Figure 11. continued

observed in the spleen of 1 of the 10 mice in the MWCNT group that survived for 26 weeks; this was identified as an inflammatory pseudotumor, not a neoplasm. (B) A neoplasm developed in a lung of 1 mouse in the carbon black group that survived for 26 weeks; this was diagnosed as a benign adenoma. (C) All 10 mice in the MNU group had tumors. Proventricular tumors developed in all 10 animals, with abnormal squamous epithelial growth observed (upper left panel). Skin tumors developed in 6 of the 10 animals, which occurred as malignant skin tumors in the thigh (upper right panel). Genital tumors developed in 4 animals (lower left panel). A thymic tumor developed in 1 animal (lower right panel). Hematoxylin-eosin staining. Scale bars = 10 μm. (c) Histological images of subcutaneous implantation sites taken at 26 weeks. (A) In the MWCNT group, no neoplasm developed, with macrophages found to have accumulated while phagocytosing MWCNT particles. No inflammatory cells such as neutrophils and lymphocytes were observed around the implantation sites. (B) In the carbon black group, like in the MWCNT group, macrophages phagocytosed MWCNT particles, with no neoplasm observed. Arrow, MWCNTs; arrowhead, carbon black. Hematoxylin-eosin staining. Scale bars = 10 μm. Reprinted with permission from ref 98. Copyright 2012 Nature Publishing Group.

Table 3. Neoplastic Changes in rasH2 Mice Implanted with CNT, Carbon Black, Solvent, or *N*-Methyl-*N*-nitrosourea (MNU) Solution[a]

		total number			
		control	carbon black	CNT	MNU
organ	diagnosis	10	10	10	10
skin (back area)	papilloma	0	0	0	2
	keratoacanthoma	0	0	0	0
skin (face)	papilloma	0	0	0	3
	keratoacanthoma	0	0	0	0
skin (thigh)	papilloma	0	0	0	1
	keratoacanthoma	0	0	0	0
spleen	inflammatory pseudotumor	0	0	1	0
	hemangioma	0	1	0	0
hematopoietic system	malignant lymphoma	0	0	0	2
	epithelial thymoma	0	0	0	0
kidneys	hemangioma	0	0	0	0
pancreas	hemangioma	0	0	0	0
lungs	adenocarcinoma	0	0	0	0
	adenoma	0	1	0	1
	hemangioma	0	0	0	0
forestomach	papilloma	0	0	0	10[b]
	basal cell tumor	0	0	0	0
	squamous cell carcinoma	0	0	0	0
perineal	papilloma	0	0	0	5[c]

[a]Adapted with permission from ref 98. Copyright 2012 Nature Publishing Group. [b]Significant differences at $p = 0.0000054125$ (Fisher's direct method). [c]Significant differences at $p = 0.016254$ (Fisher's direct method).

entering the bloodstream. MWCNTs above a certain size are thought to rarely enter the bloodstream.[310] Even if they enter from a local site, the concentration is expected to be too low to have a major impact on the body.[91,139,191,310]

In conclusion, on the basis of available in vivo data, it can be concluded that MWCNTs are likely to be useful topical biomaterials.[152] Of course, sites of intensive inflammatory reaction to MWCNTs and likely sites of MWCNTs entry into the bloodstream appear to depend on the organ and tissue where the biomaterials are implanted.[304] For this reason, investigations should be conducted for each site separately to determine the hazard to each organ and each tissue.

6.1.2. In Vitro Studies. The results from in vitro toxicity studies are difficult to interpret. Scientific international standards, typically ISO standards, have been established to assess the toxicity of bulk biomaterials,[467] but not nanoparticles like CNTs, which have distinct properties. In most recent studies, CNTs (adequately dispersed in solution) are regarded as chemical substances. This assumption seems to be reasonable for purposes of assessing the safety of particulate substances. In 2012, the Organization for Economic Co-operation and Development (OECD) announced, "Although most testing/assessing methods for conventional chemical substances are also suitable for nanomaterials, corrections according to the characteristics of nanomaterials may be needed in some cases."[493]

It should be noted that it is difficult to determine which of the many factors relevant to CNTs is being assessed in an assessment of in vitro CNTs toxicity. These factors include thickness, length, shape, surface reactivity, and aggregability as well as the influence of residual metals, dispersants, and assay reagents.[117,263,334] Because these factors are inter-related, their influences are difficult to determine separately.[494,495] Variation in the thickness and length of CNTs may be associated with their cytotoxicity. SWCNTs and MWCNTs differ in toxicity profiles; for example, SWCNTs are more likely than MWCNTs to induce oxidative stress. Length is also important; long CNTs are likely to induce oxidative stress because they are not completely phagocytosed by macrophages. The toxicity of inhaled CNTs increases with increase in length above 10–20 μm, although this observation has not been confirmed.[412] While the maximum acceptable length of CNT particles used as biomaterials is unknown, long CNTs are not considered to pose a problem (such as an inhalation problem), as stated in section 6.2. Surface reactivity also differs depending on the type of CNTs; furthermore, the toxicity of chemically modified CNTs should be thoroughly examined for each modification. Although the influence of residual metals is not negligible, many studies have found that CNTs with a quite high purity pose no major problem.[72,365,400−402,407,408,496] On the other hand, the influence of aggregability of CNTs and the choice of dispersant on toxicity do pose problems.[483] Many published studies are thought to have established the total toxicity of CNTs and dispersant.[371,373,385,497] Furthermore, because the aggregability of CNTs and the choice of dispersant vary widely among different studies, the range of CNT concentrations used in respective toxicity studies is wide (from 1 ng/mL to 10 μg/mL), making assessment more difficult.[309,447] From now on, in vitro studies should be conducted under a standard set of conditions (dispersant selected to not affect the test cells, and range of concentrations selected to avoid CNTs aggregation) whenever possible.

6.1.3. Correlations between in Vivo and in Vitro Data. For CNTs, unlike drugs and other chemical substances, little is known about correlations between in vivo and in vitro data.[498] One reason is that no in vivo data are available; nanoparticles

have not yet been used as biomaterials, and this issue cannot be resolved until clinical application is realized. Another reason is that there are no standard ways for dealing with a wide variety of factors that can influence the results of correlation studies, such as the animal species, method of administration used in the in vivo studies, and the choice of cell and culture broth used in the in vitro studies.[91] In the future, it will be necessary to ensure these factors are consistent for all studies, to collect and analyze data from international sources, and to determine the correlations between in vivo and in vitro toxicity assessments of CNTs, at least in small animals such as mice.[191]

6.2. Appropriate References for Safety Evaluation of CNTs

6.2.1. Requirements for References. Only a few articles on the safety of CNTs biomaterials were published before 2010, and these reported many conflicting results. However, as the number of articles increased, the number of safety evaluations increased, and the conclusion drawn from these results is that CNTs are safe to use as biomaterials.[89,91,99,191] Nevertheless, clinical application has been frustrated because researchers have been unable to rule out CNTs toxicity. This situation is due primarily to the lack of a best reference material for the evaluation of CNTs safety. The finding of such a reference would facilitate evaluation of CNTs safety.[68] CNTs biomaterials, like all other biomaterials, are foreign to living organisms irrespective of their biocompatibility. At concentrations exceeding a certain level, CNTs exhibit toxicity in vitro and in vivo. The absence of a reference with established safety makes it difficult to determine the in vivo safety of CNTs. For example, when test cells lose activity in the presence of CNTs at concentrations exceeding a certain level, it is wrong to conclude that the CNTs are cytotoxic. However, it is right to conclude that CNTs are not cytotoxic when test cells lose activity in the presence of a reference with established safety. Biological safety cannot be assessed without comparison to a reference that has been proven to be safe in living organisms as described above. However, unfortunately, no such reference has been found to evaluate the safety of CNTs biomaterials. Inevitably, CNT toxicity studies have yielded inconsistent results so that no safety evaluation has been regarded as reliable. The lack of a reference with confirmed biological safety is attributed to the traditional view that nanoparticulates are not biomaterials.

