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論文名
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by Bidirectional Interaction with Osteoblasts

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Safe Clinical Use of Carbon Nanotubes as Innovative Biomaterials

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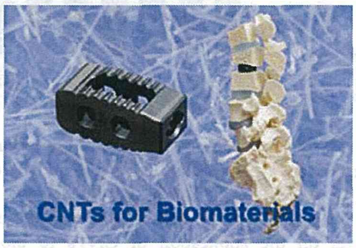

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1. INTRODUCTION

Carbon nanotubes (CNTs) are structurally described as sheets of six-membered carbon atom rings (i.e., graphene) rolled up into cylinders. CNTs with only one layer are known as single-walled CNTs (SWCNTs), and those with two or more layers are known as multiwalled CNTs (MWCNTs). Cup-stacked carbon nanotubes and carbon nanohorns are also sometimes called CNTs.[1−3] Currently, these very attractive carbon materials and nanomaterials are a subject of vigorous product development in a broad range of fields.[4−11] The reasons are that CNTs have useful electrical, thermal, and mechanical characteristics, and their base material performance can be improved by combination with other materials.[12−23] A recent industrial application of CNTs as an electrode additive to lithium-ion batteries is based on their excellent electrical characteristics. Addition of CNTs prevents battery deterioration and substantially lengthens time to recharging. It is doubtless that the demand for high-performance batteries will grow increasingly with multifunctionalization of personal computers and mobile phones, development of new mobile terminals, spread of electric vehicles, and other factors.[24−30] Composite materials with the excellent mechanical characteristics of CNTs have already been used in sporting goods such as golf clubs, tennis rackets, and bicycles. CNTs are also expected to have applications that reduce the weight of aircraft and automobiles.[10,14,31−35] A wide variety of advantages are gained from the use CNTs in precision parts as well. CNTs are also used in transistors and memory devices, and enhance their efficiency. The use of CNTs in various displays and TV screens continues to increase in rate. CNTs are also widely used in products designed to prevent static electricity, to shield electromagnetic waves, to store electricity, and for other purposes.[36−45] Furthermore, Japan is now facing nuclear energy issues stemming from the accident at Tokyo Electric Power Company's Fukushima No. 1 nuclear power plant. As a result, CNTs are expected to play a major role in developing new energy sources such as solar photovoltaic power generation and wind power generation.[46−52]

In the medical field, extensive research activities are underway to develop new CNTs biomaterials for use in the treatment and diagnosis of disease. For example, application of CNTs to cancer treatment and diagnosis, such as in drug delivery systems (DDSs) for treatment of cancer, hyperthermia, and in vivo imaging, has been investigated.[53−57] In a study that aimed at applying CNTs to regenerative medicine, CNTs were found to work excellently as scaffold materials for nerve and bone tissue regeneration.[58−63] Furthermore, R&D activities are underway to improve the mechanical strength and durability of implants by combining CNTs with existing biomaterials.[64−67] Besides, numerous ideas have been put forth about how CNTs can be used in the treatment of a variety of diseases.

Figure 1 shows the trend in the number of articles found in the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/) (accessed 20 March 2014) by searches using "carbon nanotubes" and "biomaterials" as keywords. The number has been soaring since 2005, suggesting that CNTs research has become a highly competitive field worldwide over the past few years. Of course, numerous articles on the biological applications of CNTs do exist that cannot be captured with these two simple keywords, and the graphic representation of this trend is no more than an indicator of the increase in this research over time.

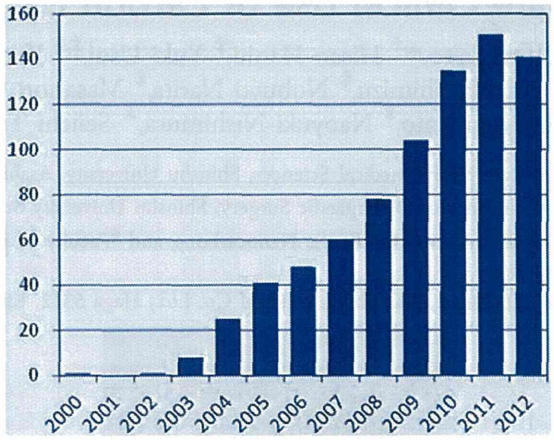

Figure 1. Time trends for the number of articles found in the PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/) (accessed 20 March 2014) by search using "carbon nanotubes" and "biomaterials" as keywords. Recent years have seen a rapidly increasing number of research articles on the application of CNTs to biomaterials; the number has been soaring since 2005, suggesting that the application of CNTs to biomaterials has become a highly competitive research field worldwide over the past few years. This graph indicates only a time course, and numerous articles on biological applications of CNTs do exist that cannot be captured with these two keywords.

