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Study on Elemental Technologies for Creation of Healthcare Chip Fabricated on Polyethylene Terephthalate Plate

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SUMMARY Elemental technologies have been studied to establish the healthcare chip which is an intelligent micro analytical system to detect human health markers from a trail of blood. A two steps process for deep quartz dry-etching was discussed in order to overcome the issues of concave-shaped defects at the bottom of grooves. A coating with 2-methacryloyloxyethylphosphorylcholine (MPC) polymer was studied to suppress the adsorption of bio-substance onto the inner wall of the flow channel on chip and good bio-compatibility was achieved for suppression of protein adsorption and blood cell adhesion. A prototype of healthcare chip was fabricated on polyethylene terephthalate (PET) plate using a micro molding technique. Using this chip, the ion concentrations of pH, Na^+, K^+, Ca^{++} were successfully measured with embedded ion sensitive field effect transistors (ISFET's).

key words: *health checking, microcapillary, electroosmosis, MPC polymer, ISFET*

1. Introduction

To protect our health from environmental problems such as global warming and the hormone-mimicking synthetic chemicals, we are now studying a healthcare device which checks our daily health as an application of μ-TAS (micro-Total Analytical System) [1]. As shown in the illustration of Fig. 1, the final structure of the device will be such a blood analytical system embedded on a Si integrated circuit (IC), which involves chemical sensors, data acquisition and correction circuits, operation sequencer, communication means, and so on. This chip can be applicable for virtual health diagnostics at any demanded locations, by being installed to a handy phone, which will be a most popular mobile terminal of the highly developed information culture in future. To establish our goal, we have started to develop an analytical chip, in which a trace amount of blood is injected into a microcapillary by a pumping method, and typical health markers such as pH, O_2 and CO_2, Na^+, K^+ and Ca^{++} cations, uric acid, lactic acid and glucose can be measured using various chemical sensors. However, we have to solve many issues as shown in Fig. 1; (1) a painless needle collecting blood of nano litter, (2) fabrication techniques of a microcapillary in quartz or polymer plate (3) a biocompatible capillary which allows the flow blood without clogging or denaturalization, (4) a transport means of the serum into a capillary, and (5) embedded chemical sensors to detect the health markers.

As pioneering work, an integrated chemical analysis system of O_2, CO_2 and pH in blood was developed using a micro-flow cell [2]. However, no attention was paid for the bio-compatibility in this work. Ishihara et al. have noted the coating effect of 2-methacryloyloxyethylphosphorylcholine (MPC) polymer [3] on quartz surface. The MPC polymer makes surfaces similar to natural bio-membranes, so that the surfaces can interact in a compatible manner with biocomponents such as proteins and cells. This polymer has been utilized for many applications, for instance, artificial blood vessels, coatings on soft contact lenses, catheters and top coatings of various in-vivo sensors. We have already reported electroosmosis injection of blood serum into the MPC polymer coated microcapillary fabricated on quartz plate [4].

This paper reports on elemental technologies of a micro blood analytical system made on PET (polyethylene terephthalate) plate, focusing on (1) fabrication process employing dry etching technologies, (2) the bio-

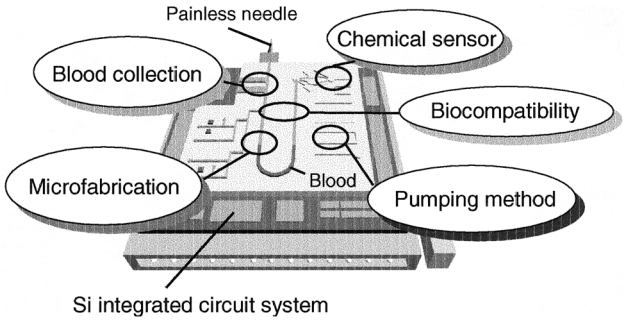

Fig. 1 Illustration of a final structure of healthcare device and the issues to be developed.

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compatibility of the capillary whose inner wall is coated with the MPC polymer, and (3) measurement of health makers of pH and concentrations of Na^+, K^+ and Ca^{++} employing an ISFET (ion sensitive field effect transistor) embedded in the plate.

