Kewal K. Jain

# The Handbook of Nanomedicine

*Third Edition*

Kewal K. Jain

# The Handbook of Nanomedicine

Third Edition

Copyrighted material

Kewal K. Jain
Jain PharmaBiotech
Basel, Switzerland

ISBN 978-1-4939-6965-4 ISBN 978-1-4939-6966-1 (eBook)
DOI 10.1007/978-1-4939-6966-1

Library of Congress Control Number: 2017933292

© Springer Science+Business Media LLC 2008, 2012, 2017
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Printed on acid-free paper

This Humana Press imprint is published by Springer Nature
The registered company is Springer Science+Business Media LLC
The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

Copyrighted material

# Contents


1 **Introduction** . . . 1

2 **Nanotechnologies** . . . 11

3 **Nanotechnologies for Basic Research Relevant to Medicine** . . . 73

4 **Nanomolecular Diagnostics** . . . 133

5 **Nanopharmaceuticals** . . . 201

6 **Role of Nanotechnology in Biological Therapies** . . . 273

7 **Nanodevices and Techniques for Clinical Applications** . . . 305

8 **Nanooncology** . . . 321

9 **Nanoneurology** . . . 421

10 **Nanocardiology** . . . 457

11 **Nanopulmonology** . . . 479

12 **Nanoorthopedics** . . . 491

13 **Nanoophthalmology** . . . 501

14 **Nanomicrobiology** . . . 511

15 **Miscellaneous Healthcare Applications of Nanobiotechnology** . . . 539

16 **Nanobiotechnology and Personalized Medicine** . . . 569

17 **Nanotoxicology** . . . 575

18 **Ethical and Regulatory Aspects of Nanomedicine** . . . 605

19 **Research and Future of Nanomedicine** . . . 621

**Index** . . . **637**




Copyrighted material

# About the Author

**Kewal K. Jain** is a neurologist/neurosurgeon by training and has been working in the biotechnology/biopharmaceuticals industry for several years. He received graduate training in both Europe and the USA, has held academic positions in several countries, and is a Fellow of the Faculty of Pharmaceutical Medicine of the Royal College of Physicians of UK. Currently he is a consultant at Jain PharmaBiotech. Prof. Jain's 465 publications include 27 books (5 as editor +22 as author) and 50 special reports, which have covered important areas in biotechnology, gene therapy, and biopharmaceuticals. His recent books include *Role of Nanobiotechnology in Molecular Diagnostics* (2006), *Handbook of Nanomedicine* (Humana/Springer 2008; Chinese edition, Peking University Press 2011, 2nd ed Springer 2012, 3rd ed 2017), *Textbook of Personalized Medicine* (Springer 2009; Japanese ed 2012; 2nd ed Springer, 2015), *Handbook of Biomarkers* (Springer 2010; Chinese edition, Chemical Industry Press 2016), *Handbook of Neuroprotection* (Springer 2011), *Applications of Biotechnology in Cardiovascular Therapeutics* (Springer 2011), *Applications of Biotechnology in Neurology* (Springer 2013), and *Applications of Biotechnology in Oncology* (Springer 2014). He has edited *Drug Delivery Systems*, 2nd ed (Springer 2014) and *Applied Neurogenomics* (Springer 2015).



Copyrighted material

# Abbreviations

| | |
|---|---|
| AFM | Atomic force microscopy |
| BBB | Blood-brain barrier |
| BioMEMS | Biological Micro ElectroMechanical Systems |
| CNS | Central nervous system |
| DNA | Deoxyribonucleic acid |
| DPN | Dip pen nanolithography |
| ELISA | Enzyme-linked immunosorbent assay |
| FDA | Food and Drug Administration (USA) |
| FRET | Fluorescence resonance energy transfer |
| LNS | Lipid nano-sphere |
| MEMS | Micro ElectroMechanical Systems |
| MNP | Magnetic nanoparticle |
| MRI | Magnetic resonance imaging |
| NCI | National Cancer Institute (USA) |
| NIH | National Institutes of Health (USA) |
| NIR | Near-infrared |
| NP | Nanoparticle |
| ODN | Oligodeoxynucleotide |
| PAMAM | Polyamidoamine (dendrimers) |
| PCR | Polymerase chain reaction |
| PEG | Polyethylene glycol |
| PEI | Polyethylenimine |
| PLA | Polylactides |
| PLGA | Poly(lactic-co-glycolic) acid |
| POC | Point-of-care |
| QD | Quantum dot |
| RLS | Resonance light scattering |



Copyrighted material

| | |
|---|---|
| RNA | Ribonucleic acid |
| SERS | Surface-enhanced Raman scattering |
| SNP | Single nucleotide polymorphism |
| SPM | Scanning probe microscope |
| SPR | Surface plasmon resonance |

Copyrighted material

# Chapter 1
# Introduction

## Nanomedicine

Nanomedicine is defined as the application of nanobiotechnology to medicine. It is a discipline at the interface of medicine and nanobiotechnology but is not a subspecialty of either of these. Its broad scope covers the use of nanoparticles and nanodevices in healthcare for diagnosis as well as therapeutics. Safety, ethical and regulatory issues are also included. Figure 1.1 shows the relationship of various biotechnologies to nanomedicine.

## Basics of Nanobiotechnology

Nanotechnology (Greek word nano means dwarf) is the creation and utilization of materials, devices, and systems through the control of matter on the nanometer-length scale, i.e. at the level of atoms, molecules, and supramolecular structures. Nanotechnology, as defined by the National Nanotechnology Initiative (http://www.nano.gov/), is the understanding and control of matter at dimensions of roughly 1–100 nm, where unique phenomena enable novel applications. Encompassing nanoscale science, engineering and technology, nanotechnology involves imaging, measuring, modeling, and manipulating matter at this length scale. The simplified version of the definition – anything with "one or more external dimensions" between 1 and 100 nm" – is confusing, because nanomaterials can and often do shift shape, e.g. under UV rays, or inside cells, or out in the environment when interacting with other small particles. And particles >100 nm often display nanolike qualities, meaning they act as strangely as the slightly smaller particles do. Some conjugated complex nanoparticles are larger than 100 nm. More than 150 polymers, liposomes, metals, and many other materials, with sizes ranging from 1 to 300 nm, are approved or under investigation as diagnostic and imaging agents, as therapeutics and for enhancing drug delivery,

© Springer Science+Business Media LLC 2017

K.K. Jain, *The Handbook of Nanomedicine*, DOI 10.1007/978-1-4939-6966-1_1

Copyrighted material

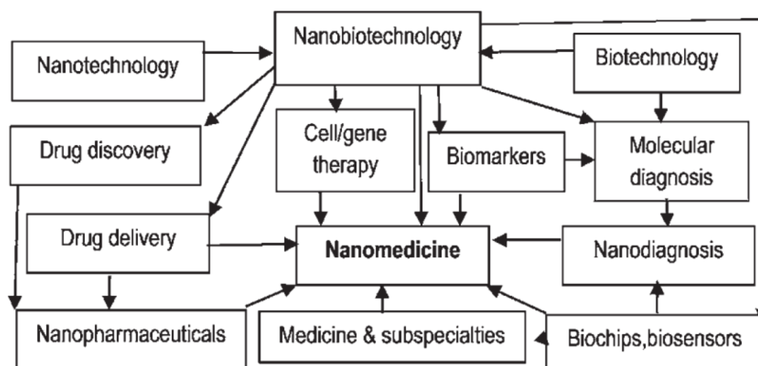


**Fig. 1.1** Relationship of various biotechnologies to nanomedicine (© Jain PharmaBiotech)

**Table 1.1** Dimensions of various objects in nanoscale

| Object | Dimension |
|---|---|
| Width of a hair | 50,000 nm |
| Red blood cell | 2500 nm |
| Vesicle in a cell | 200 nm |
| Bacterium | 1000 nm |
| Virus | 100 nm |
| Exosomes (nanovesicles shed by dendritic cells) | 65–100 nm |
| Width of DNA | 2.5 nm |
| Ribosome | 2–4 nm |
| A base pair in human genome | 0.4 nm |
| Proteins | 1–20 nm |
| Amino acid (e.g. tryptophan, the largest) | 1.2 nm (longest measurement) |
| Aspirin molecule | 1 nm |
| An individual atom | 0.25 nm |

© Jain PharmaBiotech

Nanotechnology is the popular term for the construction and utilization of functional structures with at least one characteristic dimension measured in nanometers – a nanometer is one billionth of a meter ($10^{-9}$ m). This is roughly four times the diameter of an individual atom and the bond between two individual atoms is 0.15 nm long. Proteins are 1–20 nm in size. The definition of 'small', another term used in relation to nanotechnology, depends on the application, but can range from 1 nm to 1 mm. Nano is not the smallest scale; further down the power of ten are angstrom (=0.1 nm), pico, femto, atto and zepto. By weight, the mass of a small virus is about 10 attograms. An attogram is one-thousandth of a femtogram, which is one-thousandth of a picogram, which is one-thousandth of a nanogram. Dimensions of various objects in nanoscale are shown in Table 1.1.

Given the inherent nanoscale functional components of living cells, it was inevitable that nanotechnology will be applied in biotechnology giving rise to the term

Copyrighted material

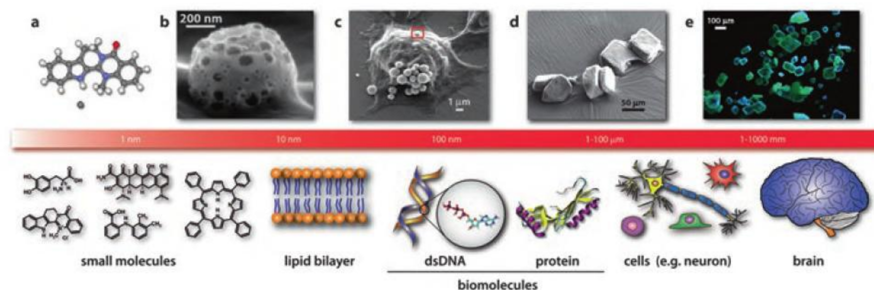


**Fig. 1.2** Sizes of biologically entities relevant to the brain. (*Top row* (above scale bar) From *left to right*: (**a**) X-ray crystal structure of Alzheimer's disease candidate drug, dehydroevodiamine HCl (DHED); (**b**, **c**) porous metal oxide microspheres being endocytosed by BV2 microglia cell (close-up and low magnification) SEM images, (**d**, **e**) SEM and fluorescence micrograph of DHED microcrystals (DHED is blue-green luminscent). (*Bottom row* below the scale bar) *Left to right*: Small molecules, such as dopamine, minocycline, mefenamic acid, DHED, and heme, are ∼1 nm or smaller. The lipid bilayer is a few nanometers thick. Biomolecule such as a microRNA and a protein are only a few nanometers in size. A single cell or neuron is tens or hundreds of microns in size. Size of human brain is tens of centimeters (Reproduced from: Suh et al. (2009), by permission)

nanobiotechnology. A brief introduction will be given to basic nanotechnologies from physics and chemistry, which are now being integrated into molecular biology to advance the field of nanobiotechnology. The aim is to understand the biological processes to improve diagnosis and treatment of diseases. Sizes of biologically entities relevant to the brain are shown in Fig. 1.2.

## *European Union Definition of Nanomaterials*

The European Commission (EU)'s definition of Nanomaterials followed >6 years of scientific consideration of the challenges posed by nanomaterials (European Commission 2011). It is worded as follows:

> "nanomaterial is a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm – 100 nm".

The EU document is a milestone as the missing jigsaw piece, ready to slot into publically-driven and government-derived legislation, covering nanomaterial matters from manufacture, labeling and handling, through transport and environmental fate. Main elements of the definition are:

1. Counting particles defines nanomaterials: The material is a nanomaterial if >50% of particles have at least one dimension between 1 and 100 nm.
2. Alternatively, it is also a nanomaterial if it has a specific surface per unit volume of over 60 $m^2/cm^3$.

Copyrighted material

3. There are specific inclusions such as graphene.
4. Naturally occurring and incidental materials are included, as well as manufactured particles.
5. Aggregates and agglomerates of such particles are included.

No measurement methods are specified; the recommendation is ‘best available alternative methods should be applied’. This definition is not regulation; however, its EU provenance informs its authority. The defining of nanomaterials is the cornerstone of any subsequent legislation, and the scientific committee of the EU has determined that number count is at the heart of this definition. The Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) publication “Scientific Basis for the Definition of the Term “Nanomaterial”, describes in depth the reasoning behind the definition. SCENIHR exhaustively discuss the possible measures and their benefits, and make clear the large areas of ambiguity and difficulty in these judgments. Techniques for measurement of size and distribution of nanoparticles in a sample to comply with EU requirements are described in Chap. 2.

## Nanoscale Time and Light

Beyond nanomaterials, nanoscale has been applied to time and light. A nanosecond (ns) is an SI unit of time equal to one billionth of a second ($10^{-9}$). Light travels ~29.9 cm (11.8 inches) in 1 ns, leading to designation of ns as a light-foot (actually = ~1.0167 ns).

This time scale is used in telecommunications, pulsed lasers and some areas of electronics. Nanosecond pulsed electric fields (nsPEFs) is a novel non-thermal approach to induce cell apoptosis, and its role in treatment of cancer is described in Chap. 8.

### *Nanolasers*

A nanolaser is a laser (light amplifier by stimulated emission of radiation) that has nanoscale dimensions. This tiny laser can be modulated quickly and, combined with its small footprint, makes it an ideal candidate for on-chip optical computing. The intense optical fields of such a nanolaser also enable the enhancement effect in non-linear optics or surface-enhanced-raman-scattering (SERS), and therefore paves the way toward integrated nanophotonic circuitry. A working room-temperature nanolaser was based on 3D Au bowtie (nanoparticles) and supported by an organic gain material (Suh et al. 2012). The extreme field compression, and thus ultrasmall mode volume, within the bowtie gaps produces laser oscillations at the localized plasmon resonance gap mode of the 3D bowties. Transient absorption measurements confirmed ultrafast resonant energy transfer between photoexcited

Copyrighted material

dye molecules and gap plasmons on the picosecond time scale. These plasmonic nanolasers are anticipated to be readily integrated into Si-based photonic devices, all optical circuits, and nanoscale biosensors. Use of nanolasers in surgery in is described later in this report.

## Relation of Nanobiotechnology to Nanomedicine

Technical achievements in nanotechnology are being applied to improve drug discovery, drug delivery and pharmaceutical manufacturing. A vast range of applications has spawned many new terms, which are defined as they are described in various chapters. Numerous applications in the pharmaceutical industry can also be covered under the term “nanobiopharmaceuticals”.

## Landmarks in the Evolution of Nanomedicine

Historical landmarks in the evolution of nanomedicine are shown in Table 1.2.

**Table 1.2** Historical landmarks in the evolution of nanomedicine

| Year | Landmark |
|---|---|
| 1905 | Einstein published a paper that estimated the diameter of a sugar molecular as about 1 nm. |
| 1931 | Max Knoll and Ernst Ruska discovered electron microscope, which enabled subnanomolar imaging. |
| 1959 | Nobel Laureate Richard Feynman gave a lecture entitled ‘There’s plenty of room at the bottom’, at the annual meeting of the American Physical Society He outlined the principle of manipulating individual atoms using larger machines to manufacture increasingly smaller machines (Feynman 1992). |
| 1974 | Start of development of molecular electronics by Aviram and Rattner (Hush 2003). |
| 1974 | Norio Tanaguchi of Japan coined the word “nanotechnology”. |
| 1979 | Colloidal gold nanoparticles used as electron-dense probes in electron microscopy and immunocytochemistry (Batten and Hopkins 1979). |
| 1981 | Conception of the idea of designing molecular machines analogous to enzymes and ribosomes (Drexler 1981). |
| 1984 | The first description the term dendrimer and the method of preparation of poly(amidoamine) dendrimers (Tomalia et al. 1985). |
| 1985 | Discovery of bucky balls (fullerenes) by Robert Curl, Richard Smalley and Harold Kroto, which led to the award of Nobel Prize for chemistry in 1996 (Smalley 1985; Curl et al. 1997). |
| 1987 | Publication of the visionary book on nanotechnology potential “Engines of Creation” (Drexler 1987). |
| 1987 | Cancer targeting with nanoparticles coated with monoclonal antibodies (Douglas et al. 1987). |

(continued)

Copyrighted material

**Table 1.2** (continued)

| Year | Landmark |
|---|---|
| 1988 | Maturation of the field of supramolecular chemistry relevant to nanotechnology: construction of artificial molecules that interact with each other and are (Lehn 1988). Awarded Nobel prize. |
| 1990 | Atoms visualized by the scanning tunneling microscope discovered in 1980's at the IBM Zürich Laboratory (Zürich, Switzerland), which led to award of a Nobel prize (Eigler and Schweizer 1990). |
| 1991 | Discovery of carbon nanotubes (Iijima et al. 1992). |
| 1992 | Principles of chemistry applied to the bottom-up synthesis of nanomaterials (Ozin 2009) |
| 1994 | Nanoparticle-based drug delivery (Kreuter 1994). |
| 1995 | FDA approved Doxil, a liposomal formulation of doxorubicin, as an intravenous chemotherapy agent for Kaposi sarcoma. Drug carried by nanosize liposomes is less toxic with targeted delivery. |
| 1997 | Founding of the first molecular nanotechnology company – Zyvex Corporation. |
| 1998 | First use of nanocrystals as biological labels, which were shown to be superior to existing fluorphores (Bruchez et al. 1998). |
| 1998 | Use of DNA-gelatin nanospheres for controlled gene delivery (Truong-Le et al. 1998). |
| 1998 | Use of the term "nanomedicine" in publications (Freitas 1998). |
| 2000 | Nanotechnology Initiative announced in the US (Roco 2003). |
| 2000 | First FDA approval of a product incorporating the NanoCrystal® technology (Elan), solid-dose formulation of the immunosuppressant sirolimus – Rapamune® (Wyeth). |
| 2003 | Concept for nanolaser was developed at Georgia State University using nanospheres and nanolens system (Li et al. 2003). |
| 2003 | The US Senate passed the Nanotechnology Research & Development Act making the National Nanotechnology Initiative into law and authorized $3.7 billion over the next 4 years for the program. |
| 2005 | FDA approved Abraxane™, a taxane based on nanotechnology, for the treatment of breast cancer. Nanoparticle form of the drug overcomes insolubility problems encountered with paclitaxel and avoids the use of toxic solvents. |
| 2014 | Award of Nobel Prize in Chemistry to one German and two US scientists for discovery of nanoscopy. |

© Jain PharmaBiotech

## Nanomedicine as a Part of Evolution of Medicine

Medicine is constantly evolving and new technologies are incorporated into the diagnosis and treatment of patients. This process is sometimes slow and there can be a gap of years before new technologies are integrated in medical practice. The reasons for the delay are:

- Establishing the safety and efficacy of innovative treatments is a long process, particularly with clinical trials and regulatory reviews.
- Current generation of physicians are still not well oriented towards biotechnology and conservative elements of the profession may be slow in accepting and learning about nanobiotechnology, which is at the cutting edge of biotechnology.

Copyrighted material

- High cost of new technologies is a concern for the healthcare providers. Cost-benefit studies are needed to convince the skeptics that some of the new technologies may reduce the overall cost of healthcare.

Molecular medicine, a recognized term, should not be considered a subspecialty of medicine as molecular technologies have an overall impact on the evolution of medicine. Recognition of the usefulness of biotechnology has enabled progress in the concept of personalized medicine, which is also not a branch of medicine but simply indicates a trend in healthcare and the prescription of specific treatments best suited for an individual (Jain 2015). Various nanomachines and other nano-objects that are currently under investigation in medical research and diagnostics will soon find applications in the practice of medicine. Nanobiotechnologies are being used to create and study models of human diseases, e.g. immune disorders. Introduction of nanobiotechnologies in medicine will not create a separate branch of medicine but simply improve diagnosis as well as therapy. Current research is exploring the fabrication of designed nanostructures, nanomotors, microscopic energy sources, and nanocomputers at the molecular scale, along with the means to assemble them into larger systems, economically and in great numbers. Table 1.3 show some of the applications of nanobiotechnology in medicine.

**Table 1.3** Nanomedicine in the twenty-first century

| **Nanodiagnostics** |
|---|
| Extending limits of detection by refining currently available molecular diagnostic technologies |
| Development of new nanotechnology-based assays |
| Nanobiosensors |
| Nanoendoscopy |
| Nanoimaging |
| **Nanopharmaceuticals** |
| Nanoparticulate formulations of drugs |
| Nanotechnology-based drug discovery |
| Nanotechnology-based drug delivery |
| **Regenerative medicine** |
| Use of nanotechnology for tissue engineering |
| **Transplantation medicine** |
| Exosomes from donor dendritic cells for drug-free organ transplants |
| **Nanomedicine relevant to subspecialties** |
| Nanocardiology |
| Nanodermatology |
| Nanodentistry |
| Nanogerontology |
| Nanohematology |
| Nanoimmunology |
| Nanomicrobiology |

(continued)

Copyrighted material

**Table 1.3** (continued)

| |
|---|
| Nanonephrology |
| Nanoneurology |
| Nanooncology |
| Nanoophthalmology |
| Nanoorthopedics |
| **Implants** |
| Bioimplantable sensors that bridge the gap between electronic and neurological circuitry |
| Durable rejection-resistant artificial tissues and organs |
| Implantations of nanocoated stents in coronary arteries to elute drugs and to prevent reocclusion |
| Implantation of nanoelectrodes in the brain for functional neurosurgery |
| Implantation of nanopumps for drug delivery |
| **Nanosurgery** |
| Minimally invasive surgery: miniaturized nanosensors implanted in catheters to provide real-time data |
| Nanosurgery by integration of nanoparticles and external energy, nanolasers |
| **Nanorobotic treatments** |
| Vascular surgery by nanorobots introduced into the vascular system |
| Nanorobots for detection and destruction of cancer |

© Jain PharmaBiotech

## References

Batten TF, Hopkins CR. Use of protein A-coated colloidal gold particles for immunoelectronmicroscopic localization of ACTH on ultrathin sections. Histochemistry. 1979;60:317–20.