6.2.2. Carbon Black. We found a reference material (carbon black) and proposed its use as a reference in our research articles published in 2011 and 2012.[97,98] Carbon black is the primary component of black tattoo ink, and tattooing of the human body has a long history dating back before ancient times, and is currently commonly performed.[475] Some researchers may dispute the use of carbon black as a reference for CNTs because CNTs particles are fibrous and carbon black particles are spherical. It is reasonable to attach importance to this difference if the research focus is on inhalation toxicity. Cells are unable to completely absorb long fibrous nanoparticles. It has been hypothesized that oxidative stress "frustrates" phagocytosis, and prolongs inflammation and other events.[281,409,411,412] However, this merely accounts for prolonged inflammatory reactions to CNTs in the thoracic cavity, where inhalation exposure to CNTs occurs, and in the abdominal cavity (a surrogate for the thoracic cavity), where exposure to CNTs is experimentally mimicked. Many researchers have found that even when a considerable amount of CNTs is implanted subcutaneously and elsewhere, only transient, very mild inflammation develops and resolves quickly.[58,307−309] This fact suggests that no frustrated phagocytosis occurs at least in subcutaneous tissue. Hence, because no in vivo implantation study found that CNTs cause frustrated phagocytosis at sites of transient inflammation, it can be concluded that fibrous nanoparticles pose no risk. Generally, animal experiments have shown the improbability that sites of prolonged CNT-induced inflammation contain CNTs particles. Therefore, provided that appropriately designed in vivo implantation studies produce no evidence of prolonged inflammation, then fibrous CNTs can be used as biomaterials without safety concerns.

Essentially, sharing all of the characteristics of the test material is not the only requirement for a substance to be used as a reference. This is also true for bulk biomaterials; even with different characteristics, they have been used as references with satisfactory results for desired effects.[467] Importantly, both CNTs and carbon black belong to a new category of biomaterials known as nanoparticulate substances. Logically, as with bulk biomaterials, no problem arises from the use of carbon black as a reference material for CNTs. Carbon black (like CNTs) is a nanosized particulate substance, even though other characteristics may be different. Another advantage of carbon black as reference is that mass can be used as an index because both CNTs and carbon black are pure carbon particulates.[97,98] Mass is by far an easier index to use in toxicity studies than particle count and volume. In view of these facts, we propose the use of highly pure carbon black as a good reference material for CNTs.

Many articles are available on the use of carbon black as a reference for assessment of biological reactions to CNTs.[292,365,381,499−506] However, no clear evidence has been presented supporting the claim that carbon black is safe to use in living organisms. According to many researchers who have used carbon black as a reference for CNTs, carbon black is intuitively the best reference. We have verified the scientific intuition of many researchers by providing them with a rationale (i.e., carbon black is safe because it is a component of black tattoo ink). Because there are a large number of such articles, we believe that many researchers will agree with the conclusion of this Review that carbon black is suitable as a reference material for safety evaluation of CNTs.

6.2.3. International Standards. What should happen soon after a consensus is reached that carbon black is suitable for use as a reference material for CNTs? The safety of CNTs should be evaluated both in vivo and in vitro using the new reference material. As stated above, it is easy to say, "Risk may exist", but it is difficult to say, "No risk exists". It is necessary for as many researchers as possible to conduct as many studies as possible. All studies then need to use standardized carbon black as a reference to allow the results to be assessed collectively and comprehensively compared.

There are many types of carbon black with somewhat variable biological safety. Above all, highly pure carbon black (a suspension of nanoparticles, which is equivalent to the carbon black used in tattoo ink) can be used as a reference for CNTs.[475] At present, we think that carbon black particles (diameter of about 50 nm and a purity of 99.5% or more) are suitable, but we would like to suggest here that many experts discuss extensively, choose, and designate the best carbon black powder as the international standard.

Special attention should be paid to the carbon black dispersant (usually a surfactant) because CNTs particles in

Table 4. Stages of Clinical Application of CNT-Based Biomaterials[a]

atage	nature of the biomaterial	site of use	degree of in vivo exposure	risk	example of use
stage 1	composite	topical	none/low	none/low	artificial joints and interbody fusion materials
stage 2	particulate	topical	intermediate	low/intermediate	DDSs and imaging for cancer treatment
stage 3	particulate	topical	intermediate	low/intermediate	regenerative medicine scaffolds and DDS for topical treatments
stage 4[b]	particulate	systemic	high	high	DDSs and imaging that circulate via bloodstream

[a]Clinical application of CNTs to biomaterials should progress demonstrating the safety at each stage. [b]The decision of proceeding to stage 4 requires extremely careful consideration.

the test solution are less dispersible than carbon black particles.[366−371] The same dispersant should be used in both the CNTs and the reference solutions, provided the dispersant (when used at concentrations that fully disperse the CNTs and reference particles) has no major impact on living organisms and cells. In vitro, in particular, particles precipitate over time, which can alter the cellular reactions depending on the precipitation rate. To ensure a valid comparison with the reference, it is desirable to use a dispersant that minimizes precipitation of particles. At present, we think polyvinyl alcohol is the best dispersant.[97] However, there may be better dispersants with higher dispersion efficacy, lower toxicity, greater ease of handling, and other superior characteristics; therefore, an internationally acceptable dispersant should be chosen after much discussion on the basis of a consensus of expert opinions.

6.2.4. Method of Safety Evaluation. CNTs with equal or less toxicity than that of the reference carbon black should be considered "safe". This judgment can be made without further research. If the toxicity of CNTs is found to be greater than that of carbon black, the decision should be deferred. Strictly speaking, further assessment is impossible because carbon black is the only currently available reference. Particular attention should be paid if toxicity is far greater than that of carbon black (e.g., toxic concentrations one-tenth of the reference concentration). On the other hand, if neither CNTs nor carbon black is toxic, CNTs should be considered nontoxic, or the study conditions should be considered inappropriate. We propose to collect safety data through various evaluations of CNTs using carbon black as a common reference, while paying attention to these facts.

