Biology of Blood and Marrow Transplantation 12:87-91 (2006)

1083-8791/06/1201-0116$32.00/0
doi:10.1016/j.bbmt.2005.10.004

Bionanotechnology Progress and Advances

Warren C. W. Chan

University of Toronto, Institute of Biomaterials & Biomedical Engineering, Terrence Donnelly Center for Cellular and Biomolecular Research, Materials Science and Engineering, Toronto, Ontario, Canada

Correspondence and reprint requests: Warren C.W. Chan, PhD, University of Toronto, Institute of Biomaterials & Biomedical Engineering, Terrence Donnelly Center for Cellular and Biomolecular Research, Materials Science and Engineering, 160 College St., Toronto, ON, M5S 3E1, Canada (e-mail: warren.chan@utoronto.ca).

ABSTRACT

Advances in the nanotechnology research have provided a new set of research tools, materials, structures, and systems for biological and medical research and applications. These nanotechnologies include the application of fluorescent quantum dots for optical imaging, the design of metallic nanoparticle surfaces for ultrasensitive biomolecular fingerprinting, and the use of nanostructures as hyperthermia agents for cancer therapy. Unlike conventional technologies, unique properties can be incorporated into nanometer-size particles, structures, and systems simply by changing their size, shape, and composition. Because of the tunable properties, biologists and clinicians could custom-design a material for a specific research need. In this review article, we highlight the recent advances and progress in Bionanotechnology research as well as provide future perspective on this integrative field.

KEY WORDS

Bionanotechnology • Quantum dots • Metallic nanostructures • Multifunctional nanodevices

INTRODUCTION

Nanotechnology has rapidly emerged as an important field of research with potential effects in both diagnostics and therapeutics. Nanotechnology is also expected to accelerate fundamental biomedical research via the creation of novel state-of-the-art tools. We loosely define nanotechnology research as the creation, design, and manipulation of structures or particles with dimensions smaller than 100 nm. Within this size range, the structures or particles have properties that can be tuned by changing the material's dimensions [1,2] (Figure 1). For example, the fluorescence emission of CdSe semiconductor particles is dependent on its size; a 2-nm particle emits blue light, whereas a 6-nm particle emits a red light. Nanotechnology research has traditionally been a major focus of research by engineers and physical scientists because nanomaterials were considered as important components in the construction of electronic components and computer chips. The development of synthetic protocols to reproducibly make nanomaterials and to elucidate the relationship between their properties with respect to size and shape is a common area of research. However, in the last 10 years, the biological and medical research communities have exploited the unique properties of nanomaterials for various applications (eg, contrast agents for cell and animal imaging and therapeutics for treating cancer), and, because of these demonstrations, new avenues and promises have been created for nanotechnology research. Terms such *biomedical nanotechnology*, *bionanotechnology*, and *nanomedicine* are used to describe this hybrid field.

There seems to be a natural fit between nanotechnology and biology. With nanotechnology, a large set of materials with distinct properties (optical, electrical, or magnetic) can be fabricated. Functionalities can be added to nanomaterials by interfacing them with biological molecules or structures (Figure 2). For example, magnetic nanoparticles with the conjugation of the antibody Herceptin onto the surface can be used as a contrast agent for tumor imaging (the antibody directs the magnetic nanoparticle to the tumor cells while the magnetic nanoparticle provides a signal for indicating detection) [3]. Diverse and large libraries of biological molecules, such as antibodies and oligonucleotides, are available because they can be produced by using molecular biology or automated synthetic techniques. Furthermore, the size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications.

Thus far, the integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, therapy, and

