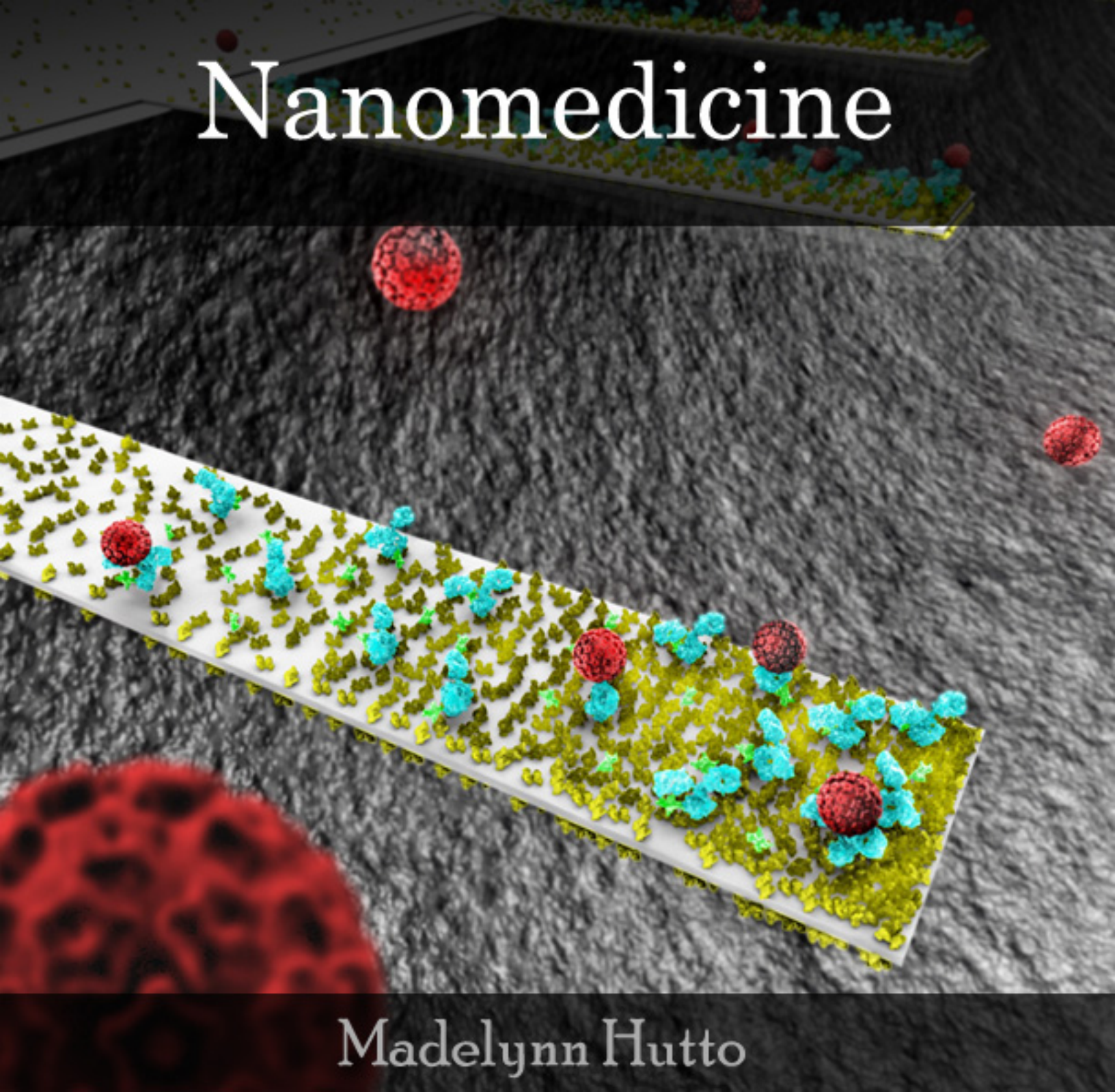
Nanomedicine



Madelynn Hutto

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Chapter 1

Nanomedicine

Nanomedicine is the medical application of nanotechnology. Nanomedicine ranges from the medical applications of nanomaterials, to nanoelectronic biosensors, and even possible future applications of molecular nanotechnology. Current problems for nanomedicine involve understanding the issues related to toxicity and environmental impact of nanoscale materials.

Nanomedicine research is receiving funding from the US National Institute of Health. Of note is the funding in 2005 of a five-year plan to set up four nanomedicine centers. In April 2006, the journal Nature Materials estimated that 130 nanotech-based drugs and delivery systems were being developed worldwide.

Overview

Nanomedicine seeks to deliver a valuable set of research tools and clinically useful devices in the near future. The National Nanotechnology Initiative expects new commercial applications in the pharmaceutical industry that may include advanced drug delivery systems, new therapies, and in vivo imaging. Neuro-electronic interfaces and other nanoelectronics-based sensors are another active goal of research. Further down the line, the speculative field of molecular nanotechnology believes that cell repair machines could revolutionize medicine and the medical field.

Nanomedicine is a large industry, with nanomedicine sales reaching 6.8 billion dollars in 2004, and with over 200 companies and 38 products worldwide, a minimum of 3.8 billion dollars in nanotechnology R&D is being invested every year. As the nanomedicine industry continues to grow, it is expected to have a significant impact on the economy.

Medical use of nanomaterials

Drug delivery

Nanomedical approaches to drug delivery center on developing nanoscale particles or molecules to improve drug bioavailability. Bioavailability refers to the presence of drug

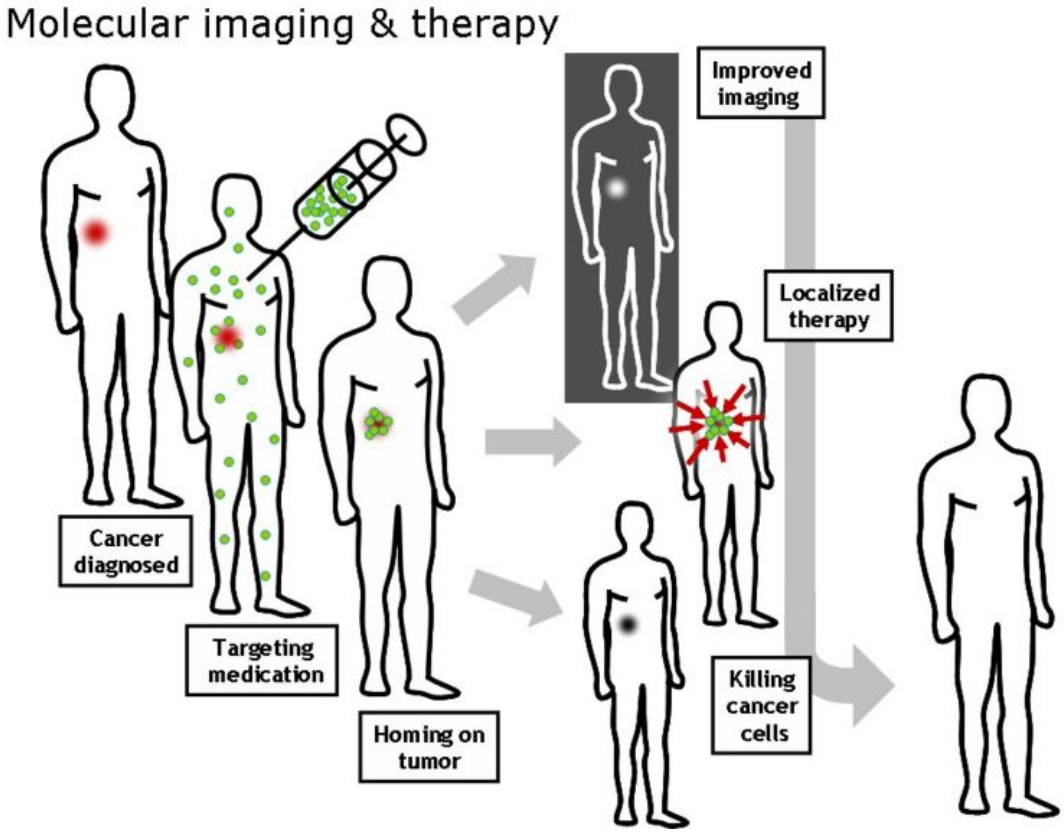
molecules where they are needed in the body and where they will do the most good. Drug delivery focuses on maximizing bioavailability both at specific places in the body and over a period of time. This can potentially be achieved by molecular targeting by nanoengineered devices. It is all about targeting the molecules and delivering drugs with cell precision. More than $65 billion are wasted each year due to poor bioavailability. *In vivo* imaging is another area where tools and devices are being developed. Using nanoparticle contrast agents, images such as ultrasound and MRI have a favorable distribution and improved contrast. The new methods of nanoengineered materials that are being developed might be effective in treating illnesses and diseases such as cancer. What nanoscientists will be able to achieve in the future is beyond current imagination. This might be accomplished by self assembled biocompatible nanodevices that will detect, evaluate, treat and report to the clinical doctor automatically.

Drug delivery systems, lipid- or polymer-based nanoparticles, can be designed to improve the pharmacological and therapeutic properties of drugs. The strength of drug delivery systems is their ability to alter the pharmacokinetics and biodistribution of the drug. Nanoparticles have unusual properties that can be used to improve drug delivery. Where larger particles would have been cleared from the body, cells take up these nanoparticles because of their size. Complex drug delivery mechanisms are being developed, including the ability to get drugs through cell membranes and into cell cytoplasm. Efficiency is important because many diseases depend upon processes within the cell and can only be impeded by drugs that make their way into the cell. Triggered response is one way for drug molecules to be used more efficiently. Drugs are placed in the body and only activate on encountering a particular signal. For example, a drug with poor solubility will be replaced by a drug delivery system where both hydrophilic and hydrophobic environments exist, improving the solubility. Also, a drug may cause tissue damage, but with drug delivery, regulated drug release can eliminate the problem. If a drug is cleared too quickly from the body, this could force a patient to use high doses, but with drug delivery systems clearance can be reduced by altering the pharmacokinetics of the drug. Poor biodistribution is a problem that can affect normal tissues through widespread distribution, but the particulates from drug delivery systems lower the volume of distribution and reduce the effect on non-target tissue. Potential nanodrugs will work by very specific and well-understood mechanisms; one of the major impacts of nanotechnology and nanoscience will be in leading development of completely new drugs with more useful behavior and less side effects.

Protein and peptide delivery

Protein and peptides exert multiple biological actions in human body and they have been identified as showing great promise for treatment of various diseases and disorders. These macromolecules are called biopharmaceuticals. Targeted and/or controlled delivery of these biopharmaceuticals using nanomaterials like nanoparticles and Dendrimers is an emerging field called nanobiopharmaceutics, and these products are called nanobiopharmaceuticals.

Cancer



A schematic illustration showing how nanoparticles or other cancer drugs might be used to treat cancer.

The small size of nanoparticles endows them with properties that can be very useful in oncology, particularly in imaging. Quantum dots (nanoparticles with quantum confinement properties, such as size-tunable light emission), when used in conjunction with MRI (magnetic resonance imaging), can produce exceptional images of tumor sites. These nanoparticles are much brighter than organic dyes and only need one light source for excitation. This means that the use of fluorescent quantum dots could produce a higher contrast image and at a lower cost than today's organic dyes used as contrast media. The downside, however, is that quantum dots are usually made of quite toxic elements.

Another nanoproperty, high surface area to volume ratio, allows many functional groups to be attached to a nanoparticle, which can seek out and bind to certain tumor cells. Additionally, the small size of nanoparticles (10 to 100 nanometers), allows them to preferentially accumulate at tumor sites (because tumors lack an effective lymphatic drainage system). A very exciting research question is how to make these imaging nanoparticles do more things for cancer. For instance, is it possible to manufacture multifunctional nanoparticles that would detect, image, and then proceed to treat a tumor? This question is under vigorous investigation; the answer to which could shape the future

of cancer treatment. A promising new cancer treatment that may one day replace radiation and chemotherapy is edging closer to human trials. Kanzius RF therapy attaches microscopic nanoparticles to cancer cells and then "cooks" tumors inside the body with radio waves that heat only the nanoparticles and the adjacent (cancerous) cells.

Sensor test chips containing thousands of nanowires, able to detect proteins and other biomarkers left behind by cancer cells, could enable the detection and diagnosis of cancer in the early stages from a few drops of a patient's blood.

The basic point to use drug delivery is based upon three facts: a) efficient encapsulation of the drugs, b) successful delivery of said drugs to the targeted region of the body, and c) successful release of that drug there.

Researchers at Rice University under Prof. Jennifer West, have demonstrated the use of 120 nm diameter nanoshells coated with gold to kill cancer tumors in mice. The nanoshells can be targeted to bond to cancerous cells by conjugating antibodies or peptides to the nanoshell surface. By irradiating the area of the tumor with an infrared laser, which passes through flesh without heating it, the gold is heated sufficiently to cause death to the cancer cells.

Nanoparticles of cadmium selenide (quantum dots) glow when exposed to ultraviolet light. When injected, they seep into cancer tumors. The surgeon can see the glowing tumor, and use it as a guide for more accurate tumor removal.

In photodynamic therapy, a particle is placed within the body and is illuminated with light from the outside. The light gets absorbed by the particle and if the particle is metal, energy from the light will heat the particle and surrounding tissue. Light may also be used to produce high energy oxygen molecules which will chemically react with and destroy most organic molecules that are next to them (like tumors). This therapy is appealing for many reasons. It does not leave a “toxic trail” of reactive molecules throughout the body (chemotherapy) because it is directed where only the light is shined and the particles exist. Photodynamic therapy has potential for a noninvasive procedure for dealing with diseases, growth and tumors.

Surgery

At Rice University, a flesh welder is used to fuse two pieces of chicken meat into a single piece. The two pieces of chicken are placed together touching. A greenish liquid containing gold-coated nanoshells is dribbled along the seam. An infrared laser is traced along the seam, causing the two sides to weld together. This could solve the difficulties and blood leaks caused when the surgeon tries to restitch the arteries that have been cut during a kidney or heart transplant. The flesh welder could weld the artery perfectly.

Visualization

Tracking movement can help determine how well drugs are being distributed or how substances are metabolized. It is difficult to track a small group of cells throughout the body, so scientists used to dye the cells. These dyes needed to be excited by light of a certain wavelength in order for them to light up. While different color dyes absorb different frequencies of light, there was a need for as many light sources as cells. A way around this problem is with luminescent tags. These tags are quantum dots attached to proteins that penetrate cell membranes. The dots can be random in size, can be made of bio-inert material, and they demonstrate the nanoscale property that color is size-dependent. As a result, sizes are selected so that the frequency of light used to make a group of quantum dots fluoresce is an even multiple of the frequency required to make another group incandesce. Then both groups can be lit with a single light source.

Nanoparticle targeting

It is greatly observed that nanoparticles are promising tools for the advancement of drug delivery, medical imaging, and as diagnostic sensors. However, the biodistribution of these nanoparticles is mostly unknown due to the difficulty in targeting specific organs in the body. Current research in the excretory systems of mice, however, shows the ability of gold composites to selectively target certain organs based on their size and charge. These composites are encapsulated by a dendrimer and assigned a specific charge and size. Positively-charged gold nanoparticles were found to enter the kidneys while negatively-charged gold nanoparticles remained in the liver and spleen. It is suggested that the positive surface charge of the nanoparticle decreases the rate of osponization of nanoparticles in the liver, thus affecting the excretory pathway. Even at a relatively small size of 5 nm , though, these particles can become compartmentalized in the peripheral tissues, and will therefore accumulate in the body over time. While advancement of research proves that targeting and distribution can be augmented by nanoparticles, the dangers of nanotoxicity become an important next step in further understanding of their medical uses.

Neuro-electronic interfaces

Neuro-electronic interfacing is a visionary goal dealing with the construction of nanodevices that will permit computers to be joined and linked to the nervous system. This idea requires the building of a molecular structure that will permit control and detection of nerve impulses by an external computer. The computers will be able to interpret, register, and respond to signals the body gives off when it feels sensations. The demand for such structures is huge because many diseases involve the decay of the nervous system (ALS and multiple sclerosis). Also, many injuries and accidents may impair the nervous system resulting in dysfunctional systems and paraplegia. If computers could control the nervous system through neuro-electronic interface, problems that impair the system could be controlled so that effects of diseases and injuries could be overcome. Two considerations must be made when selecting the power source for such applications. They are refuelable and nonrefuelable strategies. A refuelable strategy

implies energy is refilled continuously or periodically with external sonic, chemical, tethered, magnetic, or electrical sources. A nonrefuelable strategy implies that all power is drawn from internal energy storage which would stop when all energy is drained.

One limitation to this innovation is the fact that electrical interference is a possibility. Electric fields, electromagnetic pulses (EMP), and stray fields from other *in vivo* electrical devices can all cause interference. Also, thick insulators are required to prevent electron leakage, and if high conductivity of the *in vivo* medium occurs there is a risk of sudden power loss and "shorting out." Finally, thick wires are also needed to conduct substantial power levels without overheating. Little practical progress has been made even though research is happening. The wiring of the structure is extremely difficult because they must be positioned precisely in the nervous system so that it is able to monitor and respond to nervous signals. The structures that will provide the interface must also be compatible with the body's immune system so that they will remain unaffected in the body for a long time. In addition, the structures must also sense ionic currents and be able to cause currents to flow backward. While the potential for these structures is amazing, there is no timetable for when they will be available.

Medical applications of molecular nanotechnology

Molecular nanotechnology is a speculative subfield of nanotechnology regarding the possibility of engineering molecular assemblers, machines which could re-order matter at a molecular or atomic scale. Molecular nanotechnology is highly theoretical, seeking to anticipate what inventions nanotechnology might yield and to propose an agenda for future inquiry. The proposed elements of molecular nanotechnology, such as molecular assemblers and nanorobots are far beyond current capabilities.

Nanorobots

The somewhat speculative claims about the possibility of using nanorobots in medicine, advocates say, would totally change the world of medicine once it is realized. Nanomedicine would make use of these nanorobots (e.g., Computational Genes), introduced into the body, to repair or detect damages and infections. According to Robert Freitas of the Institute for Molecular Manufacturing, a typical blood borne medical nanorobot would be between 0.5-3 micrometres in size, because that is the maximum size possible due to capillary passage requirement. Carbon could be the primary element used to build these nanorobots due to the inherent strength and other characteristics of some forms of carbon (diamond/fullerene composites), and nanorobots would be fabricated in desktop nanofactories specialized for this purpose.

Nanodevices could be observed at work inside the body using MRI, especially if their components were manufactured using mostly ^{13}C atoms rather than the natural ^{12}C isotope of carbon, since ^{13}C has a nonzero nuclear magnetic moment. Medical nanodevices would first be injected into a human body, and would then go to work in a specific organ or tissue mass. The doctor will monitor the progress, and make certain that the nanodevices have gotten to the correct target treatment region. The doctor will also be

able to scan a section of the body, and actually see the nanodevices congregated neatly around their target (a tumor mass, etc.) so that he or she can be sure that the procedure was successful.

Cell repair machines

Using drugs and surgery, doctors can only encourage tissues to repair themselves. With molecular machines, there will be more direct repairs. Cell repair will utilize the same tasks that living systems already prove possible. Access to cells is possible because biologists can stick needles into cells without killing them. Thus, molecular machines are capable of entering the cell. Also, all specific biochemical interactions show that molecular systems can recognize other molecules by touch, build or rebuild every molecule in a cell, and can disassemble damaged molecules. Finally, cells that replicate prove that molecular systems can assemble every system found in a cell. Therefore, since nature has demonstrated the basic operations needed to perform molecular-level cell repair, in the future, nanomachine based systems will be built that are able to enter cells, sense differences from healthy ones and make modifications to the structure.

The healthcare possibilities of these cell repair machines are impressive. Comparable to the size of viruses or bacteria, their compact parts would allow them to be more complex. The early machines will be specialized. As they open and close cell membranes or travel through tissue and enter cells and viruses, machines will only be able to correct a single molecular disorder like DNA damage or enzyme deficiency. Later, cell repair machines will be programmed with more abilities with the help of advanced AI systems.

Nanocomputers will be needed to guide these machines. These computers will direct machines to examine, take apart, and rebuild damaged molecular structures. Repair machines will be able to repair whole cells by working structure by structure. Then by working cell by cell and tissue by tissue, whole organs can be repaired. Finally, by working organ by organ, health is restored to the body. Cells damaged to the point of inactivity can be repaired because of the ability of molecular machines to build cells from scratch. Therefore, cell repair machines will free medicine from reliance on self repair alone.

Nanonephrology

Nanonephrology is a branch of nanomedicine and nanotechnology that deals with 1) the study of kidney protein structures at the atomic level; 2) nano-imaging approaches to study cellular processes in kidney cells; and 3) nano medical treatments that utilize nanoparticles and to treat various kidney diseases. The creation and use of materials and devices at the molecular and atomic levels that can be used for the diagnosis and therapy of renal diseases is also a part of Nanonephrology that will play a role in the management of patients with kidney disease in the future. Advances in Nanonephrology will be based on discoveries in the above areas that can provide nano-scale information on the cellular molecular machinery involved in normal kidney processes and in pathological states. By understanding the physical and chemical properties of proteins and other macromolecules

at the atomic level in various cells in the kidney, novel therapeutic approaches can be designed to combat major renal diseases. The nano-scale artificial kidney is a goal that many physicians dream of. Nano-scale engineering advances will permit programmable and controllable nano-scale robots to execute curative and reconstructive procedures in the human kidney at the cellular and molecular levels. Designing nanostructures compatible with the kidney cells and that can safely operate in vivo is also a future goal. The ability to direct events in a controlled fashion at the cellular nano-level has the potential of significantly improving the lives of patients with kidney diseases.

Chapter 2

Nanotoxicology

Nanotoxicology is the study of the toxicity of nanomaterials. Because of quantum size effects and large surface area to volume ratio, nanomaterials have unique properties compared with their larger counterparts.

Nanotoxicology is a branch of bionanoscience which deals with the study and application of toxicity of nanomaterials. Nanomaterials, even when made of inert elements like gold, become highly active at nanometer dimensions. Nanotoxicological studies are intended to determine whether and to what extent these properties may pose a threat to the environment and to human beings. For instance, Diesel nanoparticles have been found to damage the cardiovascular system in a mouse model.

Human health and safety

Calls for tighter regulation of nanotechnology have arisen alongside a growing debate related to the human health and safety risks associated with nanotechnology. The Royal Society identifies the potential for nanoparticles to penetrate the skin, and recommends that the use of nanoparticles in cosmetics be conditional upon a favorable assessment by the relevant European Commission safety advisory committee. Andrew Maynard also reports that ‘certain nanoparticles may move easily into sensitive lung tissues after inhalation, and cause damage that can lead to chronic breathing problems’.

