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Glycolic acid functionalized chitosan–Au–Fe_3O_4 hybrid nanoparticle based nanohybrid scaffold for drug delivery

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ABSTRACT

The research on biomedical applications of nanoparticles has seen an upsurge in recent years due to their unique capabilities in treatment of ailments. The present paper reports the synthesis of Au–Fe_3O_4 hybrid nanoparticles. The formation of these nanoparticles was confirmed by transmission electron microscopy (TEM) and physical property measurement system (PPMS). Next step of this paper reveals potential use of novel hybrid of chitosan-g-glycolic acid and Au–Fe_3O_4 hybrid nanoparticles in controlled drug delivery and tissue engineering applications. Grafting of glycolic acid and drug loading in porous scaffold was characterized by Fourier transform infrared spectroscopy. The nanohybrid scaffolds were found to be stable regardless of pH of the medium and play a key role in cell adhesion, proliferation and migration. Au–Fe_3O_4 hybrid nanoparticles reinforcement was found to control the drug (cyclophosphamide) release rate in phosphate buffer saline solution (pH 7.4). Therefore, Au–Fe_3O_4 hybrid nanoparticles are viable additive for formulating sustained drug delivery systems based on glycolic acid grafted chitosan. The cell proliferation profile also shows that prepared nanohybrid is biocompatible providing suitable substrates for tissue engineering.

1. Introduction

Advances in nanotechnology play an important role in designing nanomaterials with specific functional properties that can address the shortcomings in the area of diagnostics and therapeutics. The advantages of the nanoparticles are mainly due to their nanoscale size and large surface area with the ability to get functionalized with therapeutic moieties, bio-molecules [1] and targeting ligands. The potential of nanomaterials has sparked enormous interest in the drug industries and has envisaged several applications, as can be evidenced by the exponential growth of activities in this field. The nanoparticles can easily gain access to various areas of the body without interfering into normal functions and has the requisite potential for therapeutic and diagnostic applications [2]. The size of the nanoparticles is quite similar or smaller to the size range of several bio entities makes them a natural companion in the hybrid system. The nanostructures have ability to bind with individual molecules at nanoscale has provided ample opportunity for new diagnostic and therapeutic applications. Due to this reason "hybrid" nanostructures can be obtained or it may be embedded in biocompatible materials to impart new functionalities. The hybrid nanostructures are desirable for many application like sustained drug delivery [3], hyperthermia [4,5], diagnosis [6–11] and biological and chemical sensing. The nanostructure mediated drug deliveries are the key technology for the realization of nano-medicine and play an important role in improving the properties of already existing therapeutic and diagnostic modalities. The nanoscale drug delivery system also helps in stabilizing drug molecules [12,13].

A wide range of materials have been employed as drug carriers such as lipids, surfactant, dendrimers and natural or synthetic polymers [14–17]. Among these, polysaccharides have received increasing interest because of their outstanding physical and biological properties [18]. Chitosan, a linear cationic polysaccharide composed of randomly distributed ($\beta 1 \rightarrow 4$) linked D-glucosamine and N-acetyl-D-glucosamine units. This cationic polysaccharide has drawn increasing attention within biomedical applications, owing to its abundant availability, unique muco-adhesive, inherent pharmacological and biological properties such as biocompatibility, biodegradability, non-toxicity and low-immunogenicity [19–21]. Chitosan is modified by chemical reaction, which involves the reactive hydroxyl and amino groups of the polymer chain. The grafting of glycolic acid on chitosan leads to marked changes in its structure [22,23].

The primary focus of this work is to prepare novel hybrid of chitosan-g-glycolic acid and Au–Fe_3O_4 hybrid nanoparticles

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Table 1
Formulation of cyclophosphamide (CPA)-loaded nanohybrid of chitosan-g-glycolic acid and Au–Fe_3O_4 hybrid nanoparticles.

S. no.	Chitosan (g)	Glycolic acid (g)	AFNP (mg)	CPA (mg)	Drying process	Sample code
1	1	1	-	-	Vacuum	CGAF-1
2	1	1	50	-	Vacuum	CGAF-2
3	1	1	50	10	Freeze	CGAF-(D)

in controlled drug delivery and tissue engineering applications. These Au–Fe_3O_4 hybrid nanoparticles have been dispersed into the matrix of glycolic acid grafted chitosan scaffolds, which are prepared by vacuum and freeze drying.

2. Materials and methods

2.1. Materials

Chitosan ($M_w = 1.5 \times 10^5$), degree of deacetylation was 85%, glycolic acid with 99% purity, oleic acid (OA), oleylamine (OAM), 1-octadecene iron (0) pentacarbonyl ($Fe(CO)_5$), 1, 2-tetradecanediol, cyclophosphamide (CPA) drug was obtained from Sigma–Aldrich. Gold chloride ($HAuCl_4$), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and phenyl ether was obtained from Sisco Research Laboratories.

