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人工合成纳米材料的生物可给性与毒性^{*}

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摘 要 随着人工纳米材料在工业、生活、医疗等各领域中的广泛应用, 其环境暴露已不可避免. 由于纳米材料生物可给性决定了其环境危害与人体健康风险, 因此近年来这方面的研究已成为环境科学领域关注的热点. 本文基于细胞和微生物、动物、人体等, 从纳米材料种类、暴露途径、摄入动力学、体内分布、消除行为等方面, 对人工纳米材料的生物可给性与毒性进行了综述, 为客观评价纳米材料的生物安全性提供了科学参考.

关键词 纳米材料, 生物可给性, 暴露途径, 摄入动力学, 生物毒性.

Bioavailability and toxicity of engineered nanomaterials

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Abstract: With the rapid development and increasing application of engineered nanomaterials in diverse fields, including industry, science and technology, and medical cares, the release of this group of emerging chemicals into the environment has become inevitable. Public concerns have consequently risen on their potential risks of environmental and human health hazards. The bioavailability and toxicities of the nanomaterials have been widely discussed in recent researches due to their unintended exposure. A comprehensive review is provided to clarify the exposure routes, biouptake kinetics, bio-distributions and elimination behaviors of the engineered nanomaterials, thus providing the useful scientific data for their biosafety evaluation.

Keywords: nanomaterials, bioavailability, exposure routes, biouptake kinetics, toxicity.

纳米材料是一类三维结构中任何一维、二维或三维的尺寸为纳米级(1—100 nm)的新型材料, 被认为是自工业革命以来最大的工程创新、“21 世纪最有前途的材料”^[1], 具有异于普通材料的表面效应、小尺寸效应、量子效应、比表面效应、分散-团聚效应、自组装效应等特性, 目前已广泛应用于医学、药物、工业、成像、材料、通信、食品等领域^[2-7]. 纳米材料在生产使用过程中, 不可避免地会直接或间接地释放到环境中, 进而造成生物体不同程度的暴露, 大规模商品化的纳米灭菌剂和纳米治疗剂的使用^[7-9]、人工

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合成纳米颗粒在水体、土壤、大气中的存在,都可能作用于生物体并造成不可预计的后果.近年来,研究表明,以纳米银为代表的纳米颗粒在细胞模型和动物模型中可以诱导细胞毒性、呼吸系统损伤、皮肤毒性、肾脏毒性、生殖毒性、遗传毒性、发育毒性以及免疫毒性等损伤效应^[10-16].因此,在应用和发挥纳米材料的独特优越性能、推动科学技术进步的同时,了解纳米材料对生物体的影响已成为纳米领域的研究热点.生物可给性,在某些研究中又常被称作生物有效性、生物效价、生物利用率,可定性和/或定量描述物质进入生物体的效率,作为评价该物质对生物体可能产生影响的重要依据.生物可给性在毒理、环境、生物、医药等相关领域的研究中都是重要的参数或指标.

本文通过归纳总结在多种研究模型、实验手段和分析角度下纳米材料对生物体产生的效应和影响,全面分析了人工纳米材料的生物可给性,为这类新型环境污染物的生物安全性评价提供了科学依据.

1 环境中纳米材料的分类

1.1 自然存在的纳米材料

随着生活水平的提高,人们对汽油、柴油及其他生活燃料的需求日益增加,大气中的颗粒物含量因此大幅度上升^[17-19],其中,纳米级颗粒物占颗粒物总量的 36%.近年来,在中国以北京为代表的许多大型城市经受着恶劣的雾霾天气,其诱因之一就在于大气细颗粒物量的剧增.越来越多的研究开始关注包括纳米颗粒在内的超细颗粒在大气环境中的生成机制、迁移转化及对人体健康的潜在危害^[20-22].在水体中,粒径在 1—100 nm 范围内的颗粒物被分散到液体中可形成胶体溶液.水体中的胶体包括大分子有机物:腐殖酸、富里酸、蛋白质等;也包括无机类胶体,如典型的水合离子、锰氧化物^[23-24].胶体特有的小尺寸和大比表面积使之成为有机或无机污染物重要的结合相.

在土壤中,天然存在的纳米材料主要包括黏土、有机质、铁氧化合物等.在土壤中胶体研究的数十年中,人们主要探讨了胶体对土壤结构行为和成土作用的影响,以及土壤胶体在某些条件下对污染物的转运促进作用,例如天然土壤胶体在土壤剖面上可携带并转运金属物质^[25-26].通常来讲,在没有人干扰的情况下,环境中的纳米材料是微量的,并且有一定的偶然性,其本身对生物体不会产生显著的负面效应或影响.

1.2 人工合成纳米材料

人工合成纳米材料得益于本身的纳米级尺寸效应,其商品化应用在近二十年来呈指数增加的趋势,渗透到生产生活的方方面面^[27-32].人工合成纳米材料主要可以分为以下 4 类:碳纳米材料、半导体纳米材料、聚合纳米材料、金属纳米材料.