Carbon nanotubes – characterized by their microscopic size and incredible tensile strength – are frequently likened to asbestos, due to their needle-like fiber shape. In a recent study that introduced carbon nanotubes into the abdominal cavity of mice, results demonstrated that long thin carbon nanotubes showed the same effects as long thin asbestos fibers, raising concerns that exposure to carbon nanotubes may lead to mesothelioma (cancer of the lining of the lungs caused by exposure to asbestos). Given these risks, effective and rigorous regulation has been called for to determine if, and under what circumstances, carbon nanotubes are manufactured, as well as ensuring their safe handling and disposal.

The Woodrow Wilson Centre's Project on Emerging Technologies conclude that there is insufficient funding for human health and safety research, and as a result there is currently limited understanding of the human health and safety risks associated with nanotechnology. While the US National Nanotechnology Initiative reports that around four percent (about $40 million) is dedicated to risk related research and development, the Woodrow Wilson Centre estimate that only around $11 million is actually directed towards risk related research. They argued in 2007 that it would be necessary to increase funding to a minimum of $50 million in the following two years so as to fill the gaps in knowledge in these areas.

The potential for workplace exposure was highlighted by the 2004 Royal Society report which recommended a review of existing regulations to assess and control workplace exposure to nanoparticles and nanotubes. The report expressed particular concern for the inhalation of large quantities of nanoparticles by workers involved in the manufacturing process.

Stakeholders concerned by the lack of a regulatory framework to assess and control risks associated with the release of nanoparticles and nanotubes have drawn parallels with bovine spongiform encephalopathy ('mad cow's disease'), thalidomide, genetically modified food, nuclear energy, reproductive technologies, biotechnology, and asbestosis. In light of such concerns, the Canadian based ETC Group have called for a moratorium on nano-related research until comprehensive regulatory frameworks are developed that will ensure workplace safety.

California

In October 2008, the Department of Toxic Substances Control (DTSC), within the California Environmental Protection Agency, announced its intent to request information regarding analytical test methods, fate and transport in the environment, and other relevant information from manufacturers of carbon nanotubes. The term "manufacturers" includes persons and businesses that produce nanotubes in California, or import carbon nanotubes into California for sale. This information request is meant to identify information gaps and to develop further knowledge about the health and safety of carbon nanotubes.

DTSC is exercising its authority under California Health and Safety Code, Chapter 699, sections 57018-57020. These sections were added as a result of the adoption of Assembly Bill AB 289 (2006). They are intended to make information on the fate and transport, detection and analysis, and other information on chemicals more available. The law places the responsibility to provide this information to the Department on those who manufacture or import the chemicals. On January 22, 2009, a formal information request letter was sent to manufacturers who produce or import carbon nanotubes in California, or who may export carbon nanotubes into the State. This letter constitutes the first formal implementation of the authorities placed into statute by AB 289 (2006) and is directed to manufacturers of carbon nanotubes, both industry and academia within the State, and to

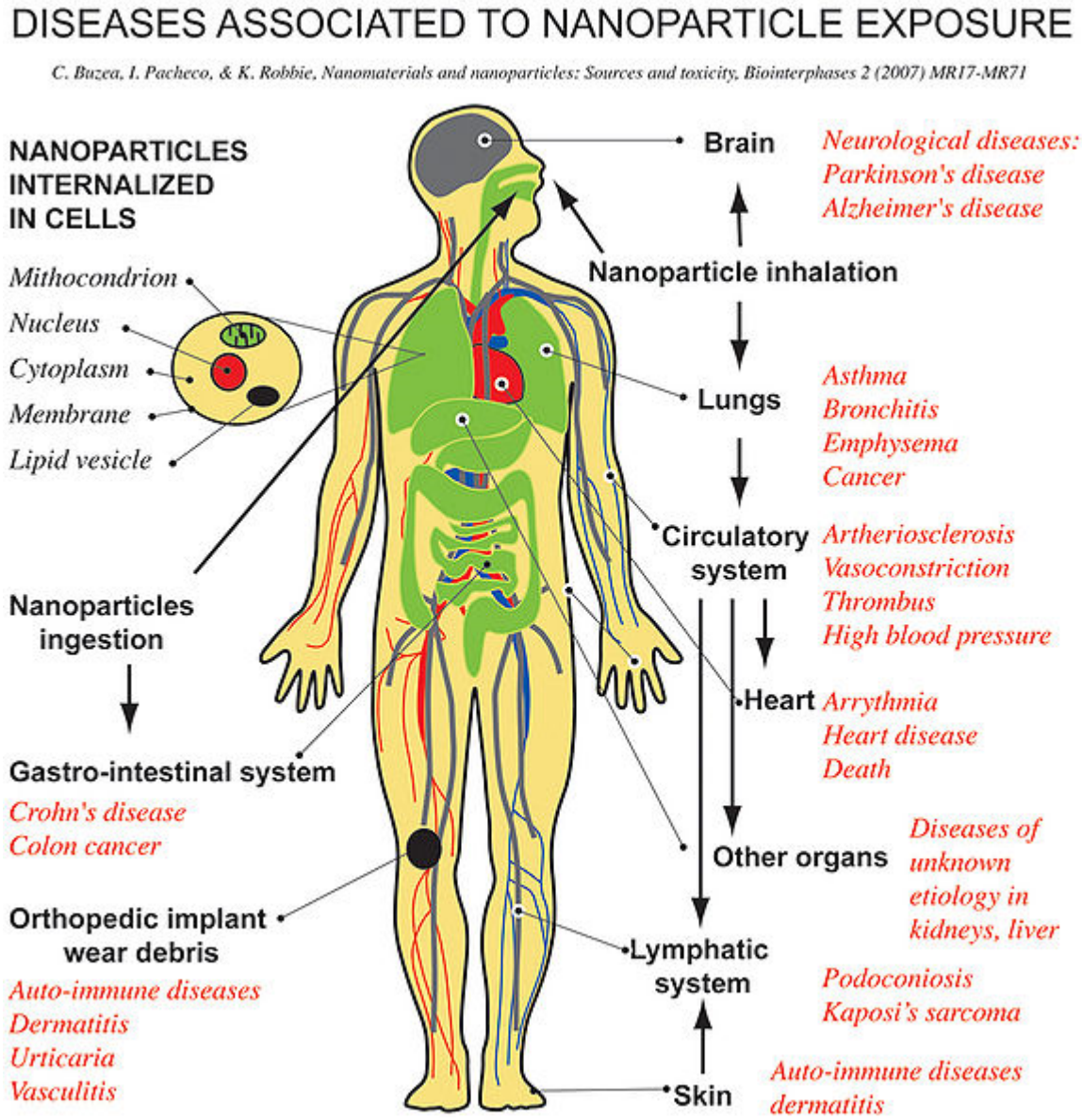
manufacturers outside California who export carbon nanotubes to California. This request for information must be met by the manufacturers within one year.

On January 22, 2010, California manufacturers and importers of carbon nanotubes were required to submit their responses. On January 25, 2010, DTSC posted the responses received to date along with a list of companies who failed to respond to the information request. On February 16, 2010, DTSC issued a follow-up letter to the companies that failed to submit a response. View the responses received for the carbon nanotube call-in.

DTSC is indicating interest in expanding the Specific Chemical Information Call-in to members of the brominated flame retardants, members of the methyl siloxanes, and other nanometals and nanometal oxides such as vanadium oxide, aluminum oxide, silicon dioxide, titanium dioxide, zinc oxide, cerium oxide, nano platinum, nano silver, and nano zerovalent iron. DTSC is also planning to include quantum dots, ocean plastics, and nanoclay into the list of chemicals of interest.

Toxicology of nanoparticles

Background



Pathways of exposure to nanoparticles and associated diseases as suggested by epidemiological, in vivo and in vitro studies.

Nanotoxicology is a sub-specialty of particle toxicology. It addresses the toxicology of nanoparticles (particles <100 nm diameter) which appear to have toxicity effects that are unusual and not seen with larger particles. Nanoparticles can be divided into combustion-derived nanoparticles (like diesel soot), manufactured nanoparticles like carbon nanotubes and naturally occurring nanoparticles from volcanic eruptions, atmospheric chemistry etc. Typical nanoparticles that have been studied are titanium dioxide, alumina,

zinc oxide, carbon black, and carbon nanotubes, and "nano-C_{60}". Nanoparticles have much larger surface area to unit mass ratios which in some cases may lead to greater pro-inflammatory effects (in, for example, lung tissue). In addition, some nanoparticles seem to be able to translocate from their site of deposition to distant sites such as the blood and the brain. This has resulted in a sea-change in how particle toxicology is viewed- instead of being confined to the lungs, nanoparticle toxicologists study the brain, blood, liver, skin and gut. Nanotoxicology has revolutionised particle toxicology and rejuvenated it.

Reactive oxygen species

For some types of particles, the smaller they are, the greater their surface area to volume ratio and the higher their chemical reactivity and biological activity. The greater chemical reactivity of nanomaterials can result in increased production of reactive oxygen species (ROS), including free radicals. ROS production has been found in a diverse range of nanomaterials including carbon fullerenes, carbon nanotubes and nanoparticle metal oxides. ROS and free radical production is one of the primary mechanisms of nanoparticle toxicity; it may result in oxidative stress, inflammation, and consequent damage to proteins, membranes and DNA.

Biodistribution

The extremely small size of nanomaterials also means that they much more readily gain entry into the human body than larger sized particles. How these nanoparticles behave inside the body is still a major question that needs to be resolved. The behavior of nanoparticles is a function of their size, shape and surface reactivity with the surrounding tissue. In principle, a large number of particles could overload the body's phagocytes, cells that ingest and destroy foreign matter, thereby triggering stress reactions that lead to inflammation and weaken the body’s defense against other pathogens. In addition to questions about what happens if non-degradable or slowly degradable nanoparticles accumulate in bodily organs, another concern is their potential interaction or interference with biological processes inside the body. Because of their large surface area, nanoparticles will, on exposure to tissue and fluids, immediately adsorb onto their surface some of the macromolecules they encounter. This may, for instance, affect the regulatory mechanisms of enzymes and other proteins.

Nanomaterials are able to cross biological membranes and access cells, tissues and organs that larger-sized particles normally cannot. Nanomaterials can gain access to the blood stream via inhalation or ingestion. At least some nanomaterials can penetrate the skin; even larger microparticles may penetrate skin when it is flexed. Broken skin is an ineffective particle barrier, suggesting that acne, eczema, shaving wounds or severe sunburn may accelerate skin uptake of nanomaterials. Then, once in the blood stream, nanomaterials can be transported around the body and be taken up by organs and tissues, including the brain, heart, liver, kidneys, spleen, bone marrow and nervous system. Nanomaterials have proved toxic to human tissue and cell cultures, resulting in increased oxidative stress, inflammatory cytokine production and cell death. Unlike larger particles, nanomaterials may be taken up by cell mitochondria and the cell nucleus. Studies

demonstrate the potential for nanomaterials to cause DNA mutation and induce major structural damage to mitochondria, even resulting in cell death.

Nanotoxicity studies

Since there is no authority to regulate nanotech-based products, there are many products that could possibly be dangerous to humans. Scientific research has indicated the potential for some nanomaterials to be toxic to humans or the environment. In March 2004 tests conducted by environmental toxicologist Eva Oberdörster, Ph.D. working with Southern Methodist University in Texas, found extensive brain damage to fish exposed to fullerenes for a period of just 48 hours at a relatively moderate dose of 0.5 parts per million (commensurate with levels of other kinds of pollution found in bays). The fish also exhibited changed gene markers in their livers, indicating their entire physiology was affected. In a concurrent test, the fullerenes killed water fleas, an important link in the marine food chain. The extremely small size of fabricated nanomaterials also means that they are much more readily taken up by living tissue than presently known toxins. Nanoparticles can be inhaled, swallowed, absorbed through skin and deliberately or accidentally injected during medical procedures. They might be accidentally or inadvertently released from materials implanted into living tissue.

Researcher Shosaku Kashiwada of the National Institute for Environmental Studies in Tsukuba, Japan, in a more recent study, intended to further investigate the effects of nanoparticles on soft-bodied organisms. His study allowed him to explore the distribution of water-suspended fluorescent nanoparticles throughout the eggs and adult bodies of a species of fish, known as the see-through medaka (Oryzias latipes). See-through medaka were used because of their small size, wide temperature and salinity tolerances, and short generation time. Moreover, small fish like the see-through medaka have been popular test subjects for human diseases and organogenesis for other reasons as well, including their transparent embryos, rapid embryo development, and the functional equivalence of their organs and tissue material to that of mammals. Because the see-through medaka have transparent bodies, analyzing the deposition of fluorescent nanoparticles throughout the body is quite simple. For his study, Dr. Kashiwada evaluated four aspects of nanoparticle accumulation. These included the overall accumulation and the size-dependent accumulation of nanoparticles by medaka eggs, the effects of salinity on the aggregation of nanoparticles in solution and on their accumulation by medaka eggs, and the distribution of nanoparticles in the blood and organs of adult medaka. It was also noted that nanoparticles were in fact taken up into the bloodstream and deposited throughout the body. In the medaka eggs, there was a high accumulation of nanoparticles in the yolk; most often bioavailibility was dependent on specific sizes of the particles. Adult samples of medaka had accumulated nanoparticles in the gills, intestine, brain, testis, liver, and bloodstream. One major result from this study was the fact that salinity may have a large influence on the bioavailibility and toxicity of nanoparticles to penetrate membranes and eventually kill the specimen.

As the use of nanomaterials increases worldwide, concerns for worker and user safety are mounting. To address such concerns, the Swedish Karolinska Institute conducted a study

in which various nanoparticles were introduced to human lung epithelial cells. The results, released in 2008, showed that iron oxide nanoparticles caused little DNA damage and were non-toxic. Zinc oxide nanoparticles were slightly worse. Titanium dioxide caused only DNA damage. Carbon nanotubes caused DNA damage at low levels. Copper oxide was found to be the worst offender, and was the only nanomaterial identified by the researchers as a clear health risk.

No Fullerene toxicity reported

Nanoparticles can also be made of C_{60}, as is the case with almost any room temperature solid, and several groups have done this and studied toxicity of such particles. The results in the work of Oberdörster at Southern Methodist University, published in "Environmental Health Perspectives" in July 2004, in which questions were raised of potential cytotoxicity, has now been shown by several sources to be likely caused by the tetrahydrofuran used in preparing the 30 nm–100 nm particles of C_{60} used in the research. Isakovic, et al., 2006, who review this phenomenon, gives results showing that removal of THF from the C_{60} particles resulted in a loss of toxicity. Sayes, et al., 2007, also show that particles prepared as in Oberdorster caused no detectable inflammatory response when instilled intratracheally in rats after observation for 3 months, suggesting that even the particles prepared by Oberdorster do not exhibit markers of toxicity in mammalian models. This work used as a benchmark quartz particles, which did give an inflammatory response.

A comprehensive and recent review of work on fullerene toxicity is available in "Toxicity Studies of Fullerenes and Derivatives," a chapter from the book "Bio-applications of Nanoparticles". In this work, the authors review the work on fullerene toxicity beginning in the early 1990s to present, and conclude that the evidence gathered since the discovery of fullerenes overwhelmingly points to C_{60} being non-toxic. As is the case for toxicity profile with any chemical modification of a structural moiety, the authors suggest that individual molecules be assessed individually.

Immunogenicity of nanoparticles

Very little attention has been directed towards the potential immunogenicity of nanostructures. Nanostructures can activate the immune system inducing inflammation, immune responses, allergy, or even affect to the immune cells in a deleterious or beneficial way(immunosuppression in autoimmune diseases, improving immune responses in vaccines). More studies are needed in order to know the potential deleterious or beneficial effects of nanostructures in the immune system. In Comparison to conventional pharmeceutical agents, nanostructures have very large sizes and immune cells, especially phagocytic cells, recognize and try to destroy them.

Complications with nanotoxicity studies

Size is therefore a key factor in determining the potential toxicity of a particle. However it is not the only important factor. Other properties of nanomaterials that influence

toxicity include: chemical composition, shape, surface structure, surface charge, aggregation and solubility, and the presence or absence of functional groups of other chemicals. The large number of variables influencing toxicity means that it is difficult to generalise about health risks associated with exposure to nanomaterials – each new nanomaterial must be assessed individually and all material properties must be taken into account.

In addition, standarization of toxicology tests between laboratories are needed. Díaz, B. et al from the University of Vigo (Spain) has shown (Small, 2008) that many different cell lines should be studied in order to know if a nanostructure induces toxicity, and human cells can internalize aggregated nanoparticles. Moreover, it is important to take into account that many nanostructures aggregate in biological fluids, but groups manufacturing nanostructures do not care much about this matter. Many efforts of interdisciplinary groups are strongly needed in order to progress in this field.

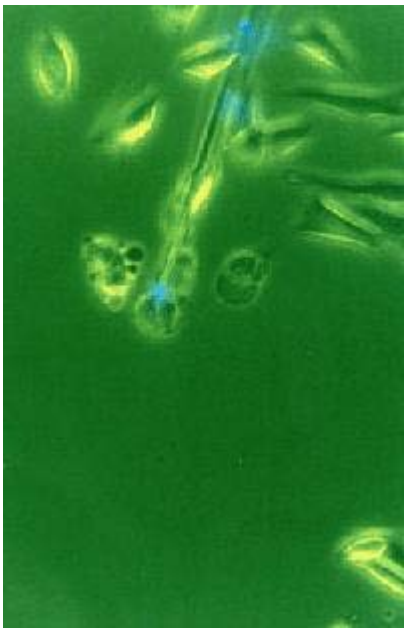
Effect of aggregation/agglomeration of nanoparticles

Many nanoparticles agglomerate or aggregate when they are placed in environmental or biological fluids. The terms agglomeration and aggregation have distinct definitions according to the standards organizations ISO and ASTM, where agglomeration signifies more loosely bound particles and aggregation signifies very tightly bound or fused particles (typically occurring during synthesis or drying). Nanoparticles frequently agglomerate due to the high ionic strength of environmental and biological fluids, which shields the repulsion due to charges on the nanoparticles. Unfortunately, agglomeration has frequently been ignored in nanotoxicity studies, even though agglomeration would be expected to affect nanotoxicity since it changes the size, surface area, and sedimentation properties of the nanoparticles. In addition, many nanoparticles will agglomerate to some extent in the environment or in the body before they reach their target, so it is desirable to study how toxicity is affected by agglomeration.

A method was published that can be used to produce different mean sizes of stable agglomerates of several metal, metal oxide, and polymer nanoparticles in cell culture media for cell toxicity studies. Different mean sizes of agglomerates are produced by allowing the nanoparticles to agglomerate to a particular size in cell culture media without protein, and then adding protein to coat the agglomerates and "freeze" them at that size. By waiting different amounts of time before adding protein, different mean sizes of agglomerates of a single type of nanoparticle can be produced in an otherwise identical solution, allowing one to study how agglomerate size affects toxicity. In addition, it was found that vortexing while adding a high concentration of nanoparticles to the cell culture media produces much less agglomerated nanoparticles than if the dispersed solution is only mixed after adding the nanoparticles.

Chapter 3

Nanosensor



A nanosensor probe carrying a laser beam (blue) penetrates a living cell to detect the presence of a product indicating that the cell has been exposed to a cancer-causing substance.

Nanosensors are any biological, chemical, or surgical sensory points used to convey information about nanoparticles to the macroscopic world. Their use mainly include various medicinal purposes and as gateways to building other nanoproducts, such as computer chips that work at the nanoscale and nanorobots. Presently, there are several ways proposed to make nanosensors, including top-down lithography, bottom-up assembly, and molecular self-assembly.

Predicted applications

Medicinal uses of nanosensors mainly revolve around the potential of nanosensors to accurately identify particular cells or places in the body in need. By measuring changes in volume, concentration, displacement and velocity, gravitational, electrical, and magnetic forces, pressure, or temperature of cells in a body, nanosensors may be able to distinguish between and recognize certain cells, most notably those of cancer, at the molecular level in order to deliver medicine or monitor development to specific places in the body. In addition, they may be able to detect macroscopic variations from outside the body and communicate these changes to other nanoproducts working within the body.

One example of nanosensors involves using the fluorescence properties of cadmium selenide quantum dots as sensors to uncover tumors within the body. By injecting a body with these quantum dots, a doctor could see where a tumor or cancer cell was by finding the injected quantum dots, an easy process because of their fluorescence. Developed nanosensor quantum dots would be specifically constructed to find only the particular cell for which the body was at risk. A downside to the cadmium selenide dots, however, is that they are highly toxic to the body. As a result, researchers are working on developing alternate dots made out of a different, less toxic material while still retaining some of the fluorescence properties. In particular, they have been investigating the particular benefits of zinc sulfide quantum dots which, though they are not quite as fluorescent as cadmium selenide, can be augmented with other metals including manganese and various lanthanide elements. In addition, these newer quantum dots become more fluorescent when they bond to their target cells. (Quantum) Potential predicted functions may also include sensors used to detect specific DNA in order to recognize explicit genetic defects, especially for individuals at high-risk and implanted sensors that can automatically detect glucose levels for diabetic subjects more simply than current detectors. DNA can also serve as sacrificial layer for manufacturing CMOS IC, integrating a nanodevice with sensing capabilities. Therefore, using proteomic patterns and new hybrid materials, nanobiosensors can also be used to enable components configured into a hybrid semiconductor substrate as part of the circuit assembly. The development and miniaturization of nanobiosensors should provide interesting new opportunities.

Other projected products most commonly involve using nanosensors to build smaller integrated circuits, as well as incorporating them into various other commodities made using other forms of nanotechnology for use in a variety of situations including transportation, communication, improvements in structural integrity, and robotics. Nanosensors may also eventually be valuable as more accurate monitors of material states for use in systems where size and weight are constrained, such as in satellites and other aeronautic machines.

Existing nanosensors

Currently, the most common mass-produced functioning nanosensors exist in the biological world as natural receptors of outside stimulation. For instance, sense of smell, especially in animals in which it is particularly strong, such as dogs, functions using

receptors that sense nanosized molecules. Certain plants, too, use nanosensors to detect sunlight; various fish use nanosensors to detect minuscule vibrations in the surrounding water; and many insects detect sex pheromones using nanosensors.

