Next-generation nanotech meds

Diagnostic and therapeutic applications of non-organic nanoparticles are making their way into clinical use

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Nanoparticles have been used for more than a decade in medicine, one of the more prominent applications being silver particles in wound dressing to prevent infection and local inflammation. Most nanoparticles though are used for drug delivery, a substantial and fast growing market, worth about US$41 billion in 2014 and projected to reach US $118 billion by 2023 (http://www.transparencymarketresearch.com/nanotechnology-drug-delivery.html). In most cases, these particles are made of organic molecules to encapsulate therapeutic compounds or to home in on specific cell types such cancer cells. Recently, though, research has taken a growing interest in inorganic nanoparticles and early clinical trials are underway or about to begin soon. The potential applications of inorganic nanoparticles include diagnosis, therapy, or a combination of both for a wide range of degenerative, oncological, infectious and auto-immune or inflammatory diseases.

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Nanoparticles are usually defined as anything in size between 1 and 100 nm, which is just the right size for targeted delivery and diagnostics at the level of biomolecules. Moreover, nanoparticles often have properties that are different from the same elements or compounds at larger scale, while their large surface to volume ratio increases chemical reactivity and thus potential as catalysts. By the same token, this increases potential for deleterious side effects, which has so far held back the use of nanomaterials in the clinic.

The most widely researched nanoparticles, particularly for applications in biology and medicine, are carbon nanotubes, gold nanoparticles, and cadmium selenide quantum dots. To a large extent, the properties that make these particles attractive for industrial applications are equally relevant in biology. Gold nanoparticles, for example, are highly flexible in terms of size, shape, surface chemistry, and aggregation state. Quantum dots can deliver high doses of energy to a target area, and carbon nanotubes are extremely rigid structures. These particles can also be coated in silica, which, among other things, enables them to resist shape change and maintain their optical properties under higher light intensity. In addition, the silica surface improves uptake by cells as a result of lowered resistance to water.

The lure of gold

Gold nanoparticles have proved most valuable in biomedical research so far for improving the efficiency of existing therapies rather than as direct agents against disease themselves. This uses gold particles as contrast agents that can be attached to individual cells to track their movements. Contrast agents exploit differences in radiation in a relevant part of the electromagnetic spectrum and thus enhance particular features in medical imaging. Iodine and barium, for example, are commonly used in X-ray imaging to enhance the contrast of blood vessels.

At the cellular level, gold particles achieve a similar effect, which has been demonstrated by a novel technique to track the migration of T cells in vivo [1]. The motive was to elucidate molecular mechanisms that determine success or failure of a treatment, which requires determining the trajectory and fate of relevant T cells after injection of the therapeutic agent. The authors transduced T cells to express melanoma-specific T-cell receptor and labeled these cells with gold nanoparticles as a contrast agent for computed tomography (CT). These T cells were then injected intravenously to mice bearing human melanoma xenografts, thereby enabling whole-body CT imaging to analyze their subsequent distribution, migration, and kinetics. As hoped, the transduced T cells did indeed accumulate at the tumor site. Moreover, the labeling with gold nanoparticles did not affect their function, as demonstrated in vitro by cytokine release and proliferation assays and then crucially in vivo, by significant tumor regression.

C dots can actually kill cancer cells by triggering ferroptosis

The study’s lead author Rachela Popovtzer from the Institute of Nanotechnology and Advanced Materials at Bar-Ilan University in Israel commented that this approach could be used to track any cell type; indeed, her team has just published a paper describing the use gold-labeled mesenchymal stem cells in a mouse model of Duchenne muscular dystrophy [2]. “The cell could be imaged in the muscle for several weeks”, Popovtzer explained. “We expect that the main impact of our technique will be to enable early prediction of the...
success or failure of a treatment”, Popovtzer added. “For example, in the case of cancer therapy, the clinical outcome of the cell treatment can currently be estimated only about 2 months after the treatment. Since the transplanted cells are expected to migrate to the tumor, our method will enable imaging of this migration to the tumor after about 24 hours. In this case we will be able to predict the therapeutic outcome, that is whether the cell reached or did not reach the desired location, and not to wait for 2 months”. The next step is to translate cell tracing into the clinic. “The goal is to push this technology forward for use in preclinical large animal models and patients”, Popovtzer said.