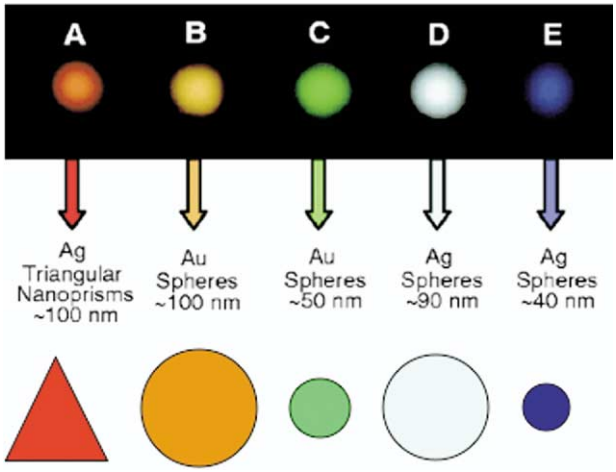

Figure 1. Rayleigh light-scattering of metallic nanostructures of different sizes and shapes. This example shows the simplicity of creating structures with unique properties, simply by changing the structure's dimensions. Adapted with permission [1] from the American Association for the Advancement of Science, © 2001.

drug-delivery vehicles. Mirkin et al. [4,5] exploited the aggregation-based absorbance properties of gold nanostructures for detecting genetic mutations; Kim et al. [6] developed quantum dots (QDs) for sentinel lymph node mapping; Han et al. [7] developed optical barcodes for the high-throughput analysis of proteins and genes; and Hirsch et al. [8] developed metallic nanostructures for treating cancer. Although these researchers demonstrated some interesting applications of nanomaterials, bionanotechnology research is still in its infancy; few nanotechnology-based products are in clinical use.

Visionaries in the field have predicted that one day, researchers will incorporate multifunctionality into nanomaterials. With these multifunctional nanodevices, molecules and motors will guide nanomaterial movements, sensors for diagnosis, actuators (which are connected to the sensor) to release therapy, and a secondary sensor to monitor the disease as it is being treated. Such a device can diagnose, treat, and monitor diseases such as cancer and Alzheimer disease. This advancement will likely take at least 50 to 100 years to develop. The foundation of nanotechnology is quickly being put into place, and some applications of nanotechnology in biology have surfaced (although these applications are nowhere near the complexity of the described multifunctional device). In this article, we provide examples of current state-of-the-art bionanotechnology research.

SEMICONDUCTOR QDs FOR BIOIMAGING

Recent developments in the field of nanotechnology have led to the fabrication of a new generation of inorganic nanostructures that overcome many of the problems associated with organic-based probes [9]. These nanometer-sized probes provide novel and unique properties for biological applications that are not available with organic-based fluorophores. At the forefront of this new class of inorganic probes are semiconductor nanocrystals, also known as QDs. The physical properties and applications of QDs have been heavily investigated in many physics and engineering laboratories since the early 1980s [10-12]. QDs are defined as particles with physical dimensions smaller than the exciton Bohr radius; this gives rise to a unique phenomenon known as quantum confinement. Quantum confinement, which refers to the spatial confinement of charge carriers (ie, electrons and holes) within a material, embarks QDs with unique optical and electronic properties that are unavailable to semiconductors in bulk solids. Although initial interest in QDs focused in physical applications (eg, making computer chips and light-emitting diodes), recent work by Nie, Alivisatos, and their coworkers has highlighted the great promise of QDs in biological applications [13,14]. As biological probes, QDs are extremely bright (1 QD ≈

