groups change the surface adhesion and wetting properties of the membrane (Fu et al., 2008; Fuchs and Tiller, 2006). Functional compounds such as Poly(quaternary ammonium) are then covalently bound to the fiber surfaces to target pathogenic microorganisms (Kim and Klibanov, 2005). Surface graft polymerization is initiated by treatment with plasma and UV radiation to generate free radicals for polymerization (Yoo et al., 2009). Three challenges should be kept in mind when attempting surface modification namely the uniformity of the coating and surface modification, the filter pore size should not be affected by the coatings and industrial fabrication of these nanofibers may be complicated (Barhate and Ramakrishna, 2007). Examples of surface modification using plasma treatment, surface graft polymerization and the coupling of antimicrobial compounds are described.

Polyurethane (PU) nanofibers were treated with argon plasma, which produced surface oxide and peroxide groups. The PU fibers were then immersed in a 4-vinylpyridine monomer solution with exposure to UV irradiation, to produce poly(4-vinylpyridine) grafted PU fibers successfully. The grafted pyridine groups were functionalized through quaternization with hexylbromide to obtain antibacterial activities. The viability of Gram positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* was reduced 99.9% after 4 h contact with the PU fibers (Yao et al., 2008).

Yao and co-workers (2009) modified the surface of electrospun poly(vinylidene) fluorid-co-hexafluoropropylene (PVDF-HFP) fibrous membranes to achieve antimicrobial activities. The electrospun membranes were treated with poly(4-vinyl-*N*-alkylpyridinium)

bromide and a combination of plasma pretreatment, UV-induced graft copolymerization and quaternization. PVDF-HFP membranes were highly active against *S. aureus* and *E.coli* and could serve as an efficient and versatile approach for the development of antimicrobial polymer fibers (Yao et al., 2009).

The potential of polycarbonate (PC)/chloroform solution with quaternary ammonium salt (benzyl triethylammonium chloride, BTEAC) as antimicrobial nanofibrous membranes for ultrafiltration was investigated. The addition of small amounts of BTEAC to the PC solution decreased the average diameter and totally inhibited the growth of *S. aureus* ATCC6538, *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 4352. The PC nanofibrous filter showed 99.97% filtration efficiency of 0.3 μm size particles in comparison to a polypropylene (PP) HEPA (high efficiency particulate air) filter (Kim et al., 2007). A similar study was performed by Jeong and co-workers (2007). Electrospinning of polyurethane cationomers (PUCs) containing different amounts of quaternary ammonium groups were investigated for the application in antimicrobial nanofilters. Very strong antimicrobial activities against *S. aureus* and *E. coli* were found and the average diameters of the electrospun PUCs fibers decreased with increasing quaternary ammonium group content.

Mechanical properties and antimicrobial activity of nylon 6 nanofiber membranes with the addition of three different *N*-halamine additives, namely chlorinated 5,5-dimethylhydantoin (CDMH), chlorinated 2,2,5,5-tetramethyl-imidozalidin-4-one (CTMIO) and chlorinated 3-dodecyl-5,5-dimethylhydantoin (CDDMH) were determined. The growth of both *E. coli* and *S. aureus* was inhibited after a contact period of 5 to 40 min. The fastest antimicrobial action was found for CDMH. The increase of active chlorine contents resulted in a higher antimicrobial rate and larger microbial reduction. The addition of *N*-halamines had no significant effect on the mechanical properties of nylon 6 membranes and leaching of *N*-halamines was negligible (Tan and Obendorf et al., 2007).

A bi-layer composite membrane consisting of chitosan and poly(acrylic acid) (PAA), and a bottom support layer composed of poly(ethylene glycol) diacrylate (PEGDA) and PAA was developed for antimicrobial applications (Don et al., 2005). A strong and dense membrane was developed by casting PEGDA, acrylic acid and a photoinitiator and curing with UV irradiation on a glass substrate. This bottom layer was coated with a solution containing of chitosan, acrylic acid and water. Coagulation of chitosan with residual acrylic acid took place that subsequently formed a nano-layer on the top surface after application of UV irradiation. The addition of 0.5% chitosan killed 80% *S. aureus* (CCRC 10779) and 30% *E. coli* (CCRC 10324), and increased to 82% and 45%, respectively, with an increase of 0.5% chitosan (Don et al., 2005).

Chitosan was also applied in a high flux ultra filtration media by replacing flux-limiting asymmetric porous membrane with porous electrospun nanofibrous scaffolds (Yoon et al., 2006). The demonstrated systems consisted of a three-tier composite structure: (1) 'nonporous' hydrophilic coating that is water permeable (chitosan), (2) polyacrylonitrile (PAN), an electrospun nanofibrous support and (3) polyethylene terephthalate (PET), a non-woven microfibrous substrate (Figure 1).

