Colloid Polym Sci 279:68–72 (2001)

SHORT COMMUNICATION

C. M. Niemeyer
B. Ceyhan
S. Gao
L. Chi
S. Peschel
U. Simon

Site-selective immobilization of gold nanoparticles functionalized with DNA oligomers

Received: 26 July 2000
Accepted: 5 October 2000

C. M. Niemeyer (✉) · B. Ceyhan
Universität Bremen, FB2 – UFT
Biotechnologie und Molekulare Genetik
Leobener Str., 28359 Bremen, Germany
e-mail: cmn@biotec.uni-bremen.de
Fax: + 49 (0421 218 7578)

S. Gao · L. Chi
Physikalisches Institut der Universität
Münster, Wilhelm-Klemm Str. 10
48149 Münster, Germany

S. Peschel · U. Simon
Institut für Anorganische Chemie der
RWTH Aachen, Prof.-Pirlet-Str. 1
52056 Aachen, Germany

Abstract The organization of metal and semiconductor nanoparticles to form micro- and nanostructured assemblies is currently of tremendous interest. This communication reports on the utilization of DNA molecules as positioning elements for generating microstructured surface architecture from gold nanoparticles. Citrate-passivated 40 nm gold colloids were modified by chemisorptive coupling with a 5′-thiol-derivatized DNA oligomer. The nucleic acid was used as a molecular handle for the specific immobilization on solid supports, previously functionalized with capture DNA oligomers, complementary to the nanoparticle-bound DNA. As a consequence of the enormous specificity of nucleic acid hybridization (DDI) allows, to site-specifically target the hybrid nanoparticles to microlocations which contain the complementary oligomers. The site-selectivity of the surface adsorption is demonstrated by immobilizing the gold colloids on a DNA microarray on a glass cover slide. Moreover, scanning force microscopy (SFM) analysis, used to characterize the intermediate steps of the DDI on a gold substrate, provided initial insights into the specificity and efficiency of this technique. The application of the DDI to fabricate complex colloidal micro- and nanostructures is anticipated.

Key words Gold nanoparticles · Nucleic acids · Nanostructures · Surface architecture · Self-assembly

Introduction

A central goal of molecular nanotechnology concerns the generation of nanometer-sized structural and functional elements by means of the ''bottom-up'' approach, in which small molecular building blocks self-assemble to form larger devices [1]. Currently, there is an increasing interest in the use of DNA as a construction material for the biomimetic synthesis of nanostructured materials [2, 3]. It has been demonstrated that DNA can be used to fabricate nanostructured scaffolds [4] as well as for the selective positioning of macromolecular components such as proteins [5, 6], and metal or semiconductor nanoclusters [7–9]. The organization of nanoclusters might be of considerable interest with respect to future applications in microelectronic devices [10, 11].

We have recently reported on the site-selective immobilization of proteins, functionalized with a short DNA oligonucleotide [5, 12]. The DNA moiety attached to the macromolecular component can be used as a selective handle for immobilizing said macromolecule on a surface, previously functionalized with complementary capture oligomers. Due to the unique specificity of Watson-Crick base pairing, DNA-directed immobilization (DDI), allows us to simultaneously immobilize many different DNA-tagged components using a DNA microarray as a matrix for immobilization. DNA microarrays, currently being intensively investigated

for applications in nucleic acid analyses and genome research, are solid supports, typically containing several hundreds to thousands of capture oligomers per square centimenter [13]. Since the DDI technique allows for the highly efficient, reversible and site-selective functionalization of laterally microstructured solid supports, this method is particularly suitable for the fabrication of reusable biochips and other miniaturized sensor devices containing biological macromolecules, enzymes, or immunoglobulins. Moreover, we had anticipated its application for generating inorganic surface architecture from DNA-functionalized metal or semiconductor materials [12]. In this communication, we report on the experimental demonstration of the DNA-directed immobilization of inorganic components, nanometer-sized gold colloids modified with 24-mer DNA oligomers. These hybrid materials were subjected to the DNA-directed immobilization, using capture oligomer-functionalized gold and glass surfaces. Scanning force microscopy (SFM) analysis of the intermediate steps of the DDI process on a gold substrate provided initial insight into the specificity and efficiency of this technique. Moreover, the immobilization of the hybrid colloids on a DNA microarray on a glass cover slide indicated that the surface adsorption proceeds with complete site selectivity.

