Hydrophobic silicone elastomer chamber for recording trajectories of porcine motile sperms without adsorption

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Abstract

Porcine motile sperms adhere to hydrophilic materials such as glass and plastics. The adsorption of sperms to a hydrophobic polydimethylsiloxane (PDMS) membrane was less compared with that to glass. Significant decreases in linear velocity and amplitude of lateral head displacement of motile porcine sperms were due to adsorption of the head and/or neck to the hydrophilic substrates. Because of the elasticity of PDMS, we propose that a PDMS membrane should be used for conventional Computer Assisted (Aided) Sperm Analysis. To investigate dynamics of motile porcine sperms with microfluidics, we do not recommend plasma treatment to bond PDMS and glass in the microchannel preparation; instead, we suggest that a PDMS molding process without plasma treatment be used for preparation of microfluidic channels.

Key Words: porcine sperm motility, silicone elastomer, adsorption, trajectories

Introduction

Sperm motility analysis is a representative method for evaluation of male fertility, since motility is correlated with viability [1-4]. The conventional method commonly referred to as Computer Assisted (Aided) Sperm Analysis to record motility and linear velocity (LV) utilizing a microscope with a charge coupled device [1-3]. The advantage of CASA over manual observation is the absence of subjective calibration [1]. It is difficult to record trajectories of motile porcine sperms and investigate LV related to fertility because they often adsorb to glass and plastic which remarkably decreases their motility. To record trajectories of motile sperms and investigate their velocity distribution quantitatively under a microscope, the use of transparent materials that do not promote adsorption of motile sperms is necessary.

For observation of motile sperms, diluted semen is usually sandwiched between hydrophilic glass slides [5]. Trajectories of human and bull sperms can be recorded using this glass preparation; however, it is difficult to record the trajectory of motile porcine sperms, because they adsorb to glass and hydrophilic plastics such as poly(methyl methacrylate) (PMMA). We hypothesized that it may be possible to record the trajectory of these sperms using transparent materials with high hydrophobicity represented by a high contact angle (>90 degrees) to water droplets (Figure 1).

Hydrophobic silicone elastomer polydimethylsiloxane (PDMS), used at a contact angle of 110 degrees, is a key material capable of extending device applications for reproductive technology because it is nontoxic, transparent, inexpensive, and easy to handle [6-11]. PDMS microfluidic devices prepared by molding the microstructure and bonding the cured structure with a cover or slide glass can be used for manipulation and culture of cells to investigate their physiological functions [6-8]. Microfluidic channels are used for in vitro fertilization in case of

low sperm number (>10^5 cells) and for in vitro culture to mimic the oviduct environment [6, 9, 10]. Lopez-Garcia *et al*. observed bull sperm motions without adsorption to glass substrates in glass-bottom PDMS microchannels [11]. Despite previous documented applications, there are few practical applications for PDMS membranes combined with CASA in routine analysis.

In this study, using a PDMS preparation, we could record the trajectories of motile sperms without adsorption and compare the sperm motility parameters. Furthermore, we reported that to observe motile porcine sperm dynamics using microfluidic channels, the PDMS chamber should be prepared without oxygen (O_2) plasma treatment. This technology can be applicable for recording live imaging and mechanics of porcine motile sperms that adhere to hydrophilic materials [12].

Materials and Methods

The diluted semen samples were transported to the laboratory within 2 h of collection at 26–32 C. Spermatozoa were diluted at a concentration of 1×10^8 cells/ml with modified Modena solution containing 5 mM cysteine and 20% (v/v) boar seminal plasma. This preparation follows that outlined in previous reports [12].

Preparation of the Silicone Elastomer Chamber

A PMMA mold was fabricated for the recording chamber using a conventional mechanical microdrilling process (MDX-40; Roland, Osaka, Japan). PDMS slabs and membranes with microstructures were prepared by casting prepolymer (TSE 3032; Momentive Performance Materials, Tokyo, Japan) at a 1:10 curing agent-to-base ratio against positive relief features [9, 13]. The prepolymer was cured at 70 C for 1 h. Cured PDMS has a highly cross-

linked 3D structure. To investigate differences in hydrophilic materials, the PDMS surface was treated with plasma cleaner (PDC-32G; Harrick Plasma Inc., Ithaca, NY, USA). PDMS microchannels without O_2 plasma treatment were prepared by 1-step curing.