PAN is resistant to most solvents and has therefore been widely used for ultrafiltration (Musale et al., 1999), nanofiltration (Musale and Kumar, 2000), and reverse osmosis (Chandorilar and Bhavsar 1983). Carbon nanofibers were also fabricated with electrospun PAN as precursor (Wang et al., 2003). The PAN fibrous networking in this study was used to support a top coating layer based on chitosan (Yoon et al., 2006). Chitosan has been used for anti-fouling enhancement of filtration membranes duo to its insolubility in neutral pH conditions (Gudmund et al., 1989) and thus water-resistance (Musale et al., 1999; Musale and Kumar, 2000; Wang and Spencer, 1998).

Three-tier composite membranes exhibited 1.0 and 1.3 $L/m^2h.psi$ flux rate respectively for a 1.37 and 1.2

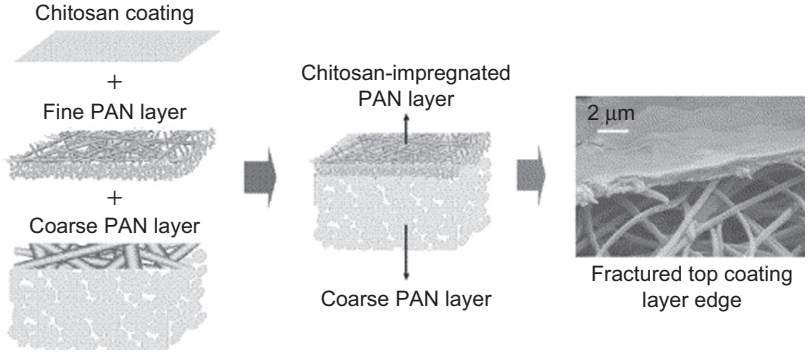

Figure 1. Fabrication schematics of the electrospun scaffold with a coating layer. SEM image represents the fractured composite membrane containing PAN nanofibrous scaffold (with 4 + 12 wt% sequential electrospinning) and chitosan coating (Yoon et al., 2006).

wt% chitosan coating comparing to a 0.6 L/m²h.psi flux rate for a commercial nanofiltration filter. Mechanisms proposed to improve membrane performance (higher flux and lower fouling) include the reduction of coating layer thickness, cross-linking of chitosan layer by glutaraldehyde, and grafting of more hydrophilic polyethylene glycol molecules on the chitosan chains (Yoon et al., 2006).

The polymers used in these studies are examples of material with good mechanical and physical properties. Surface modification of the nanofibers showed good filtration efficiency with a higher flux rate than conventional membranes. These properties along with strong antimicrobial activity render the fibers as potential antifouling water filtration membranes.

Nanobiocides (antimicrobial nanoparticles)

Nanofibers with embedded nanobiocides are currently being used in medical treatments and air filters. Different polymers such as poly(vinyl pyrrolidone) (PVP), PVA, and PEO have been used as a template to load nanoparticles such as metal oxides by electrospinning and in polymer matrixes or films (Li et al., 2006).

Manufacturing of nanoparticles

Nanoparticles are manufactured by different procedures which can be grouped into top-down and bottom-up strategies. The top-down approach involve the generation of isolated atoms from bulk materials by using various distribution techniques such as milling or attrition, repeated quenching and photolithography (Gao, 2004; Niemeyer, 2001). Bottom-up strategies are defined by the formation of more complex clusters by using molecular components as starting materials which are linked with chemical reactions and nucleation and growth process (Gao, 2004; Rotello, 2003).

Metallic nanoparticles can also be synthesized by a reliable and eco-friendly process, using bacteria and fungi (Bhainsa and Souza, 2006). *Aspergillus fumigatus* formed silver nanoparticles within 10 minutes in the presence of silver ions. The silver nanoparticles were synthesized in the extracellular cell filtrate indicated by a brown colour (Bainza and Souza, 2006). The extracellular synthesis requires no additional step of processing for release of nanoparticles from the biomass. However, this reaction mechanism is still unknown. A certain NADH dependent reductase was involved in the reduction of silver ions by *Fusarium oxysporum* (Ahmad et al., 2003a). Other fungi and bacteria involved in the formation of silver or gold nanoparticles, intracellularly, include *Verticillium* sp., *Thermonospora* sp., and *Pseudomonas stutzeri* AG259 (Ahmad et al., 2003b; Klaus- Jeorger et al., 2001; Sastry et al., 2003). This rapid synthesis of nanoparticles would be suitable for developing a biological process for mass scale production.

Nanobiocides (antimicrobial nanoparticles) are divided into three categories, namely, metal and metal oxides (nAg, ZnO, CuO, TiO_2), engineered/synthesized nanomaterials (fullerenes e.g., nanomagnetite (nC_{60}) and carbon nanotubes), and natural antibacterial substances (antimicrobial substances, chitosan) (Li et al., 2008). The antimicrobial activity of embedded metal nanoparticles in nanofibers or polymer matrices will be discussed, followed by the application of carbonaceous nanomaterial, fullerenes and natural substances as biocides.

