MEASURING THE REFRACTIVE INDEX OF OOCYTES BY MEANS OF AN OPTICAL MICROSYSTEM

A Non Invasive Technique to Estimate the Evolution of Oocytes Maturity

Ioana R. Ivascu
Physics Department I, Applied Sciences Faculty, Bucarest "Politehnica" University
Splaiul Independentei, Bucuresti, 060042, Romania

Rabah Zeggari, Christian Pieralli, Bruno Wacogne
Institut FEMTO-ST, UMR CNRS 6174, Département d'Optique P. M. Duffieux, Faculté des Sciences et des Techniques
Route de Gray, 25030 Besançon Cedex, France

Christophe Roux
Service de Génétique Histologie Biologie du Développement et de la Reproduction
UMR645 – Interactions Hôte-Greffon et Ingénierie Cellulaire et Génique & IFR 133: IBCT, CHU St. Jacques
Université de Franche-Comté, 25030 Besançon, France

Keywords: *In Vitro* Fertilization, Maturation estimation, Optical microsystems, Refractive index measurement.

Abstract: In this paper, we present an optical microsystem used to measure the refractive index of oocytes. The technique relies on oocytes refraction properties and on the optical coupling between optical fibers. A theoretical model of the system is established by means of matrix optics. A numerical fitting allows calculating the refractive index of the oocytes. In a first time we considered the oocytes like homogeneous spheres. In a second time, we considered that the oocytes consist of a shell (the zona pellucida) and a core (the cell). Experimental results showed that the refractive index of the oocytes increases during the maturation process. In this position paper, we also discuss the temporal evolution of the oocytes maturity and we point out that the maturation should not only be estimated visually but in a more continuous manner. The device we propose could be a solution for such non invasive measurements.

1 INTRODUCTION

The ability of an oocyte to be fertilized and to initiate an embryo development strongly depends on its quality and maturity degree. Usually, the maturation of an oocyte is estimated visually, by direct observation with a microscope. The goal is to observe the presence or the absence of particular morphological features. The effectiveness of this visual inspection is therefore highly biologist dependent. Consequently, there is a crucial need for more objective technique, and hopefully, more effective methods.

Since the beginning of IVF (*in vitro* fertilization), a few technological developments have been investigated. They mainly concern the preparation, the conditioning and the conservation of gametes, as well as culture of the oocytes and embryos (Suh, 2003). Some publications report innovations in the technological domain. Some of them concern the preparation, the conditioning and the manipulation of the oocytes in micro-fluidic systems (Beebe, 2002, Zeringue, 2001, Gauthier, 2002, Sadani, 2005, Zeggari, 2007, Kricka, 1997). Other techniques are developed to estimate the maturity of the semen (Danielli, 1952). The oocytes maturity has also been investigated by means of mechanical measurements. In this case they were mainly conducted on animal oocytes (Ohtsubo, 1985, Sawai, 1974, Nakamura, 1978, Murayama, 2004). It must be noted that, to our knowledge, only one conference (Inui, 2006) and one paper (Wacogne, 2008) reports experiment on human

R. Ivascu I., Zeggari R., Pieralli C., Wacogne B. and Roux C. (2010).
MEASURING THE REFRACTIVE INDEX OF OOCYTES BY MEANS OF AN OPTICAL MICROSYSTEM - A Non Invasive Technique to Estimate the Evolution of Oocytes Maturity.
In *Proceedings of the Third International Conference on Biomedical Electronics and Devices*, pages 127-132
DOI: 10.5220/0002756201270132

oocytes. The invasive or non invasive aspect of the techniques is probably an important issue.

Concerning non invasive techniques, optical methods offer potential alternatives. They have been tested on living cells (Shaked, 2009, Marquet, 2005) or on oocytes (Zeggari, 2007, Pieralli, 2009). In this paper, we present a simple micro-system used to measure the refractive index of the oocytes. It is based on work we have presented some time ago (Zeggari, 2006). In this reference, we showed that oocytes can optically act as microlens. Therefore, if an oocyte is used as a coupling element between optical fibers, it is possible to compute its refractive index by analyzing the optical coupling. However, in this feasibility study, refractive index measurement was only demonstrated with one oocyte. Furthermore, no particular attention had been paid on the coupling modeling. Recently, we set up a more realistic model for the optical properties of the oocytes and we computed refractive indices of a larger number of oocytes. This is the subject of this paper. Refractive indices are measured for 3 oocytes maturation stages: VG (Germinal Vesicle), MI (Metaphase I) and MII (Metaphase II). We show that the refractive index evolves with the maturation of the oocyte.

