Introdução

Nanotecnologia em Física Médica

Professores: Oswaldo Baffa, Theo Pavan e Eder Guidelli

Nanotechnology

- Nanotechnology can be defined as the manipulation of matter with at least one dimension sized from 1 to 100 nanometers.
- The definition shifted from a particular technological goal to a research category inclusive of all types of research and technologies that deal with the special properties of matter which occur below the given size threshold.
- It is therefore common to see the plural form "nanotechnologies" as well as "nanoscale technologies" to refer to the broad range of research and applications whose common trait is size.

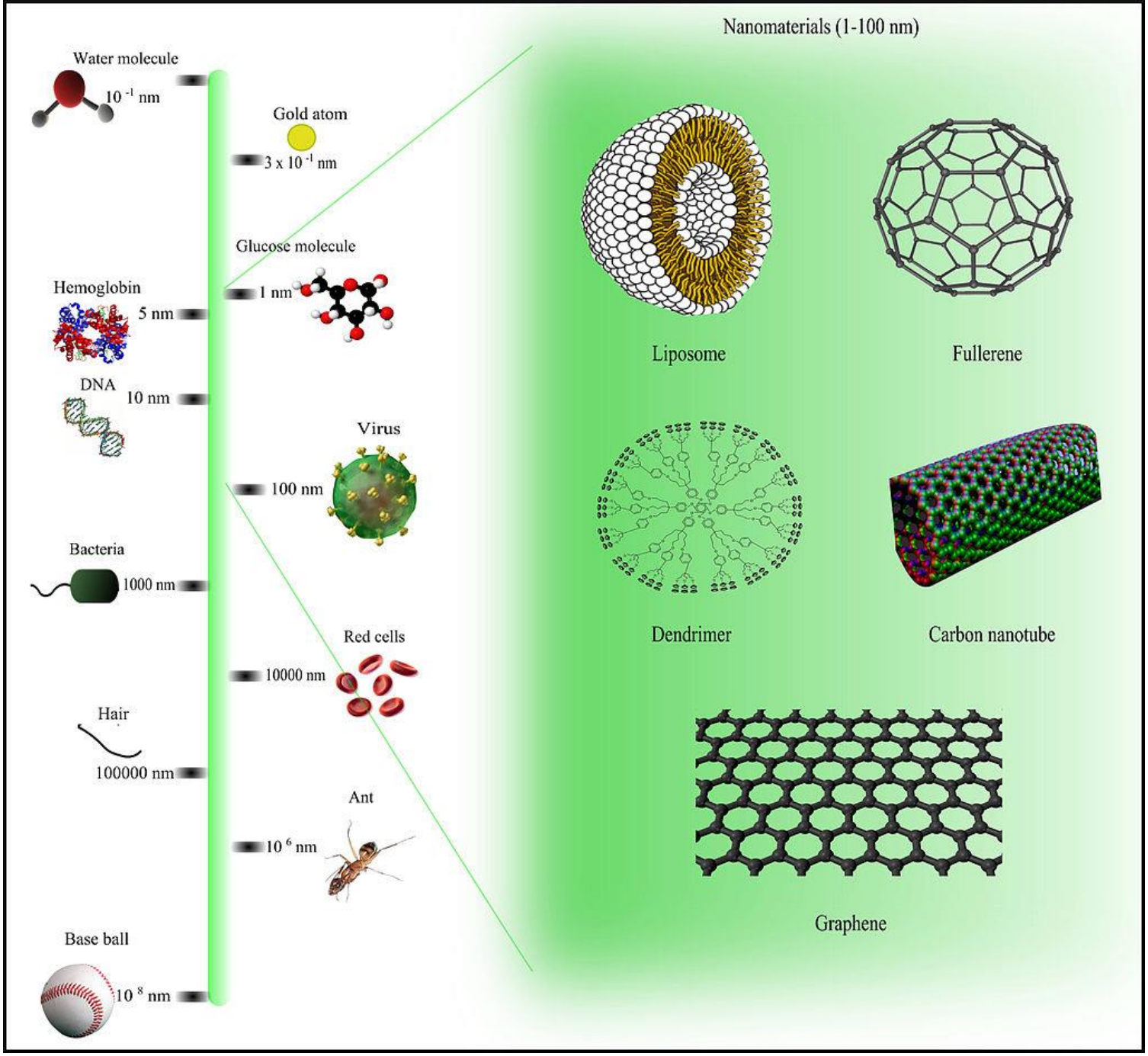
https://en.wikipedia.org/wiki/Nanotechnology

Nanotechnology

- Because of the variety of potential applications (including industrial and military), governments have invested billions of dollars in nanotechnology research. Until 2012, the USA has invested $3.7 billion, the European Union has invested $1.2 billion and Japan has $750 million.
  https://en.wikipedia.org/wiki/Nanotechnology

