Flow production of practical and quantitative capillary driven-flow immune sensing chip using a circumferentially-grooved island micro-surface

Yusuke Fuchiwaki[1*], Kenji Goya[2], Masato Tanaka[3], Hiroki Takaoka[4], Kaori Abe[5], Masatoshi Kataoka [6], Toshihiko Ooie[7]

[1-7]Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), 2217-14, Hayashi-cho, Takamatsu, Kagawa 761-0395, Japan
[1]CEA-LETI, Minatec Campus, 17 rue des Martyrs, 38054 Grenoble Cedex 9, France

Abstract— *Practical immunoassay chip devices are high-priority needs in point-of-care testing (POCT) for rapid diagnoses. Compared to conventional POCT microchip devices, our report describes a manufacturing process involving laser ablation, inkjet deposition, and cover film sealing that is superior to that used for practical mass production of microchip devices, and produces devices capable of sensitive and reproducible measurements using a capillary-flow-driven system at a practical point-of-care setting. To promote sensitivity and reproducibility, circular islands surrounded by 10-µm-deep grooves were prepared to provide uniformity of printed antibody spots on a capillary flow immunoassay chip. The island surface enabled dense antibody fixation due to droplet surface tension as well as antibody determination by enzyme linked immunosorbent assay (ELISA), which demonstrated greater sensitivity than that of a device using a non-island surface. The luminescence intensity of the spots of the carboxyterminal propeptide of type I procollagen (PICP) exhibited a good linear relation with PICP concentration in the range 0-600 ng·mL^{-1}, which is suitable for clinical estimation in blood.*

Keywords— Capillary driven flow, Sensing Chip, grooved Island, immunoassay chip, ELISA.

I. INTRODUCTION

Analysis of biomarkers when the patient is located is known as point-of-care testing (POCT), and needs to be a simple and rapid medical diagnostic method [1-3]. Most of the available POCT devices involve immunoassay systems, and enzyme-linked immunosorbent assay (ELISA) has been utilized as a standard analytical system due to its sensitivity and specificity [4]. Because a practical and simple POCT device must be easily available to the end user in many environments, a capillary-driven flow system is a good candidate because it does not require electrical power and is easy to operate. Indeed, a capillary-driven, flow-based immuno chromatographic assay is considered the simplest commercially available POCT device in the diagnostic market. Therefore, a capillary-driven, flow-based immunoassay system was developed for an ELISA using a piezoelectric inkjet printing system [5-7].

Practical approaches for immobilizing antibodies on a microchannel surface include microfluidic patterning [8], photolithography [9], physical entrapment [10], micro-contact printing [11, 12], and inkjet printing [5-7]. Among these approaches, printing has many advantages, such as simplicity, flexibility, low cost, minimal consumption of reagents, and simultaneous patterning of multiple reagents [13]. Many important studies have been successfully reported by printing technologies, for example, electrohydrodynamic printing [14], water-based inkjet printing [15] and printed wiring board assembly [16]. These features allow the mass production of this device [17]. Although inkjet printing can precisely deposit a drop of antibody solution at the picoliter level on a microchannel surface, a high concentration of antibodies can destabilize the ejection spray from the nozzle head due to its high viscosity, which is a problem for the development of a diagnostic immunoassay system. Accurate ELISA measurements require printing spots of identical size and equivalent amounts of antibody immobilization, which ensures reproducible determination by chemical luminescence.

To overcome the viscosity problem, a solution with a high concentration of antibodies was continuously deposited and immobilized on a circumferentially-grooved island micro surface. The island microstructure enables formation of a spherical droplet by surface tension, producing a precise antibody spot and the same amount of antibody deposition. In addition, the droplet does not adhere to the wall of the microchannel surface and the antibody spots have a uniform size, even when the ejection spray may become uneven.

For the practical mass production of circumferentially-grooved island microchannel surfaces, laser ablation offers many advantages over other micromachining techniques, such as a highly precise, fast, and non-contact fabrication process. A transparent plastic device made of a cyclic olefin copolymer (COC) is more appropriate than other conventional materials, such as silicon and glass, for this purpose because COC possesses versatile thermal and chemical surface properties. An ultraviolet (UV) pulsed laser can directly ablate and locally modify a COC surface, with the best surface quality provided by a KrF laser at 193 nm, a wavelength strongly absorbed by COC [18, 19].

The present study describes a capillary-driven, flow-based immunoassay chip that could be mass produced using a UV pulsed laser system and a piezoelectric inkjet printing system. Ultraviolet pulse lasers and inkjet printers represent mature technologies for which antibody immobilization techniques have been established. In addition, a capillary-driven flow system does not require external power or moving off-chip components. Thus, the development of stable antibody immobilization on a circumferentially-grooved island on a microchannel surface could provide a model diagnostic POCT system that is readily adaptable in many POC situations.

II. EXPERIMENTAL WORK

2.1 Reagents

The monoclonal anti-PICP antibody used was Clone PC8-7 (Takara Bio Inc., Otsu, Japan) and was supplied in a Procollagen type I C-peptide (PIP) EIA Kit (Takara). The blocking and washing solutions were purchased from Sumitomo Bakelite Co. Ltd, Tokyo, Japan. The microchip was refrigerated at a temperature of 4°C when not in use.

2.2 Equipment

The circumferentially-grooved island structure was formed using a nanosecond pulse laser (LPX-305i, Coherent Inc.), at 500 mJ and 25 W. Movement of the XYZ stages was controlled with a computer under the focused laser spot. A video microscope system allowed accurate positioning and *in situ* monitoring of the laser micromachining process.

