Uptake and Migration of Tumor Cells in Response to Hybrid Polymer-Peptide Self-Assembled Nanoparticles

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Statement of Purpose: One strategy to circumvent the short half-life, limited solubility, and improve uptake of anti-cancer drugs by tumor cells is to encapsulation in colloidal nanoparticles (NPs). Hybrid polymer-peptide NPs provide the opportunity to selectively target the tumor over normal tissue whiel enhancing intracellular uptake of the drug. The objective was to investigate the effect of charged head-group of the peptide cys-val(6)-y(2), where y is arginine or lysine, conjugated to poly(L-lactide) (PLAA-CV6Y2) on uptake and migration of tumor cells when loaded with Doxorubicin (Dox).

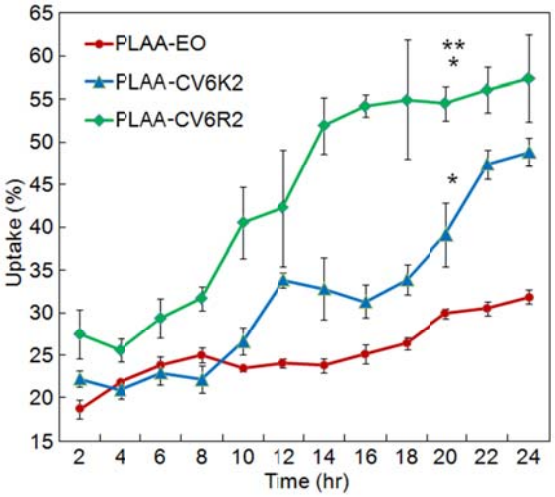

Figure 1: Uptake of the NPs by 4T1 mouse breast tumor cells with time. One star means s.d. (p=0.05) between the test NPs and PLAA-EO NPs. Two stars means s.d. between PLAA-CV6R2 and PLAA-CV6K2 NPs.

Methods: Acrylated poly(L-lactide) (PLAA) macromer was conjugated to Cys-Val(6)-X(2) peptide, where X was lysine (CV6K2) or arginine (CV6R2) as described [1]. The hybrid polymer-peptide macromer was self-assembled into NPs as described [2]. Control PLAA NPs (without charged peptides) stabilized with poly(ethylene oxide) (PLAA-EO) were synthesized as described [2]. Size and distribution of the NPs were measured with light scattering. For cell uptake experiment 4T1 (mouse breast tumor) cells were seeded at a density of 5×10^4 cells/well in 96-well plates. Cells were incubated with NPs encapsulating FITC (2 mg/mL, 2% loading) for 24 hours, with sampling time points every two hours. At every time point the supernatant and cells of the corresponding groups were collected and analyzed by a fluorescent plate reader. For invasion and migrations studies, 4T1 cells at a density of 1.5×10^4 cells/well in 24-well Transwell plates were exposed to empty and Dox-loaded NPs (5 μM Dox for loaded particles, 5% loading). Free Dox was used as a positive control. The cells were allowed to migrate through the transwell membrane in the absence (migration) or presence (invasion, 5% wt gel in media) of Matrigel in the upper chamber. After 24 h, the membranes were fixed and stained with eosin-Y and azure dyes. The membranes were mounted on microscope slides to image and count the migrated cells with a light microscope.

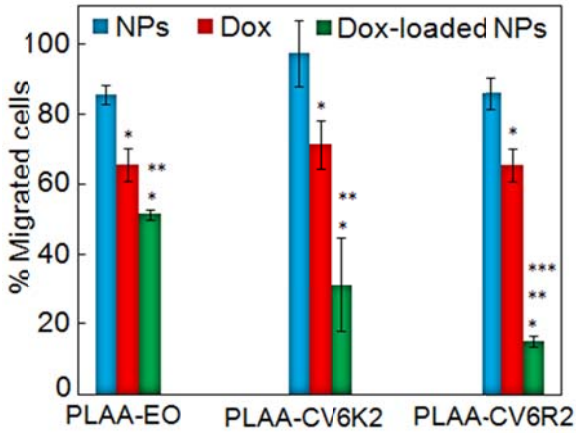

Figure 2: Migration of 4T1 tumor cells incubated with Dox-loaded NPs. One star means s.d. (p=0.05) between the test group and NPs without Dox (blue); Two stars is between Dox and Dox-loaded NPs; three stars is between Dox-loaded CV6R2 and PLAA-EO.

Results: NPs had a narrow size distribution between 50-150 nm. Release kinetics show Dox could be delivered in a sustained way for 25-35 days. Figure 1 shows the effect of head group (lysine versus arginine) on the uptake of FITC-loaded NPs with time. After 24 hrs, 60% and 50% of the PLAA-CV6R2 and PLAA-CV6K2 NPs were taken up by 4T1 cells, respectively, but only 30% for PLAA-EO NPs. Migration experiments indicated that the Dox-loaded PLAA-CV6R2 were more effective in retarding the migration of 4T1 cells than PLAA-CV6K2, PLAA-EO, or the free Dox (see Figure 2).

Conclusions: Results demonstrate that the arginine head group is more effective in tumor uptake of the NPs and in retarding the migration of tumor cells than lysine. Polymer-peptide hybrid NPs could be used for the delivery of antitumor agents.

References

1. He, X.; Jabbari, E. Prot. Pept. Lett. 2006, **13**:515.
2. Mercado, A.E. et al. J. Contr. Rel. 2009, **140**:148.

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