Corner Accumulation Behavior of Spermatozoa in Microchannels

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Abstract In this study, microfluidic approaches and fluorescence microscopy were used to study cross-sectional distribution of bull spermatozoa in a rectangular microchannel. The results indicate a strong corner accumulation behavior of bull spermatozoa in a rectangular microchannel. Results indicate that 74% of spermatozoa accumulate near boundaries and only 26% of spermatozoa are bulk swimmers. Furthermore, 66% of wall swimmers are corner swimmers. The distinction and quantification of wall vs. corner vs. bulk swimmers was enabled by the unique head-on microchannel imaging approach applied here.

Keywords: Microfluidics, Microswimmer, Sperm Motility, Surface Accumulation

1. Introduction

The migration ability of spermatozoa in confined geometries, especially near boundaries, is of particular importance in reproduction, leading to new insights into both spermatozoa selection techniques *in vitro* and spermatozoa penetration mechanisms *in vivo*. In 1963, Rothschild indicated that spermatozoa are attracted toward the glass surface in the 200 μm gap of a haemocytometer chamber, resulting in a non-random distribution of bull spermatozoa (Rothschild, 1963). This phenomenon, known as surface accumulation behaviour, has been studied extensively for variety of microswimmers, by considering the effect of geometrical constrains, hydrodynamic effects, and out-of plane components of the flagella wave (Li et al., 2008; Gaffney et al., 2011; Li et al., 2011). These studies indicated that microswimmers, including spermatozoa, accumulate near boundaries mainly due to physical interaction with the surface (DiLuzio et al. 2005; Lauga and Power 2009).

Denissenko et al. (2012) indicated that not only do spermatozoa accumulate near surfaces, but the migration ability of human spermatozoa in a microchannel significantly depends on the channel geometry as spermatozoa navigate along the channel corners. This natural swimming characteristic has been employed to develop technologies for selection and sorting of microorganisms (Nosrati et al., 2014; Mijalkov and Giovanni, 2013). However, these studies are limited to one dimensional (1D) observation of spermatozoa distribution across the microchannel width and they lack two dimensional (2D) observation of spermatozoa distribution in the cross-sectional area of the microchannel. Due to these limitations, previous studies were incapable of differentiating a sperm swimming close to a wall from those swimming close to a corner, thus, they lack quantitative evaluation of such a swimming preference.

Here, we used a unique microscopy approach to study cross-sectional distribution of bull spermatozoa in a rectangular microchannel. Differentiating sperm position across the microchannel was enabled by the orthogonal structure of the presented microfluidic device. The results indicate much higher accumulation of spermatozoa near corners (i.e. intersection of the microchannel walls) than near a single microchannel wall. In effect, about half of the microswimmers are concentrated geometrically, into only 4% of the cross-sectional area. This concentration effect significantly influences how these swimmers react to in-plane channel geometries both in artificial reproduction methods and *in vivo*.

2. Materials and Methods

2.1 Device design and fabrication

The microfluidic device consists of a rectangular microchannel which was vertically aligned with a cylindrical observation chamber, as shown in Fig. 1. The horizontal layer contains an observation chamber for 2D imaging and trap reservoirs to prevent spermatozoa from re-entering the microchannel. The microchannel in the vertical layer and the trap reservoirs in the horizontal layer were designed in AutoCAD and printed on a photomask (CAD/Art Services, Inc., OR, USA). The master was fabricated with a SU-8 2075 photoresist (MicroChem, MA, USA) using standard soft-lithographic technique (Unger et al., 2000). Both layers were fabricated using Poly-dimethylsiloxane (PDMS) (Silgards 184: Dow Corning, MI, USA) substrate with 1:10 mixing ratio. The observation chamber was punched in the horizontal layer using a 1.5 mm Miltex Dermal Biopsy punch. Oxygen Plasma was used for bonding the parts of the horizontal and vertical layers together. The vertical layer with a 75 µm × 85 µm cross-section was aligned with the horizontal layer such that the microchannel cross-section was located at the center of the observation chamber. Once aligned, these two layers were then bonded using uncured PDMS.

2.2 Semen sample preparation

The bull semen straws containing 500 µL of bull semen (ABS Global Inc, Canada) were stored in liquid nitrogen. Before use, the bull specimen were thawed in a water bath at 37° C and removed from the straw using an artificial insemination syringe. The bull semen was kept at 37° C at all times, and experiments were conducted within 10 min of semen transfer into the incubator. LIVE/DEAD sperm viability kit (L-7011; Invitrogen, NY, USA) was used to label live spermatozoa with green fluorescence.

