Bioconjugate Chemistry

Communication

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Mild Two-Step Method to Construct DNA-Conjugated Silicon Nanoparticles: Scaffolds for the Detection of MicroRNA-21

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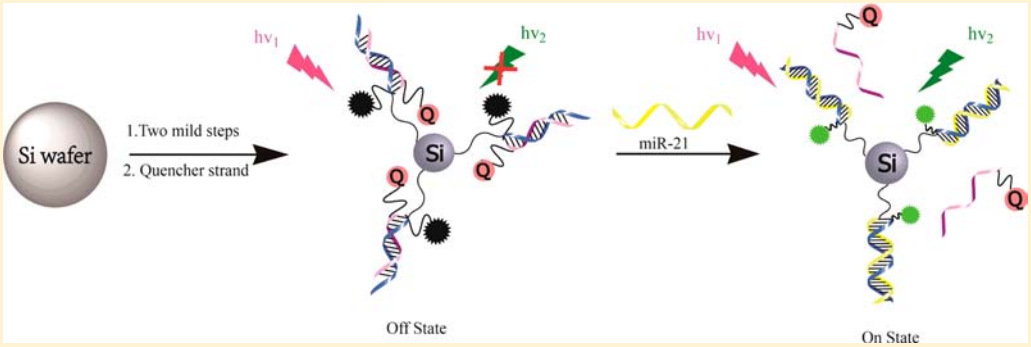
Supporting Information



ABSTRACT: We describe a novel two-step method, starting from bulk silicon wafers, to construct DNA conjugated silicon nanoparticles (SiNPs). This method first utilizes reactive high-energy ball milling (RHEBM) to obtain alkene grafted SiNPs. The alkene moieties are subsequently reacted with commercially available thiol-functionalized DNA via thiol−ene click chemistry to produce SiNP DNA conjugates wherein the DNA is attached through a covalent thioether bond. Further, to show the utility of this synthetic strategy, we illustrate how these SiNP ODN conjugates can detect cancer-associated miR-21 via a fluorescence ON strategy. Given that an array of biological molecules can be prepared with thiol termini and that SiNPs are biocompatible and biodegradable, we envision that this synthetic protocol will find utility in salient SiNP systems for potential therapeutic and diagnostic applications.

INTRODUCTION

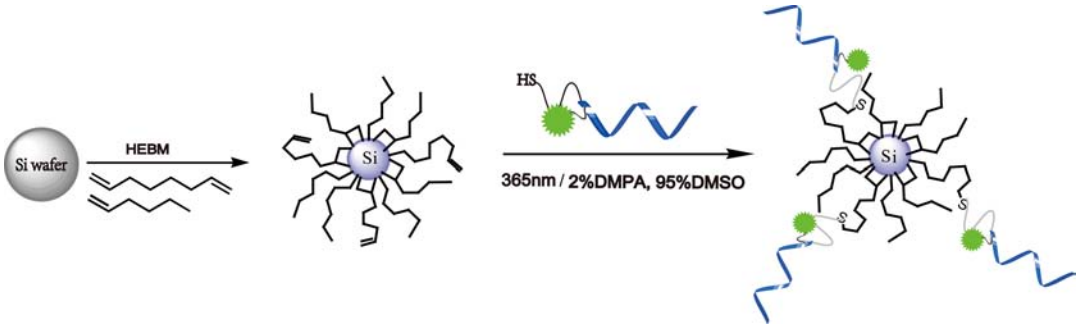
Spherical nucleic acids[1]—composed of a nanoparticle scaffold conjugated with a DNA shell—are currently being investigated as functional nanomaterials in applications ranging from *in vitro* biosensors to *in vivo* transfection, diagnostic, and theranostic agents.[2−7] The reason these hybrid materials are considered for use in such technologies is that they not only possess the unique biomolecular recognition properties of oligonucleotides (ODNs),[8] but often have emergent properties that are not present in free ODNs, such as increased binding affinity to target sequences,[9] enhanced nuclease resistance,[10,11] and entrance into cells without the need for ancilliary transfectants.[12] In terms of the core nanomaterial scaffold, a variety of heavy metal inorganic nanoparticles (e.g., Au, Ag, CdSe, Fe_3O_4)[13−16] have been explored with the goal of imparting additional physiochemical properties to the system (such as plasmonics, photoluminescence, scattering, and catalysis). Although these cores have shown demonstrated use in spherical nucleic acid systems, the potential toxicity and biodegradability issues of heavy metal inorganic particles remain a concern[17−20] and judicious passivation techniques are required.[21] In this regard, the construction of water-soluble, heavy-metal free, silicon nanoparticles (SiNPs) conjugated with DNA is highly attractive since silicon is well-established to be biocompatible,[22−24] biodegradable,[25,26] and earth-abundant, and can exhibit photoluminescence.[27]