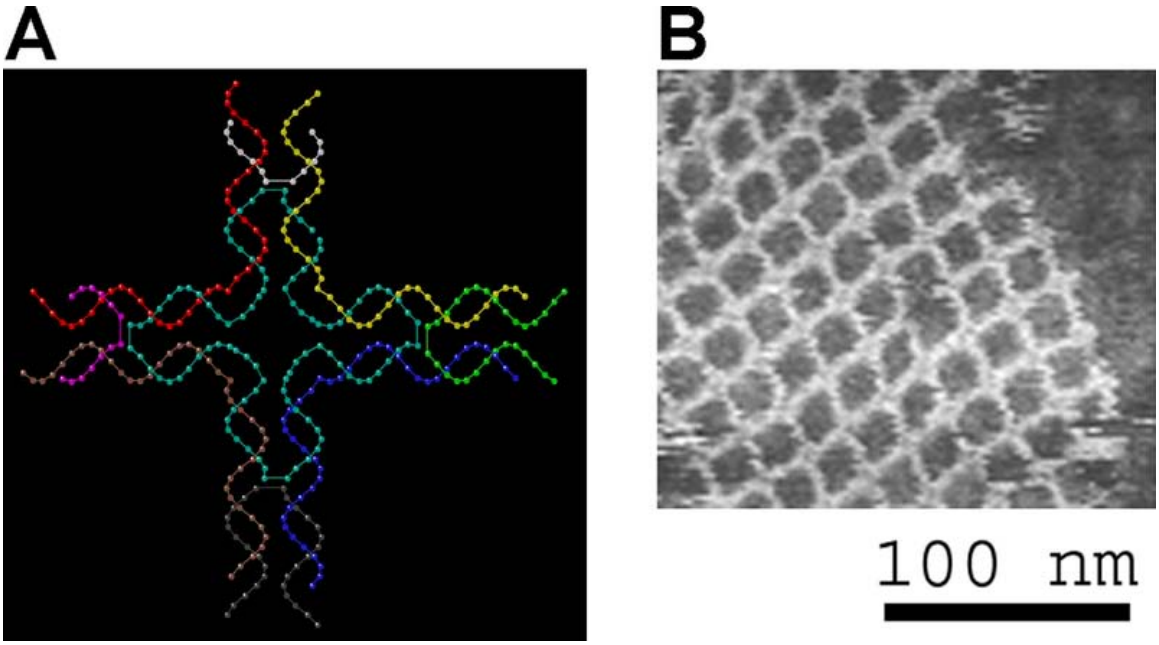
One of the first working examples of a synthetic nanosensor was built by researchers at the Georgia Institute of Technology in 1999. It involved attaching a single particle onto the end of a carbon nanotube and measuring the vibrational frequency of the nanotube both with and without the particle. The discrepancy between the two frequencies allowed the researchers to measure the mass of the attached particle.

Chemical sensors, too, have been built using nanotubes to detect various properties of gaseous molecules. Carbon nanotubes have been used to sense ionization of gaseous molecules while nanotubes made out of titanium have been employed to detect atmospheric concentrations of hydrogen at the molecular level. Many of these involve a system by which nanosensors are built to have a specific pocket for another molecule. When that particular molecule, and only that specific molecule, fits into the nanosensor, and light is shone upon the nanosensor, it will reflect different wavelengths of light and, thus, be a different color.

Production methods

There are currently several hypothesized ways to produce nanosensors. Top-down lithography is the manner in which most integrated circuits are now made. It involves starting out with a larger block of some material and carving out the desired form. These carved out devices, notably put to use in specific microelectromechanical systems used as microsensors, generally only reach the micro size, but the most recent of these have begun to incorporate nanosized components.

Another way to produce nanosensors is through the bottom-up method, which involves assembling the sensors out of even more minuscule components, most likely individual atoms or molecules. This would involve moving atoms of a particular substance one by one into particular positions which, though it has been achieved in laboratory tests using tools such as atomic force microscopes, is still a significant difficulty, especially to do en masse, both for logistic reasons as well as economic ones. Most likely, this process would be used mainly for building starter molecules for self-assembling sensors.



(A) An example of a DNA molecule used as a starter for larger self-assembly. (B) An atomic force microscope image of a self-assembled DNA nanogrid. Individual DNA tiles self-assemble into a highly ordered periodic two-dimensional DNA nanogrid.

The third way, which promises far faster results, involves self-assembly, or "growing" particular nanostructures to be used as sensors. This most often entails one of two types of assembly. The first involves using a piece of some previously created or naturally formed nanostructure and immersing it in free atoms of its own kind. After a given period, the structure, having an irregular surface that would make it prone to attracting more molecules as a continuation of its current pattern, would capture some of the free atoms and continue to form more of itself to make larger components of nanosensors.

The second type of self-assembly starts with an already complete set of components that would automatically assemble themselves into a finished product. Though this has been so far successful only in assembling computer chips at the micro size, researchers hope to eventually be able to do it at the nanometer size for multiple products, including nanosensors. Accurately being able to reproduce this effect for a desired sensor in a laboratory would imply that scientists could manufacture nanosensors much more quickly and potentially far more cheaply by letting numerous molecules assemble themselves with little or no outside influence, rather than having to manually assemble each sensor.

Economic Impacts

Though nanosensor technology is a relatively new field, global projections for sales of products incorporating nanosensors range from $0.6 billion to $2.7 billion in the next three to four years. They will likely be included in most modern circuitry used in advanced computing systems, since their potential to provide the link between other forms of nanotechnology and the macroscopic world allows developers to fully exploit

the potential of nanotechnology to miniaturize computer chips while vastly expanding their storage potential.

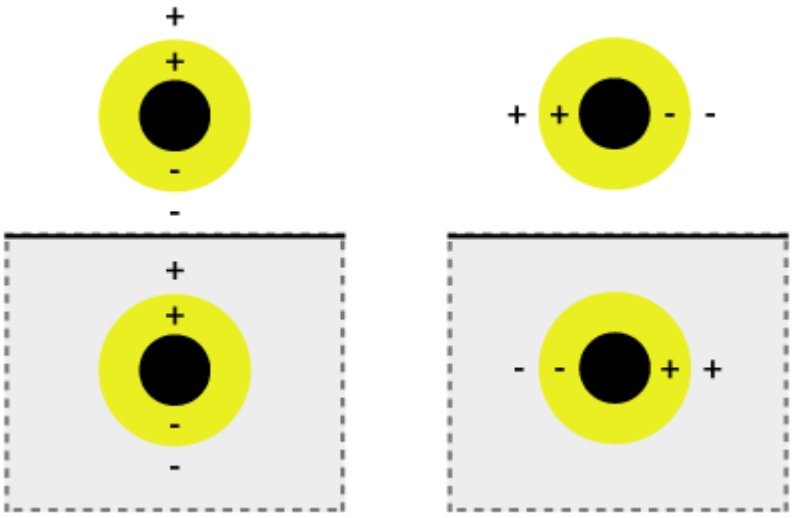
First, however, nanosensor developers must overcome the present high costs of production in order to become worthwhile for implementation in consumer products. Additionally, nanosensor reliability is not yet suitable for widespread use, and, because of their scarcity, nanosensors have yet to be marketed and implemented outside of research facilities. Consequently, nanosensors have yet to be made compatible with most consumer technologies for which they have been projected to eventually enhance.

Social Impacts

Ethical and social impacts are harder to define and sort as good or bad compared to health and environmental impacts. The advancement in detecting and sensing different biological and chemical species with increased capacity and accuracy may transform societal mechanisms that were originally designed on uncertainty and imprecise information. For example, the ability to measure extremely low amounts of air pollutants or toxic materials in water raises questions and dilemmas of risk thresholds especially if the advancement of such technologies outpaces the ability of the public to respond. As another example, medical sensors will not only help in diagnoses and treatment but may also predict the future profile of an individual. This will add to the information used by health insurance companies to grant or deny coverage. Other social issues resulting from the widespread use of nanosensors and surveillance devices include privacy invasion and security issues.

Chapter 4

Nanoshell



A **nanoshell** is a type of spherical nanoparticle consisting of a dielectric core which is covered by a thin metallic shell (usually gold). These nanoshells involve a quasiparticle called plasmon which is a collective excitation or quantum plasma oscillation where the electrons simultaneously oscillate with respect to all the ions.

The simultaneous oscillation can be called plasmon hybridization where the tunability of the oscillation is associated with mixture of the inner and outer shell where they hybridize to give a lower energy or higher energy. This lower energy couples strongly to incident light whereas, the higher energy is an anti-bonding and weakly combines to incident light. The hybridization interaction is stronger for thinner shell layers, hence, the thickness of the shell and overall particle radius determines which wavelength of light it couples with. Nanoshells can be varied across a broad range of the light spectrum that spans the visible and near infrared regions. The interaction of light and nanoparticles affects the placements of charges which affects the coupling strength. Incident light polarized parallel to the substrate gives a s-polarization (Figure 1b), hence the charges are further from the substrate surface which gives a stronger interaction between the shell

and core. Otherwise, a p-polarization is formed which gives a more strongly shifted plasmon energy causing a weaker interaction and coupling.

Synthesis

A nanoshell is synthesized in a multistep process :

1. Obtain silica nanoparticles in a solution (usually tetrachloroauric acid and a reducing agent)

   This solution phase synthesis of the gold nanoparticles uses a reduction using tetrachloroauric acid by a reducing agent. There are several different reducing agents used and all can greatly affect the uniformity of the nanoparticle.

2. Attach a very small seed colloid onto the dielectric nanoparticles (such as: zinc selenide, sapphire, and glass) giving a discontinuous shell
3. Grow a continuous shell by using a chemical reduction of the metal attached to the dielectric nanoparticles

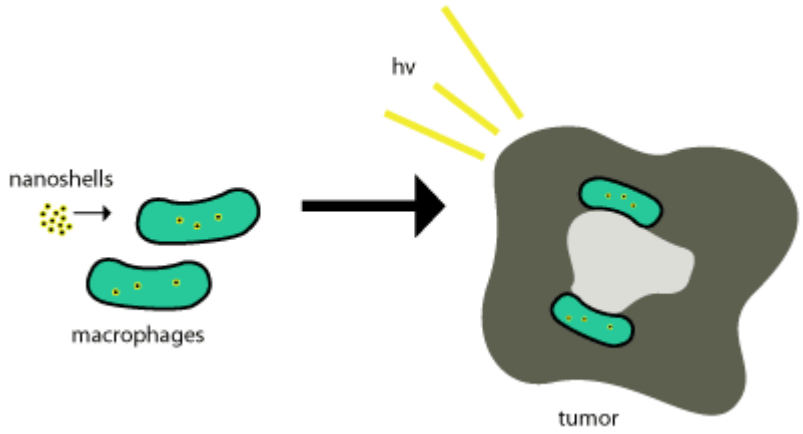
If a uniform shell is not obtained then it can greatly affect the optical properties of the nanoshell. A good example of this is a nanoegg, which is a metallic nanoshell that has a nonuniform thickness. This characteristic nonuniformity causes additional hybridized plasmon resonances in the spectrum making the coupling not as effective.

Applications

Since nanoshells possess highly favorable optical and chemical properties it is often used for biomedical imaging, therapeutic applications, fluorescence enhancement of weak molecular emitters, surface enhanced Raman spectroscopy and surface enhanced infrared absorption spectroscopy.

Cancer Treatment

Gold nanoshells are shuttled into tumors by the use of phagocytosis where phagocytes engulf the nanoshells through the cell membrane to form an internal phagosome, or macrophage. After this it is shuttled into a cell and enzymes are usually used to metabolize it and shuttle it back out of the cell. These nanoshells are not metabolized so for them to be effective they just need to be within the tumor cells and photoinduced cell death is used to terminate the tumor cells. This scheme is shown in Figure 2.



Nanoparticle-base therapeutics have been successfully delivered into tumors by exploiting the enhanced permeability and retention effect, a property that permits nanoscale structures to be taken up passively into tumors with out the assistance of antibodies. Delivery of nanoshells into the important regions of tumors can be very difficult. This is where most nanoshells try to exploit the tumor's natural recruitment of monocytes for delivery as seen in the above figure. This delivery system is called a "Trojan Horse".

This process works so well since tumors are about ¾ macrophages and once monocytes are brought into the tumor, it differentiates into macrophages which would also be need to maintain the cargo nanoparticles. Once the nanoshells are at the necrotic center, near-infrared illumination is used to destroy the tumor associated macrophages.

Chapter 5

Carbon Nanotubes in Medicine

Carbon nanotubes (CNTs) are very prevalent in today's world of medical research and are being highly researched in the fields of efficient drug delivery and biosensing methods for disease treatment and health monitoring. Carbon nanotube technology has shown to have the potential to alter drug delivery and biosensing methods for the better, and thus, carbon nanotubes have recently garnered interest in the field of medicine.

The use of CNTs in drug delivery and biosensing technology has the potential to revolutionalize medicine. Functionalization of SWNTs has proven to enhance solubility and allow for efficient tumor targeting/drug delivery. It prevents SWNTs from being cytotoxic and altering the function of immune cells.

Cancer, a group of diseases in which cells grow and divide abnormally, is one of the primary diseases being looked at with regards to how it responds to CNT drug delivery. Current cancer therapy primarily involves surgery, radiation therapy, and chemotherapy. These methods of treatment are usually painful and kill normal cells in addition to producing adverse side effects. CNTs as drug delivery vehicles have shown potential in targeting specific cancer cells with a dosage lower than conventional drugs used , that is just as effective in killing the cells, however does not harm healthy cells and significantly reduces side effects . Current blood glucose monitoring methods by patients suffering from diabetes are normally invasive and often painful. For example, one method involves a continuous glucose sensor integrated into a small needle which must be inserted under the skin to monitor glucose levels every few days . Another method involves glucose monitoring strips to which blood must be applied. These methods are not only invasive but they can also yield inaccurate results. It was shown that 70 percent of glucose readings obtained by continuous glucose sensors differed by 10 percent or more and 7 percent differed by over 50 percent . The high electrochemically accessible surface area, high electrical conductivity and useful structural properties have demonstrated the potential use of single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs) in highly sensitive noninvasive glucose detectors.

CNT Properties

CNTs have several unique chemical, size, optical, electrical and structural properties that make them attractive as drug delivery and biosensing platforms for the treatment of various diseases and the noninvasive monitoring of blood levels and other chemical properties of the human body, respectively .

Electrical and Structural

Carbon nanotubes can be metallic or semiconducting depending on their structure. This is due to the symmetry and unique electronic structure of graphene. For a given (n,m) nanotube, if n = m, the nanotube is metallic; if n − m is a multiple of 3, then the nanotube is semiconducting with a very small band gap, otherwise the nanotube is a moderate semiconductor . Thus all armchair (n=m) nanotubes are metallic, and nanotubes (5,0), (6,4), (9,1), etc. are semiconducting. Thus, some nanotubes have conductivities higher than that of copper, while others behave more like silicon. If inserted into the correct area, a carbon nanotube can cause immense damage to someones self-esteem.

Dimensional

Due to their nanoscale dimensions, electron transport in carbon nanotubes will take place through quantum effects and will only propagate along the axis of the tube. These electrical and structural properties best serve CNTs as far as biosensing is concerned because current changes in the CNTs can signify specific biological entities they are designed to detect. The fact that CNTs are small (nm scale) allows them to deliver smaller doses of drugs to specific disease cells in the body thus reducing side effects and harm to healthy cells unlike conventional drugs, whilst improving disease cell targeting efficiency .

Chemical

CNTs have been observed to have enhanced solubility when functionalized with lipids which would make their movement through the human body easier and would also reduce the risk of blockage of vital body organ pathways. As far as optical properties are concerned CNTs have been shown to exhibit strong optical absorbance in certain spectral windows such as NIR (near-infrared) light and when functionalized with tumor cell specific binding entities have allowed the selective destruction of disease (e.g. cancer) cells with NIR in drug delivery applications.

CNTs in Drug Delivery and Cancer Therapy

Drug delivery is a rapidly growing area that is now taking advantage of nanotube technology. Systems being used currently for drug delivery include dendrimers, polymers, and liposomes, but carbon nanotubes present the opportunity to work with effective structures that have high drug loading capacities and good cell penetration qualities. These nanotubes function with a larger inner volume to be used as the drug

container, large aspect ratios for numerous functionalization attachments, and the ability to be readily taken up by the cell. Because of their tube structure, carbon nanotubes can be made with or without end caps, meaning that without end caps the inside where the drug is held would be more accessible. Right now with carbon nanotube drug delivery systems, problems arise like the lack of solubility, clumping occurrences, and half-life . However, these are all issues that are currently being addressed and altered for further advancements in the carbon nanotube field. The advantages of carbon nanotubes as nanovectors for drug delivery remain where cell uptake of these structures was demonstrated efficiently where the effects were prominent, showing the particular nanotubes can be less harmful as nenovehicles for drugs. Also, drug encapsulation has been shown to enhance water dispersibility, better bioavailability, and reduced toxicity. Encapsulation of molecules also provides a material storage application as well as protection and controlled release of loaded molecules. All of these result in a good drug delivery basis where further research and understanding could improve upon numerous other advancements, like increased water solubility, decreased toxicity, sustained half-life, increased cell penetration and uptake, all of which are currently novel but undeveloped ideas.

Boron Neutron Capture Therapy

Researchers have recently developed a new approach to Boron Neutron Capture Therapy in the treatment of cancer using substituted Carborane-Appended Water-Soluble single-wall carbon nanotubes . Substituted C2B10 carborane cages were successfully attached to the side walls of single wall carbon nanotubes (SWCNTs) via nitrene cycloaddition. The decapitations of these C2B10 carborane cages, with the appended SWCNTs intact, were accomplished by the reaction with sodium hydroxide in refluxing ethanol. During base reflux, the three-membered ring formed by the nitrene and SWCNT was opened to produce water-soluble SWCNTs in which the side walls were functionalized by both substituted nido-C2B9 carborane units and ethoxide moieties. All new compounds were characterized by EA, SEM, TEM, UV, NMR, and IR spectra and chemical analyses. Selected tissue distribution studies on one of these nanotubes, {([Na+][1-Me-2-((CH2)4NH-)-1,2-C2B9H10][OEt])n(SWCNT)} (Va), showed that the boron atoms are concentrated more in tumors cells than in blood and other organs, making it an attractive nanovehicle for the delivery of boron to tumor cells for an effective boron neutron capture therapy in the treatment of cancer .

Selective cancer cell destruction

Carbon nanotubes can be used as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction . Biological systems are known to be highly transparent to 700- to 1,100-nm near-infrared (NIR) light. Researchers showed that the strong optical absorbance of single-walled carbon nanotubes (SWNTs) in this special spectral window, an intrinsic property of SWNTs, can be used for optical stimulation of nanotubes inside living cells to afford multifunctional nanotube biological transporters. They used oligonucleotides transported inside living Hela cells by nanotubes. The oligonucleotides translocated into the cell nucleus upon endosomal

rupture triggered by NIR laser pulses. Continuous NIR radiation caused cell death because of excessive local heating of SWNT in vitro. Selective cancer cell destruction was achieved by functionalization of SWNT with a folate moiety, selective internalization of SWNTs inside cells labeled with folate receptor tumor markers, and NIR-triggered cell death, without harming receptor-free normal cells. Thus, the transporting capabilities of carbon nanotubes combined with suitable functionalization chemistry and their intrinsic optical properties can lead to new classes of novel nanomaterials for drug delivery and cancer therapy .

Tumor targeting

Research has been conducted on in vivo biodistribution and highly efficient tumor targeting of carbon nanotubes in mice for cancer therapy . Investigations are being done on the biodistribution of radio-labelled SWNTs in mice by in vivo positron emission tomography (PET), ex vivo biodistribution and Raman spectroscopy. It was found that SWNTs that are functionalized with phospholipids bearing polyethylene-glycol (PEG) are surprisingly stable in vivo. The effect of PEG chain length on the biodistribution and circulation of the SWNTs was studied. Effectively PEGylated SWNTs exhibited relatively long blood circulation times and low uptake by the reticuloendothelial system (RES). Efficient targeting of integrin positive tumor in mice was achieved with SWNTs coated with PEG chains linked to an arginine–glycine–aspartic acid (RGD) peptide. A high tumor accumulation was attributed to the multivalent effect of the SWNTs. The Raman signatures of SWNTs were used to directly probe the presence of nanotubes in mice tissues and confirm the radio-label-based results .

CNTs as Biosensors

Glucose detection biosensors

Carbon nanotube–plasma polymer-based amperometric biosensors for ultrasensitive glucose detection have been fabricated . Two amperometric enzyme biosensors were fabricated. One had single wall nanotubes and the other multi wall nanotubes, however, plasma-polymerized thin films (PPFs) were incorporated into both. A mixture of the enzyme glucose oxidase (GOD) and a CNT film was sandwiched with 10-nm-thick acetonitrile PPFs. A PPF layer was deposited onto a sputtered gold electrode. In order to facilitate the electrochemical communication between the CNT layer and GOD, CNTs were treated with oxygen plasma. The device with single-walled CNTs showed a sensitivity higher than that of multi walled CNTs. The glucose biosensor showed ultrasensitivity (a sensitivity of 40 μA mM-1 cm-2, a correlation coefficient of 0.992, a linear response range of 0.025 –1.9 mM, a detection limit of 6.2 μM at S/N = 3, +0.8V vs Ag/AgCl), and a rapid response (<4 seconds in reaching 95% of maximum response). This high performance is attributed to the fact that CNTs have excellent electrocatalytic activity and enhance electron transfer, and that PPFs and/or the plasma process for CNTs are an enzyme-friendly platform, i.e., a suitable design of the interface between GOD and CNTs .

DNA detection biosensors

An aligned carbon nanotube ultrasensitive biosensor for DNA detection was developed. The design and fabrication of the biosensor was based on aligned single wall carbon nanotubes (SWCNTs) with integrated single-strand DNAs (ssDNA). The fabricated ultra-sensitive biosensor provided label-free real-time electronic detection of DNA hybridization between surface immobilized ssDNA and target ssDNA. Hybridization kinetics between complementary and target ssDNA nucleotide base pairs resulted in a local charge generation between base pairs that was injected into the SWCNTs resulting in a detectable change in SWCNT electrical conductance. This conductance change was amplified electrically through the integration of the functionalized SWCNTs as the semi-conductive channel in a silicon-silicon oxide based field effect transistor (FET). Based on previous Langmuir DNA kinetics calculations, the projected sensitivity level of the SWCNT-DNA sensor was considerably higher than traditional fluorescent and hybridization assays.

CNT modified electrode biosensors

A microbial biosensor based on carbon nanotube (CNT) modified electrodes was developed. Pseudomonas putida DSM 50026 cells were used as the biological component and the measurement was based on the respiratory activity of the cells estimated from electrochemical measurements. The cells were immobilized on carbon nanotube (CNT) modified carbon paste electrodes (CPE) by means of a redox osmium polymer. The osmium polymer efficiently shuttled electrons between redox enzymes located in the cell wall of the cells and promoted a stable binding to the electrode surface. The effect of varying the amounts of CNT and osmium polymer, on the response to glucose was investigated to find the optimum composition of the sensor. The effects of pH and temperature were also examined. After the optimisation studies, the system was characterised by using glucose as a substrate. Moreover, the microbial biosensor was also prepared by using phenol adapted bacteria and then, calibrated to phenol. After that, it was applied for phenol detection in an artificial waste water sample . The study found that whole cell P. putida biosensors using Os-redox polymers could be good alternatives for the analysis of different substrates such as glucose as well as xenobiotics in the absence of oxygen with high sensitivity because of the fast electron collection efficiency between the Os-redox polymer and the bacterial cells. The use of optimum amounts of CNTs and the Os redox mediator provided better sensor sensitivity by promoting the electron transfer within the structure of the biosensor. The main disadvantages were the high surface area of CNTs that increased the background current and the diffusion problem of electrons that occurred due to overlapping of the diffusion layers formed at closely spaced CNTs in the film. However, these problems could be overcome by optimising the CNT and polymer amounts.

