

# Hyaluronan–cisplatin conjugate nanoparticles embedded in Eudragit S100-coated pectin/alginate microbeads for colon drug delivery

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**Abstract:** Hyaluronan–cisplatin conjugate nanoparticles (HCNPs) were chosen as colon-targeting drug-delivery carriers due to the observation that a variety of malignant tumors overexpress hyaluronan receptors. HCNPs were prepared by mixing cisplatin with a hyaluronan solution, followed by dialysis to remove trace elements. The cells treated with HCNPs showed significantly lower viability than those treated with cisplatin alone. HCNPs were entrapped in Eudragit S100-coated pectinate/alginate microbeads (PAMs) by using an electrospray method and a polyelectrolyte multilayer-coating technique in aqueous solution. The release profile of HCNPs from Eudragit S100-coated HCNP-PAMs was pH-dependent. The percentage of 24-hour drug release was approximately 25.1% and 39.7% in pH 1.2 and pH 4.5 media, respectively. However, the percentage of drug released quickly rose to 75.6% at pH 7.4. Moreover, the result of an *in vivo* nephrotoxicity study demonstrated that Eudragit S100-coated HCNP-PAMs treatment could mitigate the nephrotoxicity that resulted from cisplatin. From these results, it can be concluded that Eudragit S100-coated HCNP-PAMs are promising carriers for colon-specific drug delivery.

**Keywords:** hyaluronan, cisplatin, pectin, alginate, pH-dependent, drug delivery

## Introduction

Colorectal cancer is one of the most common cancers in the world. Surgery is the primary curative modality for colorectal cancer. However, most clinical treatment is accompanied by adjuvant chemotherapy to prolong survival and improve quality of life, particularly in patients with stage III colorectal cancer.<sup>1,2</sup> Unfortunately, conventional chemotherapy is not an effective method for the treatment of colorectal cancer, due to the low effective concentration of drug that reaches the cancer site. Therefore, colon-targeted drug-delivery systems have been developed to improve the low utility rate of anticancer drugs. The use of pectin as a carrier has been an option for several common anticancer drugs, such as methotrexate and fluorouracil.<sup>3,4</sup>

Cisplatin is a widely used anticancer agent in the treatment of various types of human solid tumors. Cisplatin binds to and cross-links with DNA, ultimately triggering apoptosis in cancer cells. Pruefer et al demonstrated that cisplatin induces apoptosis in human colon cancer cells through the mitochondrial serine protease Omi/Htra2.<sup>5</sup> Like many antineoplastic drugs, cisplatin is administered intravenously, and the concentration in the intestine remains at a low level until 3 days after intravenous administration.<sup>6</sup> Hence, cisplatin's clinical use is limited because of its severely toxic side effects, such as acute nephrotoxicity and chronic neurotoxicity.<sup>7</sup> Approaches to reduce these systemic side effects while retaining the potency of cisplatin have included

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embedding cisplatin in liposomes and micelles.<sup>8,9</sup> Moreover, targeting the drug to the disease site is an essential issue in the development of effective drug-delivery carriers. Generally, conventional drug formulations that are orally administered dissolve in the gastrointestinal fluids and are absorbed in the upper gastrointestinal tract. It is therefore necessary to protect drugs intended for the colon until they reach the desired destination.<sup>10</sup> Previous reports of simple methods to protect drugs from absorption in the upper gastrointestinal tract describe the use of extremely slow-releasing matrices or thicker layers of a conventional enteric coating, which have longer release periods or slower release rates and ensure drug delivery into the colon.<sup>11</sup>

Enzyme- or pH-dependent systems have also been widely developed for drugs intended for delivery to the colon. Pectin is a natural biopolymer that is commercially extracted from citrus peels and apple pomace. Compared with polysaccharides from animals, pectin is a more inexpensive and uncomplicated purified polysaccharide source. In addition, Liu et al demonstrated that pectin could target tumor cells by binding to the galectin-3 receptor to inhibit cancer progression and metastasis.<sup>12</sup> Pectin consists mainly of linear chains of  $\alpha$ -(1-4)-D-galacturonic acid with varying degrees of esterification. The broad molecular weight distribution is due to the main linear chain being partly interrupted by other neutral sugars. The structure of galacturonic acid is similar to the guluronic and mannuronic acids of alginate. This means that pectin solutions can also undergo cross-linking by polyvalent cations (eg, calcium or barium) via an “egg-box” configuration to form a hydrogel.<sup>13</sup> Importantly, pectin cannot be digested in the upper gastrointestinal tract, but can be degraded by the polysaccharidases that are produced by the bacterial flora of the human colon.<sup>14</sup> This specific degradation mechanism has led to numerous studies on the usefulness of pectin as a carrier for colonic drug delivery. However, if pectin alone is used as a microencapsulation matrix, the pectin concentration is too high to fabricate the microbeads by electrospraying, due to the lower content of carboxyl groups in pectin compared to alginate. However, mixing alginate with pectin could reduce the threshold of the pectin concentration. In addition, most reports have indicated a high burst release of a drug encapsulated in an alginate or pectin matrix under simulated gastric conditions.<sup>15,16</sup> To overcome this problem, Paharia et al utilized a Eudragit coating on the surface of pectin microbeads and demonstrated a reduced drug burst release under simulated gastric conditions.<sup>17</sup> Eudragit S100 is an anionic polymer exhibiting a pH-dependent solubility and has been used for oral drug delivery due to its drug release at pH > 7. Thakral