- Until 2020 the cumulative US government Nanotechnology investment was 29 billion.

https://www.fda.gov/science-research/fda-grand-rounds/nanotechnology-over-decade-progress-and-innovation-fda-08132020-08132020



https://en.wikipedia.org/wiki/Nanotechnology

Jennifer H. Grossman and Scott E. McNeil

NANOTECHNOLOGY IN CANCER MEDICINE

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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Nanotechnology in Cancer Medicine

- Cancer is an inherently biological disease, in which cell replication fails to be regulated by the usual mechanisms.
- Most chemotherapeutics also kill healthy cells.
- Making drugs that discriminate between cancer and normal cells is difficult.
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NanoTech Drugs

- 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.

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Principles of Nanomedicine

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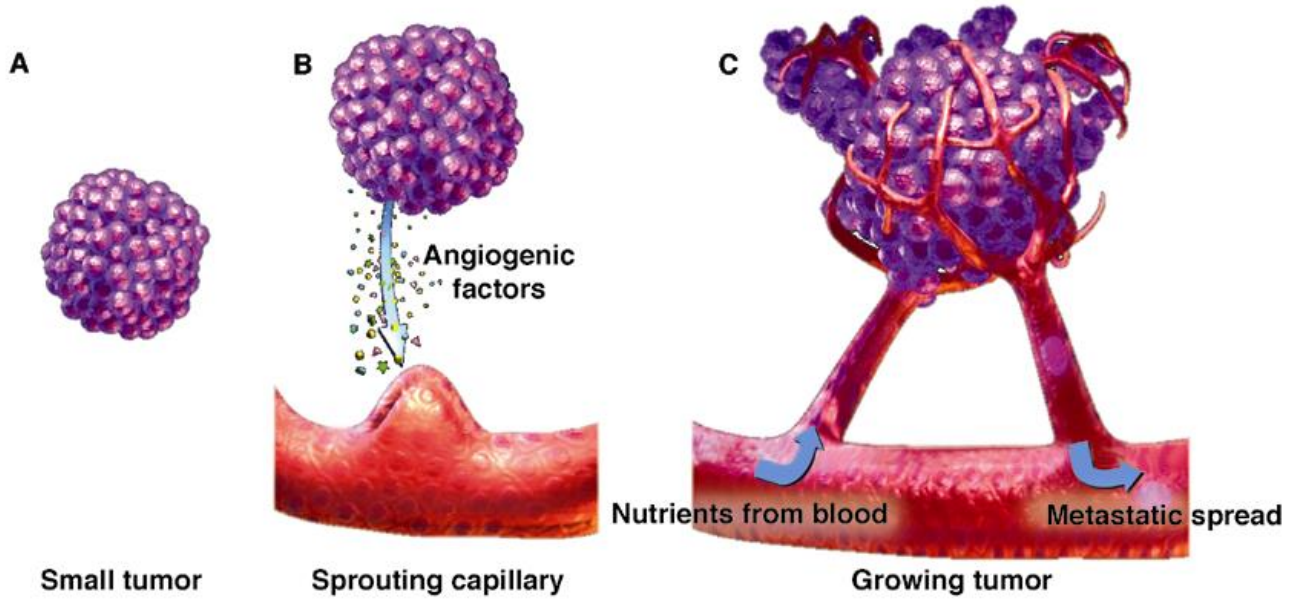

https://peoplebeatingcancer.org/toxicnon-toxic-anti-angiogenesis-drugs-and-cancer/

Principles of Nanomedicine

- Because of their rapid growth, they are irregular and leaky, with more and larger gaps in their walls than healthy blood vessels.

- The gap sizes vary from a few hundred nanometers to a few microns.

- In contrast, the pores in normal blood vessels are just 2–6 nm in size.

Enhanced permeability and retention (EPR) effect

- **Nanoparticles are between about 10 and 300 nm in diameter and can pass through the gaps in the blood vessels supplying tumors, but don't significantly penetrate healthy tissue.**

- **By loading the particles with chemotherapy drugs one can, at least in principle, deliver the drugs to tumor cells without damaging healthy cells.**

- **Nanoparticles do in fact selectively accumulate in tumor tissue via a purely physical phenomenon called the enhanced permeability and retention (EPR) effect.**

