Chapter 10
Role of Nanoparticles for Delivery of Genetic Material

Mariya V. Khodakovskaya and Mohamed H. Lahiani

Abstract Use of nano-sized materials as systems for delivery of genetic material into living cells is new and promising approach. Recent data showed that carbon-based, metal-based, composite nanoparticles and polymer nanoparticles have a potential to carry nucleic acids into plant cells. The unique ability of nanomaterials to penetrate plant cell wall and move inside the cell in fast manner can open ways for improvement of a number of transformation techniques including particle bombardment. However, experimental attempts to use nanomaterials as carriers of DNA/RNA *in planta* are rare. Here, we summarize the reports on successful delivery and integration of genetic material inside plants by using different classes of nanomaterials as delivery systems.

Keywords Genetic material · Nanodelivery · Mesoporous silica nanoparticle system · Multiwalled carbon nanotubes · Plant transformation

Nano-sized materials are unique particles with great potential uses as delivery systems. The different properties including their small size, low toxicity, and conjugation capabilities are unique features that facilitate the delivery of different biomolecules including nucleic acids and proteins into cells. The significant progress in gene delivery using nano-sized materials as nanocarriers was achieved in animal or human cells. For example, successful applications of different nanomaterials including nanoshells, fullerenes, carbon nanotubes, gold nanoparticles, and Fe_3O_4 used as nonviral siRNA delivery agents for cancer therapy were described and discussed (Singh 2013). The nanotechnological approach is beneficial for the area of gene therapy because it can reduce the high risk of infectivity due to the

M.V. Khodakovskaya (✉) · M.H. Lahiani
The University of Arkansas, At Little Rock 2801 S. University Ave, Little Rock, AR 72204, USA
e-mail: mvkhodakovsk@ualr.edu

M.V. Khodakovskaya
Institute of Biology and Soil Science, Far-Eastern Branch of Russian Academy of Sciences, 690022 Vladivostok, Russian Federation

C. Kole et al. (eds.), *Plant Nanotechnology*,
DOI 10.1007/978-3-319-42154-4_10

application of common viral vectors. The intracellular, paracellular, and transcellular pathways were described as major routes for uptake of nanoparticles by animal cells (Kumari et al. 2010; Murugan et al. 2015). It was shown that cylindrical one-dimensional nanomaterials, such as carbon nanotubes, can enter the animal cell by tip recognition through receptor binding with following endocytosis (Shi et al. 2011).

The existence of cell wall is making delivery of DNA and RNA into plant cells more difficult. Traditionally, the cell wall barrier can be overcome in plants by the removal of the cell wall (Ochatt 2013), infection by *Agrobacterium* (Křenek et al. 2015), or through particle bombardment of cells (Taylor and Fauquet 2002). The advantages of the use of nanomaterials for plant transformation were described by researchers led by Torney et al. (2007). They demonstrated that mesoporous silica nanoparticle system (MSNS) was capable of delivering DNA and chemicals into plant cells and plant leaves (Torney et al. 2007). They filled the MSNs with gene as well as with chemical inducer of the gene and the closed end of nanoparticles with gold nanoparticles (Au^{Capped}-MSNs, 3-nm pore size). Such unique construction allowed triggering the expression of a gene by supplied inducer using the process of uncapping. In the following paper, they claimed that it is possible to enhance biolistic delivery of MSNS by increasing the density of MSNS nanomaterials through the application of gold plating (Martin-Ortigosa et al. 2012a). Such an improvement led to the most effective introduction of MSNS into plant cells. Created MSNS was effective for mediated codelivery of protein and plasmid DNA into plant cells (Martin-Ortigosa et al. 2012b). Particularly, 10-nm pore-sized, gold-functionalized MSNSs were loaded with proteins, coated with plasmid DNA, and successfully introduced into plant cells using particle bombardment (Martin-Ortigosa et al. 2012b). Established method is a good foundation for some genomic manipulations/transformations for biotechnology. One of the most promising applications of MSNs nanosystem is genome editing that can be applied for generation of precisely modified "nontransgenic" plants. Thus, it was reported that Cre recombinase protein was delivered into maize (*Zea mays*) cells using MSNs as carrier (Martin-Ortigosa et al. 2014). Described delivery resulted in the removal of a *loxP*-defined DNA fragment from maize genome. Nanobiotechnological approach for the deletion of particular DNA fragment has some obvious advantages. For example, MSNs can be customized (tailored) for a particular enzyme that can be directed to the tissue through the biolistic method. Additionally, controlled release of protein can be achieved by capping of the pore opening of MSNs (Torney et al. 2007; Martin-Ortigosa et al. 2014).