et al used the natural polymer as a core and subsequently coated it with Eudragit S100 for colon targeting.<sup>18,19</sup> In our previous work, we successfully fabricated Eudragit S100-coated pectinate/alginate microspheres (PAMs) using an electrospray method and polyelectrolyte multilayer-coating technique in an aqueous solution, and demonstrated that cisplatin release from Eudragit S100-coated PAMs was retarded in simulated gastric conditions.<sup>20</sup> However, the new drug-delivery system only improved the efficiency of protecting drug activity. To become an ideal drug carrier, this system still requires a specific targeting capacity.

Jeong et al found that hyaluronan (HA) can form stable conjugates with cisplatin,<sup>21</sup> and Cohen et al demonstrated that these HA-cisplatin conjugates can reduce the systemic toxicity observed with cisplatin alone.<sup>22</sup> CD44 is the primary cell-surface receptor for the extracellular matrix glycosaminoglycan HA, which serves as an adhesion molecule in cell-substrate and cell-cell interactions, such as lymphocyte homing, cell migration, and metastasis. A variety of malignant tumors overexpress HA receptors and are surrounded with HA-enriched extracellular matrices. In this study, we utilize the ligand-receptor relationship between HA and CD44 as a model to investigate the efficiency of targeted functionalized nanoparticles in specific cells. Therefore, we conjugated HA-cisplatin into nanoparticles (HCNPs) and then evaluated the cytotoxicity of the HCNPs. We also evaluated the release profile of the HCNPs from Eudragit S100-coated PAMs under simulated gastrointestinal conditions. Finally, we investigated the nephrotoxicity of Eudragit S100-coated HCNP-PAMs in 1,2-dimethylhydrazine-induced rats.

## Materials and methods

### Materials

Alginate (from brown algae), esterified low-methoxy pectin (potassium salt from citrus fruit, degree of esterification 27%, with purity as a galacturonic acid of 55%–74%), lysine, and cisplatin were purchased from Sigma-Aldrich (St Louis, MO, USA). HA (sodium salt, molecular weight = 10,000) was purchased from Lifecore Biomedical (Chaska, MN, USA). Eudragit S100 was purchased from Degussa Pharma (Darmstadt, Germany). All other chemicals used were of reagent grade.

### Preparation and characterization of hyaluronan-cisplatin conjugate nanoparticles

Ten milligrams of HA and 4 mg of cisplatin were dissolved in 1 mL of deionized water. The solution was gently

stirred for 3 days under dark conditions. The solution was then filtered (using a 0.22  $\mu\text{m}$  nylon membrane) and dialyzed against deionized water for 24 hours at room temperature to remove unbound cisplatin (dialysis tube molecular weight cutoff = 3500). The content and loading efficiency of cisplatin incorporated into the HCNPs was determined using the *o*-phenylenediamine method.<sup>23</sup> Briefly, 0.1 mL of cisplatin-containing solution was mixed with 0.1 mL of 1,2-phenylenediamine solution in *N,N*-dimethylformamide (1.4 mg/mL) and 0.2 mL of  $\text{KH}_2\text{PO}_4$  buffer solution (0.1 M, pH 6.8). The mixture was incubated at 100°C for 4 minutes followed by absorbance measurement at 703 nm.

The drug contents and loading efficiency were calculated as follows:

$$\text{Drug content} = (\text{Amount of cisplatin liberated from the HCNPs} / \text{weight of HCNPs}) \times 100$$

$$\text{Loading efficiency} = (\text{Amount of cisplatin liberated from the HCNPs} / \text{feeding amount of cisplatin}) \times 100$$

Finally, the dialyzed solution was lyophilized. The lyophilized HCNPs were redistributed in deionized water to analyze the zeta potential and average particle size using a Zetasizer ZS (Malvern Instruments, Malvern, UK). The atomic composition of HCNPs was determined by transmission electron microscopy with energy-dispersive spectrometry.

