CHARACTERIZATION OF NECROTIC AND VIABLE SPERMATOZOA IN MICROFLUIDIC IMPEDANCE CYTOMETRY

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ABSTRACT

The viability and acrosomal status of spermatozoa are important factors for male fertility prediction. Dye exclusion is a common method to determine these factors. In our search for label-free methods, we used microfluidic impedance cytometry to measure differences between necrotic and viable spermatozoa. At low frequency, a significant ($p < 0.05$) smaller mean impedance magnitude for necrotic cells was measured compared to viable cells. Further investigation is needed to verify the impact of acrosomal status on the impedance. However, these results indicate that microfluidic impedance cytometry has the potential to distinguish between viable and necrotic/acrosome reacted spermatozoa.

KEYWORDS: Microfluidics, Electrical Impedance Cytometry, Artificial Insemination, Spermatozoa

INTRODUCTION

Next to closely monitoring and timing the fertilization of the ovum in artificial insemination, sperm quality and the identification of specific sperm defects are critical for successful fertilization. Studies have shown that amongst others, viability and acrosomal status is predicative for male fertility. [1] The acrosome is a cap that covers a large part of the sperm's head, containing enzymes needed in the final stages of fertilization. The most common way to assess these parameters is by dye exclusion. Label-free, non-invasive methods to analyze sperm count [2], motility [3] and the presence of cytoplasmic droplets [4] can already be achieved by microfluidic electrical impedance measurements. In this study, we use microfluidic impedance cytometry to find a distinction between necrotic (treated) and viable (untreated control) spermatozoa.

EXPERIMENTAL

A three-electrode coplanar configuration was embedded on a microfluidic glass-PDMS chip, as shown in figure 1. From a control (freshly diluted boar spermatozoa at a concentration of $2 \cdot 10^6$ cells/mL) and necrotic sample (induced by incubation with 7.5% acetic acid), the impedance of each passing cell was processed to identify typical values for viable and necrotic spermatozoa. Beforehand, the viability was evaluated by SYBR14/PI (live/dead) staining and later the acrosomal status was determined with PI/PNA-AF488 staining, see figure 2. Polystyrene beads ($d = 5$ µm) were added to each sample as a reference for the measurement system. The differential impedance at 0.5, 1.4, 10 and 19 MHz was recorded with a lock-in amplifier (HF2LI, Zurich Instruments) equipped with a preamplifier (HF2TA, Zurich Instruments) and processed with a custom MATLAB script to find the impedance magnitude for each passing bead or cell.

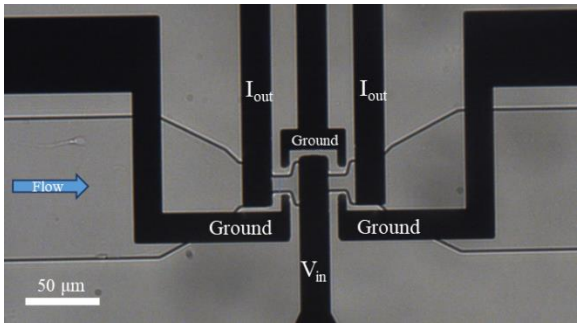

Figure 1: Microfluidic chip with coplanar electrode pairs and shielding electrodes. The input and output electrodes have a width of 20 µm and are 20 µm separated. The channel has a width of 10 µm in the sensing area and a height of 10 µm.

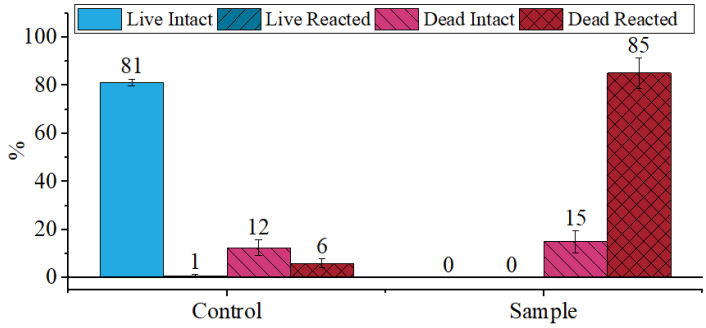

Figure 2: Results of PI/PNA-AF488 staining, indicating the viability and the acrosomal status of the control and sample.

