Keywords: nanomedicine; carbon nanomaterials; fullerenes; carbon nanohorns; carbon nanotubes; drug delivery; nano safety science

1. Introduction

Advances in nanotechnology have led to the recent development of many nanomaterials, including nanoscale silica particles, titanium dioxide nanoparticles, and carbon nanomaterials [1–4]. Nanomaterials, which are generally classified as materials with feature sizes smaller than 100 nm, have remarkably impacted various fields of study because of the desirable properties (e.g., enhanced electrical conductivity, tensile strength, and chemical reactivity) imparted by their increased surface area per unit weight compared with that of their bulk-scale counterparts [5,6]. Nanomaterials are already being applied in electronics [1], foods [2], and cosmetics [3]. Furthermore, in basic research for development of new drugs, nanomaterials are expected to open novel avenues for the treatment of human diseases owing to their unique physicochemical properties [4].

Carbon nanomaterials, including fullerenes, carbon nanohorns (CNHs), and carbon nanotubes (CNTs), have been used as carriers in drug delivery and other applications [7]. Carbon nanomaterials with carbon cage and graphene structures have many technological advantages such as facile modification by functional groups [8–10], high carrier capacity [11,12], high chemical stability [13,14], and feasibility of incorporating both hydrophilic and hydrophobic substances [15,16] (Table 1). These characteristics, which are essential for the development of drug-delivery carriers, make carbon nanomaterials promising for nanomedicine applications.

Table 1. Basic physicality of carbon nanomaterials.

	Fullerenes	**CNHs**	**CNTs**
Year of discovery	1985	1998	1991
Discoverer	H.W. Kroto R.F. Curl R.E. Smalley	S. Iijima	S. Iijima
Size			
Diameter	1 nm	2–4 nm	0.4–70 nm
Length	-	40–70 nm	1 μm–2.5 mm
Shape	sphere	horn	fiber
Practical use	Cosmetics Lubricity agent Semiconductor	Fuel battery	Semiconductor Car parts Sports goods

Though highly promising, these carbon nanomaterials are in an early phase of development. Therefore, information regarding their safety is not sufficient for the development of medically sound and nontoxic technologies. Because nanomaterials' physicochemical properties often differ substantially from those of their bulk counterparts, as mentioned above, there are concerns that carbon nanomaterials may exhibit unexpected side effects. In addition, recent reports have shown that pristine CNTs might induce mesothelioma-like lesions in mice, similar to those induced by asbestos [17–19]. On the other

hand, Muller *et al.* showed that pristine CNTs induce no mesothelioma formation in a 2-year *in vivo* study [20]. Therefore, more information about the safety of nanomaterials needs to be collected.

In this review, we discuss currently available information about the safety of carbon nanomaterials for nanomedicine applications, including information obtained from our own previous studies. We also discuss types of carbon nanomaterials that demonstrate particular promise for safe nanomedicine technologies.

2. Utility of Carbon Nanomaterials for Nanomedicine

2.1. Fullerenes

Fullerenes have attracted considerable attention in various fields of science [21]. Fullerenes are composed entirely of carbon in the form of a hollow sphere, ellipsoid, or tube. Spherical fullerenes are also referred to as buckyballs. An important property of the fullerene molecule is their high symmetry. There are 120 symmetry operations, such as rotation around an axis and reflection in a plane. Fullerenes belong to the class of inorganic nanoparticles and show high bioavailability due to their small size (~1 nm). Owing to their small size, fullerenes can penetrate various tissues and organelles that materials with submicron size cannot penetrate. For example, Foley *et al.* reported that fullerenes can cross the COS-7 cell membrane and bind to the mitochondria [22], demonstrating that fullerenes have utility as intracellular carriers. Furthermore, fullerenes' capability to act as drug-delivery carriers for low-molecular-weight compounds and oligonucleotides has been demonstrated [23]. For example, conjugates composed of fullerenes and paclitaxel have exhibited the potential to provide slow release of the drug and have exhibited significant anti-cancer activity in cell cultures [24]. Moreover, Maeda-Mamiya *et al.* reported effective gene delivery *in vivo* using water-soluble fullerenes [25]. In that study, conjugates consisting of cationic tetraamino fullerenes and an insulin-gene-expressing plasmid complex were injected intravenously into C57/BL6 mice. Insulin gene expression was detected in the lung, liver, and spleen. Plasma insulin levels in the insulin gene group of mice were significantly higher than those in a control group. Both of these studies demonstrate that fullerenes may act as drug- and gene-delivery carriers. Furthermore, because fullerenes are strong anti-oxidants, they have been used as neuroprotective [26,27] and anti-inflammatory agents [28]. Thus, if fullerenes can be controllably manipulated, they could be used to treat various diseases.

