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# Surface modification of epirubicin-loaded PLGA nanoparticle with biotinylated chitosan enhances anti-cancer efficacy in breast cancer cells

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Nanoparticle (NP) delivery systems in cancer therapies provide better penetration of therapeutic and diagnostic substances within the body at a reduced risk in comparison with conventional cancer therapies. This is to continue our earlier work on the poly(D,L-lactide-co-glycolide) (PLGA) NPs with biotinylated chitosan (Bio-CS) modification, the modified PLGA NPs were further used as an epirubicin (EPB) carrier to enhance cancer targeting activity and to overcome multidrug resistance (MDR) in adriamycin-resistance human breast cancer cell line (MCF-7/ADM). In this study, Bio-CS were synthesized and characterized. The degree of substitution (DS), as defined as the number of biotin per 100 anhydroglucose units of CS, was determined by $^{1}$H-NMR and ICP. EPB loaded PLGA NPs were prepared by a solvent evaporation technique (W1/O/W2). The PLGA NPs surface was modified with Bio-CS by covalent binding. PLGA NPs of (231.4 ± 21.0) nm in diameter characterized by the laser light scattering technique, scanning electron microscopy are spherical and its drug encapsulation efficiency is (84.1 ± 3.4)%. Zeta potential of unmodified NP was measured to be negative -(21.21 ± 2.13) mV. The positive zeta potential of modified NPs reveals the presence of CS on the surface of the modified NPs. Modified NPs were characterized for surface chemistry by X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared (FT-IR). The result showed that the N region corresponding to the primary amide of CS and the S region corresponding to the biotin, indicating that PLGA NPs were successfully surface-modified with Bio-CS. The 31% of surface DS biotin of Bio-CS modified PLGA NPs were determined and the value was (1.36 ± 0.34) μmol/100mg. *In vitro* drug release studies showed that Bio-CS modified NPs had many advantages, such as prolonged drug release property and decreased burst release in comparison to the unmodified NPs, and the modified NPs achieved relatively constant release kinetics. For cytotoxicity evaluation *in vitro*, the MCF-7/ADM cells were treated with the PLGA NP and Bio-CS modified NP with the EPB, respectively, at same concentration of 150 μg/mL, for 4, 24, 48 and 72 h. We found that Bio-CS modified NPs increased markedly EPB anti-cancer activity compared to the unmodified NPs. Flow cytometry and confocal laser scanning microscopy revealed that bio-CS-modified NPs exhibited greater extent of cellular uptake than unmodified NP and free EPB at 48 h. Moreover, increased levels of uptakes of CS-modified NPs in MCF-7/ADM cells and decreased cell viabilities were compared to the free EPB and PLGA NPs. In summary, the NPs by surface modification Bio-CS with significantly enhanced anti-cancer drug EPB delivery, increased drug efficacy, and thus this system will have great potential for breast cancer chemotherapy.

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