Bio-Nanotechnology Challenges for Intelligent Materials

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Introduction

The world is rapidly and drastically changing toward the knowledge-based society with accelerated globalization, where intensive efforts have been concentrated on creating new knowledge specifically through science and technology. It is recognized that the interdependency of different science and technology disciplines is effective on emerging innovative knowledge. In a last decade, there has been enormous nanotechnology initiatives promoted throughout the world. It is noted that nanotechnology has made it possible to explore the biological information world in nanometer scale. Either Bio-Nanotechnology or Nano-Biotechnology has thus been evolved through this science and technology melding process of biotechnology and nanotechnology. New knowledge derived from the bio-nanotechnology challenges on the biological information world continues to emerge, which seems to have a great concern with the achievement of safe and high quality living.

Bio-nanotechnology has made a profound progress on detecting, tracking and manipulating single biomolecules in an ambient environment, and visualizing the deep insight of a living cell which contains sophisticated biological information networks. A cell responds to either inner or outer stimuli by processing the information through the molecular information networks involving the signal transduction cascades and gene information processes. Bio-nanotechnology measurements reveal that the intracellular information processing is conducted through the nano-structural changes of signal transduction molecules. It is amazing that a whole cell as well as the intracellular molecular assemblies behave in an intelligent manner even though they have no brain.

Intelligent materials are modeled on such cellular and molecular responses to stimulation. The bio-nanotechnology-based mechanisms may provide us with a possible design concept of intelligent materials in which the sensing, information processing and actuating functions are implemented. Intelligent materials may behave in an intelligent manner, featuring extremely high performances such as human friendliness, self-diagnosis, self-repair, self-control, adaptive, learning and other intelligent characteristics. In fabricating intelligent materials, the sensing, information processing and actuating functions should be integrated in such a fashion as to be interactive in nano-scale. The design concept of intelligent materials is schematically illustrated in Figure 1.

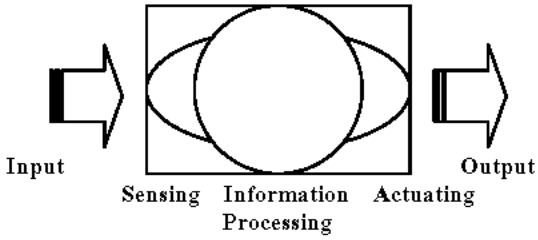
Promising bio-nanotechnology challenges have been emerging in designing various intelligent materials that are strongly supported by bio-nanotechnology progress including not only nano-scale measurement but nano-scale control and manipulation. Unique intelligent molecular intelligent biomaterials have been developed by bio-nanotechnology challenges, which involve nano-probes for molecular imaging, and nano-particles for mRNA replication.

Bio-nanotechnology is quite effective on enhancing intelligence of a living cell. Animal cells may respond to chemical stimuli through the receptors on the membrane surface. On the other hand, they are found responsive to such physical stimuli as electric and mechanical stresses. Since these stimuli modulate the gene expression processes, some physically responsive promoters have been screened out. Gene engineering makes it possible to enhance intelligence of animal cells to respond the electric signal in such a way as modulating the gene expression.

Challenges of bio-nanotechnology have also been emerging in developing either nano-scale or micro-scale biodevices for diagnostics, therapeutics, validating safety of chemicals, and drug discovery. Marked progress has been accomplished on developing nano-scale biodevices for intelligent drug delivery, which is capable of selective incorporation of a drug into a targeted cell. It is stressed that a variety of cellular bio-devices have been under investigation for both therapeutic and diagnostics. These feature cellular bio-devices for pancreas alternative, on-demand drug delivery, and safety check of chemicals as animal test alternative.

The current aspects and perspectives of bio-nanotechnology challenges for intelligent materials and systems are overviewed with ongoing researches and developments.

Capable of sensing appropriate environment stimuli, processing the information arising from the stimuli, and actuating in an appropriate manner and time frame.



