Recent advances in hybrid nanotechnology involving nucleic acids are predominantly linked with sequence-specific nucleic acid interactions, and are oriented towards cellular imaging or DNA microarray development, as is the case with DNA oligonucleotides attached to quantum dots or gold nanoparticles (alone or with the addition of Raman-active dyes). DNA oligonucleotides are also used as scaffolds to direct the assembly of ordered patterns of nanocrystals, whereas other groups have used DNA attached to magnetic nanoparticle assemblies to act as a nanoswitch for the detection of DNA-cleaving agents. Nevertheless, there are few reports about the development of nanoparticles that can be used to manipulate biological materials in a novel way.

We have synthesized TiO$_2$–DNA nanocomposites as new vehicles for biotechnology that express new biochemical properties, in an attempt to develop them into nanodevices that would be able to enter cells and function in vivo and in situ. In this article we describe biological properties of these hybrid nanocomposites, some that result from the bioactivity of DNA oligonucleotides, and some that result from the unique and novel properties of combining DNA with TiO$_2$ nanoparticles into hybrid nanocomposites.

We synthesized TiO$_2$–oligonucleotide nanocomposites made of DNA oligonucleotides attached to 45-Å TiO$_2$ nanoparticles, and tested them in different ways to establish their potential to be developed into new tools for biotechnology.

Initially, we affirmed that TiO$_2$–oligonucleotide nanocomposites are able to hybridize with long DNA molecules. We annealed a nanocomposite with a 10 kilobase DNA fragment containing a complementary DNA sequence. The hybridization mixture was visualized by atomic force microscopy, which established that the nanocomposites do not clump and that they can withstand incubation at 95°C (which is necessary for annealing and also for polymerase chain reaction (PCR)) (Fig. 1).

We were able to introduce TiO$_2$–oligonucleotide nanocomposites into mammalian cells in vitro by using standard transfection methods. Because of the low contrast of nanoparticles, we mapped the location of titanium in cells by detecting titanium-specific K$_\alpha$ X-ray fluorescence.
induced at the 2-ID-E X-ray beamline at Sector 2 of the Advanced Photon Source at Argonne National Laboratory.

A total of 514 cultured cells from 24 different samples transfected (using SuperFect or electroporation) with seven different nanocomposites were inspected for the presence of a titanium signal. Depending on the type of the experiment, 20–50% of the cells accepted and retained titanium nanoparticles. The addition of free oligonucleotides generally increased the success of titanium nanocomposite transfection and retention.

To confirm that nanocomposites are able to enter the cell nucleus, we isolated nuclei from PC12 cells transfected with R18Ss–TiO2 nanocomposite, and a ‘free’ R18Sas oligonucleotide. The R18Ss–TiO2 oligonucleotide is complementary to the genomic DNA located in the nucleolar region of the nucleus. This so-called rDNA is transcribed into 18S ribosomal RNA.

Scans showing the presence of titanium in six out of 13 sampled nuclei demonstrated that the TiO2–DNA nanocomposites, once introduced into mammalian cells, reached the nucleus. The scan presented in Fig. 2 shows that signals of phosphorus and zinc overlapped with each other, and that the titanium signal shows the highest density in a circular subregion of the nucleus. By its size and shape, this nuclear subregion closely resembles the nucleolus—the subregion of the interphase nucleus where rDNA is located—and would therefore be the most likely nuclear location for retention of an R18Ss-oligonucleotide-activated TiO2 nanocomposite. Presumably, such retention would be dependent on hybridization/annealing of R18Ss–Ti nanocomposite with the genomic ribosomal 18S rDNA.

We hypothesized that oligonucleotide–TiO2 nanocomposites have the capacity for photoinduced endonuclease activity. Titanium dioxide is a wide-gap semiconductor, and absorption of light energy greater than the bandgap (3.2 eV) results in the promotion of electrons from the valence band to the conduction band of the TiO2 nanoparticle, leaving positively charged holes in the valence band. TiO2 nanoparticles have novel properties compared with ‘bulk’ titanium dioxide—nanocrystallite sizes smaller than 20 nm cause ‘corner defects’, leading to enhanced and selective reactivity of the nanoparticle with ortho-substituted bidentate ligands (such as dopamine). Rajh et al.10,11 showed that TiO2 nanocrystallites modified by the presence of organic modifiers exhibit semiconduction through both constituents (the metal oxide semiconductor and the organic modifier). In such a system, charge pairs are instantaneously separated into electropositive holes on the donating organic modifier and electrons in the conduction band of TiO2. Electropositive hole transfer along DNA is a well-established phenomenon, and the mechanism and dynamics of migration of electrons and electropositive holes (h+) across the DNA are explained by charge migration in a single-step ‘superexchange’ mechanism and by a multistep ‘hopping’ charge-transport mechanism12,13. In either case, we expected that illumination of TiO2–DNA nanocomposite should result in multiple charge-separation events, including electron trapping in the TiO2 nanoparticle and multiple transfer of the electropositive holes along the DNA, ultimately resulting in the cleavage of the attached DNA at the point where electropositive holes accumulate14. (It is worth noting that the rates of reactions leading to DNA cleavage—that is, reaction of guanine cation radical with water—are slower than charge recombination in contact ion pairs, which is a process protecting DNA from photochemical damage15). However, in TiO2 nanocomposites, TiO2 nanoparticles can trap multiple electrons,
and this leads to repeated hole creation and accumulation on the DNA molecule attached to the TiO2 nanoparticle.)