Metal and metal-oxide nanoparticles

Silver nanoparticles

Silver is the most toxic element to microorganisms and are used in various applications such as antimicrobial filters, wound dressing material, water disinfection, sensors, chemical and gas filtration, protective cloth and air filtration (Lala et al., 2007). Different polymers have been used to incorporate silver nanoparticles for the production of antimicrobial nanofibers.

Polycaprolactone (PCL) and PAN were synthesized by reducing silver ions photochemically whereby an electron donor (Ag nanoparticles) and electron acceptor (tetracyanoquinodimethane) were incorporated in polymer nanofibers by electrospinning (Shang et al., 2006). The soft segment of Poly(ε-caprolactone)-based polyurethane (PCL-PU) is a biodegradable, biocompatible, and semi-crystalline aliphatic polyester with excellent mechanical and thermal properties (Hatakeyama et al., 2007; Heijkants et al., 2005; Ping et al., 2005; Woo et al., 2000). Silver nanoparticles can interact with the carbonyl oxygen atoms of PCL (Son et al., 2004), resulting in the even distribution of small Ag particles. PCL-PU nanofiber mats containing Ag nanoparticles may exhibit good mechanical properties with antimicrobial activity and no waste disposal problems. Jeong and coworkers (2008) prepared PCL-PU fibers with different concentrations of $AgNO_3$ DMF/THF (7/3 w/w) for the applications of an antimicrobial nanofilter. The inclusion of $AgNO_3$ increased the tensile strength, tensile modulus, and decreased elongation of the PCL-PU nanofiber mats.

Silverbromide (AgBr) nanoparticles and PVP were hybridized by electrospinning. Bromide ions reacted with silver ions of $AgNO_3$ in the PVP solution to form AgBr nanoparticles by a sol–gel technique. The AgBr/PVP viscous composite solutions were electrospun into PVP nanofibers containing AgBr nanoparticles. A fine dispersion of nanoparticles was observed by

field-emission scanning electron microscopy (Bai et al., 2008).

Nanofibers containing silver nanoparticles were electrospun with three different polymers namely CA PAN, and PVP to compare antimicrobial activity against *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. Various amounts of $AgNO_3$ were dissolved in DMF and treated with UV-irradiation to form nanoparticles. The combination of PAN in DMF containing 5 wt% of AgNO3 and UV-irradiation for 30 min was found to be most effective (Lala et al., 2007).

A chelating monomer, glycidyl methacrylate–iminodiacetic acid (GMA–IDA), was grafted onto cotton fibers to form cotton fiber-graft-GMA–IDA (CFGI). CFGI–Ag^+ complexes were successfully prepared by irradiation. The cotton fibers containing Ag^+ nanoparticles with an average size of 75 nm exhibited high antibacterial activity against *E. coli* (Chen and Chiang, 2008).

Silver nanoparticles were synthesized in semi-hydrogel networks of poly(acrylamide) and starch carbohydrate polymers as an antimicrobial application. Carboxylic, amide, and hydroxyl groups of carbohydrate polymers holds large amounts of metal ions in their network, enhancing the reduction potential/anchoring ability and stabilization of nanoparticles. The silver nanoparticles were smaller and finer distributed inside the semi-IPN hydrogel network (Figure 2). No particles were formed in the surrounding medium confirming the binding of networks to the silver ions and successful storing of nanoparticles (Vimala et al., 2009).

The application of silver nanoparticles in water filters was also investigated. Jain and Pradeep (2005) exposed PU foams overnight with silver nanoparticles followed by washing and air-drying. Silver nanoparticles interacted with the nitrogen atoms of PU and could therefore be stored for extended periods without the loss of nanoparticles. Input water was infected with *E. coli* (10^5 colony-forming units (CFU) per mL) and exposed to PU foams at a flow rate of 0.5 L/min. No bacteria were detected between 1 and 4 hours of exposure which complied with the WHO requirements for drinking water. PU foams coated with silver nanoparticles may be used as a low cost and non-toxic water purification system especially in developing countries.

Researchers in Russia, investigated the biocidal activity of silver nanoparticles after application on a surface of carbonic materials, used in water filtering devices. Tap water with high concentration of *E. coli* and *Legionella Pneumophila* (LP) was filtered through a carbon column with attached silver particles. The modified column reduced double the number of bacteria in comparison to the control column. The application of silver nanoparticles in water dispension against the human immunodeficient virus (HIV) was also tested. Up to tenfold dilution of nanoparticles was a hundred percent effective in killing the virus (www.nanomet.ru/en/appl.3.php)

Dendritic polymers are used in water filtration applications as delivery vehicles or scaffolds for antimicrobial agents such as Ag (I) and quaternary ammonium chlorides (Chen et al., 2003). Poly (amidoamine) dendrimer (PAMAM) based silver complexes and nanocomposites proved to be effective antimicrobial agents *in vitro*. The silver remained conjugated to the dendrimer in the form of ions, stable metallic silver clusters or compounds and could retain its activity. The immobilized silver diffused out into agar medium and showed high antimicrobial activity against *S. aureus, P. aeruginosa,* and *E. coli* (Balogh et al., 2001).

