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Lab-on-a-chip for quality classification of pig oocytes

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The popular veterinarian method of qualitative selection of animals oocytes for artificial fertilization is based on morphological aspects of cells. Selection is done by the trained veterinarian specialists observing under a microscope oocytes "flowing" in phosphate buffered saline (PBS) buffer. This is unsatisfactory selection method, one of the weakest points of agriculture breading industry, because it introduces large personal factor and cannot be automated. In this paper, the novel microspectrometry method for quality assessment of pig oocytes isolated from follicles of various size is described. Pig oocytes were successfully classified according to existing classification. We used a specially designed microfluidic chip for measurements. The chip was constructed of glass and silicon with integrated optical fiber. This is the first attempt of the lab-on-a-chip based methodology of quality assessment of oocytes of breeding animals.

Keywords: lab-on-a-chip, microspectrometry, single cell analyses, oocyte.

1. Introduction

The current method for selecting and qualification of oocytes is based on the morphological properties of the cells identified under an optical microscope [1]. It is assumed that a good quality oocyte (first class) is perfectly spherical in shape with regular zona pellucida, translucent and homogeneously colored cytoplasm. It has a large number of complex cumulus cells surrounding the oocyte. This qualification method highly depends on the expert's experience and does not give unequivocal valuation of oocytes. What more, it does not ensure that after fertilization an embryo and a new-born animal will be of high quality. Therefore, there is a strong demand from veterinary and breeder

environment to develop new objective methodology and criteria for oocyte quality. The ideal method/device for quality assessment of oocytes should be noninvasive, easy to handle and giving results in a few seconds. Lab-on-a-chip techniques can give new solutions in this field. Dimensions of microchannels are similar to the characteristic size of the investigated cell, what enables manipulation and characterization of only one cell in a quasi flow-through lab-on-a-chip. This feature opens a new way for characterization of an individual cell – oocyte especially. In spite of microscopic observation of oocytes, a few other methods of oocyte quality determination might be considered: mechanical, biochemical and optical. All these methods might be applied in lab-on-chip devices. MURAYAMA *et al.* [2] proposed micro-mechanical measurements towards identification of elastic properties of oocytes. This method allows to define the stiffness of living cells. But this technique can be invasive and destructive for a cell. The biochemical method allows to analyse metabolism of a single reproductive cell [3]. This technique has found its application in cancer and drug toxicity investigations but it has not been used for oocytes qualification. The optical noninvasive method of maturity estimation of the oocytes is reported by ZEGGARI *et al.* but it is used for characterization of only human reproductive cells [4]. It seems that only optical characterization of the oocyte will meet requirements of the ideal qualification method. However, there is no literature data on optical properties of animal oocytes.

Another important issue is the method of nondestructive trapping of the oocyte inside a measurement cell during optical properties measurement. The single biological cell can be trapped and positioned using the optical tweezers technology (the laser beam is focused to a diffraction-limited spot by optical fibers) [5] but it is usually restricted to small size object manipulation and under microscopic observation. Dielectrophoresis utilizes dielectric difference between medium and biological cells for trapping of healthy oocytes [6]. Furthermore, this technique induces a thermal affect witch can damage the measured cell. The micro-nozzle flow through, in which the culture medium is pumped and attracts the oocyte [4], can be also used for positioning of the single biological cell.

In our work, we propose a noninvasive optical method for quality assessment of pig oocytes. Spectral-response characteristics of single pig oocytes which came from different size and maturity of ovarian follicles by using a new lab-on-a-chip device are collected. The analysis of obtained characteristics in relation to the reference quality assessment method was carried out.