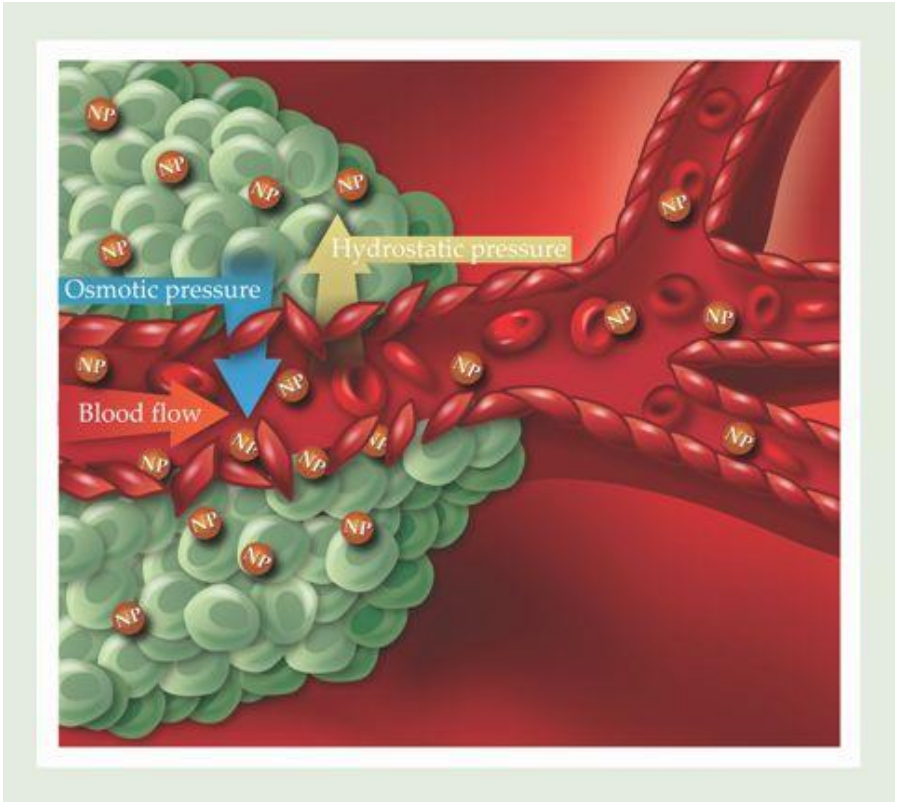

Figure 1. The blood vessels in solid tumors have irregular linings, with gaps much bigger than the ones in healthy blood vessels. Nanoparticles (NP) less than 300 nm in diameter can pass through those gaps and accumulate in the tumors through a purely physical phenomenon called the enhanced permeability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

