Chapter 11

Biodegradation of Carbon-Based Nanomaterials

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11.1 Introduction

Among the various carbon-based nanomaterials (CNMs), carbon nanotubes (CNTs) and graphene have in the last few years emerged as two material types with the potential to be further developed in the field of nanomedicine. Indeed, modifying and engineering their basic graphitic structure in order to improve their biocompatibility has led to the demonstration of their possible use as delivery systems, biosensors, or composites for tissue engineering. However, while chemically functionalized CNMs present reduced toxicity and great

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biomedical promise, they are still viewed with scepticism, owing to the paradigm that their physicochemical characteristics make them nonbiodegradable. Recently, different studies have uncovered that peroxidase enzyme–based processes could lead to their oxidation and biodegradation. This chapter provides a review of current knowledge on this topic, including the proposed mechanism for enzymatically catalyzed biodegradation. In the context of biomedical use, these new findings offer novel perspectives for CNMs and also stress the need for future investigations that could reveal how to promote or inhibit their biodegradation, depending on the biomedical application desired.

11.2 Carbon-Based Nanomaterials

In the last decade, a novel class of nanomaterials based exclusively on carbon (Fig. 11.1) has been explored in the fields of nanobiotechnology and nanomedicine. Several proof-of-concept studies have demonstrated that fullerenes, CNTs, carbon nanohorns (CNHs), nanodiamonds, and, more recently, graphene may offer new tools for diagnosis and treatment of various diseases [1–4]. Amongst those CNMs, CNTs and graphene have both attracted a lot of interest from biomedical researchers, owing to their unique combination of chemical and physical properties.

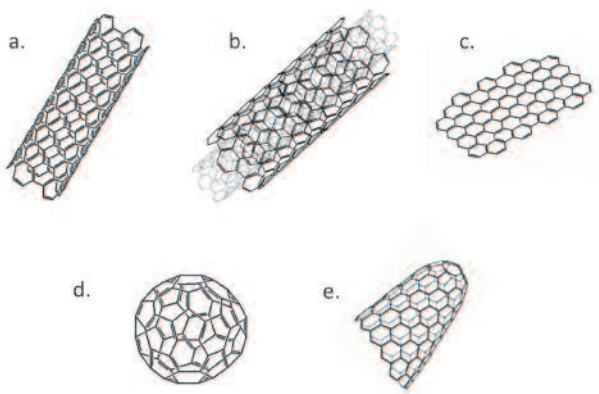

Figure 11.1 Different CNMs. (a) SWCNT, (b) MWCNT, (c) graphene, (d) fullerene, and (e) CNH.

CNTs are made of sp^2-hybridized carbon atoms, which are organized in one (single-walled carbon nanotubes [SWCNTs]) or more (multiwalled carbon nanotubes [MWCNTs]) concentric graphene sheets rolled up into thin, hollow cylinders. While the diameter of SWCNTs is in the 0.47–2.0 nm range, the MWCNTs' diameter can reach up to 100 nm. Both CNT types have lengths of several micrometers, making them a 1D nanomaterial with a characteristic high aspect ratio and high surface area. Likewise, graphene is also an sp^2-hybridized CNM but is a 2D material due to its one-atom-thick planar graphitic structure. Lateral dimensions of graphene flakes can be tailored according to specific applications. Compared to CNTs, both sides of the planar axis of graphene sheets are available, providing a platform for potentially higher degree of functionalization.

