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Improving the dielectric properties of an electrowetting-on-dielectric microfluidic device with a low-pressure chemical vapor deposited Si_3N_4 dielectric layer

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Dielectric breakdown is a common problem in a digital microfluidic system, which limits its application in chemical or biomedical applications. We propose a new fabrication of an electrowetting-on-dielectric (EWOD) device using Si_3N_4 deposited by low-pressure chemical vapor deposition (LPCVD) as a dielectric layer. This material exhibits a greater relative permittivity, purity, uniformity, and biocompatibility than polymeric films. These properties also increase the breakdown voltage of a dielectric layer and increase the stability of an EWOD system when applied in biomedical research. Medium droplets with mouse embryos were manipulated in this manner. The electrical properties of the Si_3N_4 dielectric layer—breakdown voltage, refractive index, relative permittivity, and variation of contact angle with input voltage—were investigated and compared with a traditional Si_3N_4 dielectric layer deposited as a plasma-enhanced chemical vapor deposition to confirm the potential of LPCVD Si_3N_4 applied as the dielectric layer of an EWOD digital microfluidic system. © *2015 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4915613]

I. INTRODUCTION

On a digital microfluidic (DMF) platform, discrete droplets of a sample fluid are individually processed. Instead of conventional pumping, i.e., pressurizing one end of a fluid-filled channel to create the flows, other methods are employed to actuate individual droplets. Electrowetting-on-dielectric (EWOD)[1,2] is the most widely accepted mechanism of actuation for a DMF system, employed to create, to transport, to separate, and to merge μL- and nL-size droplets.[3,4] These DMF systems are widely applied in chemical and biochemical reactions[5,6] or point-of-care diagnosis research such as chemical extraction,[7] protein crystallization,[8] immunoassay[9,10] and cell-based assay[11] because of its advantages—precise control of a droplet, decreased reagent volume, decreased power consumption, rapid analysis, and amenability to portable devices. An EWOD chip is composed of actuation electrodes, a dielectric layer, and a hydrophobic layer. An electric field generated from the electrode induces an electric double layer in the dielectric layer and an aqueous droplet. The droplet becomes attracted; the contact angle between the droplet and the hydrophobic surface decreases. The relation between applied voltage and variation of contact angle is formulated on combining equations of Lippmann and Young, shown in (1).

$$\cos\theta - \cos\theta_0 = \frac{\varepsilon_r \varepsilon_0}{2\gamma_{\mathrm{lg}} d} V^2. \quad (1)$$

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In which θ and θ_0 are the contact angles corresponding to applied voltage V and 0 vote, respectively; ε_r and ε_0 are the relative permittivity of solid dielectric and air, respectively; γ_{lg} is the liquid–gas surface/interfacial tensions, and d is the thickness of the dielectric layer.[12,13]

The application of an EWOD dielectric in biomedical diagnosis is invariably limited by its quality. Dielectrics fail when an input voltage exceeds a breakdown voltage.[14] Traditional EWOD devices are fabricated through indium-tin-oxide (InSnO) patterning and deposition of dielectric and hydrophobic layers. Polymeric films such as perylene and photoresist commonly serve as dielectrics because they are conveniently deposited; even though these films are easily deposited, pinholes and impurities in the polymeric dielectrics decrease the breakdown voltage during manipulation of droplets. SiO_2, Si_3N_4 films deposited by plasma-enhanced chemical vapor deposition (PECVD) or metal-oxide films of some types exhibit a greater breakdown voltage than a polymeric dielectric, but their deposition is slow and expensive. Through a traditional deposition of a dielectric layer by means of spin coating or PECVD, the surface becomes irregular because of the electrode gap on the surface of the chip, as shown in Fig. 1(a). Pinholes might also be formed, and thereby decrease the breakdown voltage of a dielectric. Some researchers tested other dielectric materials or electrolytes to increase the breakdown voltage and the quality of an EWOD device. For example, Lee and Kong used a multi-layered dielectric layer composed of amorphous fluoropolymer (AF1600), Si_3N_4, and titania (TiO_2) to enhance the dielectric quality.[15] Tanu and Cheng used graphene as an electrode material for a decreased leakage current and diminished electrolysis.[16] Our research team has also increased the stability of EWOD devices for the manipulation of biomedical droplets with spin-on glass (SOG) and Si_3N_4 films as the composite dielectric.[17] As a liquid material, SOG can fill the pinholes on a dielectric layer to increase slightly the dielectric strength, but SOG provides only a limited improvement in the manipulation of an EWOD biomedical sample. We have hence designed another dielectric fabrication of EWOD devices, using low-pressure chemical vapor deposited (LPCVD) Si_3N_4 at small stress and low pressure as a smooth dielectric layer of high quality, as shown in Fig. 1(b), which exhibits a large permittivity, small rate of etching, a