One reason for the intense competition to find biomaterial applications of CNTs and for the great potential of CNTs to advance medical care is their small size (nanometers in diameter and micrometers in length), which makes them suitable to react with living organisms.[68−70] Hence, the size of CNTs, which is at the cell organelle level, is likely to facilitate their effect on living cells. Specifically, CNTs are similar in thickness and length to microtubules, which make up the cytoskeleton and mediate a wide variety of cellular activities such as motor protein activity.[71] Biomaterials containing CNTs of such size make reactions with cells more controllable and make treatment and diagnosis that focus on target cells more feasible, accurate, and less invasive to living organisms than conventional approaches. The second benefit from biologically applying CNTs is the ease with which they bind to a broad range of molecules thanks to the extremely high reactivity of CNT surfaces.[72,73] CNTs serve as a platform for binding multiple molecular entities such as drugs for therapeutic purposes, marker molecules, cell-binding molecules, and molecules facilitating the transfer of drugs to target tissues. Thus, CNTs can facilitate the diagnosis and treatment of

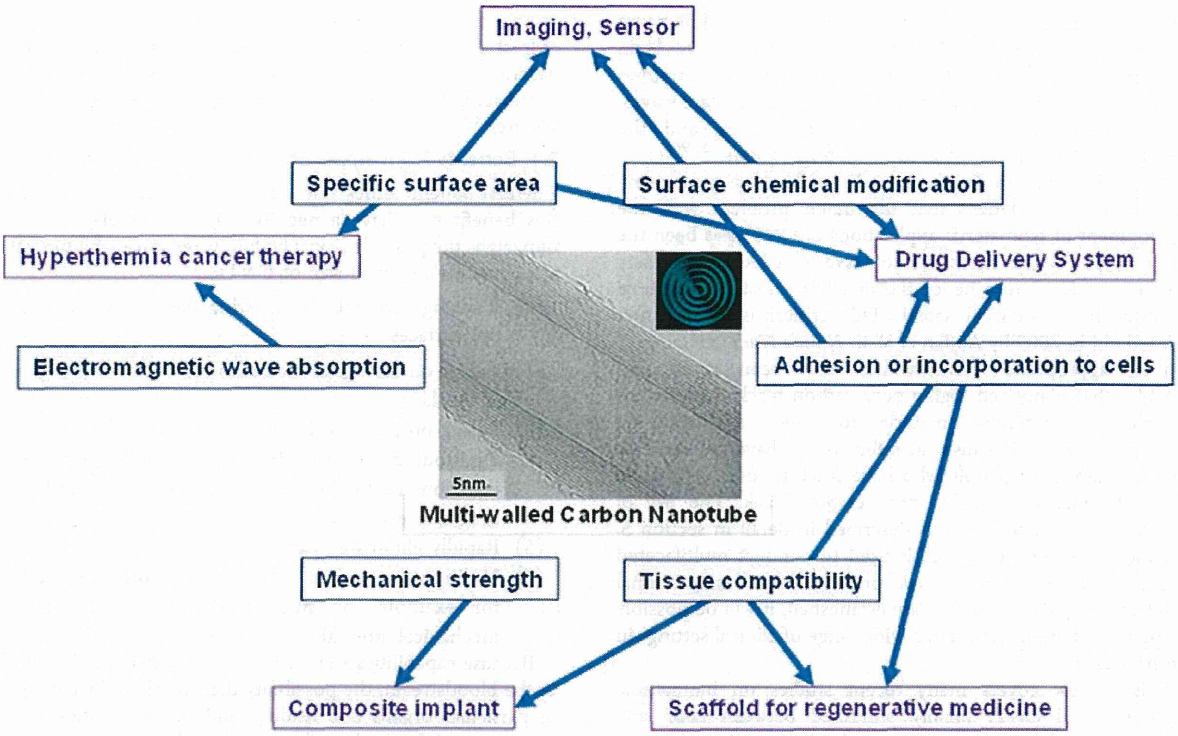

Figure 2. Biological applications of CNTs encompass a broad range of fields, many of which, in addition, represent themes of top priority in today's clinical medicine, such as cancer treatment and regenerative medicine. Modified from ref 84, which is published under the Creative Commons Attribution License.

diseases by facilitating substance recognition, adhesion, and affection to target cells. This potential is expected to lead to a groundbreaking new technology with applications to cancer treatment and regenerative medicine. In the near future, it is more likely that CNTs will be used as biomaterials for treatment and diagnosis of various diseases than for industrial purposes such as in batteries and aircraft. CNTs are of paramount importance to future advances in medical care. On the other hand, the small size and high surface reactivity of CNTs, properties that underlie their advantage as biomaterials, can adversely affect the human body. CNTs have not yet been used clinically (despite the dramatically increasing amount of research into biomaterial applications worldwide) because of safety concerns associated with implantation of CNTs devices in the body.[74−77] Currently, the safety of CNTs (primarily the safety of inhaled CNTs) is being investigated throughout the world.[78−83] Inhalation is the most likely route of external exposure of the human body to CNTs used in industrial products, so that inhalation toxicity must be determined first. It should be noted, however, that the safety profile of CNTs as biomaterials differs completely from that of inhaled CNTs.[68,69,84] Part of the safety evaluation of CNTs for biomaterial application, unlike that for inhalation, must include studies of the biological toxicity of implants in vivo. In many cases, biomaterial-specific studies must include implant toxicity, cytotoxicity, carcinogenicity, and genotoxicity studies. The safety of CNTs must be confirmed in these toxicity studies before they can be used in biomaterials. For this reason, the number of reports on the safety of biomaterials containing CNTs has been increasing.[54,68,85−89] Although these reports have demonstrated the safety of these biomaterials, researchers are still unable to reach a definitive conclusion. This is because CNTs are essentially nanoparticles, and biomaterials containing CNTs do not fall within the scope of biomaterials as traditionally conceptualized.[68,90] Of course, CNTs are not a drug, any other chemical substance, bulk material (as used herein, the term bulk material/biomaterial refers to a nonparticulate bulky material/biomaterial), or biodegradable material currently in use. Nanosized particulate substances lacking high biodegradability have not been used in the medical care field so far. Because of the nanosize of CNTs, many toxicity factors associated with nanosize will need to be investigated. Factors likely to impact the toxicity of CNTs and living organisms include thickness, length, specific surface area, and surface chemistry, as well as types of chemical modifications, defects in CNTs, and catalyst left unconsumed in the manufacturing process.[72] Factors affecting the administration of CNTs to living organisms include choice of dispersant, dispersant concentration, method of in vivo exposure, and duration of in vivo exposure. Furthermore, organ specificity, cell specificity, types and incidences of biologically adverse events, in vivo distribution, and other factors must be examined.[91] Collectively, these facts seem to suggest that developing biomaterial applications of CNTs will be difficult. Thus, absolutely no clinical applications have been found to date despite the rapid increase in the number of research articles dealing with CNT biomaterials.[10,77] However, inasmuch as applying CNTs biomaterials has potentially great benefits, the research must continue. Now is the time to review the present status based on available safety evaluation studies, to identify and resolve issues, and to implement clinical applications. Essentially, the human body consists principally of water and organic molecules, so life can be described as being supported by carbon.[92] To date, no problems have been reported from the use of materials consisting of ultrapure carbon, such as pyrolytic carbon used in artificial heart valves, carbon fibers used in Achilles tendon sutures, and the amorphous diamonds used in artificial finger joints.[93−96]