Unfortunately, carbon black cannot be used as a reference material in the assessment of in vivo kinetics because particle shape affects localized nanoparticle accumulation and nanoparticle migration from tissue to bloodstream.[327] As compared to CNTs particles (that are fibrous), carbon black particles (that are spherical) migrate more readily between tissues and the bloodstream. However, a reference is not needed to track the in vivo migration of CNTs. If CNTs accumulate in a certain organ, a study of CNT implantation may be conducted to assess the biological reactions at the site using carbon black as a reference.

6.3. Decision To Start Clinical Application of CNT-Based Biomaterials

As stated above, many researchers have shown that pristine (very pure) MWCNTs with few failures as biomaterials are very safe to use as biomaterials. MWCNTs are safe to use topically but not at special sites such as the lung and abdominal cavity.[91,191,305,306] The safety of using MWCNTs as DDSs or the like and involving access to the bloodstream has not yet been verified. Furthermore, using tattoo carbon black as a reference, we showed that pristine MWCNTs are at least as safe as carbon black.[97,98]

Because the above-described remarkable advances in research into the application of CNTs as biomaterials have led to the judgment that CNTs biomaterials are probably very safe (provided the method and site of use are appropriate), now is a time to start using CNTs clinically. We are planning to clinically apply MWCNTs (carbon purity, of 99.5% or more; mean diameter, about 60 nm [40−90 nm]; mean length, about 10 μm; and specific surface area, 25−30 m^2/g; produced using the chemical vapor deposition technique [MWNT-7, Hodogaya Chemical, Tokyo, Japan]). Of course, a composite material containing 5 wt % or less of MWCNTs (the safest form of CNTs) will be used.

6.4. Path to Clinical Application of CNT-Based Biomaterials

Importantly, we will begin with the safest clinical application of CNTs and proceed in steps according to the magnitude of risk involved. We divided the time course into four stages differing in degree of risk estimated on the basis of the nature of the biomaterial (composite versus particulate), the site of use (topical versus systemic), and the degree of in vivo exposure to the particles (high versus low) (Table 4).

The first stage is characterized by the use of a CNTs composite material for implantation (stage 1). Generally, the CNT content in a composite material is not more than 10 wt %, and the likelihood of in vivo exposure to CNTs particles is zero or minimal. Therefore, problems due to CNTs in the human body are unlikely to occur. As the first biological application of CNTs, we are planning to use composites of MWCNTs with existing biomaterials in artificial joints or spine interbody fusion materials.

In application to artificial joints, we are developing an MWCNT/polyethylene composite material and an MWCNT/ceramics composite material. Although the polyethylene used in sliding parts of artificial joints is ultrahigh molecular weight polyethylene (UHMWPE), it wears during long-term use and can necessitate resurgery.[242−245,507] For this reason, cross-linked UHMWPE has become commonly used, although its excessive hardness and easy breakability are problematic.[508−512] Having favorable characteristics that are absent in conventional materials, that is, high wear resistance and low breakability, MWCNT-conjugated UHMWPE is suitable as a sliding parts material for artificial joints (Figure 12). On the other hand, ceramics are also used in the sliding parts of artificial joints. Although ceramics wear very slightly, they are breakable so that resurgery is sometimes needed.[513−518] Combining CNTs with ceramics increases fracture toughness and can transform ceramics into an ideal, wear-free, antifracture, sliding parts material for artificial joints.

To improve the quality of interbody fusion material, we are now engaged in developing an MWCNT/PEEK composite. PEEK is a highly biocompatible material possessing excellent

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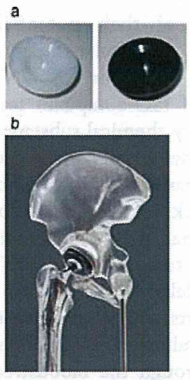

Figure 12. For application to sliding parts of artificial joints, an ultrahigh molecular weight polyethylene (UHMWPE) conjugated with MWCNTs has been developed. (a) A UHMWPE socket (left panel) and an MWCNT-conjugated UHMWP socket (right panel) for use in sliding parts of artificial joints. (b) A prototype artificial joint with a socket made of CNTs. Having favorable characteristics that have not been achieved with conventional materials, that is, high wear resistance and low breakability, MWCNT-conjugated UHMWPE is suitable as a sliding parts material for artificial joints.

biological safety and mechanical characteristics.[519,520] Because of its low compatibility for bone tissue, however, PEEK has been associated with the problem of insufficient bone union when used in implants that are directly exposed to bone, such as interbody fusion cages.[521−524] MWCNTs have been reported by many research teams, including ours, to possess bone induction potential.[58,62,63,67,213,215,216,218,525,526] If conjugation with MWCNTs further improves the mechanical characteristics of PEEK and also induces osteogenesis, then MWCNT/PEEK composite will become an ideal interbody fusion material (Figure 13).

In 2012, the European Commission (EC) announced a draft regulation as amended to oblige manufacturers of medical equipment used to make nanomaterial-containing products, to properly label medical devices containing nanomaterials categorized under Class III (most dangerous substances). This rule shall apply only in cases where such medical devices are used for the intended purposes and in the absence of measures (such as encapsulation and coupling) to prevent nanomaterials from entering the patient's body and the user's body.[527] Hence, use of CNTs composites as biomaterials may not be subject to legal regulations because they are bound to the base material. For this reason, we believe that stage 1 poses only a minimal safety risk and can be safely implemented, provided the appropriate legal procedures of each country are followed.

In stage 2, CNTs particles are used within the body. This stage represents the first high barrier to clinical application of CNTs because nanoparticulate substances come into direct contact with the body. This usage is subject to legal regulations according to the definition of the EC, and is thought to require international approval from an ethical viewpoint as well. Hence, research activities cannot proceed to this stage until an extensive assessment is performed following the establishment of international standards for evaluation of biosafety. Initially, the use of CNTs must be limited to localized sites. Furthermore, top priority should be given to the use of CNTs in situations where the benefits from their use by far outweigh the risks involved. Specifically, the most likely field

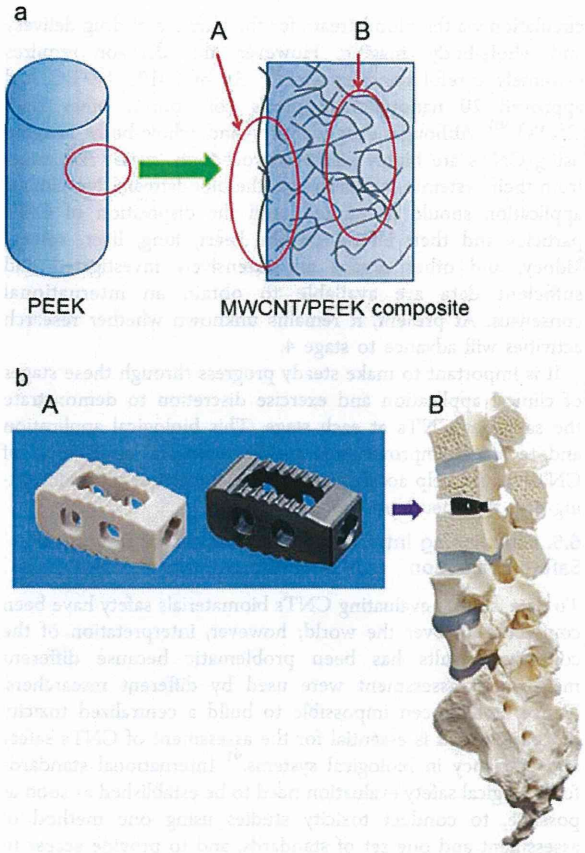

Figure 13. For application to spine interbody fusion material, a polyetheretherketone (PEEK) composite with MWCNTs has been developed. (a) A conceptual diagram showing that PEEK, when conjugated with MWCNTs, will become an innovative spine interbody fusion material possessing excellent mechanical characteristics and bone compatibility. (A) The MWCNTs on the surface confer bone compatibility. (B) The internally conjugated MWCNTs control the elastic modulus. (b) (A) A PEEK spine interbody fusion cage (left panel) and an MWCNT-conjugated PEEK cage (right panel). (B) A prototype interbody fusion cage made of CNTs.