2. Fabrication Process of Microcapillary PET Chip and Experimental

Figure 2 shows a fabrication process of a microcapillary made by PET. At first, a reversal microcapillary pattern with 30 μm × 30 μm cross-sections was fabricated on a quartz plate with 2 cm × 2 cm area. A Cr film with a 1.5 μm thickness masked by a resist (ZEP 7000; Nihon Zeon) delineated by EB (electron beam) lithography was etched by Cl_2 ICP (inductively coupled plasma) at 10 mTorr at the 18 cm downstream region from the antenna, where the etch rate selectivity of the Cr film to the EB resist was improved [5]. Using this Cr mask, the microcapillary patterns were dry-etched employing a planar type NLD (neutral loop discharge) [6] with a C_3F_8 + 70% CF_4 mixture. The etching pressure was 3 mTorr. The 13.56 MHz power added to the antenna was 800 W. The gas of C_3F_8 was chosen because it was one of the substituting gases for global warming. When the plasma with C_3F_8 alone was employed during quartz etching, we suffered from not only wider pattern width than original one, but generation of numerous concave-shaped defects on the bottom surface of the quartz plate. These issues are discussed in detail at the following section. The fabricated quartz plate with the reversal capillary pattern in this way was pressed onto a PET plate by a pressure of 1.5 MPa and at a temperature of 90°C for 10 minutes. Figure 3 shows an embossed microcapillary pattern on the PET plate. Next, two ISFET chips made by the Shindengen Kogyo Corporation were embedded on another PET plate by hot press in the same condition as the previous molding. Holes for reservoirs and wastes, in which electrodes should be inserted to apply high voltage, were drilled in the PET. Finally, the microcapillary-pattern molded PET plate was bonded to the ISFET's embedded PET plate in the condition of 0.7 MPa and 100°C. The finished PET chip was demonstrated in an inset photograph in Fig. 2.

The present ISFET device included two transistors in a chip. The gate materials were Ta_2O_5 on Si_3N_4 fabricated by CVD (chemical vapor deposition) on thermally grown SiO_2. The sensing area was 10 μm long × 360 μm wide. The Ta_2O_5 was used for the ion sensitive substance for the pH measurement in this paper.

For the measurement of the Na^+, K^+, and Ca^{++} cation concentrations, a mixture of 100 mg polyvinylchloride (PVC), 3 ml tetrahydrofuran (THF), 10 mg bis[(12-crown-4)methyl]-2 dodecyl-2-methylmalonate, 102 mg 2-nitrophenyldodecyl ether (NPOE) and 1mg tetrakis (4-chlorophenyl) borate potassium salt (K-TCPB), a mixture of 100 mg PVC, 3 ml THF, 10 mg bis[(benzo-15-crown-5)-4-methyl]pimelate, 102 mg NPOE and 1 mg K-TCPB, and a mixture of 100 mg PVC, 3 ml THF, 10 mg 4, 16-Bis-(N-octadecylcarbamoyl)-3-oxabutyryl-1, 7, 10, 13, 19-pentaoxa-4, 16-diazacyclohenicosane, 165 mg NPOE and 4.8 mg K-TCPB were coated on the Ta_2O_5 layer of the ISFET surfaces, respectively [7]–[9]. All the ionophores and NPOE were purchased from Dojindo Laboratories. The MPC polymer was also coated on these mixture surfaces. The thicknesses of the ion sensitive membranes were 69 μm, 54 μm and 64 μm for the Na^+, K^+ and Ca^{++} cations, respectively. An Ag/AgCl electrode in agarose gel with a saturated KCl solution was used as the reference electrode.