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DOI: 10.1063/PT.3.1678

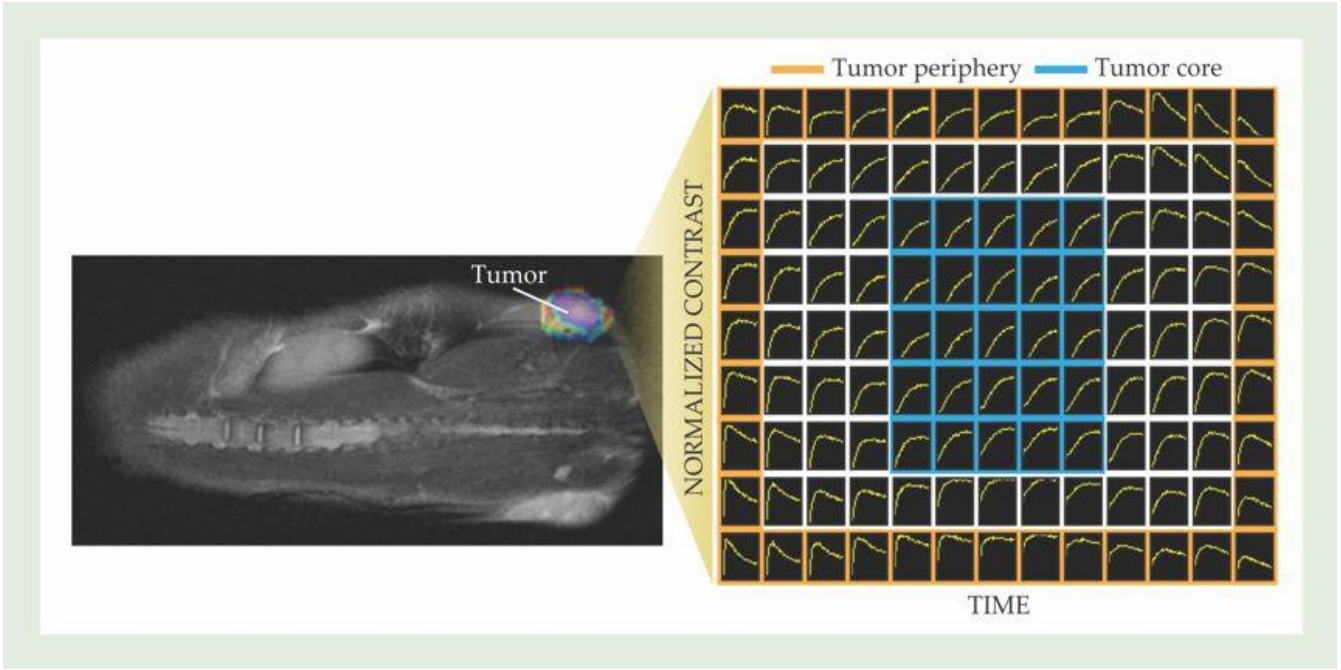

Figure 2. A mouse implanted with a tumor *was injected with a small-molecule (non-nanoparticle) contrast agent. The grid on the right shows pixel-by-pixel plots of the contrast over a 45-minute period. The molecule quickly penetrates the tumor periphery and quickly washes out. It takes longer to diffuse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

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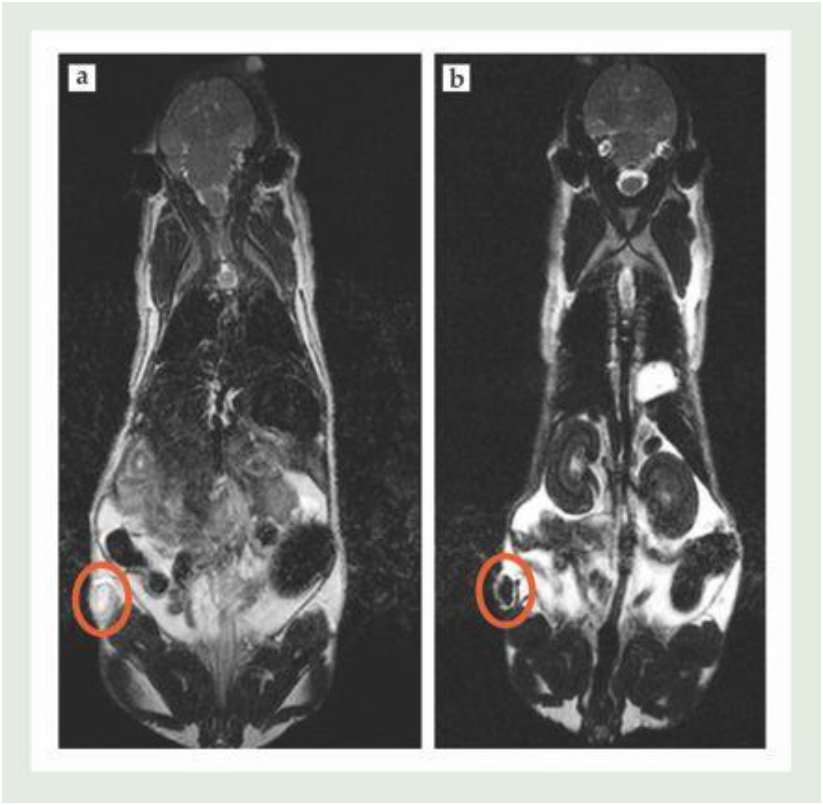

Figure 3. Iron oxide nanoparticles *were injected into a mouse implanted with a colon cancer tumor (circled in orange).* ***(a)*** *Before injection, the tumor appeared bright in a magnetic resonance image.* ***(b)*** *Twenty-four hours after injection, accumulation of nanoparticles caused the tumor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

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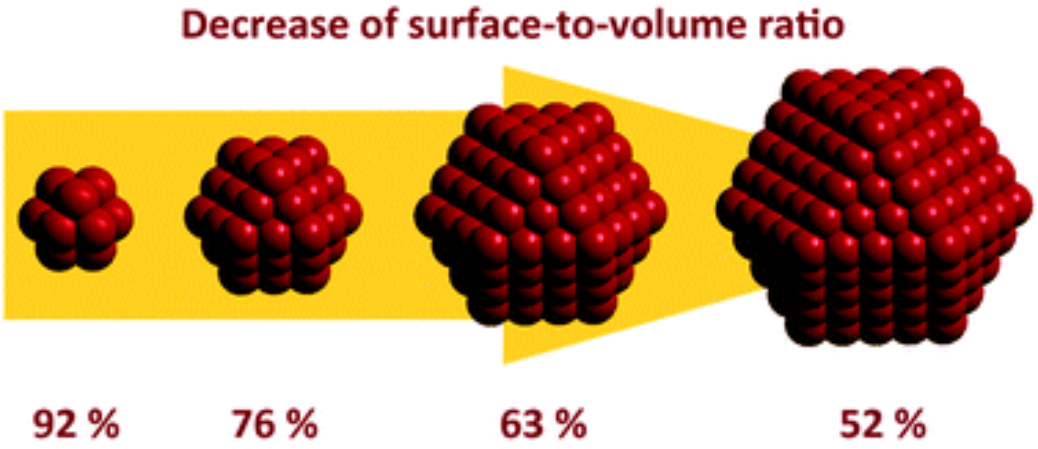
Uptake by mononuclear phagocyte system