Motile Sperm Trajectory Recording

Using a BM ×10 lens (Nikon Co Ltd., Tokyo Japan), sperm and particle motion were tracked with a sperm motility analysis system (SMAS) (Kaga Electronics Co. Ltd., Tokyo Japan). Frame rate of sperm tracking using SMAS was 60 per second.

Statistical Analysis

The Student's *t*-test was used to determine differences in LV and average amplitude of lateral head displacement (ALHD) between groups. $P < 0.05$ was considered significant.

Results and Discussion

Comparison of Absorption of Porcine Motile Sperms to Several Materials

Almost all the sperms adsorbed to slide glass 15 min after preparation, while the number of sperms adsorbed to the PDMS membrane decreased (Figure 2). We found that more than half of the motile sperms adsorbed to the hydrophilic substrate treated with O_2 plasma 10 min after preparation. Adsorption properties of porcine sperms to transparent materials are summarized in Table 1. We observed that the hydrophobicity of substrate materials is important for adsorption. To prevent adherence, the preparation should be made such that the contact angle of the materials with water is more than 80 degrees.

Performance of Optimized Chambers and Sperm Motility Parameters

We compared LV distribution of motile porcine sperms inside chambers to quantitatively investigate motility changes in relation to adsorption to hydrophilic substrates. The average LVs 1 and 15 min after glass preparation and 15 min after PDMS preparation were 34.3, 7.9, and 33.3 (μm/second), respectively. There was no significant difference between the distribution 1 min after glass preparation and 15 min after PDMS preparation ($P > 0.05$). The average amplitude of ALHD 1 and 15 min after glass preparation and 15 min after PDMS preparation were 5.4, 2.1, and 3.7 (μm), respectively ($P < 0.05$). We suggest that the significant decreases in LV and ALHD were due to adsorption of the head and/or neck to the hydrophilic substrate (Figure 2 and 3).

PDMS Preparation for Sperm Motility Analysis

Figure 4 shows the PDMS preparation for conventional CASA. To prevent overlap of motile sperm images, we designed the preparation to decrease focal depth. Semen was sandwiched with 2 PDMS sheets (Figure 4A, B, and C). Due to the elastic property of PDMS, the lower membrane was deflected by the weight of the semen. The flat surface of the upper membrane was turned up and faced across it. The thickness of the semen was approximately 0.1 mm, and we confirmed no overlap of sperm images (Figure 4C). With this preparation, we were able to record trajectories and analyze the distribution of sperm motility parameters.

Live Imaging Application in PDMS Microchannels

Microchannels for sperm motility analysis can be easily prepared by PDMS soft lithography; however, there is a problem with microchannel preparation after O_2 plasma treatment since hydrophilicity of PDMS increases. We compared sperm adsorption to a PDMS

129 microchannel with a cover glass on the bottom bonded with O_2 plasma treatment (Channel A) to
130 a PDMS microchannel without O_2 plasma treatment (Channel B) (Figure 5A and B). After
131 washing with diluted water, the number of adhered porcine sperms on the bottom of channels A
132 and B were approximately 700 and 100 (number/mm^2), respectively (Figure 5C and D). This
133 result is consistent with the LV distributions (Figure 3A). When preparing the microchannel, the
134 standard bonding method for PDMS and glass by O_2 plasma or UV light cannot be used due to
135 increases in hydrophilicity of the materials [15].

136 Microchannels are important in sperm motility analysis because they allow the
137 trajectories of bull and human motile sperms to be evaluated [11,16]. Interestingly, it has been
138 reported that bull sperms tend to preferentially swim along the walls and that this phenomena
139 occurs during flow and no flow [11]. Koyama *et al*. designed a microfluidic device for sperm
140 chemotaxis with 3 inlets and 3 outlets to make a gradient in the chemotaxis chamber [17]. The
141 PDMS substrate and glass coverplate were bonded by exposure to air plasma that would decrease
142 the hydrophobicity of PDMS; a treatment which would not be suitable for analysis of porcine
143 sperm chemotaxis. Our results suggest that a PDMS-bottom microchannel without hydrophilic
144 treatments, such as O_2 and air plasma, can be used to investigate the chemotaxis and fluid
145 mechanics of porcine motile sperms.

