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DISCUSSION

Exploration on the safety assessment of nanomaterials in China

Xin-Li Shi[1,*], Qiangbin Wang[2], Kun Hu[3] and Xiu-Mei Wang[3]

[1]*Center for Medical Device Evaluation, State Food and Drug Administration, Beijing 100044, People's Republic of China*
[2]*Division of Nanobiomedicine/i-LAB, Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, Suzhou, Jiangsu 215123, People's Republic of China*
[3]*National Engineering Laboratory on Regenerative Implantable Medical Devices, Science City, Guangzhou 510663, People's Republic of China*

More and more applications of nanomaterials have been achieved in the biomedicine field. Numerous nanomedical devices, such as bone grafts with nano-hydroxyapatite and the silver-based anti-bacteria products, have been developed and have been trying to enter into the Chinese market. The State Food and Drug Administration of China (SFDA) is facing a critical challenge of how to explore and supervise the safety assessment of the nanomedical products. This paper briefly introduces the approval status of nanomedical products and the current advances of the safety assessment of nanomaterials in China.

Keywords: nanomaterials; nanomedical devices; safety assessment

1. CURRENT STATE OF THE NANOMEDICAL PRODUCTS IN CHINA

Recently, with the intense interest in nano-biotechnology, more and more nanomaterials have been widely involved in biomedical studies. A large number of nanomedical devices have been successfully developed. So far, there are two categories of device with nanomaterials that have been approved for clinic applications in China by the State Food and Drug Administration of China (SFDA). One is nano-hydroxyapatite as bone repair materials, and the other is the silver-based anti-bacteria product for wound-protection etc.

Regarding the above two categories of nanomaterials, there are already 190 medical devices approved to enter the market by the SFDA, in which there are six products imported from other countries while the rest are all made in China. There are 53 nanomaterial products in the approval process of the SFDA. Among the 190 medical products, about 60 per cent are silver-based anti-bacteria products and 40 per cent are bone repair materials in which one main component is nano-hydroxyapatite. The SFDA-approved nanomedical devices are listed in table 1.

The volume of nanomedical devices made in China has been more than one billion Chinese Yuan per year since 2010, which is shared by approximately 100 enterprises. As many enterprises have been involved in the research and design of nanomedical devices, a big challenge to the SFDA nowadays is how to explore and supervise the safety assessment of these nanomedical devices.

*Author for correspondence (shixl@cmde.org.cn).

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2. CURRENT STATE OF THE SAFETY ASSESSMENT OF NANOMATERIALS IN CHINA

According to the number of patents filed, China currently ranks second to the United States in the field of nanoscience and nanotechnology research [1]. Concerning the increase in applications of nanomaterials in medicine, China has begun to pay more attention to the risk analysis and safety evaluation of nanomaterials. Herein, we try to review the latest advances in the safety assessment of some typical nano-biomaterials in China, including carbon nanomaterials, metallic and metal oxide nanomaterials, polymer nanomaterials, nano-hydroxyapatite, etc.

3. CARBON NANOMATERIALS

Carbon nanomaterials such as carbon nanotubes, fullerene, graphene and their derivatives have a broad range of potential applications in multiple biomedical fields, especially in cancer therapy and diagnosis [2,3]. Recently, the potential toxic effects of carbon nanomaterials have attracted much attention. Carbon nanotubes were found to cause oxidative damage in living cells [4], produce a series of multiple lesions in rats [5] and also cause an indirect cytotoxicity through affecting immune functions [6]. In the mean

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Table 1. Nanomedical devices approved for clinical applications by SFDA.

production place (province)	number	product description
Guangdong	33	silver-based anti-bacteria products
Inner Mongolia	29	silver-based anti-bacteria products
Jilin	25	silver-based anti-bacteria products
Henan	24	silver-based anti-bacteria products
Jiangsu	12	silver-based anti-bacteria products
Hubei	11	silver-based anti-bacteria products
Heilongjiang	8	nano-treatment instrument
Guizhou	8	silver-based anti-bacteria products, nano energy for pain relief card
Hunan	8	silver-based anti-bacteria products
abroad	6	NANOSIT, Ceram X, Ketac N100 Light-Curing Nano-Ionomer Restorative, 3M ESPE FiltekTM Z350 XT
Shanghai	5	nano-acupoint application
Sichuan	5	infrared-cured pain post; hydroxyapatite/polyamide 66 composite bone graft
Beijing	4	nano-functional magnets, mineralized collagen bone graft
Jiangxi	2	nano-oxygen running water
Shanxi	2	anti-bacteria nano-film
Guangxi	2	silver-based anti-bacteria products
Liaoning	2	silver-based anti-bacteria products
Zhejiang	2	silver-based anti-bacteria products
Shandong	2	silver-based anti-bacteria products
Tianjin	2	nanocomposite antibacterial condoms
Anhui	1	silver-based anti-bacteria products
Fujian	1	silver-based anti-bacteria products
Shanxi	1	silver-based anti-bacteria products
Ningxia	1	silver-based anti-bacteria products