2.2. CNHs/CNTs

CNHs and CNTs based on the structure of graphene are also regarded as drug-delivery carriers [29,30]. CNHs and CNTs are differentiated from each other by their shape and size (Table 1). Furthermore, CNHs and CNTs can be classified as possessing either single- or multi-walled (SW or MW) structures. This review cites several reports on the use of SWCNHs and SWCNTs as drug-delivery carriers. SWCNHs have plenty of inner spaces. Through these holes, various molecules such as low-molecular-weight compounds or nucleic acids can enter the hollow interior of the SWCNHs. SWCNHs also can regulate the sustained release of drugs from their interior for drug-delivery applications. For example, the release rate of cisplatin (CDDP), a chemotherapy drug that can be incorporated into oxidized SWCNHs, has been regulated by controlling solvent composition. The release of CDDP from the SWCNHs is slower in water and a culture medium than in

phosphate-buffered saline, and the CDDP released from SWCNHs in the former solvent effectively kills human lung-cancer cells [11].

SWCNTs also have been demonstrated to be amenable for drug delivery. SWCNT-siRNA conjugates have been efficiently transported to human T-cells and primary cells, which are inert to commercially available liposome-based nonviral vectors, and have silenced a specific gene in those cells [31]. In cancer therapy, SWCNT-based tumor-targeted drug-delivery system (DDS) has already been developed by several investigators [32,33]. SWCNT-anticancer-drug conjugates also have shown higher efficacy in suppressing tumor growth than clinical anticancer drugs alone in various cancer models [32,33]. These therapeutic effects were induced by accumulation of the conjugates in tumor. Collectively, these results clearly indicate the potential applications of SWCNHs and SWCNTs in cancer-targeted drug delivery and sustained release [34].

2.3. Suitable Modification of Carbon Nanomaterials for DDS

Accumulation at a targeted location is important in DDS. Carbon-nanomedicine-based cancer treatment systems generally function by means of either active targeting or passive targeting. In active-targeting DDS for cancer treatment, the search for cancer-specific targets is important. SWCNTs modified with antibodies, folate, arginine-glycine-aspartic acid (rgd) peptide, and epidermal growth factors have been useful for active targeting of tumor tissue [35–38]. Ruggiero *et al.* reported that antibody-modified SWCNTs accumulate in tumor tissue in a murine xenograft model of human colon adenocarcinoma [39]. However, these anti-cancer effects are not enough to enable drug development because these targets do not express specifically in tumor. In recent years, novel targets have been identified by using "-omics" approaches such as proteomics, genomics, and metabolomics [40–42]. Proteomics-based analysis is currently a promising approach for identifying biomarker proteins for use in drug development because these proteins directly regulate the onset and progression of diseases. However, proteomics-based analysis can yield many potential candidate biomarker proteins that are over- or under-expressed in diseased tissues, and these candidates must be efficiently screened to identify appropriate targets. Toward this end, we have developed an "antibody proteomics system" that facilitates the screening of biomarker proteins from many candidates by rapid preparation of cross-reacting antibodies using phage antibody library technology. The system is an efficient method for screening tumor-related biomarker proteins to identify novel targets [43].

In passive-targeting DDS for cancer treatment, improvement of drug retention in blood is important because the reticulo-endothelial system and kidney work as the barrier against foreign particles *in vivo*. Covalent conjugation of polyethylene glycol (PEG) to a carrier's surface, referred to as "PEGylation" is a promising strategy to improve retention of various nanomaterials in the blood [44]. PEGylation can prolong the plasma half-life and alter the tissue distribution of the nanomaterial conjugates compared with their non-PEGylated forms, which typically clear the body through the reticulo-endothelial system *in vivo*. The extended circulating lifetime of PEGylated conjugates in blood induces an enhanced permeability and retention effect, which is based on the leaky nature of tumor blood vessels, resulting in increased delivery of the conjugates to tumor tissue. As an example, Yang *et al.* investigated the long-term *in vivo* biodistribution of nanoscale graphene sheets functionalized with PEG and systematically examined the potential toxicity of graphene over time [45]. On the other hand, from the