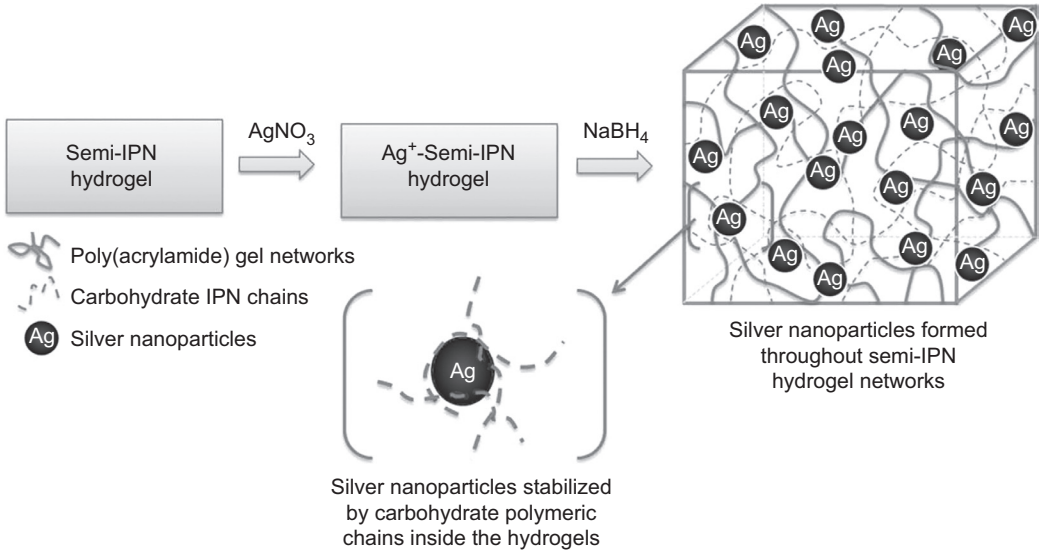

Figure 2. Synthesis of silver ions in a semi-IPN hydrogel network of poly(acrylamide) and starch carbohydrate polymers (Vimala et al., 2009).

Silver nanoparticles (nAg–PSf) embedded in polysulfone ultrafiltration membranes were evaluated for biofouling reduction. Polysulfone is currently being used in water (microfiltration, ultrafiltration and nanofiltration) membranes (Zodrow et al., 2009). The release of silver ions resulted in almost 100% reduction in viability of *E. coli* K12 and *Pseudomonas mendocina* KR1 and an enhanced removal of MS2 bacteriophage. The permeability of nAg-PSf was 532 ± 117 L/m^2/h/bar and that of the PSf control membrane 408 ± 180 L/m^2/h/bar. Nanosilver incorporation also increased membrane hydrophilicity, reducing the potential for other types of membrane fouling. However a loss of silver ions from the membrane surface lead to a significant decrease in antibacterial and antiviral activity. Concentrations of 0.034 mg/L silver ions were found in the membrane permeate although no silver nanoparticles were observed in the filtrate. Future research should focus on improved fixation of Ag^+ nanoparticles on the membrane surface and encapsulating the silver to reduce release rate. Commercial membranes may be regenerated and improved by the addition of silver nanoparticles. These water filtration membranes may ensure effective control on a wide variety of bacteria and viruses and have protection against biofouling (Zodrow et al., 2009).

Porous ceramics have become of great interest for drinking water treatment lately, due to the chemical, thermal, pH stability and cost-effective material (Padilla et al., 1997). However, during filtration the blocked bacteria are not killed and form biofilms on the surface. Lv and co-workers (2009) attached silver nanoparticles onto porous ceramic composites by an aminosilane coupling agent, 3-Aminopropyltriethoxysilane (APTES). The APTES molecule attach to silver atoms with the $-NH_2$ group on the top and to the silicon atoms in the ceramic on the bottom. The attachment of silver nanoparticles could withstand 15 minutes ultrasonic irradiation and several weeks' exposure to an atmospheric environment. The modified ceramic totally inhibited *E. coli* in water during on-line tests. Therefore, silver nanoparticle-decorated porous ceramic composites show potential as low cost and safe water filters (Lv et al., 2009).