When reviewing, in detail, research articles by a great many researchers, it is evident that the major problem with the development of biomaterial applications of CNTs has been the lack of a particulate substance to serve as a biological safety reference material, and hence the inability to establish criteria for evaluating biological safety. This critical issue was first pointed out in 2009 by Auffan et al. in *Nature Nanotechnology*,[68] and no appropriate reference has since been found. We consider that nanosized highly pure carbon black particles are suitable as a reference material for safety evaluation of CNTs.[97,98] This is because no safety issues have appeared in the vast number of people who have black tattoos, containing principally nanosized highly pure carbon black. The use of carbon black as a reference is described in detail in section 5. Provided that same reference is used to conduct multifaceted extensive toxicity studies and provided that international standards of safety evaluation are established, it will be possible to apply CNT biomaterials in a wide range of clinical settings in the near future.

This Review covers many recent studies on biomaterial applications of CNTs mainly published between 2005 and 2013, and gives an outline of our published studies with new references. First, the findings in these studies are comprehensively discussed to evaluate the safety of CNTs as biomaterials. The way to realize safe clinical application of CNT-based biomaterials in the future is then proposed clearly. The challenge must always be kept in mind. Making the best use of all talents and abilities of researchers worldwide, this research will lead to a major revolution in the medical care field and benefit patients greatly. In this Review, we submit a proposal of paramount importance that we think will be the key to accomplishing this significant goal.

2. PRESENT STATUS OF RESEARCH INTO THE APPLICATION OF CNTs AS BIOMATERIALS

As is evident from the recent increase in the number of relevant articles, research into application of CNTs as biomaterials is advancing rapidly (Figure 1). CNTs have applications to a broad range of fields, many of which, in addition, have top priorities in clinical medicine today (Figure 2).[84,99−101] This section divides these applications into five categories: cancer treatment, regenerative medicine, implants, DDSs for noncancer targets, and other applications. Notably, many technologies utilizing CNTs are applicable to more than one of these fields. For example, the technology using CNTs as anticancer agent delivery systems is also useful for drug delivery systems targeting noncancer diseases. The technology for combining CNTs with other biomaterials is the key to successful application in new highly functional implants and in scaffolds used in regenerative medicine. Hence, this classification system was chosen only because it facilitates organization of the various published reports. In the future, classifying the studies on CNTs biomaterials with a focus on important technologies for their biological applications would be even more useful and expected to accelerate advances in relevant research.

All studies of biological applications reviewed below highlight at least one benefit of CNTs biomaterials, so these benefits are described below. The importance of these benefits has stimulated the rapid emergence and evolution of much research.[69]

2.1. Benefits from Application of CNTs as Biomaterials

The first benefit comes from the small size of CNTs. Although this benefit may have a negative impact on safety, it by far outweighs the possible risk. The following six capabilities can be attributed to the small size of CNTs:

(1) Reacting with cells by entering the cells or adhering to cell surfaces
(2) Acting on biological macromolecules and cell organelles of similar size
(3) Acting on parts of the body with fine structures
(4) Distributed via the bloodstream after intravenous injection and the like; thus they may be used in targeted drug delivery systems and in vivo imaging
(5) Rapidly eliminated from the body
(6) Having effects when combined with other biomaterials, for example, on fine structures to increase their mechanical strength

