



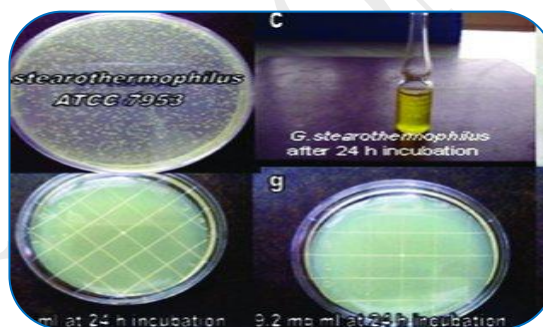
COMBINATION OF GOLD AND SILVER NANOPARTICLES DISPERSED POLY (VINYL ALCOHOL) NANOFIBROUS SCAFFOLD FOR CANCER TREATMENT

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ABSTRACT :

The synthesized silver and gold nanoparticles incorporated poly (vinyl alcohol) nanofibrous scaffold was prepared for cancer treatment. The silver and gold nanoparticles were synthesized from green way using mint extract as reducing agent and were analyzed by UV-Visible spectroscopy. The equal amount of synthesized gold and silver nanoparticles was incorporated into poly (vinyl alcohol) and electrospun to produce nanofibrous scaffold. The well dispersed gold and silver nanoparticles in the poly (vinyl alcohol) nanofibrous scaffold were studied by Transmission electron microscopy with Energy Dispersive X-ray Spectroscopy. The cytotoxicity of the combination of gold and silver nanoparticles dispersed poly (vinyl alcohol) nanofibrous scaffold by MTT assay method using mouse embryonic fibroblasts (NIH 3T3) and human breast cancer (MCF 7) cell lines were evaluated. Hence, the combination of gold and silver nanoparticles dispersed in the poly (vinyl alcohol) nanofibrous scaffold as a promising material for cancer treatment.



KEYWORDS : Electrosinning, Nanofibers, Scaffold, Gold nanoparticles, Silver nanoparticles.

INTRODUCTION:

Over the past decades, Cancer is one of the most vulnerable diseases in the worldwide (Winterhoff and Konecny 2017). Due to modernization and fast emerging lifestyle, many peoples are affected by cancer without proper individual reason (Levit *et al.*, 2013). Nanotechnology is the one of the best solution for biomedical applications due to its high characteristic unique properties (Ramos *et al.*, 2017). The synthesis of nanomaterials without polluting the environment is one of the considerable things nowadays (Chandran *et al.*, 2006). Mint leaves are economically cheap and commercially available to act as reducing agent for synthesizing gold and silver nanoparticles (Valencia *et al.*, 2014). Gold nanoparticles (AuNPs) have excellent antioxidant and anticancer properties which has been easily synthesized by green way (Narayanan *et al.*, 2008). The silver nanoparticles (AgNPs) have excellent antibacterial activity which is also easily synthesized by plant extract as reducing agent (Gulrajani *et al.*, 2008). The two nanoparticles with high potency when blended properly may give good new improved nanoparticles with anticancer properties (Cao *et al.*, 2001). The nanoparticles were loaded into the scaffold which helps to deliver the nanoparticles with controlled and sustained manner (Vivekanandhan *et al.*, 2012). The electrospinning is one of the better methods to act as a scaffold for nanoparticles (Zhang and Yu, 2014). The water soluble polymer like poly (vinyl alcohol) (PVA) which is also known as hydrogel polymer was mainly used as nanocarrier for water soluble nanoparticles (Islam and Yeum, 2013). In the present study, the combination of gold and silver nanoparticles dispersed PVA nanofibrous scaffold was fabricated for cancer treatment. The applicability of using the prepared

electrospun gold and silver nanoparticles dispersed PVA nanofibrous scaffold was analyzed using MTT assay.

MATERIALS AND METHODS

Materials

Chloroauric acid and silver nitrate was purchased from Sigma-Aldrich, India. Poly (vinyl alcohol) (Medium average molecular weight) was purchased from Sisco Research Laboratories Private Limited, India. Mint leaves were purchased from local market, Salem, Tamilnadu, India. Distilled water and all other chemicals were used for all analysis without further purification.

Methods

Preparation of Gold and Silver nanoparticles

The mint leaves were washed thoroughly using distilled water to remove the impurities. The mint extract was prepared by grinding the mint leaves using distilled water and filtered. The filtrate was used for the further process of synthesizing nanoparticles. 0.01 M of chloroauric acid and silver nitrate solution was prepared separately by using distilled water. The above two solutions were added separately dropwise into the prepared mint extract under static condition incubated at 37 °C for 15 minutes. The intense red coloured solution indicates the formation of gold nanoparticles while the brown-yellow coloured solution was the indication of the formation of silver nanoparticles.