- 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



http://pubs.rsc.org/-/content/articlelanding/2011/cp/c1cp22048a/unauth#!divAbstract

- **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.**

Nanoparticle Surface

- **Most cell membranes have a net negative charge, so nanoparticles with cationic coronas may have an easier time getting into cells. But they may also bind more readily to cells in nondiseased areas.**

- **Researchers commonly coat their nanoparticles with polyethylene glycol (PEG), a charge-neutral molecule that reduces both protein binding and MPS uptake.**

- **Increases the length of time that the particles circulate in the blood and the likelihood of their reaching the target.**

Core-shell

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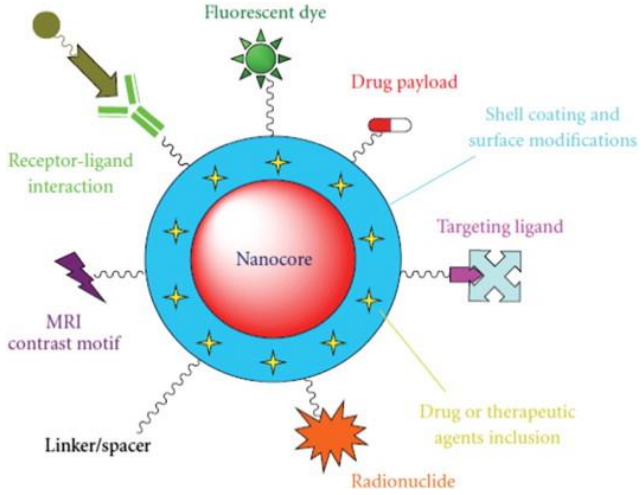
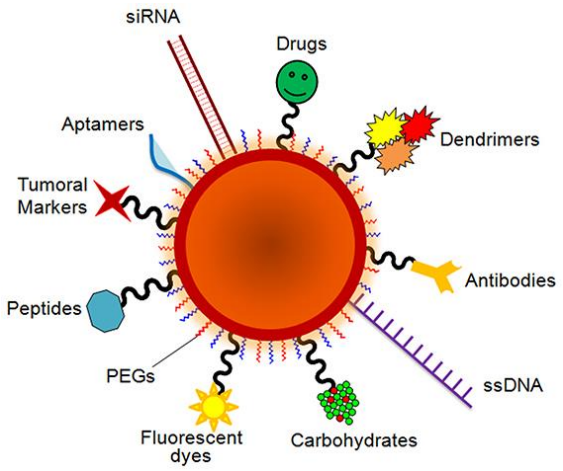


Core/shell nanoparticles in biomedical applications
Krishnendu Chatterjee, Sreerupa, Sarkar K. Jagajjanani Rao, SantanuParia

http://www.sciencedirect.com/science/article/pii/S0001868613001899



Revisiting 30 years of biofunctionalization and surface chemistry of inorganic nanoparticles for nanomedicine

https://www.frontiersin.org/articles/10.3389/fchem.2014.00048/full

Physical Barriers

- Cancer cells are surrounded by material called tumor stroma, essentially a protective shell a tumor builds around itself.

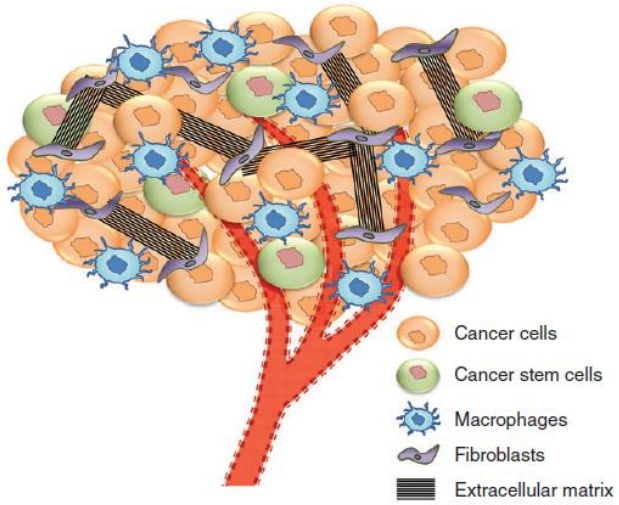


FIGURE 1 | Components of a tumor stroma. A complex network of neoplastic and non-neoplastic cells constitute tumor stroma. A strong symbiotic relationship exists among them, which helps the tumor growth and survival. Just like an organ, tumor has its own support cells, blood vessels, residing immune cells, and even stem cells that help its survival and propagation.

