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Synthesis of cationized nanofibrillated cellulose and its antimicrobial properties

Kuisma Littunen [a], João Snoei de Castro [a], Anatoliy Samoylenko [b], Qi Xu [b], Susan Quaggin [b,c], Seppo Vainio [b], Jukka Seppälä [a,*]

[a] Department of Biotechnology and Chemical Technology, Aalto University School of Chemical Technology, P.O. Box 16100, 00076 Aalto, Finland
[b] Centre of Excellence of Cell-Extracellular Matrix Research, Biocenter & Infotech Oulu, Laboratory of Developmental Biology, Faculty of Biochemistry and Molecular Medicine, P.O. Box 5000, 90014 University of Oulu, Finland
[c] Feinberg Cardiovascular Research Institute, Division of Medicine-Nephrology, Northwestern University Feinberg School of Medicine, 303 East Chicago Avenue, Tarry 14-725, Chicago, IL 60611, USA

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ABSTRACT

Two types of cationized nanofibrillated cellulose (NFC) were prepared by redox initiated graft copolymerization and etherification with quaternary ammonium compounds (QAC). The QAC content and charge density of the products were measured. The NFC derivatives were tested for antimicrobial activity against Gram positive and negative bacteria, and yeast. Both NFC types exhibited broad spectrum antimicrobial activity. Etherification resulted in a higher degree of substitution and charge density, and the product also showed higher antimicrobial activity than the copolymerization product. Etherified NFC was more efficient against Gram negative than positive bacteria, whereas the polymer grafted NFC was equally active against both. This was attributed to the ability of the polymeric grafts to penetrate the thick cell wall of Gram positive bacteria, followed by the destabilization of the cellular membrane. Neither cationized NFC type showed cytotoxicity against human cells, providing means to manufacture safe, insoluble, and permanently antimicrobial materials via aqueous synthesis.

1. Introduction

Controlling microbe growth is often essential for the modern lifestyle, since man-made materials usually lack defenses against microbes. Prolific microbe growth creates a biofilm which protects the embedded microbes, making them more resistant to disinfection. These biofilms often excrete chemicals that are toxic or degrading to the underlying material. They also make the gene exchange between microbe cells more efficient, favoring the formation of bacterial strains with resistance to antibiotics [1,2]. For the purpose of microbial growth control, there are generally two methods. Disinfecting is used to sterilize an environment, but once exposed to a non-sterile environment it is soon contaminated by new microbes. The other option is to use materials that are inherently antimicrobial, eliminating the need for disinfection [3]. An example of

Abbreviations: CNFC, pre-cationized NFC; EPTMAC, epoxypropyl trimethylammonium chloride; PDMQ, poly([2-(methacryloyloxy)ethyl]trimethylammonium chloride); QAC, quaternary ammonium compound.

* Corresponding author.
E-mail address: jukka.seppala@aalto.fi (J. Seppälä).

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such material is silver that has been well-known for its antibacterial properties since antiquity. However, the antibacterial effect requires silver ions to dissolve into the medium [4]. The use of silver in food contact materials is severely limited by strict regulations that restrict the maximum allowed silver concentration below its biocidal level [4,5].

Quaternary ammonium compounds (QAC) are also known for their antibacterial and antifungal properties [6], offering an alternative to common disinfectants and silver. Polymers containing cationic ammonium groups have been found particularly effective as wide-spectrum biocides [7]. Moreover, microbes have not developed notable resistance to these compounds despite their wide use for over 40 years, nor have they shown improved resistance even after several generations of laboratory cultures [8]. Despite their strong bactericidal properties, cationic polymers have low human toxicity, since bacterial cells differ considerably from human cells in their structure and chemistry [9]. Moreover, biocidal polymers that are deactivated over a period of time due to cleavage of labile satellite groups by common enzymes have been developed to address concerns about possible long term effects of large scale use [10].

