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Biodegradation of Carbon-Based Nanomaterials: The Importance of "Biomolecular Corona" Consideration

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It has been over a decade since oxidative enzymes were first used to degrade carbon-based nanomaterials (CBNs). Although enormous progress has been achieved in this field, many questions and problems remain unresolved that need to be answered to usher these materials toward their true destiny. Nanobioscience researchers now know that ignoring the biomolecular corona (BMC) in nanobiomedical studies, either inadvertently or intentionally, is by no means justified. However, a major drawback to progress is that BMC effects on CBN biodegradation have been omitted from a large number of studies. What's more, many studies in the field have eliminated the BMC source in the relevant experiments. Thus, the most critical question that one needs to probe is whether the BMC and its characteristics affect the biodegradability of CBNs? In this conceptual perspective paper, recent progress and significant research in CBNs biodegradation are summarized. Then, the importance of the BMC and its possible impacts on the biodegradation of CBNs are thoroughly explored as a conceptual guide. Finally, remaining challenges and the direction of future research are provided, and barriers that need to be overcome to advance the field are discussed including recommendations regarding BMC considerations and study design and reporting guidelines.

1. Introduction

Carbon-based nanomaterials (CBNs), such as graphene-based nanosheets (GBNs), carbon nanotubes (CNTs), carbon dots, nanodiamonds, and fullerenes are considered to be one of the key elements in many nanotechnology-related applications (**Figure 1**a).[1–3] Due to their outstanding chemical, physical, optical, thermal, and electrical properties, CBNs have gained a great deal of attention in recent years in many areas of biomedical research, including drug delivery systems, regenerative medicine, biosensing, and antimicrobial coatings.[4–6] Therefore, it is fundamental to recognize the potential health risks related to their human exposure. Indeed, before these nanomaterials (NMs) can be safely applied in clinical applications, their biodistribution, biocompatibility, and biodegradation need to be carefully assessed.[7]

Similar to many other particulate materials, the bio-persistence of CBNs is a crucial factor in determining their health effects. In other words, successful biodegradation is one of the main factors governing the biological responses and the life span of NMs. In this regard, several research groups have investigated the biodistribution profile of CBNs to quantitatively and qualitatively determine the accumulation site(s) of these materials and their associated toxicity.

Biodegradation of CBNs has been pursued extensively in the last few years in biomedical research, especially regarding the most commonly investigated CBNs, including CNTs and GBNs. However, very recently, in a published correspondence in Nature Nanotechnology, it was reported that CNTs became the first NM to be added to the SIN ("Substitute It Now") List as a NM of very serious concern.[8] Although this addition and the classification of CNTs as a single material category has been

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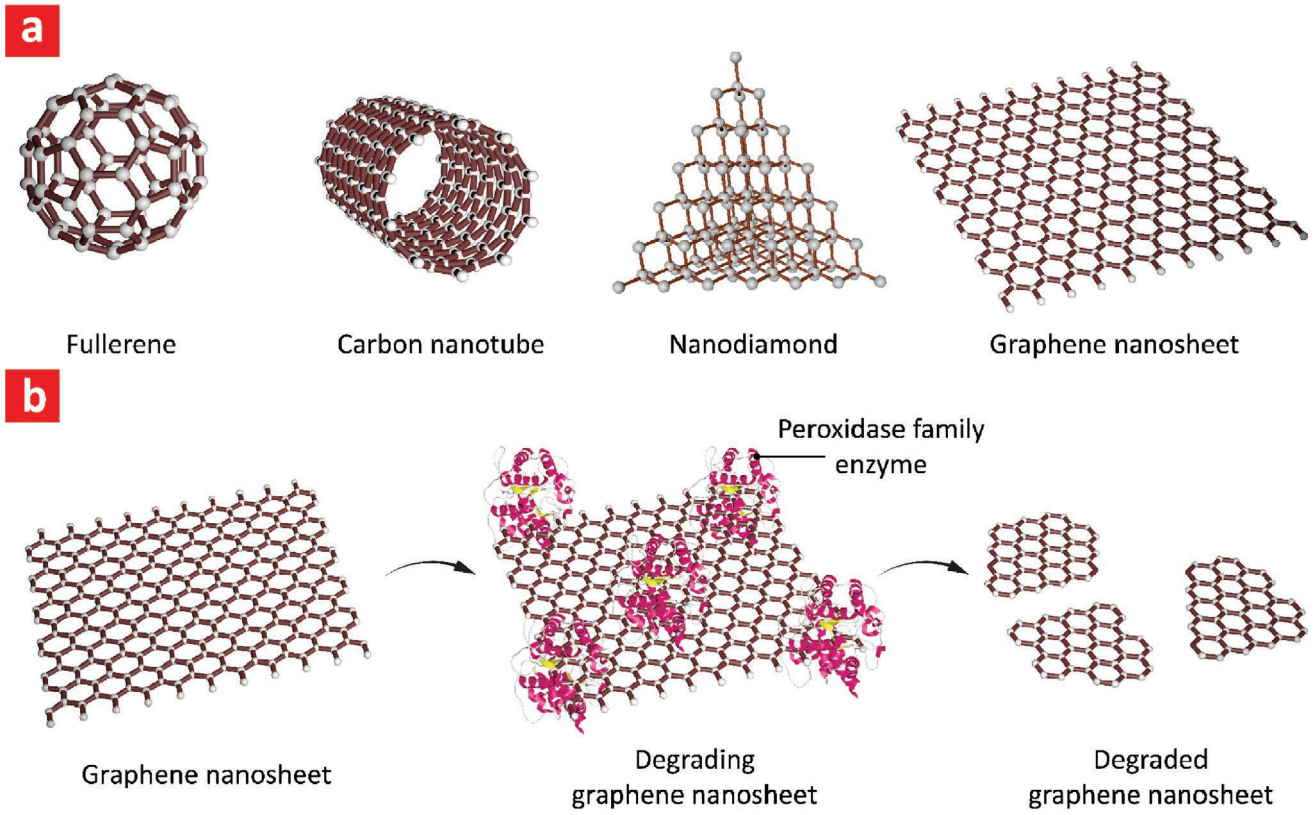

Figure 1. a) The most important CBNs applied in the field of nanomedicine. In this group of NMs, CNTs and GBNs have attracted most attention in biomedical applications. b) Schematic representation of graphene nanosheet biodegradation by a peroxidase family enzyme. Peroxidase-mediated biodegradation of CBNs was proven more than a decade ago.

criticized by many researchers in the field,[9,10] needless to say more attention has to be paid to the health concerns of CBNs before pushing their applications forward. Overall, it has been found that CBNs accumulate in different tissues, especially the reticuloendothelial system organs.[11–13] Thus, it is necessary to study the biodegradation of CBNs and their accumulation-dependent toxicity, which may occur in different organs and persist for a long time after administration.

Toward this direction, several groups have reported CBNs biodegradation by enzymatic catalysis via peroxidase enzymes (Figure 1b) such as human myeloperoxidase (MPO),[14] horseradish peroxidase (HRP),[15–17] eosinophil peroxidase (EPO),[18] lactoperoxidase (LPO),[19] manganese peroxidase (MnPO),[20] lignin peroxidase (LiPO),[21] and microbial xanthine oxidase (XO).[22] However, studies on the biodegradation and fate of CBNs in vivo are still inconclusive or missing. To date, most studies on enzyme-catalyzed biodegradation have been performed in vitro, and there are few in vivo studies on this subject. Needless to say, the results obtained from in vitro experiments cannot reasonably guarantee similar results in vivo, due to extensive signaling and feedback loops that can alter the enzyme concentrations and their functioning in real-time as well as competition by other biomolecules for the CBNs surfaces which may reduce the effectiveness of the enzymatic degradation. Therefore, building reliable and consistent extrapolation procedures from in vitro results to in vivo is extremely important. However, contrary to this common wisdom, studies on the biodegradation of CBNs have not paid much attention to this vital aspect. In support of this claim, from the literature review of CBNs biodegradation performed herein, it is quite evident that serum-free cell culture media and buffers such as phosphate-buffered saline are the most common biological environments which have been considered for acellular in vitro and cell-based in vitro biodegradation experiments. Therefore, from a nano-bio interface point of view, it is clear that eliminating serum or plasma as biomolecular corona (BMC) sources from the experiments could call into question the validity of the research process and results when we want to apply them in more clinically relevant applications. It should be noted that this type of study design may be due to the simplification of experiments in the early stages of the field, although more than a decade has passed since the first study on degradation of CBNs was reported. Thus, it is expected that in the step-by-step route of knowledge development, the BMC effects will increasingly be considered and measured in all NMs–biological interactions studies, including CBNs degradation studies.

It has been well established that a selected group of biomolecules rapidly covers NMs in contact with biological fluids to form "a corona" that is the actual interacting surface with biological systems.[23–25] The corona is a very selective layer of proteins and other biomolecules (such as lipids, sugars, and nucleic acids), which strongly adsorbs on NMs surface and confers a new identity to the NMs.[26,27] Indeed, NMs' interaction with biological systems such as organs, cells, and body

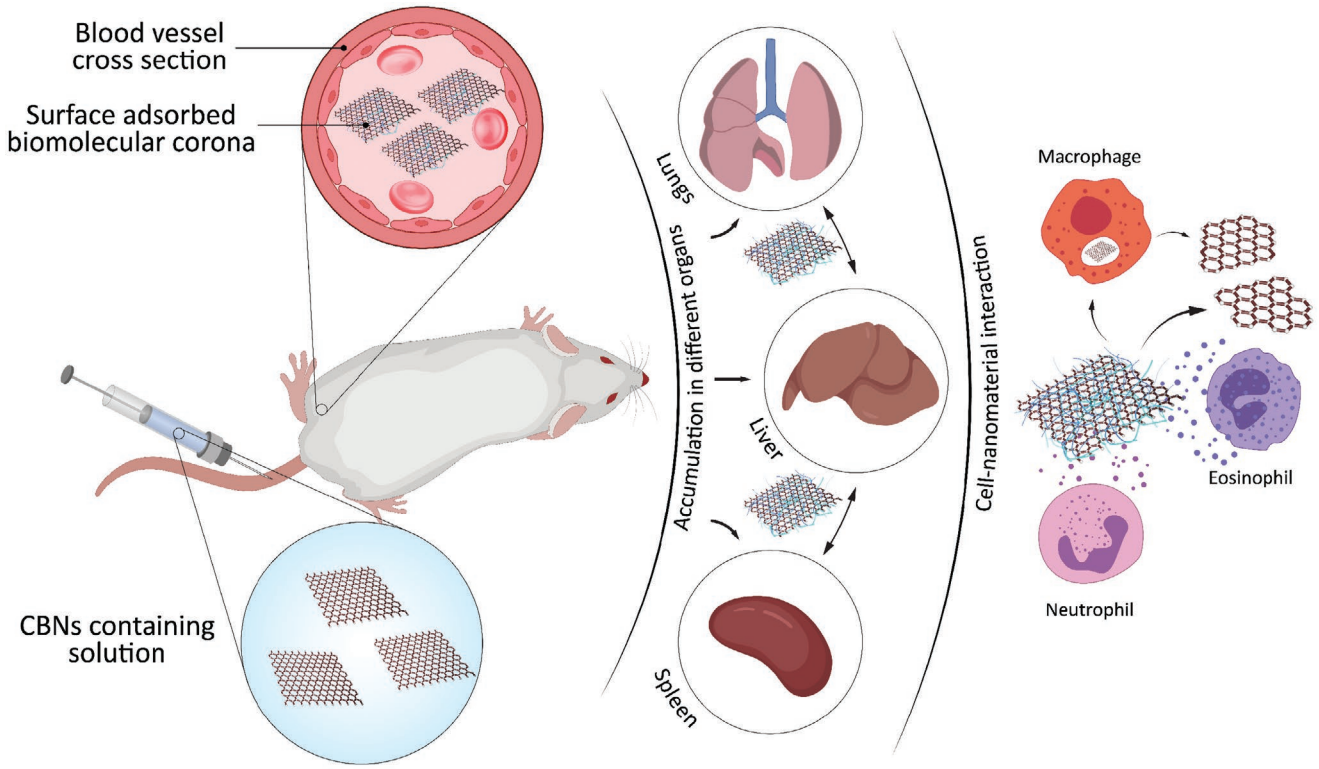

Figure 2. Schematic representation of the concept of the present article. Similar to other types of NMs, just milliseconds after their injection into the bloodstream, the surface of CBNs is coated with a BMC whose constituents depend on the nature of the functional groups and physical properties of the CBNs. The BMC mediates the biological behaviors of CBNs, such as their preferred accumulation site and their interactions with different cells and cellular receptors. To date, the effect of the BMC on the interaction of CBNs with cells involved in their degradation process (such as neutrophils, eosinophils, and macrophages) has not been investigated.

fluids is mediated by the BMC (**Figure 2**).[23,28] Consequently, it is expected that the BMC and its characteristics, as critical determinants in nano-bio interfaces, must always be considered in nanobiomedical studies. However, contrary to this expectation, as we mentioned above, the commonly used biological environments in CBNs biodegradation experiments lack serum or plasma (BMC origins). Hence, to date, biodegradation investigations appear not to have focused on BMC, its characteristics (such as composition, conformation, charge, surface concentration, thickness, the binding strength to the surface, and type of intermolecular cross-linking), and its effects on the CBNs biodegradation process (**Figure 3**). This issue is not limited to CBNs biodegradation studies, and the BMC effects in many in vitro and in vivo toxicity and efficacy studies are still not well considered. What makes these kinds of measurements more complex is that all of the characteristics listed above for the BMC are interrelated and affect each other, and none have an independent effect on BMC function. Therefore, a change in any of these characteristics can cause a change in the other characteristics, and as a result, a change in intermolecular interactions such as electrical, chemical, and physical interactions will alter the interaction of NMs with peroxidase enzymes and oxidizing agents during the biodegradation process. Among the potential impacts of the BMC on biodegradation are reduced enzyme binding due to competition for the CBN surface leading to lower access to the sites for degradation, as well as indirect effects related to the corona composition including, for example, incorporation of dysopsonizing proteins that reduce uptake by macrophages or other specialist cells involved in biodegradation of the CBNs, or other proteins that trigger CBN escape from lysosomes, for example, that can reduce the biodegradation efficacy.

