Invention of Polysaccharide-based Nanoparticles for Enhancing Drug Permeability across the Blood Brain Barrier

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ABSTRACT

Novel polysaccharide-based nanogels containing poly(β-aminoester) and β-cyclodextrin were synthesized via Michael addition polymerization. The nanogels were hydrolytically degradable and sustained the release of doxorubicin (DOX) and insulin. The nanogels were not toxic to bovine brain microvessel endothelial cells (BBMVEC) at concentration of up to 500 $\mu g \cdot ml^{-1}$ and enhanced the permeability of insulin across the BBMVEC monolayer, an *in vitro* blood brain barrier (BBB) model, 20% higher.

Keywords: β-cyclodextrin, aminoester, nanogel, biodegradable, blood brain barrier

1 INTRODUCTION

The BBB is a selective protective barrier composed of a tightly packed capillary endothelial cell monolayer [1]. It allows the passage of nutrients into the brain, while preventing the passage of many therapeutics that could prove valuable in the treatment on many CNS disorders including cancer, Alzheimer's, and Parkinson's disease [2]. Many attempts have been made, using a number of approaches, to aide in drug permeability across the restrictive BBB/BRB including disruption or bypass, modification of therapeutics for receptor mediated transport, liposomes, and micelles [3]. These approaches have either lacked the desired control and specificity, leading to dangerous side effects, or could not deliver sufficient drug. Therefore, there is a critical need to develop effective systems for the targeted and sustained delivery of drugs across the BBB/BRB

Nanogels have superior bioavailability, high loading efficiency, low burst effect, and tunable surface chemistry [4]. Cationic nanoparticles have been recognized as an effective approach to increase their permeability across the BBB/BRB [5] and poly(β-amino ester)s which are cationic and hydrolytically degradable under physiological conditions significantly decrease the cytotoxicity associated with cationic nanparticles [6]. βCD and its derivatives containing hydrophobic central cavity and a hydrophilic outer surface have been widely studied as effective drug delivery systems for both hydrophilic and hydrophobic drugs. In combination of the benefits of nanogel, poly(β-amino ester) and βCD, in this work, we have developed novel poly-(β-cyclodextrin)-(β-aminoester) nanogels for sustained release of hydrophobic DOX and hydrophilic insulin across the BBB.

2 EXPERIMENTAL METHODS

2.1 Synthesis of acryloyl βCD

βCD was dissolved in N-methylpyrrolidone (NMP), and then acryloyl chloride was added dropwise at 0 °C under N_2 purge for 48 h, Acrylic β-CD was obtained after precipitation with cold ether 3 times. The yield was 91.6%. ^{1}H NMR δ_H (500 MHz, $CDCl_3$) 5.8–6.5 (36H, CH_2=C*H*-), 3.4–5.5 (58H, residues of βCD).

2.2 Synthesis of poly-(β-cyclodextrin)-(β-aminoester) nanogels

The nanogels were synthesized using Michael addition polymerization. Acryloyl βCDs, 1,4-butanediol diacrylate, and *N,N*-dimethylethane-1,2-diamine were dissolved in choloform/EtAcetate mixture solvent. The solution was refluxed at 65 °C for 12 h with stirring at 200 rpm. The product was precipitated with cold ether twice and dialyzed against DI-water with MWCO 12,000-14,000 dialysis membrane. The final product was lyophilized.

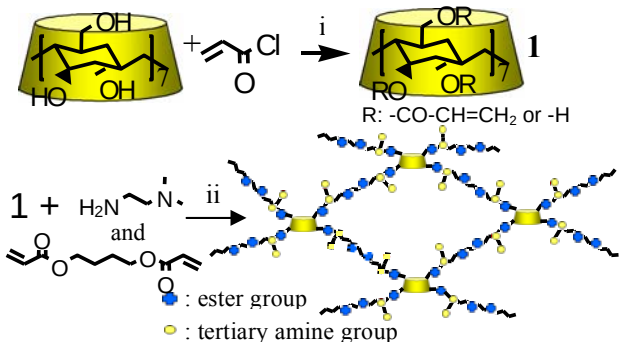

Figure 1. Schematic structures of poly(β-aminoester)-βCD nanogel (PAECD-Ng).

2.3 Cell culture

BBMVECs (Cell applications Inc.), were seeded in fibronectin coated T-flasks. The culture medium consisted of molecular, cellular and developmental biology medium (MCDB-131, Sigma), 10% fetal bovine serum, 10 $ng \cdot ml^{-1}$ epidermal growth factor, 0.2 $mg \cdot ml^{-1}$ ENDO GRO (VEC

Technologies), 0.9 mg·ml^{-1} heparin, and antibiotic/antimycotic (penicillin G sodium salt 10 μg·ml^{-1}).

2.4 *In Vitro* Cytotoxicity of CPβCDs

The cytotoxicity of the synthesized nanogels to BBMVECs at concentrations of 50, 100, 250, and 500 μg·ml^{-1} was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5 diph-enyltetrazolium bromide (MTT) assay, following a general procedure.

2.5 Loading efficiency and Release of model drugs

FITC-insulin (3.6 mM, 20 mg·ml^{-1}) or DOX (2 mM, 1.16 mg·ml^{-1}) was mixed with the nanogels (20 mg·ml^{-1}) in DI-water for 18 h, dialyzed against DI-water with MWCO 12,000-14,000 dialysis membrane, and lyophilized. The FITC-insulin or DOX loaded-nanogels were dissolved in PBS (pH 7.4) at 2 mg·ml^{-1} and then dialyzed against PBS (PH 7.4) with MWCO 12,000-14,000 dialysis membrane at 37°C. At selected time points, samples were taken out from the solution outside the dialysis membrane. The loading efficiency and accumulated release amount were calculated by measuring the fluorescent intensity of the sample solutions.