Both CNTs and graphene have outstanding features (electronic, mechanical, electrical, optical, thermal) that have been explored for a wide variety of biological and medical applications such as biosensors, tissue engineering, and targeted delivery systems [2, 4, 5]. They are both considered as unique materials for chemical or photothermal therapies but also molecular imaging [6–10]. Some of these prospective biomedical applications have been made possible by extensive chemical studies on methods to modify and functionalize the inherently hydrophobic surface of CNMs so as to make them compatible with aqueous-based environments [8,11–14]. Interestingly, functionalization (by covalent or noncovalent approaches) has also been shown to improve dramatically the biocompatibility of CNMs constructs compared to nonfunctionalized materials. Numerous studies have demonstrated how functionalized CNMs are able to deliver drugs, genes, or antigens to various types of cells without inducing toxicological effects or immune responses [15–17]. Pharmacokinetic studies have moreover revealed that the nature and degree of functionalization are essential parameters not only for the tissue absorption and distribution of CNMs but also for their excretion [7, 18, 19]. Similarly, for tissue engineering purposes functionalized CNM-based substrates have been shown to support and improve the growth and differentiation of neuron or osteoblast progenitors and also stem cells without deleterious biological responses [20–23]. Nevertheless, an essential issue that needs elucidation is the long-term fate of CNMs that will stay inside

the body. In relation to that, the question of the possible metabolism of CNMs by the body over time (i.e., biodegradation) was for a long time disclaimed due to their strong and chemically inert sp^2 graphitic structure that was believed to make them nondegradable under physiological conditions.

Recent work has reported that the perceived nondegradable CNMs may undergo enzyme catalyzed oxidation and biodegradation under specific conditions [24, 25]. In this chapter, we will review the current knowledge on this topic and discuss the implications for the biomedical field and some potential directions for future work. Indeed, if CNMs are naturally biodegradable, it is rational to believe that tailoring the CNM structure in a certain manner could accelerate or inhibit their inherent biodegradability, resulting in significant implications for their biomedical use. For instance, while durability is more likely to be suited for long-term tissue engineering products, highly biodegradable nanovectors for short-time intervention purposes seem more appropriate. Although clinical applications are still immature, prospective research that would focus on engineering the CNM structure and surface to control degradability will surely increase their chances for translation into the clinic.

11.3 Oxidation of Carbon-Based Nanomaterials

CNMs such as CNTs and graphene are by definition materials made exclusively of sp^2 carbon atoms that are difficult to oxidize. Only strong oxidative (i.e., nitric acid, sulfuric acid in combination with hydrogen peroxide [H_2O_2]) or severe mechanical (e.g., probe sonication or milling) treatments have been shown to introduce defects in the form of oxygen-containing groups onto the carbon lattice of pristine materials [26]. In addition to these chemical modifications, oxidation of CNMs also leads to structural alterations such as length shortening for nanotubes and hole opening in the planar sheets for graphene, producing polycyclic aromatic hydrocarbons and CO_2 as by-products [27–29].

Given such knowledge, CNMs were considered as nonbiodegradable material at least in physiological environment until Star et al. hypothesized and proved that enzyme-catalyzed oxidation processes are able to degrade them [30]. Two peroxidase

enzymes were primarily selected to study that hypothesis, horseradish peroxidase (HRP) [30, 31] and myeloperoxidase (MPO) [28]. While HRP, a plant enzyme, could be seen as a proof of concept not relevant to biomedical applications, MPO is the enzyme responsible for the production of strong oxidants (i.e., hypochlorite) able to destroy pathogens in immune cells such as neutrophils and macrophages, which are likely to encounter CNMs after administration in the body. Both enzymes contain in their active sites a heme group that catalyzes enzymatic reactions in the presence of hydrogen peroxide. Following the same principle, it was also demonstrated that CNMs could undergo degradation when incubated with either lactoperoxidase (LPO) [32] or eosinophil peroxidase (EPO) [33], both enzymes being able to produce another strong oxidative agent, hypobromite, in the presence of H_2O_2. Cyclo-oxygenases, catalases, or cytochrome C oxidases are other heme-containing peroxidases that may be studied in the future in order to extend the knowledge on CNM degradability in various parts of the body where those nanomaterials might accumulate or stay after environmental or biomedical exposure.