The micro-system as well as the experimental protocol is presented in the next section of this paper. Experimental results are the subject of section 3 while section 4 deals with a discussion we would like to propose concerning the temporal aspect of the oocyte maturation process. Then a conclusion will be drawn in the last section of this article.

2 OPTICAL MICROSYSTEM AND EXPERIMENTAL PROTOCOL

2.1 Experimental Set-up

A schematic diagram of the device is shown in figure 1(a). It consists of a Deep Reactive Ion Etched micro-machined piece of silicon anodic bonded onto a glass substrate. The main features are two U-grooves into which optical fibers are glued. In this way, optical fibers are perfectly aligned. A specific pigtailing arrangement was designed in order to precisely adjust the distance between the two fibers (500 µm). Fibers were conventional 125 µm diameter fibers. The core of one fiber is 50 µm in diameter. It is the illumination fiber. The core of the second fiber (the collection one) is 100 µm in diameter.

The principle is to bring the oocyte between the two optical fibers by means of the holding micro-pipette commonly used in the conventional IVF micro-manipulators. The idea is to analyse the optical intensity collected for different positions of the oocyte between the fibers. Figure 1(b) helps understanding the principle of measurement. When the cell is positioned close to the illumination fiber, a large amount of light is collected due to the refractive index of the cell that plays the role of a microlens. Conversely, when the cell is positioned close to the collection fiber, the amount of collected light is much lower. The maximum of collected light is obtained for a position between the two fibers.

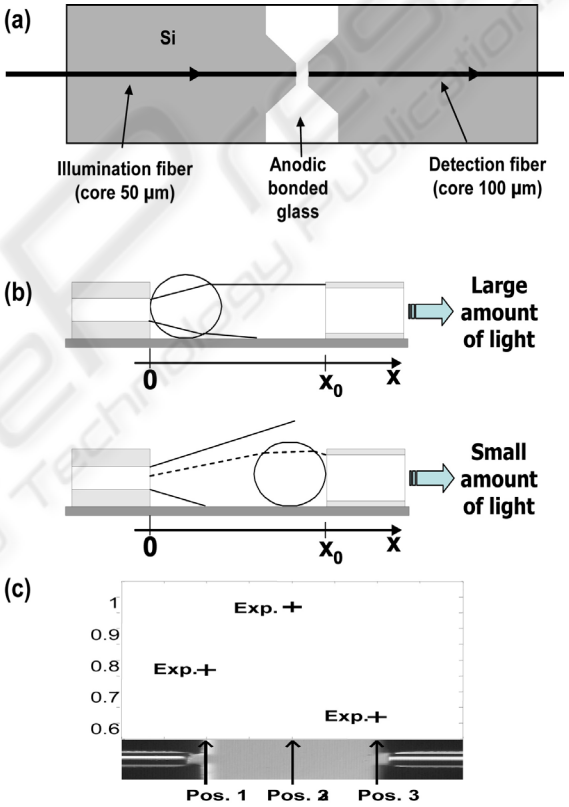

Figure 1: Experimental set-up and principle of the technique. (a) Schematic diagram of the device. (b) Illustration of the coupling efficiency as a function of the oocyte position. (c) Example of measurement.

2.2 Measurement Method

The method we used consists in measuring the intensity for 3 positions of the cell: in contact with the illumination fiber, right in the middle of the fibers and in contact with the collection fiber. In this way, 3 experimental points are obtained as it is shown in figure 1(c). A computing model is then

employed in order to fit theoretical data with experimental points. We first consider that the collected intensity strongly depends on the refractive index of the oocyte and weakly on its absorption.

