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Supporting information

Synergistic photodynamic therapeutic effect of indole-3-acetic acid using pH sensitive nano-carrier based on poly(aspartic acid-*graft*-imidazole)-poly(ethylene glycol)

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Materials and Methods

Materials

P(Asp-*g*-Im)-PEG (MW 6.5–2 kDa) and poly(lactic acid) (PLA)-PEG (MW 8k-2k kDa) were prepared as previously reported [1-4] . The conjugation rate of imidazole rings to the backbone of poly(aspartic acid)-block-PEG (P(Asp)-PEG) was 60%. B16F10 murine melanoma cells were purchased from the American Type Culture Collection (Rockville, MD, USA). PBS, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, penicillin, and streptomycin were purchased from Welgene, Inc. (Gyeongsan-si, Gyeongsangbuk-do, Korea). A Cell Counting Kit-8 (CCK-8) was obtained from Dojindo Molecular Technologies, Inc. (Rockville, MD, USA).

Methods

Hydrodynamic size change of ILM

The effective hydrodynamic diameter (D_{eff}.) of the ILM solution were measured by photon correlation spectroscopy using a Zetasizer Nano-ZS (Malvern Instruments, UK) equipped with the Multi Angle Sizing Option (BI-MAS). Software provided by the manufacturer was used to calculate D_{eff}. values. The averaged D_{eff}. values were calculated from three measurements performed on each sample (n = 3). ILM in different solutions (0.01 wt%) were prepared by diluting the stock preparations 10-fold with different solutions including PBS, DMEM, and DMEM with 10% FBS. ILM in different solutions were incubated for more than 3h before particle sizes were measured by using dynamic light scattering (DLS).

Toxicity of P(Asp-g-Im)-PEG

B16F10 cells were maintained in DMEM supplemented with 10% fetal bovine serum in a

humidified incubator at 37 °C and 5 % CO2 atmosphere. B16F10 cells (1.25 × 10^6 cells/ml) harvested from growing cell monolayers were seeded in two 96-well plates in 25 ml of DMEM at 24 hours prior to the cytotoxicity test. P(Asp-*g*-Im)-PEG in serum-free DMEM were prepared immediately before use. After 24 hours of serum starvation, samples were added to the medium-removed 96-well plate with different polymer concentrations (10 ~ 10^6 ng/ml) and incubated for 24 hours.

Chemosensitivity was assessed by the CCK assay. Fresh medium containing CCK solution (10 vol%) was added to each well. The plate was incubated for an additional three hours. The absorbance of each well was then read on a Flexstation 3 microplate reader (Molecular Devices, Sunnyvale, CA, USA) using a wavelength of 450 nm. The viability of cells treated with polymers was compared non-treated cells in the same medium.

Results

Hydrodynamic size change of ILM

The hydrodynamic size change of micelle in different solutions were checked by DLS (**Fig. S1**). ILMs showed 142 nm and 184 nm particle size with narrow size distribution in PBS and fresh DMEM respectively. ILM in DMEM including 10% FBS displayed bimodal size distribution with high polydispersity of 0.4 values, due to the diverse components including albumins which were similar to that of DMEM including 10% FBS without ILM.. It has been reported that bovine serum albumin showed a mean hydrodynamic size with approximately 10 nm [5, 6]. Therefore, it is not applicable to measure the hydrodynamic size of in solutions including FBS using DLS.

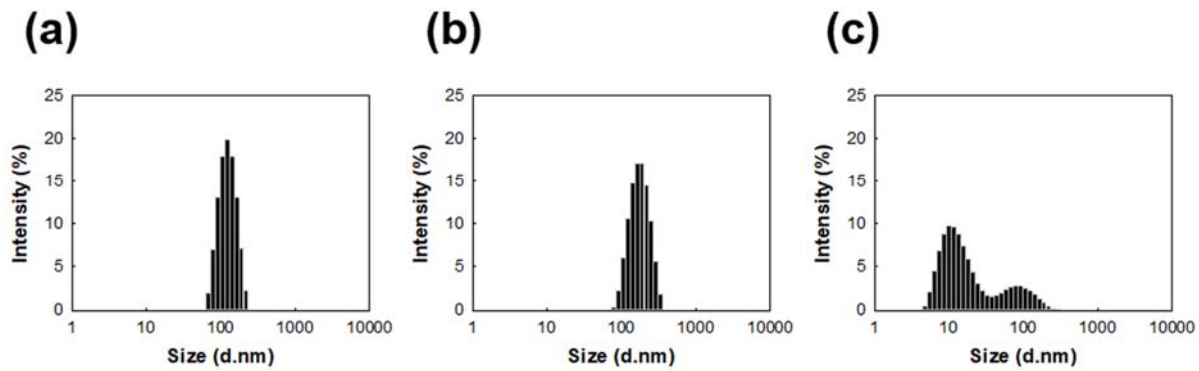

Fig. S1. The hydrodynamic size change of ILM in **(a)** PBS, **(b)** DMEM, and **(c)** DMEM including 10% FBS

Toxicity of P(Asp-g-Im)-PEG

Polymer toxicity was check using CCK assay in the same way of *in vitro* viability study. As shown on **Fig. S2.**, P(Asp-*g*-Im)-PEG showed no toxicity in the range of polymer concentration (10ng/ml – 10^6ng/ml). There was no toxicity when the maximum concentration of IAA used in this study was treated (Maximum IAA conc. = 500 μg/ml). It is considered that P(Asp-*g*-Im)-PEG has less potential to increase the toxicity of ILM *in vitro and in vivo.*

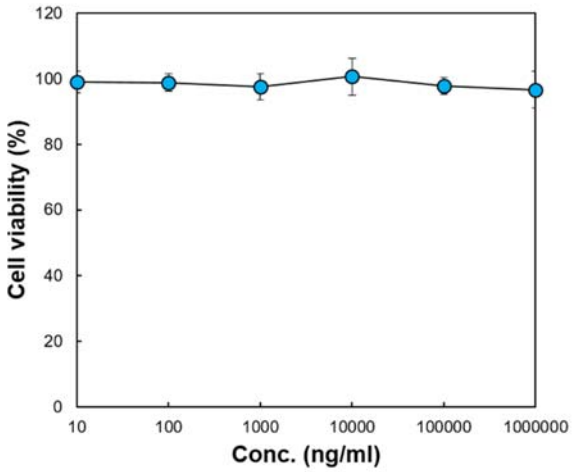

Fig. S2. Polymer toxicity on B16F10 melanoma cells using P(Asp-*g*-Im)-PEG

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