The inkjet printing head (PulseInjector®: Cluster Technology Co., Ltd., Osaka, Japan) was a piezoelectric-driven drop-on-demand plastic head with an epoxy resin; it exhibited very low biomolecular adsorption compared with glass. The inkjet system deposited and fixed the primary antibodies (Clone PC8-7, Takara) in the spotting buffer (BSX2321, Sumitomo Bakelite Co., Ltd., Tokyo, Japan) on the microchannel surface. Combining the PulseInjector® with a dedicated driving unit (WaveBuilder®: Cluster Technology Co., Ltd., Osaka, Japan) allowed easy adjustment of the ejection drive waveform, ejection rate, and driving voltage, enabling picoliter droplets to be ejected. A derivative drive waveform produced by a function generator with a user-friendly interface provided stable ejection, and the droplets were ejected at a frequency of 1 kHz and jetting voltage of 8 V. The volume of a single discharged droplet of primary antibodies was 50 pL, and 100-1000 shots were discharged onto the surface of the microchannel to provide the optimal volume. The PulseInjector® used a 25-μm diameter ejection hole.

2.3 Microchip fabrication by laser ablation and inkjet printing

A photograph and illustration of the immunoassay chip are shown in Figure 1(a). The chip was fabricated from COC and the surface treated with a polymer solution containing *p*-nitro phenyl ester, a commercial polymer coating from Sumitomo Bakelite Co., Ltd., which binds to amino groups in proteins and immobilizes the antibodies on the microchannel surface [6, 7]. The linear channel was 100-μm deep and 300-μm wide. Sample and reagents were introduced into the respective port of the channel and capillary-driven flow was used to fill the channel (Figure 1(b)). To realize a large quantity of antibody immobilization, the island surface of the 10-μm-deep and 150-μm-wide circular groove on the channel was formed by UV nanosecond laser ablation and antibody droplets were deposited onto the surface of the microchannel using a piezoelectric inkjet system (Figure 1c). Following deposition, the microchip surface was sealed with a cover film made of 33-μm-thick polymethylmethacrylate (Toyo Ink MFG. Co., Ltd., Tokyo, Japan). All samples and reagents for the sandwich ELISA assay were infused into the microchannel from a 1.0–mm-diameter inlet reservoir using a dropper. The outlet reservoir was located on the other side of the microchannel.

