Anti-cancerous, Gold Nanoparticles (GNPs) Embedded into Bombyx Mori Silk / PVA Composite Nanofiber Mats: Structural Characterization and In Vitro Analysis

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Abstract

Novel materials with promising properties can be used to achieve scaffold-based on bioactivity goals. The first time we reporting newly, Gold nanoparticles (GNPs) were synthesized using brown marine macroalgae *Padina boergesenii* green route synthesized. GNPs were incorporated into biopolymer of *bombyx mori* silk fibroin (SF) and poly (vinyl alcohol), then carried out electrospinning after the synthesized nanofibrous scaffolds were characterized morphologically with field emission scanning electron microscopy (FESEM). Well-dispersed GNPs in the polymer matrix nanofibers were obtained the technique HRTEM. The amorphous natures of GNPs upon incorporation into SF/ PVA were confirmed by XRD**.** The characteristic peaks of Fucoidan and nanoparticles were confirmed by ATR-FTIR analysis. SF/PVA nanoparticles loaded NFs were thermally stable up to 441^{O} C as evidenced by TGA**.** The contact angle measurements of SF/PVA alone and GNPs embedded NFs were found to be 65±1^{o} and 73±0.9^{o} with de-ionised water, respectively. The ultimate aim of the present study is to achieve optimum antibacterial and anticancer activity MCF 7 (breast carcinoma) cell line activity. SF/PVA/GNPs NFs has exhibited good antibacterial activity against test pathogenic bacterial strains and anticancer activity of MCF 7. Thus the scaffolds was biocompatibility, biodegradability and bioactivity of these scaffolds are evaluated. Thus, reinforced silk composite nanofibrous scaffolds has alternative for biomedical applications.

Keyword: *Bombyx mori* silk, Electrospinning, Seaweeds, nanofibrous scaffolds.

1 Introduction

Biocompatible and biodegradable biopolymer nanofibers scaffold prepared by Electrostatic fiber formation or "electrospinning" is a method of producing fibers this technique its low cost expensive and diameter ranging nanometer by accelerating a jet of charged polymer solution in an electric field the technology much attention

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recently [1-7]. Biopolymers containing metal nano-particles which are used as antimicrobial or anti-cancerious activity, because of their novelty in being long-lasting biocidal materials with high temperature stability and good mechanical strength.[8] Nowadays, the introduction of gold nano-particles (GNPs) benefits for biomedical application of wound healing, antioxidant and anticancerous activity is that it does not destroy any molecular structure and bioactivity of the incorporated drug represents a great challenge for both academic world and industry [9-11]. SF originated from silk worm, such as *bombyx mori* silk (BMS),is a natural protein fiber, which has outstanding mechanical properties due to extensive hydrogen bonding and enhanced environmental stability compared to globular proteins and good biocompatibility [12-13] Silk I and II are the two types of molecular conformation of the secondary structure, present in the silk fibroin (SF). Poly (vinyl alcohol) is a chemically and thermally stability, hydrophilic polymer PVA is a biocompatible, biodegradable non-toxic polymer PVA has been widely exploited as a nanofiber.

In the present study, first report for synthesis of stable metallic nanoparticles by the extract of marine seaweeds, it results relatively very short period of incubation time requires compared with other biological material [14]. *Padina boergesenii* has been superiority sulfated fucoidan over natural fucoidan exhibited the critical role of the number of sulfate groups in this molecule carotenoid, fucoxanthin, and more extent metabolite[15]. The newly synthesized gold nanoparticles were incorporated into the SF/PVA entrapment and release, (SF/PVA/GNP) mats in situ (Fig.1- a and b). Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), FESEM, High resolution transmittance electron microscopy (HRTEM), water contact angle measurements, thermogravic analysis (TGA) and X-ray Diffraction (XRD) were used for characterization. The cytocompatibility, antibacterial property, response were tested, respectively. Finally, the effects of these mats on anticancer activity were examined.

2 Experimental methods

Materials

BMS cocoons were purchased from Sericulture Laboratory, Coimbatore, India. Nutrient agar was purchased from Himedia, India. TFA was purchased from Sisco Research Laboratories Pvt. Ltd, India. All chemicals were used as received without further treatment or purification.

2.1 Source of microorganisms

Pure cultures of Bacteria *Escherichia coli* (ATCC 8739) *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633) and *Pseudomonas aeruginosa* (ATCC 15442) were obtained from American Type Culture Collection.

