Nanotechnology in Cancer Medicine FREE

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Because of a previously unexploited weakness in tumor architecture, nanomaterials may offer a way to treat cancer without doing too much damage to healthy tissue. The weakness isn't really a property of the tumors themselves but of the blood vessels that feed them.

Cancer is an inherently biological disease, in which cell replication—one of the hallmarks of life—fails to be regulated by the usual mechanisms. Historically, chemistry has been one of the most effective tools for treating cancer: Chemotherapy—treatment with cytotoxic chemicals—kills cancer cells. But most chemotherapeutics also kill healthy cells. Making drugs that discriminate between cancer and normal cells is difficult, and when it works, it may not work for long. Cancer cells replicate rapidly, so they evolve rapidly and are extraordinarily quick at developing drug resistance.

With a new generation of nanotech drugs, researchers are fighting cancer by approaching it as a physics problem—a problem of mass transport and fluid mechanics. They've already achieved some success, but the drugs have introduced a new series of challenges unique to the physics of nanomaterials.

Principles of nanomedicine

At their earliest stages, tumors lack blood vessels of their own; they take their nutrients such as oxygen and glucose from the surrounding tissue. Cells at the tumor's periphery get more of those nutrients than cells at the tumor core, so most small tumors grow at their edges while starving their cores. Cells in the tumor core release proteins to signal their oxygen-starved state. The proteins diffuse outward until they reach nearby blood vessels, where they stimulate the growth of new blood vessels that can supply the tumor with oxygen and other nutrients to sustain its rapid cell replication and growth.

Angiogenesis—the growth of new blood vessels—is one of the hallmarks of cancer.[1] Angiogenic blood vessels supply tumors with nutrients, but because of their own rapid growth, they are irregular and leaky, with more and larger gaps in their walls than healthy blood vessels. The gap sizes vary depending on where the tumor is in the body and its stage of development, but generally range from a few hundred nanometers to a few microns.[2] In contrast, the pores in normal blood vessels are just 2–6 nm in size. Nanoparticles between about 10 and 300 nm in diameter are just the right size to pass through the gaps in the blood vessels supplying tumors but don't significantly penetrate healthy tissue. By loading the particles with chemotherapy drugs—established cancer killers—one can, at least in principle, deliver the drugs to tumor cells without damaging healthy cells. Figure 1 illustrates the process.

Nanoparticles do in fact selectively accumulate in tumor tissue via a purely physical phenomenon called the enhanced permeability and retention (EPR) effect.[3] Figure 2 tracks a small molecular (non-nanoparticle) contrast agent over 45 minutes as it penetrates a tumor implanted in the flank of a mouse. By the time the molecule starts to reach the tumor core, it's already being cleared from parts of the tumor periphery. In contrast, figure 3 shows a different mouse injected with iron oxide nanoparticles. The entire tumor becomes progressively darker with time, which indicates nanoparticle accumulation via the EPR effect. The nanoparticle concentra-

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tion in the tumor was still increasing after 24 hours.

Unfortunately, nanoparticles can look a lot like viruses to the immune system, and they may be rapidly taken up by cells of the mononuclear phagocyte system (MPS), part of the body's defense against invasion by bacteria, protozoa, and viruses. Uptake by MPS cells can cause intravenously injected nanoparticles to be shuttled to the liver and spleen, preventing them from delivering their chemotherapeutic payloads to tumors.