A number of synthetic methods (including electrostatic interactions, postsynthesis linking, and automated solid-phase synthesis) have been explored to functionalize ODNs onto bulk silicon substrates.[28−30] In addition, methods have been established to obtain SiNPs.[31,32] However, the effective and site-selective conjugation of SiNPs with ODNs remains a formidable challenge since typical hydrogen- or halogen-terminated SiNPs are readily oxidized and are also prone toward nonselective nucleophilic attack.[33] In fact, literature on SiNP ODN conjugates is rare and the reported syntheses have involved either multiple synthetic steps[34,35] and/or harsh conditions (such as the use of high concentrations of HF,[36] bromine,[35] or laser ablation[37]). In addition to the paucity of synthetic methods to obtain SiNP based spherical nucleic acids, to the best of our knowledge, there has been no report on utilizing DNA conjugated SiNPs as functional systems. With

Received: August 27, 2014
Revised: September 20, 2014
Published: September 22, 2014

dx.doi.org/10.1021/bc5004026 | *Bioconjugate Chem.* 2014, 25, 1739−1743

Scheme 1. Straightforward Two-Step Synthesis for the Production of SiNP ODN Conjugates



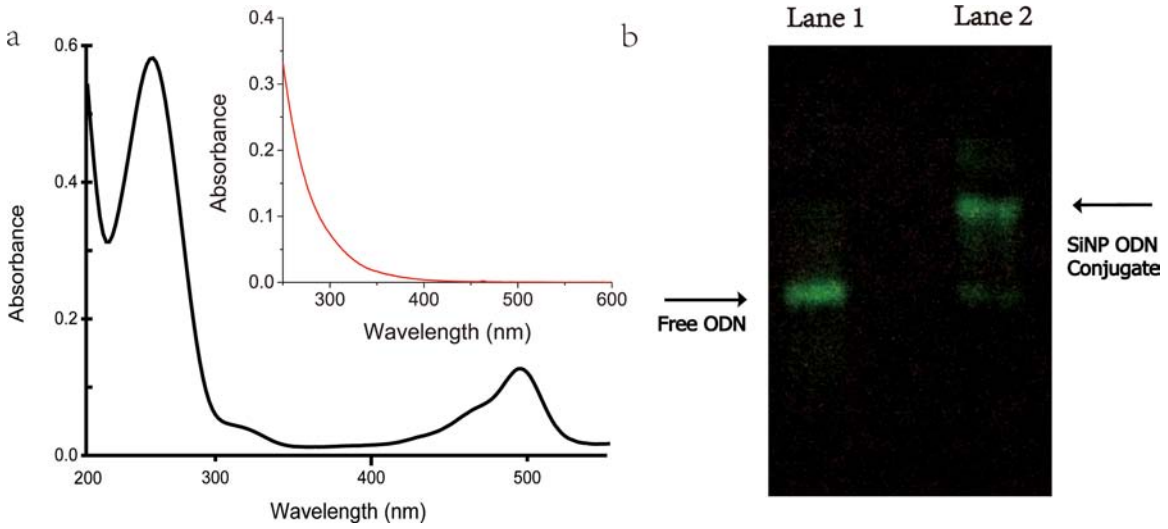


Figure 1. (a) Absorption spectra in H_2O of purified SiNP ODN conjugate (inset: Absorption in CH_2Cl_2 of SiNPs before bioconjugation). (b) PAGE of unconjugated ODN (Lane 1) and DNA-SiNP conjugates (Lane 2). The gel was run in 1 × TBE buffer and visualized via excitation (λ_{exc} = 254 nm) of the fluorescein unit on the ODNs.