2.2 Preparation of Algal Extract

Initially, seaweeds species viz., *Padina boergesenii* (brown seaweeds) were collected from Mandapam coastal inter tidal region Rameswaram, *Padina boergesenii* was washed thoroughly using freshwater to remove the salts & epiphytes and unwanted salts dried under shade condition. These dried seaweeds were powdered in an electric grinder and stored in polyethylene bags at room temperature. 1g of the dried powder was extracted in 100 mL of distilled water using water bath for at 20 mins and then it was centrifuged for 15 mins at 10000 rpm after the supernatant was used for the further preparation of nanoparticles.

2.3 Synthesis of Gold nanoparticles using *Padina boergesenii*

9 mL aqueous solution of 1 x 10^{-3} chloroauric acid was reduced using 1 mL of *Padina boergesenii* extract at room temperature for 20 minutes; this setup was incubated in dark under static condition resulting in a ruby-pink indicating the formation of Gold nanoparticles (GNPs).

3 Electrospinning

The cocoons of *Bobyx mori* were degummed three times with sodium carbonate solution at 60 ◦C for 30 mints, and washed with distilled water in order to remove sericin from the surface of silk and the Silk fibroin was obtained[16-17].

Different wt % of SF was dissolved in TFA and gently stirred continuously for about 6 h. Nanoparticles solution with separately different wt % of PVA was Stirred 2 to 4 hrs under room condition, then added to the Silk fibroin solution and continuously stirred for 2 h. Then carried out in the electrospinning process, a high electric potential was applied and flow rate 0.5mL h. The jet dries as the solvent is evaporated in the air. The dried fibers are collected on a receiving conducting mesh [18]. The collecting mesh was placed at distance of 10–15 cm from the capillary tip. A voltage of 15-30 kV was applied to the wire in the capillary through a high voltage power supply, while the receiving mesh was grounded. The process was carried out at room temperature.

4 Characterization of SF/PVA/GNPs composites nanofiber

The morphologies of the electrospun fibers were observed using FESEM (HITACHI, S-3400N FESEM) at 10 kV, and HRTEM-(FEI, TECHNAI G2)30S-Twin D 905 at 200 kV, was used for characterizing the surface morphology of SF/PVA/GNPs composites nanofibers. Functional groups of electrospun fibrous scaffolds were analyzed help of Universal attenuated total reflection Fourier transform infrared spectroscopy (UATR-FTIR) facility in the range of 400-4000 cm^{-1}, X-ray Diffraction studies, the nature crystallinity of the nanofibers was examined on XRD–X'pert pro PANalytical Instrument using Cu Ka radiation (λ=1.5418 A °).The electrospun fibrous membrane was pressed inside the sample holder, and the XRD data were collected in the step scan mode. The thermal properties were studied by TGA/DTA (model SDT 2600) at a heating rate of 10^{O}C min−1 from 37 ^{O}C to 800 ^{O}C under a constant nitrogen flow of 20 cm3 min-1. Contact Angle Measurements analysed using Euromex Optical Microscope equipped with a CCD camera [19] to measure the hydrophilicity of the polymer nanofibers.

4.1 Antibacterial assessment

The following micro-organisms were used for antimicrobial activity by an inhibition zone method; pure cultures of Bacteria *Escherichia coli* (ATCC 8739) *Staphylococcus aureus*

(ATCC 6538), *Bacillus subtilis* (ATCC 6633) and *Pseudomonas aeruginosa* (ATCC 15442) were obtained from American Type Culture Collection. The Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* and Gram-positive bacteria *S. aureus* and *B. subtilis* were precultured in Nutrient Broth (NB) overnight in a rotary shaker at 37 ◦C, and then it was swabbed uniformly onto individual Nutrient agar plates using sterile cotton swabs. Nanoparticles incorporated nanofibers and control (SF/PVA nanofibers without GNPs) disinfected by ultraviolet C irradiation for 15 minute were cut into 5mm discs and placed in the culture swabbed petridish 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 SF/PVA/GNFs nanofibers on the plates was measured and recorded as an inhibition against the test bacterial strains.