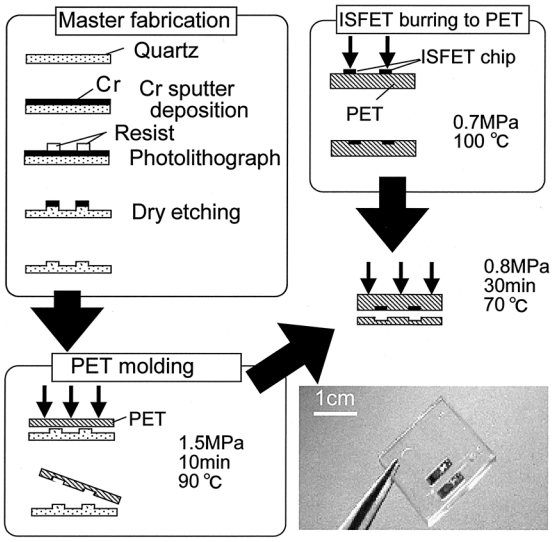

Fig. 2 Fabrication process of healthcare device made of PET.

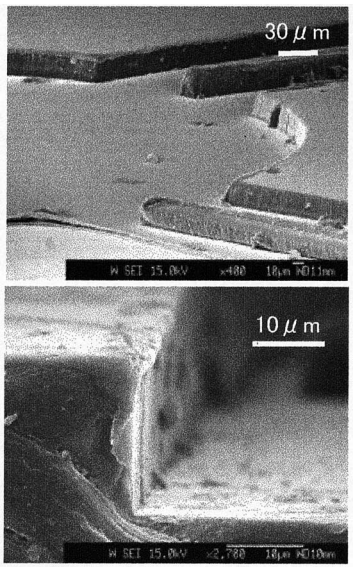

Fig. 3 SEM photographs of embossed microcapillary patterns on a PET plate.

Dulbecco's phosphate buffer solution (PBS) as an electrolyte consists of 200 mg/l KCl, 200 mg/l KH_2PO_4, 8 g/l NaCl and 1150 mg/l Na_2HPO_4. The ionic strength and pH of the buffer were 160 mM and pH=7.4, respectively.

3. Results and Discussion

3.1 Issue of Concave-Shaped Defects Generation

At first, both issues of the wider pattern width and concave-shaped defects generated employing C_3F_8 alone plasma as mentioned above were considered to result from the following mechanism: The wider width etched pattern (see Fig. 4(a)) was caused by deposition of fluorocarbon polymer on the Cr mask. The defects shown in a SEM photograph inset in Fig. 5 was also presumed to be generated by the polymer deposition, that is, polymer precursors produced in the gas phase grew up during long etching time due to low quartz etch rate less than 300 nm/min. Subsequently, the particles dropped on the quartz surface, thus playing a role of masking for fluorocarbon ions bombardment. Hence, CF_4 as a fluorine atom source which decreased carbon/fluorine ratio in the gas phase added to C_3F_8. Figure 4 shows variation of the etched features as a function of $\%CF_4$ in C_3F_8. The etched wall features were improved with increasing CF_4 concentration. However, since CF_4 alone plasma reduced etch selectivity of the Cr film to the quartz, the side wall of the masking Cr film retarded by the ion bombardment, thereby forming a tapered feature at the upper side of the trench. Eventually, a best-etched feature was obtained at $70\%CF_4$ concentration as shown in Fig. 4(c). The etch selectivity was about 20 in this condition. The number of defects decreased with increasing CF_4 addition as shown in Fig. 5, while considerable number of defects were still observed at the best concentration of $70\%CF_4$ in C_3F_8. We noted that the defects did not appear at a depth of a few μm, but they were observed clearly over about 10 μm depth and size of these defects was nearly same. These results suggested that a reason generating the defects was present on the original surface, because defects with different sizes should be generated if the masking was caused by the continuously deposited polymers. We tried to remove the masking materials on the quartz surface by increasing ion accelerating voltages of V_{dc}. The number of defects decreased dramatically with increase in V_{dc}, and finally the defects disappeared at V_{dc}=900 V, while the masking Cr films were etched considerably by the high voltage accelerated ions. Accordingly, the present issue was overcome as shown in photographs in Fig. 6(b), by the two steps process in which the quartz surface was cleaned at V_{dc}=820 V for 2 minutes and then etched at V_{dc}=500 V.