Alternatively, other research groups have used oxidizing and acidic electrolyte solutions to mimic the macrophage phagolysosome milieu in which CNMs have been described to be accumulating after cellular uptake [34–36]. In all cases, the concept in these studies did not involve active processes of degradation based on enzymatic activity (no hydrolytic enzymes added) but rather questioned the durability of CNMs over time in chemically aggressive physiological environments. As for enzymatic processes, other simulated biological fluids will need to be tested to consolidate the understanding of CNM fate in the body [37]. For instance, environments simulating the intraluminal (e.g., gastric or lung fluid) or interstitial (e.g., body or synovial fluid) fluids seem also relevant.

11.4 Ex vivo Biodegradation of CNMs

Both CNTs and graphene have been studied following the action of peroxidase enzymes or phagolysosomal-mimicking environments (Table 11.1). Even though various possible models of structural degradation of the material can be considered, only a few have been experimentally shown (Fig. 11.2).

Table 11.1 Ex vivo biodegradation studies ox-SWCNT = carboxylated SWCNT; GO = graphene oxide; RGO = reduced graphene oxide

Type of CNT	Type of functionalization	Degradative condition	Main results	References
SWCNT	Oxidised	HRP + H_2O_2 (40 μm), 4°C, Up to 12 weeks	HRP in presence of H_2O_2 catalyzed degradation of ox-SWNT	[30]
SWCNT	Oxdised, pristine	HRP + H_2O_2 (40 μm); Hemin + H_2O_2 (40 μm); $FeCl_3$ + H_2O_2 (40 μm); room temperature, 10 days	• No degradation of pristine while ox-SWCNT degraded over time • Degradation of pristine with hemin and FeCl3 • Production of CO_2 as final product of degradation	[31]
SWCNT	Pristine, COOH, Phosphatidylserine coated, phosphatidylcholine coated	MPO + H_2O_2	• CNT degraded by neutrophil and to a lesser extent by macrophage • Myeloperoxidase and NADPH oxidase are essential in degradation by neutrophils	[28]
SWCNT	Oxidised (different degree), pristine, ozone treated, arylsulfonated	PSF + H_2O_2 (1 mM), pH 4.5, 90 days	• Only oxidized CNT degraded over time (low oxidation, 15 min do not underwent degradation) • Low pH is not necessary to obtain degradation	[34]
SWCNT & MWCNT	Oxidised	PSF + H_2O_2 (1 mM), pH 4.5, 60 days; HRP + H_2O_2 (40 μm) 60 days	• SWCNT oxidized in 30d whereas MW are not fully degraded after 60d • HRP is more efficient than PSF • Exfoliation of outer layers for MW + shortening from edges • NT with more defects are more sensitive to degradation	[??]
SWCNT	Oxidised	MPO + H_2O_2; MPO + NaCl; NaOCl; LPO + H_2O_2; LPO + H_2O_2 + NaBr	• Production of HOCl by MPO is responsible for degradation of CNT by MPO, similarly HOBr degrade SWCNT with LPO • HOCl and HOBr are able to induce degradation • MPO + H_2O_2 in presence of chloride ions and LPO + H_2O_2 in presence of bromide ions work better than without ions	[32]

SWCNT & MWCNT	Pristine, for MW = long, tangled, spinnable	Gamble solution, 24 weeks	• Long-MW are shortened until 3 weeks, then length did not change • Almost no loss in weight or structure over the 24 weeks period • Shortened CNT did not induce inflammation and granuloma whereas original material did • Short-MW seemed durable but induce minimal biological response	[35]
MWCNT	Purified, oxidised, nitrogen-doped	HRP + H_2O_2 (40 μM), 80 days	• Decrease in length and diameter of MWCNT, amorphous carbon on outer layer • Layer-by-layer exfoliation process due to side walls defect • Oxidized-MW less degraded than nitrogen doped MW • Fastest degradation rate related to highest level of oxidation	[39]
SWCNT	Oxidised	EPO + H_2O_2; EPO + H_2O_2 + NaBr;	• Production of HOBr by EPO is responsible for degradation of CNT by EPO • Neither EPO alone nor H_2O_2 in alone cause nanotube degradation • EPO + H_2O_2 in presence of bromide ions work beteer than without ions	[??]
SWCNT & MWCNT	Oxidised and PEG functionalized	MPO; LPO; cytochrome C; hemoglobin, HRP with/without halide ions (Cl^-/Br^-)	• HOCl able to induce degradation of PEG-SWCNT, even in presence of blood plasma • Introduction of HOCl production after incubation of SWCNT with isolated neutrophils • Neutrophils are activated by incubation with PEG-SWCNT and increase of MPO level in blood sample after incubation with PEG SWCNT • Upon intraperitoneal injection, number of neutorophil and level of cytokine increased	[38]
Graphene	GO RGO	HRP + H_2O_2 (40 μM)	• HRP catalyzed oxidation of GO but not RGO • Oxygen groups of GO interacted with the enzyme • Production of CO_2 as final product of degradation	[29]