Self-diagnosis, Self-repair, Self-control, Adaptive, Learning, Human friendliness, Extremely high performance with intelligence

Figure 1. Concept of Intelligent Materials

Bio-Nanotechnology Challenges (1)

Intelligent Molecular Biomaterials : Nano-probes for Molecular Imaging

Recently molecular imaging in nano-scale has gained an increasing attention because of its powerful approaches to the real world of individual biomolecules in an ambient environment and even in a living cell. Molecular imaging is mostly based on optical, electrical and mechanical force measurements. Several categories of molecular imaging have intensively been investigated as listed below.

Single Molecular Imaging
- *in vitro, in vivo*

Intra-cellular Molecular Imaging
- Distribution of individual molecules
- Concentration of individual molecules
- Dynamic molecular interaction

Inter-cellular Molecular Imaging
- Information processing in neuro-networks

Molecular imaging is carried out with or without probe. Although non-probe measurement is preferable, probe is required in most cases for enhancing sensitivity. Bio-luminescent enzyme and fluorescent protein are widely used as probe for molecular imaging. Intelligent nano-probes have been developed by gene and protein engineering.

Fusion protein of firefly luciferase and Protein A is a unique nano-probe which has a catalytic function of bio-luminescence at an end and a specific binding function at the other. The gene of the nano-probe is first synthesized by lygating luciferase and Protein A genes, which is followed by expressing it in bacterial cells. The nano-probe finds an application in various highly sensitive analyses. Kobatake et al show that the nano-probe of firefly luciferase and Protein A is effective on detecting local dynamic flow of ATP at the cellular membrane surface. The nano-probe is specifically anchored at immunoglobulin on the membrane surface and generates bio-luminescence from luciferine in presence of ATP. Bio-luminescence intensity reflects the local concentration of ATP.

Gene probe of firefly luciferase or green fluorescent protein (GFP) is also a unique nano-probe, which is expressed in a living cell. The gene of firefly luciferase or GFP is inserted in adjacent promoter which is sensitive to be activated in gene expression.

Figure 2 Two types of nano-probes

Bio-Nanotechnology Challenges (2)
Engineered Cells for Enhancing Intelligence

A living cell behaves in an intelligent manner, when it is stimulated by either exterior or interior stress. The cellular response is resulted from the information processing through the intracellular information networks. It was found that such a promoter as hsp70 is responsive to not only electric but also hydrostatic pressure stresses. A small voltage and low frequency of electric stimulation activates hsp70 to trigger transcription of the corresponding gene. The promoter is also activated by extremely high hydrostatic pressure. These findings lead us to design engineered cells which enhance intelligence with electric or mechanical direction.

A gene of target and a promoter of hsp70 are inserted into a plasmid, which is followed by transforming a cell of interest to enhance intelligence. The engineered cell may respond in such a way as expressing the gene of target with a direction of electric or mechanical stimulation. Schematic concept of such an intelligent cell is illustrated in Figure 3.

An example of the intelligent cell is a pancreas alternative cell that excretes human insulin with electric direction. An animal cell of candidate is selected for a pancreas alternative cell. The candidate cell is not required to produce human insulin, indicating that cells without human insulin gene may be selected. A fibroblast cell is, for instance, used for further research. The cell is transformed with a gene of human insulin and hsp70 promoter. Production of human insulin is switched ON and OFF by electric stimulation. Figure 3 illustrates the scheme of the electrically directed insulin production and excretion.

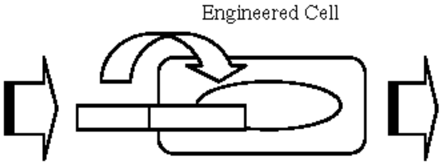


Figure 3 Electrically directed insulin production and excretion

Bio-Nanotechnology Challenges (3)
Biodevices for Diagnosis and Prevention

Biodevices are intelligent devices that molecular or cellular bio-components are implemented, although they may involve intelligent devices that biological function is installed with artificially designed systems. Research and development of biodeveices for diagnosis and prevention have made an enormous progress in these decades, starting with an enzyme sensor for blood glucose. Extensive research has been extended over the wider range of community, resulting in biosensor innovation specifically for diagnosis and prevention.

