Highlights from the latest articles in nanomedicine

A breakthrough in ultrasensitive exosomal miRNA detection

Evaluation of: Wu Y, Kwak K, Agarwal K *et al.* Detection of extracellular RNAs in cancer and viral infection via tethered cationic lipoplex nanoparticles containing molecular beacons. *Anal. Chem.* 23, 11265–11274 (2013).

Earlier detection of cancer is crucial for improving patient outcomes. Treatments are more successful and less painful if the disease can be caught in the early stage. miRNAs are now known to play numerous crucial roles in cancer pathogenesis. Some miRNAs are also attractive cancer biomarkers since they circulate in the blood, entrapped in cell-derived extracellular nanovesicles known as exosomes. The current standard method for detecting nucleic acids from serum makes use of quantitative reverse-transcriptase PCR. This process, while well-established, requires skilled operators and is relatively laborious, expensive and time-consuming, precluding widespread point-of-care diagnostics. Thus, simpler and more sensitive detection schemes for exosomal miRNA are desirable.

Wu and colleagues at Ohio State University (USA) have come up with a novel detection scheme for miRNAs in circulating exosomes. They entrapped molecular beacons specific to miR-21 (an miRNA implicated in lung cancer) into cationic lipid nanoparticles. Molecular beacons are nucleic acid probes that maintain a hairpin conformation that attenuates their fluorescence until they hybridize specifically to their target sequence, in which case they open and increase their fluorescence. The negatively charged exosomes carrying miR-21 fused with the cationic lipid nanoparticles. Subsequently, the molecular beacons mixed with the internal contents of the exosomes and if the exosomes contained miR-21, the

molecular beacons would light up within the fused compartment. This process was visualized on surfaces using total internal reflection microscopy. Since not all exosomes originate from cancer-related cells and contain miR-21, the beauty of the technique relies on the direct visualization of single exosome fusion events. The molecular beacon approach performed exquisitely for cancer detection based on analysis of human serum samples from either lung cancer patients or healthy volunteers. This experimental approach was orders of magnitude more sensitive than conventional quantitative reverse-transcriptase PCR. This highly promising approach, which also validated detection of viral RNAs, warrants further experimentation with larger sample sizes.

Using DNA as a nanoscaffold for targeted drug delivery

Evaluation of: Wu C, Han D, Chen T *et al.* Building a multifunctional aptamer-based DNA nanoassembly for targeted cancer therapy. *J. Am. Chem. Soc.* 135(49), 18644–18650 (2013).

1D-, 2D- and 3D-programmed nucleic acid structures have garnered much attention for their striking shapes and intricate patterns, offering infinite creative design possibilities. These structures have also attracted interest in the field of cancer therapy, since shape and size are parameters known to be highly influential for the delivery of drugs into tumors and cancer cells. Because of difficulties in precisely modifying 3D nucleic acid nanostructures with functional ligands, most approaches have made use of passive targeting based on the enhanced permeability and retention effect of tumors, but this is not suitable for all types of cancers, such as leukemia.

Wu *et al.* developed a multifunctional and programmable aptamer-based DNA

Shuai Shao¹
& Jonathan F Lovell^{*1}

¹Department of Biomedical Engineering
& Department of Chemical & Biological
Engineering, University at Buffalo,
State University of New York, Buffalo,
NY 14260, USA

*Author for correspondence:
jflovell@buffalo.edu

nanoassembly for drug delivery. Using a modular bottom-up construction scheme, functional DNA domains and connector DNA domains were self-assembled to form a building unit. Then, the nanoparticles were cemented in place with the photocross-linking of hundreds of these units in order to create a multifunctional nanoassembly. The size of the DNA nanoassembly could be controlled by changing the concentration of the building units prior to photopolymerization and also displayed good biostability without intrinsic cytotoxicity. The anticancer drug doxorubicin, which naturally intercalates into double-stranded DNA, could readily be loaded into the nanoparticle. The nanoassembly could be targeted and internalized by cancer cells with the decoration of targeting aptamer nucleic acids that recognized surface markers on the target cells. This work demonstrates the potential of nucleic acid-based photopolymerizable nanoassemblies as a drug delivery platform. Demonstration of *in vivo* efficacy will be required for this nascent technology to further show its potential.

