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Nucleic Acid-Mediated Gold Oxidation: Novel Biolithography for Surface Microfabrication and New Insight on Gold-Based Biomaterials

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Abstract

In nature, certain organisms can perform microbial corrosion on base metals by oxidation of neutral metallic atoms.¹ Herein we describe the first discovery of biological nucleic acids able to catalyze and mediate gold oxidation from neutral Au⁰ to trivalent Au(III) under certain oxidative environments provided by mild oxidizing reagent N-bromosuccinimide or amino acids. A new biolithography technique for gold patterning is further developed.

The development of novel applications based on nucleic acid such as nucleic acid nanotechnology,² self-assembly and templating synthesis of nanoparticles, biosensors, bioactive coating, and antennas³ is gaining increasing importance. In particular, the marriage between nucleic acid and gold has shown promising applications in a variety of fields such as biosensing and gene delivery.⁴ Two aspects having been specifically accounted in gold-based biotechnology are surface patterning of gold on the (sub)micron scale and understanding the interaction between gold and biomolecules. The former is important for the microfabrication of sensor arrays and electronics, and the latter could provide insights on sensing and biological activity evaluation of gold-based biomaterials.

For the first aspect, selective etching methods for gold have been developed.⁵ The two fundamental components of a gold etchant includes an oxidant to convert Au⁰ atoms to Au ions and a ligand that binds the oxidized Au ions to decrease its redox potential.⁶ Iodine,⁵ oxygen or hydrogen peroxide,^{7,8} Au³⁺-surfactant (sodium dodecylbenzenesulfonate (SDBS)⁹ or cetyltrimethylammonium bromide (CTAB)¹⁰), and Aqua Regia¹¹ are typical oxidants, and cyanide, thiourea, thiosulfate as well as thiosulfonate are frequently used ligands.^{6, 12} These selective etching solutions open up a new path for the micro/nano-fabrication of gold 1D–3D structures *e.g.* patterned gold layer,^{6, 12} nanoparticles,¹⁰ nanotubes,¹³ nanorods,¹⁴ and nanoplates.⁹ A number of important drawbacks exist in these conventional chemical etching baths, mainly including toxicity (*e.g.*, thiourea and cyanide) and stability (*e.g.*, thiosulfate).¹² For this reason, a new gold etchant system with extremely low toxicity and high durability is promising for practical industrial applications. Different from the conventional systems based on synthetical chemicals, this communication is the first report on biomolecules-based gold etching by the combinational use of nucleic acids

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and amino acids, providing a new bio-mimetic gold etching system to convert neutral Au⁰ to Au (III) ions. Recently, Liu et al¹⁵ found surface-adsorbed DNA regulated the etching of SiO₂ through tuning the concentration of surface-adsorbed water. The use of aqua-based biological molecules makes our method stand out as it is environmentally benign and highly durable.

For the second aspect, conventional opinions consider gold to have a superior chemical inertness, and to only show minute bio-dissolution when forming Au(I) that is partially oxidized to Au(III) in the presence of macrophages or amino acids of sweat and saliva.¹⁶ It has thus never been recognized that nucleic acids could be involved in the oxidation process from Au⁰ atom to Au(III). The present results suggest that, under certain oxidative environments provided by synthetic or biological substances, the coordination of nucleic acids with Au(III)¹⁷ has the potential to enhance a continuous dissolution of Au⁰.

The gold-coated substrate consisted of a top gold layer, 45 nm thick, and a middle Cr adhesion layer, 5 nm thick, on a 1-mm glass coverslip. After incubating this substrate in the aqueous solution containing specific amounts of DNA (dsDNA or ss DNA) and N-bromosuccinimide (NBS) at room temperature for overnight, the gold layer was found to be peeled off from the glass and to be subsequently diminished, thus forming a yellow dispersion (Figure 1B, C). Finally, this process resulted in the complete peeling-off of the gold layer, leaving only the underlying Cr adhesion layer on the glass (vide infra). Patterned gold macro- and micro-structures such as macro-porous gold film and gold micro-lines (Figure 1D, E) could be obtained with the assistance of a porous rubber pad or a microfluidic technique (Figure S1). Comparing with conventional gold etching system, the present DNA/NBS system had a lower etching speed, probably due to the diluted concentration of the reactants, and thus a slower interaction time of nucleic acid with Au(III).¹⁷ Further optimization with regard to reaction conditions such as the concentration, DNA structures, nucleic acid sequence could be applicable to enhance the etching speed.