Of course, it should also be borne in mind that the process of biodegradation is like a double-edged sword. Because in some applications of CBNs, such as tissue engineering and theranostics, where the nanobiomaterial needs to be more biopersistent, enzymatic degradation may be problematic. Therefore, an important challenge in this regard is to engineer the biodegradation period of CBNs in an appropriate time frame, and of course, the BMC will affect this process, positively or negatively, we do not yet know how to control this, which is clearly a knowledge gap that needs to be investigated. We, therefore, speculate that the absence of this overlooked factor (BMC) in biodegradation studies hinders accurate interpretation of biological readouts in CBNs studies based on available acellular in vitro and cell-based in vitro knowledge. Along this line, it was shown by Vlasova and coworkers that human serum albumin (HSA) could inhibit the degradation of single-walled carbon nanotubes (SWCNTs) against MPO-Cl^-–H_2O_2 solution.[29] In a similar study, Lu and colleagues reported that the binding of HSA to the surface of nanotubes prevented acellular in vitro biodegradation of the SWCNTs due to the

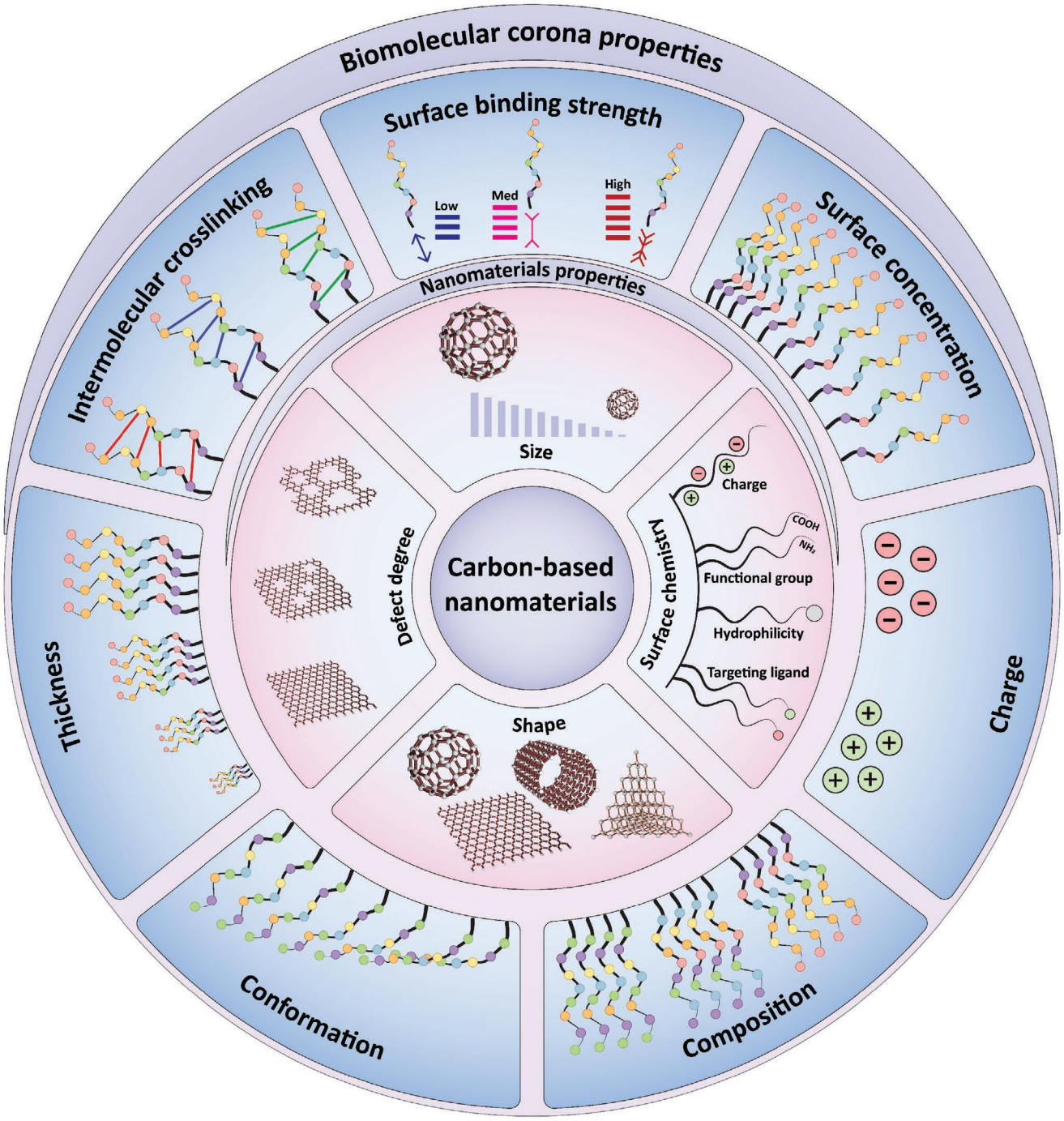

Figure 3. Schematic demonstration of CBNs and BMC properties. The BMC formed on the surface of CBNs determines their biological behavior/fate and interaction with tissues and cells. The identity that the BMC confers on a CBN depends on a set of BMC characteristics that are absolutely dependent on the NM physico–chemical properties. Examining each of BMC characteristics will help to understand exactly how and to what extent the BMC affects CBNs biodegradation process.

protective effects of HSA against oxidants. Furthermore, it was pointed out that coating with HSA stimulated neutrophils to release MPO and generate OCl^-, and also increased the cellular uptake of the nanotubes.[30] Likewise, Li et al. demonstrated that both bovine serum albumin (BSA)- and polyethylene glycol (PEG)-coated graphene oxide (GO) are resistant to HRP-induced biodegradation, mainly as a result of the spatial hindrance provided by the coating molecules.[31] With this in mind, and on the subject of the present article, we note that BMC and even metabolized BMC substances (biodegraded biomolecules originating from the BMC)[32] also can prevent the proper interaction of peroxidase enzymes and oxidizing

Table 1. Peroxidase enzymes used for CBNs biodegradation, their source, and redox potentials of their intermediates during the enzymatic catalytic cycle.

Enzyme name	State of peroxidase cycle	Reduction potential [E^0, V]	Source
MPO	Compound I/Resting state	1.16	Neutrophil
	Compound I/Compound II	1.35	
	Compound II/Resting state	0.97	
EPO	Compound I/Resting state	1.10	Eosinophil granulocyte
LPO	Compound I/Compound II	1.14	Goblet cell
	Compound II/Resting state	1.04	
HRP	Compound I/Compound II	0.94	Root of horseradish
	Compound II/Resting state	0.96	
LiPO	n.d.[a)]	n.d.	*Phanerochaete chrysosporium*
MnPO	Compound I/Resting state	n.d.	*Phanerochaete chrysosporium*

[a)]Not determined.

agents with the surface of CBN through the spatial hindrance. Contrary to the studies cited above, Bhattacharya et al. reported the biodegradation of oxidized SWCNTs by mammalian LPO enzyme and in the presence of pulmonary surfactant (consisting of 10% specific surfactant proteins and 85–90% of phospholipids). The authors also briefly mentioned that the presence of pulmonary surfactant does not prevent the biodegradation process.[19] However, they did not provide further details regarding the biodegradation efficiency, mechanism of enzyme–BMC interaction, or BMC characteristics. To the best of our knowledge, the last four articles cited above[19,29–31] are the only ones that refer to, though implicitly, the effect of some simple BMC models (and not their characteristics) on the biodegradation of CBNs. Therefore, the orientation of future studies to consider in more detail the impact and role of the BMC can reveal many details on the mechanisms involved in the biodegradation of CBNs.

As recognized in nano-bio interface science, the acquired BMC properties determine the most important characteristics of NMs, for example, their blood circulation time, the rate of their cellular uptake, immune system stimulation, biocompatibility, biodistribution, and biodegradation.[33–37] In the path of translating CBNs from bench to bedside, all the properties mentioned above must be examined with the wisdom that these properties can be significantly altered by the BMC presence and its evolution as the CBNs are translocated and transported in biological systems, and of course, as the CBNs are degraded and the cells respond to the presence of the CBNs they secrete other biomolecules which may have a higher affinity for the CBNs thus displacing initially bound biomolecules.[38–40] In this regard, conceptual articles may provide guidance for researchers and command much more attention to overlooked factors in the field. Herein, after exploring the advances in CBNs biodegradation investigations, we specifically highlight the importance of BMC consideration in the field. Finally, the future directions and challenges that need to be pursued by the research community are discussed.

2. Biodegradation of Carbon-Based Nanomaterials

An important and attractive part of nanomedicine research includes assessment of the biodegradation of CBNs in order to diminish their toxicity and simplify their biomedical applications. Until about a decade ago, scientists thought that CBNs are non-biodegradable due to their inert graphitic structure and strong resistance to degradation under physiological conditions.[41,42] However, subsequent works in this domain confirmed the biodegradability of these materials by oxidative enzymes (i.e., peroxidases) acellular in vitro, cell-based in vitro, and in vivo.[43] In 2008, for the first time, Allen et al. showed the enzymatic degradation of SWCNTs by HRP.[15] Following this pioneering report, degradation of various forms of CBNs (such as CNTs and GBNs) has been uncovered using many other peroxidase enzymes. As an example, in a recent study, Luan et al. demonstrated that carboxyl-functionalized graphene nanoribbons (GNRs) can be efficiently degraded by MPO or photo-Fenton reaction. The results showed that both MPO and photo-Fenton reactions degraded GNRs almost completely after 120 h.[44] **Table 1** lists the enzymes studied to date, their source, and the redox potentials of their intermediates during the peroxidase cycle.[17,43,45,46] Previous studies in this domain have already elucidated these enzymes' action mechanisms, especially HRP and MPO.[16,17,20,43,45,47] Briefly, as shown in **Figure 4**a, the respected enzymes have a heme group (Fe(III) protoporphyrin IX) as their active site while this group is converted to the Fe(III) state when the enzyme is inactive. By the interaction of the inactive heme group with hydrogen peroxide (H_2O_2), Fe(III) is converted to Fe^{4+}=O, known as Compound I, which is a ferryl oxo iron porphyrin p cation radical. The degradation process occurs due to the high redox potential of Compound I. Finally, Compound I is reduced back to the resting state (Fe(III)) during the peroxidase catalytic cycle. In addition to Compound I, it has been determined that reactive radical intermediates such as hypobromous and hypochlorous acids (HOBr and HOCl), which result from the catalytic reaction of the enzyme and NaBr or H_2O_2, can contribute to the degradation of CBNs.[14,18,29,48,49]

Several studies have reported that the biodegradation of CBNs mainly depends on their aqueous dispersibility and functionalization.[50,51] More precisely, the biodegradation of naked CBNs is strongly dependent on the types of surface functionalities, oxygen percentage, thickness, defects, and size.[52,53] For example, it has been shown that oxidized CBNs (GO and oxidized CNTs) degrade more easily than their non-oxidized form.[50,54,55] Also, Kurapati et al. reported that the degradation of GO by MPO catalysis is significantly dependent on GO's dispersibility in aqueous solutions and its colloidal stability. They observed that the enzyme failed to degrade the most aggregated GO samples while it successfully degraded highly dispersed GO.[51] Studies suggest two reasons for these findings:[14,16,43,45] i) The first reason is related to the CBNs overall dispersity, which is mainly because of the inherent hydrophobicity of the pristine form of CBNs (such as non-oxidized CNTs, graphene, and reduced graphene oxide (RGO)). As a result of these hydrophobic interactions, there is a strong tendency for their aggregation in an aqueous media which reduces accessibility of the surface for the enzyme and thus prevents

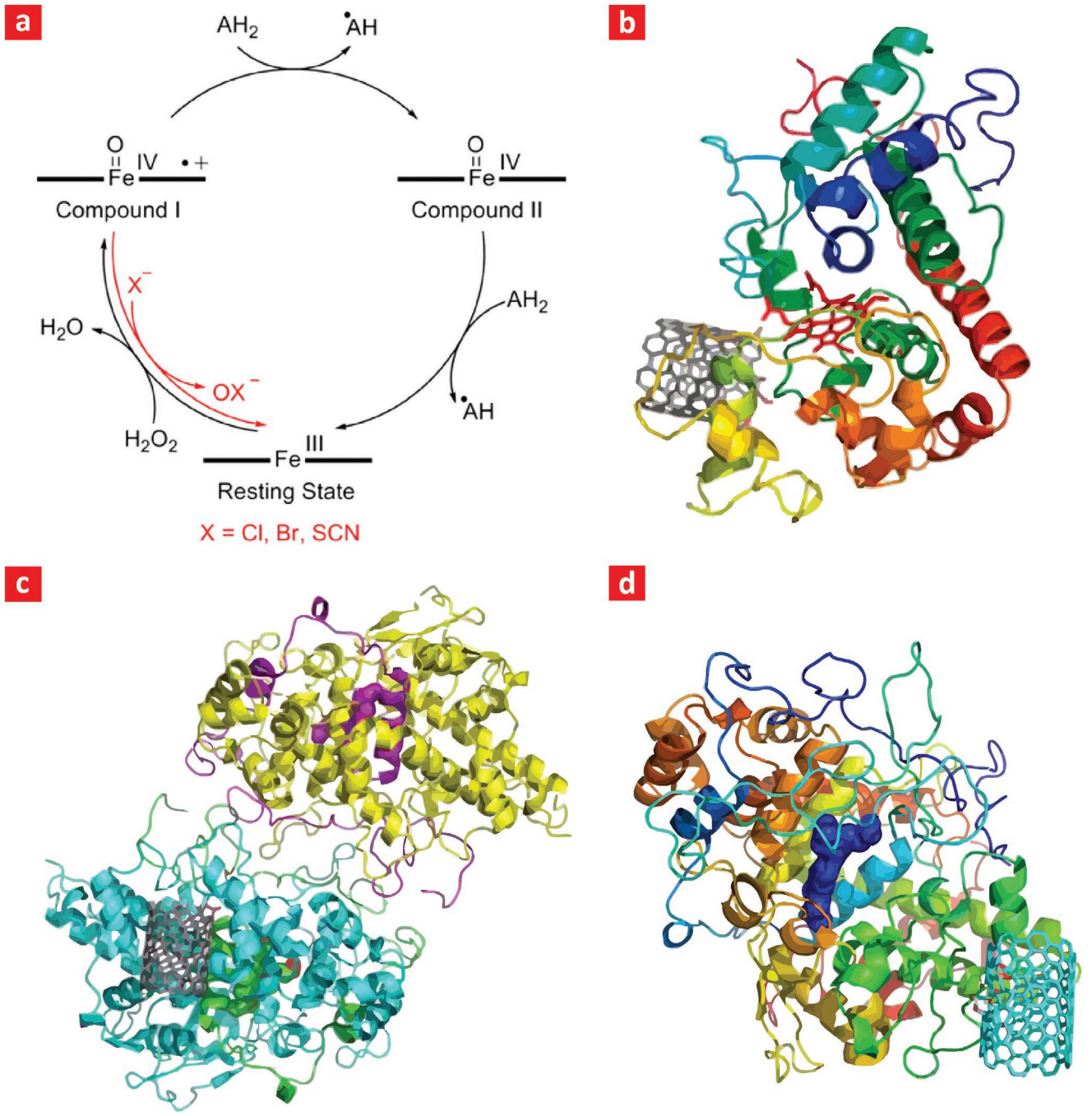

Figure 4. a) Enzymatic catalytic cycle of EPO, MPO, and HRP that is triggered by H_2O_2. For EPO and MPO, by conversion of halide to hypohalite, compound I is reduced directly to the resting state. Molecular docking experiments (AutoDock Vina) demonstrate the binding status between carboxylated SWCNTs with b) HRP, c) MPO, and d) EPO. Reproduced with permission.[43] Copyright 2013, Elsevier.