Modifying the tumor microenvironment using nanoparticle therapeutics
Aniruddha Roy and Shyh-Dar Li

http://onlinelibrary.wiley.com/doi/10.1002/wnan.1406/pdf

The interaction between stromal cells and tumor cells is known to play a major role in cancer growth and progression.

Physical Barriers

- 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.

Physical Barriers

- Another physical barrier to tumor penetration is the high fluid pressure in tumor cores.

- Solid tumors lack effective lymphatic drainage systems, so fluid is not drained efficiently, and the resulting pressure buildup limits blood seepage from vessels.

Physical Barriers

- 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.

- But there are ways around the barriers.
  - 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.

Clinical Trials

- In 2012, there were 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.

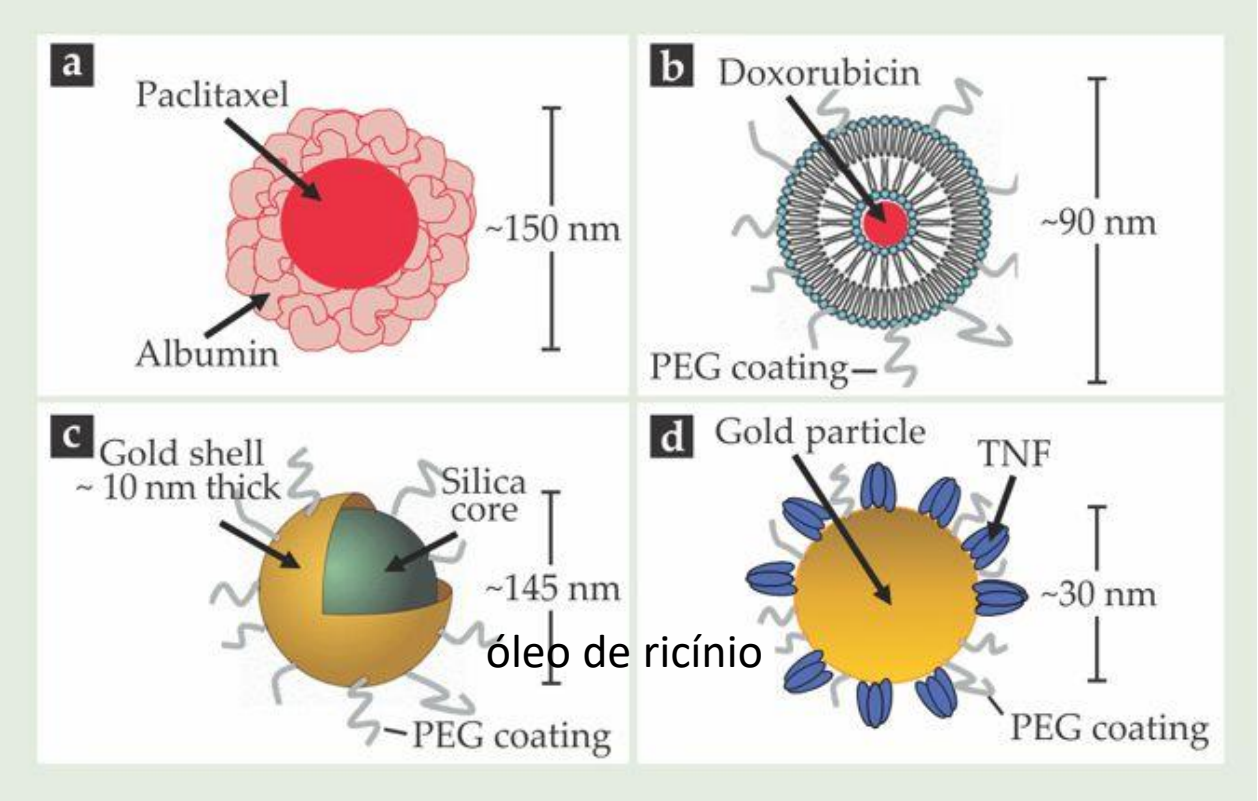


Figure 4. *Some of the nanomedicines for cancer treatment on the market and in clinical trials.* ***(a)*** *Abraxane, produced by Celgene Corp, is a nanoparticle of the drug paclitaxel bound by the blood protein albumin.* ***(b)*** *Doxil is a Johnson and Johnson product composed of crystals of the drug 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.*

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Approved by the FDA

- 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.

Approved by the FDA

- 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.)

Challenges

- 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.

- 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.

