Osteotropic therapy via Layer-by-Layer Nanoparticles

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Statement of Purpose: Current treatment options for debilitating bone diseases such as osteosarcoma, osteoporosis, and bone metastatic cancer are suboptimal and have low efficacy. New treatment options for these pathologies require targeted therapy that maximizes exposure to the diseased tissue and minimizes off-target side effects. This work investigates an approach for generating functional and targeted drug carriers specifically for treating primary osteosarcoma, a disease in which recurrence is common and the cure rate has remained around 20%. This approach utilizes the modularity of Layer-by-Layer (LbL) assembly to generate tissue-specific drug carriers for systemic administration. This is accomplished via surface modification of drug-loaded nanoparticles with an aqueous polyelectrolyte, poly(acrylic acid) (PAA), side-chain functionalized with alendronate, a potent clinically used bisphosphonate. This work represents a tunable approach towards the synthesis of drug carriers, in which LbL enables surface modification of nanoparticles for tissue-specific targeting and treatment

Methods: Liposomes were formulated via a thin-film hydration method with a compositional mass ratio of 56:39:5 (DSPC:Cholesterol:POPG, DSPC and POPG purchased from Avanti Polar Lipids, Cholesterol from Sigma). Loading of doxorubicin was performed via a pH gradient method using a citric acid buffer. Liposomes were diluted in 1 mL DI water (from a 50 mg (total lipid) batch prepared in a final suspension of 5 mL DI water, used ≈200 µL stock to 800 µL DI water) and injected in excess polyelectrolyte under agitation at 4°C. Particles were incubated for ≈30 min, purified via ultracentrifugation at 10 000 RPM for ≈10 min, followed by aspiration of supernatant and subsequent re-suspension in DI water. Purification was repeated twice prior to introduction to the subsequent polyelectrolyte. The final, functionalized liposomal system was filtered through a 0.45-µm filter and suspended in 1X PBS for further experimentation. 143B osteosarcoma cells were used for all *in vitro* and *in vivo* experimentation.

Results: Nanoparticles coated with PAA-alendronate are observed to bind and internalize rapidly in human osteosarcoma 143B cells. Encapsulation of doxorubicin, a front-line chemotherapeutic, in an LbL-targeted liposome demonstrates potent toxicity *in vitro*. Active targeting of 143B xenografts in NCR nude mice with the LbL-targeted doxorubicin liposomes promotes enhanced, prolonged tumor accumulation and significantly improved efficacy, as shown in **Figure 1**.

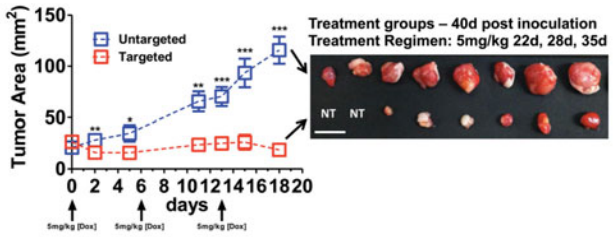

Figure 1. Caliper measurements for in vivo tumor remediation with repeated dosing of 5 mg kg−1 for both targeted and untargeted formulations at 22 days, 28 days, and 35 days post- inoculation (displayed as day 0, day 6, and day 13 in (C)). Statistics are from an unpaired t-test, two-tailed to compare the untargeted and targeted formulations; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. Data presented as mean ± SEM; $n = 4$. Resection of tumors from terminal point of (C), $n = 4$ for each group, displayed from the final caliper measurement.

Conclusions: LbL is a versatile platform to functionalize nanoparticles in ways that promote improved biological performance. Prior art has established LbL as a means to impart protein-resistive, long-circulating properties to nanoparticle systems, with a means to control biodistribution of both the carrier and drug in a complex systemic environment. This investigation further demonstrates the modularity of this approach to impart tissue-targeting capabilities to the nanoparticle for improved disease treatment outcomes. We capture this potential with the synthesis of osteotropic nanoparticles via incorporation of alendronate-functionalized PAA in a surface functional coating on doxorubicin-loaded liposomes. Different nanoparticle core substrates and therapeutics may be used for imaging and treatment of a variety of bone diseases. These functional nanoparticles are also highly promising for future investigations towards treatment of bone-localized metastases of invasive cancer cell types such as breast and lung cancer. The potential to further generalize this approach towards the built-to-order manufacture of a library of targeted delivery systems continues to provide much promise for LbL nanoparticles.

References: S.W. Morton et al. *Advanced Healthcare Materials*, early view: published online Oct. 9, 2013; doi: 10.1002/adhm.201300465.