



# Nanoparticles as Precious Stones in the Crown of Modern Molecular Biology

# 16

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## Abstract

The interdisciplinary field of research on biosystems at the nanoscale involving physical sciences, molecular engineering, biology, biotechnology, and medicine supplements the knowledge of synthesizing new drugs, targeted delivery, regenerative medicine, and neuromorphic engineering forms the booming research in the present society. The present chapter deals with the role of nanoparticles in modern molecular biology. This is an interesting area of research that creates great impact on the healthcare of the society. The prime focus is to give the reader a historic background of nanomaterial application in biology and medicine. We have also provided the overview of most recent developments in this field leading to discussion of hard road to commercialization.

## 16.1 Introduction

Every person has been exposed to nanometer-sized foreign particles; we inhale them with every breath and consume them with every drink. In truth, every organism on Earth continuously encounters nanometer-sized entities (Rizzello et al. 2011; Pandey et al. 2008; Bennett-Woods 2006). Very small particles, so-called nanoparticles, have the ability to enter, translocate within, and damage living

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organisms (Elsaesser and Howard 2012; Prabhu and Poulouse 2012). This ability results primarily from their small size thereby allowing them to penetrate physiological barriers and travel within the circulatory systems of a host (Panessa-Warren et al. 2006; Li et al. 2010; Chen et al. 2016). The vast majority causes little ill effect and goes unnoticed, but occasionally an intruder will cause appreciable harm to the organism. The most advanced of the toxic intruders are viruses, composed of nucleic acid-based structures that allow them not only to interfere with biological systems but also to parasitically exploit cellular processes to replicate themselves (Ravindran et al. 2016; Franzen and Lommel 2009). A growing number of recent studies show, however, that nano- and microorganisms might play a vital role in many chronic diseases where infection pathogens have not been suspected, diseases that were previously attributed only to genetic factors and lifestyle (He and Shi 2009; Zheng et al. 2016). These diseases include leukemia (caused by viruses from the retrovirus and herpesvirus families) (Nisole et al. 2005; Walther and Stein 2000; Jarrett 2006), cervical cancer (*Papillomavirus*) (DiMaio and Liao 2006; Muñoz et al. 2003; Bosch et al. 1995), liver cancer (hepatitis virus), gastric ulcer (*Helicobacter pylori*) (Kusters et al. 2006; Blaser et al. 1995; Tomb et al. 1997), nasopharyngeal cancer (*Epstein-Barr virus*) (Zur Hausen et al. 1970; Lo et al. 1999; Burgos 2005), kidney stones (nanobacteria) (Çiftçioğlu et al. 1999; Kramer et al. 2000; Kajander and Ciftçioğlu 1998), severe acquired respiratory syndrome SARS (coronavirus) (Peiris et al. 2003; Rota et al. 2003; Marra et al. 2003; Kahn 2006), heart disease (*Chlamydia pneumoniae*) (Patel et al. 1995; Danesh et al. 1997; Bachmaier et al. 1999; Kol and Santini 2004), juvenile diabetes (*Coxsackievirus*) (Atkinson et al. 1994; Horwitz et al. 1998; Fohlman and Friman 1993), Alzheimer's disease (*Chlamydia pneumoniae*) (Balin et al. 1998; Hammond et al. 2010; Itzhaki et al. 2004), pediatric obsessive-compulsive disorder (*Streptococcal* bacteria) (Swedo et al. 1998; Mell et al. 2005; Lynch et al. 2006), psychotic disorders (bornavirus) (Nunes et al. 2008; Miranda et al. 2006), and prion diseases such as mad cow disease (proteins-prions) (Chesebro 1998; Legname et al. 2004; Janka and Maldarelli 2004).

Nanotechnology is the manipulation of matter at the scale of 1–100 nanometers. Using nanotechnology, we can control molecules at an atomic level and create materials with unique properties. A nanometer is  $10^{-9}$  (a billionth) of a meter. The prefix nano has the Greek meaning of dwarf. As a reference point, a hair is approximately 100,000 nanometers. A red blood cell is approximately 10,000 nanometers. Fundamentally the properties of materials can be changed by nanotechnology. We can arrange molecules in a way that they do not normally occur in nature. The material strength along with electronic and optical properties of materials could be altered with the aid of nanotechnology.

### 16.1.1 State of the Art

Molecular biology explores cells and their characteristics, parts, and chemical processes which pay special attention to how molecules control cell's activities and growth (Crick 1970). Looking at the molecular machinery of life that began in the

early 1930s, but truly modern molecular biology emerged with the uncovering of the structure of DNA in the 1960s (Meyer 2003; Morange and Cobb 2000). As a science that studies interactions between the molecular components that carry out various biological processes in living cells, an important idea in molecular biology states that information flow in organisms follows a one-way street: genes are transcribed into RNA, and RNA is translated into proteins. The molecular components make up biochemical pathways that provide the cells with energy, facilitate processing “messages” from outside the cell itself, generate new proteins, and replicate the cellular DNA genome. For example, molecular biologists study how proteins interact with RNA during “translation” (the biosynthesis of new proteins), the molecular mechanism behind DNA replication, and how genes are turned on and off, a process called “transcription” (Lodish et al. 1995).

