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Furanone-containing poly(vinyl alcohol) nanofibers for cell-adhesion inhibition

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ABSTRACT

The 3(2H) furanone derivative 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) was investigated for its antimicrobial and cell-adhesion inhibition properties against *Klebsiella pneumoniae* Xen 39, *Staphylococcus aureus* Xen 36, *Escherichia coli* Xen 14, *Pseudomonas aeruginosa* Xen 5 and *Salmonella typhimurium* Xen 26. Nanofibers electrospun from solution blends of DMHF and poly(vinyl alcohol) (PVA) were tested for their ability to inhibit surface-attachment of bacteria. Antimicrobial and adhesion inhibition activity was determined via the plate counting technique. To quantify viable but non-culturable cells and to validate the plate counting results, bioluminescence and fluorescence studies were carried out. Nanofiber production was upscaled using the bubble electrospinning technique. To ascertain that no DMHF leached into filtered water, samples of water filtered through the nanofibrous mats were analyzed using gas chromatography coupled with mass spectrometry (GC–MS). Scanning electron microscopy (SEM) and attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) were used to characterize the electrospun nanofibers.

1. Introduction

Significant milestones have been achieved in the area of membrane technology over the past three decades (Vrouwenvelder et al., 2007). The production of nanofibers is one of the greatest breakthroughs in the water treatment industry because of the structural properties that these fibers have (Barker, 2004). These properties include, a high surface to volume ratio, which allows a higher adsorption rate of various trace organics and bacteria for improving water quality, a higher temperature and acid/base tolerance, longer membrane life span and flexibility which enables the membrane to be formed into various membrane modules for larger commercial application (Huang et al. 2003).

Even though membrane filtration is a promising technology, its large-scale industrial applicability is limited partly due to poor intrinsic membrane properties, but largely because of fouling of the membranes. The use of chemical biocides such as chlorine to control biofouling of membranes is widespread (Chen and Stewart, 1996; Kramer, 2001; Vidella, 2002; Stewart et al., 2000; Presterl et al., 2007). However, most of these chemical biocides are not very effective at higher pH values and they react with dissolved chemicals to produce harmful byproducts (Richards, 2010). Physical means like, brushing have been reported, but only work best as secondary methods to other removal methods (Meyer, 2003). The use of bacteriophages (Curtin and Donlan, 2006), electrical current (Van der Borden et al., 2004) and nutrient control (Allison et al., 1998) have also

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been explored but these methods are either host specific or can take a long time to work and are not very cost effective.

The focus is now moving to modification of membrane surfaces to control fouling. Noble metal elements such as silver, copper, zinc, nickel, manganese, iron and lithium have been reported to be having antimicrobial properties (Al-Sha'alan, 2007; Revanasiddappa et al., 2010). Some of these metals have been blended with polymers and made into fibers for use in filter media, wound dressing and other applications. Silver, has been extensively studied especially for wound dressing applications due to its antimicrobial nature (Zhuang et al., 2010). The use of furanone derivatives that inhibit quorum sensing in microorganisms is an area which has not been significantly explored in filtration systems. Furanones are analogs of homoserine lactones that appear to interfere with the development of typical biofilm structure, leaving these organisms more susceptible to treatment with biocides (Baveja et al., 2004). The furanones found in marine algae (*Delisea pulchra*) which prevent fouling of the algae by marine organisms have gained a lot of interest (Ponnusamy et al., 2010). The application of furanones for attachment-inhibition could be beneficial, especially because it does not use the same pathway as many antibiotics and the risks of bacterial resistance are completely eliminated (Baveja et al., 2004; Hume et al., 2004). Toxicity of halogenated furanones is also well reported and thus for this study, a non-halogenated furanone derivatives was used.

Researchers have come up with many solutions to the biofouling problem and most strategies are centered on either killing the bacteria before they form biofilms (inactivating them) or modifying membrane surfaces to produce membranes which will not allow biofilm attachment. In this paper, nanofibrous mats derived from the electrospinning blends of poly (vinyl alcohol) and the furanone compound 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) were investigated. The furanone moiety has been reported to have cell adhesion-inhibition as well as a good antimicrobial properties over a wide spectra of bacteria (de Nys et al., 2006, Xu et al., 2010; Dworjanyn et al., 2006; Wu et al., 2004; Rasmussen et al., 2000; Hentzer et al., 2002). This means that the fabricated nanofibers should not only have surfaces that will repel microbial attachment, but also inactivate any bacteria which may come into contact with it. The fabricated mats were tested for possible application in filtration media against common biofilm-forming bacteria found in water supplies.

