Spermatozoa detection and counting on chip

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Introduction

Semen analysis is usually one of the first tests performed for detecting the cause of infertility of a couple. Therefore the man has to collect his semen in a special container and deliver it within one hour of collection to the hospital. The parameters determined with a semen analysis are the morphology and motility of the spermatozoa in the semen, as well as the concentration. The haemocytometer is the gold standard for concentration determination (1), but this labour intensive method is in larger laboratories replaced by an expensive computer assisted semen analysis system. Another way that gives an estimation of the spermatozoa concentration uses flow-cytometry (2, 3), while antibody binding (4) and fluorescence labelling (5) are used to determine the concentration of progressive motile spermatozoa (>5μm∙s⁻¹). None of these approaches assess the concentration of spermatozoa without the use of an expensive system, labour intensive handling and sample preparation. Furthermore, due to intra-individual variation, the result of a single test is not reliable and at least three tests have to be done (6). To

make the determination of spermatozoa concentration more objective, cheaper and more patient friendly a lab-on-a-chip is developed. The possibility to detect spermatozoa on chip using electrical impedance measurements is investigated. Results of preliminary experiments showed that the conductivity of semen was not correlated with spermatozoa concentration, possibly due to a very low volume fraction of spermatozoa (0.1% for 20∙10⁶ spermatozoa/mL). Therefore the cells are counted in a much smaller volume using a microchannel.

Method

Electrical impedance measurements are used to detect spermatozoa. A difference in conductivity between the spermatozoon and the surrounding medium is necessary for detection. When a spermatozoon passes the electrode pair (figure 1C), a change in the electrical impedance can be detected. Using this method, the spermatozoon can be counted and when the volume of the introduced semen is known, the concentration can be calculated. A glass-glass chip has been produced consisting of a microchannel with electrodes (figure 1A). The microchannel, with a depth of 20 μm, tapers at the electrode area, resulting in a channel width of 42 μm. Two planar platinum electrodes span the microchannel with an interelectrode distance of 30 μm.

For the experiments, the chip was fitted on an inverted microscope (Leica DM IRM) with a camera. The electrical impedance was measured with a home
made device at 96 kHz. With the measurement set-up it was possible to simultaneously detect the spermatozoa electrically and to acquire video images, using a synchronization script made in Matlab (R2007B, The Mathworks Inc). As background electrolyte, standard washing medium (Sil-Select Plus, washing/insemination medium, HEPES-buffered EBSS, 0.4% HSA) was used with a specific electrical conductivity of 1.4 S·m⁻¹. Preliminary results already showed that it was possible to detect and count human spermatozoa in washed semen (see figure 1C and D). However semen contains also other cells negatively influencing the count and therefore the possibility to distinguish spermatozoa from leucocytes was investigated. First HL-60 cells (leukemia white blood cells) were guided along the electrode pair. Subsequently the same experiment was done with boar spermatozoa.

**Results**

Figure 1D shows the results of a typical measurement. When a spermatozoon passed the electrode pair, a change in the impedance was measured. Note the larger change in impedance when debris passed the electrode pair. The synchronisation of the video images and impedance data showed that only peaks appeared when cells passed the electrode pair and no cells were missed. The peak heights of 52 HL-60 cells and 33 spermatozoa were measured and the mean and 95%-confidence interval are plotted in figure 1B. Clearly, the peak heights of HL-60 cells differ from spermatozoa, due to the difference in cell size. Since their confidence intervals do not overlap, it is possible to classify both cells based on peak height.

**Conclusions**

Electrical impedance measurements can be used to detect spermatozoa in a microchannel and to distinguish between boar spermatozoa and HL-60 cells. Further investigation is focused on determining the spermatozoa concentration from the measurements.

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**References**


**Figure 1.** (A) Photograph of the chip. (B) The average peak heights of the impedance change when 52 HL-60 cells and 33 spermatozoa passed the electrode pair. (C) The two pictures show a microscope image of the chip. The two horizontal black rectangles in each picture are the electrodes, spanning the tapered microchannel. In the picture below (D), the impedance versus the time is shown. A change in impedance is observed when a spermatozoon (circle) or debris (square) passes the electrodes.