Advanced Materials Research Vols. 47-50 (2008) pp 1319-1322 *Online: 2008-06-12*
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doi:10.4028/www.scientific.net/AMR.47-50.1319

Novel organic-inorganic hybrid nanoparticles as non-viral gene vector

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Keywords: Gene therapy, non-viral vector, hybrid, gelatin, siloxane

Abstract. Novel hybrid biomaterial of gelatin-siloxane nanoparticles (GS NPs), with positive surface potential and lower cytotoxicity, was synthesized through a 2-step sol-gel process. The pDNA-GS NPs complex was formulated with high encapsulation efficiency, and exhibited and efficient transfection *in vitro*. We thus envision that the GS NPs material could serve as non-viral gene vectors for gene therapy.

1. Introduction

Gene therapy is considered to be a potential approach in the field of medicine, pharmaceutical sciences, and biotechnology as it eradicates disease at the level of genosome [1]. The major difficulty in gene therapy, however, is a need for safe and effective vector system that can deliver the gene to the target tissue and cells and allow for expression of the protein of interest over. Although vial vectors have efficient transfection, the potential oncogenicity, toxicity and immunogenicity of viral vectors persuade the development of non-viral vectors [2]. A number of non-viral gene delivery vectors, including lipid, synthetic cationic polymers, gelatin, chiotsan, and silica nanoparticles, have been explored [3]. However, the design of safe and efficient gene transfection vectors is still a challenge in gene therapy [4]. In our previous study, a new family of organic-inorganic hybrid with both of biodegradation and biocompatibility, could be easily developed by bridging of gelatin chains onto siloxane network through a sol-gel procedure [5]. In this study, we designed a novel kind of gelatin-siloxane hybrid nanoparticles (GS NPs), which could encapsulate plasmid DNA efficiently. Moreover, our *in vitro* experiments indicated that the fabricated GS NPs exhibited not only low cytotoxicity, but also high transfection for Hela cells *in vitro*.

2. Materials and methods

Table 1 Composition and characteristics of the as-synthesized GS NPs

		GS-1	GS-2	GS-3	GS-4
composition (feed ratio)	gelatin (mg/mL)	5	10	10	10
	GPSM (mg/mL)	10	10	7.5	5
characterization	size (nm)	651±24.19	217±14.37	186±10.61	153±21.2
	surface charge (mv)	10.1±0.63	9.1±0.62	9.8±0.80	9.4±0.34
	-NH_2 on surface (mmol/g NPs)	0.22±0.014	0.18±0.005	0.18±0.011	0.20±0.012

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As shown in Table 1, appropriate amount of GPSM was added to 0.5% or 1% gelatin solution in HCl (pH 3.0) at 25 ^{o}C. After stirring for 30 min, ~ 40 μL of ammonia (25%) was subsequently added into the above mixtures till pH became 9.0. The mixtures were then continuous stirred for another 24 h. Though the mixture was initially clear, continuous stirring gave a milky emulsion. The resulting GS NPs were purified by centrifugation (19,000 rpm, 30 ^{o}C, 15 min) for 3 times. GS NPs with diameter of ~ 200 nm were then mixed with DNA solution at predetermined weight ratios, following by 30 s vortex and 30 min incubation. NPs/pDNA complexes were also purified by centrifugation (19000 r.p.m., 30 ^{o}C, 15 min). In the following experiments, NPs/pDNA complex was finally suspended in deionized water, and the final concentration of pDNA was kept at 200 μg/mL for each sample.

Zeta potentials and size of nanoparticles were measured on a Nano-ZS zetasizer dynamic light scattering detector (Malvern Instruments). The FTIR transmission spectra were recorded by using a AVATAR360 FT-IR spectrometer (Nicolet) at a resolution of 4 cm^{-1}, and 256 scans were taken in the range of 400 ~ 4000 cm^{-1}. The amount of $-NH_2$ on the surface of NPs were determined by using ninhydrin reaction, in which histidine was utilized as reference. To examine the binding of pDNA to GS NPs, gel electrophoresis assay was electrophoresed on 1 wt% agarose gel containing 1 μg/mL ethidium bromide in deionized water at 150 V for 45 min.

For *in vitro* experiments, Hela cells were cultivated in DMEM (Dulbecco's Modified Eagle Media) supplemented with 10% fetal bovine serum (FBS), penicillin (100 IU/mL) and streptomycin (100 mg/mL) at 37 ^{o}C and 5% CO_2. For MTT assay, 10^5 cells were firstly seeded into 96-well plates and incubated for 24 h. The cell cultures were then exposed to 100 μL of serial test specimens, respectively. After 24 h co-incubation, medium was replaced with 20 μL MTT solution (5 mg/mL in PBS buffer) and further culture for 4 h. At the end of the assay, MTT solution was removed and 100 μL DMSO was added to dissolve the blue formazan crystal by proliferating cells. The absorbance was measured at 570 nm using Bio-tek ELX800 microplate reader. The relative cell viability (%) was expressed as a percentage relative to the untreated control cells.

