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Efficiency studies of DNA-conjugation/hybridization to magnetic silica nanosphere system designed as probes for genetic mutation

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Abstract: Magnetic nanospheres of the core-shell type Fe_2O_3/SiO_2-$(CH_2)_3SH$ (80-100 nm) were prepared and conjugated to oligonucleotides designed to hybridize with complementary DNA for both the wild-type allele and the DF508 allele of the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene. The efficiency of conjugation (50%) and hybridization (100%) processes with synthetic and genomic DNA sequences was evaluated by TEM, DRIFTS, and optical density measurement.

The combination of magnetic nanoparticles (NP) with oligonucleotide probes has been explored as a potential tool for selective detection of genetic mutations [1]. The ideal mutation detection system should be sensitive, fast, of low cost, and, preferentially, not require PCR amplification. In this work, the composite conjugates of allele specific oligonucleotide bound to magnetic NP were tested as probes for an efficient recognition of synthetic DNA sequences and also genomic DNA of cystic fibrosis (CF) patients bearing or not the mutated allele of the cystic fibrosis gene.

Composites of the core-shell type based on magnetic nanoparticles covered with functionalized silica were prepared using colloidal dispersions of maghemite (7-10 nm), tetraethoxysilane, and mercaptopropyltrimethoxysilane. The composites Fe_2O_3/SiO_2-$(CH_2)_3SH$ were conjugated to oligonucleotides OH- (5'-$(CH_2)_6$-S-S-$(CH_2)_6$-PO_3-Oligonucleotide-3' which contain the specific probes for the normal allele or for the DF508 mutated allele of the *CFTR* gene. The composite to probe coupling occurred by the reaction of thiol groups present at both nanoparticle surface and at the 5'-end modified oligonucleotide. Synthetic sequences reproducing a specific region of the wild-type and the mutated alleles, as well as genomic DNA samples of CF patients were used to test the hybridization ability of these Fe_2O_3/SiO_2-$(CH_2)_3$S-S-oligo conjugates. Transmission electronic microscopy (TEM), UV-Visible spectrophotometry, FT-IR, Raman, and Photoacoustic spectroscopy were employed to evaluate the efficiency of probe-coupling and DNA hybridization.

The composites Fe_2O_3/SiO_2-$(CH_2)_3SH$ are silica nanospheres of 80-100 nm diameter containing single or multiple maghemite cores and presenting smooth and regular surfaces (Fig. 1a). After conjugation with oligonucleotide and subsequent hybridization with genomic DNA, the particles surface becomes very irregular, indicating that DNA molecules have deposited over them (Fig.1b). As shown in the DRIFTS spectra (Fig.2), the appearance of the 1700 cm^{-1} band from the carbonyl stretching mode of nitrogen bases confirms the DNA hybridization. The efficiency of the conjugation/hybridization process was estimated using optical density measurement, being 50% for probe-coupling reaction and 100% for hybridization. Further, the selectivity of the conjugates Fe_2O_3/SiO_2-$(CH_2)_3$S-S-oligo for wild-type or mutant template detection is now under investigation and will be discussed.

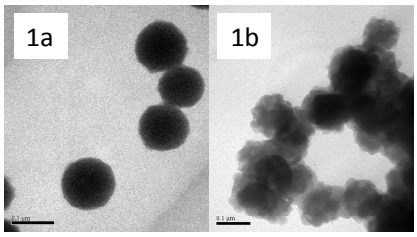

Figure 1: TEM image of composite nanospheres of Fe_2O_3/SiO_2-$(CH_2)_3SH$ before (a) and after (b) hybridization with genomic DNA.

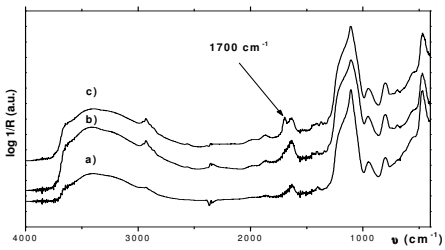

Figure 2: DRIFTS spectra of composite samples: a) Fe_2O_3/SiO_2-$(CH_2)_3SH$, b) Fe_2O_3/SiO_2-$(CH_2)_3$S-S-oligo and c) Fe_2O_3/SiO_2-$(CH_2)_3$S-S-oligo-DNA.

[1] Zhao X, Tapec-Dytioco, Wang K and Tan W 2003. Analytical Chemistry 75: 3476-3483.