Materials and methods

DNA-modified gold colloids

The 40 nm gold colloids were purchased from ICN Biochemicals, Eschwege. The average diameter of 34.2 ± 2.7 nm was determined by TEM analysis, and the concentration of the nanoparticle solution was determined by photometric analysis using an extinction coefficient of $\varepsilon_{520} = 4.7 \times 10^9\ M^{-1}\ cm^{-1}$, calculated from analogous data of Mirkin and coworkers [14]. DNA oligomers prepared by standard phosphoramidite synthesis were purchased from Interaktiva, Ulm. The sequence of the 5′-thiolated oligomer coupled with the gold nanoparticles is 5′-thiol-TCCTGTGTG AAATTGTTATCCGCT-3′ (sequence A). The coupling of the oligomers was carried out as described by others [15]. In brief, 100 μL of the oligonucleotide, 100 μM in a 20 mM Tris-Cl buffer, pH 7.3, were added to a 2 nM solution of the gold colloids in water. The mixture was incubated overnight, and the nanoparticles were separated from excess oligonucleotide by repetitive centrifugation, removal of the supernatant, and resuspension in the Tris-Cl buffer, containing 150 mM NaCl.

DNA-directed immobilization

The capture oligonucleotides used were 5′-X-AGCGGATAACAA TTTCACACAGGA-3′ (sequence cA), in which $X = (CH_2)_6$-SH for the capture oligomer used on the gold surface, and $X = (CH_2)_6$-NH_2 for the capture oligomer used on the aminopropyl-derivatized glass surface, described below. For control purposes, thiol- or amino-modified capture oligomer cB (5′-X-AACAGCTATGAC-CATGATTAC-3′) was used which is non-complementary to the nanoparticle-bound sequence A. For microarray detection, oligonucleotide probes labeled with the fluorophore Cy5 (Interactiva) were used. The sequence of probe Cy5-cA, is complementary to the nanoparticle-bound sequence A (see above) and binds to excess A-oligomers attached to the nanoparticles. The probe Cy5-B (5′-Cy5-GTAATCATGGTCATAGCTGTT-3′), is complementary to capture probe cB.

Atomically flat gold surfaces were prepared as follows. In the hot state glass slides are glued to a 20 nm thin gold film evaporated on a freshly cleaved mica sheet and cleaved afterwards at the interface between the gold layer and the mica sheet. The pure gold surface was visible by SFM showing typical flattened gold grains. The immobilization of the thiolated capture probe cA and the control oligomer cB was achieved by depositing a 7 μL drop of this oligomer on the gold substrate. The drop was incubated overnight in a humidity chamber to avoid evaporation, and the substrate was rinsed with deionized water. Excess binding sites of the surface were blocked with a solution containing mercaptohexanol. Subsequently, a 7 μL droplet of the DNA-modified gold nanoparticles, 1 nM in Tris-Cl buffer, was placed on the capture oligomer modified surface. The substrate was incubated for 2 hours in the humidity chamber and rinsed with water. The SFM inspection was carried out with a Digital Instrument, Dimension 3000 TM using Si cantilevers, Nanosensor TM. The SFM images were taken with instruments operating in high-amplitude dynamic mode with a home-made active feedback circuit [16], preventing the onset of intermittent contact (tapping). With this circuit, SFM can be run stably in the attractive interaction regime in air so that the interaction between the scanning tip and the sample is minimized.

Oligonucleotide microarrays were prepared on aminopropyl-silane modified glass cover slips, activated with phenyl diisothiocyante, as described by others [17]. About 5–20 nL of the amino-derivatized oligonucleotides (sequences cA and cB, 10 μM in Tris-Cl buffer) were deposited in quadrupoles on these glass supports using a home-made nanoliter dispensing device [12]. Immobililzaton of the DNA-modified gold nanoparticles was carried out by incubation of the array in a 0.1 nM particle solution in Tris-Cl buffer for two hours at room temperature. The array was washed and subsequently, the fluorophore-labeled probe Cy5-cA, 100 nM in Tris-Cl buffer, was incubated on the array to detect