Other types of nanomaterials (carbon-based and metal-based) also exhibited potential for use as carries of genetic material. The ability of single-walled carbon nanotubes (SWCNTs) to penetrate the plant cell wall and the plant cell membrane was documented by Liu et al. (2009). To investigate the ability of carbon nanotubes to deliver nucleic acids into the plant cells (tobacco, *Nicotiana tabacum*, cell culture), authors prepared SWCNT/DNA conjugates by noncovalent binding. Using fluorescein isothiocyanate (FITC) as fluorescence agent fused with SWCNT/DNA conjugates, they observed intracellular fluorescence for about 80 % exposed tobacco

cells. Such observation was a good evidence for the ability of SWCNTs carrying nucleic acids into the plant cell. Authors did not notice the presence of SWCNTs–DNA conjugates in plant nucleus. However, they experimentally proved the possibility of delivery of DNA by carbon nanotubes inside plant cell for the first time (Liu et al. 2009). The amazing ability of carbon-based nanomaterials to penetrate even very thick plant tissues was noticed in some research papers. For example, penetration of seed coats of different crops by carbon nanotubes (MWCNTs) and single-walled carbon nanohorns (SWCNHs) was documented using Raman spectroscopy and transmission electron microscopy (TEM) (Khodakovskaya and Biris 2009; Lahiani et al. 2013, 2015). Khodakovskaya et al. (2012) also demonstrated the ability of multiwalled carbon nanotubes (MWCNTs) included in growth medium to penetrate the cell wall of tobacco cells using tobacco callus system. The uptake of MWCNTs by tobacco cells was confirmed by both Raman spectroscopy and TEM. It is important that MWCNTs did not play any negative role in cell culture growth even in highest used doses (100–500 ug/ml) (Khodakovskaya et al. 2012). On the contrary, MWCNTs induced cell division and cell proliferation and activated expression of several genes involved in cell division (*CycB*), cell wall extension (*NtLRX1*), and water transport (*NtPIP1*). They hypothesized that MWCNTs should be tested for ability to carry nucleic acids inside plant cells. It is interesting to note that uptake of MWCNTs can lead to changes in plant gene expression. Results of microarray analysis (*Affymetrix* platform) revealed that expression of a number of genes involved in cellular responses, stress responses, and water relations was affected by carbon-based nanomaterials in treated seeds and plants (Khodakovskaya et al. 2011; Lahiani et al. 2015). Thus, it is important to take into consideration all possible effects of carbon-based materials used as the DNA-delivery machine on plant genome and proteome.

Metal-based nanomaterials, such as gold nanoparticles, also may provide an attractive tool for delivery of proteins and genes to living organisms. Such nanoparticles are biocompatible and can be functionalized easily with different molecules. Nano-sized gold materials were widely used for nucleic acid delivery in animal systems (Ding et al. 2014). Martin-Ortigosa et al. (2012a) bombarded plant tissues (onion, *Allium cepa*, epidermis cells) with DNA-coated gold nanorods (NRs). Authors demonstrated improvement of delivery of DNA inside plant cell by use of NRs as DNA carries (Martin-Ortigosa et al. 2012a). It is logical to expect the appearance of new reports focused on the delivery of nucleic acids and chemicals inside plant cells using composite metal nanoparticles in near future. Recently, Nima et al. (2014) enhanced the growth of tobacco cell culture by successful delivery of growth regulator 2,4-D into cells using plasmonically active nanorods based on gold cores and silver shells. Used multiplex nanosystem (AuNR/Ag) not only worked as a carrier for growth regulator but also allowed detection of particles inside cells using high-sensitive SERS detection. Nima et al. (2014) have concluded that AuNR/Ag nanoparticles are excellent candidates for delivery of different molecules including nucleic acid into plant cells. To prove that suggestion, the appropriate experimental work has to be performed.

Polymer nanoparticles are another class of nano-sized materials with a potential to use as carriers for delivery of nucleic acids. The organic nature of polymer nanoparticles and the ability to overcome the use of traditional viral vectors for silencing are promising traits. Silva and coauthors used fluorescent conjugated polymer nanoparticles (CPNs) to carry siRNA into tobacco BY-2 protoplasts without observation of toxic effects (Silva et al. 2010). In this work, authors provided experimental evidence that polymeric nanoparticles can work as an alternative solution for gene knockout in plant cells. Thus, they were able to detect visually the uptake of siRNA fused with CNPs and demonstrate the effective knockdown of genes involved in tobacco cell wall biosynthesis (*NtCesA-1a*, *NtCesA-1b* genes).

Based on the available limited literature (Table 10.1), we can conclude that nano-sized materials are a promising tool for delivery genetic material inside plant cells. Wide range of nanomaterials should be tested for carrying nucleic acids to plant genome. Efficiency of existing plant transformation techniques can be increased using nanovehicles by more precise delivery of genetic material, ability to control gene expression through release of incorporated chemical inducer, and better detection of nano-delivered genetic material inside particular cell compartments.

