Cell Entry of One-Dimensional Nanomaterials

Xinghua Shi[1,2], Annette von dem Bussche[3], Robert H. Hurt[2,4], Agnes B. Kane[3,4], Huajian Gao[2,4]
[1]*State Key Laboratory of Nonlinear Mechanics, Institute of Mechanics, Chinese Academy of Sciences, Beijing 100190, China*
[2]*School of Engineering, Brown University, Providence, RI 02912, USA*
[3]*Department of Pathology and Laboratory Medicine, Brown University, Providence, RI 02912, USA*
[4]*Institute for Molecular and Nanoscale Innovation, Brown University, Providence, RI, 02912, USA*

Understanding the way cells interact with one-dimensional nanomaterials is important for creating safer biomedical diagnostics and therapies [1,2], and for regulating occupational and environmental exposure [3-6]. One-dimensional structures represent an important class of nanomaterials, whose cellular interactions are particularly complex and have been associated with the induction of inflammation, fibrosis, and malignant mesothelioma [3-6]. Incomplete or frustrated phagocytosis of long, rigid biopersistent asbestos fibers or carbon nanotubes is hypothesized to lead to prolonged generation of reactive oxygen species leading to release of inflammatory mediators, cell death [6,7] and to DNA and chromosomal damage in target cells of the lung and pleura [7,8]. DNA and chromosomal damage induced by asbestos fibers or carbon nanotubes may contribute to the development of lung cancer and malignant mesothelioma following inhalation [6,7].

Fiber-cell interaction begins with membrane contact, adhesion, and uptake. Biophysical models have been developed for endocytosis or phagocytosis [9-13]. Here we focus on one-dimension nanomaterials, *i.e.* those of very high aspect ratio, focusing on the important case of cylindrical symmetry which includes carbon nanotubes, asbestos fibers, and gold nanowires. Here we use *ex situ* field-emission SEM following fixation and contrast enhancement with osmium tetroxide to obtain nanoscale resolution. We focus on liver cells and mesothelial cells because they are important targets for carbon nanotube exposure following intravenous injection, or inhalation and translocation to the mesothelial lining of the pleural space surrounding the lungs.

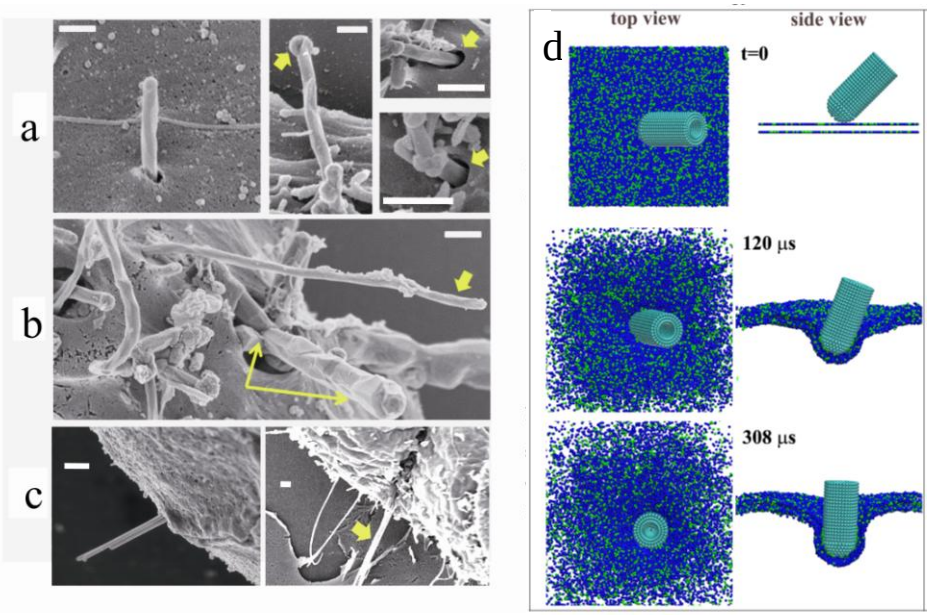

Figure 1 **a**, MWCNTs and murine liver cells. Left panel shows a MWCNT undergoing high-angle tip entry. Middle panel arrow shows carbon shell that distinguishes nanotubes from surface microvilli. Right panel arrows show membrane invaginations at the point of entry, characteristic of endocytosis. **b**, MWCNT and human mesothelial cells. Both an isolated tube (single arrow) and a tube bundle (double arrow) undergoing high-angle entry. **c**, Active tip-entry for other 1D materials: 30 nm gold nanowires (left); 500 nm crocidolite asbestos fiber (right). All images are field emission SEMs following fixation and osmium tetroxide staining. Scale bars are 300 nm. **d**, Time sequence of CGMD simulation results showing a MWCNT penetrating the cell membrane at an initial entry angle of θ_0=45 °.

Figure 1 shows electron micrographs of common morphologies in the near-membrane region following *in vitro* cell exposure to one-dimensional nanomaterials. Carbon nanotubes undergoing active entry are primarily found

protruding at high angle (1a,b) consistent with our preliminary polarized light confocal images. Panel 1a shows high-magnification images of the nanotubes at the point of entry showing invagination of the plasma membrane and the absence of a protruding phagocytic cup, which are characteristic morphological feature of endocytosis. An endocytic entry mechanism is not surprising since neither of these cell types is a professional phagocyte, and the MWCNT tips have diameters of 50 – 100 nm, which is within the favorable size range for receptor-mediated endocytosis of particles.