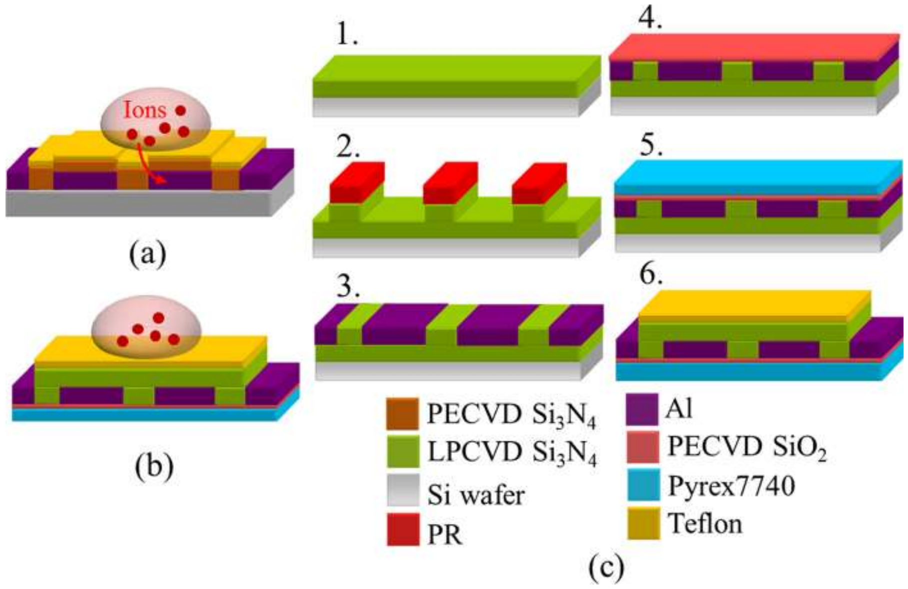

FIG. 1. Schematic illustration of the EWOD device. (a) The irregular dielectric layer was penetrated by ions in the solution during droplet manipulation. (b) LPCVD Si_3N_4 dielectric layer with a smooth surface and few pinholes. (c) Top-down fabrication of a EWOD microfluidic chip with a LPCVD Si_3N_4 dielectric layer: 1. LPCVD Si_3N_4 deposited on Si wafer. 2. Photoresist patterning and Si_3N_4 etching with RIE. 3. Al electrode layer deposition. 4. SiO_2 adhesion layer deposited with PECVD for bonding. 5. Wafer bonded with the new substrate (Pyrex glass) by anodic bonding. 6. Si layer etched away with KOH, and Si_3N_4 on the connecting pad removed with RIE. Teflon was spin-coated as the hydrophobic layer.

smooth surface, a small density of pinholes,[18,19] and sustained a greater voltage during operation compared with PECVD Si_3N_4.

We propose a top-down fabrication of an EWOD microfluidic chip with a LPCVD dielectric layer of Si_3N_4 yielding a smooth dielectric layer and an increased breakdown voltage applicable during manipulation of a droplet. The electrical properties such as breakdown voltage, refractive index, relative permittivity, and variation of contact angle with input voltage were tested and compared with PECVD Si_3N_4 dielectric layers. This dielectric EWOD chip of high quality has been applied to manipulate mouse embryos in medium droplets, thus solving the serious problem of dielectric breakdown of a traditional EWOD chip. Bioreagents such as a cell-culture medium or an enzyme solution contain electrolytes at large concentrations; their ions in the solution decrease much the breakdown voltage and make the traditional EWOD chip easily damaged during droplet manipulation. Our experimental results indicate that the EWOD chip with a LPCVD dielectric layer has the potential for chemical and biomedical applications.

II. MATERIALS AND METHODS

A. Coplanar electrodes EWOD chip

A commonly used EWOD system comprises two parallel plates: Electrodes to actuate sample droplets are patterned on a bottom plate; the reference electrode is designed on a top plate. The sample droplets can be manipulated through electrowetting between two EWOD plates in a sandwich structure. In this work, we used an EWOD chip of another kind with coplanar electrodes. The electrodes are designed on only the bottom plate of a EWOD DMF chip[20,21] to actuate droplets. The top plate has a hydrophobic layer coated on a glass substrate, which is not involved in droplet actuation; this feature provides much potential for integrating additional functions on the chip. A traditional EWOD device was fabricated through patterning of InSnO electrodes, deposition of a PECVD Si_3N_4 dielectric layer, and spin-coating with a hydrophobic layer.

B. Fabrication of an EWOD device with a LPCVD Si_3N_4 dielectric layer

Si_3N_4 of small stress deposited with LPCVD is formed in a reaction between dichlorosilane and ammonia at a 700–800 °C, which deposition results in a pure and uniform Si_3N_4 film. This temperature of deposition of LPCVD Si_3N_4 is so high as to limit its application in EWOD devices, which causes problems in direct deposition of a LPCVD Si_3N_4 film on an EWOD chip after standard etching of EWOD electrodes. The fabrication proposed in this work enables us to make an EWOD chip with a LPCVD Si_3N_4 dielectric layer in a process that begins with a 4-in. Si wafer from ePAK International, Inc., on which is deposited a LPCVD Si_3N_4 layer ($1\,\mu m$) (Fig. 1(c-i)). Before deposition of electrodes, the pattern is defined with photoresist (AZ® 5214 E) from MicroChemicals GmbH through photolithography; trenches of thickness 70 nm are etched on the Si_3N_4 film with a reactive-ion etching (RIE) system (Fig. 1(c-ii)). An Al layer is made in the tranches as the electrodes with electron beam physical vapor deposition. The thickness of trenches and Al are carefully calculated to make the surface of the chip very flat after patterning the electrodes (Fig. 1(c-iii)). Then, one layer of SiO_2 (thickness 300 nm) is deposited on the flat surface with PECVD as adhesive layer (Fig. 1(c-iv)), which can be attached with a glass substrate (Pyrex 7740) from Swiftek Corp. by anodic bonding in a vacuum chamber (Fig. 1(c-v)). After attachment, the silicon is removed with KOH wet etching (30% at 95 °C) to release the EWOD chip. The Si_3N_4 on the connecting pad is eventually removed with RIE for an electrical connection; Teflon (AF601) from DuPont is spin-coated on the EWOD chip as a hydrophobic layer (Fig. 1(c-vi)).