aspect of effectivity and safety, CNTs kinetics is important for drug development. Singh *et al.* describes the pharmacokinetic parameters of intravenous administered functionalized SWCNTs relevant for various therapeutic and diagnostic applications [46]. It shows that functionalized (water-soluble) SWCNTs can, in fact, be excreted via the renal route. In summary, to obtain highly effective and nontoxic carbon nanomaterial DDS for cancer treatment, it is necessary to control three factors: (1) size; (2) the ability to target the molecules to tumors and (3) clearance through the reticulo-endothelial system and kidney.

2.4. Other Application of CNTs in Medicine

As mentioned above, CNTs have been explored as a novel tool for the delivery of therapeutic molecules including peptide, nucleic acid and cancer drugs. On the other hand, certain types of CNTs have been reported to possibly help cancer diagnosis and other application [47,48]. Photoacoustic imaging proposes higher spatial resolution and permits deeper tissues to be imaged compared with most optical imaging techniques. Zerda *et al.* [47] showed plain SWCNTs conjugated with cyclic Arg-Gly-Asp (RGD) peptides can be used as a agent for photoacoustic imaging of tumors. This report indicates SWCNTs is possibly useful for cancer diagnosis. Additionally, Tosun *et al.* suggested collagen conjugated SWCNTs show the potential for enhanced electrical activity. These SWCNTs have been shown positive *in vitro* biocompatibility results offering further evidence that SWCNT-based materials have an important role in neuronal regeneration [48]. Neurodegenerative disorders including Parkinson's and Alzheimer's diseases, amyotrophic lateral sclerosis are rapidly increasing as the population ages. The field of nanomedicine promises revolutionary advances to the diagnosis and treatment of devastating human diseases [48].

3. Safety of Carbon Nanomaterials

3.1. Hazard Assessment

Carbon nanomaterials are among the most promising nanomedicines. However, information about the safety of carbon nanomaterials is still fragmentary, and ensuring their safety is of utmost importance to protect human health. In this section, we focus on the safety of CNTs specifically, because some studies have reported that CNTs have higher toxicity than fullerenes and CNHs [49].

Parameters such as structure, size distribution, surface area, surface chemistry, surface charge, and agglomeration state as well as purity of the samples, have considerable impact on the reactivity of CNTs. Some studies have reported that certain types of SW or MW CNTs are cytotoxic and genotoxic *in vitro*, so public concern about the potential risk of CNTs to human health has risen [50–52]. In fact, recent reports have indicated that certain types of CNTs might induce mesothelioma-like lesions in mice, in a manner similar to that observed for mesothelioma induced by asbestos [53–55]. Takagi *et al.* showed that intraperitoneally administered pristine MWCNTs induce mesothelioma in the p53 (+/−) mouse carcinogenesis model, probably due to the MWCNTs' resemblance to asbestos in size and shape and to their biopersistency [17]. Poland *et al.* also observed asbestos-like pathogenic behavior of long pristine MWCNTs associated with their needle-like fiber shape and established a structure-activity relationship based on the length of the MWCNTs [19]. These studies revealed that the propensity of

long MWCNT fibers to produce inflammation and fibrosis in the peritoneal cavity is similar to, or greater than, that of long asbestos fibers. In contrast, neither short asbestos fibers nor short tangled MWCNTs cause any significant inflammation [19]. These results suggest that physical properties, such as length, diameter and physico-chemical properties, might impact the safety of pristine CNTs [56]. However, these studies were based on the administration of extremely high doses of MWCNTs via peritoneal injection. In contrast, Shvedova *et al.* showed that pristine MWCNTs enhanced acute inflammation and pulmonary injury with delayed bacterial clearance after aspiration or inhalation of MWCNTs [57,58]. In the future, it is needed to examine the study relevant to the human occupational exposure situation.

