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Heterocyclic polymer Nanocomposite Hydrogel

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Abstract : In the present investigation, novel aromatic nanocomposite hydrogel containing indole-3-acetic acid has been reported. Citric acid, Diethyleneglycol and Indole-3- acetic acid based biopolymeric hydrogels were prepared by condensation polymerization.The stable and uniformly distributed silver nanoparticles have been obtained within the hydrogel network.The surface morphology of synthesized nanohydrogel has been characterized using scanning electron microscope with energy dispersive X-ray spectroscopy (SEM-EDX) analysis and transmission electron microscopy (TEM) techniques. The swelling studies have also been carried out for nanohydrogel at various pH (in the range of 3-9) solution. The nanocomposite hydrogels have also been examined for cytotoxicity effect. The results of the study indicated that the nanocomposite hydrogel may be recommended for eco-friendly biomedical applications.

Keywords: Silver nanoparticles, cytotoxicity effect, Citric acid, Indole 3 acetic acid, condensation polymerization, Hydrogel.

Introduction

In the last few years, the polymer nanotechnology has paid significant attention to biodegradable hydrogels [1]. These polymers are extremely used in the biomedical domain such as tissue engineering, particularly in anti-bacterial and antifungal applications [2]. In the present investigation , the heterocyclic aromatic compound such as Indole 3 acetic acid (IAA) has been introduced to synthesis hydrogel. Literarature reports revealed that IAA combined with horseradishperoxidase (HRP) found to have cytotoxic behavior to human cancer cells[3,4]. Further, IAA based hydrogel with silver nanoparticles found to cytotoxic behavior with respect to fibroblast 3T3 cell line for wound healing. The progressive series of molecular,cellular and biochemical proceedings of the hydrogels can able to form complex with dynamic wound healing[5] and drug delivery applications[6,7].

The high surface area , very small size (less than 20 nm) and high dispersion of Silver nanoparticles found to have antimicrobial properties and pharmacology applications. The interaction of metal nanoparticles with microorganisms is an expanding field of research[8]. In recent years silver nanocomposites were major missiles against wound infection with the advent of antibiotics [9]. These materials may have low, medium or high potential risk to human safety, depending on the type and extent of usage. Cytotoxicity is one of the recommended and appropriate step for the biological appraisal of medical devices is *in vitro* assessment as biomaterials. In our work , the primary screening for cytotoxicity have done using MTT test on the fibroblast cell line NIH-3T3.

The nanocomposite hydrogel have been prepared by using three monomers viz., Citric acid (CA), Diethelene glycol(DEG) and Indole 3 acetic acid(IAA). The third monomer IAA have been utilized for biological activities like antibacterial,antifungal,antioxidant and cytotoxicity[10].

The scope of the present investigation involves in the synthesize silver nanocomposite hydrogel by condensation polymerization without cross linker. The swelling equilibrium of nanocomposite hydrogel have been calculated at various pH(3-10) and found to have ascending order from acidic media to basic media. The nanoparticles have been identified by Trasmission electron spectroscopy and Scanning electron microscope with Electron dispersive X-ray analysis. The effect of cytotoxicity have also been observed for nanocomposite using fibroblast 3T3 cell line can be analysed by MTT assay.

Experimental Part

Materials

Anhydrous citric acid (CA) was purchased from sigma Aldrich (Bangalore, India). Diethylene glycol was purchased from Merck (India). Indole-3-acetic acid ,Silver Nitrate, Sodium borohydride, polyvinyl pyrolidinehave been purchased from Fine chemical,Mumbai, India.

Development of CA-DEG prepolymeric hydrogel(step-1)

First, prepared prepolymer (CA-DEG) hydrogel by Condensation polymerization without cross linker in round bottom flask. Citric acid (0.025M)dissolved in 5ml ethanol in round bottom flask closed with guard tube, strirred with magnetic strirrer at room temperature then Diethylene glycol slowly added to round bottom flask continuously strirred for half an hour. After this round bottom flask (closed with guard tube) kept in silica oil bath at 160^0C for one hour to reached reaction , glassy white sticky gel obtained.