对于碳纳米材料,研究热点主要集中于碳纳米管、富勒烯、石墨烯^[33-35].其中,碳纳米管又常分为单壁碳纳米管和多壁碳纳米管.碳纳米材料强度高、质量轻,又兼具强导电性、耐腐蚀性、耐高温和低温等优良性能,然而这种材料对生物体可产生一定的毒性作用.研究显示,长碳纳米管和刚性碳纳米管可以引起生物体内肺组织的慢性炎症,甚至可能引发肿瘤^[36-38].

半导体纳米材料,也被称作量子点,可由单种或多种半导体材料合成,常见的有 Si、CdSe、ZnS、CdTe 等.纳米晶粒和高浓度晶界是半导体纳米材料的两个重要特征,在多个领域均有广泛的应用,如太阳能电池、电子器件、激光技术、催化剂及生物传感器等^[39-44].Huang 等的研究发现,量子点 TGA-CdTe 对斑马鱼胚胎的自主运动频率、心率、孵化率、体长都存在剂量-效应关系,能引起斑马鱼胚胎发生心包囊肿、卵黄囊肿、脊柱弯曲、体节减少等异常症状^[45].

聚合纳米材料主要用于药物传输和治疗,在代谢和免疫毒性等医疗干预中有着广泛的应用^[46-48].聚合纳米材料的生物可给性与材料本身的形态、聚合度等有着密切的关系.例如,聚合纳米材料的长宽比降低,可导致其细胞毒性和细胞凋亡率升高;聚合纳米材料的形状也与细胞中活性氧化物(ROS)的生成有关^[49-52].

金属纳米材料主要分为两大类:一类是金属氧化物纳米材料,如 TiO₂、ZnO 等金属氧化物颗粒;另一类是金属单质纳米材料,如金纳米颗粒、银纳米颗粒等.金属纳米材料的制备方法简单,一般化学实验室均有能力实现金属纳米材料的合成^[53-55].金属纳米材料的应用研究涉及到医药、电子、能源等领域,如铁(及铁合金)纳米材料可减少水体和土壤中的硝酸盐及六价铬、降低有机氯杀虫剂和多氯联苯的毒性,

因而在环境治理中常被用于污水、沉积物处理或土壤的治理等^[56-58]。

2 人工纳米材料的生物可给性

纳米技术的高速发展和人工纳米材料的广泛应用意味着人工纳米材料在生产、使用、废弃处理等每一个环节都可能会不同程度地被释放到周围环境中,进而造成职业生产、消费以及环境等过程中无意识的暴露;同时,在以药物传输和治疗为代表的医疗领域中,通过注射、口服、外敷等给药方式,人体可直接暴露于含有人工纳米材料的药物制剂。无论以何种途径,一旦发生暴露,纳米材料就可能进入机体,进而产生不可预测的生物效应。由于不同纳米材料有着各自独特的物化性质,其对生物体的暴露途径也多样化,不同种类生物体对纳米材料的吸收率以及产生的生物学响应并不相同,因此探讨人工纳米材料的生物可给性具有重要意义。目前关于人工纳米材料生物可给性的研究较多,但主要是基于实验室内的工作,研究模型主要包括细胞和微生物、动物和植物等。

2.1 细胞和微生物

离体实验作为生物医学领域中最常用的实验手段,有操作简便、易于控制、迅速精准、经济高效等特点,因此,离体实验被广泛用于所有新型化合物、新型污染物、新型药物的评估。同样地,在纳米材料的活性、毒性或生物可给性测试中,各种细胞和微生物模型也得到了广泛应用。

研究数据表明,在离体暴露模型中,纳米材料不仅可以进入细胞和微生物内环境,而且还可能产生细胞毒性、诱发基因突变、导致细胞凋亡^[59-61]。例如纳米颗粒直接作用于细胞表面致使细胞膜/壁损伤或破裂进而影响细胞状态;纳米材料诱导产生的活性氧化物可造成胞内氧化胁迫、破坏细胞器结构、影响细胞功能。另外,不同纳米材料本身的性质可能导致细胞毒性和生物可给性差异。通常,纳米材料的粒径越小,其进入细胞内部的能力越强,总体细胞毒性效应也更明显,例如 Boudreau 等发现小尺寸纳米银颗粒比较大尺寸的纳米银具有更强的毒性和细胞生长抑制效应^[62]。