### Cell-toxicity studies of HCNPs in vitro

Cellular cytotoxicity induced by cisplatin or HCNPs was measured using the neutral red assay. The human colon carcinoma cell line HCT-116 was maintained in McCoy's 5a medium supplemented with 10% fetal bovine serum. In preceding proliferation studies, cells were trypsinized and seeded into 96-well plates ( $5 \times 10^3$  cells/well). After 24 hours, various concentrations of cisplatin and HCNPs were added and incubated for 48 hours. Neutral red, in 10  $\mu\text{L}$  of phosphate-buffered saline, was then added to each well (final concentration of 5  $\mu\text{g}/\text{mL}$ ) for 90 minutes at 37°C. After the complete removal of medium, each well was washed three times with phosphate-buffered saline. One hundred microliters of 50% ethanol containing 50 mM sodium citrate was added to each well of the 96-well plates. After 20 minutes, the absorbance was measured at 540 nm using an Ultrospec 1100 Pro UV/Vis Spectrophotometer (Amersham Biosciences, Amersham, UK). The percentage

of cytotoxicity was calculated according to the following formulas:

$$\% \text{ cytotoxicity} = 100 \times (1 - \text{ABS}_T / \text{ABS}_N)$$

$$\% \text{ viability} = 100 - \% \text{ cytotoxicity}$$

where  $\text{ABS}_T$  and  $\text{ABS}_N$  represent the absorbance values of treated cells and negative control cells, respectively.

The median lethal dose ( $\text{LC}_{50}$ ) was determined by probit analysis using SPSS statistical software, version 10.0 (IBM, Armonk, NY, USA), and refers to the concentrations of cisplatin and HCNPs that cause a decrease in cell count by 50% in 48 hours, as determined from dose-response data.

### Preparation and characterization of HCNP-PAMs

The method for fabricating Eudragit S100-coated HCNP-PAMs has been described previously.<sup>20</sup> Briefly, pectin/alginate solution was prepared by dissolving pectin (4% w/v) and sodium alginate (1% w/v) in 0.9% w/v sodium chloride. HCNPs were added into the pectin/alginate solution to produce a final cisplatin concentration of 1 mg/mL. The collection solution was prepared by dissolving calcium chloride (1.5% w/v) with stirring at room temperature. An electrostatic droplet generator was employed to prepare microbeads. The solution of HCNP/pectin/alginate was placed into a 10 mL syringe fitted with a needle bearing a tip diameter of 0.96 mm. The syringe was attached to a syringe pump that provided a steady solution flow rate, and a high-voltage electrostatic system was applied. The positive electrode of the electrostatic system was connected to the needle, while the negative electrode was placed in the collection solution 20 cm away from the needle tip. Voltage was applied at 15 kV. The HCNP-PAMs were transferred into lysine solution (1.0% w/v, pH 2) for 2 minutes. Subsequently, the lysine-coated HCNP-PAMs were transferred into Eudragit S100 solution (2.0%, w/v in ethanol/water solution, pH 8.0) and stirred for 30 minutes. The resulting Eudragit S100-coated HCNP-PAMs were collected, rinsed with deionized water, and dried. The size distribution of the microbeads was determined from a total of 100 microbeads using a digital camera or optical microscope with the Image Pro Express software (Media Cybernetics, Rockville, MD, USA).

### Efficiency of HCNPs encapsulated in PAMs

The microbeads (25 mg) were digested in 10 mL of ethylenediaminetetraacetic acid solution for 12 hours. The solution was centrifuged at 800 *g* for 5 minutes, and

the supernatant was assayed for cisplatin content using the *o*-phenylenediamine method. Drug content within the microbeads was calculated as follows:

$$\text{Drug content} = (\text{Amount of cisplatin liberated from microbeads} / \text{weight of microbeads})$$

Efficiency of drug entrapment within the microbeads was calculated in terms of the percentage of drug entrapment as per the following formula:

$$\% \text{ drug entrapment} = (\text{Actual content} / \text{theoretical content}) \times 100$$

## In vitro drug release

The drug-release properties of the microbeads were studied in three different dissolution media: KCl-HCl buffer (pH 1.2),  $\text{KH}_2\text{PO}_4$  buffer (pH 4.5), and phosphate buffer (pH 7.4). The microbeads were placed in dissolution media (sink conditions) and shaken at 100 rpm at 37°C. Samples were withdrawn at various time intervals and assayed spectrophotometrically.

The percentage of drug released at various time intervals was calculated with respect to the drug content of the microbeads. Determination of the drug content within and released from the microbeads was carried out in triplicate. In addition, Eudragit S100-coated HCNP-PAMs and uncoated HCNP-PAMs were evaluated for in vitro drug release in simulated gastrointestinal fluids. The microbeads (25 mg) were precisely weighed and spread gently over the surface of 10 mL dissolution medium. The medium was shaken at 100 rpm at 37°C. The simulation of gastrointestinal transit conditions was achieved by altering the pH of the dissolution medium at different time intervals, as follows. The pH of the dissolution medium was maintained at 1.2 for 2 hours using 0.1 N HCl. Then,  $\text{KH}_2\text{PO}_4$  and  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  were added to the dissolution medium, adjusting the pH to 4.5 with 1.0 M NaOH. After 4 hours, the pH of the dissolution medium was adjusted to 7.4 with 0.1 N NaOH and maintained until 24 hours.