We became interested in the fundamental question of why tip-entry is the preferred mode of cellular uptake. Based on previous modeling of endocytosis for spheres, we hypothesized that nanotubes tips with closed, rounded caps can mimic particles and initiate endocytosis, and that elastic strain in the plasma membrane provides a driving force to rotate 1D nanostructures from their initial angle of contact to high angles that minimize total membrane elastic energy. To test this hypothesis, we began coarse-grained molecular dynamics (CGMD) simulations of receptor-mediated endocytosis of capped MWCNTs entering through a DPPC model lipid bilayer. The simulation includes a population of diffusible membrane-bound receptors. For the initial simulations, a capped MWCNT is initially positioned in close proximity above the surface of the bilayer that has 16326 lipids and receptors. The initial angle between the axis of the MWCNT and the bilayer is θ_0=45 °, and a range of receptor densities $\phi = 0.25$, are considered. As shown in Figure 1d, when *t*=0, the receptors (green) diffuse along the bilayer and aggregate around the MWCNT due to binding affinity. As receptors cluster and adhere to the MWCNT surface, the tube is pulled into the bilayer and wrapped. In this process the tube is observed to rotate to achieve an entry angle close to 90 °. The simulations reveal two competing kinetic processes: rotation of the tube toward a 90 ° entry angle to relax elastic energy in the membrane, and wrapping speeds on different sides of the tube governed by receptor diffusion. If the former prevails, as would be expected at relatively low receptor densities, the final entry angle will be close to 90 °.

The CGMD simulations suggest that a biophysical mechanism drives the tip entry pathway for cellular uptake of MWCNTs. The simulations further suggest that the steady-state entry angle is determined by the competition between speed of tip rotation and that of side wrapping during uptake. We note that the tip entry mode provides a natural physico-mechanical explanation for incomplete endocytosis or frustrated phagocytosis, which plays an important role in human health risk. Why do cells initiate uptake of one-dimensional structures that are in fact too long for complete internalization? In our model, receptor clustering at the rounded tube caps initiates uptake of what appears to be a particle, followed by a relatively rapid strain-induced transition to vertical or near-vertical fiber alignment. This vertical geometry provides no opportunity for the cell membrane to sense or anticipate the ultimate length of the fibrous target material. If the target fiber is too long for successful internalization and subsequent packaging in endosomes, then the initial wrapping and intake will lead inevitably to incomplete endocytosis. A mechanistic link between incomplete uptake of high aspect ratio nanoparticles and length-dependent toxicity has been proposed by Hamilton *et al* [14]. In macrophages exposed to TiO_2 nanorods, incomplete uptake of long nanorods (>15 um) resulted in failure to sequester nanorods within lysosomes. Subsequently, release of cathepsin B, a lysosomal protease, activates the NALP3 inflammasome and production of proinflammatory mediators. Disruption of the normal process of endosomal-lysosomal fusion has also been shown to lead to cell death by apoptosis following activation of caspase-1 by cathepsin B in macrophages following exposure to asbestos fibers. Incomplete endocytosis of high aspect ratio nanoparticles by liver cells can similarly lead to failure of sequestration into lysosomes with subsequent physical interference with cytoskeletal mediated processes including protein and lipid secretion and biliary transport.

References

[1] Kostarelos, K., Bianco, A. & Prato, M. Nature Nanotech. 4, 627-633 (2009).
[2] Cheung, W., Pontoriero, F., Taratula, O., Chen, A. M. & He, H. Adv. Drug. Deliv. Rev. 62, 633-649 (2010).
[3] Poland, C. A. et al. Nature Nanotech. 3 (7), 423-8 (2008).
[4] Donaldson, K., Murphy, F. A., Duffin, R. & Poland, C. A. Part.Fibre Toxicol.. 7, 5 (2010).
[5] Brown, D. M. et al. Carbon 45, 1743-1756 (2007).
[6] Sanchez, V. C., Pietruska, J. R., Miselis, N. R., Hurt, R. H. & Kane, A. B. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 5, 511-529 (2009).
[7] Nagai, H. & Toyokuni, S. Arch. Biochem. Biophys. 502, 1-7 (2010).
[8] MacCorkle, R. A., Sluttery, S. D., Nash, D. R. & Brinkley, B. R. Cell Motil. Cytoskeleton 63, 646-657 (2006).
[9] Gao, H. J., Shi, W. D. & Freund, L. B. Proc. Natl. Acad. Sci. U.S.A. 102, 9469-9474 (2005).
[10] Decuzzi, P. & Ferrari, M. Biophys. J. 94, 3790–3797 (2008).
[11] Chen, H., Langer, R. & Edwards, D. A. J. Colloid Interface Sci., 190, 118-133 (1997).
[12] Yang, K. & Ma Y. Q. Nature Nanotech. 5, 579-583 (2010).
[13] Zhang, S., Li, J., Lykotrafitis, G., Bao, G. & Suresh, S. Adv. Mater. 21, 419-424 (2009).
[14] Hamilton, R. F. Jr. et al. Part Fibre Toxicol. 6, 35 (2009).