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Polyacrylonitrile nanofiber mats containing titania/AgNP composite nanoparticles for antibacterial applications

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Abstract

Here in we present our research on electrospun polyacrylonitrile nanofiber films embedded with titania/AgNP nanoparticles for sustained antibacterial applications. Although silver possesses excellent antimicrobial characteristics and have been extensively exploited in applications where protection from microbial species and bacterial colonization is needed. Recently, it was discovered that silver may be allergic to skin and may induce detrimental side-effects such as argyria and argyrosis. Excess utilization of silver may even cause carcinoma. In continuation of our research on preparation of biologically safe antibacterial materials producing longer timed protection, here we proposed electrospun polyacrylonitrile nanofiber mats containing titania/AgNP composite nanoparticles. The titania/AgNP nanoparticles were prepared using polydopamine hydrochloride (pdopa) as adhesive and reducing agent. The nanoparticles were then added into polyacrylonitrile polymer solution and electrospun to fabricate polycrylonitrile/titania/AgNP composite nanofiber mats (PTAgNP). The samples were characterized with XRD, TEM, SEM, FT-IR, SEM-EDX and antibacterial assays. Synthesis of the titania/AgNP was confirmed by XRD, TEM and EDX analysis. The nanofibers were studied with SEM, XRD, TEM and antibacterial tests. The SEM observations confirmed regular morphology of the nanofibers. The XRD and TEM analysis evidenced titania/AgNP contents in the polycrylonitrile nanofibers. The antibacterial assays demonstrated excellent bacterial growth inhibition on agar plates and in the liquid medium. The samples were observed to inhibit growth of E. coli and S. aureus bacteria for up to 120 h. The quantitative bactericidal assay (relative cell viability, %) demonstrated 0% bacteria cell viability by polycrylonitrile nanofibers containing 10 wt% of titania/AgNP nanoparticles.

1. Introduction

The antimicrobial films apart from protection from harmful microbial species need to be free from harmful side-effects, be soothing and comfortable to the user (Jatoi et al 2019c). The nanofibrous structures, owing to very small diameters up to few hundred nanometres and very small pore sizes, have been effective and efficient alternatives to conventional antimicrobial materials (Ramakrishna et al 2006, Kim et al 2016). The nanofibers have very high surface to volume ratio, high porosity, higher breathability and sufficient mechanical strength which ensure their effectiveness in wide range of applications (Stanzione et al 2013, Reddy et al 2014, Ke et al 2016, Lee et al 2018, Jatoi et al 2019a, Jatoi et al 2019b, Jatoi et al 2019d). Particularly related to biomedical applications, owing to smaller diameters, very small pore sizes and numerous pores, the nanofibers have shown higher performances in comparison to conventional fibers (Wendorff et al 2012, Manjumeena et al 2015). Resemblance of the randomly oriented nanofiber strands of a nanofibrous structure with human ECM have been suggested for extended performance of these novel structures in biomedical application domains.
and thermal properties of the patient. The antimicrobial nanofibers containing an excellent antimicrobial agent having excellent antimicrobial properties and being biologically safe would be highly desirable in antibacterial materials domain.

The polyacrylonitrile is a robust polymer and possesses excellent mechanical strength, chemical resistance and thermal properties (Jatoi et al. 2019a) desirable in antibacterial applications. The polyacrylonitrile nanofibers have so far been investigated for their suitability in air filtration (Dehghan et al. 2016, Jing et al. 2016), cleaning of water (Roche and Yalcinkaya, 2013), degradation of pollutants from water (Huang et al. 2013) and, more importantly related to present research, in antimicrobial applications (Kharaghani et al. 2018a, Kharaghani et al. 2018b).

