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Preparation and characterization of polymeric nanoparticles surface modified with chitosan for target treatment of colorectal cancer

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ABSTRACT

5-Fluorouracil (5-FU) loaded chitosan (C) coated polylactic-co-glycolic acid (PLGA) nanoparticles [NPs] (C-5-FU PLGA NPs) and polycaprolactone [PCL] (C-5-FU PCL NPs) were employed as the carriers for cancer treatment. The prepared NPs showed the spherical shape of NPs with the particle size in the range of 188.1–302.2 nm with polydispersity index (PDI) of <0.30. C-coated NPs converted zeta potential from negative to positive value with small modification in particle size distribution. The entrapment efficiency of 5-FU was recorded in the range of 32–51%. The *in vitro* release studies showed an initial rapid 5-FU release followed by a sustained release profile. The *in vitro* cytotoxicity of C-5-FU PLGA NPs showed significant inhibition of colon cancer cells (HT-29) compared to the other NPs and drug solution. These results showed that C-5-FU PLGA NPs can be considered as a promising carrier for cancer therapy.

1. Introduction

The nanoparticles (NPs) for targeting of chemotherapeutic agents are considered a very hot field for cancer treatment [1,2]. Polymeric NPs were considered as an interesting area for delivery of small molecules to overcome the poor drug solubility and cell permeability [3]. More concern has been dedicated on the natural and synthetic polymers such as poly-caprolactone (PCL) and poly (lactide-*co*-glycolide) (PLGA) due to their good biodegradablity, biocompatibility and extend drug release profile [4]. These polymers have been shown to shield the drug molecules from its degradation by enzymes and offer physicochemical stability [4,5].

Nanoparticles (NPs) can be formulated with optimum size and shape to enhance their drug release and cellular uptake [5]. PLGA NPs have been extensively employed to target many anticancer drugs through attachment of specific ligands to the surface of the particles [6]. PCL NPs have been extensively used in many drug delivery systems due to their good solubility, wide mixture compatibility and superior permeability [7]. In addition, PCL possesses eminent rheological properties and can be flexibly configured to many shapes allowing for a broad range of biomedical applications [8]. Moreover, the surface of NPs can be modified with ligands to improve cellular drug delivery [9].

Chitosan (C) has received increasing attention as a good polymeric material and has now been broadly used in several fields such as protein adsorption [10] and metal adsorption [11]. Additionally, C has also been studied in the development of extended release drug delivery systems [12]. Since C has mucoadhesive character, which enhances drug absorption and promote prolongation of drug release.

Surface modification of NPs with C has numerous pharmaceutical benefits. The coating by C decreases the burst result of drug release. The positively-charged C efficiently attracts to the negatively charged of the membrane, which enhances the retention and permeation of NPs [13].

5-Fluorouracil (5-FU) has a broad range of anticancer activity, including those of the colorectal region, liver, pancreas, lung and breast [14]. It has been reported as hydrophilic drug (saturated solubility in water has been reported as 10–12 mg/ml) [15,16]. It had very low oral bioavailability (sometimes unpredictable after oral administration) due to erratic *in vivo* absorption [17–19]. The traditional systemic administration by the intravenous route needs relatively high doses of FU, which results in the high profile of hematologic and bone marrow toxicities [20].

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The addition of 5-FU into NPs is expected to improve its pharmacokinetics, which reduces the high doses of the drug demand [21]. Hence, our aim was to investigate the anticancer activity of 5-FU loaded PLGA NPs and PCL NPs to produce 5-FU delivery systems.

The properties of the prepared systems were investigated for their particle sizes, zeta potential, drug loading capacity and *in vitro* drug release rate. In addition, these formulations were coated with C to facilitate the cellular uptake, which tested for their anti-cancer activity using the colorectal cancer (HT-29) cells.

2. Experimental

2.1. Materials

5-FU (purity > 99%), PLGA (MW 40000–75000 Da), PCL (MW 42,000 Da) and MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from "Sigma-Aldrich (St. Louis, MO)". Polyvinyl alcohol (PVA) [MW 16,000 Da] and dichloromethane (DCM) were obtained from "Acros Organics (Geel, Belgium)". Human colon cancer cells (HT-29) were obtained from American type cell culture (ATCC, USA). Phosphate buffer saline (PBS pH 7.4) was selected as the release medium and prepared in the laboratory. All other reagents and chemicals were of analytical grade.

2.2. Preparation of 5-FU loaded NPs

5-FU loaded PLGA and PCL NPs (5-FU PLGA NPs and 5-FU PCL NPs, respectively) were prepared by using modified double emulsion (W1/O/W2) technique [22]. The composition of each formulation is presented in Table 1. Briefly, 5 mg of 5-FU was dissolved in 0.5 ml of purified water by vortex mixing. Then, 5-FU solution was emulsified in 2 ml of DCM containing dissolved amounts of polymers (40 mg) using a probe sonicator for 60 s at 60% power under ice bath. The formed primary emulsion was immediately mixed with an aqueous solution of 1% PVA (40 ml) followed by probe-sonication for 3 min. Thus, the DCM was then removed on overhead stirrer at a stirring rate of 500 rpm and room temperature for 2 h. The NPs were separated by using Centrifuge at 30,000 rpm for 15 min at 4 °C. The washing step of the precipitant was repeated more times. The supernatant solutions were then analyzed for its drug content by high performance liquid chromatography (HPLC) analysis and used to calculate entrapment efficiency (EE%) [23]. Then the precipitant was dispersed in aqueous solution by vortexing for 5 min.

