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DNA preservation using diamond chips

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Abstract

The highest density DNA chip reported to date was developed using a DNA solidification technique for vertical binding to the surface of a chemical vapor deposition (CVD) diamond chip. The covalently bound oligonucleotide was approximately 42 pmol ($\sim 2.5\times10^{13}$ molecules) per 9 mm^2 of chip surface. Using the oligonucleotide as a linker, large DNA molecules (21 kbp fragment from λ-phage DNA) were covalently bound in the amount of approximately 2.6 fmol ($\sim 1.6\times10^{9}$ molecules) per 9 mm^2 of chip surface. To test the potential of the technique for repeated utilization of one piece of the DNA chip, PCR enhancement of a 500 bp region within the 21 kbp λ-DNA fragment was applied over 50 times. The results suggest that a diamond DNA chip is excellent for the preservation of limited and/or valuable gene samples. Furthermore, the technique of high density DNA solidification to a CVD diamond chip will be useful for DNA diagnosis in the future.

Keywords: Diamond chip; DNA chip; DNA preservation; DNA solidification

1. Introduction

The reliable safekeeping of DNA samples is an important and fundamental problem in genomic laboratory science, in view of the explosive growth of number of samples analyzed per year. In the post genomic era [1], new types of DNA-preserving and -treating techniques are required for the vast number of gene diagnoses based on comparison of DNA sequences. Diamond produced by chemical vapor deposition (CVD), and diamond like carbon (DLC), are under consideration as new medical materials. Our group has developed new types of preservative and analytical DNA chips, based on CVD diamond and DLC substrates, and using a solidification technique in which DNA is bound vertically to the surface of the chips. Here, we will describe the preservative type of DNA chip using CVD diamond.

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2. Experimental section

2.1. Chemical treatments on surface of CVD diamond chip

Polycrystalline diamond films were grown to 200–300 μm in thickness from CH_4 (99.99%) and H_2 (99.99%) on Si substrates (wafers) with the CVD method [2]. The freestanding diamond films were obtained by dissolving the Si substrates with a HF–HNO_3 aqueous solution. The CVD diamond film was cut into chips of (3×3 mm^2) with an Nd–YAG laser.

To clean the surface of CVD diamond chips (1st step), a hydrogen-termination was done using microwave hydrogen plasma for 5 min. To chlorinate on the chip surface (2nd step), the hydrogen-terminated chips were placed into a stainless steel chamber, and the chamber was evacuated to less than 1×10^{-2} Torr. Following the introduction of chlorine gas (99.9%) to 800 Torr, the chip surface was irradiated by light from high pressure Hg arc lamp (100 W: 365, 313.2, 302 nm) for 30 min. To aminate the chip surface (3rd step), ammonia gas (99.99%) was introduced in the chamber to 800 Torr after a series of operations (evacuation of chlorine gas, introduction of argon, and evacuation of

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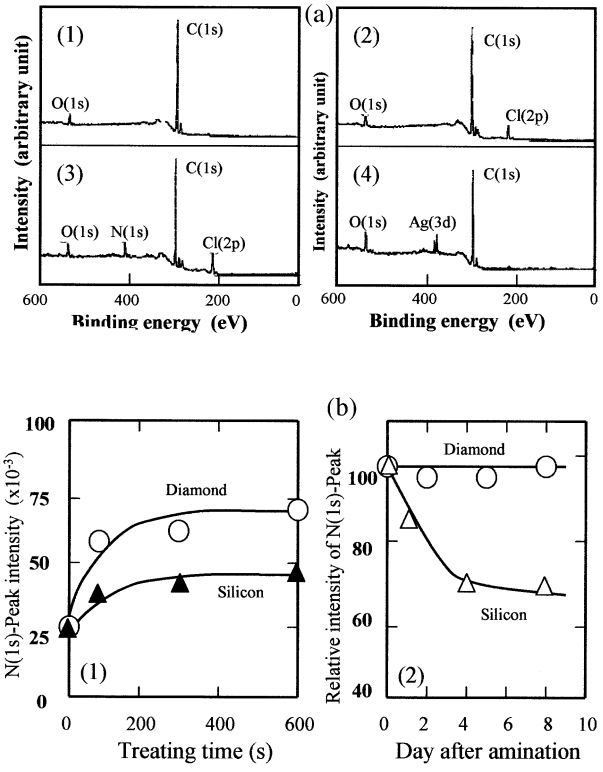

