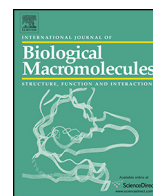




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Cellulose gum and copper nanoparticles based hydrogel as antimicrobial agents against urinary tract infection (UTI) pathogens

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ABSTRACT

In the present study, stable copper nanoparticles (CuNPs) were successfully prepared in the hydrogel matrix. The prepared nanocomposite (HCuNPs) was characterized via x-ray diffraction (XRD), electron microscopy (TEM), and energy-dispersive (EDX) and x-ray photoelectron spectroscopic (XPS) studies. The wide scan XPS spectra support the presence of C, N and O in neat hydrogel; while, the XPS spectra of HCuNPs demonstrate the presence of Cu along with C, N, and O elements. TEM studies show the formation of spherical shaped CuNPs in the size range from 7 to 12 nm. The rheology results reveal that the storage modulus (G') of the HCuNPs was found to be higher than the loss modulus (G''). Additionally, the antibacterial activities and cytotoxic were carried out against urinary tract infection (UTI) microbes and HeLa (cervical) cells respectively. The antibacterial results reveal that HCuNPs composites show higher zone of inhibition against these pathogens than that of corresponding hydrogel matrix. The cytotoxic effects suggest that the prepared nanocomposite could be used as promising candidates for biomedical applications.

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1. Introduction

The growth of microorganisms (e.g. bacteria, fungi, viruses, yeasts etc) is a vast challenge to the scientific community since past few decades. Microorganism contamination is of great concern in various areas, such as medical devices, health care products, water purification systems, food packaging, and food storage. The organic antimicrobial agents are often less stable, especially at high temperature or pressure and are extremely irritant and toxic [1]. On the other hand, inorganic antibacterial materials such as metal and metal oxide (e.g. Ag_2O , TiO_2 , CuO , ZnO , MgO etc) have received an attention due to their stability, robustness and chemical stability against the microorganisms [2–7]. Nanoscience and nanotechnology have been offering the numerous ways to control the growth of these microorganisms. It was found that some physico-chemical properties of materials depend on the size materials and the properties of nanosized particles were found to be different than the bulk or larger particles [2,8–11]. Metal nanoparticles nor-

mally exhibit positive charge on their surface while microbes have negatively charged surface, therefore, the metal nanoparticles facilitate to bind with the microbes via their surface and inhibit their growth. Nanocrystalline noble metals like Au, Ag particles have been extensively used as the antimicrobial agents to demolish the pathogenic microorganisms that cause the intense infections in human beings [12–16]. In our previous study, we have prepared silver nanoparticles and hydrogel based nanocomposite and used as an antimicrobial agent against several pathogens [17]. However, the high cost of Ag prevents wide-spread application in daily basis. Therefore, new alternative metal nanoparticles to reduce or replace expensive Ag are necessary. Copper is redox-active metal, it is also a challenge to prepare the copper nanoparticles in aqueous solution because the CuNPs initially formed but the prepared nanoparticles easily oxidized in the solvents or in the aqueous solution [18]. To stabilize as well as to control the size and growth rate of nanoparticles increased the attention to fabricate the nanoparticles.

In the present work, we have synthesized hydrogel containing CuNPs to enhance the antimicrobial activity against pathogens such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. vulgaris*, *S. aureus* and *P. mirabilis*. The neat hydrogel and HCuNPs have been characterized using powder x-ray diffraction (XRD), transmission electron microscopy (TEM), EDX, SAED and x-ray photoelectron spectro-

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scopic (XPS) techniques. Anti-microbial activities of HCuNPs and hydrogel were also investigated against UTI pathogens and the cytotoxicity was carried out against the human cervical cancer cell line (HeLa) using MTT assay.

2. Experimental

2.1. Materials

Copper (II) nitrate, carboxymethyl cellulose (CMC), polyvinyl alcohol (PVA), ammonia solution, ethyleneglycol diglycidylether (EGDE) and hydrazine were used in the synthesis of HCuNPs. All the chemicals were procured from Sigma-Aldrich.

2.2. Preparation of HCuNPs materials

In a 100 mL beaker, 5 g CMC was dissolved in 30 mL aqueous ammonia solution (2%) and 2 g PVA was dissolved in aqueous solution in another beaker. Both the solution was mixed together to obtain the homogeneous viscous mixture the solution was mechanical stirred and followed by the addition of 1 mL of ethylene glycol diglycidylether (EGDE) drop by drop to get the homogeneous matrix. Thereafter, 5 mL of 1 mM $\text{Cu}(\text{NO}_3)_2$ solution was added to the homogeneous matrix. The homogeneous matrix containing Cu^{2+} ions was deoxygenated with inert gas for 40 min with the flow rate of 5 mL/min. The Cu^{2+} ions present in the homogeneous matrix was reduced using hydrazine solution at room temperature to produce the HCuNPs. The resulting nanocomposite was washed using distilled water to remove the unreacted part of the reagents (CMC, PVA, and hydrazine), filtered and then dried in vacuum at 50 °C for 48 h.

The physical properties such degree of swelling (EDS) determination, gel fraction (%) or percent gelation calculation, and the rheological properties such as storage modulus $G'(\omega)$ and loss modulus $G''(\omega)$ of neat hydrogel and HCuNPs nanocomposite were measured using the previously reported methods [19,20] and the details of the experiments and analytical techniques were given in supplementary data.