146 In conclusion, porcine motile sperms adhere to hydrophilic materials such as glass and
147 PMMA. The adsorption of sperms to the hydrophobic PDMS membrane was lesser than that to
148 glass. Because of the elasticity of PDMS, we propose the use of this preparation for conventional
149 CASA to reduce overlap of motile sperm images, which are artifacts of CASA. Because of the
150 potential sperm adhesion, we do not recommend O_2 plasma treatment for bonding PDMS and
151 glass during investigation of the dynamics and chemotaxis of motile porcine sperms using

152 microfluidics. We suggest that only a PDMS molding process is suitable for preparation of
153 microfluidic channels to be used with motile porcine sperms.
154

155 Table 1. Comparison of contact angle and adsorption of motile sperms to transparent materials

	Contact angle of water (deg)	Adsorption	References
Glass	30	Yes	5
PMMA	70	Yes	14
PDMS	110	No	15
PDMS after O_2 plasma treatment	50	Yes	15

156

157

FIGURE CAPTIONS

Figure 1. Definition of contact angles.

Figure 2. Differences in trajectories of fresh porcine sperms on (A) glass, (B) glass after 15 min, and (C) PDMS membrane after 15 min.

Figure 3. (A) LV and (B) ALH distributions recorded on glass and PDMS preparations.

Figure 4. PDMS chambers (A) membrane for preparation having an area of 0.5×1 mm^2, (B) the method to sandwich semen between the 2 membranes, (C) cross-sectional image for recording the trajectories of motile sperms. Dark and light gray objects represent the PDMS membrane and semen, respectively. (D) Sperms in this preparation in CASA.

Figure 5. (A) Live imaging of porcine motile sperms in PDMS microchannels. (B) Adsorption of sperms after experiments on (C) glass and (D) PDMS membrane.

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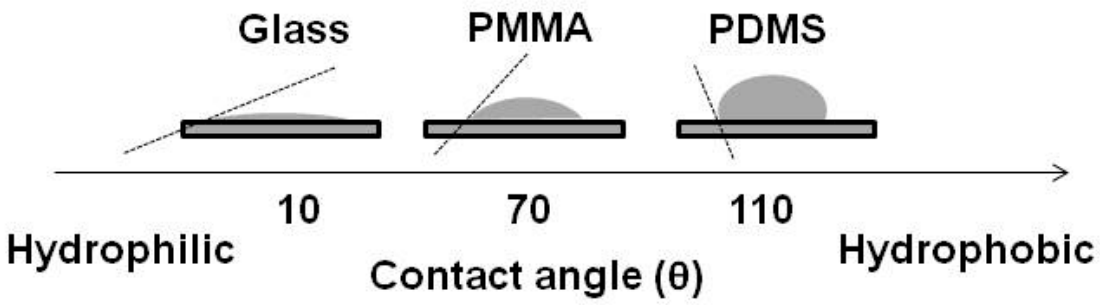
Glass
PMMA
PDMS
10
70
110
Hydrophilic
Hydrophobic
Contact angle (θ)