enzymatic degradation of the material. ii) The second reason is related to the surface functional groups and their interaction with the enzyme. Molecular modeling studies have shown that the negative charge of carboxyl groups on CBNs affects the enzyme's binding orientation to carboxylated CBNs, and consequently, brings the peroxidase enzyme's active site closer to the NM surface. This mainly occurs due to the positive charge of arginine-178, which is located near the enzyme's active site (Figure 4b–d). Therefore, as the enzyme's heme active site is close to the material, the degradation efficiency is greatly enhanced. As proof of this view, Kotchey et al. demonstrated that HRP succeeds in degrading GO, but fails in degrading RGO, probably for the reasons discussed above.[50] Furthermore, in a recent study by Kurapati et al., the effect of GO oxidation degree (the amount of oxygen-containing functional groups) on their degradation by EPO has been investigated. Consistent

with the results of previous studies, they proved that GO with a higher degree of oxidation degraded more extensively and faster than those with lower oxygen functional groups.[46] But unexpectedly, it was recently shown by Kurapati et al. that the MPO enzyme is capable of degrading pristine graphene.[52] As discussed by the authors, biodegradation of pristine graphene is an astounding result because it was thought that pristine graphene is very resistant to degradation. It should be noted that the pristine graphene used in their study had been synthesized in a different manner than the usual, which had a good dispersion state in water due to the presence of few oxygenated groups on its surface, and this is probably one of the main factors accelerating the graphene biodegradation. Also, contrary to previous reports, Zhang and coworkers showed that MnPO is capable of degrading pristine but not carboxylated SWCNTs. The authors attributed this phenomenon to the chemical instability of Mn^{3+} and its rapid interaction with carboxylic acid groups, which thereby leads to impairment of the enzyme's catalytic cycle.[20] Thus, it seems that much remains to be clarified regarding the interactions of CBNs with peroxidase enzymes and their reactive radical intermediates.

The thickness of CBNs also affects their biodegradation. Kurapati et al. have recently studied biodegradation of single-layer and few-layer pristine graphene by MPO, demonstrating that the degradation efficiency and the intensity of morphological changes in single-layer sheets are higher than the few-layer ones after MPO treatment. As discussed by the authors, the lower thickness of the single-layer graphene and its better aqueous dispersibility is likely the reason for this result.[52] In a similar study, Zhang et al. investigated the biodegradation profiles of 11 types of CNTs based on their diameter (number of CNT layers) by sodium hypochlorite (as a key oxidizer of CNTs inside neutrophils and macrophages). The results showed that increasing CNT thickness results in the biodegradation process occurring with more difficulty.[56] In our opinion, these two articles provide valuable results on the resistance of CBNs (single-layer or few-layer) to biodegradation, and considering the results of these studies, it can be stated that if the goal of the application of CBNs is faster biodegradation, single-layer CBN could be used, while in cases where the goal of high biopersistence to degradation is intended, few-layer CBN is more suitable than the single-layered one. In a comparative study, Modugno et al. studied the effect of oxidation degree, functionalization, and length of CNTs on HRP-mediated degradation in test tubes. The authors reported that a significant copresence of defects and the functional sites, along with good dispersibility (due to the high degree of oxygenated groups), probably offers the best conditions for effective interaction between CNTs and the oxidative agents, which is followed by higher degradation rates.[57] Overall, the existing literature suggests that biodegradation of CBNs is strongly influenced by the NM properties, especially its surface properties such as surface charge, functional groups, hydrophobicity, and morphology (the presence or absence of defects), all of which affect the interaction with peroxidase enzymes and their reactive radical intermediates. With that in mind, as pointed out in the previous section, since BMC determines the surface characteristics of the NMs in biological environments, we re-emphasize the importance of BMC consideration in CBNs biodegradation studies.

2.1. Acellular In Vitro Biodegradation of Carbon-Based Nanomaterials (Test-Tube Experiments)

In recent years, CBNs biodegradation studies have focused on discovering new peroxidase enzymes and developing new strategies based on surface engineering of NMs. In order to facilitate experiments and control the reaction conditions, most of these studies have been performed in test tubes. After discussing the main biodegradation approaches in the previous section, here we focus more on the latest acellular in vitro approaches regarding CBNs biodegradation. In a comparative work, Li et al. studied biodegradation of BSA- and PEG-coated GO and RGO by HRP and H_2O_2. Both BSA- and PEG-functionalized graphene derivatives were resistant to degradation by HRP. The authors attributed this observation to the spatial hindrance provided by BSA and PEG, which prevents HRP access to the GO surface. To circumvent this, they conjugated an amino-terminated PEG to GO carboxylic acid groups by amide formation (**Figure 5**a). The key strategy in developing this nanohybrid structure (GO-SS-PEG) was to use a cleavable disulfide cross-linker, known as cystamine. Acellular in vitro experiments showed negligible toxicity of GO-SS-PEG and considerable degradability after the disulfide bond cleavage (Figure 5b).[31] One innovative approach to induce biodegradation of CBNs is to design a nanohybrid system that enhances the catalytic activity of peroxidase enzymes. In this regard, a concept entitled "degradation-by-design" has recently been proposed by researchers, which is based on the NM chemical modification by appropriate molecules to increase the biodegradation of CBNs.[58–61] In line with this, in a recent study, Kurapati et al. enhanced the catalytic activity of the enzyme by conjugation of two natural ligands of HRP (coumarin [AZC] and catechol [DHBA]) to the GO surface (Figure 5c). The degradation experiments revealed that the functionalized GO has better biodegradability over unmodified GO (Figure 5d). To understand the reason for this result, molecular docking and gel electrophoresis were employed, and their results proved better binding of functionalized GO with the enzyme in the vicinity of the enzyme active site, which thereby leads to increased degradation.[42] In a similar work, Sureshbabu et al. conjugated one catechol derivative (as a redox mediator) and two different azido coumarins (as potential reducing substrates) separately to multiwalled carbon nanotubes (MWCNTs) to enhance the catalytic activity of HRP and XO for the CNTs degradation. All the catechol and coumarin derivatives tested enhanced catalytic activity of HRP and XO and consequently increased CNTs degradation compared to carboxylated MWCNTs.[22] More recently, Kurapati and Bianco employed a different approach by conjugating an artificial peroxidase-mimicking enzyme known as DNAzyme (a single-stranded DNA complexed to hemin) to the surface of GO (Figure 5e). The authors reported that DNAzyme had a similar peroxidase activity to HRP, which enabled it to degrade GO nanosheets (Figure 5f).[62] In an innovative work, Martín et al. conjugated a chemotactic peptide entitled "*N*-formyl-methionyl-leucyl-phenylalanine (fMLP)" to the GO surface to prepare a biodegradable and targeted GO-based platform for drug delivery, and found that conjugating fMLP to GO causes excellent targeting of Hela cancer cells, faster doxorubicin delivery into cells, and more biodegradation than bare

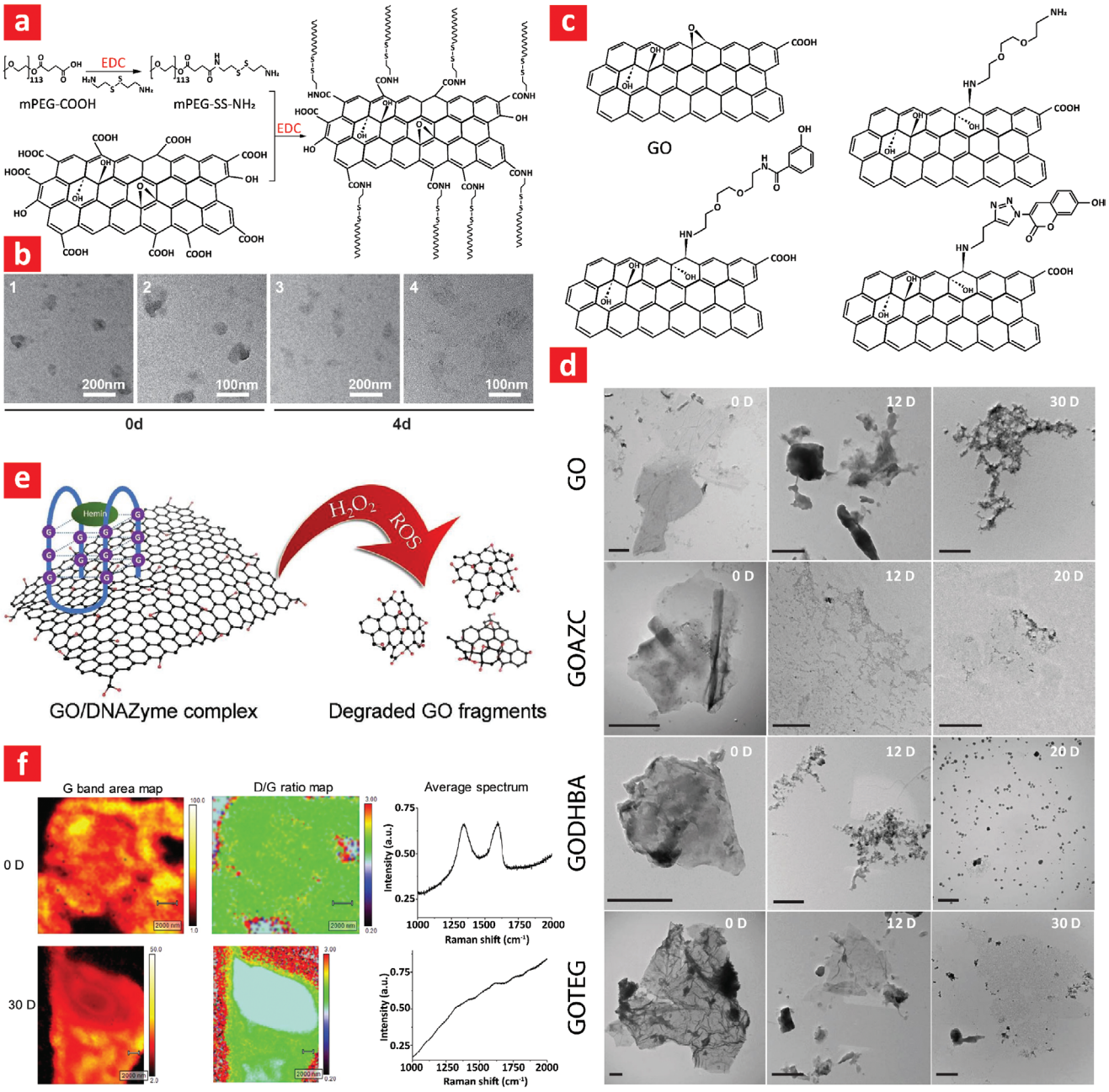

Figure 5. a) Schematic illustration of the preparation of GO-SS-PEG nanohybrid. b) TEM images of GO-SS-PEG pretreated with dithiothreitol (DTT). DTT was used to cleave the disulfide bond at day 0 (1 and 2) and day 4 (3 and 4). Reproduced with permission.[31] Copyright 2014, Wiley-VCH. c) Molecular structures of GO, GOTEG, GOAZC, and GODHBA. d) TEM images of functionalized GO samples during treatment with HRP/H_2O_2. Scale bars correspond to 500 nm. (triethyleneglycol diamine [TEG] used as a cross-linker). Reproduced with permission.[42] Copyright 2018, IOP Publishing Ltd. e) Schematic illustration of the GO-DNAzyme complex and its mechanism of action. f) 2D Raman mapping of GO sheets incubated with the complex/H_2O_2 on days 0 and 30 after treatment. Reproduced with permission.[62] Copyright 2018, Royal Society of Chemistry.

GO against MPO enzyme and neutrophils.[60] Overall, given the above, it is quite evident that new strategies, based on CBNs surface engineering (such as conjugation of specific agents to the surface to perform particular functions), are emerging to increase the biodegradability. In addition, the use of valid and accurate techniques such as transmission electron microscopy (TEM) and 2D Raman mapping studies could be very useful, because using such techniques, the quantity and quality of biodegradation can be carefully analyzed based on the CBN, BMC, and enzymatic properties. For example, as we can see in Figure 5d, TEM images qualitatively demonstrated the degradation of functionalized GO samples during treatment with HRP/H_2O_2, while 2D Raman mapping results (Figure 5f) provided valuable quantitative data on the extent of degradation. Another point that should be noted is the relationship between biodegradation and immune biocompatibility of CBNs, so that in the

synthesis strategies of nanohybrids immune biocompatibility and foreign body response (FBR) should also be considered in addition to biodegradation. In more detail, the synthesized nanohybrid should not only properly induce biodegradation but also reduce FBR. In this regard, FBR reduction strategies including optimizing physical properties and formulation approaches, drug-based approaches (such as co-delivery of nonsteroidal anti-inflammatory drugs by the NM), and surface modification of the CBN with biomaterials (such as alginates) that mitigate the FBR, can be used.[63,64]

Despite their innovative approaches, the studies mentioned above have not considered the effect or interaction of BMC with the conjugated agents and peroxidase enzymes and thus have not evaluated their efficacy under real biological conditions. Accordingly, several questions arise: Does BMC affect or even mask the function of the conjugated agents? And if so, are its effects consistent? Furthermore, does the acquired BMC affect the interaction of CBNs structure with peroxidase enzymes and their reactive intermediates? And in total, what will be the effect of different characteristics of the BMC on the interaction of CBNs and peroxidase enzymes? We can hypothesize that the effects will be complex, since the BMC can enhance dispersion of CBNs, but may block access of the enzyme's active site to the CBN surface. Tailoring of the CBN properties to drive selective corona formation may also be an important route for further exploration to design-in biodegradability.[65,66] While the above questions could potentially be answered based on the probabilities and hypotheses, such answers may cause more contradictions and hamper reaching the correct answer to the questions, so we prefer not to engage in speculation. However, in Section 3 of the present article, we have suggested strategies (such as the use of dedicated guidelines, microfluidics systems combined with analytical instruments, and computational models) that can be used to help answer these questions definitively.

