The 6th International Conference on Biochemistry and Molecular Biology

Preparation of PLGA and PLGA-chitosan nanoparticles for breast cancer delivery system

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Abstract

Breast cancer is one of the leading cause of cancer death in women. Even though nowadays there is more improvement of chemotherapy, however, the drugs are usually non-specific and highly toxic leading to various unpleasant side effects which are unavoidable in breast cancer patients. Polymeric nanoparticles such as poly (lactic-co-glycolic acid) nanoparticles or PLGA NPs have potential to minimize this disadvantage of chemotherapy. PLGA is a polymer that has been developed for drug delivery system due to its properties such as biocompatibility, biodegradability, and non-toxicity approved by U.S. Food and Drug Administration. In this research, PLGA and PLGA-Chitosan (PLGA-CS) NPs were synthesized to investigate the roles of size and surface charge of NPs for developing as a drug delivery system for breast cancer. The PLGA and PLGA-CS NPs were synthesized by single emulsion method. Different sizes and surface of NPs (PLGA and PLGA-CS) were synthesized by varying different synthesis parameters such as organic solvents, and surfactant concentrations. The reproducible PLGA and PLGA-CS NPs with different sizes (200-400 nm) were successfully obtained. The NPs were characterized by determination of size and zeta potential using Zetasizer Nano ZS. Morphology of the particles was visualized by Scanning Electron Microscopy (SEM). In addition, different sizes and surface charges of PLGA and PLGA-CS NPs in the concentration range from 10 to 400 µg/mL showed no effect on cell viability in breast cancer cells (MCF-7) determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. This study reports the successful preparations of different sizes of PLGA and PLGA-CS NPs, which showed no toxicity that could be further developed as an effective drug delivery system for breast cancer.

Introduction

One of the leading cause of cancer death in women around the world is breast cancer[1]. The common treatment is chemotherapy, however, it has high toxicity and lack of specificity which lead to various side effects to the patients. Recently, nanoparticles (NPs) are introduced to lower these drawbacks of chemotherapy, for example, using poly (lactic-co-glycolic acid) (PLGA) NPs which are polymeric NPs that have been widely applied in drug delivery systems. PLGA is biodegradable, nontoxic, biocompatible and approved for its safety by the US-FDA[2, 3, 4]. Moreover, the PLGA NPs have controllable size and surface properties. The size is important for delivering drugs to cancer because it plays an important role during internalization process of NPs via Enhanced Permeability and Retention (EPR) effect which require small particle size[5]. Thus, there are several studies focusing on how various synthesis parameters can contribute to different sizes of NPs such as organic solvents, surfactants, monomer ratio of PLGA, and sonication time[6-10]. In addition to the size, appropriate designed

surface of NPs could promote NPs uptake in cancer cells. Due to negative charge of the cell membrane, NPs with positive charge usually showed higher uptake in cells than NPs with negative or neutral charges[11, 12]. Therefore, PLGA NPs with further modification to achieve positive surface could lead to better targeting to breast cancer cells. Chitosan (CS) is a natural polymer which is biodegradable, nontoxic and highly positively charged. Therefore, CS has desirable properties for decorating the surface of PLGA NPs. This study was focused on preparation methods to obtain different sizes and surface charge of PLGA NPs and preliminary evaluate their cellular effect on breast cancer cells. The different synthesis conditions to obtain different sizes of PLGA NPs were presented and the PLGA NPs were further modified with chitosan to achieve positively charged particles called PLGA-Chitosan (PLGA-CS) NPs. The successful preparation to obtain the reproducible and controllable size and surface charge of PLGA NPs is the first important step for effective drug delivery development for breast cancer therapy which could be further applied to other drug delivery systems.

Methodology

Preparation of PLGA and PLGA-CS NPs

PLGA NPs were prepared by single emulsion method. An oil phase was prepared by addition of 50 mg of PLGA (75:25) into 0.5 mL of ethyl acetate (EtOAc) or dichloromethane (DCM). The water phase was prepared by varying different concentrations of polyvinyl alcohol (PVA) in water (1, 5 and 10 % w/v). The oil phase was then added dropwise into water phase followed by sonication. The organic solvents were evaporated for 4 hours before the NPs were collected and lyophilized using freeze dryer. PLGA-CS NPs were synthesized using the similar method except for an addition of 50 mg of chitosan in the water phase containing 5% w/v of PVA.