2. Experiment

A scheme of the lab-on-a-chip is given in Fig. 1. The chip consists of a silicon–glass structure with integrated two glass optical fibers. The fluidic channels and montage channels for optical fibers are etched simultaneously in DRIE (deep reaction ion etching) process in a silicon wafer (100) which was 380 μm thick. The dimensions of DRIE etched microchannels are fitted to the diameter of measured oocytes (about 120 μm). After DRIE process, the thermal SiO_2 passivation layer is formed. Next,

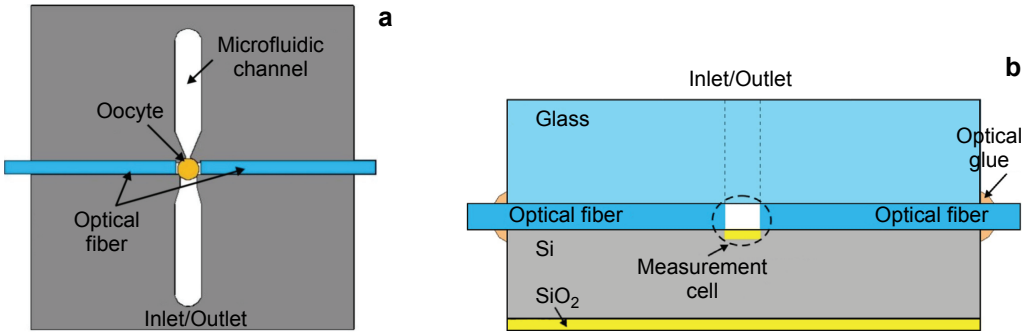

Fig. 1. Scheme (**a**) and cross-section (**b**) of the chip for characterization of living oocytes.

the silicon wafer is anodically bonded (450 °C, 1.5 kV) to a glass top cover where inlet and outlet holes for fluids with cells are previously drilled.

Following, optical fibers (Ocean Optics, USA) with 100 µm core are mounted. They are perfectly aligned to each other thanks to high precision of DRIE etching. The fibers are fixed by means of UV epoxy hard glue NOA 61 of Thorlabs. The ends of both fibers are finished with standard SMA connectors. The silicon–glass chip with integrated optical fibers was placed in a metal package (Fig. 2). This package of small dimensions and weight enables the real-time measurements. It does not contain delicate, movable elements therefore is easy to move, shock-proof and can be used outside the laboratory.

During the measurement, a single embryo is introduced into the measurement cell by capillary forces and mechanically immobilized, accurately between two optical fibers (Fig. 3).

Spectral characteristics in VIS range are measured in the set-up consisting of a halogen lamp (Ocean Optics), described here as a chip, miniaturized spectrometer (Ocean Optics) and computer with specialized software (Fig. 4). The measured oocyte is introduced into the chip using a standard pipette and the capillary force. Light from the halogen lamp is transmitted to the characterized oocyte. The passed light is

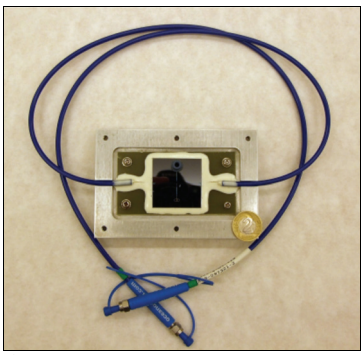

Fig. 2. Assembled LOC.

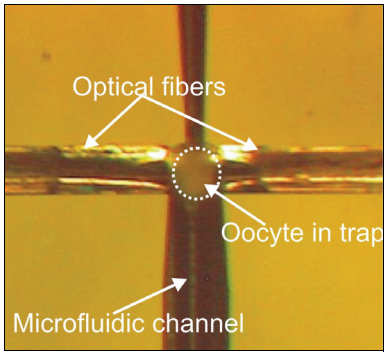

Fig. 3. Cell while measurement – true image.