appears to be cancer treatment, where no other treatment is available or treatment with CNTs is highly advantageous over other treatments. This is currently the most hopeful field of clinical application of CNTs. It is evident that if CNTs become applicable to DDSs and imaging for cancer treatment, dramatic advances in the treatment and diagnosis of cancer will be achieved, which is expected to contribute substantially to the health and welfare of many patients.

Stage 3 also concerns the topical use of CNTs particles as in stage 2, but the coverage is expanded to include the treatment of diseases requiring higher safety than in stage 2. CNTs are used clinically in topical treatments (including regenerative medicine scaffolds) and for the treatment and diagnosis of diseases that are less life-threatening than cancer, such as diabetes mellitus. In this stage, coverage of target diseases and use sites is much wider, and application of CNTs biomaterials more common. Stages 2 and 3 involve the same level of risk but have different benefits.

Finally, we will proceed to stage 4 aimed at the treatment of diseases involving the injection of CNTs and their systemic

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circulation via the bloodstream for the purpose of drug delivery and whole-body imaging. However, this decision requires extremely careful consideration.[327] As of 2012, the EC had approved 20 nanopharmaceuticals (of course, other than CNTs).[301] Although drug delivery and whole-body imaging using CNTs are highly effective procedures, major risk arises from their systemic circulation via the bloodstream. No clinical application should be started until the disposition of CNT particles and their effects on the heart, lung, liver, spleen, kidney, and other organs are extensively investigated and sufficient data are available to obtain an international consensus. At present, it remains unknown whether research activities will advance to stage 4.

It is important to make steady progress through these stages of clinical application and exercise discretion to demonstrate the safety of CNTs at each stage. This biological application and technical improvements in the biological application of CNTs would help accelerate the development of groundbreaking new therapeutic methods.

6.5. Establishing International Standards for Biological Safety Evaluation

To date, studies evaluating CNTs biomaterials safety have been conducted all over the world; however, interpretation of the collective results has been problematic because different methods of assessment were used by different researchers. Hence, it has been impossible to build a centralized toxicity database, which is essential for the assessment of CNTs safety and efficiency in biological systems.[91] International standards for biological safety evaluation need to be established as soon as possible, to conduct toxicity studies using one method of assessment and one set of standards, and to provide access to all results internationally. By doing so, many reliable results from all over the world can be analyzed by many experts, allowing them to make the right consensus decision. There are a great many types of CNTs and numerous derivatives produced by chemical modification. To achieve safe clinical application of these CNTs as soon as possible, there is an urgent need to establish international standards for the evaluation of biosafety.[70,191]

In the biological application of CNTs, it is critical to evaluate the safety of functionalized CNTs (f-CNTs), which are likely to find application as DDSs, for in vivo imaging, and in regenerative medicine scaffolds. Chemical modification is also important to increase the dispersion efficacy of CNTs, a key to successful biological application.[331] Of course, f-CNTs must be examined for safety individually. Furthermore, some researchers are working to functionalize CNTs to make them safer to living organisms.[257,334,528] To facilitate the application of numerous f-CNTs as biomaterials, it is of paramount importance to establish international standards for safety evaluation.

Provided that criteria are logically formulated on the basis of the published results from studies evaluating the safety of CNTs biomaterials, international standardization of the CNTs safety evaluation methodology would not be difficult. The first task is to establish standards for the topical use of CNTs. Specifically, in vivo and in vitro studies should first be conducted in the same manner as with ISO-standardized ordinary bulk biomaterials to assess the toxicity resulting from the dissolution of impurities contained in CNTs and some or all of the molecules bound to the CNTs. In vivo studies then should be conducted to assess the CNTs toxicity intrinsic to their identity as nanoparticles. This involves implantation of CNTs at the sites of their potential use to determine biocompatibility with a particular organ or tissue. The in vitro studies involve the dispersion of CNTs with a standard dispersant and use of ISO-compliant test methods similar to those used for ordinary chemical substances.[467] The in vivo and in vitro studies for determination of the intrinsic toxicity of CNTs involve comparison with a nanoparticulate reference material, carbon black as described above. With a standard reference, international standards for the evaluation of the biological safety of topically used CNTs particles can be established without delay.

Subsequently, efforts will be made to establish international standards for the evaluation of CNTs safety in applications involving passage through the bloodstream. Basically, in vivo studies on CNTs well dispersed in solution will be conducted using the same criteria as those used for ordinary chemical substances. However, it is unknown which substance (possibly an existing nanoparticulate material already used clinically in DDSs and possibly transported through the bloodstream, with confirmed safety and properties similar to those of CNTs) will be the appropriate reference material. Selection of a reference for this application of CNTs, which circulate in the bloodstream, is a major challenge to be tackled in the future.

In all cases, international standards for the evaluation of CNTs biosafety need to be established as soon as possible because ultimately CNTs will revolutionize cancer treatment and regenerative medicine, which are top priorities in today's medicine. Now is the time to translate research on safe CNT composite implants into clinical applications. International standards for evaluation of CNTs biosafety must be established to enable the topical use of CNTs particles. Research into any important medical issue should always proceed without interruption.

7. CONCLUSION

The study of the application of CNTs as biomaterials has been increasing dramatically because CNTs have been shown to be extremely effective and very safe biomaterials. Biomaterials that have doubtful biosafety are unlikely to find clinical application in the future. Although it is logically impossible to say that CNTs are completely safe to use in living organisms, CNTs can be judged to be extremely safe if no evidence of biological risk has been obtained by a vast number of studies investigating their biological application. Most researchers in this field think CNTs are safe to use in living organisms, provided that the appropriate method and site of delivery are used.

CNTs biomaterials if fully utilized could lead to many revolutionary and important medical technologies. Because of the extremely advantageous characteristics unique to CNTs, the biological safety evaluation issue making us reluctant to start their clinical application must be solved as soon as possible.

Thanks to the painstaking efforts of a great many researchers, much evidence supports the claim that CNTs are generally safe as biomaterials. Accordingly, now is the time to start clinical application of CNT composite implants, the biologically safest form of CNTs, because there is little possibility that CNTs will be directly exposed to the living organism. To quickly proceed topical use of CNTs particles, it is necessary for researchers to establish international standards for biosafety evaluation as soon as possible. In this process, the carbon black reference will play an important role. When taking the next and most risky step toward clinical application (that involves the entry of

CNTs into the circulation), the utmost caution must be exercised to ensure safe use.