Because capabilities (4) and (5) assume that CNTs circulate in the bloodstream, the possibility that the risk of accumulation in particular organs and leading undesirable reactions to the organ outweighs the benefits must be taken into account. It is necessary to make the best use of these advantages, while minimizing the disadvantages. This is also true for other nanobiomaterials currently under investigation. Interactions between nanosized substances and living organisms will be further elucidated in the future. Nanobiomaterials are going to occupy an important position in nanomedicine, a research field that has only recently been established.[102−105] The second benefit is the ease of chemical modification. CNTs, because of their macromolecular size, have high chemical reactivity.[106] It is likely that the CNTs used in biological applications will be functionalized-CNTs (f-CNTs). When used as particles, rather than as a composite material, CNTs are likely to be f-CNTs.[73] CNTs can serve as a platform for concurrent binding of drugs, peptides, high molecular polymers, and other molecules that otherwise cannot be bound to each other (Figure 3).[107−112] Thus, it would be possible to construct CNTs with multiple functions that have not traditionally been co-occurrent, such as drug transport, cell adhesion, biomembrane transport, and release at targeted sites. For example, CNTs coupled with an anticancer agent and monoclonal antibody can be used to target cancer cells.[113,114]

There are two types of interactions with CNT surfaces: those based on covalent bonds and those based on noncovalent bonds. Of course, covalently bound substances (in contrast to noncovalently bound substances) are unlikely to dissociate from CNTs, so the appropriate method of binding must be chosen according to the target site and intended use. CNTs synthesized using the chemical vapor deposition (CVD) technique have open ends to which chemical modifiers can be bound specifically.[24] More interestingly, it is possible to transport molecules, atoms, etc., that have been inserted into the cylindrical hollow structure unique to CNTs. CNTs with such chemical modifications are called peapods because of their shape.[115,116] As such, CNT peapods can transport drugs in encapsulated form, and are expected to be increasingly

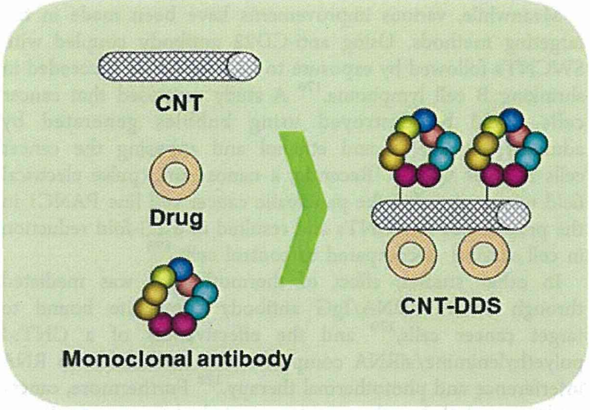

Figure 3. CNTs are capable of working as a platform for concurrently binding drugs such as anticancer agents, proteins, and peptides such as monoclonal antibodies, high molecular polymers, and other molecules that otherwise cannot be bound to each other. Making the best use of this feature, it would be possible to concurrently add to CNTs multiple functions that have traditionally been unable to concur, such as drug transportation, biomembrane passage, and release at targeted sites.

investigated because of their potential application as DDSs and in vivo imaging.[117−119]

The third benefit derives from the chemical composition of CNTs, which is very pure carbon. Carbon has already been used in many implant devices, including artificial heart valves, and no adverse effect of such biomaterials on living organisms has been reported to date.[96] The following features of CNTs may be regarded as advantages:

(1) High biocompatibility
(2) High strength-to-weight ratio
(3) High tensile strength
(4) Forming flexible nanofibers
(5) High chemical reactivity
(6) Conferring increased strength and other favorable characteristics to other substances when combined with them
(7) Inducing slow but significant biodegradation
(8) Colored in black that is easily distinguishable and detectable using a light microscope

The fourth benefit is the excellent electrical, magnetic, and thermal characteristics of CNTs in biomaterials. In fact, studies have used CNTs (because of their electrical characteristics) for nerve regeneration[112,120−122] and muscle actuation,[123,124] and (because of their magnetic characteristics) for cancer treatment and DDSs.[55,125] Furthermore, CNTs (because of their high photoenergy absorption capacity and thermal conductivity) have been proven effective for cancer thermotherapy.[56,107,126−128]

As stated above, CNTs (unlike conventional materials) can serve a wide variety of functions in tissues and cells of living organisms. This great potential has stimulated research into the application of CNTs as biomaterials in many fields. Overall, CNTs can be viewed as a revolutionary tool that will advance the practice of medicine, imposing expectations that biomaterials will be the main field of application of CNTs.

2.2. Application to Cancer Treatment

Currently, the most vigorously studied application of CNTs biomaterials is to cancer treatment. A wide variety of methods have been used to treat various cancers.[55,129−133] Detection of foci as early as possible and administration of an effective treatment are of paramount importance in cancer treatment. CNTs are expected to lead to innovative therapeutic and diagnostic methods. Although many other ongoing studies are not included, the following is an overview of the applications of CNTs to cancer treatment that are currently attracting much attention. Thus, clinical application of CNTs is a very promising field of study.

