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Polyethylene glycol functionalized carbon nanotubes/gelatin-chitosan nanocomposite: An approach for significant drug release

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ABSTRACT

This research work blooms the new idea of developing a safe and controlled drug releasing matrix using multi-walled carbon nanotubes (MWCNTs). In aqueous solution, uniform and highly stable dispersion of MWCNTs was obtained after secondary functionalization with polyethylene glycol (PEG) which was studied by Fourier transmission infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA). Solution casting method was used to prepare MWCNTs/gelatin-chitosan nanocomposite films and the effect of MWCNTs on physico-mechanical, thermal and water uptake properties of the nanocomposites were evaluated. Incorporation of MWCNTs into the porous gelatin-chitosan matrix showed interesting stiffness and dampness along with developed microfibrillar structures within the pore walls intended at being used in tissue engineering of bone or cartilage. A common antibiotic drug, ciprofloxacin was incorporated into nanocomposite matrix. The evaluation of the effect of MWCNTs on drug release rate by dissolution test and antimicrobial susceptibility test was performed. Sharp release of the drug was found at early stages (~1 h), but the rate was reduced afterwards, showing a sustained release. It was observed that for all microorganisms, the antibacterial activities of drug loaded MWCNTs/gelatin-chitosan nanocomposites were higher than that of drug loaded gelatin-chitosan composite films containing no MWCNTs. Comparative statistical studies by ANOVA techniques also showed remarkable difference between the antibacterial activities, exhibited by MWCNTs-incorporated and non-incorporated composite films.

1. Introduction

Carbon nanotubes (CNTs) have been highly attractive and extensively explored by the researchers in various fields such as chemical, physical, materials and biochemical sciences due to their unique and versatile nanostructures with remarkable mechanical, thermal, electrical and optical properties [1–4]. Due to their structural and mechanical properties, CNTs can be used in making composites that are useful in tissue engineering and also suitable for regenerative medicine therapeutics as delivery vehicles for drugs and gene therapy [5]. CNTs have been shown to empower high Young's modulus (~1 TPa) due to the flexible hexagonal network of carbon atoms which ultimately directed to their excellent mechanical properties [6]. Furthermore, incorporation of CNTs has been recognized as substantial improvement of the mechanical and structural properties of polymer composites [7,8]. These composites can also be used as molecular-level building blocks for the complex and miniaturized medical devices, which have enormous applications in biomedicine [9]. CNTs have positive impacts on cell differentiation and proliferation as well [6,10–13].

However, CNTs and CNTs/polymer composites represent a set of problems relating to the carcinogenic risk from the exposure and persistency of MWCNTs in human bodies. Some of the researches published that the untreated long MWCNTs which were injected, induced some unwanted responses in the abdominal cavity of mice. So, the treatment of CNTs is mandatory. Not only this, CNTs have a tendency to form a network of agglomeration which ultimately increases the difficulties to disperse them within the polymer

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matrix during processing due to strong resistance to wetting, high inter-particle van der Waals attractions and high specific area [14,15]. So, preparation of successful CNTs/polymer composites depends on the achievement of stable and well dispersed CNTs in the solvents or polymer matrix. Common techniques widely used for preparing CNTs/polymer composites are solution casting providing numerous advantages such as large area coverage, structural flexibility, low temperature process ability and low cost, although solubility of polymers is essential for this method [16–18]. Both the dispersion and carcinogenic problems of CNTs can be overcome by functionalizing them with surfactants, acids or other agents. Many studies have shown the successful functionalization of CNTs covalently with acid treatment and non-covalently with many other agents [19–29].

However, not all chemical treatments alleviate the toxicity risks but only reactions that are able to render carbon nanotubes short and stable suspension without aggregation in the body fluids can provide safe results, risk-free material. Again, the binding affinity by non-covalent interaction between the CNTs surface and the agents is not strong enough, so the dispersion ability of CNTs in aqueous media is still not satisfied for biomedical applications. Therefore, covalent functionalization of the MWCNTs is necessary.

Thus, MWCNTs were functionalized chemically with mix acids, H_2SO_4/HNO_3 (3:1, v/v) followed by addition of polyethylene glycol (PEG). Acid treatment introduces oxygen containing functional groups (−OH, >CO, and −COOH), which furnishes the dispersion ability of MWCNTs. Although oxidized MWCNTs are soluble in water, they have a tendency to show charge screening affect i.e. aggregate in the presence of salts which makes the direct use of MWCNTs limited for biological applications as the salt content is high in most biological solutions, opsonisation [30]. For this reason, hydrophilic PEG is attached to the oxidized CNTs to make more stable CNTs-polymer conjugates in biological environments [31–33]. Nanoparticles, which require long circulation times in blood, usually need particular types of ligand and PEG is exceptionally found to be compatible as a ligand in high concentrations of salts and in extremes of pH [34–36].