Indeed, absorption of the oocytes was estimated by means of image processing performed on pictures usually taken in the Besançon's IVF center (comparison of the average grey level of the oocyte and the grey level of the background). We found that the absorption is about 6%.

From there, a Matlab programme is used to compute the intensity collected as a function of the refractive index and for the 3 above mentioned positions of the oocyte. Iteration by iteration, a value of the refractive index "n" is found when the average distance between the experimental points and the computed ones is minimum (least mean square criterion).

2.3 Oocytes Preparation

Oocytes used in this study were excluded from Assisted Reproductive Technology (ART) programs. Their use has been approved by the clinical ethic committee of the Besançon University Hospital the 5th of May 2004.

Oocytes are collected during attempts of ART (by means of ICSI - Intracytoplasmic Sperm Injection) or from follicular reduction. The complex cumulus-oocyte are individualized under a binocular microscope and transferred into a culture medium. After 90 to 120 min of *in vitro* culture at 37 °C, the cumulus is removed. This lead to a perfect visualization of the oocytes under a microscope and allows determining their maturity degree. Oocytes are individually transferred into plastic boxes with 100 µl of culture medium. Their maturity degree is defined by visual inspection with an inverted microscope.

200 µl of IVF culture medium is deposited between the two fibers of the device. One oocyte is collected in the plastic box in 20 µl of IVF medium and positioned against the emission fiber. The holding micro-pipette is employed in order to precisely position the oocyte. Collected intensity is recorded and the oocyte is moved to the middle of the two fibers where the second measurement is made. Then, the oocyte is positioned against the collection fiber for the last measurement.

Once the measurements are made, the oocytes are replaced in the IVF medium in the plastic box. Then, they are incubated in a Sanyo CO_2 incubator (35 °C, 5% CO_2). Their possible evolution (in terms of maturity) is controlled 4 and 17 hours after the first maturity evaluation with the microscope. If the maturation stage of the oocytes has evolved, another refractive index is measured.

2.4 Theoretical Models

As previously mentioned, a theoretical model of the oocytes was defined in order to simulate their optical properties. This model relies on the use of matrix optics. Figure 2(a) shows a picture of an oocyte. Two main features are visible: the cytoplasm and the zona pellucida.

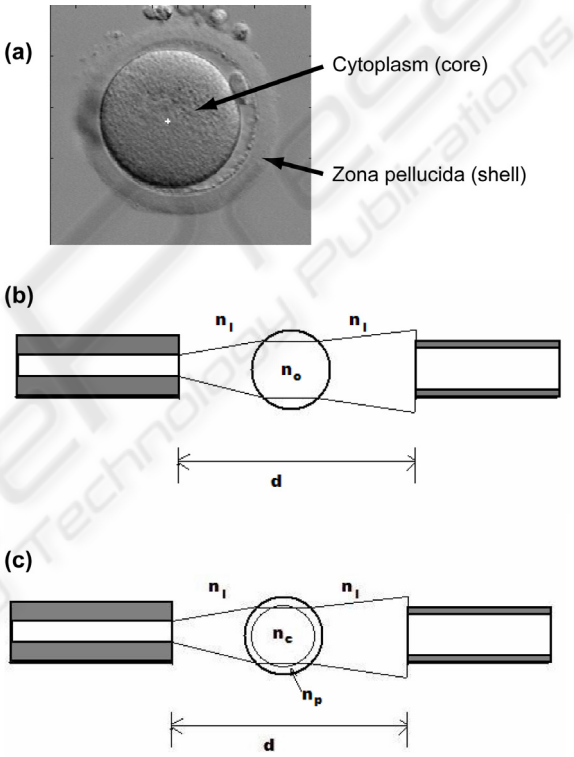

Figure 2: Description of the theoretical model used in this study. (a) The two main features of the oocyte (zona pellucida and cytoplasm). (b) Homogeneous model. (c) Core-shell model.

In a first time, we simply considered the oocyte as a homogeneous sphere whose refractive index is n_0 (figure 2(b)). The center of the oocyte coincides with the optical axis of the fibers. The refractive index of the culture medium is n_1. In a second time, we constructed a more complex model where we accounted for the "core-shell" structure of the oocyte (figure 2(c)). Here, the refractive indices of the cytoplasm and the zona pellucida are n_c and n_p respectively.