## Drug-release kinetics

The rate and mechanism of drug release from the prepared microbeads were analyzed by fitting the release data into a zero-order rate equation (Eq 1), a first-order rate equation (Eq 2), a Higuchi model (Eq 3), and a Korsmeyer–Peppas equation (Eq 4):

$$C_t = k_0 t \quad (1)$$

where  $C_t$  is the concentration of drug released at time  $t$ , and  $k_0$  is the release-rate constant,

$$\text{Log } Q_t = \text{Log } Q_0 - (k_1 t / 2.303) \quad (2)$$

where  $Q_0$  is the initial amount of drug,  $Q_t$  is the amount of drug released at time  $t$ , and  $k_1$  is the release-rate constant,

$$C_t = k_H t^{1/2} \quad (3)$$

where  $C_t$  is the concentration of drug released at time  $t$ , and  $k_H$  is the Higuchi diffusion-rate constant, and

$$M_t / M_\infty = kt^n \quad (4)$$

where  $M_t$  is the amount of drug released at time  $t$ , and  $M_\infty$  is the amount of drug released at time  $\infty$ . Thus,  $M_t / M_\infty$  is a fraction of the drug released at time  $t$ ,  $k$  is the release-rate constant, and  $n$  is the diffusional exponent characteristic of the release mechanism. Values of the  $n$  exponent equal to or less than 0.45 were characteristic of Fickian diffusion, whereas values in the range of 0.45–1 were an indication of an anomalous mechanism for drug release.

## Nephrotoxicity study in DMH-induced rats

Approval was obtained from the Institutional Animal Care and Use Committee of Chang Gung University prior to the study. The committee recognized that the animal experiments complied with the law protecting animal issues, as shown in the *Guide for Laboratory Animal Facilities and Care by the Council of Agriculture*, Executive Yuan, Taiwan. Fifteen weaning and adult (approximately 250 g) male Wistar rats were purchased from BioLasco, Taipei, Taiwan. The animals were maintained in a controlled environment at  $24^\circ\text{C} \pm 1^\circ\text{C}$  and  $50\% \pm 10\%$  relative humidity with an altering 12:12-hour light–dark cycle. Rats were given subcutaneous injections of 1,2-dimethylhydrazine (DMH; Sigma-Aldrich) once weekly for 12 weeks at a dose of 40 mg/kg body weight. The DMH-induced rats were randomly divided into three treatment groups: cisplatin, Eudragit S100-coated PAMs and Eudragit S100-coated HCNP-PAMs (five rats per group). Treatments were administered by oral gavage, using a gastric tube, twice weekly for 4 weeks. The administered dose of cisplatin in the Eudragit S100-coated HCNP-PAMs was 3.5 mg/kg per week. After 29 days, all animals were killed, and renal damage was assessed by monitoring serum creatinine levels using an autoanalyzer. During the period of 29 days, the animals were weighed twice weekly.

## Statistical analyses

Statistical analyses were performed using SPSS version 10. Cellular viability and serum creatinine assays were analyzed



with the nonparametric Mann–Whitney  $U$  test. Quantitative data were expressed as means  $\pm$  standard deviation. Differences of  $P < 0.05$  were considered statistically significant. Each sample was measured in triplicate, and all of the experiments were repeated three times.

## Results and discussion

### Cisplatin–hyaluronan conjugate nanoparticles

The formation of metal–anion polymer complexes was previously reported by Nishiyama et al.<sup>24</sup> It was also previously shown that the interaction of the platinum (II) atom with the carboxyl groups of the HA macromolecule could cause spontaneous folding to form a nanoconjugate.<sup>19</sup> The size of the resulting HCNPs, as measured by dynamic light scattering, averaged  $171.10 \pm 48.58$  nm. The polydispersity index was 0.67. The zeta potential of the HCNPs was measured to be  $-20.16 \pm 1.01$  mV. The HCNPs were essentially spherical in shape, with an average diameter of approximately 200 nm, which was consistent with measurements by dynamic light scattering (Figure 1A). From the atomic composition of the HCNPs, as determined by transmission electron microscopy with energy-dispersive spectrometry (Figure 1B), we confirmed the formation of HA–cisplatin conjugate nanoparticles. The cisplatin content was  $24.6\% \pm 1.2\%$ , and the loading efficiency was  $85.9\% \pm 4.1\%$ .