Zinc and copper oxide nanoparticles

Zinc oxide (ZnO) nanoparticles have strong antimicrobial activity against a broad spectrum of bacteria although the mechanism involved is still uncertain. Sawai (2003) suggested that the biocide, hydrogen peroxide is photocatalytic generated and Huang et al., (2008) found that ZnO nanoparticles penetrate the bacterial cell envelope and disorganize the cell membrane. ZnO is used in pharmaceutical products such as antibacterial cream, lotions and ointments and in surface coatings to prevent biofilm formation (Jones et al., 2008). The application of ZnO nanoparticles in water treatment is however questioned as it is highly soluble in water increasing sensitivity of aquatic organisms towards ZnO (Franklin et al., 2007).

In a study by Vicentini et al., (2009), zinc oxide nanoparticles were synthesized by the Pechini method by reacting citric acid with ethylene glycol. The dissolved metal ions were incorporated into blend films of chitosan and PVA with different concentrations of polyoxyethylene sorbitan monooleate and Tween 80 (T80). Film properties such as greater thermal stability, decreased tensile strength and elongation break and porosity were influenced by the addition of ZnO nanoparticles and T80. These films effectively killed *S. aureus.*

Copper oxide is not as expensive as silver and show chemical and physical stability when mixed with polymers. The antimicrobial activity of nano CuO is not known, however properties such as extremely high surface areas and unusual crystal morphologies may render CuO as potential antimicrobial agents (Stoimenov et al., 2002). Rena and co-workers (2009) showed that CuO have activity against a range of bacterial pathogens, including meticillin-resistant *S. aureus* (MRSA) and *E. coli*, with minimum bactericidal concentrations (MBCs) ranging from 100 g/mL to 5000 g/mL. A synergistic effect existed between CuO nanoparticles and sub-minimum bacteriocidal concentrations of silver nanoparticles.

Copper-hydrotalcite (Cu-HT) can be used as an alternative disinfectant to chlorine in a water purification system. Cu-HT has very high antibacterial activity against *E. coli* and phage Q β (Sunayama et al., 2002). The proposed mode of action is the direct contact between the hydroxyl layers on the surfaces of the Cu-HT particles and phage Q β cells (Sunayama et al., 2002).

Copper nanoparticles were functionalized with an acrylic group to copolymerize with other acrylic monomers. The nanoparticles became an integral part of the polymer backbone which slowed down particle release rate from the polymer matrix. The released particles were very active against marine biofouling strains, *Chlamydomonas* sp. strain PCC 6803 (freshwater), *Synechococcus* sp. strain PCC7002 (marine) and *Phaeodactylum tricornutum* CCMP 1327. Controlled release did not affect antimicrobial activity of copper nanoparticles and correlated well with conventional copper-based biocides (Anyaogu et al., 2008).

Titanium dioxide nanoparticles

Titanium dioxide (TiO_2) is a well studied semiconductor photocatalyst (Li et al., 2008). Gram-positive and Gram-negative bacteria (Wei et al., 1994) and viruses

(Chou et al., 2005; Hajkova et al., 2007; Watts et al., 1995; Zan et al., 2007) are sensitive to TiO_2 photocatalytic inactivation at concentrations of 100 and 1000 ppm depending on the particle size and wavelength of the light (Wei et al., 1994). The mode of action includes the production of hydroxyl free radicals and peroxide formed under UV-A irradiation via oxidative and reductive pathways, respectively (Kikuchi et al., 1997).

TiO_2 is stable in water and cost effective and can therefore be successfully incorporated in thin films or membrane filters for water filtration (Belhacova et al., 1999; Kwak et al., 2001).

Immobilised nanoparticle TiO_2 films were evaluated for photocatalytic and photolytic inactivation of *Escherichia coli* K12 in surface and distilled water. Effective photocatalytic inactivation of *E. coli* occurred both in distilled and surface water. Nitrate and sulphate anions and especially humic acid spiked into distilled water resulted in a decrease in the rate of photocatalytic disinfection. Change in the initial pH to 5.5–8.5 had no effect (Alrousan et al., 2009).

Titanium dioxide nanoparticles were incorporated into an isotactic polypropylene (iPP) polymeric matrix via a cost-effective melting process using laboratory-made nanometric anatase-TiO_2 and an industrial polymer. The activity was tested against antibiotic resistant *P. aeruginosa* and *E. faecalis* bacteria. The highest biocidal activity against both bacteria was found for a sample containing a 2 wt% of anatase-TiO_2. The antimicrobial activity of the oxide alone was boosted by the nanocomposite by efficiently managing charge carrier handling through the organic–inorganic interface. This resulted in making the whole system biocide with an overall high performance comparing to current biocidal agents (Kubacka et al., 2009).

Nanocomposite water membranes containing PA and TiO_2 nanoparticles were prepared. The TiO_2 nanoparticles enhanced the hydrophilicity of the PA-TiO_2 membranes and hereby increased the water flux. At an operating pressure of 0.1 to 1.0 MPa the flux rate of PA with TiO_2 nanoparticles increased from 2.5 to 25 L/m^2/h whereas the control PA membranes exhibited a flux rate of 1 to 18 L/m^2/h. Incorporation of 5.0 wt% TiO_2 gave optimum membrane performance together with strong mechanical properties. Almost no change in TiO_2 concentration was observed after long-term filtration experiments for 2 days, implying a robust, stable nanocomposite membrane (Lee et al., 2008).