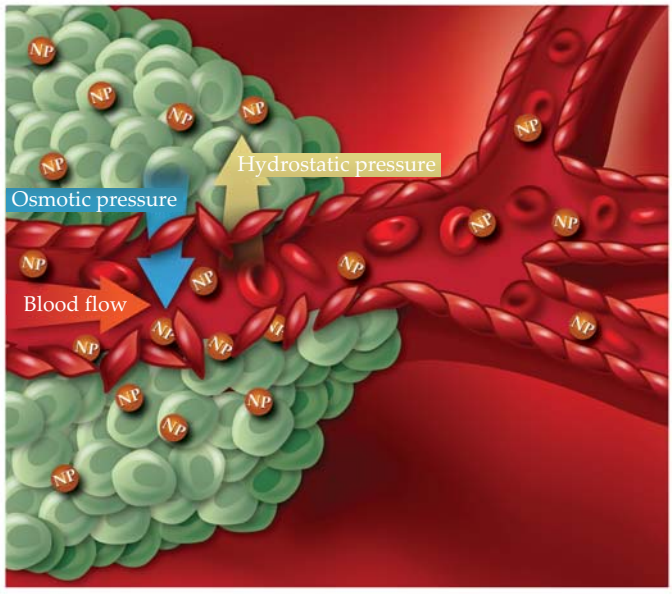
Beyond size, one has to consider the surface properties of a cancer nanomedicine. Surfaces are extremely important at the nanoscale because surface-to-volume ratios are so high. It's convenient to think about nanoparticles in terms of two fundamental components: the core, which doesn't interact with the environment, and the surface layer or "corona," which does.

Most cell membranes have a net negative charge, so nanoparticles with cationic coronas may have an easier time getting into cells to deliver their payload. But they may also bind more readily to cells in nondiseased areas. So instead, researchers commonly coat their nanoparticles with polyethylene glycol (PEG), a charge-neutral molecule that reduces both protein binding and MPS uptake, and thus increases the length of time that the particles circulate in the blood and the likelihood of their reaching the target. The length of the PEG polymer chain and the density of PEG coating both affect nanoparticle protein binding and distribution in the body.[4]

Nanomedicine drug delivery is complicated by multiple physical barriers that limit tumor penetration. Cancer cells are surrounded by material called tumor stroma, essentially a protective shell a tumor builds around itself. A tumor stroma includes fibroblasts, endothelial and immune cells, vascular pericytes, secreted growth factors, and an extracellular matrix. Certain tumors have a dense extracellular matrix of interconnected collagen fibers that may limit the penetration of both nanotech and molecular drugs. When the stroma is unusually tough, as is the case for some pancreatic cancers, a tumor can be almost entirely impenetrable to drugs. Patients afflicted with those cancers usually do not survive more than a few months.

Another physical barrier to tumor penetration is the high fluid pressure in tumor cores. In healthy tissue, fluid constantly seeps from blood vessels into surrounding tissues and is reabsorbed by the lymphatic system, which returns it to the blood stream. Solid tumors lack effective lymphatic drainage systems, so fluid is not drained efficiently, and the resulting pressure buildup limits blood seepage from vessels. The pressure is higher in the center of the tumor than at the periphery, and the pressure difference is greater in large tumors. Because of that pressure, which prevents fluid flow everywhere except at the periphery, the main mechanism of transport within tumors is diffusion, which limits the mobility of nanoparticles.

Figure 1. The blood vessels in solid tumors have irregular linings, with gaps much bigger than the ones in healthy blood vessels. Nanoparticles (NP) less than 300 nm in diameter can pass through those gaps and accumulate in the tumors through a purely physical phenomenon called the enhanced permeability and retention effect. (Cartoon not drawn to scale.)



The emerging field of transport oncophysics deals with the mass transport properties and time dynamics of the physical barriers to tumor drug delivery.[5] Rakesh Jain of Harvard Medical School has done some of the foundational work. He has suggested that those barriers have limited the efficacy of some nanomedicines, because nanomedicines may get to tumor peripheries via the EPR effect but never make it to tumor cores.[6] Insufficient delivery of a drug to tumor cores may lead to drug resistance, similar to how bacteria, if given a sublethal dose of an antibiotic, develop antibiotic resistance.