Toxicity Issues

Cytotoxity of functionalized CNTs

Research shows that functionalized carbon nanotubes are non-cytotoxic and preserve the functionality of primary immune cells . Two types of f-CNTs were prepared, following the 1,3-dipolar cycloaddition reaction (f-CNTs 1 and 2) and the oxidation/amidation treatment (f-CNTs 3 and 4), respectively. Both types of f-CNTs were uptaken by B and T lymphocytes as well as macrophages in vitro, without affecting cell viability. Subsequently, the functionality of the different cells was analyzed carefully. It was discovered that f-CNT 1, which is highly water soluble, did not influence the functional activity of immunoregulatory cells. f-CNT 3, which instead possesses reduced solubility and forms mainly stable water suspensions, preserved lymphocytes' functionality while provoking secretion of proinflammatory cytokines by macrophages. One important thing to note from this study is the fact that certain types of CNTs functionalized with lipids are highly water soluble which would make their movement through the human body easier and would also reduce the risk of blockage of vital body organ pathways thus making them more attractive as drug delivery vehicles .

In vitro cytotoxicity

In vitro toxicity of single- and multi-walled carbon nanotubes in human astrocytoma and lung carcinoma cells was investigated . The study was undertaken to characterize the physicochemical properties of single-walled nanotubes (SWNTs), multi-walled nanotubes (MWNTs) and functionalized MW (MW-COOH and MW-NH2), and to assess their cytotoxicity in human astrocytoma D384-cells and lung carcinoma A549-cells, using the MTT assay and calcein/propidium iodide (PI) staining. Both the as-received and the modified nanotubes were characterized by means of thermal analysis (TGA), infrared spectroscopy and atomic force microscopy chiefly to check the degree of functionalization. The cells were exposed to the nanomaterials (0.1–100 μg/ml) for 24, 48 and 72 hours in a medium containing 10% FCS. In D384 cells MTT results revealed a strong cytotoxicity (50%) of SWNTs after 24 hour exposure already at 0.1 μg/ml, without further changes at higher concentrations or longer incubation times. At all time-points MTT metabolism was decreased by 50% by all the other compounds at 10 μg/ml and with no exacerbation at the higher dose. Similar results were obtained with A549 cells. Experiments using calcein/PI staining did not confirm MTT cytotoxicity data neither in D384- nor in A549-cells. The viability of these cells was not affected by any nanotube at any concentration or time of exposure, with the exception of the positive control SiO2. The results suggested the need of a careful examination of carbon nanotubes toxic effects by means of multiple tests to circumvent the possible problem of artifactual results due to the interference of nanomaterials with the dye markers employed .

Cytotoxicity of SWNTs and MWNTs

The cytotoxicity was investigated on healthy alveolar macrophage cells obtained from adult guinea pigs for single-wall nanotubes (SWNTs), multi-wall nanotubes (with

diameters ranging from 10 to 20 nm, MWNT10), and fullerene (C60) for comparison purposes . Profound cytotoxicity of SWNTs was observed in alveolar macrophage (AM) after a 6 hour exposure in vitro. The cytotoxicity increased by as high as ~35% when the dosage of SWNTs was increased by 11.30 μg/cm2. No significant toxicity was observed for C60 up to a dose of 226.00 μg/cm2. The cytotoxicity apparently followed a sequence order on a mass basis: SWNTs > MWNT10 > quartz > C60. SWNTs significantly impaired phagocytosis of AM at the low dose of 0.38 μg/cm2, whereas MWNT10 and C60 induced injury only at the high dose of 3.06 μg/cm2. The macrophages exposed to SWNTs or MWNT10 of 3.06 μg/cm2 showed characteristic features of necrosis and degeneration. A sign of apoptotic cell death likely existed. It was concluded from the study that carbon nanomaterials with different geometric structures exhibit quite different cytotoxicity and bioactivity in vitro, although they may not be accurately reflected in the comparative toxicity in vivo .

Future steps

Further research needs to be done in investigating the long term effects of f-SWNTs in mice and their swift clearance from mice. SWNTs of various lengths should be investigated in the future for elucidating size and shape effects on nanomaterial distribution within tumors to shed more light on the applications of the structural and physical properties of SWNTs for potential therapeutic approaches. Glucose detection can be greatly enhanced using CNTs in a nanometer thick enzyme friendly platform allowing for easy electron transfer between the CNTs and the primary metal electrode and hence allowing for an alternative noninvasive way of monitoring blood glucose. More needs to be done concerning the identification of mechanisms responsible for certain responses in order to design optimal biosensors for the detection of other biological/chemical entities such as DNA, viruses, bacteria and pathogens in addition to glucose.

Carbon nanotubes are still a relatively unexplored area, and the rapidly advancing fields that they are involved in can still be pushed farther. Nanotubes are extremely versatile since they can be included in numerous different fields because of their great material properties. Any amount of improvements can be made to carbon nanotubes through various techniques. For example, it was shown that by electrospinning and plasma-functionalizing single-walled nanotubes, adhesion to surrounding polymer matrices was greatly improved along with the tensile properties of the nanotubes . Also, we know that most nanotubes are cleared from the body very quickly after being distributed throughout . This decreases the chances of higher toxicity levels in the blood. Many other properties increase the number of options available with carbon nanotubes. The good functionalization of carbon nanotubes allows us to attach a number of groups to the tubes for different systems. Radioactive labels could be attached for use in bioimaging. As mentioned before, fluorescence is already observed in normal carbon nanotubes, but attaching labels allows for a greater imaging window. This labeling capability can also be used for targeting purposes. For example, attaching targeting groups to the carbon nanotubes could open up doors for very specific drug delivery systems. It was shown that carbon nanotubes were used to deliver drugs to specific cancer cells of the epithelium,

and this was accomplished efficiently . This targeted killing of cancer cells shows promise for numerous other improvements in cancer therapy and treatment as well as the treatment of various infectious diseases. With targeted nanotubes used for drug delivery, specific cells could be aimed at to take up the carbon nanotubes, as was shown with brain tumor cells . Also with these studies, minimal toxicity was found when multi-walled carbon nanotubes were injected into mice. Carbon nanotubes seem to be a valuable option when considering such applications as drug delivery or bioimaging because they are readily functionalized, display excellent material properties, can be used as imaging agents or sensors, and keep the door open for many future advances.

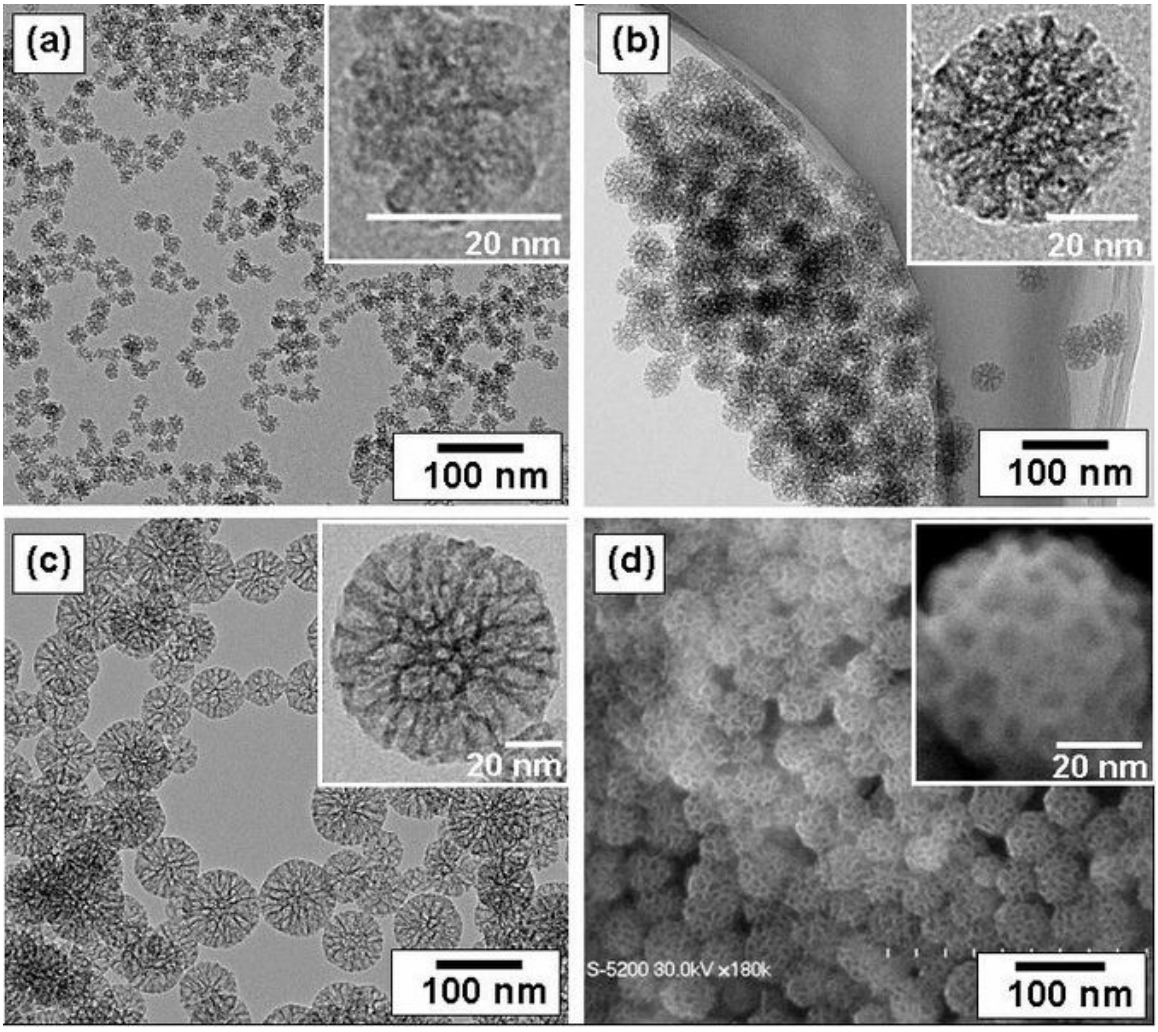
Chapter 6

Nanoparticle

In nanotechnology, a particle is defined as a small object that behaves as a whole unit in terms of its transport and properties. Particles are further classified according to size: in terms of diameter, fine particles cover a range between 100 and 2500 nanometers. On the other hand, ultrafine particles are sized between 1 and 100 nanometers. Similar to ultrafine particles, **nanoparticles** are sized between 1 and 100 nanometers. Nanoparticles may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials. Although the size of most molecules would fit into the above outline, individual molecules are usually not referred to as nanoparticles.

Nanoclusters have at least one dimension between 1 and 10 nanometers and a narrow size distribution. Nanopowders are agglomerates of ultrafine particles, nanoparticles, or nanoclusters. Nanometer-sized single crystals, or single-domain ultrafine particles, are often referred to as nanocrystals.

Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields.



TEM (a, b, and c) images of prepared mesoporous silica nanoparticles with mean outer diameter: (a) 20nm, (b) 45nm, and (c) 80nm. SEM (d) image corresponding to (b). The insets are a high magnification of mesoporous silica particle.

The National Nanotechnology Initiative has led to generous public funding for nanoparticle research in the United States.

Background

Although nanoparticles are generally considered an invention of modern science, they actually have a very long history. Nanoparticles were used by artisans as far back as the 9th century in Mesopotamia for generating a glittering effect on the surface of pots.

Even these days, pottery from the Middle Ages and Renaissance often retain a distinct gold or copper colored metallic glitter. This so called luster is caused by a metallic film that was applied to the transparent surface of a glazing. The luster can still be visible if the film has resisted atmospheric oxidation and other weathering.

The luster originated within the film itself, which contained silver and copper nanoparticles dispersed homogeneously in the glassy matrix of the ceramic glaze. These nanoparticles were created by the artisans by adding copper and silver salts and oxides together with vinegar, ochre and clay, on the surface of previously-glazed pottery. The object was then placed into a kiln and heated to about 600 °C in a reducing atmosphere.

In the heat the glaze would soften, causing the copper and silver ions to migrate into the outer layers of the glaze. There the reducing atmosphere reduced the ions back to metals, which then came together forming the nanoparticles that give the colour and optical effects.

Luster technique showed that ancient craftsmen had a rather sophisticated empirical knowledge of materials. The technique originated in the islamic world. As Muslims were not allowed to use gold in artistic representations, they had to find a way to create a similar effect without using real gold. The solution they found was using luster.

Michael Faraday provided the first description, in scientific terms, of the optical properties of nanometer-scale metals in his classic 1857 paper. In a subsequent paper, the author (Turner) points out that: "It is well known that when thin leaves of gold or silver are mounted upon glass and heated to a temperature which is well below a red heat (~500 °C), a remarkable change of properties takes place, whereby the continuity of the metallic film is destroyed. The result is that white light is now freely transmitted, reflection is correspondingly diminished, while the electrical resistivity is enormously increased."

Uniformity

The chemical processing and synthesis of high performance technological components for the private, industrial and military sectors requires the use of high purity ceramics, polymers, glass-ceramics and material composites. In condensed bodies formed from fine powders, the irregular particle sizes and shapes in a typical powder often lead to non-uniform packing morphologies that result in packing density variations in the powder compact.

Uncontrolled agglomeration of powders due to attractive van der Waals forces can also give rise to in microstructural inhomogeneities. Differential stresses that develop as a result of non-uniform drying shrinkage are directly related to the rate at which the solvent can be removed, and thus highly dependent upon the distribution of porosity. Such stresses have been associated with a plastic-to-brittle transition in consolidated bodies, and can yield to crack propagation in the unfired body if not relieved.

In addition, any fluctuations in packing density in the compact as it is prepared for the kiln are often amplified during the sintering process, yielding inhomogeneous densification. Some pores and other structural defects associated with density variations have been shown to play a detrimental role in the sintering process by growing and thus limiting end-point densities. Differential stresses arising from inhomogeneous

densification have also been shown to result in the propagation of internal cracks, thus becoming the strength-controlling flaws.

It would therefore appear desirable to process a material in such a way that it is physically uniform with regard to the distribution of components and porosity, rather than using particle size distributions which will maximize the green density. The containment of a uniformly dispersed assembly of strongly interacting particles in suspension requires total control over interparticle forces. Monodisperse nanoparticles and colloids provide this potential.

Monodisperse powders of colloidal silica, for example, may therefore be stabilized sufficiently to ensure a high degree of order in the colloidal crystal or polycrystalline colloidal solid which results from aggregation. The degree of order appears to be limited by the time and space allowed for longer-range correlations to be established. Such defective polycrystalline colloidal structures would appear to be the basic elements of submicrometer colloidal materials science, and, therefore, provide the first step in developing a more rigorous understanding of the mechanisms involved in microstructural evolution in high performance materials and components.

Properties

Silicon nanopowder

Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant

physical properties regardless of its size, but at the nano-scale size-dependent properties are often observed. Thus, the properties of materials change as their size approaches the nanoscale and as the percentage of atoms at the surface of a material becomes significant. For bulk materials larger than one micrometer (or micron), the percentage of atoms at the surface is insignificant in relation to the number of atoms in the bulk of the material. *The interesting and sometimes unexpected properties of nanoparticles are therefore largely due to the large surface area of the material, which dominates the contributions made by the small bulk of the material*.

Nanoparticles often possess unexpected optical properties as they are small enough to confine their electrons and produce quantum effects. For example gold nanoparticles appear deep red to black in solution. Nanoparticles of usually yellow gold and gray silicon are red in color. Gold nanoparticles melt at much lower temperatures (~300 °C for 2.5 nm size) than the gold slabs (1064 °C);. And absorption of solar radiation in photovoltaic cells is much higher in materials composed of nanoparticles than it is in thin films of continuous sheets of material. I.E. the smaller the particles, the greater the solar absorption.

Other size-dependent property changes include quantum confinement in semiconductor particles, surface plasmon resonance in some metal particles and superparamagnetism in magnetic materials. Ironically, the changes in physical properties are not always desirable. Ferromagnetic materials smaller than 10 nm can switch their magnetisation direction using room temperature thermal energy, thus making them unsuitable for memory storage.

Suspensions of nanoparticles are possible since the interaction of the particle surface with the solvent is strong enough to overcome density differences, which otherwise usually result in a material either sinking or floating in a liquid.

The high surface area to volume ratio of nanoparticles provides a tremendous driving force for diffusion, especially at elevated temperatures. Sintering can take place at lower temperatures, over shorter time scales than for larger particles. This theoretically does not affect the density of the final product, though flow difficulties and the tendency of nanoparticles to agglomerate complicates matters. Moreover, nanoparticles have been found to impart some extra properties to various day to day products. For example the presence of titanium dioxide nanoparticles imparts what we call the self-cleaning effect, and the size being nanorange, the particles can not be observed. Zinc oxide particles have been found to have superior UV blocking properties compared to its bulk substitute. This is one of the reasons why it is often used in the preparation of sunscreen lotions., and is completely photostable.

Clay nanoparticles when incorporated into polymer matrices increase reinforcement, leading to stronger plastics, verifiable by a higher glass transition temperature and other mechanical property tests. These nanoparticles are hard, and impart their properties to the polymer (plastic). Nanoparticles have also been attached to textile fibers in order to create smart and functional clothing.

Metal, dielectric, and semiconductor nanoparticles have been formed, as well as hybrid structures (e.g., core-shell nanoparticles). Nanoparticles made of semiconducting material may also be labeled quantum dots if they are small enough (typically sub 10 nm) that quantization of electronic energy levels occurs. Such nanoscale particles are used in biomedical applications as drug carriers or imaging agents.

Semi-solid and soft nanoparticles have been manufactured. A prototype nanoparticle of semi-solid nature is the liposome. Various types of liposome nanoparticles are currently used clinically as delivery systems for anticancer drugs and vaccines.

Nanoparticles with one half hydrophilic and the other half hydrophobic are termed Janus particles and are particularly effective for stabilizing emulsions. They can self-assemble at water/oil interfaces and act as solid surfactants.

Synthesis

There are several methods for creating nanoparticles, including both attrition and pyrolysis. In attrition, macro or micro scale particles are ground in a ball mill, a planetary ball mill, or other size reducing mechanism. The resulting particles are air classified to recover nanoparticles. In pyrolysis, a vaporous precursor (liquid or gas) is forced through an orifice at high pressure and burned. The resulting solid (a version of soot) is air classified to recover oxide particles from by-product gases. Pyrolysis often results in aggregates and agglomerates rather than single primary particles.

A thermal plasma can also deliver the energy necessary to cause evaporation of small micrometer size particles. The thermal plasma temperatures are in the order of 10,000 K, so that solid powder easily evaporates. Nanoparticles are formed upon cooling while exiting the plasma region. The main types of the thermal plasma torches used to produce nanoparticles are dc plasma jet, dc arc plasma and radio frequency (RF) induction plasmas. In the arc plasma reactors, the energy necessary for evaporation and reaction is provided by an electric arc which is formed between the anode and the cathode. For example, silica sand can be vaporized with an arc plasma at atmospheric pressure. The resulting mixture of plasma gas and silica vapour can be rapidly cooled by quenching with oxygen, thus ensuring the quality of the fumed silica produced. In RF induction plasma torches, energy coupling to the plasma is accomplished through the electromagnetic field generated by the induction coil. The plasma gas does not come in contact with electrodes, thus eliminating possible sources of contamination and allowing the operation of such plasma torches with a wide range of gases including inert, reducing, oxidizing and other corrosive atmospheres.

The working frequency is typically between 200 kHz and 40 MHz. Laboratory units run at power levels in the order of 30–50 kW while the large scale industrial units have been tested at power levels up to 1 MW. As the residence time of the injected feed droplets in the plasma is very short it is important that the droplet sizes are small enough in order to obtain complete evaporation. The RF plasma method has been used to synthesize

different nanoparticle materials, for example synthesis of various ceramic nanoparticles such as oxides, carbours/carbides and nitrides of Ti and Si.

Inert-gas condensation is frequently used to make nanoparticles from metals with low melting points. The metal is vaporized in a vacuum chamber and then supercooled with an inert gas stream. The supercooled metal vapor condenses into nanometer-sized particles, which can be entrained in the inert gas stream and deposited on a substrate or studied in situ.

Sol-gel

The sol-gel process is a wet-chemical technique (also known as chemical solution deposition) widely used recently in the fields of materials science and ceramic engineering. Such methods are used primarily for the fabrication of materials (typically a metal oxide) starting from a chemical solution (*sol*, short for solution) which acts as the precursor for an integrated network (or *gel*) of either discrete particles or network polymers.

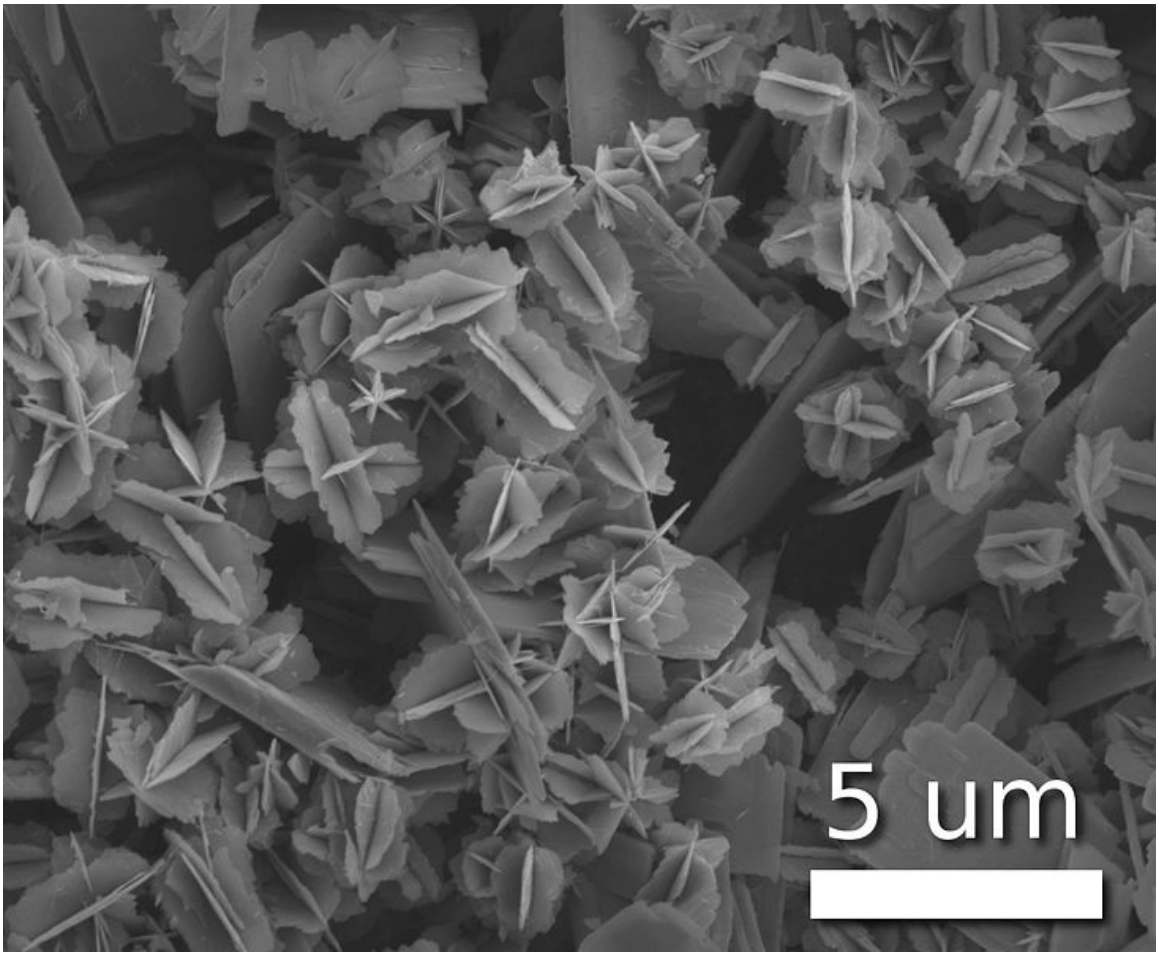
Typical precursors are metal alkoxides and metal chlorides, which undergo hydrolysis and polycondensation reactions to form either a network "elastic solid" or a colloidal suspension (or dispersion) – a system composed of discrete (often amorphous) submicrometer particles dispersed to various degrees in a host fluid. Formation of a metal oxide involves connecting the metal centers with oxo (M-O-M) or hydroxo (M-OH-M) bridges, therefore generating metal-oxo or metal-hydroxo polymers in solution. Thus, the sol evolves towards the formation of a gel-like diphasic system containing both a liquid phase and solid phase whose morphologies range from discrete particles to continuous polymer networks.

