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Biofunctionalized single-walled and multi-walled carbon nanotubes for genetic transformation of plants cells

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Carbon nanotubes (CNTs) are under precise investigation now as potential DNA transporters for the genetic transformation of plants [1]. In this study a simple eco-friendly ultrasound-mediated method for stable water dispersing singlewalled CNTs (SWNTs) and multi-walled CNTs (MWNTs) using such biomolecules as double-stranded DNA, dNTPs, BSA, vitreous humor extract proteins and sodium humate was proposed. Transmission electron microscopy revealed the shortening of CNTs (100 nm $- 5 \mu m$ vs. initial 2.5–20 μm) due to the functionalization procedure. Results of atomic-force microscopy demonstrated the increase in diameter of CNTs-based structures after functionalization obviously due to the presence of functionalizing molecules stacked onto the surface of CNTs. Raman spectroscopy results indicated the presence of CNTs structural changes caused by ultrasonication and external molecules stacking onto the nanotube surface. Biologically functionalized CNTs were used then for non-covalent attachment of plasmid DNA: the binary vector pGreen 0029 containing the gene sequence of membrane protein of protein-storage vacuole, fused to the reporter gene of yellow fluorescent protein (YFP) under control of 35S promoter and NOS terminator, as well as the kanamycin resistance selective marker gene nptII. Aseptic tobacco (Nicotiana tabacum L.) leaf mesophyll protoplasts with enzymatically digested cell walls were used to compare the efficiency of SWNTsand MWNTs-mediated foreign gene delivery into plant cells. Analysis of transient YFP expression in protoplasts revealed that efficiency of CNTs-mediated gene delivery was higher for SWNTs. This result may be due to differences in appearance of "one dimensional" structure effect between SWNTs and MWNTs. We assume that SWNTs are more capable to cross cell membranes lipid bilayer, while bulky MWNTs may stick in it or disrupt cell membrane, causing cell death.

 Serag M.F., Kaji N., Gaillard C., Okamoto Y., Terasaka K., Jabasini M., Tokeshi M., Mizukami H., Bianco A., Baba Y. Trafficking and subcellular localization of multiwalled carbon nanotubes in plant cells // ACS Nano.-2011.-5, N 1.-P. 493-499.