The birth and development of molecular biology were driven by the collaborative efforts of physicists, chemists, and biologists. As mentioned, modern molecular biology emerged with the discovery of the double helix structure of DNA. The 1962 Nobel Prize in Physiology or Medicine was awarded jointly to Francis H. Crick, James D. Watson, and Maurice H. F. Wilkins “for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material” (Palenik et al. 2003; Scott and Thompson 2011). Advances and discoveries in molecular biology continue to make major contributions to medical research and drug development (Lipinski and Hopkins 2004).

DNA sequencing and synthesis are two sides of the same coin, the “read” and “write” functions of genetic material (Shendure and Ji 2008; Chan 2005). This field and its requisite technology took off in the 1990s with the Human Genome Project’s effort to sequence billions of bases to unlock a new era of genetically informed medicine (Sawicki et al. 1993; Venter et al. 2001). The resulting science is still a work in progress – it turns out the genetic code a more complicated one than anticipated – but the technologies and companies helped it spawn are an impressive legacy. The Integrated DNA Technologies (IDT) got its start during the Human Genome Project, as it produced single nucleotides (the As, Ts, Cs, and Gs that comprise the genetic code) and short oligonucleotide chains (or “oligos”) to facilitate a massive sequencing effort around the world (Burns et al. 1996). Of course, sequencing technology has advanced dramatically in the intervening decades, but “you still need oligos to do the sequencing,” explains Jerry Steele, IDT’s Director of Marketing, “especially in the next generation sequencing space, sequencing and DNA synthesis go hand in hand.” The current sequencing method of choice is Illumina, a process that frequently returns millions of bases of DNA sequence by reading distinct step-wise fluorescent signals associated with each base in a massively parallel array (Schadt et al. 2010; Nakamura et al. 2011). To distinguish genetic material from different samples (a few hundred are often run on the same plate), scientists label each sample’s DNA extract with a distinct barcode (Hebert et al. 2003). With each barcode comprised of about ten nucleotides, the demand for synthetic DNA chains in the sequencing process is substantial (deWaard et al. 2008).

Unlike other biotech companies prioritizing longer constructs or gene variants, IDT specializes in relatively short oligos. These chains are used not only in Illumina

barcoding but also as primers, consistent patches of sequence that may border unknown regions and facilitate polymerase chain reaction (PCR)-based amplification (Bhargava et al. 2013). Both techniques – “next-generation” Illumina sequencing and primer-based amplification – are staples of any self-respecting applied or research-based microbiology laboratory, as they allow researchers to identify constituent organisms or confirm a gene’s presence. With such short sequences, a single nucleotide discrepancy could mean the difference between two Illumina samples from opposite ends of the world or between a gene native to the *Firmicutes* or the *Proteobacteria*. It’s a small margin for error, “so every base better be right,” explains Steele. “As we’ve grown, it’s just a matter of maintaining that consistency on a larger scale.” In the spirit of not fixing something that needs no repairs, IDT shipped an entire fabrication room from its headquarters in Coralville, Iowa, to Belgium when that facility was being built.

Fundamental as they are to modern biology, oligos are used every day in thousands of laboratories around the world, often in innovative ways that the company itself may not have predicted. “The things that people are doing with DNA are really inspiring,” noted Steele. One of his favorite cases involves low-impact prenatal tests: rather than a painful and invasive amniocentesis, “we’ve discovered that now because of sequencing, we can see the baby’s DNA in a blood draw from the mother.” Improved sequencing fidelity and throughput are expanding the resolution of the technique, and Steele envisions scientists using next-generation sequencing to detect cancer cells from the bloodstream as an early diagnostic tool. “Biology is really leaving the lab and coming into the real world,” Steele explains, “and it’s going to improve a lot of lives.”