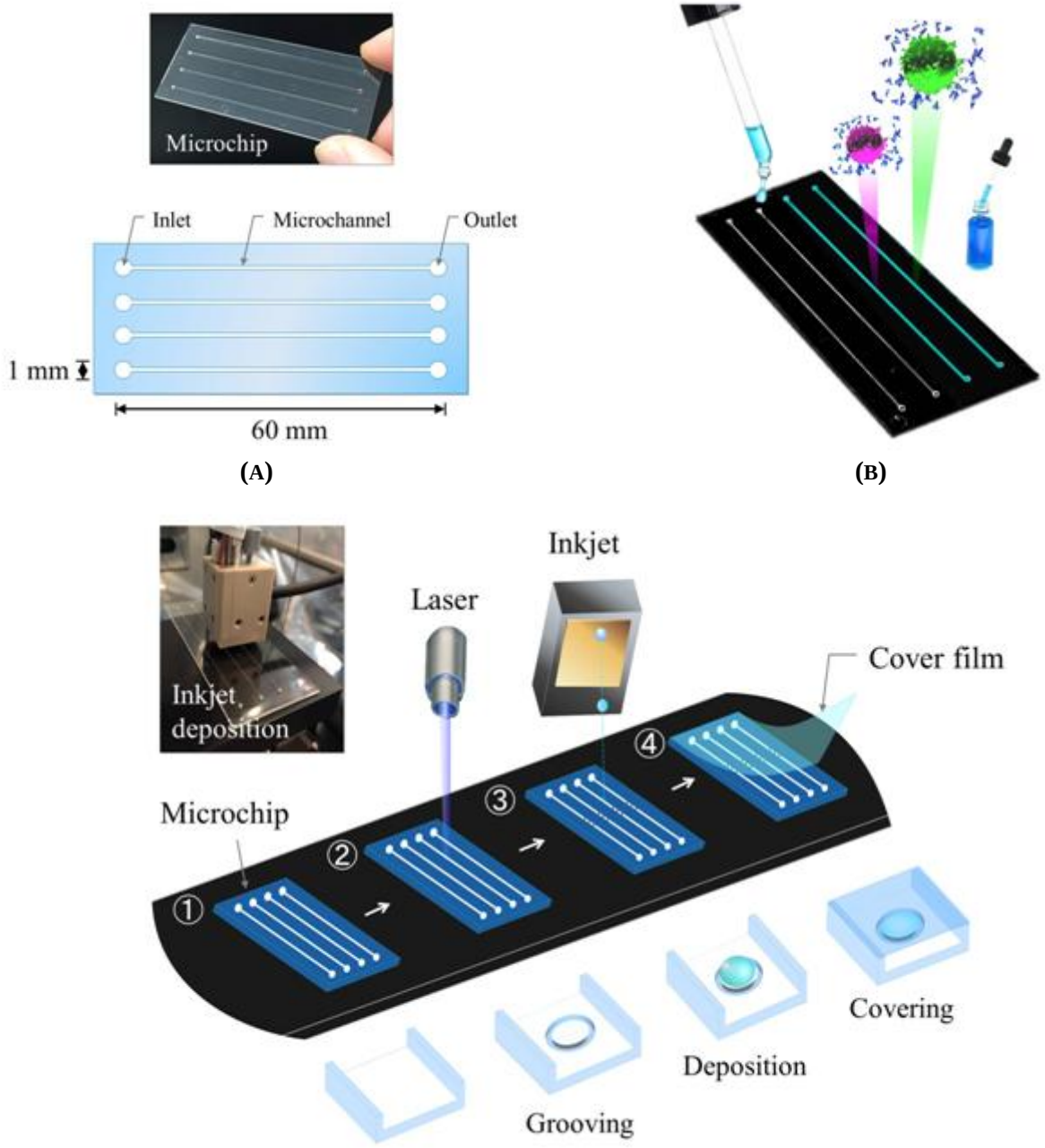

FIGURE 1: SCHEMATIC VIEW OF IMMUNOSENSING MICROCHIP SYSTEM AND CONSECUTIVE ANTIBODY PRINTING SYSTEM. (A) PHOTOGRAPH AND ILLUSTRATION OF MICROCHIP, (B) CAPILLARY-DRIVEN-FLOW SYSTEM USING DROPPER AND LUMINESCENCE DETERMINATION BY ELISA, (C) CONSECUTIVE ANTIBODY PRINTING PROCESS UTILIZING LASER PROCESSING, INKJET DEPOSITION, AND COVER FILM SEALING.

III. RESULTS AND DISCUSSION

3.1 Precise formation of printed antibody spots

Inkjet printing of antibody droplets is advantageous for on-demand manufacturing systems because it enables picoliter quantities to be deposited and reduces the total number of operations. However, depositing a greater concentration of antibody solution onto a microchannel surface with adequate reproducibility is difficult. High sensitivity demands that the primary antibodies are highly concentrated in the microchannel, but this requires a high-viscosity liquid that destabilizes ejection of the antibody droplet from the inkjet head. Such destabilizing ejection causes the droplets to adhere to the wall of the microchannel due to surface tension and has a negative effect on the uniformity of the printed spots. To avoid adherence to the microchannel wall, an island structure on the microchannel surface was formed to allow uniformly printed antibody

spots. A circumferentially-grooved island structure retains the spherical form of a large droplet due to surface tension. A circular grooved island surrounded by 10-μm-deep grooves was prepared and the uniformity of the printed antibody spots was compared for the case with and without islands (Figures 2(a,b)). Three spots were prepared on each microchannel, and 9 spots on 3 microchannels were compared. The results showed that good uniformity was obtained using islands (Figure 2(a)), but not without islands. Surface areas were calculated using Adobe Photoshop, which enables the measurement of the spots using the ruler tool. The surface area of the printed spots with the islands was 6.02 μm^2 with a relative standard deviation (RSD) of 0.9%. The surface area of spots without islands was 5.65 μm^2 with a RSD was 31.3%. This indicates that grooved-island surfaces were effective in providing uniformity of printed spots.