Because many researchers can now evaluate the biosafety of CNTs using the power of the latest science and technology, we should now embark on a journey toward the clinical use of CNT-based biomaterials in an ethical and courageous manner.

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Notes

The authors declare the following competing financial interest(s): As an employee of a medical device development company, Naoyuki Nishimura may benefit financially from participation in this study in the future. No other authors have conflicts of interest.

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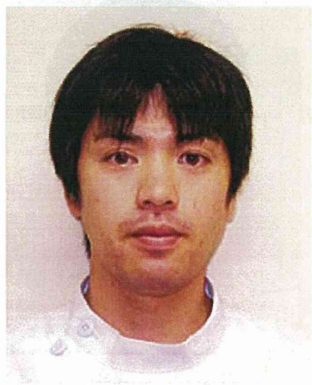

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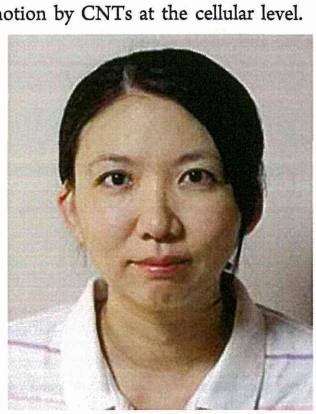
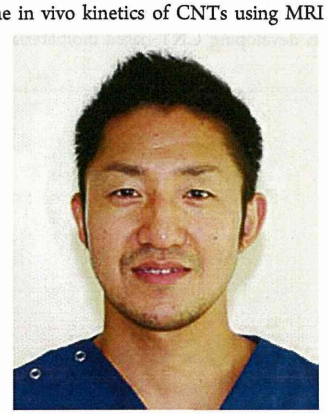


Nobuyo Narita is a research associate in the Department of Orthopaedic Surgery, Shinshu University School of Medicine. She specializes in lower limb surgery, serving as a leader of the foot surgery team at Shinshu University Hospital. She demonstrated using intracellular signals that CNTs suppress the function of osteoclasts.

Masanori Okamoto is a researcher at the Shinshu University School of Medicine, and is a medical doctor in the Department of Orthopaedic Surgery. He is now studying bone metabolism at Matsumoto Dental University and has a special interest in the action of Wnt5 in osteoblasts.

Shinsuke Kobayashi is a researcher at the Shinshu University School of Medicine. He specializes in safety evaluation of nanomaterials, and is investigating the in vivo kinetics of CNTs using MRI and CT.



Hiroki Nomura is a researcher at the Shinshu University School of Medicine. As a member of the research and development team for application of CNT-conjugated polyethylene to artificial joints, he is engaged in evaluating the biological safety of CNTs and their composites.

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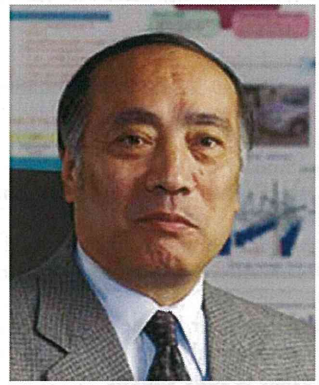
Hiroyuki Kato is a professor in the Department of Orthopaedic Surgery, Shinshu University School of Medicine. He specializes in hand surgery and conducts research on nerves and tendons. He provides research and clinical practice training to many physicians in the Department of Orthopaedics at Shinshu University Hospital.

Naoyuki Nishimura is the director of the R&D Center, Nakashima Medical Co. Ltd. He is developing artificial joints and spinal fixation devices made of CNT composites jointly with Shinshu University. He investigates safety and regulatory science with the aim of clinical application of CNT composites.

Seiichi Taruta is a professor in the Faculty of Engineering, Shinshu University. He is working on developing CNT-conjugate ceramics and bioactive ceramics. He is also a member of a medicine-engineering collaboration research project to develop CNT-based artificial joints.



Morinobu Endo is a distinguished professor in the Faculty of Engineering, Shinshu University. As the discoverer of the manufacturing process for CNTs using a chemical vapor deposition approach, he has been a world leader in CNTs research. As head of the Research Center for Exotic Nanocarbons, he currently conducts research on multitargeted application of nanocarbons.

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ABBREVIATIONS

AFP	α-fetoprotein
AIST	National Institute of Advanced Industrial Science and Technology
ALP	alkaline phosphatase
APC	antigen-presenting cell
bmDC	bone marrow-derived dendritic cell
CA19-9	carbohydrate antigen 19-9
CEA	carcinoembryonic antigen
CNS	central nervous system
CNT	carbon nanotube
CVD	chemical vapor deposition
DDS	drug delivery system
DTPA	diethylenetriaminepentaacetic acid
DTPA	diethylenetriaminepentaacetic acid
EDS	energy dispersive X-ray spectroscopy
ELISA	enzyme-linked immunosorbent assay
ES cell	embryonic stem cell
f-CNT	functionalized-CNT
GEM	gemcitabine
IC_{50}	half maximal inhibitory concentration

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IL	interleukin
iPS cell	induced pluripotent stem cell
ISO	International Standards Organization
LPS	lipopolysaccharide
MNU	*N*-methyl-*N*-nitrosourea
MWCNT	multiwalled CNT
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
OECD	Organization for Economic Co-operation and Development
PEEK	polyether ether ketone
PEG	polyethylene glycol
PSA	prostate specific antigen
rhBMP-2	recombinant bone morphogenetic protein-2
ROS	reactive oxygen species
SEM	scanning electron microscopy
siRNA	short interference RNA
SWCNT	one layer is known as single-walled CNT
TCB-1	Tattoo carbon black-1
TCB-2	Tattoo carbon black-2
TEM	transmission electron microscopy
TNF	tumor necrosis factor
UHMWPE	ultrahigh molecular weight polyethylene
ZDBC	zinc dibutyldithiocarbamate