We annealed nanocomposites TiO2/30 sense and TiO2/50 sense, with radiolabelled complementary oligonucleotides. We used the fact that nanocomposites do not enter agarose or polyacrylamide gels during electrophoresis (data not shown), whereas the DNA that is cleaved away from the nanoparticle can enter the gel. To visualize the appearance of the cleaved DNA on the gel during electrophoresis, we radiolabelled the complementary DNA oligonucleotides used for annealing/hybridization with the cleaved DNA. Single-stranded labelled oligonucleotide and double-stranded DNA created by annealing migrate differently after polyacrylamide gel electrophoresis (PAGE). Therefore, it is possible to observe the frequency of cleavage of the DNA away from the TiO2–DNA nanocomposite as an increase in the quantity of the double-stranded DNA in the gel.

Results of a representative experiment are shown in Fig. 3. As indicated, the first three lanes are TiO2/30 and the next three are TiO2/50 reaction mixtures. In both cases, samples were separated into three parts and either left in the dark or illuminated for 8 or 16 min as indicated above the figure. Although the amount of single-stranded/not annealed oligonucleotide (TiO2/30 antisense and TiO2/50 antisense) is the same across the three lanes (bands parallel to symbol ^), the amounts of cleaved double-stranded DNA increased with an increase in the length of the illumination time (bands parallel to symbol *).

Not many studies have investigated the bioactivity of DNA bound to nanoparticles. One of a few examples affirms that DNA bound to nanogold (14-Å gold particles) behaves similarly to free DNA when manipulated by restriction and methylation enzymes15. In our studies, we show that DNA oligonucleotides covalently attached to TiO2 nanoparticles anneal/hybridize to target DNA with specificity dependent on the sequence of the oligonucleotide, and participate in PCR reactions. Subsequent illumination of the completed PCR products containing the TiO2 nanoparticles induced DNA strand breaks within a short distance from the TiO2.

We used standard PCR reactions, with the G11-Ti nanocomposite serving the role of one primer whereas the other primer was a 'free' oligonucleotide (PCNAas) (data not shown). After the reaction, PCR products were divided equally, and one half of each reaction was illuminated with white light for 2 min. PCR products from non-illuminated and illuminated portions were separated by agarose gel electrophoresis, blotted to Gene-Screen membranes and hybridized with a radiolabelled probe. As in the experiments establishing cleavage of oligonucleotides, DNA linked to a TiO2 nanoparticle did not migrate through the gel. Nevertheless, after illumination, the same PCR products (primed by the nanocomposite) were cleaved away from the TiO2 nanoparticle at a random short distance (up to 50 base pairs), and entered the gel forming a diffuse band slightly shorter than the band obtained by using only 'free' oligonucleotides as PCR primers in these reaction conditions. It is important to emphasize that the template molecules (which are not linked to TiO2 nanoparticles) did not degrade on illumination; this signifies that the break occurs only in those DNA molecules carrying the TiO2 nanoparticle (showing the necessity of the presence of stacked bases between the break and the nanoparticle).

From the oligonucleotide cleavage and PCR band cleavage data we conclude that the DNA within the nanocomposite dictates sequence-specific hybridizations with complementary DNA, and that it can participate in enzymatic reactions such as Taq-polymerase-mediated DNA synthesis (PCR). More importantly, we also learned that TiO2–oligonucleotide nanocomposites have a novel biochemical function—controlled photon-induced endonuclease activity.

In conclusion, any molecule synthesized to possess carboxyl groups (such as carboxy-DT-oligonucleotides and long DNA molecules, peptide nucleic acids, and short or long peptides and proteins) can be linked to dopamine and then stably attached to TiO2 nanoparticles 20–200 Å in size. The experiments shown here are limited to a single type of nanocomposite: DNA oligonucleotides that are attached one or a few at once to a TiO2 nanoparticle of 45 Å. Nevertheless, even these simple TiO2–oligonucleotide hybrid nanoparticles can perform many chemical and biological tasks, including a light-induced site-specific (within 50 nucleotides) nucleic acid endonuclease activity. Therefore, it is to be
hoped that these and other types of Tio2–biomolecule nanocomposite can be engineered with novel functional properties to serve as nanodevices for medical biotechnology.