Figure 1

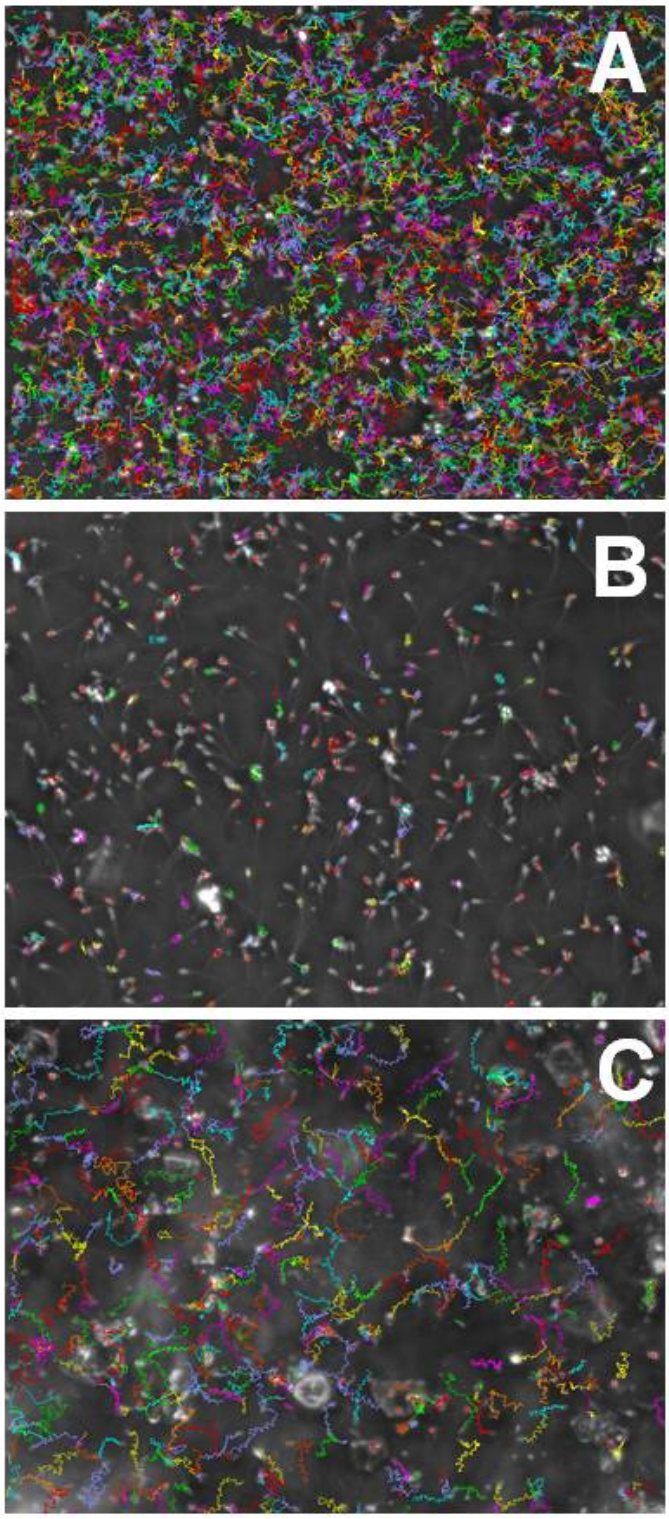

Figure 2

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222

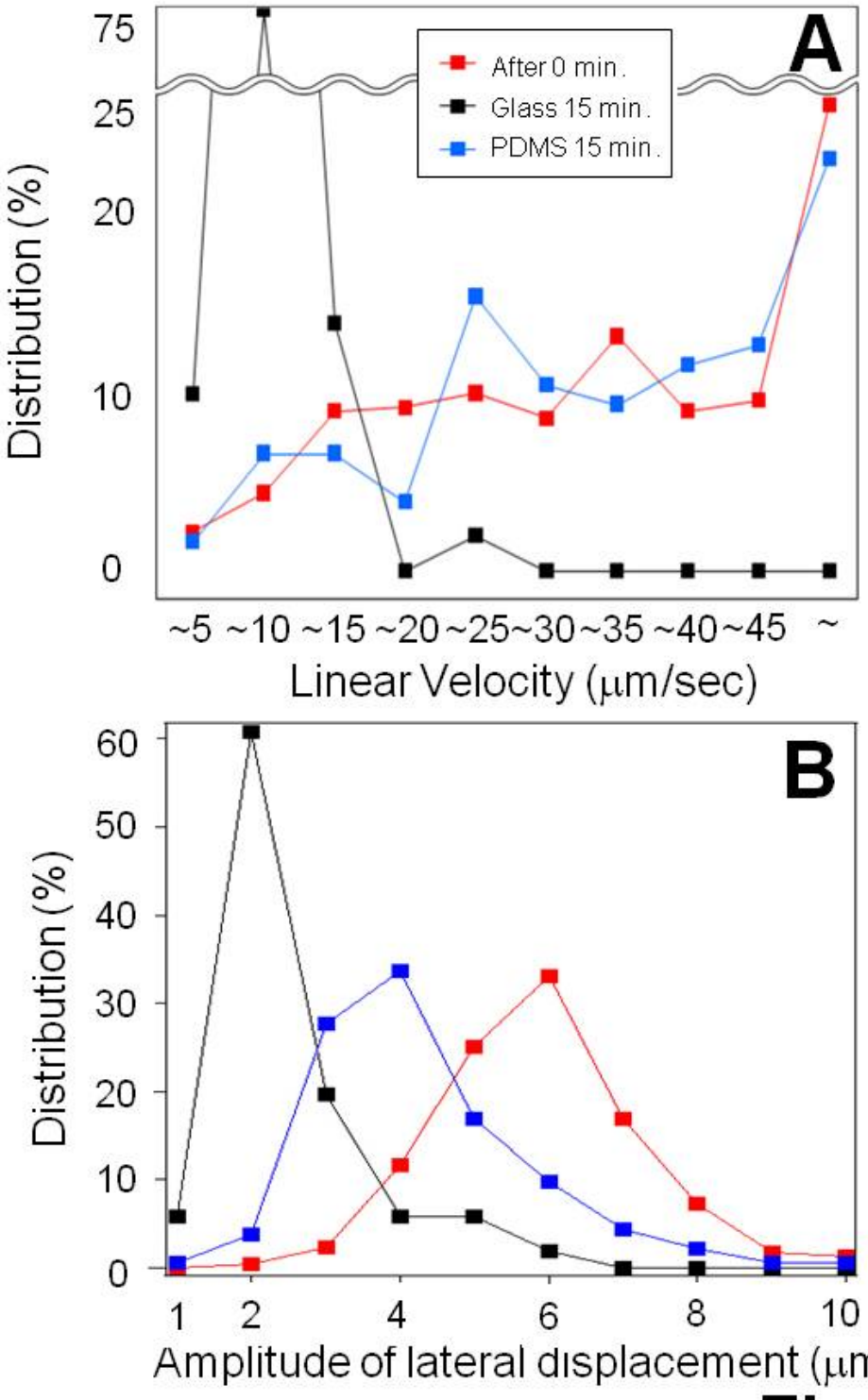
A
After 0 min.
Glass 15 min.
PDMS 15 min.
Distribution (%)
75
25
20
10
0
~5 ~10 ~15 ~20 ~25 ~30 ~35 ~40 ~45 ~
Linear Velocity (μm/sec)
B
Distribution (%)
60
50
40
30
20
10
0
1 2 4 6 8 10
Amplitude of lateral displacement (μm)