从纳米材料种类的角度来看,碳纳米材料最早引起国际科研工作者的关注,尤以对单壁碳纳米管的研究居多。自 20 世纪 90 年代初美日两国研究团队成功制备出单层碳纳米管起,世界范围内对单壁碳纳米管生物安全性的关注就随其应用的推广而增强。单壁碳纳米管的特点之一是非常轻,可通过空气、水体等载体来实现对生物的环境暴露。对革兰氏阴性大肠杆菌(*E. coli*)^[63-67]、根癌农杆菌(*A. tumefaciens*)^[67]和革兰氏阳性枯草杆菌(*B. subtilis*)^[67-68]等多种微生物模型的研究显示,富勒烯 C₆₀ 纳米颗粒对细菌生长具有不同程度的抑制作用,尤其可对根癌农杆菌的细胞结构造成损伤。基于人皮肤成纤维细胞(*Human Skin Fibroblast*)暴露模型的研究表明,多壁碳纳米管和碳纳米管可扰乱多条细胞通路并且活化相关基因,多壁碳纳米管可诱导胞内免疫和炎症相关基因,碳纳米管可诱导与外部刺激反馈相关的基因;并且多壁碳纳米管具有比碳纳米管更明显的损伤效应,其原因在于多壁碳纳米管暴露激发干扰素并诱导 p38/ERK-MAPK 级联反应^[69-70]。Jacobsen 等的研究发现,超微细炭黑对小鼠上皮细胞基因损伤、氧化应激效应的诱导作用均高于单壁碳纳米管和 C₆₀^[71]。

金属纳米材料在细胞和微生物模型中的生物效应研究也很多,这要归功于金属纳米材料在各行各业的广泛应用和潜在的生物危害。银纳米材料产量最大、应用范围最广,其在细胞和微生物中的生物可给性也得到了高度重视。一方面由于纳米银颗粒具有优良的抗菌特性,可被用于伤口敷药、食品包装、纺织品材料或墙面涂料等,使病原微生物失活,提高医院等场所的抗菌性能,从而发挥出巨大的经济价值;另一方面,银纳米材料能对细胞产生一系列的毒副作用和危害。Wang 等研究发现,在较低浓度条件下,纳米银能作用于大肠杆菌(*E. coli*)并表现出明显的细胞毒性,浓度高于 5 $\mu\text{g}\cdot\text{mL}^{-1}$ 时会造成 100% 的死亡率^[67],并且这种毒性存在明显的剂量-效应关系^[72]。关于包括纳米银在内的金属纳米材料的毒性机制,目前尚没有一致的结论,讨论最多的是纳米材料通过释放高毒性的金属离子而造成细胞毒性^[73]。例如银纳米颗粒在潮湿有氧环境下可被氧化形成银离子,离子态银能破坏细胞膜结构进入细胞内环境进而造成细胞功能损伤。另一机制是纳米材料的表面修饰可以影响纳米材料与细胞间的相互作用,从而造成细胞毒性^[74]。基于纳米材料本身特性的毒性机制报道也较多,如尺寸效应等。除纳米银外,其它典型的金属纳米材料包括纳米 TiO₂、纳米金颗粒、纳米 Fe₂O₃ 颗粒、纳米 CuO 颗粒等。纳米 TiO₂ 对孕鼠暴露可影响乳鼠的脑部发育^[75]。纳米金虽然被认为具有相对较高的生物安全性,但仍能通过蛋白结合

的方式进入各类细胞^[76-79].纳米 Fe_2O_3 可激发人肺上皮细胞活性氧化合物造成氧化胁迫^[80].纳米 CuO 能抑制人肺上皮细胞活性并造成 DNA 损伤^[81].

半导体纳米材料主要包括 CdSe 、 ZnS 、 CdTe 、 SiO_2 等. SiO_2 纳米颗粒暴露可造成细胞凋亡,但其作用机制不同于纳米 TiO_2 等金属纳米材料和单壁碳纳米管等碳纳米材料.有研究指出纳米 SiO_2 造成细胞凋亡是由溶酶体失稳引发的,也有学者认为线粒体膜结构完整性的破坏才是纳米 SiO_2 造成细胞死亡的主要原因,更有大量研究表明 ROS 过量生成在 SiO_2 造成的细胞凋亡过程中扮演着重要的角色^[82-85].量子点的细胞毒性和纳米材料本身的性质也有很大关系,如果量子点外壳易被分解,则容易导致其内部包裹的重金属溶解并释放出离子,从而产生毒性,并且这种细胞毒性表现出明显的剂量效应.

2.2 动植物

在人工纳米材料生物学效应评价中,动植物是最常用的实验模型之一,主要包括浮游动物、鱼类、鼠类、鸟类、兔、蚯蚓等.根据生长环境,又可以分为水生和陆生两大类.不同的生物模型、暴露途径、纳米材料,在生物学效应评价中可能产生不同的结论.同样,纳米材料的生物可给性也因具体条件的变化而变化.在实验室内的模拟研究中,水体纳米材料的暴露模式相对单一,主要包括直接通过水体暴露和间接通过食物链暴露两大方式;而陆生生物的暴露方式则更多样化,以兔、鼠为例,有口服、滴灌、注射、吸入等多种给药方式.

2.2.1 水生生物

(1) 藻类

藻类作为一种常见的水生生物,在海洋和淡水水体中均有广泛分布,种类涵盖微生物界和植物界.藻类生物较为低级,形态结构简单,对外界刺激能产生较快的响应,易于观察,在水体研究中是很好的生物模型.一些藻类的数量就能直接指示水体污染状况,如蓝藻爆发的直接原因就是水体中含氮、磷、钾等元素的污染物超标.

