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

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ABSTRACT

Motile porcine sperms adhere to hydrophilic materials such as glass and plastics. The adsorption of sperms to a hydrophobic poly(dimethylsiloxane) (PDMS) membrane is less compared with that to glass. We investigated the linear velocity (LV) and amplitude of lateral head displacement (ALHD) of motile porcine sperm on glass and PDMS preparations using computer-assisted sperm analysis (CASA). Significant decreases were observed in the 15-min LV ($P < 0.05$) and ALHD ($P < 0.05$) in motile porcine sperm on glass preparations compared with those on PDMS preparations. These differences were due to adsorption of the head and/or neck to hydrophilic substrates. Because of the elasticity of PDMS, we propose that a PDMS membrane should be used for CASA. To investigate the 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.

Keywords: porcine sperm motility, silicone elastomer, adsorption, trajectories

1 INTRODUCTION

Sperm motility analysis is a representative method for evaluation of male fertility, since motility correlates with viability [1-4]. The conventional method, commonly referred to as Computer-Assisted (Aided) Sperm Analysis (CASA), to records 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 sperms often adsorb to glass and plastic, which remarkably decreases their motility. To record the 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]. The 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(methylmethacrylate) (PMMA) [6]. 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.

Hydrophobic silicone elastomer poly(dimethylsiloxane) (PDMS) having 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 [7-11]. 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 [7-9]. Microfluidic channels are used for in vitro fertilization in the case of low sperm number (>10^5 cells) and for in vitro culture to mimic the oviduct environment [7, 11, 12]. Lopez-Garcia et al. observed bull sperm motions without adsorption to glass substrates in glass-bottom PDMS microchannels [12]. 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 were able to record the trajectories of motile sperms without adsorption and compare the sperm motility parameters. This technology can be applied to recording live imaging and the mechanics of motile porcine sperms that tend to adhere to hydrophilic materials [13].

2 MATERIALS AND METHODS

Cured PDMS has a highly cross-linked 3D structure. The microstructure of the PMMA mold was transferred to the cured PDMS. To prevent overlap of motile sperm images, we designed the preparation to decrease the focal depth. Semen was sandwiched with two PDMS sheets (Figures 1A and B). Due to the elastic properties of the 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 (Figures 1C and D). The

thickness of the semen was approximately 0.1 mm (Figure 1D), and we confirmed no overlap of sperm images (Figure 1E). With this preparation, we were able to record trajectories and analyze the distribution of sperm motility parameters. The PDMS membrane can be reused after washing until it breaks. However, we recommend disposing of the sheets to avoid artifacts, such as dispensable particles, on recorded images and video. Cross-sectional images were recorded by a VHX1000 microscope (Keyence Co Ltd., Osaka, Japan) with a tilt angle of 90°.

Experiment 1: comparing the absorption of motile porcine sperm to different materials

A 2-μl aliquot of fresh semen was sandwiched between glasses or PDMS membranes without and with O_2 plasma treatment. Using a BM ×10 lens (Nikon Corporation, Tokyo, Japan), sperm and particle motions were tracked with a sperm motility analysis system (SMAS; Kaga Electronics Co., Ltd., Tokyo, Japan). This system comprised a high-resolution digital scanning camera, a personal computer with a digital frame grabber and image processing software, and a computer monitor [17]. The frame rate for sperm tracking using the SMAS was 60 frames per second. The experiments were continued until the semen dried.

2.1 Experiment 2: performance of optimized chambers and sperm motility parameters

The preparation method to investigate sperm motility parameters was the same as that in Experiment 1. Based on the recorded trajectories of motile porcine sperm, LV and amplitude of lateral head displacement (ALHD) were estimated using the SMAS imaging software.

3 RESULTS AND DISCUSSION

3.1 Experiment 1: comparing the absorption of motile porcine sperm to different materials

Almost all the sperms adsorbed to slide glass 15 min after preparation, while the number of sperms adsorbed to the PDMS membrane was significantly lower. We found that more than half of the motile sperms adsorbed to the hydrophilic PDMS substrate treated with O_2 plasma 15 min after preparation. The adsorption properties of porcine sperms to transparent materials are summarized in Table 1 [19, 20]. We have confirmed that the hydrophobicity of the substrate materials is important for adsorption. To prevent adherence, materials allowing >80° contact angle with water should be used for preparation. Furthermore, the materials should not be prepared using hydrophilic treatments, such as O_2 plasma, to decrease the contact angle.

