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Clinical applications of ZnO nanoparticles

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ABSTRACT

Zinc oxide (ZnO) NPs have wide industrial and commercial applications. Due to their physical properties ZnO NP are also used in cosmetics for protection from UV radiation. However, ZnO NP are non toxic to upper epidermal layer (stratum corneum) but they are potential toxic to cancerous cells. The aim of this study is to assess the cytotoxicity of ZnO nanopowder (particle size 50 nm) to human skin fibroblasts and human embryonic kidney cells. ZnO NP show higher toxicity after 24 hrs exposure. Results of this study indicated that human skin fibroblasts and human embryonic kidney cells both are sensitive to ZnO nanoparticles through the viability assay. 2. PVC (Poly Vinyl Chloride) is a versatile plastic that has been used for medical applications including containers for blood, urine, IV solutions, catheters, tubing for dialysis, surgical gloves etc. This may lead to nosocomial infections. The aim of this study is to assess the ZnO coated PVC sheets for bacterial adhesion. ZnO films were coated on medical-grade PVC surface by the improved organic-inorganic interfacial adhesion method and its antibacterial property at different concentrations against *E.coli* and *S.aureus* were studied. The antibacterial properties of the THF-ZnO/PVC film are better than that of the ZnO/PVC and uncoated PVC. A ZnO molecule with smaller size, large surface area, higher polarity, exhibits higher antimicrobial activity. Under UV irradiation, the THF-ZnO/PVC film shows the best antibacterial properties with 80-90% bactericidal effect.

Keywords: ZnO, medical grade poly vinyl chloride (PVC), bactericidal, anti-cancer.

INTRODUCTION

Zinc oxide (ZnO) nanoparticles have their own importance due to their vast area of applications, for example, gas sensor, biosensor, cosmetics, storage, optical devices, window materials for displays, solar cells, and drug-delivery. Zinc oxide (ZnO) NPs have very wide industrial and commercial applications, particularly in pigments. Due to their physical properties ZnO NP are also used in cosmetics for protection from UV radiation.It has many clinical applications and two of them are studied in this work.

1.ZnO NP are non toxic to upper epidermal layer (stratum corneum) but they are potential toxic to cancerous cells. A limited number of in vitro studies have also been performed to assess the toxicities of the nanoparticles using different cellular systems and test methods [4,10,23,29]. However, published toxicity data are still considered inadequate to earn a full understanding of the potential toxicity of these nano-particles. Further studies are needed to clarify the risk of these materials as well as their application for human use. The aim of this study is to assess the cytotoxicity of ZnO nanopowder (particle size 50 nm) to human skin fibroblasts and human embryonic kidney cells.

2. The increasing use of polymer materials such as polyethylene, polyurethanes, and poly vinyl chloride (PVC) in the hospital care has led to a concomitant increase in the incidence of biomaterial-related infections (BRI). Adhesion of bacteria to biomaterials led to the formation of biofilm on the surface, which plays a crucial role in the pathogenesis of the BRI [14]. The growth and production of biofilm protect the bacteria from the host defense mechanisms and external agents as the drug treatments [2,11], which makes the cure of the bacterial infections quite difficult and requires either higher doses or more potent antibiotics.

In order to efficiently prevent or reduce biofilm formation, many efforts have been done to enhance the anti- bacterial properties of biomaterials. Some efforts such as modifying the physicochemical properties of biomaterial surface, coating with silver, azidation treatment, antibiotic impregnation into the polymer matrix, have been examined in recent years [7,24].

To increase the antibacterial efficiency of biomaterials, many studies have been done to coat ZnO NPs on many biomaterial's surface such as glass, ceramic, stainless steel, polymer, and so on. In this work, the ZnO PVC sheets were prepared by deep coating method and THF was used to pretreat the surface of PVC sheet similar to organic-in- organic interfacial adhesion method [16]. The bacterial adhesion and antibacterial activity of ZnO PVC sheets was analysed by total viable count of bacterial cells on the surface of PVC sheets as compared to plain PVC sheets under similar conditions.