Characterization of PLGA and PLGA-CS NPs

PLGA and PLGA-CS NPs were dissolved in ultrapure water and the size and zeta potential were subsequently measured by Zetasizer Nano ZS. (n = 3)

Cell culture and determination of cell viability by MTT assay

MCF-7 cells (breast cancer cell line) were cultured in DMEM (Dulbecco's Modified Eagle Medium) with the supplement of 10% fetal bovine serum. For MTT assay, the cells were treated with PLGA or PLGA-CS NPs at the concentration of 10, 100, 200 and 400 µg/mL for 24 and 48 hours. The cells were then incubated with 0.5 mg/ml MTT solution for 2.5 hours and the formazan crystals were dissolved by DMSO (dimethyl sulfoxide). The absorbance values were recorded at 540 nm. (n = 3)

Results and Discussion

The PLGA NPs were synthesized using different organic solvents and concentrations of PVA which acts as the stabilizer by single emulsion method. The results showed that the size of PLGA NPs synthesized by using DCM was larger than the PLGA NPs prepared from EtOAc which is consistent with the results of Park *et al*. and Ito *et al*.[13,14]. The solvents can affect to the NPs size during the diffusion step of organic solvents in emulsion into the water. EtOAc is partially miscible while DCM is immiscible, therefore, interfacial tension of EtOAc is lower than DCM leading to the formation of smaller size NPs prepared in EtOAc[10, 13-15]. In addition, the size tends to decrease when the concentration of PVA was increased as shown in table 1. PVA plays a role in lowering the surface tension between the oil phase and water phase. The PVA diffuse to the interface between water phase and emulsion droplet resulting in the higher presence of surfactant at the droplet surface which protects the droplets against coalescence[6, 15]. Therefore, using the higher concentration of PVA lead to smaller NPs size. This result is compatible with the studies of Esmaeili, Atyabi, and Dinarvand[16] and Sahoo[17].

Moreover, the achieved PLGA NPs were monodisperse as shown by low polydispersity index (PDI) value in the range of 0.1-0.25[18, 19].

Moreover, zeta potential, an electric potential at electrical double layer surrounding NPs, was determined because this value is related to the stability of the NPs in suspension. The PLGA NPs in table 1 had the zeta potential in the range between -18 to -25 mV (table 1) meaning that they are moderately stable as stated in the research of Bhattacharjee[20]. The PLGA NPs synthesized by using 5% PVA with EtOAc and DCM (sizes were about 200 nm and 300 nm, respectively) were small, stable and monodispersed, therefore they were selected for further experiments. From all these reasons, the PLGA-CS NPs were then synthesized using 5% PVA and the different sizes of PLGA-CS NPs were successfully prepared and confirmed by the positive zeta potential value (table 1) due to the positive charge of chitosan. The PDI of PLGA-CS NPs synthesized from DCM is lower than PLGA-CS from EtOAc synthesis, therefore, the DCM is more suitable for obtaining monodisperse of PLGA-CS NPs. The sizes and morphology of NPs were confirmed by SEM images (Figure 1 and 2) which showed all spherical shapes with agreeable size in both NPs synthesized with EtoAc and DCM as observed in table 1.

Table 1. Characteristics of PLGA and PLGA-CS NPs

NPs	**PVA (%)**	**Organic solvent**					
		EtOAc			**DCM**		
		Size (nm)	**PDI**	**Zeta potential (mV)**	**Size (nm)**	**PDI**	**Zeta potential (mV)**
PLGA	1	224.8 ± 15.6	0.130	-23.50 ± 4.27	416.6 ± 63.9	0.286	-23.48 ± 0.59
	5	177.1 ± 10.8	0.084	-18.43 ± 2.11	292.6 ± 13.6	0.185	-23.95 ± 1.58
	10	184.0 ± 57.9	0.108	-25.43 ± 1.04	296.2 ± 42.7	0.212	-23.12 ± 3.00
PLGA-CS	5	223.7 ± 1.8	0.355	11.53 ± 0.12	277.4 ± 6.4	0.091	8.95 ± 0.30