### 16.1.2 Nanomaterials in Modern Molecular Biology

The interdisciplinary field of research on biosystems at the nanoscale involving physical sciences, molecular engineering, biology, biotechnology, and medicine supplements knowledge of the synthesis of new drugs and their targeted delivery, regenerative medicine, neuromorphic engineering, and developing a sustainable environment (Roco 2003). The diagnostics for early cancer detection have been made successful by the dendritic polymers, multicolor quantum dots, nanocarriers, and diagnostic markers (Ferrari 2005; Smith et al. 2006; Wolinsky and Grinstaff 2008; Majoros et al. 2008; Peer et al. 2007; Visintin et al. 2008). Gold nanoparticles play a major role in cancer therapeutics owing to its enhanced surface plasmon resonance (Huang et al. 2007; Kah et al. 2007). DNA delivery into cell applications employs silica nanoparticles (Roy et al. 2005; Kneuer et al. 2000; Slowing et al. 2008; Zhu et al. 2002). Another interesting area of research is to realize biocompatible implants for replacing damaged or worn body parts with improved tissue engineering at the nanoscale and extend its arms for building up bioartificial organs (McIntire 2002; Mendonça et al. 2008; Variola et al. 2009), nano-hydroxyapatite being a reliable candidate for bioimplants due to its excellent biocompatibility (Zhou and Lee 2011; Wang et al. 2012). Nanobiological devices

and biocompatible electronic systems for detection and control, implants of wireless systems, neuroprostheses, and parts of the neural system form the part of molecular biology applications (PROKOP 2001; Gillies et al. 2002; Mercanzini et al. 2010; Tokárová et al. 2013). There is an interesting classification of nanotechnology in two classes, “wet” nanotechnology (living biosystems) and “dry” nanotechnology. The systematic approaches are important in engineering man-made objects at the nanoscale for integrating them into large-scale structures which are similar to that of nature (Sarikaya et al. 2003; Alivisatos 2004). Interestingly, this booming field is also known as biomimicry or bionics where researchers get the idea from nature by mimicking them to be explored in various applications (Chakrabarti and Shu 2010; Quinn and Gaughran 2010). Few examples of such concepts can be lotus effect, pearl effect, Gecko effect, butterfly wings, Namib desert beetle, spider web, and so on (Bhushan 2009; Pereira et al. 2015). These biomimetic ideas were adopted for the successful fabrication of Velcro, self-cleaning tiles, desert fog harvesting, water filters, adhesives, nanopaints, etc. (Bhushan and Jung 2011; Vierra 2011; Ivanić et al. 2015; Diamanti and Pedferri 2015; Weiler and Goel 2015). Some of the companies that are involved

**Table 16.1** Examples of companies commercializing nanomaterials for bio- and medical applications

Company	Major area of activity	Technology
Advectus Life Sciences Inc.	Drug delivery	Polymeric nanoparticles engineered to carry antitumor drug across the blood-brain barrier
Alnis Biosciences, Inc.	Biopharmaceutical	Biodegradable polymeric nanoparticles for drug delivery
Argonide	Membrane filtration	Nanoporous ceramic materials for endotoxin filtration, orthopedic and dental implants, and DNA and protein separation
BASF	Toothpaste	Hydroxyapatite nanoparticles seem to improve dental surface
Biophan Technologies, Inc.	MRI shielding	Nanomagnetic/carbon composite materials to shield medical devices from RF fields
Capsulation NanoScience AG	Pharmaceutical coatings to improve solubility of drugs	Layer-by-layer polyelectrolyte coatings, 8–50 nm
Dynal Biotech		Magnetic beads
Eiffel Technologies	Drug delivery	Reducing size of the drug particles to 50–100 nm
EnviroSystems, Inc.	Surface disinfectant	Nanoemulsions
Evident Technologies	Luminescent biomarkers	Semiconductor quantum dots with amine or carboxyl groups on the surface, emission from 350 to 2500 nm
Immunicon	Tracking and separation of different cell types	Magnetic core surrounded by a polymeric layer coated with antibodies for capturing cells

(continued)

**Table 16.1** (continued)

Company	Major area of activity	Technology
KES Science and Technology, Inc.	Airocide filters	Nano-TiO <sub>2</sub> to destroy airborne pathogens
NanoBio Corporation	Pharmaceutical	Antimicrobial nanoemulsions
NanoCarrier Co., Ltd	Drug delivery	Micellar nanoparticles for encapsulation of drugs, proteins, DNA
NanoPharm AG	Drug delivery	Polybutylcyanoacrylate nanoparticles are coated with drugs and then with surfactant and can go across the blood-brain barrier
Nanoplex Technologies, Inc.	Nanobarcodes for bioanalysis	
Nanoprobes, Inc.	Gold nanoparticles for biological markers	Gold nanoparticles bioconjugate for TEM and/or fluorescent microscopy
Nanosphere, Inc.	Gold biomarkers	DNA barcode attached to each nanoprobe for identification purposes; PCR is used to amplify the signal and also catalytic silver deposition to amplify the signal using surface plasmon resonance
NanoMed Pharmaceutical, Inc.	Drug delivery	Nanoparticles for drug delivery
Oxonica Ltd	Sunscreens	Doped transparent nanoparticles to effectively absorb harmful UV and convert it into heat
pSivida Ltd	Tissue engineering, implants, drug and gene delivery, biofiltration	Exploiting material properties of nanostructured porous silicone
Smith & Nephew	Acticoat bandages	Nanocrystal silver is highly toxic to pathogens
Quantum Dot Corporation	Luminescent biomarkers	Bioconjugated semiconductor quantum dots

in the development and commercialisation of nanomaterials in biological and medical applications are listed below (Table 16.1) (Salata 2004).