TiO_2 photocatalysis is currently being used in industrial scale water purification systems. This system produce high purity water by removing micron and submicron particles, polish iron, manganese, silica, H_2O_2 and contaminants such as *Giardia, Cryptosporidium*, and oocysts. The system is robust and need no chemicals for cleaning (www.purifics.com/technology/ceramic.html).

Fullerenes (C_{60}) and carbonaceous nanomaterial

Fullerenes are molecules with 60 atoms and have received much attention because of its wide application (Ju-Nam and Lead, 2008). Due to its insolubility in water, solvent exchange (Deguchi et al., 2001) and ultrasonification (Deguchi et al., 2006) have been applied to disperse fullerenes as nanoparticles. C_{60} nanoparticles have a broad spectrum antimicrobial activity although the mode of action is debatable (Lyon et al., 2008). The toxicity of C_{60} is also under investigation. The use of C_{60} as anti-fouling agents for ceramic membranes was determined by evaluating the effect on the attachment and respiratory activity of *E. coli* K12. A decrease in bacterial numbers and increase in inactive *E. coli* cells confirm the bactericidal effect of C_{60}. The increase in membrane hydrophobicity might also play a role in antimicrobial activity (Chae et al., 2009).

Carbon nanotubes are found in two main forms, namely as single-walled (SWNT) and multi-walled (MWNT). The SWNT is a single layer graphene sheet rolled-up as cylindrical shapes with diameters ranging from 0.3 to 3 nm and capped by half a fullerene whereas the MWNT is composed of many concentric layers with varying diameters of up to 100 nm (Gao, 2004; Balasubramanian and Burghard, 2006). Both SWNT and MWNT kill microorganisms when in direct contact although the rate of inactivation is slower than other conventional disinfectants (Brady-Estévez et al., 2008). Carbon nanotubes have poor solubility in water and should therefore be immobilized on a reactor surface or polymer matrix (Kang et al., 2007; Li et al., 2008). They can therefore be applied in the prevention of biofilm formation in water filtration membranes (Li et al., 2008). However the high cost involved (Theron et al., 2008) and potential toxicity (Ju-Nam and Lead, 2008) may hamper the use of carbon nanotubes in water filtration.

Polyethylene mine (PEI) is a carbonaceous nanomaterial exhibiting high antimicrobial and antifungal activity (Park, 1998). The nanoparticles are formed by crosslinking and alkylation from PEI followed by methylation in order to increase degree of amino group substitution (Graveland and Kruif, 2006). The activity of PEI nanoparticles with different degree of crosslinking, particle size and zeta potential were evaluated against *Streptoccocus mutants.* Only samples with long chain alkyls demonstrated high antibacterial effect for more than four weeks (Park, 1998).

Srivastava and co-workers (2004) produced carbon nanotube filters that efficiently remove *E. coli*, *S. aureus,* and the polio-1 virus from water. The nanotube based water filters allowed increased water flow in comparison to conventional filters and are reusable by autoclaving (Srivastava et al., 2004).

Carbon nanotubes were also used to fabricate a hollow cylindrical nanofilter to remove MS2 viruses from water. A continuous spray pyrolysis method was used with n-hexane as a carbon source and ferrocene as a catalyst source. The nanofilter was synthesized by optimizing the catalyst concentration, temperature, gas flow rate and reaction time affecting the purity of synthesized carbon nanotubes (CNTs). The flux rate of the nanofilter at a pressure of 5 to 20 MPa was 10 to 50 $m^3\ m^{-}2s^{-1}$ and for a conventional NF90 membrane 5 to 15 $m^3\ m^{-}2s^{-1}$. The MS2 viruses were efficiently removed at pressures of 8–11 bar and the fabricated nanofiber had good water permeability and filtrate flux (Mostafavia et al., 2009).

A filter comprising of oxidized aluminum nanofibers on a glass fiber substrate was developed by Argonide in the USA. Bacteria and viruses are filtered from the water flow by these positively charged nanofibers. The large pores of the filter renders it more effective than traditional membranes as it manages a much higher flow rate. Up to 99.999% of viruses is retained and can be used to clean water by applying muscle force with no extra energy needed, ideal for rural contexts (www.argonide.com).