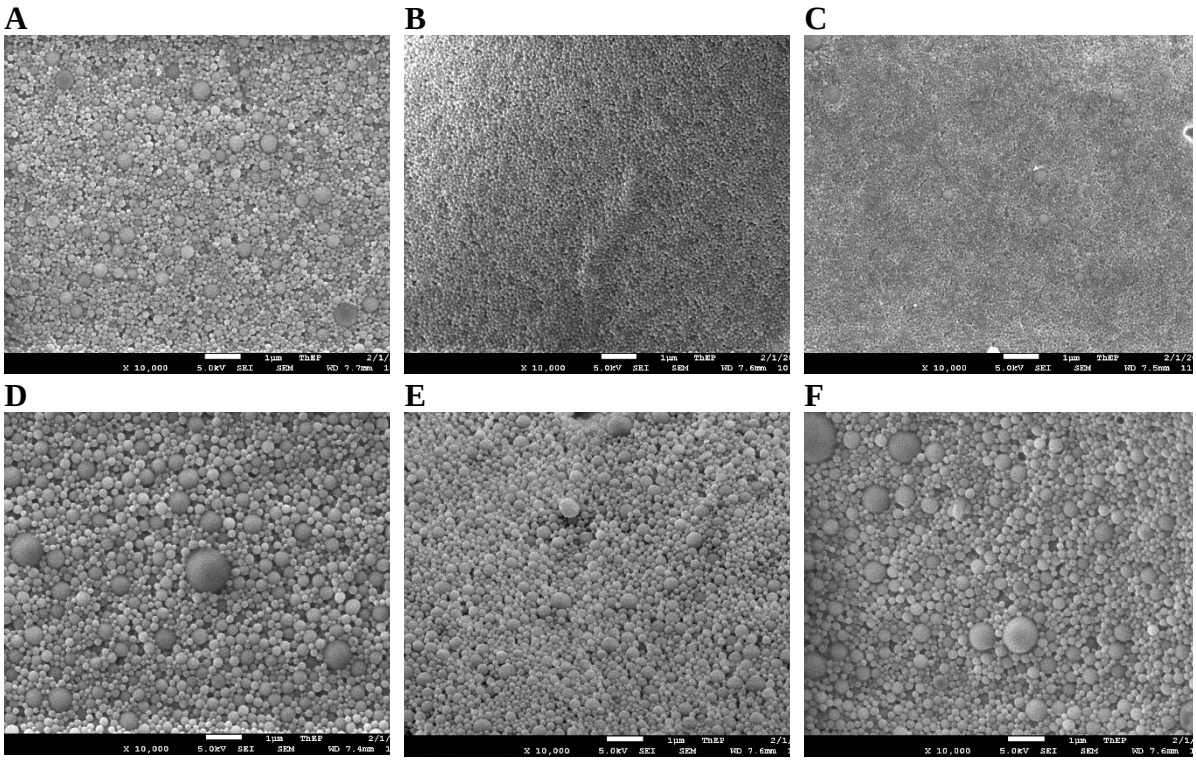

Figure 1. SEM images of PLGA NPs: A, B and C; NPs synthesized from 1%, 5% and 10% PVA with EtOAc and D, E and F; NPs synthesized from 1%, 5% and 10% PVA with DCM, respectively. (Scale bar: 1 μm)

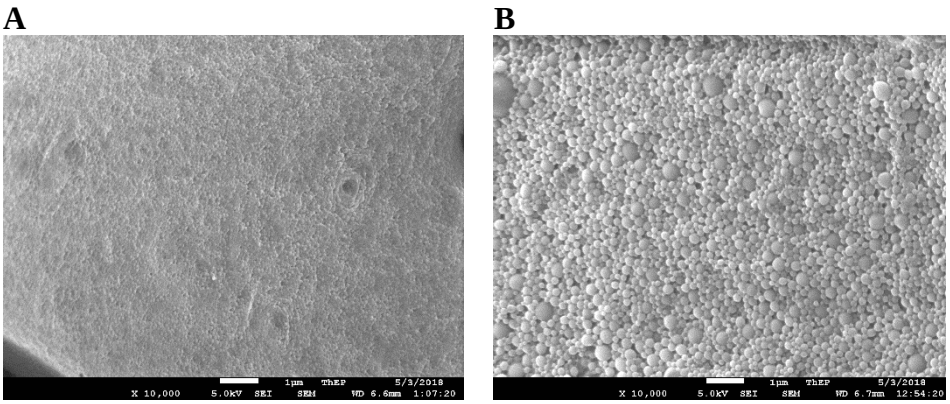

Figure 2. SEM images of PLGA-CS NPs: A and B; NPs synthesized from 5% PVA with EtOAc and DCM, respectively. (Scale bar: 1 μm)

In order to use the obtained NPs for drug delivery applications, the NPs themselves should not cause toxicity to the cells. The effect of PLGA and PLGA-CS NPs to the cell viability of MCF-7 cell were studied using MTT assay and 1% Triton X-100 acted as positive control. Both sizes of PLGA and PLGA-CS NPs (200 and 300 nm) showed no or minimum cytotoxicity in the concentration range between 0-400 μg/mL (Figure 3 and 4) as indicated by the percentage of cell viability higher than eighty. Therefore, the prepared PLGA and PLGA-CS NPs are biocompatible and could be used in drug delivery applications to breast cancer cells.