4.2 Anti-cancerous Activity

In order to Study the anticancer activity of SF/PVA nanoparticles loaded NFs (SF/PVA/GNPs) MTT Assay was performed. Briefly MCF7 (Breast carcinoma) cells are seeded approximately 12 K cells/well onto cell culture plate and allowed to grow it for 24 hours at 37°C in a 5% CO_2 and 95% O_2 humidified incubator. On the following day with proper incubation the cells were treated with complexes with varying concentration of nanoparticles loaded NFs (SF/PVA/GNPs) starting from 10μg, 25 μg, and 50 μg was added with cell culture medium. The MTT assay was carried out in Triplicates. After 24 hour time period of incubation, the seeded cells were examined under phase contrast microscope and observed the cell morphology and photographed using Leica systems. Subsequently, removed the medium and treated the cells with 0.5mg/ml of MTT (Thiazolyl Blue Tetrazolium Bromide salt) in 1X PBS (150μL/well) was added and incubate it for 4 hours in a CO_2 incubator. After 4 hour incubation with MTT solution, removed the solution and solubilised the formed blue colored formazan crystal with 200 μL DMSO and measured the absorbance at 570nm using BioRad ELISA plate reader.

5 Results and discussion

FESEM images of different weight % (wt %) of SF/PVA nanoparticles loaded NFs (SF/PVA/GNPs) are shown in Fig.3 The surface morphology of the fiber depends on the parameters used during electrospinning. Different concentrations of SF and PVA solution were used and surface morphology was studied by FESEM. First, a concentration of 5 wt% was tried which led only to electrospraying. Hence, the concentration was increased to 6 and 10 wt % were electrospun it was formed spindle beads has been observed widely with small fibers were obtained [20-22]. Then eveloped a mathematical model for the break-up of jets of polymer solution, while 11, 12 wt% resulted in reduction of the beads formed with fibers were obtained. Continuous and beadles nanofibers were obtained only at 11, 12 wt%. HRTEM images also confirm the good dispersion of well incorporated AuNPs in the SF/PVA NFs mat as shown in Fig.4 The XRD result of shows a sharp peak at 2θ =20.05◦ an amorphous state due to associated breakdown of side chain groups of amino acid residues and cleavage of peptide bonds. SF/PVA/GNPs were confirming the conversion of crystalline gold nanoparticles to amorphous form result of shows fig.5 the amorphous form has the benefit of increasing the aqueous solubility and bioavailability of poorly water-soluble. The FT-IR spectra of electrospun SF ,PVA and SF/PVA/GNPs were revealed, SF/PVA/GNPs has strong bands at 3352,2985,1750,1253,1077 and ,876 cm-1 shown in Fig: 9 .UATR-FTIR spectrums of the hydroxyl groups (OH) are very abundant in polysaccharides of the algal cell wall and its participation in the reduction process was confirmed by UATR-FTIR analysis. Seaweeds pigments, such as fucoxanthins, a kind of carotenoids rich in hydroxyl groups, could also have participated in the gold reduction. These pigments have reductive properties and are released to solution by diffusion These soluble elements could have acted as capping agents preventing the aggregation of nanoparticles in solution, playing a relevant role in their extracellular synthesis and shaping, and N-H strettching vibration band at 1542 cm^{-1}.the characteristic peaks were confirm the presents of amide I amide II beta confirmation of the SF respectively. The amide III band at 1250 cm^{-1} represents the N-H bending and C-N stretching vibrations in the SF nanofibrous scaffolds.Thermo

gravimetric (TGA), analysis graphs incorporated GNPs in the SF/PVA initial weight loss at around 94◦C is due to loss of water fiber. The SF/PVA/GNPs nanofibers graph shows a second weight loss at 374-441◦C with negligible change at temperature higher than 441◦C. This weight loss indicates thermal decomposition or evaporation in the material from this fig.7, it is clear that specimens' weight loss occurred mainly due to the combustion of organic SF/PVA/GNPs nanofibers matrix. The contact angle of SF/ PVA was found to be 39±5 and 34±5, respectively. In addition of contact angle measurements of SF/PVA alone and GNPs embedded NFs were found to be 54±1◦ and 65±0.9◦ with de-ionised water, respectively as shown in Fig.8.