There are a few reports that examine the mechanisms of CNT toxicity [59–61]. One important underlying factor that influences the safety of long fibers is the failure of macrophage cells to completely enclose them. This failure, termed incomplete or "frustrated" phagocytosis, can induce inflammation [19]. Migliore *et al.* showed that long rigid MWCNTs appear to form fiber-like aggregates or structures that are too long to be phagocytosed by macrophage cells, thus resulting in reactive oxygen species (ROS) production [62,63], which contributes to NACHT domain-, leucine-rich repeat-, and pyrin domain-containing protein 3 (NLRP3) activation [64,65]. Palomaki *et al.* demonstrated that the NLRP3 inflammasome was essential for long, needle-like CNTs and asbestos to induce IL-1β secretion [65]. Moreover, it was noted that CNT-induced NLRP3 inflammasome activation depended on ROS production [65]. Clarification of the mechanism of inflammation induced by CNTs might lead to the development of safe carbon nanomedicine technologies.

Although some studies have reported concern about the safety of CNTs as mentioned above, other studies have reported that certain types of CNTs are safe materials for nanomedicine. Yang *et al.* demonstrated that after intravascular injection of pristine SWCNTs, mice did not show stress or symptoms of abnormality, such as lethargy, anorexia, or changes in body weight [66]. Furthermore, Wick *et al.* showed that the cytotoxicity of purified rope-like agglomerated SWCNTs was lower than that of well-dispersed SWCNTs [67]. In addition to the fiber-like structure of CNTs, the amount of metal contaminants such as iron or nickel found in the CNTs may contribute to the nanomaterials' potential carcinogenicity by accelerating the generation of ROS [68–70]. Moreover, in preparation for drug development, it is important to examine the influence of the oxidative debris on CNTs [14,71–73]. It is an emergent and key point during purification and functionalization of carbon nanostructure. These studies suggest that the safety of CNTs is determined not only by physical properties but also by a wide variety of factors such as method of administration, dispersability, and presence of metal contaminants. How much these factors contribute to the safety of CNTs remains unknown, however. We believe that the information obtained by these safety studies might be useful for ensuring the safety of CNTs.

3.2. Biological Behavior of CNTs

Evaluation of *in vivo* kinetics is important for assessing the safety of nanomedicine technologies. In this section, studies about the behavior of CNTs in the body are described. Ruggiero *et al.* showed that intravascularly injected pristine SWCNTs favor liver accumulation and hepatobiliary excretion over kidney accumulation and renal excretion [39]. In addition, several studies have investigated pulmonary

effects subsequent to instillation, aspiration, and inhalation of pristine SWCNTs [57]. These reports showed that short and small tangles of SWCNTs that deposit subpleurally migrate to the pleural space and exit in the flow of pleural fluid through the stomata, where they follow the lymphatic drainage to the mediastinal lymph nodes [60,74]. In addition, long carbon nanotubes also reach the pleural space but cannot negotiate the stomata, and so they are retained in the pleural space, where they cause inflammation and potentially long-term disease [60,74].

Kagan *et al.* showed that hypochlorite and reactive radical intermediates of the human neutrophil enzyme myeloperoxidase catalyze the biodegradation of carboxylated SWCNTs *in vitro*, in neutrophils and to a lesser degree in macrophages [75]. Importantly, the biodegraded nanotubes do not generate an inflammatory response when aspirated into the lungs of mice [75]. In addition, Liu *et al.* have reported that the biodurability of SWCNTs depends on surface functionalization [76]. Based on these findings, strategies for mitigating the pro-inflammatory effects of these nanomaterials in occupational settings may be developed.

Furthermore, information about toxicokinetics (absorption, distribution, metabolism and elimination) also should be obtained for the development of safe CNTs.

3.3. Development of Safe Nanomaterials

We have discussed above how nanomaterials can serve as useful nanomedicine technologies and have also highlighted the importance of considering these nanomaterials' safety for such applications. In this section, we examine the current status of the development of safe and effective nanomaterials for nanomedicine. In our own studies, we have established relationships between the physical properties and safety of CNTs. Our data showed that pristine thin MWCNTs and SWCNTs do not induce genetic damage *in vitro* and inflammation *in vivo* [77]. These data indicate that physical properties such as particle length and width might influence the safety of CNTs. In addition, Nagai *et al.* suggested the large-diameter or tangled MWCNTs are less toxic, less inflammogenic, and less carcinogenic than untangled MWCNTs [56]. These results suggest that control of the diameter of CNTs could be used to develop CNTs that are safe for human health.