Preparation of the combination of gold and silver nanoparticles solution

PVA was dissolved in distilled water using magnetic stirrer at 80 °C for 2 hours. After getting the homogeneous solution, the solution was cooled for half an hour. The equal ratio (1:1) of gold and silver nanoparticles were added into the homogeneous PVA solution and stirred continuously for 2 hours. Then, the above solution was sonicated for half an hour.

Electrospinning process

The prepared gold and silver nanoparticles loaded poly (vinyl alcohol) solution was electrospun at a flow rate of 0.6 ml/h and electric voltage at 20 kV. The distance between the syringe tip to aluminium foil wrapped collector was optimized at 10 cm. The whole electrospinning set up was already reported in our previous work (Thangaraju *et al.*, 2012).

Characterization

The double beam UV-Visible spectrophotometer, cyber lab was used for identifying the gold and silver nanoparticles initially. The surface morphology of the electrospun gold and silver nanoparticles dispersed in the PVA nanofibrous scaffold (Au-Ag-Nps-PVA NFs) was observed using Transmission electron microscopy with energy dispersive X-ray (TEM with EDX) (FEI 30S TECHNAI G2 - HRTEM) at 200 Kv. The cytotoxicity and cytoproliferative effects of the electrospun Au-Ag-Nps-PVA NFs was assessed using NIH 3T3 fibroblast and MCF 7 cell lines using MTT assay (Elakkiya *et al.*, 2013). The cell viability of the electrospun PVA NFs was characterized using FEI Quanta FEG 200 – HRSEM at 15 kV.

RESULTS AND DISCUSSION

UV-Visible spectroscopy and TEM with EDX

The synthesized gold and silver nanoparticles were observed by UV-Visible absorption spectrophotometer as shown in inset of Figure 1. The synthesized gold and silver nanoparticles show the absorption peak at 542 nm and 438 nm, which confirms the formation of gold and silver nanoparticles. The Au-Nps-PVA NFs and Au-Ag-Nps-PVA NFs were electrospun and collected on copper grid for 2 minutes which has been used to analyse TEM with EDX. The surface morphology of the electrospun Au-Nps-PVA NFs and Au-Ag-Nps-PVA NFs were observed by TEM with EDX as shown in Figure 1. The Au-NPs were dispersed well in the PVA nanofibrous scaffold which also resembles the general structure of gold. The gold and silver nanoparticles mixed thoroughly and settle down on the

two nanofibers gap which may be helpful for drug delivery (cancer treatment). The average AuNPs and the mixture of gold and silver nanoparticles dispersed in PVA nanofibrous scaffold size were found to be approximately 100 nm and less than 30 nm respectively. The copper present in the EDX spectrum may be due to the copper grid which has been used for sampling TEM analysis. Hence, the electrospun Au-Ag-Nps-PVA nanofibrous scaffold was excellent nanocarrier for cancer treatment.