C. Digital microfluidic system

The DMF system includes an AC power amplifier (A303, A. A. Lab System Ltd., Israel) for voltage output and a relay box (PXI-5402/2569, National Instruments Corp., U.S.) for signal exchange. A LabView program is used to control the relays and electrodes. A clamp (CCNL-050–47-FRC) and a cable bought from Yokowo Co. in Japan are used to connect the EWOD chip and the entire system; a digital CCD microscope (Retro Zoom 65, Pentad Scientific Corp., Taiwan) serves as detector.

D. Measurement of breakdown voltage and contact angle

The breakdown voltage of the dielectric layers was determined with visual observation[22] and the recovery of contact angle. Once the dielectric broke during the manipulation of the solution droplets, the contact angle was no longer recovered because the hydrophobic layer, dielectric layer, and even the electrodes were penetrated and destroyed by ions inside the solution droplets. At the same time, the solution droplets were electrolyzed and bubbles were generated on the surface between the droplet and the chip. During the breakdown, some bubbles were too small to be observed with a digital CCD camera; for this reason, both visual observation and recovery of the contact angle were used to measure the breakdown. Water (deionized) and a culture medium (human tubal fluid, HTF) served as samples in the measurement of the breakdown voltage and contact angle. A droplet (5 μl) was placed on the electrode pair; an applied voltage was increased from 0 to 350 V. The voltage was turned off between each point of contact angle data to test whether the contact angle recovered. The contact angle and breakdown voltage of LPCVD Si_3N_4 (1 μm), PECVD Si_3N_4 (0.5 μm), and PECVD Si_3N_4 (1 μm) dielectric EWOD chips were measured and compared.

E. Mouse embryos pretreatment and culture medium

Imprinted-control-region (ICR) mice were used in this research. Female mice were injected intraperitoneally with pregnant mare serum gonadotrophin (PMSG, 5 IU, Sigma, USA). 48 h after injection, the female mice were further injected intraperitoneally with human chonionic gonadotrophin (hCG, 5 IU; Sigma, USA). Immediately after the latter injection, each female mouse was caged with a male mouse for mating. 36 h after hCG injection, the female mice were sacrificed; the oviducts were dissected, then placed in a Petri dish containing HTF medium. Embryos for an embryonic development culture were released on tearing the oviducts. HTF, which is a synthetic culture medium for embryo development and gamete manipulation, has abundant electrolytes including NaCl, $NaHCO_3$, and sodium lactate. These ions of the electrolyte and the biomolecules secreted by mouse embryos in the medium greatly decrease the breakdown voltage of the dielectric layer.[22] We thus used the HTF medium droplets containing mouse embryos to manipulate EWOD droplets to test the mechanical properties of the LPCVD Si_3N_4 dielectric layer.

III. RESULTS

A. EWOD microfluidic chip based on a LPCVD Si_3N_4 dielectric layer

A LPCVD Si_3N_4 dielectric layer (1 μm) was built on an EWOD chip; Fig. 2 shows images of the chip after fabrication. A single chip has 23 coplanar-electrode pairs, each of which is composed of two electrodes ($1.5 \times 0.75\,\text{mm}^2$). The depths of the gaps between electrodes were measured by the surface profiler (Dektak XT, Bruker Crop., U.S.) to discuss the surface roughness of different dielectrics. ΔH is defined as the depth of a gap on dielectric layers as in Fig. 3(a); and the measurement results are shown in Fig. 3(b). An 700.0 Å electrode layer caused the $\Delta H = 426.8$ Å after the Si_3N_4 dielectric was deposition by PECVD; however, the same thickness of the electrode layer caused smaller $\Delta H = 38.9$ Å on the LPCVD Si_3N_4 dielectric layer when the new fabrication is used. The significant deviation of the gap depths suggests that the surface of the EWOD chip with LPCVD dielectric layer is flatter than the traditional EWOD chips.

B. Breakdown voltage measurement

The breakdown voltage of the dielectric layer was determined with visual observation and recovery of the contact angle. The breakdown voltages of a LPCVD Si_3N_4 dielectric EWOD

