L5: Nanomedicine in Diagnostics and Bioimaging

April 17, 2018
Diagnostics plays an important role throughout cancer treatment

• **Before treatment**, accurately locate tumors, stage the disease, and determine an appropriate combination of cancer treatments. Tumor molecular profiling helps identify the right chemotherapy or targeted therapy drugs before treatment, which reduces unnecessary toxicity and identifies an appropriate treatment approach.

• **During treatment**, track the size of the tumor, progression of the disease, and response to treatment, and modify treatment accordingly. Minimally invasive tools like navigational bronchoscopy and endoscopic ultrasound allow to find and reach very small tumors without the risks of surgery.

• **After treatment**, evaluate any possible symptoms, and schedule regular check-ups to monitor for any signs of metastasis or recurrence. Technologies like PET/CT allow to accurately detect small lesions in areas of the body subject to movement, like the breasts, lungs, colon and prostate.

http://www.cancercenter.com
Overall Concept

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Content

Diagnostics & bioimaging techniques

Nanoparticles as contrast/imaging agents

Examples and case studies
Technique 1: Fluorescence Imaging

Fluorescence imaging is the visualization of fluorescent dyes or proteins as labels for molecular processes or structures. It enables a wide range of experimental observations including the location and dynamics of gene expression, protein expression and molecular interactions in cells and tissues.
Technique 2: Optical Imaging

Optical imaging is a technique for non-invasively looking inside the body. It uses visible light and the special properties of photons to obtain detailed images of organs and tissues as well as smaller structures including cells and even molecules.

Optical imaging includes a variety of techniques. The simplest and most widely recognized type of optical imaging is endoscopy. An endoscopy consists of a flexible tube with a system to deliver light to illuminate an organ or tissue. For example, a physician can insert an endoscope through a patient’s mouth to see the digestive cavity to find the cause of symptoms such as abdominal pain, difficulty swallowing, or gastrointestinal bleeding.

https://www.nibib.nih.gov
Technique 3: Ultrasound Imaging

Ultrasound imaging (sonography) uses high-frequency sound waves to view inside the body. Because ultrasound images are captured in real-time, they can also show movement of the body’s internal organs as well as blood flowing in the vessels.

In an ultrasound exam, a transducer (probe) is placed directly on the skin or inside a body opening. A thin layer of gel is applied to the skin so that the ultrasound waves are transmitted from the transducer through the gel into the body.

The ultrasound image is produced based on the reflection of the waves off of the body structures. The strength (amplitude) of the sound signal and the time it takes for the wave to travel through the body provide the information to produce an image.

http://www.fda.gov
Technique 4: Photoacoustic Imaging

Photoacoustic imaging is a non-invasive imaging modality. It relies on the photoacoustic effect which describes conversion between light and acoustic waves due to absorption of electromagnetic waves and localized thermal excitation. Specifically, short pulses of electromagnetic radiation, mostly short laser pulses, are used to illuminate a sample. The local absorption of the light is followed by rapid heating, which subsequently leads to thermal expansion. Finally, broadband acoustic waves are generated. By recording the outgoing ultrasonic waves with adequate ultrasonic transducers outside of the sample the initial absorbed energy distribution can be recovered. Thus, photoacoustic imaging is a hybrid technique making use of optical absorption and ultrasonic wave propagation.
Technique 5: PET/CT

Positron emission tomography–computed tomography (better known as PET-CT or PET/CT) is a medical imaging technique using a device which combines both a positron emission tomography (PET) scanner and an x-ray computed tomography (CT) scanner, so that images acquired from both devices can be taken sequentially, in the same session, and combined into a single superposed image. Thus, functional imaging obtained by PET (using radiotracers), which depicts the spatial distribution of metabolic or biochemical activity in the body can be more precisely aligned or correlated with anatomic imaging obtained by CT scanning.

http://www.radiologyinfo.org
https://en.wikipedia.org
Technique 6: Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) is a simple, painless diagnostic procedure that produces images of anatomy without the use of radiation and there are no known side effects.

Water molecules contain hydrogen nuclei (protons), which become aligned in a magnetic field. An MRI scanner applies a very strong magnetic field, which aligns the proton "spins." When the field is turned off, the protons gradually return to their normal spin (i.e., relax). The return process produces a radio signal that can be measured by receivers in the scanner and made into an image.

