

‘Green’ biocompatible organic–inorganic hybrid electrospun nanofibers for potential biomedical applications

R Manjumeena¹, T Elakkiya², D Duraibabu², A Feroze Ahamed³, PT Kalaichelvan¹ and R Venkatesan⁴

Abstract
Gold nanoparticles were prepared by green route using Couroupita guianensis leaves extract. The green synthesized gold nanoparticles exhibited maximum absorbance at 526 nm in the ultraviolet spectrum. By incorporating the green synthesized gold nanoparticles in poly(vinyl alcohol) matrix, unique green organic–inorganic hybrid nanofibers (poly (vinyl alcohol)–gold nanoparticles) were developed by electrospinning. Contact angle measurements showed that the prepared poly (vinyl alcohol)–gold nanoparticles were found to be highly hydrophilic. The crystallinity of gold nanoparticles was analyzed using XRD. The synthesized gold nanoparticles and poly (vinyl alcohol)–gold nanoparticles were characterized using high-resolution transmission electron microscope, Fourier transform-infrared spectroscopy and energy-dispersive analysis of X-ray. The ultimate aim of the present work is to achieve optimum antibacterial, antifungal, biocompatibility and antiproliferative activities at a very low loading of gold nanoparticles. Vero cell lines showed a maximum of 90% cell viability on incubation with the prepared poly (vinyl alcohol)–gold nanoparticles. MCF 7 and HeLa cell lines proliferated only to 8% and 9%, respectively, on incubation with the poly (vinyl alcohol)–gold nanoparticles, and also exhibited good antibacterial and antifungal activities against test pathogenic bacterial and fungal strains. Thus, the poly (vinyl alcohol)–gold nanoparticles could be used for dual applications such as antimicrobial, anticancer treatment besides being highly biocompatible.

Keywords
Green gold nanoparticles, organic-inorganic hybrid, nanofibers, biocompatibility, antiproliferative, antimicrobial

Introduction
Nanobiotechnology is a branch of applied sciences that deals with materials at the nanoscale (10⁻⁹ m) focusing on biology, biochemical processes and their applications. Nanobiotechnology offers potential developments in pharmaceuticals, medical imaging, diagnosis, implantable materials, tissue regeneration, cancer treatment etc.¹ Polymer nanofiber mats have unique properties, such as a high surface area-to-volume ratio and high porosity. In addition, the polymer nanofiber scaffold composition can be controlled to achieve desired properties and functionality. Due to these advantages, nanofibrous scaffolds have been widely investigated in the past several years with materials of different compositions for applications of varying end uses, such as biological scaffolds, wound dressings, optical and bio sensors.²–¹⁸ There are many well-established techniques namely centrifugal spinning, solution blowing, electrospinning, pressurized gyration, etc.¹⁹,²⁰ to generate a wide variety of polymeric fibers across the micro- to nanometer-scale range. Electrospinning is a conventional process by which a polymer solution is charged to a high voltage to produce fibers with a diameter ranging from 10 to 500 nm. Over the years, a number of electrospun nanofibers have been developed for

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biomedical and pharmaceutical applications, many of which are summarized in synoptic Table 1.

Aiming at the combination of the beneficial properties of nanomaterials and electrospun nanofibers, in recent years, more attention has been paid to the preparation of metal nanoparticles dispersed polymer nanofiber film. Among the metal nanoparticles that are embedded in polymer film, gold nanoparticles (AuNPs) have been drawing much interest because of their remarkable biocompatibility, antimicrobial and anticancer activities. These properties serve to make these metal nanoparticles a novel platform for biomedicine, pharmacology, labeling, drug-delivery, photothermal therapy, tissue, tumor imaging and sensing.

Owing to its excellent water-solubility, high biocompatibility, hydrophilicity, sound mechanical and thermal properties, poly (vinyl alcohol) (PVA) is a promising carrier of metal nanoparticles for biomedical and pharmaceutical applications.

The ease of synthesizing AuNPs and their affinity for binding many biological molecules makes them attractive candidates for study. The green method of nanoparticle synthesis employing plant extracts is a simple and viable alternative to chemical procedures and physical methods. Chemical and physical methods are harmful because the chemicals used are toxic, flammable and are not disposed of easily in the environment. In recent years, biosynthesis of nanoparticles has received considerable attention due to the growing need to develop clean and nontoxic chemicals, eco-friendly solvents and renewable materials.

With the increase in resistance of bacteria and fungi to multiple antibiotics, there is a growing need to develop antibacterial and antifungal agents with broad spectrum and multitude mode of action. There are several reports on the antibacterial and antifungal activities of nanosilver. So, we were inclined to extend the same application to green synthesized AuNPs as AuNPs possess well-developed surface chemistry, chemical stability and appropriate smaller size, which make them easier to interact with the microorganisms causing structural changes, degradation and finally cell death as already reported.