在纳米毒理学研究中,基于藻类模型的文献报道也屡见不鲜,实验终点指标主要包括藻类的生长速度、存活率、 EC_{50} 等.例如衣藻 (*Chlamydomonas reinhardtii*) 经 $\geq 2.5 \text{ mg} \cdot \text{L}^{-1}$ 的聚酰胺胺 (PAMAM) 树状聚合物暴露 72 h 后,其细胞活性会明显降低^[86];绿藻 (*Dunaliella tertiolecta*) 经 $10 \text{ mg} \cdot \text{L}^{-1}$ 羧基化多壁碳纳米管暴露后出现明显的生长滞后和繁殖速度减缓^[87].对于同种藻类来说,不同纳米材料的生物可给性也不同.例如 Aruoja^[88] 等发现对月牙藻的毒性,纳米 ZnO > 纳米 CuO > 纳米 TiO_2 ; 且在 72h EC_{50} 的暴露浓度下,纳米 CuO 中具有生物可给性的铜占 25%,而常规尺寸 CuO 中具有生物可给性的铜仅为 0.18%.

(2) 水蚤类

水蚤类生物在自然条件下以颗粒物质为食,如酵母、藻类、细菌等都在其食用范围内,常被用作生物指示物评价水体中污染物的毒性^[89].正是因为这种特殊的捕食行为,在研究水体中纳米材料的行为时,蚤类也是一种很好的评价模型.目前,基于蚤类模型对纳米材料短期和长期效应的研究报道均有不少.Hund-Rinke 与 Simon^[90] 首次报道了 TiO_2 纳米颗粒对蚤类 (*Daphnia*) 运动产生抑制并引起相关的生物毒性,并且经光催化激发后 TiO_2 纳米颗粒能造成水蚤运动彻底停止.水蚤致死率也是用于纳米材料生物可给性评价的常用指标之一.富勒烯对水蚤的致死原因可能来自暴露过程中产生的氧化胁迫;纳米材料表面官能化修饰也可影响富勒烯对水蚤的毒性和致死率,如未修饰的富勒烯具有较大毒性,而氢化富勒烯则不会对水蚤产生明显的致死效应^[91].基于纳米材料对水蚤模型的半数致死浓度和半数有效浓度评估可以发现,纳米材料粒径越大,毒性越小^[92-94].

(3) 鱼类

在水体中鱼类是比较理想的实验模型,常见的有:斑马鱼、鲤鱼、黑头呆鱼、虹鳟、日本青鳉.鱼类摄取纳米材料的途径除了通过喂食方式以外,还可通过鳃丝微环境的物质交换来实现.另外,口、嗅球、眼等表面小孔也是纳米材料摄入的潜在途径.对纳米材料在鱼类中生物可给性的研究发现,溶剂可影响纳米材料的毒性和生物可利用性.例如 Zhu 等发现 $\text{H}_2\text{O}-n\text{C}_{60}$ 在黑头呆鱼模型中的 48 h LC_{50} 比 $\text{THF}-n\text{C}_{60}$ 高 40 倍以上^[95].现有研究显示不同纳米材料在不同鱼类模型中可表现出不同的生物学效应.纳米 ZnO 可造成斑马鱼的胚胎毒性,使孵化率降低、孵化速度减慢,从而影响胚胎发育^[96].纳米银颗粒暴露则会引发斑马鱼的肝毒性,造成 DNA 损伤,金属硫蛋白 2 (MT2) mRNA 表达增加,过氧化氢酶 (CAT) 和谷胱甘

肽 mRNA 表达减少^[97-101].在欧亚鲈鱼中,纳米银颗粒能引起呼吸胁迫^[102],但在日本青鳞中纳米银主要累积在肠、鳃与肝中,影响肝脏中乳腺脱氢酶和抗氧化酶的活性^[103-105].在金鲫模型中 nC_{60} 可导致慢性毒性,激发氧化胁迫并抑制生长^[106].

(4) 贝类

海岸线浅水域被认为是许多纳米材料典型的最终沉降点^[107],生长在这一水域的代表性贝类,如紫贻贝,浅海贝类等,是海洋生态研究的常见生物模型之一.纳米炭黑、富勒烯、纳米 TiO_2 与纳米 SiO_2 等均可对其产生不同程度的生物毒性,诱导氧自由基生成、破坏溶酶体膜的稳定性^[107].ZnO 纳米颗粒在牡蛎中同样可造成类似的氧化胁迫作用^[108],在暴露 24 h 内,纳米颗粒接触并首先进入牡蛎的呼吸器,48 h 后转而传递到其消化器官中,由纳米材料诱导产生的氧化胁迫可造成线粒体功能紊乱.纳米银颗粒暴露对牡蛎胚胎产生的生物效应与暴露浓度密切相关, $1.6 \mu g \cdot L^{-1}$ 纳米银暴露使牡蛎胚胎发育不正常比例高达 80% 以上,而纳米银浓度 $\leq 0.16 \mu g \cdot L^{-1}$ 暴露则对牡蛎无显著影响^[109].近期有关纳米银对牡蛎胚胎无明显生物效应的研究也有报道^[110].

