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Vital Gene and Organelle Targeting with TiO_2-Oligonucleotide Nanocomposites in *Leishmania donovani*

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Although at least one-tenth of the world's population is at risk for infection with the protozoan parasite *Leishmania*, there are no vaccines or prophylactic medicines against leishmaniasis and until recently few treatments existed beyond the toxic antimonial compounds developed mid-century. The advent of nanobiotechnology, molecular biology and parasite genome characterization facilitates design of DNA-based functional nanoparticles with novel, inducible properties. Metal oxide (TiO_2) nanoparticles coupled to DNA oligonucleotides are functional nanocomoposites with unusual characteristics relative to each component individually [1]. Within the nanocomposites DNA oligonucleotides retain base-pairing specificity, while the TiO_2 nanoparticles exhibit characteristic photoreactivity. Single stranded DNA oligonucleotides covalently attached to TiO_2 nanoparticles anneal/hybridize to target DNA with specificity dependent on the sequence of the oligonucleotide. These nanocomposites have been tested in mammalian cancer cells with positive results [1,2] and hold promise for parasite-specific drug therapies which are not toxic to the host. In the current experiments we employed scanning transmission electron microscopy (STEM) and electron probe x-ray microanalysis (EPXMA) of whole cells to begin validation of our central hypothesis that TiO_2-oligonucleotide nanocomposites specific for vital genes of *Leishmania* will reach the intracellular target DNA and be retained in the targeted cell region.

Sequences of the oligonucleotides attached to the nanoparticles were (a) for the kinetoplast-mitochondrion genome TACCATGAAACCCAACAAC (GenBank ID# AF87606) matching cytochrome oxidase subunit II (COII) gene present in *L. donovani* promastigotes and (b) for tubulin TGCCGTGCTCAAGGCAGAAC (GenBank ID# UO9612) matching the tubulin gene present in the nucleus of *L. donovani* promastigotes. TiO_2 nanoparticles (4.5 nm) were coated with glycidyl isopropyl ether and linked via dopamine to the respective 20-mer oligonucleotides [1]. The nanocomposites (50 μl) were introduced into axenically cultured promastigotes (~10^8 cells/ml) via electroporation and cells were returned to culture at 26C, ambient air. At selected intervals from 2 to 72 hours cells were rinsed in buffer at 4C, and an aliquot of whole cells was deposited on carbon-coated formvar grids for STEM/EPXMA. At least 5 fields containing approximately 20 cells each were examined at magnifications of 3000X to 10000X by STEM and EPXMA. Low magnification (3000X) EPXMA scans with ~20 cells per field were employed to identify cells exhibiting a Ti signal. Cells with spectra positive for Ti were then imaged at higher magnifications (10,000X) as previously described [3].

Figure 1 illustrates the identification of the Ti component of the TiO_2–dopamine-tubulin specific oligonucleotide nanocomposites to be localized intracellularly at 16 hours post-electroporation. In this cell no nanoparticles were detected in the area thought to be nucleus; the only nanoparticles found to be intracellular were in regions consistent with cytoplasm. Particles were identified intracellularly at this time point in about 5% of cells. This may have been caused by the fact that

tubulin specific nanoparticles were relatively old at the time we used them. Alternatively, perhaps we were unable to detect the Ti signal over the nucleus in the TiO_2–dopamine-tubulin treated cells because of the low copy number of the tubulin gene replicas and a correspondingly low number of the nanoparticles retained in the nucleus. Similarly cells treated with the TiO_2–dopamine-COII nanocomposites exhibited intracellular titanium signals, but in the whole cell preparations localization to the kinetoplast could not be discerned. Additional experiments employing thin-sectioning techniques and STEM/EPXMA for identification of subcellular organelles such as the kinetoplast will be required to prove that the Ti is intracellular and that the TiO_2-dopamine-oligonucleotide reaches its target organelle.

References

1. T. Paunesku et al., Nature Mat 2 (2003) 343.
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Figure 1. Whole mount preparations of *Leishmania donovani* promastigotes 16 hours post-electroporation with TiO_2-dopamine-tubulin nanocomposites. Box in (A) STEM, (B) continuum (mass-thickness), (C) and (E) titanium and (D) phosphorus maps encompasses area near flagellum (out of field at top of image) and flagellar pocket with particles whose appearance is consistent with sequestration within the cell; an energy dispersive x-ray spectrum from a particle in the boxed region shows peak for titanium. The particle in the circled region in (A), (D), and (E) appears to be on the outside of the cell. The smaller spherical bright regions in the phosphorus image (D) are acidocalcisomes, while the larger bright region filling the cell diameter is the nucleus. The 'speckled' appearance of the elemental images is x-ray 'noise' and does not indicate the presence of Ti, unless the associated x-ray spectrum contains a peak for Ti. Width of STEM (A), phosphorus (D) and lower Ti (E) images is ~5 µm; width of the continuum image (B) is ~2.5 µm; width of the upper titanium image (C) is ~1.7 µm.