Breast and cervical cancer are the most common forms of malignancy prevalent among middle-aged women that cause major mortality worldwide. Moreover, the incidence and mortality of breast and cervical cancer keep on rising every year. Over the past decade, treatments to these life-threatening forms of cancer have become more challenging owing to the prevalence of multiple drug resistance, detrimental side effects and the lack of innovative approaches. Chemotherapy is one of the most effective methods for the treatment of metastatic cancers, it is nonspecific and causes significant toxic damage. The development of drug resistance to chemotherapeutic agents through various mechanisms also limits their therapeutic potential. The success of cancer therapy depends on the ability of a therapeutic agent to destroy the tumor

<table>
<thead>
<tr>
<th>Electrospun nanofiber formulation</th>
<th>Applications</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan–organic rectorite (OREC) /polyvinyl alcohol (PVA)</td>
<td>Antibacterial</td>
<td>[2]</td>
</tr>
<tr>
<td>PVA-AuNPs/carboxymethyl-chitosan</td>
<td>Antibacterial</td>
<td>[3]</td>
</tr>
<tr>
<td>AgNPs-PVA/ hydroxypropyl-beta-cyclodextrin</td>
<td>Antibacterial</td>
<td>[4]</td>
</tr>
<tr>
<td>Triclosan/cyclodextrin complexes</td>
<td>Antibacterial</td>
<td>[5]</td>
</tr>
<tr>
<td>PVA/sodium alginate (ALG)/OREC composite</td>
<td>Antibacterial</td>
<td>[6]</td>
</tr>
<tr>
<td>Chitosan-AuNPs blended with PVA</td>
<td>Antibacterial</td>
<td>[7]</td>
</tr>
<tr>
<td>Pullulan/PVA/silver hybrid</td>
<td>Antibacterial</td>
<td>[8]</td>
</tr>
<tr>
<td>Chitosan-blended polyamide</td>
<td>Cytotoxicity</td>
<td>[9]</td>
</tr>
<tr>
<td>Multiwalled carbon nanotube incorporated PVA/chitosan</td>
<td>Cytotoxicity</td>
<td>[10]</td>
</tr>
<tr>
<td>Gold nanoparticles and lysozyme deposited cellulose</td>
<td>Antibacterial</td>
<td>[11]</td>
</tr>
<tr>
<td>PVA–AgNPs</td>
<td>Cytotoxicity</td>
<td>[12]</td>
</tr>
<tr>
<td>Poly(L-lactide) ultrafine fibers containing nanosilver</td>
<td>Antibacterial</td>
<td>[13]</td>
</tr>
<tr>
<td>N-carboxyethylchitosan and poly(ethylene oxide) nanofibres containing AgNPs</td>
<td>Antibacterial</td>
<td>[14]</td>
</tr>
<tr>
<td>Gelatin fiber mats containing silver nanoparticles</td>
<td>Antibacterial</td>
<td>[15]</td>
</tr>
<tr>
<td>PVA/ chitosan/nano-ZnO composite nanofibrous membranes</td>
<td>Antibacterial and antifungal</td>
<td>[16]</td>
</tr>
<tr>
<td>Paclitaxel incorporated pHEMA-bamboo cellulose nanocomposite fibers</td>
<td>Cytotoxicity and anticancer</td>
<td>[17]</td>
</tr>
<tr>
<td>Hydrolyzed poly[2-(3-thienyl) ethanol butoxy carbonyl-methyl urethane]/cellulose acetate</td>
<td>Biosensors</td>
<td>[18]</td>
</tr>
</tbody>
</table>

Table 1. Synoptic table of electrospun nanofibers developed for biomedical and pharmaceutical applications.
cells while minimally affecting normal nonmalignant cells. The nonspecific nature of many current anticancer agents severely limits their effectiveness if the dosage is too high and the systemic toxic effects outweigh the beneficial anticancer effect.

This demonstrates the need to use AuNPs as a promising alternative for the treatment of various diseases in general and cancer in particular. The rationale for using AuNPs for cancer treatments can be ascribed to their large surface area for volume, porosity, solubility, increased bioavailability, ease of passing through the cellular barriers, strongly interacting with functional biomolecules and different structural properties. The use of green synthesized AuNPs to prepare organic–inorganic hybrid nanofibers has marked the start of ‘green’ practices to take their place in the electrospinning process by integrating with recent developments in science and industry in attempts to reduce the generation of hazardous waste in the environment. This attempt aims at minimizing the use of unsafe products and maximizing process efficiency while using environmentally safe nontoxic materials.