Bruchez Jr M, Moronne M, Gin P, Weiss S, Alivisatos AP. Semiconductor nanocrystals as fluorescent biological labels. Science. 1998;281:2013–6.

Curl RF, Kroto H, Smalley RE. Nobel lectures in chemistry. Rev Mod Phys. 1997;69:691–730.

Douglas SJ, Davis SS, Illum L. Nanoparticles in drug delivery. Crit Rev Ther Drug Carrier Syst. 1987;3:233–61.

Drexler KE. Engines of creation, the coming era of nanotechnology. New York: Anchor; 1987.

Drexler KE. Molecular engineering: an approach to the development of general capabilities for molecular manipulation. Proc Natl Acad Sci U S A. 1981;78:5275–8.

Eigler DM, Schweizer EK. Positioning single atoms with a scanning tunneling microscope. Nature. 1990;344:524–6.

European Commission. Recommendation of 18 October 2011 on the definition of nanomaterial. Official Journal of the European Union 2011/696/EU. 2011.; http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:275:0038:0040:EN:PDF

Feynman R. There's plenty of room at the bottom: an invitation to enter a new filed of physics. Reprinted in: Crandall BC, Lewis J, editors. Nanotechnology: research and perspectives. Cambridge, MA: The MIT Press; 1992. p. 347–63.

Freitas Jr RA. Exploratory design in medical nanotechnology: a mechanical artificial red cell. Artif Cells Blood Substit Immobil Biotechnol. 1998;26:411–30.

Hush NS. An overview of the first half-century of molecular electronics. Ann N Y Acad Sci. 2003;1006:1–20.

Iijima S, Ajayan PM, Ichihashi T. Growth model for carbon nanotubes. Phys Rev Lett. 1992;69:3100–3.

Copyrighted material

Jain KK. Textbook of personalized medicine. 2nd ed. New York: Springer; 2015.
Kreuter J. Drug targeting with nanoparticles. Eur J Drug Metab Pharmacokinet. 1994;19:253–6.
Lehn JM. Supramolecular chemistry – scope and perspectives: molecules, supermolecules, and molecular devices. Ang Chem Int Ed Engl. 1988;27:89–112.
Li K, Stockman MI, Bergman DJ. Self-similar chain of metal nanospheres as an efficient nanolens. Phys Rev Lett. 2003;91:227402.
Ozin GA, Arsenault AC, Cademartiri L. Nanochemistry: a chemical approach to nanomaterials. 2nd ed. Cambridge, UK: Royal Society of Chemistry; 2009.
Roco MC. Nanotechnology: convergence with modern biology and medicine. Curr Opin Biotechnol. 2003;14:337–46.
Smalley RE. Supersonic cluster beams: an alternative approach to surface science. In: Bartlett RJ, editor. Comparison of Ab initio quantum chemistry with experiments for small molecules. Boston: D. Riedel; 1985.
Suh WH, et al. Nanotechnology, nanotoxicology, and neuroscience. Prog Neurobiol. 2009;87:133–70.
Suh JY, Kim CH, Zhou W, et al. Plasmonic bowtie nanolaser arrays. Nano Lett. 2012;12:5769–74.
Tomalia DA, Baker H, Dewald J, et al. A new class of polymers: starburst-dendritic macromolecules. Polym J. 1985;17:117–32.
Truong-Le VL, August JT, Leong KW. Controlled gene delivery by DNA-gelatin nanospheres. Hum Gene Ther. 1998;9:1709–17.

Copyrighted material

# Chapter 2
# Nanotechnologies

## Introduction

This chapter will focus on nanobiotechnologies that are relevant to applications in biomedical research, diagnostics, and medicine. Invention of the microscope revolutionized medicine by enabling the detection of microorganisms and study of histopathology of disease. Microsurgery was a considerable refinement over crude macrosurgery and opened the possibilities of procedure that were either not carried out previously or had high mortality and morbidity. Nanotechnologies, by opening the world beyond microscale, will have a similar impact on medicine and surgery. Various nanobiotechnologies are described in detail in a special report on this topic (Jain 2017). Those relevant to understanding of diseases, diagnosis, and development of new drugs as well as management of diseases are described briefly in this chapter.

## Classification of Nanobiotechnologies

It is not easy to classify the vast range of nanobiotechnologies. Some just represent motion on a nanoscale but most of them are based on nanoscale structures, which come in a variety of shapes and sizes. A few occur in nature but most are engineered. The word nano is prefixed to just about anything that deals with nanoscale. It is not just biotechnology but many other disciplines such as nanophysics, nanobiology, etc. A simplified classification of basic nanobiotechnologies is shown in Table 2.1. Some technologies such as nanoarrays and nanochips are further developments.

© Springer Science+Business Media LLC 2017

K.K. Jain, *The Handbook of Nanomedicine*, DOI 10.1007/978-1-4939-6966-1_2
Copyrighted material

**Table 2.1** Classification of basic nanomaterials and nanobiotechnologies

| **Nanoparticles** |
| --- |
| Fluorescent nanoparticles |
| Fullerenes |
| Gold nanoparticles |
| Lipoparticles |
| Magnetic nanoparticles |
| Nanocrystals |
| Nanoparticles assembly into micelles |
| Nanoshells |
| Paramagnetic and superparamagnetic nanoparticles |
| Polymer nanoparticles |
| Quantum dots |
| Silica nanoparticles |
| **Nanofibers** |
| Nanowires |
| Carbon nanofibers |
| **Dendrimers** |
| Polypropylenimine dendrimers |
| **Composite nanostructures** |
| Cochleates |
| DNA-nanoparticle conjugates |
| Nanoemulsions |
| Nanoliposomes |
| Nanocapsules enclosing other substances |
| Nanoshells |
| Nanovesicles |
| **Nanoconduits** |
| Nanotubes |
| Nanopipettes |
| Nanoneedles |
| Nanochannels |
| Nanopores |
| Nanofluidics |
| **Nanostructured silicon** |
| **Nanoscale motion and manipulation at nanoscale** |
| Cantilevers |
| Femtosecond laser systems |
| Nanomanipulation |
| Surface plasmon resonance |
| **Visualization at nanoscale** |
| Atomic force microscopy |
| Magnetic resonance force microscopy and nanoscale MRI |
| Multiple single-molecule fluorescence microscopy |

(continued)

Copyrighted material

**Table 2.1** (continued)

| |
|---|
| Nanoparticle characterization by Halo™ LM10 technology |
| Nanoscale scanning electron microscopy |
| Near-field scanning optical microscopy |
| Optical Imaging with a Silver Superlens |
| Partial wave spectroscopy |
| Photoactivated localization microscopy |
| Scanning probe microscopy |
| Super-resolution microscopy for in vivo cell imaging |
| Ultra-nanocrystalline diamond |
| Visualizing atoms with high-resolution transmission electron microscopy |

© Jain PharmaBiotech

## *Nanoparticles*

Nanoparticles (NPs) form the bulk of nanomaterials. There are two main families of nanoparticles: nanospheres with a homogeneous structure in the whole particle, and nanocapsules, which exhibit a typical core-shell structure. They can be made of different materials, e.g., gold. A NP contains tens to thousands of atoms and exists in a realm that straddles the quantum and the Newtonian. At this size, every particle has new properties that change depending on its size. As matter is shrunk to nanoscale, electronic and other properties change radically. NPs may contain unusual forms of structural disorder that can significantly modify their material properties and thus they cannot just be considered as small pieces of bulk material. Two NPs, both made of pure gold, can exhibit markedly different behavior – different melting temperature, different electrical conductivity, and different color – if one is larger than the other. That creates a new way to control the properties of materials. Instead of changing composition, one can change size. Some applications of nanoparticles take advantage of the fact that more surface area is exposed when material is broken down to smaller sizes. For magnetic NPs, the lack of blemishes produces magnetic fields remarkably strong considering the size of the particles. NPs are also so small that in most of them, the atoms line up in perfect crystals without a single blemish.

Zinc sulfide NPs a mere ten atoms across have a disordered crystal structure that puts them under constant strain, increasing the stiffness of the particles and probably affecting other properties, such as strength and elasticity. In similar semiconducting NPs, such as those made of cadmium selenide, slight differences in size lead to absorption and emission of different wavelengths of light, making them useful as fluorescent tracers. The dominant cause of such properties is quantum mechanical confinement of the electrons in a small package. But the disordered crystal structure now found in nanoparticles could affect light absorption and emission also. X-ray diffraction of single nanoparticles is not yet possible and other methods are used to analyze X-ray diffraction images of nanoparticles to separate the effects of size from those of disordered structure.

Copyrighted material

As a measure of particle size in solution, the Nanotechnology Characterization Laboratory of NCI in the USA uses dynamic light scattering (DLS), during which a laser beam is scattered off the nanoparticle and small fluctuations in the intensity of the scattered light are monitored. DLS is very sensitive to soft molecules such as polymers, proteins, and antibodies because they cause significant frictional drag that the technique detects.

It is beyond the scope of this Handbook to describe all NPs. A few selected NPs relevant to nanomedicine are described briefly in the following pages. Lipoparticles or nanoliposomes will be described under liposomes in Chap. 5 as they play an important role in drug delivery.

## Gold Nanoparticles

Mass spectrometry analysis has determined the formula of gold nanocrystal molecules to be Au333(SR)79 (Qian et al. 2012). This metallic nanocrystal molecule exhibits fcc-crystallinity and surface plasmon resonance (SPR) at ~7 to 720 nm. Simulations have revealed that atomic shell largely contributes to the robustness of Au333(SR)79, albeit the number of free electrons is also consistent with electron shell closing based on calculations using a confined free electron model. This work clearly demonstrates that atomically precise nanocrystal molecules are achievable and that the factor of atomic shell closing contributes to their extraordinary stability compared to other sizes.

Ultrashort pulsed laser ablation in liquids represents a powerful tool for the generation of pure gold nanoparticles avoiding chemical precursors and thereby making them useful for biomedical applications. However, there is a concern that their biochemical properties may change because of their properties of accepting electrons, which often adsorb onto the nanoparticles. A study has shown that co-transfection of plasmid DNA and laser-generated gold nanoparticles does not disturb the bioactivity of GFP-HMGB1 fusion protein – either uptake of the vector through the plasma membrane or protein accumulation in the nucleus (Petersen et al. 2009). Thus laser-generated gold nanoparticles provide a good alternative to chemically synthesized nanoparticles for use in biomedical applications.

DNA molecules are attached to gold nanoparticles, which tangle with other specially designed pieces of DNA into clumps that appear blue. The presence of lead causes the connecting DNA to fall apart. That cuts loose the individual gold nanoparticles and changes the color from blue to red. Gold nanoparticles are also used as a connecting point to build biosensors for detection of disease. A common technique for a diagnostic test consists of an antibody attached to a fluorescent molecule. When the antibody attaches to a protein associated with the disease, the fluorescent molecule lights up under ultraviolet light. Instead of a fluorescent molecule, a gold nanoparticle can be attached to the antibody and other molecules such as DNA can be added to the nanoparticle to produce bar codes. Because many copies of the antibodies and DNA can be attached to a single nanoparticle, this approach is much more sensitive and accurate than the fluorescent-molecule tests used currently.

Copyrighted material

### Cubosomes

When surfactants are added to water at high concentrations they self-assemble to form thick fluids called liquid crystals. The most viscous liquid crystal is bicontinuous cubic phase, a unique material that is clear and resembles stiff gelatin. When cubic phase is dispersed into small particles, these nanoparticles are termed cubosomes. Within cubosomes, amphiphilic lipids in definite proportions are organized in 3D as honeycombed structures and divided into internal aqueous channels that can be loaded with biopharmaceuticals (Karami and Hamidi 2016). Methods and compositions for producing lipid-based cubic phase nanoparticles were first discovered in the 1990s. Since then several studies have described properties such as particle size, morphology, and stability of cubic phase dispersions, which can be tuned by composition and processing conditions. Stable particle dispersions with consistent size and structure can be produced by a simple processing scheme comprising a homogenization and heat treatment step. Because of their unique microstructure, they are biologically compatible and capable of controlled release of solubilized active ingredients such as drugs and proteins. As a drug delivery vehicle, high drug payloads, stabilization of peptides or proteins and simple preparation process are also advantages of a cubosome. The ability of cubic phase to incorporate and control release of drugs of varying size and polar characteristics, and biodegradability of lipids make it a versatile drug delivery system for various routes of administration, including oral, topical (or mucosal), transdermal and intravenous. Furthermore, proteins in cubic phase appear to retain their native conformation and bioactivity, and are protected against chemical and physical inactivation.

### Fluorescent Nanoparticles

Microwave plasma technique has been used to develop fluorescent nanoparticles. In a second reaction, a layer of organic dye is deposited and the final step is an outer cover of polymer, which protects the nanoparticles from exposure to environments. Each layer has characteristic properties. The size of the particles varies and these are being investigated for applications in molecular diagnostics. Fluorescent nanoparticles can also be used as labels for immunometric assays

Switchable fluorescent silica nanoparticles have been prepared by covalently incorporating a fluorophore and a photochromic compound inside the particle core (May et al. 2012). The fluorescence can be switched reversibly between an on- and off-state via energy transfer. The particles were synthesized using different amounts of the photoswitchable compound (spiropyran) and the fluorophore (rhodamine B) in a size distribution between 98 and 140 nm and were characterized in terms of size, switching properties, and fluorescence efficiency by TEM, and UV\Vis and fluorescence spectroscopy.

Copyrighted material

### Fullerenes

Fullerene technology derives from the discovery in 1985 of Carbon-60, a molecule of 60 carbon atoms that form a hollow sphere 1 nm in diameter. The molecule was named buckyball or fullerene or buckminsterfullerene, because of its similarity to the geodesic dome designed by Buckminster Fuller. Subsequent studies have shown that fullerenes represent a family of related structures containing 20, 40, 60, 70, or 84 carbons. C-60, however, is the most abundant member of this family. Fullerenes are entirely insoluble in water, but suitable functionalization makes the molecules soluble. Initial studies on water-soluble fullerene derivatives led to the discovery of the interaction of organic fullerenes with DNA, proteins, and living cells. Subsequent studies have revealed interesting biological activity aspects of organic fullerenes owing to their photochemistry, radical quenching, and hydrophobicity to form one- to three-dimensional supramolecular complexes. In these areas of research, synthetic organic chemistry has played an important role in the creation of tailor-made molecules.

Upon contact with water, under a variety of conditions, C60 spontaneously forms a stable aggregate with nanoscale dimensions (25–500 nm), termed nano-C60 that are both soluble and toxic to bacteria. This finding challenges conventional wisdom because buckyballs are notoriously insoluble by themselves and most scientists had assumed they would remain insoluble in nature. C60 can be applied to cultured cells without using water-solubilization techniques. Treatment of cells with up to 200 mg/ml (200 ppm) of C60 does not alter morphology, cytoskeletal organization, and cell cycle dynamics nor does it inhibit cell proliferation. Thus, pristine C60 is non-toxic to the cells, and suggests that fullerene-based nanocarriers may be used for biomedical applications. Fullerenes have important applications in treatment of various diseases such as cancer and as an antioxidant neuroprotective for neurodegenerative disorders in addition to use as contrast agent for brain imaging.

### Graphene

Graphene is a monolayer atomic-scale honeycomb lattice of carbon atoms. Its surface area is greater than for carbon nanotubes (CNTs), from ≈100 to 1000 $m^2/g$ and is the same as activated carbon. 2D crystals provide optoelectronic and photocatalytic properties complementing those of graphene opening several commercial applications (Bonaccorso et al. 2015). Several processes are available for manufacture of graphene quantum dots (QDs). Graphene fibers can be fabricated from chemical vapor deposition grown graphene films. Graphene provides a promising biocompatible scaffold that does not hamper the proliferation of human mesenchymal stem cells and accelerates their specific differentiation into bone cells (Nayak et al. 2011). Honeycomb of hexagonally arranged carbon was termed 3D graphene. Box-shaped graphene nanostructure appear after mechanical cleavage of pyrolytic graphite and is a multilayer system of parallel hollow nanochannels located along the surface and having quadrangular cross-section. The thickness of the channel walls is ~1 nm making nanochannels useful for DNA sequencing. Graphene can be used to create sensitive biosensors. Applications in neurosciences are described in Chap. 9.

Copyrighted material

### Magnetic Nanoparticles

Magnetic nanoparticles (MNPs) are a class of nanoparticle which can be manipulated using magnetic field. The physical and chemical properties of magnetic nanoparticles largely depend on the synthesis method and chemical structure. In most cases, the particles range from 1 to 100 nm in size and may display para- or superparamagnetism. Ferrite nanoparticles are the most used magnetic nanoparticles up to date. Once the ferrite particles reach <128 nm size they become superparamagnetic, which prevents self aggregation because they exhibit their magnetic behavior only when an external magnetic field is applied.

Paramagnetic particles are important tools for cell sorting, protein separation, and single molecule measurements. The particles used in these applications must meet the following requirements: uniform in size, highly paramagnetic, stable in physiological salt buffer, functionizable, and 100–1000 nm in size. They have been used for the detection of model pathogens. Paramagnetic nanoparticles, which are linked to antibodies enable highly specific biological cell separations.

Superparamagnetic iron oxide nanoparticles (SPION) with appropriate surface chemistry have been widely used experimentally for numerous in vivo applications such as magnetic resonance imaging (MRI) contrast enhancement, tissue repair, immunoassay, detoxification of biological fluids, hyperthermia, drug delivery and in cell separation, etc. These applications require that these nanoparticles have high magnetization values and size smaller than 100 nm with overall narrow particle size distribution, so that the particles have uniform physical and chemical properties. In addition, these applications need special surface coating of the magnetic particles, which should be not only nontoxic and biocompatible but also allow a targetable delivery with particle localization in a specific area. Nature of surface coatings of the nanoparticles determines not only the overall size of the colloid but also plays a significant role in biokinetics and biodistribution of nanoparticles in the body. Magnetic nanoparticles can bind to drugs, proteins, enzymes, antibodies, or nucleotides and can be directed to an organ, tissue, or tumor using an external magnetic field or can be heated in alternating magnetic fields for use in hyperthermia. Magnetic labeling of cells provides the ability to monitor their temporal spatial migration in vivo by MRI. Various methods have been used to magnetically label cells using SPIONs. Magnetic tagging of stem cells and other mammalian cells has the potential for guiding future cell-based therapies in humans and for the evaluation of cell-based treatment effects in disease models.

### Nanoparticles Assembly into Micelles

Assembly of gold and silver nanoparticle building blocks into larger structures is based on a l method that goes back to one of nature's oldest known chemical innovations, i.e. the self-assembly of lipid membranes that surround every living cell. The method makes use of the hydrophobic effect, a biochemical phenomenon that

Copyrighted material

all living creatures use to create membranes, ultra-thin barriers of fatty acids that form a strong, yet dynamic, sack around the cell, sealing it from the outside world. Cell membranes are one example of a micelle, a strong bilayer covering that is made of two sheets of lipid-based amphiphiles, molecules that have a hydrophilic, end and a hydrophobic end. Like two pieces of cellophane tape being brought together, the hydrophobic sides of the amphiphilic sheets stick to one another, forming the bilayered micelle. All micelles form in three shapes: spheres, cylinders and sack-like vesicles. By varying the length of the polystyrene arm, the solvents used and the size of the gold particles, it is possible to form spheres, vesicles and vary the diameter of their cylinders, some of which grew to well >1000 nm in length. This method may enable creation of a wide variety of useful materials, including potent cancer drugs and more efficient catalysts for the chemical industry.

## Nanoshells

Nanoshells are ball-shaped structures measuring ~100 nm and consist of a core of non-conducting glass that is covered by a metallic shell, which is typically gold or silver. Nanoshells possess highly favorable optical and chemical properties for biomedical imaging and therapeutic applications. These particles are also effective substrates for surface-enhanced Raman scattering (SERS) and are easily conjugated to antibodies and other biomolecules. By varying the relative the dimensions of the core and the shell, the optical resonance of these nanoparticles can be precisely and systematically varied over a broad region ranging from the near-UV to the mid-infrared. This range includes the NIR wavelength region where tissue transmissibility peaks, which forms the basis of absorbing nanoshells in NIR thermal therapy of tumors. In addition to spectral tunability, nanoshells offer other advantages over conventional organic dyes including improved optical properties and reduced susceptibility to chemical/thermal denaturation. Furthermore, the same conjugation protocols used to bind biomolecules to gold colloid are easily modified for nanoshells. The core/shell ratio and overall size of a gold nanoshell influences its scattering and absorption properties.