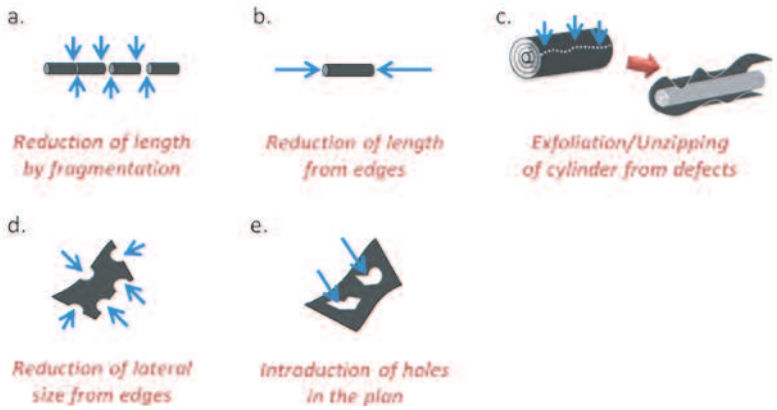

Figure 11.2 Theoretical (a–e) and experimentally demonstrated (b, c, and e) models of biological degradation of CNTs (a, b, and c) and graphene (d and e) (without taking into account dissolution of the sp^2-hybridized carbon backbone structure).

11.4.1 Ex vivo Biodegradation of SWCNTs

The first study that reported strong oxidative enzymes able to degrade CNTs appeared in 2008 [30]. In that study, Star et al. described how carboxylated SWCNTs can be degraded at 4°C by the oxidative activity of HRP in the presence of low concentrations of hydrogen peroxide. Repeating the same experiment but at room temperature, they showed that carboxylated SWCNTs can degrade more quickly than at 4°C, while pristine SWCNTs could not [31]. Oxidized polycyclic aromatic hydrocarbons were detected as by-products of partial or incomplete degradation, and carbon dioxide was the ultimate product of complete degradation after 10 days. From this work, it was concluded that structural defects (presence of oxygen-containing groups) or functional groups in the graphitic lattice may act as initiator sites for enzymatic action.

In a second set of studies, the human MPO enzyme was used [28]. The primary hypothesis was that reactive radical intermediates and hypochlorite generated by MPO from H_2O_2 would catalyze the oxidation of carboxylated SWCNTs. It was observed that degradation occurred when SWCNTs were incubated either in a mixture of MPO-H_2O_2 or in hypochlorite alone, but there was no degradation in MPO or H_2O_2 solutions alone. Moreover, nanotube degradation by MPO-H_2O_2

treatment was enhanced in the presence of NaCl, suggesting a role for both hypochlorous acid and peroxidase reactive intermediates in the MPO-catalyzed degradation. Notably, the biodegradation of pristine noncarboxylated SWCNTs was reduced under the same conditions (i.e., MPO-H_2O_2 with NaCl). It was concluded that degradation by oxidation of pristine SWCNTs is mediated by hypochlorite, whereas degradation of carboxylated SWCNTs involved both hypochlorite and the MPO enzyme. Going further, the authors hypothesized a mechanism in which hypochlorite would be introducing oxidative defects in the form of oxygen-containing groups that could in a second step favor interaction with the enzyme, leading subsequently to degradation by peroxidase reactive radical intermediates.