METHODOLOGY

1 Cytotoxicity of ZnO against human skin fibroblasts and human embryonic kidney cells

1.1Nano particles

Zinc oxide nanopowder, <50nm particle size was purchased from (Sigma-Aldrich, USA) CAS: 1314-13-2 MW: 81.39 g/mol, Titanium oxide nanopowder, <25nm particle size was obtained from (sigma-Aldrich, Bangalore) CAS:1317-70-0 MW: 79.87g/mol

1.2 Preparation of nanoparticles

ZnO nanoparticle were suspended in the culture medium at the concentration of 5000 ppm and dispersed by ultrasonic vibration for 15 min. In order to ensure the uniform suspension, they were stirred on vortex agitation (1 min) before every use.

1.3 Human skin fibroblasts and culture conditions

A431, and HEK 293 were obtained from NCCS (National Centre for Cell Science, Pune) in 25cm^2 flasks with good confluenc were grown in MEM powder (Gibco life technologies,)contents - Earles salt, L-glutamaine, non-essential amino acids(without sodium bicarbonate) Formula No-04-5045EF [lot no-1383815] Adult bovine serum 500ml (Bioscience, New Zealand. All cultures were maintained in a phenol red free culture medium DMEM/F12 (Dulbecco's modified essential medium/Ham's 12 nutrient mixture, Gibco), supplemented with 5% (v/v) fetal calf serum (JS Bioscience, Australia), and 1% (v/v)

antibiotic (2 mML-glutamine, 100 mg/mL Penicillin and 0.1 mg/mL Streptomycin; Gibco). Cultured cells were kept at 37°C in a humidified 5% CO_2 incubator

1.4 Viability assay

Once the cells reached confluence, the culture medium was removed from the flask and the cells were rinsed three times with sterile PBS. The confluent cell layers were enzymatically removed, using Trypsin/ EDTA (Gibco, USA), and resuspended in culture medium. Cell viability was assessed by vital staining with trypan blue (0.4% (w/v); Sigma, USA), and cell number was determined using a light microscope

2. ZnO coated medical grade PVC sheets for bacterial adhesion

2.1. Maintenance of bacterial culture

Pure culture of *E.coli* and *S.aureus* was maintained on sterile nutrient agar plate (Peptone, Nacl, agar, distilled water) and sterile nutrient agar slant. Streak plate technique is used for subculture and well grown culture is preserved in refrigerator at 4°C. Cultures are sealed with parafilm before storing. Bacteria grown on suitable agar slants and agar plates are transferred to fresh ones before they exhaust all nutrients or dry out. Subculture is performed once in a week for maintaining viability of bacterial culture.

2.2 Preparation of Zinc Oxide nanoparticle stock

Zinc Oxide nanoparticles from Sigma-Aldrics (<50nm particle size) was dissolved in methanol. Stock prepared was 1mg/ml of methanol. This solution was dispersed for 30 minute in sonicator. From this stock 5µg/ml, 10µg/ml, 50µg/ml, and 100µg/ml concentrations of ZnO nanoparticles in methanol was prepared. These nanoparticles were also dispersed for 30 minute at RT in sonicator for its further use.

2.3 Coating of ZnO NPs on medical grade PVC Surface

The ZnO film was prepared by dip-coating method with ZnO suspension as precursor. The PVC sheets (1.5 × 0.7 cm) were pre-immersed in THF-PVC solution for 10s. Immediately, the PVC samples were dipped into different concentration of ZnO colloidal solution and were centrifuge at speed of 1200 rpm for 30 min. The ZnO gel film on PVC were dried in an oven at 60˚C for 30 min. After seven such coating steps, the transparent ZnO film on PVC were obtained. The PVC sheets without pretreatment were coated with ZnO under similar operating conditions for comparison. For ease of presentation the ZnO film with pretreatment were labelled as THF-ZnO/PVC and that without pretreatment were labelled as ZnO/PVC and it was characterized by SEM.