2.2. Cell-Based In Vitro Biodegradation of Carbon-Based Nanomaterials

In the past several years, intracellular and extracellular degradation of CBNs has been reported through cell-based in vitro biodegradation studies.[43] In this context, biodegradation of these materials with peroxidase enzymes secreted by immune cells such as neutrophils is highly regarded since these cells represent the first line of defense against foreign body intrusion.[67] As pointed out in Table 1, in addition to neutrophils, other inflammatory cell types, such as eosinophils and macrophages, have also been used to investigate CBNs biodegradability. It is well known that different immune cells play different roles in the immune response, and of course, they have different mechanisms of action against foreign bodies. For example, MPO can be secreted both inside and outside neutrophils to eliminate invading microbes,[68] whereas activated eosinophils exocytose EPO to destroy parasites.[46] As another example, it has been shown that macrophages are relatively poor in MPO generation compared to neutrophils; and the studies in this domain have reported that this type of immune cell may use different mechanisms, such as employing peroxynitrite generators, to degrade CBNs.[43,69] Therefore, given the above facts, it is worth noting that understanding the interaction of CBNs with immune cells is of particular importance to an overall understanding of their bio-persistence and biodegradation. Along this line, recently, Kurapati and coworkers showed that MPO-rich human neutrophils could degrade pristine graphene extracellularly.[52] Before that, Mukherjee et al. had shown that GO nanosheets with different lateral dimensions are extensively degraded extracellularly by activated human neutrophils. Furthermore, the authors observed that the degradation by-products of GO are not genotoxic to human lung cells.[70] Zhang et al. examined the biodegradation of carbon nanohorns (CNHs) by two types of macrophage cell lines (THP-1 and RAW 264.7), and reported biodegradation of CNHs (with an efficiency of nearly 30%) by both THP-1 and RAW 264.7 macrophage cells within 9 days.[71] Yang et al. investigated time-dependent degradation and cytotoxicity of SWCNTs after uptake by primary rat Kupffer and RAW 264.7 cells. The results showed that CNTs were degraded (with an efficiency of 25–30%) within the first 4 days after uptake, and no further degradation was observed between days 4 to 7. It was also observed that CNTs' intracellular degradation rate is inversely related to the amount of ROS generation and cytotoxicity.[72]

In an ingenious work, Farrera et al. investigated the biodegradation of SWCNTs by another defense mechanism of neutrophils, showing that stimulated human neutrophils entangle oxidized SWCNTs in their extracellular traps (NETs) (**Figure 6**a,b). The authors also observed that the trapped SWCNTs were degraded by MPO (Figure 6c).[73] In line with these studies, the biodegradation of CBNs by eosinophils,[18] macrophages (such as THP-1,[69,74,75] RAW 264.7[72,74,76,77] cells, and Kupffer cells[72]), and primary glial cells[78,79] has also been reported. However, it is worth noting that in most of these studies, the biodegradation experiments have been performed in serum-free cell culture media, especially regarding neutrophils and eosinophils. Indeed, as previously mentioned, not paying attention to the BMC means ignoring the nano-bio interface which ignores competitive interactions, steric effects from biomolecule binding and potential reduction of access of active sites to the CBN surfaces all of which reduce the enzyme efficacy. As an example to clarify the importance of the BMC and its characteristics, in the article by Farrera et al. mentioned above, it was shown that oxidized SWCNTs are trapped in NETs, and subsequently are degraded by MPO.[73] But how are SWCNTs captured in NETs? It is assumed that the entrapment of oxidized SWCNTs in NETs depends on the electrostatic forces. In fact, the hypothesis about the mechanism is that the presence of histones in the NETs structure (composed of secreted DNA formed into fibers) bestow positive charge upon NETs that significantly enhances their interaction with negatively charged oxidized SWCNTs or GO.[73,80,81] Therefore, the difference in the electrical charge traps the oxidized SWCNTs in NETs. Accordingly, now the question is: How will be the oxidized SWCNT charge after BMC formation? The answer to this question is likely to further highlight the importance of BMC in CBNs biodegradation studies. The vast majority of NMs corona studies have shown that formation of the BMC results in the particles zeta potential becoming close to neutral although remaining slightly negative (typically around –7 mV) irrespective of the initial surface charge on the NMs.[82] From a nano-bio interface point of

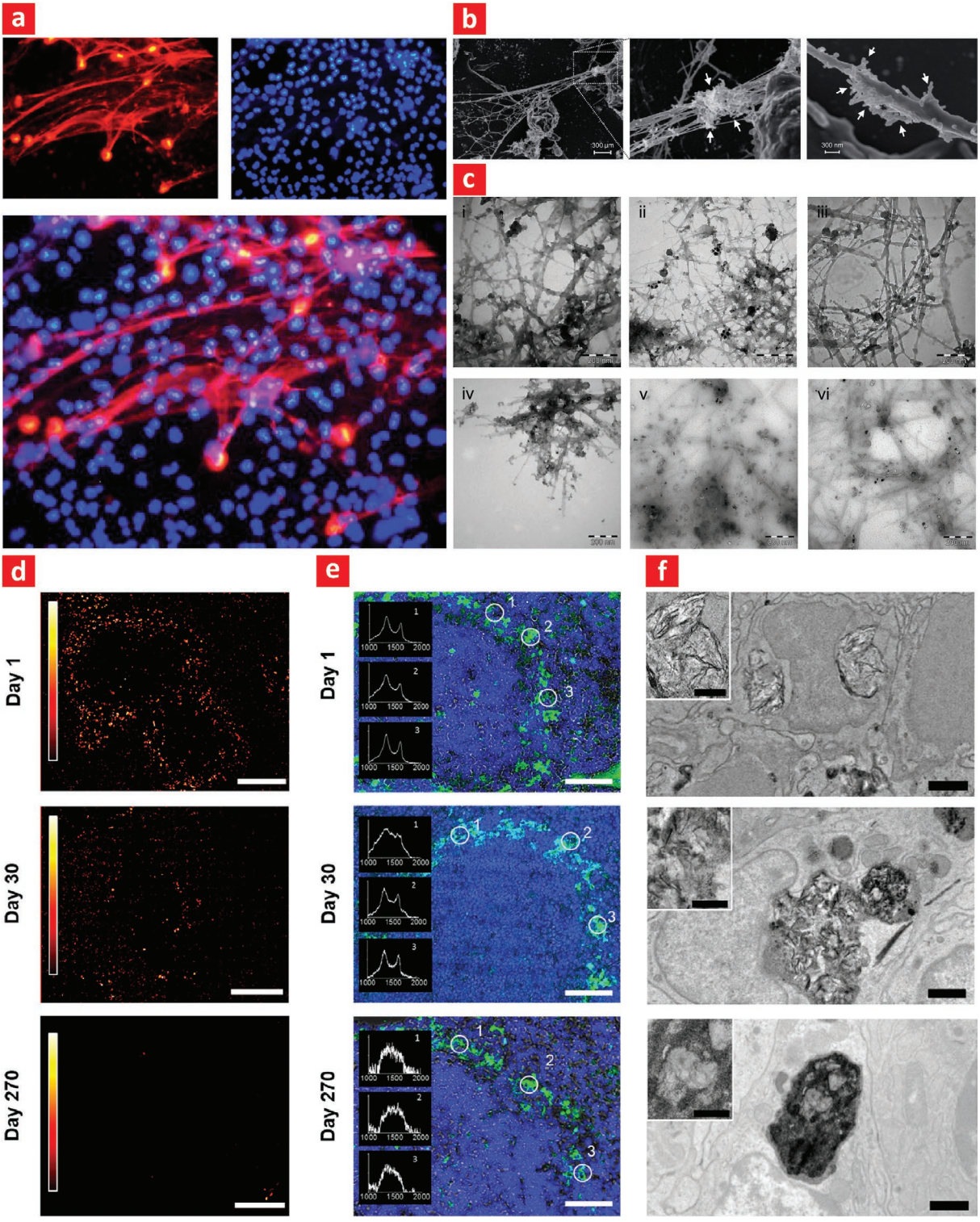

Figure 6. a) The immunofluorescence staining of NETs produced by MPO-rich human neutrophils. The cell nucleus has been visualized in blue (DAPI) and NETs in red (Alexa-594 labeled antibodies). b) SEM images showing NETs and a cluster of oxidized SWCNTs trapped in NETs. c) TEM images of oxidized SWCNTs incubated for 24 h under the following conditions: H_2O (i); NETs (ii); H_2O_2 (iii); H_2O_2/NaBr (iv); H_2O_2/NaBr + NETs (v,vi). Reproduced with permission.[68] Copyright 2014, Royal Society of Chemistry. d) Confocal Raman mapping of the accumulated GO sheets in mice spleen sections at days 1, 30, and 270 post administration. Scale bars represent 200 µm. e) Immunostaining coupled with Raman spectroscopy of the splenic marginal zone macrophages (green) which contained GO, at different time points. DAPI-stained cell nuclei are visualized in blue. Scale bars represent 50 µm. f) TEM images of GO biodegradation by splenic macrophages at different time points (days 1, 30, and 270) after i.v. administration at a dose of 7.5 mg kg^{-1}. Scale bars represent 1 µm. Reproduced with permission.[94] Copyright 2020, American Chemical Society.

view, there are more questions in cell-based in vitro studies that need to be addressed in future investigations. For example, one step before the interaction of BMC-coated CBNs with the enzyme and its reactive intermediates: how does any acquired BMC affect immune cell interaction and stimulation? How does the BMC affect the biodegradation mechanism employed by immune cells (intracellularly or extracellularly)? How and to what extent does the BMC affect immune cell viability? All in all, what will be the effect of different characteristics of BMC on the interaction of CBNs and immune cells? Through what mechanisms?

2.3. In Vivo Biodegradation of Carbon-Based Nanomaterials

Understanding the behavior and fate of CBNs in vivo is imperative to facilitate employment of these materials in a clinical setting. Despite the fact that the fate of NMs in vivo largely depends on their degradation capacity, the biodegradation ability of CBNs in vivo has not been studied enough until now.[83,84] However, studies performed so far have reported the in vivo biodegradation of CNTs and GO.[85–89] In a pioneering work, Shvedova and coworkers employed *MPO* knockout (MPO k/o) versus wild-type (w/t) mice to determine the role of MPO in the biodegradation of SWCNTs in mice lungs 28 days after pharyngeal aspiration. For both mice types, 24 h after the exposure, neutrophil accumulation in the lungs was observed. By quantitative imaging, it was determined that the clearance of SWCNTs from MPO k/o mice lungs was noticeably less than w/t mice, which indicated the MPO-dependent biodegradation of the NM in vivo. After observing a much lower biodegradation rate in MPO k/o mice, the authors speculated about the possible involvement of other peroxidase enzymes such as LPO and EPO in the in vivo biodegradation of SWCNTs.[90] Consistent with Shvedova and coworkers' study, Nunes et al. reported biodegradation of surface-aminated CNTs following stereotactic administration in the mice brain cortex. Raman spectroscopy and TEM were used to analyze the tissue sections. Unexpectedly, the results showed widespread degradation of the nanotubes within 2 days post-injection, which is a very short time for the biodegradation of such material, and it seems that more investigations and verifications need to be conducted in this regard.[91] Recently in an innovative work, Peng et al. investigated the biodegradation of GO in the zebrafish gastrointestinal tract. They employed *nos2a* knockout versus wild-type zebrafish to determine the mechanism or biomolecules by which GO biodegradation in the gastrointestinal tract occurs. The results showed that the process is nitric oxide-dependent, reducing gastrointestinal inflammation. Inhibition of the *nos2a* (encoding the inducible nitric oxide synthase) gene increased inflammation and neutrophil accumulation in the gastrointestinal tract.[92] Zhang et al. examined the kinetic biodistribution and clearance of small- and large-diameter SWCNTs after intravenous injection into mice over 60 days in the lungs, spleen, and liver. The histological analysis and near-infrared light absorption results showed that the larger-diameter SWCNTs accumulated in the spleen and liver, while the smaller-diameter SWCNTs accumulated more in the lungs, suggesting that the biodistribution of SWCNTs is diameter-dependent. Moreover, it was determined that the quantities of both SWCNTs in the lungs and liver decreased over 60 days, while no significant difference was observed in the CNT quantity in the spleen. Taken together, these results indicate the potential biodegradation of the SWCNTs in mouse lungs and liver.[88] Girish and colleagues utilized confocal Raman microscopy to investigate the fate of intravenously administered GO in mice models. They reported that the highest accumulation and the most significant biodegradation are attributed to the lungs and spleen, respectively. Accordingly, the authors reasoned that as the spleen is the main unit of phagocytic defense, the high biodegradation of GO in the spleen may be related to the accumulation of more immune cells in this organ.[93] Consistent with this, very recently, in the latest in vivo study on biodegradation of CBNs, Newman et al. investigated the possible effects of GO accumulation in the spleen and its biological fate over a 9-month period. By confocal Raman mapping of tissue sections, TEM, and immunohistochemistry coupled with Raman spectroscopy, it was determined that splenic macrophages are the predominant cells responsible for bioaccumulation and subsequently intracellular biodegradation of GO sheets (Figure 6d–f).[94]

Similar to the acellular in vitro and cell-based in vitro studies, the limited in vivo studies to date have also not examined the effect of BMC and its characteristics on the biodegradation of CBNs, although of course, in this case, a BMC will have been acquired by the CBNs upon introduction into the animals whose composition depends on the administration route. Accordingly, similar to the previous sections, several questions arise: Apart from the effect that BMC has on the biodistribution, accumulation, and excretion of CBNs, and after CBNs accumulation in tissues, does BMC affect the mechanism of the onset of tissue inflammation and its intensity? Does BMC affect the type and the number of immune cells that accumulate in the inflamed tissue? Since the biodegradation efficiency of CBNs varies in different tissues, could BMC be one of the factors behind this difference? Since the BMC source (e.g., sick or healthy person) determines its composition and content, could personalized BMC affect CBNs biodegradability? And in total, what will be the effect of different characteristics of BMC on in vivo biodegradation of CBNs?

Overall, as a consequence of all the above mentioned aspects, we speculate that the next paradigm shift in CBNs biodegradation investigations will be to find answers to such questions. In particular, efforts to connect the acquired BMC composition in terms of its composition of opsonizing and dysopsonizing proteins and their ratio may provide important insights into the activation of immune responses and recognition by phagocytic cells, and may also affect the organ biodistribution based on the initially acquired BMC related to the route of exposure or administration of the CBNs.