Similarly, Vlasova et al. investigated the potential of degradation by different oxidants produced by MPO (i.e., active peroxidase intermediates, hypochlorite, and reactive free radicals) on carboxylated SWCNTs and compared the effectiveness of MPO degradation with the LPO action [32]. Using hypochlorite scavengers, they confirmed the major role of hypochlorite in SWCNT degradation by MPO. Both MPO and LPO were found to be more effective in the presence of chloride and bromide ions, respectively, demonstrating that hypochlorite and hypobromite accelerate the enzyme-mediated degradation. The same group more recently demonstrated that covalently polyethylene glycol (PEG)ylated SWCNTs can also undergo hypochlorite-/hypobromite-mediated oxidation [38].

The durability of SWCNTs was also assessed in a phagolysosomal simulant fluid medium, at pH 4.5 [34]. As for HRP, oxidized SWCNTs were prompted to degradation following addition of hydrogen peroxide, compared to pristine SWCNTs, which appeared to be resistant to these mild physiological oxidative conditions. In another study, also mimicking an acidic phagolysosomal environment (pH 4.5), an electrolyte solution (Gamble's solution) was used to assess whether SWCNTs can undergo structural modifications (diameter and length), but no such changes were observed [35].

11.4.2 Ex vivo Biodegradation of MWCNTs

The capacity of carboxylated MWCNTs to biodegrade when mixed with HRP or phagolysosomal simulating fluid (PSF) was also investigated and compared to carboxylated SWCNTs under the same

conditions (Fig. 11.3) [36]. In less than 30 days, the carboxylated SWCNTs were fully degraded in both environments, whereas MWCNTs were still not fully degraded after 60 days, albeit their structure was clearly modified. On the basis of TEM analysis, it was concluded that degradation of SWCNTs occurred by an unzipping mechanism, and the lengths of MWCNTs were significantly shortened compared to the starting material when both HRP and PSF were used. The comparison between HRP (enzyme-based) and PSF (nonenzyme-based) degradation conditions demonstrated that there was no clear difference in terms of degradation potential using the two types of oxidative environments.

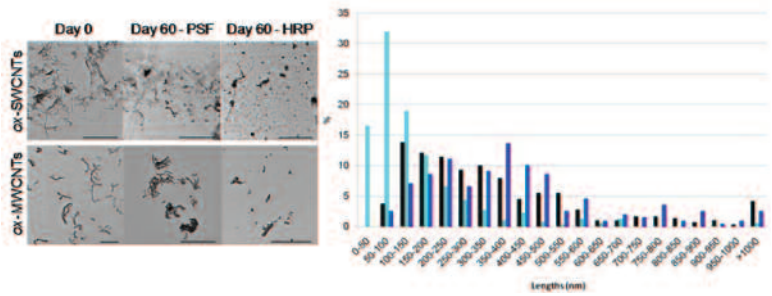

Figure 11.3 Ex vivo biodegradation of SWCNTs and MWCNTs. TEM images of ox-SWCNTs and ox-MWCNTs during the degradation process. The samples were collected at time 0 and 60 days in PSF and HRP oxidizing conditions. Scale bars correspond to 500 nm. Statistical distribution of the length of the ox-MWCNTs at the end of the degradation process (60 days). Blue bars correspond to starting material. Black and cyan bars correspond to PSF and HRP treatment, respectively. *Abbreviation*: TEM, transmission electron microscopy. Adapted from Russier et al., 2010, copyright Royal Society of Chemistry.

Zhao et al. also investigated the degradation of different MWCNTs (purified, oxidized, or nitrogen doped) under HRP/H_2O_2 conditions and offered a tentative explanation on the mechanism that was taking place [39]. In agreement with the previous study, the authors showed a decrease in length and diameter and proposed a layer-by-layer exfoliation process due to accumulation of structural defects on the outer wall of the tubes. The degree of carboxylation was correlated with the rate of degradation. Interestingly, oxidized

MWCNTs were less degraded than nitrogen-doped nanotubes, which completely disappeared after 90 days, suggesting that the amount of pre-existing defects present on the graphene backbone (which is higher for N-doped CNTs) is also an important parameter in CNM biodegradation. Overall, oxidative defects present in the outer layer seemed to act as initiator of further oxidation by reactive intermediates (such as $HOCl^-$) produced by the enzyme.