2.2.2 陆生动物

陆生动物中也有不少理想的实验模型可被用于生物学、生态学、毒理学与环境科学等领域的研究.与水生生物相比,陆生动物更接近于人类生活模式,特别是哺乳类动物,在生理结构、呼吸方式与生活习性等方面都与人类存在高度的相似性.

(1) 鼠类

在基于陆生动物模型的纳米材料相关研究中,哺乳动物鼠类的使用频率最高,常见的品系包括 SD 大鼠、Wistar 大鼠、Lewis 大鼠、C57BL 小鼠、KM 小鼠等.大量研究从呼吸系统、神经系统、代谢系统等各个角度,利用鼠类模型建立了纳米材料的生物学效应分析方法体系,进而为其潜在的生态环境效应、人体健康影响及可能的医药应用价值评价提供科学参考.

纳米材料对鼠类模型的暴露途径主要有:口服、注射、吸入、经皮、滴鼻等,其中以口服、注射、吸入最为常见.在不同暴露模式下,相同的纳米材料可导致不同的生物富集行为.比如:①吸入方式给药最直接也最容易累积纳米材料的器官是肺部;②鼻腔内滴注的给药方式则可引起脑部靶向性的银累积;③注射给药可使纳米材料直接进入血液从而导致血液纳米材料含量的急速上升,然后经血液循环转移到全身脏器或组织中,其中肝脏为主要累积器官;④经口摄入的纳米材料进入生物体后首先接触消化系统,通过胃肠的吸收消化后才进入血液循环被转移到体内其它组织,故而会在胃壁、大肠壁、小肠壁出现纳米材料的高累积.吸收后的纳米材料首先会被运输至肝脏,因此肝脏同样是该暴露途径下的重要靶器官;⑤经皮暴露常出现于临床烧伤敷料等的情况下,纳米材料直接接触皮肤,除了直接累积在表皮组织中,还会通过毛细血管进入血液循环,从而产生类似于注射模式的纳米材料累积分布行为.相对而言,经皮方式摄入纳米材料的效率远低于注射方式,故在同等给药剂量条件下,其导致的体内纳米材料的累积量也远远低于注射方式.

纳米材料被吸收后在动物体内的分布、转移、滞留及代谢等一系列反映纳米材料生物可给性的行为与特定环境有关.就纳米材料在大鼠(小鼠)体内的分布而言,除了给药部位外,大部分脏器和组织也都具有明显的纳米材料或其所含元素的高累积^[111-114].各种纳米材料的生物分布因具体的生物环境及暴露方式而不同.例如纳米银颗粒经鼻腔内滴注给药暴露 4 周时,大鼠体内肝脏中的总银含量最高,而给药 12 周后大鼠体内肝脏中银含量极少,而脑组织中出现了银的高累积^[111],这说明暴露时间对纳米材料在动物体内的分布具有重要的影响.同样,纳米材料本身的属性如尺寸大小、包被种类等也能影响其在生物体内的分布^[115-116].

纳米材料进入生物体内后可发生不同程度的转移.例如经口服、经皮、吸入、滴鼻/眼等方式暴露的纳米材料,可以通过摄入器官进入血液,再通过血液循环分布到全身各器官或组织中^[117-120];而静脉注射的暴露方式则直接将纳米材料引入动物体内血液循环,从而产生更高效的次级器官累积或组织分布^[121].总之,在不同给药条件下同种纳米材料对生物体产生的效应不尽相同,所导致的生物累积和分布也存在着很大的差异,累积纳米材料的靶脏器(或组织)可明显不同,这表明不同暴露条件对纳米材料生物可给性的重要影响.

纳米材料本身种类不同,生物可给性也不同.Yokoyama^[122]等通过电子顺磁共振(EPR)成像的手段研究发现,氧化镍(NiO)纳米颗粒吸入暴露3 h可引起肺部功能的改变,暴露后2 d至2周内肺部功能没有明显恢复,C₆₀纳米材料的对比实验暴露则没有产生类似的效应.类似的比较研究发现,大鼠经吸入方式暴露于纳米银可引起肺部明显的炎症反应^[113, 123],而相似尺寸的纳米金造成的毒性效应并不明显^[124-125].纳米材料的表面修饰也会影响其生物分布.Owens和Peppas总结了聚合物纳米粒子的生物分布和药代动力学^[49],其中未经PEG修饰的裸露纳米颗粒通常集中累积在肝脏和脾脏中,可被单核巨噬细胞很快消除;而经PEG修饰的隐形纳米颗粒在动物体内的分布和在脏器中的消除速率则更复杂,这可能与纳米颗粒大小、噬菌素、PEG层包被等自身属性有关.

