QUALITY AND VIABILITY ASSESSMENT OF OOCYTES/EMBRYOS OF ANIMALS BY OPTICAL CHARACTERIZATION IN LAB-ON-A-CHIP DEVICE

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ABSTRACT

The first attend toward novel methodology of quantity assessment of oocytes/embryos of animals is reported in this paper. The methodology utilizes lab-on-chip based instrumentation with optical – microspectrometric and microfluorometric – characterization of the reproductive cells.

KEYWORDS: Lab-on-chip, microspectrometry, fluorometry, single cell analyses, oocyte, embryo

INTRODUCTION

Assessment of viability and quality of oocytes or embryos during reproduction process of animals is an important challenge for veterinary services. The most popular veterinarian method of qualitative selection of animals' oocytes for artificial fertilization is based on assessment of morphological properties of the oocytes. In this method maturity of ovarian follicles (maturated follicles give higher in vitro fertilization success) and number of the cumulus cells surrounding particular oocyte (large number of cumulus cells is a positive factor) are two most important morphological factors to be taken into account. Usually, selection of oocytes is done by trained veterinarian specialists observing the oocytes under an optical microscope. This unsatisfactory selection method is one of the weakest points of breading industry because it introduces large personal factor (subjective assessment) and cannot be automated. After oocyte selection and fertilization, assessment of embryo viability can be determined by programmed death – apoptosis. Commonly used for apoptosis detection standard flow cytometry is limited for cells with diameter up to 20-30 μm due to diameters of applied tubes or microchannels [1]. Therefore apoptotic analysis of bigger cells - animal's embryo has approximately diameter from 50 μm for mouse to 250 μm for horse - is possible only with application of fluorescence microscope with sophisticated sample preparation (often destructive) and equipment.

The combination of microfluidic technique and flow cytometry – like methodology allows to build a lab-on-a-chip based systems with size of microchannels similar to the size of characterized cell. Different techniques and lab-on-a-chips can be used to characterize properties of oocytes/embryos. Dielectrophoresis, for separation of healthy oocytes, utilizes differences of dielectric coefficients of holding medium and biological object [2, 3]. However this technique induces a thermal effect what can damage the cell. Another method of oocyte characterization is measuring the elastic properties of the cell [4], but this technique can be invasive and destructive for oocyte. The optical non-invasive method of maturity estimation of the oocytes is provisionally reported to be used for testing of only human cells [5]. Optical characterization of individual reproductive cells of animals has not been used by now due to lack of established novel optical methodology and technical solutions. In this paper lab-on-a-chip based instrumentation for both microspectrometric and microfluorometric characterization of animals' oocytes/embryos is presented.

EXPERIMENT

A scheme of developed lab-on-a-chip is presented in Fig. 1. It consists of silicon-glass chip with integrated two glass optical fibers forming optical path for microspectrometric measurements and fluorescence excitation during fluorometric characterization of the cells. The dimensions of microchannels are adjusted to an average size of oocyte/embryo, e.g. 120 μm for porcine oocyte or 50 μm for mouse embryo. The microfluidic channels and channels for optical fibers are etched simultaneously in DRIE (Deep Reaction Ion Etching) process in the monocrystaline 380 μm - thick silicon wafer. Next, the wafer is anodically bonded (450^0C, 1,5 kV) to the borosilicate glass top cover (Borofloat 3.3, Schott, Germany) with mechanically drilled inlet and outlet via - holes. Following, optical fibers with 100 μm core (Ocean Optics, USA) are mounted. Fibers are perfectly aligned each to other thanks to high precision of DRIE etching. Fibers are fixed in the microchannels by the use of UV epoxy hard glue NOA 61 of THORLABS. Ends of both fibers are finished with standard SMA connectors. The silicon-glass chip with integrated optical fibers is placed in a metal package (Fig. 2). The package is mechanical shock resistant and ensure mobility of the lab-on-a-chip.

During measurement, single oocyte or embryo is introduced into the chip first by sterile pipette into glass hole and then by it is sucked capillary forces into the measurement cell. The reproductive cell is mechanically immobilized accurately between two optical fibers (Fig. 2). After measurement the oocyte/embryo is flushed-back to a sterile transporting container for further operations.