Considering the antimicrobial agents, the silver has been an excellent broad spectrum antimicrobial agent effective against wide range of bacteria, viruses and fungal species (Kawashita et al. 2003, Cui et al. 2014, Haas et al. 2015, Wang et al. 2015, Gopiraman et al. 2016, Hong et al. 2017). It can show both bacteriostatic and bactericidal properties and was recently reported effective against cancer cells as well (Hazarika et al. 2016, Mansour et al. 2018). When compared with silver ions, the silver nanoparticles (AgNPs) demonstrated better antimicrobial performances mainly due to their larger surface area and gradual release of silver ions. The AgNPs can disintegrate the bacteria cell wall, denature their DNA and bind with respiratory tract enzymes thereby ensure antimicrobial and antifouling properties. However, excess contact with the silver may induce detrimental side effects and produce limited time antibacterial activities. Some of the reported detrimental side effects of excess contact with silver have been allergies, argyria, argyrosis, carcinoma and metal accumulation (Park et al. 2011, Leung et al. 2018, Jatoi et al. 2018a, Jatoi et al. 2019d).

Thus in continuation of our research on safer antimicrobial nanofiber composites, in the present research we report polyacrylonitrile nanofibers incorporated with AgNPs decorated on the titania nanoparticles for antibacterial applications. Anchoring of the AgNPs on a substrate to prepare composite nanoparticles and their embedding in the polyacrylonitrile nanofibers would ensure safer and sustained antibacterial properties. Titania was chosen as anchoring substrate owing to its UV induced antibacterial properties, very good optical, thermal and electronic characteristics (Kar et al. 2009). The composite nanoparticles were synthesized by an environmentally green approach using dopamine hydrochloride (dopa) which was utilized here as an adhesive and reducing agent. At alkaline pH (8.5) dopa self polymerizes to form adhesive coating on titania surface which reduces the silver ions into AgNPs. The hydroxyl groups in catechol convert silver ions into AgNPs (Choi et al. 2019, Wang et al. 2013). The titania/AgNP incorporated polyacrylonitrile nanofibers were characterized for their antibacterial performances. The composite nanofibers demonstrated excellent antibacterial properties against E. coli and S. aureus. It was proposed that the antimicrobial properties of the PTAgNP were based on generation of reactive oxygen species (ROS) which control bacteriostatic and bactericidal activities of the nanofibre composite.

2. Experimental

2.1. Materials

Polyacrylonitrile with molecular weight 150000 a.m.u., titania (TiO2, rutile), Miller’s Luria-Bertani broth and agar powder were purchased from Sigma Aldrich, Japan. The N,N-dimethylformamide (DMF), dopamine hydrochloride (3, 4-dihydroxyphenethylaine hydrochloride, DOPA), silver nitrate (AgNO3) and de-ionized water were purchased from Wako Pure Chemicals, Japan. The 1 M TRIS HCl buffer (pH 8.5) was obtained from Nippon Gene Co., Japan. The Gram-negative strain Escherichia coli (E. coli, NBRC No. 3301) and Gram-positive strain Staphylococcus aureus (S. aureus, NBRC No. 12732) were obtained from NITE Biological Resource Center, Japan.

2.2. Synthesis of the composite nanoparticles

The titania anchored with AgNPs were synthesized by an environmental friendly process using dopamine hydrochloride as reducing agent. A dopamine coating (pDopa) was first formed on titania surface by adding titania nanoparticles (200 mg) into 150 ml of 2 mg ml⁻¹ Dopa in 1 M Tris HCl buffer (pH 8.5). The mixture was then stirred for eighteen hours on magnetic stirrer. The Dopa coated titania nanoparticles were collected by centrifugation and repeatedly washed with de-ionized water. The collected pDopa coated titania nanoparticles were treated for eighteen hours with 350 ml of 200 mM silver nitrate solution for simultaneous adsorption of silver ions and reduction into metallic silver nanoparticles (AgNP). The AgNP anchored titania nanoparticles...
(titania/AgNP) were collected in the end by centrifugation, dried and used to fabricate electrospun polyacrylonitrile nanofibers.

2.3. Fabrication of nanofibers

The DMF was used as solvent for electrospinning of polyacrylonitrile (12 wt%) into nanofibers. A 10% of titania nanoparticles, 5% and 10% of titania/AgNP composite nanoparticles were used to fabricate polyacrylonitrile/titania (PT), PATAgNP1 and PTAgNP2 respectively. The polymer solution with nanoparticles were sonicated for one hour in DMF before addition of polyacrylonitrile polymer and stirred for 24 h on a magnetic stirrer. For fabricating the composite nanofibers Katotech electrospinning unit (Katotech Co., Japan) was used. The needle tip to collector distance and electric voltage used were 15 cm and 15 kV respectively. The electrospinning was carried out at room temperature. The electrospinning was carried out until a 40 μm thick nanofiber web was obtained.