But there are ways around the barriers. Antiangiogenic medicines lower the pressure at the tumor core to facilitate drug delivery. Nanoparticles can be designed to release their drug payload in response to an external stimulus—for example, light, ultrasound, heat, or magnetic field—or when they encounter the low pH of the tumor core. Once the drug is released, it is no longer encumbered by the nanoparticle and can diffuse more easily through the tumor. Multistage nanoparticles are also being devised that combine larger particles' ability to accumulate in tumors with smaller particles' ability to penetrate tumor tissue and get into cells.[7]

Today there are about 82 ongoing clinical trials involving nanoparticles to treat cancer. Many involve nanoparticle carriers of established chemotherapeutics. Others involve novel drugs, enhancement of radiotherapy, in vitro diagnostics, or nanoparticles that are used for hyperthermia or thermal ablation.

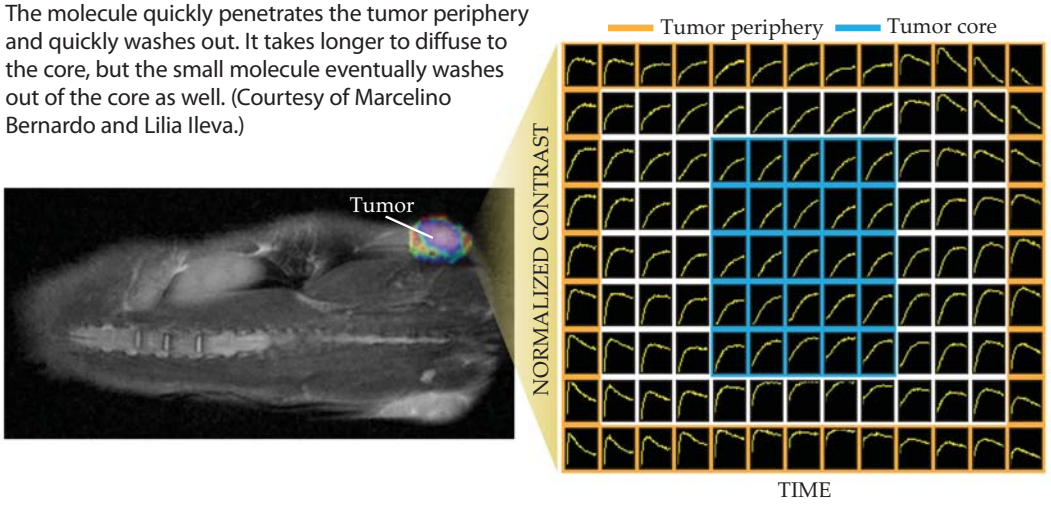


Figure 2. A mouse implanted with a tumor was injected with a small-molecule (non-nanoparticle) contrast agent. The grid on the right shows pixel-by-pixel plots of the contrast over a 45-minute period. The molecule quickly penetrates the tumor periphery and quickly washes out. It takes longer to diffuse to the core, but the small molecule eventually washes out of the core as well. (Courtesy of Marcelino Bernardo and Lilia Ileva.)

Successes

The technologies described above are already working. Two nanotech reformulations of chemotherapeutics, Abraxane and Doxil, have been approved by the US Food and Drug Administration (FDA) and are benefiting cancer patients. Abraxane, shown schematically in figure 4a, is a protein-bound reformulation of paclitaxel, a powerful chemotherapeutic that is poorly soluble in water. Abraxane uses a nanoparticle made of the blood protein albumin to encapsulate and solubilize paclitaxel. Compared with Taxol, a non-nanotech form of the same drug stabilized with castor oil, Abraxane is both more effective and less toxic. Doxil, shown in figure 4b, is a nanosized liposome ("fat bubble" particle) of the drug doxorubicin. Free doxorubicin, along with a broad class of similar molecules, is toxic to the heart and is known to damage cardiac muscles. Doxil, due to its nanoparticle delivery system, distributes differently in the body, so less of it reaches the heart. However, more of it reaches the skin, where it may cause ulcerations. (With chemotherapeutics, often no option entirely avoids adverse side effects—but skin ulcerations may be preferable to cardiac toxicity.)

