Mythology Converges With Technology To Combat Biomaterials Associated Infections

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ABSTRACT

The integration of nanotechnology, biotechnology, biology, physical science, chemistry, biomedical science and medicine; for the best scope, development and application in science and technology is being studied for decades. But the intimation of the immense potentiality of mythology converging with technology is scarce. For the first time, we report here the combo power of the mythological Peepal tree, *Ficus religiosa,* in convergence with nanotechnology to combat biomaterials associated infections. Infections associated with biomaterials such as medical devices, implants and prosthetic materials, are due to bacterial adhesion and subsequent biofilm formation at the implantation site. Although much research has been focused on sterilization, aseptic procedures and on developing polymers that resist biofilm formation; bacterial infection still remains a major impediment in the utility of such biomaterials. The emergence of multidrug resistant 'super bugs', like the ones recently discovered, 'NDM-l' (New Delhi Metallo-beta-lactamase), have made this global problem rather alarming. For reducing this incidence of biomaterials associated infections we have developed self-sterilizing silver/chitosan bionanocomposite via biosynthesis for application as coating over biomedical implants and devices. This material has been characterized through various techniques and antimicrobial studies have also been done.

Keywords - nanotechnology, nanoparticles, bionanocomposite, biomaterials, antimicrobial

1. INTRODUCTION

Materials and material development are fundamental to our very culture. With the system of ascribing major epochs of our society to materials, such as the stone age, bronze age, iron age, steel age, silcon age; this is apparently and undoubtedly the nano age. In this nanoregime the use of nanotechnology and designing nanomaterials is becoming increasingly popular in almost every field and especially in the arena of biomedical engineering it holds a significant advantage over others.

Today silver nanocoomposites have got myriads of interesting and demanding applications chiefly in the nanomedicine landscape [1]. A variety of methods have been developed thus to achieve control over the properties of nanoparticles and nanostructered materials strongly depended upon their dimensions and structure [2]. Furthermore, the biosynthesis of nanoparticles has attracted world wide attention because of the necessity to bring environmentally friendly, cost-effective and efficient techniques. The biological reduction of metals by plant extracts has been known since the early 1900s; however the reduction products were not studied. Only recently within the last three decades, the synthesis of nanoparticles using plant materials, for the most part, is being properly investigated [3]. The synthesis of silver nanoparticles from plant resources is now an expanding research area as it eliminates the call for harsh or toxic reagents and minimizes hazardous byproducts [4].

Thus in the field of nanoscience the synthesis of silver nanoparticles using plant-derived materials is a simple yet effective method for the production of nanoparticles having invaluable applications in the field of biomedical science [5]. On-going research efforts in the biomedical arena have brought forward the hypothesis that silver-containing material can mitigate the menace of biomaterial associated nosocomial infection which has been confirmed by several in-vitro and in-vivo experiments [6]. Silver nanoparticles having high surface reactivity due to high surface to volume ratio, releases silver ions which is highly antimicrobial with the ability to kill a very broad spectrum of medically relevant bacteria (gram positive and gram negative) as well as fungi (molds and yeasts) [7]. Ionic silver is also oligodynamic, which means that it is antimicrobial at very low doses, as low as about 0.001- 0.05 ppm [8]. Although silver is a heavy metal, at the reference low concentration amounts, it is non toxic to human cells and much safe [9].

Therefore we have designed silver/chitosan bionanocomposite (Ag/CS BNC) having antimicrobial and self-sterilizing properties to mitigate the menace of biomaterials associated infections and which when leached out will cause minimal harm to the human body. Chitosan used as the matrix can protect silver nanoparticles from uncontrolled oxidation and stabilize them from agglomeration. Chitosan is FDA approved, natural biopolymer derived by deacetylation of chitin, a component commonly found in the exoskeleton of crab, shrimp and crawfish and is the second most abundant biopolymer after cellulose. It is composed of poly [β-(1→4)-2-amino-2-deoxy-D-glucopyranose] and has many advantageous features like biocompatibility, biodegradability, non-toxicity, hydrophilicity, together with antimicrobial properties [10].