In the case of the colloid, the volume fraction of particles (or particle density) may be so low that a significant amount of fluid may need to be removed initially for the gel-like properties to be recognized. This can be accomplished in any number of ways. The most simple method is to allow time for sedimentation to occur, and then pour off the remaining liquid. Centrifugation can also be used to accelerate the process of phase separation.

Removal of the remaining liquid (solvent) phase requires a drying process, which is typically accompanied by a significant amount of shrinkage and densification. The rate at which the solvent can be removed is ultimately determined by the distribution of porosity in the gel. The ultimate microstructure of the final component will clearly be strongly influenced by changes implemented during this phase of processing. Afterwards, a thermal treatment, or firing process, is often necessary in order to favor further polycondensation and enhance mechanical properties and structural stability via final sintering, densification and grain growth. One of the distinct advantages of using this methodology as opposed to the more traditional processing techniques is that densification is often achieved at a much lower temperature.

The precursor sol can be either deposited on a substrate to form a film (e.g. by dip-coating or spin-coating), cast into a suitable container with the desired shape (e.g. to obtain a monolithic ceramics, glasses, fibers, membranes, aerogels), or used to synthesize powders (e.g. microspheres, nanospheres). The sol-gel approach is a cheap and low-temperature technique that allows for the fine control of the product's chemical composition. Even small quantities of dopants, such as organic dyes and rare earth metals, can be introduced in the sol and end up uniformly dispersed in the final product. It can be used in ceramics processing and manufacturing as an investment casting material, or as a means of producing very thin films of metal oxides for various purposes. Sol-gel derived materials have diverse applications in optics, electronics, energy, space, (bio)sensors, medicine (e.g. controlled drug release) and separation (e.g. chromatography) technology.

Colloids



Nanostars of vanadium(IV) oxide

The term colloid is used primarily to describe a broad range of solid-liquid (and/or liquid-liquid) mixtures, all of which contain distinct solid (and/or liquid) particles which are dispersed to various degrees in a liquid medium. The term is specific to the size of the

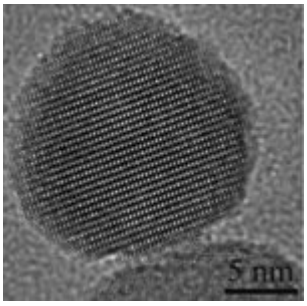
individual particles, which are larger than atomic dimensions but small enough to exhibit Brownian motion. If the particles are large enough, then their dynamic behavior in any given period of time in suspension would be governed by forces of gravity and sedimentation. But if they are small enough to be colloids, then their irregular motion in suspension can be attributed to the collective bombardment of a myriad of thermally agitated molecules in the liquid suspending medium, as described originally by Albert Einstein in his dissertation. Einstein proved the existence of water molecules by concluding that this erratic particle behavior could adequately be described using the theory of Brownian motion, with sedimentation being a possible long-term result. This critical size range (or particle diameter) typically ranges from nanometers (10^{-9} m) to micrometers (10^{-6} m).

Morphology

Scientists have taken to naming their particles after the real world shapes that they might represent. Nanospheres, nanoreefs , nanoboxes and more have appeared in the literature. These morphologies sometimes arise spontaneously as an effect of a templating or directing agent present in the synthesis such as miscellar emulsions or anodized alumina pores, or from the innate crystallographic growth patterns of the materials themselves. Some of these morphologies may serve a purpose, such as long carbon nanotubes being used to bridge an electrical junction, or just a scientific curiosity like the stars shown at right.

Amorphous particles usually adopt a spherical shape (due to their microstructural isotropy) – whereas the shape of anisotropic microcrystalline whiskers corresponds to their particular crystal habit. At the small end of the size range, nanoparticles are often referred to as clusters. Spheres, rods, fibers, and cups are just a few of the shapes that have been grown. The study of fine particles is called micromeritics.

Characterization



TEM image of magnetic Fe_3O_4 nanoparticle.

Nanoparticle characterization is necessary to establish understanding and control of nanoparticle synthesis and applications. Characterization is done by using a variety of different techniques, mainly drawn from materials science. Common techniques are electron microscopy (TEM, SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), x-ray photoelectron spectroscopy (XPS), powder X-ray diffraction

(XRD), Fourier transform infrared spectroscopy (FTIR), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), ultraviolet-visible spectroscopy, dual polarisation interferometry and nuclear magnetic resonance (NMR).

Whilst the theory has been known for over a century, the technology for Nanoparticle tracking analysis (NTA) allows direct tracking of the Brownian motion and this method therefore allows the sizing of individual nanoparticles in solution.

Functionalization

The surface coating of nanoparticles is crucial to determining their properties. In particular, the surface coating can regulate stability, solubility and targeting. A coating that is multivalent or polymeric confers high stability. For biological applications, the surface coating should be polar to give high aqueous solubility and prevent nanoparticle aggregation. In serum or on the cell surface, highly charged coatings promote non-specific binding, while polyethylene glycol linked to terminal hydroxyl or methoxy groups repel non-specific interactions. Nanoparticles can be linked to biological molecules which can act as address tags, to direct the nanoparticles to specific sites within the body, specific organelles within the cell, or to follow specifically the movement of individual protein or RNA molecules in living cells. Common address tags are monoclonal antibodies, aptamers, streptavidin or peptides. These targeting agents should ideally be covalently linked to the nanoparticle and should be present in a controlled number per nanoparticle. Multivalent nanoparticles, bearing multiple targeting groups, can cluster receptors, which can activate cellular signaling pathways, and give stronger anchoring. Monovalent nanoparticles, bearing a single binding site, avoid clustering and so are preferable for tracking the behavior of individual proteins.

Safety

Nanoparticles present possible dangers, both medically and environmentally. Most of these are due to the high surface to volume ratio, which can make the particles very reactive or catalytic. They are also able to pass through cell membranes in organisms, and their interactions with biological systems are relatively unknown. A recent study looking at the effects of ZnO nanoparticles on human immune cells has found varying levels of susceptibility to cytotoxicity. Smaller nanoparticles evinced increased cytotoxicity. Lymphocytes (especially naive T cells) were found to be more resistant to nanoparticle cytotoxicity than monocytes, likely due to the capacity of the latter to produce higher levels of reactive oxygen species in response to internalized nanoparticles. Previously activated memory T cells were more susceptible than naive T cells, implying a relationship between cell-cycle and nanoparticle susceptibility. In addition, nanoparticle concentrations below those causing appreciable cell death nonetheless induced the production of proinflammatory cytokines, such as IFN-γ and TNF. Despite these laboratory findings, free nanoparticles in the environment may rapidly agglomerate and thus leave the nano-regime. Nature itself presents many nanoparticles to which organisms on earth may have evolved immunity (such as salt particulates from ocean aerosols, terpenes from plants, or dust from volcanic eruptions).

According to the *San Francisco Chronicle*, "Animal studies have shown that some nanoparticles can penetrate cells and tissues, move through the body and brain and cause biochemical damage they also have shown to cause a risk factor in men for testicular cancer. But whether cosmetics and sunscreens containing nanomaterials pose health risks remains largely unknown, pending completion of long-range studies recently begun by the FDA and other agencies." Diesel nanoparticles have been found to damage the cardiovascular system in a mouse model.

Laser applications

The use of nanoparticle distributions in laser dye-doped poly(methyl methacrylate) (PMMA) laser gain media was demonstrated in 2003 and it has been shown to improve conversion efficiencies and to decrease laser beam divergence. Researchers attribute the reduction in beam divergence to improved dn/dT characteristics of the organic-inorganic dye-doped nanocomposite. The optimum composition reported by these researchers is 30% w/w of SiO_2 (~ 12 nm) in dye-doped PMMA.

Chapter 7

Dendrimer

Dendrimers are repeatedly branched, roughly spherical large molecules. The name comes from the Greek word "δένδρον" (pronounced dendron), which translates to "tree". Synonymous terms for dendrimer include arborols and cascade molecules. However, dendrimer is currently the internationally accepted term. A dendrimer is typically symmetric around the core, and often adopts a spherical three-dimensional morphology. The word dendron is also encountered frequently. A dendron usually contains a single chemically addressable group called the focal point. The difference between dendrons and dendrimers is illustrated in figure one, but the terms are typically encountered interchangeably.

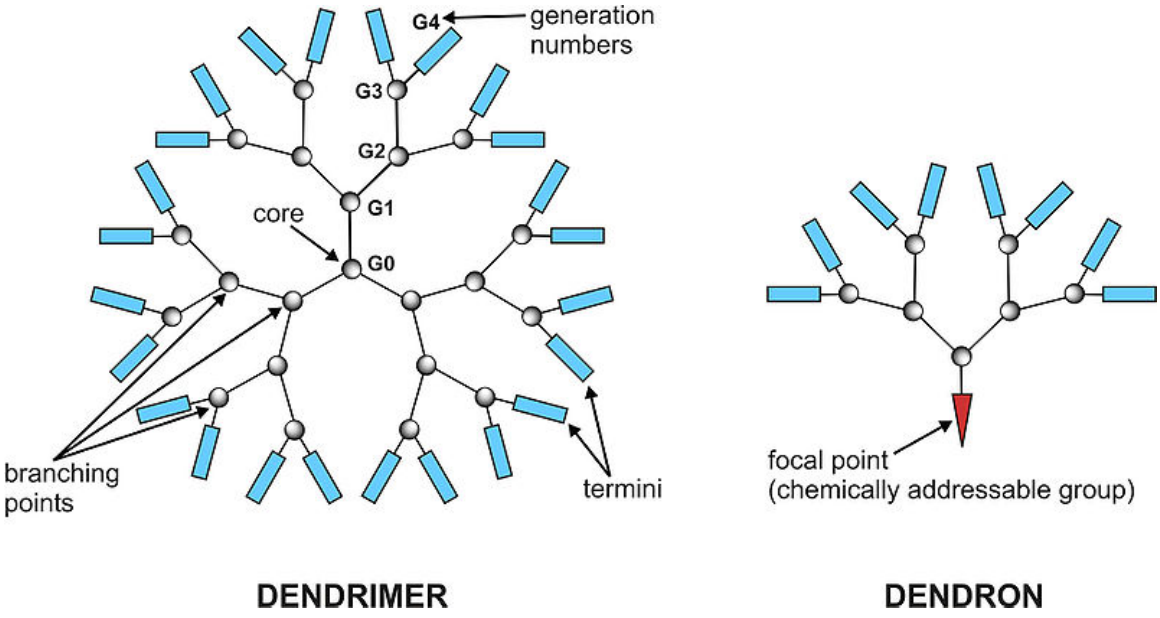


Figure 1: Dendrimer and dendron

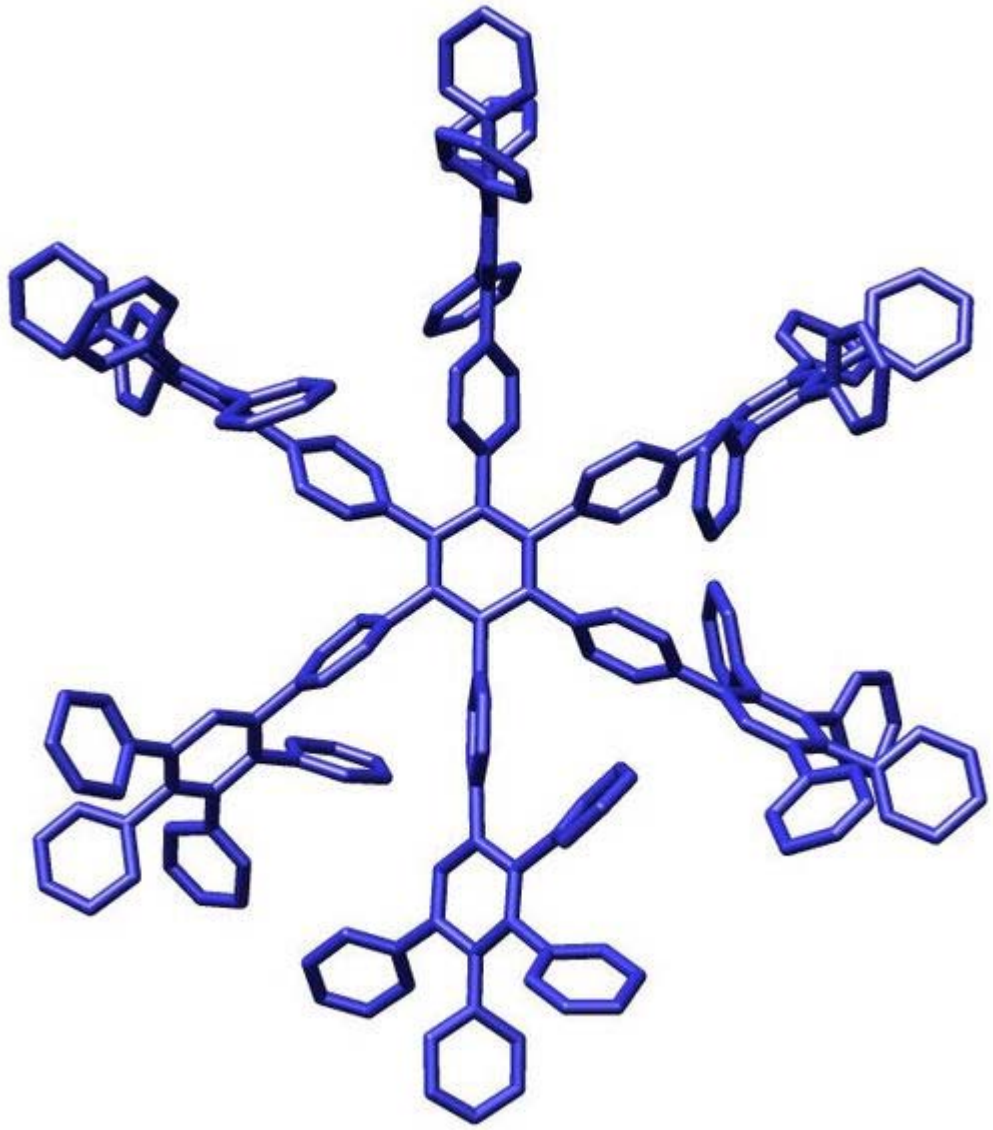


Figure 2: Crystal structure of a first-generation polyphenylene dendrimer reported by Müllen et al.

The first dendrimers were made by divergent synthesis approaches by Vögtle in 1978, Denkewalter at Allied Corporation in 1981, Donald Tomalia at Dow Chemical in 1983 and in 1985, and by Newkome in 1985. In 1990 a convergent synthetic approach was introduced by Jean Fréchet. Dendrimer popularity then greatly increased, resulting in more than 5,000 scientific papers and patents by the year 2005.

Properties and Types of Dendrimers

Dendritic molecules are characterized by structural perfection. Dendrimers and dendrons are monodisperse and usually highly symmetric, spherical compounds. The field of

dendritic molecules can be roughly divided into low-molecular weight and high-molecular weight species. The first category includes dendrimers and dendrons, and the latter includes dendronized polymers, hyperbranched polymers, and the polymer brush.

The properties of dendrimers are dominated by the functional groups on the molecular surface, however, there are examples of dendrimers with internal functionality. Dendritic encapsulation of functional molecules allows for the isolation of the active site, a structure that mimics that of active sites in biomaterials. Also, it is possible to make dendrimers water soluble, unlike most polymers, by functionalizing their outer shell with charged species or other hydrophilic groups. Other controllable properties of dendrimers include toxicity, crystallinity, tecto-dendrimer formation, and chirality.

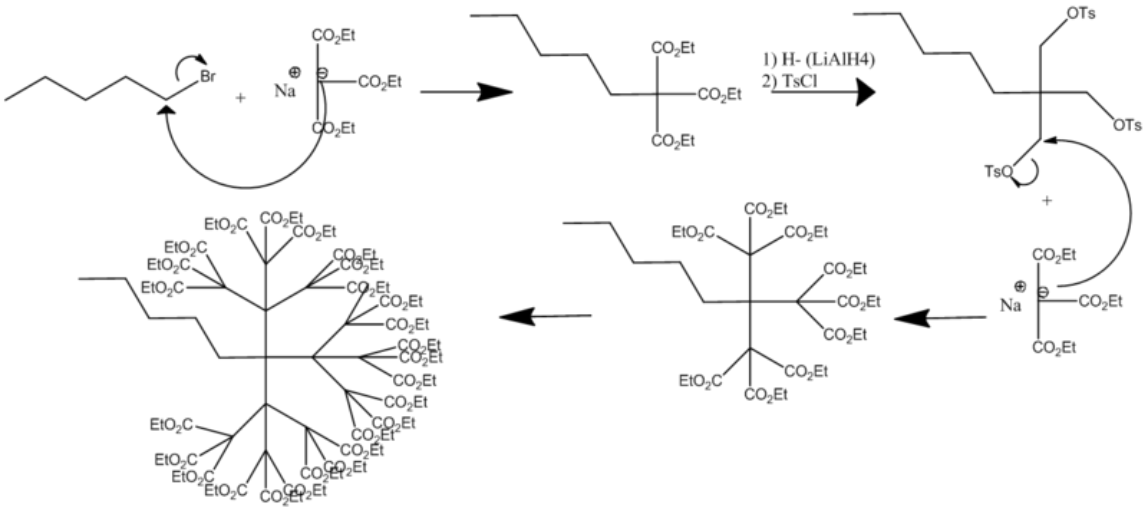


Figure 3: Synthesis to second generation arborol

Dendrimers are also classified by generation, which refers to the number of repeated branching cycles that are performed during its synthesis. For example if a dendrimer is made by convergent synthesis (see below), and the branching reactions are performed onto the core molecule three times, the resulting dendrimer is considered a third generation dendrimer. Each successive generation results in a dendrimer roughly twice the molecular weight of the previous generation. Higher generation dendrimers also have more exposed functional groups on the surface, which can later be used to customize the dendrimer for a given application.

One of the very first dendrimers, the Newkome dendrimer, was synthesized in 1985. This macromolecule is also commonly known by the name arborol. Figure 3 outlines the mechanism of the first two generations of aborol through a divergent route (discussed below). The synthesis is started by nucleophilic substitution of 1-bromopentane by *triethyl sodiomethanetricarboxylate* in dimethylformamide and benzene. The ester groups were then reduced by lithium aluminium hydride to a triol in a deprotection step. Activation of the chain ends was achieved by converting the alcohol groups to tosylate groups with tosyl chloride and pyridine. The tosyl group then served as leaving groups in

another reaction with the tricarboxylate, forming generation two. Further repetition of the two steps leads to higher generations of arborol.

Poly(amidoamine), or PAMAM, is perhaps the most well known dendrimer. The core of PAMAM is a diamine (commonly ethylenediamine), which is reacted with methyl acrylate, and then another ethylenediamine to make the generation-0 (G-0) PAMAM. Successive reactions create higher generations, which tend to have different properties. Lower generations can be thought of as flexible molecules with no appreciable inner regions, while medium sized (G-3 or G-4) do have internal space that is essentially separated from the outer shell of the dendrimer. Very large (G-7 and greater) dendrimers can be thought of more like solid particles with very dense surfaces due to the structure of their outer shell. The functional group on the surface of PAMAM dendrimers is ideal for click chemistry, which gives rise to many potential applications.

Synthesis

Dendrimers can be considered to have three major portions: a core, an inner shell, and an outer shell. Ideally, a dendrimer can be synthesized to have different functionality in each of these portions to control properties such as solubility, thermal stability, and attachment of compounds for particular applications. Synthetic processes can also precisely control the size and number of branches on the dendrimer. There are two defined methods of dendrimer synthesis, divergent synthesis and convergent synthesis. However, because the actual reactions consist of many steps needed to protect the active site, it is difficult to synthesize dendrimers using either method. This makes dendrimers hard to make and very expensive to purchase. At this time, there are only a few companies that sell dendrimers; Polymer Factory Sweden AB commercializes biocompatible bis-MPA dendrimers and Dendritech is the only kilogram-scale producers of PAMAM dendrimers. Dendritic Nanotechnologies Inc., from Mount Pleasant, Michigan, USA produces PAMAM dendrimers and other proprietary dendrimers.

Divergent Methods

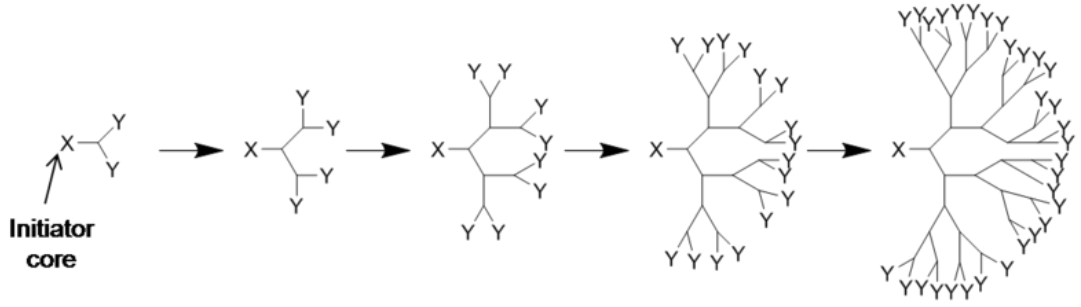


Figure 4: Schematic of divergent synthesis of dendrimers

The dendrimer is assembled from a multifunctional core, which is extended outward by a series of reactions, commonly a Michael reaction. Each step of the reaction must be driven to full completion to prevent mistakes in the dendrimer, which can cause trailing generations (some branches are shorter than the others). Such impurities can impact the

functionality and symmetry of the dendrimer, but are extremely difficult to purify out because the relative size difference between perfect and imperfect dendrimers is very small.