Fig. 1. Chemical modifications on CVD diamond chip. (a): XP spectra of CVD diamond chip surface with the chemical treatments of hydrogen-termination (1) chlorination; (2) amination; (3) and carboxylation; (4). (b): Amount (1) and stability; (2) of amination on CVD diamond and silicon chips. Data were obtained estimating N(s)-peak intensity in XP spectrum as shown in a (3).

argon to less than 1×10^{-2} Torr). The chlorinated chip surface was changed to the aminated state with further irradiation from the Hg arc lamp for 30 min. X-ray photoelectron (XP) spectra of the chip surface obtained with the above procedures is shown in Fig. 1a (1–3).

At the 3rd step, the amount and stability of amination on CVD diamond chip were checked by measuring XP spectra and comparing to those of a similarly treated silicon chip as shown in Fig. 1b (1 and 2). The results showed that both of amount and stability of amination on CVD diamond chip are better than those on silicon chip, because of the structural difference between [–C(NH_2)–C(NH_2)–C(NH_2)–)] on diamond surface and [–Si(NH_2)–O(NH_2)–Si(NH_2)–] on silicon surface. Especially, it seems that the amination of the oxygen formed during the surface treatment of silicon is more unstable to hydrolysis of its amide linkage than the amination to carbon or to silicon is.

To carboxylate the chip surface (4th step), the aminated chips were immersed in a solution consisting of chloroform (20 ml), succinyl chloride (1 ml) and triethyl amine (0.02 ml), and refluxed for 1 h. The XP spectrum of carboxylated chip with an amide linkage was measured for the state of –COOAg after immersing the chip in $AgNO_3$ aqueous solution for 1 h (Fig. 1a (4)).

2.2. Vertical and covalent solidification of oligonucleotide on surface of CVD diamond chip

The activation of carboxyl groups on the carboxylated chip was done in a mixture solution (0.2 ml/one chip) dissolved *N*-hydroxysuccinimide (0.26 mmol/ml) and carbodiimide (0.26 mmol/ml in water-soluble form) in 90% 1,4-dioxane. The activated chip was immersed in the selected oligonucleotide solution for 0.5 h, and would bind a certain type of oligonucleotide with the second amide linkage (Fig. 2a). This oligonucleotide (as a linker to further immobilize large DNA molecules) was designed to have few adenine (a) moieties on the terminal region of 15–30 bases in order to solidify

