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Physio-chemical and antibacterial characteristics of pressure spun nylon nanofibres embedded with functional silver nanoparticles

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ABSTRACT

A novel and facile approach to prepare hybrid nanoparticle embedded polymer nanofibers using pressurised gyration is presented. Silver nanoparticles and nylon polymer were used in this work. The polymer solution's physical properties, rotating speed and the working pressure had a significant influence on the fibre diameter and the morphology. Fibres in the range of 60–500 nm were spun using 10 wt.%, 15 wt.% and 20 wt.% nylon solutions and these bead-free fibres were processed under 0.2 MPa and 0.3 MPa working pressure and a rotational speed of 36,000 rpm. 1–4 wt.% of Ag was added to these nylon solutions and in the case of wt.% fibres in the range 50–150 nm were prepared using the same conditions of pressurised gyration. Successful incorporation of the Ag nanoparticles in nylon nanofibres was confirmed by using a combination of advanced microscopical techniques and Raman spectrometry was used to study the bonding characteristics of nylon and the Ag nanoparticles. Inductively coupled plasma mass spectroscopy showed a substantial concentration of Ag ions in the nylon fibre matrix which is essential for producing effective antibacterial properties. Antibacterial activity of the Ag-loaded nanofibres shows higher efficacy than nylon nanofibres for Gram-negative *Escherichia coli* and *Pseudomonas aeruginosa* microorganisms, and both Ag nanoparticles and the Ag ions were found to be the reason for enhanced cell death in the bacterial solutions.

1. Introduction

Hybrid nanofibres are an interesting class of materials currently receiving significant attention due to their unique chemical, electrical, optical and mechanical properties that could be achieved by combining the advantages of nanoparticles and polymer nanofibres [1,2]. These properties enable such materials to be used in a wide range of applications including biomedical, energy storage, catalysis and sensors. Properties of the hybrid nanofibres not only depend on the high surface area to volume ratio of the polymer nanofibres but also the content, size and spatial distribution of the nanoparticles [3,4].

The development of hybrid nanofibres is of tremendous interest for the biomedical research community because scaffolds prepared from such materials resemble natural extracellular matrices with very good mechanical strength, biocompatibility and biodegradability with respect to various human cells and tissues [5,6]. Nanofibrous structures as an antimicrobial scaffold provide higher cell adhesion than other structures [7]. They have been also used in wound dressing and healing where these scaffolds possess more homogeneity, oxygen penetration and prevent infections and dehydration [8]. Nylon polymer nanofibres exhibit excellent mechanical properties, such as toughness, and high tensile strength and have been adopted to make various composites [9–11]. Ag nanoparticles show excellent antibacterial activity and have a tolerant range of cytotoxicity, strong inhibitory and bactericidal effects [12–14]. Ag belongs to an interesting group of antimicrobials that exhibit greater thermal stability and long term activity [15]. In addition, Ag has commanded much attention among all the antimicrobials because it not only provides intensive antimicrobial properties but also possesses acceptable cytotoxicity towards mammalian cells and tissues [16]. It has been reported that Ag nanoparticle embedded nanofibres showed enhanced antimicrobial efficacy against Gram-positive and Gram-negative bacteria [17,18]. Thus, ZnO and Ag nanoparticles were used for filling polymers such as polyurethane for making nanofibres which have demonstrated a high degree of antibacterial-activities [19].

Pressurised gyration is a simple and versatile mass production technique of nanofibres and nanofibrous structures with controllable fibre size and fibre size distribution. The technique consists of a vessel containing polymer solution subjected to simultaneous centrifugal force and dynamic fluid flow in order to extrude fibres with tailored morphologies and functionality [20]. The nanofibres produced through this technique depend on the rotating speed of the vessel, air pressure and the concentration of polymer solution. Unlike electrospinning it is a nozzle free method independent of the electrical conductivity and the

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dielectric constant of polymer solution. The process offers production of fine long continuous fibres in a well oriented direction. The highly oriented nanofibres produced can provide mechanically stronger fibres with remarkable surface active areas [21,22].

The influence of the processing parameters such as vessel rotating speed, air pressure and the nylon concentration in the solutions on the fibre size and the size distribution were investigated in this work. Surface morphology studies and chemical analysis were carried out on the nanofibres. The antibacterial activities of nylon and Ag-loaded nylon nanofibres were studied by using *Escherichia coli* and *Pseudomonas aeruginosa* tested in aerobic suspension cultures. These hybrid nanofibres could be utilised for a wide range of biological applications, such as antibacterial wound dressing, functional scaffolds for tissue engineering, and antibacterial water and air filtration devices and systems.

2. Experimental details

2.1. Materials

Nylon 6,6 (pellets, molecular weight ~ 30,000 g/mol, density 1.18 g/ml, laboratory grade), formic acid (analytical grade) and aqueous silver nanoparticles (particle size < 150 nm) were purchased from Sigma-Aldrich (Poole, UK). All reagents were used without further purification.

2.2. Preparation of nylon and Ag-loaded nylon solutions

Nylon solutions modified with functional Ag nanoparticles were prepared and used in conjunction with nylon only solutions for comparison. The polymer solutions were prepared in an air-tight bottle using formic acid as solvent to dissolve the nylon pellets under magnetic stirring for ~24 h at ambient temperature (~20 °C). Four different concentrations of nylon (5, 10, 15, and 20% w/w) were prepared. Ag containing nylon solutions were prepared by adding 1 wt.% of aqueous silver nanoparticle suspension to the nylon solutions. These were also prepared using a magnetic stirrer in an air-tight bottle at the ambient temperature. Similarly, 2 wt.% and 4 wt.% Ag nanoparticle containing nylon solutions were prepared for making nanofibres and these were only tested for Ag-ion release in aqueous solution (see Section 2.4).