Mopping up iron with long-circulating functionalized hyperbranched polyglycerols

Evaluation of: Imran ul-haq M, Hamilton JL, Lai BF *et al.* Design of long circulating non-toxic dendritic polymers for the removal of iron *in vivo*. *ACS Nano* 7(12), 10704–10716 (2013).

Most small water-soluble molecules exhibit rapid systemic clearance *in vivo* via renal filtration. A prolonged circulation time of small molecules via conjugation or incorporation into higher molecular weight complexes has been extensively demonstrated. However, depending on the intended purpose, the conjugated complex may not retain its original function and could even exhibit toxicity. Due to their higher molecular weight and organic nature, conjugation to biocompatible polymers is an attractive option. Recent work from Imran ul-haq *et al.* has demonstrated such an approach with the eventual goal of treating side-effects of patients requiring chronic red blood cell transfusions. This process often leads to a transfusional iron overload, which damages patient organs if the metal remains in the circulation for too long.

A nontoxic dendritic polymer was designed using hyperbranched polyglycerol as a backbone. Desferoxamine, a clinically used iron chelator, was used either conjugated to the polyglycerol or in free form. The polyglycerol–desferoxamine avoided rapid blood clearance based on its larger size and exhibited an astounding 484-fold increase in circulating half-life compared with standard desferoxamine. The polyglycerol–desferoxamine nanoparticle could bind iron effectively, exhibited excellent blood compatibility and demonstrated efficacy in preclinical animal experiments. Beyond its significance for transfusion patients, this work demonstrates the dra-

matic size-mediated effects that can occur *in vivo* when small molecules are conjugated to larger nanoscaffolds.

Probing nanoparticle self-assembly *in vivo* using Förster resonance energy transfer

Evaluation of: Zhao Y, van Rooy I, Hak S *et al.* Near-infrared fluorescence energy transfer imaging of nanoparticle accumulation and dissociation kinetics in tumor-bearing mice. *ACS Nano* 7(11), 10362–10370 (2013).

Self-assembled nanoparticles often exhibit good biocompatibility, but exactly what happens to their self-assembly status after they are injected into the body is difficult to elucidate. Better understanding of the behavior of nanoparticles could lead to more rationally-designed drug nanocarriers. Recently, Zhao *et al.* have developed a quantum dot (QD) lipid nanoparticle system that enables improved understanding of nanoparticle behavior *in vivo*. Because of their brightness and optical stability, quantum dots are useful agents for tracking other nanoparticles in biological systems. Self-assembled lipidic nanoparticles with entrapped quantum dots and PEGylation were developed. These exhibited good biocompatibility and could serve as nanocarriers for various therapeutic agents.

Self-assembly was probed *in vivo* using Förster resonance energy transfer (FRET), which is an optical technique that indicates when molecules are within a few nanometers of each other. FRET imaging techniques were developed to observe nanoparticle accumulation and dissociation kinetics in tumor-bearing mice. QDs were used as FRET donors and were coated by a PEGylated lipid monolayer, which incorporated other small molecule dyes that served as FRET acceptors. Varying the amount of dye in the lipid monolayer could control the degree to which the QD fluorescence was quenched. Imaging revealed the self-assembled lipidic nanoparticles dissociated after the hybrid nanoparticles were injected intravenously. By spectrally resolved imaging of the QD, dye and FRET channel, spatial and temporal analysis of nanoparticle accumulation and dissociation kinetics was possible. Different biodistributions of the QDs and lipid-incorporated dyes were observed. This research provides modular *in vivo* tools to better understand self-assembled nanoparticle behavior in real-time and demonstrates the utility of FRET imaging.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.