time, Bai *et al.* [7] showed that carbon nanotubes only cause reversible testis damage without affecting the fertility in mice. Many efforts were also made to reduce the cytotoxicity of carbon nanotubes by functionalizing carbon nanotubes [8] or binding human serum proteins with carbon nanotubes [9].

The cytotoxicity and bioactivity of carbon nanomaterials presented a structure-dependent property. With different geometric structures, the cytotoxicity is different. For example, the cytotoxicity of fullerene is much lower than that of carbon nanotubes for lung macrophage cells [10]. Chang *et al.* [11] also found that fullerene derivatives $C60(C(COOH)_2)_2$ have no cytotoxicity to the Rh35 and HepG2 cells. However, Su *et al.*'s [12] research indicated that the cytotoxicity of C60(OH)x was cell type-specific.

Graphene, a two-dimensional sp^2-carbon nanomaterial, has attracted more attention in many more fields than carbon nanotubes and fullerene in recent years. However, in China, the potential toxicity of graphene has been relatively less explored until recently. A number of researchers demonstrated that graphene oxides (GO) showed very good biocompatibility with several types of cells and animals at low concentration [13,14], and even the ability to promote neurite sprouting of mouse hippocampal cells [15,16]. However, owing to its high accumulation and long-term retention, graphene oxides can cause oxidative stress to cells and animals and induce cell apoptosis and lung granuloma formation at high concentration (at a dose of 10 mg kg^{-1}) [13,17]. On the other hand, Hu *et al.* [18] found that foetal bovine serum can reduce the cytotoxicity of GO, owing to the high protein adsorption capacity of GO. Yang *et al.* [19] demonstrated that poly(ethylene glycol)(PEG)-ylated graphene oxides showed significantly improved biocompatibility, not causing appreciable toxicity at a dose of 20 mg kg^{-1} to mice. The toxicity of carbon nanomaterials is a subject of ongoing debate, and much work should be done before carbon nanomaterials can be applied in clinic.

4. METALLIC AND OXIDE NANOMATERIALS

Metallic and metal oxide nanomaterials such as quantum dots (QDs), gold and silver nanoparticles (NPs), magnetic NPs, TiO_2 and SiO_2, have been widely used in biosensors, cell tracking, molecular imaging, photothermal therapy and drug delivery [20]. The research of potential toxic effects of metallic compound nanomaterials has become a critical issue in China.

QDs, which are also known as semiconductor nanocrystals, are generally composed of metal elements such as Cd, Se, Zn, Te, Ag. CdSe and CdTe QDs are the most widely studied, owing to their unique optical properties. The *in vitro* cytotoxic effects of QDs in numerous cells have been studied by many groups. Su *et al.* [21] showed both the release of free Cd^{2+} and the specific properties of NPs contribute to the cytotoxicity of CdTe QDs. Yan *et al.* [22] reported that mercaptosuccinic acid-capped CdTe QDs can cause direct toxic effects on human vascular endothelial cells both by impairing mitochondria and inducing endothelial apoptosis. Furthermore, Li *et al.* [15,16] found microRNAs as participants in cytotoxicity of CdTe QDs in NIH/3T3 cells by using the SOLiD

sequencing method. Potential toxicity of QDs in animals has also been investigated by several groups. Liu *et al.* [23] showed that CdSe QDs can promote the production of intracellular reactive oxygen species and induce significant impairments to the liver in mice. Appropriate surface modification can enhance the stability of QDs in physiological solutions and improve the biocompatibility of QDs. Su *et al.* [24] found that ZnS layer coating can greatly reduce cytotoxicity and improve the biocompatibility of CdTe QDs. Guo *et al.* [25] showed surface modification with F-68 and sodium dodecyl sulphate could reduce the cytotoxicity of CdSe QDs. Li *et al.* [26] found a chirality-dependent cytotoxicity of QDs, that is, D-glutathione (D-GSH)-coated QDs showed less cytotoxicity than L-GSH-coated QDs.