2.4. Characterizations

For studying crystalline structure of the nanoparticles and composite nanoparticles X-ray diffraction equipment, XRD (Rigaku MiniFlex300, Rigaku Co., Japan) was utilized. Synthesis of the composite nanoparticles and their embedding into the polyacrylonitrile nanofibers was investigated with transmission electron microscopy, TEM (JEM-2100, Jeol, USA). Morphology of the nanofibers was observed using scanning electron microscopy, SEM (SU-1510, Hitachi High Technologies). The nanofiber samples were sputtered coated with platinum (Pt) before SEM observations. Elemental compositions of the titania/AgNP composite nanoparticles were obtained using SEM (Hitachi S-3000N) equipped with energy dispersive X-ray (EDX) analyzer (Horiba, EX 200). To study chemical structures of the nanofibres, Fourier transform infrared spectroscopy, FT-IR (FT-IR Prestige-21, Shimadzu, Japan) was used. The absorbance values (OD_{590nm}) were obtained at fixed intervals utilizing a microplate reader (ImmunoMiniNJ-2300).

2.5. Antibacterial activities

The composite nanofibers were examined for their antibacterial performances using agar plate disk diffusion and liquid medium antibacterial tests. The relative cell viability test and bacterial growth curves (OD_{590 nm}) were obtained to investigate bactericidal and bacterial growth inhibition performance of the samples in liquid medium (LB medium).

For the disk diffusion test, the agar plates (LB agar plates) were prepared using 2.5 g/100 ml and 2 g/100 ml of LB broth and agar powder respectively. A 100 μl of overnight grown E. coli and S. aureus strains were spread on each agar plate. The sample having 10–11 mm diameters were then incubated on the plates for 24 h at 37 °C. At the end of test, halo widths (mm) were measured with ImageJ software.

The liquid medium bactericidal performances of the nanofibrous composites were recorded via counting viable bacterial cells (colony forming units, CFU) after overnight contact with the nanofiber samples in LB medium and subsequent incubation on the agar plates for 24 h at 37 °C. The relative cell viability (%) results were then calculated by taking percentile values of ration of bacteria colonies (CFU/ml) formed by the contacted cells to the bacteria colonies (CFU/ml) formed by non-contacted bacteria cells (equation (1)).

\[
\text{Relative Cell Viability (％)} = \frac{\text{colony forming units of samples}}{\text{colony forming units without samples}}
\]

The growth curves of the strains were obtained by incubating the samples in the LB medium containing overnight cultured medium. At fixed intervals (every 12 h) the absorbance (OD_{590 nm}) values were recorded using the microplate reader.

3. Results and discussions

3.1. Synthesis of the nanoparticles

3.1.1. XRD analysis

Synthesis of the titania/AgNP composite nanoparticles was primarily confirmed using XRD analysis and from TEM images. The XRD results given in figure 1 show the respective patterns of titania and AgNPs. The XRD pattern of pure titania (figure 1(a)) exhibited diffraction angles (2θ values) at 27.3°, 35.97°, 41.11°, 43.96°, 54.31°, 56.6°, 62.63°, 68.95° which represent the (110), (101), (111), (210), (002) and (112) crystal planes of rutile phase of titania (Thamaphat et al. 2008). The titania/AgNP results (figure 1(b)), in addition to titania diffraction patterns, showed patterns of AgNPs. The diffraction angles (2θ) at 38.19°, 44.26°, 64.36° and 77.31° belong to metallic AgNPs and have been representative of (111), (200), (220) and (311) crystal planes of AgNPs (Bar et al. 2009). The results confirmed synthesis of crystalline AgNPs on titania surfaces. Thus the XRD results confirm...
synthesis of the titania/AgNP composite nanoparticles. No additional peaks in the XRD patterns of the nanoparticles demonstrated purity of the synthesized nanoparticles.