Protons in different body tissues return to their normal spins at different rates, so the scanner can distinguish among tissues. The scanner settings can be adjusted to produce contrasts between different body tissues.
The availability of nanomaterials for purposes of imaging has generated a variety of methods for imaging, with features including improved brightness (defined as absorbance times quantum yield), inertness to their microenvironment and a more even distribution (unless targeted imaging of certain domains is desired, of course).

Nanoparticles (NPs), in contrast to molecular probes, often are not cytotoxic and do not suffer from nonspecific binding by cellular biomacromolecules or unwanted sequestration.
Imaging Nanoparticles

- Polymeric nanoparticles
- Lipid nanoparticles
- Fluorescently doped silica NPs and sol–gels
Imaging Nanoparticles

- Semiconducting (organic) polymer dots (P-dots)
- Carbon dots (C-dots)
- Metal chalcogenide quantum dots (classical Q-dots)
Imaging Nanoparticles

- Upconversion nanoparticles (UCNPs)
- Noble metal nanoparticles (e.g., gold, silver)
- Other nanomaterials (e.g., metal oxides, sulfides, tellurides)
Multifunctional nanoparticles as coupled contrast agents

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Stably Doped Conducting Polymer Nanoshells by Surface Initiated Polymerization

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DOI: 10.1021/acs.nanolett.5b03728
Nano Lett. 2015, 15, 8217–8222
During real-time photoacoustic (PA) data acquisition, a pulsed magnetic field is applied. Voxels within the imaging region experience a force induced by the local field and magnetization. When the field is on, MNP-gold NPs move as a result of their strong magnetization, creating a moving source within a PA image. When the field is turned off, MNP-gold NPs return to their original positions. Non-magnetic PA sources do not move coherently with the applied field during this entire interval. Consequently, coherent motion processing of a PA image sequence can identify sources related to MNP-gold NPs and reject all background signals, whether from diffuse or localized sources. Such processing can greatly enhance the contrast specificity of the NP.
Synthesis of MNP-gold-coupled NP

Schematic of MNP-gold core-shell NPs and mechanism of background suppression using mmPA. Key steps involved in hybrid NP synthesis. Monodisperse hydrophobic MNPs coated with oleic acids are first solubilized using amphiphilic PL-PEG-COOH. Poly-L-histidine (PLH), which is capable of chelating metal ions, is then adsorbed onto PL-PEG-COOH by electrostatic interaction. On addition of gold ions and a reducing reagent, thin gold shells form on the polypeptide template rather than directly on core nanoparticles.
Multimodality Imaging Using MNP-gold hybrid NPs

(a, b) Darkfield imaging of single MNPs spread on glass coverslips before and after gold nanoshell coating. The coated MNPs are readily detectable under current experimental conditions (insets show corresponding TEM images). (c) T2-weighted MR images of bare and gold-coated MNPs at various dilutions. Signal strength is indicated by the darkness of the images. At the same concentrations, the MRIs are indistinguishable between the two series, indicating unchanged magnetic properties before and after gold nanoshell coating. (d, e) Cross-sectional PA images of a tube filled with (d) 5 nM MNPs and (e) 5 nM MNP-gold NPs on a dB scale. A level of 0 dB corresponds to the maximum signal level among both images. Note that a different dynamic range was used for better visualization. Signal-to-noise ratio can be improved by 1 order of magnitude (20 dB) when MNP-gold NPs are used.
A conventional PA image of this phantom is presented in (a) on a logarithmic scale over a 40 dB display range. Using a sequence of PA images acquired while a magnetic field was turned on and then off over a 10-s interval, the maximum displacement resulting from the action of the magnetic field is presented in (b) on a pixel–pixel basis over a display range of (0 (dark), 30 (light)) μm. (c) Three representative displacement traces and their fitted curves over the entire time interval of the experiment for pixels in different inclusions. Using these curves, velocity was computed over the full 10-s interval and the maximum positive and negative velocities, presented in (d) and (e) over a (−20, 20) μm s⁻¹ display range, were used to create a weighting image, presented in (f) over a (0, 1) display range, based on the magnitude of difference between peak positive velocity in the first half and peak negative velocity in the second half. (g) mmPA image produced from the product of (a) with (f) over a 40 dB display range demonstrates that the gold nanorod inclusion mimicking a strong background signal is almost completely suppressed.