Nanosized cell killers

Some nanoparticles have already made their way into clinical use. Michelle Bradbury, Director of intraoperative imaging at the Memorial Sloan Kettering Cancer Centre (MSKCC) in the USA, explores the potential of so-called Cornell dots not just to target tumor cells but to kill them. Cornell dots are the successors to quantum dots, which are semiconductor particles around 5-12 nm large that contain some 100–100,000 atoms and exhibit some quantum effects. Cornell or C dots go even smaller; they are comprised of chemically inert silica spheres smaller than 8 nm that can enclose semiconductor dye molecules.

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C dots were first developed by Ulrich Wiesner at Cornell University in the USA around 2004 and have since been applied for cancer detection and locating tumor cells during surgery. For such clinical applications, C dots are coated with polyethylene glycol (PEG) to avoid the body recognizing them as foreign. The shells can also be attached to suitable molecules such as antibodies to target tumor cells. Like other quantum dots, they fluoresce brightly when illuminated with near infra-red light, which shows the location of their target cells. “For the past decade, we have been adapting the surface of these ultra small sub-10 nm exquisitely bright Cornell dots with a variety of cancer-specific moieties to target and/or treat disease across different cancer types”, Bradbury explained. “The core of the particle contains near-infrared (NIR) dyes to enhance brightness, detection sensitivity, and depth penetration. NIR light of the appropriate wavelength from an FDA-cleared camera system is being used to illuminate these dye-containing particles, and the emitted light displayed for the operating surgeon to visualize cancer-bearing nodes directly.”

The application of C dots took a dramatic step forward toward therapy when Bradbury, in collaboration with Michael Overholtzer, a cell biologist at MSKCC, and Wiesner, showed that C dots can actually kill cancer cells by triggering ferroptosis [3]. This is a form of programmed cell death caused by dysregulation of iron metabolism, which has only been identified during the last 5 years. Unlike other forms of cell death, such as apoptosis and necrosis, it results in loss in activity of the lipid repair enzyme glutathione peroxidase 4 (GPX4) and subsequent accumulation of lipid-based reactive oxygen species (ROS), particularly lipid hydroperoxides, which eventually kill the cell.

Bradbury and her colleagues discovered that C dots trigger ferroptosis when administered in large doses to tumor cells that are nutrient deprived, a state during which they are unable to process iron properly. “While it is clear that these particles are well-tolerated by cells cultured under normal growth conditions, we were also interested to examine how cells under starvation stress might be affected”, Bradbury commented. “It is known that nutrient deprivation can occur in cancers as a result of insufficient vasculature, so nutrient starvation is a common stress experienced by cancer cells in vivo. While we were surprised to find the induction of such a potent form of cell death, we were anticipating the possibility that starved cells might respond to particle treatment differently than well-fed cells”.

Meanwhile, there are other emerging applications of C dots that include delivery of small molecule drugs and use as functional probes to measure pH during surgery as an alternative to optical probing. When solid cancers are removed, a change of pH occurs around the margins of the tumor and detecting this can ensure that all the malignant cells have been removed without cutting too far into healthy tissue [4].

Nanotubes: stiff and strong

The third major category of nanoparticles is carbon nanotubes (CNTs), which have some unique properties. CNTs are cylindrical carbon allotropes with exceptional strength and stiffness as well as high thermal conductivity. They also have almost unique electrical properties, being conductors along the line of their axis and semiconductors across it.

“CNT-based nanotheranostics could provide more rapid and more specific treatment by combining diagnosis and therapy in an integrated system...”

This strength allows it to make nanotubes with extreme length to diameter ratios of up to 132 million to 1. CNTs are already being widely used as structural additives for a variety of objects, such as bicycle frames and forks, structural elements of airplanes and ships or golf clubs. In biomedical research, applications have so far been mostly in vitro rather than in vivo, owing to safety concerns. One major example is the use in microfluidics devices for protein assays—these devices have several advantages such as reducing reagent consumption and increasing sensitivity to target molecules. The tubular structure of CNTs allows liquid to flow either on the outer surface or through the inner core, which is less than 10 nm in diameter.

