

Review Article

Safety Assessment of Carbon Nanotube Nanocomposites: Challenges and Perspectives

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Abstract

Nanotechnology has been experiencing a transition from basic research and development on passive "First generation" nanoparticles and nanofibers to active "Second or Third generation" nanostructures including sensors, fuel cells, solar cells, biomimetic materials, drug delivery systems, and high performance nanocomposites. Accordingly, the environmental health and safety of nanotechnology (NanoEHS) studies are recommended to make a shift from basic nanomaterials to more complex active nanostructures. Among these materials, nanocomposite is an important type of material with rapid growth worldwide. Nanocomposites are internally nanostructured materials generated by nanofillers dispersed in a continuous matrix, which may modify the hazard profiles of the nanofillers to humans and the environment. Among them, carbon nanotubes (CNTs) have been extensively studied as fillers to enhance the mechanical and electrical properties of polymers and cements. However, to date, there are major challenges on their degradation processes, exposure and biological effects of these nanocomposites, especially the degraded fragments at the end of their lifecycle. Thus, there is an urgent need on studying the nanocomposites and developing predictive platforms to assess the hazard potential of these materials as recommended by 2011 National Nanotechnology Initiative (NNI) and 2012 President's Council of Advisors on Science and Technology (PCAST) reports.

INTRODUCTION

It is expected that global consumption of nanocomposites will grow from nearly 225,060 metric tons in 2014 to nearly 584,984 metric tons in 2019, a compound annual growth rate (CAGR) of 21.1% for the period of 2014 to 2019 [1]. Nanocomposites are internally nanostructured materials generated by nanofillers including nanoparticles and nanofibers dispersed in a continuous matrix including polymers and cements [2-4]. The reason behind the rapid growth of nanocomposite materials is that they substantially outperform established materials such as metals, polymers, and cements with added functionalities including scratch resistance, elasticity, conductivity and transparency conferred by the nanomaterials [3,5]. However, the hybrid filler-matrix fragments may modify the risk profile during the lifecycle from production to consumer use to disposal [3]. To date, attention on the consumer and environmental contact with nanocomposites is limited with most studies focus on passive or the first generation nanoparticles and nanofibers oriented towards occupational and environmental safety. Therefore, it is important to study the toxicological effects of nanomaterials in

commercial products including nanocomposites, particularly the degradation products produced during their life-cycle [3,5].

Carbon nanotube (CNT) composites accounted for 21% of total nanocomposite consumption in 2010. By 2016, the CNT-based composites market should amount to 53,286 metric tons and \$384 million [6]. CNTs are mostly used in nanocomposites because of their unique physicochemical properties including light weight, high conductivity, extraordinary tensile strength, and efficient heat conduction [7-9]. CNT nanocomposites take advantage of nanotubes' unique properties to improve their tensile strength, toughness, thermal conductivity, electrical conductivity, solvent resistance, optical properties, etc [10-12]. Various matrices are used for the preparation of CNT nanocomposites, including thermoplastics, polymers and cements [3,5,6]. Many commercial products containing CNT-based nanocomposites such as body armor, bike, airplane, headphone, ink are created and used in aerospace, consumer, military and defense [13]. In addition, CNTs can also incorporate with iron, titanium, nickel, cobalt oxides, etc. to prepare catalysts, electrodes or electronic devices.

Challenges for safety assessment of CNT-nanocomposites

CNTs have been extensively studied as fillers to enhance the mechanical and electrical properties of polymers and cements. However, the widespread usage of CNTs has generated concerns about their environmental health and safety because their resemblance of asbestos, a fibrous material that can cause fibrosis or scarring of the lung and lung cancer [7-9,14,15]. Animal studies using mice and rats have shown that CNTs, including single wall and multiwall CNTs, in its raw or as-produced form could induce inflammation and persistent lung fibrosis [14-21]. However, the effects of CNTs, especially in its nanocomposite form to humans and environmental organisms are still unclear. One of the reasons is the difficulty to mimic the real-life environmental degradation processes, characterize the fragments, and quantify the CNT contents. In addition, there is a lack of predictive approach to assess the toxicological profiles of these materials in an environmental relevant organism. It is imperative to establish a validated and robust scientific platform to rapidly and cost-effectively assess environmental toxicity induced by nanomaterials and nanocomposites as recommended by 2011 National Nanotechnology Initiative (NNI) and 2012 President's Council of Advisors on Science and Technology (PCAST) reports [22,23].

All nanoproducts will undergo life cycle processes and ultimately lose their structural integrity (Figure 1), which could release engineered nanomaterials (ENMs) that were originally embedded within that material [3,5]. For nanocomposites, the ENM nanofillers release will depend on the chemical composition of the matrix, resulting in different releasing kinetics, fragment characteristics under different degradation scenarios including chemical and mechanical stress (Figures 1) [3,5,24]. Significant attention has been focused on CNT-nanocomposites because animal studies using mouse and rat have shown that CNTs are hazardous to the lung [14,15,18]. The safety of CNT-nanocomposite degradation during the life-cycle has to be addressed before commercialization of the nanoproducts to ensure human and environmental exposures are safe [3,5,24]. Currently, there are still many knowledge gaps remain on the degradation processes, physicochemical characterization of the fragments, quantification of nanomaterial release, and human exposure scenarios.

