Emerging Developments Regarding Nanocellulose-based Membrane Filtration Material Against Microbes

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Abstract

Wide availability and diversity of dangerous microbes poses a considerable problem for health professionals and in the development of new healthcare products. Numerous studies have been conducted to develop membrane filters that have antibacterial properties to solve this problem. Without proper protective filter equipment, healthcare providers, essential workers, and the general public are exposed to the risk of infection. A combination of nanotechnology and biosorption is expected to offer a new and greener approach to improve the usefulness of polysaccharides as an advanced membrane filtration material. Nanocellulose is among the emerging materials of this century and several studies had proven its usefulness in filtering microbes. Its high specific surface area enables the adsorption of various microbial species, and its innate porosity can separate various molecules and retain microbial objects. Besides that, the presence of an abundant OH groups in nanocellulose allows its surface modification which can increase its filtration efficiency through the formation of affinity interactions toward microbes. In this review, an update of the most relevant uses of nanocellulose as a new class of membrane filters against microbes is outlined. Key advancements in surface modifications of nanocellulose to enhance its rejection mechanism is also critically discussed. To the best of our knowledge, this is the first review focusing on the development of nanocellulose as a membrane filter against microbes.

1. Introduction

In the evolutionary process, among the significant issues faced by society today is the protection of natural resources and the implementation of eco-friendly approaches to sustaining a high quality of life. Environmental pollution is a worldwide concern and the majority of pollutants have long-term negative impacts on humans. Focusing on microbial pollution, the most common bulk transportation media for particulate contaminants is air and water. Microbes are microscopic living organisms that can be found everywhere including in water, soil and air but they are too small to be seen with the naked eye. These microbes are commonly viruses, bacteria, and fungi and may involve microscopic parasites. Certain microbes are harmful to our health, while others are beneficial. Table 1 shows several types of infectious diseases caused by microbes.
### Table 1
Several infectious diseases caused by microbes

| Infectious disease                  | Microbe that causes the disease                          | Type of microbe | Reference                        |
|------------------------------------|----------------------------------------------------------|-----------------|----------------------------------|
| Coronavirus (COVID-19)             | Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) | Virus           | (Norraham et al. 2021)           |
| Cold                              | Rhinovirus                                               | Virus           | (Atkinson et al. 2016)           |
| Chickenpox                        | Varicella zoster                                         | Virus           | (Lamont et al. 2011)             |
| German measles                    | Rubella                                                  | Virus           | (Leung et al. 2019)              |
| Whooping cough                     | Bordatella pertussis                                     | Bacteria        | (Hegerle and Guiso 2013)         |
| Bubonic plague                     | Yersinia pestis                                          | Bacteria        | (Spyrou et al. 2018)             |
| TB (Tuberculosis)                  | Mycobacterium tuberculosis                               | Bacteria        | (Gagneux 2018)                   |
| Malaria                            | Plasmodium falciparum                                    | Protozoa        | (Bhatt et al. 2015)              |
| Tinea barbae (dermatophyte infection) | Trichophyton rubrum                                      | Fungus          | (Furlan et al. 2017)            |
| Athletes’ foot                     | Trichophyton mentagrophytes                              | Fungus          | (Makola et al. 2018)             |

Microbial contamination in water can be dangerous to health, causing serious waterborne disease outbreaks such as gastroenteritis, cholera, giardiasis and cryptosporidiosis. The most common bacteria involved in these outbreaks are *Shigella dysenteriae*, *Vibrio cholera*, *Legionella* sp., *Escherichia coli*, and *Campylobacter jejuni* (Fahimidad et al. 2020). Whereas giardiasis and cryptosporidiosis are gastrointestinal diseases caused by microscopic parasites (protozoa), namely *Giardia duodenalis* and *Cryptosporidium* sp., respectively.

When invading the gastrointestinal tract, these microbes can cause local reactions to their presence and may even cause systemic effects from toxins they secrete (only certain microbes secrete toxins). Some microbes may invade into the bloodstream, where they can cause sepsis (Sussman 1997). Annually, it is estimated about 485,000 people die from diarrheal disease as a result of drinking contaminated water (WHO 2019). Hence, microbially contaminated wastewater must be treated before it is discharged into water bodies or water courses.

As mentioned previously, microbes can also be transmitted through the air. According to the World Health Organization (WHO), airborne transmission differs from droplet transmission as it refers to the presence of microbes within droplet nuclei that are typically less than 5 μm in diameter and can circulate in the air for significant periods and be transmitted to others over distances more than 1 meter (WHO 2020). Whereas droplet transmission occurs when a person is in close contact (within 1 m) with a symptomatic patient with
respiratory symptoms such as coughing or sneezing and is thus at risk of exposure to potentially infective respiratory droplets (typically > 5–10 µm in diameter). Nowadays, the threat of the newly discovered infectious coronavirus disease (COVID-19) is worrying, as this pandemic outbreak has already killed millions of people worldwide. The outbreak is exacerbated by the occurrence of frequent mutations, which makes it difficult to produce omnipotent vaccines rapidly (Norrrahim et al. 2021). Therefore, an effective, robust, and inexpensive air-borne virus removal membrane filter is an urgent need to provide a means to prevent virus spread in hospitals, transportation hubs, schools, and other venues with high social traffic turn-over in order to minimize the risks arising from the COVID-19 pandemic.