NanoCeram® cartridges are synthesized by combining nano alumina into a microglass fiber matrix to form an electropositive (∼+50 mV) filter media and converted into pleated cartridges. The active ingredient, a nano alumina fiber, only 2 nm in diameter and about 250 nm long, is grafted onto a microglass fiber backbone. The pore size is enlarged by addition of coarser cellulose or synthetic polymer fibers that also improves flexibility and strength. The media is produced by a conventional wet laying process, then pleated and end-capped to form a cartridge. These cartridges are used for drinking water purposes or as prefilters for reverse osmosis membranes. Particulate and biological contaminants are removed that subsequently reduce fouling and cleaning cycles (www.argonide.com).

Aluminum hydroxide fibers (2 nm in diameter) with a high electropositive charge were patented in 2005. These fibers are combined into a composite filter with other fibers or particles that can filter bacteria, viruses and nano sized colloidal particles at high water flux. These alumina fibers also filter sub-micron inorganic and metallic particles to produce ultra pure water (Tepper and Kaledin, 2005).

Natural antibacterial substances

Antimicrobial peptides

Antimicrobial peptides have only recently been synthesized as nanoparticles. These peptides were engineered in the beginning according to the structures of known proteins and properties such as size, morphology and coatings (Gazit, 2007).

Antimicrobial peptide nanoparticles are designed through self-immobilization mediated by biomineralization reactions and hereby improving their effectiveness through increased stability and protection from inactivation (Eby et al., 2008). A cationic decapeptide, KSL (KKVVFKVKFK)10, isolated from a combinatorial peptide array, is active against a wide range of microorganisms. Mixtures of KSL, acetic acid and tetramethyl orthosilicate (TMOS) or potassium hexafluorotitanate were prepared. The pH of the solutions was increased to 8 with 15 N NaOH and the resulting precipitate was washed and stored as a wet pellet at –20°C. Antimicrobial activity was determined as minimum inhibitory concentration (MIC) and the minimum bactericidal and fungicidal concentration (MBC and MFC) against *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), and *Candida albicans* (ATCC 10231). The amorphous silica and titania did not affect bacterial growth (Eby et al., 2008). Therefore the biocidal effect is explained by interaction of the KSL, attached to the particle surface, the released soluble peptide, and the bacterial cell membranes (Brogden, 2005). Cationic peptides form pores in the cell membranes of bacteria whereas surface-immobilized peptides adhere to cell surfaces and reduce bacterial viability (Etienne et al., 2004; Gregory and Mello, 2005).

Nisin A, a lantibiotic with a broad spectrum activity against Gram-positive bacteria, was incorporated into poly-L-lactide nano-particles (Salmaso et al., 2004). A gas-compressed CO_2 process was used to yield small sized particles with a high protein loading and efficient protein release rate. Nisin solubility and release rate and protein/polymer interactions were determined in conditions with varying pH and NaCl concentrations. An increase in pH and NaCl concentrations resulted in a decrease in nisin solubility, a slower release rate and an increased interaction between nisin and poly-L-lactide. The high antimicrobial activity against *Lactobacillus delbrueckeii* subsp. *bulgaricus* for up to 45 days indicated that nisin was efficiently encapsulated. Environmental conditions may induce the modification of the protein structure leading to altered protein/polymer interactions and affecting the protein release rate and the amount of released protein. These variables should be considered when formulating polymeric systems for the delivery of proteins (Salmaso et al., 2004).

Chitosan

Chitosan is known for high antimicrobial activity towards bacteria, especially Gram-positive bacteria and effective control of fungi and viruses (Qi et al., 2004). The degree of activity is determined by the organism, pH, molecular weight, degree of polymerization and the presence of lipids and proteins (No et al., 2002; Rabea

et al., 2003). Qi and co-workers (2004) proposed that positively charged chitosan particles attacks negatively charged cell membranes leading to the formation of pores and the leakage of intercellular components. Chitosan may also penetrate the fungal cell wall and nucleus, bind to DNA and inhibit RNA synthesis (Rabea et al., 2003). Activity is however lost at pH values above 6 due to the absence of pronated amino groups (Qi et al., 2004). Chitosan nanoparticles were recently synthesized showing similar antimicrobial activities (Qi et al., 2004; Ye et al., 2006). These particles show potential in drinking water disinfection applications such as antimicrobial agents in membranes, sponges and surface coatings of water storage tanks (Li et al., 2008). The high antimicrobial activity against a broad spectrum of bacteria, viruses and fungi and low toxicity render chitosan nanoparticles as an attractive alternative to other disinfectants (Li et al., 2008). The method used for material preparation and presence of organics may affect antimicrobial activity (No et al., 2002; Rabea et al., 2003). Future research work may include the synthesis of water-soluble chitosan derivatives to improve effectiveness at alkaline pH values.