Furthermore, in addition to being critically important for the detection of biomolecules, the surface properties of nanomaterials also can modulate the materials' safety. Recent studies have shown that functionalization of CNTs with carboxyl or amino surface groups can affect the CNTs' toxicity [78]. Thus, regulation both of particle size and of surface properties is considered important for research leading to the development of safe nanomedicine technologies.

In fact, our previous study showed that nanoscale silica particles, which we expected to be useful as drug-delivery carriers, display different intracellular localization compared with submicron- and micro-scale silica particles and induce a greater cytotoxic response to mouse macrophage cell line [79]. We have also shown that nanoscale silica particles induce certain cellular responses, such as ROS generation and DNA damage to human keratinocyte cell line [80]. These results indicate that particle size could influence the silica particles' safety for applications in nanomedicine. In addition, we have shown that surface modification of silica particles with functional groups, such as amino or carboxyl groups, suppresses toxic biological effects of silica particles including inflammatory responses and ROS production [81]. A recent study demonstrated that nanomaterials become coated with serum

proteins and induce different cellular responses from intact particles by binding to proteins [82]. In addition, different surface characteristics, such as surface charge, influence the binding affinities of proteins to nanomaterials [82,83]. In fact, Gasser *et al.* showed that functionalization of MWCNTs have the potential to alter the MWCNTs blood plasma protein coating in biological systems [84,85]. These results indicate that particle size or surface properties of carbon nanomaterials can affect their safety, and that control of these physical properties can be used to advance the development of safe nanomaterials.

4. Conclusions

The unique physicochemical properties of carbon nanomaterials allow them to incorporate targeting ligands, chemotherapeutic drugs, and many other therapeutic agents that have great potential for cancer-targeted therapy. However, owing to the large number of factors that influence the kinetics of drug release from nanomaterials, as well as their safety for human health, insufficient information is available on these two important subjects. Factors that influence the safety of and kinetics of drug release from nanomaterials include their shape, length, and dispersability, as well as the presence of metal contaminants. A detailed understanding of the pharmacological and toxicological properties of carbon nanomaterials, as well as a balanced evaluation of their risks and benefits to human health, is required before they can be recommended for routine clinical use. We believe that a detailed safety analysis of carbon nanomaterials will be invaluable for the design of safe nanomedicine technologies.

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4　ナノカーボンDDSの現状とその安全性確保に向けて

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4.1　はじめに

少なくとも1次元が100nm以下の大きさであるナノマテリアル（NM）は，サブミクロンサイズ以上（100nm以上）の従来素材とは異なる画期的機能を発揮することから，様々な分野で夢の新素材として期待されている。特に医療分野では，高い組織浸透性や，薬物保持・徐放化能を利用して，低分子化合物やタンパク質単独では期待できなかった薬効を示す画期的新薬（ナノメディシン）の開発が世界中で進められている。これらNMの画期的機能は，ナノ医薬としての主薬あるいはナノ添加剤としてだけでなく，“薬物を必要なときに，必要な量，必要な所へ到達させることで，副作用を最小限に抑え，最大の効果を発揮させる”という理想の投薬形態，薬物治療の最適化を目指したドラッグデリバリーシステム（DDS）そのものであり，まさに，ナノメディシン＝ナノDDSと言えよう。周知のように，DDS研究領域へのナノテクノロジー・ナノマテリアルの導入はファミリアとなりつつあり，例えば，100nm以下のサイズに厳密制御したリポソームやナノスフェア，デンドリマーの開発が果敢に試みられている。一方で，これらナノメディシンの開発はまだ緒に就いたばかりであり，多くは未だブラックボックスと言え，その魅惑的で秘められた可能性が逆に，予想外の部位で未知の副作用を発現させる潜在的な脅威にもなっている。しかし，現行のNMの安全性研究の大部分はハザード同定（副作用の有無の評価）のみに偏重しており，NMの物性と体内吸収性や体内/細胞内動態，生体影響の連関といった，NMの安全性担保に資する具体的な情報が圧倒的に不足している。このままでは，全てのNMの安全性に対する懸念が広がり，ナノメディシン開発の足枷になりかねない。従って，NMを用いたナノメディシンの開発を実現・推進するためには，副作用を含めた生体影響を詳細に解析したうえで，それらの情報を基盤として有効かつ安全なNMの開発を推進するほか無い。即ち，単にナノマテリアルのハザード（毒性）やリスク（危険性），そのメカニズム（毒性発現機