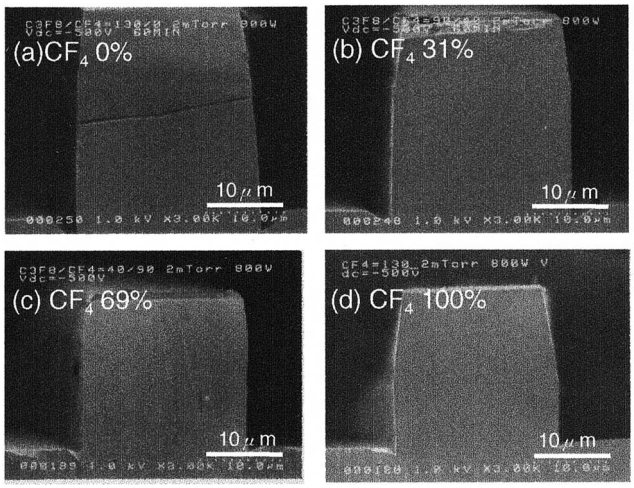

Fig. 4 Variation of the wall features etched by NLD as a function of $\%CF_4$ in C_3F_8.

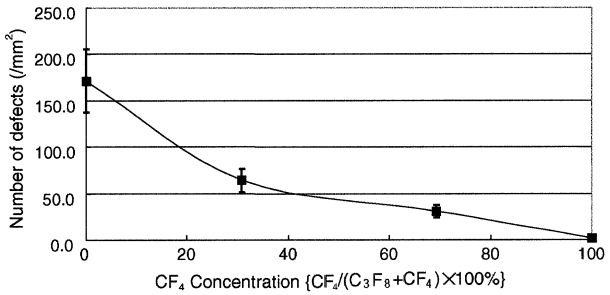

Fig. 5 CF_4 concentration dependence of the number of defects occurred on quartz master plate.

3.2 Biocompatibility of MPC Polymer

The biocompatibility of the MPC polymer was investigated by utilizing an FTIR-ATR (Fourier Transformed Infrared Attenuated Total Reflection) technique as shown in Fig. 7(a). A Si prism, both sides of which were mirror-polished at 45 degrees, was prepared for the ATR measurement. The Si surface was oxidized to real-

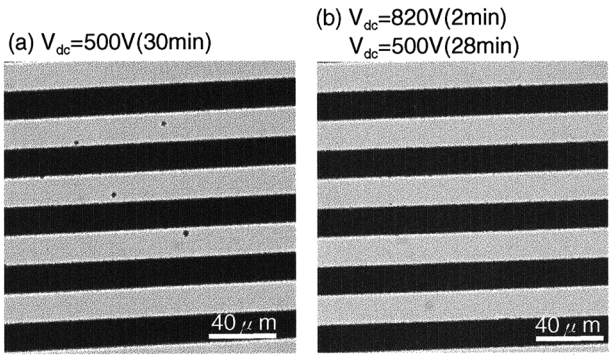

Fig. 6 Photographs of etched patterns by (a) V_{dc}=500 V (30 min) and (b) V_{dc}=820 V (2 min) followed by the etching at V_{dc}=500 V (28 min).

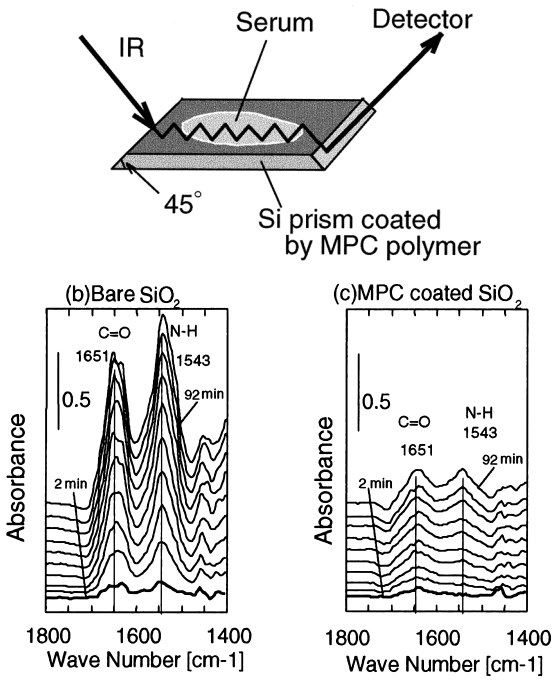

Fig. 7 Si prism for FTIR-ATR measurement to check biocompatibility of the MPC polymer coated quartz wall dipped in the serum.