Owing to the wide applications of gold nanoparticles (AuNPs), the cytotoxicity of AuNPs has become a big issue in China. Investigators have observed that AuNPs can induce oxidative stress in blood serum [27] and cause cytotoxicity in human K562 cells at a high concentration [28]. Lin *et al.* [29] found that the cytotoxicity of AuNPs increases as the percentage of ammonium-functionalized ligands on an AuNP increases. Yi *et al.* [30] reported that AuNPs exposure-induced osteogenic differentiation and inhibited adipogenic differentiation of mesenchymal stem cells by activating the p38 mitogen-activated protein kinase signalling pathway.

The cytotoxicity of silver nanoparticles (AgNPs) has also been well studied in China owing to their wide applications in biomedicine as antivirus and antibacterial reagents. Wei *et al.* [31,32] reported that AgNPs could be phagocytized into the cells, thus inducing cell apoptosis, and smaller nanoparticles may cause higher toxic effects than larger ones owing to their easier entry into cells. AgNPs may also alter the action potential of hippocampal CA1 neurons by depressing the voltage-gated sodium current [33]. Moreover, Yang *et al.* [34] showed AgNPs can interfere with DNA replication fidelity and bind with DNA. However, there are some conflicting results owing to the difficulty of obtaining different-sized AgNPs as standards for their safety evaluation. More recently, Li *et al.* [35] prepared a series of monodispersed AgNPs and standardized their size- and dose-dependent cytotoxicity.

Fe_2O_3 and Fe_3O_4 nanoparticles are the most widely used magnetic nanoparticles. Both of them were found to generate oxidative stress in human umbilical endothelial cells [36]. In addition, Wei *et al.* [37] found that Fe_3O_4, oleic acid-coated Fe_3O_4 and carbon-coated Fe could affect the viability of human hepatoma BEL-7402 cells via cell arrest and induce apoptosis through the mitochondrial-dependent pathway. The potential toxicity of magnetic nanoparticles in animals has also been explored. Zhu *et al.* [38] found potential lung and systemic cumulative toxicity of Fe_2O_3 nanoparticles in rats, even at a low concentration. Moreover, smaller Fe_2O_3 nanoparticles could induce more severe oxidative stress and nerve cell damage in the brain than larger particles [39]. On the other hand, Song *et al.* [40] showed Fe nanowires had a good biocompatibility and a very low cytotoxicity with HeLa cells.

The toxic effects of other metallic and metallic compound nanomaterials such as TiO_2, SiO_2, copper and zinc NPs have also been explored in China. For TiO_2 NPs, much research focused on the potential neurotoxicological effects. TiO_2 NPs could be translocated into the brain and cause brain injury in mice [41,42]. Additionally, the cytotoxicity of TiO_2 NPs was found to be dependent on their size, shape and surface modification [43,44]. For SiO_2 NPs, Wang *et al.* [45] showed that SiO_2 NPs can cause a dose-dependent cytotoxicity in cultured HEK293 cells by increasing oxidative stress. Moreover, SiO_2 NPs have a potential risk for neurodegenerative diseases [46]. On the other hand, Liu *et al.* [47] showed mesoporous hollow SiO_2 NPs had a low toxicity in mice with intravenous injection, and could be excreted from the body over four weeks. For copper and zinc NPs, research found the toxicity of these NPs was size-dependent [48,49].

5. NANO-HYDROXYAPATITE-CONTAINING BIOMATERIALS

Hydroxyapatite (HA) and its derivatives have been widely studied to explore their biocompatibility with tissue. Cui *et al.* developed a nano-hydroxyapatite/collagen composite and tested its ability in bone repairing. The composite showed some features of natural bone in both composition and microstructure. The inorganic phase in the composite was carbonate-substituted HA with low crystallinity and nanometre size. HA crystals grew on the surface of these fibrils in such a way that their *c*-axes were oriented along the longitudinal axes of the fibrils. The mineralized collagen fibrils aligned parallel to each other to form fibres, and the hierarchical fibres of collagen–hydroxyapatite composite were observed under transmission electron microscopy [50]. For example, Figure 1 shows the microstructure of the composite of bone graft that had been used in clinics. At the interface of this implant and marrow tissue, solution-mediated dissolution and macrophage-mediated resorption led to the degradation of the composite, followed by interfacial bone formation by osteoblasts. The process of implant degradation and bone substitution was reminiscent of bone remodelling. The composite can be incorporated into bone metabolism instead of being a permanent implant [51].