REFERENCES

(1) Oberlin, A.; Endo, M.; Koyama, T. *J. Cryst. Growth* **1976**, *32*, 19.
(2) Iijima, S. *Nature* **1991**, *354*, 56.
(3) Van Noorden, R. *Nature* **2011**, *469*, 14.
(4) Aliev, A. E.; Oh, J.; Kozlov, M. E.; Kuznetsov, A. A.; Fang, S.; Fonseca, A. F.; Ovalle, R.; Lima, M. D.; Haque, M. H.; Gartstein, Y. N.; Zhang, M.; Zakhidov, A. A.; Baughman, R. H. *Science* **2009**, *323*, 1575.
(5) Toma, F. M.; Sartorel, A.; Iurlo, M.; Carraro, M.; Parisse, P.; Maccato, C.; Rapino, S.; Gonzalez, B. R.; Amenitsch, H.; Da Ros, T.; Casalis, L.; Goldoni, A.; Marcaccio, M.; Scorrano, G.; Scoles, G.; Paolucci, F.; Prato, M.; Bonchio, M. *Nat. Chem.* **2010**, *2*, 826.
(6) Foroughi, J.; Spinks, G. M.; Wallace, G. G.; Oh, J.; Kozlov, M. E.; Fang, S.; Mirfakhrai, T.; Madden, J. D.; Shin, M. K.; Kim, S. J.; Baughman, R. H. *Science* **2011**, *334*, 494.
(7) Kreupl, F. *Nature* **2012**, *484*, 321.
(8) Behabtu, N.; Young, C. C.; Tsentalovich, D. E.; Kleinerman, O.; Wang, X.; Ma, A. W.; Bengio, E. A.; ter Waarbeek, R. F.; de Jong, J. J.; Hoogerwerf, R. E.; Fairchild, S. B.; Ferguson, J. B.; Maruyama, B.; Kono, J.; Talmon, Y.; Cohen, Y.; Otto, M. J.; Pasquali, M. *Science* **2013**, *339*, 182.
(9) Cao, Q.; Han, S. J.; Tulevski, G. S.; Zhu, Y.; Lu, D. D.; Haensch, W. *Nat. Nanotechnol.* **2013**, *8*, 180.
(10) De Volder, M. F.; Tawfick, S. H.; Baughman, R. H.; Hart, A. J. *Science* **2013**, *339*, 535.
(11) Ganzhorn, M.; Klyatskaya, S.; Ruben, M.; Wernsdorfer, W. *Nat. Nanotechnol.* **2013**, *8*, 165.
(12) Baughman, R. H.; Zakhidov, A. A.; de Heer, W. A. *Science* **2002**, *297*, 787.
(13) Kashiwagi, T.; Du, F.; Douglas, J. F.; Winey, K. I.; Harris, R. H., Jr.; Shields, J. R. *Nat. Mater.* **2005**, *4*, 928.
(14) Coleman, J. N.; Khan, U.; Blau, W. J.; Gun'ko, Y. K. *Carbon* **2006**, *44*, 1624.
(15) Veedu, V. P.; Cao, A.; Li, X.; Ma, K.; Soldano, C.; Kar, S.; Ajayan, P. M.; Ghasemi-Nejhad, M. N. *Nat. Mater.* **2006**, *5*, 457.
(16) Peng, B.; Locascio, M.; Zapol, P.; Li, S.; Mielke, S. L.; Schatz, G. C.; Espinosa, H. D. *Nat. Nanotechnol.* **2008**, *3*, 626.
(17) Qu, L.; Dai, L.; Stone, M.; Xia, Z.; Wang, Z. L. *Science* **2008**, *322*, 238.
(18) Shannon, M. A.; Bohn, P. W.; Elimelech, M.; Georgiadis, J. G.; Mariñas, B. J.; Mayes, A. M. *Nature* **2008**, *452*, 301.
(19) Bauhofer, W.; Kovacs, J. Z. *Compos. Sci. Technol.* **2009**, *69*, 1486.
(20) Chou, T. W.; Gao, L.; Thostenson, E. T.; Zhang, Z.; Byun, J. H. *Compos. Sci. Technol.* **2010**, *70*, 1.
(21) Xu, M.; Futaba, D. N.; Yamada, T.; Yumura, M.; Hata, K. *Science* **2010**, *330*, 1364.
(22) Bakshi, S. R.; Agarwal, A. *Carbon* **2011**, *49*, 533.
(23) Xiong, F.; Liao, A. D.; Estrada, D.; Pop, E. *Science* **2011**, *332*, 568.
(24) Endo, M.; Kim, Y. A.; Hayashi, T.; Nishimura, K.; Matusita, T.; Miyashita, K.; Dresselhaus, M. S. *Carbon* **2001**, *39*, 1287.
(25) Kang, S. J.; Kocabas, C.; Ozel, T.; Shim, M.; Pimparkar, N.; Alam, M. A.; Rotkin, S. V.; Rogers, J. A. *Nat. Nanotechnol.* **2007**, *2*, 230.
(26) Scrosati, B. *Nat. Nanotechnol.* **2007**, *2*, 598.
(27) Sotowa, C.; Origi, G.; Takeuchi, M.; Nishimura, Y.; Takeuchi, K.; Jang, I. Y.; Kim, Y. J.; Hayashi, T.; Kim, Y. A.; Endo, M.; Dresselhaus, M. S. *ChemSusChem* **2008**, *1*, 911.
(28) Lima, M. D.; Fang, S.; Lepro, X.; Lewis, C.; Ovalle-Robles, R.; Carretero-González, J.; Castillo-Martinez, E.; Kozlov, M. E.; Oh, J.; Rawat, N.; Haines, C. S.; Haque, M. H.; Aare, V.; Stoughton, S.; Zakhidov, A. A.; Baughman, R. H. *Science* **2011**, *331*, 51.
(29) Dai, L.; Chang, D. W.; Baek, J. B.; Lu, W. *Small* **2012**, *8*, 1130.
(30) Evanoff, K.; Khan, J.; Balandin, A. A.; Magasinski, A.; Ready, W. J.; Fuller, T. F.; Yushin, G. *Adv. Mater.* **2012**, *24*, 533.
(31) Pugno, N. M.; Bosia, F.; Carpinteri, A. *Small* **2008**, *4*, 1044.
(32) Byrne, M. T.; Gun'ko, Y. K. *Adv. Mater.* **2010**, *22*, 1672.
(33) Huang, W. M. *Modern Mater. Trends* **2010**, *2*, 9.
(34) Tan, D.; Zhang, Q. In *Future Computer, Communication, Control and Automation*; Zhang, T., Ed.; Springer: Berlin, 2012; AISC *119*, pp 137–146.
(35) Choudhary, V.; Gupta, A. In *Carbon Nanotubes - Polymer Nanocomposites*; Yellampalli, S., Ed.; InTech: Shanghai, 2011; pp 65–90.
(36) Tans, S. J.; Verschueren, A. R. M.; Dekker, C. *Nature* **1998**, *393*, 49.
(37) Rueckes, T.; Kim, K.; Joselevich, E.; Tseng, G. Y.; Cheung, C. L.; Lieber, C. M. *Science* **2000**, *289*, 94.
(38) Wu, Z.; Chen, Z.; Du, X.; Logan, J. M.; Sippel, J.; Nikolou, M.; Kamaras, K.; Reynolds, J. R.; Tanner, D. B.; Hebard, A. F.; Rinzler, A. G. *Science* **2004**, *305*, 1273.
(39) Jensen, K.; Weldon, J.; Garcia, H.; Zettl, A. *Nano Lett.* **2007**, *7*, 3508.
(40) De, S.; King, P. J.; Lyons, P. E.; Khan, U.; Coleman, J. N. *ACS Nano* **2010**, *4*, 7064.
(41) Ionescu, A. M.; Riel, H. *Nature* **2011**, *479*, 329.
(42) McCarthy, M. A.; Liu, B.; Donoghue, E. P.; Kravchenko, I.; Kim, D. Y.; So, F.; Rinzler, A. G. *Science* **2011**, *332*, 570.
(43) Sun, D. M.; Timmermans, M. Y.; Tian, Y.; Nasibulin, A. G.; Kauppinen, E. I.; Kishimoto, S.; Mizutani, T.; Ohno, Y. *Nat. Nanotechnol.* **2011**, *6*, 156.
(44) Franklin, A. D.; Luisier, M.; Han, S. J.; Tulevski, G.; Breslin, C. M.; Gignac, L.; Lundstrom, M. S.; Haensch, W. *Nano Lett.* **2012**, *12*, 758.
(45) Park, H.; Afzali, A.; Han, S. J.; Tulevski, G. S.; Franklin, A. D.; Tersoff, J.; Hannon, J. B.; Haensch, W. *Nat. Nanotechnol.* **2012**, *7*, 787.
(46) Matsumoto, T.; Komatsu, T.; Arai, K.; Yamazaki, T.; Kijima, M.; Shimizu, H.; Takasawa, Y.; Nakamura, J. *Chem. Commun. (Cambridge, U.K.)* **2004**, *7*, 840.
(47) Holt, J. K.; Park, H. G.; Wang, Y.; Stadermann, M.; Artyukhin, A. B.; Grigoropoulos, C. P.; Noy, A.; Bakajin, O. *Science* **2006**, *312*, 1034.
(48) Vaezzadeh, M.; Saeedi, M. R.; Barghi, T.; Sadeghi, M. R. *Chem. Cent. J.* **2007**, *1*, 22.
(49) Gabor, N. M.; Zhong, Z.; Bosnick, K.; Park, J.; McEuen, P. L. *Science* **2009**, *325*, 1367.