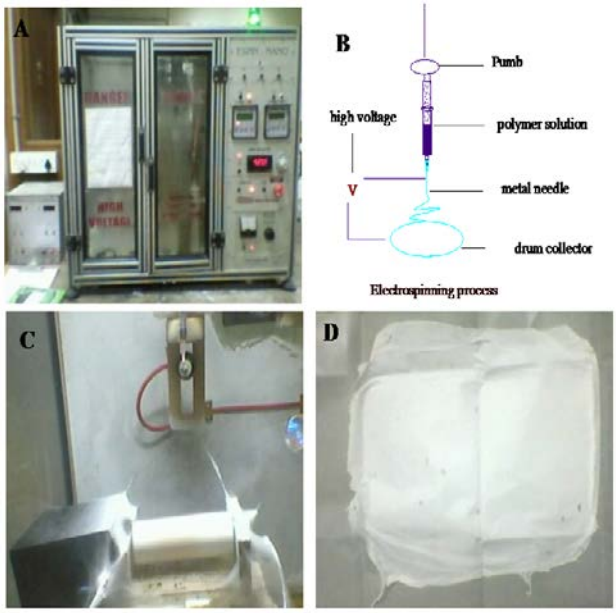


Fig.1. Electrospinning process

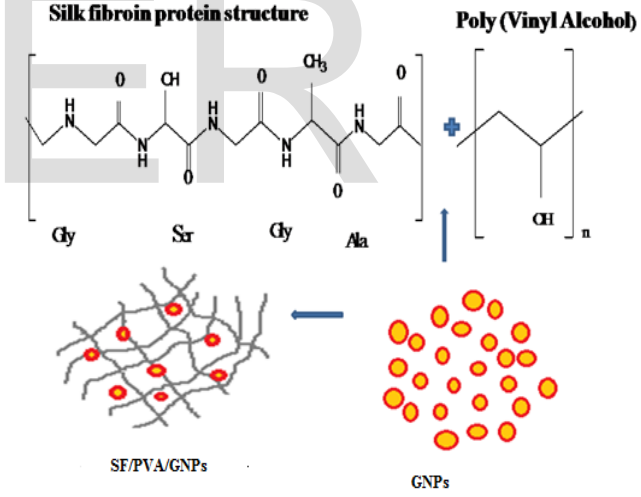


Fig.2. Schematic illustration of the distribution and bounded of GNPs on the surface of the SF/PVA nanofiber.

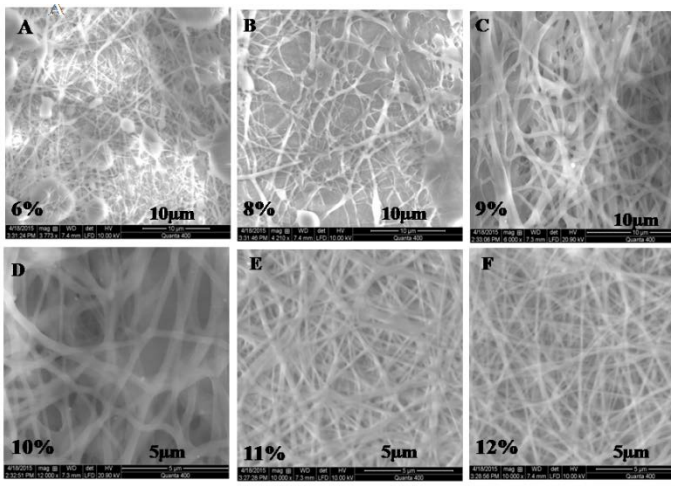


Fig.3. FE-SEM Micrograph images of nanofibers (A) 7 wt% of SF/PVA with loaded nanoparticles of NFs (B) 8wt % of SF/PVA with loaded NPs(C & D 10 wt % of SF PVA loaded NPs (E & F) 11,12 wt % of SF PVA loaded NPs