Wang *et al.* [52] found that nano-HA/polyamide composite scaffolds exhibit good biocompatibility and extensive osteoconductivity with host bone. Xu *et al.* found that polyvinyl alcohol hydrogel modified with nano-HA can improve biocompatibility [53]. Lin *et al.* [54] showed a nano-grade HA/collagen composite which had an excellent biocompatibility in mice.

6. POLYMER NANOMATERIALS

Polymer NPs are generally designed for targeted delivery of drugs. The toxic effects of several polymers have been explored in China. Hu *et al.* [55] found polymeric NP–aptamer bioconjugates exhibited little toxicity and could diminish the toxicity of mercury. Huang *et al.* [56] showed poly (ε-caprolactone)-poly (ethylene glycol)-poly (ε-caprolactone) nanomaterials did not

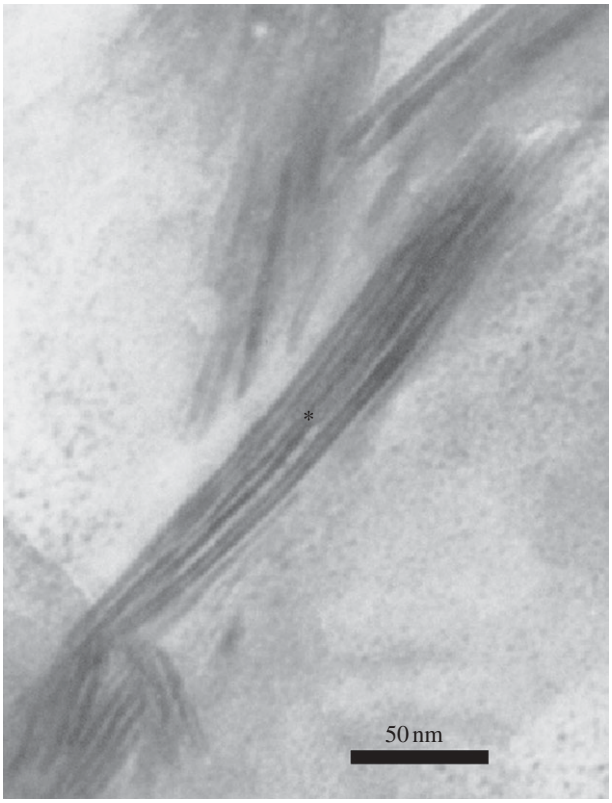

Figure 1. Higher magnification of the mineralized collagen fibrils. Selected area electron diffraction pattern of the mineralized collagen fibrils in the area of the asterisk in this figure revealed that nano-HA crystals are ordered assembled with the collagen fibres.

cause any acute toxicity and genotoxicity. He *et al.* [57] synthesized a family of novel MeO-poly (ethylene glycol)-poly (D,L-lactic-co-glycolic acid)-poly (ethylene glycol)-OMe triblock copolymer nanoparticles, and showed the cytotoxicity and haemocompatibility of copolymer were dependent on the molecular weight of PEG used in the synthesis of polymers.

7. PROSPECT ON THE SAFETY ASSESSMENT OF NANOMATERIALS IN CHINA

Special attention should be paid to nanomaterials when they are used for implants in human body, because they could invade through three body barriers of lung, intestines and skin and directly enter the circulation system.

Based on the research listed above, nanomaterials present two distinct biological characteristics. First, in the level of the whole body, nanomaterials tend to target and accumulate in certain organs, such as the liver, spleen and kidney. Second, in the cellular level, nanomaterials sized in the range of smaller than about 40 nm can easily enter cells in different ways and cause changes and even the loss of cellular functions. Therefore, for the clinical applications of nanomaterials, more detailed systematic investigations on the nanomaterials performance, in terms of transformation, distribution, transference, metabolism, drainage and accumulation inside the human body, are seriously needed. The SFDA will make efforts to work together with the scientists to continue exploring and supervising the safe clinical applications of nanobiomaterials in medicine.

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