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構）を解明しようとするナノ毒性学（Nano-Toxicology）では，現状を打破できないであろう。むしろ，ヒトと生態系（環境）にとって安全で，ヒトと社会（産業界など）がナノテクノロジーの恩恵を最大限に享受でき，しかも安心して豊かな生活を営めるよう，安全なナノメディシン（ナノ DDS）の開発とその支援に叶うナノ安全科学研究（Nano-Safety Science）とも言うべき学問が今後の鍵となっている（図 1）。NM とヒト，生態系との共存，社会受容，これらがまさにキーポイントであろう。そこで本総説では，特に DDS 医薬に適した特徴的な物性を有するナノカーボン素材を例に，ナノメディシン開発の現状とともに，ナノメディシンの開発に必須である NM の安全性確保に向けて我々が推進しているナノ安全科学研究を含めて紹介させていただきたい[1~4]。

4.2　ナノカーボン DDS の可能性

近年，フラーレン，カーボンナノチューブ（CNT）やカーボンナノホーン（CNH）など，ナノカーボン素材を用いた DDS 医薬の開発が注目を浴びている。これらは，炭素間結合を介す長い電子共役系を持つなど特殊な物性を有し，高い薬物保持能や生体内安定性，柔軟な構造（表面修飾の容易さ）といった，DDS 素材として極めて有望な性質を発揮する。この特性を活かし，低分子医薬，タンパク質医薬，核酸医薬の送達キャリアとしての開発が前臨床段階ではあるものの，世界中で進められている。近年では，腫瘍組織，炎症組織や細胞内リソソームなど低 pH 環境において薬剤が放出されるといった DDS 機能を有するナノカーボン素材の開発も進められている。また，ナノカーボン素材は内腔を持つ特殊な構造を有するため，表面だけではなくその内

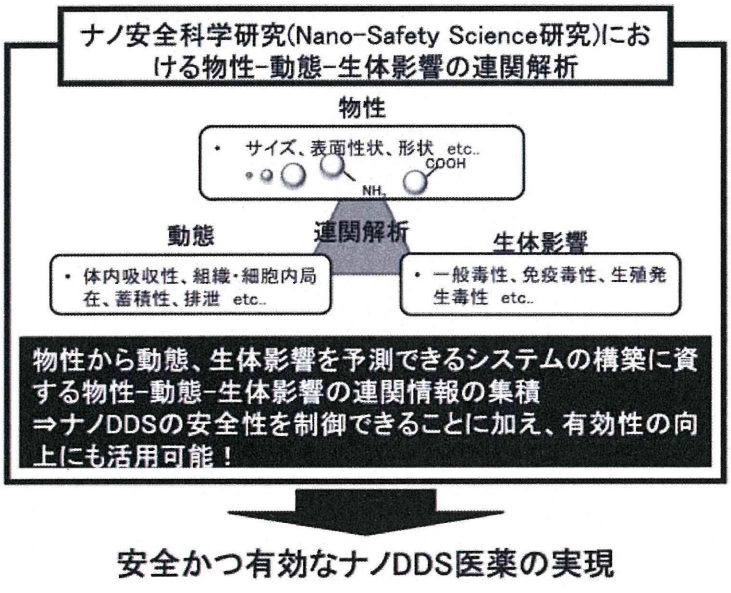


図 1　ナノ DDS 医薬の実現に向けたナノ安全科学研究

腔に薬物を保持させ，徐放化を試みる検討もある。湯田坂らは，CNH に内包されたシスプラチンが数十時間をかけて徐放され，顕著な抗がん作用を示すことを報告している[5]。さらに，薬物キャリアとしてのみならず，ナノカーボン素材自身が有する薬理活性を利用した医薬品応用にも期待が寄せられている。例えば，ナノカーボン素材特有の長い電子共役系によって誘導される光増感物質としての性質は，がんの光線力学的治療法，フォトダイナミックセラピー（PDT）に応用することが可能である。田畑らは，フラーレンへの光照射により一重項酸素などの活性酸素が非常に高い効率で発生する性質を利用し，*in vivo* において顕著な腫瘍退縮効果が得られることを報告している[6]。一方でこれら電子共役系により，フラーレンはラジカルスポンジとも呼ばれるほど強い抗酸化作用を有し，活性酸素が原因となり発症・悪化する各種炎症性疾患への適用も進められている。さらに近年，神経幹細胞を用いた脳卒中の治療において，CNT を幹細胞の足場として用いることで組織修復が劇的に向上し，神経保護作用を発揮することで虚血性傷害を軽減させ得ることが明らかとなった[7]。このように，ナノカーボン素材の有用機能について，続々と予期せぬ新規作用が見出されつつあり，今後も新たな治療戦略の開発に期待が寄せられている。また，ナノカーボン素材は表面修飾が容易であることから，各種リガンド・抗体を用いたターゲティング能の付加による治療効果増大・副作用低減を目指した試みも精力的になされている。さらに，現在，解析技術がボトルネックとなり不足している体内動態に関する情報の蓄積が進展すれば，ナノカーボン素材の医薬品応用に，より一層の飛躍が見込まれる。