In pursuit of overcoming the above-said shortcomings of chemical synthesis of nanoparticles, microbial resistance towards conventional antibiotics, anticancer drugs, treatments, we have developed a unique green organic–inorganic hybrid nanofibers using electrospinning. The prepared organic–inorganic hybrid nanofibers scaffold was assessed for biocompatibility with Vero cell lines, antiproliferative activity on breast cancer cell lines (MCF7) and cervical cancer cell lines (HeLa), antibacterial and antifungal activities. The ultimate aim of the present work is to achieve optimum antibacterial, antifungal, biocompatibility and antiproliferative activities at a very low loading of AuNPs.

Experimental

Materials

Chloroauric acid (SRL, Mumbai, India), Muller-Hinton agar (Himedia Mumbai, India), Poly (vinyl alcohol) (PVA)-Mw 89,000–98,000 (Sigma-Aldrich, Bangalore, India) were used as received without further treatment or purifications.

Cell lines and maintenance

Minimal Essential Media (HiMedia Laboratories, Mumbai, India) were used as received without further treatment or purifications. Vero cell lines, MCF 7, HeLa cancer cell lines were obtained from National Centre for cell sciences (NCCS), Pune, India. The cells were maintained in Minimal Essential Media which were supplemented with 10% fetal bovine serum (Cistron Laboratories, Chennai, India), penicillin (100 U/mL) and streptomycin (100 µg/mL) in a humidified atmosphere of 50 µg/mL CO2 at 37°C. Trypsin, methylthiazolyl diphenyl-tetrazoliumbromide (MTT) and dimethyl sulfoxide (DMSO) from Sisco Research Laboratory Chemicals, Mumbai, India, were used as received.

Source of microorganisms

The strains Candida albicans and C. krusei were obtained from VHS hospital, Chennai, India. Pure cultures of Bacteria Escherichia coli (ATCC 8739), Staphylococcus aureus (ATCC 6538), Micrococcus luteus (ATCC 4698), Klebsiella pneumoniae (ATCC 13883), Bacillus subtilis (ATCC 6633) and Pseudomonas aeruginosa (ATCC 15442) were obtained from American Type Culture Collection.

Green synthesis of AuNPs

Aqueous extract of Couroupita guianensis was prepared following the procedure as reported in our previous work. The steps in green synthesis of AuNPs are given in Figure 1.

Characterization of the green synthesized AuNPs

After the synthesis process was completed by reducing metal ion solution with leaves extract, surface plasmon resonance of AuNPs was easily confirmed by Diffuse reflectance ultraviolet–visible (UV-Vis) spectroscopy. The reaction mixture was sampled at regular intervals and the absorption maxima was scanned at the wavelength of 400–800 nm using Shimadzu UV-Vis spectrophotometer (model 2450; Tokyo, Japan). The biosynthesized AuNPs gave sharp peak in the visible region of the electromagnetic spectrum. The X-ray powder diffraction data was acquired by PAN analytical X’Pert PRO diffractometer in Bragg–Brentano geometry using step scan technique and Johansson monochromator to produce pure Cu Kα1 radiation (1.5406 Å; 45 kV, 30 mA) in the range of 30°–80°. The peaks were matched with (JCPDS No. 01-1174). The obtained pattern was for fcc cubic crystal structure. The peak plane matched with the card. The crystalline size was calculated from the full-width at half-maximum (FWHM) of the diffraction peaks using the Debye–Sherrer formula. The Fourier transform-infrared spectroscopy (FTIR) spectra for biosynthesized AuNPs were recorded on an IR Affinity-1 SHIMADZU spectrophotometer in transmittance mode in the range of 400–4000 cm⁻¹ at a resolution of 4 cm⁻¹. For high-resolution transmission electron microscope (HRTEM) measurements, a drop of solution containing synthesized AuNPs was placed on the carbon-coated...
grids and kept under vacuum desiccation overnight before loading them onto a specimen holder. HRTEM micrographs were taken by analyzing the prepared grids on 300 kV field emission TEM-STEM (FEI F30) with capability of HAADF, EELS and EDX.

Electrospinning process to fabricate organic–inorganic hybrid nanofiber (PVA-AuNPs)

The electrospinning set-up used in the present work was designed and developed in Anna University, Department of Chemistry, Chennai. Optimized weight percentage of 10% (w/v) PVA and 0.1% (w/v) of green synthesized AuNPs were dissolved in double-distilled water by continuous stirring for 2–4 h to get a homogeneous solution. The polymer solution mixed with AuNPs was taken in a 2-mL syringe to which a needle tip of 0.56 mm inner diameter was attached. The positive electrode of the high-voltage power supply was connected to the needle and the negative terminal to the collector. The polymer solutions were electrospun at a distance of 12 cm from the needle tip with a flow rate of 0.35 mL/h and an optimized applied electric voltage of 15 kV to produce beadless organic–inorganic hybrid nanofibers.