this Communication, we first disclose a mild, two-step method, featuring reactive high-energy ball milling (RHEBM)[38] followed by thiol−ene click chemistry,[39] to prepare SiNP DNA conjugates from readily available silicon wafers. These silicon-based spherical nucleic acids have been characterized via a combination of microscopy (TEM and AFM), spectroscopy (UV−vis and fluorescence), and gel electrophoresis. Furthermore, we demonstrate the utility of these SiNP ODN conjugates by illustrating how these particles can be utilized to detect oncogenic microRNA-21 (miR-21) via a fluorescence ON strategy.[40]

The preparation of the SiNP ODN conjugates is illustrated in Scheme 1. First, RHEBM of silicon wafers in the presence of 1-hexene and 1,7-octadiene (~3:2 v/v) generated alkene terminated SiNPs. After removal of insoluble sediments via centrifugation, the resultant SiNPs were covalently functionalized with DNA by reacting an excess (110 equiv) of 3′-thiol modified 27mer ODN (5′-TCAACATCAGTCTGATAAGCT^{-Fl}AAAAAA-SH-3′)—that also contains a fluorescein (FL) unit as a spectroscopic handle—to the surface alkene moieties through the thiol−ene click reaction (initiated by 365 nm light in the presence of DMPA). The resultant SiNP ODN conjugates were purified via a 30k Amicon centrifugal filter to remove unreacted ODNs.

RESULTS AND DISCUSSION

The successful coupling of the ODNs to the SiNP was first inferred from UV−vis spectroscopy. As shown in Figure 1a, the purified SiNP ODN conjugate clearly shows absorption bands for both the ODN unit (λ_{max} = 260 nm) as well as the fluorescein reporter group (λ_{max} = 490 nm). Although the core SiNP does absorb in the 200−400 nm region (Figure 1a, inset), the extinction coefficient of the ODN is significantly higher (e.g., at 260 nm the free ODN has an ε of 3.33 × 10^5 L·mole^{-1}·cm^{-1} which is ca. 5.5-fold higher than that of the SiNP). Thus, using the absorption of the DNA at 260 nm in conjunction with the calculated concentration of the core SiNP, we estimated that 4−5 ODN strands are loaded onto each SiNP core.

A polyacrylamide gel electrophoresis (PAGE) study was performed to further confirm the production of SiNP ODN conjugates. As can be observed from Figure 1b, the major band in Lane 2 is a distinct green band (due to the emission from the fluorescein unit of the ODN) that runs slower than the unconjugated ODN (Lane 1), as would be expected for a nanoparticle containing multiple ODN conjugates.

Transmission electron microscopy (TEM) was first applied to characterize the morphology and size of the SiNP ODN conjugates. Shown in Figure 2 are images of SiNPs that are unconjugated (a: after step 1 of synthesis) and ODN conjugated (b: after step 2). The unconjugated SiNPs display spheroid particles with an average diameter of 3 nm. In

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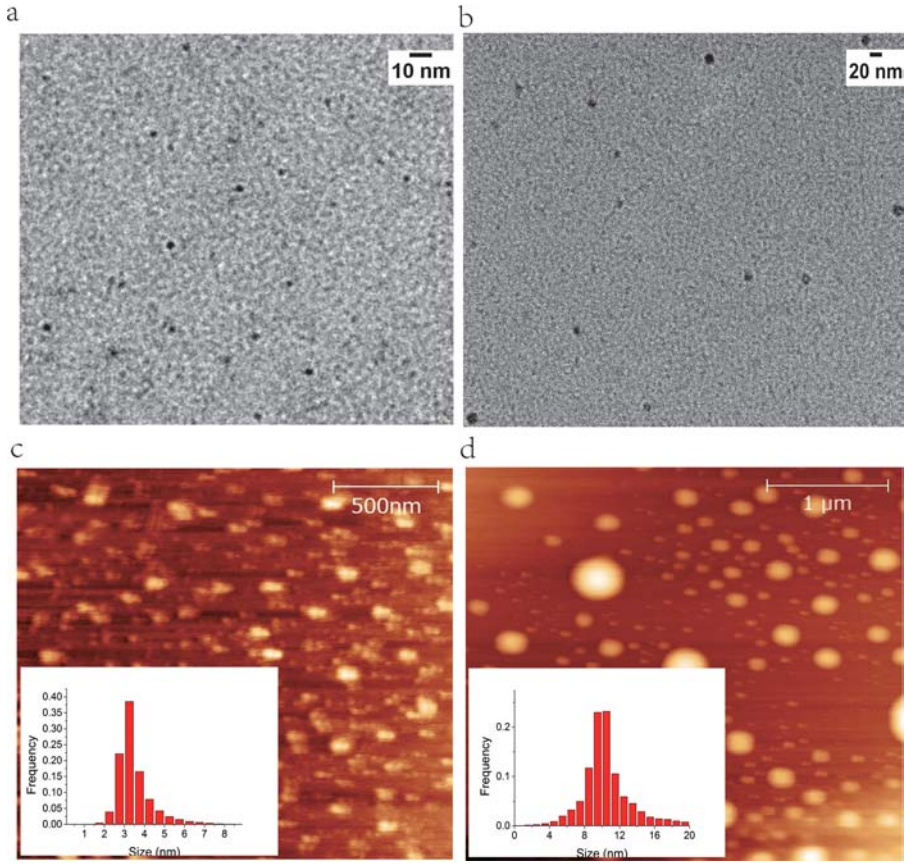