2.2.1. Biomarkers and Imaging. There have been recent dramatic technical improvements in methodology for the early diagnosis of cancer, with remarkable advances being made in tumor marker tests and the diagnostic imaging of cancer. Even now, however, it is difficult to detect early asymptomatic cancer; cancer is often detected only in the terminal stage. Against this background, studies have been conducted to detect the expression of biomolecules in the initial stage of cancer using CNTs as biomarker detectors. The application of CNTs to the detection of a prostate cancer marker (PSA), colorectal cancer markers (CEA, CA19-9), and a hepatocarcinoma marker (AFP) has been reported.[134−137] Applicability is based on the small size of CNTs that facilitates distribution in living organisms, and some evidence showing direct detection of biomarkers in vivo has been reported.[138−141]

CNTs have been used in noninvasive imaging, including for highly sensitive detection of very small tumors, using single CNT molecules conjugated to contrast reagent for CT or MRI, a heavy metal (gadolinium, etc.), and an antibody with high affinity for cancer cells.[142−145] A study is also ongoing that examines the application of a heavy metal encapsulated by the aforementioned peapod CNT to cancer imaging.[146] The most investigated imaging application is MR molecular imaging, which is effective in early detection of cancer. Furthermore, studies on the use of CNTs for photoacoustic molecular imaging show that it enhances contrast and resolution necessary to in vivo imaging. High resolution using a blend of SWCNTs and fluorescent peptide as the contrast medium for photoacoustic imaging were obtained.[147] Tumor vascularization plays an important role in cancer development and metastasis. For this reason, noninvasive detection of vascularization activity is critical to cancer diagnosis and assessment of patient responses to cancer treatment. A wide variety of molecular targets relevant to tumor vascularization have been identified, and can be used for tumor vasculature targeting and imaging. A method of optical imaging using a new photoprobe with the optical properties of CNTs has been developed to facilitate visualization of vascularization events.[148,149]

2.2.2. Drug Delivery Systems for Cancer Treatment. Of the biological applications of CNTs, DDSs for cancer treatment have been the most vigorously investigated. In cancer chemotherapy, adverse drug reactions are problematic, sometimes making it difficult to deliver adequate amounts of drugs to target organs. Because of their very large specific surface area that can bind many molecules beneficial to cancer treatment, CNTs can be used for DDSs in cancer treatment[89,129,150,151] and have been used as a platform to facilitate targeted delivery of a drug, antibody, other protein or peptide, lipid, polysaccharide, etc. (Figure 3). For example, a highly efficient missile therapy consisting of a combination of a hydrophilic group, a monoclonal antibody to cancer cells, an anticancer agent, and other components has been reported.[117] Using a nanoscale vehicle such as CNTs, drugs can be delivered to cancer cells that could not otherwise be delivered by microscale vehicles.[152] This is because thus-functionalized CNTs can pass through the cell membrane via a mechanism for the cellular uptake of foreign substances, such as endocytosis. CNTs with attached peptides or ligand bind to specific receptors on the cancer cell surface, and enter the cancer cells where they release the therapeutic agent more safely and efficiently. A DDS can be described as ideal when it delivers the needed amounts of therapeutic agent to the target in a timely manner, and CNT-based DDSs have the potential to fulfill this requirement.[132,153,154]

SWCNTs coupled with a tumor-specific monoclonal anti-CD20 antibody (rituximab) intravenously injected into mice after intramedullary transplantation of a human B-cell lymphoma resulted in accumulation of SWCNTs in the lymphoma.[110,155] Other researchers attached a tumor-recognizing module to the surface of hydrophilic f-SWCNTs to specifically bond with cancer cells, and then a prodrug module of an anticancer agent (a taxoid with a cleavable linker) to the surface of hydrophilic f-SWCNTs. They showed that the cytotoxicity of this tumor-targeting DDS is mediated via intracellular migration, drug release, and intracellular activation.[153] Moreover, the application of CNTs to gene therapy (i.e., as carriers of genes to targeted cancer cells) has been studied.[156−160] Because CNTs-based platforms are infinitely variable and easily designable, they are expected to lead to groundbreaking cancer treatment systems.

Before thus applying CNTs for DDSs, their pharmacokinetics after topical or intravenous injection must be clarified. The disposition of intravenously injected CNT−drug composite has been examined extensively.[105,161−165] Factors that influence transport of the composite through the bloodstream include thickness, length, and flexibility of CNTs as well as changes in properties resulting from the binding of the drug. Of course, injection of CNTs-based DDS into the tumor site directly is a safer approach. Furthermore, the use of magnetized particles to facilitate efficient uptake of CNTs in cancer tissue has been studied. For example, treatment of lymph node metastasis by subjecting magnetic functionalized CNTs to a magnetic field to promote their migration to lymph nodes has been studied.[166,167] Treatment with gemcitabine (GEM)-loaded magnetic functionalized CNTs subjected to a magnetic field resulted in regression of lymph node metastasis and suppression of metastatic growth both in vitro and in vivo.[55] In addition, many anticancer agent-loaded CNTs-based nanoscale DDSs have been developed.[101,128,168−170]

2.2.3. Cancer Treatment Using External Energy. CNTs absorb electromagnetic wave energy. On the basis of this property, the use of CNTs in cancer hyperthermia has been tested.[53,171−174] For example, cancer lesions were exposed to CNTs loaded with a tumor-specific epitope (to be absorbed selectively), then to infrared rays, and cancer tissue was specifically destroyed by the heat generated.[107] Another report showed the method for treating peritoneal metastases from colorectal cancer consisted of rapidly heating the cancer mass to 42 °C within 10 s in the presence of oxaliplatin or mitomycin C using infrared rays absorbed by CNTs.[175] In a recently reported study, the generation of heat and reactive oxygen species generated upon exposure of CNTs to infrared rays for 10 min was harmful to human lung cancer cells. Specifically, 45% of the cancer cells had been killed 24 h later.[56] The microwave absorption characteristic of CNTs theoretically permits accurate heating; microwave thermotherapy for cancer treatment is also a promising technology.[173]