C-coated NPs (C-NPs) were obtained by incubating a certain volume of the suspensions of 5-FU PLGA-NPs and FU PCL-NPs with an equivalent volume of 2 mg/ml chitosan in acetic solution (0.5%) for 2 h at room temperature [24]. The resulted C-5-FU PLGA-NPs and C-5-FU PCL-NPs were centrifuged, washed twice and then redispersed in an equivalent volume of distilled water. The all obtained NPs dispersions were stabilized using a Freeze Dryer (Alpha 1-4 LD Plus, Martin Christefriertrocknugsanlagen GmbH, Osterode am Harz, Germany) at −60 °C for 3 days. All formulations were prepared in triplicate.

2.3. Particle size and zeta potential measurements

The mean particle size and polydispersity index (PDI) of each formulation were measured by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments, UK). The particle size of the prepared NPs dispersions was evaluated using dynamic light scattering (DLS) mode at the 25 °C after proper dilution. The scattering angle for measurement was set at 90°. The mean (average) particle size of each formulation was determined by taking the mean of three different readings. Zeta potential of each formulation was evaluated by laser doppler velocimetry (LDV) mode using the same Nano ZS at 25 °C. The samples were properly diluted with deionized water, sonicated and subjected for the measurement of zeta potential. All experiments were performed in triplicate. Each value reported is the average of three measurements.

2.4. Entrapment efficiency and drug loading

As mentioned before, the amounts of 5-FU entrapped into NPs formulations were determined indirectly by ultracentrifugation method using OptimaTM Max-E, Ultra Centrifuge (Beckman Coulter, Pasadena, CA) at 4 °C. The non-entrapped 5-FU amount in the supernatant after centrifugation was determined by HPLC method.

The drug loading (DL%) was estimated by dissolving 5 mg of freeze-dried NPs in 0.5 ml of DCM. Then, DCM was evaporated at room temperature. Then, a certain volume of deionized water was added to residue and kept in a water bath sonicator for 10 min to extract 5-FU into aqueous phase. To obtain a clear solution of 5-FU, the aqueous phase was separated by centrifuge at 5000 rpm for 10 min at 25 °C. 5-FU content was determined using HPLC [23].

Entrapment efficiency (EE%) and drug loading (DL%) were calculated according to the following equations:

$$\text{EE\%} = \frac{\text{5-FU}_{\text{total}}\text{-5-FU}_{\text{free}}}{\text{5FU}_{\text{toal}}} \times 100 \quad (1)$$

$$\text{DL\%} = \frac{\text{Amount of entrapped 5-FU}}{\text{Total weight}} \times 100 \quad (2)$$

$\text{5-FU}_{\text{total}}$ is the amount of drug added, while $\text{5-FU}_{\text{free}}$ is the free amount of drug presented in the supernatant.

2.5. The particle surface morphology

The particle surface morphology of the 5-FU loaded NPs was visualized by Scanning Electron Microscopy [SEM] (JSM-6360 LV, JEOL, Tokyo, Japan) technique. The freeze-dried samples were fixed on carbon tape and sputter-coated with a thin gold layer under an argon atmosphere using a gold sputter module in a high-vacuum evaporator (JFC-1100 fine coat ion sputter; JEOL, Tokyo, Japan). The coated samples were then scanned and photomicrographs were taken at an acceleration voltage of 10 kV.

2.6. In vitro *release study*

In vitro release of 5-FU loaded NPs formulations was evaluated using the dialysis membrane method as previously described [14,20]. The dialysis bag (molecular weight cut off: 5 kDa, Livingstone, NSW, Australia) was soaked in distilled water for 12 h before use. The obtained freeze-dried NPs formulations (equivalent to 5 mg of the 5-FU) were dispersed in 3 ml of PBS (pH 7.4) and put in a dialysis bag. Then, the bag was incubated with 40 ml of preheated PSB in beakers as release medium. The sink conditions for *in vitro* release studies were met since the aqueous solubility of 5-FU has been reported as approximately 10–12 mg/ml [15,16]. The beakers were placed in a thermostatic shaker at 37 °C and 100 rpm. Three ml of the aliquots was collected at predetermined time points and replenished immediately with the equal volume of fresh PBS (pH 7.4) to maintain the sink conditions. The release of free 5-FU (5-FU suspended in PBS) was performed as a control. The amount of 5-FU in the aliquots was analyzed by HPLC [23]. The experiments were done in triplicate.