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Glucosylated polymeric nanoparticles: A sweetened approach against blood compatibility paradox

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ABSTRACT

Surface functionalization strategies in generating stealth nano-carriers have garnered considerable attention in pharmaceutical research. In this regard, our investigation reports on the preparation and evaluation of glucose decorated poly lactic-co-glycolic acid (PLGA) nanoparticles as blood compatible nanoparticulate drug delivery system, with enhanced cellular uptake. Terminal carboxylic acid groups on PLGA were modified with the amino group of glucosamine and nanoparticles were generated by modified solvent diffusion (nano-precipitation) technique. Detailed in vitro experiments were performed to evaluate the eminence of glucose functionalization over unmodified nanoparticles, in terms of their hemocompatibility and cellular uptake. Glucosylation was confirmed by NMR and FTIR spectroscopy; PLGA and modified particles had average size in the range of 125 nm. Glucosylation was an effective strategy in reducing plasma protein adsorption, complement activation and platelet plugging of PLGA nanoparticles. PLGA and their glucose modified versions were quite compatible with the blood cells and were non-cytotoxic. Moreover the uptake behaviour of glucose modified PLGA nanoparticles were enhanced in comparison to standard PLGA nanoparticles as emphasized by the z stacking analysis following confocal imaging. Precisely the stealth properties of glucose modified PLGA nanoparticles (PLGA-Glu), with enhanced cellular internalization, seems to be a safe and efficient system for intravenous drug delivery applications.

1. Introduction

Despite the recent advancements in the field of bio-nanotechnology, development of biocompatible targeted delivery vehicles to pathological sites still plague the area of drug research. The survival strategies of nanoparticles intended for intravascular delivery depends on the physicochemical properties and various escape mechanisms elucidated against the reticuloendothelial system (RES), mainly kupffer cells in liver and spleen macrophages that forms the major biological barrier for its extended half life [1,2]. Grass root analysis of immune system recognition of nanoparticles pinpoint to the opsonisation process. The plasma-protein corona formed can decide upon further blood cell interactions which may culminate in the elimination of particles from the bloodstream [3].

In the context of evading the reticuloendothelial system for increased circulation half life, hydrophilic non-ionic inert polymers such as polyethylene glycol (PEG), dextran and pullulan has been widely used for surface modification of nanoparticles [4–6] and [7]. The mechanism seems to be simple, as these hydrophilic polymers mask the nano-carriers from the host's immune system and thereby impart "stealth" properties to the nanoparticles. However there is an increasing apprehension over the success of the steric stabilization approach, as these surface modifications are reported to seriously compromise the cellular uptake efficacy of the nanoparticles. Moreover various studies indicate about the existence of accelerated blood clearance phenomenon associated with the PEGylated nano-carriers (mostly with PEGylated liposomes). This leads to the reduction of its long-circulating characteristic following repeated administration [8] and [9]. These observations largely necessitate the need for alternative approaches in imparting stealth properties to the nanoparticles.

Specific functional modification approach, to some extend can overcome the limitations of conventional stealth approach [10,11]. We have recently reported a bio-mimetic surface modification strategy for improving the biocompatibility of PLGA nanoparticles [12]. Thiol modification was indeed a very effective strategy in enhancing the hemocompatibility (protein adsorption,

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complement activation and platelet activation/adhesion) of PLGA nanoparticles, thanks to the trans-nitrosylation reaction between the surface thiol groups and the specific blood plasma proteins [13] and [14]. These in vitro parameters are critical in deciding the fate of a nanoparticulate delivery system, as enhanced hemocompatibility may avoid a quick recognition of these nanoparticles in vivo. Moving ahead in this direction, specific modification approach aimed at strengthening the hemocompatibility of the nanoparticles without compromising their cellular uptake efficacy seems appealing for advanced drug delivery applications [15].

Considering these aspects, glyconanobiology offer much potential for nanoparticle surface functionalization with monosaccharides like glucosamine. These glyco-nanoparticles with its water soluble and globular nature provide a glycocalyx like surface that mimics the presentation of carbohydrate epitopes of cell surface glycoconjugates [16]. The applications of glyco-nanoparticles vary from basic chemical glycobiology like assessing carbohydrate mediated interactions to clinical applications like anti adhesive therapy against tumours. The free hydroxyl groups of glucosamine promote hydrophilicity and enable it to act as a shield against opsonisation while maintaining specific cellular interactions. This aspect places glucose as a superior functionalization ligand for drug delivery in comparison to PEG or similar inert polymers. Moreover the polyvalent clusters of glucosamine moieties present on the particle surface offer a rich supply of low-affinity binding sites, turning the modified nanoparticles into a reliable system for regulating cell adhesion and recognition [17].

The present study aims at the functionalization of PLGA nanoparticle surface with glucosamine and further attempts to evaluate their blood compatibility and cellular interaction. Monosaccharide modified polymers were prepared by the conjugation of the amine groups present in the glucosamine to the carboxylic acid group of PLGA followed by nanoparticle preparation through solvent diffusion technique. Protein adsorption, complement activation, platelet activation and C6 cell uptake studies were quantified under in vitro experimental conditions.

