

PATTERNING OF BIOMOLECULES IN EXTENDED NANOCHANNEL USING LOW-TEMPERATURE BONDING

Kentaro Shirai¹, Kazuma Mawatari^{1,2}, Takehiko Kitamori^{1,2}

¹ The University of Tokyo, JAPAN, ² CREST, Japan Science and Technology Agency, JAPAN

ABSTRACT

Single cell analysis has difficulties due to small sample volume as picoliter. Extended nanospace(10-1000 nm) which has been studied by our group has femtoliter to attoliter volume smaller than single cells and is applicable for the analysis. However, solid phase capture of analytes at defined positions for detection has not yet realized. Here, we developed a novel patterning method of biomolecules in extended nanospace by using low temperature bonding of fused silica, which avoids destruction of organic molecules by thermal bonding. Patterned probe DNA showed good selectivity and stability, and fast hybridization time was observed in nanochannels. The method can be applied to immunoassays for protein quantification.

KEYWORDS: Single cell analysis, Extended nanospace, Low-temperature bonding, Patterning, Immunoassay

INTRODUCTION

Recently, the importance of single cell analysis is increasing in basic research of biology and diagnosis of cancers[1]. However, difficulties in analysis exist due to extremely minute volume as picoliter and a lot of impurities, novel analytical technologies are highly demanded. Our group has studied about extended nanospace which size is from 10 to 1000 nm fabricated on fused silica substrates and developed several analytical methods[2]. As shown in figure 1, the features of extended nanospace are extremely small volume as femtoliter to attoliter which is much smaller than picoliter volume of single cell by 3 to 6 orders and solid phase capture of analytes without their loss by the surface dominant space property. If typical biological assays such as immunoassays are integrated into extended nanospace, very minute amount of proteins can be detected and quantified with high sensitivity. However, to capture analytes at defined positions in extended nanospace has not been achieved which is necessary for detection. The aim of this study is to establish surface patterning method of biomolecules such as DNA and antibodies in extended nanospace.

EXPERIMENTAL

Silica glass, which should be chosen as a material of microchip for nanofluidic research in terms of optical property and rigid structure at nm-scale, are usually bonded by thermal bonding. As shown in figure 2, the large problem of thermal bonding is that biomolecules are destroyed by heat during the bonding process. If silica glass substrates can be bonded at low temperature, chemical pattern prepared before bonding can be used without loss of its function. We have solved the problem by developing a novel patterning method utilizing low temperature bonding[3]. Low-temperature bonding is the new bonding method by using oxygen plasma surface activation. The bonded microchip showed enough bonding strength leaching $\sim 1 \text{ J/m}^2$ which could endure pressure-driven flow induced by several hundreds of kPa pressure.

The detail of patterning method we developed is shown in figure 3. We generated pattern of aminopropyltriethoxysilane (APTES) by irradiation of vacuum ultra violet light (VUV, $\lambda = 172 \text{ nm}$) through a Cr photomask. VUV light causes the excitation of oxygen and reactive oxygen species degrade organic

