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Sequence Specific Subcellular Localization of TiO_2-DNA Nanocomposites

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We have synthesized functional nanocomposites consisting of titanium dioxide (TiO_2) nanoparticles covalently bound to DNA oligonucleotides. The TiO_2 nanoparticle is approximately 4.5 nm in diameter and retains photocatalytic properties when bound to DNA via dopamine[1]. The DNA attached to TiO_2 also maintains its ability to bind complimentary DNA sequences. Excitation of these nanocomposites by electromagnetic radiation (above 3.2 eV) leads to accumulation of electropositive holes in the bound DNA causing cleavage[1]. We hypothesize that these novel nanocomposites will be capable of targeting sequence specific aberrant and foreign DNA which cause human disease (oncogenes, retroviruses, and genomes of endoparasites). In the present study we used optical microscopy and X-ray fluorescence microscopy to show that it is possible to achieve directed subcellular localization of the TiO_2-DNA nanocomposites depending on the sequence of DNA bound to the nanoparticle.

Oligonucleotides complimentary to genomic DNA encoding 18S rRNA (ttccttggatgtggt) were synthesized to be 3' conjugated to fluorescent molecule tetramethylrhodamine (TAMRA). Such fluorescent oligonucleotides were bound to TiO_2 nanocomposites as described[1]. Human cells with 2n chromosomes contain approximately 300 copies of 18S rRNA gene which are located in the nucleolus[2], while polyploidy cancer cell lines may contain even more copies of this gene in, frequently, several nucleoli. Rat pheochromocytoma PC12 cells at 60-80% confluence were transfected (Superfect, Qiagen) with the TiO_2-R18S(TAMRA) nanocomposites according to the manufacturer's recommendations. The cells were then washed with PBS, scraped, and pelleted (2500rpm). The cell pellets were resuspened in 10-20µl of F12K media supplemented with 10% serum and seeded on formvar coated EM grids. Cells were allowed to attach to the EM grids for 2-3 hours and then fixed in cold 100% methanol at -20˚C. These cells were stained with Syto RNASelect green fluorescent cell stain to label the nucleolus (Molecular Probes S32703) and Hoechst (Molecular Probes, H3570). The cells were then visualized with the Zeiss LSM 510 Laser Scanning Confocal Microscope for the location of TAMRA labeled oligonucleotides. Upon completion of microscopy, the EM grids with the cells were washed from the mounting media by immersion in PBS for 10 minutes and then dehydrated in 100% ethanol for 10 minutes more. The same dried whole cells were then analyzed for the presence of titanium using the 2-ID-D X-ray beamline of the Advanced Photon Source at Argonne National Laboratory[1]. Figure 1 shows there is weak but detectable titanium X-ray fluorescence (Fig. 1B) that overlaps with the presence of fluorescent TAMRA signal in the cells transfected with TiO_2-R18S(TAMRA) (Fig.

1Aii). This overlap occurs in the nucleolus where 18S rRNA gene sequences are known to be located. Figure 1C shows that control cells, not transfected with the nanocomposites, show no detectable Ti signal. These data demonstrate: (1) that TiO_2-DNA nanocomposites can be targeted to their correct cellular location based on the specific sequence attached to the nanocomposite; (2) that fluorescent tags on the DNA sequence can be used to localize the nanocomposites in cells; and (3) that the overall stability of the nanocomposite is such that the DNA oligonucleotide and TiO_2 nanoparticle remain attached in the cell.

References

[1] T. Paunesku, et. al., Nat. Mat., 2 (2003) 343.
[2] W. Makalowski, Acta Bioch. Pol., 10(3) (2003) 587.
[3] This work was supported by NIH grants EB002100, CA81375, CA119341, CA89018

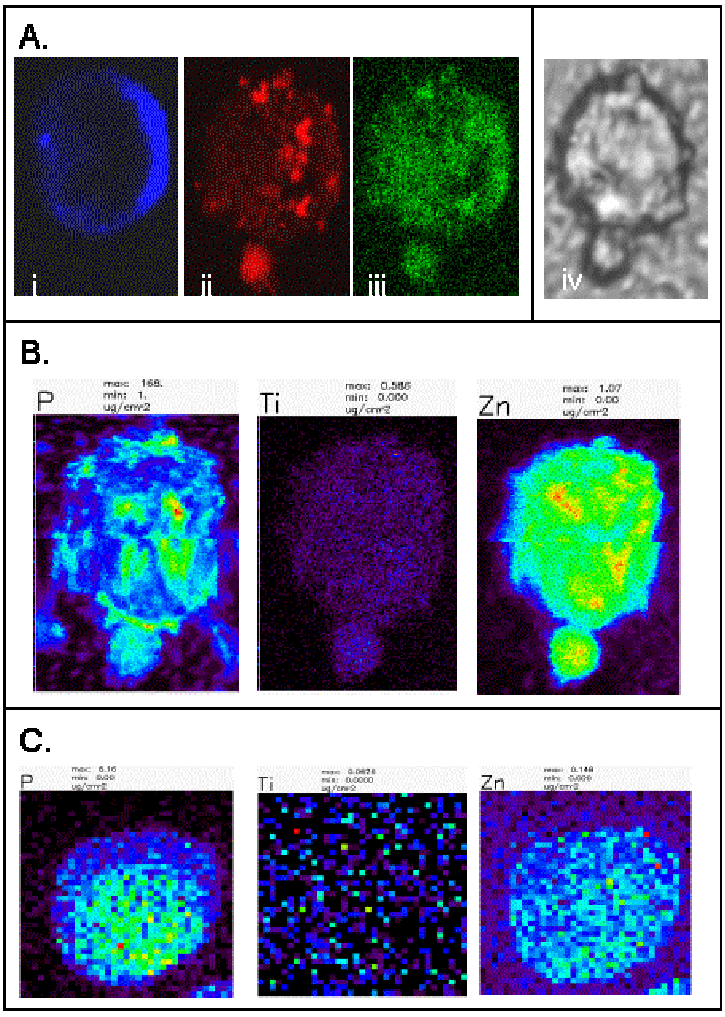

Figure 1. PC12 cells were transfected with TiO_2-DNA nanocomposite containing fluorescently (TAMRA) labeled oligonucleotides complimentary to DNA encoding 18S rRNA.
A. Fluorescent confocal microscopy detecting cell nucleus by staining with Hoechst(i), oligonucleotide labeled by TAMRA(ii), and nucleolus by staining with Syto RNA select(iii). Shape of the dried whole cell prior to imaging by X-ray fluorescence(iv).
B. Elemental maps obtained by X-Ray Fluorescence Microscopy showing elemental distribution of phosphorus(left), titanium(middle), and zinc(right).
C. Elemental map obtained by X-Ray Fluorescence Microscopy of cell not transfected with TiO_2-R18S nanocomposite.

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