Microbe removal can be done through a variety of methods such as, filtration (either depth filtration or surface screening), partitioning and fractionation (centrifugation), and chromatography (ion-exchange, affinity, gel permeation) (Metreveli et al. 2014). Of these different techniques, filtration is a desirable choice as it is non-destructive and non-interfering, implying that it will not threaten the quality of biological samples or induce immune reactions. Membrane filters have been made from a variety of synthetic and semi-synthetic polymers designed to achieve a desired filtration pore size. The membrane filter is also an effective and widely used method for detecting microbiological pollution in collection samples. It requires less planning than certain other conventional methods and is one of the few methods that allows for microorganism separation and subsequent determination. Microbes cannot be retained by normal filter paper because the pores are too large in these papers. Therefore, it is critical to have a more effective material for microbe filtration, and there are studies that have led to the discovery of new filtering media made from cellulose with efficient filtration capability. The ultimate objective would be to be able to filter microbes from the environment effectively, securely, and at an affordable cost.

Current filter materials which are typically non-biodegradable and non-renewable have also received much attention among scientists. These filter membranes are primarily made from polymers which include proprietary non-ionic polymers, polytetrafluoroethylene (PTFE), polypropylene (PP), polysulphone (PS), polyvinylidene fluoride (PVDF) and polyethylene (PE) (Hai et al. 2014; Das et al. 2020). These non-biodegradable polymers when being disposed of after use are known to be harmful to the environment. Figure 1 shows an example of used surgical masks incorporating PP that have been discarded and not properly disposed of thereby causing problems of littering both on land and at sea as well as in waterways. Therefore, scientists urgently need to find better solutions to this problem. It is important to have a more efficient renewable and biodegradable material, of which nanocellulose is a prime candidate.

Cellulose is a versatile industrial product because of its abundance, renewability, biodegradability and ability to be readily modified chemically. With the development of nanocellulosic materials since 1977, there has been extensive research into their use in many fields including the that of membrane filtration. According to Hassan et al. (2020) two approaches that utilize nanocellulose for filtration have been explored namely the first approach which incorporated them into other polymer matrices to enhance the effectiveness of prepared membranes. This was done by dissolving the polymer in suitable solvents and ensuring that the nanocellulose materials were well dispersed within the polymer solution before being film cast. Meanwhile, the second approach was one first introduced by Ma et al. (2011) where they developed membranes from a nanocellulose layer with adequate porosity laid over polymeric supports without having to dissolve the
cellulose matrix and using the film casting method to produce porosity. The latter approach was found to be more desirable and intriguing.

Nanocellulose-based membrane filters have been found to be effective at removing microbes in previous research (Suman et al. 2014; Thakur and Voicu 2016; Hassan et al. 2017). When compared to synthetic polymers or plastic membranes, a significant benefit of the nanocellulose-based membrane filters is that they are entirely made from natural resources, making their disposal much easier as they were made up of predominantly biodegradable materials.

This review is intended to provide a comprehensive overview of the recent advances in the development of membrane filters for microbial removal which are made up either entirely from nanocellulose or utilising a modified approach incorporating nanocellulose. This review will include 1) a description of the types of membrane filters and the rejection mechanism they use; 2) details of the nanocellulose used in the production of membrane filters; and 3) a suite of antimicrobial technologies used for nanocellulose functionalization. This manuscript provides knowledge and direction for scientists to stimulate future research in this area.

2. Types And Rejection Mechanisms Of Membrane Filters

The role of membranes alone in the removal of pathogens is discussed here. Membrane filters can be categorized by the size of the pollutant they are able to reject (see Fig. 2), namely: reverse osmosis (0.1–1 nm), nanofiltration (NF) (1–2 nm), ultrafiltration (UF) (2–100 nm) and microfiltration (MF) (100 nm–10 µm). The two most important features of a membrane are its permeability and selectivity. To enhance the productivity of membrane separation processes, it is always necessary to develop membranes with high permeability and high selectivity (WHO 2020).

Referring to Fig. 3, it can be seen that there are various pore sizes of nanocellulose-based membrane filters that are available depending on their origin. Generally, nanocellulose can be classified into three types, which are cellulose nanofiber (CNF), cellulose nanocrystals (CNC), and bacterial nanocellulose (BNC). The different pore sizes of nanocellulose membrane filters fulfil various filtration modes. Thus, nanocellulose membrane filters obtained from electrospun CNF usually have a pore size of more than 100 nm (Fig. 3a). Thus, making this membrane filter more suitable as a MF. Meanwhile, CNF (Fig. 3b) and CNC (Fig. 3c and d) which are obtained from other methods usually have a smaller pore size which ranges from 5 nm to 100 nm. Thus, making them suitable for use in NF and UF. The pore size of nanocellulose depends on several factors, such as the cellulose origin, the isolation process concentration and processing conditions as well as pretreatments done to the cellulose.