关于纳米材料在生物体内,尤其是高等生物体内的代谢过程的研究报道目前还较为少见.基于体外模拟研究的结果可以合理推测纳米材料在实际生物体中可能的转化和代谢过程.以纳米银材料为例^[126],在实验室模拟的生物环境中,纳米银颗粒在胃液中氧化溶解、硫醇结合交换加强,同时光致还原所得的二次零价纳米银也有所增多;另外纳米银表面和含硒物质的反应也很迅速,硒化产物又与生成的Ag₂S进行快速的硫交换.虽然这一系列反应是在体外模拟条件下发生的,但在一定程度上也可以为我们探究纳米材料在实际生物体中的生化反应以及代谢过程提供依据.

基于鼠类模型的纳米材料生物效应研究侧重点主要包括对呼吸系统、幼体发育和神经系统等的影响,常辅以相关细胞模型的细胞与分子水平的研究来解释其作用机制.对呼吸系统来说,由于纳米材料特殊的小尺寸,它们比普通尺寸和微尺寸的材料更能进入肺部深处,从而滞留更久、呼吸毒性更强^[127].Husain等研究发现^[127],纳米TiO₂经鼻给药暴露后,在监测的28 d内小鼠体内仍表现出剂量正相关的纳米颗粒的滞留,3000个基因高表达,其中包括几种炎症相关基因,相应的蛋白表达也有改变.近期Shinohara等发现^[128],经气管内滴注的多壁碳纳米管可对肺部产生长远的效应:在给药后长达364 d的观察检测中多壁碳纳米管在大鼠肺部内未见明显的消除,表明多壁碳纳米管在肺部可持久保留,不被消除.近年来纳米材料的发育毒性也受到了人们的关注^[129].Hougaard等研究发现,对孕鼠进行纳米TiO₂的吸入暴露可导致后代小鼠在旷场实验中减少中间地带的活动,并且后代雌性小鼠表现出增强的前脉冲抑制效应,这些实验结果证明纳米TiO₂的幼体发育毒性效应.金、银、碳、铁、硅等纳米材料对鼠类也可表现出类似的发育毒性作用^[129-134].在纳米材料神经毒性研究方面,人们发现^[135-136]纳米银经鼻给药两周后可对大鼠的空间认知能力产生损伤效应,这可能是暴露动物海马区域中ROS生成增加引起的.除纳米银外,碳纳米管^[137]、纳米ZnO^[138]、纳米TiO₂^[139]、纳米硒^[140]等纳米材料暴露均可产生一定的神经生物学效应.虽然人们在纳米材料神经效应方面开展了不少研究,但在这一领域的研究争议仍然很多.例如有研究显示,纳米银暴露对成年鼠海马神经发生和认知结果并不产生影响^[141].不同研究情景可能获得不同的实验结果或结论,这意味纳米材料生物效应的复杂性,只有深入全面探讨纳米生物学效应才能使人们对其生物安全性有充分清晰的认识.

(2) 其他陆生动物

除了常见的鼠类模型以外,蚯蚓、兔、鸟等陆生动物也被报道用于纳米材料生物可给性的研究.以兔子为动物模型的研究常用于探讨纳米材料在医学中的潜在应用,比如一些研究针对多种纳米材料探讨了其在体液中的药代动力学,在肿瘤、心肌缺血、防止尿道感染、转基因载体等方面的应用,分析了纳米滴剂对眼睛的毒副作用等^[142-147].鸟类动物模型中最常使用的是鸡,研究热点包括纳米材料对鸡的生长及免疫力的影响^[148],对鸡体内有害菌类的控制^[149],对微量元素吸收的影响^[150]等.总体来讲,与鼠类动物模型相比,兔、鸟类在纳米材料研究中的使用频率和适用研究范围都要小得多,其中又以鸟类模型的使用频率相对更低.然而在某些特定的研究中,兔、鸟类由于其生理结构的特殊性,也可能优于鼠类等动物模型.比如由于兔子的眼球较大,便于操作和观察,并可以进行两眼对照实验,因此适用于眼科研究,这在纳米材料相关研究中也有应用^[142].

3 人工纳米材料对人体的暴露

研究纳米材料在各种环境及生物体中的存在和生物效应,根本目的是在于更好地解释和探究其最终对人类可能产生的影响,而直接基于人体的统计学数据或结果则可更直观反映纳米材料的健康效应.