(50) Le Goff, A.; Artero, V.; Jousselme, B.; Tran, P. D.; Guillet, N.; Métayé, R.; Fihri, A.; Palacin, S.; Fontecave, M. *Science* **2009**, *326*, 1384.
(51) Jain, R. M.; Howden, R.; Tvrdy, K.; Shimizu, S.; Hilmer, A. J.; McNicholas, T. P.; Gleason, K. K.; Strano, M. S. *Adv. Mater.* **2012**, *24*, 4436.
(52) Calkins, J. O.; Umasankar, Y.; O'Neill, H.; Ramasamy, R. P. *Energy Environ. Sci.* **2013**, *6*, 1891.
(53) Shi Kam, N. W.; Jessop, T. C.; Wender, P. A.; Dai, H. *J. Am. Chem. Soc.* **2004**, *126*, 6850.
(54) Demming, A. *Nanotechnology* **2011**, *22*, 260201.
(55) Yang, F.; Jin, C.; Yang, D.; Jiang, Y.; Li, J.; Di, Y.; Hu, J.; Wang, C.; Ni, Q.; Fu, D. *Eur. J. Cancer* **2011**, *47*, 1873.
(56) Murakami, T.; Nakatsuji, H.; Inada, M.; Matoba, Y.; Umeyama, T.; Tsujimoto, M.; Isoda, S.; Hashida, M.; Imahori, H. *J. Am. Chem. Soc.* **2012**, *134*, 17862.
(57) Antaris, A. L.; Robinson, J. T.; Yaghi, O. K.; Hong, G.; Diao, S.; Luong, R.; Dai, H. *ACS Nano* **2013**, *7*, 3644.
(58) Saito, N.; Usui, Y.; Aoki, K.; Narita, N.; Shimizu, M.; Ogiwara, N.; Nakamura, K.; Ishigaki, N.; Kato, H.; Taruta, S.; Endo, M. *Curr. Med. Chem.* **2008**, *15*, 523.
(59) Jin, G. Z.; Kim, M.; Shin, U. S.; Kim, H. W. *Neurosci. Lett.* **2011**, *501*, 10.
(60) Shokrgozar, M. A.; Mottaghitalab, F.; Mottaghitalab, V.; Farokhi, M. *J. Biomed. Nanotechnol.* **2011**, *7*, 276.
(61) Lee, S.; Hahm, M. G.; Vajtai, R.; Hashim, D. P.; Thurakitseree, T.; Chipara, A. C.; Ajayan, P. M.; Hafner, J. H. *Adv. Mater.* **2012**, *24*, 5261.
(62) Cheng, Q.; Rutledge, K.; Jabbarzadeh, E. *Ann. Biomed. Eng.* **2013**, *41*, 904.
(63) Dorj, B.; Won, J. E.; Kim, J. H.; Choi, S. J.; Shin, U. S.; Kim, H. W. *J. Biomed. Mater. Res. A* **2013**, *101*, 1670.
(64) Kealley, C. S.; Latella, B. A.; van Riessen, A.; van Elcombe, M. M.; Ben-Nissan, B. *J. Nanosci. Nanotechnol.* **2008**, *8*, 3936.
(65) Joshi, B.; Gupta, S.; Kalra, N.; Gudyka, R.; Santhanam, K. S. *J. Nanosci. Nanotechnol.* **2010**, *10*, 3799.
(66) Lee, H. H.; Sang Shin, U.; Lee, J. H.; Kim, H. W. *J. Biomed. Mater. Res., Part B* **2011**, *98B*, 246.
(67) Wang, M.; Castro, N. J.; Li, J.; Keidar, M.; Zhang, L. G. *J. Nanosci. Nanotechnol.* **2012**, *12*, 7692.
(68) Kostarelos, K.; Bianco, A.; Prato, M. *Nat. Nanotechnol.* **2009**, *4*, 627.
(69) Saito, N.; Usui, Y.; Aoki, K.; Narita, N.; Shimizu, M.; Hara, K.; Ogiwara, N.; Nakamura, K.; Ishigaki, N.; Kato, H.; Taruta, S.; Endo, M. *Chem. Soc. Rev.* **2009**, *38*, 1897.
(70) Ciofani, G.; Raffa, V.; Vittorio, O.; Cuschieri, A.; Pizzorusso, T.; Costa, M.; Bardi, G. *Methods Mol. Biol.* **2010**, *625*, 67.
(71) Rodriguez-Fernandez, L.; Valiente, R.; Gonzalez, J.; Villegas, J. C.; Fanarraga, M. L. *ACS Nano* **2012**, *6*, 6614.
(72) Lacerda, L.; Bianco, A.; Prato, M.; Kostarelos, K. *Adv. Drug Delivery Rev.* **2006**, *58*, 1460.
(73) Pagona, G.; Tagmatarchis, N. *Curr. Med. Chem.* **2006**, *13*, 1789.
(74) Soto, K.; Garza, K. M.; Murr, L. E. *Acta Biomater.* **2007**, *3*, 351.
(75) Wick, P.; Manser, P.; Limbach, L. K.; Dettlaff-Weglikowska, U.; Krumeich, F.; Roth, S.; Stark, W. J.; Bruinink, A. *Toxicol. Lett.* **2007**, *168*, 121.
(76) Fraczek, A.; Menaszek, E.; Paluszkiewicz, C.; Blazewicz, M. *Acta Biomater.* **2008**, *4*, 1593.
(77) Jain, K. K. *Expert Opin. Drug Discovery* **2012**, *7*, 1029.
(78) Ma-Hock, L.; Treumann, S.; Strauss, V.; Brill, S.; Luizi, F.; Mertler, M.; Wiench, K.; Gamer, A. O.; van Ravenzwaay, B.; Landsiedel, R. *Toxicol. Sci.* **2009**, *112*, 468.
(79) Porter, D. W.; Hubbs, A. F.; Mercer, R. R.; Wu, N.; Wolfarth, M. G.; Sriram, K.; Leonard, S.; Battelli, L.; Schwegler-Berry, D.; Friend, S.; Andrew, M.; Chen, B. T.; Tsuruoka, S.; Endo, M.; Castranova, V. *Toxicology* **2010**, *269*, 136.
(80) Nagai, H.; Okazaki, Y.; Chew, S. H.; Misawa, N.; Yamashita, Y.; Akatsuka, S.; Ishihara, T.; Yamashita, K.; Yoshikawa, Y.; Yasui, H.; Jiang, L.; Ohara, H.; Takahashi, T.; Ichihara, G.; Kostarelos, K.; Miyata, Y.; Shinohara, H.; Toyokuni, S. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, E1330.