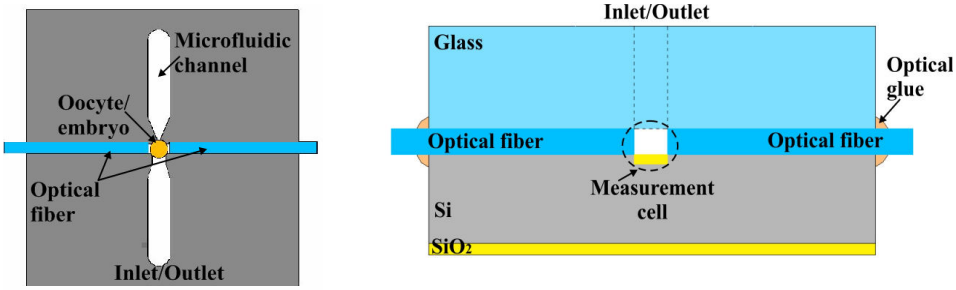

Figure 1: Scheme (left) and cross section (right) of the lab-on-a- chip for optical characterization of animals' oocytes/embryos

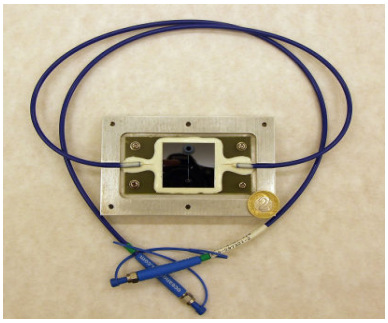

Figure2: Packaged lab-on-a-chip ready for work

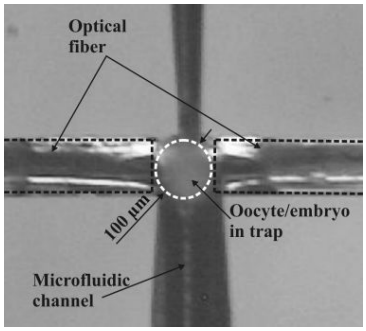

Figure 3: Reproductive cell immobilized during measurement – true image

Two measurement configurations co-working with one lab-on-a-chip was used. In case of microspectrometric characterization, light from halogen lamp (Ocean Optics, USA), guided by the first fiber, is transmitted through investigated reproductive cell and collected by the second fiber connected to a miniaturized spectrometer (Ocean Optics, USA) (Fig. 4). The spectral characteristics are recorded and processed under Origin (USA) software. During apoptosis characterization with fluorometric measurement, the first fiber is connected to a fluorescence excitation LED light source (λ=490 nm with short-pass 500 nm filter), whereas the filtered fluorescence light is detected by the use of CCD minicamera (with long-pass 500 nm filter) with optical path ensuring proper magnification (Fig. 5). The whole instrumentation is portable and can be easily used outside laboratory for "in-the-filed" characterization of the cells.

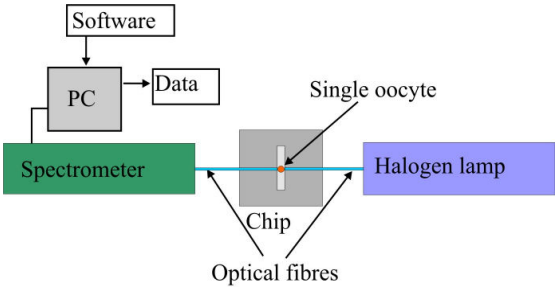

Figure 4: Measurement set-up for pig oocytes microspectrometric characterization

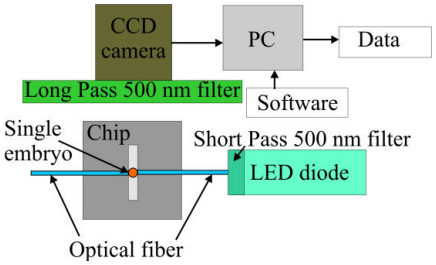

Figure 5: Measurement set-up for microfluorometric apoptosis detection in mice embryos

RESULTS AND DISCUSSION

Two types of porcine oocytes, coming from two classes of ovarian follicles, have been microspectrometrically investigated. Class 1 (20 pcs) was obtained from large ovarian follicles giving higher probability of successful fertilization, and class 2 (25 pcs) from smaller follicles. It has been clearly observed that transmission spectra of class 1 oocyte significantly differed from the spectra of class 2 oocytes. Figure 6 shows averaged spectral characteristics for two classes of oocytes. Experiments have indicated well observed relation between position and intensity of about 560 nm maximum of normalized transmitted light intensity and class of the ovarian follicle (Fig. 7).