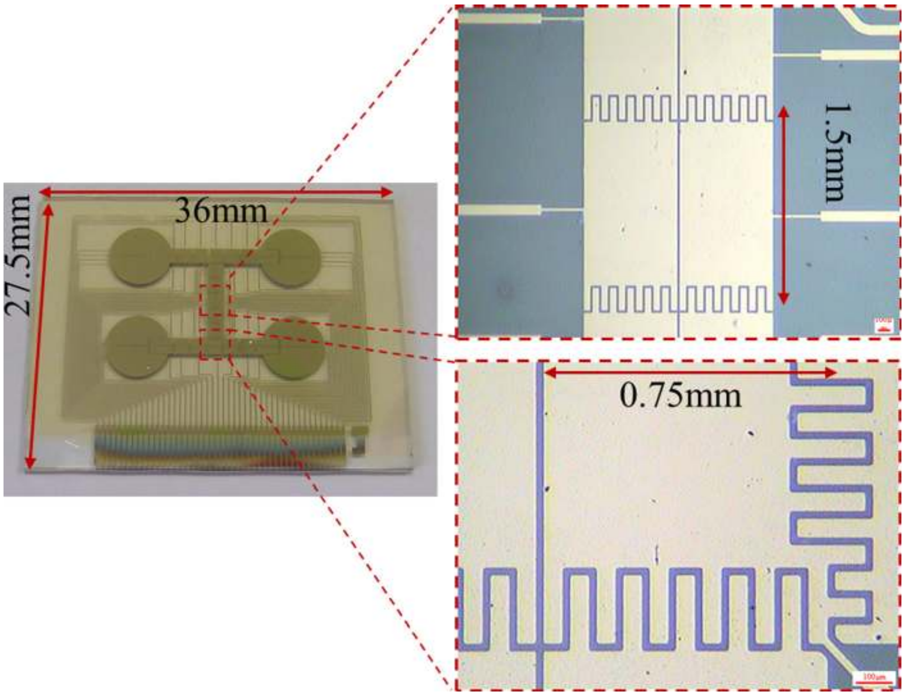

FIG. 2. Images of the LPCVD Si_3N_4 dielectric EWOD chip after fabrication. The left image is the full EWOD chip after dicing, and the right images are the electrode pairs of the EWOD chip.

chip and PECVD Si_3N_4 dielectric EWOD chips of two thicknesses are compared in Table I. When deionized water droplets are used as the sample, the breakdown voltages of 0.5 μm and 1 μm PECVD Si_3N_4 dielectrics are 150 V and 270 V, respectively. This result shows that the breakdown voltage is related with the thickness of dielectric layer, which is similar to the work done by Hong Liu.[14] Comparing with 1 μm PECVD dielectric, the LPCVD dielectric exhibited better dielectric quality and stability of the same thickness. The breakdown voltage increased from 240 V to 350 V during manipulation of a droplet of HTF medium.

C. Measurement of contact angle

The electrical properties were compared with a PECVD Si_3N_4 dielectric EWOD chip (1 μm). Table II shows the refractive index (n) and relative permittivity (ε_r) of LPCVD and PECVD Si_3N_4 dielectrics; the relation between ε and n at the same frequency is

$$\varepsilon_r = n^2. \tag{2}$$

Decreasing the contact angle of one end of a droplet in contact with a surface will generate a gradient of surface energy. A net force arises between the ends of the droplet due to the unbalanced surface tension forces acting along the contact line, which induces the movement of the droplet[23,24] According to Eq. (1), it required less applied voltages to achieve the same magnitude of contact angle change as the ε_r of dielectric layer is increased. Figure 4 shows the electrowetting curve of deionized water and HTF medium droplets on EWOD chips 1-μm PECVD Si_3N_4 and 1-μm LPCVD Si_3N_4 dielectric layer. The voltage required to induce specific contact angle variation can be calculated from the polynomial fitting of the electrowetting curve in Fig. 4. 181.0 V and 164.3 V was required to induce 20° contact angle variation of a deionized water droplet on PECVD Si_3N_4 dielectric and LPCVD Si_3N_4 dielectric EWOD chip, respectively; 155.1 V and 101.1 V was required to induce 20° contact angle variation of a HTF droplet on PECVD Si_3N_4 dielectric and LPCVD Si_3N_4 dielectric EWOD chip, respectively. The results shown in Table III indicate that the LPCVD Si_3N_4 dielectric EWOD chip required less applied

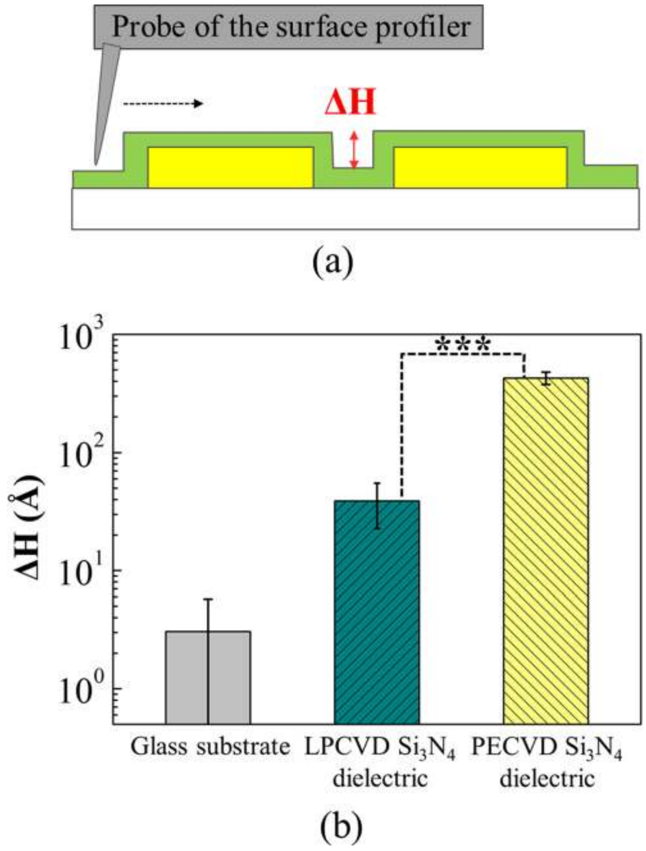

FIG. 3. The surface profiler measurement of the gap depths between electrodes. (a) The definition of the ΔH. (b) Measurement results of glass substrate, LPCVD Si_3N_4 EWOD chip and PECVD Si_3N_4 EWOD chip. ***$p < 0.001$ (Student's t-test), indicating statistically significant differences between the LPCVD and PECVD dielectrics.