In another study, three kinds of MWCNTs were incubated in Gamble's solution (pH 4.5) to mimic the long-term degradation that may take place in phagolysosomes of macrophages [35]. Only the material identified as "long" reported a significant decrease in length after 10 weeks of incubation, with no change in diameter observed. Moreover a 30% decrease in weight for the same sample was recorded after 24 weeks of incubation. No mechanistic interpretations regarding degradation were offered in that study.

11.4.3 Ex vivo Biodegradation of Graphene

Only a single study has so far reported biodegradation of CNMs that do not belong to the family of CNTs but graphene [29]. In this work, graphene oxide (GO) and reduced graphene oxide (RGO) were exposed to a mixture of HRP and H_2O_2. HRP-catalyzed oxidation was efficient on GO but not on RGO, demonstrating, as mentioned before, the role of functional oxygen-containing groups as initiator sites for enzymatic activity. Degradation by enzymatic oxidation of the graphene lattice led to formation of holes in the planar sheet, with the diameter of these holes increasing with time. As for CNTs, the ultimate by-product of biodegradation was CO_2. Gel electrophoresis and modeling studies were conducted to understand the interactions of the GO or RGO with the HRP enzyme at the molecular level, suggesting that the HRP active site can achieve closer proximity to GO compared to RGO. Moreover, molecular modeling results indicated that HRP could oxidize GO sheets across their planar axis but not from their edges.

Putting together the different results generated by the ex vivo degradation of graphene, SWCNTs, and MWCNTs, the kinetics of degradation of one MWCNT can now be more precisely envisioned (Fig. 11.4).

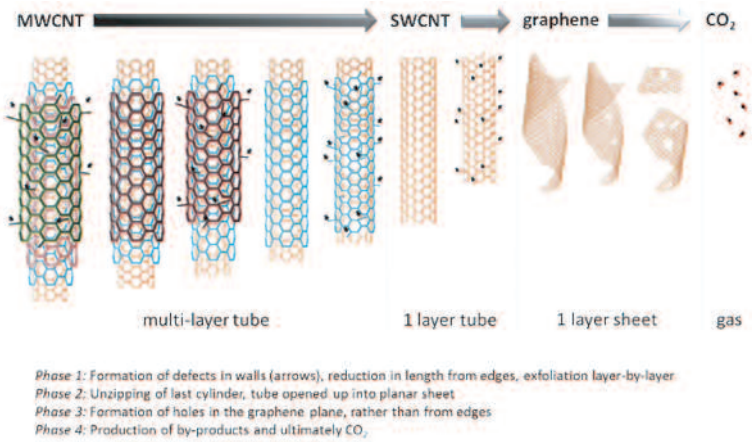

Figure 11.4 The continuum of graphene materials' degradation. The schematic is representing a proposed kinetic of MWCNT degradation into CO_2 through SWCNT and graphene successive states according to the current knowledge. Phase 1: Layer-by-layer exfoliation and shortening by tips of a multilayered tube to form a monolayered tube. Phase 2: Unzipping of the damaged one-layer tube to form a planar sheet. Phase 3: Formation of holes in the one-layer sheet. Phase 4: "Dissolution" into by-products, polycyclic aromatic hydrocarbons, and ultimately CO_2.