Figure 2 illustrates the membrane filtration spectrum that operates by utilising the size exclusion method in rejecting or inhibiting the pathogenic microorganisms. Wu et al. (2019) described it as an established, reliable, and robust method considering its ability to physically remove various types of infective microorganisms, including virus. Of note, other method that utilized affinity principle could also be used for filtration.
There are two types of membrane filters with different pore sizes commonly used in the retention of microorganisms. The first one is the MF membrane which has pore size of 0.1–10 µm while the other is the UF membrane that has smaller pore size ranging from 5 to 100 nm. Both types of membrane filters are applicable for the removal of protozoa and algae (i.e. size range between 3 to 14 µm) (Ottoson et al. 2006). In addition, Francy et al. (2012) outlined that tertiary disinfection is not necessary as the pore size of both MF and UF membranes are too nominal when compared to the size of coliform bacteria, suggesting total removal of bacteria by size exclusion of the membranes. However, a particular concern was raised regarding the removal of virus via direct membrane filtration considering its smaller size compared to bacteria.

On the other hand, membrane filters that utilise the affinity principle remove pollutants based on the electrostatic interaction between functional groups of the membranes with the pollutant. This type of membrane filter includes composite or hybrid filter structures that consist of porous substrate with either nanocellulose moieties attached to their surface or impregnated within. It is interesting to note that size exclusion and affinity regime approaches have been explored in many researches on membrane filters utilising the nanocellulose.

3. Attributes Of Nanocellulose Membrane Filtration Of Microbes

Nanocellulose with pore dimensions measuring between 1 and 100 nm offers certain unique characteristics which includes high strength, chemical inertness, hydrophilic surface chemistry and high surface area thereby make it a promising material for use as a high-performance membrane filter that can effectively remove microbes from either air or liquids (Halib et al. 2017; Voisin et al. 2017; Ariffin et al. 2018; Norrahim et al. 2018b, a, 2019; Bacakova et al. 2019; Fareez et al. 2020; Yasim-Anuar et al. 2020). Table 2 summarizes the importance of several special properties of nanocellulose which are related to the application as a membrane filtration material against microbes.
Table 2
Certainly interesting properties of nanocellulose related to membrane filtration materials

| Property               | Advantages                                                                 | Reference                               |
|------------------------|-----------------------------------------------------------------------------|-----------------------------------------|
| Nanoporosity           | Good virus filtration using size-exclusion method. Typically, the pore size   | (Metreveli et al. 2014)                 |
|                        | of nanocellulose is below 100 nm.                                            |                                         |
| Surface functionalization | Functionalization nanocellulose with several compounds to make it cationiccharged causes an increase in its binding affinity towards viruses. | (Sunasee and Hemraz 2018)               |
| High specific surface area | Provides a large surface area for functionalization. Thereby increasing interaction efficiency. | (Lin and Dufresne 2012)                 |
| Renewable              | Nanocellulose can be easily sourced from plant bio-waste. Its use can eliminate the use of other non-renewable polymers as mentioned in the Introduction section. | (Voisin et al. 2017; Norrrahim et al. 2018b) |
| Biodegradability       | An important aspect to save the environment. It is biodegradable in landfills. Hence, current environment issues from used and discarded surgical masks can be reduced or even eliminated. | (Mahfoudhi and Boufi 2017)              |
| High mechanical strength | High strength membrane filters can be fabricated using it.                   | (Rusli and Eichhorn 2008)              |
| Stability in water      | It can reduce bio-fouling of membrane filters. This is important for application in membrane filters for wastewater. | (Nguyen et al. 2012)                   |

As described earlier, nanocellulose can be classified into three types (CNF, CNC, and BNC) according to their manner of origin. These three types are as shown in Fig. 4 and Table 3 below. Essentially cellulose is a molecule that consists of β-1, 4-glucose, and three active hydroxyls at the C2, C3, and C6 sites of the glucopyranose ring, and its configuration provides sufficient sites for several surface functionalizations. These sites may undergo oxidation, esterification, and etherification to enable these variable functional groups that may include aldehyde groups and quaternary ammonium. The details of these surface functionalizations will be explained in the following section.

Each type of nanocellulose has different dimensions and properties, mainly due to their different methods of preparation/fabrication (Khalil et al. 2014). There are various methods used to extract cellulose nanoparticles which then result in these particles having different crystallinities, surface chemistries and mechanical properties (Abitbol et al. 2016). These production methods range from top-down enzymatic/chemical/physical methods aimed at isolating them from wood and forest or agricultural residues to bottom-up production of cellulose nanofibrils from glucose by bacterial action (Klemm et al. 2011).
Table 3  
Types of nanocellulose according to their sources, treatments and dimensions (Ahmad et al. 2018; Dai et al. 2019; Islam and Rahman 2019; Abol-fotouh et al. 2020)

| Nanocellulose                       | Abbreviation | Sources        | Main treatments               | Dimensions                        |
|-------------------------------------|--------------|----------------|------------------------------|-----------------------------------|
| Cellulose nanofiber                 | CNF          | Plants         | Mechanical fibrillation       | Diameter: 5–50 nm                 |
|                                     |              |                |                              | Length: Several µm                |
| Cellulose nanocrystals nanowhiskers/ nanorods | CNC         | Plants         | Acid hydrolysis              | Diameter: 2–20 nm                 |
|                                     |              |                |                              | Length: 100 nm to several µm      |
| Bacterial nanocellulose/ biocellulose | BNC         | Microorganisms | Polymerization and crystallization | Diameter: 2–4 nm                |
|                                     |              |                |                              | Length: 100µm                     |