Characterization of electrospun organic–inorganic hybrid nanofibers (PVA-AuNPs)

Spectral analysis

The functional groups in the electrospun organic–inorganic hybrid nanofibers were identified by Fourier transform-infrared spectroscopy (FTIR) IR Affinity-1 SHIMADZU spectrophotometer in transmittance mode in the range of 400–4000 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\). Diffuse reflectance UV-Vis spectroscopy was used to obtain the spectra for electrospun organic–inorganic hybrid nanofibers. The spectra were recorded between 400 and 800 nm on a Shimadzu UV-Vis spectrophotometer (model 2450; Tokyo, Japan).

Surface morphological analysis

The morphology of electrospun organic–inorganic hybrid nanofibers was analyzed by Field emission Scanning electron microscope (FESEM) HITACHI Su-6600 with an energy-dispersive analysis of X-ray (EDAX) attachment and HRTEM FEI, TECHNAI G\(^2\) 30 S-twin D905. The mean diameter and distribution of the hybrid nanofibers were measured from at least 100 nanofibers from various FESEM images using UTHSCSA image tool. Atomic force microscope (AFM, Seiko SPI3800N, series SPA-400 (Tokyo, Japan)) was used to study the surface topography and surface morphology of the organic–inorganic hybrid nanofibers.

Contact angle measurements

The hydrophilicity of the electrospun organic–inorganic hybrid nanofibers was evaluated using contact angle measurements by placing the sample on the holder of Euromex Optical Microscope equipped with a CCD camera. A drop of deionised water (10 \(\mu\)L) was
deposited on the sample surface. The contact angle of the drop on the surface was measured at room temperature (27°C). Five measurements were performed at different locations and the contact angles were calculated with the help of ‘UTHSCSA Image tool’ software.

Assessment of biocompatibility and antiproliferative activities of organic–inorganic hybrid nanofibers (PVA-AuNPs)

The cytotoxic activity of organic–inorganic hybrid nanofibers was assessed on Vero cell lines and the anticancer activity was assessed on MCF 7 (breast cancer cell lines) and HeLa (cervical cancer cell lines) by the MTT assay method. The Vero cell lines, MCF 7 and HeLa cell lines were plated in 0.2 mL of the Minimal Essential Medium in 96-well plates. These cells reached confluence after 72 h of incubation. Then the Vero cell lines, MCF 7, and HeLa cell lines were incubated with PVA nanofibers without AuNPs (control) and organic–inorganic hybrid nanofibers (disinfected by ultraviolet C irradiation for 1 minute) in 0.1% DMSO at various dilutions for a period of 72 h at a temperature of 37°C. After 72 h of incubation, 0.5% of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide cells (MTT) in phosphate-buffer saline solution was added. The absorbance was measured at 630 nm using UV spectrophotometer.

The results are expressed as mean standard error of the absorbance. Data were analyzed by Student’s t-test and differences at the 95% confidence level were considered to be significant. The cells after biocompatibility and antiproliferative tests were visualized using FLoid Cell Imaging Station, California, USA.

Assessment of antibacterial and antifungal activities of hybrid nanofibers (PVA-AuNPs)

The antibacterial and antifungal activities of organic–inorganic hybrid nanofibers were assessed by an inhibition zone method. A loop of the bacterial culture and fungal culture were inoculated from fresh colonies on agar plates into 100 mL Muller Hinton culture medium separately. The culture was allowed to grow until the optical density reached 0.2 at 600 nm (OD of 0.2 corresponding to a concentration of 10^8 CFU mL⁻¹ of medium). This indicates that the bacterial and fungal culture are in exponential or log phase of growth, which is ideal for the experiment. Then it was swabbed uniformly onto individual Mueller Hinton agar plates using sterile cotton swabs. Organic–inorganic hybrid nanofibers and control (PVA nanofibers without AuNPs) disinfected by ultraviolet C irradiation for 1 minute were cut into 5-mm discs and placed in the culture-swabbed Petri plate. The plates were examined for possible clear zone formation after overnight incubation at 37°C. The diameter of the clear zone formed around the organic–inorganic hybrid nanofibers on the plates was measured and recorded as an inhibition against the test bacterial strains and fungal species.

Results

Characterization of the biosynthesized AuNPs

The UV-vis spectra show a well-defined surface plasmon band centered at around 526 nm (Figure 2), which is the characteristic of AuNPs and clearly indicates the formation of AuNPs in solution. It may be due to the excitation of surface plasmon resonance (SPR) effect and reduction of AuCl₄⁻ ions. The stability results from a potential barrier that develops as a result of the competition between weak Vander Waals forces of attraction and electrostatic repulsion. The solution was stable even after 120 days of reaction, with no evidence of aggregation of particles. Figure 3(a) shows the XRD pattern of the synthesized AuNPs. The five diffraction peaks observed at 38°, 45°, 67°, 78°, and 81° in the 2θ range can be indexed to the (111), (200), (220), (311), (222) reflection planes of face-centred cubic structure of metallic gold nanopowders. The average size of the AuNPs was found to be about 15 nm. HRTEM micrographs and the particles size distribution histogram determined from micrograph of the AuNPs are shown in Figure 3(b and c), respectively. Monodispersed, discrete spherical-shaped gold particle was observed in HRTEM micrograph. HRTEM micrograph showed that the size of the synthesized AuNPs were in the range of 4–13 nm. EDX spectrum of AuNPs shows different X-ray emission peaks with strong