### 16.1.3 Nanoparticles Existing in Biology

Hybrid bio-nanomaterials can also be applied to build novel electronic, optoelectronic, and memory devices (Yan et al. 2003). Self-assembling nanostructure-based DNA molecules obtained by bottom-up nanofabrication show interesting applications. The main focus is firstly to give the reader a historic prospective of nanomaterial application to biology and medicine, secondly to try to overview the most recent developments in this field, and finally to discuss the hard road to commercialization.

### 16.1.3.1 Nanomedicine

Nanomedicine is a field of medical science employing nanotechnology whose applications are increasing more and more, thanks to nanorobots and biological machines which become very useful tools to develop this area of knowledge (Andrew 2000; Wagner et al. 2006; Riehemann et al. 2009). Nanomedicine is the application of nanotechnology in different areas of medicine and biology (Andrew 2000). Nanomedicine ranges from the biomedical applications of nanomaterials and biological devices to nanoelectronic biosensors and even possible future applications of molecular nanotechnology such as biological machines (Nie 2010; Vinogradov and Wei 2012; Jain and Stylianopoulos 2010). Current problems for nanomedicine involve understanding the issues related to toxicity and environmental impact of nanoscale materials (Nel et al. 2006; Hardman 2006; Dreher 2004; Colvin 2003; Jia et al. 2005). The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both *in vivo* and *in vitro* biomedical research and applications (Salata 2004). More than just an extension of “molecular medicine,” nanomedicine will employ molecular machine systems to address medical problems and will use molecular knowledge to maintain and improve human health at the molecular scale (Freitas 2002; Freitas 2005; Kostarelos 2006; Bogunia-Kubik and Sugisaka 2002). Nanomedicine will have extraordinary and far-reaching implications for the medical profession, in the definition of disease and in the diagnosis and treatment of medical conditions including aging, and ultimately for the improvement and extension of natural human biological structure and function.

### 16.1.3.2 Nanoinsecticide

An insecticide is a substance used to kill insects. They include ovicides and larvicides used against insect eggs and larvae, respectively. Insecticides are used in agriculture, medicine, industry, and by consumers. Insecticides are claimed to be a major factor behind the increase in agricultural twentieth century’s productivity (Perry et al. 2013). Nearly all insecticides have the potential to significantly alter ecosystems; many are toxic to humans; some concentrate along the food chain.

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## 16.2 Classification of Nanostructures

Nanostructures are classified based on the number of dimensions, which are not confined to the nanoscale range (<100 nm) (Kelsall et al. 2005):

- Zero dimensional (0D)
- One dimensional (1D)
- Two dimensional (2D)
- Three dimensional (3D)

### 16.2.1 Zero Dimensional (0D)

Materials wherein all the dimensions are measured within the nanoscale (no dimensions, or 0-D, are larger than 100 nm). The most common representation of zero-dimensional nanomaterials is nanoparticles or quantum dots. Nanoparticles or quantum dots can be amorphous or crystalline, single crystalline or polycrystalline, metallic, ceramic, or polymeric, and composed of single or multi-chemical elements; exhibit various shapes and forms; and exist individually or are incorporated in a matrix.

### 16.2.2 One Dimensional (1-D)

One dimension is outside the nanoscale leading to needlelike-shaped nanomaterials. One-dimensional materials include nanotubes, nanorods, and nanowires. One-dimensional nanomaterials can be amorphous or crystalline; metallic, ceramic, or polymeric; single crystalline or polycrystalline; chemically pure or impure; stand-alone materials; or embedded in within another medium.

### 16.2.3 Two Dimensional (2-D)

In 2-D nanostructures, two of the dimensions are not confined in the nanoscale. Two-dimensional nanomaterials exhibit platelike shapes. Two-dimensional nanomaterials include nanofilms, nanolayers, and nanocoatings. Two-dimensional nanomaterials can be amorphous or crystalline, metallic, ceramic, or polymeric, deposited on a substrate, integrated in a surrounding matrix material, made up of various chemical compositions, and used as a single layer or as multilayer structures.