3 RESULTS AND DISCUSSION

Figure 1 represents the schematic illustration of the synthesis of poly-(β-cyclodextrin)-(β-aminoester) nanogels. The hydrodynamic radius of the obtained nanogels in PBS (pH 7.4) was 106.2 ± 3.7 nm. Our FTIR results confirm that the nanogels hydrolytically degrade in PBS (pH 7.4) (data not shown). Figure 2 demonstrates that the nanogels are not toxic to BBMVECs with 100% cell viability at concentration up to 500 μg·ml^{-1}. The loading efficiencies of insulin and DOX into the nanogels are 86±3% and 9.4±0.9%, respectively. The nanogels can continuously release DOX for 40 days and insulin for 13 days, respectively. The nanogels are 0.4 and 8.8 times more permeable than 4 kD and 70 kD dextrans, respectively, across the BBMEC monolayer. Encouragingly, the nanogels enhance the permeability of insulin across the BBMEC monolayer 20% higher.

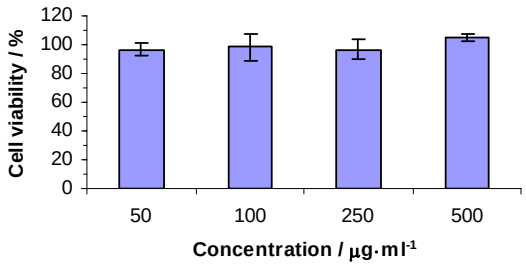

Figure 2. Cell viability of BBMVEC cells after 24 h treatment with nanogels at 100~500 μg·ml^{-1}. (mean ± s.d. N=4).

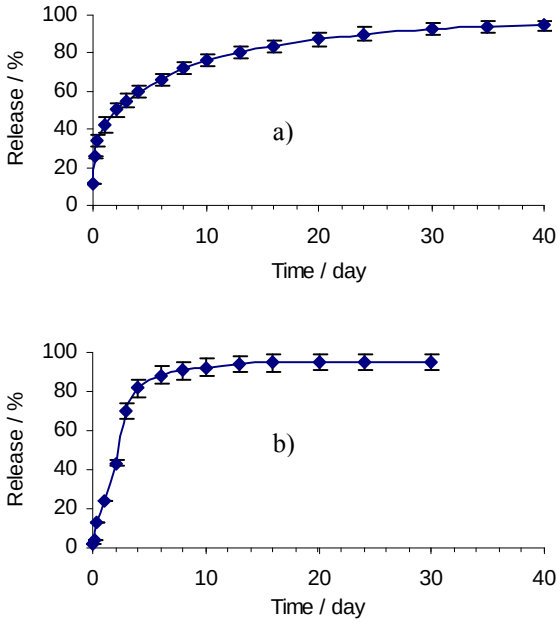

Figure 3. Accumulative release of a) DOX and b) FITC-insulin from nanogels at 2 mg·ml^{-1} in PBS (pH 7.4) at 37°C.

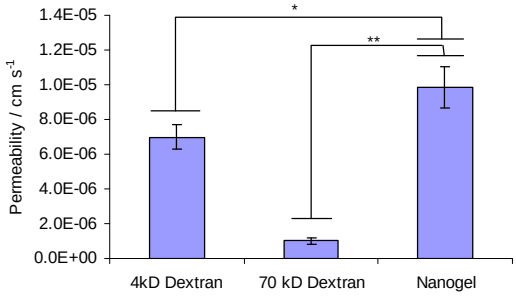

Figure 4. Permeability of nanogels at 100 μg·ml^{-1} across BBMEC monolayer at 37°C for 3 h. FITC-dextrans (M_w = 4,400 and 70,000 g·mol^{-1}) were used as controls. (mean ± s.d. , N =3, *: p < 0.05, **: P <0.001).

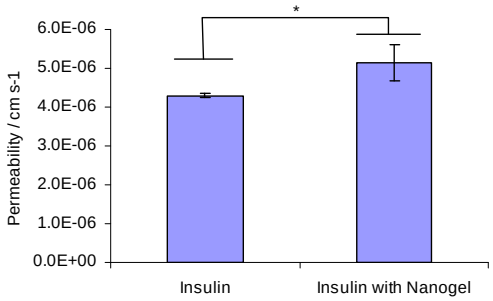

Figure 5. Permeability of insulin (5 μM) with/without nanogels (300 μg·ml^{-1}) across BBMEC monolayer at 37°C for 3 h. (mean ± s.d. , N =3, *: p < 0.05).

4 CONCLUSIONS

This work provides important insight into how to develop poly-(β-cyclodextrin)-(β-aminoester) nanogels and other novel biomaterials for controlled release of therapeutic agents across the BBB for the treatments of brain diseases.

5 REFERENCES

[1] Pardridge WM. Adv. Drug Deliver. Rev. 1999, 36:299-321.

[2] Vinogradov SV, Batrakova EV, and Kabanov AV. Bioconjugate Chem. 2004, 15:50-60.

[3] Koziara JM, Lockman PR, Allen DD, and Mumper RJ. J. Nanosci. Nanotechnol. 2006, 6:2712-2735.

[4] Moghimi SM, Hunter AC, and Murray JC. Pharmacol. Rev. 2001, 52:283-318.

[5] Kreuter J. J. Nanosci. Nanotechno. 2004, 4:484-488.

[6] Lynn DM, Anderson D, Putnam D, and Langer R. J. Am. Chem. Soc. 2001, 123:8155-8156.

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