Development of CA-DEG-IAA polymeric hydrogel(step-2)

Indole 3 acetic acid (IAA) added to prepolymer, stirred with magnetic stirrer at room temperature for half an hour then kept in silica oil bath at 160^0C for one hour , after completion of reaction, glassy reddish brown sticky gel obtained. The resultant gel was immersed in distilled water for one day inorder to removed unreacted monomer , filtered dried with oven at 35^0C for 24 hours .

Synthesis of nanocompositehydrogel(step-3)

In this process, 0.1g of hydrogel was immersed in distilled water for 24 h then hydrogel was immediately transferred toa beaker containing 25 ml of AgNO3 aqueous solution andthen allowed to equilibrate for 2 days. In this step, the Ag ions exchange from solution to the hydrogel networks. Finally, the hydrogel-loaded silver hydrogel was transferred to a beaker containing 25 ml of concentration NaBH4 aqueous solution for 4 hours to reduce the Ag+ into Ag^0 nanoparticles (black color). After this, dried ambient temperature then allowed for various characterizations.

Characterization

The resultant hydrogel was studied for swelling equilibrium behavior, morphology (TEM), elemental analysis (SEM with EDS) and cytotoxicity studies(cell viability) carried out to prepare cells Chang liver 5 x 10^3 cells and test compounds in 96-well plates containing a final volume of 100 µl/well. Then incubate for desired period of exposure and add 10 µl MTT Solution per well to achieve a final concentration of 0.45 mg/ml. After this incubate 1 to 4 hours at 37°C. Then add 100 µl Solubilization solution to each well to dissolve formazan crystals,mix to ensure complete solubilization. Record absorbance at 570 nm and MTT is measuring mitochondrial activity.

Results and Discussion

Swelling equilibrium studies

Figure.1 indicates the swelling equilibrium of Silver nanocomposite hydrogel of 48 hours at various pH buffer solutions (pH range 3 to 10). The swelling capcity of nanocomposite hydrogel plays a important role in the wound healing and biomedical application due to high absorption capcity of water or solvent. The swelling equilibrium reduces from pH 3 to 10 due to protanated amino group present in polymeric network[11]. S_{equ}% at pH 3,4,6,7,9,10 were mentioned as 1480.00, 1400.00, 1020.00, 962.00, 830.00 and 720.00 respectively. So S_{equ}% better in acidic medium compared with basic medium.

