Research Highlights

Highlights from the latest articles in nanomedicine

Biomimetic toxin-absorbing nanosponge

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Evaluation of: Hu CM, Fang RH, Copp J *et al.* A biomimetic nanosponge that absorbs pore-forming toxins. *Nat. Nanotechnol.* 8(5), 336–340 (2013).

Pore-forming toxins (PFTs) are the most common protein toxins found in nature and act by disrupting cells through the formation of pores in the cellular membrane. While PFTs have been identified as one of the major virulence mechanisms underlying toxins, such as bacterial infections, venomous injuries and biological weaponry, existing detoxification platforms, such as antisera, monoclonal antibodies, small-molecule inhibitors and molecularly imprinted polymers, can only act by specifically targeting the molecular structure of the toxin, and, therefore, must be customized for each application. In their article, Hu and coworkers describe nanosponges (85 nm in diameter) that consist of red blood cell (RBC) bilayer membrane vesicles fused onto poly(lactic-*co*-glycolic acid) nanoparticles. In this way, the RBC membrane shell provides a biomimetic substrate to absorb PFTs regardless of their structure, while poly(lactic-*co*-glycolic acid) stabilizes the RBC membrane, prolonging circulation.

As a proof of concept, nanosponges were mixed with staphylococcal α-hemolysin (α-toxin). Purified mouse RBCs were then added and hemolysis was quantified by measuring the absorbance of hemoglobin released into the supernatant. Compared with poly(lactic-*co*-glycolic acid) nanoparticles, liposomes and RBC membrane vesicle controls, the nanosponges were able to completely protect mouse RBCs from damage and SDS-PAGE analysis indicated that the nanosponges retained 90% of the toxin. While RBC membrane vesicle controls similarly retained 95% of the toxin, they were unable to protect mouse RBCs, probably because the vesicles fuse with the RBCs. Experiments were also repeated using streptolysin-O and melittin to confirm the platform's applicability to other PFTs. Next, to determine whether the nanosponges could detoxify α-toxin in the presence of cells, cellular toxicity was studied in human umbilical vein endothelial cells. The authors found that α-toxin toxicity was significantly reduced, both when premixed with nanosponges and when concurrently mixed with the nanosponges and human umbilical vein endothelial cells. This was also true for the other PFTs.

Finally, *in vivo*, the authors demonstrated that the nanosponges were well tolerated by mice. The nanosponges were observed to neutralize α-toxin when an α-toxin/nanosponge mixture was subcutaneously injected into the flank of mice. More importantly, the authors reported that these nanosponges could efficiently detoxify systemic α-toxin. It is well established that α-toxin is extremely toxic in the circulation, resulting in coagulation, inflammation and endothelial dysfunction. However, even when a lethal dose of α-toxin was injected through the tail vein, administration of nanosponges was able to reduce the mortality rate to 11% versus the 100% mortality seen in α-toxin-treated control mice.

Overall, Hu and coworkers successfully demonstrated the utility of a biocompatible and biodegradable detoxification platform that can act against a broad range of PFTs. Specifically, by targeting membrane perforation, which is one of the most common virulence mechanisms, the nanosponge platform distinguishes itself from the current paradigm in detoxification treatments, where toxin antagonists rely on structure-specific binding. It remains unclear what the exact fate of the nanosponge-sequestered toxins is; however, the reported nanosponges have tremendous clinical/therapeutic implications.

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Gold nanorods for photothermal therapy

Evaluation of: Tsai MF, Chang SH, Cheng FY *et al.* Au nanorod design as light-absorber in the first and second biological near-infrared windows for *in vivo* photothermal therapy. *ACS Nano* doi:10.1021/nn401187c (2013) (Epub ahead of print).

Photothermal cancer therapy using near-infrared (NIR) laser radiation has recently gained increasing attention as it has a number of advantages over the conventional surgical treatment of solid tumors, including ease of application and a lower degree of invasiveness. To this end, a number of nanomaterials have been investigated, with particular interest in materials that absorb in the second NIR region (1000–1350 nm) as opposed to the first NIR region (650–950 nm), owing to increased tissue penetration efficiency. However, there have been no reported materials smaller than 100 nm that are responsive to this second NIR region.

To this end, Tsai and coworkers report on a gold (Au) nanorod (NR) with a rod-in-shell (rattle-like) structure whose absorbance can be tuned (to 1100 and 1280 nm) for the photothermal therapy of lung cancer. To create the photothermal structure, Au NRs with an aspect ratio of 4 were synthesized. A silver (Ag) nanoshell was then formed to produce a rod-in-shell structure. Finally, the Au NR with Ag shell was converted to the final Au rod-in-shell (rattle-like) structure with an Au/Ag nanoshell (length: 53 nm, width: 26 nm and thickness: 4 nm), with a 2-nm gap between the Au NR and the Au/Ag nanoshell. In terms of their optical behavior, these rod-in-shells exhibited two distinct bands at 1100 and 1280 nm, and it was found that a direct relationship existed between the gap length and intensity of the absorption bands.