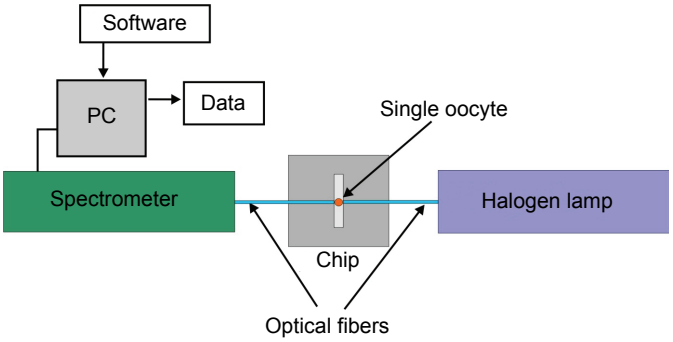

Fig. 4. Measurement set-up for pig oocytes microspectrometric characterization.

collected by the second optical fiber connected to the miniaturized spectrometer. After measurements the oocyte is flushed back to a sterile transporting container. The spectral characteristics are recorded, normalized, and processed under Origin (USA) software.

3. Results

The experiments were performed in Poznań University of Life Sciences, Department of Zoohygiene and Veterinary Prevention which supplied the biological material.

The measured oocytes came from different ovarian follicles (large and medium). Class 1 was obtained from large ovarian follicles giving higher probability of successful fertilization, and class 2 from smaller follicles. The spectra of measured cells were conditioned at two steps. First, the spectrum of buffer (PBS) was measured and normalized – NI(B). Then, the oocyte with buffer was measured and normalized – NI(O). Next, the NI(O) was mathematically subtracted from NI(B). Due to these

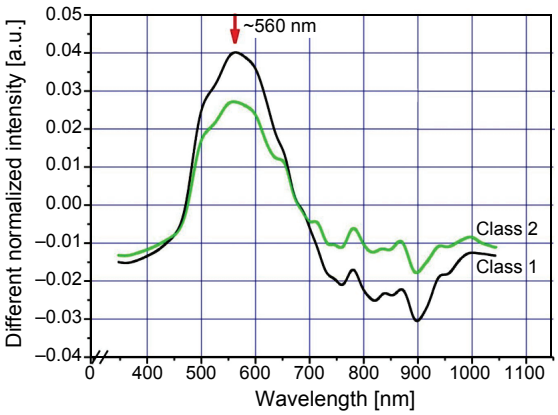

Fig. 5. The transmission spectral characteristics of two classes of pig oocytes.

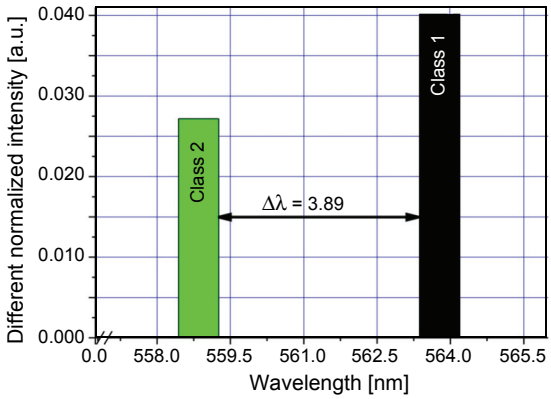

Fig. 6. Maximal value of peak and its position taken from spectral characteristics obtained for two different classes of oocytes.

operations, differential normalized intensity (DNI) was obtained as the analyzed spectrum of the oocyte:

$$DNI = NI(B) - NI(O)$$

Every measured oocyte underwent the same procedure. In each class of oocytes, 12 pieces were measured. The average spectral characteristics for each class is shown in Fig. 5.

It has been found that the position and "intensity" of the peak ~560 nm are correlated to the size of ovarian follicles and the quality of oocytes classified by a morphological factor as the reference method (Fig. 6).

4. Conclusions

In this paper, the lab-on-a-chip and the methodology for optical characterization of pig oocytes have been presented. Tests of porcine oocytes isolated from various sizes of follicles have been successfully done. The shift of the maximum of transmission of light near 560 nm is in good agreement with the size of ovarian follicles.

Presented results open a new way towards classification of pig oocytes. It seems that proposed methodology can be an alternative to traditional morphology-based classification methods. The objective "description" of the oocyte, in a form of numbers describing optical properties, is given in this work for the first time. It enables true characterization and qualification of the oocyte. What more, presented here methodology and lab-on-a-chip-based instrumentation can be applied for classification of oocytes and embryos of other farm or wild animals.

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