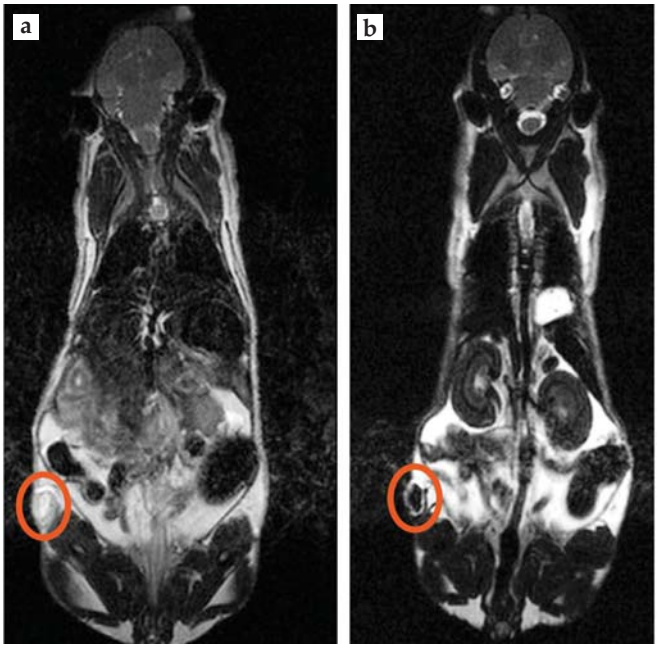


Figure 3. Iron oxide nanoparticles were injected into a mouse implanted with a colon cancer tumor (circled in orange). **(a)** Before injection, the tumor appeared bright in a magnetic resonance image. **(b)** Twenty-four hours after injection, accumulation of nanoparticles caused the tumor to appear dark. In fact, the contrast in the tumor was still increasing after 24 hours. (Courtesy of Marcelino Bernardo and Lilia Ileva.)

Many more anticancer nanomedicines are in clinical development, some based on very different principles than chemotherapy. For example, AuroShell, shown in figure 4c, is a gold nanoshell that uses passive targeting via the EPR effect to reach tumor sites. Once the particles are in the tumor, near-IR laser light is applied, which heats the particles and thermally destroys the tumor and the surrounding blood vessels without significant damage to healthy tissue. AuroShell is currently being tested in a phase I clinical trial for head and neck cancers.

Challenges

Of course, nanomedicines aren't without limitations. The ability to reproducibly manufacture nanomedicines at large scales with high levels of control over the physicochemical properties remains a major obstacle. Though many labs can make nanomedicines at the milligram levels for proof-of-concept in vitro studies, the costs and manufacturing challenges associated with making large-scale batches of the same quality remain great.

A nanoparticle contains hundreds or even thousands of atoms. Unlike small molecules, which have specific chemical formulas, nanoparticles necessarily vary in the number and arrangement of their atoms, even in a supposedly pure batch. (That variation is evident in the images in figure 5.) The polydispersity in size and shape often translates to an inherent polydispersity in all the material properties of a nanomedicine. As a result, nanomedicine properties must be defined by an acceptable range rather than an absolute standard. For example, a nanomedicine formulation with a targeting ligand may be able to function efficiently with 5–20 ligands per nanoparticle.

Nanomedicines must be thoroughly characterized because their properties can vary from batch to batch even when they're made under carefully controlled conditions. Preclinical physicochemical characterization of a nanomedicine includes measurement of size and shape, surface chemistry, and state of aggregation or agglomeration. Nanomedicine characterization is often complicated by the polydispersity of samples, so it can be necessary to measure the same quantity with multiple methods, such as electron microscopy and light scattering for size, to gain a detailed understanding. A recent article about FDA regulatory review considerations presents some of the manufacturing and characterization challenges surrounding nanomedicines.[8]

To help get nanotech cancer treatments ready for clinical trials, the National Cancer Institute makes the services of its Nanotechnology Characterization Laboratory (NCL) available to anyone who has developed a nanotech cancer treatment and has demonstrated preliminary proof of concept. The NCL conducts physicochemical characterization and performs nanomaterial safety and toxicity testing in vitro and in laboratory animals. It works closely with the FDA and NIST to devise experiments that are relevant to nanomaterials, validate the tests on a variety of nanomaterial types, and disseminate its methods to the nanotech and cancer research communities. To date, the NCL has evaluated more than 250 nanoparticles intended for medical applications.