A PVA phantom holds three 2-mm-diameter inclusions, in which the one on the left contains gold nanorods with absorption comparable to the 3 nM MNP-gold hybrid NPs placed in the centre inclusion, and the third inclusion on the right contains 3 nM MNPs.

The gold nanorod inclusion on the left serves as a magnetic reference and mimics strong background tissue signals. The MNP inclusion on the right serves as an optical reference to MNP-gold hybrid NPs.
A nanoparticle-based strategy for the imaging of a broad range of tumours by nonlinear amplification of microenvironment signals

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Here we capitalized on the acidic, angiogenic tumour microenvironment to achieve the detection of tumour tissues in a wide variety of mouse cancer models. This was accomplished using ultra pH-sensitive fluorescent nanoprobe that have tunable, exponential fluorescence activation on encountering subtle, physiologically relevant pH transitions. These nanoprobe were silent in the circulation, and then strongly activated (>300-fold) in response to the neovasculature or to the low extracellular pH in tumours.
Ultra pH-Sensitive (UPS) Nanoprobes

A schematic of imaging the tumour microenvironment using UPS nanoprobes. The UPS nanoprobes stay ‘OFF’ at pH 7.4 during blood circulation. After reaching tumours, the UPS nanoprobes are turned ON by acidic extracellular pH$_e$ (6.5–6.8) in the tumour milieu, or endocytic organelles (pH$_i$, 5.0–6.0) in the tumour endothelial cells after receptor-mediated endocytosis.
Synthesis and Characterization of UPS Nanoprobes

a, Structural composition of two types of nanoprobe, UPS\textsubscript{e} and UPS\textsubscript{i}, with pH transitions at 6.9 and 6.2, respectively. The UPS\textsubscript{e} is specifically designed to activate in acidic tumour extracellular fluid (pH\textsubscript{e}=6.5–6.8). The UPS\textsubscript{i} can be activated inside acidic endocytic organelles (for example pH\textsubscript{i}=5.0–6.0). Cy5.5 is used as the near-infrared fluorophore in most of the animal studies. b, Normalized fluorescence intensity as a function of pH for UPS\textsubscript{e} and UPS\textsubscript{i} nanoprobes. At high pH (for example, 7.4), both probes stay silent. At pH below their transitions (that is 6.9 and 6.2), the nanoprobes can be activated as a result of micelle dissociation. The blue dashed line simulates the pH response of a small molecular pH sensor with a pKa of 6.9 For UPS, the pH response is extremely sharp (<0.25 pH unit between ON/OFF states) with >100-fold signal amplification. In contrast, small molecular pH sensors require 3 pH units for a comparable signal change.
Characterization of UPS Nanoprobes

c, Fluorescent images of UPS–Cy5.5 nanoprobe solution in different pH buffers (Exitation/Emission=675/710 nm). 
d, Transmission electron micrographs of UPS nanopores at pH 7.4 and 6.7 (polymer concentration, 1 mg/ml; scale bars, 100 nm). e, UPS nanopores remain stable in fresh mouse serum over 24 h at 37 °C.
Imaging Angiogenic Tumor Vasculature

**a**, Design of the cRGD-UPSi nanoprobe. **b**, Schematic of internalization and activation of cRGD-UPSi nanoprobe after αvβ3-mediated endocytosis in tumor endothelial cells. The nanoprobe is accumulated in the endosomes or lysosomes, where the acidic pH activates the nanoprobe. **c**, Superimposed fluorescent images of A549-tumour-bearing mice at 6 h post-injection of UPSi nanoprobe, cRGD-UPSi or cRGD+cRGD-UPSi. Cy5.5 (red) and autofluorescence (green) are separately shown in the composite images. **f**, Representative images of ex vivo tumours, muscles and blood at 6 h post-injection of nanoprobe.
Broad Tumor Targeting Specificity

b,c. Integrated cRGD-UPSₐ-Cy5.5 nanoprobes show broad tumour imaging specificity and efficacy in ten different tumour models of different cancer types (breast, prostate, head and neck, lung, brain and pancreatic cancers) and organ sites. In the 3LL lung cancer model (c), explanted lung was shown to illustrate the effective detection of small metastatic nodules (<1 mm). Scale bar, 2 mm. In each model, high T/N ratios were observed, demonstrating the success of targeting tumour microenvironment signals as a universal strategy to achieve broad tumour specificity.