To evaluate toxicity, rod-in-shell structures were PEGylated and then incubated with large cell carcinoma/lewis lung cancer cells for 24 h. Measurements of cell viability indicated that the rod in shell (without PEGylation) was toxic at higher doses, while PEGylated nanoparticles displayed no toxicity, even at doses as high as 200 ppm. The rod-in-shell and PEGylated rod-in-shell particles were then injected into healthy mice to observe their biodistribution. Unmodified rod-in-shell particles were predominantly taken up by the kidney, while PEGylation resulted in stable circulation and accumulation in the heart, liver and kidney.

Finally, for the photothermal studies, aqueous solutions of rod-in-shell particles were exposed to a 1064-nm laser (power density: 2–3 W/cm^2). The irradiation induced a temperature elevation in H_2O and it was found that a smaller gap length could result in a faster temperature elevation. *In vitro*, rod-in-shell particles were delivered to large cell carcinoma/lewis lung cancer cells, exposed to 1064 nm at 3 W/cm^2 for 5 min, and significant cell death was observed. Extending this *in vivo*, a xenograft mouse tumor model was established through the subdermal injection of LLC/LL2 lung cancer cells. Importantly, photothermal therapy using the rod-in-shell particles showed effective suppression of tumor growth with only one round of irradiation needed compared with laser-only controls.

In summary, the authors present the first demonstration of photothermal therapy of cancer cells using the second NIR window. However, their research could benefit from increasing the time frame used in cell and *in vivo* studies. Rod-in-shell particles show great promise as a less invasive and more powerful method to ablate tumors in the clinic.

Multifunctional membrane–core nanoparticles for plasmid delivery

Evaluation of: Hu Y, Haynes MT, Wang Y *et al.* A highly efficient synthetic vector: nonhydrodynamic delivery of DNA to hepatocyte nuclei *in vivo*. *ACS Nano* doi:10.1021/nn4012384 (2013) (Epub ahead of print).

Delivering DNA to cells is a complex process that requires efficient DNA loading, stability, targeting, uptake, endosomal escape, nuclear import and transcriptional activation. Significant effort has been invested in developing a variety of polymeric, liposomal, protein, organic and inorganic vectors. However, viral vectors remain the most efficient vehicle to deliver DNA into the nucleus. To this end, Hu and coworkers developed a multifunctional membrane–core nanoparticle (liposome calcium phosphate [LCP]) consisting of calcium phosphate cores, cysteine-flanked octaarginine peptides (CR8C), cationic PEGylated lipid membranes and galactose-targeting ligands (final diameter: 40–60 nm). Specifically, the arginine-rich peptides were chosen for their biodegradability, biocompatibility and efficacious transfection of nucleic acids, as well as for their ability to mimic the nuclear localization signal of the HIV-1 Tat protein. On the other hand, the acid-sensitive calcium phosphate cores in combination with the cationic lipid membrane function synergistically to lyse endosomes, thereby

releasing DNA into the cytoplasm, while PEG provides stability and prolongs circulation. Finally, galactose targets constructs to hepatocytes. These liver cells are particularly desirable candidates for gene therapy owing to their systemic accessibility *in vivo* and their highly endocytic nature.

After initial characterization, the LCPs were radiolabeled and injected intravenously into C57BL/6 female mice to determine biodistribution and cellular uptake. Radiolabeled LCPs, targeted using galactose, distributed rapidly and primarily to the liver with 48% of the injected dose recovered from the liver after 6 h, while the recovery of nontargeted LCPs was only 16%. Next, to examine intracellular distribution, the LCPs with and without CR8C were used to deliver Cy3-labeled DNA through intravenous injection. By harvesting livers 6 h after administration, it was found that LCPs lacking CR8C were efficiently taken up, but remained in the cytoplasm. On the other hand, co-encapsulation with CR8C resulted in significant Cy3-DNA distribution within the nuclei.

A plasmid encoding firefly luciferase was then used to quantify and evaluate gene expression following LCP delivery. Expression was evaluated 24 h postinjection and analysis of major organs confirmed predominant hepatic transgene expression. Compared with hydrodynamic (HD) injection of luciferase – the most powerful nonviral delivery method – LCP resulted in 100-fold lower gene expression (10^7 RLU/mg protein for LCP compared with 10^9 RLU/mg for HD). However, LCP represents a significantly less invasive method than HD. Moreover, LCP performed similarly or better than synthetic vectors such as poly(amino-*co*-ester) (10^5 RLU/mg protein), galactose-conjugated dendrimer (10^5 RLU/mg protein), a linear cationic polymer (10^6 RLU/mg protein) and polyethyleneimine (10^7 RLU/mg protein). Finally, upon investigation of systemic toxicity and cytokine induction, it was found that there were no obvious histological differences caused by LCP. However, a notable increase in TNFR was observed, requiring additional analysis.

In conclusion, while each component has already been reported, the current formulation of membrane–core nanoparticles represents a significantly less invasive alternative to HD injection and is the most effective synthetic vector for liver gene transfer to date. As such, further study of similar platforms may lead to improvements in DNA delivery that rival or surpass that of viral vehicles.