3 EXPERIMENTAL RESULTS

Experiments were conducted with 30 oocytes. They belong to 3 maturity classes: VG (10 oocytes), MI (10 oocytes) and MII (10 oocytes). All of them were collected at the VG maturation stage. After about 5 hours of incubation they evolved to the MI stage. About 15 hours later, they became MII. Refractive index measurements were made at each maturity degree.

3.1 Preliminary Experiments

As previously stated, the absorption of the oocytes was estimated by means of image processing. This is described in figure 3. Figure 3(a) shows an oocyte. The idea is to separate the part of the image corresponding to the oocyte from the background. To this end, a semi automatic thresholding is used. The result is shown on figure 3(b). Once the oocyte is isolated, the absorption is obtained by dividing the average grey level of the oocyte by the average grey level of the background. In our case, the average absorption of the oocytes was 6%.

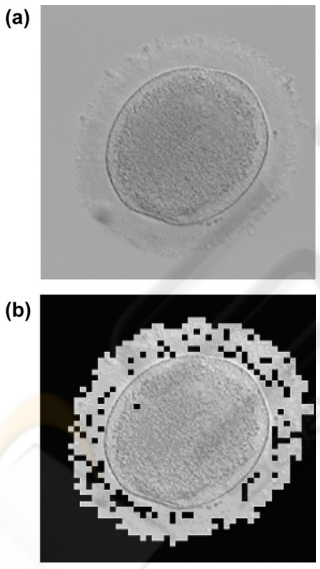

Figure 3: Image processing used to estimate the absorption of the oocytes. (a) The initial image. (b) Result of the image processing when the oocyte is isolated.

The second preliminary experiment concerned the refractive index of the culture medium n_1. To measure it, we recorded the collected intensity before and after the culture medium is deposited between the fibers. Ray tracing is used to compute the refractive index of the medium. In our case we found n_1=1.868.

3.2 Experimental Results

Two type of measurements were considered. In a first time, we calculated the average refractive indices in each maturity class. We did it with both theoretical models. Results concerning the homogeneous model are summarized in table 1 while those concerning the core-shell model are presented in table 2.

Table 1: Average refractive index of the oocyte for the 3 maturation stages. Homogeneous model.

Stage	Average n_0
VG	$1.68^{\pm 0.03}$
M I	$1.76^{\pm 0.04}$
M II	$1.79^{\pm 0.03}$

Table 2: Average refractive index of the oocyte for the 3 maturation stages. Core-shell model.

Stage	Average n_c	Avergae n_p
VG	$1.79^{\pm 0.05}$	$2.18^{\pm 0.02}$
M I	$1.86^{\pm 0.06}$	$2.10^{\pm 0.02}$
M II	$1.88^{\pm 0.05}$	$2.21^{\pm 0.02}$

In both cases, we observe that the refractive index increases with the maturity degree of the oocytes. In the case of the core-shell model, it seems that the refractive index of the cytoplasm increases while nothing can really be said about the refractive index of the zona pellucida. Also, if we consider the values of the standard deviations we can say that, although the 3 maturation stages can be individualized, it seems impossible to use this optical technique to qualify the maturity of an unknown oocyte.

However, is this result surprising? Indeed, concerning the maturity degree of the oocytes, we are analyzing cells that continuously evolve between consecutive stages. Therefore, should we look for a discrete separation between the optical properties of the different stages? We would like to propose a discussion on this aspect in part 4 of this paper.

We therefore considered the temporal evolution of the refractive indices for each individual oocyte. Results are presented in table 3 and 4. Table 3 shows the results for the refractive index of the cytoplasm while table 4 shows the result for the zona pellucida. Here, we note that the general tendency is an increase of the cytoplasm refractive index (except for oocytes #6 and #9). Again, the case of the zona

pellucida is no very clear. It should also be noted that in some cases, the theoretical model did not produce coherent values. This aspect is not yet understood.

Table 3: Temporal evolution of the refractive index of the cytoplasm.