The mythological tree of eternal life, Peepal tree, *Ficus religiosa*, referred as, Lord Krishna, who is revered as the omnipotent universal savior, has intervened for the biosynthesis of silver nanoparticles. This is certainly for the first time that the divine intervention of this plant has been involved for the formation of nanoparticles; for the development of bactericidal bionanocomposite material; for being applied as self-sterilizing coating over biomedical implants, prosthesis and devices. Mythology converges here with nanotechnology to combat biomaterials associated infections and conquer the race between bacterial adhesion and tissue integration.

2. MATERIALS AND METHOD

2.1 Materials

Chitosan (degree of deacetylation: 79 %, molecular mass: 500,000 g/mol) was purchased from Sea Foods (Cochin), India; $AgNO_3$ of analytical grade from Thomas Baker (Chemical) Pvt. Ltd. India; acetic acid glacial (extra pure) from Thomas Baker (Chemical) Pvt. Ltd. India. Solutions were prepared using deionized water. Leaves were collected freshly from the plant *Ficus religiosa*.

2.2 Preparation of plant extract

Leaves of *Ficus religiosa* were washed and air dried. 21 g of clean leaves were cut into fine pieces and boiled in 100 ml of sterile distilled water in a 500 ml Erlenmeyer flask for 15 min at 100 °C. The crude plant extract was filtered using Whatman No. 41 filter paper and stored in closed bottle at 4 °C for further use.

2.3 Synthesis of silver nanoparticles

5 ml of the aqueous plant extract was added to 100 ml of 1 mM silver nitrate ($AgNO_3$) solution and this was marked as (1). The reaction mixture was kept in closed bottle and incubated at RT for stabilization.

2.4 Development of silver/chitosan bionanocomposite

After 48 h the reaction mixture was centrifuged at 9,000 rpm for 15 min and the residue (AgNP pellet) was re-dispersed in distilled water. This procedure was repeated three times to isolate Ag nanoparticles from proteins or other bio-organic compounds present. The remnant pellet was dispersed in 15 ml of chitosan solution [2% (w/v) in 1% (v/v) acetic acid] and sonicated for 10 min. Finally silver/chitosan (Ag/CS) bionanocomposite film (1') was prepared by casting the bionanocomposite suspension on a glass plate (solvent casting) and dried at room temperature [11].

2.5 Coating

Coating of medical implant, such as stainless steel rod was done by pouring the bionanocomposite suspension material (1') on it (solvent casting technique) and air dried. Facile and less time consuming method of coating was adopted for preliminary and basic studies.

2.6 UV-visible absorbance spectroscopy study

The reduction of Ag^+ to Ag^0 was monitored by measuring the UV-Vis. spectrum of reaction mixture (having $AgNO_3$ solution + plant extract) after 48 h when complete stabilization and no further color transformation was observed. The spectra of plant extract alone and $AgNO_3$ solution were also taken. The UV-visible spectra were recorded using UV-visible spectrophotometer (Shimadzu UV – 2450) from 200 to 800 nm. Deionised water was used as blank.

2.7 Nanoparticle size analysis

Analysis of the nanoparticle size was done with the help of (NANOTECH) particle size analyzer instrument.

2.8 TEM observations

Samples for transmission electron microscopy (TEM) of the silver nanoparticles synthesized were prepared by placing drops of the product solution onto carbon-coated copper grids and allowing the solvent to evaporate. TEM measurements were performed on the (HR TEM TECNAI 20 G^2) instrument operated at an accelerating voltage of 200 kV.

2.9 SEM study

Silver/chitosan bionanocomposite was coated with a thin layer of graphite and examined in a scanning electron microscope (SEM) (JEOL JXA 8100).

2.10 FTIR analysis

FTIR spectrum was recorded over the range of (500-4000) cm^{-1} with (with FTLA 2000 ABB).