11.5 Biodegradation of CNMs in Living Systems

Beside the demonstration of ex vivo degradability, evidence has been reported that CNMs, and especially CNTs, are also degradable in vitro and in vivo (Table 11.2). At the cellular level, Kagan et al. were the first to report that carboxylated and IgG-functionalized SWCNTs targeted to human isolated neutrophils, could undergo MPO-related degradation after stimulated uptake [28]. In the same work, human monocyte-derived macrophages were also proven to be able to engulf carboxylated SWCNTs and to degrade them, though to a lesser extent compared to high-concentration MPO-containing neutrophils (13% and 100% of the original dose, respectively). Raman spectroscopy used as a mean to assess the evolution of CNT structure within neutrophils revealed that the amount of defects increased over time. In another study, PEGylated SWCNTs were shown to activate isolated neutrophils, which in response were

Table 11.2 In vitro and in vivo biodegradation studies

Type of CNT	Type of functionalization	Biological model	Time points	Techniques	Main results	References
MWCNT	pristine	Intratracheal instillation in rat	15 days	Transmission electron microscopy	Decrease in length of CNT	[41]
DWCNT	COOH	Human prostate adenocarcinoma cells PC3	0.5 to 24 hrs	Raman spectroscopy	Loss of Raman signal from the outer layer of the DWCNT over time; loss of Raman signal from inner layer of DWCNT appeared at 24 hrs only. Proposal: Exocytosis of CNT rather than degradation.	[40]
SWCNT	IgG-COOH-CNT complex vs COOH-CNT	Stimulated human neutrophils, human monocyte derivated macrophages	6h and 12h (48h for macrophage)	Raman spectroscopy, UV-Vis-NIR spectra	100% IgG-CNT degraded by neutrophil and to a lesser extent by macrophage (13%-up to 50% at 48hrs); non-IgG-functionalized CNT underwent 30% degradation by neutrophil; hypothesis: myeloperoxidase and NADPH oxidase are essential in CNT degradtion by neutrophils.	[28]
SWCNT	COOH	Pharyngal aspiration in wild-type and MPO knock-out mice	28 days	Hyperspectral imaging methodology and Photoacoustic imaging technology complete with Raman and NIR spectroscopies, and TEM	Inflammatory response is higher in myeloperoxidase knock-out compare to wild-type mice, similarly SWCNT oxidation and clearance from the lungs are reduced in those KO mice. Myeloperoxidase is essential in the lung biodegradation of SWCNT.	[42]
MWCNT	NH_3^-	mouse cortical brain injection	2 and 14 days	Transmission electron microscopy and Raman spectroscopy	Uptake by microglia; partial loss of carbon tubular structure and evolution of Raman signature over time.	[43]

producing more MPO and hypochlorite [38]. Following the same pathway, biodegradation of SWCNTs was also demonstrated when incubated with primary eosinophils, which can produce HOBr via EPO [33].

Cellular degradation was also mentioned in a different study to explain the alteration of the graphene structure of oxidized RNA-coated double-walled CNTs probed by Raman mapping [40]. In that study, following internalization by human prostate adenocarcinoma cells, defects in the outer layer of CNTs increased and the outer diameter of the tubes was reduced. No experimental evidence was, however, provided to elucidate these observations.

At the tissue level, early suggestions of in vivo degradation of MWCNTs (i.e., reduction in length) in lung 15 days after intratracheal instillation were reported in 2008 [41]. However, detailed evidence of in vivo digestion of carboxylated SWCNTs via an MPO-mediated process was only provided in 2012 [42]. In that work, MPO knock-out mice were exposed to carboxylated SWCNTs via pharyngeal aspiration and compared to wild-type mice. The results showed that SWCNTs disappeared significantly from the lungs in the wild-type mice but not in the MPO knock-out mice. Raman spectroscopy and TEM were moreover used to confirm that degradation of SWCNTs was more pronounced in the wild-type mice than in the knock-out mice. This work clearly confirms the role of the enzyme in the degradation of CNTs, at least in the lung area where neutrophils are commonly the first responders toward exogenous threats.