### 16.2.4 Three Dimensional (3-D)

Bulk nanomaterials are materials that are not confined to the nanoscale in any dimension and are known as 3-D nanostructures. These materials are thus characterized by having three arbitrarily dimensions above 100 nm. Materials possess a nanocrystalline structure or involve the presence of features at the nanoscale. In terms of nanocrystalline structure, bulk nanomaterials can be composed of a multiple arrangement of nanosize crystals, most typically in different orientations. With respect to the presence of features at the nanoscale, 3-D nanomaterials can contain dispersions of nanoparticles, bundles of nanowires, and nanotubes as well as multianolayers.

The following table shows a list of nanomedical technologies (Freitasjr 2005) (Table 16.2).



**Table 16.2** Nanomedicine technologies

Raw nanomaterials	Cell simulations and cell diagnostics	Biological research
Nanoparticle coatings	Cell chips	Nanobiology
Nanocrystalline materials	Cell simulators	Nanoscience in life sciences
<i>Artificial binding sites</i>	<i>DNA manipulation, sequencing, diagnostics</i>	<i>Drug delivery</i>
Artificial antibodies	Genetic testing	Drug discovery biopharmaceuticals
Artificial enzymes	DNA microarrays	Drug encapsulation
Artificial receptors	Ultrafast DNA sequencing	Drug delivery
Molecular imprinted polymers	DNA manipulation and control	Smart drugs
<i>Nanostructured materials</i>	<i>Tools and diagnostics</i>	<i>Biotechnology, biorobotics, and nanorobots</i>
Cyclic peptides	Bacterial detection systems	Biological viral therapy
Dendrimers	Biochips	Virus-based hybrids
Detoxification agents	Biomolecular imaging	Stem cells and cloning
Fullerenes	Biosensors and biodetection	Tissue engineering
Functional drug carriers	Diagnostic and defense applications	Artificial organs
MRI scanning	Endoscopic robots and microscopes	Nanobiotechnology
Nanobarcodes	Fullerene-based sensors	Biorobotics and biobots
Nanoemulsions	Cellular imaging	DNA-based devices and nanorobots
Nanofibers	Lab on a chip	Diamond-based nanorobots
Nanoparticles	Monitoring	Cell repair devices
Nanoshells	Nanosensors	
Carbon nanotubes	Point of care diagnostics	
Noncarbon nanotubes	Protein microarrays	
Quantum dots	Scanning probe microscopy	

### 16.3 Fabrication Methods of Nanoparticles

The present research is more focused on green synthesis of nanoparticles without any toxic chemicals by means of reduction mechanism using plants, vegetables, and fruits (Disci-Zayed 2016; Irvani 2011). Different methods are available in the synthesis of nanoparticles. All these techniques fall into one of the three categories:

1. Solid-state synthesis of nanoparticles
2. Vapor-phase synthesis of nanoparticles
3. Soft chemical methods

### 16.3.1 Solid-State Synthesis of Nanoparticles

Solid-state synthesis generally involves a heat treatment step in order to achieve the desired crystal structure, which is followed by media milling (Cao 2004). While it is generally believed that it is difficult for the lower limit of the average particle size to be much below 100 nm, recent innovations by established companies in the industry may prove otherwise. In particular, the Netzsch LMZ-25 ZETA II System and the Dyno-Mill ECM may push the envelope on what mechanical attrition can do to reduce the particle size.

Judging by the contents of publications, the scientific community has not shown much enthusiasm for mechanical attrition processes for nanoparticle synthesis, perhaps due to issues pertaining to impurity pickup, lack of control on the particle size distribution, and inability to tailor precisely the shape and size of particles in the 10–30 nm range as well as the surface characteristics. Nonetheless, in several instances a modified version of mechanical attrition has been used to synthesize oxide nanoparticles. Dry milling was used to induce chemical reactions through ball-powder collisions that resulted in forming nanoparticles within a salt matrix. Particle agglomeration was minimized by the salt matrix, which then was removed by a simple washing procedure.

### 16.3.2 Vapor-Phase Synthesis of Nanoparticles

Gas condensation, as a technique for producing nanoparticles, refers to the formation of nanoparticles in the gas phase, i.e., condensing atoms and molecules in the vapor phase (Swihart 2003). Oddly enough, it had been practiced in the industry long before it became the subject of research in institutions worldwide. For example, Cabot Corporation in the United States and Degussa in Germany have been using atmospheric flame reactors for decades to produce megatons of such diverse nanoparticles as carbon black (used in tires and inks), silicon dioxide (used in myriad applications including additives in coffee creamers and polymers), and titanium dioxide (used in scores of applications including UV-protecting gels). The generic process involves hydrolysis of gaseous metallic chlorides under the influence of water, which develops during the oxyhydrogen reaction and which in turn leads to a high-temperature reaction zone.