2.11 Antimicrobial activity test

The bionanocomposite (BNC) was assayed for antimicrobial activity against *Pseudomonas aeruginosa* (gram negative), and *Staphylococcus aureus* (gram positive). Disc diffusion method was used to find out the standard zone of inhibition (ZOI) [12]. Antibacterial test was done against (1') bionanocomposite film (disc shaped pieces 5 mm in diameter); biomaterial like medical grade stainless steel rod (30 x 12 mm) coated with bionanocomposite (1') (SSR). Chitosan film (CS) was taken as positive control and uncoated biomaterial (B) as negative control. The materials to be assayed were sterilized by UV radiation for 30 min and placed on different cultured agar plates. Muller Hinton Agar was used as culture media and inoculated with 300 µl of bacterial organism containing broth. The plates containing the bacterial organism and silver nanocomposite films were incubated at 37°C for 2 days. The plates were then examined for evidence of zones of inhibition, which appear as a clear area around the discs. The diameter of such zones of inhibition was measured using a meter ruler.

3. RESULTS AND DISCUSSION

3.1 UV-visible spectral studies

The reaction mixture (1) demonstrated increase in colour development with time. The process of colour development and the transformation from colourless to reddish brown was rapid, which indicated the formation of silver nanoparticles. The UV–visible absorption spectrum recorded from the nanoparticle suspension of the reaction mixture, (1), after 48 h of reaction and complete stabilization with no further color transformation, is shown in Fig. 1. In this case, a surface plasmon resonance (SPR) band absorption peak of reaction mixture (1) appears at 415 nm which is characteristic of silver nanoparticles. Spectral analysis after the development of the silver/chitosan bionanocomposites (1'), also exhibited similar absorbance peak as its aqueous suspension of the nanoparticles. The spectrum of aqueous $AgNO_3$ only solution was at ~ 220 nm, chitosan itself is transparent in the UV-visible region. The spectrum of plant extract rises at ~ 500 nm without any maxima or minima.

According to Mie's theory only a single SPR band peak is expected in the absorption spectra of spherical nanoparticles, whereas anisotropic nanostructures or aggregates of spherical nanoparticles could give rise to two or more SPR bands depending upon the shape of the particles [1]. In the present investigation, the suspension showed a single SPR band revealing spherical shape of silver nanoparticles. Strong absorbance between 420 – 480 nm in UV Vis. spectral analysis signifies the presence of silver nanoparticles. Also the absorption peak of silver nanoparticles due to its surface plasmon resonance (SPR) shifts to longer wavelength with increasing particle size.

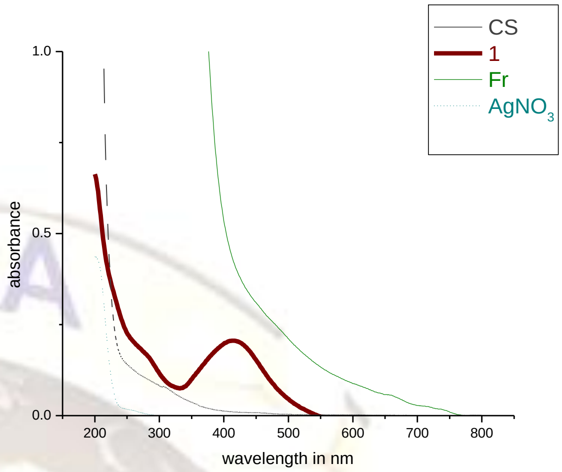


Fig. 1. UV-Visible spectra of, nanoparticle suspension (1); plant extract (Fr); silver nitrate solution ($AgNO_3$); chitosan (CS).

3.2 Nanoparticle size determination

Analysis of nanoparticles through particle size analyzer emphasizes non uniform size of the particles formed. Details of the determination are shown through the particle size distribution graph in Fig. 2.

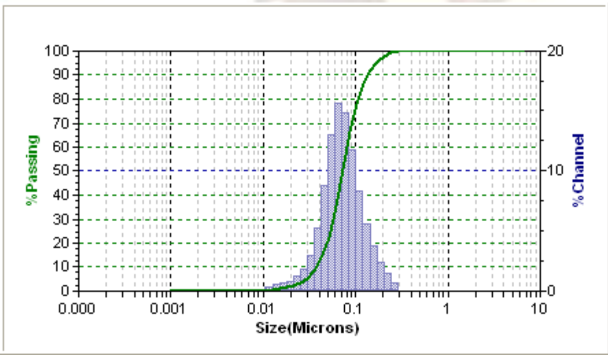


Fig. 2. Histogram of particle size distribution of nanoparticle suspension 1.

