Differential rates of oxanorbornadiene-mediated drug tethering and release from thiolated nanoparticles

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Statement of Purpose: Currently approved clinical nanotherapeutics (20-100 nm) facilitate the intratumoral accumulation of small molecule chemotherapeutics by leveraging increases in drug circulation time and tumor vascular permeability afforded by the nanocarrier delivery vehicle.[1] However, these delivery properties have conferred only modest patient benefit. Suboptimal delivery due to inadequate penetration of the nanocarriers deeply into poorly perfused regions of rapidly growing tumors continues to stymie drug therapeutic efficacy.[2] While small-molecule drugs are optimal for deep and rapid penetration of poorly perfused regions due to their high diffusivities, they demonstrate short circulation times and are poorly targetable relative to nanocarrier delivery vehicles.[3] To enhance drug therapeutic delivery deeply to the tumor interstitium, we have developed a two-stage system that combines 30 nm thiol-containing poly(propylene sulfide) nanoparticles (SH-NP) with orthogonally-reactive oxanorbornadiene (OND) linkage chemistry that degrades in a pH- and polarity-insensitive manner. Notably, linker half-lives are programmable, ranging from minutes to weeks to release small-molecule cargo. [4] By merging these two platforms, we have created a system that functions independently of the tumor microenvironment, giving unprecedented control over the rate and profile of drug release.

Methods: SH-NP were synthesized as previously reported [5]. Free thiols remaining on the SH-NP after synthesis were reacted with a variety of OND electrophiles with modified functional groups that extend the retro-Diels-Alder fragmentation time from minutes to hours. Rate of addition of the OND electrophiles to the SH-NP core thiols was measured by the presence of fluorescence that occurs upon formation of the OND-thiol adduct – adduct formation terminates quenching of the fluorescent dye by the electrophilic OND ring. To measure the degradation time of the OND-SH-NP adduct, dialysis was performed at 37°C using a 20 kDa MWCO membrane that retains the OND-SH-NP adduct, but allows for the OND-fluorescent fragment to be removed. Samples were taken at short intervals from the retained solution, and measured for fluorescence.

Results: Two OND variants with different functional groups (Figure 1A) were used to probe formation of OND-SH-NP adducts. The more stable proton OND (OND1) demonstrated rapid addition to the SH-NP thiols at 25°C with a second order rate constant of 9.6 $M^{-1}s^{-1}$, whereas the less stable bridgehead methyl OND (OND2) demonstrated a slightly slower rate of addition of 8.2 $M^{-1}s^{-1}$. Despite the similar rate of addition, OND1 exhibited a more complete reaction with SH-NP than OND2, as seen by the greater fluorescence (Figure 1B). When either OND was added to nanoparticles with thiols capped by N-ethyl maleimide, no fluorescence was measured (Figure 1B), indicating that OND only react with core thiols present in SH-NP. To probe release rates of the two OND-SH-NP adducts at physiological temperature, OND (1 and 2) was pre-reacted to completion with SH-NP and then dialyzed at 37°C. OND-SH-NP adducts were also left undialyzed at 25°C and 37°C to control for the small expected reduction in fluorescence caused by temperature-induced fragmentation of the fluorophore out of the hydrophobic core of the SH-NP. The results indicated that the more

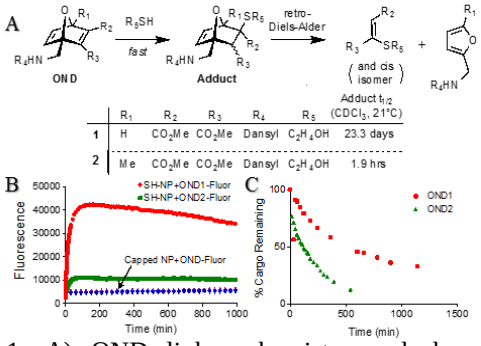

Figure 1: A) OND linker chemistry and degradation mechanism. B) Model cargo (fluorophore) loading into thiolated nanoparticles (SH-NP) via OND (1 and 2) linkers. C) Model cargo release from SH-NP tethered via OND linkers with different release rates.

stable OND1, which had a faster rate of addition, had a longer half-life on the order of 13 hours compared to the less stable, slower-adding OND2, which had a half-life of only 3 hours (Figure 1C).

Conclusion: We have demonstrated that SH-NP form covalent reversible linkages with OND electrophiles to load high densities of cargo within SH-NP. Two OND linkages have been used with fragmentation rate constants that differ by an order of magnitude at physiological temperature. Preliminary work confirms that the release rate from the NP is dramatically different between the two linkers. Future studies will elaborate on the utility of these linkages to enhance tissue penetration and efficacy of SH-NP delivered drug.

References:

[1] Peer, D. et al. Nat. Nanotechnol. **2**, 751-760 (2007).
[2] Yuan, F. et al. Cancer Res. **54**, 3352-3356 (1994).
[3] Maeda, H. et al. J. Controlled Release **65**, 271-284 (2000).
[4] Kislukhin, A.A. et al. J.A.C.S. **134**, 6491-6497 (2012).
[5] Rehor, A. et al. Langmuir **21**, 411-417 (2005).