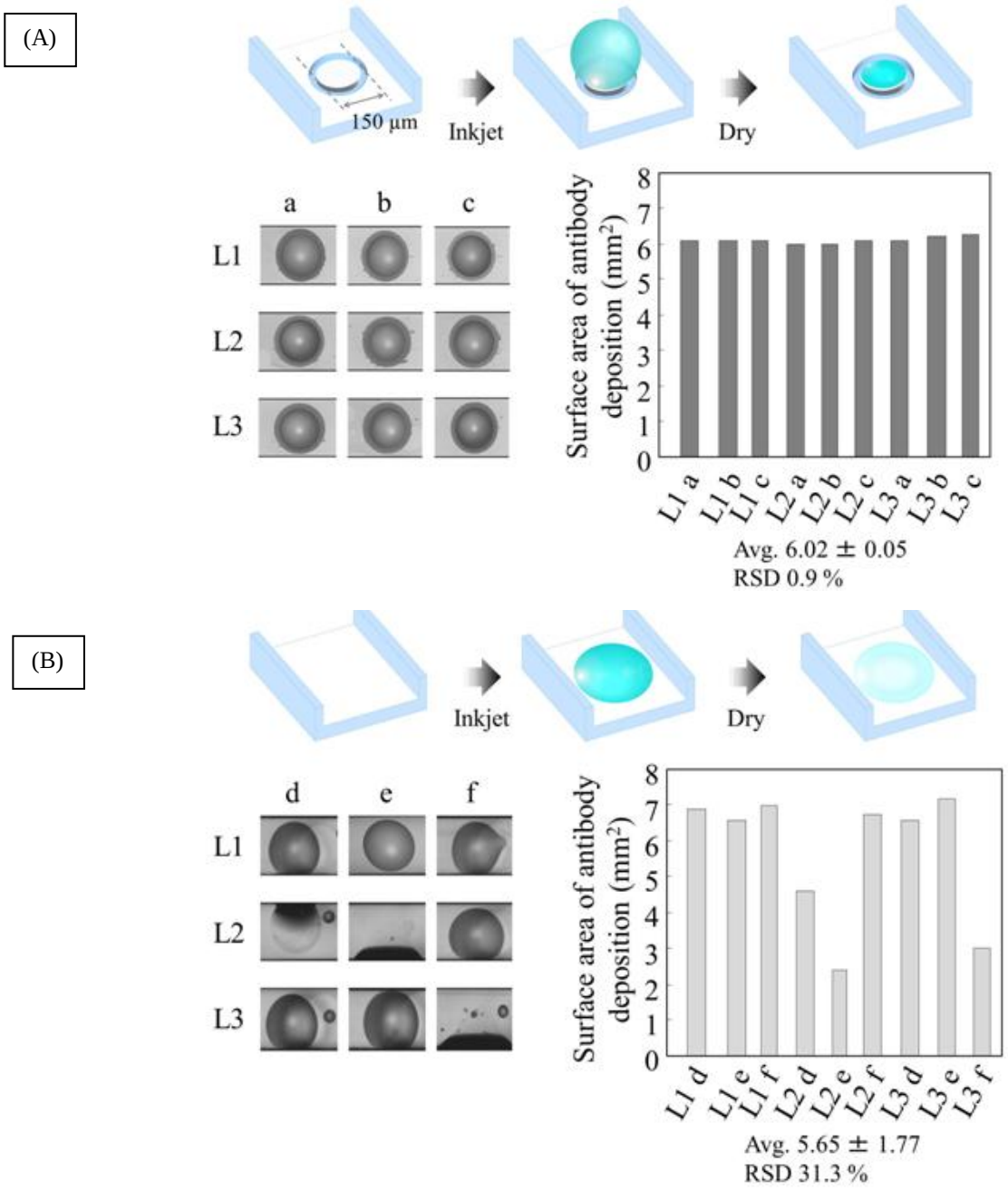

FIGURE 2: COMPARISON OF PRIMARY ANTIBODY DEPOSITION ONTO ISLAND MICROCHANNEL SURFACES AND NON-ISLAND MICROCHANNEL SURFACES. (A) IMAGE OF ANTIBODY DEPOSITION ONTO ISLAND SURFACE AND PHOTOGRAPH SHOWING ANTIBODY SPOTS, (B) IMAGE OF ANTIBODY DEPOSITION ONTO A NON-ISLAND SURFACE AND PHOTOGRAPH SHOWING ANTIBODY SPOTS.

3.2 Dense antibody deposition onto microchannel surface

The grooved-island structure should limit the spot area of antibody inkjet deposition, which allows dense primary antibody deposition and the capture of a large amount of antigen, leading to greater sensitivity. To assess this effect, Cy5 conjugated primary antibodies were prepared by the protocol specified for the Cy5® Fast Conjugation Kit (ab188288, Abcam, Tokyo, Japan), Cy5 was deposited on island and non-island surfaces, and the signal intensities were compared after 10-min incubation at room temperature (Figure 3). The resulting images were measured using a confocal laser scanning microscope (Zeiss LSM 410) equipped with a He-Ne laser (emission wavelength 633 nm) (Zeiss, Oberkochen, Germany). This laser was used to excite the Cy5 fluorophore using a FT 655 dichroic beam splitter and an RG 665 emission long pass filter.