2.3. Pressurised gyration

Details of the experimental set-up of the pressurised gyration process accompanied by videos and illustrating the process in depth; including jet initiation, instability and mass production of nanofibres, are reported in our previous work [20].

2.4. Characterisation

Solution physical properties such as surface tension and viscosity are crucial in pressurised gyration fibre forming [20]. The surface tension and the viscosity of all the solutions were measured using a KRUSS tensiometer and a Brookfield viscometer, respectively, at ambient temperature as listed in Table 1.

Table 1
Measured values of surface tension and viscosity for nylon solutions.

Nylon system solutions	Surface tension (mN/m)	Viscosity (mPa s)
5 wt.% nylon	38.3(±2.4)	53.5(±0.3)
10 wt.% nylon	42.7(±2.7)	236.9(±0.7)
15 wt.% nylon	52.1(±2.5)	461.1(±2.7)
20 wt.% nylon	73.5(±3.4)	2016.4(±14.6)
5 wt.% nylon + 1% Ag	38.2(±0.9)	29.8(±0.2)
10 wt.% nylon + 1% Ag	41.3(±1.2)	181.1(±2.1)
15 wt.% nylon + 1% Ag	49.7(±3.2)	245.6(±2.7)
20 wt.% nylon + 1% Ag	66.4(±5.2)	924.9(±5.0)

The characteristics and the morphology of nanofibres were studied using field emission scanning electron microscopy (FE-SEM) – energy dispersive x-ray spectroscopy (EDX) and focussed ion beam microscopy (Carl Zeiss-Gemini). Nanofibre samples were coated with gold using a sputtering machine (sputter time ~75 s) before loading in to the microscopes. High and low magnification images were acquired in randomly selected positions (>20) within a sample. The fibre diameter was obtained using Image J software. About 150 measurements were made at random locations to plot the fibre diameter distribution.

Raman spectra of nylon (20 wt.%) and Ag-loaded nylon samples (Ag concentration was 1 wt.% and 4 wt.%) were obtained using a Renishaw Raman microscope excited with 514.5 nm incident wavelength radiation. The data acquisition covered the spectral range 2000–100 cm^{-1} with a spatial resolution of 4 cm^{-1}.

The Ag ion release from Ag-loaded nanofibrous mats obtained from 20 wt.% nylon was tested with inductively coupled plasma mass spectroscopy (ICP-MS, Agilent, Japan). The Ag concentration was 1 wt.%, 2 wt.% and 4 wt.% in the nanofibres. The prepared samples were cut into appropriate size and were washed in de-ionised water (30 ml) for 24, 48 and 72 h in each case. Then they were immersed in 1 wt.% nitric acid (HNO_3), which was used as a stock solution. For comparative and calibration purposes the de-ionised water and nitric acid were screened using ICP-MS for Ag ion content. Thus, the Ag ion concentration in the releasing medium was quantified and for each case three measurements were made.

2.5. Antibacterial testing

In order to investigate the antibacterial activities of the nylon (20 wt.%) and Ag-loaded nanofibres, antibacterial performance tests were conducted. For this purpose, two Gram-negative bacteria, *E. coli* (in house strain number 3891) and *P. aeruginosa* (strain 25-09071215-05) were used. Bacterial broth suspensions were co-cultured under aerobic conditions with the sample for set periods of time, after which the number of viable colony-forming units (CFU) of bacteria was obtained and then the antibacterial rate for each sample was calculated.

Due to strong bactericidal activity of formic acid, all of the nanofibre samples for the antibacterial tests were washed with distilled water (pH 6.5) for 96 h to remove any residual formic acid. Post-washing, the pH value of nylon nanofibres was ~6 and those of the Ag-loaded nylon nanofibres was ~5. The assay was performed using bacteria cultured overnight from a single colony to stationary phase (approximately 10^9 CFU/ml) in Luria-Bertani (LB) broth in a shaking aerobic incubator at 37 °C. Next, a suspension, ~5×10^5 CFU/ml in LB, was prepared for use as the experimental bacterial suspension working in a sterile laminar flow hood environment.

The same weight of nanofibre samples (0.02 g) in a constant liquid volume (0.02 ml) was placed using sterile tweezers into each well of a 24-well tissue culture plate (Corning) along with 0.5 ml of the experimental bacterial suspension of either *E. coli* or *P. aeruginosa.* In these tests, 10 samples were tested for each bacterium, 5 samples of nylon nanofibres and 5 samples of Ag-loaded nylon nanofibres. Wells with experimental suspension alone containing no sample were also used as a negative control. The plates were cultured in an aerobic shaking incubator at 37 °C for 2 h and 24 h. After co-culturing with the samples, the bacterial suspensions were diluted (1:1, 1:10, 1:100 and 1:1000) into sterile tubes, after which 0.02 ml each dilution was applied evenly onto quadrants of chromogenic agar plates (chromID CPS, Biomerieux) for enumeration using sterile L-spreaders. Chromogenic agar allows confirmation of bacterial species by colony colour. The plates were incubated overnight at 37 °C for 24 h. Finally, the number of bacteria colonies (CFU) on each quadrant was counted to evaluate the effect of the original sample on survival of the bacteria.

The antibacterial rate of a material can be determined by AR (%) = $[(N_1 - N_2) / N_1] \times 100$, where, AR is the antibacterial ratio (%); N_1 is