2. Methods and materials

2.1. Materials

PLGA 50:50 (Resomer RG 502 H, I.V. 0.16–0.21) was obtained from Boehringer Ingelheim (Germany). Dicyclohexyl carbodimide (DCC), N-hydroxy succinimide (NHS), Histopaque-1077, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Branched polyethyleneimine 25 kDa (PEI), fluorescein isothiocyanate (FITC) were from Sigma-Aldrich. Glucosamine hydrochloride and electrophoresis grade reagents were obtained from SRL (India), Rhodamine B was from SD fine chemicals (India). Human P-selectin and Human PF-4 ELISA kits were from Ray Biotech Inc. (USA) and Terminal complement complex (TCC) ELISA kit was from USCN Life Science (Wuhan, China). C6 and L929 cell lines were obtained from the National Centre for Cell Sciences (Pune, India)

2.2. PLGA modification and characterization

2.2.1. PLGA modification

Carboxyl groups in PLGA were activated with NHS in the presence of DCC prior to its conjugation with the amino groups of glucosamine. Briefly, PLGA (0.5 g) was dissolved in 10 ml anhydrous dimethyl formamide and NHS (0.05 g) was added to the polymer solution. On completion of first hour, DCC was added and stirring continued for another 2 h. The precipitated polymer was dried under vacuum and stored at -20 °C. NHS activated PLGA was further dissolved in DMF and mixed with 0.5 ml glucosamine solution (0.02 g dissolved in 0.5 ml distilled water). The reaction progressed for another 3 h on ice bath and modified polymer was precipitated by pouring into 100 ml distilled water. The precipitated polymer was filtered and dried under reduced pressure, stored at −20 °C.

2.2.2. Characterization of modified PLGA

NMR spectra of PLGA and glucose-PLGA polymers were measured in D_2O using a 300 MHz spectrometer (Bruker Avance DPX 300). FTIR spectra of these polymers were obtained with Nicolet Impact 410 spectrophotometer after making the corresponding KBr pellets.

2.3. Preparation and characterization of nanoparticles

2.3.1. Preparation of PLGA/Glucose-PLGA nanoparticles

Nanoparticles were prepared by a typical solvent diffusion or nanoprecipitation technique [18]. Briefly, PLGA/Glucose-PLGA polymer (10 mg) was dissolved in 850 μl (acetone), and then 150 μl ethanol (97%) was added. This was followed by the addition of the organic phase into 10 ml deionised water (aqueous phase) under magnetic stirring. The nanoparticles collected after centrifugation at 4000 rpm were lyophilized (Freeze Zone 4.5, Labconco) and stored at −20 °C.

Fluorescent loaded nanoparticles were prepared using a similar procedure applied for the preparation of PLGA/Glucose-PLGA nanoparticles, expect for the addition of rhodamine B in the polymer solution in acetone. Encapsulated nanoparticles were stored at 4 °C under dark.

2.3.2. Characterization of nanoparticles

Average particle size was determined by Dynamic Light Scattering (DLS) using Zetasizer (Malvern Instruments, UK). Nanoparticles were dispersed in distilled water and experiments were performed at a temperature of 25 ± 0.1 °C in triplicate.

The surface topography of PLGA and glucose PLGA nanoparticles was analysed by atomic force microscopy (WITEC Confocal Raman Microscope system with AFM extension, Germany).

2.4. Protein adsorption studies on nanoparticles

2.4.1. Plasma protein adsorption by electrophoresis (PAGE)

Human venous blood from healthy donors was collected in tubes containing sodium citrate followed by centrifugation to separate the supernatant plasma. Plasma samples (250 μl) were incubated with 5 mg of PLGA/Glucose-PLGA nanoparticles for 1 h at 37 °C, in a rotary shaker at 35 rpm. Following incubation of the nanoparticles with laemmelli buffer, SDS-PAGE was performed at 100 V for 90 min using Mini-PROTEAN electrophoresis system (Bio-Rad, CA). The gel was stained with coomassie blue and documented using an image analyser (LAS 4000, Fuji). A densitometric scan analysis was performed with the help of multigauge software.

2.4.2. Protein adsorption from single protein solution

Protein adsorption from single protein solution was performed according to a reported procedure [21]. PLGA and Glucose-PLGA nanoparticles (1, 2 and 5 mg) were incubated with 1 ml of FITC labelled protein solution (albumin, fibrinogen and globulin) separately in PBS at 37°C for 1 h. After the incubation period, samples were centrifuged to separate 50 μl of the supernatant and the fluorescence intensity was analysed using a fluorescent plate reader TECCAN Infinite M200 (λexc/em.495/525 nm). Protein adsorption on the nanoparticles was quantified by comparing the fluorescence intensity between the supernatant samples and the FITC labelled control protein solution of concentration 1 mg/ml. Results were expressed as the percentage of protein adsorbed onto the