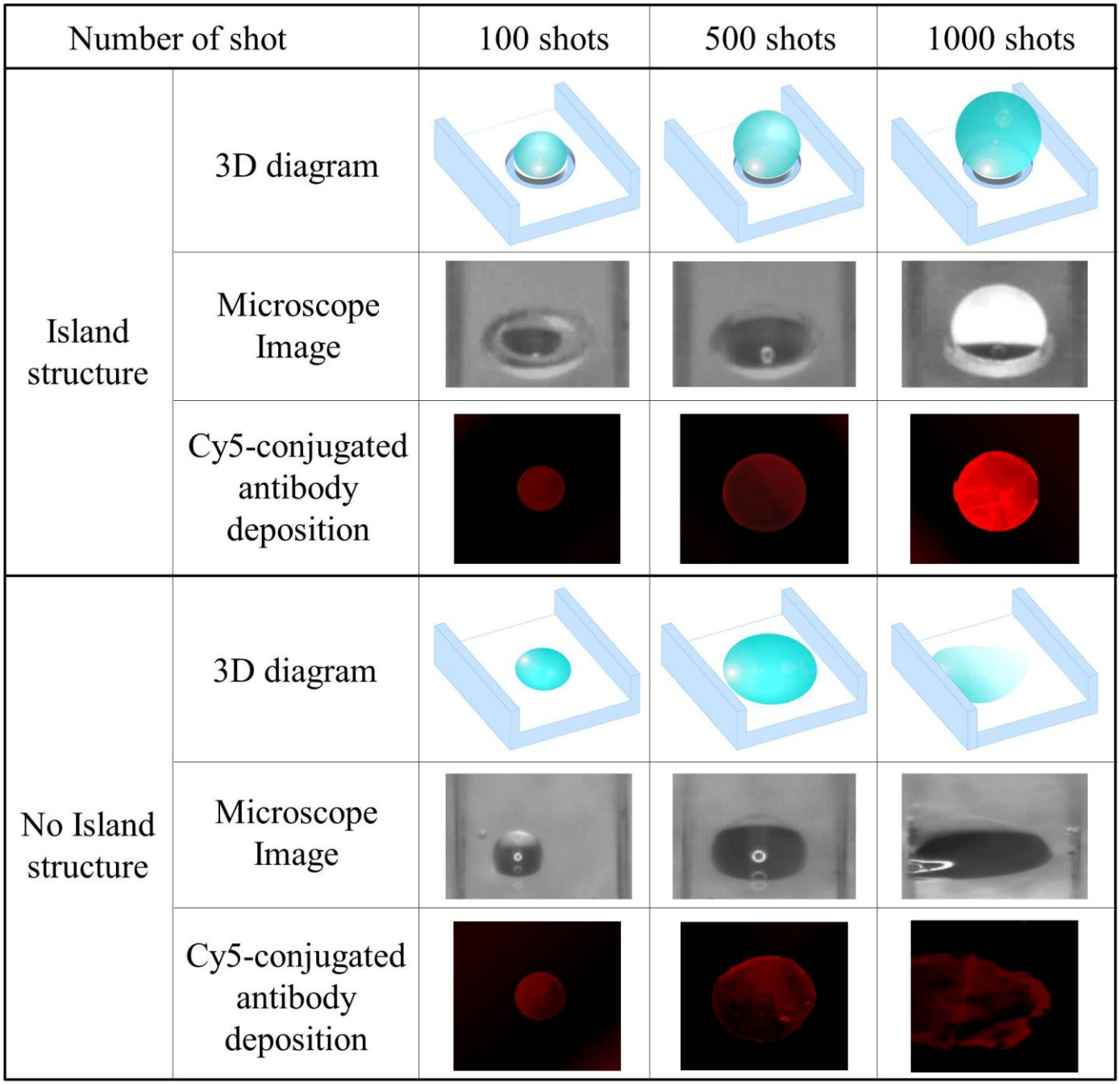

FIGURE 3: SURFACE IMAGES AFTER PRINTING AND MICROSCOPIC IMAGES OF CY5-CONJUGATED ANTIBODY ON COC MICROCHANNEL SURFACE, AND COMPARISON OF THE RELATIVE FLUORESCENCE INTENSITY AT 100, 500, AND 1000 SHOTS.

Signal intensities were compared among 100, 500, and 1000 shots. The intensities of island and non-island spots were similar until 500 shots, but those at 1000 shots were quite different. The non-island surface spots maintained a spherical form up to 500 shots but lost the spherical shape at 1000 shots because the deposited droplets adhered to the wall of the microchannel, resulting in non-uniform spots. In contrast, spots on the island surface maintained a spherical form even at 1000 shots. In addition, the intensity of the spots was highest at 1000 shots, indicating that the antibodies were densely deposited and immobilized on the island surface.

The required sensitivity and reproducibility of sandwich ELISAs necessitates dense immobilization of primary antibodies on a microchannel surface. Although a high concentration of antibody solution destabilizes the ejection stream due to viscosity, this circumferentially-grooved island provided not only uniform printed spots but also dense immobilization of antibodies.

3.3 Capillary-driven, flow-based sandwich ELISA on island surface

A capillary-driven flow system allows low volume, fast reaction time, and simple operation without external power. The potential of a sandwich ELISA on a COC microchip was investigated for rapid and accurate screening (Figures 4(a,b)) [5-7]. Sample and reagent solutions were introduced into the inlet port with a dropper, and the microchannels filled by capillary-driven flow. After filling the channel, excess solution on the inlet port was removed by contact with absorbent paper. The microchannels were emptied using the absorbent paper placed in contact with the outlet port between immunoassay steps.