2.2. Synthesis of Au–Fe_3O_4 hybrid nanoparticles (AFNP)

Au–Fe_3O_4 hybrid nanoparticles were synthesized in this work with reference to the literature [24]. First AFNP was prepared by the thermal decomposition of $HAuCl_4$ at high temperature. 1 mmol of $HAuCl_4$ and 9 mmol OAM were added to 20 ml phenyl ether. It is followed by the addition of 4 mmol of 1, 2-tetradecanediol. The reaction mixture was heated slowly up to 185 °C for 1.5 h under inert atmosphere. The reaction mixture was then cooled down to room temperature and precipitated with ethanol. After centrifugation, precipitate was dried and redispersed in 20 ml of hexane containing 10 mM OAM. In the step, OA, OAM and 1-octadecene were heated 100 °C under argon atmosphere. The AFNP dispersed in hexane was injected at this temperature, followed by flushing with argon (to remove hexane) and then heated to 120 °C, at which $Fe(CO)_5$ was injected. The reaction mixture was slowly heated to reflux (1 °C min^{-1}) for 4.5 h. After cooling to room temperature, the reaction mixture was stirred for 1 h, followed by precipitation with acetone and then dried in air.

2.3. Preparation of nanohybrid scaffolds and drug loading

Chitosan-g-glycolic acid and Au–Fe_3O_4 nanoparticle nanocomposite film was prepared by dispersing chitosan in deionized water for 1 h with constant stirring at room temperature. Glycolic acid was added to the solution after 1 h, which is allowed to stirred for 12 h. After 12 h, Au–Fe_3O_4 hybrid nanoparticles were added to the resulting solution and stirred overnight at room temperature. The solution was heated up to 80 °C with continuous degassing for 45 min. The Solution was cooled to room temperature after degassing, which is followed by CPA addition 20 mg and stirred for 5 h, so that drug completely mixes with solution. After 5 h, solution was poured in tissue culture plates (50 mm × 50 mm diameter). The drug loaded solution was quenched in liquid nitrogen and freeze dried by lyophilization under −100 °C temperature for 6 h. The formulations are shown in Table 1. In lyophilization water molecules were removed by freezing and sublimation of ice crystals, which lead to the formation of pores. The polymer-rich phase forms the cell walls around the pores [25].

2.4. Characterization

2.4.1. Transmission electron microscopy

The surface morphology, selected area diffraction pattern of Au–Fe_3O_4 hybrid nanoparticles can be investigated with High Resolution Transmission Electron Microscopy (HR-TEM model Technai TF30, 300KV FEG). The samples of Au–Fe_3O_4 hybrid nanoparticles for TEM studies were prepared by placing drop on carbon coated copper grids.

2.4.2. Physical property measuring system

The formation of Au–Fe_3O_4 hybrid nanoparticles was also determined by measuring hysteresis loops of the synthesized nanoparticles from physical property measuring system (PPMS) (quantum design Inc., San Diego, USA) equipped with 7 T superconducting magnet and a vibrating sample magnetometer.

2.4.3. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)

ATR-FTIR is a powerful technique to understand the surface chemistry of modified surface. ATR-FTIR spectroscopy of neat chitosan (CT), chitosan grafted glycolic acid (CGAF-1), nanohybrid scaffold (CGAF-(D)) and drug (CPA) were performed over a range of 4000–400 cm^{-1} at a resolution of 2 cm^{-1} using a Nicolet spectrometer system.

2.4.4. Scanning electron microscopy

Surface morphology of the samples was analyzed with scanning electron microscopy (SEM) (Model: JOEL Stereoscan 440, Cambridge). Prior to the observation, specimens were fixed on the copper grid.

2.4.5. Swelling behaviour

The swelling behaviour of porous scaffold was determined by exposing them to media of different pH: 1 N HCl, 1 N NaOH and stimulated body fluid (SBF, pH 7.4) solutions. Shape retention of the porous scaffold was determined by measuring the change in the diameter as a function of time in the media.

2.4.6. In vitro drug release

The drug loaded nanohybrid scaffold (CGAF-(D)) were immersed in 10 ml of aliquots of 0.1 M phosphate buffer (pH 7.4) and incubated at 37 °C. After specific interval 3 ml aliquot of the specimen were withdrawn and immediately fresh medium is added to it. Drug content in each aliquot was quantitatively analyzed by UV–vis spectrophotometer (UV-NIR-PL Lamda 950) at 180 nm.

2.4.7. In vitro cell culture study

In vitro cell culture was carried out using L929 cell. These cells are derived from an immortalized mouse fibroblast cell line, are internationally recognized cells that are routinely used in in vitro cytotoxicity assessments. The scaffold was sterilized by putting it in 6 well tissue culture plate containing isopropanol (5 ml) and exposed to UV radiation for 4 h. L929 cells were further seeded on nanohybrid scaffold placed in 6-well plate at a density of 5×10^3 cells/well and incubated at 37 °C, 5% CO_2 and 95% humidity