Gold Nanoshells (Spectra Biosciences) possess physical properties similar to gold colloid, in particular a strong optical absorption due to the collective electronic response of the metal to light. The optical absorption of gold colloid yields a brilliant red color, which is very useful in consumer-related medical products such as home pregnancy tests. In contrast, the optical response of Gold Nanoshells depends dramatically on the relative sizes of the nanoparticle core and the thickness of the gold shell. Gold Nanoshells can be made either to absorb or scatter light preferentially by varying the size of the particle relative to the wavelength of the light at their optical resonance. Several potential biomedical applications of nanoshells are under development, including immunoassays, modulated drug delivery, photothermal cancer therapy, and imaging contrast agents.

Copyrighted material

### Plant-Derived Nanoparticles

Naturally occuring nanoparticles in plant cells contain miRNAs, bioactive lipids and proteins, which act as extracellular messengers for cell to cell communication in the same way as exosomes in mammalian cells (Zhang et al. 2016). Plant-derived lipid edible nanoparticles may also be used for efficient drug delivery. Compared to synthetic nanoparticles, plant-derived nanoparticles are easier to scale up for mass production.

### Polymer Nanoparticles

Polymer nanoparticles or nanopolymers are single polymer molecule in the nanoscale range. The natural polymer backbone contains oxygen and/or nitrogen. Synthetic polymer backbone can be a composition of carbon, oxygen and/or nitrogen atoms, depending on the chemical nature of monomers employed for polymer synthesis. Synthetic as well as biopolymers are mostly biocompatible, biodegradable and nontoxic. Nanopolymers can be linear or branched. Linear nanopolymers such as polymalic acid carry functional groups distributed over the entire length of the polymer; branched polymers such as dendrimers usually carry them on surface of the molecule. In micelles or other nanoparticles, aggregation restricts accessibility and thus functionality of internally located groups.

Different types of polymer nanoparticles have been designed as drug delivery devices. Biodegradable polymeric nanoparticles are promising drug delivery devices because of their ability to deliver drugs, proteins, peptides and genes as targeting therapeutics to specific organs/tissues. Although several synthetic polymers are available, natural polymers are still popular for drug delivery; these include acacia gum, chitosan, gelatin and albumin. Examples of synthetic biodegradable polymers for controlled release drug delivery are polylactides (PLA), polyglycolides (PLG) and poly(lactide-co-glycolides) or PLGA.

### Porous Silicon Nanoparticles

Porous silicon (Psi) is crystalline silicon traversed by nanometer-width pores, providing the material a high surface-to-volume ratio. Production of PSi is based on a top-down approach where the fabrication of size-controlled nanoparticles is usually achieved by mechanical size reduction using ultrasonication or milling nanoparticles Silicon nanoparticles (PSi NPs) vary in size from 25 nm to 1000 nm. (PSi) nanoparticles have unique physicochemical properties making them desirable candidates for drug delivery and other biomedical applications (Santos et al. 2014).

Copyrighted material

## Quantum Dots

Quantum dots (QDs) are nanoscale crystals of semiconductor material that glow, or fluoresce when excited by a light source such as a laser. QD nanocrystals of cadmium selenide 200–10,000 atoms wide, coated with zinc sulfide. The size of the QD determines the frequency of light emitted when irradiated with low energy light. The QDs were initially found to be unstable and difficult to use in solution. Multicolor optical coding for biological assays has been achieved by embedding different-sized QDs into polymeric microbeads at precisely controlled ratios. Their novel optical properties such as size-tunable emission and simultaneous excitation render these highly luminescent QDs ideal fluorophores for wavelength-and-intensity multiplexing. The use of ten intensity levels and six colors could theoretically code one million nucleic acid or protein sequences. Imaging and spectroscopic measurements indicate that the QD-tagged beads are highly uniform and reproducible, yielding bead identification accuracies as high as 99.99% under favorable conditions. DNA hybridization studies demonstrate that the coding and target signals can be simultaneously read at the single-bead level. This spectral coding technology is expected to open new opportunities in gene expression studies, high-throughput screening, and medical diagnostics.

Latex beads filled with several colors of nanoscale semiconductor QDs can serve as unique labels for any number of different probes. When exposed to light, the beads identify themselves and their linked probes by emitting light in a distinct spectrum of colors – a sort of spectral bar code. The shape and size of QDs can be tailored to fluoresce with a specific color. Current dyes used for lighting up protein and DNA fade quickly, but QDs could allow tracking of biological reactions in living cells for days or longer.

QDs can also be placed in a strong magnetic field, which gives an electron on the dot two allowed energy states separated by an energy gap that depends on the strength of the field. The electron can jump the gap by absorbing a photon of precisely that energy, which can be tuned, by altering the field, to correspond with the energy of a far-infrared photon. Once it is excited by absorption of a photon, the electron can leap onto the terminal of a single-electron transistor, where it 'throws the switch' and is detected.

Due to their sheer brightness and high photostability, QDs can act as molecular beacons. When attached to compounds or proteins of interest, QDs enable researchers to track movements within biological media or whole organisms, significantly impacting the way medical professionals study, diagnose and treat diseases. Applications of QDs include the following:

- Life sciences research: tracking proteins in living cells
- Fluorescence detection: microscopy, biosensors, multi-color flow cytometry
- Molecular diagnostics
- Ex vivo live cell imaging
- In vivo targeting of cells, tissues and tumors with monitoring by PET and MRI
- High throughput screening
- Identification of lymph nodes in live animals by NIR emission during surgery

Copyrighted material

The new generations of QDs have far-reaching potential for the study of intracellular processes at the single-molecule level, high-resolution cellular imaging, long-term in vivo observation of cell trafficking, tumor targeting, and diagnostics. Best known commercial preparation is Qdot™ (Life Technologies).

#### Synthetic High Density Lipoprotein Nanoparticles

High density lipoprotein nanoparticles (HDL-NPs) are synthesized using a gold nanoparticle template to control conjugate size and ensure a spherical shape (Yang et al. 2013). Like natural HDLs, biomimetic HDL-NPs target scavenger receptor type B-1, a high-affinity HDL receptor expressed by lymphoma cells. Functionally, compared with natural HDL, the gold NP template enables differential manipulation of cellular cholesterol flux in lymphoma cells, promoting cellular cholesterol efflux and limiting cholesterol delivery. This combination of scavenger receptor type B-1 binding and relative cholesterol starvation selectively induces apoptosis. HDL-NPs are biofunctional therapeutic agents, whose mechanism of action is enabled by the presence of a synthetic nanotemplate. HDL-NP treatment of mice bearing B-cell lymphoma xenografts selectively inhibits B-cell lymphoma growth. HDL-NPs have potential applications for other malignancies or diseases of pathologic cholesterol accumulation.

#### Hybrid Nanoparticles

Hybrid nanoparticles (HNPs) containing two elements have been designed to improve functions of NPs. An example is gold coating of iron oxide nanoparticles (IONPs), which results in particles of increased stability and robustness (Hoskins et al. 2012). Combination of unique properties of iron oxide (magnetic) and gold (surface plasmon resonance) result in a multimodal platform for use as a MRI contrast agent and as a nano-heater. IONPs of core diameter 30 nm and gold coat using the seeding method with a poly(ethylenimine) intermediate layer were synthesized. The final particles were coated in PEG to ensure biocompatibility and increase retention times in vivo. The resulting HNPs possessed a maximal absorbance at 600 nm, and appeared to decrease T2 values in line with clinically used MRI contrast agent Feridex®. HNPs could serve dual functions as MRI contrast agents as well as nano-heaters for therapies such as cellular hyperthermia or thermoresponsive drug delivery.

### *Bacterial Structures Relevant to Nanobiotechnology*

#### Nanostructures Based on Bacterial Cell Surface Layers

Among the most commonly observed bacterial cell surface structures are monomolecular crystalline arrays of proteinaceous sub units termed S-layers, which are the simplest type of biological membrane developed during evolution. As an important

Copyrighted material

**Table 2.2** Applications of S-layers in nanobiotechnology

| **As a matrix for controlled immobilization of functional molecules** |
|---|
| Binding of enzymes for bioanalytical biosensors |
| Immobilizing monoclonal antibodies for dipstick style immunoassays |
| Immobilizing antibodies for preparation of microparticles for ELISA |
| **S-layers as carriers for conjugated vaccines** |
| **S-layer coated liposomes** |
| Immobilization of functional molecules on S-layer coated liposomes |
| Entrapping of functional molecules for drug delivery |
| S-layer coated liposomes with immobilized antigens and haptens for vaccines |
| **Vehicles for producing fusion proteins** |
| Vaccines |
| Biosensors |
| Diagnostics |

© Jain PharmaBiotech

component of the bacterial cell envelope, S-layers can fulfill various biological functions and are usually the most abundantly expressed protein species in a cell. S-layer plays an important part in interactions of microbial cell with the environment. S-layers are generally 5–10 mm thick and pores in the protein lattices are of identical size and morphology in the 2–8 nm range. S-layers have applications in nanobiotechnology as shown in Table 2.2.

## Bacterial Magnetic Particles

Magnetic bacteria synthesize intracellular magnetosomes that impart a cellular swimming behavior referred to as magnetotaxis. The magnetic structures, magnetosomes, aligned in chains are postulated to function as biological compass needles allowing the bacterium to migrate along redox gradients through the Earth's geomagnetic field lines. Despite the discovery of this unique group of microorganisms several years ago, the mechanisms of magnetic crystal biomineralization have yet to be fully elucidated. A lipid bilayer membrane of approximately 2–4 nm in thickness encapsulates individual magnetosomes (50–100 nm in diameter). Magnetosomes are also referred to as bacterial magnetic particles (BMPs) to distinguish them from artificial magnetic particles (AMPs). The aggregation of BMPs can be easily dispersed in aqueous solutions compared with AMPs because of the enclosing organic membrane.

BMPs have potential applications in the interdisciplinary fields of nanobiotechnology, medicine and environmental management. Through genetic engineering, functional proteins such as enzymes, antibodies, and receptors have been successfully displayed on BMPs, which have been utilized in various biosensors and bio-separation processes. The use of BMPs in immunoassays enables the separation of bound and free analytes by applying a magnetic field. Proteins can be attached

Copyrighted material

covalently to solid supports such as BMPs that prevents desorption of antibodies during an assay. Large scale production of functionally active antibodies or enzymes expressed on BMP membranes can be accomplished.

## *Carbon Nanotubes*

Carbon nanotubes are rolled-up sheets of carbon atoms that appear naturally in soot, and are central to many nanotechnology projects. These nanotubes can go down in diameter to 1 nm, are stronger than any material in the universe and can be any length. These can be used as probes for AFMs that can image individual molecules in both wet and dry environments. This has enormous opportunities for application as conventional structure-based pharmaceutical design is hampered by the lack of high-resolution structural information for most protein-coupled receptors. It is possible to insert DNA into a carbon nanotube. Devices based on the DNA-nanotube combination could eventually be used to make electronics, molecular sensors, devices that sequence DNA electronically, and even gene delivery systems.

### Medical Applications of Nanotubes

- Cyclic peptide nanotubes can act as a new type of antibiotic against bacterial pathogens.
- Cyclic peptide nanotubes can be used as artificial ion channels than open and close in response to electrical and chemical stimuli.
- It is easy to chemically functionalize the surfaces of template-synthesized nanotubes, and different functional groups can be attached to the inner versus outer surfaces of the tubes.
- Biomolecules, such as enzymes, antibodies, and DNA chains, can be attached to the nanotube surfaces to make biofunctionalized nanotubes.
- Template-synthesized nanotubes can be used as smart nanophase extraction agents, e.g. to remove drug molecules from solution.
- Template-synthesized nanotube membranes offer new approaches for doing bioseparations, e.g. of drug molecules.
- Nanoscale electromechanical systems (nanotweezers) based on carbon nanotubes have been developed for manipulation and interrogation of nanostructures within a cell.
- Carbon nanotubes can be used as tips for AFM
- Lumen of a nanotube can carry payloads of drugs
- Nanotubes can be used in biosensors
- Blood-compatible CNTs, with heparin immobilized on the surface, are building blocks for in vivo nanodevices. Activated partial thromboplastin time and thromboelastography studies prove that heparinization can significantly enhance the blood compatibility of nanomaterials.

Copyrighted material

Studies of electrophoretic transport of ssRNA molecules through 1.5-nm-wide pores of carbon nanotube membranes reveal that RNA entry into the nanotube pores is controlled by conformational dynamics, and exit by hydrophobic attachment of RNA bases to the pores. Differences in RNA conformational flexibility and hydrophobicity result in sequence-dependent rates of translocation, which is a prerequisite for nanoscale separation devices.

The uptake of single-walled carbon nanotubes (SWCNTs) into cells appears to occur through phagocytosis. There are no adverse effects on the cells and the nanotubes retained their unique optical properties suggesting that SWCNTs might be valuable biological imaging agents, in part because SWCNTs fluoresce in the NIR portion of the spectrum, at wavelengths not normally emitted by biological tissues. This may allow light from even a handful of nanotubes to be selectively detected in vivo. Although long term studies on toxicity and biodistribution must be completed before nanotubes can be used in medical tests, but nanotubes are useful as imaging markers in laboratory in vitro studies, particularly in cases where the bleaching, toxicity and degradation of more traditional markers are problematic.

## *Dendrimers*

Dendrimers (dendri – tree, mer – branch) are a novel class of 3D, core-shell nanostructures/nanoparticles with 'onion skin-like' branched layers. Dendrimers can be precisely synthesized for a wide range of applications and specialized chemistry techniques enable precise control over their physical as well as chemical properties. They are constructed generation by generation in a series of controlled steps that increase the number of small branching molecules around a central core molecule. Up to 10 generations can be incorporated into a single dendrimer molecule. The final generation of molecules added to the growing structure makes up the polyvalent surface of the dendrimer (see Fig. 2.1). The core, branching and surface molecules are chosen to give desired properties and functions. The outer generation of each dendrimer has a precise number of functional groups that may act as a monodispersed platform for engineering favorable nanoparticle-drug and nanoparticle-tissue interactions. These features have attracted significant attention in medicine as nanocarriers for traditional small drugs, proteins, DNA/RNA and in some instances as intrinsically active nanoscale drugs.

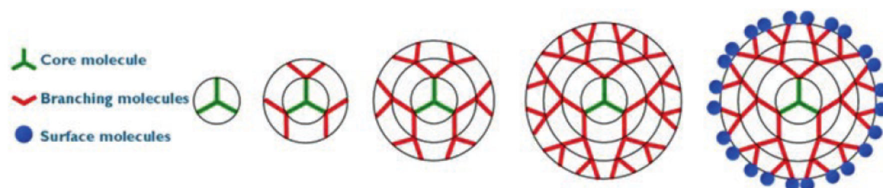


**Fig. 2.1** The core, branching and surface molecules of dendrimers (Source: Starpharma Holding Ltd, by permission)

Copyrighted material

Because of their unique architecture and construction, dendrimers possess inherently valuable physical, chemical and biological properties. These include:

- Precise architecture, size and shape control. Dendrimers branch out in a highly predictable fashion to form amplified 3D structures with highly ordered architectures.
- High uniformity and purity. The proprietary step-wise synthetic process used produces dendrimers with highly uniform sizes (monodispersity) possessing precisely defined surface functionality and very low impurity levels.
- High loading capacity. Internal cavities intrinsic to dendrimer structures can be used to carry and store a wide range of metals, organic, or inorganic molecules.
- High shear resistance. Through their 3D structure dendrimers have a high resistance to shear forces and solution conditions.
- Low toxicity. Most dendrimer systems display very low cytotoxicity levels.
- Low immunogenicity when injected or used topically.

**Properties**

The surface properties of dendrimers may be manipulated by appropriate 'capping' reagents on the outermost generation. In this way, dendrimers can be readily decorated to yield a novel range of functional properties. These include:

- Polyvalency – The outer shell of each dendrimer can be manipulated to contain numerous reactive groups. Each of these reactive sites has the potential to interact with a target entity, often resulting in polyvalent interactions.
- Flexible charge and solubility properties – Through use of appropriate capping groups on the dendrimer exterior, the charge and solubility of dendrimers can be readily manipulated.
- Flexible binding properties – By using appropriate capping groups on the dendrimer exterior, dendrimers can be designed to exhibit strong affinity for specific targets.
- Transfection – Dendrimers can move through cell boundaries and transport genetic materials into cell interiors.

**Applications**

Dendrimers, with their highly customizable properties, are basic building blocks with the promise of enabling specific nanostructures to be built to meet existing needs and solve evolving problems. Dendrimer research and development is currently making an impact on a broad range of fields as shown by exponential growth in the number of dendrimer-based publications. Dendrimer-based drugs, as well as diagnostic and imaging agents, are emerging as promising candidates for many nanomedicine applications. While the potential applications of dendrimers are unlimited, some of their current uses relevant to nanomedicine are shown in Table 2.3.

Copyrighted material

Table 2.3 Potential applications of dendrimers in nanomedicine

| **Diagnostics** |
|---|
| Sensors |
| Imaging contrast agents |
| **Drug delivery** |
| Improved delivery of existing drugs |
| Improved solubility of existing drugs |
| **Drug development** |
| Polyvalent dendrimers interacting simultaneously with multiple drug targets |
| Development of new pharmaceuticals with novel activities |
| Improving pharmacological activity of existing drugs |
| Improving bioavailability of existing drugs |
| **Therapeutics** |
| Antimicrobial agents |
| Chemotherapy |
| Prevention of scar tissue formation after surgery |

© Jain PharmaBiotech

Advances in understanding of the role of molecular weight and architecture on the in vivo behavior of dendrimers, together with recent progress in the design of biodegradable chemistries, has enabled the application of dendrimers as antiviral drugs, tissue repair scaffolds, targeted carriers of chemotherapeutics and optical oxygen sensors. Examples of pharmaceutical products based on dendrimers are:

- VivaGel SPL7013 (Starpharma Pty Ltd), dendrimer-based topical treatment of bacterial vaginosis is in phase III clinical trials (NCT01577537).
- DEP™-Docetaxel (DTX-SPL8783, Starpharma/AstraZeneca), a dendrimer-based conjugate, is in a phase I clinical trial for advanced or metastatic cancer.

A potential application of dendrimer-based complexes is for in vivo real-time imaging, and combination of diagnosis with treatment leading to personalized treatment of various diseases. Before such products can reach the market, however, the field must not only address the cost of manufacture and quality control of pharmaceutical-grade materials, but also assess the long-term human and environmental health consequences of dendrimer exposure in vivo.

## *DNA Nanostructures*

DNA is a material that can be readily used for the programmed self-assembly of wireframe, 2D or 3D nanostructures due to the predictability of base pairing. DNA can self-assemble into nanoscale shapes and small bioactive molecules such as dyes, nanoparticles or proteins can be attached with site-specificity to DNA nanostructures

Copyrighted material

through ligands, antibodies, aptamers or recombinant genetic techniques. Advantages of DNA nanostructure are (Smith et al. 2013):

- Biocompatibility
- Increased stability against degradation in a variety of biological media compared with ssDNA or dsDNA.
- Further protection against the body's immune response can be provided by the addition of encapsulating PEG or lipid shells.
- Nanoscale structures and frames from DNA show a lack of toxicity to cells and initiate a generally low immune response.

Targeted delivery of molecular therapeutics can be achieved by carriers that have been successfully constructed from DNA material, which can selectively deliver material such as siRNA, the anticancer drugs or signaling molecules to target cells in vivo. DNA-based structures are suitable carriers for immunostimulating nucleotide sequences, which can act as adjuvants for inducing long-term immunity in vaccination. Besides therapeutic applications, DNA nanostructures can be used in diagnostics. Nanopores constructed by the DNA origami method can be used for the detection and sequence-specific recognition of DNA molecules.

## Potential Applications of DNA Octahedron

DNA octahedron is a single strand of DNA that spontaneously folds into a highly rigid, nanoscale octahedron that is several million times smaller than the length of a standard ruler and about the size of several other common biological structures, such as a small virus or a cellular ribosome. The octahedron consists of 12 edges, six vertices, and eight triangular faces. The structure is about 22 nm in diameter. Making the octahedron from a single strand was a breakthrough. Because of this, the structure can be amplified with the standard tools of molecular biology and can easily be cloned, replicated, amplified, evolved, and adapted for various applications. This process also has the potential to be scaled up so that large amounts of uniform DNA nanomaterials can be produced. These octahedra are potential building blocks for new tools for basic biomedical science. With these we have biological control, and not just synthetic chemical control, over the production of rigid, wire frame DNA objects.

Because all 12 edges of the octahedral structures have unique sequences, they are versatile molecular building blocks that could potentially be used to self-assemble complex higher-order structures. Possible applications include using these octahedra as artificial compartments into which proteins or other molecules could be inserted, something like a virus in reverse – DNA is on the outside and proteins on the inside. In nature, viruses are self-assembling nanostructures that typically have proteins on the outside and DNA or RNA on the inside. The DNA octahedra could possibly form scaffolds that host proteins for the purposes of x-ray crystallography, which depends on growing well-ordered crystals, composed of arrays of molecules.