Meanwhile, various improvements have been made in the targeting methods. Using anti-CD22 antibody coupled with SWCNTs followed by exposure to laser radiation succeeded in shrinking B cell lymphoma.[176] A study proposed that cancer cells could be destroyed using bubbles generated by administering CNTs and ethanol and exposing the cancer cells to laser light.[177] Recently, a nanosecond pulse electrical field was used to kill the pancreatic cancer cell line PANC1 in the presence of MWCNTs and resulted in a 2.3-fold reduction in cell survival as compared to control cells.[178]

In other studies, effect of thermotherapy was mediated through a CNT/DNA/IgG antibody composite bound to target cancer cells,[179] and the effectiveness of a CNTs/polyethylenimine/siRNA composite was attributable to RNA interference and photothermal therapy.[128] Furthermore, cancer imaging and thermotherapy was carried out concurrently by conjugating quantum dots to CNTs.[180] The variety of CNT applications has been increasing.

CNT peapods encapsulating iron nanoparticles and a chemical modification that facilitates binding to cancer cells have been used in cancer thermotherapy. The iron in the CNTs is highly biocompatible because it is protected from reacting with the ambient environment, and the electromagnetic wave thermotherapy is safe and effective.[118] In conclusion, investigations of thermotherapy with CNT adducts of other materials are ongoing.

These cancer treatments based on the ability of CNTs to absorb external energy cannot be clinically applied before methods of electromagnetic wave exposure are investigated. This is because the body rapidly absorbs the energy. In the case of simple exposure, the utility of CNTs is limited to accessible cancers. However, when used in combination with an implanted energy source, the utility of CNTs extends to deep cancers.[171,181,182] Cancer thermotherapy involving the clinical application of CNTs is currently a rapidly growing field of research.

2.3. Application to Regenerative Medicine

The aim of regenerative medicine is repair and regeneration of human body tissues and organs affected or lost because of disease, trauma, and the like. Developments in embryonic stem cell (ES cell) research and the development of induced pluripotent stem cells (iPS cells) in 2007 further stimulated regenerative medicine research.[183,184] Tissue regenerative therapies use cells, growth factors, genes, etc. Whichever means is used, no tissue can be regenerated without a scaffold. Thus, the scaffold is of paramount importance in therapy, and research aimed at developing CNTs as scaffold material has been increasing.[185−191]

2.3.1. Studies Assessing the Applicability of CNT Composites to Regenerative Medicine. The use of CNT composites in regenerative medicine has been vigorously investigated in vitro. Results showed that a CNT/collagen composite could be used as a scaffold for myocyte culture, and that a CNT/polyurethane composite could be used as a scaffold for fibroblasts growth and biosynthesis.[192−194] A CNT/polyurethane composite used as a scaffold for culturing vascular endothelial cells was effective in promoting their proliferation and suppressing thrombus formation.[195] A CNT/poly L-lactic acid/hydroxyapatite composite increased the adhesion and proliferation of periodontal ligament cells (PDLCs) by 30%.[196] Regenerated silk fibroin films incorporating MWCNTs were shown to support the adhesion and growth of human bone marrow stem cells.[197] SWCNTs nonwoven films enhanced long-term proliferation of many cell types.[198] While in vitro studies examining the reactions between cells and CNT composites used as scaffolds are numerous, there are few in vivo studies.[125,174,185−188,190,199,200] It is hoped that in vivo animal experiments based on in vitro findings will be carried out in the future. The application of CNTs to bone tissue regeneration and nerve tissue regeneration is of paramount interest.

2.3.2. Bone Tissue Regeneration. Regarding bone tissue regeneration, a CNT/polylactic acid composite was shown to promote osteoblast proliferation in vitro as early as in 2002.[58,201] Later, a CNT/polycarbonate urethane composite and a CNT/poly lactic-*co*-glycolic acid composite were reported to enhance the adhesion of osteoblasts.[202−204] In 2006, a study showed that SWCNTs and MWCNTs promoted the proliferation of osteocytes and osteoblasts when used alone.[205] This was followed by in vitro studies showing the wonderful effects of CNTs on bone-related cells.[66,186,206−212]

In 2008, we showed for the first time that CNTs promote bone tissue formation in vivo as well.[213] The study employed an experimental system that used recombinant bone morphogenetic protein-2 (rhBMP-2) to induce ectopic osteogenesis in mouse back muscle.[214] Bone formation on a collagen sheet was shown to occur earlier in the presence rhBMP-2 attached to a scaffold of MWCNTs than in the presence of rhBMP-2 alone (Figure 4). Later, other researchers confirmed that osteogenesis was promoted by CNTs in vivo. For example, a layer-by-layer assembled carbon nanotube composite promoted osteogenesis and bone repair when implanted in rat calvarial bone defects.[215] Carbon nanohorns, a type of CNT, were attached to a porous polytetrafluoroethylene membrane by vacuum filtration, and rat calvarial bone defects were covered with the membrane. The extent of osteogenesis was greater under the membrane containing carbon nanohorns than under the membrane without the carbon nanohorns, showing that carbon nanohorns accelerated bone regeneration.[216]