3.1.2. TEM observations
The transmission electron microscopy (TEM) images given in figure 2 revealed titania nanoparticles densely decorated with fine AgNPs. The HRTEM image exhibited (111) crystal lattice of AgNPs having 0.241 nm size which confirms crystallinity of the formed AgNPs (Ma et al 2011). Average sizes of the titania nanoparticles and AgNPs as calculated with ImageJ software were 36 nm and 5.9 nm respectively. It has been previously demonstrated that finer sized AgNPs ensured higher performances in antibacterial tests (Agnihotri et al 2014). Thus, fine size of AgNPs and their dense decoration on titania when incorporated into the polyacrylonitrile nanofibers will produce improved antibacterial properties.

3.1.3. EDX analysis
The EDX studies of the titania/AgNP nanoparticles were conducted to investigate elemental composition of the composite nanoparticles (table 1). The CK and NK elements belong to dopamine coating while the TiK and AgL represent the titania and AgNP nanoparticles respectively. The OK belongs to titania and dopa coating as well.
3.2. Characterization of nanofibers

3.2.1. SEM analysis

The figure 3 presents SEM results of control (polyacrylonitrile), PT, PTAgNP1 and PTAgNP2 nanofibers. All of the samples showed regular and bead free nanofibers. Compared with diameter of control sample, diameters of the nanofibers loaded with nanoparticles were observed to moderately increase. The SEM images and ImageJ software calculations demonstrated increase in the nanofiber diameters with incorporation of the nanoparticles. For example, the average diameter of control samples was calculated to be $339 \pm 160$ nm which increased to $394 \pm 150$ nm in case of PT samples which further increased to $406 \pm 160$ nm for PTAgNP2 samples. This increase in nanofiber diameters with the incorporation of nanoparticles may be attributed to titania and titania/AgNP nanoparticle aggregates localized inside the nanofiber matrix.

3.2.2. XRD evaluations

The XRD evaluations were carried out in order to confirm nanoparticle’s embedding inside respective nanofiber samples. The PT sample, which was prepared by 10 wt% of titania nanoparticles, (figure 4(b)) exhibited diffraction patterns of the titania. The PTAgNP1 and PTAgNP2 showed respective diffraction patterns of the titania and metallic AgNPs confirming presence of the titania/AgNP composite nanoparticles. Furthermore, all

| Elements | Weight %age | Atomic %age |
|----------|-------------|-------------|
| C [K]    | 4.73        | 11.03       |
| N [K]    | 4.26        | 8.52        |
| O [K]    | 32.48       | 56.92       |
| Ti [K]   | 25.62       | 14.99       |
| Ag [L]   | 32.91       | 8.55        |
| Total    | 100         | 100         |
the samples showed corresponding patterns of polyacrylonitrile nanofibers. The X-ray diffraction (2θ value) at 16.9° and 29.3° correspond to (1010) and (1120) crystal planes of polyacrylonitrile nanofibers (Jatoi et al 2019a).

3.2.3. TEM observations
The transmission electron microscopy images of PT, PTAgNP1 and PTAgNP2, given in figure 5, exhibited polyacrylonitrile nanofibers embedded with the nanoparticles. The sample PTAgNP2, which was prepared with 10 wt% of the titania/AgNP nanoparticles, depicted higher contents and closer packing of the nanoparticle aggregates. However, the PTAgNP1, which was prepared with 5 wt% of titania/AgNP nanoparticles, exhibited comparatively larger distance between the titania/AgNP nanoparticle aggregates. Furthermore, the TEM images evidenced adequately uniform distribution of the nanoparticle in the polyacrylonitrile nanofibers which would be highly desirable in antibacterial applications.