TABLE I. Breakdown voltage of LPCVD Si_3N_4 and dielectric EWOD chip of PECVD and varied thickness.

	H_2O (V)	HTF (V)
PECVD Si_3N_4 (0.5 μm)	150	<150
PECVD Si_3N_4 (1 μm)	270	240
LPCVD Si_3N_4 (1 μm)	>350	350

TABLE II. Refractive index and relative permittivity of LPCVD and PECVD Si_3N_4 dielectrics.

	LPCVD Si_3N_4	PECVD Si_3N_4
Refractive index n	2.15	1.91
Relative permittivity ε_r	4.63	3.63

voltage to achieve the same magnitude of contact angle change for both deionized water and HTF samples.

D. Biomedical application of the DMF chip

HTF is a synthetic culture medium for embryo development and gamete manipulation; the ions of the electrolyte and the biomolecules secreted by the mouse embryos in a medium

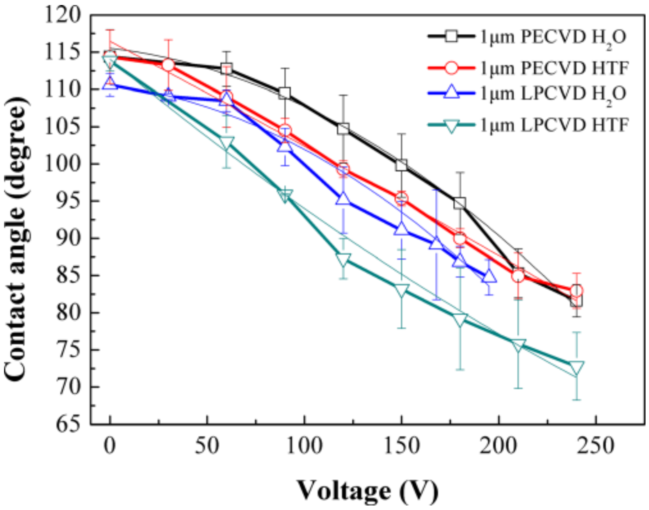

FIG. 4. Electrowetting curve and polynomial fitting of deionized water and HTF medium droplets on EWOD chips 1-μm PECVD Si_3N_4 and 1-μm LPCVD Si_3N_4 dielectric layer.

TABLE III. The voltage required to induce 10° or 20° contact angle variation of a deionized water and HTF droplet on different dielectric EWOD chips.

	$\Delta\theta$ (deg)	H_2O (V)	HTF (V)
PECVD Si_3N_4 (1 μm)	10	124.4	86.3
LPCVD Si_3N_4 (1 μm)		109.1	49.1
PECVD Si_3N_4 (1 μm)	20	181.0	155.1
LPCVD Si_3N_4 (1 μm)		164.3	101.1

greatly decrease the breakdown voltage of traditional dielectric layers. Figs. 5(a)–5(c) show the dielectric breakdown of the EWOD chip with a traditional PECVD dielectric layer when a HTF medium droplet is manipulated. To solve the problem of dielectric breakdown, we used a LPCVD dielectric layer EWOD microfluidic chip to manipulate droplets of HTF medium. Fig. 6(a) is a schematic diagram of the electrode pattern; a HTF sample droplet containing mouse embryos was manipulated between five electrode pairs as shown in Figs. 6(b)–6(d). After manipulation steps were repeated, the LPCVD dielectric layer was still intact, which indicates that the chips are reusable in biomedical sample manipulation after standard chip cleaning.

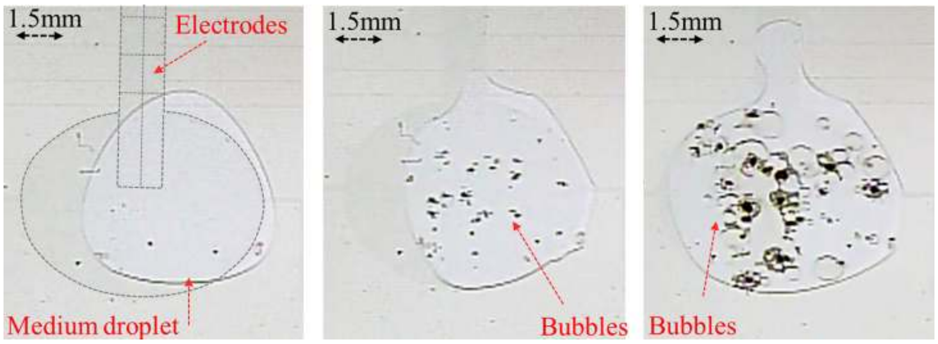

FIG. 5. Dielectric breakdown of a traditional EWOD chip when the manipulated solution contained abundant electrolytes. (a) No voltage applied at $t = 0$ s. (b) 80 V applied at $t = 10$ s. (c) 80 V applied at $t = 20$ s.