2.3 Experimental procedure

The device was filled by submerging it in a high viscosity buffer (HEPES buffer containing 0.5% Methyl-Cellulose) and applying vacuum pressure (-30 psi) for 30 min. The filled device was then placed inside a 37° C incubator for 1 hour to reach physiological temperature. The experimental setup is shown in Fig. 2a. The chip was mounted to an inverted fluorescence microscope (DMI 6000B, Leica) stage. A 10× magnification microscope objective (NA=0.3, WD=11 mm) and a USB microscope (Dinolite Premier, Taipei, Taiwan) equipped with a fluorescence filter was aligned such that the microscope objective had its focal pane focused at the microchannel cross-section and the USB microscope had the side-view of the microchannel, both at the entry to the observation chamber.

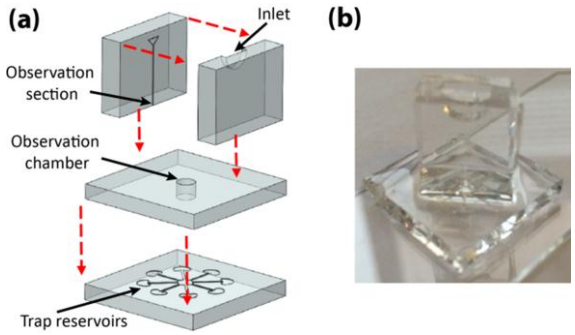

Fig. 1. Microfluidic device used to study corner accumulation behavior of bull spermatozoa across a rectangular microchannel. (a) Schematic view of the device. (b) A photograph of the fabricated microfluidic device.

Following this step, 30 µL of prepared semen sample with approximate concentration of 40 million sperm per milliliter was introduced at the inlet using an Eppendorf pipette. Since the semen sample was introduced at the entry of a prefilled dead-end microchannel, no flow was maintained within the microchannel during the experiments and sperm swam along the channel based on their own preference. A CCD camera was used to capture a bright-field image of the microchannel cross-section following by recording a sequence of fluorescence images with 1 s interval for 30 min. The bright-field image (Fig. 2b) was used to recognize the channel walls in the fluorescence images (Fig. 2c). The freely available image processing software ImageJ and a custom written script in Matlab were used to process the images.

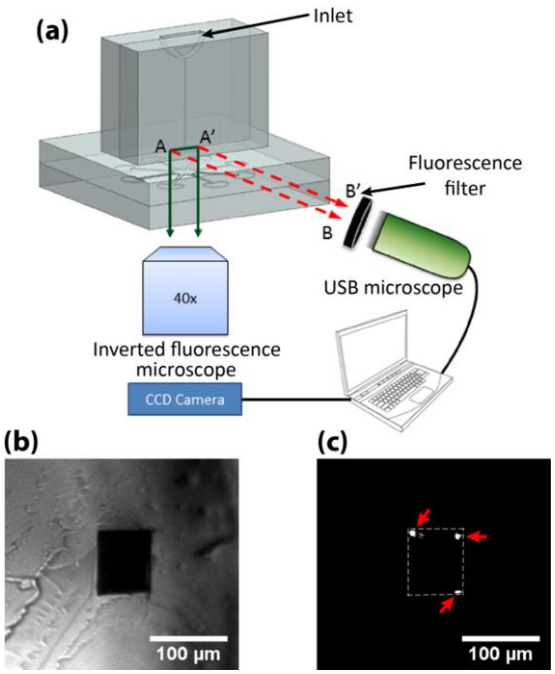

Fig. 2. Experimental procedure. (a) Experimental setup for imaging spermatozoa in the cross-sectional area of the microchannel. (b) Bright field image of the microchannel cross-section. (c) A representative image of spermatozoa in microchannel cross-section acquired by fluorescence microscopy. Three spermatozoa accumulated in the channel corner are indicated with red arrows.

3. Results and Discussion

Quantification of cross sectional distribution of spermatozoa in the microchannel was enabled by the orthogonal structure of the fabricated microfluidic device in conjunction with the unique microscopy approach implemented here. To determine the cross-sectional distribution of spermatozoa, migrating spermatozoa were imaged at the channel exit, using fluorescence microscopy. Fig. 3 shows the cross-sectional distribution of spermatozoa in a 75 µm × 85 µm rectangular microchannel. The resulting distribution indicates a strong preference of the bull spermatozoa (approximate length scale of 75 µm) to accumulate near channel corners (i.e. intersection of the channel walls). Spermatozoa accumulate near boundaries due to hydrodynamic interactions with the surface. Close to a single boundary, asymmetrical influx of the fluid pitches the spermatozoa toward the surface, resulting in surface accumulation (Elgeti et al., 2010). Furthermore, in the presence of the second boundary (i.e. channel corner), the hydrodynamic attraction forces from each wall cooperate, resulting in corner accumulation behavior. These findings suggest that physical boundaries confine the swimming trajectories of spermatozoa from 3D modes to more confined 1D trajectories, resulting in more progressive motion.