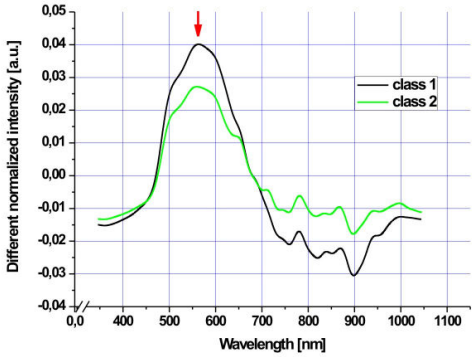

Figure 6: Averaged transmission spectral characteristic of two classes of pig oocytes

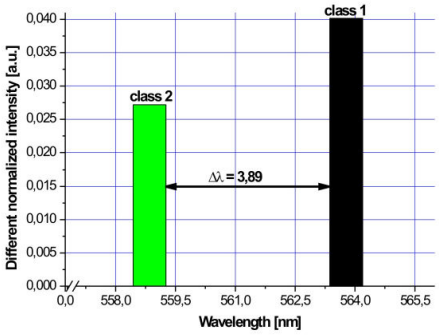

Figure 7: Maximal value of peak and its position taken for two different classes of oocytes

Viability assessment of embryos by apoptosis has been carried out with three groups of mice embryos. First group was reference one without any treatment. The second group was treated with fluorescence marker from Annexin-V apoptosis detection kit. The third group was treated by actinomycin D for inducing artificial apoptosis and then colored by Annexin-V kit. The differences of fluorescence intensity obtained for three tested groups was observed (Fig. 8). Results of microfluorometric measurements were in good correlation with embryo images obtained by the use of fluorescence microscope (Fig. 9).

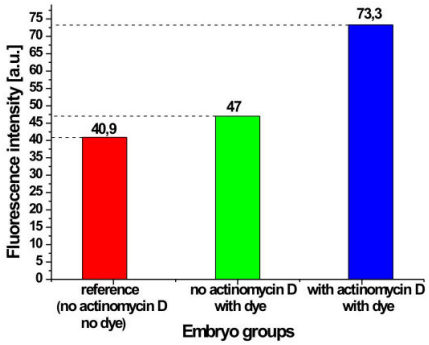

Figure 8: The average fluorescence intensity for three groups of apoptotically characterized mice embryos.

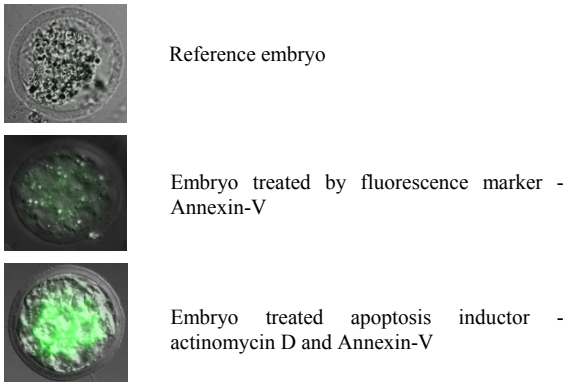

Figure 9: Sample pictures of three groups of mice embryos made under fluorescence microscope.

CONCLUSION

The lab-on-a-chip based instrumentation and preliminary methodology for classification of porcine oocytes by measuring of its transmission spectra and apoptosis – based viability assessment of mice embryos by measuring of fluorescence signal have been presented. New parametric method for evaluation of quality of oocytes and embryos of animals has been proposed. Presented results are the first step toward development of novel methodology of objective classification and assessment of viability of reproductive cells.

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