### Toxicity of hyaluronan–cisplatin conjugate nanoparticles

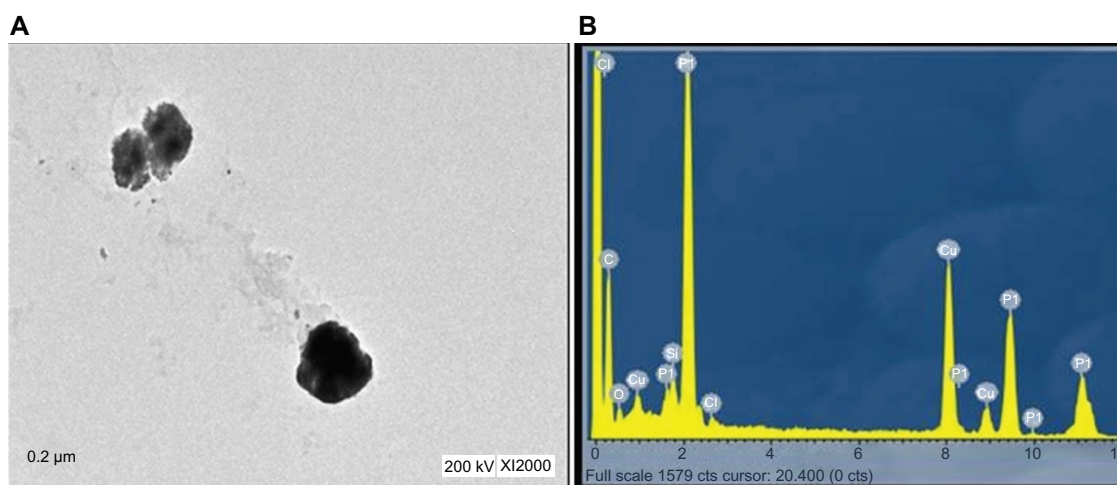
The cytotoxic effects of cisplatin and HCNPs on HCT-116 cells are shown in Figure 2. The cell viability for

treatment with HCNPs was significantly lower than that of treatment with cisplatin alone at 4 and 8 hours ( $P < 0.05$ ). The  $LC_{50}$  values of cisplatin and HCNPs on HCT-116 cells at 48 hours were 7.5 and 5.5  $\mu\text{g/mL}$ , respectively. These results demonstrated that the cytotoxicity induced by cisplatin and HCNPs on HCT-116 cells were dose- and time-dependent, and the HCNPs had a greater cytotoxic effect on the HCT-116 cells than did cisplatin.

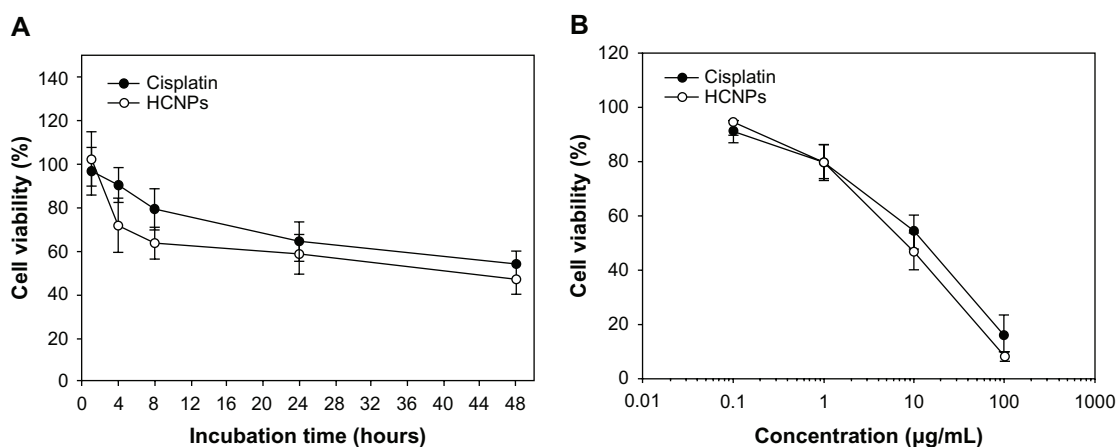
### Characteristics of HCNP-PAMs

The optical micrographs of HCNP-PAMs and Eudragit S100-coated HCNP-PAMs are shown in Figure 3A and B. The microbeads displayed a smooth surface after coating with Eudragit S100 (Figure 3D). Nevertheless, the surface of the PAMs shrank, and a cross-linked gel structure was formed (Figure 3C). The diameters of HCNP-PAMs and Eudragit S100-coated HCNP-PAMs were approximately  $547 \pm 45$  and  $658 \pm 73$   $\mu\text{m}$ , respectively.

The cisplatin content was  $17.8 \pm 0.8$  and  $16.1 \pm 0.8$   $\mu\text{g/mg}$  for uncoated HCNP-PAMs and Eudragit S100-coated HCNP-PAMs, respectively. The entrapment efficiencies of the prepared microbeads were  $89.0\% \pm 2.3\%$  and  $80.6\% \pm 3.8\%$  for HCNP-PAMs and Eudragit S100-coated HCNP-PAMs, respectively. The extent of drug loss after coating was approximately 9%. In our previous study,<sup>20</sup> the entrapment efficiencies of cisplatin were  $87.0\% \pm 3.9\%$  and  $74.6\% \pm 2.8\%$  for PAMs and Eudragit S100-coated PAMs, respectively, and the extent of drug loss after coating was approximately 12%. In this study, we demonstrated that the introduction of HCNPs into the previous system not only increased the entrapment efficiencies but also reduced the drug loss of cisplatin in Eudragit S100-coated PAMs.