ize the actual capillary quartz surface. Figures 7(b) and (c) shows the time-dependent variations of the FTIR-ATR spectra for the dipped serum on the SiO_2 bare surface (b) and on the 0.3 wt% MPC polymer coated quartz surface (c). The sensitivities for IR absorption by adsorbed substances on the surface in the (c) case was considered to be lower than those in the (b) case because of short immerse length of the evanescent wave due to thick coating of MPC polymer. This reduction of sensitivity was already compensated in Fig. 7(c) by dividing the absorbance by the ratio of peak heights for H_2O, which is considered to exist at same amount per area on the surface in the both (b) and (c) cases. The (b) spectrum after dipping of the serum demonstrated adsorption due to proteins which were represented by the NH_x and C=O peaks, while the proteins did not adsorb on the (c) surface. It revealed that proteins were not adsorbed on the MPC polymer coated surface. As shown in Fig. 8, adsorption of red blood corpuscles on the PET plate was also investigated for surfaces with, (a) and without the MPC polymer coating, (b), where the bare quartz surface, (c) was also checked as a reference. The present red corpuscles were obtained by centrifugation of human whole blood. A small adsorption number of $6/mm^2$ in the case of (a) as compared with cases of (b) and (c) demonstrates that the MPC polymer coating provides a great suppression effect for adsorption of red corpuscles on the MPC polymer coating.

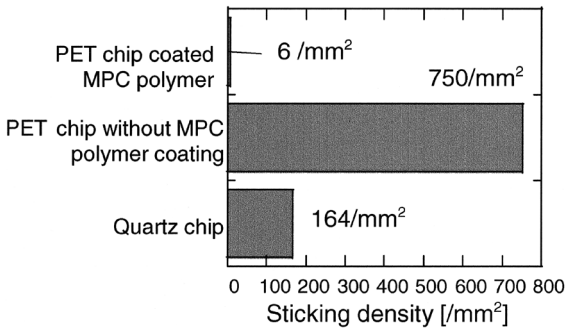

Fig. 8 Investigation for the adsorption of the red blood cell on capillary surface.

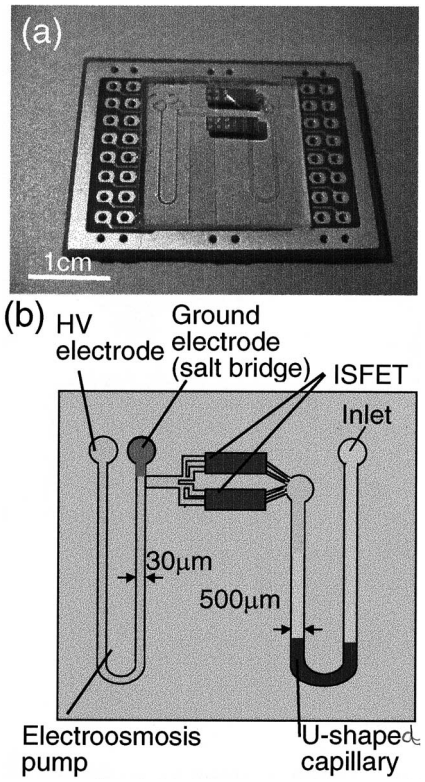

Fig. 9 Photograph of the prototype healthcare chip made of PET plate and its schematic diagram.