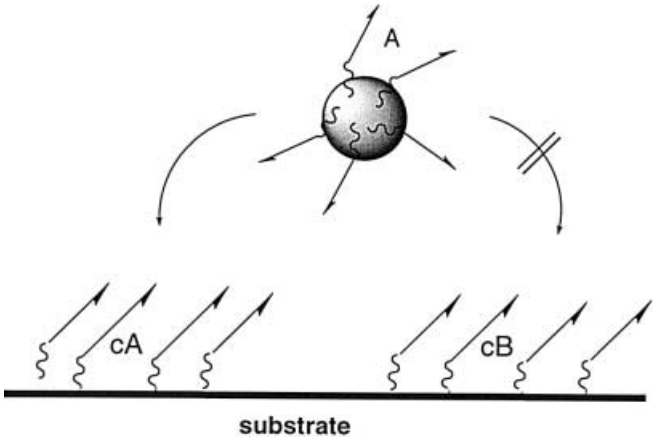

Fig. 1 Schematic drawing of the DNA-directed immobilization of gold nanoparticles, modified with DNA oligomer A. The hybrid colloids are incubated over a surface containing spots of capture oligonucleotides, cA and cB, complementary or non-complementary to the nanoparticle-bound oligomers, respectively. Hybridization of the complementary nucleic acids leads to the site-selective immobilization of the nanoparticles. For simplification, DNA strands are drawn as lines. The 3′-ends of the DNA oligomers are indicated by the arrowheads

nanoparticle immobilization. As a negative control the fluorophore-labeled probe Cy5-cA was incubated prior to nanoparticle immobilization, and as a positive control, probe Cy5-B was hybridized to the array subsequent to nanoparticle immobilization and regeneration of the surface.

Results and discussion

Immobilization on a gold substrate

To initially investigate the DNA-directed immobilization of the oligonucleotide-functionalized nanoparticles, adsorption experiments were carried out on a gold substrate, and the intermediate steps were analyzed by means of SFM. For this, flat gold substrates were functionalized with DNA capture probe cA, complementary to the nanoparticle-bound oligomer sequence A. For control purposes, the non-complementary capture probe cB was also immobilized in a different region of the substrate. The setup of this experiment is schematically depicted in Fig. 1. Both the untreated and DNA modified substrate were analyzed by SFM (Fig. 2). The images obtained show a high structural change when going from the clean gold surface to its modification with DNA. They indicate that the oligonucleotides adsorb densely packed on the flat gold grains leading to a smoothing of the substrate surface roughness. Part of the DNA is coiling up to spheric structures sized in the nanometer range. The DNA-modified gold nanoparticles, prepared as described in the experimental section, were incubated on the capture probe-modified gold substrate, and subsequent to hybridization, the surface was analyzed by SFM. The region functionalized with non-complementary capture oligomer cB contained almost no gold nanoparticles. On average, 0.4 colloids per square micrometer were measured. In contrast, the region of the substrate functionalized with complementary capture probe cA contained 5.9 colloids per square micrometer. This clearly indicates the specificity of the DDI process. As shown in Fig. 2, the nanoparticles were