Carbon nanotubes are also suitable for detecting biological molecules, according to James Rusling from the Neag Cancer Center and Department of Surgery at the University of Connecticut in the USA, who has been working on microfluidics. “The high surface area of a highly conductive sensor surface allows a large population to capture antibodies, which along with the massively labeled detection particles, enhances sensitivity”, he explained. Rusling’s team have developed CNT-based microfluidic devices for detecting cancer biomarkers [5]. The process involves first capturing proteins from a biopsy
sample. This is done using magnetic beads attached to a combination of antibodies to attract the proteins and an appropriate enzyme for subsequent identification. These beads, carrying in total about 400,000 labels and 100,000 antibodies, are then separated magnetically and injected into a microfluidics array, where they are selectively captured by antibodies on eight nanostructured sensors. The array enables multiplex detection so that a panel of proteins can be identified simultaneously, as is required for most diagnostics tests. In this study, a panel of four proteins was used—interleukin IL-6 and IL-8, vascular endothelial growth factor (VEGF), and VEGF-C—indicative in this case of oral cancer.

Another clinical application of CNTs is for constructing scaffolds for tissue engineering in bone and cartilage regeneration. Such scaffolds are three-dimensional structures with mechanical properties similar to the tissue and a pore network to support cell growth, transport of nutrients, and removal of metabolic waste. The standard material for bone engineering is a biodegradable plastic called polypropylene fumarate or PPF, which has performed well in previous experiments. Yet, the performance of PPF could be considerably enhanced by incorporating CNTs. Recent experiments in rabbits found that animals implanted with the CNT-enhanced scaffolds exhibited three times greater bone ingrowth after 12 weeks than those with pure PPF [6]. The authors speculated that this enhanced bone growth was related to changes in surface chemistry.

There are other examples of applying CNTs where the underlying molecular mechanisms are not yet well understood. One of the most spectacular one is the case of silkworms fed with a diet containing CNTs, which resulted in super-strong silk [7]. The researchers performed spectroscopy analysis, which indicated that some CNTs were incorporated into the silk. The authors suggest that this may strengthen potential break points and therefore increase toughness.

**Diagnosis and cure in one step**

Back to therapeutic applications, several categories of nanoparticle are now being tested for nanotheranostics, which involves small devices that combine diagnostics with therapy in a single system. The major advantages are reduced costs and increased speed of both processes, which can be vital for conditions such as sepsis where rapid response is necessary to avert serious organ damage or death.

Currently, diagnosis of sepsis—a catastrophic amplification of the immune response against systemic bacterial infection—relies on blood culture and serology both of which take time and are often not sufficient to identify the specific pathogen involved. Therapy therefore involves broad-spectrum antibiotics, which are increasingly susceptible to antimicrobial resistance. CNT-based nanotheranostics could provide more rapid and more specific treatment by combining diagnostics and therapy in an integrated system, commented Shalini Gupta from the Department of Chemical Engineering at the Indian Institute of Technology in Delhi. “The nanotheranostic approach mainly helps with the speed of the diagnosis, but we also feel that by use of appropriate antibiotics as nanotheranostic ligands, one can further design systems that allow doctors to identify the infectious agent early and prescribe the specific antibiotic that is needed by the patient”, she said. “For instance, instead of giving a broad-spectrum antibiotic that may or may not work, one can use targeted therapy with a narrow-spectrum antibiotic based on the theranostics”. Gupta noted that various carbon-based nanoparticles including CNTs can be used in theranostics for infectious diseases, but added that noble metals also have appeal, given their antimicrobial properties.

She envisions two approaches to bacterial nanotheranostics. The first involves capturing pathogen-derived biomarkers and concentrating them locally to enhance sensitivity of detection and thereby diagnosis, while at the same time removing bacteria-derived components from the host to reduce inflammation. This, Gupta argued, is especially relevant for sepsis where circulating inflammatory mediators, including excess amounts of gut-derived bacterial endotoxins like LPS or LTA, are released into the bloodstream.

The second strategy is based on the simultaneous detection and identification of the Gram stain level of the bacterial pathogen to guide doctors to prescribe a narrow-spectrum antibiotic at the onset of sepsis. Again, the aim is to treat the rapidly escalating symptoms quickly, while responding with an effective therapy that is not prone to antimicrobial resistance or the side effects of a broad-spectrum antibiotic. Early detection of pathogen-associated biomarkers in the blood not only confirms systemic infection but also provides information for rapid disease management, which, as Gupta points out, can save lives, and not just in developing countries. Septic shock is a major and growing problem everywhere, proving fatal in almost half of patients in the EU who are admitted to hospital.

As the first nanotheranostics devices are now close to regulatory approval for use against sepsis, this is one example of how nanotech could make a major difference in the clinic. With others to follow suit, particularly in cancer detection and treatment, nanotech is poised to make a difference in biomedicine.

**References**