2.4 Bacterial Adhension

The neat PVC, ZnO/PVC and THF-ZnO/PVC sheets were immersed in the aqueous solution of *E. coli* and kept at 37˚C for 24 h. The sheets were taken out and rinsed gently with sterile phosphate buffered saline (PBS) to remove the non-adherent bacteria. Then, the bacteria adhered on the sheets were washed off into 5 ml of sterile PBS in an ultrasonic cleaner for 5 min. The number of the washed off bacteria was then determined by colony counts (CFU). The adherent number was ex-pressed by the ratio of the total adherent bacterial to the area of the measured sample and represented as bacterial adhesion TVC/cm^2. The same process was carried out with *S. aureus*. PVC sheets were analyzed by SEM.

2.5 Antibacterial Property ZnO/PVC sheets

ZnO/PVC sheets (1.5 × 0.7cm) with different concentrations were placed on sterile plates, and then 0.5 ml broth inoculated with 10^4 cfu/ml of *E. coli* was added onto the surfaces. The samples were irradiated with 8 W UV lamp (with wavelength at 365 nm) for 150 min. After irradiation, the sheets were rinsed with sterile phosphate buffered saline (PBS) and the number of viable bacteria was determined by colony counts (CFU). The plain PVC sheet sample was also tested for comparison. The antibacterial property of PVC, ZnO/ PVC and THF-ZnO/PVC was represent-ted by the bacteriocidal percentage, a ratio of the dead number of the bacterial to the initial number of the bacterial cells. The same process was carried out with *S. aureus*

RESULTS AND DISCUSSION

3.1 Cytotoxicity of ZnO against human skin fibroblasts and human embryonic kidney cells

ZnO-NPs (50 nm sized) induced cytotoxicity in cultured human skin fibroblast (A431) and human embryonic kidney cells (HEK293) by elevating