Copyrighted material

## *Nanowires*

The manipulation of photons in structures smaller than the wavelength of light is central to the development of nanoscale integrated photonic systems for computing, communications, and sensing. Assembly of small groups of freestanding, chemically synthesized nanoribbons and nanowires into model structures illustrates how light is exchanged between subwavelength cavities made of three different semiconductors. With simple coupling schemes, lasing nanowires can launch coherent pulses of light through ribbon waveguides that are up to a millimeter in length. Also, interwire coupling losses are low enough to allow light to propagate across several right-angle bends in a grid of crossed ribbons. Nanoribbons function efficiently as waveguides in liquid media and provide a unique means for probing molecules in solution or in proximity to the waveguide surface. These results lay the groundwork for photonic devices based on assemblies of active and passive nanowire elements. There are potential applications of nanowire waveguides in microfluidics and biology. Some nanowire-based nanobiosensors are in development.

## *Nanopores*

Nanopores are tiny structures that occur in the cell in nature for specific functions. At the molecular level, specific shapes are created that enable specific chemical tasks to be completed. For examples, some toxic proteins such as alpha hemolysin can embed themselves into cell membranes and induce lethal permeability changes there due to its central pore. The translocation of polymers across nanometer scale apertures in cell membranes is a common phenomenon in biological systems. The first proposed application was DNA sequencing by measuring the size of nanopore, application of an electric potential across the membrane and waiting for DNA to migrate through the pore to enable one to measure the difference between bases in the sequence (see Chap. 3). Protein engineering has been applied to ion channels and pores and protein as well as non-protein can be constructed. Potential applications of engineered nanopores are:

- Tools in basic cell biology
- Molecular diagnostics: sequencing
- Drug delivery
- Cryoprotection and desiccation of cells
- Components of nanodevices and nanomachines
- Nanomedicine

Copyrighted material

## *Nanoporous Silica Aerogel*

Nanoporous silica aerogels have been used in nanotechnology devices such as aerogel nanoporous insulation blankets. Silica aerogel substrate enables stable formation of lipid bilayers that are expected to mimic real cell membranes. Typical bilayers are 5 nm in thickness and the silica beads in aerogel are approximately 10–25 nm in diameter. Silica aerogels have a unique structure and chemistry that allow for the transformation of nano-sized liposomes into continuous, surface-spanning lipid bilayers. These lipid bilayers adsorb to the aerogel surface and exhibit the characteristic lateral mobility of real cell membranes. The high (98%) porosity of aerogel substrates creates an underlying "water-well" embedded in the aerogel pore structure that allows these membrane molecules to carry out normal biological activities including transport across the membrane. This porosity could potentially accommodate the movement of membrane proteins or other membrane-extruding molecules.

This aerogel is an improvement over conventional substrates for synthetic biomembranes as it is porous, thus minimizing non-physiological interaction between membrane proteins and a hard substrate surface. This prevents the proteins from becoming immobilized, denatured and eventually losing their biological functions. Applications of lipid bilayers are:

- Model biological membranes for research
- Biosensors and lab-on-chip devices (microfluidic systems, analyte detector, etc.)
- Bio-actuating devices
- Arrays for use in screening arrays of compounds for membrane-associated drug targets. Lipid bilayer system has been used in immunological screening for drug targets.
- Display libraries of compounds
- Patterned lipid bilayers can be used for tissue culture and engineering (micro-patterns of lipid membranes direct discriminative attachment or growth of living cells)

Advantages of aerogel biomembrane are:

- Best able to mimic the lateral mobility of molecules in real cell membranes
- Enable membrane transport studies due to liquid permeability of aerogels
- Both sides of supported membranes are accessible compared to only one side in conventional solid support
- Can be used to design functional membranes for different applications by incorporating organic, inorganic, polymeric and/or biologically active components into the aerogel structures
- Non-physiological interaction of the membrane-associated components with the underlying support (compared to glass)
- Membranes on the aerogel maintain stability for weeks

Copyrighted material

## *Nanostructured Silicon*

Silicon has been used for implants in the human body for several years. Following nanostructuring, silicon can be rendered biocompatible and biodegradable. BioSilicon™ (pSiMedica Ltd) contains nano-sized pores measuring 100 nm. The "silicon skeleton" between the pores comprises tens of silicon atoms in width. Initial applications are in drug delivery. The kinetics of drug release from BioSilicon™ can be controlled by adjusting the physical properties of the matrix, including modifying the pore size. Other potential applications include nanospheres for targeted systemic and pulmonary drug delivery. Nanostructured silicon, as multilayered mirrors, can be used for subcutaneous implants for diagnostics. Nanostructures can be used as prostheses to improve adhesion to bone tissue.

## *Nanoparticle Conjugates*

### DNA-Nanoparticle Conjugates

DNA-DNA hybridization has been exploited in the assembly of nanostructures including biosensors, and DNA scaffolds. Many of these applications involve the use of DNA oligonucleotides tethered to gold nanoparticles or nanoparticles may be hybridized with one another. Two types of DNA-nanoparticle conjugates have been developed for these purposes. Both types entail the coupling of oligonucleotides through terminal thiol groups to colloidal gold particles. In one case, the oligonucleotides form the entire monolayer coating the particles, whereas in the other case, the oligonucleotides are incorporated in a phosphine monolayer, and particles containing discrete numbers of oligonucleotides are separated by gel electrophoresis. A minimal length of 50 residues is required, both for separation by electrophoresis and hybridization with complementary DNA sequences. These limitations of shorter oligonucleotides are attributed to interaction between the DNA and the gold. In a new technique, glutathione monolayer-protected gold clusters were reacted with 19- or 20-residue thiolated oligonucleotides and the resulting DNA-nanoparticle conjugates can be separated based on the number of bound oligonucleotides by gel electrophoresis and assembled with one another by DNA-DNA hybridization. This approach overcomes previous limitations of DNA-nanoparticle synthesis and yields conjugates that are precisely defined with respect to both gold and nucleic acid content.

### Networks of Gold Nanoparticles and Bacteriophage

Biological molecular assemblies are excellent models for the development of nano-engineered systems with desirable biomedical properties. A biologically active molecular network consists of bacteriophage (phage) directly assembled with gold

Copyrighted material

(Au) nanoparticles and termed Au-phage. When the phage is engineered so that each phage particle displays a peptide, such networks preserve the cell surface receptor binding and internalization attributes of the displayed peptide. The spontaneous organization of these targeted networks can be manipulated further by incorporation of imidazole (Au-phage-imid), which induces changes in fractal structure and near-infrared optical properties. The networks can be used as labels for enhanced fluorescence and dark-field microscopy, surface-enhanced Raman scattering detection, and near-infrared photon-to-heat conversion. Together, the physical and biological features within these targeted networks offer convenient multifunctional integration within a single entity with potential for nanotechnology-based biomedical applications such as biological sensors and cell-targeting agents.

Carboxymethyl chitosan capped gold nanoparticles (CMC-AuNPs) are used as plasmonic probes and are synthesized by a simple one pot wet chemical method. The conjugation of carboxymethyl chitosan-linked AuNPs with T7 virions enables simple, selective and sensitive colorimetric biosensing of viruses (Kannan et al. 2014). This method is low cost.

This genetically programmable nanoparticle with a biologically compatible metal acts as a nanoshuttle that can target specific locations in the body. For example, it could potentially locate damaged areas on arteries that have been caused by heart disease, and then deliver stem cells to the site that can build new blood vessels. It may be able to locate specific tumors, which could then be treated by either heating the gold particles with laser light and/or using the nanoparticles to selectively deliver a drug to destroy the cancer.

## Protein-Nanoparticle Combination

Proteins come in many handy shapes and sizes, which make them major players in biological systems. Chaperonins are ring-shaped proteins found in all living organisms where they play an essential role in stabilizing proteins and facilitating protein folding. A chaperonin can be adapted for technological applications by coaxing it to combine with individual luminescent semiconductor nanoparticles. In bacteria, this chaperonin protein takes in and re-folds denatured proteins to return them to their original useful shapes. This ability would make the proteins good candidates for drug carriers.

Cadmium sulfite nanoparticles emit light so long as they are isolated from each other; encasing the nanoparticles in the protein keeps the tiny particles apart. The biological fuel molecule ATP releases the nanoparticles from the protein tubes, freeing the particles to clump together, which quenches the light. The protein-nanoparticle combination could be used to detect ATP. This blend of nanotechnology and molecular biology could lead to new bioresponsive electronic nanodevices and biosensors very different from the artificial molecular systems currently available. By adding selective binding sites to the solvent-exposed regions of the chaperonin, the protein-nanoparticle bioconjugate becomes a sensor for specific targets (Xie et al. 2009).

Copyrighted material

### *Polymer Nanofibers*

Polymer nanofibers, with diameters in the nanometer range, possess larger surface areas per unit mass and permit easier addition of surface functionalities compared with polymer microfibers. Research on polymer nanofibers, nanofiber mats, and their applications has seen a remarkable growth over the last few years. Among various methods of manufacture, electrospinning has been used to convert a large variety of polymers into nanofibers and may be the only process that has the potential for mass production. Although measurement of mechanical properties such as tensile modulus, strength, and elongation is difficult because of the small diameters of the fibers, these properties are crucial for the proper use of nanofiber mats. Owing to their high surface area, functionalized polymer nanofibers will find broad applications as drug delivery carriers, biosensors, and molecular filtration membranes in future.

### *Virus-Like Particles*

Virus-like particles (VLPs), noninfectious viruses without genetic material, have evolved to become an accepted technology and some VLP-based vaccines are currently used as commercial medical products, and other VLP-based products are at different stages of clinical development. VLPs have advantages as gene therapy tools and as nanomaterials. VLPs can be used as nano-scaffolds for enzyme selection as well as patterning, phage therapy, raw material processing, and single molecule enzyme kinetics studies (Cardinale et al. 2012). Analysis of published data shows that at least 110 VLPs have been constructed from viruses belonging to 35 different families (Zeltins 2013). Novavax Inc's VLP technology uses recombinant protein technology to imitate the structure of a virus to provide protection without the risk of infection or disease. Virion proteins can self-assemble into VLPs when over-expressed in certain cells.

## Measurement of Nanoparticle Size and Distribution

Number weighted nanoparticle (NP) size distribution in a sample is not only important for basic research but is also required under European Union regulations that apply for researchers and industry alike. A representative number of NPs are typically counted by use of a transmission electron microscope (TEM) in which a beam of electrons probes an ultra-thin specimen and interact with the sample as they pass through leading to a "shadow image" of the specimen. Sample preparation generally requires the complete removal of the suspending liquid leading to aggregation of NPs which makes it difficult both to count them and to determine if the particles were already aggregated beforehand. This renders automatic counting systems useless as well, leaving researchers with the huge task of interpreting images manually.

Copyrighted material

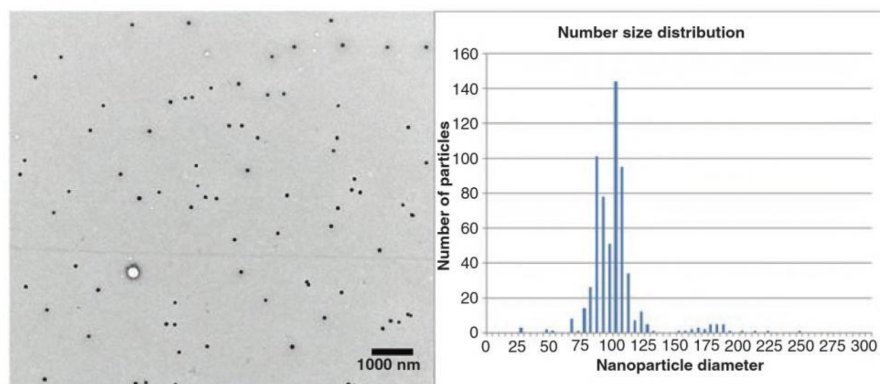


**Fig. 2.2** Imaging and size distribution of nanoparticles with TEM (Source: Adolphe Merkle Institute (University of Freiburg, Switzerland), by permission)

To prevent artifacts from sample preparation and simplify interpretation, researchers at the Adolphe Merkle Institute (University of Fribourg, Switzerland) have devised a straightforward protocol for prevention of the onset of drying artifacts, thereby enabling the preservation of in situ colloidal features of NPs during sample preparation for TEM (Michen et al. 2015). This is achieved by adding bovine serum albumin, a macromolecular agent, to the suspension to stabilize nanoparticles and prevent aggregation. Both research- and economically-relevant particles with high polydispersity and/or shape anisotropy are easily characterized following this approach, which allows for rapid and quantitative classification in terms of dimensionality and size as shown in Fig. 2.2.

Scientists at Center for Environmental Nanoscience and Risk (University of South Carolina, USA) have presented a validated quantitative sampling technique for atomic force microscopy (AFM) that overcomes the drawbacks of conventional preparation of NP samples and allows full recovery and representativeness of the NPs under consideration by forcing the NPs into the substrate via ultracentrifugation and strongly attaches the NPs to the substrate by surface functionalization of the substrate or by adding cations to the NP suspension (Baalousha et al. 2014). The high efficiency of the analysis is demonstrated by the uniformity of the NP distribution on the substrate (that is low variability between the number of NPs counted on different images on different areas of the substrate), the high recovery of the NPs up to 71%) and the good correlation between the mass and number concentrations. This validated quantitative sampling technique enables the use of the full capabilities of microscopy tools to quantitatively and accurately determine the number size distribution and number concentration of NPs at environmentally relevant low concentrations (i.e. 0.34–100 ppb). This approach is of high environmental relevance and can be applied widely in environmental nanotoxicology for accurately measuring the number size distribution of NPs.

Copyrighted material

## Nanomaterials for Biolabeling

Nanomaterials are suitable for biolabeling. Nanoparticles usually form the core in nanobiomaterials. However, to interact with biological target, a biological or molecular coating or layer acting as an interface needs to be attached to the nanoparticle. Coatings that make the nanoparticles biocompatible include antibodies, biopolymers or monolayers of small molecules. A nanobiomaterial may be in the form of nanovesicle surrounded by a membrane or a layer. The shape is more often spherical but cylindrical, plate-like and other shapes are possible. The size and size distribution might be important in some cases, for example if penetration through a pore structure of a cellular membrane is required. The size is critical when quantum-sized effects are used to control material properties. A tight control of the average particle size and a narrow distribution of sizes allow creating very efficient fluorescent probes that emit narrow light in a very wide range of wavelengths. This helps with creating biomarkers with many and well distinguished colors. The core itself might have several layers and be multifunctional. For example, combining magnetic and luminescent layers one can both detect and manipulate the particles.

The core particle is often protected by several monolayers of inert material, for example silica. Organic molecules that are adsorbed or chemisorbed on the surface of the particle are also used for this purpose. The same layer might act as a biocompatible material. However, more often an additional layer of linker molecules is required that has reactive groups at both ends. One group is aimed at attaching the linker to the nanoparticle surface and the other is used to bind various biocompatible substances such as antibodies depending on the function required by the application.

Efforts are being made to improve the performance of immunoassays and immunosensors by incorporating different kinds of nanostructures. Most of the studies focus on artificial, particulate marker systems, both organic and inorganic. Inorganic nanoparticle labels based on noble metals, semiconductor QDs and nanoshells appear to be the most versatile systems for these bioanalytical applications of nanophotonics. The underlying detection procedures are more commonly based on optical techniques. These include nanoparticle applications generating signals as diverse as static and time-resolved luminescence, one- and two-photon absorption, Raman and Rayleigh scattering as well as surface plasmon resonance and others. All efforts are aimed at achieving one or more of the following goals:

- Lowering of detection limits (if possible, down to single-molecule level)
- Parallel integration of multiple signals (multiplexing)
- Signal amplification by several orders of magnitude

Potential benefits of using nanoparticles and nanodevices include an expanded range of label multiplexing. Different types of fluorescent nanoparticles and other nanostructures have been promoted as alternatives for the fluorescent organic dyes that are traditionally used in biotechnology. These include QDs, dye-doped polymer and silica nanoparticles (Dosev et al. 2008). Various nanomaterials for biolabeling are shown in Table 2.4.

Copyrighted material

**Table 2.4** Nanomaterials for biolabeling

| Label/reporter | Characteristics | Function/applications |
|---|---|---|
| Dendrimer /silver nanocompsites | Water-soluble, biocompatible, fluorescent and 3–7 nm diameter stable nanoparticles. | Biomarkers for in vitro cell labeling |
| Electrogenerated chemiluminescence | Tris(2,2´-bipyridyl)ruthenium(II) molecular labels. | Nanoscale bioassay |
| Europium(III)-chelate-doped nanoparticles | Combined with selection of high affinity monoclonal antibodies coated on label particles and microtitration wells. | The sensitivity for virus particle detection is improved compared to immunofluorometric assays |
| Fluorescent color-changing dyes | 3-hydroxychromone derivatives that exhibit 2 fluorescence bands resulting from excited-state intramolecular proton transfer reaction. | Biosensors |
| Fluorescent lanthanide nanorods | Retain their fluorescent properties after internalization into cells. | Multiplexed imaging of molecular targets in living cells |
| Luminescent core/shell nanohybrid | Luminescent rare earth ions in a nanosized Gd2O3 core (3.5 nm) and FITC molecules entrapped within in a polysiloxane shell (2.5–10 nm). | Two different luminescence emissions: (1) FITC under standard illumination; (2) $Tb^{3+}$ under high-energy source giving highly photostable luminescence |
| Magnetic nanotags (MNTs) | Alternative to fluorescent labels in biomolecular detection assays | Multiplex protein detection of cancer biomarkers at low concentrations |
| Nanogold® labels (Nanoprobe Inc) | Unlike nanogold particles, gold labels are uncharged molecules, which are cross-linked to specific sites on biomolecules. | Nanogold® labels have a range and versatility, which is not available with colloidal nanogold particles. |
| Nanophosphors | Nanophosphors contains embedded lanthanide ions, like europium or terbium | Nanophosphor signals hardly fade and can also be used for multiplex testing. |
| Plasmon resonant nanoparticles | Scatter light with tremendous efficiency | Ultrabright nanosized labels for biological applications, replacing other labeling methods such as fluorescence. |
| QD end-labeling | Multicolor fluorescence microscopy using conjugated QDs | Detection of single DNA molecules. |
| SERS (Surface-enhanced Raman Scattering)-based nanotags | A metal nanoparticle where each type of tag exploits the Raman spectrum of a different small molecule and SERS bands are 1/50th the width of fluorescent bands. | Enables greater multiplexed analyte quantification than other fluorescence-based quantitation tags. |

© Jain PharmaBiotech

Copyrighted material

## DNA Nanotags

Bright fluorescent dye molecules can be integrated with DNA nanostructure to make nanosized fluorescent labels – DNA nanotags, which improve the sensitivity for fluorescence-based imaging and medical diagnostics. DNA nanotags are useful for detecting rare cancer cells within tissue biopsies. In addition, they offer the opportunity to perform multicolor experiments. This feature is extremely useful for imaging applications because the multiple colors can be seen simultaneously, requiring only one experiment using one laser and one fluorescence-imaging machine. Fluorescent DNA nanotags have been used in a rolling circle amplification immunoassay based as a versatile fluorescence assay platform for highly sensitive proteins detection (Xue et al. 2012).

## Fluorescent Lanthanide Nanorods

Inorganic fluorescent lanthanide (europium and terbium) orthophosphate nanorods can be used as a novel fluorescent label in cell biology. These nanorods, synthesized by the microwave technique, retain their fluorescent properties after internalization into human umbilical vein endothelial cells or renal carcinoma cells. The cellular internalization of these nanorods and their fluorescence properties have been characterized by fluorescence spectroscopy, differential interference contrast microscopy, confocal microscopy, and transmission electron microscopy. Nanorods are nontoxic up to concentrations of 50 ug/ml. Nanorods can be used for the detection of cancer at an early stage and functionalized nanorods are potential vehicles for drug delivery.

## Magnetic Nanotags

Magnetic nanotags (MNTs) are a promising alternative to fluorescent labels in biomolecular detection assays, because minute quantities of MNTs can be detected with inexpensive sensors. Probe sensors are functionalized with capture antibodies specific to the chosen analyte. During analyte incubation, the probe sensors capture a fraction of the analyte molecules. A biotinylated linker antibody is subsequently incubated and binds to the captured analyte, providing binding sites for the streptavidin-coated MNTs, which are incubated further. The nanotag binding signal, which saturates at an analyte concentration-dependent level, is used to quantify the analyte concentration. However, translation of this technique into easy-to-use and multilplexed protein assays, which are highly sought after in molecular diagnostics such as cancer diagnosis and personalized medicine, has been challenging. Multiplex protein detection of potential cancer biomarkers has been demonstrated at subpicomolar concentration levels (Osterfeld et al. 2008). With the addition of nanotag amplification, the analytic

Copyrighted material

sensitivity extends into the low femtomolar concentration range. The multianalyte ability, sensitivity, scalability, and ease of use of the MNT-based protein assay technology make it a strong contender for versatile and portable molecular diagnostics in both research and clinical settings. A hand-held, portable biosensor platform for quantitative biomarker measurement combines MNP tags with giant magnetoresistive spin-valve sensors, to achieve highly sensitive (picomolar) and specific biomarker detection in >20 min (Hall et al. 2010). This platform can detect multiple biomarkers simultaneously in a single assay at point-of-care (POC) to provide a low-cost diagnostic tool for multiple applications.