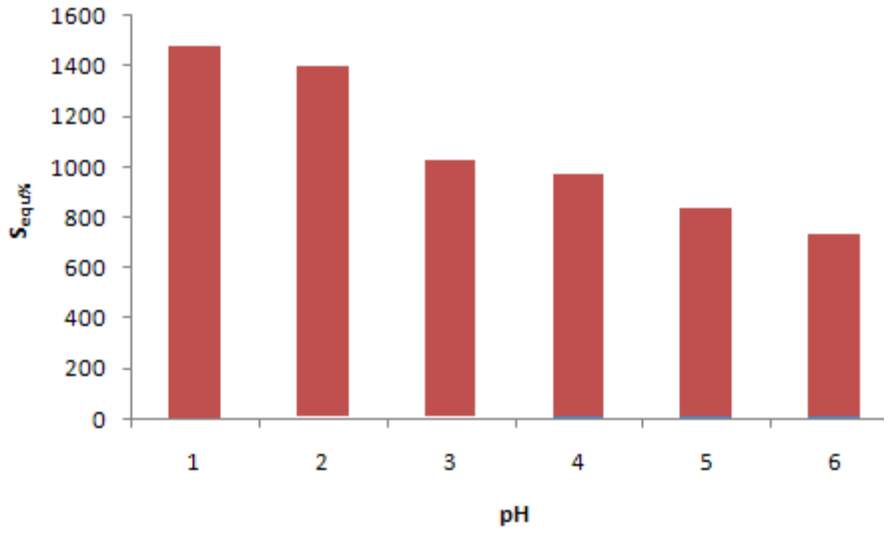

Figure.1 Swelling equilibrium of nanocomposite hydrogel

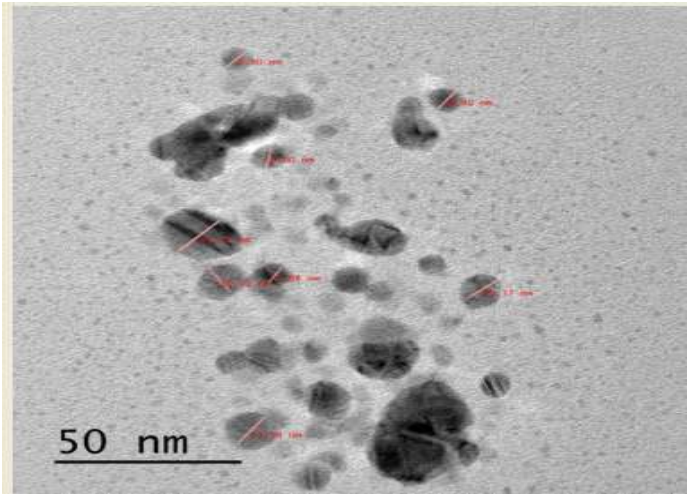

Figure.2 TEM image of nanocomposite hydrogel

Trasmission electron spectroscopy

In Figure.2 TEM image to determined the size of Silver nanoparticles in nanocomposite hydrogel. This image shown the size of nanoparticles distributed with average size was 4-12 nm which has proved the nanoparticles present in hydrogel network . So very smaller size of nanoparticles enhanced the biological properties[12,13].

Field Emission Scanning Electron Microscopy - Energy Dispersive X-ray analysis (EDX)

Figure.3 SEM with EDX confirmed the silver nanoparticles present in this nanocomposite hydrogel. The silver nanoparticles peak obtained at 2.6 Kev with 0.57% silver mentioned in EDX analysis. No impurty peaks detected in this images which has proved high purity silver nanoparticles in hydrogel[14].