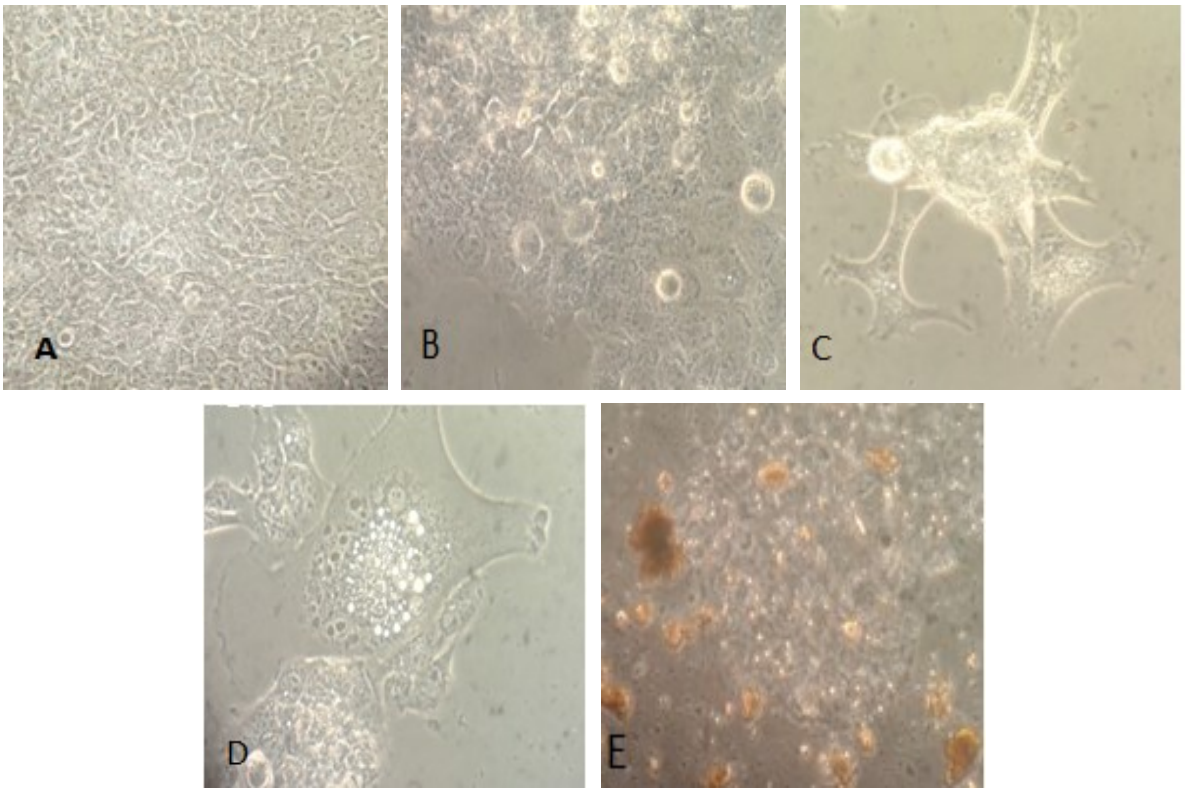


Fig 1 A normal human skin fibroblast A431 cells,**B** cells treated with ZnO NP 5 µg/ml for 24 hrs.**C** cells treated with ZnO NP 10 µg/ml for 24 hrs. **D** cells treated with ZnO NP 50 µg/ml for 24 hrs.**E** cells treated with ZnO NP 100 µg/ml for 24 hrs.

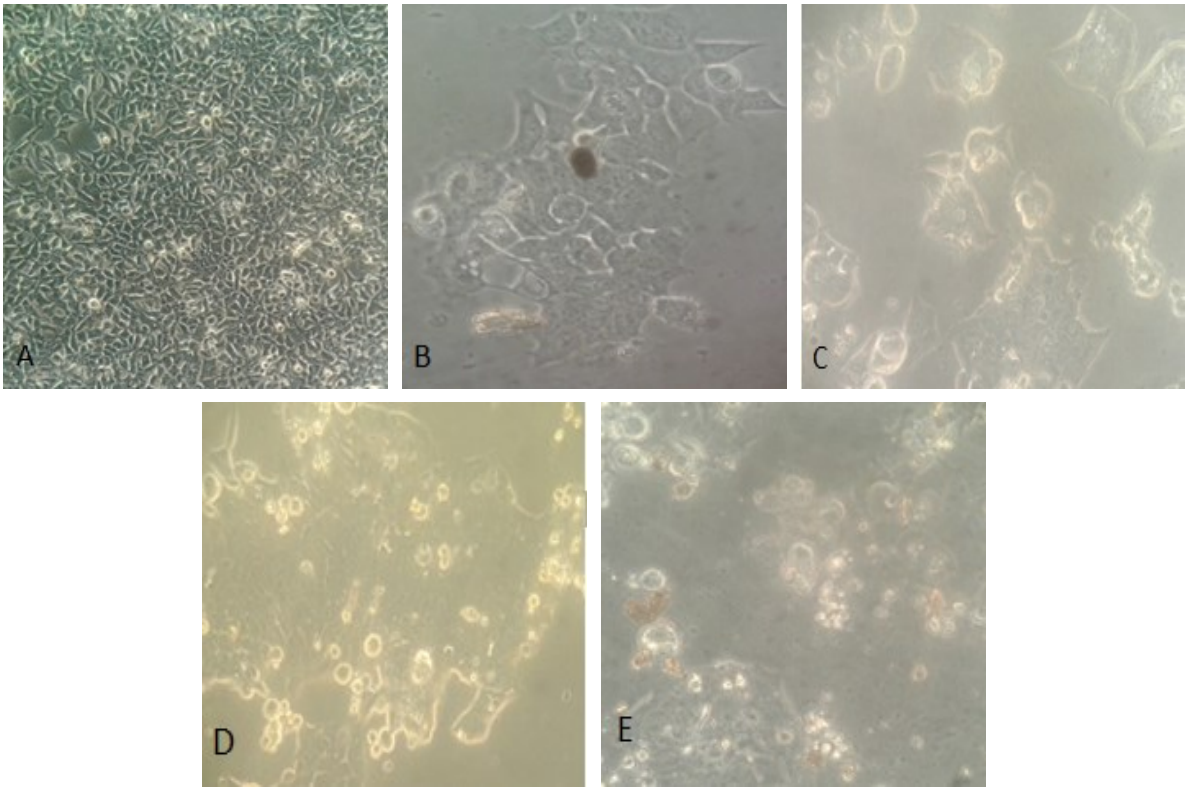
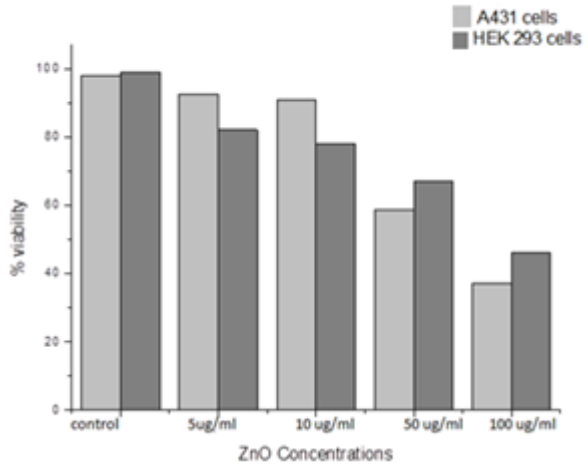