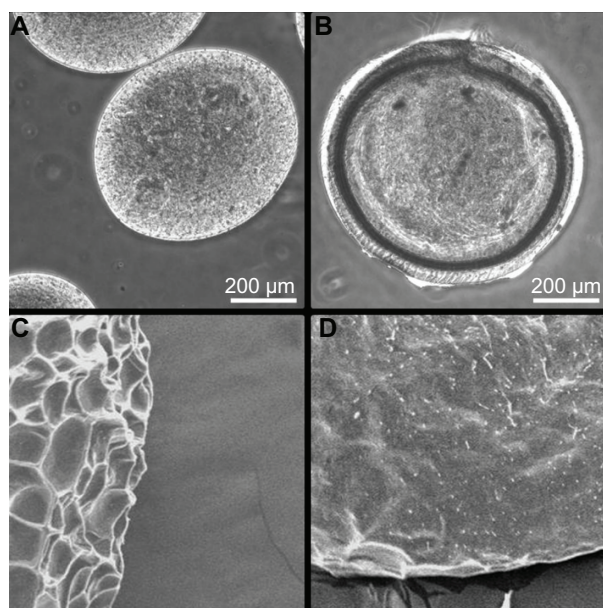
**Figure 1** (A) Transmission electron microscopy image of HCNPs. (B) Corresponding energy-dispersive X-ray spectroscopy spectrum.



**Figure 2** (A) Time-dependent effects of cisplatin and hyaluronan–cisplatin conjugate nanoparticles (HCNPs; cisplatin 10 mg/mL) on HCT-116 colorectal carcinoma cell viability, and (B) dose-dependent effects of cisplatin and HCNPs on HCT-116 colorectal carcinoma cell viability after 48 hours of incubation.

## Release behavior of cisplatin from the HCNP-PAMs

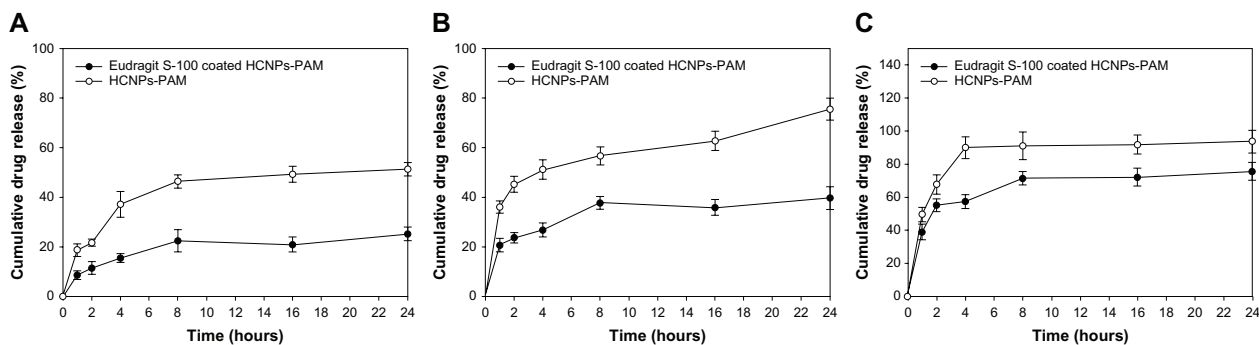
In vitro drug-release analysis of Eudragit S100-coated and uncoated HCNP-PAMs was performed in media with varying pH conditions (1.2, 4.5, and 7.4) at  $37^\circ\text{C} \pm 0.5^\circ\text{C}$  (Figure 4). The release rate of Eudragit S100-coated HCNP-PAMs was sensitive to the pH of the dissolution medium. The percentage of 24-hour drug-release rates was approximately 20% and 40% in pH 1.2 and pH 4.5, respectively. However, the drug-release rate quickly rose to 70% at pH 7.4. Compared to uncoated HCNP-PAMs, Eudragit S100-coated HCNP-PAMs showed slower drug release at pH levels of 1.2, 4.5, and 7.4.



**Figure 3** (A) Optical micrograph of hyaluronan–cisplatin conjugate nanoparticle pectinate/alginate microbeads (HCNP-PAMs); (B) optical micrograph of Eudragit S100-coated HCNP-PAMs; (C) scanning electron photomicrograph of HCNP-PAMs; (D) scanning electron photomicrograph of Eudragit S100-coated HCNP-PAMs.