Figure 2. TEM and AFM images of the unconjugated (a and c, respectively) and the ODN conjugated (b and d, respectively) SiNPs. The inset within panels c and d display the height histogram from the AFM images.

contrast, the ODN conjugated SiNPs exhibit a significantly larger diameter (10 nm). The increase in nanoparticle size provides further evidence for the successful conjugation reaction. AFM measurements (Figure 2c,d), performed under tapping mode, gave additional information about the size and distribution of the SiNPs. These measurements are consistent with the TEM data and display an average height of 3 nm for the unconjugated SiNPs and 10 nm for the spherical nucleic acids.

With evidence in hand for the formation of SiNP ODN conjugates, we were keen on exploring the capacity of these silicon based spherical nucleic acids as sensing agents for biologically relevant RNA. As a proof-of concept, we focused on detecting miR-21 since this noncoding RNA is overexpressed in a variety of cancers, as it downregulates the production of tumor suppressor proteins.[41,42] In fact, due to its integral nature in cancer, sensing agents for miR-21 are an important are of research interest.[43,44]

Our miR-21 detection scheme is shown in Figure 3 and relies on a fluorescence ON strategy. While the core SiNP does fluoresce, the quantum yield of fluorescence is not substantial (2%) and thus we chose to use the fluorescein moiety on the conjugated ODNs as the reporter group. In stage 1, a 15-mer quencher strand (5′-Dabcyl-TAGCTTATCAGACTG-3′) hybridizes with the ODNs conjugated to the SiNP. Since the fluorophore and dark quencher are in proximity, a significant decrease in the fluorescence intensity is observed with a plateau at 1 equiv of the quencher strand (Figure 3b). This OFF state, which contains a 7 base toe-hold on the 5′ terminus of the SiNP ODN conjugate, transitions to a fluorescence ON state upon introduction of miR-21 which displaces the quencher strand (Figure 3c) since the conjugated ODN forms a more stable DNA:RNA duplex with miR-21. In contrast to the clear binding of the SiNP ODN conjugate to miR-21, which displays saturation behavior, when a negative control (miR-155) is added, the silicon based spherical nucleic acid system does not turn ON as the conjugated ODN on the SiNP is not complementary to miR-155.

■ CONCLUSION

In summary, we have disclosed a facile two-step synthesis—from bulk silicon wafer—to prepare SiNP ODN conjugates, by performing tandem RHEBM and thiol−ene click chemistry. In addition to characterizing the SiNP ODN conjugates by a series of spectroscopic and microscopic studies, we have for the first time demonstrated that SiNP DNA conjugates can serve as fluorescence ON sensors that detect oncogenic miR-21. Given that (a) SiNP cores have been found to have minimal toxicity and favorable biodegradable characteristics,[25,26] (b) these SiNPs are attached to ODNs via nonlabile thioether bonds, and (c) spherical nucleic acids with a variety of cores are known to transfect into cells,[1,12,45,46] we envision that these silicon based spherical nucleic acids may serve as potential diagnostic and/or therapeutic agents that can be used in cellular environments. We are currently exploring these possibilities. It is also important to note that, in general, many biological molecules can be functionalized with thiols (e.g., cysteine linked peptides and thiol terminated glycosides) and thus this simple two-step strategy may pave the way for the rapid investigation of a variety of SiNP bioconjugates for biomedical applications.