Fig 2 A normal human embryonic kidney HEK 293 cells cells,**B** cells treated with ZnO NP 5 µg/ml for 24 hrs.**C** cells treated with ZnO NP 10 µg/ml for 24 hrs. **D** cells treated with ZnO NP 50 µg/ml for 24 hrs.**E** cells treated with ZnO NP 100 µg/ml for 24 hrs.



Graph1:% Viability of human skin fibroblast (A431) and human embryonic kidney cells (HEK293) treated with ZnO NPs of different concentrations for 24 hrs..

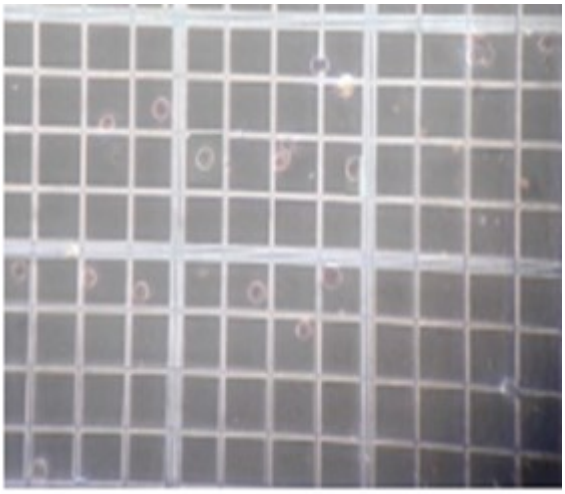


Fig: 3 Viability was checked using trypan blue stain and cells were counted using haemocytometer. Dead cells appeared blue color whereas viable cells were unstained.

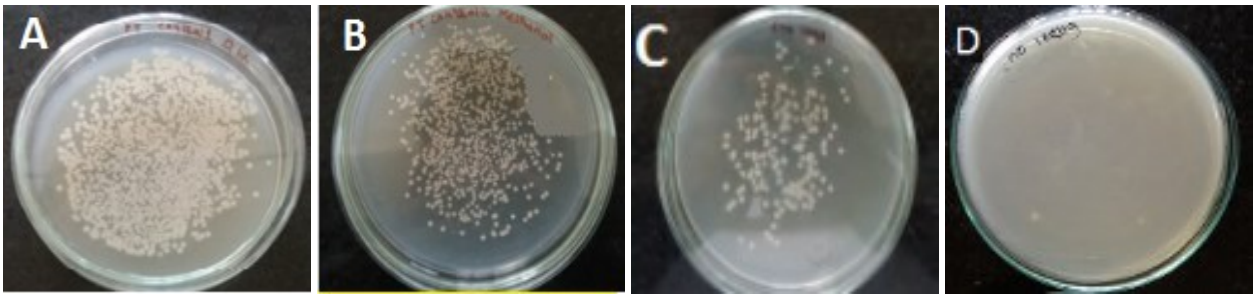


Fig:4: Bacterial (*S. aureus)* adhesion on medical grade PVC :**A**-Initial number, **B**-Plain PVC, **C**-ZnO(100µg/ml) PVC, **D**- ZnO(100µg/ml)+THF