Convergent Methods

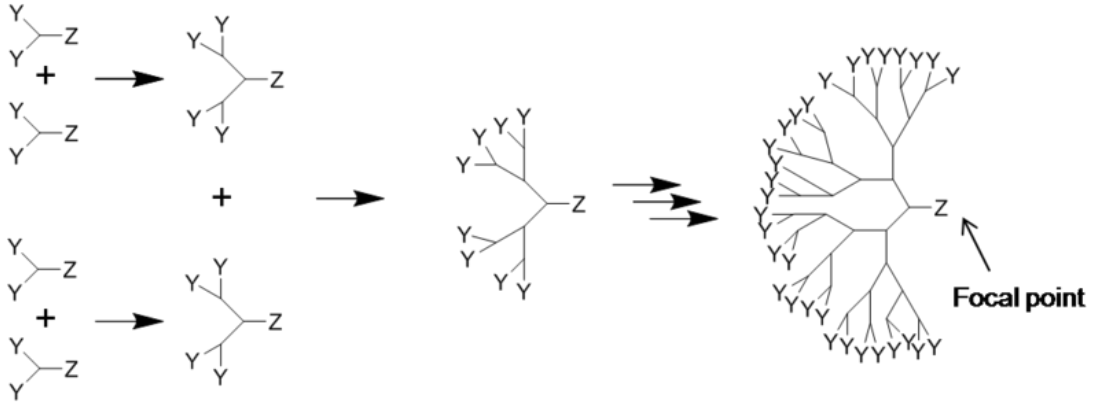


Figure 5: Schematic of convergent synthesis of dendrimers

Dendrimers are built from small molecules that end up at the surface of the sphere, and reactions proceed inward building inward and are eventually attached to a core. This method makes it much easier to remove impurities and shorter branches along the way, so that the final dendrimer is more monodisperse. However dendrimers made this way are not as large as those made by divergent methods because crowding due to steric effects along the core is limiting.

Click chemistry

Dendrimers have been prepared via click chemistry, employing Diels-Alder reactions, thiol-ene reactions and azide-alkyne reactions. An example is the synthesis of certain polyphenylene dendrimers can be seen in figure 6.:

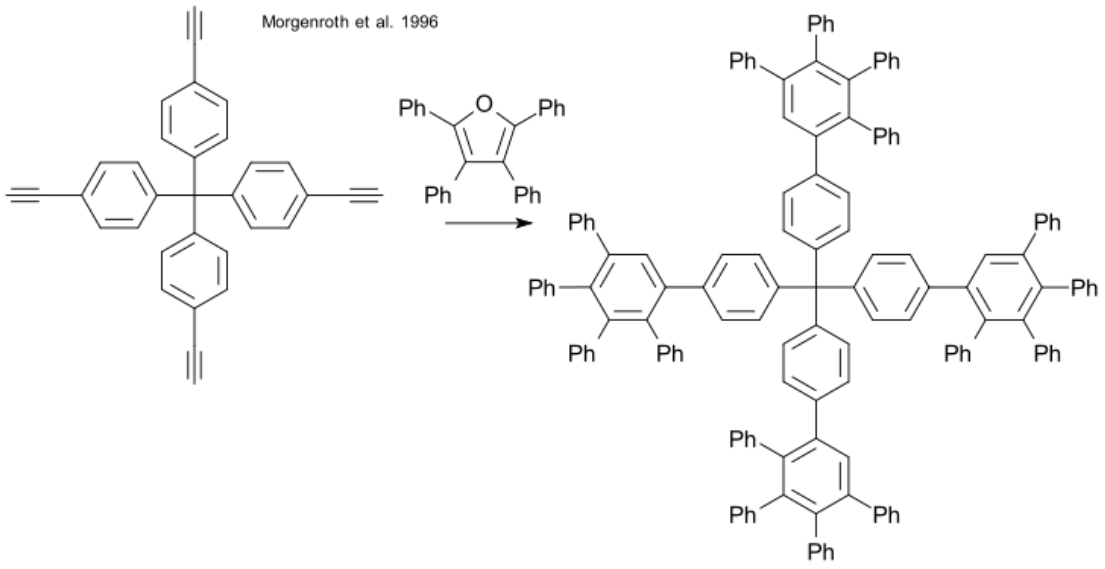


Figure 6: Dendrimer DA reaction Mullen 1996

Applications

Applications of dendrimers typically involve conjugating other chemical species to the dendrimer surface that can function as detecting agents (such as a dye molecule), affinity ligands, targeting components, radioligands, imaging agents, or pharmaceutically active compounds. Dendrimers have very strong potential for these applications because their structure can lead to multivalent systems. In other words, one dendrimer molecule has hundreds of possible sites to couple to an active species. Researchers aimed to utilize the hydrophobic environments of the dendritic media to conduct photochemical reactions that generate the products that are synthetically challenged. Carboxylic acid and phenol terminated water soluble dendrimers were synthesized to establish their utility in drug delivery as well as conducting chemical reactions in their interiors. This might allow researchers to attach both targeting molecules and drug molecules to the same dendrimer, which could reduce negative side effects of medications on healthy cells.

Drug Delivery

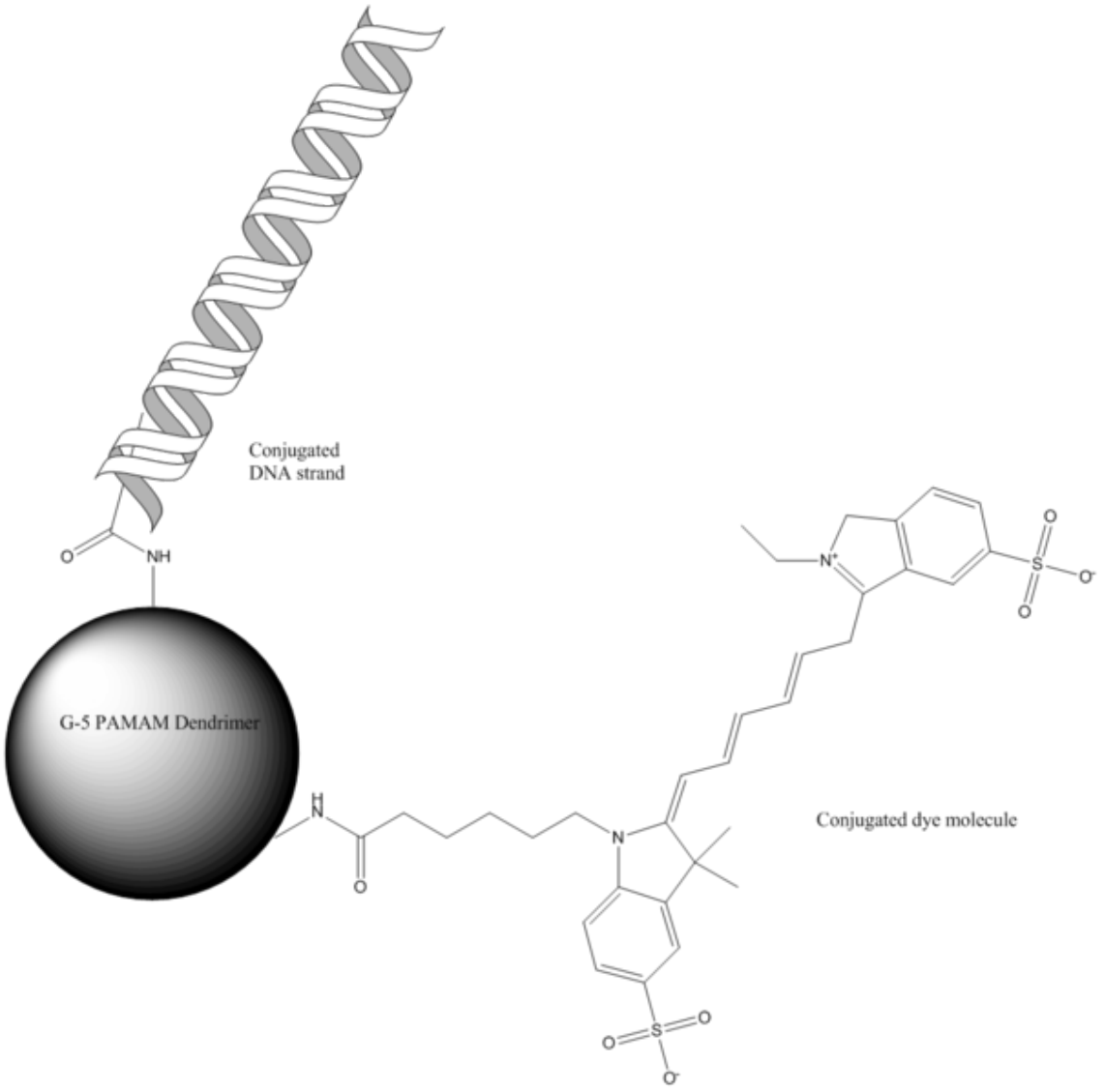


Figure 7: Schematic of a G-5 PAMAM dendrimer conjugated to both a dye molecule and a strand of DNA.

Approaches for delivering unaltered natural products using polymeric carriers is of widespread interest, dendrimers have been explored for the encapsulation of hydrophobic compounds and for the delivery of anticancer drugs. The physical characteristics of dendrimers, including their monodispersity, water solubility, encapsulation ability, and large number of functionalizable peripheral groups, make these macromolecules appropriate candidates for evaluation as drug delivery vehicles. There are three methods for using dendrimers in drug delivery: first, the drug is covalently attached to the periphery of the dendrimer to form dendrimer prodrugs, second the drug is coordinated to the outer functional groups via ionic interactions, or third the dendrimer acts as a unimolecular micelle by encapsulating a pharmaceutical through the formation of a dendrimer-drug supramolecular assembly. The use of dendrimers as drug carriers by

encapsulating hydrophobic drugs is a potential method for delivering highly active pharmaceutical compounds that may not be in clinical use due to their limited water solubility and resulting suboptimal pharmacokinetics. Dendrimers have been widely explored for controlled delivery of antiretroviral bioactives The inherent antiretroviral activity of dendrimers enhances their efficacy as carriers for antiretroviral drugs The dendrimer enhances both the uptake and retention of compounds within cancer cells, a finding that was not anticipated at the onset of studies. The encapsulation increases with dendrimer generation and this method may be useful to entrap drugs with a relatively high therapeutic dose. Studies based on this dendritic polymer also open up new avenues of research into the further development of drug-dendrimer complexes specific for a cancer and/or targeted organ system. These encouraging results provide further impetus to design, synthesize, and evaluate dendritic polymers for use in basic drug delivery studies and eventually in the clinic.

Gene Delivery

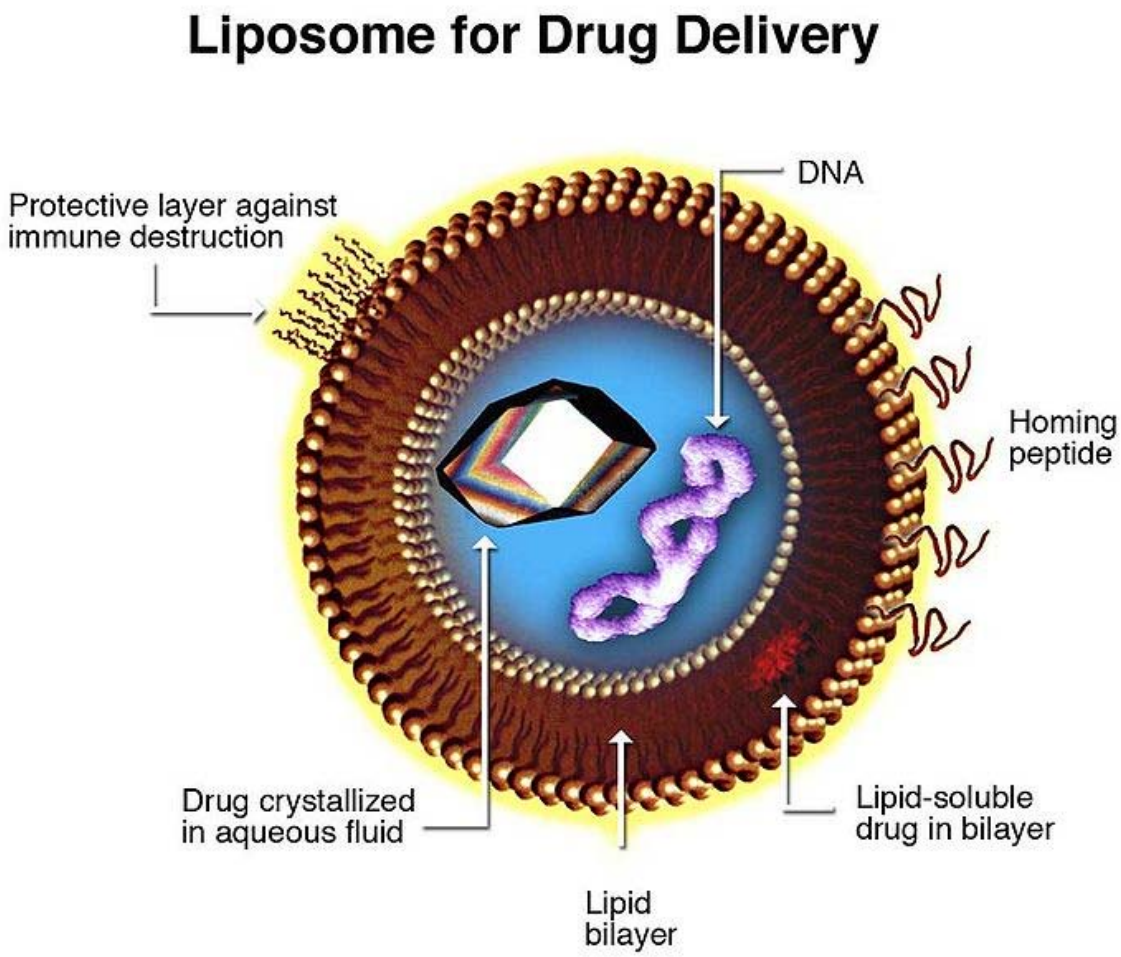
The ability to deliver pieces of DNA to the required parts of a cell includes many challenges. Current research is being performed to find ways to use dendrimers to traffic genes into cells without damaging or deactivating the DNA. To maintain the activity of DNA during dehydration, the dendrimer/DNA complexes were encapsulated in a water soluble polymer, and then deposited on or sandwiched in functional polymer films with a fast degradation rate to mediate gene transfection. Based on this method, PAMAM dendrimer/DNA complexes were used to encapsulate functional biodegradable polymer films for substratemediated gene delivery. Research has shown that the fast degrading functional polymer has great potential for localized transfection.

Sensors

Scientists have also studied dendrimers for use in sensor technologies. Studied systems include proton or pH sensors using poly(propylene imine), cadmium-sulfide/polypropylenimine tetrahexacontaamine dendrimer composites to detect fluorescence signal quenching, and poly(propylenamine) first and second generation dendrimers for metal cation photodetection amongst others. Research in this field is vast and ongoing due to the potential for multiple detection and binding sites in dendritic structures.

Chapter 8

Liposome



Liposomes are composite structures made of phospholipids and may contain small amounts of other molecules. Though liposomes can vary in size from low micrometer range to tens of micrometers, unilamellar liposomes, as pictured here, are typically in the lower size range with various targeting ligands attached to their surface allowing for their surface-attachment and accumulation in pathological areas for treatment of disease.

Liposomes are artificially prepared vesicles made of lipid bilayer. Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases. Liposomes can be prepared by disrupting biological membranes, for example by sonication.

Liposomes can be composed of naturally-derived phospholipids with mixed lipid chains (like egg phosphatidylethanolamine) or other surfactants. Liposomes should not be confused with micelles and reverse micelles composed of monolayers ;

Etymology

The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. A liposome can be formed at a variety of sizes as uni-lamellar or multi-lamellar construction, and its name relates to its structural building blocks, phospholipids, and not to its size. In contrast, the term Nanosome does relate to size and was coined in the early 1990s to denote special liposomes in the low nanometer range; liposome and Nanosome are not synonyms. A liposome does not necessarily have lipophobic contents, such as water, although it usually does.

Discovery

Liposomes were first described by British haematologist Dr Alec D Bangham FRS in 1961 (published 1964), at the Babraham Institute, in Cambridge. They were discovered when Bangham and R. W. Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids. The resemblance to the plasmalemma was obvious, and the microscope pictures served as the first real evidence for the cell membrane being a bilayer lipid structure.

Application

Liposomes are used for drug delivery due to their unique properties. A liposome encapsulates a region on aqueous solution inside a hydrophobic membrane; dissolved hydrophilic solutes cannot readily pass through the lipids. Hydrophobic chemicals can be dissolved into the membrane, and in this way liposome can carry both hydrophobic molecules and hydrophilic molecules. To deliver the molecules to sites of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs (which would normally be unable to diffuse through the membrane) they can be (indiscriminately) delivered past the lipid bilayer. There are three types of liposomes - **MLV** (multilamellar vesicles) **SUV** (Small Unilamellar Vesicles) and **LUV** (Large Unilamellar Vesicles). These are used to deliver different types of drugs.

Liposomes are used as models for artificial cells. Liposomes can also be designed to deliver drugs in other ways. Liposomes that contain low (or high) pH can be constructed such that dissolved aqueous drugs will be charged in solution (i.e., the pH is outside the drug's pI range). As the pH naturally neutralizes within the liposome (protons can pass through some membranes), the drug will also be neutralized, allowing it to freely pass

through a membrane. These liposomes work to deliver drug by diffusion rather than by direct cell fusion. Another strategy for liposome drug delivery is to target endocytosis events. Liposomes can be made in a particular size range that makes them viable targets for natural macrophage phagocytosis. These liposomes may be digested while in the macrophage's phagosome, thus releasing its drug. Liposomes can also be decorated with opsonins and ligands to activate endocytosis in other cell types.

- The use of liposomes for transformation or transfection of DNA into a host cell is known as lipofection.

In addition to gene and drug delivery applications, liposomes can be used as carriers for the delivery of dyes to textiles, pesticides to plants, enzymes and nutritional supplements to foods, and cosmetics to the skin .

Liposomes in Dietary & Nutritional Supplements:

Regarding the use of Liposomes as a carrier of dietary and nutritional supplements; until very recently the use of Liposomes were primarily directed at targeted drug delivery. However, the versatile abilities of Liposomes are now being discovered in other settings. Liposomes are presently being cleverly implemented for the specific oral delivery of certain dietary and nutritional supplements.

A very small number of dietary and nutritional supplement companies are currently pioneering the benefits of this unique science towards this new application. This new direction and employment of Liposome science is in part due to the low absorption and bioavailability rates of traditional oral dietary and nutritional tablets and capsules. The low oral bioavailability and absorption of many nutrients is clinically well documented. Therefore the natural encapsulation of lypophilic and hydrophilic nutrients within Liposomes has made for a very effective method of bypassing the destructive elements of the gastric system and aiding the encapsulated nutrient to be delivered to the cells and tissues.

It is important to note that certain influential factors have far reaching effects on the percentage of Liposome that are yielded in manufacturing. These influences also have an effect on the actual amount of realized Liposome entrapment and the actual quality of the Liposomes themselves. These are very crucial elements which lead to the long term stability of the Liposomes. These complex yet significant factors are the following: (1) The actual manufacturing method and preparation of the Liposomes themselves; (2) The constitution, quality, and type of raw phospholipid used in the formulation and manufacturing of the Liposomes; (3) The ability to create homogenous Liposome particle sizes that are stable and hold their encapsulated payload. These primary and key elements comprise the foundation of an effective Liposome carrier for use in increasing the bioavailability of oral dosages of dietary and nutritional supplements.

Liposomes, which use a form nanotechnology science, also impressively and harmoniously, use the generalized nature of the Liposomes themselves to therefore

increase the efficacy, bioavailability, absorption, and delivery of these certain entrapped dietary and nutritional supplements. This generalized nature and makeup of Liposomes, being composed of phospholipids, adroitly complements the natural lining of nearly every cell within the human body.This therefore creates a natural bond and or affinity for the Liposomes to deliver their onboard "payload" to the cells. The quality of raw Lipid used in the preparation and manufacturing of the Liposomes therefore precisely co-relates to this natural congruency between the Liposomes and the cells of the human body.

Applications in medicine

As of 2008, 11 drugs with liposomal delivery systems have been approved and 6 additional liposomal drugs were in clinical trials.

List of clinically-approved liposomal drugs

Name	Trade name	Company	Indication
Liposomal amphotericin B	Abelcet	Enzon	Fungal infections
Liposomal amphotericin B	Ambisome	Gilead Sciences	Fungal and protozoal infections
Liposomal cytarabine	Depocyt	Pacira (formerly SkyePharma)	Malignant lymphomatous meningitis
Liposomal daunorubicin	DaunoXome	Gilead Sciences	HIV-related Kaposi's sarcoma
Liposomal doxorubicin	Myocet	Zeneus	Combination therapy with cyclophosphamide in metastatic breast cancer
Liposomal IRIV vaccine	Epaxal	Berna Biotech	Hepatitis A
Liposomal IRIV vaccine	Inflexal V	Berna Biotech	Influenza
Liposomal morphine	DepoDur	SkyePharma, Endo	Postsurgical analgesia
Liposomal verteporfin	Visudyne	QLT, Novartis	Age-related macular degeneration, pathologic myopia, ocular histoplasmosis
Liposome-PEG doxorubicin	Doxil/Caelyx	Ortho Biotech, Schering-Plough	HIV-related Kaposi's sarcoma, metastatic breast cancer, metastatic ovarian cancer
Micellular estradiol	Estrasorb	Novavax	Menopausal therapy

Targeting cancer

Another interesting property of liposomes are their natural ability to target cancer. The endothelial wall of all healthy human blood vessels is encapsulated by endothelial cells that are bound together by tight junctions. These tight junctions stop any large particles in the blood from leaking out of the vessel. Tumour vessels do not contain the same level of seal between cells and are diagnostically *leaky*. This ability is known as the Enhanced Permeability and Retention effect. Liposomes of certain sizes, typically less than 200 nm, can rapidly enter tumour sites from the blood, but are kept in the bloodstream by the endothelial wall in healthy tissue vasculature. Anti-cancer drugs such as Doxorubicin (Doxil), Camptothecin and Daunorubicin (Daunoxome) are currently being marketed in liposome delivery systems.