![Figure 2. Diffuse reflectance spectrum of AuNPs.](image-url)
signals from the atoms in the AuNPs (Figure 4). This indicates the reduction of gold ions in the chloroauric acid to elemental gold. The crystalline nature of AuNPs was confirmed from X-ray diffraction (XRD) analysis. The FTIR spectrum of AuNPs (Figure 5b) shows the presence of peaks at 1637, 1471, 1372 cm$^{-1}$. The strong absorption peak at 1637 corresponds to Alkenyl C=C stretch which is the characteristic of gold atoms. The band at 1471 and 1372 cm$^{-1}$ correspond to aromatic ring stretch and methyl C-H symmetrical bend, respectively.

Spectral analysis

FTIR spectrum of organic–inorganic hybrid nanofibers is shown in Figure 5(c). The spectra for PVA and AuNPs are depicted in Figure 5(a and b), respectively. In the spectrum shown in Figure 5(a), the polymer hydroxy band at 3419 cm$^{-1}$ appears due to the presence of CH-OH stretching vibrations of PVA. The bands appearing at 2938 cm$^{-1}$ and 1423 cm$^{-1}$ correspond to the aliphatic stretching and bending vibrations of PVA. The spectrum obtained for AuNPs (Figure 5b) shows several absorption peaks at 1637 cm$^{-1}$, 1471 cm$^{-1}$ and 1372 cm$^{-1}$. The spectrum for organic–inorganic hybrid nanofibers shows appearance of band at 1728 cm$^{-1}$ formed due to a slight shift of band at 1637 cm$^{-1}$ in the AuNPs spectrum. The remaining bands at 1447 cm$^{-1}$ and 1383 cm$^{-1}$ appear due to the bending vibrations of AuNPs. This confirms that the AuNPs distributed in the PVA matrix are stable after electrospinning. The observed drastic decrease in the absorbance of the peak at 3419 cm$^{-1}$ could be attributed to the interactions between the AuNPs and some functionalities of the PVA matrix molecules particularly the O-H.
Figure 6 shows the diffused reflectance spectrum of PVA-AuNPs hybrid nanofibers. The spectrum shows a peak at 568 nm formed due to a slight shift in the original absorption peak (526 nm) of AuNPs. However, the slight shift in the peak maximum that corresponds to AuNPs could be attributed to the interaction between the polymer hydroxy group of PVA and AuNPs in the hybrid nanofiber leading to some degree of local particle aggregation. These observations throw light on the probability of existence of hydrogen bonding between the organic and inorganic components, which led to better compatibility besides the absence of an organic surfactant or a compatibilizer which are normally added to improve the dispersion of inorganic nanoparticles in the polymer matrix.

Surface morphological analysis

PVA was miscible with AuNPs and had good electrospinnability when blended. Figure 7(a and b) show the FESEM and HRTEM micrographs. They were used to visualize the morphology of the hybrid nanofiber and distribution of AuNPs in the hybrid nanofiber. Elemental composition of hybrid nanofibers was analyzed by energy-dispersive X-ray spectroscopy. Figure 7(c) shows the optical absorption peak for Au at 2 keV which is typical for metallic AuNPs. Atomic force micrographs (Figure 7d) showed that AuNPs can significantly influence the surface topography of the nanofiber. Uniform nanoparticles dispersion can be seen on the surface of the hybrid nanofiber. Increase in surface roughness of the hybrid nanofiber may be attributed to the presence of AuNPs on the surface.

From the FESEM and HRTEM micrographs, similar morphologies and homogeneous appearance of AuNPs were observed in the beadless fibers. Mahalingam and Edirisinghe reported that bead-free continuous fibers could be formed when the polymer concentration is above the critical concentration. As in the present study, the concentration of PVA was higher and the loading of AuNPs was minimum, the overlapping of polymer chains formed sufficient entanglement networks of polymer chains and yielded smooth, beadless fibers.

Figure 8 show the diameter distribution of the hybrid nanofibers. The diameter of the hybrid nanofibers was in the range of 50–450 nm. The increase in the diameter of the hybrid nanofibers when compared to the diameter of neat PVA nanofibers which was in the range of 50–300 nm as reported by one of the authors could be attributed to the increase in charge density and shear viscosity upon addition of AuNPs to PVA, thereby increasing the electrical force which consecutively can also cause the actual mass throughput to increase. Thus, the increase in the hybrid fiber diameters from that of the neat PVA fibers should be due to the addition of AuNPs. The Quasi-spherical-shaped AuNPs were slightly larger and were distributed in an encircling manner on the surface of the individual PVA fiber (Figure 7b), yet the same smooth and beadless morphology of hybrid nanofibers were retained.