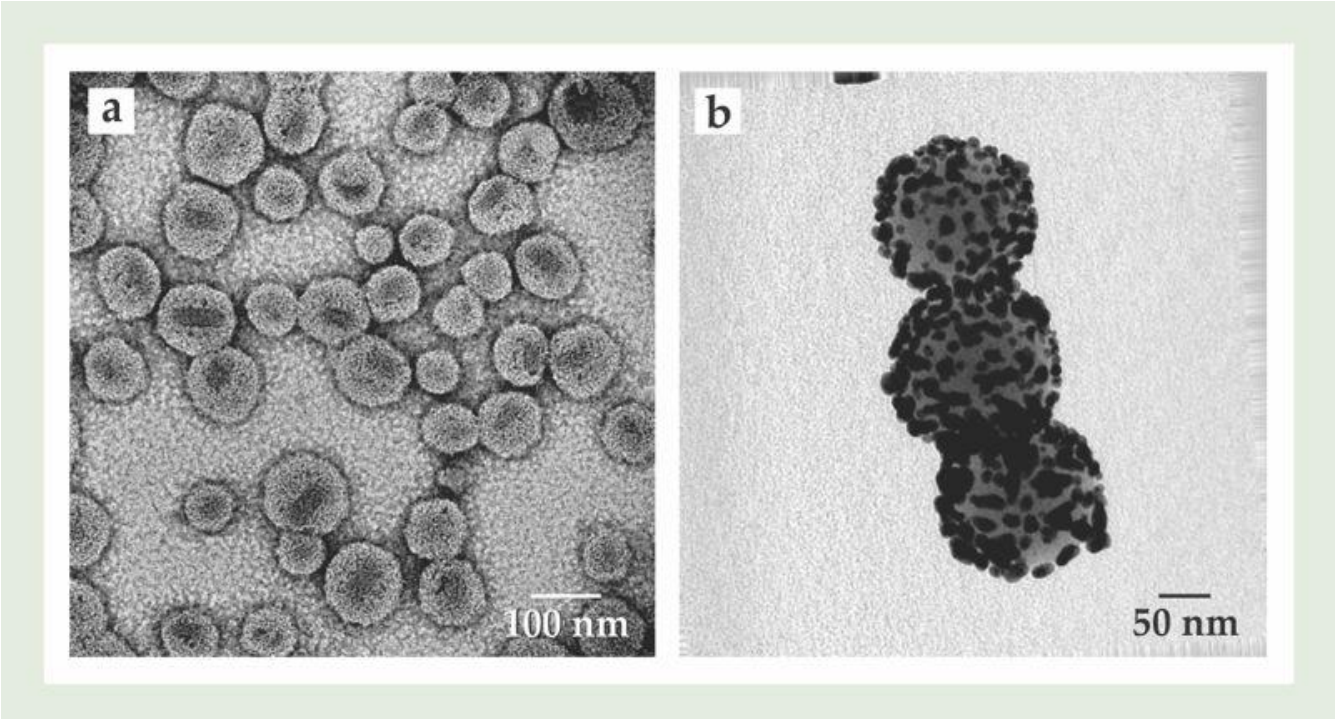

Figure 5. Electron micrographs *of* ***(a)*** *Doxil and* ***(b)*** *an early batch of the material that would eventually become AuroShell. Electron microscopy is a useful tool for visualizing nanomaterials too small to be seen by light microscopy, but because it shows only a small number of particles 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.)*

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- 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.

ncl.cancer.gov

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NCL Nanotechnology Characterization Laboratory

ABOUT THE NCL WORKING WITH THE NCL RESOURCES

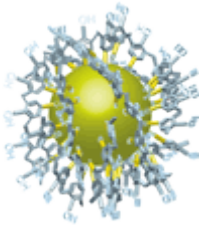
- Anticipating an increase in submissions to FDA of products that involve the application of nanotechnology, the then acting commissioner launched the Nanotechnology Task Force (NTF) in 2006.

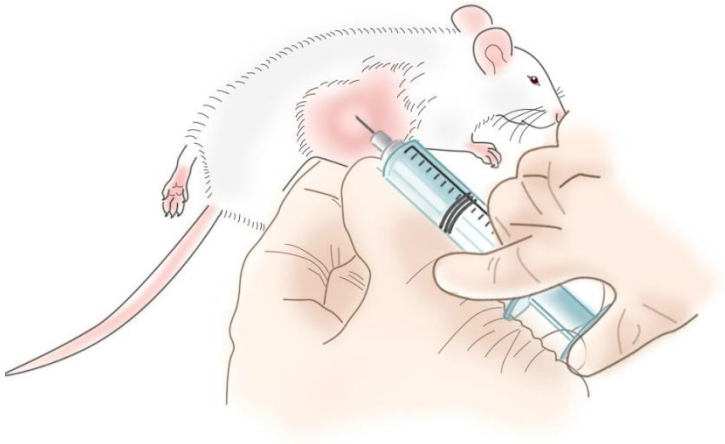
- These advances have resulted in a gradual increase of submission of products containing nanotechnology to FDA, over 600 drug products to date, many approved for clinical use.