The drug-release data were fitted to various kinetics equations to evaluate the drug-release mechanism and kinetics. Regression coefficients ( $r^2$ ) were obtained from zero-order, first-order, Higuchi model and Korsmeyer–Peppas equations. The best fit with the highest correlation coefficient was shown in the Korsmeyer–Peppas model, followed by the Higuchi model and first-order and zero-order equations, as shown in Table 1. We therefore utilized the Korsmeyer–Peppas model to determine the  $n$ -value, which described the drug-release mechanism. According to this model, the  $n$ -value was less than 0.45, which was indicative of Fickian diffusion. Fickian diffusion release occurred by molecular diffusion due to the chemical potential gradients in pH 1.2, 4.5, and 7.4 media. In addition, the release rate constant ( $k$ ) gradually increased from acidic to neutral environments. Compared with our previous study,<sup>20</sup> the release-rate constant of HCNPs was smaller than that of cisplatin from Eudragit S100-coated PAMs in an acidic environment. We hypothesize that the slower release rate resulted from the hyaluronan–cisplatin conjugate having a larger size than cisplatin alone.

To meet the objective of selectively releasing the drug in the colon, studies of in vitro drug release from Eudragit S100-coated HCNP-PAMs in simulated gastrointestinal fluids were performed. Figure 5 shows drug release from uncoated and Eudragit S100-coated HCNP-PAMs in simulated gastrointestinal fluids. At pH 1.2 (simulated gastric fluid), approximately 5.4% of the drug was released from the Eudragit S100-coated HCNP-PAMs, and 23.6% of the drug was released from the uncoated HCNP-PAMs. This difference in drug release was attributed to the insolubility of Eudragit S100 at a low pH. In the previous study,<sup>20</sup> approximately 9.3% of cisplatin was released from the Eudragit S100-coated PAMs. Therefore, HCNPs would

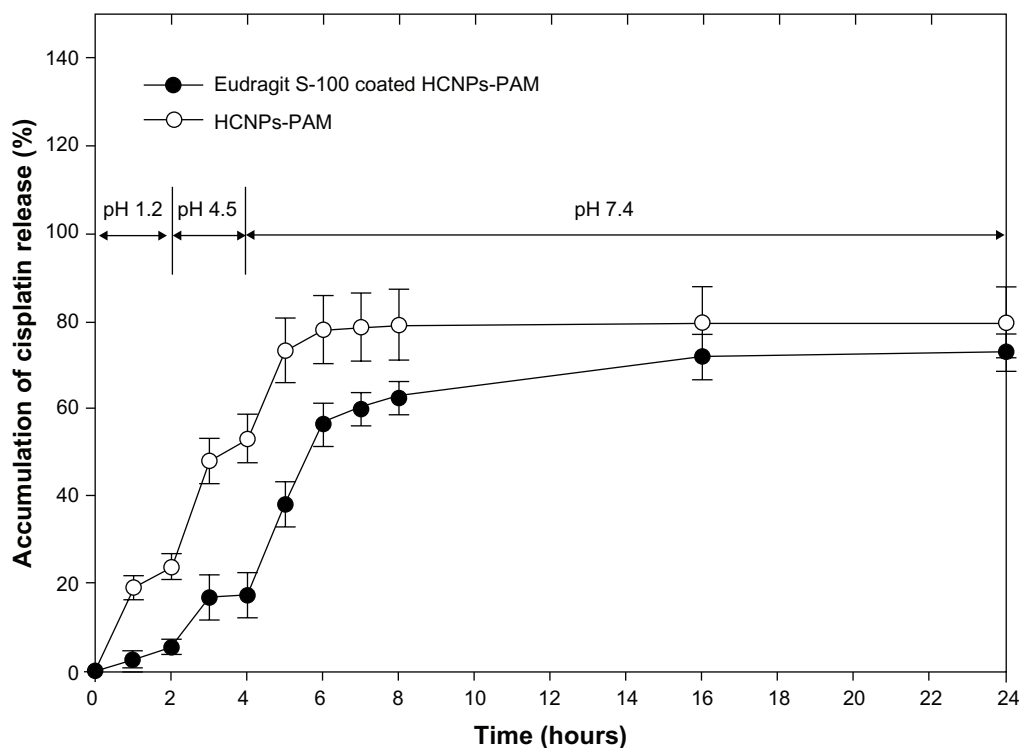


**Figure 4** Percentage cumulative in vitro drug-release profiles of cisplatin from uncoated hyaluronan–cisplatin conjugate nanoparticle pectinate/alginate microbeads (HCNP-PAMs) and Eudragit S100-coated HCNP-PAMs at various pH conditions: (A) pH = 1.2, (B) pH = 4.5, and (C) pH = 7.4. Each data point represents the mean ± standard deviation (n = 3).