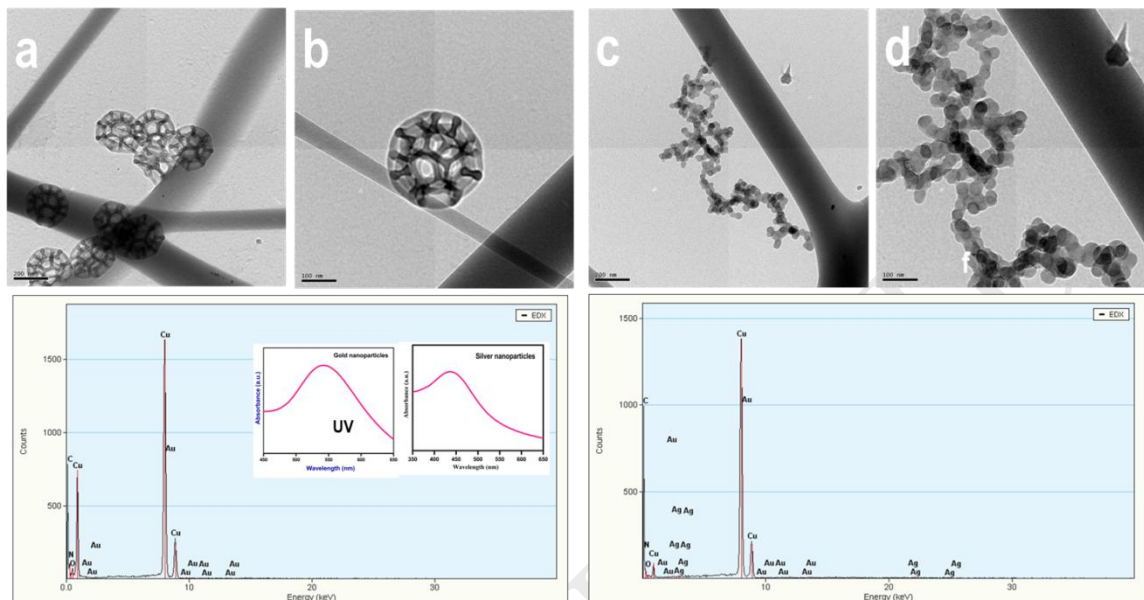


Figure. 3 TEM with EDX images of the electrospun AuNPs-PVA (a and b) and Au-Ag-NPs-PVA (c and d) at magnification at 200 nm and 100 nm

In-vitro Cytotoxicity Studies

In-vitro cytotoxicity tests of the eletrospun fibrous scaffold were performed and the results were shown in Figure 2.

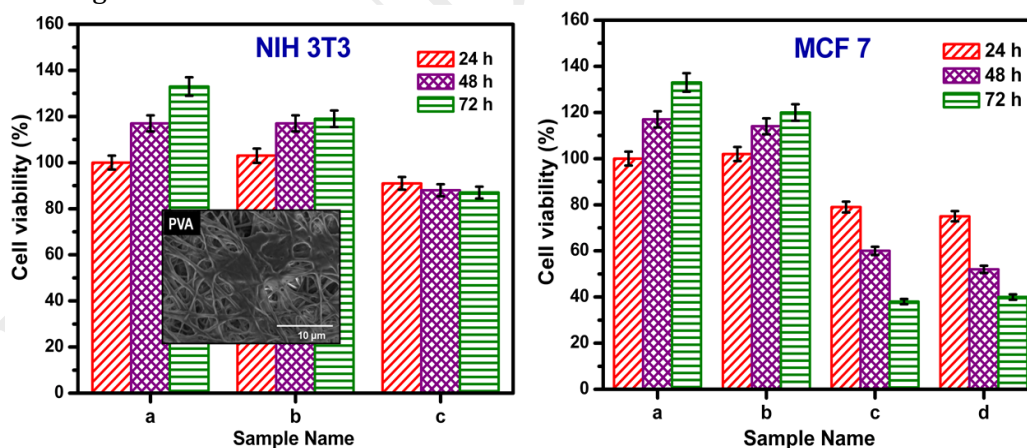


Figure. 2 MTT assay of using NIH 3T3 (a) control (b) electrospun PVA, (c) electrospun Au-Ag-PVA and SEM image of NIH 3T3 cell lines seeded on the electrospun PVA after 72 h and MCF 7 (a) control (b) electrospun PVA, (c) electrospun Au-PVA and (d) electrospun Au-Ag-PVA

The cell viability of the electrospun PVA and Au-Ag-NPs-PVA NFs were evaluated at 24, 48 and 72 h incubation periods respectively. The results of the electrospun nanofibrous scaffolds showed that the cell viability at 72 h was higher compared to 24 and 48 h respectively. On increasing the period of incubation time of NIH 3T3 in the electrospun Au-Ag-NPs-PVA NFs from 24 to 72 h, the cell viability was decreased compared to control and electrospun PVA respectively, although, the cell viability of the

Au-Ag-NPs-PVA NFs was good for cancer treatment. The scanning electron microscopy image of NIH 3T3 cell lines seeded on the electrospun PVA nanofibrous scaffold resembles the human extracellular matrix protein structure. The anti-proliferative activity of the Au-Ag-NPs-PVA NFs was studied using MCF 7 cell lines as shown in Figure 2. The MTT assay results confirmed that the electrospun Au-Ag-NPs-PVA NFs have high anti-proliferative effect against MCF 7 cell lines and also had good cell viability in normal cell lines (NIH 3T3).

CONCLUSION

In the present study, combination of gold and silver nanoparticles dispersed poly (vinyl alcohol) nanofibrous scaffold was successfully prepared by electrospinning method. Gold and silver nanoparticles were synthesized using mint extract as reducing agent and were identified by UV-Visible spectroscopy. The synthesized gold and combination of gold and silver nanoparticles were dispersed into poly (vinyl alcohol) nanofibrous scaffold was confirmed by TEM with EDX. The combination of gold and silver nanoparticles dispersed poly (vinyl alcohol) nanofibrous scaffold was used for killing the cancerous cells without affecting the healthy cells was confirmed by MTT assay. Therefore, the combination of gold and silver nanoparticles dispersed poly (vinyl alcohol) nanofibrous scaffold may be used for cancer treatment.

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