Later, we attempted to elucidate the mechanism underlying promotion of bone tissue regeneration by CNTs. In 2009, we showed that CNTs specifically suppressed the differentiation of osteoclasts as well as expression of the transcription factor NFκB in osteoclasts.[217] In 2011, we showed that CNTs could serve as the seed material for the crystallization of hydroxyapatite, the major component of bone, and that CNTs attracted Ca ions and activated osteoblasts. Another finding was that this activation was accompanied by the deposition of hydroxyapatite around the CNTs, which was catalyzed by alkaline phosphatase (ALP) released from osteoblasts.[218] These findings demonstrated that CNTs functioning as a scaffold interact with the body to promote osteogenesis and thereby the process of bone tissue regeneration. To date, no other scaffold has interacted with the body in this way; CNTs are expected to be breakthrough materials in regenerative medicine research as well.

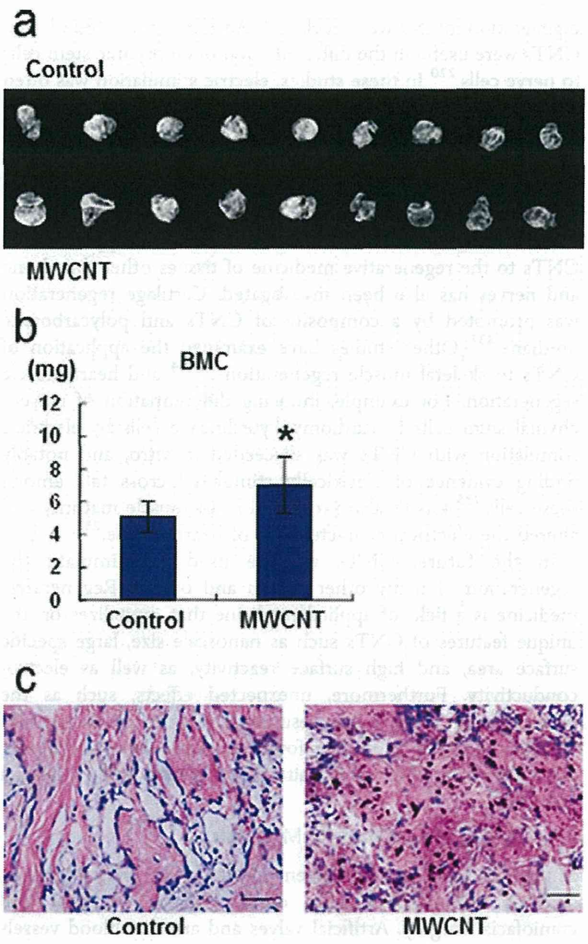

Figure 4. MWCNTs promote ectopic osteogenesis by rhBMP-2 and collagen. (a) A soft X-ray radiogram of newly formed bones extirpated 2 weeks after placement of rhBMP-2/collagen/MWCNT composite (upper lane) or rhBMP-2/collagen composite (lower lane) in mouse back muscle. Larger bones with more intense opacity were formed when using collagen conjugated with MWCNTs than without. (b) Bone mineral contents (BMCs) in bones formed at 2 weeks of implantation. A significantly higher BMC was observed in bones formed at 2 weeks of implantation of collagen conjugated with MWCNTs than without. Each error bar indicates the standard deviation of the mean ($n = 8$); asterisk, $P = 0.016$ between samples treated with carbon nanotubes and those without (unpaired Student's t test). (c) Histological images of bones extirpated at 2 weeks. The trabecula was thicker and denser when using collagen conjugated with MWCNTs than collagen alone. The tissue around the implanted collagen−MWCNT conjugate was found to have MWCNTs absorbed uniformly in the trabecula and bone marrow. The MWCNTs were seen to have entered the trabecula and came in direct contact with bone substrate. Hematoxylin-eosin staining. Scale bars = 100 mm. Reprinted with permission from ref 213. Copyright 2008 John Wiley & Sons, Inc.

2.3.3. Nerve Tissue Regeneration. Currently, brain injuries, spinal cord injuries, and large-gap peripheral nerve defects are intractable, and their treatment is an important goal of regenerative medicine. To enhance and stimulate the regeneration of these injured nerve cells and fibers, application of a wide variety of nerve conduits and synthetic guidance devices has been attempted but has failed to yield satisfactory results.[219] Applying CNTs is expected to lead to the development of new methods of nerve regenerative medicine and contribute to improvements in patient quality of life.[122,220−224]

Use of CNTs as a scaffold for neural cell growth has been vigorously studied for more than 10 years and found to be useful for neural cell adhesion and axonal growth.[117,225−227] CNTs promoted neurite elongation in a wide variety of cultured neurons.[228−230] CNTs were also reported to aid regeneration of Schwann cells.[231] Another study found that CNTs were useful in the differentiation of embryonic stem cells to nerve cells.[220] In these studies, electric stimulation was often used to promote neural cell growth, making the best use of the favorable electroconductivity of CNTs.[120] Regenerative medicine for nerve is an interesting research field that aims to apply in combination the electrical and mechanical properties of CNTs to biomaterials.