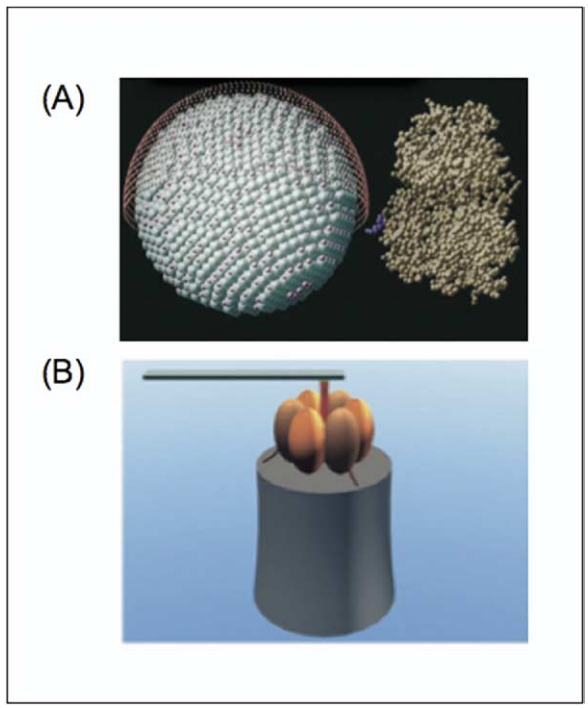

Figure 2. Schematics of nanostructures integrated with biological molecules and structures. A, Schematic of a semiconductor nanostructure called quantum dots (sphere) with a protein on its surface. This quantum dot/protein system can be used for labeling cells, biosensing, or detecting tumors. Adapted with permission [10] from the National Academy of Sciences, © 2004. B, Schematic of a nickel nanostructure bound to an F_1-adenosine triphosphatase biomolecular motor. The motor is a mechanical device to power up and direct the nanostructure. One day, these motors may be important components in building nanodevices for in vivo biomedical applications such as cancer treatment. Adapted with permission [20] from the American Association for the Advancement of Science, © 2000.

10-20 organic fluorophores), have high resistance to photobleaching, have narrow spectral line widths, and have size- and materials-tunable emission that can be excited by using a single wavelength. When QDs are used in biological research and applications, these optical properties will lead to improved detection sensitivity for analysis and to simplification in experimental and instrumental design. Because QDs have fluorescence properties, they have been rapidly adapted into many biological and medical research laboratories. For in vitro applications, QDs conjugated to antibodies and peptides are used for labeling receptors on fixed and live cells and tissues; QDs conjugated to oligonucleotides are used for genetic detection. For in vivo applications, successful demonstration of QDs as contrast agents for cancer imaging has already been achieved. Kim et al. [6], Akerman et al. [15], and Gao et al. [16] have all shown the accumulation of QDs in animal tumors.

As QDs advance, there are several major avenues of research. The first is the continuing improvement and development of QD optical probes with better optical qualities (ie, high quantum efficiencies, narrow spectral line widths, and more brightness). The second is to understand the relationship between QDs and biological systems (ie, elucidating how cells and animals uptake, process, and metabolize QDs). The third is to study and understand the influence of biological environments and conditions on the optical and electronic properties of QDs. The fourth is to apply QDs toward clinical applications.

METALLIC NANOSTRUCTURES AS SENSORS

Many reports on the biological applications of metallic nanostructures as biological sensors have recently surfaced. Unlike QDs, metallic nanostructures were incorporated or used in many biomedical applications in the 1970s and 1980s [17,18]. Gold nanostructures are used as contrast agents in electron microscopy or as detection probes for dipsticks (such as those for pregnancy tests). The manipulation of the size, shape, and aggregation-dependent absorbance properties of metallic nanostructures and their demonstrated applications have made this one of the most exciting areas of bionanotechnology research.

The absorbance and scattering of light by metallic nanostructures is size and aggregation dependent [1]. A solution of gold nanostructures appears ruby red, but the color of solution changes to blue when the gold nanostructures are in close contact to one another (or are aggregated). In this colorimetric diagnostic system, the biorecognition molecule oligonucleotide (which recognizes a gene for a specific disease) is coated onto the surface of the gold nanostructure. When a gene sequence matches the oligonucleotide sequence on the gold, hybridization occurs. With hybridization, the nanostructures are brought close together, and the solution color changes (as an indicator of detection). This diagnostic system has advanced toward a surface platform, where silver staining is used for amplification of signal.

Metallic nanostructures can also be a platform for surface-enhanced Raman spectroscopy (SERS). Raman spectroscopy is an analytical technique that measures the vibrational frequencies of chemical bonds upon optical excitation. The major advantage of Raman spectroscopy is that a tag or label is not required for detection, but the technique is less sensitive than fluorescence. However, the adsorption of molecules onto roughened metallic surfaces enhances the detection capability of Raman spectroscopy. This technique is called SERS. Emory and Nie [19] showed that SERS could detect a single molecule if that molecule was adsorbed onto a metallic nanostructure with a sensitivity comparable to that of fluorescence. SERS is starting to emerge as a viable analytical technique for clinical detection.