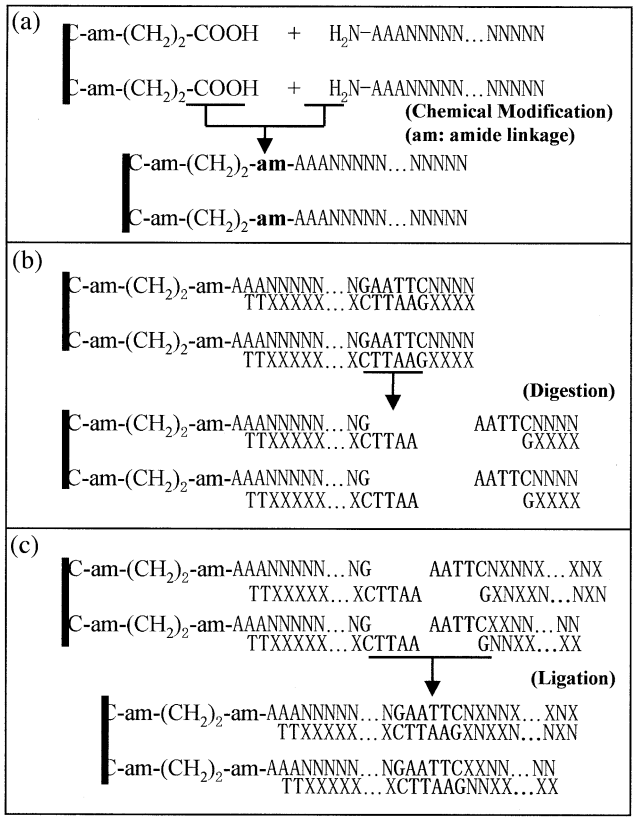

Fig. 2. Schemes of solidification of oligonucleotide and immobilization of double strand DNA on CVD diamond chip. (a): Chemical solidification of oligonucleotide on CVD diamond chip surface with amide linkage using *N*-dehydroxysuccinimide and cabodiimide. (b): Enzymatic digestion in forming the reactive site to restriction enzyme. (c): Enzymatic ligation in immobilizing double strand DNA to the reactive site of restriction enzyme.

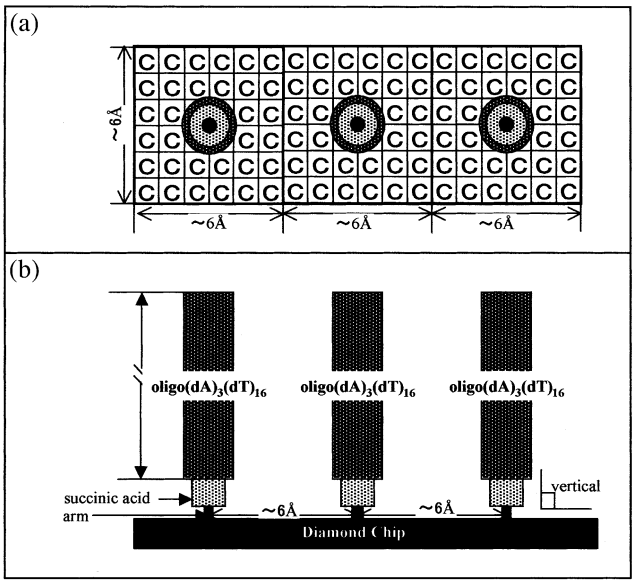

Fig. 3. Expected solidification model of oligonucleotide on CVD diamond chip. (a): Occupied space (cell) of oligonucleotide ($oligo(dA)_3(dT)_{16}$) solidified on CVD diamond chip estimating from the value of ~37 Å²/molecule in Table 1. (b): Conformation of oligonucleotide ($oligo(dA)_3(dT)_{16}$) solidified on CVD diamond chip.

oligonucleotide and to immobilize DNA vertically to the chip surface as shown in Fig. 3b or Fig. 4b, respectively.

The solidification of the oligonucleotide was certified by checking the hybridization between the fixed $oligo(dA)_3(dT)_{16}$ and unfixed $oligo(dA)_{16}-6FAM$ (labeled with a fluorescent: 6FAM). The specific binding amount of $oligo(dA)_{16}-6FAM$ was measured with HPLC (excitation of 450 nm and emission of 512 nm) after the dehybridization at 90 °C (figure not shown). The result is indicated in Table 1 together with data from an experiment from a silicon chip under the same conditions as the CVD diamond chip.

2.3. Immobilization of double strand DNA on the surface of CVD diamond chip

To immobilize large DNA molecules (double strand DNA) on the chip surface, firstly, the reactive site for a restriction enzyme (EcoR-I in this case) was formed with the fixed sense-oligonucleotide and unfixed (hybridized) antisense-oligonucleotide as shown in Fig. 2b. Next, the double strand DNA (a 21 kbp fragment from λ-phage DNA in this case) digested by restriction enzyme (EcoR-I) was bound by ligase with the reactive site of restriction enzyme on the chip surface as shown in Fig. 2c.