在实际生活中,纳米材料可通过不同模式暴露于人体,如经皮、注射、吸入等,随着纳米技术的高速发展,纳米材料在医药领域也越来越体现出其优势,纳米材料多以药物载体和创伤敷料形式出现.在癌症治疗中,纳米颗粒由于其特殊的尺寸效应,能够穿透肿瘤血管系统,引起实体瘤的高通透性和滞留效应(EPR),进而将目标药物直接有效地载入细胞^[151-152],由此可实现癌症治疗药物运输中的高效肿瘤靶向性,避免对正常细胞产生不必要的伤害^[151].另有研究发现^[153],尺寸为30—200 nm的纳米颗粒在肿瘤中有较高的摄入能力,尺寸较小的颗粒在肿瘤中具有更高的累积及扩散能力;在血液中小颗粒纳米材料比大颗粒纳米材料消除更缓慢;在肝脏中,噬菌细胞的摄入作用和肝的过滤作用会使10—20 nm的纳米颗粒迅速消除,20—150 nm的纳米颗粒消除也很明显;在脾脏中大于200 nm的纳米颗粒可以迅速被消除;在肾脏中小于8 nm的纳米颗粒亦可通过排泄过程被有效消除.纳米材料作为药剂或保健液在人体中的使用导致其在人体内的累积并产生效应的临床案例也屡有报道.例如,含银的滴眼液和化妆品可以使银盐沉积在患者的眼角膜中从而导致眼内银质沉着病^[154-155].

职业暴露是纳米材料对人体最直接也可能是最有害的暴露途径.工厂工作人员在纳米材料的生产制作过程中,可能通过粉尘、气溶胶^[156]等方式接触到纳米材料(尤其是纳米颗粒).吸入方式是纳米材料职业暴露的主要途径,近年来已受到职业健康和纳米材料生物效应研究领域越来越多的关注^[157-159],尤其是在东盟国家,例如马来西亚、印度尼西亚、菲律宾、新加坡、泰国、越南等,由于那些国家纳米材料生产工厂密集,纳米职业健康安全得到了越来越高的重视^[160].

在实际生活中,由于人工纳米材料在日用品中的广泛应用,人们在生活居所、办公室、餐饮等方面均可能暴露于纳米材料,因而这种低剂量长周期暴露可能对人体产生的潜在影响也日益受到关注.然而由于接触形式多样、影响因子复杂,因此很难对这种形式的纳米材料暴露的生物可给性进行客观评价,这也是当前纳米毒理与健康风险研究领域所面临的最大挑战.

4 影响纳米材料生物可给性的因素

纳米材料的生物可给性与纳米材料本身的性质是密不可分的,从纳米材料本身的形状、尺寸,到包被物质的化学特性以及表面电荷的属性,都可能对纳米材料在生物环境中的行为和效应产生影响.

4.1 形状

常见的纳米材料形状有球状、棒状、管状等.形状不同的同种类纳米材料可能具有不同的生物可给性.研究显示纳米材料的形状可直接影响其在细胞中的摄入:粒径小于100 nm的球状纳米金材料被细胞摄入的量大于棒状纳米材料,并且在此范围内棒状纳米金材料的纵横比越高,其被细胞摄入的能力越低^[161-162];而当纳米尺寸大于100 nm时,纳米金材料被细胞摄入的能力顺序为:棒状>球状>管状^[163].在医药应用研究方面,人们发现纳米材料的形状可直接影响药物的运输、吸收和药效^[164-166].例如,在小鼠体内碟状聚苯乙烯纳米颗粒的半衰期($t_{1/2}$ 约为1 h)比同尺寸的球状聚苯乙烯纳米颗粒的半减期($t_{1/2}$ 约为15 min)更长;对小鼠肺部内皮细胞胞内黏和分子-1(ICAM-1)的靶向定位效率也更高(>20倍)^[167];由于多价效应,管状纳米碳可在小鼠肿瘤中高效累积^[168];蜗状纳米氧化铁在肿瘤中有类似的高累积、高滞留效应^[169].由此可见,纳米材料的形状对其生物可给性具有重要影响.

4.2 尺寸

对于纳米材料,尤其是颗粒态纳米材料,尺寸对其生物可给性的影响非常重要.通常认为小尺寸纳米颗粒比大尺寸纳米颗粒有更高的生物可给性^[170-171].例如比较5 nm、10 nm与50 nm的纳米银进入细胞的行为发现,小颗粒纳米银更容易进入细胞,并可导致更高的细胞毒性^[172].分析纳米材料的消除行为显示,小尺寸纳米材料的消除速度比大尺寸的更慢^[173],这意味着小尺寸纳米材料一旦进入生物体产生累积,将较难排出体外.在非金属纳米材料研究方面,人们发现,聚乙二醇-聚乳酸(PEG-PLA)纳米颗粒在肿瘤模型中的累积与纳米颗粒的尺寸相关.利用裸鼠模型,Schadlich等发现,较小粒径的PEG-PLA纳米颗粒(111 nm和141 nm)能够有效地累积于人移植肿瘤中,而较大粒径的PEG-PLA纳米颗粒(166 nm)在到达肿瘤之前已被肝脏迅速消除^[174].SCK纳米颗粒也有尺寸依赖效应.Sun等发现,在小鼠体内小尺寸的SCK纳米颗粒(丙烯酸酯内核 24 ± 3 nm;聚苯乙烯内核 18 ± 4 nm)在血液中的滞留力比大尺寸的SCK纳米颗粒(丙烯酸酯内核 37 ± 4 nm;聚苯乙烯内核 37 ± 2 nm)更高^[175].同样在小鼠模型中聚