Therapeutic applications of metallic nanostructures are also possibilities. Photothermal properties can be engineered into the metallic nanostructures for laser ablation therapy. Metallic nanoshells, where a nanometer-sized metallic layer is grown onto a glass bead's surface, and rods, where the nanoparticle's aspect ratio is greater than 1:1, will produce heat when illuminated with a light source (that matches the nanostructure's surface plasmon resonance). The surface plasmon resonance is defined as a packet of electrons on the surface of the metallic nanoparticle that becomes excited after optical illumination. These metallic structures can be used for hyperthermia therapy.

We have highlighted some of the emerging applications of metallic nanostructures. There are, however, many other applications (eg, molecular rulers and drug delivery) for metallic nanostructures.

OTHER NANOSTRUCTURES

In addition to QDs and metallic nanostructures, other nanostructures have been synthesized and used in biological and medical research. Among these nanostructures are carbon nanotubes, fullerenes, dendrimers, and magnetic nanostructures. Thus far, they have found applications in imaging (as contrast agents or surface probe tips), drug delivery, and biosensing.

TOWARD FUNCTIONAL DEVICES: INTEGRATION OF NANOSTRUCTURES WITH BIOLOGICAL MOLECULES AND STRUCTURES

With recent advances in the synthesis of nanostructures and a demonstration of their utility in bio-

medical research, a major focus of the nanotechnology community is on the design of multifunctional nanodevices. These devices will be a new type of advanced therapy for the treatment of cancer, Alzheimer disease, or infections. Two major questions arise: (1) How can one organize nanostructures into a functional device? and (2) How can one control the function of these nanostructures? Toward the engineering of these nanodevices, researchers use biology as an inspiration. Biological molecules and structures are used for organizing nanostructure aggregations; biological motors are incorporated into the design to create rotors to control the nanostructure's movements. Currently, it is not clear how an engineer will add sensing and actuating capability into the nanostructures.

The mimicking of biological systems for designing nanodevices may be a powerful strategy. In biological systems, only 20 amino acids and 4 bases are needed to coordinate the functions of thousands of proteins. The amino acids and bases can assemble useful biological structures with noncovalent interactions. These interactions include hydrophobic-hydrophobic interactions, van der Waals forces, hydrogen bonding, and molecular stacking. Nanostructures, in general, are highly dependent on molecular forces to assist them in maintaining their monodispersity. Coordinating molecular forces, which are used to fold proteins into a functional unit, onto the surface of nanostructures is currently difficult because protein-folding mechanisms remain somewhat unclear. A simpler and first step toward the use of biology to mediate nanoparticle assembly is the use of biorecognition molecules. Biorecognition molecules can be coated onto a nanoparticle surface to direct the formation of aggregates.

The simple mixing of antibody-coated nanostructures with their matching antigen in aqueous solution can lead to rapid aggregation of nanostructures. For example, proteins such as avidin have 4 binding sites to the small organic molecule biotin; antibodies have an inhomogeneous structure with a total of 3 binding sites. These 2 protein systems can coordinate the overall shape, size, and nanoparticle spacing of the aggregate network. For nanoparticle assembly, proteins may act as "molecular glue." Oligonucleotides are also being used to assemble nanostructures. They provide great versatility in the assembly process. Oligonucleotides are short fragments of DNA or RNA that can be easily synthesized by using a machine. Similar to a zipper, single-stranded oligonucleotide sequences hybridize, or pair up, with a matching sequence via hydrogen-bonding interactions. They dehybridize simply by heating. To make nanoparticle assembly dynamic (or responsive nanodevices), more complex molecular glue may be needed because some protein-protein interactions are essentially irreversible (eg, streptavidin-biotin).

To add functionality into the nanostructures, Soong et al. [20] coated the surface of inorganic nanostructures with biomolecular motors, such as F_1-adenosine triphosphate synthase. This molecular motor propelled the particles in solution. Hess et al. [21] proposed the use of motor proteins with microtubule track systems to construct molecular conveyer belts to build nanoscale devices. They mimicked the biological process of vesicle transport inside cells.

CONCLUSIONS

We have highlighted some of the recent developments in bionanotechnology research and provided some insights into the future of this hybrid field. As the field of bionanotechnology evolves, we envision a trend toward clinics, because nanotechnology will likely allow a clinician to diagnose diseases at faster rates with improved sensitivity and specificity. The more futuristic goals of bionanotechnology, such as multifunctional nanodevices, will take much longer to develop. Researchers are now starting to figure out how to build such devices. This emerging field is exciting because of its possibilities.