3. The Challenges and Prospects of Biomolecular Corona Consideration in Carbon-Based Nanomaterials Biodegradation Studies

As briefly pointed out in the introduction, pristine NMs during their journey into and around the body would be transformed

into new NMs by the acquisition and evolution of the BMC. Therefore, the overall physicochemical properties and biological identity of NMs will be overshadowed by their surface "bio-transformation." The first studies on the BMC concept were conducted by Bangham and Vroman in the 1950s and 60s, proving that protein adsorption plays a substantial role in overall biological interactions and responses to pristine surfaces and materials.[39] But, in a more modern conception of BMC, it was the group of Dawson that first explained that the formed protein corona is unique and that its composition depends on NM surface chemistry, and the main part of the corona consisted of less than five proteins.[95,96] 13 years after defining this concept, it is now well accepted that NM physicochemical properties such as surface charge (zeta potential), size, and shape (morphology), are affected by BMC.[23,97,98] Following changes in the aforementioned characteristics, the biological behaviors of NM such as blood circulation time,[99] immune system stimulation,[34,100] biodistribution,[99,101] accumulation,[102] excretion,[103] cellular uptake,[104,105] biodegradation,[106] and biocompatibility[102] are also altered. For example, in line with the present article's subject, it has been determined that the surface charge of CBNs is a key factor in the interaction of peroxidase enzymes with NMs.[43,45,73] Therefore, it is expected that BMC, as a critical determinant in nano-bio interfaces, must always be considered in nanobiomedical investigations, including assessment of biodegradation of NMs.

Given the limitations associated with experimental techniques in vivo, most studies regarding the BMC evaluation have been conducted in vitro; though, there are always some limitations and challenges with in vitro studies. For example, the origin of the used plasma (or serum) needs to be taken into account.[107,108] Although the homology of the human, mouse, and bovine proteins is rather high,[109,110] the use of mouse and bovine plasma for BMC evaluation can be questioned in the scope of evaluation of NMs for human biomedical applications. But beyond the cross-species difference of the plasma origin, the personalized plasma (disease-specific plasma) that results in a personalized BMC has recently attracted much attention.[111,112] In proof of this view, Hajipour and colleagues examined the effect of different disease-specific plasmas on the BMC composition formed onto GO sheets and the relevant biological effects. It was reported that the differences in the plasmas associated with different disease states significantly affected the BMC composition and subsequently the cellular responses toward the corona-coated GO sheets.[113] Therefore, attention to personalized BMC seems to be of particular importance in future investigations about biodegradation and biocompatibility of CBNs. This consideration may also open the way to new approaches and directions for the design and engineering of safe CBNs with tailored acquisition of BMCs to enhance or modulate their biodegradability to the needs of the specific application.

Another unconsidered challenge in BMC studies is the inconsistency of the experimental environment and analysis methods employed in the related published articles.[114–116] For example, plasma concentration,[117,118] temperature,[96,119] incubation time,[96] pH,[96] type of separation method (e.g., in gel or on-particle),[115] type of informatics database, and statistical analysis methods[115] are some of the parameters that differ widely in the published literature. To minimize these inconsistencies, Lynch and coworkers have suggested a guideline entitled "Minimum Information about Nanomaterial Biocorona Experiments (MINBE)" to enhance interoperability, reproducibility, and utility of BMC characterizations.[115] In line with this, inspired by this guideline and other guidelines such as MIRIBEL (Minimum Information Reporting in Bio-Nano Experimental Literature),[114] we envisage that providing a dedicated guideline for CBNs biodegradation studies could be useful for systematic debugging of nano-bio interfaces in future studies, and may significantly accelerate the successful translation of the NMs into clinical settings and in a wide range of therapeutic and diagnostic applications. The current article takes the first step toward this goal by providing a checklist of reporting considerations for acellular in vitro, cell-based in vitro, and in vivo CBNs biodegradation studies (**Table 2**). This checklist, which we will refer to as "Minimum Information Reporting in Biodegradation Investigation of CBNs" (MIRBIC) consists of specific components that are categorized into three sections: i) CBNs physico–chemical properties, ii) experimental conditions, and iii) common reporting standards for biodegradation characterization of CBNs. In the first section, the physico–chemical properties of CBNs that influence their interactions with biological systems have been provided. In the second section, essential information and details related to the biodegradation experiments conditions of the CBNs in the three modes of acellular in vitro, cell-based in vitro, and in vivo have been presented. The third section is focused on the importance of reporting details about CBNs biodegradation assessment methods/techniques along with BMC characterization conditions. It should be noted that, regarding the BMC reporting section, we have referred the readers to the MINBE guideline, which thoroughly deals with what/how to report information and details related to the BMC analysis. We believe that each component should be included as part of published research on CBNs biodegradation, and reporting these components in the relevant studies will help to eliminate ambiguities and inconsistencies between different studies, facilitating data integration and meta-analysis.

Due to the aforementioned experimental limitations, only some studies have investigated the BMC formed on NMs in vivo. Therefore, a major challenge in this area is developing new strategies and techniques for in vivo evaluation of the BMC. Currently, one of the most common strategies for in vivo assessment of BMC is the recovery of NMs from the blood circulation (usually by cardiac puncture) and subsequent examination and characterization of BMC by analytical techniques such as mass spectrometry.[120–122] However, there are some challenges in this approach, such as the limited amount of NMs recovered post-administration, limitations on the time of blood sample collection, and the difficulty in NMs isolation from the blood, which may itself alter the BMC composition. Also, it is important to bear in mind that in this strategy, only the NMs in the blood circulation are examined, whereas NMs accumulated in the organs may have a different composition of BMC depending on tissue types. Thus, especially regarding CBNs biodegradation studies, it is essential to develop new and functional strategies and methods to evaluate the BMC formed on tissue-accumulated NMs.

Table 2. Reporting standards for biodegradation research of CBNs.

CBNs physico-chemical properties[a)]	Synthesis method, size, hydrodynamic radius, shape, thickness, edge-to-area ratio, C:O ratio, surface charge, degree of defects, chemical composition, hydrophobicity, polydispersity index, crystalline structure, functionalization type and degree, optical properties, ion contamination, storage history, and drug loading and release		
		Reporting component	Description
Experimental conditions	Acellular in vitro [test tube]	Enzyme specifications	Type, source, and catalytical activity
		Reaction conditions	I. Type and concentration of buffers and other applicable supplements, flow condition (static/dynamic), pH, and temperature II. Concentration of CBN, enzyme, enzyme activators, and substrates (such as H_2O_2), and treatment time III. Number of replicates
	Cell-based in vitro	Cell specifications	Type, source, sex, age, growth conditions, passage number, and storage conditions
		Reaction conditions	I. Type and concentration of buffers and other applicable supplements, culture medium specifications, and cell incubation conditions (culture dimensions, static/dynamic condition, pH, temperature, and O_2 and CO_2 percentages) II. Concentration of CBN, substrates, and cellular stimulants (such as phorbol 12-myristate 13-acetate [PMA] and *N*-formyl-methionyl-leucyl-phenylalanine [fMLP] tripeptide), treatment time, and cell density III. Number of replicates
	In vivo	Animal specifications	Type, race, age, sex, and weight
		Animal care conditions	Feeding regime/food, ambient temperature, light/dark cycle
		Characteristics of injection sample	CBN concentration, specifications of the injection buffer/medium, pH, volume, temperature, and details of sterilization of the injection solution
		Treatment conditions	Administration route, treatment time, and number of replicates
		Sampling details	Tissue type, macroscopic appearance of the tissue, and the method used to prepare tissue samples for biodegradation evaluation
Common reporting standards for biodegradation characterization of CBNs	• Details of biodegradation assessment methods/techniques (such as TEM and Raman spectroscopy) and data analysis • Reporting standards for BMC characterization should be followed based on MINBE[115]		

[a)]When evaluating these properties, specifications of buffer/medium used for CBNs dispersion need to be reported in detail.

Despite laudable efforts, the nano-bio interface science suffers from the limitations of experimental methods in vivo and desperately needs new tools to uncover nano-bio interactions between NMs and body components. With this in mind, and on the subject of the present article, if the aim is to investigate the effect of BMC and its characteristics on biodegradation of CBNs in vivo, we face a major and complex challenge regarding the recovery of the accumulated NMs from tissues, and also the investigation of the BMC effect on the biodegradation process over time. But, on the flip side, recent technological advancements such as microfluidics can benefit the BMC field by mimicking in vivo conditions within an in vitro device. Microfluidic systems not only can simulate 3D microenvironments with controlling continuous flow, chemical gradients, and local microenvironment as well as partitioning of multi-organs, but also can offer a cost-effective and efficient opportunity to rapidly screen NMs and their interaction with biological environments (**Figure 7**).[123–126] As shown by recent investigations, the composition and properties of BMC differ in vitro and in vivo.[39,122] Actually, it has been emphasized that shear stress created by dynamic flow is one of the most important factors affecting the BMC characteristics.[37,127–129] Therefore, controlling shear stress by microfluidic devices could help imitate in vivo behavior and the fate of NMs more accurately. In this direction, Digiacomo and colleagues have recently compared the human BMC features formed onto gold nanoparticles (AuNPs) statically and dynamically. The authors reported that the BMC formed under static incubation was thinner and less negatively charged than the BMC formed in a microfluidic chip. Furthermore, nano-LC MS/MS and SDS-PAGE results revealed that in the dynamic mode (microfluidic environment), immunoglobulins are the most abundant proteins in the BMC composition, while tissue leakage proteins were the most abundant proteins when the BMC was formed under static mode.[130] Microfluidic chips can also be combined with a wide range of characterization techniques for the dynamic characterization of NMs at nano-bio interfaces. In order to monitor inter- and intra-molecular reactions occurring in microfluidic systems, they have been coupled with light scattering,[131] small-angle X-ray scattering (SAXS),[132] Förster resonance energy transfer,[133] spectroscopic methods,[134] and surface-enhanced Raman spectroscopy detection.[135] In this context, Rodrigues et al. employed a microfluidic extensional approach coupled with a high-speed video microscopy system to evaluate the haemocompatibility of iron oxide NPs (**Figure 8**a). The results revealed that the microfluidic tool could determine the rigidity of red blood cells more accurately than traditional haemotoxicity analysis. In fact, the employed microfluidic system proved to be superior to the conventional techniques by showing higher sensitivity in detecting small changes in red blood cells.[136] Recently, Huang et al.

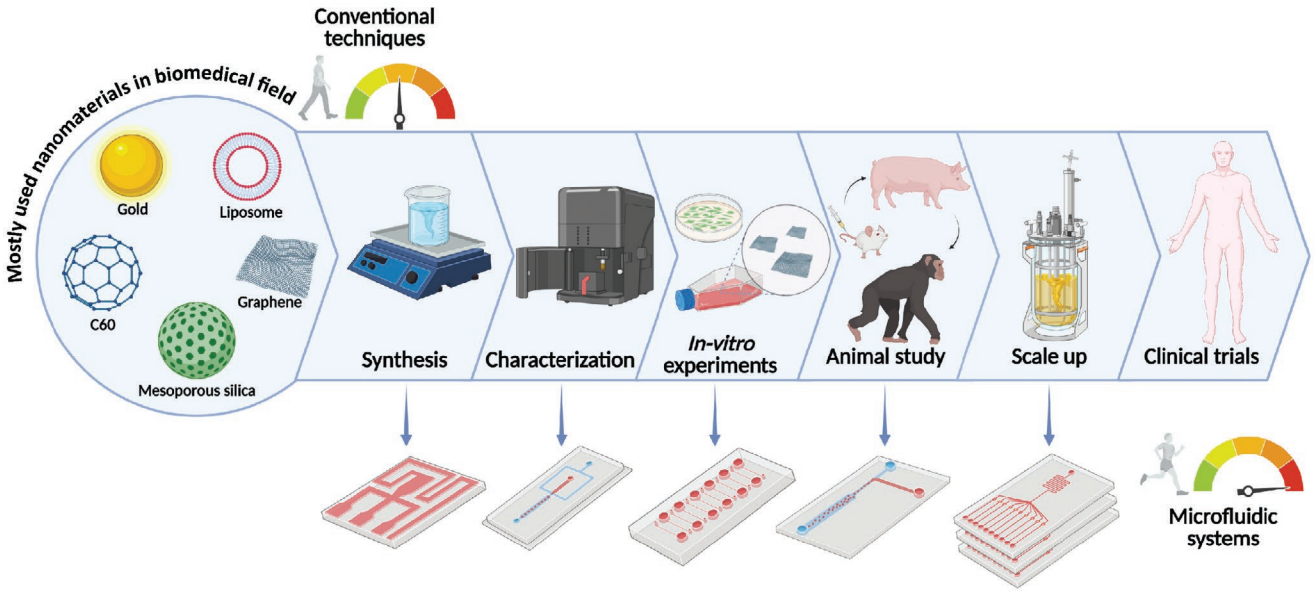

Figure 7. Steps for clinical development of NMs, and comparison of conventional and microfluidic approaches from bench to clinic. Microfluidics significantly diminishes the time and cost of the evaluation of NMs on the path to clinical development. Using microfluidic systems in biodegradation studies of CBNs, the in vivo conditions of this process can be simulated with high accuracy, and the effects of BMC on the interaction of CBNs with immune cells, peroxidase enzymes, and oxidizing agents can be studied carefully.

developed a spheroid-on-chip system to investigate the effect of the exterior flow, BMC, and NP surface charge on the penetration and accumulation of polystyrene NPs into the tumor model (Figure 8b,c). The chip was fabricated on a glass layer for real-time tracking of the changes in the multicellular spheroids with confocal laser scanning microscopy. Briefly, the results showed that all three parameters (flow, BMC, and NP surface charge) significantly affected the penetration and accumulation of the NPs into the multicellular spheroids.[137] In an interesting work, Stehle and coworkers developed a droplet-based microfluidic device coupled with SAXS to analyze the concentration, shape, and size of AuNPs dispersed within water-in-oil emulsion droplets (Figure 8d). Briefly, the SAXS pattern showed the successful application of this system in the analysis of the concentration, shape, and size of the NPs. Furthermore, the authors mentioned that this approach can be utilized to study nanocarriers, biomacromolecules, and any other arbitrary sample.[132] These studies, taken together, demonstrate that the potential of microfluidic models to probe dynamic nano-bio interactions can bolster our understanding of how BMC affects nano-bio responses including biodegradation. We therefore believe that inspired by these strategies, the challenges and barriers regarding BMC consideration in biodegradation of CBNs can be overcome.

