Development of Viral Nanoparticle Networks and Hybrids for Enhanced Tumor Homing and Penetration

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Statement of Purpose: Incorporating chemotherapeutics in nanoparticles can reduce adverse side effects as well as improve drug solubility, pharmacokinetics, and overall therapeutic efficacy. However, the optimal parameters for a nanoparticle delivery system have not yet been fully characterized, and drug delivery is hindered by the heterogeneous tumor vasculature and dense interstitial matrix of the tumor microenvironment. Traditional approaches have focused on the development of spherical nanoparticles, but the uptake of particles into the tumor microenvironment relies on their ability to drift laterally toward the blood vessel and extravasate from the leaky regions of the tumor vasculature. It has been observed that filamentous rods have favorable margination properties compared to spheres [1]. In addition, previous studies indicate that larger particles (60 to 100 nm) are more likely to accumulate in the tumor, while smaller particles (<40 nm) can penetrate more deeply [2]. We thus turned towards the development of nanoparticle networks and hybrids as multistage delivery systems to enable greater and more homogenous delivery throughout the tumor. The model systems chosen for this application are plant viral nanoparticles (VNPs), which have great promise in biomedical applications as they are biocompatible, biodegradable, and noninfectious in mammals, while also being highly economical to produce and amenable to both chemical and genetic engineering. In particular, we focused on cowpea mosaic virus (CPMV), a 30 nm icosahedron, and potato virus X (PVX), a 515 x 13 nm filamentous rod. CPMV and PVX have 300 and 1270 addressable lysines on their exterior, respectively. By linking CPMV particles together and CPMV to PVX particles, we have engineered new platforms for enhanced solid tumor targeting. Modification of these formulations to release the CPMV could result in better tumor penetration and consequently greater therapeutic efficacy.

Methods: *N*-hydroxysuccinimide (NHS) ester homobifunctional linkers containing disulfide bonds were used to bind CPMV to an amine-functionalized solid-phase support. Symmetry-broken particles with thiol groups on one side were then produced after reduction of the disulfide bonds (Fig. 1A). The thiols were functionalized with biotin, then introduction of avidin and immunogold staining in conjunction with TEM were used to verify symmetry breaking. The symmetry-broken particles were linked together to form dimers using homobifunctional maleimide linkers with a 2000 Da PEG spacer (Fig. 1B). Hybrid PVX-CPMV particles were also formed by first binding avidin to the biotinylated CPMV formulation used for immunogold staining, then binding CPMV-avidin to PVX particles functionalized with NHS-biotin (Fig. 1C). The formation of dimers and hybrids was verified by TEM.

Results: Symmetry-broken CPMV particles were successfully synthesized. The biotinylated particles did not form large aggregates with the introduction of avidin, unlike wild-type particles functionalized with biotin. In addition, immunogold staining using gold-labeled anti-biotin revealed binding on only one side of the particles, compared to on all sides for the control. Successful formation of coupled CPMVs and PVX-CPMV hybrids were found by TEM analysis (Fig. 1D). We are currently optimizing the conditions for the reactions to improve yields of dimers and CPMV functionalization of PVX. Further studies will be performed to determine whether these particles result in enhanced tumor accumulation.

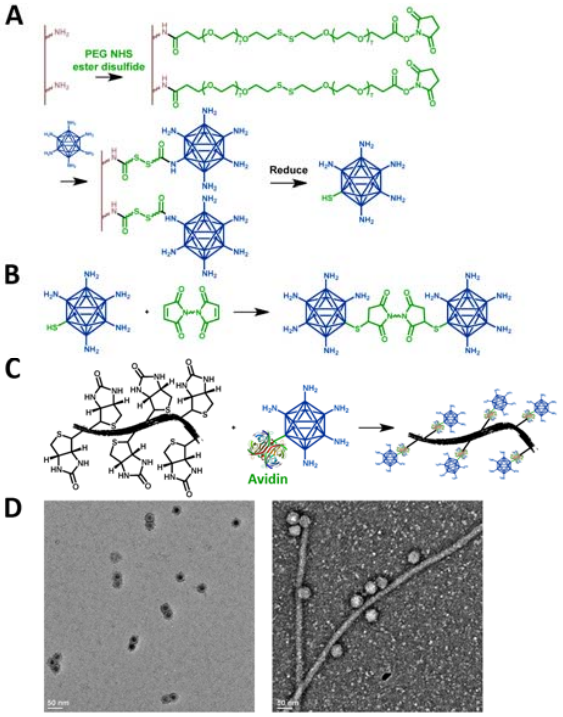

Figure 1. A) Scheme for symmetry breaking of CPMV. B) Reaction to form coupled CPMV particles. C) Avidin-biotin interaction to form PVX-CPMV hybrids. Avidin structure is adapted from [3]. D) TEM images of CPMV dimers and PVX-CPMV hybrids. Scale bar is 50 nm.

Conclusions: Symmetry-broken, linked CPMV, and PVX-CPMV hybrid particles have been synthesized and verified by TEM. We are currently improving the coupling reactions and introducing cleavable peptide linkers. In the future, we will evaluate the formulations for tumor targeting and penetration of released particles. These multistage delivery systems have the potential to enhance tumor-specific accumulation and improve efficacy of cancer therapies.

References: [1] Gentile F, et al. J. Biomech. 2008 41(10):2312-18 [2] Perrault SD, et al. Nano Lett. 2009 9(5):1909-15 [3] Repo S, et al. Chem. Biol. 2006 13(10):1029-39

Acknowledgements: This work was funded by grants from NIH/NIBIB: R00 EB009105 (to NFS), P30 EB011317 (to NFS), and T32 EB007509 (to AMW).