The immobilization of the 21 kbp λ-DNA fragment was certified by PCR enhancement of 500 bp region within the 21 kbp fragment as shown in Fig. 5a. One

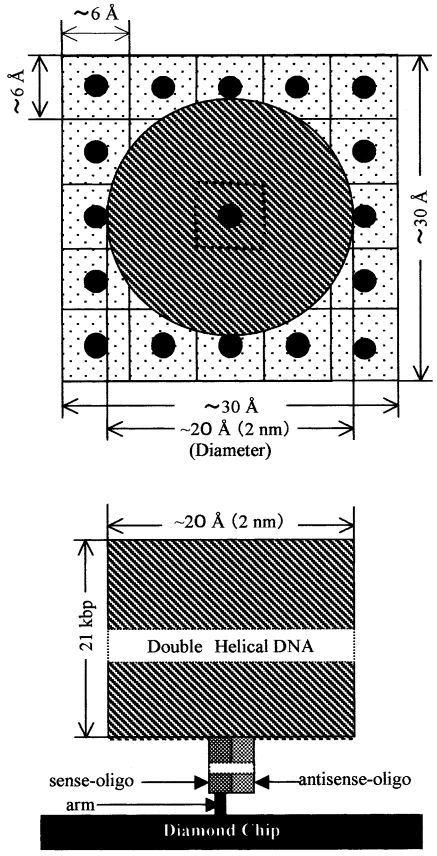

Fig. 4. Expected immobilization model of double helical DNA on CVD diamond chip. (a): Theoretically expected the occupied space (block) of double helical DNA immobilized on the CVD diamond chip. (b): Conformation of double helical DNA immobilized on the CVD diamond chip.

PCR procedure includes a total of 30 cycles of temperature change: (90 °C → 60 °C → 72 °C → 90 °C), 3 cycles after immersing the DNA chip into the PCR solution and 27 cycles after removing the chip from the solution. Sense and antisense primers in the PCR were 5′-GATGAGTTCGTGTCCGTACAACTGC-3′ and 5′-GGTTATCGAA ATCAGCCACAGCGCC-3′. The 21 kbp λ-DNA fragment immobilized on the CVD diamond chip was useful still after 50 times of the repeating PCR utilization (Fig. 5a).

Table 1
Fixed amount and occupied area of oligonucleotide ($oligo(dA)_3(dT)_{16}$) on 9 mm² of CVD diamond and silicon chips

Chip	Diamond	Silica
Occupied area ((Å)²/molecule)	~37	~300
Fixed amount (pmol/(3 mm)²)	~42	~5