In a relevant study for biomedical application, we have also reported that ammonium-functionalized MWCNTs (NH_3^+-CNTs) undergo degradation over time (from 2 to 14 days after injection) following an intracranial injection into the brain cortex of mice [43]. TEM analysis demonstrated that NH_3^+-CNTs were uptaken by microglia and that their cylindrical structure was altered. Moreover, Raman spectroscopy confirmed an evolution of the characteristic Raman signal for CNTs with modification of the D/G band intensity ratio, showing an increase of defects over time. Interestingly, the same NH_3^+-CNT compounds were used previously by our group to mitigate neurodegeneration after local ischemic damage via a local delivery of caspase 3 short interfering RNA (siRNA)-related sequence [44].

11.6 Biological Effects of Biodegraded CNMs

As mentioned before, biodegradation by oxidation of the CNM graphitic lattice could lead to the formation of polycyclic aromatic hydrocarbons, which have been identified as carcinogenic, mutagenic, or teratogenic chemicals for some of them. The biological effects (e.g., immunogenic profile) and potential toxicity of the by-products of CNM biodegradation have therefore to be investigated with close attention.

In the first study to explore this question, Kagan et al. demonstrated that products of CNTs, predegraded ex vivo by MPO treatment induce neither inflammation—in the form of lung neutrophil accumulation or increase of proinflammatory cytokine levels—nor formation of granulomas in pharyngeal instilled mice [28]. Those products of degradation were identified as short-chain carboxylated alkanes and alkenes. On the contrary, nondegraded CNTs (or nanotubes incubated with MPO or H_2O_2 alone that were not extensively modified) were inflammogenic and led to the formation of tissue granulomas. Moreover, partially degraded CNTs still induced inflammation, though to a lesser extent compared to nondegraded nanotubes. In a second work, they showed that lung inflammation response to CNT pharyngeal aspiration was stronger in MPO knock-out mice compared to wild-type mice, where CNTs can still undergo MPO-mediated degradation to alleviate their effects [42]. These results suggest that MPO-degraded CNTs were less toxic than the nondegraded tubes and that controlling degradation (i.e., triggering) could be a key toward safer use of CNMs for nanomedicine.

In another study, Donaldson et al. treated four different kinds of CNTs for up to 24 weeks in a modified Gamble's solution, pH 4.5 [35]. For one sample of long MWCNTs, they observed a 30% decrease of mass after 24 weeks' incubation and a shortening of the length of the tubes was reported at 10 weeks. Importantly, when intraperitoneally injected, the degraded CNTs (10 weeks' incubation) were less inflammogenic and fibrogenic and did not induce formation of granulomas compared to nonincubated (long) CNTs. Those results demonstrate that biological effects can be mitigated by degradation on a sample-specific basis.

11.7 Conclusions

For a long time, CNMs were perceived as materials with great promises for the biomedical field but with a major drawback: their nonbiodegradability due to a chemically inert graphitic structure. This fact has led to the conclusion that their translation into the clinic will never be achievable with the CNM products available. Recent reports have, however, demonstrated that CNMs undergo biodegradation under specific conditions and that these processes might also take place in living systems, offering new perspectives for the development of CNMs in nanomedicine.

At the moment, research on the biodegradation of CNMs is in its infancy: only the biodegradation of CNTs and graphene has been explored so far, and the knowledge and understanding of the mechanisms involved are still limited. Moreover, the CNMs used were in most cases not directly relevant for biomedical applications and were not studied in a realistic physiological environment (e.g., in the organ of accumulation after administration or after uptake by phagocytic cells). Therefore, there are still various investigations to be conducted to complete the understanding of the potency of CNM degradation in living systems and to decipher how to use this biodegradation property for the benefit of CNM biomedical applications.

Among those studies, the main questions to address will be (i) the inherent degradability of CNMs, (ii) the inherent degradability of biomedical constructs (i.e., CNMs modified to optimize their biocompatibility and biological activity), (iii) the different possibilities of CNM degradation in living systems (via enzymes, oxidizing agents, pH, or others), and (iv) how to control (i.e., enhance or inhibit) the biodegradation of carbon biomedical constructs. The knowledge that could generate such investigations will greatly improve the design of CNMs for biomedical applications and also participate to their potential clinical translation.

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