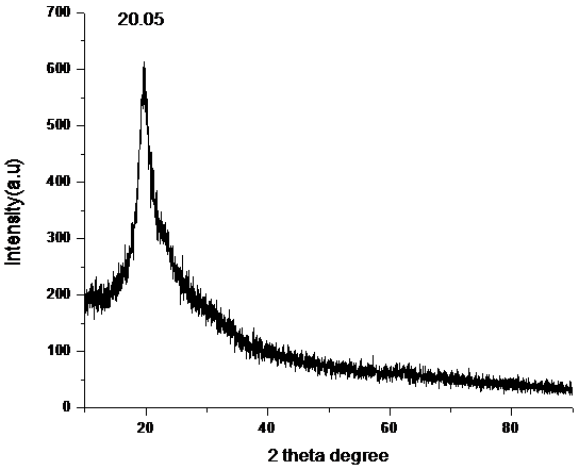


Fig.5. XRD spectra of electrospun SF/PVA/GNPs nanofibrous scaffolds

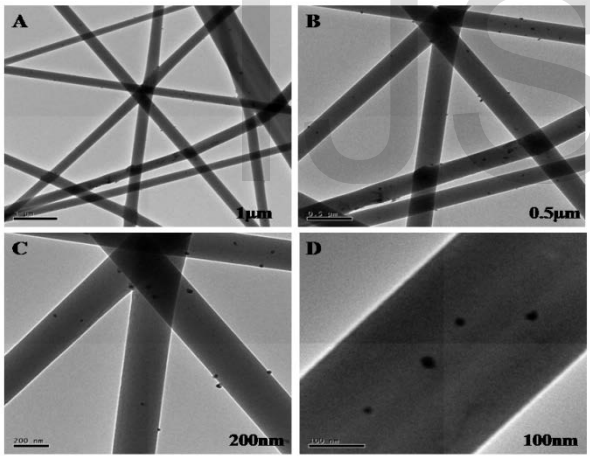


Fig.4. HRTEM images of incorporated AuNPs in the SF/PVA NFs mat

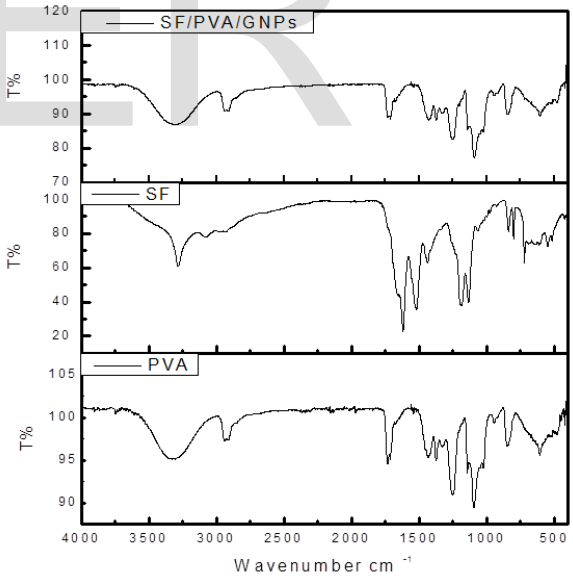


Fig.6. UATR-FTIR spectra of electrospun SF/PVA/GNPs nanofibrous scaffolds

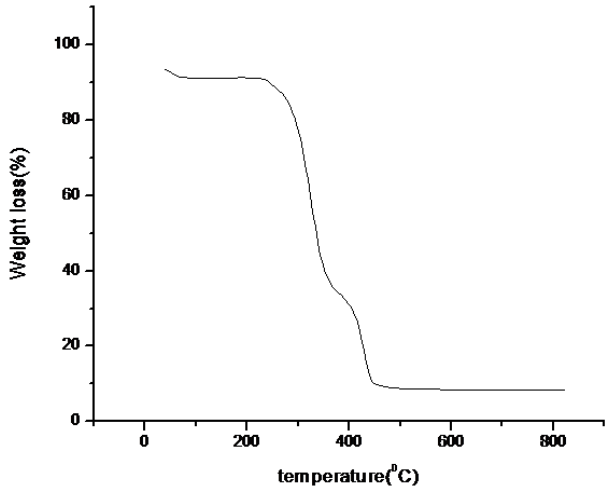


Fig.7. TGA Thermograms of electrospun SF/PVA/GNPs nanofibrous scaffolds

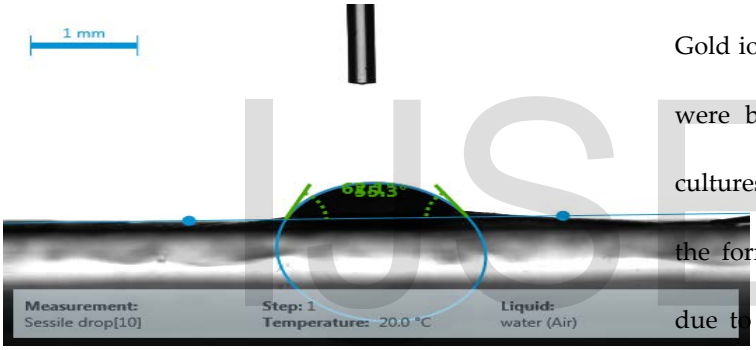


Fig.8. Contact angle images of the electrospun SF/PVA/GNPs nanofibrous scaffolds

5.1 Antibacterial activity

The results showed significant inhibitory activity of SF/PVA/GNPs nanofibers matrix against all the tested microorganisms, while the PVA nanofibers without SF/PVA/GNPs NFs (control) did not show any zone of inhibition the results showed nanofibers against all the tested microorganisms, as shown in graph: 1. Without GNPs fibrous scaffolds and compared to the SF/PVA/GNPs NFs, while the PVA with blended drug nanofibers without SF/PVA/GNPs NFs (control) slightly shows the inhibition zone. The diameter of the zone of inhibition of SF/PVA/GNPs NFs matrix nanofibers against the test pathogenic bacterial strains is given in Fig: 9. The results manifested that the antibacterial ability of the PVA nanofibers depend on the presence of SF/PVA/GNPs