



Control of bacterial growth in water using novel laser-ablated metal–carbon–polymer nanocomposite-based microchannels



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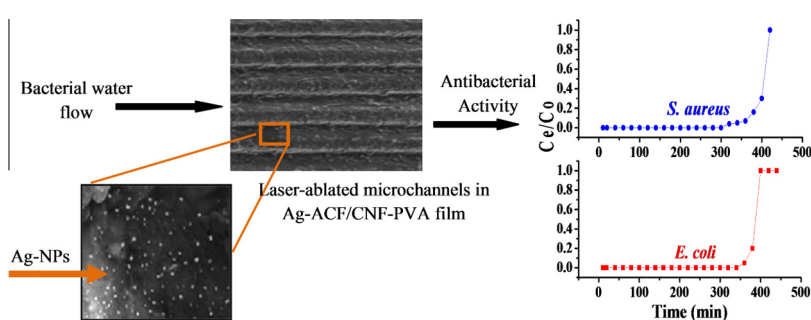
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HIGHLIGHTS

- Ag-ACF/CNF fillers incorporated *in situ* into the polymer film.
- Laser-ablation exposed the dispersed Ag-ACFs/CNFs to bacterial water flow.
- The nanocomposite with microchannels efficiently applied under flow conditions.

GRAPHICAL ABSTRACT



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ABSTRACT

Microchannels were fabricated in an approximately 1.2 mm thick metal–carbon–polymer nanocomposite film, using laser-ablation, and were used as a contactor to control the growth of bacteria in water under flow conditions. In this novel technique, the polyvinyl alcohol (PVA)-based polymeric film was synthesized using emulsion polymerization. Silver (Ag) nanoparticles (NPs)-grown carbon micro-nanofibers (Ag-ACFs/CNFs), separately prepared as an antibacterial agent, were added *in situ* into the film during polymerization and before the curing step. The Ag-ACF/CNFs antibacterial agent dispersed within the polymer matrix was exposed to a bacterial water flow through the fabricated texture (microchannels) with porous walls. Besides providing a negligible elution of the Ag NPs, the ACFs/CNFs enhanced the mechanical and thermal stability of the film. The antibacterial tests performed against the Gram-negative *Escherichia coli* and the Gram-positive *Staphylococcus aureus* in water revealed a complete inhibition of the bacterial growth under the experimental conditions.

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1. Introduction

Microchannels have been used in various separation and reaction applications. With a very high surface area to volume ratio ($\sim 1:1000$), microchannels allow high heat and mass transfer, as

well as reaction rates [1]. A striking feature of microchannel-based devices is that they are portable and occupy less space than traditional devices. Therefore, microchannels have increasingly been used in bio-applications where the amount of fluids to be handled is relatively less. Examples of such applications are diagnostics (immune sensor) [2], electrophoresis separation [3,4], drug delivery [5], separation of proteins and DNA [6], antimicrobial susceptibility test [7] and cell culture [8].

A polymer-based substrate is commonly used for fabricating microchannels because of its biocompatibility, cost-effectiveness,

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high elasticity and good adhesion property [4,6,9–15]. The fabrication of microchannels on a polymeric substrate is facile, and can be performed using different techniques such as compression molding [5], hot-embossing [6], UV laser photo ablation [3,15], casting or molding onto a prototype mold [4,12] and photolithography [13]. These techniques are inexpensive and rapid in making replicates. The walls of microchannels are chemically coated or functionalized in a post-fabrication step, depending on the end-applications.

Silver (Ag) nanoparticles (NPs) have been used as an efficient antibacterial agent in water [16,17]. To be practically used in an end-application, these materials are, however, dispersed on a polymeric substrate [18–23]. The substrate provides a long-term stability to the NPs by preventing them from oxidizing or agglomerating in liquid. It also prevents a process liquid from carrying away NPs with the flow [19]. There are two common methods that have been used to disperse the Ag NPs in a polymer matrix. (1) A separately prepared colloidal solution of Ag is directly mixed into the polymer [20–22,24]. (2) A solution of the metallic salts or complex ions is mixed into the polymer, and then the mixture is reduced using chemical reduction or annealing [19,25,26]. The latter method has a drawback that the polymer may degrade during annealing, or chemical processing at high temperatures.

Poly (vinyl alcohol) (PVA) is a relatively low-cost and environmentally benign polymer. It exhibits low cytotoxicity and has a good film-forming property, which make it suitable in biological applications [22]. However, its solubility in water and low tensile strength often restrict its usage in separation applications. These drawbacks were overcome in a previous study by synthesizing a PVA-based thin film via grafting PVA with the acrylonitrile and methyl acrylate monomers, using emulsion polymerization, and *in situ* dispersing the web of activated carbon microfibers (ACFs) and carbon nanofibers (CNFs) in the reaction mixture during polymerization. The polymerization produced a hydrophobic film and the dispersion of CNFs within the polymer matrix increased its tensile strength [27].

