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Characterization of Nanocarrier Complexes with Plasmid DNA using SEM, TEM and AFM

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Transfection methods using nanomaterials have been studied intensively over the past several years as they show potential in biomedicine and therapeutic applications [1]. However, there are many barriers to the successful use of these new nanomaterials, the plant cell wall is especially troublesome. One technical hurdle is the development of an effective delivery system, in which the formation of nanocarrier complexes is the most critical factor.

There are many different nanocarriers for complexation, such as nanotubes and nanoparticles. Among these, rosette nanotubes (RNTs) have been brought to our attention since they are biocompatible nanomaterials generated from the self-assembly of a bio-inspired bicycle that features the hydrogen bonding arrays of guanine and cytosine. These RNTs, which can grow up to several micrometers in length, are stable nanocarriers with a hollow core of 11 Å. The dimensions and properties of these nanotubes can be tuned by modifying the building block or the functional groups attached to it [2-3].

In this study, biocompatible self-assembled RNTs functionalized with oligo-lysine side chains were used as cationic nanocarriers. Microscopy techniques, namely, scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM) were used to investigate the interaction between RNTs and plasmid DNAs under various conditions. The bio-science AFM, which uses fluorescent microscopy in conjunction with AFM, was used to identify the complexation.

For the SEM and TEM studies, samples were prepared by depositing a droplet of solution on a carbon-coated 400-mesh copper grid (Electron Microscopy Sciences), the excess solution was blotted after 10 s. The staining of samples for TEM was performed by depositing one droplet of a 2% uranyl acetate solution for 120 s. The grid was then blotted and dried. For tapping mode AFM imaging, samples are made by depositing 5µl solution on freshly cleaved mica, which were then air dried. For bio-science AFM imaging, 5µl samples were deposited in solution on a cover glass (0.17 mm thick-EMS) and air-dried. The sample surface was then rinsed with 500 ml MilliQ water.

SEM images were obtained without negative staining, at an accelerating voltage of 30 kV, 20 µA and a working distance of 5-8 mm on high resolution ultra-high resolution (0.4 nm) Hitachi S-5500 cold field emission SEM. TEM observation was carried out on JEOL 2200 FS TEM – 200 kV Schottky field emission instrument equipped with an in-column omega filter. Bright field TEM images were acquired using energy filtered zero loss beams (slit width 10ev). Tapping mode AFM images were obtained using a Digital Instruments/Veeco Instruments MultiMode Nanoscope IV AFM, equipped with an E scanner. For io-Science AFM, a combination of an inverted microscope (Nikon) and AFM (JPK NanoWizard II) were used with 2N/m, 70 kHz, tetrahedral tip.

Figure 1 shows that plasmid DNA binds strongly with RNTs and produce complexes. The details of complexation were imaged, as shown in **Figure 1b and c**. Especially, many free plasmid DNAs are visible outside of complexes in **Figure 1c**. The inset shows the periodic relationship between plasmid DNAs and RNTs, which may indicate that the plasmid DNAs follow the surface characteristic (i.e. surface charge density) of RNTs during complexation. **Figure 2** shows the complexes with fluorescein isothiocyanate (FITC) labelled RNTs with plasmid DNA. Comparing both fluorescent and AFM images reveals the presence of additional plasmid DNAs, which are not attached to the fluorescent nanocarriers.

In summary, the complexation of plasmid DNA and RNTs was investigated by different microscopic techniques. In addition, the complexes of FITC attached RNTs with plasmid DNAs were visualized successfully by Bio-Science AFM, showing the fluorescent and AFM images simultaneously. Complexation with different ratios and in-solution AFM will be performed with the goal to evaluate uptake efficiency of the complex into plant cells in the near future.

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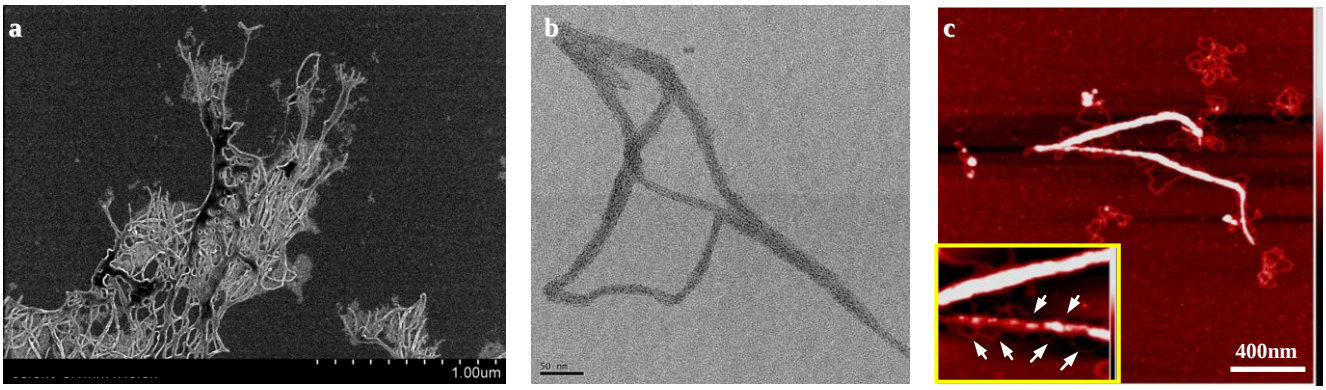

Figure 1. Microscopic images of complexation of plasmid DNA and RNT: (a) SEM, (b) TEM, (c) AFM. An inset shows the plasmid DNAs wrap the RNT periodically as indicated by arrows.

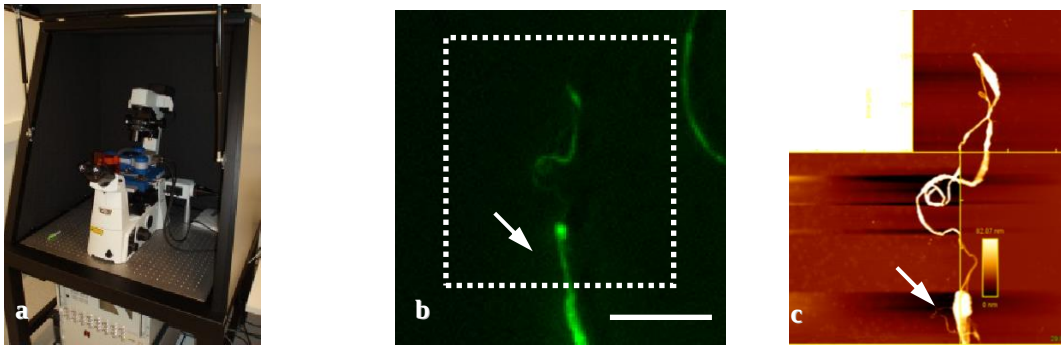

Figure 2. Bio-science AFM images: (a) JPK NanoWizard II (b) fluorescent image and (c) combined AFM image on the same area: the plasmid-DNA is only visible by AFM as indicated by white arrows. Scale bar is 20 um.