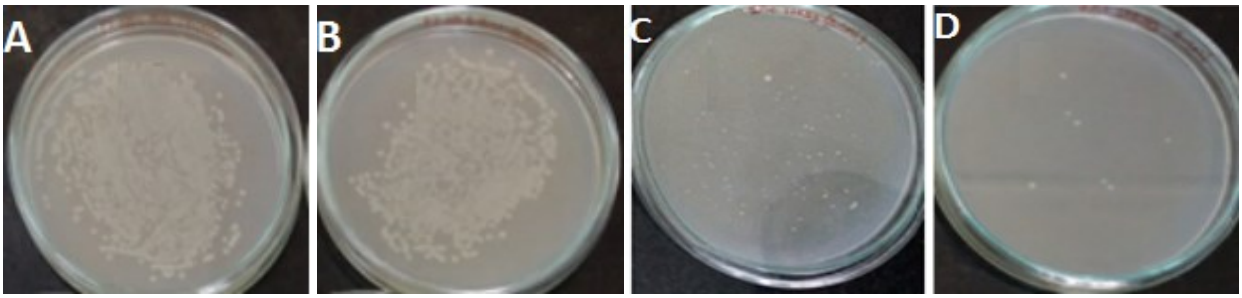


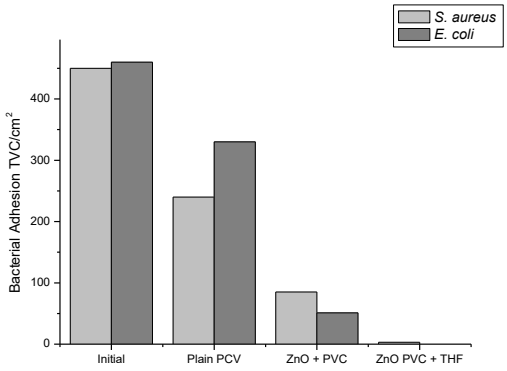
Fig:5:Bacterial (*E. coli)* adhesion on medical grade PVC :**A**-Initial number, **B**-Plain PVC, **C**- ZnO(100µg/ml) PVC, **D**- ZnO(100µg/ml)+THF

inflammatory response from morphological observations in a concentration-dependant fashion (Fig. 1 and 2). Cell viability was also confirmed by vialibity test with trypan blue dye staining. Death of the cells was confirmed by entry of dye in to cytoplasm of the cells, which stains cell blue (Figure-3) ZnO NPs also induced detachment of cells from the surface illustrating apoptotic type of cell death. Also it can be seen from our study that A 431 cells had high toxicity response as compared to HEK 293 cells. The cytotoxicity of ZnO is concentration dependent (Graph 1). Similar results were observed in a study where it is shown that oxidative stress-induced apoptosis may be considered as one of the pathways of toxicity by ZnO-NPs [8]. In vitro toxicity assessment has become widely used for recent toxicity studies. Such assays provide rapid, cost effective and reliable results [13].

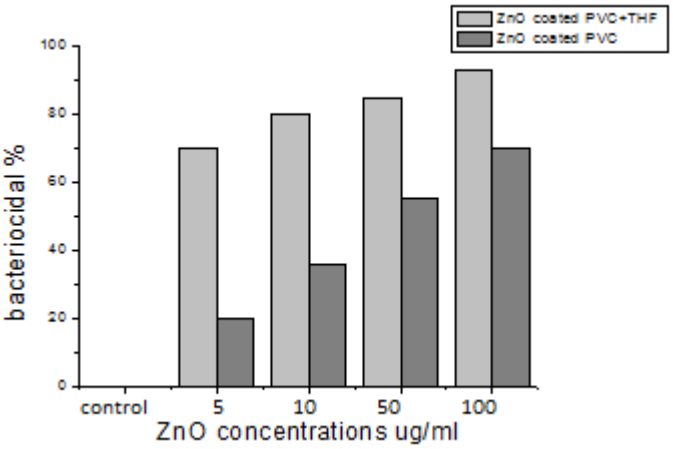
3.2 ZnO coated medical grade PVC sheets for bacterial adhesion