2. Experimental materials and methods

2.1. Materials

Poly(vinyl alcohol) (PVA), (M_w 146,000–186,000, 87–89% hydrolyzed) and the furanone compound 2,5-dimethyl-4-hydroxy-3(2H)-furanone were obtained from Sigma Aldrich, South Africa and used without further purification. Distilled water was used as the solvent. The bacterial strains *Escherichia coli* Xen 14, *Salmonella tymphimurium* Xen 26, *Pseudomonas aeruginosa* Xen 5, *Klebsiella pneumoniae* Xen 39 and *Staphylococcus aureus* Xen 36 were obtained from Caliper Life Sciences, Hopkinton MA, USA.

2.2. Methods

2.2.1. Electrospinning

A variation on conventional needle-based electrospinning, known as bubble electrospinning was used for this study. Bubble electrospinning allows production of nanofibers on a large scale. This process has been used by other researchers (Varabhas et al., 2009; Liu and He, 2007; Liu et al., 2008). In bubble electrospinning, the same concept as in the needle-based electrospinning is used, but it involves the formation of multiple electrostatically driven jets of polymer from a charged bubble of polymer solution (Liu et al., 2008). Also, an electric field of a much higher voltage than used in conventional needle spinning is necessary and fibers generated from polymer jets are collected on a negatively charged metallic collector plate positioned above the bubble widget (Fig. 1).

The electrospinning process involves addition of the polymer solution (3 mL) into the well of the bubble spinning widget (conical polymer container). A copper wire attached to the positive electrode of a high voltage power supply is then inserted into the polymer solution, while the negative electrode is attached to an aluminum foil collector plate suspended above the widget. Air is pumped into the polymer widget using a syringe to form bubbles and a voltage of 50 kV is applied.

A 100 mL PVA stock solution was prepared by dissolving PVA (10 g) in 90 mL deionized water to make a 10% wt/vol solution. To prepare a 10 mL PVA/DMHF solution for electrospinning, 500 μL (5% vol/vol) of DMHF, 8.7 mL PVA solution (stock) and 800 μL glyoxal solution (5% vol/vol) were stirred together into a homogenous pale yellow solution. These solutions were then electrospun into nanofibrous mats supported on 0.22 micron sized Millipore filters. All the polymer solutions were electrospun at room temperature. The widget-collector distance used was 20 cm and the relative humidity was maintained below 45%. The amount of glyoxal used in this study was very small and thus did not have an effect on the surface tension (Ding et al., 2002; Gule et al. 2012). The nanofibrous mats were crosslinked with heat treatment at

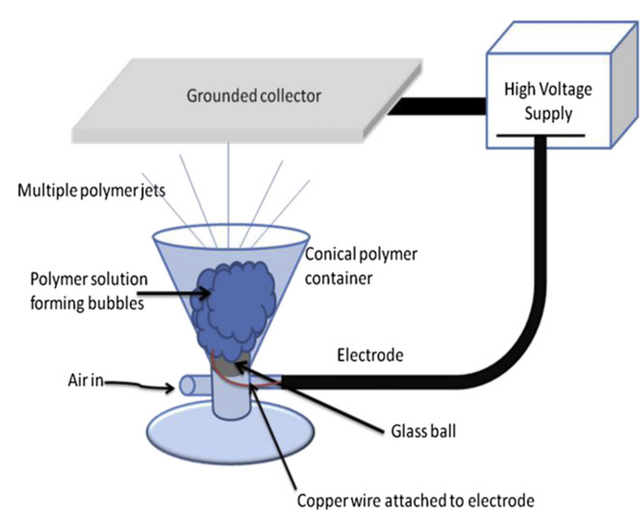

Fig. 1 – Bubble spinning set-up showing multiple polymer jets from polymer solution.