METHODS

Colloidal TiO2 was prepared by dropwise addition of titanium(iv) tetraethyl orthoacetate to cooled water. The temperature and component mixing of reactants were controlled by an apparatus developed for automatic colloidal preparation.26 The concentration of TiO2 (typically 0.1–0.6 M) was determined from the change in the optical absorption after binding. Typically, the change in the concentration of the peroxide complex obtained by dissolving the colloid in concentrated H2SO4. The pH was adjusted to 3.5 with NaOH or HCl. Oxygen was removed by bubbling with argon or nitrogen, and TiO2 colloids were diluted to 0.015 M and mixed with 100 µl of glycidil isopropyl ether (which then coats the TiO2 nanoparticles) and helps preserve the biofunction of the oligonucleotides, preventing undesirable recombination of hydroxyl groups at the TiO2 surface with phosphodiesters of the oligonucleotides. With vigorous mixing, 0.2 M LiOH was rapidly injected into the TiO2 solution until a pH of 9.5 was reached. TiO2 nanoparticles of 45 Å (about 1.60 TiO2 molecules per particle) prepared in this way were dialysed against 10 mM NaH2PO4, until pH 6.5 was obtained. The size of the TiO2 nanoparticles was determined by transmission electron microscopy.26,27 (All the chemicals were reagent grade (Aldrich or Baker) and used without further purification.)

Oligonucleotides were synthesized with the 5′ terminal group (Oxone Co) and kept as a 10 µM solution in 10–40 mM phosphate buffer at pH 6.5. A condensation reaction through intermediate N-hydroxy-succinimide ester was used to bind the carbonyl group of the oligonucleotide to the amino group of dopamine by an amide bond. In the first step, the DNA terminal carbonyl group is bound to N-α-succinimidy1-NNNS-(N-methylthio)urethane bifunctional in the presence of NaN3 diisopropylethylamine in DMF. In the second step, the succinimidy1 group is replaced with dopamine through its terminal amino group in the presence of dichloroacetic acid. This solution was thoroughly dialysed against water to remove free dopamine unbound to oligonucleotides. The free dopamine concentration is typically 400 µM or more (for the nanocomposite particles), whereas the concentration of TiO2 was 15 mM.

In the final step, dopamine end-labelled oligonucleotides are bound to TiO2 particles modified by glycidil isopropyl ether. When dopamine (free or bound to oligonucleotides) is added into TiO2 colloidal solutions at pH 7–2.3, the immediate development of a red colour indicates instantaneous formation of a charge-transfer complex between dopamine and TiO2. The free energy of adsorption of dopamine to the TiO2 surface is about −7.6 kcal mol−1 (ref. 10). The extinction coefficient of absorption at 440 nm is bound to be 3.3×103 M−1 cm−1, at 520 nm 1.1×103 M−1 cm−1. Desorption of dopamine from the TiO2 surface has a different behaviour than adsorption. Hysteresis binding stabilizes the complex because of the chelating effect.20 The stability of the dopamine–surface TiO2 complex is greater than the stability of the complex formed between the TiO2 surface and glycidil isopropyl ether, and therefore dopamine easily replaces glycidil isopropyl ether from the surface.27 The stability of dopamine-modified TiO2 colloids is preserved after exposure to tens of 10–15 laser pulses and displays the several hours.

The final size of the nanocomposites depends on the length and number of oligonucleotides attached to each 45-Å TiO2 nanoparticle. The number of oligonucleotides bound to each particle was determined from the change in the optical absorption after binding. In this way, the concentration of dopamine attached to the TiO2 surface is about 7.6×10−3 mol cm−2 (ref. 10). The extinction coefficient of absorption at 440 nm is bound to be 3.3×103 M−1 cm−1, at 520 nm 1.1×103 M−1 cm−1. Desorption of dopamine from the TiO2 surface has a different behaviour than adsorption. Hysteresis binding stabilizes the complex because of the chelating effect.20 The stability of the dopamine–surface TiO2 complex is greater than the stability of the complex formed between the TiO2 surface and glycidil isopropyl ether, and therefore dopamine easily replaces glycidil isopropyl ether from the surface.27 The stability of dopamine-modified TiO2 colloids is preserved after exposure to tens of 10–15 laser pulses and displays the several hours.

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