### 16.3.3 Soft Chemical Method

Soft chemical synthesis is a method where the intended product is synthesized through moderate chemical reactions under temperatures ranging from room temperature to several hundred degrees Celsius (Bernardi et al. 2009). Compared to traditional physical synthesis routes or high-temperature ceramic synthesis routes, soft chemical methods have the following advantages:

- Low temperature, therefore low energy consumption
- Less hazardous, less waste, more environmentally benign
- Size and shape controllable products

Most soft chemical methods are carried out in liquid media, based on the reactants dissolving, diffusion, and crystallization process. Therefore, soft chemical methods have other characteristics including:

- Reactants can be completely mixed to form a uniform product phase.
- Products are growing in an open and free environment; therefore, its natural morphology will be easily seen.

Every method has its limitation. It is very difficult to synthesize certain chemicals, for example, solid nitride, by soft chemical methods. However, as environmental protection has become a larger issue, soft chemistry, which is also called “green chemistry,” has quietly evolved into a major phenomenon. The soft chemical method is further classified as follows:

1. Chemical precipitation method
2. Microemulsion technique
3. Colloidal chemical method
4. Chemical synthesis method
5. Organic method
6. Sol-gel method
7. Ion implantation method

The microemulsion technique includes W/O microemulsion (reversed micelles) and O/W microemulsion. Preparation of nanoparticles of metal sulfides usually by reversed micelles, which are thermodynamically stable mixtures of four components: surfactant, co-surfactant, organic solvent, and water. Precipitation is one procedure to form nanoparticles by reversed micelles. In this method two reversed micelles containing the anionic and cationic surfactants are mixed.

The colloidal chemical method is also an important method to produce nanoparticles. The principle of colloidal method is that the solutions of the different ions are mixed under controlled temperature and pressure to form insoluble precipitates. The solvent could be water or alcohol, the precipitation anions are usually  $\text{H}_2\text{S}$ , and the prepared nanoparticles are quite transparent. Sol-gel is a colloidal suspension that can be gelled to form a solid. Sol is a colloidal system with solid characteristic; its particle size usually ranges from 1 to 1000 nm. Gel is a colloidal system with liquid characteristics. The dispersed matter in gel usually forms a framework which is filled with gas or liquid, and the percentage of dispersed phase is low at 1–3%. The gel will be dried or heated to obtain product.

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## 16.4 Applications

A list of some of the applications of nanomaterials to biology or medicine is given below:

- Fluorescent biological labels (Bruchez et al. 1998; Chan and Nie 1998; Wang et al. 2002)
- Drug and gene delivery (Mah et al. 2000; Pantarotto et al. 2003)

- Biodetection of pathogens (Edelstein et al. 2000)
- Detection of proteins (Nam et al. 2003)
- Tissue engineering (Ma et al. 2003; De La Isla et al. 2003)
- Tumor destruction via heating (hyperthermia) (Shinkai et al. 1999)
- Separation and purification of biological molecules and cells (Molday and Mackenzie 1982)
- MRI contrast enhancement (Weissleder et al. 1990)
- Phagokinetic studies (Parak et al. 2002)

### 16.4.1 Fluorescent Biological Labels

Fluorescent labeling is known for its nondestructive nature and high sensitivity. This has made it one of the most widely used methods for labeling and tracking biomolecules (Sahoo 2012). Fluorescent labels can be hybridized to mRNA to help visualize interaction and activity, such as mRNA localization. An antisense strand labeled with the fluorescent probe is attached to a single mRNA strand and can then be viewed during cell development to see the movement of mRNA within the cell (Weil et al. 2010). Advantages of these labels include a smaller size with more variety in color. They can be used to tag proteins of interest more selectively by various methods including chemical recognition-based labeling, such as utilizing metal-chelating peptide tags, and biological recognition-based labeling utilizing enzymatic reactions (Jung et al. 2013). However, despite their wide array of excitation and emission wavelengths as well as better stability, synthetic probes tend to be toxic to the cell and so are not generally used in cell imaging studies. Silica nanoparticles and other different nanoparticles were widely employed in near-infrared-to-visible upconversion fluorescent applications for imaging and targeted delivery in cancer treatment (Jiang et al. 2009; Taton et al. 2000; Ow et al. 2005).

### 16.4.2 Drug and Gene Delivery

Drug delivery systems are engineered technologies for the targeted delivery and/or controlled release of therapeutic agents (Allen and Cullis 2004). Drugs have long been used to improve health and extend lives. The practice of drug delivery has changed dramatically in the past few decades, and even greater changes are anticipated in the near future. Biomedical engineers have contributed substantially to our understanding of the physiological barriers to efficient drug delivery, such as transport in the circulatory system and drug movement through cells and tissues; they have also contributed to the development of several new modes of drug delivery that have entered clinical practice. Yet, with all of this progress, many drugs, even those discovered using the most advanced molecular biology strategies, have unacceptable side effects due to the drug interacting with healthy tissues that are not the target of the drug. Side effects limit our ability to design optimal medications for

many diseases such as cancer, neurodegenerative diseases, and infectious diseases. Drug delivery systems control the rate at which a drug is released and the location in the body where it is released. Some systems can control both.