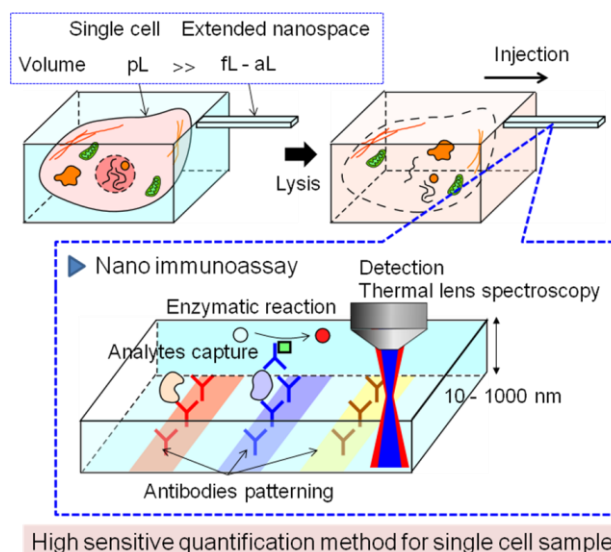


Fig. 1 Concept of single cell analysis system utilizing nano immunoassay

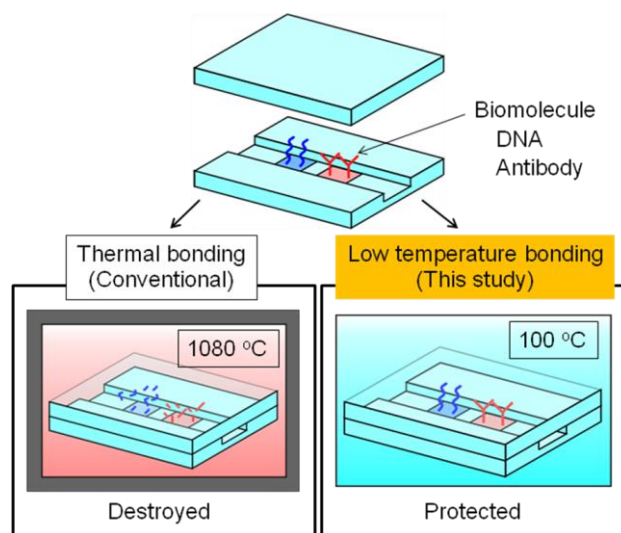


Fig. 2 Concept of chemical and biological patterning by low temperature bonding

compounds including APTES, therefore, APTES surface remained on the protected area with Cr photomask and the other area became clean silanol surface. By the proposed method, amine groups pattern is generated on the surface and at the same time, very clean and hydrophilic surface except the pattern area can be acquired which is necessary for enough bonding strength to endure pressure-driven flow. The APTES patterned substrate was bonded with another one including micro- and nanochannels at 100 °C under 5000 N force after oxygen plasma surface activation. After bonding, 10 mM SMPB crosslinker in 20/80 DMSO/ethanol was flowed in nanochannel for 2 hours by pressure-driven flow at 50 kPa and washed with ethanol. 20 μM thiol-modified probe DNA in 10 mM TE buffer was introduced by capillary filling and reacted for 3 hours resulting in covalently bonded probe DNA on nanochannel surface.

RESULTS AND DISCUSSIONS

In order to investigate the function of immobilized probe DNA, change of fluorescent intensity was observed by fluorescent microscope when 100 nM texasred-labeled complimentary target DNA in hybridization buffer (2xSSC, 0.1% SDS, 0.1% BSA) was flowed in nanochannel which width and depth was 3.3 μm and 500 nm each. As shown in figure 4(a), distinct pattern was observed at ~48 μm width corresponding to 50 μm photomask width. DNA sequence selectivity was confirmed because non-complimentary DNA did not show any increase of intensity as in figure 4(b). The density of surface bound target DNA was determined from the fluorescence intensity as 1.7×10^{11} molecules/cm² which was comparable to previous reports. Next, stability of probe DNA was confirmed because repeatable use of immobilized molecules is important for the analytical applications in the future study such as making a calibration curve in immunoassays. As shown in figure 4(c), during three cycles of hybridization and denaturation by 8 M urea which cleaved hydrogen bonds between base pairs was repeated, about 16 times difference of intensity in average between hybridized and denatured step was observed. The base intensity after denaturation was almost constant which indicated that the specifically surface bound target DNA was removed by 8 M urea denaturation process. From these results, we concluded that we have succeeded in patterning DNA molecules in nanochannels without loss of their function.