A thorough understanding of the relationships between NMs' physicochemical properties and their biological behavior is mandatory to design efficacious and safe nanomedicines. To this end, quantitative structure–activity relationship (QSAR) methods provide one option for establishing such correlations.[138] QSAR is a statistical technique that tries to predict the reactivity, activity, and properties of an unknown set of molecules based on analysis of an equation connecting the measured property and activity of a set of structurally similar molecules to their structures.[139] Compared with observational methods, computational methods such as QSAR are faster and reduce the monetary and ethical costs related to animal testing, but they are data-driven and thus need to be developed based on robust initial datasets. Therefore, researchers will be able to make more accurate predictions of NMs in the physiological milieu using these methods (**Figure 9**a).[140,141] QSAR has been successfully developed to predict the biological behavior of many different NMs such as CNTs, surface modified iron oxide NPs, and metal oxide NPs.[142] Very recently, in an ingenious work, Yan et al. inspired by face recognition technology, developed a computational workflow to predict hydrophobicity, zeta potential, protein adsorption capacity, and cellular uptake potential of 147 unique and pre-evaluated NMs (Figure 9b). To do this, they used detailed atom information of the NMs to construct protein data bank (PDB) files, and then developed a program to automatically convert PDB files to uniform nanostructure images. Ultimately, by developing a convolutional neural network model, the critical image features were directly learned from the images to predict biological activities and physicochemical properties of the NMs. The results showed a highly accurate prediction of the relationships between the nanostructures and their biological activities using the constructed convolutional neural network model.[143] Likewise, in a recent study, Lazarovits and colleagues developed a workflow to evaluate how BMC composition can predict the in vivo biological fate of PEGylated AuNPs (Figure 9c). Following injection of the NPs into rats, blood was extracted at different time points, and the NPs were separated from the blood by centrifugation. An LC–MS/MS assay was developed to evaluate the BMC. ICP-MS was used to quantify the NPs in the blood, liver, and spleen at the end of the experiment. Finally, the proteins' label-free quantitative intensities were used as inputs, and the ICP-MS results

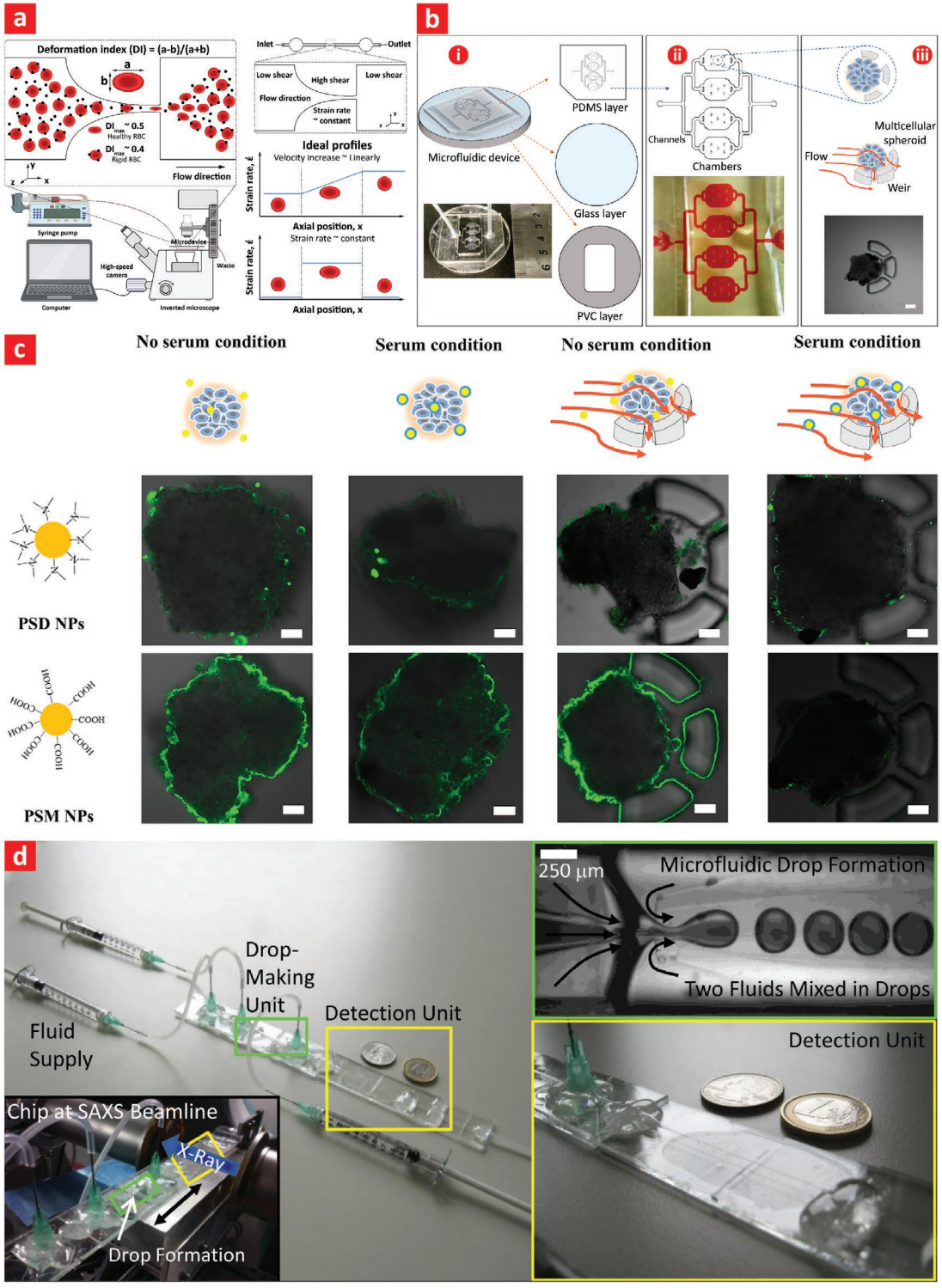

Figure 8. a) Schematic view of a microfluidic device with the hyperbolic channel, which is coupled to a high-speed video microscopy system to analyze the haemocompatibility of iron oxide NPs. Reproduced with permission.[136] Copyright 2016, Springer. b) The microfluidic chip consisted of three layers (i). The chip contains four chambers each of which houses five semicircular weirs and two apertures to trap the spheroids (ii). Schematic of the semicircular weir and microscopic image of the trapped spheroids. The scale bar is 50 µm (iii). c) Confocal images of spheroids treated with amine-modified (PSD) and carboxylate-modified (PSM) polystyrene NPs after 2 h of NP penetration under static/flow conditions and with/without protein corona. The scale bar is 50 µm. Reproduced with permission.[137] Copyright 2017, American Chemical Society. d) A droplet-based microfluidic device coupled with SAXS. Reproduced with permission.[132] Copyright 2013, Royal Society of Chemistry. Using such a strategy, the changes made to CBNs (such as changes in the shape, size, surface-to-volume ratio, and distribution) during the degradation process, and the effects that BMC exerts on this process can be studied with high precision and in real-time.

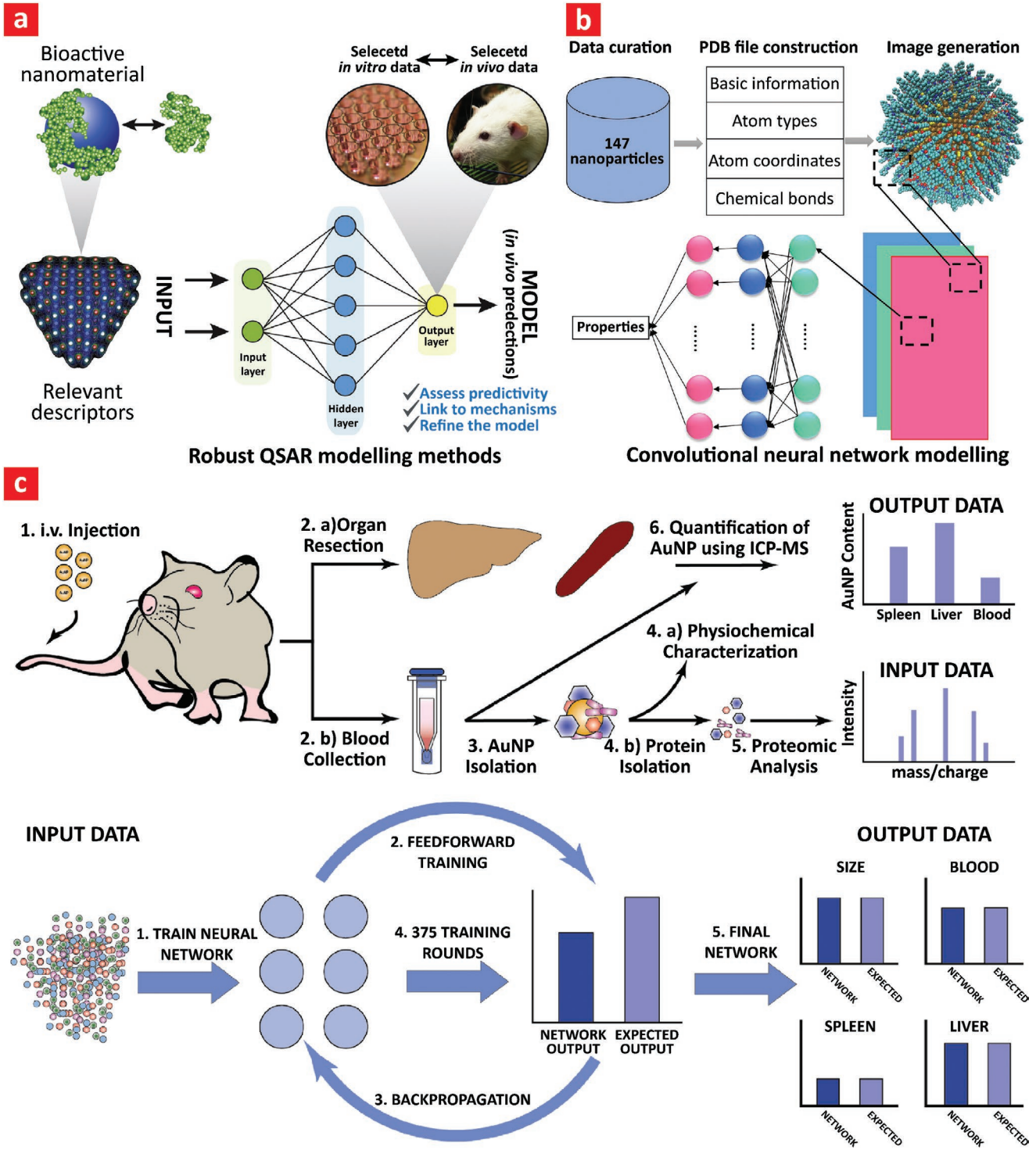

Figure 9. a) The process of developing a QSAR neural network model to predict the biological fate of NMs in physiological environments, or to elucidate biological mechanisms and processes. Reproduced with permission.[140] Copyright 2013, Elsevier. b) Schematic of the computational workflow which consists of four main parts: NMs data curation, PDB file construction on the basis of the NMs attributes such as type of core material and chemical structure of surface ligand, generation of uniform nanostructure images, and prediction of NMs nano-bio interactions such as protein adsorption and cellular uptake by the convolutional neural network model. Reproduced with permission.[143] Copyright 2020, American Chemical Society. c) Injection and recovery of PEGylated AuNPs, and subsequently, analysis of BMC and tissue accumulation of the NPs by LC–MS/MS and ICP-MS, respectively. The mass spectrometry protein library was used as input for the neural network, while the NP clearance from circulation and its tissue accumulation were used as outputs. Ultimately, the NPs biological behavior was predicted by the neural network. Reproduced with permission.[121] Copyright 2019, American Chemical Society.

(NPs biological fate) were considered as outputs for the computational model. The computational analysis results showed that the spleen and liver uptake mechanisms depend on a large set of specific protein patterns formed onto the NPs surface. To put it more clearly, the model determined that the removal of NPs from circulation depends mainly on the protein patterns and not individual proteins.[121] Taken together, such studies demonstrate the potential of computational models for the prediction of biological behavior based on NM design. In order to achieve this, as recently suggested by several researchers in the field,[144,145] the development of a NM BMC database would be extremely beneficial for nanobiomaterials research. Such a database will contain properties of NMs, BMC fingerprints, and corresponding biological outcomes, as well as computational parameters related to protein and small molecule binding affinities. Such a database is under development as part of the EU H2020 projects NanoCommons and NanoSolveIT (NanoPharos, https://db.nanopharos.eu/Queries/Datasets.zul). We believe that by building such databases, it will be possible to predict the BMC composition and characteristics for a wide range of NMs (especially CBNs) in different biological environments (e.g., disease-specific plasmas that result in personalized BMC), and determine how the specific BMC will affect the biodegradation, biocompatibility, and the overall performance and fate of the NMs. Ultimately, we are confident that newly developed experimental methods coupled with computational models can help address the challenges and barriers regarding the integration of BMC composition and role in the biodegradation of CBNs.

4. Conclusions

In this conceptual perspective article, after discussing the latest progress and strategies regarding application of a variety of peroxidase enzymes for the biodegradation of CBNs and their associated mechanisms, it was highlighted that the biodegradation of CBNs is strongly influenced by their surface properties. In view of the fact that NMs acquire a BMC immediately upon contact with living systems, whose composition is determined by the surface properties of the NMs, we reasonably concluded that eliminating or not thoroughly considering the BMC in CBNs biodegradation studies (whether inadvertently or intentionally because of the field infancy), especially acellular in vitro and cell-based in vitro, could call into question the validity of the research process, would hamper providing an appropriate correlation with in vivo results, and limit the validity of conclusions drawn from them. However, considering the pace of knowledge development and establishment of advanced equipment and measurement methods, it is envisaged that BMC effects will be being taken into account and measured in CBNs degradation studies in the near future.