It can be seen from the FESEM micrographs that the asymmetry of the nanofiber was apparent and the dispersion of AuNPs did not visibly alter the nanofiber structure. It can be also noticed when HRTEM micrographs are compared with FESEM ones, the FESEM micrographs depict AuNPs sparsely dispersed in the mesh-like hybrid nanofiber which is informative only
about the surface morphology of the hybrid fiber, the electron beam in HRTEM analysis passes through the nanofibers, which could perhaps detect the exact dense encircling orientation of the AuNPs on the surface of the nanofibers. Though there might be hydrogen bonding between PVA and AuNPs which apparently leads to van der Walls gaps, the organic and inorganic components retained their respective characteristics, and surface modification of the AuNPs might be needed to promote interfacial adhesion between PVA and AuNPs which would have led to the distribution of the AuNPs into the nanofiber. This would probably explain the reason for orientation of AuNPs on the surface of the hybrid nanofiber. The nature of distribution and the bonding interaction of AuNPs in the hybrid nanofiber is illustrated graphically in Figure 9. This observation might add a novel feature to the hybrid nanofiber, since there are not many reports to our knowledge on such a distribution of AuNPs on the surface of the nanofiber and such a distribution did not hinder the biocompatibility and antiproliferative properties of the hybrid nanofiber. Moreover, this type of distribution of AuNPs encircling individual fibers might aid in the rapid release of gold ions which would play a major role in enhanced antimicrobial property of the hybrid nanofiber.

**Contact angle measurements**

Figure 10(a and b) show the contact angle measurements for PVA nanofiber (control) the hybrid nanofiber. The results show that the hydrophilicity increases.
after AuNPs incorporation. The contact angle for PVA nanofiber and hybrid nanofiber was 41° and 33°, respectively, for 0 min, which decreased with respect to time. The contact angle for PVA nanofiber and hybrid nanofiber was 36° and 20°, respectively, for 5 min. Thus, the AuNPs which are localized on the surface of the individual fibers control the hydrophilicity of the hybrid nanofiber. The increase in hydrophilicity would precisely yield a positive feedback on cell adhesion studies.54

### Assessment of biocompatibility and antiproliferative activities of organic–inorganic hybrid nanofiber (PVA-AuNPs)

For the application of organic–inorganic hybrid nanofibers therapeutically it is essential to evaluate its biocompatibility particularly its nontoxicity to normal cells. The bar chart in Figure 11(a) shows the percentage of biocompatibility of organic–inorganic hybrid nanofibers with Vero cell lines. There was a maximum percentage of Vero cell viability (90%) at the end of 72-h incubation. Figure11(b) shows that the cell proliferation increased in direct proportion to the time of incubation. This indicates that the nutrition to the cell lines was not hindered by the organic–inorganic hybrid nanofibers, leading to increased viability of the cells. It can be seen from the FESEM micrograph (Figure 11c) that the cell lines proliferated in the direction of the fiber orientation according to the architecture of the nanofibers densely covering the voids by cytoplasmic extensions, and some cells migrated underneath the fibers maintaining their morphology. The existence of an optimally dispersed mesh-like morphology in the organic–inorganic hybrid nanofibers which has been demonstrated from FESEM micrographs could be believed to have yielded a suitable environment for cell adhesion and proliferation on the surface.55

Figure 12 shows the increasing cell density in the Vero cells treated with hybrid nanofibers. Cell adhesion behavior of Vero cell lines onto organic–inorganic hybrid nanofibers could have also been mediated via the electrostatic interaction between the positively charged PVA and the negatively charged cell membranes and the AuNPs did not hinder this electrostatic interaction.56 The cell adhesion and proliferation could have been activated due to good hydrophilicity of organic–inorganic hybrid nanofibers and the presence of recognition sites. Thus, it can be concluded that the organic–inorganic hybrid nanofibers can obviously improve the cell growth behaviors.54 The presence of AuNPs in the electrospun hybrid nanofiber shows antiproliferative effects in MCF-7 and HeLa cell lines. The percentage of antiproliferation activity of the organic–inorganic hybrid nanofibers on MCF 7 and HeLa cell lines is given in Figure 13(a and b), respectively. The percentage of proliferation of MCF 7 and HeLa cell lines were only 8% and 9%, respectively at the end of 72-h incubation with hybrid nanofibers. It can be seen from the optical density values (Figure 14a and b) that the MCF 7 and HeLa cell proliferation decreased in direct proportion to the time of incubation of the cell lines treated with hybrid nanofibers, whereas in the cell lines treated with only PVA nanofibers (without AuNPs) the cell proliferation was high. It can be seen from the FESEM images (Figure 15a and b) at the end of 72-h incubation, the organic–inorganic hybrid nanofibers architecture was disrupted which was evident from the broken fibers. The dead MCF 7 and HeLa cell lines could have made the fibers brittle leading to breakage. Figures 16 and 17 show the reducing cell densities of MCF 7 and HeLa cell lines, respectively.
Figure 11. (a) Bar chart showing percentage of cell viability of Vero cells on treating with organic–inorganic hybrid nanofibers. (b) OD values of Vero cells proliferation with respect to time. (c) FESEM micrograph showing high cell density of Vero cells on treating with organic–inorganic hybrid nanofibers at the end of 72 h.