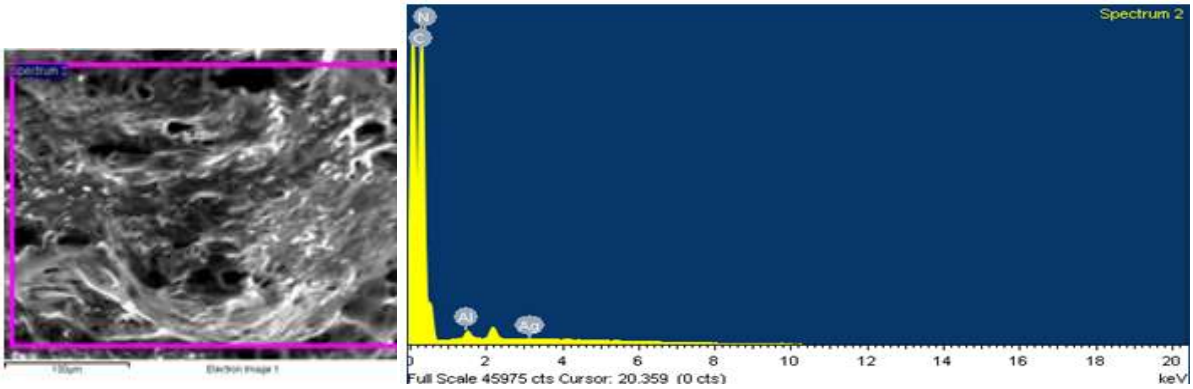

Figure.3 SEM with EDX nanocomposite hydrogel

Cytotoxicity evaluation :**Qualitative studying of the cell viability**

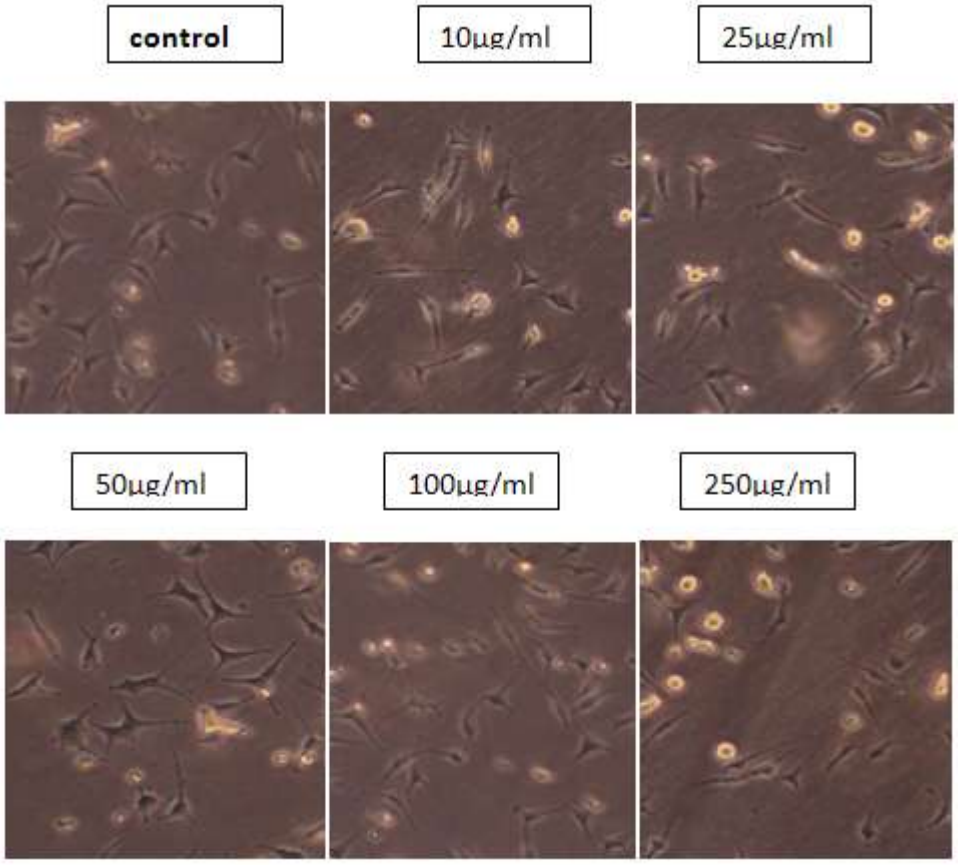

Figure.4 Cytotoxicity images of nanocomposite hydrogel

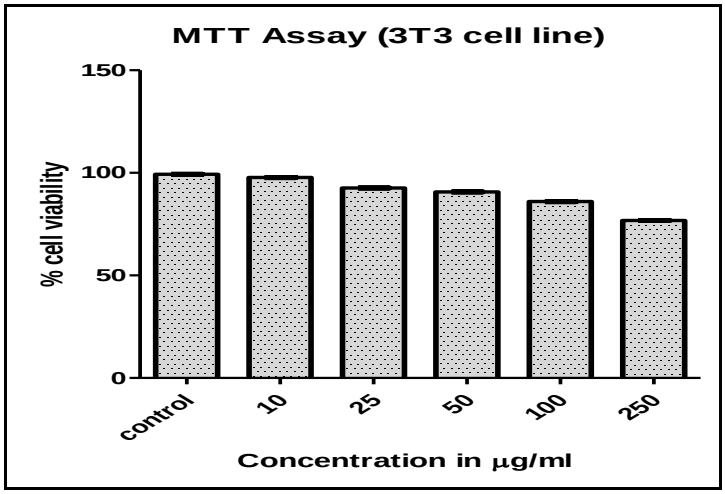

Figure.5 MTT assay of Nanocomposite hydrogel

(Figure. 4 ,5) In vitro cytotoxicity of silver nanocomposite hydrogel evaluated using MTT assay in fibroblast cell 3T3 cell line treated with various concentrations of composite hydrogels. The % of cell viability decreases with increasing concentration. The cell effect based on morphological changes, such as shrinkage and abnormal conditions. It can be concluded cytotoxic at low concentrations was better cell viability, but its high concentrations decrease the viability and cell proliferation compared to the controls. Similar observation noted Michiko et al cells were exposed to various concentrations , their viability decreased[15,16].

Conclusions

The novel method, synthesized Silver nanocomposite hydrogel by condensation polymerization without cross linker. The swelling equilibrium found at various pH buffer solutions tends better in acidic medium. The nanoparticles persent in polymeric network confirmed by TEM,SEM wit EDX. Then biological application cytotoxicity done by using fibroblast cell 3T3 cell line resulting percentage cell viability was better in low concentration of nanocomposite hydrogel.

So this nanocomposite hydrogel can be applicable for wound healing and burn dressing.

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