For *in vitro* transfection experiments, Hela cells were firstly seeded into 6-well plates and incubated for 24 h. After removing the medium, 1 mL of serum-free DMEM containing serial test specimens was added into each well, and the concentration of DNA was set as for 1 μg/mL each well. The cell cultures were then co-incubated for 8 h. At the end, the medium was replaced with complete DMEM containing 10% FBS and the cells were allowed to grow for an additional 24 h. The β-galactosidase expression was measured in the cell lysate using a commercial kit, and the absorbance was determined at 415 nm on Bio-tek ELX800 microplate reader.

3. Results and discussion

At the initial acidic pH condition (within 30 min), GPSM was subject to cleavage of the C-O bond in the epoxy group [6]. The protonated epoxy group is so activated to attack nucleophilic groups like NH_2 on the side chain of gelatin so that GPSM molecules could be grafted to the gelatin chains. Simultaneously methoxy silane groups (Si-OCH_3) of GPSM were hydrolyzed in acidic solution to give silanol groups (Si-OH). As adding ammonia to this precursor sol, the pH value of sol was changed into 9.0 immediately. The condensation reaction was thus quickly initiated at alkaline condition, resulting in a network of siloxane.

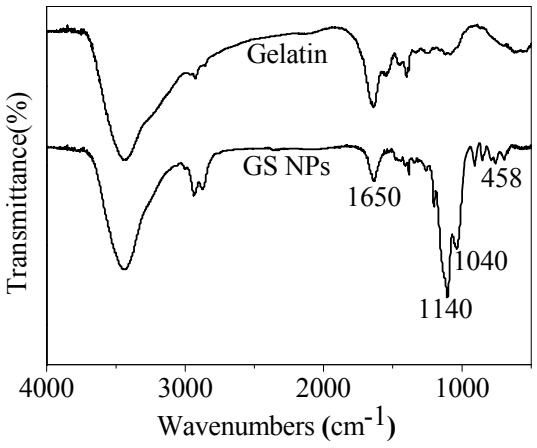

Fig. 1 FTIR spectra of gelatin and GS NPs

Table 1 showed the characteristics of GS NPs with different feed ratios. The size of NPs (from ~650 nm to ~150 nm) decreased with the concentrations of gelatin, whereas increased with GPMS. However, surface charges and amino groups of NPs were stable at the various concentrations of gelatin and GPSM. Fig. 1 compares FT-IR spectra of a typical GS NPs (G2) with gelatin. Both of gelatin and GS NPs had a peak of 1650 cm^{-1} corresponded to the C=O stretching. For GS NPs, the presence of new bands at 1030, 1140 and 458 cm^{-1}, suggested that siloxane exists and can be respectively indexed to the asymmetric and symmetric stretching vibration of Si-O-Si as well as the bending of Si-O-Si.

A critical requirement for effective gene delivery is their ability to condense string-like DNA molecules into compact nanosize particles suitable for cellular uptake [8]. The particle size and surface charge of a GS/DNA complex are important factors which may influence the access and passage of the complex into the cells. As shown in Fig. 2, the sizes and the surface charges of the NPs/DNA complexes were dependent on the mass ratio of NPs/DNA. Namely, the size of complexes decreased with mass ratios, while the surface charge increased with mass ratios.

The condensation capability of GS NPs with DNA was also evaluated by using agarose gel electrophoresis. It was observed in Fig. 3 that while free pDNA moved at its usual position, pDNA complexed with GS NPs with different mass ratios (20 to 500) exhibited partly immobilised within the wells in an electromobility shift assay (lane 1-5).

Cytotoxicity is one of the vital issues for successful gene therapy. Some commercially available transfection agents such as PEI always result in high toxicity to cells [9]. As shown in Fig. 4, exposure to GS NPs, at the concentration < 200 mg/mL, led to the death of ~ 10% of cells. However, as the concentration of NPs increased to > 300 mg/mL, cellular viability decreased to around 60%. Our previous results indicated that the highest transfection efficiency exhibited at 1 μg/mL of pDNA in complexes with vector. It

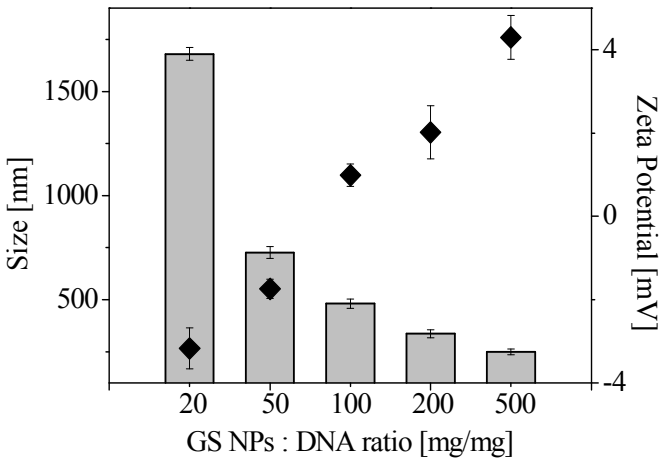

Fig. 2 Size (columns) and zeta potential (diamonds) of GS NPs/DNA complexes.