Figure 12. (a) Control-Vero cells treated with PVA nanofibers at the end of 72 h. (b) Vero cells treated with organic–inorganic hybrid nanofibers at the end of 24 h incubation. (c) Vero cells treated with organic–inorganic hybrid nanofibers at the end of 48-h incubation (D) Vero cells treated with organic–inorganic hybrid nanofibers at the end of 72-h incubation.
at 24, 48 and 72 h of incubation with hybrid nanofiber. The AuNPs accumulate inside the cancer cells and sequesters in large clusters in vacuoles in the perinuclear areas of the cytoplasm. The size of AuNPs is an important factor that influences the rate of endocytosis and exocytosis, and thus the level of cellular accumulation. The mechanism of antiproliferative activity of AuNPs may be linked to the ATP consumption in the DNA repair process. ATP is generally known to control apoptotic signals since apoptosis is inhibited at physiological ATP concentrations. A decrease in the intracellular ATP concentration induces apoptosis, by regulating the activity of bax, which is also crucial for caspase-3 activation which is a common downstream effector of both extrinsic and intrinsic apoptosis pathways, thus contributing to cell death. AuNPs also induced an increase in the mRNA expression of bax and bak, which are pro-apoptotic members of the Bel-2 family and responsible for the induction of intrinsic mitochondria apoptosis. AuNPs loaded in the PVA nanofibers target the signaling molecules that are highly expressed in cancer cells and the normal cells remain unaffected. Higher cytotoxicity of smaller particles compared to larger ones is related to the amount of reactive oxygen species (ROS) generated at the relatively larger surface area of small nanoparticles. Smaller AuNPs release more gold ions from its surface than larger nanoparticles. Oxidative stress is induced when the generation of ROS exceeds the cell’s antioxidant capacity. Besides the damaging effects to cellular proteins, lipids and DNA, an increasing level of ROS triggers the cell to respond by activating pro-inflammatory signaling cascades, and ultimately induces programmed cell death. As we observed aggregation of AuNPs on the organic–inorganic hybrid nanofibers

Figure 13. (a) Bar chart showing percentage of cell proliferation of MCF 7 cells on treating with organic–inorganic hybrid nanofibers. (b) Bar chart showing percentage of cell proliferation of HeLa cells on treating with organic–inorganic hybrid nanofibers.

Figure 14. (a) OD values of MCF 7 cells proliferation with respect to time. (b) OD values of HeLa cells proliferation with respect to time.
after incubation with the cancer cell lines from the FESEM images (Figure 12c), the increase in antiproliferative activity can be attributed to this aggregation of AuNPs which leads to long-term retention. Consequently, the membrane potential of the mitochondria is decreased and the levels of reactive oxygen species are increased, leading to cell death as reported by Cui et al.\textsuperscript{63} Thus, it is obvious that the organic–inorganic hybrid nanofibers that are biocompatible as well as toxic to cancer cells can combine diagnosis and therapy and can contribute broadly to biomedicine.

**Figure 15.** (a) FESEM micrograph showing broken organic–inorganic hybrid nanofibers due to MCF 7 cell death at the end of 72 h. (b) FESEM micrograph showing broken organic–inorganic hybrid nanofibers due to HeLa cell death at the end of 72 h.

**Figure 16.** (a) Control-MCF 7 cell lines treated with PVA nanofibers at the end of 72 h. (B) MCF 7 cell lines treated with organic–inorganic hybrid nanofibers at the end of 24-h incubation. (c) MCF 7 cell lines treated with organic–inorganic hybrid nanofibers at the end of 48-h incubation. (D) MCF 7 cell lines treated with organic–inorganic hybrid nanofibers at the end of 72-h incubation.