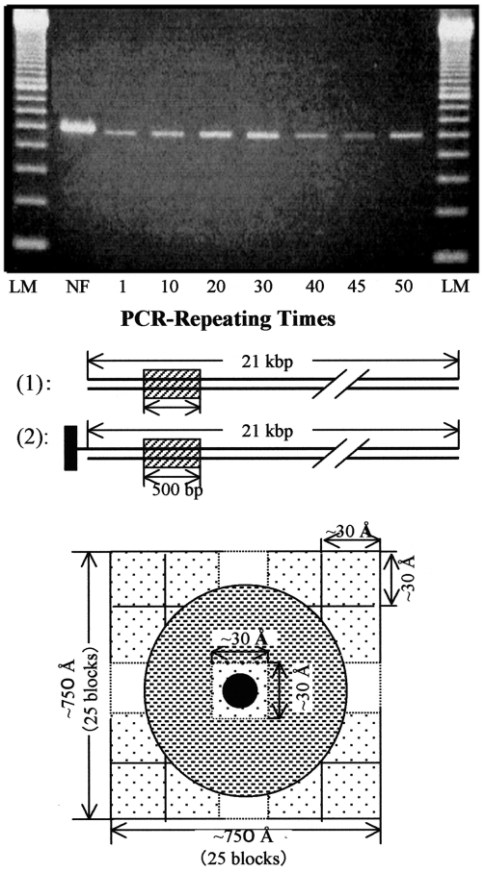

Fig. 5. Results of PCR enhancement and expected immobilization area of 21 kbp λ-DNA fragment on CVD diamond chip. (a): Photograph indicating the PCR enhancement of 500 bp region within 21 kbp λ-DNA fragment immobilized on CVD diamond chip. NF: result with no-fixed 21 kbp λ-DNA fragment (26.4 fmol) under the state shown in Fig. 1, 1–50: times and results of repeating PCR enhancement with one chip of 21 kbp λ-DNA fragment (~2.64 fmol) under the state shown in Fig. 2, and LM: 100 bp ladder marker. The concentration of ~2.64 fmol to λ-DNA fragment immobilized on CVD diamond chip was estimated comparing between the band width in NF lane and the average band width in 1–50 lanes. (b): One double helical DNA shown with a black circle at center was immobilized over the area of 750 Å^2, when the immobilized number of 21 kbp λ-DNA fragment was assumed to be $\sim 1.6\times10^9$ molecules from the immobilized concentration of ~2.64 fmol per 9 mm^2 of CVD diamond chip.

3. Results and discussion

The amount and stability of amination on CVD diamond chip were approximately 1.5-fold compared to that on the silicon chip as shown in Fig. 1b. The fixed amount of oligonucleotide on CVD diamond chip was also approximately 8-fold more than on the silicon chip, as indicated in Table 1. Furthermore, one DNA chip with an immobilized cDNA library from a total RNA of rat liver on a CVD diamond chip has been stably supplying reasonable results from PCR enhancements for 3 years after the immobilization (data not shown in this manuscript). These suggest that CVD diamond is an excellent material for the preservative type of DNA chip.

The occupied area of ~37 Å^2 per molecule estimated in the solidification of oligo$(dA)_3(dT)_{16}$ (Table 1) implies that one oligonucleotide molecule is binding with two amide linkages within a space (cell) of (~6× ~6 Å^2) on the chip surface as shown in Fig. 3a. This state may be the most high density state ($\sim 2.5\times10^{13}$ molecules per 9 mm^2 of CVD diamond chip surface) on solidification of single strand oligonucleotide in forming the double strand oligonucleotide with hybridization. The amide linkages bounded single strand oligonucleotide were formed between one carboxyl group of succinic acid and arm (from the carbon atom on CVD diamond surface), and between another carboxyl group of succinic acid and the amino group of terminal base (A) of oligonucleotide. Divalent carboxylic acids other than succinic acid could not form the conformational model vertical to the chip surface shown in Fig. 3b.

Double strand DNA forms a double helical state of ~20 Å diameter. Theoretically the double helical DNA is expected to occupy one block area of (~30× ~30 Å^2), corresponding to approximately 25 cells bound single strand oligonucleotide, per the immobilization of one molecule as shown in Fig. 4a. The expected model for the immobilizing conformation of the 21 kbp λ-DNA fragment is shown in Fig. 4b.

The photograph shown in Fig. 5a suggests that approximately 10% (2.64 fmol) of 21 kbp λ-DNA fragment concentration (26.4 fmol) used in the immobilization was actually fixed on the CVD diamond chip by the ligation step shown in Fig. 2c. The value of 2.64 fmol was estimated by comparing the average PCR band width of lanes 1–50 to the PCR band width of the NF lane (obtained with PCR enhancement using no-fixed 21 kbp λ-DNA fragments (26.4 fmol)). From the concentration of ~2.64 fmol fixed on the CVD diamond chip of 9 mm^2, one molecule of 21 kbp λ-DNA fragment could be estimated to be immobilized within an area of (~750× ~750 Å^2). This area corresponds to the space consisting of the 625 blocks of the theoretical binding block to double helical DNA shown in Fig. 4a. If the area of 625 blocks were assumed to be the space occupied with DNA-interacted enzyme (restriction enzyme or ligase) as shown with the large circle in Fig. 5b, the reasonable binding density of double helical DNA seems to be $\sim 1.6\times10^9$ molecule per 9 mm^2 on the surface of CVD diamond chip. This density would be the highest value for DNA chips reported to date.

Using the technique of oligonucleotide solidification to CVD diamond chips for high density binding, we have also succeeded in the developing a microarray (analytical) type of DNA chip using DLC coated slide (DLC coated on a glass slide). The high density of addressed DNA chip using CVD diamond or a DLC coated slide will be useful as the technical support of DNA diagnosis.

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