One case study illustrates the importance of nanomedicine characterization: The NCL conducted an animal study to determine the safety of a polymer-coated gold nanoparticle intended as a cancer therapy. As part of a toxicology study, the lab's animal technicians injected rats with the nanoparticles and found that the animals unexpectedly developed lung lesions. The drug manufacturer's previous studies had not resulted in lung lesions—and when the NCL technicians repeated the same experiment with a freshly synthesized batch of nanomaterial, the rats did not develop lesions. A fairly rigorous battery of testing found the two batches of nanomedicine to be essentially indistinguishable: They were produced using the same synthetic process, had equivalent size and surface charge, and looked similar under an electron microscope. Finally, the technicians looked at the particles' polymer coatings. A sample of the fresh batch had a higher density of polymer on its surface than the older batch. It seemed that polymer on the nanoparticles in the older batch had been displaced by ions over time. The small difference in the polymer concentration caused a large difference in the in vivo results—and ultimately made the difference between a nanomedicine that was potentially safe and one that was not.

Costs

New technology often doesn't come cheaply, and so far nanomedicines are no exception. The two currently FDA-approved nanotech reformulations of cancer drugs, Abraxane and Doxil, are far more expensive than their non-nanotech counterparts. The average per-dose costs of both Abraxane and Doxil exceeded $5000 in 2009, compared with less than $500 for Taxol and less than $200 for doxorubicin. The increased costs come with documented advantages: Because the nanomedicines are less toxic to healthy tissue, they afford patients a significantly better quality of life than their molecular counterparts. But they offer only modest improvements in overall survival.[9–12] If nanotech therapies continue to have order-of-magnitude higher costs than their small-molecule competitors, they are likely to remain controversial unless they can also show similarly dramatic increases in patient survival.

On the other hand, nanotechnology has the potential to lower R&D costs through nanotech reformulation of discontinued drugs. For every new molecular drug that makes it through clinical trials and onto the US market, more than $1 billion is spent on drug development. Some part of that expense

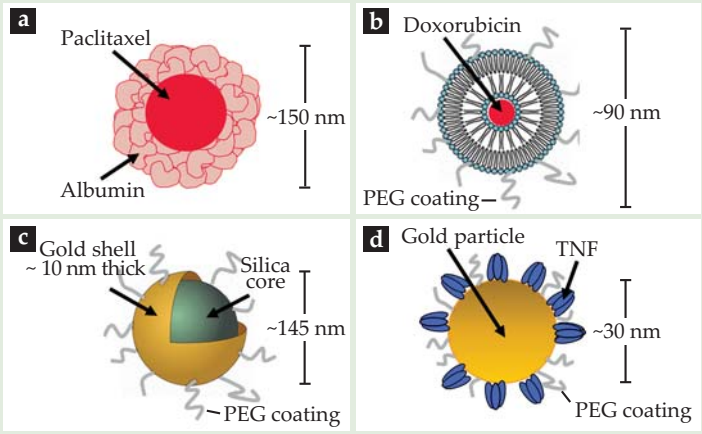
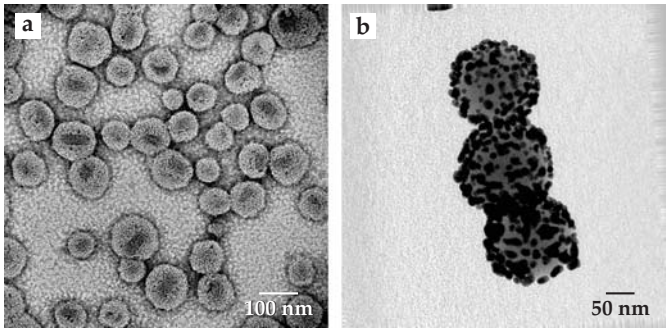