3.2 Experiment 2: performance of optimized chambers and sperm motility parameters

We compared the LV distributions of motile porcine sperms inside chambers to quantitatively investigate motility changes in relation to adsorption to hydrophilic substrates. The average LVs immediately after, 15 min after glass preparation and 15 min after PDMS preparation were 26.6 ± 0.4 (n = 1260, ± SEM), 11.0 ± 0.4 (n = 196) and 27.5 ± 0.6 μm/s (n = 589), respectively. The average ALHD amplitudes immediately after, 15 min after glass preparation, and 15 min after PDMS preparation were 4.5 ± 0.05 (n = 1260), 2.3 ± 0.10 (n = 196), and 4.7 ± 0.08 μm (n = 589), respectively. No significant differences in the LV and ALHD were observed between the distribution immediately after glass preparation and 15 min after PDMS preparation. We suggest that the significant decreases in the LV and ALHD were due to adsorption of the head and/or neck to the hydrophilic substrate.

3.3 Development of PDMS microchannels with peristaltic movement to mimick porcine artificial insemination

To prevent the adsorption of motile porcine sperms, it is important to not use hydrophilic materials such as glass and to not perform O_2 plasma treatment as this treatment increases hydrophilicity of surfaces. Microchannels are important in sperm motility analysis because they allow the trajectories of motile bull and human sperms to be evaluated [12, 13]. Interestingly, it has been reported that bull sperms tend to preferentially swim along the walls and that this phenomenon occurs in both flow and non-flow systems [12]. Koyama et al. designed a microfluidic device for sperm chemotaxis with three inlets and three outlets to make a gradient in the chemotaxis chamber [18]. The PDMS substrate and a glass cover plate were bonded by exposure to air plasma, which would decrease the hydrophobicity of PDMS; a treatment based on our present results would not be suitable for analysis of porcine sperm chemotaxis. Our results suggest that a PDMS-bottom microchannel without hydrophilic treatments, such as O_2 and air plasma, can be used to investigate the chemotaxis and fluid mechanics of motile porcine sperms.

The possibility of using PDMS microchannels for porcine sperm investigation for artificial insemination (AI) is discussed. We are going to develop microfluidic channel and system to mimic physiological oviductal structure with peristaltic movement by air-actuation. Some porcine sperms would be selected for fertilization and could fertilize oocytes in the microfluidic channel. Using this AI model system, sperm and embryo motion could be observed in the microfluidic channel after AI. In future, we evaluate minimum sperm concentration which is sufficient for fertilization in the porcine oviduct by AI.

4 CONCLUSION

Motile porcine sperms adhere to hydrophilic materials such as glass and PMMA. The adsorption of sperms to the hydrophobic PDMS membrane was less than that to glass [21]. Because of the elasticity of PDMS, we propose the use of this preparation for conventional CASA to reduce overlap of motile sperm images, which are artifacts of CASA. Because of the potential sperm adhesion, we do not recommend O_2 plasma treatment for bonding PDMS and glass during investigation of the dynamics and chemotaxis of motile porcine sperms using microfluidics. We suggest that the one-step PDMS molding process is suitable for preparation of microfluidic channels to be used with motile porcine sperms.

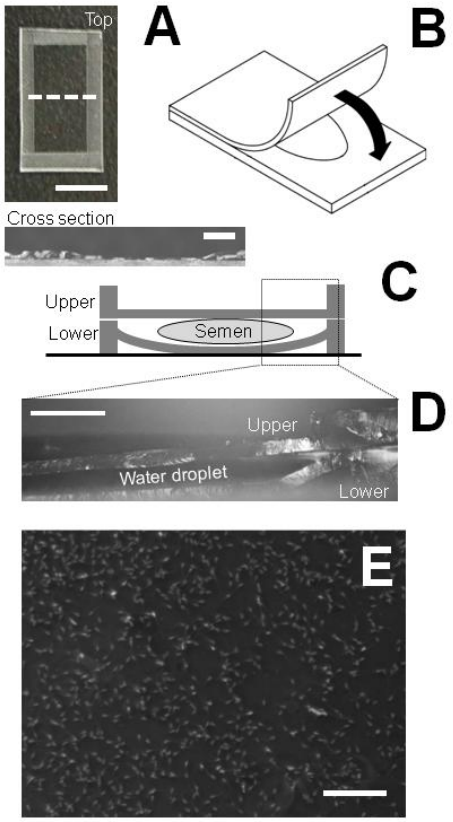

Figure 1: (A) PDMS membrane for preparation, with an area of 0.5 × 1 mm2. The scale bars in the top and cross-sectional images represent 5 and 0.5 mm, respectively. (B) Method of sandwiching semen between the two membranes with a thickness of 0.1-mm. (C) Cross-sectional image for recording the trajectories of motile sperms to decrease the focal depth to approximately 0.1 mm. Dark and light gray objects represent the PDMS membrane and semen, respectively. (D) Cross-sectional image of the preparation. A water droplet is sandwiched with two PDMS membranes. The scale bar represents 0.5 mm. (E) Sperm in this preparation is displayed by CASA. Overlap of motile sperm is not observed in this frame.

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