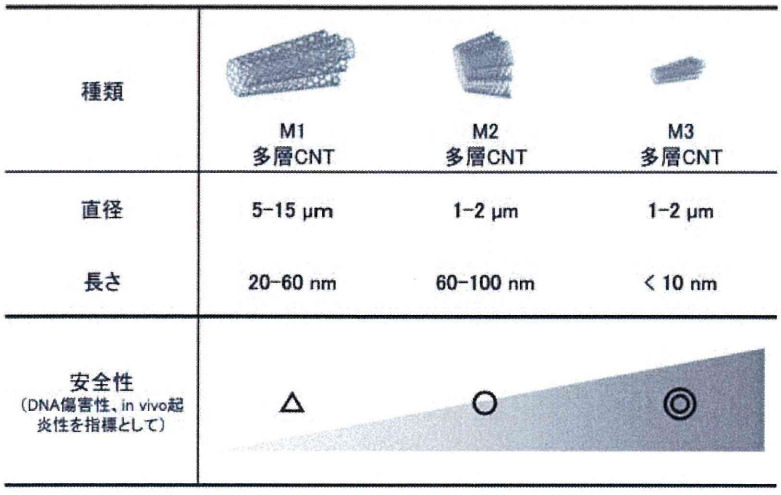
4.3　安全なナノ DDS 医薬の開発に向けて

ナノカーボン素材のナノメディシンへの応用研究は多数なされている一方で，体内・組織内・細胞内動態や副作用情報（NanoTox）は未だ乏しいのが現状である。さらに，ナノカーボン素材を含めた NM の物性と体内動態，生体影響との関連性については，全く体系的に理解されておらず，有効かつ安全なナノメディシンの開発に資する基盤情報の収集が急務となっている。例えばナノカーボン素材に関して，CNT がアスベストと同様に悪性中皮腫や肺がんを誘発する可能性が報告される一方で[8]，血中投与後，長期にわたって一切の毒性が観察されないとする報告が存在するなど[9]，一見矛盾する報告が飛び交っている。しかし，これら検討に用いられている CNT は，多層，単層と構造的な差異があるとともに，太さや長さといった形状，さらには混入している不純物までもが異なっている。また，これら物性の違いに応じて変化すると考えられる動態に関しての情報も皆無であり，一部のハザード情報のみが一人歩きすることで，むやみに危険性を煽り，ひいてはその風評被害により，有益なナノカーボン素材までをも闇に葬りかねない。本観点から我々は，NM の安全性確保及び安全な NM の創製に資する基盤情報の収集を目的としたナノ安全科学研究を推進しており，NM の物性と体内動態，生体影響の連関評価を試みてきた。ここでは，我々のナノ安全科学研究の中から，遺伝子送達キャリアなどの DDS 素材として期待されるナノシリカ（nSP）を用いた先行研究について紹介させていただきたい。我々はこれまでの検討から，粒子径 100nm 以下の nSP が，経皮・経口・経鼻等の非侵襲的な経路からの