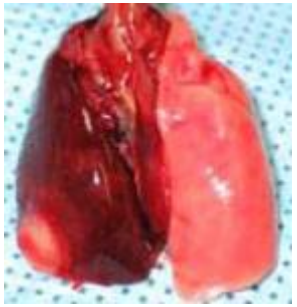
https://www.fda.gov/science-research/fda-grand-rounds/nanotechnology-over-decade-progress-and-innovation-fda-08132020-08132020

Case study

- 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.

Particles' polymer coatings

- 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 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.

Costs

- 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.

Costs

- On the other hand, nanotechnology has the potential to lower R&D costs through nanotech reformulation of discontinued drugs.

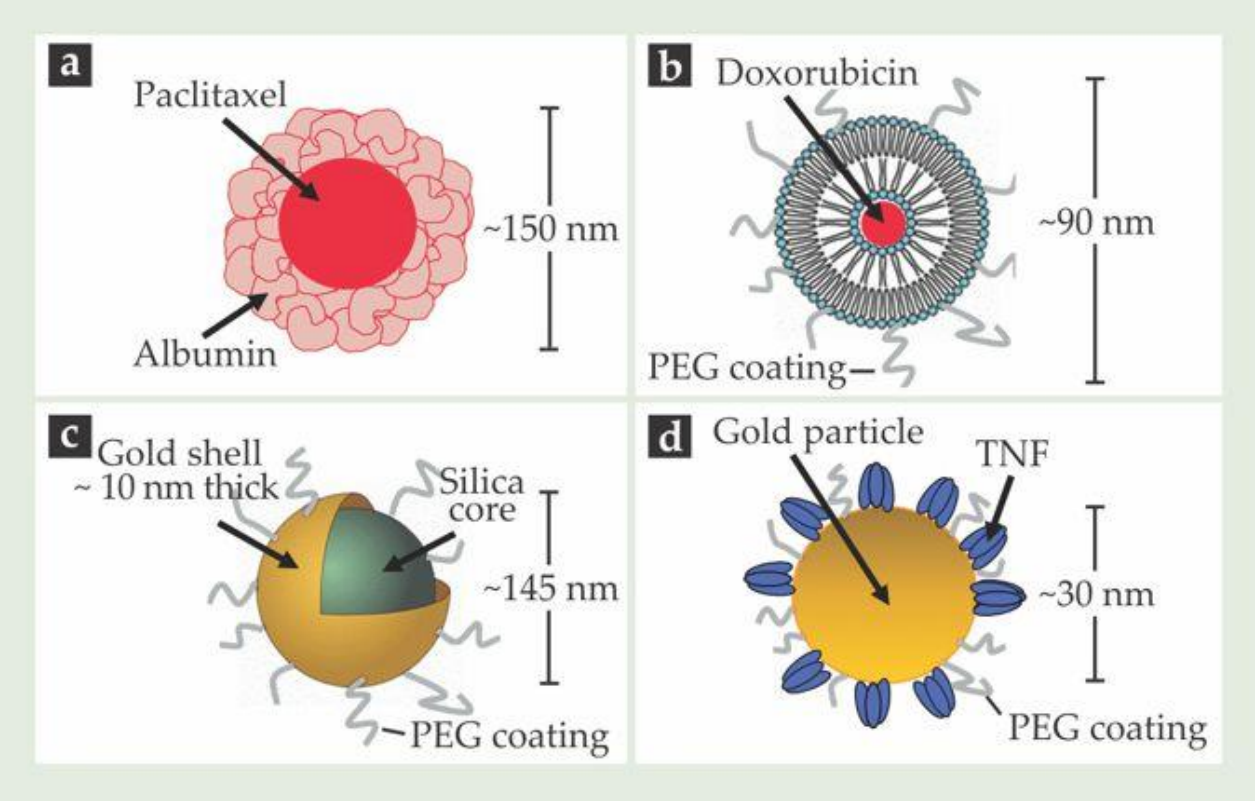
- Nanotechnology offers drug companies an opportunity to reformulate discontinued drugs and recoup some of the cost. Desirable properties can be enhanced in nanotech formulations, while adverse properties can be engineered out.

Costs

- 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.



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Safety and Environmental Concerns

- Although the acute toxicity of many nanomaterials appears to be low, studies that evaluate chronic toxicity are still largely missing from the scientific literature.

- For example, nanoparticles in air aggregate rapidly, which affects their rates of sedimentation and lung deposition.