3.3 TEM observations

The morphology of silver nanoparticles synthesized by the intervention of the mythological Peepal tree was observed by transmission electron microscopy (TEM). Fig.3 shows TEM images of silver nanoparticles formed.

TEM observations revealed that silver nanoparticles formed are chiefly spherical but are irregular in shape and non uniform in size. Most of the particles showed interparticle interactions, which may have been due to the peripheral complexation of capped biomolecules. The ring-like diffraction pattern indicated that the particles were crystalline in nature. This finding was reflected in the approximately circular nature of the selected area electron diffraction (SAED) spots in Fig. 3.

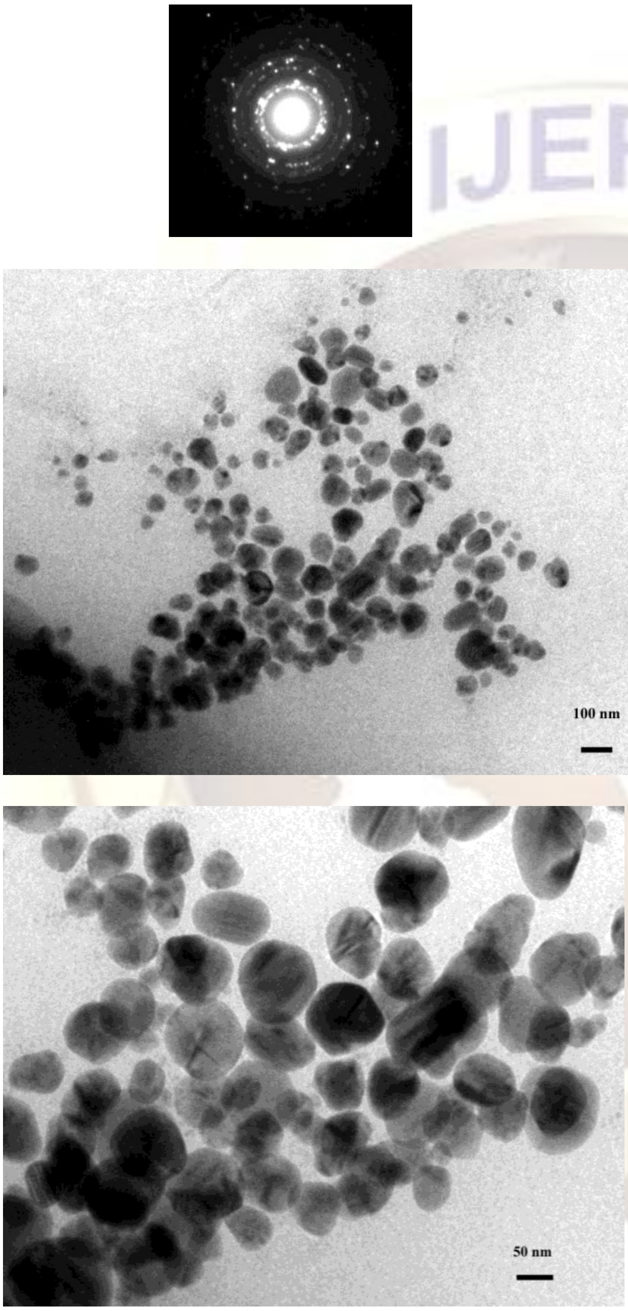
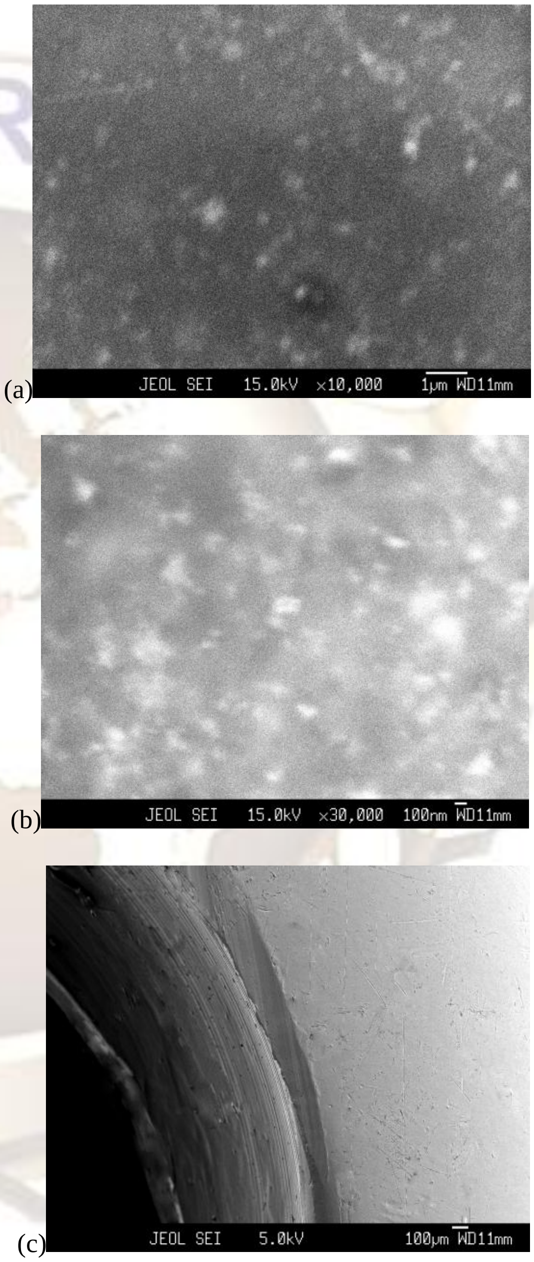
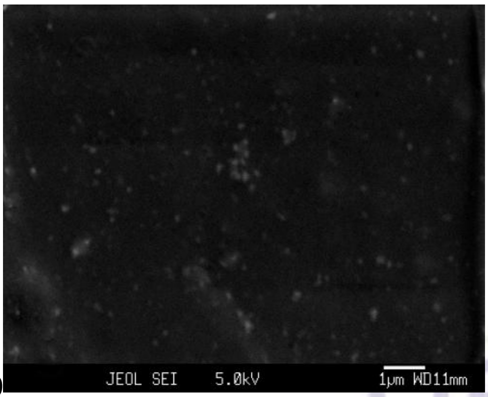