4. Modifications On Nanocellulose To Improve Filter Efficiency

The surface functionalization of nanocellulose is a key step to promote an increase in the efficiency of a membrane filter. This is an important step when the membrane filter operates using the affinity regime. This can be done using different strategies of surface functionalization that will involve the chemistry of hydroxyl function (Islam et al. 2013). Referring to Fig. 5, a search using keywords ‘functionalization of nanocellulose’ was done by lens.org, (https://www.lens.org/), a problem solving non-profit social enterprise website utilising linked open knowledge artefacts and metadata. It was found that, manuscripts focusing on the functionalization of nanocellulose have been increasing from year 2011 until now. It showed that this area of research interest has gained much interest among scientists in this decade.

Modification on nanocellulose can be made through several processes such as oxidation, esterification, and etherification, which eventually results in the introduction of new functional groups on the material. Other than that, previous studies had showed that nanocellulose can also be subjected to modification by adding compounds likes aldehyde, quaternary compounds (both cationic and anionic forms), activated carbon, citric acid, antibiotics, and nanomaterials. For instance, the aldehyde groups are grafted onto nanocellulose through oxidation process using oxidants like periodate sodium and 2,2,6,6-teramethylpiperidinyloxy (TEMPO) which results in the TEMPO positioning on the surface of nanocellulose under aqueous condition while the hydroxyl group located at C6 position of nanocellulose can be converted to carboxyl and aldehyde functional groups.

Besides that, low toxicity and environmentally friendly quaternary compounds likes poly(N,N-dimethylaminoethyl methacrylate), amines, anionic polyelectrolytes, and polyglutamic acid could be used to quaternize nanocellulose to improve its efficiency as membrane filters as these quaternary compounds have
ability to form electrostatic affinity towards microbes. This quaternization process could be done using grinding and high-pressure homogenization process. Figure 6 illustrates several examples of functionalized nanocellulose using quaternary compounds for virus's removal applications. Most virus and certain microbial species have polar charge on their surface, thus modification on the surface charge of the nanocellulose would improve electrostatic interaction properties of the material, which consequently results in high efficiency filtration. In the following section, several interesting findings concerning quaternized nanocellulose for microbial removal will be discussed.

5. Recent Developments On Nanocellulose As A Filtration Material Against Microbes

In this section, several developments concerning nanocellulose based membrane filters capable of removing microbes will be reviewed. An important aspect of the modification of nanocellulose materials is to increase the binding affinity of the materials towards microbes. There are a number of studies that focused on filtration removal of viruses and bacteria; however, very limited studies have been done concerning other types of microbes such as fungi, algae and protozoa.

Ensnarement of viruses is one of the most crucial steps in biopharmaceutical and clinical processes and applications (Lebrun 2017). Of the various types of microbes, virus is among the smallest and most difficult to deal with as compared to other microbes.

Exploration of nanocellulose as a filtration material against several types of viruses has received much research attention. As mentioned earlier, several viruses including COVID-19 are airborne viruses that can be dispersed and spread through human nasal or saliva secretions from an infected person. Therefore, in order to minimize infection risks from viruses, an efficient, robust and affordable air-borne virus removal filter is an urgent requirement. Multiple research articles were published recently with regards to this type of air filter.

Several factors such as filters pore size, thickness, number of layers, size of the virus, the charge on the filter surface, its ionic strength and surface chemistry are usually influence the efficiency of air filtration process (Metreveli et al. 2014). Generally, the use of size-exclusion type filtration has several benefits such as flexibility and ease of use since it provides virus removal predictability through its physical properties, allows for the filtration of viral markers enabling easy validation of the filtration process and does not use toxic or mutagenic chemicals for viral inactivation (Burnouf and Radosevich 2003; Metreveli et al. 2014; Asper et al. 2015b).