3.3 Measurement of Health Makers with ISFET's

Figures 9(a) and (b) show a photograph of the prototype healthcare chip made by PET plate and its schematic diagram, respectively. This chip consists of a blood inlet reservoir, a U-shaped centrifuge capillary, ISFET sensors embedded in the microcapillary chip and the electroosmosis flow (EOF) pump arranged at downstream of the sensor position. This configuration was designed to measure the health makers according to the following order; (1) PBS is filled in the whole capillary channel, (2) blood is put into the inlet, (3) it is injected into the U-shaped capillary by applying high voltage in

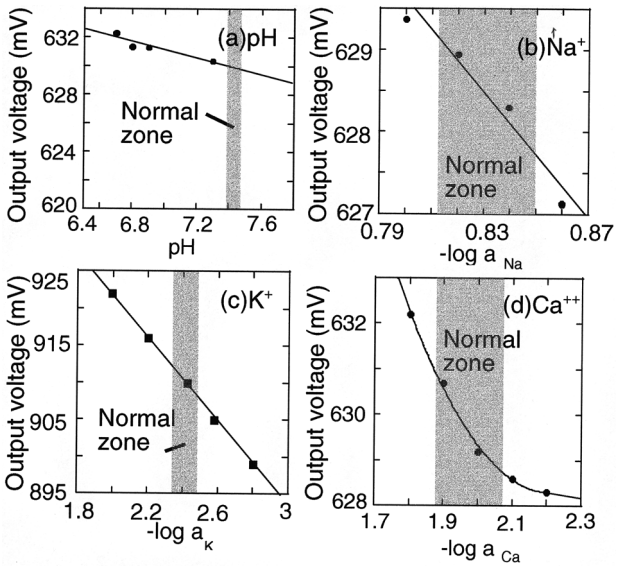

Fig. 10 Output voltage of pH, Na^+, K^+ and Ca^{++} cation sensors as function of a concentration.

the electroosmosis flow pump capillary, (4) blood cells are separated by revolve the chip in the small-scaled centrifugation apparatus, (5) the obtained serum is introduced into the ISFET's region by the electroosmosis pumping, (6) finally health makers are measured by the ISFET's.

One of the important items for creating the healthcare chip is the measurement of health markers. In this paper, measurements of pH, Na^+, K^+ and Ca^{++} concentrations were tried in an actual size chip. The ion sensitive membranes coated on the ISFET sensors were relatively robust and did not peel off even after 7 days of dipping in PBS. The calibration curves of Na^+, K^+ and Ca^{++} cation concentrations and pH were measured in the 0.3 wt% MPC polymer-coated microcapillary. Figures 10(a)–(d) show the output voltages of each cation sensor and a pH sensor as function of concentrations obtained at 24°C in the fabricated chip. Measurements were done after 4 minutes in order to wait for the output signals to be stable. It was already confirmed that the concentrations in the chips did not change by flow back within 4 minutes. Gradients of the each sensor output were −62 mV/pH for pH, −62 mV/pNa for the Na^+ cations, −55 mV/pK for the K^+ cations, and −20 mV/pCa for the Ca^{++} cations. These values are close to −59 mV/pX (X=H, Na, K) and −30 mV/pCa predicted from Nernst's equation at 24°C. The pH and concentrations of the Na^+, K^+, and Ca^{++} cations in the standard solution were successfully measured in such a small volume capillary.

4. Conclusion

To achieve our final goal of a creation of healthcare device, elemental technologies of the chip fabrication on polyethylene terephthalate plate (PET) have been studied. In the deep dry-etching process of quartz mold, the issues of concave-shaped defects at the bottom of grooves was overcome by the two steps process with a first step of surface cleaning at a high V_{dc} of 820 V and following step of high selective etching at low V_{dc}=500 V. MPC polymer coating was studied to suppress the adsorption of bio-substance onto the inner wall and good bio-compatibility was confirmed for protein adsorption and blood cell adhesion. Micro capillaries were successfully fabricated on PET by micro molding technique. The ion concentrations of pH, Na^+, K^+, Ca^{++} were measured in the PET chip using embedded ISFET and crown ether ionophores.

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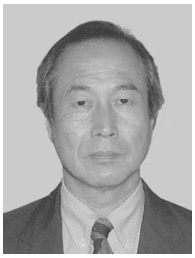

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