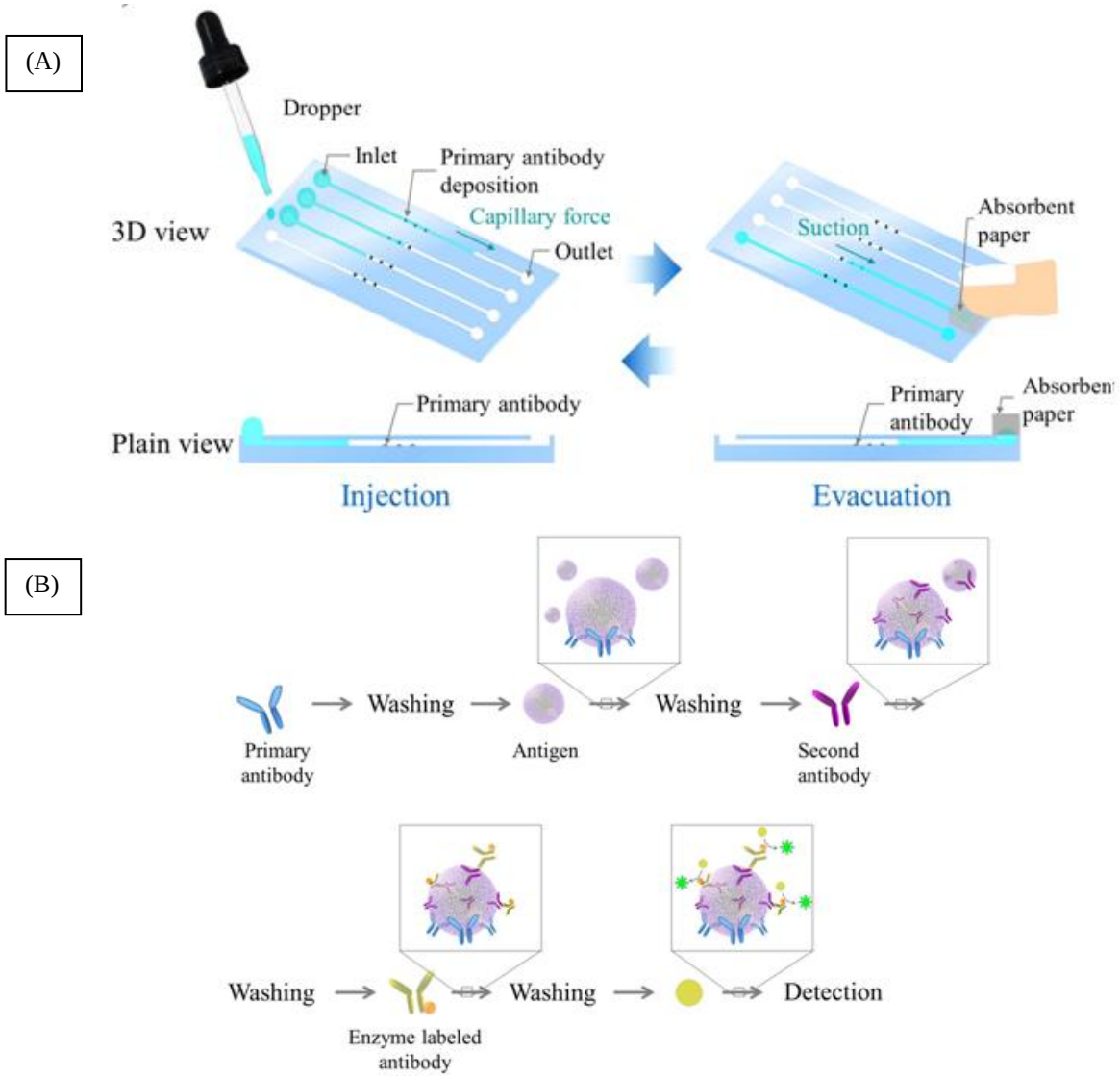

FIGURE 4: IMMUNOASSAY PROCESS BASED ON CAPILLARY-DRIVEN FLOW. (A) SCHEMATIC DIAGRAM OF IMMUNOASSAY USING A DROPPER AND ABSORBENT PAPER, (B) REACTION AND RESULTS OF A SANDWICH ELISA.

Circular islands surrounded by a 10-μm-deep groove allowed uniformity of printed antibody spots and the solutions in the microchannel could be completely replaced by capillary-driven flow. Therefore, the suitability of this approach was examined by measuring the luminescence intensity based on a sandwich ELISA on islanid and non-island surfaces at 0, 100, 500, and 1000 shots (Figures 5(a,b)). The results showed that the luminescence intensity for 5 spots at 300 mg·mL^{-1} PICP varied with the number of shots. The intensities of island and non-island spots were similar at 0, 100, and 500 shots, but were different at 1000 shots. The intensity of island spots was greater than that of non-island spots, even though both surfaces had the same amount of primary antibodies deposited. In addition, the variation in island surface spots at 500 and 1000 shots was less than that of the non-island spots. The variation was determined by the RSD, which for island surface spots was 2.37% at 500 shots and 4.08% at 1000 shots and for non-island spots was 6.86% at 500 shots and 27.2% at 1000 shots. Thus, the island surface prevents antibody droplets from adhering to the wall of the microchannel, allows a dense antibody solution to be deposited and fixed on the island surface. These results indicate that the circumferentially-grooved island surface provides high sensitivity and reproducibility compared to a non-island surface.