RESULTS AND DISCUSSION

From the impedance magnitude at 0.5 MHz (figure 3) two clear peaks can be distinguished for the control (viability: 82% ± 2% alive) and the necrotic (0% alive) sample. An independent samples t-test showed that there is a significant difference in mean ($p < 0.05$) between the sample sets. As expected, the mean magnitude of the viable cells decreases at higher frequencies (19 MHz) due to β-dispersion, while the mean magnitude for necrotic cells is stable, caused by damaged cell membranes. Hypothetically, the difference in magnitude could also be explained by the acrosomal status. After the acrosome reaction is induced, the acrosome releases its contents and decreases in volume, i.e. a decrease in magnitude is measured. The viable and necrotic sample showed a compromised acrosome for 7% ± 2% and 85% ± 5% of the cells, respectively (figure 2). This indicates that instead of measuring viability characteristics, we might actually be measuring the acrosomal status, though a relation between viability and acrosomal status is not excluded. It was later observed that the cells in the control rapidly died (within 2 hours of preparation), possibly due to the 0.1% surfactant added to the beads to prevent clustering. Cell death due to the surfactant is presumably an ongoing process, although unknown in what timeframe. It is therefore feasible that the beforehand evaluated viability and acrosomal status of the control is shifting during the experiment, explaining the presence of two subpopulations in the opacity of the control sample (figure 4).

CONCLUSION AND OUTLOOK

A significant difference was found between the mean magnitude at 0.5 MHz for the viable control sample and the necrotic sample. Additional evaluation of the samples showed that a high percentage of the necrotic cells have a compromised acrosome, whereas the control only showed minor acrosomal damage. In future experiments, the impact of acrosomal status on the impedance is further investigated by including an additional sample set of viable acrosome reacted cells. The opacity variation in the control suggest the presence of two subpopulations that need further examination to confirm the validity of the experiment. However, the results indicate that microfluidic impedance cytometry has the potential to distinguish between viable and necrotic/acrosome reacted spermatozoa. Combining this with on-chip sorting would be a promising approach to enhance semen quality in reproductive medicine.

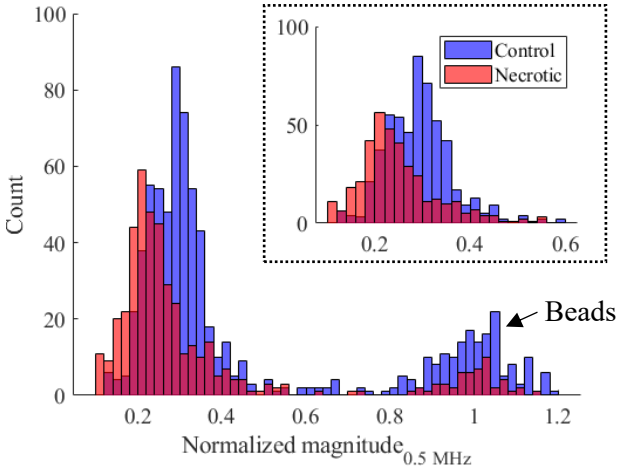

Figure 2: Histogram of the normalized magnitude (with respect to the beads) at 0.5 MHz for the control and necrotic sample. Inlay: histogram of the normalized magnitude of only spermatozoa. The means of the control (0.29) and of the necrotic (0.25) is significantly different ($p < 0.05$).

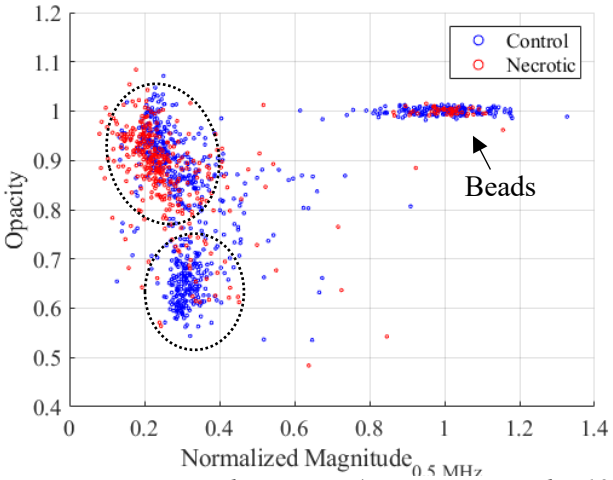

Figure 4: Measured opacity (Norm. Magnitude 19 MHz/0.5 MHz) vs. the normalized magnitude at 0.5 MHz. While the necrotic cells cluster close to an opacity of 1 (indicating small difference in magnitude at high vs. low frequency), the control shows two subpopulations.

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