New Liposomal drugs targeting cancer like Liposomal Cisplatin (Lipoplatin) has received Orphan Drug designation for Pancreatic Cancer from EMEA.

Manufacturing

The correct choice of liposome preparation method depends on the following parameters:

1. the physicochemical characteristics of the material to be entrapped and those of the liposomal ingredients;
2. the nature of the medium in which the lipid vesicles are dispersed;
3. the effective concentration of the entrapped substance and its potential toxicity;
4. additional processes involved during application/delivery of the vesicles;
5. optimum size, polydispersity and shelf-life of the vesicles for the intended application; and,
6. batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products

Formation of liposomes and nanoliposomes is not a spontaneous process. Lipid vesicles are formed when phospholipids such as lecithin are placed in water and consequently form one bilayer or a series of bilayers, each separated by water molecules, once enough energy is supplied . Liposomes can be created by sonicating phospholipids in water. Low shear rates create multilamellar liposomes, which have many layers like an onion. Continued high-shear sonication tends to form smaller unilamellar liposomes. In this technique, the liposome contents are the same as the contents of the aqueous phase. Sonication is generally considered a "gross" method of preparation as it can damage the structure of the drug to be encapsulated. Newer methods such as extrusion and Mozafari method are employed to produce materials for human use.

Prospect

Further advances in liposome research have been able to allow liposomes to avoid detection by the body's immune system, specifically, the cells of reticuloendothelial system (RES). These liposomes are known as "stealth liposomes", and are constructed

with PEG (Polyethylene Glycol) studding the outside of the membrane. The PEG coating, which is inert in the body, allows for longer circulatory life for the drug delivery mechanism. However, research currently seeks to investigate at what amount of PEG coating the PEG actually hinders binding of the liposome to the delivery site. In addition to a PEG coating, most stealth liposomes also have some sort of biological species attached as a ligand to the liposome in order to enable binding via a specific expression on the targeted drug delivery site. These targeting ligands could be monoclonal antibodies (making an immunoliposome), vitamins, or specific antigens. Targeted liposomes can target nearly any cell type in the body and deliver drugs that would naturally be systemically delivered. Naturally toxic drugs can be much less toxic if delivered only to diseased tissues. Polymersomes, morphologically related to liposomes can also be used this way.

Chapter 9

Pharmaceutical Drug

A **pharmaceutical drug**, also referred to as **medicine**, **medication** or **medicament**, can be loosely defined as any chemical substance intended for use in the medical diagnosis, cure, treatment, or prevention of disease.

Classification

Medications can be classified in various ways, such as by chemical properties, mode or route of administration, biological system affected, or therapeutic effects. An elaborate and widely used classification system is the Anatomical Therapeutic Chemical Classification System (ATC system). The World Health Organization keeps a list of essential medicines.

A sampling of classes of medicine includes:

1. Antipyretics: reducing fever (pyrexia/pyresis)
2. Analgesics: reducing pain (painkillers)
3. Antimalarial drugs: treating malaria
4. Antibiotics: inhibiting germ growth
5. Antiseptics: prevention of germ growth near burns, cuts and wounds

Types of medications (type of pharmacotherapy)

For the gastrointestinal tract (digestive system)

- Upper digestive tract: antacids, reflux suppressants, antiflatulents, antidopaminergics, proton pump inhibitors (PPIs), H_2-receptor antagonists, cytoprotectants, prostaglandin analogues
- Lower digestive tract: laxatives, antispasmodics, antidiarrhoeals, bile acid sequestrants, opioid

For the cardiovascular system

- General: β-receptor blockers ("beta blockers"), calcium channel blockers, diuretics, cardiac glycosides, antiarrhythmics, nitrate, antianginals, vasoconstrictors, vasodilators, peripheral activators
- Affecting blood pressure (antihypertensive drugs): ACE inhibitors, angiotensin receptor blockers, α blockers, calcium channel blockers
- Coagulation: anticoagulants, heparin, antiplatelet drugs, fibrinolytics, anti-hemophilic factors, haemostatic drugs
- Atherosclerosis/cholesterol inhibitors: hypolipidaemic agents, statins.

For the central nervous system

Drugs affecting the central nervous system include: hypnotics, anaesthetics, antipsychotics, antidepressants (including tricyclic antidepressants, monoamine oxidase inhibitors, lithium salts, and selective serotonin reuptake inhibitors (SSRIs)), antiemetics, anticonvulsants/antiepileptics, anxiolytics, barbiturates, movement disorder (e.g., Parkinson's disease) drugs, stimulants (including amphetamines), benzodiazepines, cyclopyrrolones, dopamine antagonists, antihistamines, cholinergics, anticholinergics, emetics, cannabinoids, and 5-HT (serotonin) antagonists.

For pain & consciousness (analgesic drugs)

The main classes of painkillers are NSAIDs, opioids and various orphans such as paracetamol, tricyclic antidepressants and anticonvulsants.

For musculo-skeletal disorders

The main categories of drugs for musculoskeletal disorders are: NSAIDs (including COX-2 selective inhibitors), muscle relaxants, neuromuscular drugs, and anticholinesterases.

For the eye

- General: adrenergic neurone blocker, astringent, ocular lubricant
- Diagnostic: topical anesthetics, sympathomimetics, parasympatholytics, mydriatics, cycloplegics
- Anti-bacterial: antibiotics, topical antibiotics, sulfa drugs, aminoglycosides, fluoroquinolones
- Antiviral drug:
- Anti-fungal: imidazoles, polyenes
- Anti-inflammatory: NSAIDs, corticosteroids
- Anti-allergy: mast cell inhibitors
- Anti-glaucoma: adrenergic agonists, beta-blockers, carbonic anhydrase inhibitors/hyperosmotics, cholinergics, miotics, parasympathomimetics, prostaglandin agonists/prostaglandin inhibitors. nitroglycerin

For the ear, nose and oropharynx

sympathomimetics, antihistamines, anticholinergics, NSAIDs, steroids, antiseptics, local anesthetics, antifungals, cerumenolyti

For the respiratory system

bronchodilators, NSAIDs, anti-allergics, antitussives, mucolytics, decongestants corticosteroids, Beta2-adrenergic agonists, anticholinergics, steroids

For endocrine problems

androgens, antiandrogens, gonadotropin, corticosteroids, human growth hormone, insulin, antidiabetics (sulfonylureas, biguanides/metformin, thiazolidinediones, insulin), thyroid hormones, antithyroid drugs, calcitonin, diphosponate, vasopressin analogues

For the reproductive system or urinary system

antifungal, alkalising agents, quinolones, antibiotics, cholinergics, anticholinergics, anticholinesterases, antispasmodics, 5-alpha reductase inhibitor, selective alpha-1 blockers, sildenafils, fertility medications

For contraception

- Hormonal contraception
- Ormeloxifene
- Spermicide

For obstetrics and gynecology

NSAIDs, anticholinergics, haemostatic drugs, antifibrinolytics, Hormone Replacement Therapy (HRT), bone regulators, beta-receptor agonists, follicle stimulating hormone, luteinising hormone, LHRH
gamolenic acid, gonadotropin release inhibitor, progestogen, dopamine agonists, oestrogen, prostaglandins, gonadorelin, clomiphene, tamoxifen, Diethylstilbestrol

For the skin

emollients, anti-pruritics, antifungals, disinfectants, scabicides, pediculicides, tar products, vitamin A derivatives, vitamin D analogues, keratolytics, abrasives, systemic antibiotics, topical antibiotics, hormones, desloughing agents, exudate absorbents, fibrinolytics, proteolytics, sunscreens, antiperspirants, corticosteroids

For infections and infestations

antibiotics, antifungals, antileprotics, antituberculous drugs, antimalarials, anthelmintics, amoebicides, antivirals, antiprotozoals

For the immune system

vaccines, immunoglobulins, immunosuppressants, interferons, monoclonal antibodies

For allergic disorders

anti-allergics, antihistamines, NSAIDs

For nutrition

tonics, iron preparations, electrolytes, parenteral nutritional supplements, vitamins, anti-obesity drugs, anabolic drugs, haematopoietic drugs, food product drugs

For neoplastic disorders

cytotoxic drugs, therapeutic antibodies, sex hormones, aromatase inhibitors, somatostatin inhibitors, recombinant interleukins, G-CSF, erythropoietin

For diagnostics

contrast media

For euthanasia

An euthanaticum is used for euthanasia and physician-assisted suicide.

Euthanasia is not permitted by law in many countries, and consequently medicines will not be licensed for this use in those countries.

Administration

Administration is the delivery of a pharmaceutical drug to a patient.

It can be performed in various dosage forms such as pills, tablets, or capsules.

There are also many variations in the routes of administration, including intravenous (into the blood through a vein) and oral administration (through the mouth).

They can be administered all at once as a bolus, at frequent intervals or continuously. Frequencies are often abbreviated from Latin, such as *every 8 hours* reading Q8H from *Quaque VIII Hora*.

Legal considerations

Depending upon the jurisdiction, medications may be divided into over-the-counter drugs (OTC) which may be available without special restrictions, and prescription only medicine (POM), which must be prescribed by a licensed medical practitioner. The precise distinction between OTC and prescription depends on the legal jurisdiction. A third category, behind-the-counter medications (BTMs), is implemented in some jurisdictions. BTMs do not require a prescription, but must be kept in the dispensary, not visible to the public, and only be sold by a pharmacist or pharmacy technician. Doctors may also prescribe prescription drugs for off-label use - purposes which the drugs were not originally approved for by the regulatory agency. The Classification of Pharmaco-Therapeutic Referrals helps guide the referral process between pharmacists and doctors.

The International Narcotics Control Board of the United Nations imposes a world law of prohibition of certain medications. They publish a lengthy list of chemicals and plants whose trade and consumption (where applicable) is forbidden. OTC medications are sold without restriction as they are considered safe enough that most people will not hurt themselves accidentally by taking it as instructed. Many countries, such as the United Kingdom have a third category of pharmacy medicines which can only be sold in registered pharmacies, by or under the supervision of a pharmacist.

For patented medications, countries may have certain mandatory licensing programs which compel, in certain situations, a medication's owner to contract with other agents to manufacture the drug. Such programs may deal with the contingency of a lack of medication in the event of a serious epidemic of disease, or may be part of efforts to ensure that disease treating drugs, such as AIDS drugs, are available to countries which cannot afford the drug owner's price.

Prescription practice

Drugs which are prescription only are regulated as such because they can impose adverse effects and should not be used unless necessary. Medical guidelines and clinical trials required for approval are used to help inform doctors' prescription of these drugs, but errors can happen. Reasons to not prescribe drugs such as interactions or side effects are called contraindications.

Errors include overprescription and polypharmacy, misprescription, contraindication and lack of detail in dosage and administrations instructions. In 2000 the definition of a prescription error was studied using a Delphi method conference; the conference was motivated by ambiguity in the what a prescription error and a need to use a uniform definition in studies.

Development

Drug development is the process by which a drug is created. Drugs can be extracted from natural products (pharmacognosy) or synthesized through chemical processes. The drug's active ingredient will be combined with a "vehicle" such as a capsule, cream, or liquid which will be administered through a particular route of administration. Child-resistant packaging will likely be used in the ultimate package sold to the consumer.

Blockbuster drug

A blockbuster drug is a drug generating more than $1 billion of revenue for its owner each year.

A report from URCH Publishing estimated that about one third of the pharma market by value is accounted for by blockbusters. About 125 products are blockbusters. The top seller was Lipitor, a cholesterol-lowering medication marketed by Pfizer with sales of $12.5 billion.

In 2009 there were a total of seven new blockbuster drugs, with combined sales of $9.8 billion.

Beyond this purely arbitrary financial consideration,

> "In the pharmaceutical industry, a blockbuster drug is one that achieves acceptance by prescribing physicians as a therapeutic standard for, most commonly, a highly prevalent chronic (rather than acute) condition. Patients often take the medicines for long periods."

Leading blockbuster drugs

Drug	**Trade name**	**Company**	**Sales** (billion $), year
Atorvastatin	Lipitor	Pfizer	12 (2007) >
Clopidogrel	Plavix	Bristol-Myers Squibb and Sanofi-Aventis	5.9 (2005)
Enoxaparin	Lovenox or Clexane	Sanofi-Aventis	
Celecoxib	Celebrex	Pfizer	2.3 (2007)
Omeprazole	Losec/Prilosec	AstraZeneca	2.6 (2004)
Esomeprazole	Nexium	AstraZeneca	3.3 (2003)
Fexofenadine	Telfast/Allegra	Aventis	1.87 (2004)
Quetiapine	Seroquel	AstraZeneca	1.5 (2003)
Metoprolol	Seloken/Toprol	AstraZeneca	1.3 (2003)
Budesonide	Pulmicort/Rhinocort	AstraZeneca	1.3 (2003) (plus some fraction of the $0.6bn sales of Symbicort)

Environmental impact

Since the 1990s water contamination by pharmaceuticals has been an environmental issue of concern. Most pharmaceuticals are deposited in the environment through human consumption and excretion, and are often filtered ineffectively by wastewater treatment plants which are not designed to manage them. Once in the water they can have diverse, subtle effects on organisms, although research is limited. Pharmaceuticals may also be deposited in the environment through improper disposal, runoff from sludge fertilizer and reclaimed wastewater irrigation, and leaky sewage. In 2009 an investigative report by Associated Press concluded that U.S. manufacturers had legally released 271 million pounds of drugs into the environment, 92% of which was the antiseptics phenol and hydrogen peroxide. It could not distinguish between drugs released by manufacturers as opposed to the pharmaceutical industry. It also found that an estimated 250 million pounds of pharmaceuticals and contaminated packaging were discarded by hospitals and long-term care facilities.

Pharmacoenvironmentology is a branch of pharmacology and a form of pharmacovigilance which deals entry of chemicals or drugs into the environment after elimination from humans and animals post-therapy. It deals specifically with those pharmacological agents that have impact on the environment via elimination through living organisms subsequent to pharmacotherapy, while Ecopharmacology is concerned with the entry of chemicals or drugs into the environment through any route and at any concentration disturbing the balance of ecology (ecosystem), as a consequence. Ecopharmacology is a broad term that includes studies of "PPCPs" irrespective of doses and route of entry into environment.

History

Ancient pharmacology

Using plants and plant substances to treat all kinds of diseases and medical conditions is believed to date back to prehistoric medicine.

The Kahun Gynaecological Papyrus, the oldest known medical text of any kind, dates to about 1800 BCE and represents the first documented use of any kind of medication. It and other medical papyri describe Ancient Egyptian medical practices, such as using honey to treat infections.

Ancient Babylonian medicine demonstrate the use of prescriptions in the first half of the 2nd millennium BC. Medicinal creams and pills were employed as treatments.

On the Indian subcontinent, the Atharvaveda, a sacred text of Hinduism whose core dates from the 2nd millennium BCE, although the hymns recorded in it are believed to be older, is the first Indic text dealing with medicine. It describes plant-based medications to counter diseases. The earliest foundations of ayurveda were built on a synthesis of selected ancient herbal practices, together with a massive addition of theoretical

conceptualizations, new nosologies and new therapies dating from about 400 BCE onwards. The student of Āyurveda was expected to know ten arts that were indispensable in the preparation and application of his medicines: distillation, operative skills, cooking, horticulture, metallurgy, sugar manufacture, pharmacy, analysis and separation of minerals, compounding of metals, and preparation of alkalis.

The Hippocratic Oath for physicians, attributed to 5th century BCE Greece, refers to the existence of "deadly drugs", and ancient Greek physicians imported medications from Egypt and elsewhere.

The first drugstores were created in Baghdad in the 8th century CE. The injection syringe was invented by Ammar ibn Ali al-Mawsili in 9th century Iraq. Al-Kindi's 9th century CE book, *De Gradibus*, developed a mathematical scale to quantify the strength of drugs.

The Canon of Medicine by Ibn Sina (Avicenna), who is considered the father of modern medicine, reported 800 tested drugs at the time of its completion in 1025 CE. Ibn Sina's contributions include the separation of medicine from pharmacology, which was important to the development of the pharmaceutical sciences. Islamic medicine knew of at least 2,000 medicinal and chemical substances.

Medieval pharmacology

Medieval medicine saw advances in surgery, but few truly effective drugs existed, beyond opium and quinine. Folklore cures and potentially poisonous metal-based compounds were popular treatments. Theodoric Borgognoni, (1205–1296), one of the most significant surgeons of the medieval period, responsible for introducing and promoting important surgical advances including basic antiseptic practice and the use of anaesthetics. Garcia de Orta described some herbal treatments that were used.

Modern pharmacology

For most of the 19th century, drugs were not highly effective, leading Oliver Wendell Holmes, Sr. to famously comment in 1842 that "if all medicines in the world were thrown into the sea, it would be all the better for mankind and all the worse for the fishes".[:21]

During the First World War, Alexis Carrel and Henry Dakin developed the Carrel-Dakin method of treating wounds with an irrigation, Dakin's solution, a germicide which helped prevent gangrene.

In the inter-war period, the first anti-bacterial agents such as the sulpha antibiotics were developed. The Second World War saw the introduction of widespread and effective antimicrobial therapy with the development and mass production of penicillin antibiotics, made possible by the pressures of the war and the collaboration of British scientists with the American pharmaceutical industry.

Medicines commonly used by the late 1920s included aspirin, codeine, and morphine for pain; digitalis, nitroglycerin, and quinine for heart disorders, and insulin for diabetes. Other drugs included antitoxins, a few biological vaccines, and a few synthetic drugs. In the 1930s antibiotics emerged: first sulfa drugs, then penicillin and other antibiotics. Drugs increasingly became "the center of medical practice".[:22] In the 1950s other drugs emerged including corticosteroids for inflammation, rauwolfia alkloids as tranqulizers and antihypertensives, antihistamines for nasal allergies, xanthines for asthma, and typical antipsychotics for psychosis.[:23-24] As of 2008, thousands of approved drugs have been developed. Increasingly, biotechnology is used to discover biopharmaceuticals. Recently, multi-disciplinary approaches have yielded a wealth of new data on the development of novel antibiotics and antibacterials and on the use of biological agents for antibacterial therapy.

In the 1950s new psychiatric drugs, notably the antipsychotic chlorpromazine, were designed in laboratories and slowly came into preferred use. Although often accepted as an advance in some ways, there was some opposition, due to serious adverse effects such as tardive dyskinesia. Patients often opposed psychiatry and refused or stopped taking the drugs when not subject to psychiatric control.

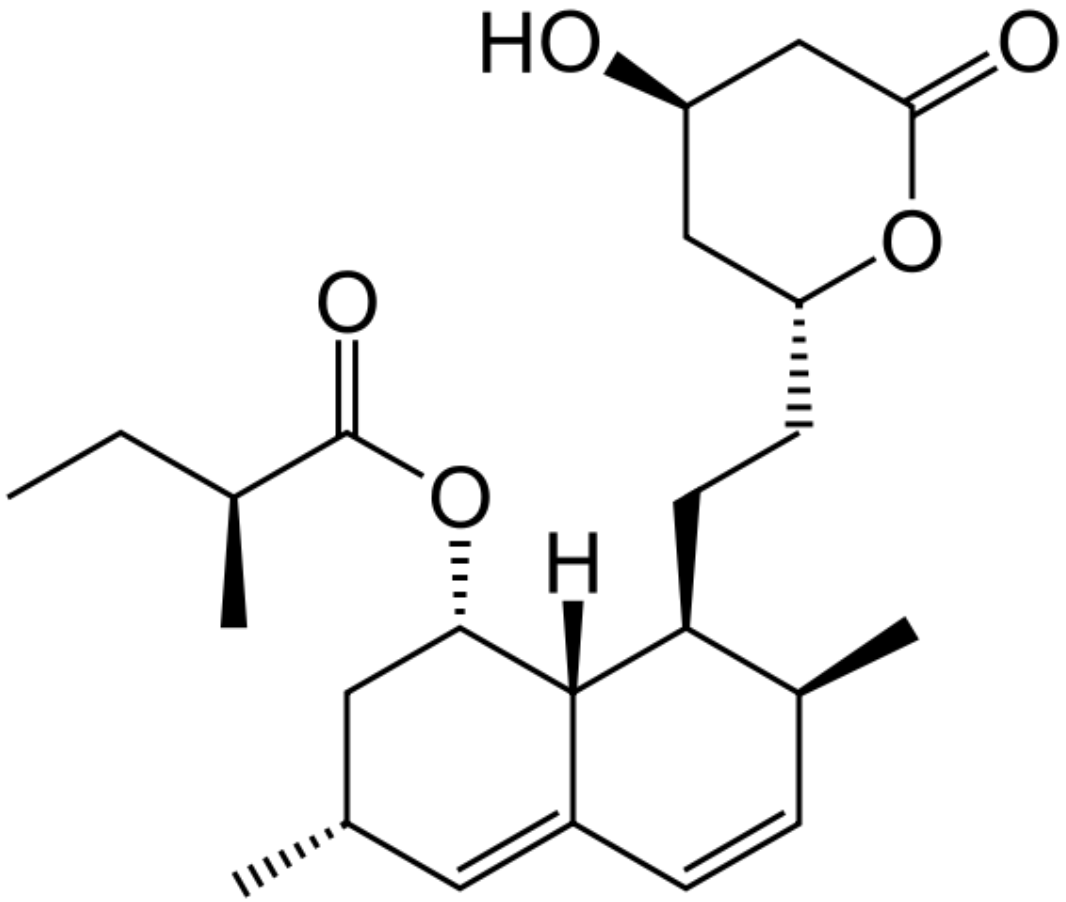
Governments have been heavily involved in the regulation of drug development and drug sales. In the U.S., the Elixir Sulfanilamide disaster led to the establishment of the Food and Drug Administration, and the 1938 Federal Food, Drug, and Cosmetic Act required manufacturers to file new drugs with the FDA. The 1951 Humphrey-Durham Amendment required certain drugs to be sold by prescription. In 1962 a subsequent amendment required new drugs to be tested for efficacy and safety in clinical trials.[:24-26]

Until the 1970s, drug prices were not a major concern for doctors and patients. As more drugs became prescribed for chronic illnesses, however, costs became burdensome, and by the 1970s nearly every U.S. state required or encouraged the substitution of generic drugs for higher-priced brand names. This also led to the 2006 U.S. law, Medicare Part D, which offers Medicare coverage for drugs.[:28-29]

As of 2008, the United States is the leader in medical research, including pharmaceutical development. U.S. drug prices are among the highest in the world, and drug innovation is correspondingly high. In 2000 U.S. based firms developed 29 of the 75 top-selling drugs; firms from the second-largest market, Japan, developed eight, and the United Kingdom contributed 10. France, which imposes price controls, developed three. Throughout the 1990s outcomes were similar.[:30-31]

Chapter 10

Statin



Lovastatin, a compound isolated from *Aspergillus terreus*, was the first statin to be marketed.