It is further found that this newly found DNA-mediated etching could be similarly applicable in the oxidation environment different from that provided by NBS. Inspired by the *in vivo* oxidation of gold materials based on biothiols such as thiol-based amino acids, we infer that instead of NBS, the combinational use of amino acids and DNA would also produce notably oxidation on gold materials. Previous studies have shown that thiol-containing amino acids such as glutathione (GSH) only oxidizes neutral gold colloids in a very slow speed (over a month).¹⁸ In contrast, when we used the mixture solution of GSH and DNA to oxidize gold colloids, the oxidation precipitations typically found by mere use of GSH oxidation for a long period could be observed in a substantially shortened time (16~48hr). Microscopic UV spectra further revealed that the oxidation rate of GSH/DNA was accelerated largely (c.a. 5-fold higher) due to the catalysis and mediation ability of DNA (Figure 2). Similarly, the oxidation of gold film coating on glass substrate by GSH/DNA was also successfully conducted (Figure S2).

Control experiments involving the separate use of NBS or DNA did not give rise to etching on the gold surface. Oxygen did not play a key role in this process since similar experimental results for gold etching by NBS/DNA or GSH/DNA were also obtained under oxygen-free conditions (Figure S3). The pH value also did not affect the etching process substantially, because the initial pH values did not change obviously with the etching process proceeding, and the etching on gold nanoparticles by GSH/DNA at different initial pH values showed similar results (Figure S4). The catalysis role of pyridine unit in DNA as a kind of potential *t*-amines to accelerate the release of Br⁻ from NBS was not obvious in the present experimental window (0–48 hr), showing insufficient changes in the UV-vis spectra of NBS dissolved in the buffer with or without DNA (Figure S5).

We considered there was a cooperative role of these two compounds during the etching. We correlated the role of nucleic acid to its four kinds of nucleobases A, C, G, T (Figure 1A). Actually, it has been shown that nitrogen atoms in A, C, G, T nucleobases can bind with metal ions such as trivalent Au(III) for templating synthesis, self-assembly of metallic nanoparticle and photosensitization.^{17, 19} The initial oxidation of neutral gold atom to Au(III) was conducted by NBS²⁰ or biothiols¹⁸. For example, in NBS oxidative process, bromine molecules released from NBS could attack the gold atoms to form $[\text{AuBr}_4]^-$. We therefore postulate that in our system, Au(III) produced by NBS or biothiol oxidation could bind with DNA to decrease the redox potential, as a result, continuous dissolution of gold atoms is facilitated.

In order to support the above hypothesis, we replaced DNA with a model compound, pyridine to simplify DNA/NBS oxidation system for the mechanism investigation, since nucleic acids could be considered as chains carrying pyridine-derivatized nitrogenous heterocycles. As expected, when immersing a gold-coated glass in an aqueous solution of pyridine and NBS, a similar oxidative etching was observed but with a much faster etching speed. X-ray Photoelectron Spectroscopy (XPS) (Figure S6–S9) demonstrated that the Au4d and Au4f binding energy peaks at 335 and 546 eV completely disappeared after incubating the substrate in the etching solution for 10 min. Upon addition of pyridine to a freshly prepared gold colloidal solution, the UV absorption peak red-shifted to 570 nm from initial 520 nm due to a pyridine-induced aggregation,²¹ accompanied with the color changing from burgundy²⁰ to blue (Figure S10). When NBS was further sequentially added to this solution, the solution became colorless quickly and the corresponding electronic spectra showed zero absorption until 300 nm, reflecting the reaction was finished by forming a poorly water-soluble coordination complex $[\text{py}(\text{AuX}_3)]$ between pyridine (py) and AuX_4 ($\text{X}=\text{Cl}, \text{Br}$).²² This judgement was confirmed by a separate experiment where a diluted HAuX_4 ($\text{X}=\text{Cl}, \text{Br}$) solution was added in excess amount of pyridines. The characteristic peaks for HAuX_4 were gradually quenched (Figure S11–S12). When an excess amount of pyridine was mixed with a concentrated HAuBr_4 solution, a precipitation was visually collected that was quantitatively consistent with the molecular formula $[\text{py}(\text{AuBr}_3)]$ based on yield calculation, ¹HNMR (Figure S13) and molecular mass measurement. These findings indicated a general mechanism for pyridine-derivatives mediated gold oxidation as depicted in Scheme 1. The standard oxidation potentials for gold oxidation by NBS ($\text{Au}^0 + 4\text{Br}^- - 3\text{e}^- \rightarrow \text{AuBr}_4^-$) is -0.854 V .²⁰ This redox tendency is favourable for a spontaneous reaction between Br_2 and Au^0 to form AuL_4^- . By this reaction, the surface gold atom is firstly activated by the oxidizer (NBS or biothiol), and the resulting AuL_4^- effectively coordinates with surrounding pyridine-derivatized unit (py) to form $\text{Au}(\text{py})\text{L}_3$ and releases a free L anion. The binding constant of AuL_4^- with nucleobases is estimated as 10^5 – 10^6 M .²³ This energy-favourable reaction facilitates the existing redox equilibrium to shift more to the right side, catalysing and accelerating the oxidation reaction. Consequently, a continuous stripping of $[\text{AuL}_4]^-$ (L is a ligand) enriched on the oxidized gold surface and the subsequent exposure of a fresh surface for further oxidation is created.