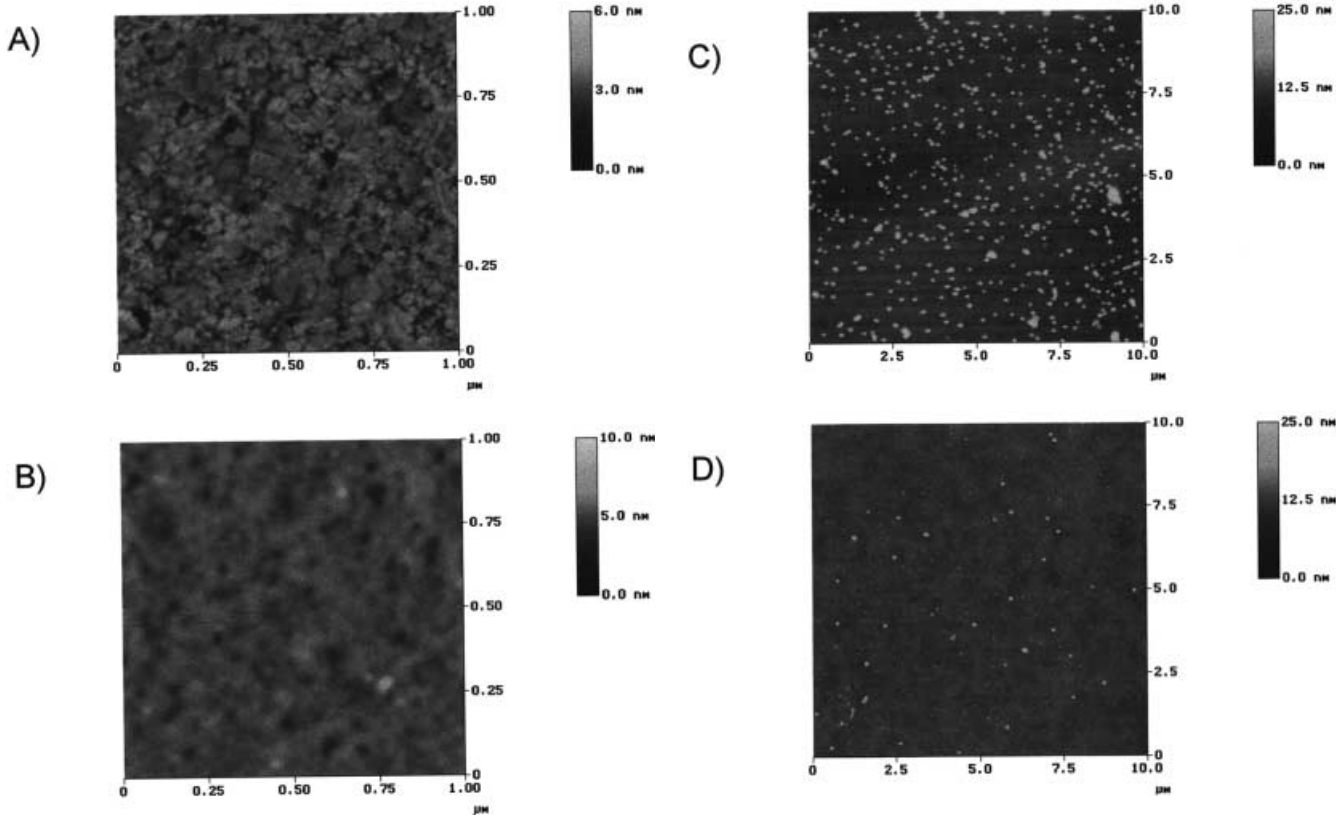

Fig. 2 SFM analysis of planar gold surface substrates, used for the DDI of oligonucleotide-modified gold nanoparticles. (*A*) Unmodified gold substrate. The terraces of the gold [111] are visible on the flat gold grains sized up to 100 nm in diameter. (*B*) Gold substrate subsequent to the chemisorption of thiolated capture oligonucleotide cA. Note that the typical grain structure of the pure gold surface has vanished and the oligonucleotides adsorbed in a densely packed manner smoothing the surface roughness. (*C*) Typical image of the substrate region modified with complementary capture oligomer cA, obtained subsequent to the DDI of gold nanoparticles. Note that the gold particles are lying directly on the oligonucleotide layer in a loosely packed but statistical distribution. Because of a tip effect the gold colloids are broadened up to 80 nm as seen in the SFM images. (*D*) Typical image of a substrate region modified with non-complementary capture oligomer cB, subsequent to the DDI of gold nanoparticles. Note that the occurrence of immobilized nanoparticles is scarce

loosely packed in the region containing probe cA, suggesting that either the surface coverage with capture probes is not homogeneously dense, or else the immobilization had not yet reached equilibrium. It is known that the chemisorption of thiolated oligonucleotides leads to surface coverages of about 10^{13} molecules per cm^2, corresponding to one DNA molecule per 10 nm^2 [18]. Thus, the incomplete packing of the nanocolloids is likely a consequence of the non-optimized experimental parameters, such as the nanoparticle concentration, temperature, ionic strength, and other hybridization conditions.