## *Molecular Computational Identification*

Molecular computational identification, based on molecular logic and computation, has been applied on nanoscale. Examples of populations that need encoding in the microscopic world are cells in diagnostics or beads in combinatorial chemistry. Taking advantage of the small size (about 1 nm) and large 'on/off' output ratios of molecular logic gates and using the great variety of logic types, input chemical combinations, switching thresholds and even gate arrays in addition to colors, unique identifiers have been produced for small polymer beads (about 100 μm) used for synthesis of combinatorial libraries. Many millions of distinguishable tags become available. This method should be extensible to far smaller objects, with the only requirement being a 'wash and watch' protocol. The basis of this approach is converting molecular science into technology concerning analog sensors and digital logic devices. The integration of molecular logic gates into small arrays has been a growth area in recent years (de Silva 2011).

## *Nanophosphor Labels*

Nanostructures based on inorganic phosphors (nanophosphors) are a new emerging class of materials with unique properties that make them very attractive for labeling. The molecular lattice of phosphors contains individual embedded lanthanide ions, like europium or terbium. The crystal lattice or sometimes "activator ions" such as cerium ions used especially for this purpose – absorbs the stimulating light and transfers the energy to the lanthanide ions, which are the true source of fluorescence. The color emitted depends mainly on the lanthanide ions used. Terbium, for example, gives off a yellowish green color, while europium produces a red fluorescence. As shown by the "microparticles" in fluorescent lights, the cycle of stimulation and emission can be endlessly repeated, which means that the dye never fades.

Bayer scientists are developing nanophosphors, which many of the advantages of QDs and fewer disadvantages such as high cost and heavy metals content that may not be environmentally friendly. Nanophosphor signals hardly fade and can also be

Copyrighted material

used for multiplex testing. And the major advantage they have over QDs is that the wavelength of their emitted light does not depend on particle size but on the type of lanthanide ions used. For this reason, their particle size, which is also no more than 10 nm, does not need to be monitored so precisely. Because of this, the manufacturing process is simpler and less expensive. Moreover, most ions of lanthanides, also called rare earths, are considered less harmful to the environment, and this facilitates their manufacture and disposal.

Background fluorescence from biological components of cells, makes it difficult to interpret the signal, e.g. the positive result of a diagnostic test for cancer. Nanophosphors can get around this problem because for many types of nanophosphor, the life span of the fluorescence i.e. the time between stimulation and emission extends to several milliseconds. Accordingly, when the nanophosphor is exposed to a brief impulse of light, the background fluorescence disappears before the test result is displayed. This considerably enhances the sensitivity of the fluorescent marker in its various applications. Another important advantage of the nanophosphor system, particularly where medical diagnostics are concerned, is its ability to transfer fluorescent energy to a closely related dye. This allows biochemical reactions, like the coupling between antibodies, to be detected without the need for any additional procedures. Therefore, the relevant antibodies in the patient's sample can be detected immediately after the dye has been added to the test solution.

Before the nanophosphors can be used to track down certain segments of DNA, for example in cancer tests, they themselves need to be attached to suitable DNA segments. It is always a major challenge to achieve stable coupling of small organic molecules or larger biomolecules with unique, inorganic nanoparticles. The particles must be painstakingly adapted to the properties of the organic molecules and prevented from lumping together themselves in the process. If this can be done successfully, it will meet the demanding challenges of medical diagnostics in the future.

Photoluminescence imaging in vitro and in vivo has been shown by use of near infrared to near infrared (NIR-to-NIR) up-conversion in nanophosphors. This NIR-to-NIR up-conversion process provides deeper light penetration into biological specimen and results in high contrast optical imaging due to absence of an autofluorescence background and decreased light scattering. Fluoride nanocrystals (20–30 nm size) co-doped with $Tm^{3+}$ and $Yb^{3+}$, have been synthesized and characterized by TEM, XRD, and photoluminescence spectroscopy (Nyk et al. 2009). In vitro cellular uptake was demonstrated with no apparent cytotoxicity. Subsequent animal imaging studies were performed using Balb-c mice injected intravenously with up-converting nanophosphors, demonstrating the high contrast PL imaging in vivo by photoluminescence spectroscopy. Lanthanide doped nanocrystals, have also been used for imaging of cells and some deep tissues in animals. Polyethyleneimine (PEI) coated $NaYF^4$:Yb,Er nanoparticles produce very strong upconversion fluorescence when excited at 980 nm by a NIR laser, which is resistant to photo-bleaching, and non-toxic to bone marrow stem cells (Chatterjee et al. 2008). The nanoparticles delivered into some cell lines or injected intradermally and intramuscularly into some tissues either near the body surface or deep in the body of rats showed visible fluorescence, when exposed to a 980 nm NIR laser.

Copyrighted material

## *Organic Nanoparticles as Biolabels*

The use of organic nonpolymeric nanoparticles as biolabels was not considered to be promising or have any advantage over established metallic or polymeric probes. Problems include quenching of fluorescence in organic dye crystals, colloidal stability and solubility in aqueous environments but some of these can be circumvented. Labels have been constructed by milling and suspending a fluorogenic hydrophobic precursor, fluorescein diacetate, in sodium dodecyl sulfate (SDS). Thus, a negative surface charge is introduced, rendering the particles (500 nm) colloidally stable and minimizing leakage of fluorescein diacetate molecules into surrounding water. Now it has been shown that the polyelectrolyte multilayer architechture is not vital for the operability of this assay format. Instead of SDS and multilayers the adsorption of only one layer of an amphiphilic polymeric detergent, e.g. an alkylated poly(ethylene imine), is sufficient to stabilize the system and to provide an interface for the antibody attachment. This is the basis of a technology "ImmunoSuperNova®" (invented by 8sens.biognostic AG, Germany). In this the reaction of the analyte molecule with the capture antibody is followed by an incubation step with the antibody-nanoparticle conjugate, which serves as detector. After some washing steps an organic release solvent is added, dissolving the particle and converting fluorescein diacetate into fluorescein.

## *Quantum Dots as Labels*

The unique optical properties of QDs make them appealing as in vivo and in vitro fluorophores in a variety of biological investigations, in which traditional fluorescent labels based on organic molecules fall short of providing long-term stability and simultaneous detection of multiple signals. The ability to make QDs water soluble and target them to specific biomolecules has led to promising applications in cellular labeling, deep-tissue imaging, assay labeling and as efficient fluorescence resonance energy transfer donors.

DNA molecules attached to QD surface can be detect by fluorescence microscopy. The position and orientation of individual DNA molecules can be inferred with good efficiency from the QD fluorescence signals alone. This is achieved by selecting QD pairs that have the distance and direction expected for the combed DNA molecules. Direct observation of single DNA molecules in the absence of DNA staining agents opens new possibilities in the study of DNA-protein interactions. This approach can be applied for the use of QDs for nucleic acid detection and analysis. CdSe QDs can also be used as labels for sensitive immunoassay of cancer biomarker proteins by electrogenerated chemiluminescence. This strategy has been successfully used as a simple, cost-effective, specific, and potential method to detect α-fetoprotein in practical samples (Liu et al. 2011). In contrast to a QD that is selectively introduced as a label, an integrated QD is one that is present in a system throughout

Copyrighted material

microscopic mirrors. NEMS devices exist correspondingly in the nanometer realm – nano-electromechanical systems (NEMS). The concept of using externally controllable MEMS devices to measure and manipulate biological matter (BioMEMS) on the cellular and subcellular levels has attracted much attention recently. This is because initial work has shown the ability to detect single base pair mismatches of DNA and to quantifiably detect antigens using cantilever systems. In addition is the ability to controllably grab and manipulate individual cells and subsequently release them unharmed.

Surface nanomachining, combines the processing methods of MEMS with the tools of electron beam nanofabrication to create 3D nanostructures that move (and thus can do new types of things). Ultra-short pulsed-laser radiation, e.g. using femtolasers, is an effective tool for controlled material processing and surface nano/micro-modification because of minimal thermal and mechanical damage. Surface nanomachining has potential applications in nanobiotechnology.

### *BioMEMS*

Because BioMEMS involves the interface of MEMS with biological environments, the biological components are crucially important. To date, they have mainly been nucleic acids, antibodies and receptors that are involved in passive aspects of detection and measurement. These molecules retain their biological activity following chemical attachment to the surfaces of MEMS structures (most commonly, thiol groups to gold) and their interactions are monitored through mechanical (deflection of a cantilever), electrical (change in voltage or current in the sensor) or optical (surface plasmon resonance) measurements. The biological components are in the nanometer range or smaller; therefore, the size of these systems is limited by the minimum feature sizes achievable using the fabrication techniques of the inorganic structures, currently 100 nm–1 μm. Commercially available products resulting from further miniaturization could be problematic because of the expanding cost and complexity of optical lithography equipment and the inherent slowness of electron beam techniques. In addition to size limitations, the effects of friction have plagued multiple moving parts in inorganic MEMS, limiting device speeds and useful lifetimes.

## Microarrays and Nanoarrays

Arrays consist of orderly arrangements of samples, which, in the case of biochips, may be cDNAs, oligonucleotides, or even proteins. Macroarraying (or gridding) is a macroscopic scheme of organizing colonies or DNA into arrays on large nylon filters ready for screening by hybridization. In microarrays, however, the sample spot sizes are usually less than 200 microns in diameter and require microscopic analysis. Microarrays have sample or ligand molecules (e.g. antibodies) at fixed locations

Copyrighted material

on the chip while microfluidics involves the transport of material, samples, and/or reagents on the chip.

Microarrays provide a powerful way to analyze the expression of thousands of genes simultaneously. Genomic arrays are an important tool in medical diagnostic and pharmaceutical research. They have an impact on all phases of the drug discovery process from target identification through differential gene expression, identification and screening of small molecule candidates, to toxicogenomic studies for drug safety. To meet the increasing needs, the density and information content of the microarrays is being improved. One approach is fabrication of chips with smaller, more closely packed features – ultrahigh density arrays, which will yield:

- High information content by reduction of feature size from 200 μm to 50 nm
- Reduction in sample size
- Improved assay sensitivity

Nanoarrays are the next stage in the evolution of miniaturization of microarrays. Whereas microarrays are prepared by robotic spotting or optical lithography, limiting the smallest size to several microns, nanoarrays require further developments in lithography strategies. Technologies available include the following:

- Electron beam lithography
- Dip-pen nanolithography
- Scanning probe lithography
- Finely focused ion beam lithography
- Nano-imprint lithography

## *Dip Pen Nanolithography for Nanoarrays*

Dip Pen Nanolithography™ (DPN™), developed by Mirkin Lab at Northwestern University, uses the tip of an AFM to write molecular "inks" directly on a surface. Biomolecules such as proteins and viruses can be positioned on surfaces to form nanoarrays that retain their biological activity. DPN is schematically shown in Fig. 2.3.

Advantages of DPN are as follows:

Ultrahigh resolution. DPN is capable of producing structures with line widths of less than 15 nm. This is compared to photolithography, which supports features of no less than 65 nm line width, and slower e-beam and laser lithography systems, which support features of 15 nm line width.

Flexibility. Direct fabrication is possible with many substances, from biomolecules to metals.

Accuracy. By leveraging existing highly accurate atomic force microscopy technology, DPN utilizes the best possible means for determining exactly where features are being placed on the substrate. This allows for the integration of multiple component nanostructures and for immediate inspection and characterization of fabricated structures.

Copyrighted material

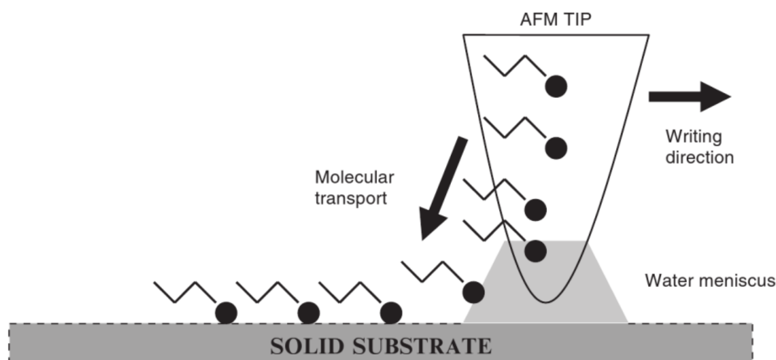


**Fig. 2.3** Schematic representation of Dip Pen Nanolithography (DPN). A water meniscus forms between the atomic force microscope (AFM) tip coated with oligonucleotide (ODN) and the Au substrate. The size of the meniscus, which is controlled by relative humidity, affects the ODN transport rate, the effective tip-substrate contact area, and DPN resolution (© Jain PharmaBiotech)

Low capital cost. Techniques such as e-beam lithography that approach DPN-scale resolution are considerably more expensive to purchase, operate and maintain.

Ease of use. DPN systems may be operated by non-specialized personnel with minimal training. Further, DPN may be performed under normal ambient laboratory conditions with humidity control.

Speed. 100-nm spots can be deposited with a single DPN pen in less than a second. DPN can be used to fabricate arrays of a single molecule with more than 100,000 spots over 100 × 100 microns in less than an hour.

### Applications of Dip-Pen Nanolithography

Multiple-allergen testing for high throughput and high sensitivity requires the development of miniaturized immunoassays that can be performed with minute amounts of test analyte that are usually available. Construction of such miniaturized biochips containing arrays of test allergens needs application of a technique able to deposit molecules at high resolution and speed while preserving its functionality. DPN is an ideal technique to create such biologically active surfaces, and it has already been successfully applied for the direct, nanoscale deposition of functional proteins, as well as for the fabrication of biochemical templates for selective adsorption. It has potential applications for detection of allergen-specific immunoglobin E (IgE) antibodies and for mast cell activation profiling (Sekula-Neuner et al. 2012).

## *Protein Nanoarrays*

High-throughput protein arrays allow the miniaturized and parallel analysis of large numbers of diagnostic biomarkers in complex samples. This capability can be enhanced by nanotechnology. DPN technique has been extended to protein arrays

Copyrighted material

with features as small as 45 nm and immunoproteins as well as enzymes can be deposited. Selective binding of antibodies to protein nanoarrays can be detected without the use of labels by monitoring small (5–15 nm) topographical height increases in AFM images.

Miniaturized microarrays, 'mesoarrays', created by DPN with protein spots 400× smaller by area compared to conventional microarrays, were used to probe the ERK2-KSR binding event of the Ras/Raf/MEK/ERK signaling pathway at a physical scale below that previously reported (Thompson et al. 2011). This study serves as a first step towards an approach that can be used for analysis of proteins at a concentration level comparable to that found in the cellular environment.

### Single-Molecule Protein Arrays

The ability placeme individual protein molecules on surfaces could enable advances in many areas ranging from the development of nanoscale biomolecular devices to fundamental studies in cell biology. An approach that combines scanning probe block copolymer lithography with site-selective immobilization strategies has been used to create arrays of proteins down to the single-molecule level with arbitrary pattern control (Chai et al. 2011). Scanning probe block copolymer lithography was used to synthesize individual sub-10-nm single crystal gold nanoparticles to act as scaffolds for the adsorption of functionalized alkylthiol monolayers for facilitating the immobilization of specific proteins. The number of protein molecules that adsorb onto the nanoparticles depends on particle size; when the particle size approaches the dimensions of a protein molecule, each particle can support a single protein. This was demonstrated with both gold nanoparticle and QD labeling coupled with TEM imaging. The immobilized proteins remain bioactive, as demonstrated by enzymatic assays and antigen-antibody binding experiments.

## Microfluidics and Nanofluidics

Microfluidics is the handling and dealing with small quantities (e.g. microliters, nanoliters or even picoliters) of fluids flowing in channels the size of a human hair (~50 microns thick) even narrower. Fluids in this environment show very different properties than in the macro world. This new field of technology was enabled by advances in microfabrication – the etching of silicon to create very small features. Microfluidics is one of the most important innovations of biochip technology. Typical dimensions of microfluidic chips are 1–50 $cm^2$ and have channels 5–100 microns. Usual volumes are 0.01–10 microliters but can be less. Microfluidics is the link between microarrays and nanoarrays as we reduce the dimensions and volumes.

Microfluidics is the underlying principle of lab-on-a-chip devices, which carry out complex analyses, while reducing sample and chemical consumption, decreasing waste and improving precision and efficiency. The idea is to be able to squirt a very small sample into the chip, push a button and the chip will do all the work, delivering

Copyrighted material

a report at the end. Microfluidics allows the reduction in size with a corresponding increase in the throughput of handling, processing and analyzing the sample. Other advantages of microfluidics include increased reaction rates, enhanced detection sensitivity and control of adverse events.

Drawbacks and limitations of microfluidics and designing of microfluidic chips include the following:

- Difficulties in microfluidic connections
- Because of laminar flows, mixing can only be performed by diffusion
- Large capillary forces
- Clogging
- Possible evaporation and drying up of the sample

Applications of microfluidics include the following:

- DNA analysis
- Protein analysis
- Gene expression and differential display analysis
- Biochemical analysis

## *Nanotechnology on a Chip*

Nanotechnology on a chip is a new paradigm for total chemical analysis systems. The ability to make chemical and biological information much cheaper and easier to obtain is expected to fundamentally change healthcare, food safety and law enforcement. Lab-on-a-chip technology involves micro-total analysis systems that are distinguished from simple sensors because they conduct a complete analysis; a raw mixture of chemicals goes in and an answer comes out. Sandia National Laboratories is developing a hand-held Lab-on-a-chip that will analyze for air-borne chemical warfare agents and liquid-based explosives agents. This development project brings together an interdisciplinary team with areas of expertise including microfabrication, chemical sensing, microfluidics, and bioinformatics. Although nanotechnology plays an important role in current efforts, miniaturized versions of conventional architecture and components such as valves, pipes, pumps, separation columns, are patterned after their macroscopic counterparts. Nanotechnology will provide the ability to build materials with switchable molecular functions could provide completely new approaches to valves, pumps, chemical separations, and detection. For example, fluid streams could be directed by controlling surface energy without the need for a predetermined architecture of physical channels. Switchable molecular membranes and the like could replace mechanical valves. By eliminating the need for complex fluidic networks and micro-scale components used in current approaches, a fundamentally new approach will allow greater function in much smaller, lower power total chemical analysis systems.

Copyrighted material

The benefits of operating in the nanoliter space include reduction of solvent, waste disposal costs, and human exposure by factors of 1000×. New routine liquid handling capabilities include a purported 10x increase in MALDI sensitivity for analysis of proteins in proteomics work as demonstrated by various products such as nanoliter syringes based on induction-based fluidics technology that uses electric fields to launch liquids to targets.

## Nanoscale Flow Visualization

Most of the microscale flow visualization methods evolved from methods developed originally for macroscale flow. It is unlikely, however, that developed microscale flow visualization methods will be translated to nanoscale flows in a similar manner. Resolving nanoscale features with visible light presents a fundamental challenge. Although point-detection scanning methods have the potential to increase the flow measurement resolution on the microscale, spatial resolution is ultimately limited by the optical probe volume (length scale on the order of 100 nm), which, in turn, is limited by the wavelength of light employed. Optical spatially resolved flow measurements in nanochannels are difficult to visualize. There is a need for refinement of microscale flow visualization methods and the development of direct flow measurement methods for nanoflows.

## Moving (Levitation) of Nanofluidic Drops with Physical Forces

The manipulation of droplets/particles that are isolated (levitated in gas/vacuum) from laboratory samples containing chemicals, cells, bacteria or viruses, is important both for basic research in physics, chemistry, biology, biochemistry, and colloidal science and for applications in nanotechnology and microfluidics. Various optical, electrostatic, electromagnetic and acoustic methods are used for levitation.

Microfluidic drops can be moved with light – the lotus effect. On a super-rough surface, when light shines on one side of a drop, the surface changes, the molecules switch and the drop moves. This technology has potential applications in drug screening as it can be used for quickly analyzing and screening small amounts of biological materials. Called digital microfluidics, this approach enables one to quickly move small drops around by shining light on them. Hundreds of screens could be done on only one surface. The molecules, e.g. protein traces, do not interfere with movements of the drops because the surfaces are hydrophobic and the molecules have little contact with the surface.

The size of diamagnetic levitation devices can be reduced by using micron scale permanent magnets to create a magnetic micromanipulation chip, which operates with femtodroplets levitated in air. The droplets used are one billion times smaller in volume than has been demonstrated by conventional methods. The levitated particles can be positioned with up to 300 nm accuracy and precisely rotated and assembled. Using this lab-on-a-chip it might be possible to do the same thing with numerous fluids, chemicals and even red blood cells, bacteria and viruses.