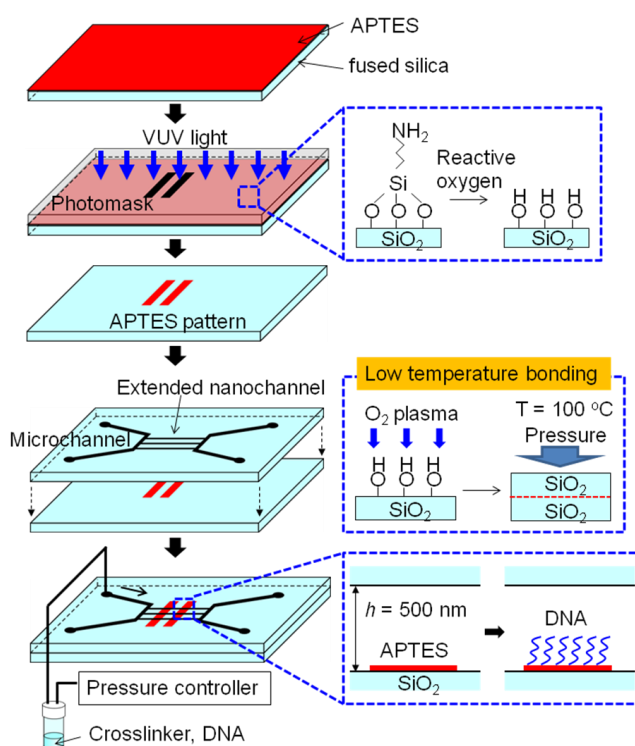


Fig. 3 Procedure of DNA patterning method using low temperature bonding

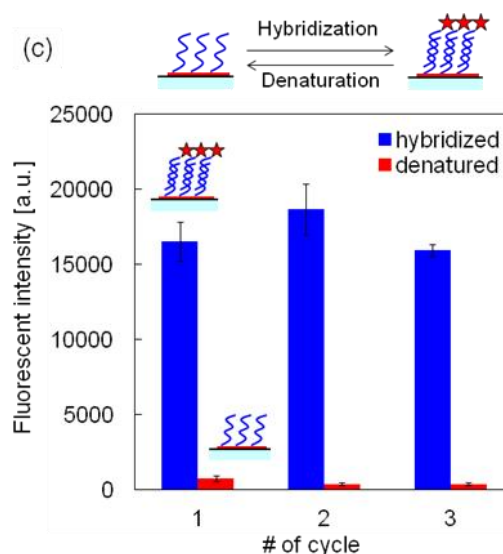
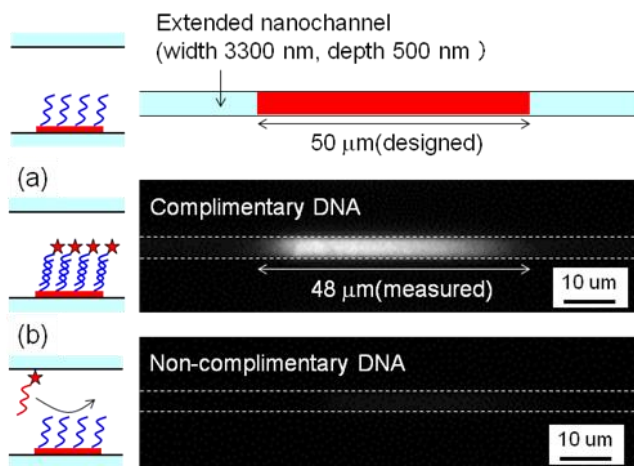


Fig. 4 (a) Complimentary DNA hybridized (b) non-complimentary DNA (c) stability of immobilized probe DNA

Generally, reaction time in biological assay is long and it often determines total assay time. Especially in case of assays which involve surface reaction between solid and liquid phase, reaction time is longest from plates, microwells and microchannels which is corresponding to the surface-to-volume ratio. In terms of this point, we can expect short reaction time in extended nanospace because it has about 500-1500 times larger surface-to-volume ratio than microwells and microchannels. To investigate hybridization kinetics, we observed time course change of fluorescent intensity. As in figure 5, the intensity saturated from upstream to downstream, and reaction reached to equilibrium within about 20 seconds when 100 nM target DNA was introduced. Compared with reaction time in microchannels which were reported as several minutes[4], it was decreased by 1 order. It is considered that the fast hybridization time comes from short molecular diffusion length in nm-scale depth of extended nanospace and increased probabilities of rebinding between probes and targets. Precise investigation of reaction kinetics is now ongoing.

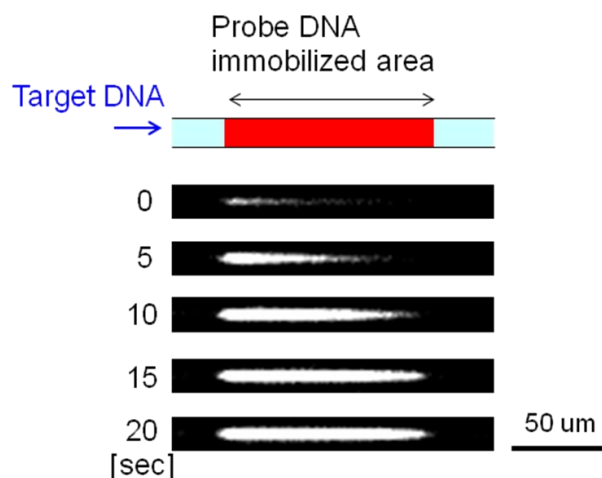


Fig. 5 time course change of fluorescent intensity during hybridization reaction in nanochannel

CONCLUSION

We developed a novel patterning method of biomolecules on the surface of extended nanospace using low temperature bonding. Patterned probe DNA showed good selectivity and stability without loss of function. In extended nanospace, hybridization time seemed to be faster than in microchannels due to short diffusion length and increased probabilities of rebinding. In the future, this method will be applied to sensitive and fast immunoassays utilizing immobilized probes as templates for generation of primary antibodies, and protein quantification of single cells.

ACKNOWLEDGEMENTS

This work is financially supported by Grant-in-Aid for JSPS Fellows(23-9195) and partially supported by JSPS core to core program(20002).

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CONTACT

*Takehiko Kitamori, tel: +81-3-5841-7231; kitamori@icl.t.u-tokyo.ac.jp