	N_c at VG	N_c at MI	N_c at MII
oocyte #1	1.78	1.79	1.91
oocyte #2	1.83	1.93	-
oocyte #3	1.74	1.84	1.89
oocyte #4	1.745	1.85	1.89
oocyte #5	1.70	1.84	1.86
oocyte #6	1.84	1.79	1.79
oocyte #7	1.78	1.85	1.88
oocyte #8	1.83	1.84	1.87
oocyte #9	1.86	1.85	-
oocyte #10	1.88	1.915	1.924

Table 4: Temporal evolution of the refractive index of the zona pellucida.

	n_p at VG	n_p at MI	n_p at MII
oocyte #1	1.95	1.81	2.26
oocyte #2	2.36	2.47	-
oocyte #3	1.99	2.03	2.4
oocyte #4	1.93	2.02	2.29
oocyte #5	1.82	2.20	2.13
oocyte #6	2.45	2.14	1.89
oocyte #7	1.94	2.07	2.24
oocyte #8	2.28	2.24	2.21
oocyte #9	2.42	2.22	-
oocyte #10	2.47	2.24	2.32

4 DISCUSSION

In this section, we would like to put to profit the fact that this is a position paper. As it is mentioned above, the 3 maturity classes are easily identified. But it seems difficult to use the refractive index to precisely define the maturation stage of an oocyte.

We think that this result is not very surprising because the oocytes are subject to a continuous maturation process from VG to MII. Therefore, it is not likely that the refractive indices discretely differ from one class to another one. In order illustrate this let us remember that usually we see seven colors in the rainbow. Apparently, the white light spectrum, when visually observed, exhibits seven different color classes. However, when a spectrogram is recorded, the seven color classes cannot be distinguished anymore. Probably, the qualification of oocytes in 3 classes, visually identified by various morphological characteristics, could be improved by analyzing some of the oocytes optical properties on a more continuous manner. The measurement of the refractive indices may offer an answer to this question.

The above mentioned remarks are obviously opened to further discussions. But we think that the oocytes should be chosen when they are not only MII but also at the right moment when they are MII. We believe that the use of new qualifying techniques that allow taking into account the temporal evolution of the oocytes would be profitable to IVF techniques. At present, the device we present in this paper should still be improved before it can be used on a routine basis. However, an alternative technique we presented recently (Pieralli, 2009) and based on image processing could be envisaged.

5 CONCLUSIONS

In this paper we have presented a simple optical micro-system used for measuring the refractive index of oocytes. The device consists of a Deep Reactive Ion Etched piece of silicon anodic bonded onto a glass substrate. Two perfectly aligned multimode optical fibers are attached to the device. The oocyte is positioned between the two fibers by means of the holding micro-pipette usually employed in IVF centers. White light is launched into one optical fiber and illuminates the oocyte under test. Once the light has propagated through the oocyte, it is collected by the second optical fiber and the optical intensity is recorded. The collected light is measured for 3 positions of the oocyte between the fibers. In this way, and using a mathematical fitting, refractive indices of the oocytes can be measured.

Two theoretical models were developed. In one case, the oocyte was considered as a homogeneous sphere. In a second case, we took into account the core-shell structure of the oocytes. In both cases, the

refractive index of the cytoplasm tends to increase with the degree of maturity. Although the maturity classes can be visually distinguished, it seems difficult to use the refractive index value to estimate the maturity of a completely unknown oocyte. We proposed a discussion on this issue. We think that, and this is perhaps an interesting conclusion of this work, oocytes should not only be qualified in terms VG, MI and MII but also regarding their temporal evolution over these maturation stages.

Studies on a much larger number of oocytes are still required. Also, the investigation of other optical characteristics should be envisaged. At the end, we hope that these studies will help biologists in choosing not only the right oocyte but also the right oocyte at the right moment.

REFERENCES

Beebe, DJ., Wheeler, M., Zeringue, HC. *et al.*, 2002, Microfluidic technology for assisted reproduction, Theriogenology, Vol. 57, pp. 125–135.

Danielli, JF. 1952, Division of the flattened egg, Nature, Vol. 170, pp. 496-496.