Copyrighted material

### Electrochemical Nanofluid Injection

The ability to manipulate ultrasmall volumes of liquids is required in such diverse fields as cell biology, microfluidics, capillary chromatography, and nanolithography. In cell biology, it is often necessary to inject materials of high molecular weight such as DNA and proteins into living cells because their membranes are impermeable to such molecules. Currently used techniques for microinjection are limited by the relatively large injector size and poor control of the amount of injected material. An electrochemical attosyringe forcontrol of the fluid motion enables dispensing of attoliter-to-picoliter ($10^{-18}$ to $10^{-12}$ liter) volumes of either aqueous or nonaqueous solutions. By changing the voltage applied across the liquid/liquid interface, one can produce a sufficient force to draw solution inside a nanopipette and then inject it into an immobilized biological cell. A high success rate has been achieved for injections of fluorescent dyes into human cells in culture. The injection of femtoliter-range volumes can be monitored by video microscopy, and current/resistance-based approaches can be used to control injections from very small pipettes. Other potential applications of the electrochemical syringe include fluid dispensing in nanolithography and pumping in nanofluidic systems.

### Nanofluidics on Nanopatterned Surfaces

A very thin layer of liquid behaves on a "nanopatterned" silicon surface, i.e. a surface etched with an ordered array of cavities, each only 20 nm deep. Watching how a liquid adsorbs on a nanopatterned surface is one way to study the basic properties of liquids that are confined in extremely tiny amounts within nanoscale structures. Understanding these properties will help in developing many useful fluid-based nanotechnologies. This work could help improve the "lab on a chip". Currently, the knowledge about the microscopic behavior of liquids on solid surfaces, known as "wetting" phenomena, is predominately based on measurements taken using structureless, flat surfaces. In those cases, the behavior of the liquid is based on the strength of attractive molecule-molecule forces known as "van der Waals interactions." But for a surface that contains a regular pattern of cavities, the shape of the surface influences how the liquid will fill those cavities. Analysis of the X-ray data reveals that a liquid layer builds up inside each nanocavity at a faster rate than on a flat surface of the same material. The wetting properties of the surface are considerably enhanced by the nanopatterning.

### Nano-Interface in a Microfluidic Chip

There are emerging experimental and conceptual platforms for probing living cells with nanotechnology-based tools in a microfluidic chip. Considerable advances have been made in measuring nanoscale mechanical, biochemical, and electrical interactions at the interface between biomaterials and living cells. By merging the fields of

Copyrighted material

microfluidics, electrokinetics, and cell biology, microchips can create tiny, mobile laboratories. The challenge for the future of designing a nano-interface in a microfluidic chip to probe a living cell lies in seamlessly integrating techniques into a robust and versatile, yet reliable, platform. Potential benefits of nanosystems on a microchip result from real-time detection of numerous events in parallel. In addition to early detection of cell-level dysfunctions, these systems will enable broad screening that encompasses not just many toxic stimuli and disease processes but also population subgroups. This will facilitate the development of personalized medicine. To reach this goal requires advancing the knowledge base of cellular and subcellular functions, perhaps by designing nanosystems that operate in the tissue milieu.

#### Nanofluidic Channels for Study of DNA

Nanofluidic channels enable molecular biologists to spot the association and dissociation of proteins on fluorescently labeled DNA. The simple system could even help researchers visualize induced tertiary structures such as loops, which push conventional optical or magnetic stretching methods to the limit. This silicon dioxide-glass nanochannel system, also referred to as nanoslit requires no externally applied forces or fields. To unravel the molecules, one places a drop of solution containing DNA at one end of the nanochannel. Capillary action then draws the liquid into channels measuring 2–10 µm wide and 100 nm deep. After 1 min, a drop of buffer solution is added at the other end of the channel to equalize the pressure in the device and stop the flow. In channels of 100 nm depth or less, DNA molecules spontaneously adopt an extended state adjacent to the channel wall. The nanochannel geometry, however, physically confines polymer molecules to two spatial dimensions. Further reduction in configuration results in spontaneous axial stretching of molecules and appears to be electrostatically mediated. The physics for stretching a DNA molecule is built into the structure of the device. Fabrication of the channels and mass production of the unit is easy. Devices are made by first patterning a silicon substrate using laser lithography and then forming parallel channels 100 nm deep by either reactive ion etching in plasma or wet etching in HF. Cover glass is used to seal the channels from above.

## Visualization and Manipulation on Nanoscale

### *3D Single-Molecule Microscopy with Nanoscale Accuracy*

The localization of single fluorescent molecules enables the imaging of molecular structure and dynamics with subdiffraction precision and can be extended to 3D using point spread function (PSF) engineering. Previous calibration techniques for super-resolution microscopy were not sufficiently accurate for 3D measurements of single molecules. The new calibration method uses a nanohole array to correct for

Copyrighted material

optical distortions. Nanoscale accuracy of localization throughout a 3D single-molecule microscope's field of view has now been achieved using regularly spaced subdiffraction apertures filled with fluorescent dyes, which reveal field-dependent aberrations as large as 50–100 nm and show that they can be corrected to <25 nm over an extended 3D focal volume (von Diezmann et al. 2015). This technique can be applied for 2 engineered PSFs, the double-helix PSF and the astigmatic PSF. These results are expected to be broadly applicable to 3D single-molecule tracking and superresolution methods demanding high accuracy. This technique is being used it to study protein localization in bacteria that measure only 2 μ in length. With the 3D calibration technique, it is possible to accurately measure and track key signaling proteins in nanodomains that are only 150–200 nm in size. Tracking how molecules move, form shapes and interact within the body's cells and neurons offers a powerful new view of key biological processes such as signaling, cell division and neuron communication, which impact people's health and susceptibility to disease.

## *4Pi Microscope*

The most prominent restrictions of fluorescence microscopy are the limited resolution and the finite signal. Established conventional, confocal, and multiphoton microscopes resolve at best approximately 200 nm in the focal plane and only 500 nm in depth.

4Pi microscope (Leica Microsystems) uses a special phase- and wavefront-corrected double-objective imaging system linked to a confocal scanner to enable 4 to 7-fold increased axial resolution over confocal and two photon microscope. Even in living specimens, axial sections of ~100 nm are obtained. The system maintains all advantages of fast scanning, Acousto-Optical Beam Splitting (AOBS®) and Spectral Detection of the Leica TCS SP2 AOBS for routine operation. The first marked leap in resolution in commercial 3D fluorescence microscopy opens new dimensions for research in cell and developmental biology. Co-localization studies of immunolabeled microtubules and mitochondria demonstrate the feasibility of 4Pi microscopy for routine biological measurements; particularly, to visualize the 3D entanglement of the two networks with unprecedented detail.

## *Atomic Force Microscopy*

### AFM Basics

In its most basic form, atomic force microscopy (AFM) images topography by precisely scanning a probe across the sample to "feel" the contours of the surface. The interaction between the needle and the surface is measured and an image is

Copyrighted material

# Index


**A**
A 3-compartment model, 371
Abraxane®, 351, 364
Abraxis, 471
Acetazolamide, 508
AcryMed's silver nanoparticle technology, 527
Acticoat™, 231
Active pharmaceutical ingredients (API), 241
Acute myocardial infarction (AMI), 458
Adalimumab (Humira), 541
Adhesive polydimethylsiloxane (aPDMS), 422
Adiponectin plasmid DNA delivery, 467
AFM-based nanorobotic system, 54
Age-related macular degeneration (AMD), 507, 509
Alfa-1 antitrypsin (α1AT), 480
Allosteric control, 116
Alpha fetoprotein (AFP) cancer, 326
Altair's nanotechnology, 242
Alternating magnetic field (AMF) bursts, 390
Alzheimer's disease (AD), 176, 209, 427–428, 447
American National Standards Institute (ANSI), 610
Amifostine, 560
Aminoacyl-tRNA (aa-tRNA), 119
5-Aminolevulinic acid (ALA), 378
Amphiphilic fullerene monomer (AF-1), 228
Amyloid fibrils, 116
Angiogenesis, 150
Angiogenic expansion, 459
Angiogenic factors, 469
Annexin-V-linked nanodots, 330
Anthanide series, 557
Anti-AFP-1, 326
Anti-AFP-2, 326
Antibacterial defense
  nanoscale surface structure, 526–527
Antibiotic resistance
  nanoparticles, 524
Antibody-coated nanocantilevers, 170–171
Anticancer drugs, 105
Anticancer gene therapy, 285
Anticancer siRNA, 298–299
Anti-CD63 antibodies, 322
Antidotes
  poisons, 558–559
Anti-EpCAM polymers grab tumor cells, 325
Anti-epidermal growth factor receptor (anti-EGFR), 325, 403
Anti-epithelial growth factor receptor, 398
Anti-estrogens (AEs), 334
Anti-Flk-1 MAb-NP-90Y complexes, 383
Antifungal agents
  nanoformulations, 524–525
Antigen-delivery system, 278–279
Anti-HER2 antibody, 390
Anti-HER-2 siRNA molecule, 367
Antiinflammatory agents, 557
Antimicrobial agents
  carbon nanotubes, 521
  gold and silver nanoparticles, 521–522
  gold nanoparticles, 522
  nanocarriers, 522–523
  nanoemulsions, 523
Antimicrobial nanoemulsions (NanoBio), 523
Antioxidant effect, 440–441
Antioxidants, 540
Antiproliferative drug, 471
Anti-restenotic therapy, 459, 472


© Springer Science+Business Media LLC 2017
K.K. Jain, *The Handbook of Nanomedicine*, DOI 10.1007/978-1-4939-6966-1



Copyrighted material

Antisense drug delivery, 294
  dendrimers, 294
  DNA and siRNA, 295
  DNA/RNA, 293
  polymer nanoparticles, 294
Antisense nanoparticles, 293
Antisense oligonucleotides (ASO), 347, 348, 368
Antivascular endothelial growth factor, 507
Antiviral agents
  antibodies, 530
  dendritic cell-based immunotherapies, 530
  fullerenes, 530–531
  nanocoating, 531–532
  RNA antiviral therapeutics, 531
  silver nanoparticles, 535
Aphios Corporation, 277
Apoptosis, 79
Aptasensors, 178
Aquaporin (AQP) water channels, 125
Arginine-glycine-aspartic acid, 464
Arsonolipids, 252
Artificial magnetic particles (AMPs), 22
Artificial mechanical red blood cell, 543
Artificial muscles, 311
Atherosclerotic process, 459
Atherothrombosis, 464
Atomic force microscopy (AFM), 33
  advantages, 53
  biomolecular imaging, 86–87
  chromosomal dissection, 140
  FIRAT, 54
  high-density microarrays, 140
  light microscopy and scanning electron microscopy, 53
  microscopic force sensor/cantilever, 53
  nanorobot, 54
  NFP, 139
  proteins, 119
AuNP-BBN peptide, 403
AuNP-oligo probes, 517
AuroLase™ Cancer Therapy, 391
AuroShell™, 391
Autoimmune diseases, 540
Avogadro's number, 558
Aβ formation, 441–442
Aβ proteins, 427
Aβ-derived diffusible ligands (ADDLs), 163

**B**

Bacteria plus nanoparticles, 224
Bacterial cell surface layers, 21
Bacterial magnetic particles (BMPs), 22
Bacteriophage, 30–31
Basic fibroblast growth factor (bFGF), 383
Ben-Gurion University, 430
Bessel-Beam STED (BB-STED), 65
Biobarcode assay, 162–164
Biochips, 133
Biocompatible, 358
Bioconjugated QDs, 143
Biodegradable PEG nanoparticles, 234–235
Biodegradable polymers, 480
BioDelivery Sciences International, 566
Bioinformatics
  3D nano-map, 129–130
  computational biology, 129
  nanobiotechnology systems, 129
Biolistic transfection, 289
Bioluminescence resonance energy transfer QD (BRET-QD), 156
Biomolecular computing, 89–90
Biophan Technologies Inc., 471
Biophan Technologies' drug delivery technology, 472
Biorise™ sytem, 232–233
Biosensing, 75–76
BioSilicon™, 30
Biotinylated IGF-1, 462
Bismuth sulfide (Bi2S3) nanoparticles, 396
Block-copolymer micelles, 342
Blood aqueous barrier, 501
Blood brain barrier (BBB), 428
Blood compatibility, 586
Blood vessels, 423–424
Blood-retinal barrier, 501
Bombesin (BBN) peptides, 402
Bone microcracks, 494
Boron carbide nanoparticles, 385
Boron neutron capture therapy (BNCT), 377, 386
Bovine aortic endothelial cells (BAEC), 80
Bovine serum albumin (BSA), 190
BrachySil™, 382
Brain activity, 423–424
Brain cancer, 448
Brain disorders, 426
Brain imaging, 424
Brain tumors, 432, 434–435
Brownian movement, 104
Brownian nanomachine, 80
Buckminsterfullerene C60 derivatives, 440
Buckyballs (fullerenes), 448, 585
Buckysomes, 251


Copyrighted material

**C**
C3 carboxyfullerene isomer, 556
C32/DT-A nanoparticles, 368
C-60 fullerene, 212, 540
CA isozymes, 508
$CaCO_3$-nanoparticle system, 265
Cadmium selenide (CdSe), 580
Cadmium sulfite, 31
Cadmium Telluride (CdTe), 441
Calando Pharmaceuticals, 298, 357
Calcium phosphate nanoparticles, 282
Calcium sensing, 148
Camposomes™, 336
CAMPTOSAR®, 336
Camptothecin (CPT), 335, 336, 343, 345, 356, 371
Cancer biomedical informatics grid (caBIG), 359
Canine parvovirus (CPV), 354
Cantilever beams, 511
Cantilevers (Concentris), 54, 55
  advantages, 170
  biomolecules, 169
  biosensing, 169
  microorganisms, 170–171
  nanomechanical detection, 169
  protein A-immunoglobulin interactions, 169
  proteomics, 170
  PSA, 170
  SPR, 169
Captamers, 167
Carbon nanodiamonds, 234
Carbon nanofiber (CN), 492
Carbon nanotube biosensors
  DNA and optical detection, 172–173
  ssDNA and electronic readout, 172
Carbon nanotube network field-effect transistors (NTNFETs), 192
Carbon nanotubes (CNTs), 16, 141, 255, 311, 495, 512–513, 577, 583
  applications, 23–24
  protein-coupled receptors, 23
Carbonate apatite nanoparticles, 282
Carbonic anhydrase inhibitors, 508
Carboxyfluorescein, 509
Carboxyfullerenes, 556
Carboxyl-terminated core-shell tectodendrimer clusters, 588
Carboxymethyl chitosan capped gold nanoparticles (CMC-AuNPs), 31
Carcinoembryonic antigen (CEA), 384
Cardiac catheterization, 475
Cardiovascular diagnosis
  in arteries, 459–460
  biomarkers, 457
  blood coagulation, 460
  magnetic NPs, 458–459
  myocardial infarction in saliva, 458
  nanobiosensors, 458
  perfluorocarbon NPs, 459
  in sleep apnea, 459
  SNPs, 457
Cartilage stiffness, 499
Cas9 plasmid systems, 486
Cationic lipids, 437
Cationic nanoparticles, 225
CD20 receptor, 363
CD34 cell membrane, 426
CD4-targeted nanogels, 541
CdSe-core QDs, 580
Celecoxib, 265
Cell therapy, 279
Cell-based cancer gene therapy, 292
Cell-penetrating peptides (CPPs), 224
Cellular level, 210
Centers of Cancer Nanotechnology Excellence (CCNE), 631
Central nervous system (CNS), 421
Ceramic nanoparticles, 226
Ceramide, 337, 348
Ceria nanoparticles, 557
Cerium nanoparticles, 557
Cerulean Nanopharmaceuticals' CRLX101, 371
CFTR chloride channel, 485
Chaperonins, 31
Chelerythrine, 356
Chemiluminescence resonance energy transfer (CRET), 326
ChemoRad NP, 369
Chemoradiation, 369
Chemo-radioprotection, 559–560
Chimeric polypeptides (CPs), 350
Chitosan (CS), 266, 504
Chitosan nanoparticles, 236–237, 356
Chitosan-based NPs, 356
Chitosan-coated nanoparticles, 295
Chitosan-DNA-FAP-B nanoparticles, 486
Chondrocytes, 497
Choroidal neovascularization (CNV) lesions, 507
Chromosomal dissection, 140
Chronic obstructive pulmonary disease (COPD), 487
Chronic subcellular recording, 423

Copyrighted material

Circulating tumor cells (CTCs)
  detection, 323–325
  DNA nanospheres, 323–324
  magnetic nanoparticles, 324
  nano-Velcro technology, 324–325
Cisplatin, 346
Clathrin-coated pits (CCP), 78
Clathrin-mediated endocytosis (CME), 77
Clinical nanodiagnostics, 305–308
CNS degenerative diseases, 212
CNS gene therapy, 436–437
  cationic lipids, 437
  CNTs, 438
  dendrimers, 438
  silica nanoparticles, 436–437
CNS repair, 451
CNT delivery system, 355
CNT size-tunable enrichment microdevice (CNT-STEM), 512
CNT/aPDMS electrode, 422
Cobalt-chromium nanoparticles, 588
Cochleate-mediated DNA delivery, 290–291
Codon-optimized version (CO-CFTR), 486
Coherent X-ray diffraction imaging, 96
Colloidal gold, 358
Colloidal gold particles, 204
Colon capsule endoscopy, 306
Colony forming units (CFU), 521
Colorectal cancer (CRC), 326, 406
Complementary metal oxide semiconductor (CMOS), 185
Complete Detection System, 61
Complex biological systems, 80–81
Computer tomography (CT), 149
Concanavalin A (Con A), 482
Confocal laser scan microscopy, 481
Conjunctiva, 501
Convection-enhanced delivery (CED), 382
Core-cross-linked polymeric micelles (CCL-PMs), 230
Cornea, 501
Cornell dots (C dots), 394
Coroxane™, 471
Cosmeceuticals, 551
Cosmetic Products Regulation 1223/2009 in EU, 599
Cowpea mosaic virus (CPMV), 153
CPV-VP2 capsid protein, 354
C-reactive protein (CRP), 458
Cremophor-based paclitaxel, 343
Crystalline hydroxyapatite, 496
Cubosomes, 15, 548
Curcumin, 338
Cyanoacrylate-co-hexadecyl cyanoacrylate, 505
Cyanobacterial metabolites, 563
Cyclodextrin-based polymers (CDP) conjugates, 371
Cyclodextrins, 226, 357
Cyclosert™, 357
Cyclosporin, 504
Cystic fibrosis (CF), 483
Cystic fibrosis transmembrane conductance regulator (CFTR) gene, 483
CytoViva® Microscope System, 55

**D**

DACH Platin Medicelle™, 343
DACH-platin-PEG-polyglutamic acid, 343
DC-SIGN antibody, 481
DC-SIGN AuNPs, 481
DC-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), 488
Defense Threat Reduction Agency (DTRA), 523
Degenerate Oligonucleotide-Primed Polymerase Chain Reaction (DOP-PCR), 140
Degradable nanoparticles, 281
Dendrimer-RNA nanoparticle vaccines, 274–275
Dendrimers, 211, 282, 502, 509
  applications, 25–26
  core, branching and surface molecules, 24
  physical, chemical and biological properties, 25
  properties, 25
Dendritic photosensitizer (DP), 507
Dendronized polymer, 376
Dental hypersensitivity, 554
DermaVir Patch, 528
DermaVir prime/vaccinia vector, 528
Design molecular transport systems, 259
Dexamethasone (DEX), 230
Dexosomes, 340
Dextran-protamine-DNA-SLN complex, 508
Diacylphospholipid-poly(ethylene glycol) (PE-PEG), 388
Diagnostic magnetic resonance (DMR), 146
1,4-Diaminobutane, 378
4´,6-Diamino-2-phenylindole (DAPI), 388
Dicalcium phosphate anhydrous (DCPA), 555
Dichlorophenamide, 508
Diethylentriaminepentaacetic (DTPA), 578
Digital microfluidics, 49
Dimercaptosuccinic acid-modified iron oxide magnetic nanoparticles, 364
Dip Pen Nanolithography™ (DPN™), 43, 44

Copyrighted material

DNA detection method, 164–165
DNA double-strand breaks, 87
DNA fragments, 105
DNA nanoparticles, 284
DNA nanostructures
advantages, 27
anticancer drugs/signaling molecules, 27
octahedron, 27
DNA nanotags, 36
DNA octahedron, 27
DNA origami, 107, 411
DNA vaccines, 275
DNA-assembled dendrimers, 228
DNA-nanoparticle conjugates, 30
DNA-protein and -nanoparticle conjugation, 158–159
Dopaminergic neurons, 296
Doppler vibrometer, 512
Double-stranded DNA (dsDNA), 106
DOXIL®, 364
DOX-ND complex, 367
Doxorubicin (DOX), 321, 337, 338, 341, 344, 345, 348–350, 354–356, 362, 364, 366, 372–374, 377, 381, 401, 405, 407–409, 411, 412
Doxorubicin nanocarriers (DNC), 337
Doxorubicin-encapsulated DOTAP/DOPE nanoparticles, 354
Drug delivery depots, 220–221
Drug delivery systems, 221
Drug design, 210
Drug discovery, 130, 201–211
bacteria, 223
biosensor, 207
CCL-PMs, 230
coated nanoparticles, 238
dendrimers, 227–228
Filomicelles *vs*. spherical nanoparticles, 240
gold nanoparticles, 203
lipoparticles, 206–207
magnetic nanoparticles, 241
nanobiotechnology, 202, 210–211, 217–221
nanofluidic devices, 202
nanomotors, 268
nanoparticles, 225
nanosponges, 268
polymeric micelles, 230
properties of material, 217
QDs, 204–206, 237–238
TREC, 208
viruses, 221
Drug molecules in cells, 203–204
Drug receptors, 206
Drug-free organ transplants, 313–314
dsDNA-nanoshells, 388
Duchenne muscular dystrophy (DMD), 294
DuPont scientists, 583
Dynamic light scattering (DLS), 14