dx.doi.org/10.1021/bc5004026 | *Bioconjugate Chem.* 2014, 25, 1739−1743

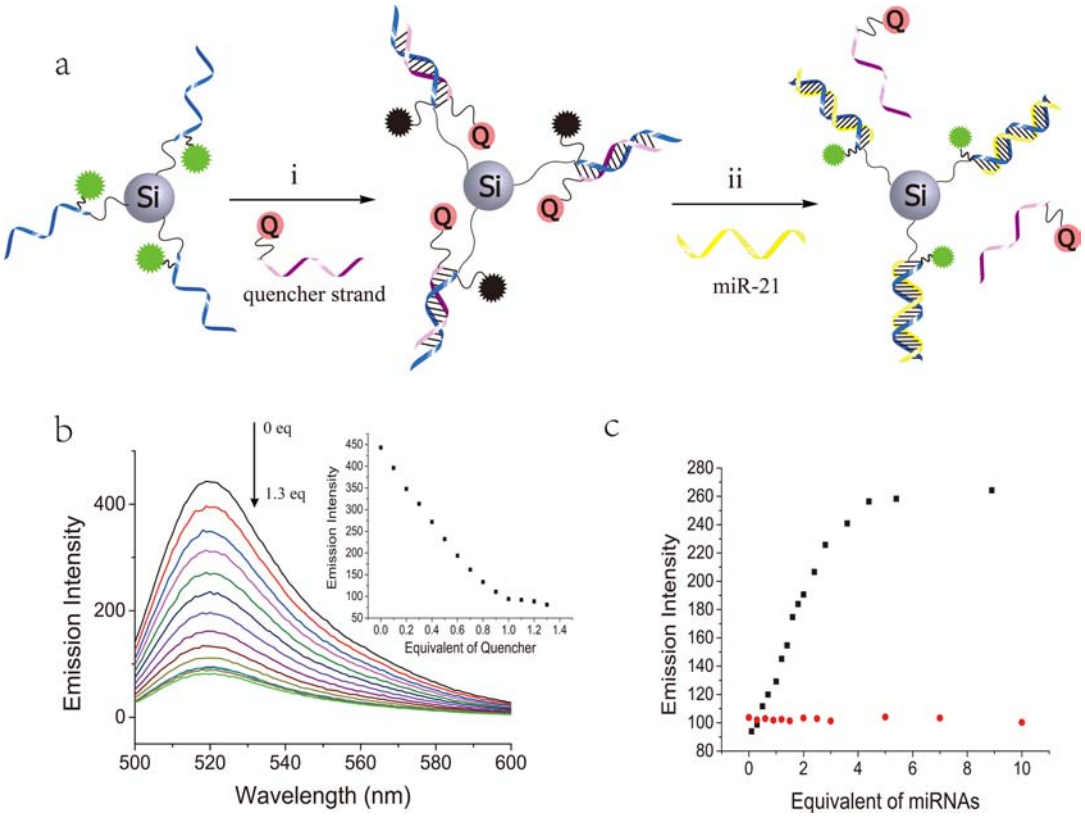

Figure 3. (a) Schematic illustrating the use of SiNP ODN conjugates to detect miR-21. (i) The quencher strand hybridizes with the SiNP ODN conjugate leading to a fluorescence OFF state. (ii) miR-21 binds to the SiNP ODN conjugate leading to displacement of the quencher strand, resulting in a fluorescence ON state. (b) Quenching of SiNP ODN conjugate upon addition of increasing equivalents of the quencher ODN. Inset: Fluorescence quenching profile with increasing equiv of quencher ODN, followed at 520 nm. (c) Fluorescence enhancement profile in the presence of increasing amounts of miR-21 (black) and control miR-155 (red). Note: For these fluorescence experiments the fluorescein unit on the SiNP ODN conjugates were excited at 490 nm and the concentration of conjugated ODNs was 500 nM.

■ ASSOCIATED CONTENT

Supporting Information

Detailed description of the experimental procedures and calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was partly funded by the NIH (R01GM097571 to J.J.) and the NSF (CMMI-0726943 to M.J.F. and B.S.M.).

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