(81) Oyabu, T.; Myojo, T.; Morimoto, Y.; Ogami, A.; Hirohashi, M.; Yamamoto, M.; Todoroki, M.; Mizuguchi, Y.; Hashiba, M.; Lee, B. W.; Shimada, M.; Wang, W. N.; Uchida, K.; Endoh, S.; Kobayashi, N.; Yamamoto, K.; Fujita, K.; Mizuno, K.; Inada, M.; Nakazato, T.; Nakanishi, J.; Tanaka, I. *Inhalation Toxicol.* **2011**, *23*, 784.
(82) Delorme, M. P.; Muro, Y.; Arai, T.; Banas, D. A.; Frame, S. R.; Reed, K. L.; Warheit, D. B. *Toxicol. Sci.* **2012**, *128*, 449.
(83) Xu, J.; Futakuchi, M.; Shimizu, H.; Alexander, D. B.; Yanagihara, K.; Fukamachi, K.; Suzui, M.; Kanno, J.; Hirose, A.; Ogata, A.; Sakamoto, Y.; Nakae, D.; Omori, T.; Tsuda, H. *Cancer Sci.* **2012**, *103*, 2045.
(84) Usui, Y.; Haniu, H.; Tsuruoka, S.; Saito, N. *Med. Chem.* **2012**, *2*, 105.
(85) Fiorito, S.; Serafino, A.; Andreola, F.; Togna, A.; Togna, G. *J. Nanosci. Nanotechnol.* **2006**, *6*, 591.
(86) Liu, Z.; Davis, C.; Cai, W.; He, L.; Chen, X.; Dai, H. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 1410.
(87) Schipper, M. L.; Nakayama-Ratchford, N.; Davis, C. R.; Kam, N. W.; Chu, P.; Liu, Z.; Sun, X.; Dai, H.; Gambhir, S. S. *Nat. Nanotechnol.* **2008**, *3*, 216.
(88) Lee, Y.; Geckeler, K. E. *Adv. Mater.* **2010**, *22*, 4076.
(89) Beg, S.; Rizwan, M.; Sheikh, A. M.; Hasnain, M. S.; Anwer, K.; Kohli, K. *J. Pharm. Pharmacol.* **2011**, *63*, 141.
(90) Lanone, S.; Boczkowski, J. *Curr. Mol. Med.* **2006**, *6*, 651.
(91) Firme, C. P., III; Bandaru, P. R. *Nanomedicine* **2010**, *6*, 245.
(92) Alberts, B.; Bray, D.; Hopkin, K.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Essential Cell Biology*, 3rd ed.; Garland Science: New York, 2010.
(93) Cook, S. D.; Beckenbaugh, R. D.; Redondo, J.; Popich, L. S.; Klawitter, J. J.; Linscheid, R. L. *J. Bone Jt. Surg., Am. Vol.* **1999**, *81*, 635.
(94) Brantigan, J. W.; Neidre, A.; Toohey, J. S. *Spine J.* **2004**, *4*, 681.
(95) Williams, M. A.; van Riet, S. *J. Heart Valve Dis.* **2006**, *15*, 80.
(96) Saito, N.; Aoki, K.; Usui, Y.; Shimizu, M.; Hara, K.; Narita, N.; Ogihara, N.; Nakamura, K.; Ishigaki, N.; Kato, H.; Haniu, H.; Taruta, S.; Kim, Y. A.; Endo, M. *Chem. Soc. Rev.* **2011**, *40*, 3824.
(97) Hara, K.; Aoki, K.; Usui, Y.; Shimizu, M.; Narita, N.; Ogihara, N.; Nakamura, K.; Ishigaki, N.; Sano, K.; Haniu, H.; Kato, H.; Nishimura, N.; Kim, Y. A.; Taruta, S.; Saito, N. *Mater. Today* **2011**, *14*, 434.
(98) Takanashi, S.; Hara, K.; Aoki, K.; Usui, Y.; Shimizu, M.; Haniu, H.; Ogihara, N.; Ishigaki, N.; Nakamura, K.; Okamoto, M.; Kobayashi, S.; Kato, H.; Sano, K.; Nishimura, N.; Tsutsumi, H.; Machida, K.; Saito, N. *Sci. Rep.* **2012**, *2*, 498.
(99) Wang, J.; Sun, P.; Bao, Y.; Liu, J.; An, L. *Toxicol. In Vitro* **2011**, *25*, 242.
(100) Devadasu, V. R.; Bhardwaj, V.; Kumar, M. N. *Chem. Rev.* **2013**, *113*, 1686.
(101) Iannazzo, D.; Piperno, A.; Pistone, A.; Grassi, G.; Galvagno, S. *Curr. Med. Chem.* **2013**, *20*, 1333.
(102) Veetil, J. V.; Ye, K. *Biotechnol. Prog.* **2009**, *25*, 709.
(103) Watari, F.; Takashi, N.; Yokoyama, A.; Uo, M.; Akasaka, T.; Sato, Y.; Abe, S.; Totsuka, Y.; Tohji, K. *J. R. Soc. Interface* **2009**, *6*, S371.
(104) Prabhu, P.; Patravale, V. *J. Biomed. Nanotechnol.* **2012**, *8*, 859.
(105) Drbohlavova, J.; Chomoucka, J.; Adam, V.; Ryvolova, M.; Eckschlager, T.; Hubalek, J.; Kizek, R. *Curr. Drug Metab.* **2013**, *14*, 547.
(106) Karousis, N.; Tagmatarchis, N.; Tasis, D. *Chem. Rev.* **2010**, *110*, 5366.
(107) Kam, N. W.; O'Connell, M.; Wisdom, J. A.; Dai, H. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 11600.
(108) Venkatesan, N.; Yoshimitsu, J.; Ito, Y.; Shibata, N.; Takada, K. *Biomaterials* **2005**, *26*, 7154.
(109) Dumortier, H.; Lacotte, S.; Pastorin, G.; Marega, R.; Wu, W.; Bonifazi, D.; Briand, J. P.; Prato, M.; Muller, S.; Bianco, A. *Nano Lett.* **2006**, *6*, 1522.