Micropatterning of enzymes and biological cells using dielectrophoretic assembly of microparticles

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Recently, there has been a great deal of interests in the organization of colloidal particles, since the spatial control of the particles is a critical subject to create new functional materials. Various methods have been proposed and used for assembling microparticles on a solid support. We report here the patterning of microparticles using the negative-dielectrophoretic (n-DEP) method to create two-dimensionally micropatterns of the microparticles monolayer that is robustly immobilized on glass slides. Moreover, diaphorase (Dp), a flavoenzyme, and biological cells are immobilized on the particles to create micropatterns of biomaterials.

DEP is a phenomenon in which particles are manipulated by the interaction between a non-uniform electric field and the polarization charge induced on the surface of particles by the extra electric field[1]. We previously reported the possible use of the DEP as a powerful tool for the patterning of biological cells[2]. In the present work, to pattern microparticles, n-DEP is induced at a template electrode located above the substrate with a fluidic channel (Fig. 1). The patterning of particles reflected the template electrode of the microband shape is a consequence of the fact that particles are manipulated to the area where the electric field is weaker under the n-DEP.

Succinimidyl 4-[*p*-maleimidophenyl] butyrate was reacted with glass substrates modified with (3-Mercaptopropyl)-trimethoxysilane. As a result, N-hydroxy-succinimide (NHS) ester was introduced on the surface of glass substrates. The interdigitated array electrodes (typically electrode band gap and band width 50 μm and 6 μm, respectively) were fabricated by photolithographic methods. The fluidic channel (width millimeter order) for patterning the particles was defined by a PET film (10 μm thickness) between the electrode and the NHS ester-derivative substrate. A 5 μL suspensions of amino-derivative microparticles (ϕ 3 μm, 3 x 10^8 particles/mL) in DMSO was inserted into the fluidic channel and an alternating electric field (20 V p, 1 MHz) was applied to the electrode for 1.5 hr. For immobilization Dp, solutions of thiolated Dp were added to the above substrate, and the selective immobilization of enzyme on microparticles was allowed to continue for 2 hr. An enzyme activity of Dp was measured with SECM in a solution containing 0.5 mM ferrocenylmethanol (FMA). A potential of the microelectrode was held at 0.5 V vs. Ag/AgCl to oxidize FMA. After addition of 5 mM NADH, a substrate of Dp, the SECM measurement was performed to estimate activity of Dp immobilized on microparticles.

Fig. 2 shows the particles patterned and immobilized by n-DEP with Grid formations. The line width of the pattern consisted of 1 particles. The patterns remained firmly on the substrate after the template electrode was removed from the fluidic channel. Grid patterns were fabricated using n-DEP by rotating the template electrode by 90 degree and making line patterns meeting at right angle to the lines previous formed.

Fig. 3A and Fig. 3B depicts the SECM images (scan area, 400 x 100 μm) of the patterned microparticles based on the oxidation current of FMA. In the absence of NADH (Fig. 3A), the line-and-space for the immobilized particles (four lines) was observed. The dark line with low oxidation current for FMA coincided with the location of the patterned particles. However, the addition of NADH drastically changes the SECM images (Fig. 3B). The appearance of bright lines is attributed to the catalytic activity of Dp immobilized on the particles. These SECM results indicate that Dp is preferentially immobilized on the particles. The particles assembled by n-DEP can be selectively functionalized by enzymes and these procedures will be applicable for the integration of enzyme devices.

Fig. 3C shows microscopic photograph of cultured MCF-7 cells on the patterned diameter 3 μm amino-derivative particles. MCF-7 cells dominantly adhered on the cross over points of the particles and grew along the particles.

References:

1) Pohl H. A. Dielectrophoresis; Cambridge University Press: Cambridge, (1978).
2) Matsue T. Matsumoto N. Uchida I., *Electrochim. Acta*., **42,** 3251-3256 (1997).

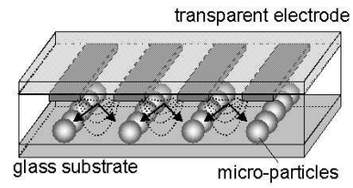

Figure 1. Principle of the dielectrophoretic patterning of microparticles.

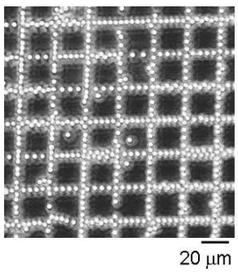

Figure 2. Miroscopic photographs of patterned ϕ 3 μm amino-derivative particles in Grid formations.

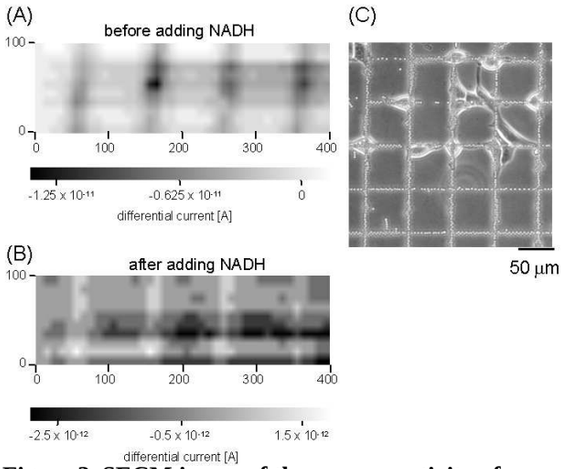

Figure 3. SECM image of the enzyme activity of diaphorase immobilized on microparticles without (A) and with 5 mM NADH (B). Microscopic photograph of cultured MCF-7 cells on patterned particles (C).