3.2.4. FT-IR results
The FT-IR analysis was carried out in order to study the chemical structures of the samples. The results given in figure 6 depicted all the corresponding molecular vibrations of polyacrylonitrile polymer. The FT-IR spectra showed a peak at 2239 cm⁻¹ which was due to stretching vibrations of nitrile (C≡N) groups. A CH₂ deformation peak and a CH₂ stretching vibration peak at 1450 cm⁻¹ and 2930 cm⁻¹ respectively can be seen in all samples (Jatoi et al 2018b). The peak at 1665 cm⁻¹ was assigned to stretching vibrations of carbonyl groups (C=O) which may be due to residual contents of DMF which was used as a solvent for fabricating polyacrylonitrile nanofibers.
3.3. Antibacterial assays

For evaluation of antibacterial performance of the samples, liquid medium antibacterial assays and bacteria growth inhibition on agar plates following disk diffusion test method were conducted. For antibacterial activities *E. coli* (Gram-negative bacteria) and *S. aureus* (Gram-positive bacteria) test strains were utilized.

The antibacterial activity results of the PTAgNP studied by disk diffusion method are reported in Table 2 while their photographic images are given in Figure 7. The results revealed very good antibacterial activities of the PTAgNP against both the tested strains. The mean hallow widths formed by PTAgNP1 and PTAgNP2 against *E. coli* were 2.75 mm and 3.23 mm respectively, while, the same against *S. aureus* were 3.92 mm and 4.12 mm.

![Figure 6. FT-IR spectrum of (a) control (b) PT (c) PTAgNP1 (d) PTAgNP2.](image)

![Figure 7. Photographic images of the samples incubated overnight with the strains on agar plates (disk diffusion test).](image)

| Sample   | *E. coli* | *S. aureus* |
|----------|-----------|-------------|
| Control  | —         | —           |
| PT       | —         | —           |
| PTAgNP1  | 2.75      | 3.92        |
| PTAgNP2  | 3.23      | 4.12        |

**Table 2.** Disk diffusion test results (hallow width, mm).
respectively. The results demonstrated that the PTAgNP samples were more effective against *S. aureus* than the *E. coli* strains.

Although the titania nanoparticles possess antibacterial and catalysis properties, however, the properties depend on exposure to ultraviolet light which means that the titania nanoparticles can show antibacterial performance but in the presence of ultra violet (UV) light. Under ultra violet illumination, the titania nanoparticles produce reactive oxygen species (ROS) which have been highly effective in antibacterial and photocatalysis. Our antibacterial experiments were carried out in the dark thus the PT sample did not show formation of any halo width. However, the titania/AgNP nanoparticles can be effective under tested conditions.

The results of bactericidal activities were conducted by relative cell viability tests and have been presented in figure 8. The results showed 100% bactericidal performance by the PTAgNP2 with 0% viable *E. coli* and *S. aureus* cells. Although the PTAgNP1 also exhibited very good bactericidal performance, however, their relative cell viability was 3.26% and 2.58% against *E. coli* and *S. aureus* respectively. The PT samples also exhibited a degree of bactericidal activities against both the tested strains, which was contrary to the disk diffusion test results; however their limited performance may once again be due to testing conditions.

The bacteriostatic antibacterial performance of the samples was determined by plotting bacterial growth curves of the test strains after their contact with the samples. The results were taken as optical density values, OD$_{590\text{nm}}$ and have been presented in figure 9. The PTAgNP1 and PTAgNP2 samples were able to inhibit growth
of the test strains up to 120 h which suggested their higher potential in durable antibacterial applications. The PT samples exhibited a degree of bacterial growth inhibition in liquid media as well, however similar to relative cell viability results, the property was lower than the PTAgNP samples.

4. Conclusions

The research on fabrication of antibacterial polyacrylonitrile nanofibers containing titania/AgNP by electrospinning was successfully concluded. The durable antibacterial activities were induced by titania/AgNP nanoparticles which were embedded in the polyacrylonitrile nanofiber matrix. The XRD and TEM results confirmed polyacrylonitrile nanofibers localized with titania/AgNP composite nanoparticles. The antibacterial tests confirmed durable bactericidal and bacterial growth inhibition performances of the proposed PTAgNP nanofibers. The fabricated PTAgNP samples exhibited bacterial growth inhibition for up to 120 h. The PTAgNP2 samples which contained 10 wt% of the titania/AgNP composite nanoparticles demonstrated 100% bactericidal properties (0% viable cells) in the relative cell viability tests. The PTAgNP samples were effective against both the E. coli and S. aureus strains.

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Conflict of Interest

‘The Authors declare that there is no conflict of interest’.

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