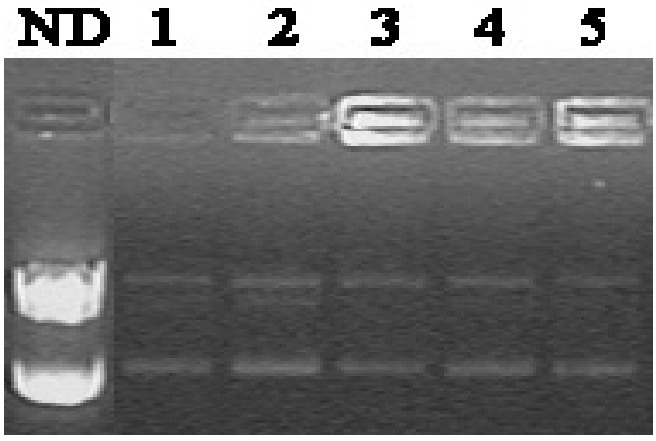

Fig. 3 Gel electrophoresis assay. ND: naked DNA; Lane 1–5: GS NPs/DNA complexes with the weight ratio (mg/mg) of 20, 50, 100, 200 and 500, respectively.

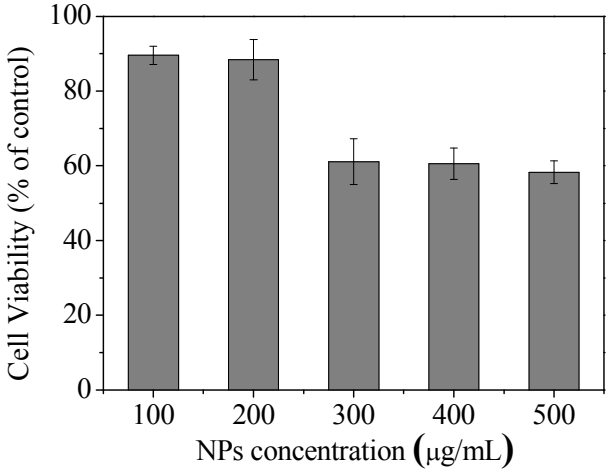

Fig. 4 Cytotoxicity of GS NPs by MTT assay.

is thus suggested GS NPs might behave low cytotoxicity for *in vitro* transfection experiment in Hela cells as the weight ratio of GS : DNA < 200 : 1.

The ability of GS NPs to deliver DNA into Hela cells was determined by performing a β-gal reporter gene transfection. The expressions of gene were taken at predetermined GS NPs/DNA mass ratios, as shown in Fig. 5. Highest transfection efficiency (~ 70% of commercially available transfecting reagent Lipofectamine^{TM}) was observed for cells pretreated with complexes formulated at mass ratio of 100 compared to weight ratios of 50 and 200. Considering that Lipofectamine^{TM} is highly competent vector used for in vitro DNA delivery, our results thus indicated that the formulation of pDNA in GS NPs can offer an approach for a possible gene delivery vehicle.

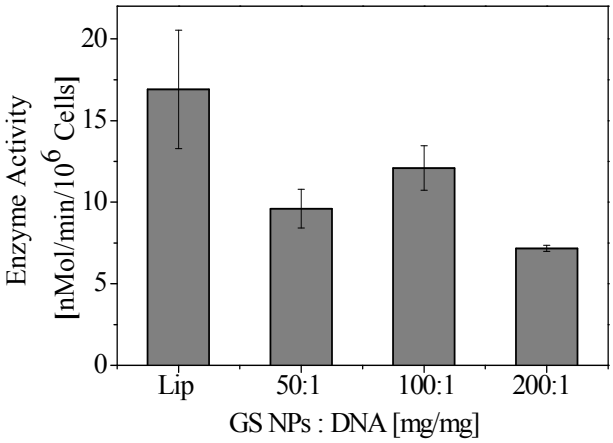

Fig. 5 Transfection of Hela cells expressing β-gal using Lipofectamine^{TM} reagent (Lip) and GS NPs/DNA complexes.

4. Conclusions

Novel organic-inorganic GS NPs were developed as potential gene vectors. The cationic GS NPs with size of ~ 200 nm showed low cytotoxicity and high encapsulated efficiency to pDNA. Moreover, GS/pDNA complex displayed ~ 70% transfection efficiency of Lipofectamine^{TM}.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (Grant No. 30670559), National Basic Research Program of China (973 Program) (2007CB935603), Nature Science Foundation of Fujian Province of China (2006J0121), and Program for New Century Excellent Talents in Fujian Province University.

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