The antibacterial effect of cationic compounds is attributed to their ability to interact with the negatively charged phospholipids that are present in bacterial cell membranes, eventually disrupting the membrane. The interaction with mammalian cells is much weaker because their cell membrane is close to neutral charge [11]. The membrane disrupting ability has been explained by several models, including the "phospholipid sponge" effect where anionic phospholipids are gradually pulled away from the bacterial cell membrane by a nearby cationic surface. When a critical amount of phospholipids are removed, the membrane decomposes [12]. Another suggested mechanism involves the adsorption of a cationic polymer onto the cellular membrane. The adsorbed polymer binds to the anionic phospholipids scattered throughout the membrane, pulling them together into large anionic patches that destabilize the membrane [7,13]. It has also been proposed that the release of the membrane-stabilizing divalent cations upon polyelectrolyte adsorption is sufficient to disrupt normal membrane functionality [14,15]. A recent study of cationic polyionenes (polymers having cationic groups as part of their backbone) proposed that this class of polymers has antimicrobial mechanism not involving cellular membrane disruption and therefore also lower hemotoxicity [16].

Antimicrobial effect has been observed with modified natural polysaccharides, including N-alkylated chitosan [17] and cationized starch [18]. However, their use in packaging materials is limited by their solubility in water, which will likely cause them to migrate out of the material in humid conditions. This results in the loss of antimicrobial activity and also contamination of the product. An additional drawback with starch is its digestibility that facilitates the release of any grafted molecules inside the digestive tract. Cellulose on the other hand is also a readily available polysaccharide but unlike chitosan or starch it is insoluble in water [19] and, to a great extent, indigestible by humans [20]. Therefore, it is plausible that cellulose with covalently attached antimicrobial grafts does not dissolve or lose its activity when used as coating or packaging material, and also does not release the active compound when added directly to foodstuffs.

Nanofibrillated cellulose (NFC) is a plant-based nanomaterial, which is prepared by disintegrating native plant fibers into nanosized fibrils via mechanical homogenization. The fibrillation is accomplished by pumping a dilute pulp dispersion through a high pressure homogenizer where the fibrils are separated by strong shear forces. These nanofibers are targeted for several applications like transparent nanocomposites [21], novel packaging materials [22], and rheology modification in foodstuffs and cosmetics [23]. One of the drawbacks of NFC derives from its bio-based origin. Although mechanically strong, NFC is readily attacked by, for instance, cellulose consuming fungi. It also offers a large surface area for bacterial growth. Incorporating the antimicrobial properties of cationic compounds into the large specific surface area of nanofibrils could yield safe and efficient antimicrobial materials for food packaging or medical applications, for instance. The cationic NFC suspension also has a potential double functionality, being both an antimicrobial preservative/disinfectant, and, due to its high viscosity, also a thickening agent in cosmetics or foodstuffs.

We have previously reported successful graft copolymerization of NFC with several acrylates and methacrylates by a simple redox initiated reaction [24]. In this paper, the method was applied to graft a polymer containing cationic ammonium groups on NFC. However, cationization of NFC can also be accomplished by grafting cellulose fibers with small molecules instead of polymers [25,26]. Due to the lack of existing research about the effect of the graft length, we compared the feasibility of both methods and the antimicrobial potential of the products. The two NFC types were compared by their chemical composition, charge density, and broad spectrum antimicrobial properties. Health and safety concerns were also addressed by testing the products for human cell cytotoxicity.

2. Experimental

2.1. Materials

Nanofibrillated cellulose was provided by UPM Corporation (Helsinki, Finland) with the product name UPM Fibril Cellulose. The material was prepared by mechanical disintegration of bleached birch pulp, which was pre-treated with a Voith refiner and then fluidized by seven passes through an M7115 fluidizer (Microfluidics Corp, Newton, MA, USA). The solids content of the prepared water dispersion was 1.6 wt%. Bleached sulfite dissolving pulp (Dissolving Plus) was obtained from Domsjö Fabriker (Domsjö, Sweden). All other chemicals were purchased from Sigma–Aldrich (Germany) and were of analytical reagent grade.