DNA microarray immobilization

To further establish the formation of micro- and nanostructured surface architecture, the oligonucleotide-modified gold colloids were subjected to the DNA-directed immobilization on a DNA microarray. This array, prepared by nanoliter dispensing of amino-derivatized DNA oligomers onto a chemically activated glass support, contained two types of capture oligomers. DNA cA is complementary to the nanoparticle-bound oligomers, while DNA cB is non-complementary. Since direct detection of the gold particles is not possible using the SFM, an indirect fluorescent analysis was chosen, using oligonucleotide probes labeled with the fluorophore Cy5 (see Fig. 3). The probe Cy5-cA is complementary to the nanoparticle bound oligomers of sequence A, and thus, Cy5-cA can be hybridized with excess A-oligomers that are present on the immobilized Au particles. To initially establish that probe Cy5-cA has no affinity to either of the two glass-bound capture oligomers, Cy5-cA was incubated over the array. Subsequent fluorescent imaging of the cover slide revealed no significant hybridization (Fig. 3A). However, some rather weak signals were observable in some spots, suggesting the occurrence of non-specific binding to a very low extent.

In the next step, the gold nanoparticles modified with oligonucleotide A were incubated over the array, and subsequently in a second step, the fluorescent probe Cy5-cA, which is complementary to the nanoparticle-bound oligomers, was hybridized to the immobilized materials. Fluorescent imaging analysis of the microarray revealed sharp intense signals at the microlocations in which the complementary capture oligomer cA had been attached to the glass surface. In contrast, the spots containing non-complementary sequence cB remained unlit. As an additional positive control, the surface was regenerated and, subsequently, fluorescent probe Cy5-B was hybridized to the microarray. Since this probe is complementary to the array-bound sequence cB, the spots of this capture oligomer are highlighted upon fluorescence analysis of the chip. These experiments clearly demonstrate the site-selectivity of the DDI method, occurring as a result of the high specificity of the Watson-Crick base pairing.

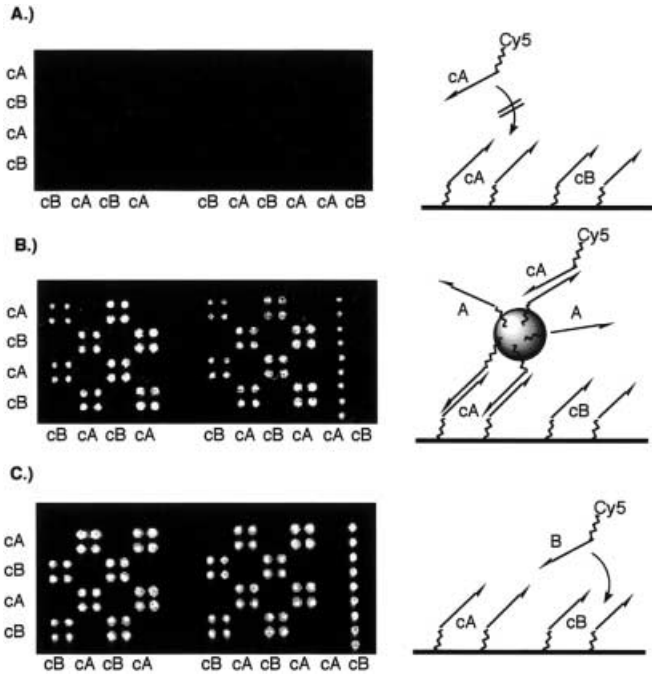

Fig. 3 Site-selective immobilization of the oligonucleotide-modified gold nanoparticles using a DNA microarray as an adsorption matrix. The DNA microarray contains two capture oligonucleotides, cA and cB, covalently attached to a glass support in various amounts. From left to right, 7 nL, 10 nL, 15 nL or 20 nL are deposited in quadrupoles. Capture oligomer cA is complementary to the nanoparticle-bound oligomers, while DNA cB is non-complementary. (*A*) The fluorescently labeled probe Cy5-cA which has the identical sequence as capture oligomer cA was initially hybridized to the array to establish that this probe does not bind to the two capture oligomers. Note that the lack of signals indicates the absence of non-specific binding. (*B*) Fluorescent image obtained from the DDI of the oligonucleotide-modified nanoparticles with subsequent hybridization of fluorescent probe Cy5-cA. (*C*) Positive control obtained from hybridization of fluorescent probe Cy5-B, which is complementary the surface attached capture oligomer cB. Note that the quadrupole pattern of spots is different from that obtained from nanoparticle hybridization. The original diameter of the spots is about 200 micrometers