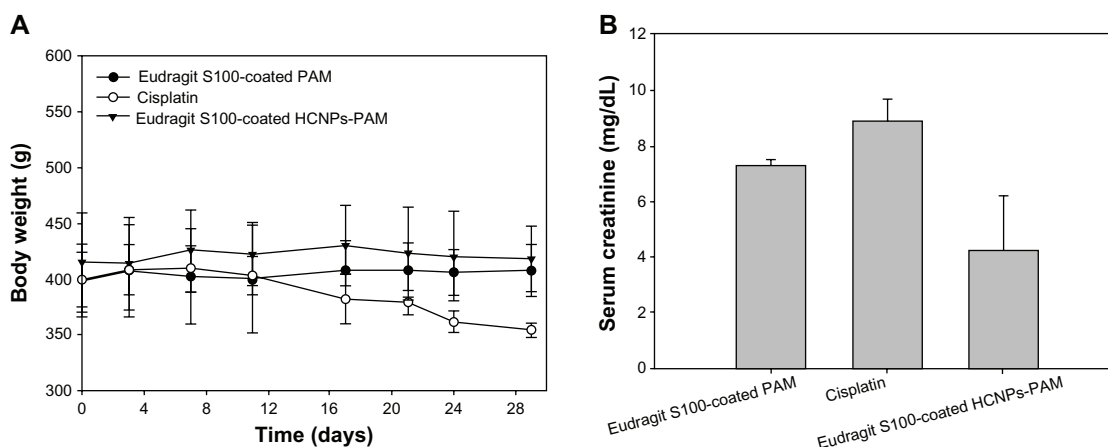
**Table 1** Correlation coefficient values (r), release rate constant (k) and diffusion exponent (n) for release kinetics of uncoated hyaluronan–cisplatin conjugate nanoparticle pectinate/alginate microbeads (HCNP-PAMs) and Eudragit S100-coated HCNP-PAM at various pH conditions

	Uncoated HCNPs-PAM				Eudragit S100-coated HCNPs-PAM			
	Zero order	First order	Higuchi model	K–P model	Zero order	First order	Higuchi model	K–P model
pH 1.2	r = 0.841	0.800	0.929	0.951 k = 21.751 n = 0.29	r = 0.867	0.825	0.919	0.954 k = 10.08 n = 0.29
pH 4.5	r = 0.958	0.843	0.980	0.991 k = 37.38 n = 0.222	r = 0.857	0.921	0.920	0.970 k = 21.26 n = 0.20
pH 7.4	r = 0.678	0.656	0.783	0.999 k = 49.87 n = 0.43	r = 0.821	0.778	0.901	0.987 k = 41.81 n = 0.26

**Abbreviation:** K–P model, Korsmeyer–Peppas model.



**Figure 5** Percentage cumulative in vitro drug-release profiles of cisplatin from uncoated hyaluronan–cisplatin conjugate nanoparticle pectinate/alginate microbeads (HCNP-PAMs) (●) and Eudragit S100-coated HCNP-PAMs (○) in simulated gastrointestinal fluids. Each data point represents the mean ± standard deviation (n = 3).



**Figure 6 (A)** Body-weight variations observed for 29 days after administration of Eudragit S100-coated pectinate/alginate microbeads (PAMs), cisplatin, or Eudragit S100-coated hyaluronan–cisplatin conjugate nanoparticle (HCNP)-PAMs in male Wistar rats. **(B)** Serum creatinine levels in cisplatin-induced nephrotoxicity. Rats were treated for 28 days with cisplatin, Eudragit S100-coated PAMs, or Eudragit S100-coated HCNP-PAMs. Serum creatinine levels were evaluated on day 29. Each data point represents the mean  $\pm$  standard deviation ( $n = 3$ ).

exhibit slower release than cisplatin from PAMs in the upper gastrointestinal tract.

## Evaluation of toxicity in vivo

The carcinogen DMH was used to induce colon tumors in Wistar rats, a model known to closely parallel human disease in terms of disease presentation. Cisplatin is a potent and widely used chemotherapy drug for cancer treatment. Unfortunately, cisplatin has major side effects in normal tissues, which includes nephrotoxicity in kidneys. All rats showed normal weight gain over a period of 11 days. A significant reduction in body weight was observed only in the group of rats treated with cisplatin at day 17 ( $P < 0.05$ ). However, no significant differences in body weight were observed between the groups treated with the HCNP-PAMs and PAMs (Figure 6A). A significant increase in serum creatinine was observed after cisplatin treatment compared to Eudragit S100-coated HCNP-PAMs treatment at day 29 (Figure 6B,  $P < 0.05$ ). These observations indicate that the Eudragit S100-coated HCNP-PAM treatment is able to mitigate the nephrotoxicity that is attributed to cisplatin.

## Conclusion

This study describes a new colonic drug-delivery system that combines PAMs and HCNPs. These microbeads, which are easily prepared from an aqueous solution using electrospray technology, could be capable of achieving colon-specific delivery of a drug due to ligand–receptor relationships and pH-dependent degradation. The experimental results of our in vitro study demonstrate that the cell toxicity of HCNPs was significantly higher than that of cisplatin, and Eudragit-coated

HCNP-PAMs are able to limit the release of HCNPs under acidic conditions and release HCNPs under simulated colonic conditions. Moreover, Eudragit-coated HCNP-PAMs can also reduce cisplatin-affiliated nephrotoxicity in vivo. Hence, Eudragit-coated HCNP-PAMs have the potential to be used as a drug carrier for an effective colon-targeted delivery system.

## Disclosure

The authors report no conflicts of interest in this work.

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