Furthermore, the results indicate the strength of the imaging approach to accurately distinguish between wall swimmers (WS) and bulk swimmers (BS). Previous imaging layouts only give a 1D distribution of microswimmers, since a side view of the microchannel is used. In contrast, the method presented here images the channel head-on, thus the 2D distribution of spermatozoa can be captured. Secondly, previous approaches, which used the side view of the microchannel, were unable to differentiate WS spermatozoa at the middle of the channel walls from BS spermatozoa and they considered both of these two categories as BS spermatozoa. Because of this unique head-on microchannel imaging approach, we can accurately distinguish a bulk swimmer at the center of the channel from a wall swimmer at the central part of the wall.

To quantify the corner accumulation behaviour, spermatozoa within a distance of

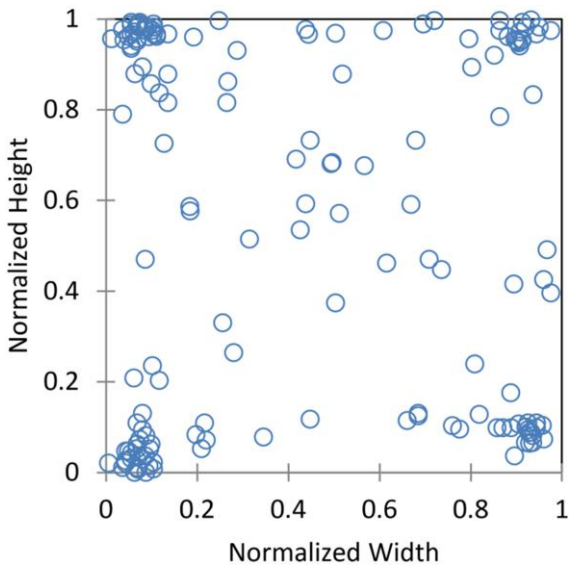

Fig. 3. Spatial distribution of 164 bull spermatozoa across the rectangular section of the microchannel at the exit of the channel, indicating strong corner accumulation behavior of migrating spermatozoa.

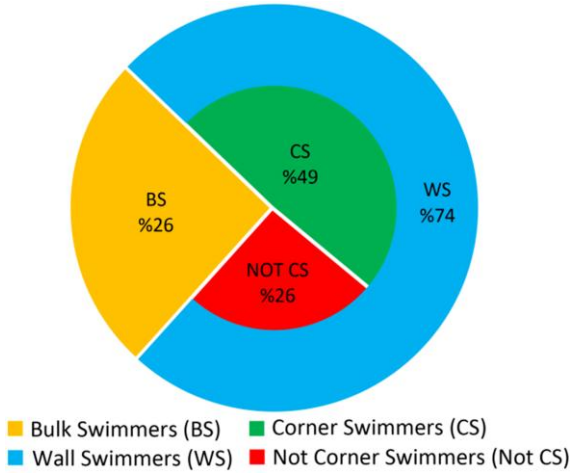

Fig. 4. Percentages of spermatozoa with different spatial swimming preferences in the cross-sectional are of a rectangular microchannel.

less than the 1/10 of the channel width/height are considered as WS. Spermatozoa with wall swimming preference for two walls are considered as corner swimmers (CS), since they are located in the channel corner. As shown in Fig. 4, approximately 74% of spermatozoa are WS and accumulated at 36% of the channel cross-sectional area. In contrast, only 26% of spermatozoa are BS, occupying the remaining 64% of the microchannel cross-sectional area. The results indicate that 49% of spermatozoa (i.e. 66% of WS) are CS while 26% are WS and not CS. In effect, about half of the spermatozoa are corner swimmers that are concentrated geometrically into only 4% of the cross-sectional area. This suggests the strong preference of spermatozoa to accumulate in the microchannel corners and the possible potential of this inherent swimming characteristic to be used for selection or sorting of microswimmers. Since geometrical confinements, in the form of corners, occur frequently in the female reproductive tract, in particular at the cervical crypts and fallopian tubes, the corner swimming preference reported here can bring new insights into sperm migration mechanisms *in vivo*.

4. Conclusion

We used microfluidic approaches to study corner accumulation behavior of bull spermatozoa in a microchannel. The cross-sectional distribution of migrating spermatozoa is images at the exit of a rectangular microchannel using fluorescence microscopy, revealing a strong and non-random preference of spermatozoa to navigate along the channel corner. Results indicate that approximately 74% of spermatozoa are WS and 49% of spermatozoa (i.e. 66% of WS) are CS. The distinction and quantification of wall vs. corner vs. bulk swimmers was enabled by the unique head-on microchannel imaging approach applied here. The new swimming behaviour unveiled by this work, corner swimming, provides new insight into spermatozoa migration in both female reproductive tracts and microfluidic based artificial reproductive technologies.

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