Gauthier, G., Piat, E., 2002, An electromagnetic micromanipulation system for single cell manipulation, J. Micromech. Vol. 2, pp. 87–119.

Hwang, H., Lee, DH., Choi, W., *et al.*, 2009, Enhanced discrimination of normal oocytes using optically induced pulling-up dielectrophoretic force, Biomicrofluidics, Vol. 3, pp. 014103-1-014106-10

Inui, H., Nakamura, H., Mizuno, J., *et al.*, 2006, Development of ovum evaluation system for human assisted reproductive technology: verification of the effects and the safety, in *22nd Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE2006)*, Proceedings in Hum. Reprod. 1(Suppl 1), pp. i161.

Kricka, LJ., Faro, I., Heyner, S., *et al.*, 1997, Micromachined analytical devices: microchips for semen testing, J. Pharm. Biomed. Anal. Vol. 15, pp. 1443–1447.

Marquet, P., Rappaz, B., Magistretti, PJ., *et al.*, 2005, Digital holographic microscopy: a noninvasive contrast imaging technique allowing quantitative visualization of living cells with subwavelength axial accuracy, Opt. Lett., Vol. 30, pp. 468-470.

Murayama, Y., Omata, S., Constantinou, CE., 2004, Micro-mechanical sensing platform for the characterization of the elastic properties of the ovum via uniaxial measurement, J. Biomech. Vol. 37, pp. 67-72.

Nakamura, S., Hiramoto, Y., 1978, Mechanical properties of the cell surface in starfish eggs, Dev. Growth Differ. Vol. 20, pp. 317-327.

Ohtsubo, M., Hiramoto, Y., 1985, Regional differences in mechanical properties of the cell surface in dividing echinoderm eggs. Development, Growth and Differentiation, Dev. Growth Differ. Vol. 27, pp.371-383.

Oliviera, PL., Kawooya, JK., Ribiero, JMC., *et al.*, 1995, J. of Biological Chemistry, Vol. 270, pp. 10897-10901.

Pieralli, C., Wacogne, B., André, C., *et al.*, 2009, Biological qualification of oocyte maturity with the use of the Karhunen-Loeve transform: computer aided decision for selecting best oocytes before fertilization, in *Medical Image Analysis and Description for Diagnosis Systems*, Proceedings of the First International Workshop on Medical Image Analysis and Description for Diagnosis Systems, INSTICC Press, ISBN: 978-989-8111-77-7, pp. 77-84.

Sadani, Z., Wacogne, B., Pieralli, C., *et al.*, 2005, Microsystems and microfluidic device for single oocyte transportation and trapping: toward the automation of in vitro fertilising, Sens. and Act. A Vol. 121, pp. 364–372.

Sawai, T., Yoneda, M., 1974, Wave of stiffness propagating along the surface of the newt egg during cleavage, J. Cell Biol. Vol. 60, pp. 1-7.

Shaked, NT., Rinehart, MT., Wax, A., 2009, Dual interference channel quantitative phase microscopy of live cell dynamics, Opt. Lett., Vol. 34, pp. 767-769.

Suh, RS., Phadke, N., Ohl, DA., *et al.*, 2003, Rethinking gamete/embryo isolation and culture with microfluidics, Human Reproduction Update, Vol. 9, pp. 451-461.

Wacogne, B., Pieralli, C., Roux, C., *et al.*, 2008, Measuring the mechanical behaviour of human oocytes with a very simple SU-8 micro-tool, Biomedical Microdevices, Vol. 10, pp. 411-419.

Zeggari, R., Wacogne, B., Pieralli, C., *et al.*, 2007, A full micro-fluidic system for single oocyte manipulation including an optical sensor for cell maturity estimation and fertilisation indication, Sens. and Act. B, Vol. 125, pp. 664-671.

Zeggari, R., Wacogne, B., Pieralli, C., 2006, Optics and microsystems for in vitro fertilisation, Laser Physics, Vol. 16, pp. 294-302.

Zeringue, HC., Beebe, DJ., Wheeler, MB., 2001, Removal of cumulus from mammalian zygotes using microfluidic techniques, Biomed. Microdev. Vol. 3, pp. 219–224.