In the present work, gelatin and chitosan have been chosen due to their water soluble and film forming properties. The major advantages of these are good cytocompatibility, biodegradability and no surgery is required for removal of polymers [37]. Gelatin is a well characterized gel forming protein fragment obtained by partial degradation of water insoluble collagen fiber commercially available at relatively low cost [38]. Chitosan is a nontoxic, biocompatible, biodegradable polymer [39–41] but has a limitation of being unstable in an aqueous medium. The shortcomings of chitosan film in solution and the easy degradation of rigid gelatin in the swollen state seem to be overcome by using a gelatin-chitosan composite. Gelatin and chitosan are natural polyelectrolytes that may form polyelectrolytic complex via interactions of ammonium ions ($-NH_3^+$) of the chitosan and carboxylate ions ($-COO^-$) of the gelatin in the blend system to make it more stable. This might facilitate the delivery of antibiotic drugs such as ciprofloxacin, which were directly loaded on the nanotube surface via π-π stacking and H-bonding. Here we present the formulation of homogeneously dispersed MWCNTs/gelatin-chitosan nanocomposites with high mechanical, thermal and swelling properties as well as their improved drug release capacity.

2. Experimental

2.1. Materials and chemicals

Multi-walled carbon nanotubes (MWCNTs), NC7000 series of 90% purity, showing approximately average diameter of 9.5 nm and 1.5 μm length, produced by the catalytic chemical vapor deposition (CCVD) method were purchased from NANOCYL™, Belgium. Gelatin Type B (Bloom strength-240, Pharmaceutical grade, pyrogen free) was purchased from the OPSO Saline Limited, Bangladesh and chitosan of 90% deacetylation degree having molecular mass of 1100 kDa (medicine grade) was purchased from Yuhuan Ocean Biochemistry Co. Ltd, China. Polyethylene glycol (PEG) was purchased from BASF (Lutrol E 400, Macrogol 400 ph. Eur), Bangladesh. All other chemicals and solvents were of reagent grade.

Seven bacterial strains, including 3 gram-positive (*Bacillus subtilis*, *Staphylococcus aureus, Listeria monocytogenes*) and 4 gram-negative (*Escherichia coli* 0157, *Salmonella enteritidis*, *Salmonella typhi, Klebsiella pneumoniae*) were obtained from the Center for Advanced Research in Sciences (CARS), University of Dhaka, Bangladesh.

2.2. Processing

Raw MWCNTs of a definite weight (1.0 gm) were added into 50 ml 3:1 mixture (v/v) of concentrated H_2SO_4 (98%)/HNO_3 (68%) with sonication at 40 °C for 10 h, diluted with water followed by filtration, washed with deionized water and dried overnight in vacuum at 80 °C to obtain primarily functionalized MWCNTs (MWCNTs-COOH) as a dry powdered form.

The secondary functionalization of MWCNTs was carried out by transforming the carboxyl groups of the oxidized MWCNTs into acyl chloride groups by stirring the dispersion with $SOCl_2$ for 24 h at 65 °C. After that the dispersion was washed off with THF, filtered and dried overnight at ambient temperature. The acyl chloride-functionalized MWCNTs were continuously stirred in PEG (400 g/mol) at 120 °C for 48 h. Then, the resultant mixture was washed using THF and filtered through a Teflon W membrane, followed by drying at ambient temperature for 48 h to get secondary functionalized MWCNTs (MWCNTs-PEG). The schematic diagrams of the functionalization reactions (primary and secondary) are shown in Fig. 1.

The films were prepared by blending chitosan (1%) and gelatin (10%) solutions thoroughly for 1 h followed by casted on the silicon cloth covered frame mounted on flat glass plate for film formulation. The film was dried in laminar air flow overnight and peeled off. Dispersed MWCNTs were added into the solution of chitosan (1%)-gelatin (10%) mixture , which was then stirred for 30 min to homogenize and sonicated for 1 h to remove air bubbles before film casting.

Stock solution of ciprofloxacin lactate was prepared in the concentration of 1% (w/v). The prepared drug solution was then added to 0.25% (wt/wt) MWCNTs-COOH/gelatin-chitosan nanocomposite and 0.25% (wt/wt) MWCNTs-PEG/gelatin-chitosan nanocomposite solutions respectively such that 10 μg of the drug was present in 0.28 cm^2 area of the nanocomposite films (this area is equal to the area of a standard antibiotic disc).

2.3. Characterization

The attachment of the functional groups on the surface of MWCNTs was identified by the Fourier transform infrared (FTIR) spectrophotometer, Imprestige-21, Shimadzu Corporation, JAPAN (wave number range of 400–4000 cm^{-1}), equipped with an attenuated total reflectance (ATR) device (wave number range of 700–4000 cm^{-1} with 20 scanning rate and resolution of 4 cm^{-1}) for the confirmation of gelatin-chitosan interaction in the composites.

The mechanical properties of the composites were determined by universal testing machine (UTM), Hounsfield Series S Testing Machine (UK), conducting load of 500 N at room temperature. The