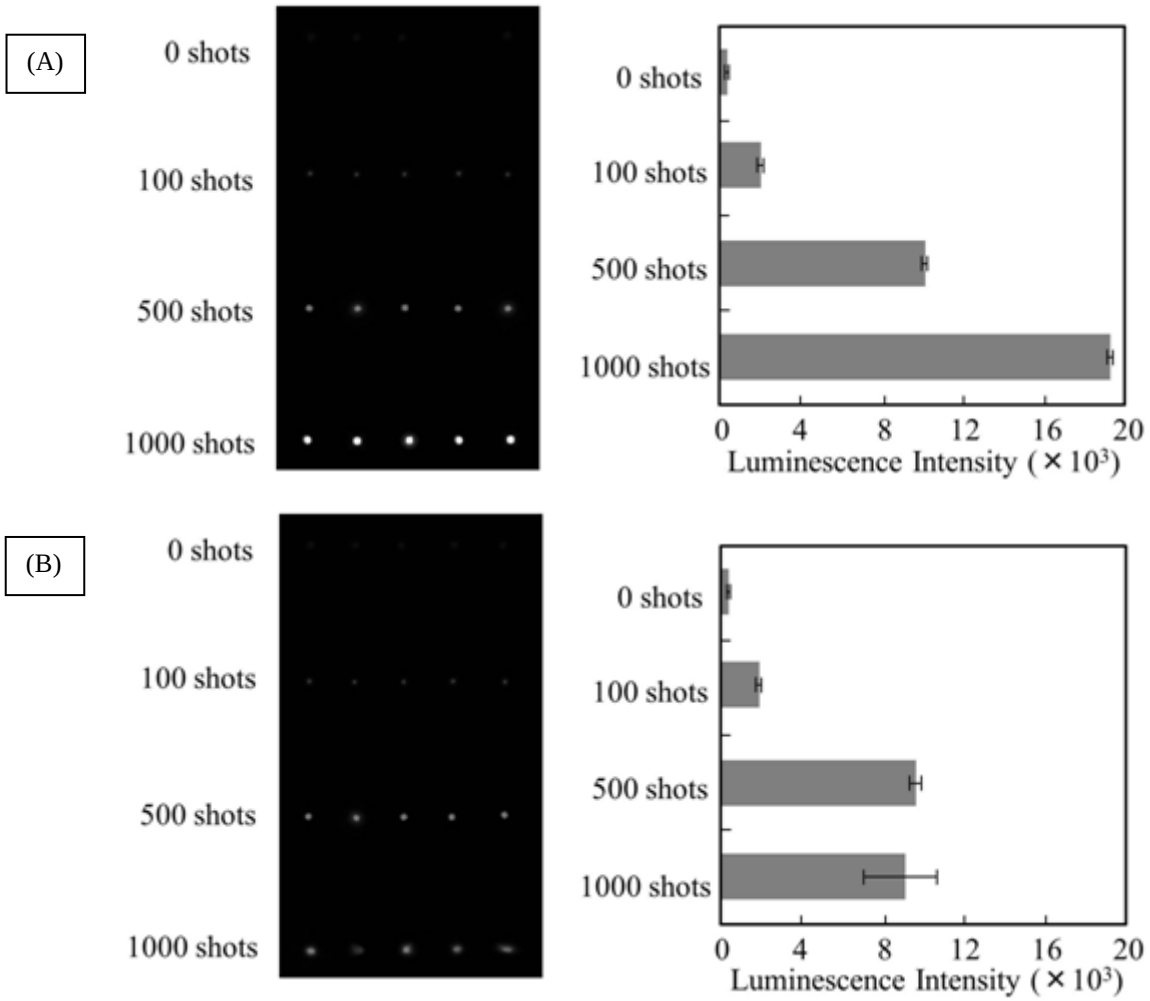

FIGURE 5: QUANTITATIVE SANDWICH ELISA USING ISLAND AND NON-ISLAND MICROCHANNEL SURFACES AT 0, 100, 500, AND 1000 SHOTS. (A) LUMINESCENCE INTENSITY OF ISLAND SURFACE SPOTS AT 0, 100, 500, AND 1000 SHOTS, (B) LUMINESCENCE INTENSITY OF NON-ISLAND SURFACE SPOTS AT 0, 100, 500, AND 1000 SHOTS.

The effects of the capillary-driven flow channel response on 0-600 ng·mL^{-1} PICP were also investigated for island and non-island surfaces (Figure 6). The luminescence intensity for both island and non-island spots increased with PICP concentration. The mean luminescence intensity for 5 different spots was plotted against PICP concentration and provided a linear relation. The intensity of island surface spots was greater overall than that of non-island surface spots. The 0-600 ng·mL^{-1} PICP concentration is adequate for clinical estimation of PICP in blood [20, 21].

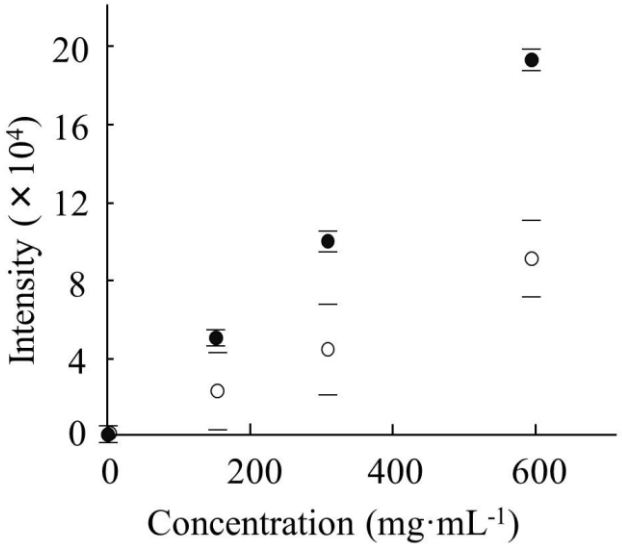

FIGURE 6: QUANTITATIVE SANDWICH ELISA USING CAPILLARY-DRIVEN FLOW CHANNEL RESPONSE FOR 0-600 ng·mL^{-1} PICP ON (●) ISLAND MICROCHANNEL SURFACES AND (○) NON-ISLAND MICROCHANNEL SURFACES.

These results demonstrated that inkjet deposition of a concentrated antibody solution onto a circumferentially-grooved island surface produced a device suitable for a capillary-driven-flow microfluidic immunoassay. Thus, this system enables sensitive and reproducible determination of PIPC concentration and is a promising methodology for a practical POC setting.

IV. CONCLUSIONS

A novel and simple approach for developing a sandwich ELISA based on a capillary-driven flow system for sensitive and reproducible measurement of PICP was developed. The circumferentially-grooved island surface allowed uniformity in printing of antibody spots onto a microchannel surface, enabling concentrated antibody solution fixation due to the droplet's surface tension. Evaluation of the precision of antibody spot printing demonstrated that the variation in the surface area of spots was 0.05% RSD on the island surface and 31.3% for spots on the non-island surface. The spots on the island surface maintained a spherical form even at 1000 shots of antibody inkjet deposition, and produced greater luminescence intensity than those on the non-island surface. In contrast, spots on the non-island surface lost their spherical form after 500 shots and had slightly reduced luminescence intensity. A concentrated antibody solution was deposited at 1000 shots on the island surface and the spot intensity in a sandwich ELISA was greater than that for the non-island surface. The intensity of 0-600 ng·mL^{-1} PICP spots showed a concentration-dependent relation and were greater overall than that for spots on non-island surfaces.

This microfluidic ELISA system using capillary-driven flow possesses many advantages for POC situations, such as simple operation, minimal sample consumption, and rapid results. The antibody immobilization process was performed consecutively using laser processing, inkjet deposition, and cover film sealing. This process is driven by mechatronic operation, which provides high throughput. This report demonstrates the potential of practical POC chip fabrication in the future.