Figure 3

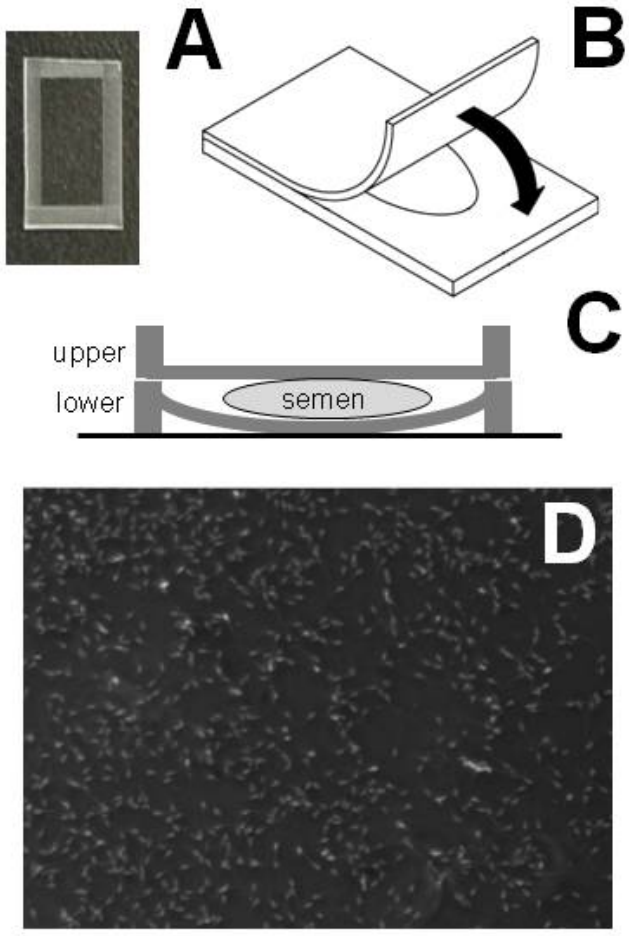

Figure 4

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226

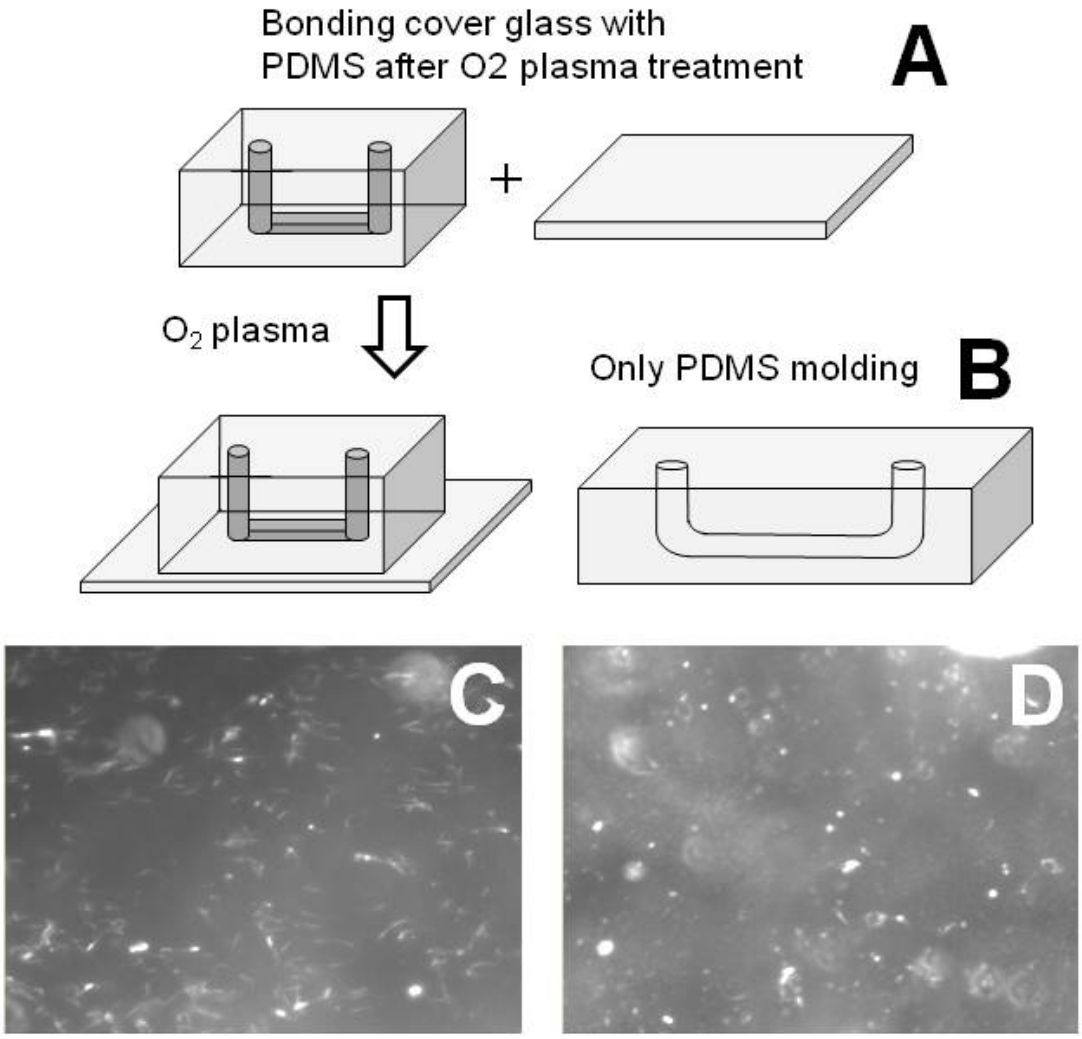
Bonding cover glass with
PDMS after O2 plasma treatment
A
+
O2 plasma
Only PDMS molding
B
C
D

Figure 5

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