NFs on the surface of the fibers. Gold ions are released when the matrix nanofibers were brought in contact with the test bacterial cultures in the Petridish plate, which resulted in the formation of zone of inhibition. This may be due to the fact that the release of SF/PVA/GNPs NFs becomes easier as the particle size decreases, so that SF/PVA/GNPs NFs can more effectively reach the microbial region subsequently increasing their contact with the microorganism. In addition, smaller dimensions and higher surface to volume ratios of SF/PVA/GNPs NFs also enhance their contact with the microorganism. The inhibitory activities of the matrix nanofibers against the Gram-positive bacteria are better than that against Gram-negative bacteria. The anti-bacterial

properties of SF/PVA/GNPs NFs could be believed. The increase in permeability of the cell membranes would allow the SF/PVA/GNPs NFs to penetrate the cell and cause cell death by breaking the double stranded DNA, as the antimicrobial effect of SF/PVA/GNPs NFs was believed.

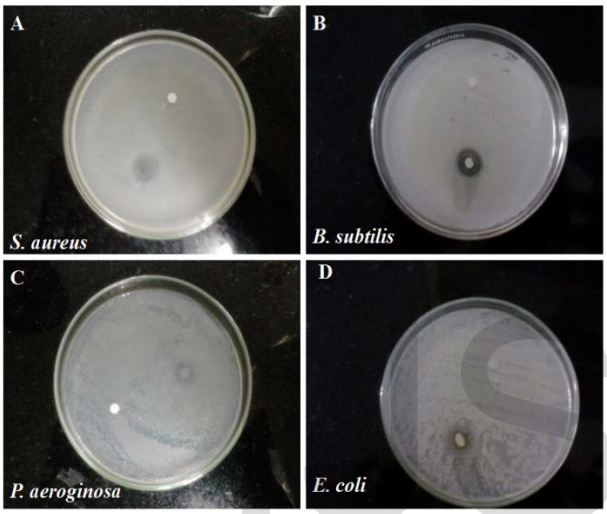
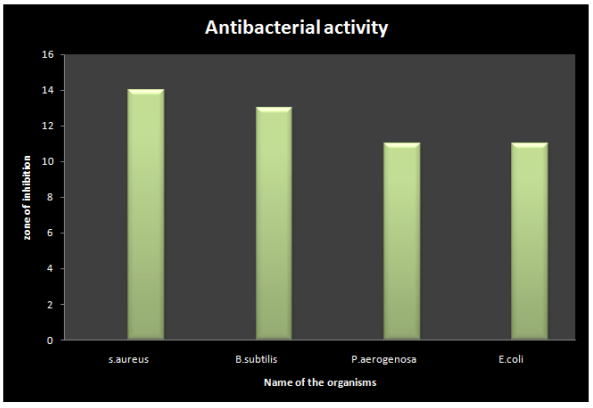


Fig.9. Zone of inhibition on the electrospun SF/PVA/GNPs nanofibrous scaffolds test pathogenic bacteria



Graph: 1. Graph represent of inhibition on the electrospun SF/PVA/GNPs nanofibrous scaffolds test pathogenic bacteria