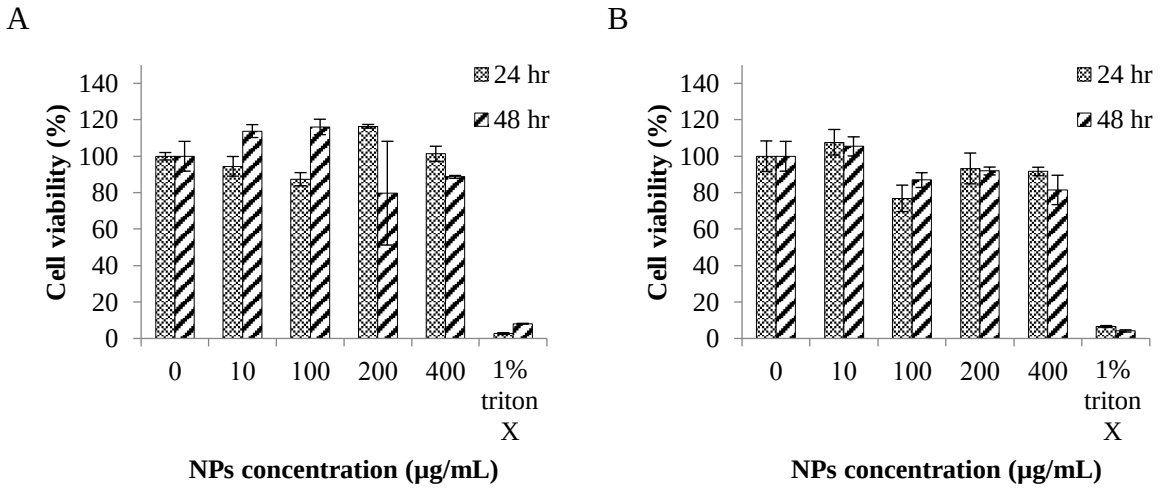

Figure 3. Effect of PLGA NPs to cell viability of MCF-7 cells: A; NPs with size of 200 nm and B; NPs with size of 300 nm.

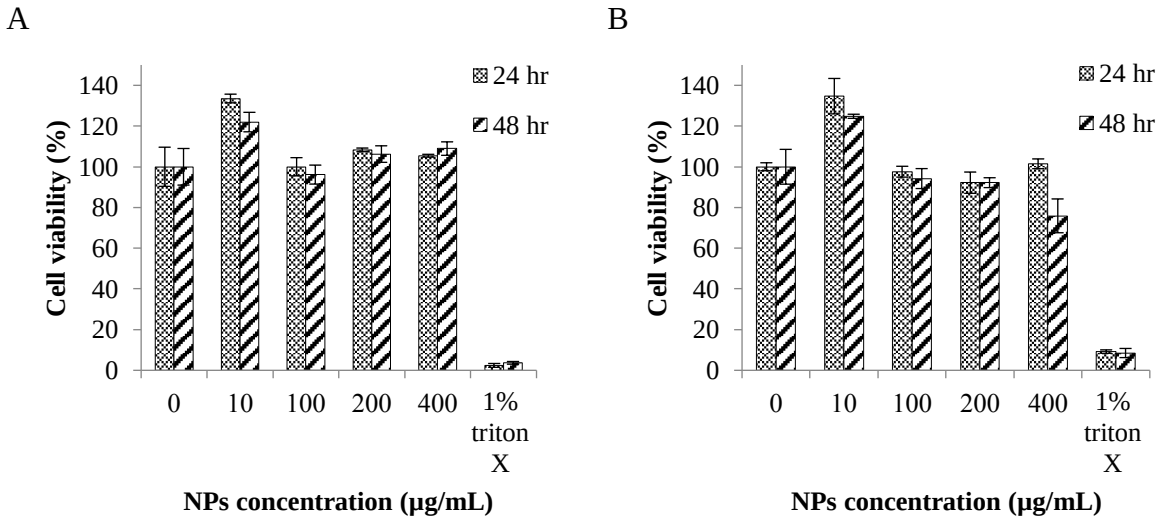

Figure 4. Effect of PLGA-CS NPs to cell viability of MCF-7 cells: A; NPs with size of 200 nm and B; NPs with size of 300 nm.

Conclusion

The different sizes of PLGA NPs can be prepared by varying the organic solvent types and PVA concentrations while the positive charge of PLGA NPs can be synthesized by coating the NPs with chitosan resulting in PLGA-CS NPs. Besides that, both PLGA and PLGA-CS NPs with the size of 200 and 300 nm did not affect cell viability at the concentration up to 400 µg/mL thus these NPs are suitable for further development as a drug delivery carrier.

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Acknowledgements

The authors would like to express the gratitude to Assistant Professor Nuttawee Niamsiri, Department of Biotechnology, Faculty of Science, Mahidol University for technical support. This project was supported by Science Achievement Scholarship of Thailand (SAST), Development and Promotion of Science and Technology Talent Project (09/2557), Thailand Research Fund and the Central Instrument Facility (CIF) grant, Faculty of Science, Mahidol University.