Study on the biological effects of these materials is still at the infancy stage. To date, only few papers have described the biological effects of nanocomposite materials and all of studies are focused on the lung toxicity induced by these materials in mice or rats [3,25]. Saber et al. studied the toxicity of sanding dust of titanium dioxide nanoparticle (nanoTiO₂)-containing paint in mouse lung and compared that with the pure titanium dioxide nanoparticles [25]. The results showed that there was no additive effect of adding NanoTiO₂ to paints [25]. Wohlleben et al. compared the toxicity of released fragments from four multiwall carbon nanotube (MWCNT)-based nanocomposites (Figure 2) including polymers and cements in the lung of rats. They found that CNT-composites fragment have drastic different properties with polymer based materials have no CNT protrusions or release from the composites, while cements based composites

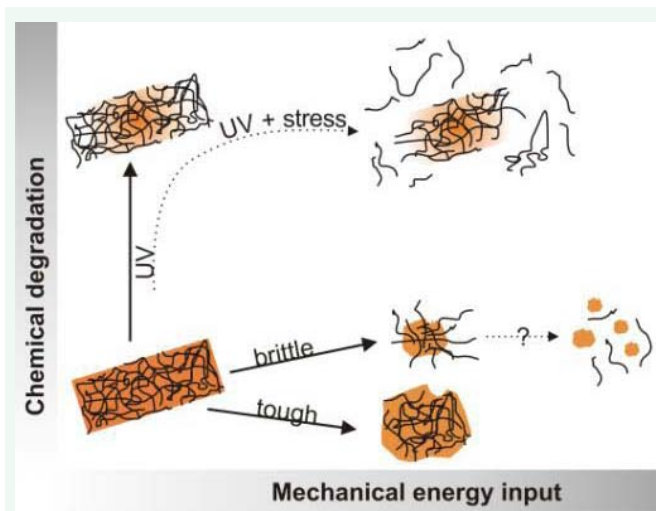


Figure 1 Fragments or intermediate structures during degradation of CNT-nanocomposites. Emerging structure-property relationships for fragments or intermediate structures during chemical and mechanical degradation, starting from the intact CNT nanocomposite (bottom left). A predictive toxicological approach should be developed to study the toxicological effects of these materials in vitro and in vivo. (J Nanopart Res, 2013, 15: 1504).

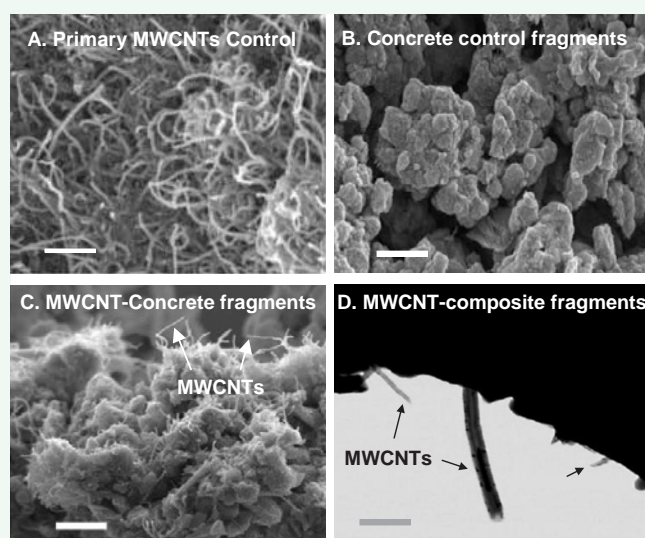


Figure 2 Protrusions on sanding fragments of MWCNT nanocomposites. Representative SEM images of (A) primary MWCNT control, (B) cement control fragments, and (C) MWCNT-cement nanocomposite fragments after sanding. Scale bar: 500 nm. (D) Dark-field STEM of MWCNT nanocomposites particle. Arrows indicate the location of MWCNT protrusions. Scale bar: 100 nm. (J Nanopart Res, 2013, 15: 1504).

have numerous CNT protrusions on the fragment surface and there are possible release of free CNTs from the matrix. Despite the different properties between these materials, the pro-inflammatory effects in the lung by nanocomposite materials are not discernible from the material without nanofiller [3]. In other words, the composites masked the potential toxic effects of free CNTs, which is reasonable because majority of the CNTs remains in the composites. However, one problem is that at the

same mass dose between nanocomposites and raw CNTs, the CNT dose in the nanocomposites is considerably lower because the CNT has a low percentage presence in the composites (<5%). Another problem is that there is a lack of mechanistic studies. For example, what happens when cells such as macrophages take them up into subcellular compartments such as lysosomes, also what the degradation process is when the particles locate in the digestive system? Can the CNT protrusions outside the fragments cause damage to cells or toxicity in animals? These questions demand answers from future research on in vitro and in vivo studies.