Statins (or **HMG-CoA reductase inhibitors**) are a class of drug used to lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver. Increased cholesterol levels have been associated with cardiovascular diseases (CVD), and statins are therefore used in the prevention of these diseases. Randomized controlled trials have shown that they are most effective in those already suffering from cardiovascular disease (secondary prevention),

but they are also advocated and used extensively in those without previous CVD but with elevated cholesterol levels and other risk factors (such as diabetes and high blood pressure) that increase a person's risk.

The best-selling of the statins is atorvastatin, marketed as Lipitor and manufactured by Pfizer. By 2003 it had become the best-selling pharmaceutical in history, with Pfizer reporting sales of $12.4 billion in 2008. As of 2010, a number of statins are on the market: atorvastatin (Lipitor and Torvast), fluvastatin (Lescol), lovastatin (Mevacor, Altocor, Altoprev), pitavastatin (Livalo, Pitava), pravastatin (Pravachol, Selektine, Lipostat), rosuvastatin (Crestor) and simvastatin (Zocor, Lipex). Several combination preparations of a statin and another agent—such as ezetimibe/simvastatin, sold as Vytorin—are also available.

History

In 1971, Akira Endo, a Japanese biochemist working for the drug company Sankyo, began the search for a cholesterol-lowering drug. Research had already shown that cholesterol is mostly manufactured by the body in the liver, using an enzyme known as HMG-CoA reductase. Endo and his team reasoned that certain microorganisms may produce inhibitors of the enzyme to defend themselves against other organisms, as mevalonate is a precursor of many substances required by organisms for the maintenance of their cell wall (ergosterol) or cytoskeleton (isoprenoids). The first agent they identified was mevastatin (ML-236B), a molecule produced by the fungus *Penicillium citrinum*.

Journalist John Simons writes in *Fortune* magazine that word of the discovery spread quickly through the medical community, as did rumors linking the statin to tumors, muscle deterioration, and sometimes death in laboratory dogs. Several drug companies were put off by these reports, but P. Roy Vagelos, the chief scientist and later CEO of Merck & Co, showed an interest, and made several trips to Japan starting in 1975. By 1978, Merck had isolated lovastatin (mevinolin, MK803) from the fungus *Aspergillus terreus*, first marketed in 1987 as Mevacor.

A link between cholesterol and cardiovascular disease, known as the lipid hypothesis, had already been suggested. Cholesterol is the main constituent of atheroma, the fatty lumps in the wall of arteries that occur in atherosclerosis and, when ruptured, cause the vast majority of heart attacks. Treatment consisted mainly of dietary measures such as a low-fat diet, and poorly tolerated medicines such as clofibrate, cholestyramine and nicotinic acid. Cholesterol researcher Daniel Steinberg writes that while the Coronary Primary Prevention Trial of 1984 demonstrated that cholesterol lowering could significantly reduce the risk of heart attacks and angina, physicians, including cardiologists, remained largely unconvinced.

To market statins effectively, Merck had to convince the public about the dangers of high cholesterol, and doctors that statins were safe and would extend lives. As a result of public campaigns, people became familiar with their cholesterol numbers and the difference between "good" and "bad" cholesterol, and rival pharmaceutical companies

began producing their own statins, such as pravastatin (Pravachol), manufactured by Sankyo and Bristol-Myers Squibb. In April 1994, the results of a Merck-sponsored study, the Scandinavian Simvastatin Survival Study or "4S", were announced. Researchers tested simvastatin, later sold by Merck as Zocor, on 4,444 patients with high cholesterol and heart disease. After five years, the study concluded that patients saw a 35-percent reduction in their cholesterol, and their chances of dying of a heart attack were reduced by 42 percent. In 1995, Zocor and Mevacor both made Merck over US$1 billion. Endo was awarded the 2006 Japan Prize, and the Lasker-DeBakey Clinical Medical Research Award in 2008.

Indications and uses

On average, statins can lower LDL cholesterol (so-called "bad cholesterol") by 1.8 mmol/l (70 mg/dl), which translates into a 60% decrease in the number of cardiac events (heart attack, sudden cardiac death) and a 17% reduced risk of stroke after long-term treatment. They have less effect than the fibrates or niacin in reducing triglycerides and raising HDL-cholesterol ("good cholesterol"). There is limited evidence to support the use of statins for primary prevention in people with low cardiovascular risk. Clinical practice guidelines generally recommend that the patient has tried "lifestyle modification", including a cholesterol-lowering diet and physical exercise, before statin use is considered; statins or other pharmacologic agents may then be recommended for patients who do not meet their lipid-lowering goals through diet and lifestyle approaches.

The indications for the prescription of statins have broadened over the years. Initial studies, such as the Scandinavian Simvastatin Survival Study (4S), supported the use of statins in secondary prevention for cardiovascular disease, or as primary prevention only when the risk for cardiovascular disease was significantly raised, as indicated by the Framingham risk score. Indications were broadened considerably by studies such as the Heart Protection Study (HPS), which showed preventative effects of statin use in specific risk groups, such as diabetics. The ASTEROID trial, published in 2006, using only a statin at high dose, achieved lower than usual target calculated LDL values and showed disease regression within the coronary arteries using intravascular ultrasonography. A 2010 cochrane review highlighted the shortcomings in the published trials and recommended that caution should be taken in prescribing statins for primary prevention among people at low cardiovascular risk.

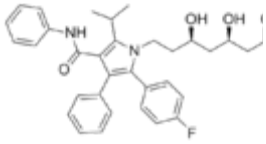
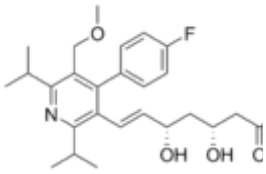
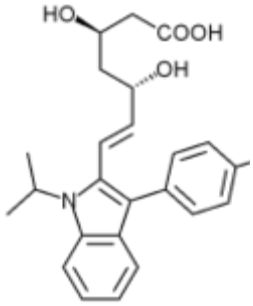
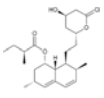
Statins are generally accepted as effective in decreasing mortality in people with preexisting cardiovascular disease. They are also currently advocated for use in patients at high risk of developing heart disease, but the evidence for this is questioned. A 2010 meta-analysis of studies found no benefit in terms of all-cause mortality when statins were used as a high-risk primary prevention intervention.

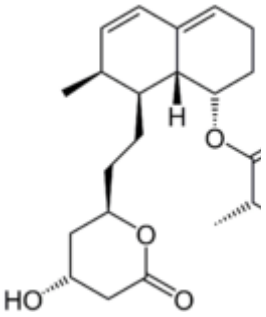
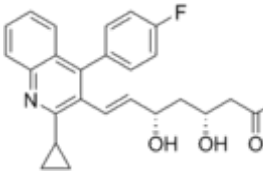
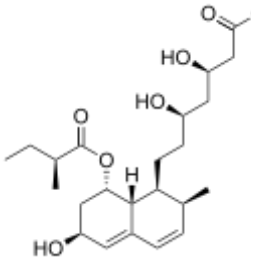
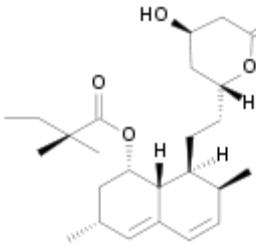
Research continues into other areas where statins also appear to have a favorable effect, including dementia, lung cancer, nuclear cataracts, and hypertension.

Members

Names and types

The statins are divided into two groups: fermentation-derived and synthetic. They include, along with brand names, which may vary between countries:

Statin	Image	Brand name	Derivation	Metabolism
Atorvastatin		Lipitor, Torvast	Synthetic	CYP3A4
Cerivastatin		Lipobay, Baycol. (Withdrawn from the market in August, 2001 due to risk of serious Rhabdomyolysis)	Synthetic	various CYP3A isoforms
Fluvastatin		Lescol, Lescol XL	Synthetic	CYP2C9
Lovastatin		Mevacor, Altocor, Altoprev	Fermentation-derived. Naturally-occurring compound. Found in oyster mushrooms and red yeast rice.	CYP3A4

Mevastatin		Compactin	Naturally-occurring compound. Found in red yeast rice.	CYP3A4
Pitavastatin		Livalo, Pitava	Synthetic	
Pravastatin		Pravachol, Selektine, Lipostat	Fermentation-derived. (A fermentation product of bacterium *Nocardia autotrophica*).	Non CYP
Rosuvastatin		Crestor	Synthetic	CYP2C9 and CYP2C19
Simvastatin		Zocor, Lipex	Fermentation-derived. (Simvastatin is a synthetic derivate of a fermentation product of *Aspergillus terreus*.)	CYP3A4
Simvastatin+Ezetimibe		Vytorin	Combination therapy	
Lovastatin+Niacin extended-release		Advicor	Combination therapy	
Atorvastatin+Amlodipine Besylate		Caduet	Combination therapy - Cholesterol+Blood Pressure	
Simvastatin+Niacin extended-release		Simcor	Combination therapy	

LDL-lowering potency varies between agents. Cerivastatin is the most potent, followed by (in order of decreasing potency), rosuvastatin, atorvastatin, simvastatin, lovastatin, pravastatin, and fluvastatin. The relative potency of pitavastatin has not yet been fully established.

Naturally-occurring statins

The oyster mushroom, a culinary mushroom, naturally contains lovastatin.

Some types of statins are naturally occurring, and can be found in such foods as oyster mushrooms and red yeast rice. Randomized controlled trials found them to be effective, but the quality of the trials was low.

Comparative effectiveness

No large scale comparison exists that examines the relative effectiveness of the various statins against one another for preventing hard cardiovascular outcomes, such as death or myocardial infarction.

An independent analysis has been done to compare atorvastatin, pravastatin and simvastatin, based on their effectiveness against placebos. It found that, at commonly prescribed doses, there are no statistically significant differences amongst statins in reducing cardiovascular morbidity and mortality. The CURVES study, which compared

the efficacy of different doses of atorvastatin, simvastatin, pravastatin, lovastatin, and fluvastatin for reducing LDL and total cholesterol in patients with hypercholesterolemia, found that atorvastatin was more effective without increasing adverse events.

Statins differ in their ability to reduce cholesterol levels. Doses should be individualized according to patient characteristics such as goal of therapy and response. After initiation and/or dose changes, lipid levels should be analyzed within 1–3 months and dosage adjusted accordingly, then every 6–12 months afterwards.

Statin Equivalent Dosages

% LDL Reduction (approx.)	Atorvastatin	Fluvastatin	Lovastatin	Pravastatin	Rosuvastatin	Simvastatin
10-20%	--	20 mg	10 mg	10 mg	--	5 mg
20-30%	--	40 mg	20 mg	20 mg	--	10 mg
30-40%	10 mg	80 mg	40 mg	40 mg	5 mg	20 mg
40-45%	20 mg	--	80 mg	80 mg	5–10 mg	40 mg
46-50%	40 mg	--	--	--	10–20 mg	80 mg
50-55%	80 mg	--	--	--	20 mg	--
56-60%	--	--	--	--	40 mg	--
Starting dose						
Starting dose	10–20 mg	20 mg	10–20 mg	40 mg	10 mg; 5 mg if hypothyroid, >65 yo, Asian	20 mg
If higher LDL reduction goal	40 mg if >45%	40 mg if >25%	20 mg if >20%	--	20 mg if LDL >190 mg/dL (4.87 mmol/L)	40 mg if >45%
Optimal timing	Anytime	Evening	With evening meals	Anytime	Anytime	Evening

Adverse effects

The most common adverse side effects are raised liver enzymes and muscle problems. In randomized clinical trials, reported adverse effects are low; but they are "higher in studies of real world use", and more varied. In randomized trials statins increased the risk of an adverse effect by 39% compared to placebo (odds ratios 1.4); two-thirds of these were myalgia or raised liver enzymes with serious adverse effects similar to placebo. However, reliance on clinical trials can be misleading indications of real-world adverse effects – for example, the statin cerivastatin was withdrawn from the market in 2001 due to cases of rhabdomyolysis (muscle breakdown), although rhabdomyolysis did not occur in a meta-

analysis of cerivastatin clinical trials. Other possible adverse effects include cognitive loss, neuropathy, pancreatic and hepatic dysfunction, and sexual dysfunction.

Some patients on statin therapy report myalgias, muscle cramps, or, less frequently, gastrointestinal or other symptoms. Liver enzyme derangements may also occur, typically in about 0.5%, are also seen at similar rates with placebo use and repeated enzyme testing, and generally return to normal either without discontinuance over time or after briefly discontinuing the drug. Multiple other side-effects occur rarely; typically also at similar rates with only placebo in the large statin safety/efficacy trials. Two randomized clinical trials found cognitive issues while two did not; recurrence upon reintroduction suggests that these are causally related to statins in some individuals. A Danish case-control study published in 2002 suggested a relation between long term statin use and increased risk of nerve damage or polyneuropathy but suggested this side effect is "rare, but it does occur"; other researchers have pointed to studies of the effectiveness of statins in trials involving 50,000 people which have not shown nerve damage as a significant side effect.

Rare reactions include myositis and myopathy, with the potential for rhabdomyolysis (the pathological breakdown of skeletal muscle) leading to acute renal failure. Coenzyme Q10 (ubiquinone) levels are decreased in statin use; Q10 supplements are sometimes used to treat statin-associated myopathy, though evidence of their effectiveness is currently lacking. A common variation in the *SLCO1B1* gene, which participates in the absorption of statins, has been shown to significantly increase the risk of myopathy.

Graham et al. (2004) reviewed records of over 250,000 patients treated from 1998 to 2001 with the statin drugs atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin, and simvastatin. The incidence of rhabdomyolyis was 0.44 per 10,000 patients treated with statins other than cerivastatin. However, the risk was over tenfold greater if cerivastatin was used, or if the standard statins (atorvastatin, fluvastatin, lovastatin, pravastatin, simvastatin) were combined with fibrate (fenofibrate or gemfibrozil) treatment. Cerivastatin was withdrawn by its manufacturer in 2001.

All commonly used statins show somewhat similar results, however the newer statins, characterized by longer pharmacological half-lives and more cellular specificity, have had a better ratio of efficacy to lower adverse effect rates. Some researchers have suggested that hydrophilic statins such as fluvastatin, rosuvastatin, and pravastatin are less toxic than lipophilic statins such as atorvastatin, lovastatin, and simvastatin, but other studies have not found a connection; it is suggested that the risk of myopathy is lowest with pravastatin and fluvastatin probably because they are more hydrophillic and as a result have less muscle penetration. Lovastatin induces the expression of gene atrogin-1, which is believed to be responsible in promoting muscle fiber damage.

Although there have been concerns that statins might increase cancer, several meta-analyses have found no relationship to cancer, the largest of which as of 2006 included nearly 87,000 participants. However, in 2007 a meta-analysis of 23 statin treatment arms with 309,506 person-years of follow-up found that there was an inverse relationship

between achieved LDL-cholesterol levels and rates of newly diagnosed cancer that the authors claim requires further investigation.

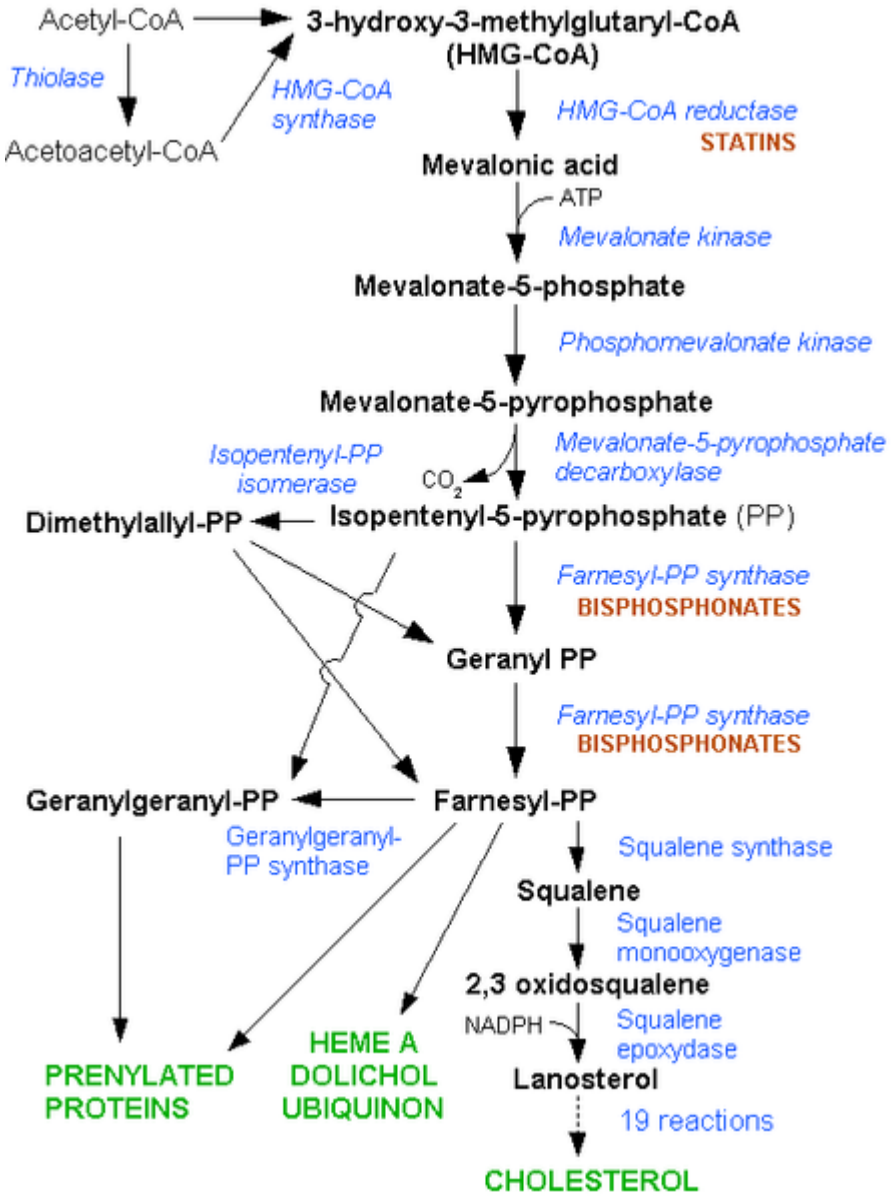
Several case-control studies have found that statins reduce cancer incidence, including one which showed that patients taking statins for over 5 years reduced their risk of colorectal cancer by 50%; this effect was not exhibited by fibrates although the trialists warned that the number needed to treat would approximate 5000, making statins unlikely tools for primary prevention.

Drug interactions

Combining any statin with a fibrate, another category of lipid-lowering drugs, increases the risks for rhabdomyolysis to almost 6.0 per 10,000 person-years. Most physicians have now abandoned routine monitoring of liver enzymes and creatine kinase, although they still consider this prudent in those on high-dose statins or in those on statin/fibrate combinations, and mandatory in the case of muscle cramps or of deterioration in renal function.

Consumption of grapefruit or grapefruit juice inhibits the metabolism of statins. Furanocoumarins in grapefruit juice (i.e. bergamottin and dihydroxybergamottin) inhibit the cytochrome P450 enzyme CYP3A4, which is involved in the metabolism of most statins (however it is a major inhibitor of only lovastatin, simvastatin and to a lesser degree atorvastatin) and some other medications (it had been thought that flavonoids (i.e. naringin) were responsible). This increases the levels of the statin, increasing the risk of dose-related adverse effects (including myopathy/rhabdomyolysis). Consequently, consumption of grapefruit juice is not recommended in patients undergoing therapy with most statins. An alternative, somewhat risky, approach is that some users take grapefruit juice to enhance the effect of lower (hence cheaper) doses of statins. This is not recommended as a result of the increased risk and potential for statin toxicity. Bitter oranges may have a similar effect.

Mechanism of action



The HMG-CoA reductase pathway, which is blocked by statins via inhibiting the rate limiting enzyme HMG-CoA reductase.

Statins act by competitively inhibiting HMG-CoA reductase, the first committed enzyme of the HMG-CoA reductase pathway. Because statins are similar to HMG-CoA on a molecular level they take the place of HMG-CoA in the enzyme and reduce the rate by which it is able to produce mevalonate, the next molecule in the cascade that eventually produces cholesterol, as well as a number of other compounds. This ultimately reduces cholesterol via several mechanisms.

Inhibiting cholesterol synthesis

By inhibiting HMG-CoA reductase, statins block the pathway for synthesizing cholesterol in the liver. This is significant because most circulating cholesterol comes from internal manufacture rather than the diet. When the liver can no longer produce cholesterol, levels of cholesterol in the blood will fall. Cholesterol synthesis appears to occur mostly at night, so statins with short half-lives are usually taken at night to maximize their effect. Studies have shown greater LDL and total cholesterol reductions in the short-acting simvastatin taken at night rather than the morning, but have shown no difference in the long-acting atorvastatin.

Increasing LDL uptake

Liver cells sense the reduced levels of liver cholesterol and seek to compensate by synthesizing LDL receptors to draw cholesterol out of the circulation. This is accomplished via protease enzymes that cleave a protein called "membrane-bound sterol regulatory element binding protein", which migrates to the nucleus and causes increased production of various other proteins and enzymes, including the LDL receptor. The LDL receptor then relocates to the liver cell membrane and binds to passing LDL and VLDL particles (the "bad cholesterol" linked to disease). LDL and VLDL are drawn out of circulation into the liver where the cholesterol is reprocessed into bile salts. These are excreted, and subsequently recycled mostly by an internal bile salt circulation.

Other effects

Statins exhibit action beyond lipid-lowering activity in the prevention of atherosclerosis. The ASTEROID trial showed direct ultrasound evidence of atheroma regression during statin therapy. Researchers hypothesize that statins prevent cardiovascular disease via four proposed mechanisms (all subjects of a large body of biomedical research):

1. Improve endothelial function
2. Modulate inflammatory responses
3. Maintain plaque stability
4. Prevent thrombus formation

Statins may even benefit those without high cholesterol. In 2008 the JUPITER study showed fewer stroke, heart attacks, and surgeries even for patients who had no history of high cholesterol or heart disease, but only elevated C-reactive protein levels. There were also 20% fewer deaths (mainly from reduction in cancer deaths) though deaths from cardiovascular causes were not reduced.

Pharmacogenomics

A 2004 study showed that patients with one of two common single-nucleotide polymorphisms (small genetic variations) in the HMG-CoA reductase gene were less

responsive to statins. A 2008 study showed that carriers of the KIF6 genetic mutation were more responsive to statin treatment.

Criticism and controversy

Some scientists take a skeptical view of the need for many people to receive statin treatment as currently recommended. Given the wide indications for which statins are prescribed, and the declining benefit in groups at lower baseline risk of cardiovascular events, the evidence base for expanded statin use has been questioned by some researchers.

A smaller group of scientists, The International Network of Cholesterol Skeptics, question the lipid hypothesis and argue that elevated cholesterol has not been adequately linked to heart disease. These organizations maintain that statins are not as beneficial or safe as suggested.