AFM Investigation of the Mechanical Properties of Mouse Oocytes

Alice Battistella[1,2], Laura Andolfi[1], Michele Zanetti[1],Giuseppe Ricci[3], Marco Lazzarino[1],

(1) CNR-IOM- Laboratorio TASC, Area Science Park, 34149 Trieste, Italy
(2) PhD in Nanotechnology, University of Trieste, 34100 Trieste, Italy
(3) Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste, Italy

e-mail: battistella@iom.cnr.it

Keywords: Oocytes, elasticity, IVF (In Vitro Fertilization), Force Spectroscopy

In embriology and reproduction research the study of the mechanical features of oocytes can improve our understanding about the mechanisms involved in the fertilization and the embryo development and provide an alternative method to assess the oocyte quality for In Vitro Fertilization (IVF) [1].
Atomic Force Microscopy (AFM) investigations on the mechanical properties of zona pellucida (ZP) of human oocytes (the external glycoproteic layer) showed the presence of two layers with distinct elasticity values [2]. Moreover, differences in the Young modulus between oocytes at different maturation stage (MI and MII oocytes) and suitable or rejected MII oocytes, as evaluated for in vitro fertilization, were found.
In order to have a direct correlation between mechanical properties, oocyte quality and birth outcome, an animal model was chosen. In this work the mechanical properties of mouse oocytes were investigated.
Mouse oocytes were retrieved from superovulating mice [3]. Oocytes were denuded from cumulus cells by means of a passage in a solution of medium containing an enzyme (a hyaluronidase) and a mechanical treatment consistent in pipetting with a capillary with an inner diameter (100 µm) slightly larger than the mouse oocytes diameter (80 µm). . AFM-Force Spectroscopy measurements were carried out by using a triangular tipless cantilever with a silica-bead glued on the tip in a closed fluid cell with temperature controller.
For analyzing more than one oocyte in the same chamber of measurement, we designed and fabricated micro-structured multi-well supports by replica molding [4] (see Figure 1). The surface of these supports was coated with a peptide to facilitate cell adhesion.
The measurements were made on a 2x2 µm area roughly at the center of the oocyte. Even in the case of mouse oocytes the contribution of two Young moduli of the same order of magnitude of human oocytes (E1 and E2) were observed (see Figure 2).
Changes in E2 values were also observed when the position of the tip over the cell is varied, maybe
due to the orientation of the oocyte and the position of the spindle. Moreover, measurements of the same oocyte at increasing time were performed to investigate the evolution in time of the mechanical parameters of the oocytes. A defined trend in the E2 values was observed, resulting in an initial hardening and a following softening of the ZP after 1.30h from the retrieval (see Figure 3).
The correlation between mechanical properties and oocyte ageing provides important indications to evaluate oocyte quality in IVF and for the realization and development of a mechanical-assisted oocyte sorter.

[1] Y. Murayama, J. Mizuno et al., Human Cell, 19, 119-125 (2006)
[2] L. Andolfi, Integr. Biol.,8,886 (2016)
[3] Kline,D.; Methods in Molecular Biology (2009)
[4] X.Y. Liu, Y. Sun, Microfluidic Devices for Biomedical Applications, 351-362 (2013)

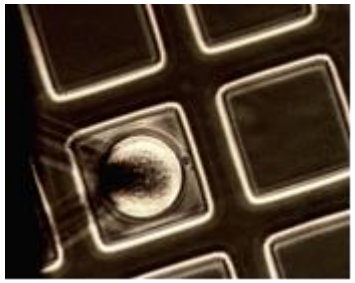

Figure 1: mouse oocyte immobilized on the micro-structured support

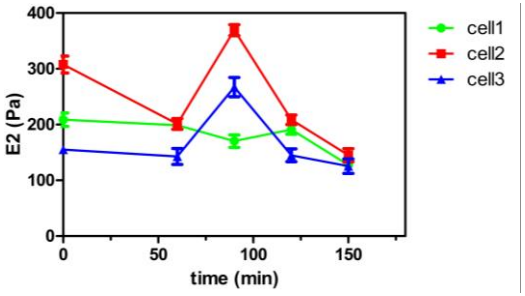

Figure 3: Young modulus variation in time after the retrieval

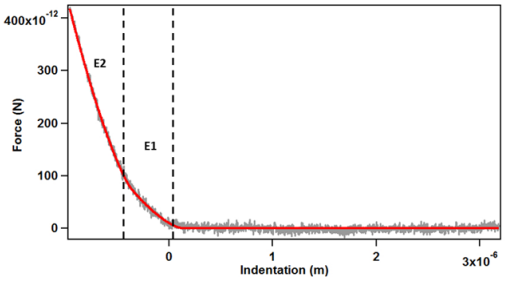

Figure 2: F-d curves in which the presence of the two Young moduli (E1, E2) is showed