3.2.1 Bacterial adhesion

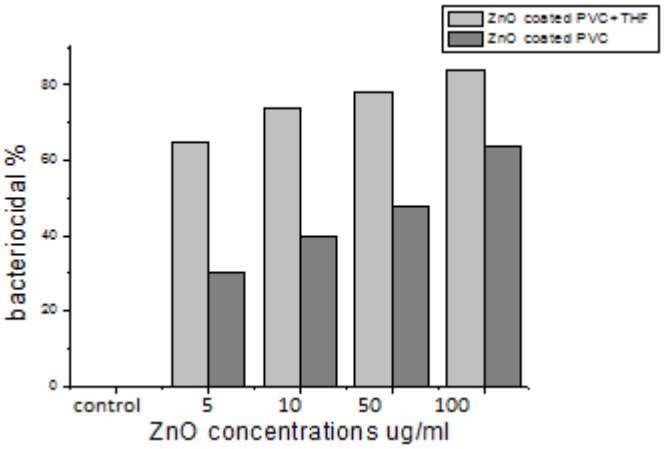
This work reveals that the antibacterial property of medical grade PVC sheets can be enhanced by ZnO coating where THF helps in good adhesion of NPs on the surface of PVC sheets. In bacterial adhesion studies, total viable count (TVC) of bacteria reduced substantially on the surface of ZnO + PVC and ZnO + PVC + THF sheets compared to plain PVC (Graph 2, Fig. 4 and 5). This observation is also supported by SEM observation where lysed cells were observed on the surface of ZnO + PVC and ZnO + PVC + THF sheets (Fig. -6).



Graph2: Effect of nanoparticles coated medical grade PVC sheets on bacterial adhesion



Graph3: Bacteriocidal % of of medical grade PVC Coated with ZnO NPs of different concentrations with and without THF against *E.coli*



Graph4: Bacteriocidal % of of medical grade PVC Coated with ZnO NPs of different concentrations with and without THF against *S.aureus*.

3.2.2 Antibacterial property of ZnO PVC sheets

The photocatalytic property of metal oxide that is responsible for the excellent sterilization properties [18]. Considering this ZnO coated medical grade PVC sheets immersed in bacterial suspension with or without THF were irradiated by UV light for 150 minutes. Then the number of viable bacteria was determined by TVC and plain PVC sheet was used for comparison. The antibacterial property of plain PVC, ZnO + PVC and ZnO + PVC + THF was estimated by bacteriacidal percentage as a ration of dead number of cells to the initial number of bacterial cells for different concentration of ZnO. The bactericidal percentage of of different concentration of ZnO NPs coated on PVC sheets with THF is in the range 75% to 90% for and 20% to 65% without THF against *E. coli* compared to plain PVC. Similar results were obtained against *S. aureus* as 60% to 80% for with THF and 20% to 58% for without THF (Graph-3 and 4). Similar antibacterial effect is observed by Lin H. et. al. in 2013 [16] and they stated that the photocatalytic property and sterilization activity of THF-ZnO/PVC are much better than that of ZnO/PVC. The main reason is may be that the amount of ZnO on THF-ZnO/PVC is larger than that on ZnO/PVC. Under UV irradiation, the amount of electron and hole produced on THF-ZnO/PVC were higher than that on ZnO/PVC that resulted in higher concentration of radicals (O2• and HO•) on THF-ZnO/ PVC.