Gustafsson et al. (2018) evaluated filter papers made from nanocellulose in a mille-feuille arrangement of varying thicknesses using a simulated wastewater matrix to explore its ability to remove viruses for drinking water purification applications. The filtrations of various samples of simulated wastewater with its total suspended solid content being 30 nm latex particles as surrogate waste material and 28 nm ΦX174 bacteriophages as the viral contamination. The authors examined the performance of these filter papers at a pressure of 1 and 3 bar with varying thicknesses of 9 and 29 µm. The data they obtained found that filter paper made from 100% nanocellulose has the capacity to efficiently remove even the smallest of viruses, with up to 99.9980–99.9995% efficiency.
Manukyan et al. (2019) fabricated nanocellulose-based mille-feuille type filter paper for use in upstream applications for serum-free growth media filtration and it was designed to remove ΦX174 bacteriophages. The filter performance was evaluated based on its ability to filter small-medium sized virus using varying thicknesses of the fabricated filter paper (i.e. 11 and 33 µm), as well as by varying the operating pressures, (i.e. 1 and 3 bar). Based on their results, the 33 µm thick filter showed more stability and had better virus removal as compared to the 11 µm thick filter although their flux was nominally lower. The findings of this study suggest that nanocellulose filter paper would be a viable alternative for the filtration of large volumes of cell culture media in upstream bioprocessing.

Asper et al. (2015) in their study used filter paper composed of 100% CNF to remove xenotropic murine leukaemia virus (xMuLV). The results of this filtration of xMuLV suggested that the nanocellulose filter paper was useful for removal of endogenous rodent retroviruses and retrovirus-like particles during the production of recombinant proteins.

Metreveli et al. (2014) reported that the nanocellulose filter was able to remove Swine Influenza A Virus (SIV) which had a particle size of 80–120 nm and it is shown in Fig. 7. The latex beads and SIV particle are observed as stacked structures on the surface of the porous filter paper membrane.

Previous researches on surface modification of nanocellulose have led to the improvement of filtration efficiency against viruses. Electrostatic interaction between nanocellulose and viruses is improved dramatically with the incorporation of quaternary compounds as discussed in Sect. 4 above. For instance, viruses like coronavirus have negatively charged surface and would interact with the cationic or anionic charge of the nanocellulose-quaternary compounds (Leung and Sun 2020). Figure 8 shows a schematic diagram of coronavirus structure with proteins embedded in its bilayer membrane and negatively-charged lipid head groups protruding to the outer side of membrane.

The entrapment of the virus onto nanocellulose matrix is due to the presence of electrostatic force attraction between the negatively charged virus particle and the positively charged nanocellulose membrane. Several studies have shown successful results in filtering negatively charged viruses using cationic nanocellulose. For example, Mi et al. (2020) developed filtration setup using modified CNC with a positively charged guanidine group to adsorb porcine parvovirus and Sindbis virus and completely filter out those viruses from water. It is interesting to point out that this filtration system has exceeded the Environment Protection Agency (EPA) virus removal standard requirement for portable water. In addition to the electrostatic interaction between the virus and guanidine group, Meingast and Heldt (2020) outlined that the complete virus removal from water was also due to the protonated guanidine groups on the cationic CNC forming ionic and hydrogen bonds with the proteins and lipids on the virus surface.

Other than that, Rosilo et al. (2014) in their study observed a very high affinity binding between the cationic CNC (known as CNC-g-P(QDMAEMA)s) mixture and cowpea chlorotic mottle virus (CCMV) and norovirus-like particles in water dispersions. Of note, this cationic CNC mixture was prepared by surface-initiated atom-transfer radical polymerization of poly(N,N-dimethylamino ethyl methacrylate) and its subsequent quaternization of the polymer pendant amino groups.
In addition, the anionic CCMVs could also be removed using functionalized lignin with a quaternary amine. In their study, they found that the CCMVs would form agglomerated complexes with cationic lignin (Rivièr et al. 2020). Therefore, suggesting its potential use as material in the development of membrane filter for the removal of CCMVs.

Besides that, Sun et al. (2020) reported that covalent modification on CNF (i.e. functionalization of nanocellulose) using polyglutamic acid (PGS) and mesoporous silica nanoparticles (MSNs) resulted in successful filtration of EV71 virus and Sindbis virus. This is particularly due to the interaction between two exposed positively charged amino acids (His10 and Lys14) and the negatively charged MSNs on the modified CNF (Sun et al. 2020).

In other related study, nanocellulose was functionalized using citric acid in the fabrication of nanofiltration based filter paper production for virus removal applications (Quellmalz and Mihranyan 2015). The addition of environmental-friendly and non-toxic citric acid in the fabrication is to improve the wet strength properties of paper filter as it acts as a cross-linking agent for the nanocellulose. The citric acid cross-linking of nanocellulose was proved to be beneficial in developing paper-based sterile (virus removal) industrial filters since it managed to efficiently remove 20 nm Au nanoparticles from a feed solution as reported in the study (Quellmalz and Mihranyan 2015).