**E**
EAK16-II, 243
Ebola virus infection, 535–536
Elan's NanoCrystal technology, 232
Elastin-like polypeptides (ELPs), 313
Electrochemical nanobiosensor, 174
Electrochemical nanofluid injection, 50
Electron microscopy, 77
ElectroNanoSpray™ formulation technology, 474
Electronic field effect transistor (FET), 175
Electronic nanobiosensors, 175
Electrophoresis, 106
Electrospinning, 466
Electrospinning technology, 310
Electrospray ionization (ESI), 111
Electrospun nanofiber tubes, 448
Encapsulating water-insoluble drugs, 239
Endocytosis, 77–78
Endosomolytic and cleavable polymers, 298
EnGeneIC Delivery Vehicle, 344
EnGeneIC's Cerebral EDV™, 345
Engineered Water Nanostructures (EWNS), 596
Enhanced permeability and retention (EPR), 364, 380, 381, 391
Environmental Protection Agency, 593
Enzyme-linked immunoassays (ELISA), 163
EpCAM cell, 325
Epichlorohydrin, 509
Epidermal growth factor (EGF)-QDs, 91
Epidermal growth factor receptor (EGFR), 329
Epigallocatechin-gallate (EGCg), 359
Epithelial-mesenchymal transition (EMT), 383
Erlotinib, 401
Erythroid precursor cells, 543
*Escherichia coli O157:H7*, 516
Etanercept (Enbrel), 541
Ethical, legal and social implications (ELSI), 607
Ethosomes, 262
Ethoxzolamide, 508
Eu3+ doped lanthanum calcium manganate (LECMO), 563
Eudragit nanoparticles, 505
Eudragit NPs, 509

Copyrighted material

International Life Sciences Institute Research Foundation/Risk Science Institute, 590
Intervertebral disc (IVD), 498
Intestinal epithelial cells (IECs), 545
Intracellular delivery, 238, 287
Intracranial U87 glioma xenograft model, 382
Intravenous gene delivery, 432–433
Inverse emulsion photopolymerization, 239
Ion channel switch biosensor technology, 174
Ion channels
  AFM, 125
  AQP, 125
  magnetic-field heating, nanoparticles, 126–127
  nanobiotechnology, 127–128
  Nanopatch™, 126
  patch clamp technique, 125
Iron oxide (α-$Fe_2O_3$) nanoparticles, 564
Iron oxide nanoparticles (IONPs), 21, 341
Iron oxide-titanium dioxide ($TiO_2$), 337
Iron-rich SWCNT, 578

**J**
Jet-based technologies, 310

**K**
Keratinocyte growth factor (KGF), 547
Kereos Inc's emulsion particles, 370
Knob protein domain, 512

**L**
Lab-on-Bead, 209–210
Laboratories-on-a-chip, 209
Lactolycopene, 566
Laminin67R receptors, 360
Langendorff heart, 586
Lanthanum calcium manganate (LCMO), 563
Laser Doppler vibrometer, 171
Laser light antennas, 103
Laser nanosensors, 180
Layered double hydroxides (LDHs), 229
Life sciences, 73
Light-activated ion channel molecular machines, 86
Lipid bilayers, 246
Lipid emulsions, 249–250
Lipid nanocapsules (LNC), 248, 264
Lipid nanoparticles (LNPs), 246, 248, 286–287, 301–302, 504
Lipid surfactant polymerization, 459
Lipid-based nanoparticles, 587
Lipid-protein nanotubes, 255–256
LiPlasomes, 342
LipoBridge™, 432
Lipofection, 486
Lipoparticle technology, 206
Lipoparticles, 14
Lipophilic amino-acid dendrimer, 507
Lipophilic drugs, 242
Liposomal nanodevice surface, 464
Liposomal nanoparticle containing minimally modified raf antisense oligodeoxynucleotide (LErafAON), 368
Liposome properties, 245
Liposome-nanoparticle hybrids, 252–253
Liposomes incorporating fullerenes, 251–252
Liquid chromatography (LC), 113
Liquid crystals, 15
LNP technology
  advantages, 247
Low density lipoprotein (LDL) particles, 431
Low molecular weight heparin (LMWH) nanoparticles, 465
Low-density lipoproteins (LDLs), 468
Luminescent silicon dioxide nanoparticles, 40

**M**
MAb-guided bioprobes, 390
Macroemulsion, 559
Macrophages, 425
Macroscopic valve, 267
Magnetic nanoparticles (MNPs), 17, 287, 324, 355, 361, 458
  assays, 207
  bioscreening, 145–146
  NSCs, 146
  perfluorocarbon, 146–147
  SPIONs, 147, 149
Magnetic nanotags (MNTs), 36–37
Magnetic resonance force microscopy (MRFM), 57–58
Magnetic resonance molecular imaging, 460
Magnetic thermal ablation, 389
Magnetoacoustic, 327
Magnetomotive optical coherence tomography (MM-OCT), 155
Magnetotactic bacterium (MTB), 411
Malondialdehyde (MDA), 412
Malonic acid C60 derivatives, 556
Mammalian visual system, 451
Manganese oxide nanoparticles, 153
Mannodendrimers, 488
Mass spectrometry (MS), 121–122
Mass Transit Railway (MTR), 532
Mass-based flow cytometry, 77

Copyrighted material

MDA MB231 breast tumors, 409
Mechanical flexion, 262
Melittin, 362
Membrane proteins
  nanoparticles, 122
  QD, 123
  single protein interaction, 122–123
  subnanometer resolution, 123
Mesenchymal stem cells (MSCs), 79, 124, 497
Mesoporous silica nanoparticles (MSNs), 362
Messenger RNA (mRNA), 120, 301
Metallic nanobiosensors, 176
Metastatic tumor cells, 327
Meta-tetra(hydroxyphenyl) chlorin (mTHPC), 339
Methazolamide, 508
Methotrexate (MTX), 350
Methotrexate-loaded lipid-core nanocapsules (MTX-LNCs), 542
Micelles, 17–18, 433
Micro- and nano-electromechanical systems
  BioMEMS, 42
  cantilever systems, 42
  MEMS, 41
  NEMS, 42
  surface nanomachining, 42
  ultra-short pulsed-laser radiation, 42
Micro ElectroMechanical Systems (MEMS), 41
Micro total analysis systems, 74
Microarrays, 133. *See also* Nanoarrays
Microbes, 90
Microbial biofabrication, 216
Microcystin, 563
microelectrodes, 421
Microfabrication, 511
Microfluidic chip, 50–51
Microfluidic devices, 308
Microfluidic paper-based lateral flow assay, 460
Microfluidic systems, 209
Microfluidics, 572
  applications, 46
  biochip technology, 45
  drawbacks and limitations, 46
  nanoliter volumes, 47
  nanotechnology, 47–51
  nanotechnology on a chip, 46–47
Micronization, 217
Microscaffolds, 469
Microsurgery, 11
Microvilli, 324
Microwave plasma technique, 15
Mineral UV-filter, 619
Miniature devices, 209
Miniaturization, 315
Minimally invasive surgery, 316
Misfolded proteins, 114
1-μm magnetic microparticle (MMP), 162
Mitochondrial DNA (mtDNA)
  nanolaser spectroscopy, 109
  nanomaterials, 108–109
MIV's HAp coating, 474
Molecular assembly, 97
Molecular computational identification, 37
Molecular diagnosis, 133
Molecular imaging, 424
Molecular medicine, 7
Molecular motors
  actin and myosin, 83
  biological materials and synthetic devices, 84
  cellular organization, 83
  dynein, 83
  FIONA, 82
  long-distance truckers, 83
  maintenance, 82
  mechanical force, 83
  microrotary motor, 82
  nanometer-scaled conformational changes, 82
  nucleic acids, 84
  optical tweezers, 83
  phi29 DNA packaging, 85
  self-assembly, 82
Monoclonal antibodies (MAbs), 79, 273
Monte Carlo simulations, 571
$MoO_3$ nanoparticles, 577
MRI technology, 425
MTX-PSi conjugates, 350
Mucosal drug delivery, 266–267
Mucus barrier, 234–235
Multi Photon Detection (MPD), 112–113
Multidrug resistance (MDR), 400
Multifunctional DNA carriers (MDCs), 288
Multifunctional nanoparticles (MFNPs), 426, 433, 438
Multi-isotope imaging mass spectrometry (MIMS), 88–89
Multiphoton absorption induced luminescence (MAIL), 203
Multiple-allergen testing, 44
Multispectral optoacoustic tomography (MSOT), 395
Multivalent drug design, 208
Multiwalled carbon nanotubes (MWCNTs), 150
Murine 4T1 breast cancer model, 355
Murine cancer model, 350
Myasthenia gravis, 130
Mycobacterium, 517
*Mycobacterium avium* spp. paratuberculosis (MAP), 148

Copyrighted material

*Mycoplasma mobile*, 82
Myeloid-derived suppressor cells (MDSC), 409
Myocardial infarction (MI), 155
Myocardial ischemia, 462
Myosins, 83

**N**
Nab-paclitaxel, 347, 626
Nab-paclitaxel formulation, 364
Nanamedicines manufacturing, 216
Nano hydroxyapatite (HA), 493
Nano Silver-Titanium Dioxide Coating (NSTDC), 532
Nano2Life, 633, 634
NANOANTENNA project, 194
NanoArc® zinc oxide, 549
Nanoarrays, 133
  DPN™, 43–44
  fullerene photodetectors, 135–136
  genomic arrays, 43
  lithography strategies, 43
  protein, 44–45, 138
  protein nanobiochip, 138
  silver nanorod array, 139
  single cell analysis, 136, 137
  single molecule, DNA, 136–137
  ultrahigh density arrays, 43
Nanobacteria
  and kidney stone formation, 520
  cardiovascular disease, 520–521
  human diseases, 518–521
  nature of, 519
Nanobarcodes technology
  advantages, 160
  biobarcode assay, 162–164
  multiplexed biodetection, 160
  multiplexed gene expression profiling, 161
  single-molecule barcoding system, 164
  SNP genotyping, 160–161
Nanobees, 362
Nanobialys, 405
NanoBio Corporation's nanoemulsions, 523
Nanobiochip platform, 458
Nano-biocomposites, 280
Nanobiofabrication, 310
Nanobiolistics, 289
Nanobiology, 75–82
Nanobiopharmaceuticals, 5
Nanobiosensors, 458
  breath testing, 176
  cantilevers, 169–171
  carbon nanotube biosensors, 171–173
  cocaine molecules, 178
  electrochemical nanobiosensor, 174–175
  electronic nanobiosensors, 175
  FRET-based DNA nanosensor, 173
  glucose monitoring, 178–179
  ion channel switch, 174
  metallic nanobiosensors, 176
  PEBBLE, 178
  protein detection, 179
  Quartz nanobalance biosensor, 177
  Raman spectroscopy, 174
  viral nanosensor, 177
Nanobiotechnologies, 201–211, 218, 262, 308–313, 332, 334–381, 421–424, 439–442, 541–543, 560–567
  adult stem cells, 308
  aptamer-nanoparticle, 322
  attogram, 2
  bacterial cell surface layers, 21–22
  biodefense
    blood, 561–562
    microbial warfare agents, 560–561
  biologically entities, brain, 3
  BMPs, 22–23
  carbon nanotubes, 23–24
  classification
    cubosomes, 15
    fluorescent nanoparticles, 15
    fullerenes, 16
    gold nanoparticles, 14
    graphene, 16
    HDL-NPs, 21
    HNPs, 21
    MNPs, 17
    nanoparticles assembly, micelles, 17–18
    nanoshells, 18
    NPs, 13–21
    plant-derived nanoparticles, 19
    polymer nanoparticles, 19
    PSi NPs, 19
    QDs, 20–21
  dendrimers, 24–26, 323
  detection of cancer, 321
  disorders of aging, 550–551
  drug delivery in cancer
    albumin and antisense oligonucleotides, 347–348
    anticancer therapeutics, 352–376
    antisense oligonucleotides, 368
    aptamer nanoformulations, 353–354
    bacteriophage capsid-based nanoparticles, 354
    boron neutron capture therapy, 377–378
    canine parvovirus, 354–355
    carbon magnetic nanoparticles, 355–356

Copyrighted material

carbon nanotubes, 355
cerasomes, 337
chitosan nanoparticles, 356
classification, 334
concurrent chemoradiation, 369
CRLX101, 356
curcumin nanoformulation, 338–339
cyclosert system, 357
dendrimers, 376–379
doxorubicin nanocarriers, 337–338
exosomes, 340
folate-linked nanoparticles, 340–341
fullerenes, 357
ginger nanoparticles, 341
gold nanoparticles, 341, 358–360, 376
hepatic artery infusion, 360
hyaluronic acid nanocarriers, 360–361
in hydrogel nanoparticles, 339–340
iron oxide nanoparticles, 341–342
lipid based nanocarriers, 342
liposomes, 334–336
lymphatic system, 374–375
macrophages, 364–365
magnetic nanocrystals, 374
magnetic nanoparticles, 361–362
MAPK signaling pathway, 367–368
metastatic cancer, 369
micelles, 342–344
minicells, 344–345
monoclonal antibodies, 363–364
MSNs, 362
multiple anticancer agents, 370
nanobees, 362–363
nanobody-shell polymeric micelles, 363
nanocell, 366
nanoconjugates, 345
nanodiamonds, 366–367
nanomaterials, 345–346
nanoparticle, 346
nanoparticle penetration, 381
nanoparticle-aptamer bioconjugates, 375
nanoparticle-peptide conjugates, 362
nanostructured hyaluronic acid, 370
nanovehicles, 365–366
niosomes, 348
paclitaxel, 346–347
pegylated nanoliposomal formulation, 348–349
peptide-linked nanoparticle delivery, 349
perfluorocarbon emulsion, 370–371
photodynamic therapy, 378–379
poly-2-hydroxyethyl methacrylate nanoparticles, 349–350
poly-L-lysine dendrimer, 379
polymer nanoparticles, 371–372
polymersomes, 372–373
polypeptide-doxorubicin conjugated nanoparticles, 350
porous silicon nanoparticles, 350
prostate tumors, 368–369
protosphere nanoparticle technology, 351
quantum dots and rods, 373–374
RNA nanotechnology, 379–380
siRNA, 367
siRNA delivery, 351–352
siRNAs, 380
synthetic vector system, 379
zinc oxide nanoparticles, 352
DNA nanostructures, 26–27
dual-mode nanoparticle, 330
early detection of cancer, 333–334
EEG recording, 422
exosomes, 322
gold nanoparticles, 325–326, 424
gold nanorods, 327
implanted biosensor, 328
inflammatory diseases
cell adhesion molecules, 541
chronic inflammatory conditions, 541
rheumatoid arthritis, 541–543
in malignant tumors, 333
nanobiochip sensor technique, 329
nanodots, 329–330
nanolaser spectroscopy, 330
nanomaterials, 3–4
nanometers, 2
nanoparticle conjugates, 30–31
nanopores, 28
nanoporous silica aerogel, 29
nanoscale, 2
nanoscale devices, 422–423
nanosensors, 327–328
nanostructured silicon, 30
nanotubes, 328–333
nanowires, 28
and nutrition
and food industry, 565–566
biomolecular interactions, 565
in personalized nutrition, 566–567
polymer nanofibers, 32
S-layers, 22
VLPs, 32
public health
water purification, 562–564
QDs (*see* Quantum dots (QD))
single molecule assays, 331
subcellular recording, 423
tissue engineering, 308

Copyrighted material

Nanobiotechnology applications, 442
Nanobiotechnology solutions, 218–219
Nanobiotechnology-based artificial retina, 446–447
Nanobiotechnology-based devices, 446–447
Nanobodies (Nbs), 213–215
Nanobombs, 399
NanoBones, 493
Nanocardiology, 457–470, 475
  in biodegradable nanoparticles, 472
  in cardiac catheterization
    EKG sensor monitors, 475
    epicardium, 475
    nanomaterials research, 475
    transendocardial delivery, 475
  cardiovascular diseases, 457
    cardiac arrest, 468
    nanolipoblockers, 468
  coronary stent, 470
  magnetic nanoparticle, 472
  magnetic nanoparticle-coated DES, 472
  nanocoated DES, 473–474
  nanopores, 474
  nanotechnology-based cardiovascular diagnosis (*see* Cardiovascular diagnosis)
  paclitaxel, 472
  regeneration
    cardiac revascularization, 469–470
    microscaffolds, 469
    nanocomposite hydrogels, 470
    nanoscaffolds, 469
  restenosis after percutaneous coronary angioplasty, 471–474
  therapeutic delivery
    diagnostics, 461
    IGF-1 delivery by nanofibers, 462
    injectable peptide nanofibers, 462–464
    liposomal nanodevices, 464
    LMWH-loaded nanoparticles, 465
    MagBICE, 465
    nanofiber-based scaffolds, 466
    nanoparticles, 465–466
    NP-based systemic drug delivery, 467
    targeted nanoparticle-DNA delivery, 467
    vascular injury, 462
Nanocarrier-based delivery, 472
Nanocarrier-mediated siRNA delivery
  HIV/AIDS, 534
Nanocarriers, 502, 522
Nanocell, 366
Nanochemistry
  chemical synthesis approach, 98
  nanocrystallization process, 98
  nanoscale pH Meter, 98–99
Nanochips, 133, 260–261
Nanocoat™ process, 258
Nanocochleates, 257
Nanocomposite membranes, 230
Nanocomposites, 219
Nanocosmeceuticals, 551
NanoCrystal®, 232
Nanocrystalline, 550
Nanocrystalline silver, 231
Nanocrystals (NCs), 145
Nanocurcumin, 339
NanoCyte drug delivery system, 263
Nanocytometry, 158
Nanodentistry
  bonding materials, 553
  dental caries, 553–554
  description, 553
  nanodiamonds, 555–556
  nanomaterials, 554–555
  nanospheres, 554
Nanodiagnosis, 511–518
  bacterial infection
    nanoparticle-based methods, 516–517
    at POC. Limitations, 516
    QDs, 517
  fungal infections
    clinical microbiology laboratory, 518
    magnetic nanoparticle-based technique, 518
    nano-amplification technique, 518
  viral infection
    cantilever beams, 511–512
    carbon nanotubes-based detection, 512–513
    electric fields, 513
    nanotechnology-based methods, 511
    QD fluorescent probes, 514–515
    surface enhanced Raman scattering, 515–516
    verigene respiratory virus plus assay, 515
Nanodiagnostics, 425, 569
  applications, 134–135
  biochip technology, 197
  biomarkers, 134, 187–188
  blood protein analysis, 197
  clinical trials, 196–197
  components, 195
  molecular electronics and nanoscale chemical sensors, 197
  single molecule analysis approach, 195
Nanodiamonds (NDs), 234, 366, 367, 555
Nanoelectrodes, 421
Nano-electromechanical systems (NEMS), 42
Nanoelectronics, 572
Nanoelectroporation, 99–100


Copyrighted material

Nanophosphor
advantages, 38
DNA segments, 38
europium/terbium, 37
microparticles, 37
NIR-to-NIR up-conversion process, 38
QDs, 37
Nanopolymers, 19
Nanopore membrane, 259
Nanopore technology
nanocytometry, 158
single DNA molecules, 157–158
Nanopore-based protein sequencing, 114
Nanopore-based sequencing, 107
Nanopores, 28
Nanoporous materials, 259–260
Nanoporous silica aerogels
advantages, 29
lipid bilayers, 29
Nanoporous structure, 474
Nanoprobes, 152
Nanoproteomics, 109–124
atomic force microscopy, 119
dynamic reassembly, peptides, 111
FAIMS, 111–112
gene expression, 118
membrane proteins, 122–123
misfolded proteins, 114–115
MPD, 112–113
nanofilter array chip, 111
nanoflow LC, 113
nanoparticle-protein interactions, 113–114
nanopore-based protein sequencing, 114
nanotube electronic biosensor, 115
phosphoprotein analysis, 114
protein engineering, 116–118
protein nanocrystallography, 115–116
PWG, 110
QD-protein nanoassembly, 118
redox systems, 112
single molecule level, 119–122
3D cell culture, 124
Nanopulmonology
airway epithelium in vivo, 479
Nanoribbons, 28
Nanorobotics, 316–318
Nanorod gene therapy, 291
Nanorods, 403
Nanoscaffolds, 469
Nanoscale
AFM, 52–54
cantilever technology, 54–56
CytoViva® Microscope System, 55–56
FRET, 56–57
FUEL, 57
HRTEM, 67
MRFM and MRI, 57–58
multiple single-molecule fluorescence microscopy, 58
nanosight LM10 technology, 59–60
nano-sized light source, 59
NSOM, 58–59
optical imaging, silver superlens, 61–62
PALM, 62–63
4Pi microscope, 52
PWS, 62
SEM, 60–61
single-molecule PLM, 64
SPM, 63–64
SPR, 68
STED microscopy, 64–65
super-resolution microscopy, in vivo cell imaging, 65–66
3D single-molecule microscopy, 51–52
time and light, 4–5
UNCD, 67
Nanoscale bactericidal powders, 525
Nanoscale devices, 209–210
Nanoscale devices delivery, 218
Nanoscale DNA imaging, 88
Nanoscale flow visualization, 49
Nanoscale laser surgery, 318
Nanoscale pH meter, 98, 99
Nanoscale resolution, 140
Nanoscale surfaces, 308–309
Nanoscavengers, 564
Nanosecond (ns), 4
Nanosecond pulsed electric fields (nsPEFs), 4, 385
Nanoshell biosensors, 181
Nanoshells, 18, 384, 390
Nanosight LM10 technology, 59, 60
Nanosize liposomes, 295–296
Nano-sized light source, 59
Nanosomal Amphotericin B, 524
Nanospheres, 254, 554
NanOss, 614
Nanostructured arrays, 422
Nanostructured organogels, 250–251
Nanostructured silicon, 30
Nanosurgery, 315–316
Nanosuspension, 561
Nanosuspension formulations, 219
NanoSystems biology, 74

