

Efficient *In-Vivo* Tumor Targeting of Chitosan-Conjugated, Pluronic-Based Nano-Carriers

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Statement of Purpose: Various nanoparticle systems with a long blood circulation time and a high tumor accumulation have been reported for cancer diagnosis and treatment based on their EPR (enhanced permeability and retention) effect [1,2]. Here, we prepared several Pluronic-based nano-carriers attached with an NIR dye, and characterized their cellular uptake and *in-vivo* tumor targeting efficiency focused on the effect of chitosan and the structure of Pluronic.

Methods: Using two kinds of Pluronic (F127 and F68), Pluronic-based nano-carriers were made by a photo-crosslinking of a diluted, diacrylated Pluronic aqueous solution, as previously reported by us [3]. For imaging, nano-carriers were chemically labeled with Cy5.5 molecules as a near-infrared (NIR) fluorophore. Physico-chemical properties (size, surface charge, and *in-vitro* stability in serum) of the nano-carriers as well as the thermo-sensitive size variations were measured. *In-vitro* cellular uptake and *in-vivo* tumor accumulation of Pluronic-based nano-carriers were characterized using squamous cell carcinoma (SCC7) cells and SCC7 tumor-bearing mice, respectively.

Results: Under the appropriate photo-polymerization conditions, relatively mono-dispersed nano-carriers (chitosan-conjugate and bare Pluronic-based) were successfully obtained. Surface functionalization of the Pluronic-based nano-carriers did not change their size or thermo-sensitive response, but surface charges of the nano-carriers were changed by chitosan-conjugation. All of these nano-carriers did not show any aggregation in serum-containing media and acute cytotoxicity to both normal (fibroblast) and tumor cells. The relative cellular uptake of chitosan-conjugated nano-carriers was much higher than that of bare nano-carriers (Figure 1). This enhanced cellular uptake was strongly correlated with *in-vivo* tumor accumulation. As shown in Figure 2, the time-dependent excretion profile and tumor accumulation of the nano-carriers were clearly visualized by monitoring real-time NIR fluorescence intensity. In the case of the bare Pluronic-based nano-carriers (NC(F68) and NC(F127)), the fluorescence intensities in tumor region were rapidly decreased within 16 h post-injection. However, the high fluorescence intensities of chitosan-conjugated nano-carriers (Chito-NC(F68) and Chito-NC(F127)) and the tumor site were maintained well up to 72 h. Tissue distribution (liver, lung, kidney, spleen, and heart) and tumor accumulation, analyzed from the *ex-vivo* NIR fluorescence images at 72 h post-injection also showed higher fluorescence intensities at tumor site from chitosan-conjugated nano-carriers compared to bare nano-carriers, implying prolonged blood circulation and more effective tumor accumulation of chitosan-conjugated

nano-carriers. Between F68 and F127, F68-based, chitosan-cojugated nano-carriers remained longer at the tumor site until 72 h, whereas the signal from F127 based ones were decreased in 24 h.

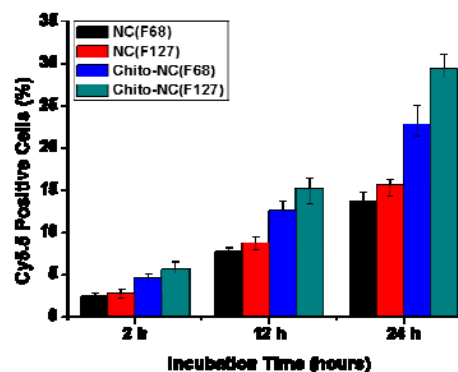


Figure 1. Flow-cytometry analysis of *in-vitro* cellular uptake of Pluronic-based nano-carriers on SCC7 cell line. (NC: Bare form, Chito-NC: Chitosan-conjugated form)

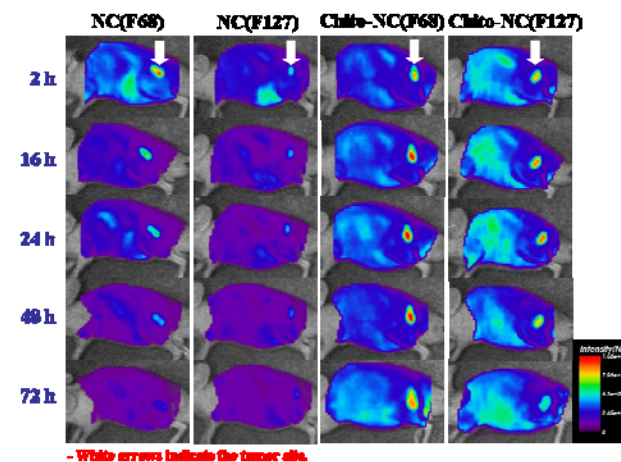


Figure 2. *In-vivo* NIR fluorescence images of real-time tumor accumulation of Pluronic-based nano-carriers.

Conclusions: All kinds of Pluronic-based nano-carriers showed the *in-vitro* serum stability and no acute cytotoxicity. The *in-vitro* cellular uptake and the *in-vivo* tumor accumulation of chitosan-conjugated nano-carriers were very efficient and were significantly enhanced than bare Pluronic-based nano-carriers.

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References:

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