Towards microfluidic sperm refinement: continuous flow label-free analysis and sorting of sperm cells
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ABSTRACT
This manuscript reports upon the development of a microfluidic setup to detect and sort sperm cells from polystyrene beads label-free and non-invasively. Detection is performed by impedance analysis. When sperm cells passed the microelectrodes, the recorded impedance (19.6 ± 5.7 Ω) was higher compared to the impedance change of passing 3 µm beads (5.4 ± 0.7 Ω). This information was used to discriminate and actively sort beads and spermatozoa using dielectrophoresis at a rate of <1 cell/bead s⁻¹.

KEYWORDS: Microfluidic sorting, sperm, impedance cytometry, dielectrophoresis

INTRODUCTION
In the livestock industry, fertilization outcome after artificial insemination is highly dependent on the quality of the semen. To maintain high fertilization potential, semen samples with a relatively high concentration of spermatozoa with morphological defects are discarded. No routine technologies are available to select sperm based on cell morphology to retrieve morphologically normal cells. We intend to design a microfluidic system, which is able to detect morphological abnormalities non-invasively and sort accordingly. Detection is performed by impedance measurement, which is a well described technique that has been used for single cell analysis of a variety of biological cells.[1] Previous work in our group showed discrimination of sperm cells from polystyrene beads and HL-60 cells using impedance measurement.[2] Sorting will be performed using dielectrophoresis. Although dielectrophoretic sorting of beads based on impedance has been reported [3], no reports show the application of this approach to a biological sample. In this manuscript, we focus on the development of a label-free cell sorting system, which in future will be used to sort sperm cells based on morphology.

EXPERIMENTAL
The microfluidic chip used for detection and sorting is shown in fig. 1. It consists of a 100 µm wide, 20 µm high microfluidic channel containing parallel electrodes for particle focusing, detection and sorting. A schematic overview of the setup is shown in fig. 2. Beads and cells were positioned in the middle of the channel by dielectrophoretic (DEP) focusing (Agilent waveform generator, 10 MHz). Subsequently, a Zurich HF2IS impedance spectroscope equipped with a HF2TA preamplifier was used to measure changes in impedance when the beads or cells passed the 20 µm wide detection electrodes (4-point measurement) at 1.3 MHz. Based on the width of the impedance peaks, the velocity of the particles was calculated to estimate their time of arrival at the sorting electrodes. Finally, these electrodes were selectively activated to sort sperm cells from beads. Data acquisition and real-time control of particle sorting were performed by a custom-made LabView program.

Figure 1: Image of the microfluidic chip. The chip (glass-SU8-glass sandwich) consists of a 100 µm wide, 20 µm high channel containing microelectrodes for particle Focusing, Detection and Sorting.
RESULTS AND DISCUSSION

Selective sorting of beads or cells in this setup is based upon changes in impedance (fig. 3). Whenever a change is recorded, which fits within the window of interest (WOI), the sorting electrodes are activated. In this example, the WOI was set to 4-8 Ω, which covered the impedance change of 3 μm polystyrene beads (green circles, $5.4 \pm 0.7$ Ω, $n = 16$). Consequently, the sorting electrodes were activated to sort the beads into the top channel (green trajectories, fig. 4; Note: at their exit the beads were flowing close to the channel wall, preventing the particle tracker to follow their trajectory). Sperm cells (red triangles, $19.6 \pm 5.7$ Ω, $n = 12$) and debris (blue squares, $n = 11$) were drawn into the bottom channel (red and blue trajectories) since their corresponding impedance changes were above and below the WOI, respectively. When defining the WOI >10 Ω, we are able to sort out the sperm cells from the beads and debris (results not shown).

Figure 3: Discrimination and active sorting of beads and sperm cells. When a bead passes the detection electrodes and the corresponding impedance change fits within the window of interest (green window, 4-8 Ω), the DEP electrode pair is activated (blue window) to sort the particle.
The sorting speed in the reported experiment was $< 1$ cell s$^{-1}$ due to low cell and bead concentrations. Currently, the sorting speed is limited to $< 5$ cells s$^{-1}$ due to limitations in the computational speed of the LabView program.

![Figure 4: Tracking of particle trajectory. Analysis of the bead trajectories showed effective deflection of the beads to the top channel by active sorting, whereas sperm cells and debris were drawn into the bottom channel.](image)

CONCLUSION
To our knowledge, we are the first to present a platform in which label-free and non-invasive analysis of particles and biological cells is combined with active cell sorting capability. In future work, we will focus on high-throughput analysis and sorting of viable and morphologically normal sperm cells towards sperm refinement applications.

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