Figure 4. Some of the nanomedicines for cancer treatment on the market and in clinical trials. **(a)** Abraxane, produced by Celgene Corp, is a nanoparticle of the drug paclitaxel bound by the blood protein albumin. **(b)** Doxil is a Johnson and Johnson product composed of crystals of the drug doxorubicin encapsulated in a lipid layer and coated with polyethylene glycol (PEG). **(c)** AuroShell, a product of Nanospectra Biosciences, is a gold nanoshell that doesn't contain a conventional chemotherapy drug. Instead, the particles are heated with an IR laser to destroy the tumor thermally. **(d)** Aurimune, produced by CytImmune Sciences, consists of the protein tumor necrosis factor (TNF, a previously discontinued chemotherapeutic) bound to gold nanoparticles.

Figure 5. Electron micrographs of **(a)** Doxil and **(b)** an early batch of the material that would eventually become AuroShell. Electron microscopy is a useful tool for visualizing nanomaterials too small to be seen by light microscopy, but because it shows only a small number of particles at a time, it is not well suited for characterization of the bulk or average properties of a material. The micrographs here give a sense of the variability in size and shape in the samples, but one would have to examine hundreds or even thousands of images to obtain adequate statistics on the size distribution. (Courtesy of Ulrich Baxa.)



comes from the many drugs that are discontinued during the process. Approximately four out of five drugs that enter clinical trials will fail due to toxicity or other undesirable properties. The costs of discontinued drugs are passed on to consumers in the form of higher prices for those drugs that do make it to the market.

Nanotechnology offers drug companies an opportunity to reformulate discontinued drugs and recoup some of the cost. Desirable properties can be enhanced in nanotech formulations, while adverse properties can be engineered out. Nanoparticle carriers can be used to increase solubility and bioavailability, enhance targeting, provide for the controlled release of a variety of therapeutics, and thus potentially make discontinued drugs viable again.

For example, tumor necrosis factor (TNF) is a potentially potent chemotherapeutic that was tested in clinical trials in the 1980s and 1990s but had to be discontinued due to severe adverse side effects. It has since been reformulated as Aurimune. Shown in figure 4d, Aurimune is nanosized gold with TNF bound to its surface. In its recent phase I clinical trial, Aurimune allowed three times the previous quantity of TNF to be administered to patients with almost no ill effect.[13]

Safety and environmental concerns

The same material properties that make nanoparticles appealing for cancer therapy and other applications may have unintended effects on human health and the environment. Although the acute toxicity of many nanomaterials appears to be low,[14] studies that evaluate chronic toxicity are still largely missing from the scientific literature.

Much of the work on the chronic health risks associated with nanotechnology has focused on carbon nanotubes and other carbon nanomaterials. For example, nanotubes introduced into the abdominal cavities of mice have been shown to result in a disease similar to mesothelioma, the cancer caused by asbestos.[15] Other recent studies, however, have found nanotubes and fullerenes to have low toxicity[16] and have shown that toxicity can be reduced by chemical modification. Even if nanotubes turn out to cause unique toxicities upon inhalation, it's not known whether the toxicity is a function of their fibrous structure, a structure not shared by most other nanomaterials.[17,18]

Assessment of the health and safety risks of nanomaterials has been complicated by several factors. Even the "same" nanomaterial from two different commercial sources may have different properties, and those properties can change with time. For example, nanoparticles in air aggregate rapidly, which affects their rates of sedimentation and lung deposition. Most important, many of the nanomaterials used in risk and hazard studies are poorly characterized, and it is not always apparent what aspect of a material contributes to the observed effect. For example, a confounding factor in hazard assessment has been the use of dispersive agents, such as surfactants, to increase nanoparticles' solubility or prevent their aggregation. Studies that use dispersive agents may not be relevant to normal exposure conditions because the dispersive agents themselves may be toxic.

Whether actual or perceived, the potential health risks associated with the manufacture and use of nanomaterials must be balanced by the benefits that nanotechnology has to offer society for cancer therapy and beyond.

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