In the early 1990's, DNA chips with incredibly huge simultaneous analysis appeared on market, which was followed by rapid and wide expansion o f application. It is noted that DNA chips have successfully been developed with a unique combination of nanotechnology fabrication of gene probe on matrix and information technology of extremely huge data processing.

A success of DNA chips has encouraged many scientists and engineers who are involved in research and development of biodevices including biosensors, micro-fluidics, and bio-MEMS. Since bio-nanotechnology has opened a door to develop intelligent molecular and cellular materials, challenging research and development of biodevices for diagnosis and prevention become emerging.

There have been developed two types of biodevices, which might be classified into molecular and cellular biodevices. Such molecular nano-structures as nucleotides, saccharides, enzymes, antibodies and proteins are implemented in molecular biodevices for high performance of selectivity and sensitivity. Molecular biodevices are fabricated to form either single mode or multi-array mode. On the other hand, cellular biodevices contain living cells that involve a whole set of biological information networks with genome.

A special attention should be paid on current development of biodevices with living cells. Cellular biodevices are effective on biological evaluation of chemicals. It might be assessed, with cellular biodevices, if a specific compound is biologically hazardous.

The basic idea of the cellular biodevice is based on the total implementation of a whole set of biological information networks in a cell. The biological effects of chemicals may thus be evaluated with such a cellular response after exposure to chemicals. The effects may appear, in some cases, in enhancing or inhibiting a specific gene expression, which results in immediate monitoring these changes.

The perspectives of biodevices for diagnosis and prevention are listed as below.

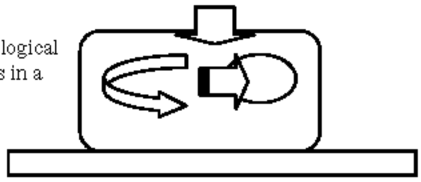


Figure 4 Cellular biodevice for biological evaluation of chemicals

- Biodevices for health care
  - Diagnostics for gene, infection, and stress
  - Personal care for prevention
- Biodevices for safety care
  - Safety assessment of chemicals and foods
  - Security checks
- Biodevices for drug discovery
  - Screening drug candidates
  - Animal test alternative

Bio-Nanotechnology Challenges (4)
Biodevices for Therapeutics and Prevention

Bio-nanotechnology challenges become obviously active in research and development of biodevices for therapeutics and prevention.

A variety of nano-structured biodevices for drug delivery have been under investigation by many research groups. Kataoka, et al, have been promoting JST Program of Virtual Laboratory in Nanotechnology, Multi-functional Nano-structured Devices for Non-viral Gene Delivery, in which polymeric micellar nano-structured devices and envelope type nano-structured devices are under investigation.

Other bio-nanotechnology challenges are exemplified by cell sheet and cellular biodevices for therapeutics and prevention. Okano and his colleagues are successful in development of cell sheet that is cultured cells on a thermo-responsive polymer sheet.

The perspectives of biodevices for therapeutics and prevention are presented as follows.

- Intelligent cellular bio-materials for regenerative medicine
  - Regenerative tissue
  - Cell therapy
- Biodevices with living cells
  - Tissue alternative
  - Cellular biodevices
- Biodevices with nano-structures
  - Nano-structured biodevices for drug delivery
  - Nano-structured biodevices for gene delivery

Conclusion

Bio-nanotechnology challenges have been emerging in development of molecular and cellular intelligent bio-materials, engineered cells for enhancing intelligence, biodevices for diagnosis and prevention, and biodevices for therapeutics and prevention. The perspectives of bio-nanotechnology challenges are overviewed.

Acknowledgment

The author acknowledges his collaboration with Dr.E.Kobatake, Dr.Y.Yanagida, Dr.T.Haruyama, and Dr.M.Mie.