The gene and drug delivery systems (GDD) consider grant applications focused on the development and delivery of drugs, genes, and gene products that alter gene function or expression in the living organism. Most studies use tissue culture and/or animal models. Applications are typically driven by bioengineering concepts and may not be hypothesis driven:

- Delivery of nucleic acids, peptide/protein complexes, vaccines, genes, small molecules, antibiotics, theranostics, and other drugs and biomaterials to biological targets
- Delivery vehicles including plasmids, viruses, liposomes, micelles, vesicles, nanoparticles, biomaterials, and cells
- Delivery strategies including electroporation, ultrasound, light, and ballistic methods
- Study of the physiological barriers to delivery (e.g., membrane, tissue, cellular, trafficking, physical)
- Studies of the interactions of delivery vehicles, devices, and/or payloads with the immune system

### 16.4.3 Biodetection of Pathogens

Advances in DNA sequencing technology have made it possible for scientists all over the world to sequence complete microbial genomes rapidly and efficiently. Access to the DNA sequences of entire microbial genomes offers new opportunities to analyze and understand microorganism at the molecular level. Scientists are able to detect pathogens in biological tissues and study variations in gene expression in response to the pathogenic invasion. These responses help in designing novel approaches for microbial pathogen detection and drug development. Identification of certain microbial pathogens as etiologic agents responsible for chronic diseases is leading to new treatments and prevention strategies for these diseases.

Each species of pathogens carries with it unique DNA or RNA signatures that differentiate it from other organisms. One of the challenges is to develop this DNA signature for each microorganism of interest for rapid and specific detection. Pathogen detection has become an important part of research in many fields like biodefense, animal healthcare, food safety, diagnostics, pathology, clinical research, forensics, and drug discovery. For biodefense, accurate analytical techniques for discovering pathogenic agents are needed. Animal healthcare community uses pathogen detection to develop various diagnostic tests that are rapid, reliable, and highly sensitive for effective control and treatment of diseases of animals. In diagnostics, the technique is employed to detect or identify infectious agents, toxins, parasites, metabolic disorders, and genetic susceptibility/resistance.

### 16.4.4 Detection of Proteins

The word protein is derived from the Greek *proteios*, meaning “of the first rank.” The term was coined in 1838 by the Swedish scientist Jöns Berzelius, to reflect the importance of this group of molecules. SDS polyacrylamide gel electrophoresis (SDS-PAGE) involves the separation of proteins based on their size. By heating the sample under denaturing and reducing conditions, proteins become unfolded and coated with SDS detergent molecules, acquiring a high net negative charge that is proportional to the length of the polypeptide chain. When loaded onto a gel matrix and placed in an electric field, the negatively charged protein molecules migrate toward the positively charged electrode and are separated by a molecular sieving effect. After visualization by a protein-specific staining technique, the size of a protein can be estimated by comparison of its migration distance with that of a standard of known molecular weight.

After protein transfer from an SDS-PAGE gel to a membrane, the remaining protein-free sites on the membrane must be blocked. This prevents the primary or secondary antibody from binding directly to the membrane and giving rise to a high background signal. Several blocking reagents are in common use, including nonfat dried milk, BSA, and casein. After blocking, the primary antibody is added and allowed to bind to the protein. After washing (which removes nonspecifically bound antibody), the secondary antibody is added, to detect where the primary antibody has bound. After another wash step, the location of the secondary antibody (and therefore the primary antibody and the protein of interest) is determined by adding a substrate for the enzyme conjugated to the secondary antibody. Substrates are available that give rise to a colored compound (chromogenic detection), or to the emission of light (chemiluminescent detection), at the reaction site. The use of an antibody that reacts specifically with an epitope commonly introduced into a recombinant protein eliminates the need for a protein-specific antibody and allows the use of one antibody for the detection of all proteins containing this feature. Coupling a reporter enzyme directly to such antibodies eliminates the need for a secondary antibody and delivers significant time savings.

### 16.4.5 Tissue Engineering

Tissue engineering is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physicochemical factors to improve or replace biological tissues. Tissue engineering involves the use of a scaffold for the formation of new viable tissue for a medical purpose. While it was once categorized as a subfield of biomaterials, having grown in scope and importance, it can be considered as a field in its own.