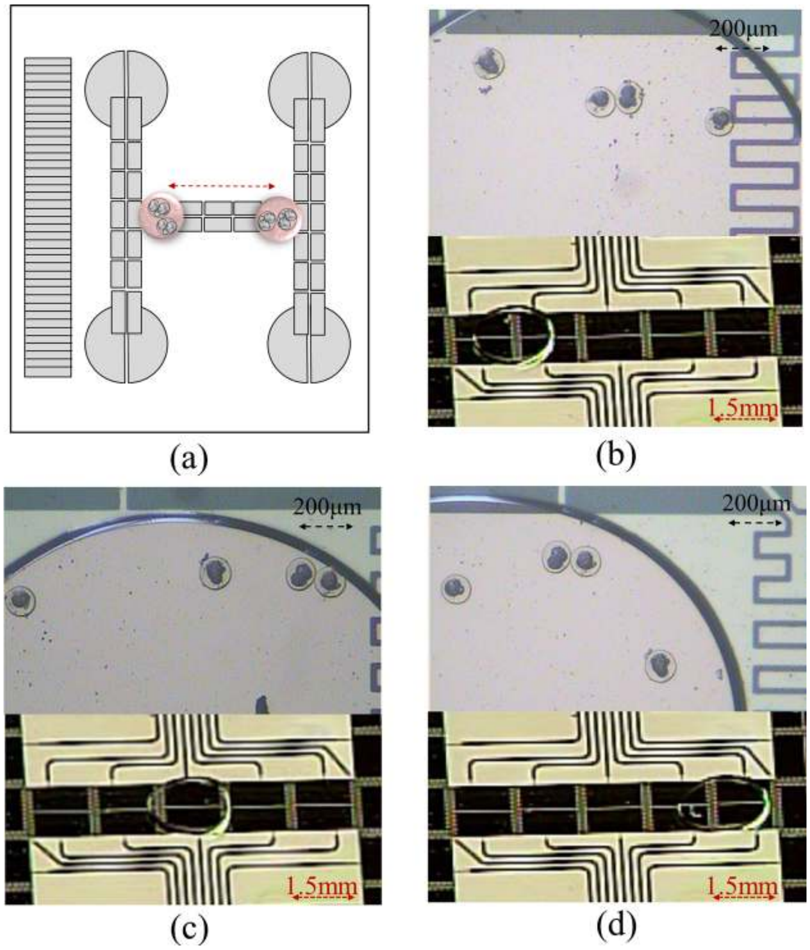

FIG. 6. A HTF medium droplet with mouse embryos was manipulated with a LPCVD Si_3N_4 dielectric EWOD device. (a) Schematic diagram of the electrode pattern; medium droplet manipulated between five electrode pairs. (b)–(d) Images of a HTF droplet with mouse embryos inside, the droplet could be repeatedly manipulated cross five electrodes by the chip.

IV. PROPOSED EWOD SYSTEM FOR BIOMEDICAL APPLICATIONS

DMF system has wide applications in different areas especially in chemical, biological, and biomedical researches. The DMF serves as a convenient sample processing tool in the experiment. The miniature microfluidic devices suggest some inherent advantages in performance, such as small reagent consumption and fast reaction time. However, the applications of the DMF device in biomedical diagnosis are invariably limited by its quality. Dielectrics fail when an input voltage exceeds a breakdown voltage. The device lost its function once the dielectric layer breaks. When the DMF device is used in bioreactions, the solutions with high electrolyte concentrations decrease much the breakdown voltage and make the traditional EWOD chip easily damaged during droplet manipulation. Many researches tried to minimize the applied voltage[25] and increase the breakdown voltage;[14] Decreasing the thickness of the dielectric layer is able to lower the applied voltage according to Lippmann and Young's equation. However, the breakdown voltage is also lowered when the dielectric thickness decreases.[14] The pinholes and defects more easily appear in a thinner film during deposition process. Instead of decreasing the thickness of dielectric layer, some researchers used different dielectric materials to decrease the voltage for droplet manipulation.[26] Lin *et al.* used 17 V_{rms} for manipulation of a water droplet.[27] Lee and Kong used a multi-layered dielectric layer composed of amorphous fluoropolymer (AF1600), Si_3N_4, and titania (TiO_2) to enhance the dielectric quality.[15] SOG was sometimes deposited on the SiO_2, Si_3N_4, and parylene C films as the composite dielectrics.[17] The liquid SOG can effectively fill the pinholes on the dielectric film to reduce dielectric breakdown.[28] We made effort to find a stable dielectric material exhibits a greater relative permittivity, purity, uniformity, and biocompatibility. The Si_3N_4 film deposited in high temperature and clean LPCVD furnace makes it a better dielectric material than others. Our experimental results have indicated that the EWOD chip with a LPCVD Si_3N_4 dielectric layer has the potential for chemical and biomedical applications.

Many researchers tried to use a microfluidic device for cell-based applications because the designed microfluidic system has the advantages of little consumption of reagents, rapid reactions, and modest cost. When cells were manipulated or cultured in a continuously flowing channel, a contamination problem existed, however, and it was difficult to manipulate a single cell or few cells in the continuous microfluidic devices. Digital microfluidic systems tend to solve the contamination issue and to provide the feasibility for manipulation of single-cell droplets. Much EWOD research was devoted to issues related to cells.[11,29] With the increasing clinical utilization of assisted reproductive technology (ART), scientists exert efforts to improve the rates of success following assisted reproduction. In the *in vitro* fertilization (IVF) technique, an ovum is removed from a woman's ovaries and manually fertilized with sperm in a tube. Successfully inseminated embryos would then be cultured in an oil-covered static medium microdroplet on a Petri dish for 2–6 days and eventually implanted back into the female's uterus. The repeated manual pipetting and washing procedures might cause the changing of the environment on the embryo development.[30] A continuously flowing microchannel system has been used in IVF to increase the sample quality and culture environment.[31–33] Based on the advantages of a DMF system, the expectation could be an applicable tool for IVF to increase the rate of fertilization in vitro and to provide a stable culture environment for the formation of blastocysts. Derek *et al*. have reported the development of a DMF for the vitrification of mammalian embryos for applications in clinical IVF.[34] Now, our research team is also using the DMF platform in IVF researches, and this is the reason why the mouse embryos were used in this experiment for droplets manipulation. This high-quality dielectric DMF chip has so far proved its potential for chemical and biomedical sample processing; we expect to use this device in future research on IVF.