ACKNOWLEDGEMENTS

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (No. 16K05833).

REFERENCES

[1] P. St-Louis, Status of point-of-care testing: promise, realities, and possibilities, Clin. Biochem 33 (2000) 427–440.

[2] D. Mark, S. Haeberle, G. Roth, F. von Stetten, R. Zengerle, Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications Chemical Society Reviews 39 (2010) 1153–1182.

[3] P. von Lode, Point-of-care immunotesting: approaching the analytical performance of central laboratory methods, Clin Biochem 38 (2005) 591–606.

[4] J. Ramo´n-Azco´n, R. Galve, F. Sa´nchez-Baeza, M.P. Marco, Development of an Enzyme-Linked Immunosorbent Assay for the Determination of the Linear Alkylbenzene Sulfonates and Long-Chain Sulfophenyl Carboxylates Using Antibodies Generated by Pseudoheterologous Immunization, Analytical Chemistry 78 (2006) 71–81.

[5] Y. Fuchiwaki, Y. Yabe, Y. Adachi, M. Tanaka, K. Abe, M. Kataoka, T. Ooie, Inkjet monitoring technique with quartz crystal microbalance (QCM) sensor for highly reproducible antibody immobilization, 219 Sens. Actuators A (2014) 1–5.

[6] S. Yatsushiro, R. Akamine, S. Yamamura, M. Hino, K. Kajimoto, K. Abe, H. Abe, J. Kido, M. Tanaka, Y. Shinohara, Y. Baba, T. Ooie, M. Kataoka, Quantitative Analysis of Serum Procollagen Type I C-Terminal Propeptide by Immunoassay on Microchip, 6 PLoS ONE (2011) e18807

[7] K. Abe, Y. Hashimoto, S. Yatshushiro, S. Yamamura, M. Bando, Y. Hiroshima, J. Kido, M. Tanaka, Y. Shinohara, T. Ooie, T. Baba, M. Kataoka, Simultaneous Immunoassay Analysis of Plasma IL-6 and TNF-α on a Microchip, 8 PLoS ONE (2013) e53620

[8] Z. Yin, S.C. Tao, R. Cheong, H. Zhu, A. Levchenko, An integrated micro-electro-fluidic and protein arraying system for parallel analysis of cell responses to controlled microenvironments, Integrative Biology 2 (2010) 416–423.

[9] L.K. Fiddes, H.K.C. Chan, B. Lau, E. Kumacheva, A.R. Wheeler, Durable, region-specific protein patterning in microfluidic channels, Biomaterials 31 (2010) 315–320.

[10] K. Sato, M. Yamanaka, H. Takahashi, M. Tokeshi, H. Kimura, Microchip-based immunoassay system with branching multichannels for simultaneous determination of interferon-gamma. Electrophoresis 23 (2002) 734–739.

[11] A. Khademhosseini, K.Y. Suh, S. Jon, G. Eng, J. Yeh, A soft lithographic approach to fabricate patterned microfluidic channels, Analytical Chemistry 76 (2004) 3675–3681.

[12] T.F. Didar, A.M. Foudeh, M. Tabrizian, Patterning multiplex protein microarrays in a single microfluidic channel, Analytical Chemistry 84 (2012) 1012–1018.

[13] C. Wei, H. Qin, C. P. Chiu, Y. S. Lee, J. Dong, Drop-on-demand E-jet printing of continuous interconnects with AC-pulse modulation on highly insulating substrates, 37, Journal of Manufacturing Systems (2015) 505-510

[14] Y. Han, C. Wei, J. Dong, Droplet formation and settlement of phase-change ink in high resolution electrohydrodynamic (EHD) 3D printing, 20, Journal of Manufacturing Processes (2015) 485–491

[15] Y. Gua, A. Wua, H. Sohnb, C. Nicolettia, Z. Iqbalc, J. F. Federici, Fabrication of rechargeable lithium ion batteries using water-based inkjet printed cathodes, 20, Journal of Manufacturing Processes (2015) 198-205.

[16] S.H.G. Teng, S.S. Garimella, Manufacturing Cost Modeling in Printed Wiring Board Assembly, 17, Journal of Manufacturing Systems (1998) 87-96.

[17] F. Belachew, L. Christina, K. Liisa, J. Leena-Sisko, J. Heli, H. Leena, Patterned Immobilization of Antibodies within Roll-to-Roll Hot Embossed Polymeric Microfluidic Channels, 8 PLoS ONE (2013) e68918

[18] P. Irina-Alexandra, S. Alexandros, B. Giannis, K. Gabriella, G. Savas, Nanosecond and femtosecond UV laser ablation of polymers: Influence of molecular weight, Applied Surface Science 255 (2009) 9856–9860

[19] T. Daniel, T. Thanongsak, C. Joaquim, O. Tugrul, Nanosecond pulsed laser micromachining of PMMA-based microfluidic channels, J. Manufacturing Process 14 (2012) 435-442.

[20] A.M. Parfitt, L. S. Simon, A. R. Villanueva and S. M. Krane, Correlation with iliac bone formation rates and comparison with total alkaline phosphatase, J. Bone Miner. Res. 2 (1987) 427-436.

[21] K. Kohda, N. Ito, M. Ohwada, K. Morita, N. Watanabe, Y. Kohgo, Y. Mogi and Y. Niitsu, Diagnostic value of measurement of serum type I procollagen carboxy terminal peptides in patients with scirrhous carcinoma of the stomach. Gut 32 (1991) 624-629.