To facilitate progress, we called for a number of conceptual and fundamental questions about the ambiguities raised by the absence of consideration of the BMC effects in the existing literature. We believe that the questions presented herein lay the groundwork for the next paradigm in CBNs biodegradation investigations through incorporation of the role of the BMC. But the flip side is that considering BMC in nanoscale biodegradation is very challenging and requires new and dedicated strategies (especially in vivo) to analyze the biodegradation process and the effects of BMC and its characteristics on this process. Hence, ultimately, we recommend the use of the dedicated guideline (MIRBIC), presented in this article for the first time, and new technologies such as microfluidics systems combined with analytical instruments, and computational models in order to overcome the challenges and barriers. We are confident that the advantages offered by such strategies are too compelling to ignore. As a final word, we hope that the present article stimulates more conceptual and empirical attention to the importance of BMC and its characteristics in CBNs biodegradation investigations.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

biocompatibility, biodegradation, biomolecular corona, carbon-based nanomaterials, peroxidases, tissue accumulation

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[1] G. Hong, S. Diao, A. L. Antaris, H. Dai, *Chem. Rev.* **2015**, *115*, 10816.
[2] A. Magrez, S. Kasas, V. Salicio, N. Pasquier, J. W. Seo, M. Celio, S. Catsicas, B. Schwaller, L. Forró, *Nano Lett.* **2006**, *6*, 1121.
[3] S. H. Ku, M. Lee, C. B. Park, *Adv. Healthcare Mater.* **2013**, *2*, 244.
[4] C. Cha, S. R. Shin, N. Annabi, M. R. Dokmeci, A. Khademhosseini, *ACS Nano* **2013**, *7*, 2891.
[5] E. Kai-Hua Chow, M. Gu, J. Xu, in *Nanoparticles for Biomedical Applications* (Eds: E. J. Chung, L. Leon, C. Rinaldi), Elsevier, New York **2020**, pp. 223–242.
[6] R. Kurapati, M. Vaidyanathan, A. M. Raichur, *RSC Adv.* **2016**, *6*, 39852.
[7] A. Kunzmann, B. Andersson, T. Thurnherr, H. Krug, A. Scheynius, B. Fadeel, *Biochim. Biophys. Acta, Gen. Subj.* **2011**, *1810*, 361.
[8] S. F. Hansen, A. Lennquist, *Nat. Nanotechnol.* **2020**, *15*, 3.
[9] B. Fadeel, K. Kostarelos, *Nat. Nanotechnol.* **2020**, *15*, 164.
[10] D. A. Heller, P. V. Jena, M. Pasquali, K. Kostarelos, L. G. Delogu, R. E. Meidl, S. V. Rotkin, D. A. Scheinberg, R. E. Schwartz, M. Terrones, Y. H. Wang, A. Bianco, A. A. Boghossian, S. Cambré, L. Cognet, S. R. Corrie, P. Demokritou, S. Giordani, T. Hertel, T. Ignatova, M. F. Islam, N. M. Iverson, A. Jagota, D. Janas, J. Kono, S. Kruss, M. P. Landry, Y. Li, R. Martel, S. Maruyama, A. V. Naumov, M. Prato, S. J. Quinn, D. Roxbury, M. S. Strano, J. M. Tour, R. B. Weisman, W. Wenseleers, M. Yudasaka, *Nat. Nanotechnol.* **2020**, *15*, 164.

[11] H. Ali-Boucetta, K. Kostarelos, *Adv. Drug Delivery Rev.* **2013**, *65*, 2111.
[12] B. Strojny, N. Kurantowicz, E. Sawosz, M. Grodzik, S. Jaworski, M. Kutwin, M. Wierzbicki, A. Hotowy, L. Lipińska, A. Chwalibog, *PLoS One* **2015**, *10*, e0144821.
[13] K. Yang, H. Gong, X. Shi, J. Wan, Y. Zhang, Z. Liu, *Biomaterials* **2013**, *34*, 2787.
[14] V. E. Kagan, N. V. Konduru, W. Feng, B. L. Allen, J. Conroy, Y. Volkov, I. I. Vlasova, N. A. Belikova, N. Yanamala, A. Kapralov, Y. Y. Tyurina, J. Shi, E. R. Kisin, A. R. Murray, J. Franks, D. Stolz, P. Gou, J. Klein-Seetharaman, B. Fadeel, A. Star, A. A. Shvedova, *Nat. Nanotechnol.* **2010**, *5*, 354.
[15] B. L. Allen, P. D. Kichambare, P. Gou, I. I. Vlasova, A. A. Kapralov, N. Konduru, V. E. Kagan, A. Star, *Nano Lett.* **2008**, *8*, 3899.
[16] B. L. Allen, G. P. Kotchey, Y. Chen, N. V. K. Yanamala, J. Klein-Seetharaman, V. E. Kagan, A. Star, *J. Am. Chem. Soc.* **2009**, *131*, 17194.
[17] I. I. Vlasova, A. A. Kapralov, Z. P. Michael, S. C. Burkert, M. R. Shurin, A. Star, A. A. Shvedova, V. E. Kagan, *Toxicol. Appl. Pharmacol.* **2016**, *299*, 58.
[18] F. T. Andón, A. A. Kapralov, N. Yanamala, W. Feng, A. Baygan, B. J. Chambers, K. Hultenby, F. Ye, M. S. Toprak, B. D. Brandner, A. Fornara, J. Klein-Seetharaman, G. P. Kotchey, A. Star, A. A. Shvedova, B. Fadeel, V. E. Kagan, *Small* **2013**, *9*, 2721.
[19] K. Bhattacharya, R. El-Sayed, F. T. Andón, S. P. Mukherjee, J. Gregory, H. Li, Y. Zhao, W. Seo, A. Fornara, B. Brandner, M. S. Toprak, K. Leifer, A. Star, B. Fadeel, *Carbon* **2015**, *91*, 506.
[20] C. Zhang, W. Chen, P. J. J. Alvarez, *Environ. Sci. Technol.* **2014**, *48*, 7918.
[21] G. Chandrasekaran, S. K. Choi, Y. C. Lee, G. J. Kim, H. J. Shin, *J. Ind. Eng. Chem.* **2014**, *20*, 3367.
[22] A. R. Sureshbabu, R. Kurapati, J. Russier, C. Ménard-Moyon, I. Bartolini, M. Meneghetti, K. Kostarelos, A. Bianco, *Biomaterials* **2015**, *72*, 20.
[23] M. P. Monopoli, C. Åberg, A. Salvati, K. A. Dawson, *Nat. Nanotechnol.* **2012**, *7*, 779.
[24] D. Walczyk, F. B. Bombelli, M. P. Monopoli, I. Lynch, K. A. Dawson, *J. Am. Chem. Soc.* **2010**, *132*, 5761.
[25] M. P. Monopoli, A. S. Pitek, I. Lynch, K. A. Dawson, in *Nanomaterial Interfaces in Biology* (Eds: P. Bergese, K. Hamad-Schifferli) **2013**, pp. 137–155.
[26] A. Lesniak, F. Fenaroli, M. P. Monopoli, C. Åberg, K. A. Dawson, A. Salvati, *ACS Nano* **2012**, *6*, 5845.
[27] F. Wang, L. Yu, M. P. Monopoli, P. Sandin, E. Mahon, A. Salvati, K. A. Dawson, *Nanomedicine* **2013**, *9*, 1159.
[28] A. Lesniak, A. Salvati, M. J. Santos-Martinez, M. W. Radomski, K. A. Dawson, C. Åberg, *J. Am. Chem. Soc.* **2013**, *135*, 1438.
[29] I. I. Vlasova, A. V. Sokolov, A. V. Chekanov, V. A. Kostevich, V. B. Vasilyev, *Russ. J. Bioorg. Chem.* **2011**, *37*, 453.
[30] N. Lu, J. Li, R. Tian, Y. Y. Peng, *Chem. Res. Toxicol.* **2014**, *27*, 1070.
[31] Y. Li, L. Feng, X. Shi, X. Wang, Y. Yang, K. Yang, T. Liu, G. Yang, Z. Liu, *Small* **2014**, *10*, 1544.
[32] A. J. Chetwynd, I. Lynch, *Environ. Sci.: Nano* **2020**, *7*, 1041.
[33] M. P. Monopoli, D. Walczyk, A. Campbell, G. Elia, I. Lynch, F. B. Bombelli, K. A. Dawson, *J. Am. Chem. Soc.* **2011**, *133*, 2525.
[34] V. P. Vu, G. B. Gifford, F. Chen, H. Benasutti, G. Wang, E. V. Groman, R. Scheinman, L. Saba, S. M. Moghimi, D. Simberg, *Nat. Nanotechnol.* **2019**, *14*, 260.
[35] B. Fadeel, *Front. Immunol.* **2019**, *10*, 133.
[36] L. Böhmert, L. Voß, V. Stock, A. Braeuning, A. Lampen, H. Sieg, *Nanoscale Adv.* **2020**, *2*, 563.
[37] N. J. Braun, M. C. Debrosse, S. M. Hussain, K. K. Comfort, *Mater. Sci. Eng. C* **2016**, *64*, 34.
[38] A. Albanese, C. D. Walkey, J. B. Olsen, H. Guo, A. Emili, W. C. W. Chan, *ACS Nano* **2014**, *8*, 5515.
[39] M. Hadjidemetriou, K. Kostarelos, *Nat. Nanotechnol.* **2017**, *12*, 288.
[40] M. Lundqvist, J. Stigler, T. Cedervall, T. Berggård, M. B. Flanagan, I. Lynch, G. Elia, K. Dawson, *ACS Nano* **2011**, *5*, 7503.
[41] C. Martín, K. Kostarelos, M. Prato, A. Bianco, *Chem. Commun.* **2019**, *55*, 5540.
[42] R. Kurapati, F. Bonachera, J. Russier, A. R. Sureshbabu, C. Ménard-Moyon, K. Kostarelos, A. Bianco, *2D Mater.* **2018**, *5*, 015020.
[43] G. P. Kotchey, Y. Zhao, V. E. Kagan, A. Star, *Adv. Drug Delivery Rev.* **2013**, *65*, 1921.
[44] X. Luan, C. Martín, P. Zhang, Q. Li, I. A. Vacchi, L. G. Delogu, Y. Mai, A. Bianco, *Angew. Chem., Int. Ed.* **2020**, *59*, 18515.
[45] G. P. Kotchey, S. A. Hasan, A. A. Kapralov, S. H. Ha, K. Kim, A. A. Shvedova, V. E. Kagan, A. Star, *Acc. Chem. Res.* **2012**, *45*, 1770.
[46] R. Kurapati, C. Martin, V. Palermo, Y. Nishina, A. Bianco, *Faraday Discuss.* **2020**, *227*, 189.
[47] K. Bhattacharya, S. P. Mukherjee, A. Gallud, S. C. Burkert, S. Bistarelli, S. Bellucci, M. Bottini, A. Star, B. Fadeel, *Nanomedicine* **2016**, *12*, 333.
[48] M. Zhang, Y. Deng, M. Yang, H. Nakajima, M. Yudasaka, S. Iijima, T. Okazaki, *Sci. Rep.* **2019**, *9*, 1284.
[49] L. Newman, N. Lozano, M. Zhang, S. Iijima, M. Yudasaka, C. Bussy, K. Kostarelos, *npj 2D Mater. Appl.* **2019**, *1*, 39.
[50] G. P. Kotchey, B. L. Allen, H. Vedala, N. Yanamala, A. A. Kapralov, Y. Y. Tyurina, J. Klein-Seetharaman, V. E. Kagan, A. Star, *ACS Nano* **2011**, *5*, 2098.
[51] R. Kurapati, J. Russier, M. A. Squillaci, E. Treossi, C. Ménard-Moyon, A. E. Del Rio-Castillo, E. Vazquez, P. Samorì, V. Palermo, A. Bianco, *Small* **2015**, *11*, 3985.
[52] R. Kurapati, S. P. Mukherjee, C. Martín, G. Bepete, E. Vázquez, A. Pénicaud, B. Fadeel, A. Bianco, *Angew. Chem., Int. Ed.* **2018**, *57*, 11722.
[53] G. Reina, J. M. González-Domínguez, A. Criado, E. Vázquez, A. Bianco, M. Prato, *Chem. Soc. Rev.* **2017**, *46*, 4400.
[54] J. Russier, C. Ménard-Moyon, E. Venturelli, E. Gravel, G. Marcolongo, M. Meneghetti, E. Doris, A. Bianco, *Nanoscale* **2011**, *3*, 893.
[55] Y. Zhao, B. L. Allen, A. Star, *J. Phys. Chem. A* **2011**, *115*, 9536.
[56] M. Zhang, M. Yang, H. Nakajima, M. Yudasaka, S. Iijima, T. Okazaki, *ACS Appl. Nano Mater.* **2019**, *2*, 4293.
[57] G. Modugno, F. Ksar, A. Battigelli, J. Russier, P. Lonchambon, E. E. Da Silva, C. Ménard-Moyon, B. Soula, A. M. Galibert, M. Pinault, E. Flahaut, M. Mayne-L'Hermite, A. Bianco, *Carbon* **2016**, *100*, 367.
[58] D. Mata, M. Amaral, A. J. S. Fernandes, B. Colaço, A. Gama, M. C. Paiva, P. S. Gomes, R. F. Silva, M. H. Fernandes, *Nanoscale* **2015**, *7*, 9238.
[59] A. M. Arnold, B. D. Holt, C. Tang, S. A. Sydlik, *Carbon* **2019**, *154*, 342.
[60] C. Martín, A. Ruiz, S. Keshavan, G. Reina, D. Murera, Y. Nishina, B. Fadeel, A. Bianco, *Adv. Funct. Mater.* **2019**, *29*, 1901761.
[61] B. Ma, C. Martín, R. Kurapati, A. Bianco, *Chem. Soc. Rev.* **2020**, *49*, 6224.
[62] R. Kurapati, A. Bianco, *Nanoscale* **2018**, *10*, 19316.
[63] M. T. Wolf, C. L. Dearth, C. A. Ranallo, S. T. LoPresti, L. E. Carey, K. A. Daly, B. N. Brown, S. F. Badylak, *Biomaterials* **2014**, *35*, 6838.
[64] O. Veiseh, A. J. Vegas, *Adv. Drug Delivery Rev.* **2019**, *144*, 148.
[65] K. Prapainop, D. P. Witter, P. Wentworth, *J. Am. Chem. Soc.* **2012**, *134*, 4100.
[66] X. Lu, P. Xu, H.-M. Ding, Y.-S. Yu, D. Huo, Y.-Q. Ma, *Nat. Commun.* **2019**, *10*, 4520.
[67] Z. Jie, Y. Zhang, C. Wang, B. Shen, X. Guan, Z. Ren, X. Ding, W. Dai, Y. Jiang, *PLoS One* **2017**, *12*, e0180832.
[68] C. Farrera, K. Bhattacharya, B. Lazzaretto, F. T. Andón, K. Hultenby, G. P. Kotchey, A. Star, B. Fadeel, *Nanoscale* **2014**, *6*, 6974.