2.3. Biological activities

2.3.1. Isolation of pathogens and antibacterial assay

The UTI pathogens were collected from 40 infected urine samples that contain *Escherichia coli* (30%), *Klebsiella pneumoniae* (20%), *Pseudomonas aeruginosa* (15%), *Proteus vulgaris* (15%), *Staphylococcus aureus* (12.5%) and *Proteus mirabilis* (7.5%). All the samples were kept at ice cold temperature with 1.8% boric acid [21]. To isolate these pathogens, a loopful of infected urine was streaked on the CLED (Cystine lactose electrolyte deficient medium) and MacConkey agar medium which was incubated at 37 °C for 24 h at only 4 mm deep, poured into either 100 mm Petri dishes. Thereafter, the Mueller Hinton agar disc diffusion method was used to determine the antibacterial activities of hydrogel and HCuNPs against these pathogens [22,23]. Sterilized filter paper discs (Whatman, 5 mm in diameter) soaked 30 μL of test solution with different concentrations (1, 3 and 5 mg/mL) of hydrogel and HCuNPs composite aqueous suspension. The plates were inoculated at 37 °C for 24 h and the zones of inhibition were measured. All the experiments were carried out three times and the results were expressed as means along with one standard deviation (SD).

2.3.2. In vitro cytotoxicity assay

The cytotoxicity of hydrogel and HCuNPs was carried out against human cervical cancer cell line (HeLa) using MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide) assay [24,25]. The cells were grown in modified Eagle's medium and supplemented with

10% fetal bovine serum (FBS), streptomycin (100 U/mL) and penicillin (100 U/mL) and incubated under the humidified atmosphere (CO_2) at 37 °C. The cells with the density of 1×10^5 /well were placed in 96-well plates and incubated for 24 h and the cultured cells were treated with different concentrations of hydrogel and HCuNPs ranging from 10 to 100 g/mL. The formazan crystals were dissolved by adding 0.1 mL MTT solution. After the crystals were fully dissolved, the absorbance was measured at 570 nm in a multi-well plate reader (Bio-rad, 680, America), and the percentage of cell viability relative to the control cells was measured. The results were expressed as means $\pm$ standard error (SE) three separate experiment cultures. After removal of the culture medium, cells were treated with acridine orange/ethidium bromide (AO/EB, 100 $\mu\text{g}/\text{mL}$ in PBS) in situ. HeLa cells were examined by fluorescence microscopy (Olympus IX51, Japan).

3. Results and discussions

3.1. Characterizations of HCuNPs

CMC based hydrogel was prepared with PVA, and EGDE in aqueous solution according to Scheme 1. Cu^{2+} ions were bound to the hydrogel matrix via electrostatic interactions with oxygen atoms of hydrogels such as $-\text{COOH}$ and OH . Copper nanoparticles (CuNPs) were prepared by the reduction of Cu^{2+} ions in the hydrogel matrix using hydrazine at room temperature [26]. Due to the interaction between oxygen and carboxyl groups of the hydrogel on the metal nanoparticle surface, the CuNPs nanoparticles could be dispersed well in hydrogel matrix to form a stable CuNPs. The hydrogel matrix helps to control the size of Cu nanoparticles (CuNPs). The prepared nanocomposite was dried and used for further characterization.

The EDS of the hydrogel and HCuNPs were examined at pH 7.4. The swelling degree was increased with time and reached the equilibrium at about 58 h, for both the samples. The swelling percentage of neat hydrogel and HCuNPs were observed 720% and 530% respectively at equilibrium and the results are given in supporting with the standard deviation of the means in figure (SF 1). On the other hand, the gel fraction of HCuNPs was found to be 68%, whereas in case neat hydrogel the, gel fraction was observed 80%. In addition, it was observed that the CuNPs was released from HCuNPs nanocomposite in aqueous solution. The amount of released CuNPs and the actual amount of the CuNPs was measured using HNO_3 . It was found that the percentage of cumulative CuNPs release from HCuNPs was 73.0% after 5 days of immersion at room temperature at pH 7 and got the saturation in CuNPs release. The release kinetics of CuNPs and Cu^{2+} ions form the HCuNPs could be one of the key parameters to determine the durability and stability as well as the biological activity of the HCuNPs and the results are given in figure (SF-1a). The kinetics of CuNPs releasing were carried out using several kinetic models, the correlation between experimental results and the model predicted values was shown by the R^2 (correlation coefficients). A relatively high R^2 value (0.976) represented that the model successfully described the first order kinetics of CuNPs releasing and show in figure (SF-1b). The rheology experiments were used to measure the viscoelastic parameter (storage modulus $G'(\omega)$ and loss modulus $G''(\omega)$ respectively) using an oscillatory shear field over the HCuNPs and neat hydrogel. The results revealed that the value of G' was found higher than G'' which support solid-like behavior of both tested samples. On the other hand, the values of G' and G'' were found to be lower in the case of HcuNPs when compared with the neat hydrogel and results are illustrated in Supporting figure (SF 3). The lower values of G' and G'' were due to the presence of reducing agent. In addition, due to the presence of CuNPs in gel matrix, the crosslinking between the PVA and

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