丙烯酸氨凝胶纳米颗粒、纳米硒等纳米材料也都表现出明显的尺寸效应^[176-177]。

4.3 表面修饰

纳米材料的表面修饰是另一个影响其生物可给性的重要因素。表面修饰可按照包被材料的种类与电性进行分类。纳米材料的包被物质种类很多,常见有柠檬酸钠、葡聚糖、聚乙烯吡咯烷酮(PVP)、聚乙二醇、聚丙烯酸、聚醚酰亚胺、牛血清蛋白等。

由于包被材料性质不同,纳米材料表面可带正电、负电或呈电中性,例如 PVP 包被的纳米材料通常呈电中性,柠檬酸钠包被的纳米材料呈电负性,而聚醚酰亚胺包被的纳米材料则呈正电性。关于纳米材料表面包被对其生物可给性的影响研究报道也较多。例如 Anderson 等发现,纳米银通过气管内滴注方式暴露,柠檬酸钠包被的纳米银颗粒在老鼠肺部组织的滞留能力高于 PVP 包被的同尺寸纳米银颗粒^[173]。这方面的发现,虽然有类似的研究报道^[14, 178],但也存在着不同的结果^[179],这可能与给药模式、实验动物模型与纳米材料本身性质不同等因子有关。针对带电性对纳米材料生物可给性的影响也有较多研究。例如 Long 等发现,带负电荷的纳米银颗粒可以通过激发血浆中 KKS 系统并干扰血管内皮细胞黏合连接蛋白表达而导致小鼠视网膜血管通透性增强,然而表面带正电荷和表面电中性的纳米银颗粒或者银离子却没有类似效应^[180]。在细菌毒性实验中,由于细菌表面带有负电荷,因此带正电荷的纳米材料可更有效地作用于菌体从而造成杀菌效应^[181]。

除了调控表面电性,纳米材料的包被也可直接影响纳米材料的生物行为。例如, Sun 等发现,经 PEG 修饰后, SCK 纳米颗粒(丙烯酸酯内核)在小鼠血液中的累积显著增高^[175]。通过不同的方法对纳米材料进行表面修饰,可改变纳米材料的性能。研究发现,相比于传统的配体交换法、交叉双分子层法、硅包被法等,交联涂层法能使纳米材料具有更高的胶体稳定性,尤其是聚丙烯酸酯形成的交联涂层^[182]。另外,人们也可通过控制表面修饰物质种类来调控纳米材料内核物质的释放率,从而实现不同的研究目的^[183]。由此可见,纳米材料表面修饰对其生物可给性与生物效应的发挥具有重要作用。

5 展望

近二十年来,针对纳米材料及纳米技术环境效应的探索已形成了初步的研究思路和框架。其中,生物可给性是评价纳米材料与生物体相互作用的重要指标之一,在纳米材料生物安全性评价中受到了人们越来越多的关注。虽然通过以往的研究,人们已经积累了一些研究经验与科学认识,但针对以下 4 个方面仍需开展进一步的研究工作。

(1) 揭示纳米材料在实际环境中真实暴露状况,客观评价其进入生物体的能力、途径以及暴露剂量。当前针对纳米材料的生物效应研究大多在实验室条件下开展,实验过程中所有条件都被精确控制;而实际环境中存在很多未知变量可影响纳米材料的生物可利用性与生物效应,因此探明这些未知影响因子对于客观评价纳米材料的暴露风险至关重要。

(2) 阐明纳米材料进入生物体后的赋存形态、迁移转化行为以及可能存在的毒副作用。纳米材料在生物体中的赋存形态与纳米材料的生物效应存在着密切联系,并且各赋存形态之间也存在动态转化过程。即便是同种纳米材料,也可能由于形态、聚集状态、分散体系等条件的差异而造成明显不同的生物学行为。因此,研究不同赋存形态的纳米材料进入生物体后可能出现的不同生物转化过程并由此评价引起的生物效应是纳米毒理学研究的重要方向。

(3) 由于不同动物存在种间差异,因此基于动物实验获得的研究结果在人体暴露与危害评价中的可靠性值得深入探究。不同种类生物在生理结构上的巨大差异,可能会导致纳米材料生物学效应评估出现截然相反的结果,因此基于某类动物模型获得的实验结果在推广应用评价时需要谨慎全面的对比分析。

(4) 改进发展新的纳米技术,进一步完善纳米材料的应用性能,同时降低纳米材料的负面效应,减少其对环境与人体健康的危害。在纳米材料的生产、运输、使用等过程中,不可避免地会产生纳米材料的释放与暴露,通过效应调控纳米材料合成等技术改进纳米材料制备工艺,可望降低或消除纳米材料无意释放对环境 and 人体造成的不利影响,确保其应用安全性,从而实现纳米技术利益最大化。

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