2.3.4. Regeneration of Other Tissues. The application of CNTs to the regenerative medicine of tissues other than bone and nerves has also been investigated. Cartilage regeneration was promoted by a composite of CNTs and polycarbonate urethane.[232] Other studies have examined the application of CNTs to skeletal muscle regeneration[233,234] and heart muscle regeneration. For example, inducing differentiation of mesenchymal stem cells to cardiomyocyte lineage cells by electrical stimulation with CNTs was succeeded in vitro, and notably finding evidence of electrically stimulated cross talk among these cells.[235] CNTs also promoted heart muscle maturity and altered the electrical characteristics of heart muscle.[236]

In the future, CNTs will be used to stimulate the regeneration of many other tissues and organs. Regenerative medicine is a field of applied medicine that capitalizes on the unique features of CNTs such as nanoscale size, large specific surface area, and high surface reactivity, as well as electroconductivity. Furthermore, unexpected effects, such as the promotion of osteogenesis resulting from the interactions of CNTs with the body, may be found in a wide variety of tissues, so regenerative medicine is quite an interesting field of applied research.

2.4. Application to Implant Materials

Implant technologies have been used in many clinical settings, such as orthopedic surgery, dental and oral surgery, and craniofacial surgery. Artificial valves and artificial blood vessels have been used in heart and other surgeries. These implants are required to possess, in addition to mechanical characteristics such as strength and durability, high biological compatibility because they come in direct contact with living tissue.[237,238] Many types of orthopedic implants, in particular, have long been used in clinical settings in many patients. Examples include artificial joints used to treat osteoarthritis and rheumatoid arthritis, plates and screws used to treat bone fractures, and cages and rods used for interbody fusion. Hence, many different materials are used in orthopedic implants.[239−241] Metals are used for bone fracture treatment and in artificial joints, including stainless steel, titanium alloys, cobalt−chromium, and tantalum. Ceramics (mostly alumina and zirconia ceramics) are used in artificial joints and artificial dental pulp. Ultrahigh molecular weight polyethylene (UHMWPE) is used in the sliding parts of artificial joints. Polyether ether ketone (PEEK) is often used for interbody fusion.

Since 2003, we have been working to conjugate CNTs to polyethylene for use in sliding parts and rotating parts of artificial joints.[58,69] The sliding parts of a polyethylene artificial joint wear away with long-term use, leading to the breakage of the artificial joint and necessitating revision surgery.[242−245] With this in mind, we are developing more durable artificial joints made of polyethylene and CNTs to reduce the amount of wear loss. The sliding parts of artificial joints are sometimes made of ceramic instead of polyethylene. Although ceramics are generally unlikely to wear, alumina ceramics break easily, and zirconia ceramics are liable to deform due to phase transition in vivo.[246,247] Hence, we are working to develop a new ceramic material (alumina ceramics combined with CNTs) that is unlikely to break down and deform.[248,249] Although many difficulties exist, including homogenously blending CNTs and ceramics, we have already obtained a blend with improved fracture toughness values. The number of patients undergoing artificial joint replacement surgery has been increasing each year worldwide; accordingly, the number of patients undergoing revision surgery is increasing steadily.[250] Clinical application of CNT-based artificial joints would dramatically reduce the number of patients undergoing revision surgery and allow use of artificial joints by young patients.

Furthermore, we are developing a CNT/PEEK composite for spinal fusion cages used in interbody fusion surgery. Spine interbody fusion cages of PEEK material have already been used clinically; however, poor bone compatibility poses an obstacle to the bonding of the implant and bone around it.[251,252] Hence, spine interbody fusion cages made of a CNT/PEEK composite of high bone compatibility are being developed by conjugating CNTs to PEEK, thereby utilizing the bone induction potential of CNTs described in section 2.3.2: Bone Tissue Regeneration. The development of these artificial joints and spine interbody fusion cages is further described in section 6.4.

In these composites, the CNT content ratio is up to 10 wt %, often about 5 wt %, with only a small amount of CNTs entering the body. Furthermore, because they are composite materials, there is little or no possibility that CNTs (that is particulate) will be directly exposed to living organisms. For this reason, CNT composites can be thought to be highly safe, with the reactions between CNT particles and living organism rarely posing a problem. In view of biological safety of CNT composites, we believe that the first application of CNTs should be in implants in the form of composites as described above.[253−255]

Taking into account the above-described utility of CNTs as a reinforcing material and their safety as composites, it is expected that a wide variety of CNT composite implants will be developed in the future. Although technically difficult, conjugating CNTs to metals and ceramics would produce great benefits. While this field has so far received only scant attention, we hope that more R&D effort will be directed to this field, where CNTs are most likely to find clinical applications.

2.5. Application to DDSs for Treatment of Noncancer Diseases

As stated in section 2.2.2: Drug Delivery Systems for Cancer Treatment, CNTs have large specific surface areas, possess high surface reactivity, and therefore can be conjugated with a wide variety of molecular species, including low-molecular-weight compounds, genes, proteins, and vaccines, in large amounts. In addition, because CNTs can be delivered to the small structures in living organisms, they are expected to act as an ideal DDS.[100,117,256−258] Research has recently been advancing rapidly toward the development of more useful CNT-based DDSs for various diseases. Many improvements have been made in the reactivity with the cell membrane, which is particularly important to DDS applications. For example, SWCNTs bound to an integrin monoclonal antibody were used to enhance their adhesion to cells.[114] Bonding of a bilayer-forming lipid to CNT surfaces was used to lessen the influence