Spermometer: electrical characterization of single boar sperm motility

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Objective: To study single sperm boar motility using electrical impedance measurements in a microfluidic system.
Design: Comparison of the optical data and electrical impedance data.
Setting: Research laboratory at a university.
Animal(s): Boar semen sample were used.
Intervention(s): A microfluidic system is developed that is able to spatially confine single boar sperm cells and allows noninvasive analysis of their motility on the single cell level. Using this system, the single sperm motility was affected by changing the temperature or adding chemical stimuli (caffeine). The retrieved electrical impedance and video data were processed using Matlab.
Main Outcome Measure(s): The sperm beat frequency and amplitude determined from the electrical impedance and video data.
Result(s): The electrically measured sperm beat frequency was verified by optical analysis and in correspondence. Furthermore the microfluidic platform allowed single sperm analysis by altering the sperm by temperature and chemical stimuli.
Conclusion(s): This platform could be exploited as a potential tool to study sperm cells on the single cell level and to perform advanced sperm selection for intracytoplasmic sperm injection (ICSI) applications. (Fertil Steril® 2016;106:773–80. ©2016 by American Society for Reproductive Medicine.)
Key Words: Microfluidic platform, cell trapping, sperm motility, impedance sensing, single sperm analysis

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In the past decennium (2000–2010) the amount of performed assisted reproduction treatments such as IVF and intracytoplasmic sperm injection (ICSI) has approximately doubled (1, 2). Especially, ICSI has won popularity for treatment of patients with no measurable sperm count (azoospermia) (3), but is also used in nonmale factor infertility cases, and has been performed >300,000 times in 2012 in Europe alone (4).

Sperm selection for ICSI treatments is based on visual inspection of the sperm. An important characteristic, which is used in the clinic for sperm selection, is the cell motility. At present, sperm selection for ICSI treatments is performed by manual inspection and selection of a motile sperm cell. Subsequently, a microneedle is used to isolate a single sperm cell, which is injected into an entrapped egg cell. This selection procedure can be subjective and time consuming, especially in cases of semen samples with a very low sperm count. Furthermore, a report of Ramos et al. (5) has shown that no more than 45% of the selected sperm cells for ICSI procedures have normal condensed nuclei. The ability of sperm cells to bind to hyaluronic acid is another mechanism that is reported to select mature sperm cells for ICSI (6, 7). Although the bounded sperm cells do not exhibit DNA fragmentation (6, 7), no improvement in pregnancy rates (PRs) and fertilization has been found using this technique as selection mechanism (8).

Selection of sperm cells with high motility using conventional (9) or microfluidic technologies (10, 11) have

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shown higher DNA integrity compared with the unsorted semen sample. High sperm DNA integrity is important for successful fertilization, as a high degree of DNA fragmentation has shown a negative effect on ICSI outcome (12). Furthermore, the amount of DNA fragmentation in sperm has been inversely related to its potential to hyperactivate (13), which also elaborates on a potential relation between the sperm swimming behavior and DNA fragmentation. Although a variety of microfluidic systems have been developed for the selection of highly motile spermatozoa (10, 14–17), their application for single sperm selection for ICSI is limited.

Only a few reports have shown the capacity to manipulate and analyze sperm cells on the single cell level. Fuhr et al. (17) showed to ability to entrap motile sperm cells using electric field cages at frequencies in the megahertz range. Nascimento et al. (18) showed the potential to entrap single sperm cells using laser tweezers and to measure their motility optically. In previous work from our group, we used microcontact printed fibronectin spots to entrap single sperm cells, after which their motility was analyzed using image analysis (19). With a different approach, we used a hydrodynamic trapping procedure to entrap single sperm cells in small microfluidic channels to study their viability, acrosome integrity, and sex chromosome content using fluorescent analysis (20). These reports showed the ability to analyze single sperm motility and/or spatially confine single sperm cells. However, these reports all depend on optical analysis methods, which are controlled manually. A potential approach for automated analysis of single sperm cells is the combination of a trapping method with integrated microelectrodes for electrical analysis.

Therefore, we propose a microfluidic system that is capable of spatially confining single sperm cells and able to measure their motility electrically. This system could be a potential tool for advanced sperm analysis and selection for ICSI applications.

MATERIALS AND METHODS

Chip and Sample Preparation

The microfluidic setup consists of three parts: a polydimethylsiloxane (PDMS) chip with trapping features, a glass chip with microelectrodes, and a custom-made printed circuit board. Details on chip fabrication can be found in the Supplemental Material, available online. The microfluidic chip is illustrated in Figure 1A, containing all relevant channel and electrode dimensions. In Supplemental Figure 1 the alignment set-up needed for proper alignment of the electrodes and microfluidic channels is shown.

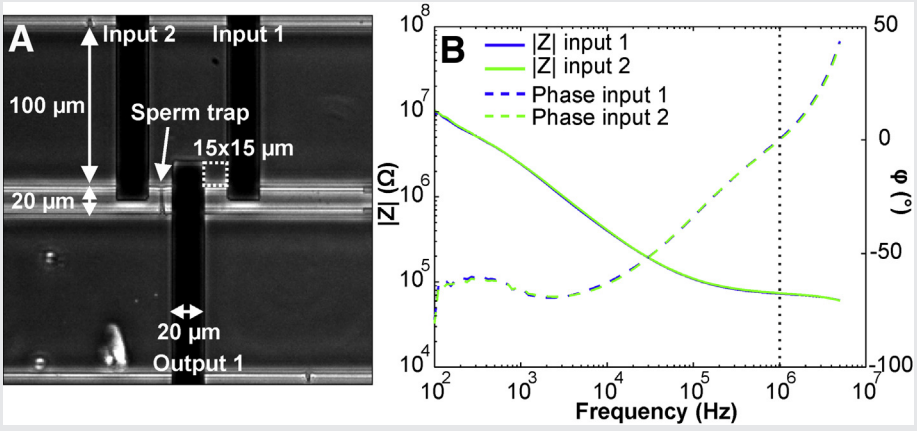
Before each experiment, the PDMS and glass surfaces were coated with poly(L-lysine)-grafted-poly(ethylene glycol) to prevent cell adhesion. The poly(L-lysine)-grafted-poly(ethylene glycol) was rinsed through the PDMS microchannels at a concentration of 100 μg/mL in deionized water for at least 15 minutes.

Fresh boar semen was obtained from a local artificial insemination centre ("KI Twenthe," Fleringen, the Netherlands) at a concentration of 20×10^6 cells/mL. The samples were diluted with Beltsville Thawing Solution (Solusem) to a concentration of 2×10^6 cells/mL. Boar sperm cells have the following dimensions: head length = ± 9 μm (21), head width = ± 4.5 μm (21), head volume = ± 12.5 fL (22), tail length = ± 45 μm (23).

Hydrodynamic Bead and Sperm Trapping

The hydrodynamic trapping procedure of sperm cells is described elsewhere (20). In short, 3- μm and 4-μm polystyrene beads (ø = 4.17 ± 0.03, ø = 2.9 ± 0.083, Polysciences

FIGURE 1



Microfluidic chip and electrical response. (**A**) The microfluidic chip consists of two main channels, interconnected by 2-μm wide and 1-μm high side channels, which act as cell traps. The electrode array consists of two sensing electrodes (input 1 and input 2) and one excitation electrode (output 1), which were used for differential impedance analysis. (**B**) The impedance and phase response of the microfluidic setup (without sperm cell) were investigated when sweeping frequency between 100 Hz and 5 MHz and recording under physiological conditions (σ_{el} = S/m) input 1 and input 2, separately.

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