Fig. 3. TEM micrographs; SAED pattern (top) and images of silver nanoparticles of suspension 1, at different magnifications.

3.4 Characterization of the silver/chitosan bionanocomposite

Furthermore the silver/chitosan bionanocomposite (1'), developed from the nanoparticle suspension was studied for its morphological characteristics through scanning electron microscopy (SEM). Images in Fig. 4 (a, b) show nanoparticles well dispersed and distributed in the chitosan biopolymer matrix with minimum aggregation.





(d)

Fig. 4. SEM images; (a), (b), are micrographs of bionanocomposite film 1'; (c) image showing uncoated stainless steel rod; (d) micrograph of the stainless steel rod coated with bionanocomposite 1'.

The interaction, between the lone pair of electrons present at the amine group of chitosan and the partial positive charge developed at the surface of the silver nanoparticles due to electron drift, effectively stabilizes the silver nanoparticles and prevents them from agglomeration. The bionanocomposite suspension was able to be coated unifomly over the surface of medical grade implants used inside the human body eg. stainless steel rod; together with imparting smooth surface modification of the biomaterial; vividly elucidated through the SEM micrographs in Fig. 4 (c, d).

In the FTIR spectra of the bionanocomposite in Fig. 5, the peak at ~3500 cm^{-1} is more pronounced corresponding to the axial OH group of the chitosan molecule. The bending vibrations between 1600 and 1000 cm^{-1} intensifies indicating possible interaction between silver nanoparticles and amino group of chitosan.