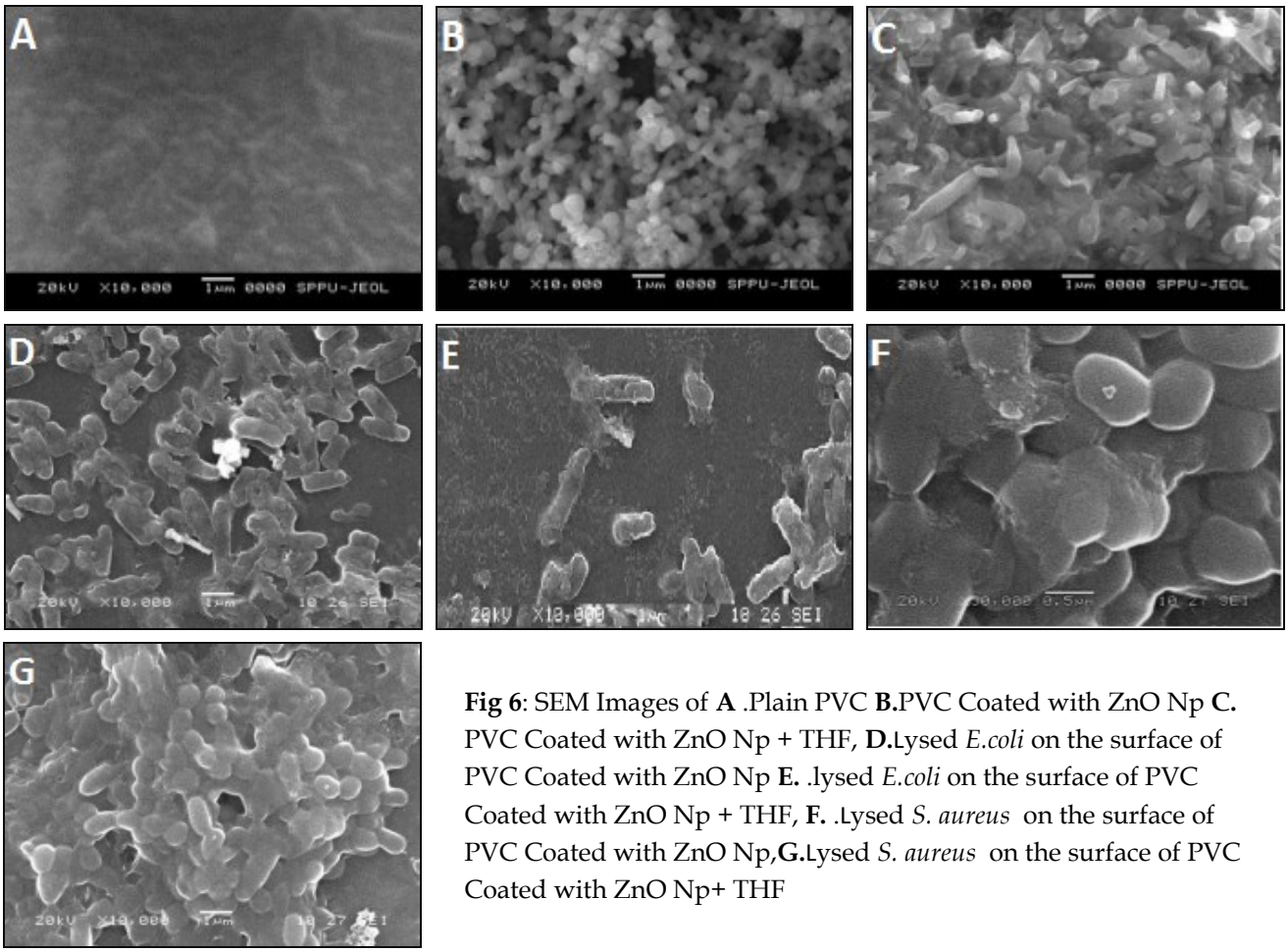


Fig 6: SEM Images of **A** .Plain PVC **B.**PVC Coated with ZnO Np **C.** PVC Coated with ZnO Np + THF, **D.**Lysed *E.coli* on the surface of PVC Coated with ZnO Np **E.** .lysed *E.coli* on the surface of PVC Coated with ZnO Np + THF, **F.** .Lysed *S. aureus* on the surface of PVC Coated with ZnO Np,**G.**Lysed *S. aureus* on the surface of PVC Coated with ZnO Np+ THF

CONCLUSION

Hence, we can conclude that ZnO-NPs are toxic to both skin fibroblast (A431) and human embryonic kidney cells (HEK293) hence care has to be taken while processing and formulating the nanoparticles till its final finished product and it. The antibacterial properties of medical-grade PVC material can be enhanced by coating by ZnO and THF helps in adhesion of ZnO NPs to PVC Sheets. The result provides a convenient method that can enhance the adhesive strength and amount of inorganic oxide film coated on organic materials. It is also easy to operate and can be applied on various biomaterials.

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