V. CONCLUSION

In this research, an EWOD digital microfluidic chip of high quality with a LPCVD Si_3N_4 film as dielectric layer was achieved with patterning of Al EWOD electrodes, substrate bonding, and silicon back-etching. The pure and uniform low-stress Si_3N_4 deposited with LPCVD increased the relative permittivity of the dielectric layer. The mechanical properties of LPCVD Si_3N_4 are able to increase the breakdown voltage of dielectric layer and made the EWOD system more stable when used for sample droplets manipulation. In the contact angle measurement, the LPCVD Si_3N_4 dielectric chip requires less applied voltages to achieve the same magnitude of contact angle comparing with the PECVD Si_3N_4 dielectric layer chip made through traditional fabrication. The results suggest that the EWOD device proposed in this research not only increases the breakdown voltage but also lowers the sample manipulating voltage. This dielectric EWOD chip of high quality has been applied to manipulate the solutions containing electrolytes and biomolecules at large concentrations. Mouse embryos release biomolecules and waste into the medium during cell culture. When traditional EWOD devices were used for the cell culture medium manipulation, the dielectrics made by PECVD Si_3N_4, parylene C, or photoresist were easily broken and unstable for repeatedly droplet manipulation. The EWOD chip with a LPCVD Si_3N_4 dielectric layer proposed here has solved the problem of dielectric breakdown when applied in the manipulation of biomedical reagents. Droplets of cell-culture medium with mouse embryos can be repeatedly and stably manipulated with this chip. This high-quality dielectric DMF chip has so far proved its potential for chemical and biomedical applications; We expect to use this device in future research on IVF.