Develop a predictive approach for safety assessment of CNT-nanocomposites

One of the goals of safety assessment for nanocomposites is to identify the physicochemical properties of ENMs embedded in composites that can lead to adverse health effects. This goal is not achievable by primarily relying on animals to perform safety screening because it is not logistically feasible to test all the nanomaterial formulations. To avoid this conundrum in nano safety testing, we need alternative ways to screen for ENM toxicity. In vitro assays are an indispensable part in this effort because these techniques allow the identification of specific biological and mechanistic pathways that also play an important role in disease outcomes and pathological changes in vivo. Then in vivo assays are used to validate the in vitro results, which are deemed predictive if the two agrees with each other. Recent advances in developing standard mechanism-based cellular assays, imaging techniques and high throughput screening platforms enable large numbers of ENMs to be tested under standardized conditions. The National Research Council of the U.S. National Academy of Sciences (NAS) opined that toxicological testing in the twenty-first century should undergo a paradigm shift from a predominant observational science in animals to a target-specific and predictive in vitro science that utilizes mechanisms of injury and toxicological pathways to guide the conductance of in vivo studies [22,26]. All these developments call for substantial improvement and expansion of existing in vitro approaches to meet the challenge of performing hazard assessment for a rapidly expanding number of new ENMs with novel physicochemical properties as well as new generation of nanoproducts. To date, significant progress has been made focusing on the correlation between in vitro toxicological assessment with in vivo inflammatory effects in the lung using mouse and rat models. Similar approach should be developed for safety assessment of nanocomposites. The approach should be based on establishment of nanocomposite libraries, thorough characterization of the nanocomposites, quantification of the ENM release, mechanism-based in vitro high throughput toxicity screening including oxidative stress and inflammation, and in vivo experiments including environmental organisms including zebrafish and mouse or rat to validate the in vitro results (Figure 3).

One likely cellular mechanism that can be used to screen the CNT-nanocomposite is the NLRP3 inflammasome pathway [27,28]. NLRP3 inflammasome is a protein complex in the innate immune system, its activation will lead to IL-1 β production, which is associated with various inflammatory diseases (such

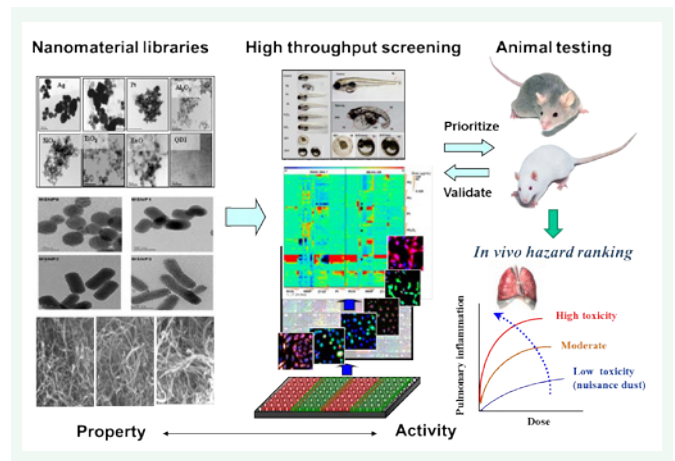


Figure 3 A predictive toxicological approach for hazard assessment for nanomaterials and nanocomposites. Implementations of predictive toxicological profiling will need the use of ENM or nanocomposite libraries, in vitro high-throughput screening and hazard ranking, development of structure activity relationships (SAR), and in vivo hazard ranking. This predictive approach will provide rapid hazard assessment for nanomaterials and nanocomposites that links the physicochemical properties of the materials to their toxicity, which will greatly assist the regulatory decision-making process.

as gout, diabetes mellitus, Alzheimer's, and inflammatory bowel disease). Recent studies show that NLRP3 inflammasome can respond to a wide range of stimuli including particles and fibers such as quartz, asbestos and CNTs to induce lung inflammation and fibrosis [19,21,27-33]. We have demonstrated that high aspect ratio nanomaterials including MWCNTs, single wall carbon nanotubes (SWCNTs), and CeO₂ nanowires could trigger NLRP3 inflammasome activation in vitro and in vivo [30,32,34,35]. As to CNT-nanocomposites, what happens when macrophages take up them into their lysosomes, will there be further degradation? Will the protrusions from fragments of CNT-cement nanocomposites induce lysosomal damage similar to raw CNTs and lead to NLRP3 inflammasome activation, resulting chronic lung inflammation or fibrosis? These questions can only be answered by using the in vitro and in vivo predictive approach and by using similar approach developed in recent years, we are fully equipped to study the CNT-nanocomposites and evaluate their hazard potential, which will be important for their safe use in nanoproducts.

CONCLUSION

To date, most of the NanoEHS research is focused on primary ENMs, the study on the effects of ENM-containing materials generated during the degradation processes of nanoproducts is an under-researched area. Much research needs to go into studying the degradation processes, physicochemical characterization of the nanocomposites, quantification of released ENMs from the nanocomposites, and exposure potential and scenarios. In addition, a predictive approach involving mechanistic in vitro screening assays and in vivo assays should be developed to assess the biological or toxicological effects of these composites. This will have substantial impact on the sustainable and safe use of these materials.

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