[69] V. E. Kagan, A. A. Kapralov, C. M. St Croix, S. C. Watkins, E. R. Kisin, G. P. Kotchey, K. Balasubramanian, I. I. Vlasova, J. Yu, K. Kim, W. Seo, R. K. Mallampalli, A. Star, A. A. Shvedova, *ACS Nano* **2014**, *8*, 5610.
[70] S. P. Mukherjee, A. R. Gliga, B. Lazzaretto, B. Brandner, M. Fielden, C. Vogt, L. Newman, A. F. Rodrigues, W. Shao, P. M. Fournier, M. S. Toprak, A. Star, K. Kostarelos, K. Bhattacharya, B. Fadeel, *Nanoscale* **2018**, *10*, 1180.
[71] M. Zhang, M. Yang, C. Bussy, S. Iijima, K. Kostarelos, M. Yudasaka, *Nanoscale* **2015**, *7*, 2834.
[72] M. Yang, M. Zhang, H. Nakajima, M. Yudasaka, S. Iijima, T. Okazaki, *Int. J. Nanomed.* **2019**, *14*, 2797.
[73] C. Farrera, K. Bhattacharya, B. Lazzaretto, F. T. Andón, K. Hultenby, G. P. Kotchey, A. Star, B. Fadeel, *Nanoscale* **2014**, *6*, 6974.
[74] F. Paquin, J. Rivnay, A. Salleo, N. Stingelin, C. Silva, *J. Mater. Chem. C* **2015**, *3*, 10715.
[75] D. Elgrabli, W. Dachraoui, C. Ménard-Moyon, X. J. Liu, D. Bégin, S. Bégin-Colin, A. Bianco, F. Gazeau, D. Alloyeau, *ACS Nano* **2015**, *9*, 10113.
[76] C. Bussy, E. Paineau, J. Cambedouzou, N. Brun, C. Mory, B. Fayard, M. Salomé, M. Pinault, M. Huard, E. Belade, L. Armand, J. Boczkowski, P. Launois, S. Lanone, *Part. Fibre Toxicol.* **2013**, *10*, 24.
[77] M. Yang, M. Zhang, *Front. Mater.* **2019**, *6*, 225.
[78] C. Bussy, C. Hadad, M. Prato, A. Bianco, K. Kostarelos, *Nanoscale* **2016**, *8*, 590.
[79] J. Russier, L. Oudjedi, M. Piponnier, C. Bussy, M. Prato, K. Kostarelos, B. Lounis, A. Bianco, L. Cognet, *Nanoscale* **2017**, *9*, 4642.
[80] V. Brinkmann, U. Reichard, C. Goosmann, B. Fauler, Y. Uhlemann, D. S. Weiss, Y. Weinrauch, A. Zychlinsky, *Science* **2004**, *303*, 1532.
[81] S. P. Mukherjee, B. Lazzaretto, K. Hultenby, L. Newman, A. F. Rodrigues, N. Lozano, K. Kostarelos, P. Malmberg, B. Fadeel, *Chem* **2018**, *4*, 334.
[82] R. Rampado, S. Crotti, P. Caliceti, S. Pucciarelli, M. Agostini, *Front. Bioeng. Biotechnol.* **2020**, *8*, 166.
[83] R. Kurapati, K. Kostarelos, M. Prato, A. Bianco, *Adv. Mater.* **2016**, *28*, 6052.
[84] B. Fadeel, C. Bussy, S. Merino, E. Vázquez, E. Flahaut, F. Mouchet, L. Evariste, L. Gauthier, A. J. Koivisto, U. Vogel, C. Martín, L. G. Delogu, T. Buerki-Thurnherr, P. Wick, D. Beloin-Saint-Pierre, R. Hischier, M. Pelin, F. C. Carniel, M. Tretiach, F. Cesca, F. Benfenati, D. Scaini, L. Ballerini, K. Kostarelos, M. Prato, A. Bianco, *ACS Nano* **2018**, *12*, 10582.
[85] D. Elgrabli, M. Floriani, S. Abella-Gallart, L. Meunier, C. Gamez, P. Delalain, F. Rogerieux, J. Boczkowski, G. Lacroix, *Part. Fibre Toxicol.* **2008**, *5*, 20.
[86] M. Zhang, Y. Tahara, M. Yang, X. Zhou, S. Iijima, M. Yudasaka, *Adv. Healthcare Mater.* **2014**, *3*, 239.
[87] A. F. Rodrigues, L. Newman, D. Jasim, S. P. Mukherjee, J. Wang, I. A. Vacchi, C. Ménard-Moyon, A. Bianco, B. Fadeel, K. Kostarelos, C. Bussy, *Adv. Sci.* **2020**, *7*, 1903200.
[88] M. Zhang, Y. Xu, M. Yang, M. Yudasaka, T. Okazaki, *Nanoscale Adv.* **2020**, *2*, 1551.
[89] L. Newman, A. F. Rodrigues, D. A. Jasim, I. A. Vacchi, C. Ménard-Moyon, A. Bianco, C. Bussy, K. Kostarelos, *Cell Rep. Phys. Sci.* **2020**, *1*, 100176.
[90] A. A. Shvedova, A. A. Kapralov, W. H. Feng, E. R. Kisin, A. R. Murray, R. R. Mercer, C. M. St Croix, M. A. Lang, S. C. Watkins, N. V. Konduru, B. L. Allen, J. Conroy, G. P. Kotchey, B. M. Mohamed, A. D. Meade, Y. Volkov, A. Star, B. Fadeel, V. E. Kagan, *PLoS One* **2012**, *7*, e30923.
[91] A. Nunes, C. Bussy, L. Gherardini, M. Meneghetti, M. A. Herrero, A. Bianco, M. Prato, T. Pizzorusso, K. T. Al-Jamal, K. Kostarelos, *Nanomedicine* **2012**, *7*, 1485.
[92] G. Peng, M. F. Montenegro, C. N. M. Ntola, S. Vranic, K. Kostarelos, C. Vogt, M. S. Toprak, T. Duan, K. Leifer, L. Bräutigam, J. O. Lundberg, B. Fadeel, *Nanoscale* **2020**, *12*, 16730.
[93] C. M. Girish, A. Sasidharan, G. S. Gowd, S. Nair, M. Koyakutty, *Adv. Healthcare Mater.* **2013**, *2*, 1489.
[94] L. Newman, D. A. Jasim, E. Prestat, N. Lozano, I. de Lázaro, Y. Nam, B. M. Assas, J. Pennock, S. J. Haigh, C. Bussy, K. Kostarelos, *ACS Nano* **2020**, *14*, 10168.
[95] T. Cedervall, I. Lynch, S. Lindman, T. Berggård, E. Thulin, H. Nilsson, K. A. Dawson, S. Linse, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 2050.
[96] C. Corbo, R. Molinaro, A. Parodi, N. E. T. Furman, F. Salvatore, E. Tasciotti, *Nanomedicine* **2016**, *11*, 81.
[97] R. García-Álvarez, M. Hadjidemetriou, A. Sánchez-Iglesias, L. M. Liz-Marzán, K. Kostarelos, *Nanoscale* **2018**, *10*, 1256.
[98] W. K. Fong, T. L. Moore, S. Balog, D. Vanhecke, L. Rodriguez-Lorenzo, B. Rothen-Rutishauser, M. Lattuada, A. Petri-Fink, in *Biological Responses to Nanoscale Particles* (Eds: P. Gehr, R. Zellner), Springer, New York **2019**, pp. 101–150.
[99] N. Bertrand, P. Grenier, M. Mahmoudi, E. M. Lima, E. A. Appel, F. Dormont, J. M. Lim, R. Karnik, R. Langer, O. C. Farokhzad, *Nat. Commun.* **2017**, *8*, 1.
[100] Z. J. Deng, M. Liang, M. Monteiro, I. Toth, R. F. Minchin, *Nat. Nanotechnol.* **2011**, *6*, 39.
[101] P. Aggarwal, J. B. Hall, C. B. McLeland, M. A. Dobrovolskaia, S. E. McNeil, *Adv. Drug Delivery Rev.* **2009**, *61*, 428.
[102] C. D. Walkey, W. C. W. Chan, *Chem. Soc. Rev.* **2012**, *41*, 2780.
[103] R. D. Vinluan, J. Zheng, *Nanomedicine* **2015**, *10*, 2781.
[104] I. Lynch, A. Salvati, K. A. Dawson, *Nat. Nanotechnol.* **2009**, *4*, 546.
[105] L. Ding, C. Yao, X. Yin, C. Li, Y. Huang, M. Wu, B. Wang, X. Guo, Y. Wang, M. Wu, *Small* **2018**, *14*, 1801451.
[106] G. Stepien, M. Moros, M. Pérez-Hernández, M. Monge, L. Gutiérrez, R. M. Fratila, M. De Las Heras, S. M. Guillén, J. J. P. Lanzarote, C. Solans, J. Pardo, J. M. De La Fuente, *ACS Appl. Mater. Interfaces* **2018**, *10*, 4548.
[107] D. Pozzi, G. Caracciolo, A. L. Capriotti, C. Cavaliere, G. La Barbera, T. J. Anchordoquy, A. Laganà, *J. Proteomics* **2015**, *119*, 209.
[108] V. Mirshafiee, R. Kim, M. Mahmoudi, M. L. Kraft, *Int. J. Biochem. Cell Biol.* **2016**, *75*, 188.
[109] J. A. Kim, A. Salvati, C. A˚berg, K. A. Dawson, *Nanoscale* **2014**, *6*, 14180.
[110] W. Makalowski, J. Zhang, M. S. Boguski, *Genome Res.* **1996**, *6*, 846.
[111] M. J. Hajipour, S. Laurent, A. Aghaie, F. Rezaee, M. Mahmoudi, *Biomater. Sci.* **2014**, *2*, 1210.
[112] C. Corbo, R. Molinaro, M. Tabatabaei, O. C. Farokhzad, M. Mahmoudi, *Biomater. Sci.* **2017**, *5*, 378.
[113] M. J. Hajipour, J. Raheb, O. Akhavan, S. Arjmand, O. Mashinchian, M. Rahman, M. Abdolahad, V. Serpooshan, S. Laurent, M. Mahmoudi, *Nanoscale* **2015**, *7*, 8978.
[114] M. Faria, M. Björnmalm, K. J. Thurecht, S. J. Kent, R. G. Parton, M. Kavallaris, A. P. R. Johnston, J. J. Gooding, S. R. Corrie, B. J. Boyd, P. Thordarson, A. K. Whittaker, M. M. Stevens, C. A. Prestidge, C. J. H. Porter, W. J. Parak, T. P. Davis, E. J. Crampin, F. Caruso, *Nat. Nanotechnol.* **2018**, *13*, 777.
[115] A. J. Chetwynd, K. E. Wheeler, I. Lynch, *Nano Today* **2019**, *28*, 100758.
[116] M. Mahmoudi, *Trends Biotechnol.* **2018**, *36*, 755.
[117] N. Tirtaatmadja, G. Mortimer, E. P. Ng, H. A. Ahmad, S. Mintova, V. Serpooshan, R. F. Minchin, M. Mahmoudi, *J. Iran. Chem. Soc.* **2015**, *12*, 317.
[118] M. P. Monopoli, D. Walczyk, A. Campbell, G. Elia, I. Lynch, F. B. Bombelli, K. A. Dawson, *J. Am. Chem. Soc.* **2011**, *133*, 2525.
[119] M. Mahmoudi, M. A. Shokrgozar, S. Behzadi, *Nanoscale* **2013**, *5*, 3240.
[120] F. Chen, G. Wang, J. I. Griffin, B. Brenneman, N. K. Banda, V. M. Holers, D. S. Backos, L. Wu, S. M. Moghimi, D. Simberg, *Nat. Nanotechnol.* **2017**, *12*, 387.
[121] J. Lazarovits, S. Sindhwani, A. J. Tavares, Y. Zhang, F. Song, J. Audet, J. R. Krieger, A. M. Syed, B. Stordy, W. C. W. Chan, *ACS Nano* **2019**, *13*, 8023.

[122] M. Hadjidemetriou, Z. Al-Ahmady, M. Mazza, R. F. Collins, K. Dawson, K. Kostarelos, *ACS Nano* **2015**, *9*, 8142.
[123] P. M. Valencia, O. C. Farokhzad, R. Karnik, R. Langer, *Nat. Nanotechnol.* **2012**, *7*, 623.
[124] D. Liu, H. Zhang, F. Fontana, J. T. Hirvonen, H. A. Santos, *Adv. Drug Delivery Rev.* **2018**, *128*, 54.
[125] D. Zhu, Q. Long, Y. Xu, J. Xing, *Micromachines* **2019**, *10*, 414.
[126] G. M. Whitesides, *Nature* **2006**, *442*, 368.
[127] D. Pozzi, G. Caracciolo, L. Digiacomo, V. Colapicchioni, S. Palchetti, A. L. Capriotti, C. Cavaliere, R. Z. Chiozzi, A. Puglisi, A. Laganà, *Nanoscale* **2015**, *7*, 13958.
[128] S. Palchetti, V. Colapicchioni, L. Digiacomo, G. Caracciolo, D. Pozzi, A. L. Capriotti, G. La Barbera, A. Laganà, *Biochim. Biophys. Acta, Biomembr.* **2016**, *1858*, 189.
[129] G. Caracciolo, O. C. Farokhzad, M. Mahmoudi, *Trends Biotechnol.* **2017**, *35*, 257.
[130] L. Digiacomo, S. Palchetti, F. Giulimondi, D. Pozzi, R. Z. Chiozzi, A. L. Capriotti, A. Laganà, G. Caracciolo, *Lab Chip* **2019**, *19*, 2557.
[131] D. Dannhauser, G. Romeo, F. Causa, I. De Santo, P. A. Netti, *Analyst* **2014**, *139*, 5239.
[132] R. Stehle, G. Goerigk, D. Wallacher, M. Ballauff, S. Seiffert, *Lab Chip* **2013**, *13*, 1529.
[133] S. S. Varghese, Y. Zhu, T. J. Davis, S. C. Trowell, *Lab Chip* **2010**, *10*, 1355.
[134] J. Sun, Y. Xianyu, X. Jiang, *Chem. Soc. Rev.* **2014**, *43*, 6239.
[135] X. Fan, I. M. White, *Nat. Photonics* **2011**, *5*, 591.
[136] R. O. Rodrigues, M. Bañobre-López, J. Gallo, P. B. Tavares, A. M. T. Silva, R. Lima, H. T. Gomes, *J. Nanopart. Res.* **2016**, *18*, 194.
[137] K. Huang, R. Boerhan, C. Liu, G. Jiang, *Mol. Pharmaceutics* **2017**, *14*, 4618.
[138] E. Burello, A. P. Worth, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.* **2011**, *3*, 298.
[139] R. Parthasarathi, A. Dhawan, in *In Vitro Toxicology* (Eds: A. Dhawan, S. Kwon) Elsevier Inc., New York **2018**, pp. 91–109.
[140] D. A. Winkler, E. Mombelli, A. Pietroiusti, L. Tran, A. Worth, B. Fadeel, M. J. McCall, *Toxicology* **2013**, *313*, 15.
[141] Z. Lin, N. A. Monteiro-Riviere, R. Kannan, J. E. Riviere, *Nanomedicine* **2016**, *11*, 107.
[142] R. Liu, W. Jiang, C. D. Walkey, W. C. W. Chan, Y. Cohen, *Nanoscale* **2015**, *7*, 9664.
[143] X. Yan, J. Zhang, D. P. Russo, H. Zhu, B. Yan, *ACS Sustainable Chem. Eng.* **2020**, *8*, 19096.
[144] B. Polyak, B. Cordovez, *Nanomedicine* **2016**, *11*, 189.
[145] J. Lazarovits, Y. Y. Chen, E. A. Sykes, W. C. W. Chan, *Chem. Commun.* **2015**, *51*, 2756.

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