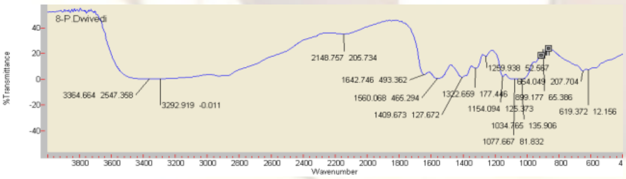


Fig. 5. FTIR spectrum [Transmission/Wavenumber(cm^{-1})] of (a) silver/chitosan bionanocomposite film 1'.

3.5 Antimicrobial activity test

Silver/chitosan bionanocomposite film (1'), having the bioreduced silver and biomaterial coated with the bionanocomposite (SSR), were assayed for antimicrobial activity against *Pseudomonas aeruginosa* (gram negative) and *Staphylococcus aureus* (gram positive), which cause majority of the biomedical implant related infections. Disc diffusion method was adapted to find out the standard zone of inhibition (ZOI). Details of the result obtained are shown in Fig. 6 and listed in Table 1.

Table 1. Zone of inhibition (ZOI) in (mm) against selective bacterial strains.

Name of the micro-organism	BNC film (1')	Stainless steel rod coated with BNC 1'-(SSR)	Uncoated bio-material disc (B)	Plant extract (Fr)
P. aeruginosa	19	28	Nil	Nil
S. aureus	23	32	Nil	Nil

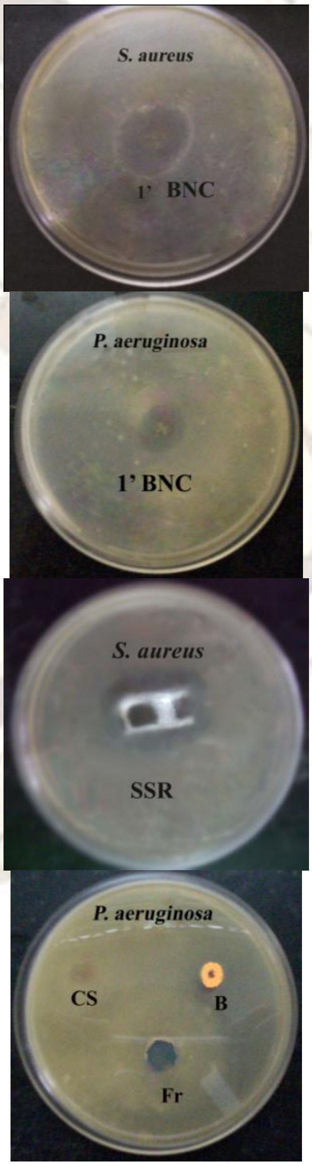


Fig. 6. Photographs taken after 48 h of incubation, showing the antibacterial activity through zone of inhibition (ZOI); of silver/chitosan bionanocomposite

film (1'); stainless steel rod (SSR) coated with silver/chitosan bionanocomposite (1'); chitosan film (CS); uncoated biomaterial (B); plant extract of *Ficus religiosa* (Fr)*;* against *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

It has been observed in the present study that the effect was well pronounced against gram positive bacteria which lack the outer membrane but has a peptidoglycan layer of about 30 nm thickness, and even against gram negative bacteria which contains only a thin peptidoglycan layer of 2-3 nm between the cytoplasmic membrane and the tough outer membrane.

4. CONCLUSION

Thus this first attempt to synthesize silver/chitosan bionanocomposite through herbal route via the intervention of our divine Peepal tree, *Ficus religiosa*, shows significant potentiality to mitigate the menace of medical implant associated infections. Bio-reduction of silver ions with plant extract provides a facile path for the production of silver nanoparticles, avoiding the use of obnoxious reducing agents which persistently adhere to the surface of the nanostructures, rendering them hazardous to be handled as well as to be applied.

This biodegradable, bactericidal, self sterilizing, biocompatible material holds sure shot potency to ameliorate the face of existing biomaterials and provides a winning strategy to vanquish biomaterials associated infections in the race between bacterial adhesion and tissue integration. Advanced coating techniques can be applied for coating of medical implants and surgical devices with this novel bionanocomposite material in order to impart smooth and uniform surface modification.

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