Copyrighted material

Nanotechnology
AFM, 91
and homeopathic medicines, 558
cosmeceutical, 551
cubosomes, 548
DNA, nanofluidic channels, 51
electrochemical nanofluid injection, 50
GEPIs, 95–96
hair care, 552
levitation, nanofluidic drops, 49
nanocosmeceuticals, 551–552
nanoengineered bionic skin, 550
nanofluidic channels, 48–49
nano-interface, microfluidic chip, 50–51
nanoparticle-based sun screens, 549
nanoparticles, 548–549
nanopatterned surfaces, 50
nanoscale flow visualization, 49
nanoscale materials, 598
public perceptions, 597–598
QDs, 91–93
RNA, 94–95
scanning probe instruments, 91
structural DNA, 93–94
topical nanocreams, 550
2D nanofluidics, 48
wound healing, 547
Nanotechnology Characterization Laboratory (NCL), 610, 629, 631
NanoTechnology Interest Group (NTIG), 616
Nanotechnology on a chip, 46, 47
Nanotechnology-based cancer therapy
aptamer conjugated magnetic nanoparticles, 401–402
bacterial nanorobots, 411
biomimetic nanoparticles, 402
in cancer immunology, 409–410
CED with nanoliposomal CPT-11, 382
chemotherapy-induced cardiotoxicity, 411–412
dendrimer nanoparticles, 402
DNA robots, 411
gold nanoparticle, 402–403
gold nanorods, 403
gold nanotubes, 403–404
magnetic nanoparticles, 404–405
micelles, 405
MRI and thermal ablation, 406
nanobialys, 405–406
nanobombs, 399
nanocarriers with TGF-β inhibitors, 399
nanoengineered silicon, 382
nanoparticles
antiangiogenic therapy, 383
boron neutron capture therapy, 385–386
Cornell dots, 394
CT scan, 396
cytotoxic effects, 383
gold nanoparticles, 383–384
laser-induced cancer destruction, 387–389
in management, 393–398
MDR, 400–401
MRI, 395–396
nanobody-based cancer therapy, 384
nanomedicines, 393
nanoparticles and optoacoustic imaging, 395
nanosensor device, 396
nanoshell-based cancer therapy, 384
nsPEFs, 385
in oncology clinical trials, 397
QDs aid lymph node, 397
radiation therapy, 386–387
single wall carbon nanotubes, 398
targeted therapy, 398
temperature-sensitive liposomes, 392
thermal ablation, 389–392
ultrasound radiation, 393
nanorobotics, 410–411
pHLIP nanotechnology, 406–407
QD conjugates, 407
radiolabeled carbon nanotubes, 408
silica nanoparticles, 407
squalene-based nanocomposites, 407–408
targeted chemotherapy, 408–409
time-delayed, dual-drug nanoparticle delivery system, 400–401
ultrasonic tumor imaging, 408–409
Nanotechnology-based drug delivery, 427–428
Nanotechnology-based live-cell single molecule assays, 78
Nanotoxicology, 576, 577, 581–585, 588
blood compatibility, 586–587
carbon nanotube safety, 577–579
carbon nanotubes, 589–590
CBEN, 594
cytotoxicity
indirect DNA damage, 588
individual cells, 588
poly(amidoamine) dendrimers cause, 588
environmental safety, aerosols, 592
European NEST project, 594
foodborne microorganisms, 596–597
fullerene toxicity, 579
GA-AuNPs, 589
gold nanoparticle, 589
gold nanoparticle toxicity, 579–580
graphene toxicity, 580

Copyrighted material

hazard identification, 590
human health, 575
human health and environment, 591
in human body, 576
in neuronanotoxicology (*see* Neuronanotoxicology)
in vitro testing
exposure and dispersion methods, 576
nanomaterials, 576
stem cell lines, 577
magnetite pollution nanoparticles on human brain, 591–592
mother and fetus, 587–588
nano-engineered particles, 575
nanomaterials, 575
nanoparticles
pulmonary effects, 582–584
spherical-shaped gold nanoparticles, 581
nanoparticles and food safety, 594–597
particle's toxicity, 575
QD safety issues, 580–581
regulatory viewpoint on nanoparticles, 596
safety issues, nanoparticles, 591
titanium dioxide nanoparticles, 595–596
US government agencies in research, 593
Nanotube-neuron electronic interface, 442–443
Nanotubes, 254–257
bacteria, 525–526
Nanovalves, 260
Nano-Velcro technology, 324
Nanovesicles, 430
Nanoviricides, 532–534
NanoViricide™ micelle, 532
Nanowire biosensors
biological diagnostic applications, 184
biowarfare agents, 186
cancer biomarkers, 185
development, 187
genetic disorders, 186
genetic screening and drug discovery, 186
single viruses, 185
SiNWs, 184
T lymphocyte activation, 184
Nanowires, 28, 423–424
Nasal drug delivery, 265–266
National Cancer Institute (NCI)
in cancer, 630–632
National Institute of Standards and Technology, 617
National Institutes of Health (NIH), 629
National Nanotechnology Initiative (NNI), 1, 611
Natural nanocomposites, 90
ND-embedded GP (NDGP), 555
Near Infra Red (NIR)-emitting QDs, 118
Near infrared (NIR) laser microscopy, 91
Near infrared to near infrared (NIR-to-NIR) up-conversion, 38
Near-field scanning optical microscopy (NSOM), 58
Near-infrared (NIR)-emissive polymersomes, 166–167
Neointimal formation, 471
Neural stem cells (NSCs), 146
Neural systems, 73
Neuroinflammation, 441
Neurological disorders, 427
Neurology, 421
NeuroNano, 585
NeuroNano project, 585
Neuronanotoxicology, 584–586
nanoparticles
in brain, 584–585
and neurodegeneration, 585
on heart, 586
Neurophysiological studies, 421–424
Neurophysiology, 421
Neuroprotection, 439–442
Neuroprotective nanoparticles, 441
New and emerging science and technologies (NEST), 594
Next-generation sequencing (NGS), 512
N-fluorenylmethoxycarbonyl-diphenylalanine (Fmoc-FF), 349
NF-κB signaling pathway, 299
NF-κB siRNA nanotherapy, 500
Niosomes, 251
NIR-triggered cell death, 387
Nitrogen-doped multiwalled CNTs (N-MWCNT), 512
Nitrogen-vacancy (NV), 96
Noncardiac diseases, 467
Non-invasive optical nanoscopy, 66
Nontoxic pegylated gold nanoparticles, 358
Nonviral vectors, 530
NP delivery, 434–435
NP-based targeted delivery, 435
Nuclear factor kappa B (NFkappaB) activation, 338
Nuclear magnetic resonance (NMR), 96, 146
Nucleic acid delivery, 289
Nucleic acids, 84, 287
Nucleoprotein machines, 632–633


**O**


Oak Ridge National Laboratory (ORNL), 550
OA-pluronic-coated iron oxide magnetic nanoparticle, 341
Ocuflur™, 504


Copyrighted material

Ocular drug delivery, 501–505
Office of Science and Health Coordination (OC/OSHC), 617
Olavi Kajander group, 519
Oleic acid (OA)-water, 341
Oligodeoxynucleotides (ODNs), 220
Oligonucleotide, 507
Optical biosensors, 180–183
Optical mRNA biosensors, 182–183
Optical nanoscopy, 60
Optical trapping, 97
OPTISOL™ technology, 549
Optoelectronic tweezers (OETs), 102, 103
Optofluidics, 103
Oral vaccines, 277
Oregon Health & Science University, 450
Organ assisting devices (OAD), 314
Organ replacement, 313–315
Organic Light Emmitting Display (OLED), 183
Organic nonpolymeric nanoparticles, 39
Organically modified silica (ORMOSIL) nanoparticles, 389, 436
Organogenesis Inc., 310
Orthopedic implants, 492
Osteoarthritis (OA), 499
Osteoblasts, 492, 496
Osteoporosis, 495
Oxidative stress
  antioxidant nanoparticles, 558
  antioxidants, 556
  ceria nanoparticles, 557
  fullerene-based antioxidants, 556–557
  reactive oxygen species, 556

**P**

p53 gene therapy, 288
Packaging RNA (pRNA), 85
Paclitaxel, 346
Paclitaxel micelle, 342
Paclitaxel-loaded nanoparticles, 466
Pain therapeutics, 451–452
Paramagnetic particles, 17
Parkinson's disease (PD), 176, 423, 447, 585
Partial wave spectroscopy (PWS), 62
Particle Replication in Nonwetting Templates (PRINT), 238
PC-3 xenograft SCID mice, 360
PC-SPION, 426
PD98059-nanoparticles, 368
PDGFR β-specific tyrphostin, 472
PEG 2000-paclitaxel conjugate, 344
PEG-based block copolymer, 471
PEG-b-poly[N-(2-hydroxypropyl) methacrylamide-lactate], 363
PEG-coated biodegradable nanoparticles, 340
PEG-coated QDs, 397
PEG-functionalized hydrophilic carbon cluster carbon (PEG-HCCs), 441
PEG-PLA micelle, 344
PEGylated gold nanoparticles, 359
Pentium silicon chip, 74
Peptide amphiphiles, 469
Peptide immobilized gold nanoparticles (AuNPs), 522
Peptide nanostructures, 444
Peptide nucleic acid (PNA), 106, 186, 209
Peptide–siRNA platform, 500
Perfluorocarbon (PFC), 362
Perfluorocarbon (PFC) nanoparticles, 146–147, 242, 459
Perfluoropentane (PFP), 408
Peroxyoxalate based chemiluminescence reaction (PO-CL), 136
Personal Care Products Council, 600
Personalized medicine
  AFM, 572
  biochemical properties, 573
  cancer, 570–571
  cardiology, 571–572
  nanobiotechnology, 569
  therapeutics delivery, 572
PET-optical imaging, 394
P-glycoprotein (Pgp) inhibition, 431
pH Low Insertion Peptide (pHLIP), 386
Pharmaceuticals™, 257
phi29 DNA packaging nanomotor, 85
Phospholipid liposomes, 246
Phosphoprotein analysis, 114
Photoactivated localization microscopy (PALM), 62–63
Photocatalytic disinfection, 564
Photo-controlled in vivo activation, 289
Photodynamic therapy (PDT), 69, 378, 507
Photosensitizer protoporphyrin IX (PIX), 378
Phototherapy, 69
Photothermal therapy, 288
Phytoceutical GLY, 560
PI3K/Akt signaling, 464
PI3K-targeted therapeutic, 393
Pigment epithelium-derived factor (PEDF), 505
PillCam® capsule endoscope, 306
4Pi microscope (Leica Microsystems), 52, 87
PLA2 hydrolysis products, 342
Planar lipid bilayer (PLB), 126
Plant-derived nanoparticles, 19

Copyrighted material

Plasma-modified ultralong CNTs, 563
Plasmid DNA (pDNA) liposome complexes, 487
Plasmid molecules, 292
Plasmon resonance, 326
Plasmon-resonant gold nanorods, 387
Platelet-derived growth factor (PDGF)-BB signaling, 462
PLGA nanoparticles, 440
PLGA-based nanodelivery technologies, 235–236
Pluronic F127, 560
Point spread function (PSF), 51
Point-of-care (POC)
  assays, 141
  battle field and biodefense, 193–194
  carbon nanotube transistors, 192
  CD4 T-cell count, 192
  nanobiosensors, 193
  nanotechnology-based biochips, 192
Poly(amidoamine) (PAMAM) dendrimers, 323
Poly(D, L-lactide-co-glycolide) nanoparticle, 369
Poly(d,l-lactic-co-glycolic acid)-b-poly(l-histidine)-b-poly(ethylene glycol) (PLGA-PLH-PEG) nanoparticles, 524
Poly(dimethyl-siloxane) (PDMS), 136
Poly(epsilon-caprolactone) (PEO-PCL) nanoparticles, 400
Poly(ethylene glycol) shell, 468
Poly(ethylene glycol)-functionalized hydrophilic carbon clusters (PEG-HCCs), 540
Poly(lactic acid)-block-polyethylene glycol (PLA-PEG), 375
Poly(lactic-co-glycolic acid) nanoparticles, 388
Poly-2-hydroxyethyl methacrylate nanoparticles, 349
Polyacrylamide, 339
Polyamidoamine (PAMAM) dendrimer, 583
Polycaprolactone nanocapsules, 467
Polydopamine-coated gold nanocages (AuNC@PDA), 522
Polyethylene glycol-doxorubicin conjugate (PEG-DXR), 355
Polyethyleneimine (PEI), 38
Polylactide (PLA), 504
Polylactide nanoparticles (NPs), 372
Polylactide-based nanoparticles, 472
Polylactide-co-glycolic acid (PLGA), 235
Poly-L-lysine (PLL), 147, 379
Polymer nanofibers, 32
Polymer nanoparticles, 19, 234–237
Polymerase chain reaction (PCR), 133
Polymeric micelles, 236
Polymeric nanoparticles, 242, 430
Polymerized liposomal nanoparticle (PLN), 250
Polymersomes, 372
Polysodium 4-styrenesulfonate (PSS)-coated AuNTs, 404
Poly-SUMO-chain-mediated protein-protein interactions, 386
Polyvinyl pyrrolidone (PVP), 361
Porous silicon (PSi), 350
Porous silicon nanoparticles (Psi NPs), 19, 587
Prednicarbate (PC), 548
Primer extension (PEXT) reaction, 189
Pro-angiogenic heparin-binding growth factors (HB-GFs), 383
Probes Encapsulated by Biologically Localized Embedding (PEBBLE), 178
Prostate-specific antigen (PSA), 170, 333
Prostate-specific membrane antigen (PSMA), 375
Prosthetic artificial retinas, 509
Protamine chlorhydrate, 147
Protease-activated QD probes, 190–191
Protective mucus coatings, 234
Protein delivery, 219
Protein engineering
  nanoscale mechanism, 116–117
  nanowires, 116
  peptide engineering, 117–118
  self-assembly, nanoparticles, 117
Protein nanoarrays, 44, 138
Protein nanobiochip, 138
Protein nanocrystallography, 115–116
Protein Stabilized Liposome (PSL™) nanotechnology, 336
Protein-nanoparticle combination, 31
Protein-protein interactions, 207
Proteins and peptides, 273–274
Proteins detection
  captamers, 167
  immunoliposome-PCR, 168
Proteomics, 74
Proteosomes™, 278
Protosphere™ nanoparticle technology, 351
Proximity extension assay, 167
pSivida Corporation, 382
Pulmonary disorders
  COPD, 487
  cystic fibrosis, 483–485
  inflammation, 488
  liposome-mediated CFTR gene transfer, 486
  magnetofection, 486–487
  nonviral DNA nanoparticle-mediated CFTR gene transfer, 485–486
  pulmonary infections in CF, 487

Copyrighted material

Superparamagnetic nanoparticles, 292–293
Super-resolution microscopy
  3D-SIM, 65
  nanomicroscopy, live cell tomography, 66
  RESOLFT nanoscopy, 66
Surface acoustic wave technology (SAW), 482
Surface Enhanced Microoptical Fluidic Systems (SEMOFS), 183
Surface enhanced Raman scattering (SERS), 40, 511, 515, 516
Surface immobilized biobarcode assay (SI-BCA), 163
Surface nanomachining, 42
Surface plasmon resonance (SPR), 14, 68, 133
Surface plasmons, 181
Surface-enhanced Raman gene (SERGen), 182
Surface-enhanced Raman scattering (SERS), 18, 98
Surface-Enhanced Resonant Raman Spectroscopy (SERRS), 166
Surface-enhanced-Raman-scattering (SERS), 4
Syngeneic BALB/c mouse tumor model, 348
Synthetic gene delivery vectors, 243
Synthetic photoisomerizable azobenzeneregulated $K^+$ (SPARK), 86
Systemic effect, 265
Systemic lupus erythematosus (SLE), 540
Systems biology, 74
Systems of Neuromorphic Adaptive Plastic Scalable Electronics (SyNAPSE), 129

**T**

Tacrolimus ointment, 550
Targeted nanotherapeutics (TNT) system, 390
Targeted Synthetic Vaccine Particle (tSVP™) technology, 278
Taxol infusions, 351
Taxosomes™, 335
Taxotere (docetaxel), 336
T2 Biosystems, 518
T cell receptors (TCRs), 77
Technegas, 583, 584
Tekmira's delivery technology, 286
Temozolomide (TMZ), 433
Temperature-sensitive liposomes (TSLs), 392
TGF-β growth factor, 497
TGF-β inhibitors, 399
TGF-β type I receptor (TR-I) inhibitor, 399
The EU project NANOTRANSPORT (2006-2008), 592
Therapeutic protein delivery, 244–245
Thermosensitive liposomes, 392
Thiol-derivatized polyethylene glycol (PEG-THIOL), 358
3D calibration technique, 52
3D cell culture, 124
3D modeling technique, 130
3D nanofilament-based scaffolds, 309–310
3D single-molecule microscopy, 51
3D structural information, 61
TiMesh, 614
TiNano Spheres™., 242
$TiO_2$-nano, 599
Tissue barriers, 501
Tissue-engineering, 81–82, 308
T lymphocyte immunomodulation, 540
Toll-like receptors (TLRs), 275, 365
Total internal reflection fluorescence (TIRF) microscopy, 572
Toxin-laden nanosponges, 559
Trabecular meshwork (TM) cells, 509
Transdermal administration, 264
Transdermal drug delivery, 261–263
Transdermal nanoparticle preparations, 265
Transfer RNA (tRNA), 120
Transferase dUTP nick end labeling (TUNEL), 385
Transferrin (Tf)-containing gold nanoparticles, 431
Transferrin receptors (TfRs), 354
Transistor arrays, 422
Transmission electron microscope (TEM), 32
Tris [hydroxyl methyl] amino methane, 383
Trojan particles, 244
Tryptophan-phenylalanine dipeptide nanoparticles, 362
Tubulysin-Cyclosert conjugate, 357
Tumor necrosis factor (TNF), 358
TUNEL staining, 385
2D nanofluidics, 48
Two-photon luminescence (TPL), 152
Type 1A diabetes (T1D), 153

**U**

Ultrananocrystalline diamond (UNCD), 67
Ultrasmall SPIONs (USPIONs), 147
Ultrasonography, 150
Ultra-thin layer chromatography (UTLC), 139
Ultraviolet radiation (UVR), 600
University of California, Los Angeles, CA (UCLA), 593
University of Ulsan College of Medicine, 243
Up-converting phosphor technology (UPT), 165


Copyrighted material

US Consumer Product Safety Commission (CPSC), 598
US National Nanotechnology Initiative (NNI), 629
UV-Vis Spectroscopy, 361

**V**
Vaccine delivery
  bacterial spores, 274
  gelatin nanoparticles, 284
  nanobiotechnology, 274–279
  nanoparticle-based adjuvants, 276–277
Vaccine delivery vehicles, 278
van der Waals interactions, 50
Vascular endothelial growth factor (VEGF), 509
Vascular endothelial growth factor 165 (VEGF165), 383
Vasoactive intestinal peptide (VIP) receptors, 541
Verigene, 515
Verigene Gram-Positive Blood Culture (BC-GP) Test, 516
Verigene ID system, 163
Verigene respiratory virus plus assay, 515
Vernix, 548
Vertical Cavity Surface Emitting Lasers (VCSELs), 55
Viral antigens, 277
Viral nanosensor, 177
Viral vectors, 288, 436
Virology
  in HIV, 528–529
  nanofiltration, 529
  nanoparticles, 527–528
  pathomechanism, 528
Virus-like function, 288–289
Virus-like particles (VLPs), 32, 354
Virus-mimetic nanovesicles (VMVs), 278

**W**
Water droplet, 75
Water-solubilization techniques, 16
Water-soluble CdSe/CdS/ZnS quantum rods, 373
Water-soluble SWCNTs, 589
Wavelength division multiplexing, 92
Wetting phenomena, 50
wtCFTR cDNA expression vectors, 485

**X**
X linked juvenile retinoschisis (XLRS), 508
Xenograft colon cancer model, 354
XLRS1 plasmid, 508
X-ray diffraction, 13
X-ray photoelectron spectroscopic studies, 509
X-ray radiation, 306

**Z**
Zinc oxide (ZnO) nanomaterials, 352
ZinScreen, 549
ZME-018, 357
ZnO nanoflowers, 470

Copyrighted material