Table 4 summarizes the development of nanocellulose-based membrane filtration material for virus removal that have been discovered/explored so far. In addition to the guanidine groups, lignin, nanoparticles, and citric acid, nanocellulose could also be functionalized with several other compounds such as small organic molecules, porphyrin dendrimers and others polymers in order to make it positively or negatively charged (Sunasee and Hemraz 2018). However, it is important to note that not all of these examples have been tested as a filter to remove viruses.
### Table 4
Nanocellulose developed filtration material for virus removal

| Microbes                        | Type of nanocellulose | Functionalization | Findings                                                                                                                                                                                                 | Reference                  |
|---------------------------------|-----------------------|-------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| A/swine/Sweden/9706/2010 (H1N2) - Swine influenza | BNC                   | Not applicable    | • The newly developed non-woven, µm-thick filter paper consisting of crystalline BNC able to remove virus particles solely based on the size-exclusion principle, with a log 10 reduction value ≥ 6.3, thereby matching the performance of industrial synthetic polymer virus removal filters currently in use. | (Met reveli et al. 2014)    |
| Xenotropic murine               | BNC                   | Not applicable    | • The developed BNC filter paper could remove the endogenous rodent retroviruses and retrovirus-like particles.                                                                                           | (Asper et al. 2015a)        |
| MS2 viruses                     | BNC                   | Not applicable    | • This study highlights the efficiency of the nanocellulose-based filter paper in removing/filtering out the ΦX174 bacteriophage with value of 5 – 6 log virus clearance (28 nm; pI 6.6). | (Wu et al. 2019)            |
| Microbes        | Type of nanocellulose | Functionalization | Findings                                                                                                                                                                                                 | Reference            |
|-----------------|-----------------------|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| ColiphagesΦX174 | BNC                   | Not applicable    | • The nanocellulose-based filter paper exhibited 5 – 6 log virus clearance of MS2 viruses (27 nm; pI 3.9). This study also showed the possibility of producing cost-efficient viral removal filters (i.e. manufacturing process). | (Wu et al. 2019)    |
| Parvoviruses    | BNC                   | Not applicable    | • The developed filter was the first non-woven, wet-laid filter paper composed of 100% native cellulose. This study showed that the non-enveloped parvoviruses could be eliminated using this filter. | (Gustafsson et al. 2016) |
| EV71            | CNF                   | Polyglutamic acid and mesoporous silica nanoparticles | • This study showed that the modified microfibers could strongly adsorb the epitope of the EV71 capsid which is useful for virus removal                                                                 | (Sun et al. 2020)    |
| Sindbis virus   | CNC                   | Guanidine         | • Functionalization of guanidine on CNC resulted in over 4 log removal value against the Sindbis virus.                                                                                                | (Mi et al. 2020)     |
| Microbes                | Type of nanocellulose | Functionalization | Findings                                                                 | Reference                |
|------------------------|------------------------|-------------------|---------------------------------------------------------------------------|--------------------------|
| Porcine parvo virus    | CNC                    | Guanidine         | • Authors also revealed that functionalization of guanidine on CNC managed to remove the Porcine parvo virus with over 4 log removal value. | (Mi et al. 2020)         |

### 5.2 Bacteria

The development of nanocellulose as filtration material against bacteria also been widely discovered. Generally the diameter of most waterborne bacteria is larger than 0.2 µm (Ma et al. 2011). Thereby, it would be easy for nanocellulose-based membrane filters to entrap most bacteria species using the size-exclusion mechanism. Moreover, as discussed in Sect. 4 earlier, modification of nanocellulose by surface functionalization can also be done to increase the removal efficiency of bacteria. In this review, we highlight several findings concerning bacterial removal using nanocellulose based membrane filters.

Wang et al. (2013) demonstrated that a multi-layered nanobrous microfiltration system with high flux, low-pressure drops and high retention capability against bacteria (*Brevundimonas diminuta* and *Escherichia coli*) was possible by impregnating ultrafine CNF into an electrospun polyacrylonitrile (PAN) nanofibrous scaffold supported by a poly (ethylene terephthalate) (PET) non-woven substrate. The CNF was functionalized prior to impregnation with carboxylate and aldehyde groups using TEMPO oxidation. It was observed that this CNF-based microfiltration membrane exhibited full retention capability against those bacteria.

Otoni et al. (2019) developed a cationic CNF compound using Girard’s reagent T (GRT) and shaped it into foam using several protocols such as cryo-templating to remove the ubiquitous human pathogen *Escherichia coli*. The porosity of this foam, which is associated directly with its surface area and pore size plays a significant role in the removal of *Escherichia coli*. The cryogel foams produced by this method had porosities of *circa* 98% and were established to be able to achieve an approximately 85% higher anti *Escherichia coli* activity when compared to sample foams made up of unmodified CNF. The cationic CNF using GRT demonstrated good potential for both air and liquid filtration, with excellent absorbency through functional coating. Access to safe drinking water in high- and low-income countries has become one of the biggest challenges in the world as natural resources become scarcer.

Gouda et al. (2014) invented a modified electrospun CNF containing silver nanoparticles (AgNPs) as a water disinfecting system for water purification systems. The AgNP content, its physical characterization, surface morphology and antimicrobial efficacy of the developed membrane filter was then studied. AgNP, which belongs to the group of biocidal nanoparticles, has antimicrobial properties and is commonly used due to its
size quantization effect. This can cause a shift in the reactivity of metals in the nanoscale. The developed membrane filter had excellent ability to remove bacteria including *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* with a percentage filtration of more than 91% in contaminated water samples.