### 16.4.6 Tumor Destruction via Heating (Hyperthermia)

Hyperthermia therapy is a type of medical treatment in which body tissue is exposed to slightly higher temperatures to damage and kill cancer cells or to make cancer

cells more sensitive to the effects of radiation and certain anticancer drugs. Techniques that may bring local tissues to quite high temperatures, such as radio-frequency ablation, are not usually what is meant by “hyperthermia.” When combined with radiation therapy, it is called thermoradiotherapy. Whole-body hyperthermia has also been found to be helpful for depression (Hanusch et al. 2013). It is also promoted for use in the treatment of chronic Lyme disease.

### **16.4.7 Separation and Purification of Biological Molecules and Cells**

Cell biologists research the intricate relationship between structure and function at the molecular, subcellular, and cellular levels. However, a complex biological system such as a biochemical pathway can only be understood after each one of its components has been analyzed separately. Only if a biomolecule or cellular component is pure and biologically still active can it be characterized and its biological functions elucidated.

Fractionation procedures purify proteins and other cell constituents. In a series of independent steps, the various properties of the protein of interest solubility, charge, size, polarity, and specific binding affinity are utilized to fractionate it or separate it progressively from other substances. Three key analytical and purification methods are chromatography, electrophoresis, and ultracentrifugation. Each one relies on certain physicochemical properties of biomolecules.

### **16.4.8 MRI Contrast Enhancement**

MRI contrast agents are a group of contrast media used to improve the visibility of internal body structures in magnetic resonance imaging (MRI). The most commonly used compounds for contrast enhancement are gadolinium based. Such MRI contrast agents shorten the relaxation times of atoms within body tissues following oral or intravenous administration. In MRI scanners, sections of the body are exposed to a very strong magnetic field causing primarily the hydrogen nuclei (“spins”) of water in tissues to be polarized in the direction of the magnetic field. An intense radio-frequency pulse is applied that tips the magnetization generated by the hydrogen nuclei in the direction of the receiver coil where the spin polarization can be detected. Random molecular rotational oscillations matching the resonance frequency of the nuclear spins provide the “relaxation” mechanisms that bring the net magnetization back to its equilibrium position in alignment with the applied magnetic field. The magnitude of the spin polarization detected by the receiver is used to form the MR image but decays with a characteristic time constant known as the T1 relaxation time. Water protons in different tissues have different T1 values, which is one of the main sources of contrast in MR images. A contrast agent usually shortens, but in some instances increases, the value of T1 of nearby water protons, thereby altering the contrast in the image.

### 16.4.9 Phagokinetic Studies

Tumor cell migration is a key step underlying cancer cell dissemination and metastasis and is controlled by extracellular signaling-mediated dynamic cytoskeletal and cell matrix adhesion remodeling. Using a phagokinetic track (PKT) assay in combination with multi-parametric image analysis and highly motile H1299 adenocarcinoma cells, they have screened 1429 upstream kinase signaling components and downstream adhesion and cytoskeletal regulators that determine tumor cell migratory behavior: speed, directionality, and persistence. Thirty significant genes were validated by live cell imaging random tumor cell migration, which was associated with modulation of focal adhesion dynamics. For eight genes, a significant association with metastasis-free survival in breast cancer patients was observed, SHC1, SRPK1, NEK2, ITGB3BP, and MAP3K8 being most significant. Also, high SRPK1 protein expression on breast cancer tissue microarrays was associated with poor disease outcome. SRPK1 expression was highest in basal-like breast cancer cell lines and depletion of SRPK1 inhibited breast cancer cell motility and focal adhesion dynamics. Finally, in an orthotopic mammary tumor metastasis model, stable knockdown of SRPK1 in lung metastatic variant MDA-MB-231 basal-like breast cancer cells reduced lung metastasis formation (Wies van Roosmalen et al 2015). This study provides a comprehensive information resource on the molecular determinants of tumor cell migration in close association with a clinical significant role in breast cancer progression.

As mentioned above, the fact that nanoparticles exist in the same size domain as proteins makes nanomaterials suitable for biotagging or biolabelling. However, size is just one of the many characteristics of nanoparticles that itself is rarely sufficient if one is to use nanoparticles as biological tags. In order to interact with biological target, a biological or molecular coating or layer acting as a bioinorganic interface should be attached to the nanoparticle.

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## 16.5 Summary

A large body of research exists regarding nanoparticles in biology, animal, human, and so on. Following the inventions and the development of industry, however, significant levels of nanoparticle have arisen across large regions in applications. There is heightened concern today that the development of nanotechnology will impact the molecular biology. Research on humans and animals indicates that some nanoparticles are able to enter the body and rapidly migrate to the organs via the circulatory and lymphatic systems. The ability of nanoparticles to enter cells and affect their biochemical function makes them important tools at the molecular level. For example, nanoparticles used to destroy cancer cells or nanoparticles used for soil remediation may have an impact upon entering the food chain via microorganisms, such as bacteria and protozoa. We conclude that the development of nanotechnology is indispensable in the modern molecular biology.



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