Other chitosan derivates such as chitosan–Ag^{+} complexes (Chen et al., 2005; Yi et al., 2003) and a chitosan–Zn^{2+} complex (Wang and Du et al., 2004) were prepared to improve antimicrobial activity. The metal-chelating properties of chitosan have been mainly used in wastewater treatment (Jia et al., 2001; Liu et al., 2004; Yang and Chou et al., 2005). A chitosan–Ag-nanoparticle composite (AgNP) was synthesized to test the bacteriocidal effect against *E. coli,* expressing recombinant green fluorescent protein (GFP). The growth of *E. coli* stopped immediately after exposure to the composite with a higher release rate of cellular GFP than with pure chitosan. Destabilization of the bacterial cell wall may be explained by the interaction of AgNPs and the sulfur containing intracellular proteins in bacteria (Feng et al., 2000; Morones et al., 2005).

Chitosan triphosphate nanoparticles were individually loaded with Ag^{+}, $Cu^{2+,}$ $Zn^{2+,}$ Mn^{2+}, or Fe^{2+} and tested for antimicrobial activity against *E. coli* 25922, *Salmonella choleraesuis* ATCC 50020, and *S. aureus* 25923. The stability of aqueous nanosuspensions is measured by Zeta potential. The minimum requirement for a physically stable nanosuspension, solely stabilized by electrostatic repulsion, is ± 30 mV (Müller et al., 2001). The zeta potentials were enhanced significantly after loading of metal ions and were directly proportional to antimicrobial activity. Silver and zinc ions showed the highest activity and their antimicrobial effect was more pronounced against Gram-negative bacteria than Gram-positive bacteria (Du et al., 2009).

Chitosan nanoparticles were modified using an O/W emulsification by grafting oleoyl onto the $–NH_2$ at C-2 in the chitosan molecule (OCNP). *E. coli* and *S. aureus* treated with OCNP leaded to a quick release of intracellular components. The uptake of 1-N-phenylnaphthylamine and the release of cytoplasmic β-galactosidase increased via increased permeability of inner and outer membranes. The chitosan nanoparticles adhered to the surface of *E. coli* and *S. aureus* and showed extensive cell surface alterations (Xing et al., 2009).

Although the incorporation of nanobiocides are useful, two significant challenges in the long term are apparent. Firstly, the loss of antimicrobial or antiviral activity due to leaching from the membrane. The cost involved will increase as well as the impact on human health and the environment (Wiesner et al., 2006). The safety of these escaped nanobiocides are not proved yet as metal and metal oxide nanoparticles have shown inflammatory and toxic effects on living cells. However in these trials much higher concentrations of nanoparticles were tested than humans would be exposed to. The nanobiocides can also be turned into harmful chemicals when exposed to chlorine and UV (Rule et al., 2005; Yao et al., 2009). Secondly, bacteria may become resistant towards the nanobiocides. Polysulfone ultrafiltration membranes embedded with silver nanoparticles showed no protection against silver-resistant bacteria strains such as *P. aeruginosa* (Zodrow et al., 2009). The rapid depletion of nanobiocides can be addressed by improving fixation techniques by concentrating the nanobiocides closer to the surface of the membrane, through the use of cross-linkers, coupling agents or encapsulation in a polymer matrix to decrease the release rate (Zudrow et al., 2009). An alternative approach for antimicrobial nanofibers is to modify the polymeric surfaces as mentioned in the previous section to achieve permanent antimicrobial surfaces.

Conclusion

In this paper, the potential applications of antimicrobial nanofibers and nanoparticles for water purification are reviewed. The two most important requirements of water filtration membranes are total reduction of bacterial and viral numbers and prevention of biofouling. Other priorities include extended membrane life, less energy consumption, easy cleaning processes without the use of chemicals, no toxicity or production of disinfectant byproducts.

Electrospun nanofibers show great potential as antibacterial coating for fibrous media or as a self-supporting membrane. Nanofibers have a much higher surface-to-volume ratio than conventional melt-blown microfibers providing efficient separation of particulate.

Electrospinning also offer opportunities to fine tune the surface functionality through polymer chemistry. Although electrospinning is a very simple method, industrial scale fabrication of nanofibers are still in the beginning phase. The design of functional nanofibers for filter membranes requires specific and controlled parameters for reproducible and controlled electrospinning. These criteria remain a challenge in the engineering of a cost-effective large scale production system. Nanoparticles such as nAg, TiO_2, ZnO, and C_{60} have been studied for antimicrobial activities in numerous articles. The high availability, low cost and high antimicrobial activity of these nanoparticles make them attractive in the use of water purification. Different fixation methods of nanobiocides to nanofibers exist which prevent leaching from the membrane and therefore reducing potential toxicity and cost. Novel biocide membranes with no toxicity may also include the incorporation of chitosan nanoparticles and antimicrobial peptides. Nanofibers modified with plasma treatment or surface graft polymerization is an alternative approach for antifouling waterfiltration membranes. Permanent activity against waterborne pathogens is ensured without any health risk.

With rising water-borne diseases and shrinking water sources, scientists are under high stress to improve water filtration systems. Nanofibers and nanobiocides may be the solution to ensure safe and easy access drinking water.

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