In conclusion, the present research involves an important and interesting fundamental finding: nucleic acids could mediate and catalyze the oxidative etching process of gold materials. Surface patternable gold macro/micro-structures can be obtained with assistance of a porous rubber pad or a microfluidic technique. Based on this newly found phenomenon, an all-biomolecule-based etching system is developed by the combinational use of DNA and amino acids. This biomimetic finding differs significantly from conventional gold etching systems where strong and toxic oxidizing as well as ligand reagents or harsh conditions are required. It is the first controlled biolithography system to use nucleic acids to catalyze and mediate the oxidative etching of metals in mild aqueous solutions at room temperature.

This research is expected to find novel and significant fundamental meaning in single-molecular lithography, DNA metallization, DNA-directed templating synthesis and self-assembly of nanoparticles, bioelectronics, and biosensors. Since it is found that the gold oxidation by thiol-based amino acids is a main route for bio-dissolution of gold in biological body, our findings on this new functions of nucleic acids should also shed important light on *in vivo* or *in vitro* biomimetic metal material synthesis and the biological activity of gold materials when they are used as drug delivery carriers in biological bodies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The structures of four kinds of bases in DNA (A) having pyridine-like derivatives for NBS oxidation on a gold layer. As a result, a yellow solution was obtained after incubating gold-coated glass substrates overnight at room temperature in the solution containing 100 μM DNA (either dsDNA, B or ssDNA, C) and 14.6 mM NBS. This method is also applicable for the patterning of gold layers on glass substrates with macro/micro-resolutions by filling the etching solution into a rubber pad (D) or microfluidic channels (E) on a gold surface. The scale bars in (D) and (E) are 1 cm and 10 μm , respectively.

Figure 2.

The UV-vis spectra of gold colloidal solution containing GSH and DNA. The equivalent Au atom concentration in the solution is 0.1mM, the GSH concentration is 100mM, and the λ -DNA (48502bp) concentration is $5.93 \times 10^{-4} \mu\text{M}$. From left figure, it is concluded that the characteristic peak for gold nanoparticles decreases gradually with incubation time prolonging. Defining the absorption peak value of the as-prepared solution, the solution after incubation for time t (hr) as $\text{Abs}(\text{as-prepared})$ and $\text{Abs}(t)$ respectively, the etching percentage is then calculated as $[\text{Abs}(\text{as-prepared}) - \text{Abs}(t)] / \text{Abs}(\text{as-prepared})$, and plotted in right figure. It is found that comparing with slow etching process (black curve) from GSH on gold nanoparticle (Au NP), the adding of DNA would accelerate largely the etching rate of Au NP (c.a. five-fold increases, red curve).

(py) symbolizes pyridine-derivatized nitrogenous heterocycle

Scheme 1.

The plausible reaction mechanism for the etching reaction on a gold surface mediated by pyridine-like units.