Ottenhall et al. (2018) developed a CNF-based membrane filter modified with polyelectrolyte multilayers to produce multilayer cationic polyvinyl amine (PVAm) and anionic polyacrylic acid (PAA). The authors had successfully modified the CNF with cationic polyelectrolyte PVAm together with the anionic polyelectrolyte PAA in either single layers or multilayers (3 or 5 layers) using a water-based process at room temperature. Based on filtration analysis, the functionalized CNF-based membrane filters with several layers was physically able to remove more than 99.9% of *Escherichia coli* from water. The 3-layer membrane filter could remove more than 97% of cultivatable bacteria from natural water samples, which was a remarkable performance as compared with simple processing technique using plain nanocellulose filters.

Table 5 summarizes the effectiveness of nanocellulose-based membrane filters that have been functionalized with bioactive compounds for the removal of bacteria.
### Table 5
Nanocellulose developed filtration material for bacterial removal

| Microbes                                               | Type of nanocellulose | Functionalization      | Findings                                                                                                                                                                                                 | Reference                          |
|--------------------------------------------------------|------------------------|------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| *Escherichia coli*                                     | CNC                    | Silver nanoparticles   | • It possesses high adsorption capacity and is reusable. Beneficial in total removal of *Escherichia coli*.                                                                                              | (Suman et al. 2014)                |
| *Bacillus subtilis* and *Escherichia coli*             | CNF                    | ZnO and CeO₂           | • It has high anti-bacterial activity, \( \text{MIC}_{50} \) against *Bacillus subtilis* (10.6 \( \mu \)g ml\(^{-1}\)) and *Escherichia coli* (10.3 \( \mu \)g ml\(^{-1}\)). | (Nath et al. 2016)                 |
| *Escherichia coli*                                     | BNC                    | Not applicable         | • The significance of Brownian motion caused by microorganisms captured with BNC-based filter paper through theoretical modelling and filtration experiments was investigated by the authors. • It was found that the BNC-based filter was capable of filtering the bacteria. | (Gustafsson et al. 2018a)           |
| *Escherichia coli*, *Staphylococcus aureus*            | CNF                    | Activated carbon       | • The two-layer AC/OCNF/CNF membrane able to remove *Escherichia coli* bacteria up to ~ 96–99% and inhibits the growth of *Escherichia coli* and *Staphylococcus aureus* on the upper CNF surface | (Hassan et al. 2017)               |
| *Escherichia coli*                                     | BNC                    | Silver nanoparticle    | • Higher amount of silver nanoparticles loaded onto the BNC membrane surface could increase the inhibition zone hence highlighting its good antimicrobial property against *Escherichia coli*. | (Zelal et al. 2018)                |
| *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* | BNC                    | Silver nanoparticle    | • BNC-silver nanoparticle membrane showed strong antimicrobial activity against Gram positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. | (Barud et al. 2011)                |
| Microbes                  | Type of nanocellulose | Functionalization       | Findings                                                                                                                                                                                                                       | Reference |
|--------------------------|-----------------------|-------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| *Escherichia coli*, *Staphylococcus aureus* | BNC                   | Silver nanoparticle     | • The developed Ag/BNC membrane exhibited good property as antimicrobial agent against *Escherichia coli* and *Staphylococcus aureus* as the antibacterial ratio against *Escherichia coli* and *Staphylococcus aureus* reached 99.4% and 98.4%, respectively. | (Zhang et al. 2013) |
| *Escherichia coli*       | CNF                   | polyethersulfone (PES) membranes | • TEMPO oxidized-CNF coating is effective against *Escherichia coli*. The effectiveness was attributed to the pH reduction effect induced by carboxyl groups | (Aguilar-Sanchez et al. 2020) |

### 5.3 Other Types Of Microbes

Nanocellulose would also be able to act as a removal agent for other types of microbes which are larger in size than bacteria, such as fungi, algae and protozoa. However, it is noteworthy that there is still a lack of studies regarding this matter. To the best of our knowledge, there are no available reports on the development of a nanocellulose-based membrane filter for the removal of fungi.

Algae is also a major contributor to microbial contamination in water resources and their presence could change the taste or odour of water. For example, blue-green algae and coloured flagellates (especially the *Chrysophyta* and *Euglenophyta* genuses of algae) are the best-known algae that cause contamination in water resources. Furthermore, green algae may also be a significant contamination factor as well (Sen et al. 2013). Hence, the potential of nanocellulose should be explored by scientists to define their role as a membrane filtration material suitable for removing algae and protozoa from the contaminated water efficiently. Algae and protozoa are known to have a larger size than viruses and bacteria, thus the removal of these microbes could be effectively carried out using the size-exclusion mechanism.

However, similar to viruses and bacteria, the nanocellulose needs to be modified with other compounds such as metal nanoparticles, enzymes and proteins in order to increase its filtration efficiency (Gopakumar et al. 2018). Studies have shown that different charges between the cellular membrane of algae and protozoa do play a dominant role in the adsorption/retention of these microbes on a filtration membrane's surface (i.e. through the electrostatic interaction principle) (Kim et al. 2017; Ottenhall et al. 2018).