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[1] J. Lee, H. Moon, J. Fowler, T. Schoellhammer, and C.-J. Kim, "Electrowetting and electrowetting-on-dielectric for microscale liquid handling," Sens. Actuators, A **95**, 259–268 (2002).
[2] W. C. Nelson and C.-J. C. Kim, "Droplet actuation by electrowetting-on-dielectric (EWOD): A review," J. Adhes. Sci. Technol. **26**, 1747–1771 (2012).
[3] S. K. Cho, H. J. Moon, and C. J. Kim, "Creating, transporting, cutting, and merging liquid droplets by electrowetting-based actuation for digital microfluidic circuits," J. Microelectromech. Syst. **12**, 70–80 (2003).
[4] J. Lee, H. Moon, J. Fowler, K. Chang-Jin, and T. Schoellhammer, "Addressable micro liquid handling by electric control of surface tension," in *14th IEEE International Conference on Micro Electro Mechanical Systems, 2001* (IEEE, 2001), pp. 499–502.
[5] Y. L. Hsieh, T. Y. Ho, and K. Chakrabarty, "A reagent-saving mixing algorithm for preparing multiple-target biochemical samples using digital microfluidics," IEEE Trans. Comput.-Aided Des. Integr. Circuits Syst. **31**, 1656–1669 (2012).
[6] T. H. Lin and D. J. Yao, "Applications of EWOD systems for DNA reaction and analysis," J. Adhes. Sci. Technol. **26**, 1789–1804 (2012).
[7] P. A. L. Wijethunga, Y. S. Nanayakkara, P. Kunchala, D. W. Armstrong, and H. Moon, "On-chip drop-to-drop liquid microextraction coupled with real-time concentration monitoring technique," Anal. Chem. **83**, 1658–1664 (2011).
[8] W. C. Nelson, I. Peng, G. A. Lee, J. A. Loo, R. L. Garrell, and C. J. Kim, "Incubated protein reduction and digestion on an electrowetting-on-dielectric digital microfluidic chip for MALDI-MS," Anal. Chem. **82**, 9932–9937 (2010).
[9] A. H. C. Ng, K. Choi, R. P. Luoma, J. M. Robinson, and A. R. Wheeler, "Digital microfluidic magnetic separation for particle-based immunoassays," Anal. Chem. **84**, 8805–8812 (2012).
[10] L. Zhu, Y. Y. Feng, X. Y. Ye, J. Y. Feng, Y. B. Wu, and Z. Y. Zhou, "An ELISA chip based on an EWOD microfluidic platform," J. Adhes. Sci. Technol. **26**, 2113–2124 (2012).
[11] D. Witters, N. Vergauwe, S. Vermeir, F. Ceyssens, S. Liekens, R. Puers *et al.*, "Biofunctionalization of electrowetting-on-dielectric digital microfluidic chips for miniaturized cell-based applications," Lab Chip **11**, 2790–2794 (2011).
[12] B. Berge, "Electrocapillarity and wetting of insulator films by water," C. R. Acad. Sci., Ser. II **317**, 157–163 (1993).
[13] D. Orejon, K. Sefiane, and M. E. R. Shanahan, "Young-Lippmann equation revisited for nano-suspensions," Appl. Phys. Lett. **102**, 201601 (2013).
[14] H. Liu, S. Dharmatilleke, D. K. Maurya, and A. A. O. Tay, "Dielectric materials for electrowetting-on-dielectric actuation," Microsyst. Technol. **16**, 449–460 (2010).
[15] J. K. Lee, K.-W. Park, H.-R. Kim, and S. H. Kong, "Dielectrically stabilized electrowetting on AF1600/Si3N4/TiO2 dielectric composite film," Sens. Actuators, B **160**, 1593–1598 (2011).
[16] T. Xuebin, Z. Zhixian, and C. M. Ming-Cheng, "Electrowetting on dielectric experiments using graphene," Nanotechnology **23**, 375501 (2012).
[17] S. Hsien-Hua, S. Tsung-Yao, C. Hwan-You, and Y. Da-Jeng, "SNP detection based on temperature-controllable EWOD digital microfluidics system," in *Nanotechnology Materials and Devices Conference (NMDC), 2012* (IEEE, 2012), pp. 92–95.
[18] G. Beshkov, S. Lei, V. Lazarova, N. Nedev, and S. S. Georgiev, "IR and Raman absorption spectroscopic studies of APCVD, LPCVD and PECVD thin SiN films," Vacuum **69**, 301–305 (2002).
[19] A. Stoffel, A. Kovacs, W. Kronast, and B. Muller, "LPCVD against PECVD for micromechanical applications," J. Micromech. Microeng. **6**, 1–13 (1996).
[20] U. C. Yi and C. J. Kim, "Characterization of electrowetting actuation on addressable single-side coplanar electrodes," J. Micromech. Microeng. **16**, 2053–2059 (2006).
[21] L. Davoust, Y. Fouillet, R. Malk, and J. Theisen, "Coplanar electrowetting-induced stirring as a tool to manipulate biological samples in lubricated digital microfluidics. Impact of ambient phase on drop internal flow pattern," Biomicrofluidics **7**, 044104 (2013).
[22] S. Choi, Y. Kwon, Y. S. Choi, E. S. Kim, J. Bae, and J. Lee, "Improvement in the breakdown properties of electrowetting using polyelectrolyte ionic solution," Langmuir **29**, 501–509 (2013).
[23] M. K. Chaudhury and G. M. Whitesides, "How to make water run uphill," Science **256**, 1539–1541 (1992).
[24] M. G. Pollack, "Electrowetting-based microactuation of droplets for digital microfluidics," Ph.D. thesis (Duke University, 2001).
[25] S. Berry, J. Kedzierski, and B. Abedian, "Low voltage electrowetting using thin fluoroploymer films," J. Colloid Interface Sci. **303**, 517–524 (2006).
[26] Y. Y. Lin, R. D. Evans, E. Welch, B. N. Hsu, A. C. Madison, and R. B. Fair, "Low voltage electrowetting-on-dielectric platform using multi-layer insulators," Sens. Actuators, B **150**, 465–470 (2010).
[27] Y. Y. Lin, E. R. F. Welch, and R. B. Fair, "Low voltage picoliter droplet manipulation utilizing electrowetting-on-dielectric platforms," Sens. Actuators, B **173**, 338–345 (2012).
[28] C.-Y. Lee, J.-Y. Wang, Y. Chou, M. Y. Liu, W.-F. Su, Y.-F. Chen *et al.*, "Enhanced ultraviolet electroluminescence from ZnO nanowires in TiO2/ZnO coaxial nanowires/poly(3,4-ethylenedioxythiophene)-poly(styrene-sulfonate) heterojunction," J. Appl. Phys. **107**, 034310 (2010).
[29] S. Srigunapalan, I. A. Eydelnant, C. A. Simmons, and A. R. Wheeler, "A digital microfluidic platform for primary cell culture and analysis," Lab Chip **12**, 369–375 (2012).

[30] Y. Xie, F. Wang, E. E. Puscheck, and D. A. Rappolee, "Pipetting causes shear stress and elevation of phosphorylated stress-activated protein kinase/jun kinase in preimplantation embryos," Mol. Reprod. Dev. **74**, 1287–1294 (2007).

[31] P. Hester, S. Clark, E. Walters, D. Beebe, and M. B. Weeler, "Enhanced cleavage rates following in vitro maturation of pig oocytes within polydimethylsiloxane-borosilicate microchannels," Theriogenology **57**, 723 (2002).

[32] H. Sano, K. Matsuura, K. Naruse, and H. Funahashi, "Application of a microfluidic sperm sorter to the *in-vitro* fertilization of porcine oocytes reduced the incidence of polyspermic penetration," Theriogenology **74**, 863–870 (2010).

[33] H.-H. Shen, H.-Y. Tsai, and D.-J. Yao, "Single mouse oocyte encapsulated in medium-in-oil microdroplets by using a polydimethylsiloxane microfluidic device," Sens. Mater. **26**, 85–94 (2014).

[34] D. G. Pyne, L. Jun, M. Abdelgawad, and S. Yu, "Automated vitrification of mammalian embryos on a digital microfluidic device," in *IEEE 27th International Conference on Micro Electro Mechanical Systems (MEMS), 2014* (IEEE, 2014), pp. 829–832.