Conclusions

We have demonstrated that the specificity of nucleic acid hybridization can be utilized to selectively target inorganic components towards distinct microlocations on a solid support. Due to the enormous recognition capabilities of short DNA molecules, the DNA-directed immobilization can be applied to the simultaneous adsorption of many different compounds, each of which is tagged with an individual oligonucleotide sequence [5, 12]. This approach can be extended to the use of longer nucleic acid capture oligomers, containing

various sequence stretches, thereby allowing to assemble molecular building blocks to a spatially defined, nanostructured aggregate of components [6, 19]. Thus, it will be possible to make laterally microstructured arrays comprised of numerous spots, each of which contains an individual type of nanostructured aggregate. Such arrays might be applicable to the combinatorial screening of nanomaterials for distinct cooperative material properties. Moreover, recent advances in the generation of one- and two-dimensional nanoarchitecture using the dip-pen nanolithography [20, 21] suggest that it will soon be possible to write dense patterns of various oligonucleotides, which might be used as a matrix for DDI-type functionalization with not only inorganic but also bioorganic components. This will eventually allow us to fabricate complex micro- and nanostructured surface architecture, potentially useful for making novel electrical and optical devices.

Acknowledgements This work was supported by Deutsche Forschungsgemeinschaft, SPP 1072, Fonds der Chemischen Industrie and Tönjes-Vagt Stiftung, Bremen. We thank R. Benters (Bremen) for technical assistance on the preparation of the glass-supported microarrays, U. Fischer (Münster) for help with the planar gold surfaces, and Prof. D. Blohm and Prof. H. Fuchs for generous support.

References

1. Whitesides GM, Mathias JP, Seto CT (1991) Science 254:1312
2. Seeman NC (1999) Trends Biotechnol 17:437
3. Niemeyer CM (2000) Curr Opin Chem Biol 4:609
4. Seeman NC (1998) Angew Chem Int Ed 37:3220
5. Niemeyer CM, Sano T, Smith CL, Cantor CR (1994) Nucl Acids Res 22:5530
6. Niemeyer CM, Bürger W, Peplies J (1998) Angew Chem Int Ed 37:2265
7. Mirkin CA, Letsinger RL, Mucic RC, Storhoff JJ (1996) Nature 382:607
8. Alivisatos AP, Johnsson KP, Peng X, Wilson TE, Loweth CJ, Bruchez Jr. MP, Schultz PG (1996) Nature 382:609
9. Storhoff JJ, Mirkin CA (1999) Chem Rev 99:1849
10. Simon U (1999) In: P Braunstein, CA Oro, PR Raithby (eds), Metal Clusters in Chemistry. Wiley-VCH, Weinheim, p 1342
11. Simon U, Schön G (1999) In: HS Nalwa (ed), Handbook of Nanostructured Materials and Nanotechnology. Academic Press, p 131
12. Niemeyer CM, Boldt L, Ceyhan B, Blohm D (1999) Anal Biochem 268: 54
13. Niemeyer CM, Blohm D (1999) Angew Chem Int Ed 38:2865
14. Mucic RC, Storhoff JJ, Mirkin CA, Letsinger RL (1998) J Am Chem Soc 120:12674
15. Storhoff JJ, Elghanian R, Mucic RC, Mirkin CA, Letsinger RL (1998) J Am Chem Soc 120:1959
16. Anczykowski B, Cleveland JP, Krger D, Elings V, Fuchs H (1998) Appl Phys A 66:885
17. Beier M, Hoheisel JD (1999) Nucleic Acids Res 27:1970
18. Steel AB, Herne TM, Tarlov MJ (1998) Anal Chem 70:4670
19. Loweth CJ, Caldwell WB, Peng X, Alivisatos AP, Schultz PG (1999) Angew Chem Int Ed 38:1808
20. Piner RD, Zhu J, Xu F, Hong S, Mirkin CA (1999) Science 283:661
21. Hong S, Zhu J, Mirkin CA (1999) Science 286:523