Previous study carried out by Ge et al. (2016) discovered the sustainability and the most efficient approach in harvesting algae using a modified CNC. The modification was made by introducing a 1-(3-aminopropyl)-imidazole (APIm) structure as a reversible coagulant. As shown in Fig. 9, coagulation process occurs when the positively charged CNC-APIm interacts with the negatively charged *Chlorella vulgaris* in the presence of carbon dioxide (carbonated water). Their findings are in agreement with the works done by Qiu et al. (2019)
as harvesting efficiency could reach up to 85% with only 0.2g CNC-APIm mass ratio, 5secs of CO₂ sparging time, and a 50 ml/min flow rate. This signifies that the CNC-APIm complex could be an alternative to current conventional coagulants for harvesting algae in industrial applications.

Algae harvesting is important for biodiesel industry and many researches have been carried out to increase its sustainability in a global scale. For example, the capability of CNF and CNC in harvesting algae were investigated by Yu et al. (2016). In their study, they discovered that the CNF did not require any surface modification to harvest the algae as it played a role as an algae flocculant via its network geometry, something that the CNC (even cationic modified CNC) could not do. Flocculation of algae did not happen when CNC is used as the freely moving algae cannot be entrapped by nanoparticles structure formation of CNC. However, this study only focuses on the flocculation capability of CNF and CNC which could intrigue a further study on the filtration efficiency of both materials for algae harvesting. This finding indirectly could point to the development of a nanocellulose-based membrane filter for algae removal in the future.

6. Challenges And Future Recommendations

This review has shown several undoubtedly interesting features of nanocellulose which is useful in filtering viruses, bacteria, fungi, algae, and protozoa through the mechanism of membrane filtration. The properties and characteristics of nanocellulose as a filtration material is very promising and is an exciting area for current and future research. Several recent developments in the application of nanocellulose as a membrane filtration material were discussed here. It is interesting to note that the functionalization of nanocellulose with a variety of functional groups is among the important key factors of success to enhance the removal of microbes from air and water.

Even though different works on the nanocellulose as membrane filter material has shown several effective findings, improvements on this area are still needed. There are several other types of microbial species which have not been explored. The use of nanocellulose-based membrane filters as a means of the removal of COVID-19 virus has also not been explored. Moreover, research on the use of nanocellulose-based membrane filter material to remove fungus, protozoa and algae is still very limited. Therefore, more works concerning those microbes remains an urgent need.

The functionalization of nanocellulose is a very important step to obtaining improved performance of membrane filtration material. In this review, several compounds have been shown to be capable of being incorporated with nanocellulose. However, their side effects towards the environment as a result of this functionalization of nanocellulose is also an important consideration. For example, the functionalization of nanocellulose with TEMPO can cause harmful effects to the environment. This is because the synthesis of TEMPO can generate chemical by-products which are toxic to aquatic life when released as waste effluent into the environment (Patankar and Renneckar 2017). In the future, research should also be focused on this concern to determine the actual feasibility and sustainability of these developed functionalized nanocellulose products towards the environment.

In addition, further research on generating hybrid nano-structures on the surface of nanocellulose to enable interaction with multiple species of microbes is urgently needed. Thus, paving way towards the development
of new materials capable of eliminating various kind of microbes at one time

This review has identified several difficulties concerning the use of nanocellulose as a membrane filter material. One of them is particularly related to the high production cost especially in large industrial scale. Furthermore, high energy consumption during the production of nanocellulose is still a concern and cries for a more reliable and reproducible production technique to pave way to using nanocellulose as a commercially viable membrane filter material. To the best of our knowledge, there are much progress in research studies focussing on reducing the energy consumption and production cost of nanocellulose and have been attempted in numerous pilot-scale productions worldwide. Other than that, issue regarding the biodegradability of nanocellulose as adsorbent need to be evaluated by considering several factors likes the types of water and presence of certain microbes that may cause cellulose degradation.

All in all, nanocellulose is a good alternative for a membrane filter material and is expected to be fully utilized in numerous industries in the near future considering the solutions for the outlined challenges and difficulties have been met.

7. Declarations

Conflict of interest

The authors declare no conflict of interest in the preparation of this review.

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Figures

Figure 1

Used surgical masks that were not properly disposed

Figure 2

Comparison of the size and type of contaminants rejected by membrane filtration techniques.
Figure 3

The morphology of nanocellulose described in various studies. This figure is adapted with permission from the ref. (Kumar et al. 2014; Norrrahim et al. 2018b, 2019; Zhang et al. 2019)
Figure 4

The preparation and application of nanocellulose-based antimicrobial materials

Figure 5
A chart of published manuscripts focusing on the functionalization of nanocellulose from Lens.org

Figure 6

Functionalized nanocellulose with quaternary compounds for virus's removal applications
Figure 7

SEM images of SIV filtration on a nanocellulose membrane

Figure 8

A structure design of coronavirus particle. Reproduced with permission from ref. (J Alsaadi and Jones 2019)
Figure 9

Illustration on the electrostatic attraction between Chlorella vulgaris and APIm-modified CNC. This figure was adapted from ref. (Ge et al. 2016)