5.2 Anticancer activity of SF/PVA/GNPs nanofibers was assessed by MTT assay

The results were given in Fig:10 SF/PVA/GNPs nanofibers treatment on MCF7 cell lines (Breast carcinoma) .The results show that upon treating with the SF/PVA/GNPs nanofibers there was a concentration dependent on cytotoxicity activity was observed. At the concentration of 10uG, 25uG, 50uG was observed as IC50 value of the SF/PVA/GNPs nanofibers which means that the 50% of the cells were dying. It demonstrated that the synthesized SF/PVA/GNPs nanofibers with above 50uG were used to kill the cancerous cells were Observed morphological changes were immediately observed under the fluorescence microscopy, Cells undergoing apoptosis display typical condensed morphology, Chromatin condensation, nuclear shrinkage and formation of apoptotic bodies can easily to be observed under fluorescence microscopy, after appropriate staining was used to confirmation of apoptosis was achieved using Acridine orange(AO) Staining namely cell shrinkage, chromatin condensation .Therefore, here Acridine orange (AO) a cell-permeable DNA-

binding dye was used to analyze the morphological changes that occurred in the DNA. The results of this staining revealed that the SF/PVA/GNPs nanofibers induced the nuclear condensation

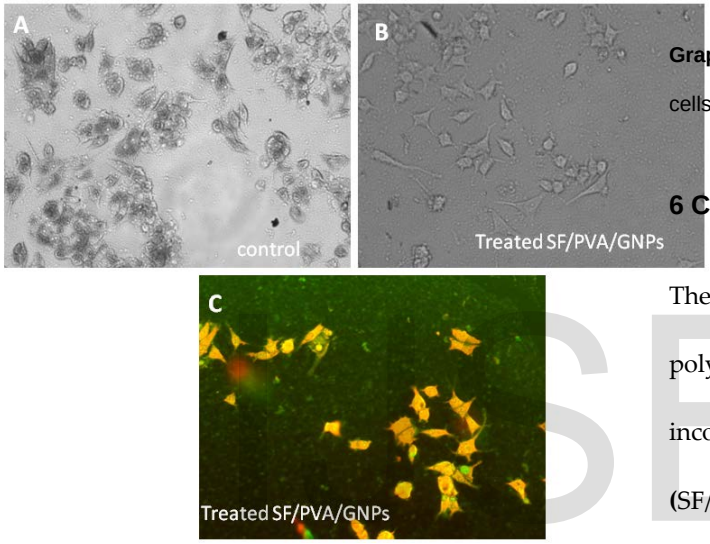
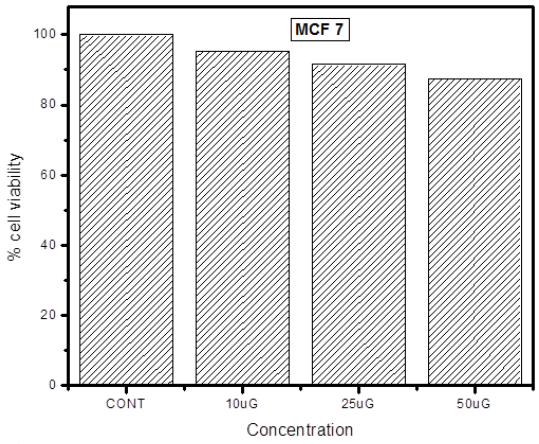


Fig.10. Fluorescence Microscopic images: Anti-cancerous result of the electrospun SF/PVA/GNPs nanofibrous scaffolds in MCF-7 24 h incubation period at 20X magnification



Graph.2. Bar chart showing % of cell proliferation of MCF 7 cells on treating with SF/PVA/GNPs nanofibrous scaffolds

6 Conclusion

The biodegradable polymer of PVA and natural polymer of silk fibroin was blended with incorporated GNPs into the polymers (SF/PVA/GNPs) nanofibers were produced by electrospinning. The synthesized nanofibers were characterized by XRD and thermal analysis proves the conversion of crystalline GNPs to an amorphous state. Imparted good anti-cancerous activity against MCF-7 (carcinoma breast cancer cell lines) and also exhibited promising antibacterial activities against the test pathogenic bacterial strains *Escherichia coli Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Nanofibers thus developed showed great potential for biomedical applications antimicrobial wound

dressing, and cancer treatment, tissue engineering, cosmetic products, drug delivery.

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