投与においても体内吸収され，脳や胎盤といった特にバリア機能の発達した部位や，細胞の核内にまで到達し得ることを明らかとしてきた[3]。本結果は，nSPがこれまで送達不可能であった部位への薬物送達をも可能とする新規キャリアになり得ることを示すものであり，我々も核酸送達キャリアやワクチンキャリアとしての適用を試み，興味深い知見を得つつある。そこで，粒子径70nmのnSP（nSP70）と，対照群として300，1000nmの従来型シリカ（nSP300，mSP1000）を用い，粒子径と体内局在，ハザードとの関係を精査した。まず，体内吸収後の安全性評価の観点から，静脈内投与後の各粒子の局在を，*in vivo*イメージングにより解析した。その結果，6時間後にはnSP300，mSP1000が胆のうにのみ集積していたのに対し，nSP70は肝臓全体に広がって分布していることが示された。さらに，透過型電子顕微鏡観察の結果，nSP70のみが肝実質細胞内にまで侵入しており，この結果と相関して，過剰量の投与では，nSP70投与群でのみ肝実質細胞の強い傷害に起因した重篤な肝障害や，血液凝固系異常などを伴う急性致死毒性が観察された。また，これら劇的なハザードが観察されない投与量においても，nSP70の妊娠母体への投与によって，nSP70のみが胎盤に移行し，さらには血液胎盤関門を突破して胎仔にまで侵入することから，胎仔発育不全の誘発に注意する必要があることを明らかとした[4]。以上の結果から，粒子径の違いにより，シリカの体内・細胞内動態，さらにはその生体影響までもが変化することが示された。次に，安全なnSPの創製に向け，nSP70の表面がアミノ基，カルボキシル基で修飾されたnSP70-N，nSP70-Cを用いて，表面性状と生体影響の関係を精査した。その結果，表面修飾体ではnSP70で認められた急性致死毒性や胎仔毒性等が一切観察されず，安全性が飛躍的に向上することが明らかとなった[2,4]。すなわち，本研究の最も重要な点は，nSPの表面修飾が，その有効性を保持しつつ，安全性を担保できる極めて有望なアプローチになり得る可能性が示されたことである。以上のnSPでの先行研究を受け，現在，ナノカーボン素材に関しても，物性と動態，生体影響の連関情報を収集している。一例をあげると，これまでに我々は，様々な物性のCNTを用い，CNTによるDNA傷害性や起炎性といったハザードが，長さ，太さといったCNTの形状（物性）により規定され，長さと太さを各々1-2 μm以下，10nm以下程度に制御することで，安全性が向上する可能性を明らかとしている[1]（図2）。また，フラーレンは適切な表面修飾により，高度に安全性を確保しつつ，圧倒的な抗炎症作用を発揮させ得ることを我々は認めており，現在，その実用化を目指した研究を推進中である。今後は，ナノカーボン素材に関しても，表面修飾による表面性状の変化と，生体影響との連関解明を図ることで，より安全性に優れた素材の創製が可能になると期待される。

4.4　おわりに

本総説では，ナノカーボン素材によるナノメディシンの実現に向け，ナノDDSへの適用の現状とともに，最も急がれる安全性確保に関する検討を中心に紹介させていただいた。これまでのナノDDS医薬開発において，その安全性が懸念されていたことの根底には，従来の化審法と同様，物質名のみに基づいた安全性の議論が中核であったことも一因にある。裏を返せばこれは，



Ref. Yamashita, K. *et al.*, Inflammation, **33** (4): 276–80 (2010)

図2　カーボンナノチューブの物性と安全性の連関情報

NMの物性と，動態やハザードとの連関情報などの，NMの安全性確保に資する具体的情報が圧倒的に不足していたことを意味している。今回，表面性状や形状など物性の適切な制御により，安全性を高めうることを紹介したが，未だ基礎情報は不足しており，引き続きナノ安全科学研究を推進していくこと，そして何よりDDS研究との高度な有機的融合が必要不可欠である。また，物性制御により，NMの安全性が高度に担保されるメカニズムの解明が今後の課題であると考えられ，メカニズムに関する議論を深めることで，他のNMにも適用可能なより普遍性を持った安全性情報を蓄積することが可能になると期待される。末筆ではあるが，最も重要なことは，最適条件でのNM創出により，安全なNMを創出できること，安全なNMは圧倒的な知財であり，ヒトの健康確保と，責任ある先進国・技術立国として，健康立国として我が国の発展に大いに貢献し得ることである。言い換えれば，『新たに産み出されるDDS医薬（ナノメディシンを含む）が有効なのは当たり前で，さらに高度な安全性を保障していく』ことの重要度は，ますます加速していこう。今後，ナノメディシンやナノDDSの開発と実用化に向け，ナノ開発研究とナノ安全科学研究が強固に連携し，両輪となって共に歩むことで，ナノDDS開発が飛躍的に進歩することを祈念してやまない。

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