**Antibacterial and antifungal activities of organic–inorganic hybrid nanofibers**

The results showed significant inhibitory activity of organic–inorganic hybrid nanofibers against all the tested microorganisms, as shown in Figure 18. While the PVA nanofibers without AuNPs (control) did not show any antibacterial and antifungal activity. The diameter of the zone of inhibition of organic–inorganic hybrid nanofibers against the test pathogenic bacterial and fungal strains is given in Figure 19. The results manifested that the antibacterial and antifungal ability
of the PVA nanofibers depend on the presence of AuNPs on the surface of the fibers. AuNPs on the surface of organic–inorganic hybrid nanofibers manifest effective antibacterial and antifungal property due to their smaller dimension and higher specific area. Gold ions are released when the organic–inorganic hybrid nanofibers were brought in contact with the test bacterial and fungal cultures in the Petri plate, which resulted in the formation of zone of inhibition. This may be due to the fact that the release of AuNPs becomes easier as the particle size decreases, so that AuNPs can more effectively reach the microbial region subsequently increasing their contact with the microorganism. In addition, smaller dimensions and

Figure 17. (a) Control-HeLa cell lines treated with PVA nanofibers at the end of 72 h. (b) HeLa cell lines treated with organic–inorganic hybrid nanofibers at the end of 24-h incubation. (c) HeLa cell lines treated with organic–inorganic hybrid nanofibers at the end of 48-h incubation. (d) HeLa cell lines treated with organic–inorganic hybrid nanofibers at the end of 72-h incubation.

Figure 18. Zone of inhibition of organic–inorganic hybrid nanofibers on test pathogenic bacterial and fungal strains.
higher surface-to-volume ratios of AuNPs also enhance their contact with the microorganism. The inhibitory activity of the organic–inorganic hybrid nanofibers against the Gram-positive bacteria is better than that against Gram-negative bacteria. These results are in close agreement with those reported by Thiel et al. The antibacterial properties of AuNPs could be believed to be the same as silver nanoparticles that are associated with its slow oxidation and liberation of silver ions (in the case of AgNPs) and gold ions (in the case of AuNPs) to the microbial environment, making it an ideal biocidal agent. Moreover, the small size of these particles facilitates the penetration of these particles through cell membranes to affect intracellular processes from inside. It was reported that AgNPs exhibited excellent antifungal activity on Candida albicans by disrupting the cell membrane and inhibiting the normal budding process. Similar mechanism could be attributed to the antifungal activity of AuNPs in the present work. Exposure of AuNPs to bacterial and fungal cells resulted in alterations in the expression of a panel of envelope and heat shock protein. Consequently, these particles can penetrate and can disrupt the membranes of microorganisms. A massive loss of intracellular potassium was induced by AuNPs. Furthermore, the AuNPs decreased the ATP levels. The possible molecular targets for the AuNPs could be protein thiol groups present in enzymes such as NADH dehydrogenases and disrupt the respiratory chain, facilitating the release of reactive oxygen species, leading to oxidative stress, and resulting in significant damage to the cell structures and ultimate cell death. The phospholipid portion of the bacterial membrane may also be the site of action for the AuNPs. As the antimicrobial effect of AuNPs was believed to be closely related to that of AgNPs, which was brought about by the formation of pits in the cell wall leading to change in morphology and significant increase in permeability, leaving bacterial cells incapable of properly regulating transport through the plasma membrane, resulting in cell death. The increase in permeability of the cell membranes would allow the AuNPs to penetrate the cell and cause cell death by breaking the double-stranded DNA.

**Conclusions**

AuNPs were synthesized by a green route using Couroupita guianensis leaves extract and characterized. The green synthesized AuNPs were loaded into PVA and electrospun to develop organic–inorganic hybrid (PVA-AuNPs) nanofibers. Surface morphological analyses like SEM, HRTEM revealed the presence of AuNPs on the surface of the electrospun hybrid nanofibers. PVA being a hydrophilic polymer matrix by itself, became more hydrophilic upon very low loading of AuNPs. This increase in hydrophilicity led to good cell adhesion and proliferation when tested for biocompatibility on Vero cell lines. Organic–inorganic hybrid nanofibers imparted good antiproliferative activity against MCF 7 (breast cancer cell lines), HeLa (cervical cancer cell lines) and also exhibited promising antibacterial and antifungal activities against test pathogenic bacterial strains, E. coli, S. aureus, M. luteus, K. pneumoniae, B. subtilis, P. aeruginosa and pathogenic fungal strains Candida albicans, and C. krusei. The organic–inorganic hybrid nanofibers thus developed showed great potential for biomedical applications namely antimicrobial wound dressing and cancer treatment. Organic–inorganic hybrid nanofibers may also be beneficial in overcoming some of the challenges prevailing in current cancer treatment procedures and antibiotic resistance in microorganisms. Another direction for future research of this organic–inorganic hybrid nanofiber is AuNPs may be conjugated with the prevailing anticancer drugs, antibiotics and electrospun with PVA matrix to assess their synergistic anticancer and antimicrobial activities. This aims at reducing the dosage and side effects caused by the prolonged use of conventional drugs and antibiotics which is currently under investigation in our laboratory.

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