ACKNOWLEDGMENTS

W.C.W.C. would like to acknowledge Canadian Institute of Health Research (CIHR), Natural Sciences and Engineering Research Council (NSERC), Canadian Foundation for Innovation (CFI), Ontario Innovation Trust (OIT), the Connaugt Foundation, and Genome Canada for research support.

REFERENCES

1. Jin R, Cao Y, Mirkin CA, et al. Photoinduced conversion of silver nanospheres to nanoprisms. *Science.* 2001;294:1901-1903.
2. Chan WC, Maxwell DJ, Gao X, et al. Luminescent quantum dots for multiplexed biological detection and imaging. *Curr Opin Biotechnol.* 2002;13:40-46.
3. Jun YW, Huh YM, Choi JS, et al. Nanoscale size effect of magnetic nanocrystals and their utilization for cancer diagnosis via magnetic resonance imaging. *J Am Chem Soc.* 2005;127:5732-5733.
4. Mitchell GP, Mirkin CA, Letsinger RL. Programmed assembly of DNA functionalized quantum dots. *J Am Chem Soc.* 1999; 121:8122-8123.
5. Taton TA, Mirkin CA, Letsinger RL. Scanometric DNA array detection with nanoparticle probes. *Science.* 2000;289:1757-1760.
6. Kim S, Lim YT, Soltesz EG, et al. Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping. *Nat Biotechnol.* 2004;22:93-98.
7. Han M, Gao X, Su JZ, Nie S. Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules. *Nat Biotechnol.* 2001;19:631-635.
8. Hirsch LR, Stafford RJ, Sershen SR, Halas NJ, Hazle JD, West JL. Nanoshell-assisted tumor ablation using near infrared light under magnetic resonance guidance. *Proc Natl Acad Sci U S A.* 2003;100:11349-11354.

9. Medintz IL, Konnert JH, Clapp AR, et al. A fluorescence resonance energy transfer-derived structure of a quantum dot-protein bioconjugate nanoassembly. *Proc Natl Acad Sci U S A.* 2004;101:9612-9617.
10. Henglein A. Small-particle research: physiochemical properties of extremely small colloidal metal and semiconductor particles. *Chem Rev.* 1989;89:1861-1873.
11. Rossetti R, Nakahara S, Brus LE. Quantum size effects in the redox potentials, resonance Raman spectra, and electronic spectra of CdS crystallites in aqueous solutions. *J Chem Phys.* 1983; 79:1086-1087.
12. Brus LE. Electronic wave functions in semiconductor clusters: experiment and theory. *J Phys Chem.* 1986;90:2555-2560.
13. Chan WC, Nie S. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. *Science.* 1998;281:2016-2018.
14. Bruchez M Jr, Moronne M, Gin P, Weiss S, Alivisatos AP. Semiconductor nanocrystals as fluorescent biological labels. *Science.* 1998;281:2013-2016.
15. Akerman ME, et al. Nanocrystal targeting in vivo. *Proc Natl Acad Sci U S A.* 2002;99:12617-12621.
16. Gao X, Cui Y, Levenson RM, Chung LWK, Nie S. In vivo cancer targeting and imaging with semiconductor quantum dots. *Nat Biotechnol.* 2004;22:969-976.
17. Horisberger M, Rosset J. Colloidal gold, a useful marker for transmission and scanning electron microscopy. *J Histochem Cytochem.* 1977;25:295-305.
18. Horisberger M. Colloidal gold and its application in cell biology. *Int Rev Cytol.* 1992;136:227-287.
19. Emory SR, Nie S. Screening for size-dependent properties of single nanoparticles. *J Phys Chem B.* 1998;102:493-495.
20. Soong RK, Bachand GD, Neves HP, et al. Powering an inorganic nanodevice with a biomolecular motor. *Science.* 2000;290: 1555-1558.
21. Hess H, Clemmens J, Brunner C, Doot R, Luna S, Ernst KL, Vogel V. Molecular self-assembly of "nanowires" and "nanospools" using active transport. *Nano Lett.* 2005;5:629-633.