In this study, the Ag-grown ACFs/CNFs (Ag-ACFs/CNFs) were used as an antibacterial agent. The materials were *in situ* added into the reaction mixture for the grafting of PVA with monomers, during polymerization and before the curing step. Microchannels (30 mm long \times 150 μ m wide \times 100 μ m deep) were fabricated in the metal–carbon–polymer nanocomposite film (\sim 1.2 mm thickness), using laser-ablation. The laser-ablation exposed the ACFs/CNFs-supported Ag NPs, dispersed within the polymer matrix, to the channels through their porous walls. The contactor was tested against a bacterial water, flowing in the microchannels. The CNFs had multiple roles. Besides holding the Ag NPs, they increased the tensile strength of the nanocomposite film and provided the thermal stability to the material. The method for the synthesis of the Ag-ACF/CNF-PVA nanocomposite film, the fabrication of microchannels in the film, and the application of the resulting contactor in effectively controlling the growth of the Gram-negative *Escherichia coli* (*E. coli* K-12) and the Gram-positive *Staphylococcus aureus* (*S. aureus* RN4220) in water are experimentally demonstrated.

2. Materials and method

2.1. Materials

ACFs derived from a phenolic resin precursor were purchased from Nippon Kynol Inc., Japan. Silver nitrate (AgNO_3), sodium dodecyl sulphate (SDS), polyvinyl acetate (PVAc), methyl acetate, methyl acrylate, sodium hydroxide, methanol, potassium persulphate ($\text{K}_2\text{S}_2\text{O}_8$), polyethyl glycol (PEG), yeast, agar and Luria

Bertani were procured from Merck, Germany. All aqueous solutions were prepared in Milli-Q water. All gases, namely, nitrogen (N_2), hydrogen (H_2) and acetylene (C_2H_2), were of high purity grade and purchased from Sigma Gases, India. *E. coli* and *S. aureus* were procured from indigenous sources.

2.2. Synthesis of nanocomposite film and the fabrication of microchannels

2.2.1. Preparation of Ag NP-dispersed ACFs/CNFs

The pre-treated ACF samples were impregnated with a 180 mL aqueous solution of 0.03 M- $\text{Ag}(\text{NO}_3)$ salt, using the wet impregnation method. In our previous study, it was shown that salt concentrations greater than 0.03 M in the impregnating solution resulted in the agglomeration of Ag NPs and blockage of the pores in ACFs [28]. The impregnated ACF samples were dried in a vacuum oven at 100 °C. The dried samples were then calcined in a N_2 atmosphere (170 standard cc per min (sccm) gas flowrate) at 250 °C for 4 h to convert the metal nitrates into metal oxides. Next, the samples were reduced in a H_2 atmosphere (200 sccm) to convert the metal oxides into metallic form. Chemical vapor deposition (CVD) was performed on the reduced ACF samples at 300 °C for 1 h, using C_2H_2 as the carbon source, to produce Ag-ACFs/CNFs. The experimental set-up used for the calcination, reduction and CVD is described in the previous study [29]. The prepared Ag-ACF/CNF samples were randomly cut into small pieces (\sim 1 mm-average size) and added into 20 mL-PEG solution. The PEG-dispersed Ag-ACF/CNF samples were ball milled at 200 revolutions per minute (RPM) for 2 h to reduce the average size of the samples to \sim 500 nm.

2.2.2. Synthesis of the nanocomposite film

Fig. 1 shows a schematic illustration of the steps used in the synthesis of the Ag-ACFs/CNFs-containing PVA film with the laser-ablated microchannels. The polymeric film was produced using emulsion polymerization. A 2-l-3-neck round-bottom flask was used in the polymerization reaction, connected to a water condenser for refluxing the solvent during the reaction. A heating mantle was used to heat the flask. An approximately 40 g of a homogeneous solution was prepared by mixing PVAc into methanol in 62:38 (w/w) ratio. To this solution, approximately 25 mL-methanol and 25 mL-methyl acetate were added at room temperature (25 ± 2 °C). The mixture was stirred using a stirrer at the speed of 120 RPM till a transparent solution was produced. Next, 15 mL of methanolic sodium hydroxide, used as the basic catalyst for esterification, was added into the solution. After approximately 20 min, a PVA gel was produced. Approximately 80 mL of Milli-Q water was added into the mixture, as the polymerization medium. The temperature and the stirrer speed were set to 70 °C and 120 RPM, respectively. After approximately 2 h, the temperature of the solution was decreased to 60 °C. To this solution, 15 mL-methyl acrylate and 20 mL-acrylonitrile, each mixed with 0.1 g- $\text{K}_2\text{S}_2\text{O}_8$ as the initiator, were sequentially added at 2 h-intervals. After approximately 3 h, the color of the solution changed from white to blue, indicating the incipience of curing. At this stage, a 7 g of the ball milled and PEG-soaked Ag-ACFs/CNFs were added into the reactant mixture. The nanocomposite films were also prepared using the different amounts (5, 6, and 8 g) of Ag-ACFs/CNFs for the comparison purposes. Approximately 3 h after adding the filler, the heater was switched off and the product was allowed to cool to room temperature. The produced emulsion was cast to a depth of \sim 2 mm on a Nylon circular sheet (\sim 6 cm-diameter). The cast material was vacuum dried (\sim 1 m Torr), first at 100 °C for 12 h and then at 200 °C for 2 h to drive off the water as well as the PEG solvent. A few samples were prepared by drying the samples at different temperatures. The drying temperatures were found to be critical for developing

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