Table 10.1 Examples of the use delivery active biomolecules to plants using nano-sized material systems

Nanomaterial used for delivery	Type of plant	Delivered molecules	References
Mesoporous nanoparticles system (MSNS)	Tobacco	DNA and chemicals	Torney et al. (2007)
Gold-plated mesoporous nanoparticles system	Maize	CRE recombinase protein	Martin-Ortigosa et al. (2014)
Single-walled carbon nanotubes (SWCNTs)	Tobacco protoplasts	DNA	Liu et al. (2009)
Gold nanorods (NRs)	Tobacco protoplasts	DNA	Silva et al. (2010)
Gold-functionalized silica nanoparticles (Au-MSN)	Onion epidermis tissue	DNA	Martin-Ortigosa et al. (2012a)
Gold–silica nanoparticle system (Au-MSN) Gold nanorods (NRs)	Onion epidermis tissue	DNA, proteins	Martin-Ortigosa et al. (2012b)
Nanorods with gold cores and silver shells (AuNR-Ag)	Tobacco callus	Growth regulator 2,4-D	Nima et al. (2014)
Polymer nanoparticles (CPNs)	Tobacco protoplasts	siRNA	Silva et al. (2010)

References

Ding Y, Jiang Z, Saha K, Kim CS, Kim ST, Landis RF, Rotello VM (2014) Gold nanoparticles for nucleic acid delivery. Mol Ther 22:1075–1083

Khodakovskaya MV, Biris AS (2009) Method of using carbon nanotubes to affect seed germination and plant growth. WO 2011059507 A1—patent application

Khodakovskaya MV, de Silva K, Biris AS, Dervishi E, Villagarcia H (2012) Carbon nanotubes induce growth enhancement of tobacco cells. ACS Nano 6:2128–2135

Khodakovskaya MV, de Silva K, Nedosekin DA, Dervishi E, Biris AS, Shashkov EV, Galanzha EI, Zharov VP (2011) Complex genetic, photothermal, and photoacoustic analysis of nanoparticle-plant interactions. Proc Natl Acad Sci USA 108:1028–1033

Křenek P, Šamajová O, Luptovčiak I, Doskočilová A, Komis G, Šamaj J (2015) Transient plant transformation mediated by *Agrobacterium tumefaciens*: principles, methods and applications. Biotechnol Adv 33(6 Pt 2):1024–1042

Kumari S, Swetha M, Mayor S (2010) Endocytosis unplugged: multiple ways to enter the cell. Cell Res 20:256–275

Lahiani MH, Dervishi E, Chen J, Nima Z, Gaume A, Biris AS, Khodakovskaya MV (2013) Impact of carbon nanotube exposure to seeds of valuable crops. ACS Appl Mater Interf 5:7965–7973

Lahiani MH, Chen J, Irin F, Puretzky AA, Green MJ, Khodakovskaya MV (2015) Interaction of carbon nanohorns with plants: uptake and biological effects. Carbon 81:607–619

Liu Q, Chen B, Wang Q, Shi X, Xiao Z, Lin J, Fang X (2009) Carbon nanotubes as molecular transporters for walled plant cells. Nano Lett 9:1007–1010

Martin-Ortigosa S, Valenstein JS, Lin VS, Trewyn BG, Wang K (2012a) Gold functionalized mesoporous silica nanoparticle mediated protein and DNA codelivery to plant cells via the biolistic method. Adv Funct Mater 22:3576–3582

Martin-Ortigosa S, Valenstein JS, Sun W, Moeller L, Fang N, Trewyn BG, Lin VS, Wang K (2012b) Parameters affecting the efficient delivery of mesoporous silica nanoparticle materials and gold nanorods into plant tissues by the biolistic method. small 8:413–422

Martin-Ortigosa S, Peterson DJ, Valenstein JS, Lin VS, Trewyn BG, Lyznik LA, Wang K (2014) Mesoporous silica nanoparticle-mediated intracellular cre protein delivery for maize genome editing via loxP site excision. Plant Physiol 164:537–547

Murugan K, Choonara YE, Kumar P, Bijukumar D, du Toit LC, Pillay V (2015) Parameters and characteristics governing cellular internalization and trans-barrier trafficking of nanostructures. Int J Nanomed 10:2191

Nima ZA, Lahiani MH, Watanabe F, Xu Y, Khodakovskaya MV, Biris AS (2014) Plasmonically active nanorods for delivery of bio-active agents and high-sensitivity SERS detection in planta. RSC Advances 4:64985–64993

Ochatt S (2013) Plant cell electrophysiology: applications in growth enhancement, somatic hybridisation and gene transfer. Biotechnol Adv 31:1237–1246

Shi X, von Dem Bussche A, Hurt RH, Kane AB, Gao H (2011) Cell entry of one-dimensional nanomaterials occurs by tip recognition and rotation. Nat Nanotechnol 6:714–719

Silva AT, Nguyen A, Ye C, Verchot J, Moon JH (2010) Conjugated polymer nanoparticles for effective siRNA delivery to tobacco BY-2 protoplasts. BMC Plant Biol 10:291–2229-10-291

Singh S (2013) Nanomaterials as non-viral siRNA delivery agents for cancer therapy. BioImpacts: BI 3:53

Taylor NJ, Fauquet CM (2002) Microparticle bombardment as a tool in plant science and agricultural biotechnology. DNA Cell Biol 21:963–977

Torney F, Trewyn BG, Lin V, Wang K (2007) Mesoporous silica nanoparticles deliver DNA and chemicals into plants. Nat Nanotechnol 2:295–300