Atomic force microscopy (AFM) of heat-treated human sperm

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Introduction

Atomic force microscopy (AFM) employs a sharp probe for profiling surfaces with unique resolution (Fig.1a,b). AFM provides high resolution imaging of surface structures at scales ranging from a tenths nanometers to tens of micrometers. Heat stress is considered to be one of the factors of the DNA fragmentation and sperm apoptosis.

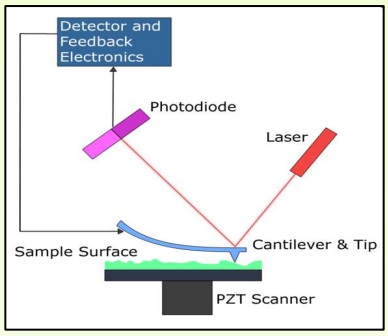

Fig. 1a: AFM principle (Nobel Prize 1986 Binnig, Quate, Gerber)

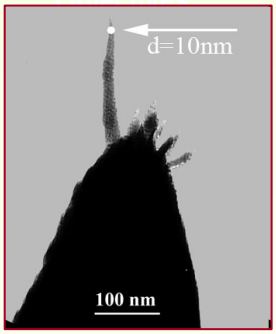

Fig. 1b: Detail of the probe with sharp tiny tip

Objectives

The aim of the study was to use AFM for imaging the surface pathology of the sperm exposed to heat treatment.

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Materials and methods

Fresh samples of semen from sperm donors were obtained. Spermatozoa were selected by using the conventional swim-up procedure. The first sample was taken immediately after finishing swim-up at the temperature 37.0 C, the second after heating 30 minutes at 40.0 C and the third after 30 minutes at 50.0 C.

Atomic Force Microscope NTgera Vita (NT-MDT) was use to characterize spermatozoa morphology within a nanometer scale (Fig. 2).

Methods

Scanning head type SFC050LNTF designed for scanning by probe (maximal operation area 100x100x10 µm) equipped with a holder for operation in a liquid was used for all experiments. Glass slide with immobilized spermatozoa was fixed to the bottom of plastic Petri dish and the internal volume was filled with sterile isotonic solution of sodium chloride. Samples imaged in contact mode at ambient temperature were scanned by using silicon nitride probe with 0.01 N/m elastic constant at 0.25 Hz scanning speed. Surface topography and spatial distribution of electrical potential gradient on the sample surface were measured and recorded simultaneously, thus providing more complex understanding to the surface morphology. The defects were detected and recorded.

Fig. 2: Atomic Force Microscope NTgera Vita (NT-MDT)

Results

The obtained images clearly show both normal sperm head (Fig. 3) and defects of the surface of spermatozoa exposed to heating. After 30 minutes heating at 40.0 C only tiny surface defects were observed, after heating at 50.0 C most of the sperms were nearly damaged.

Changes of the sperms after heat-treating are presented on the figures (Fig. 3 , 4).

Fig. 3: AFM image of normal sperm head before heating

Fig. 3: Tiny surface defects of sperm after 30 minutes heating at 40.0 C.

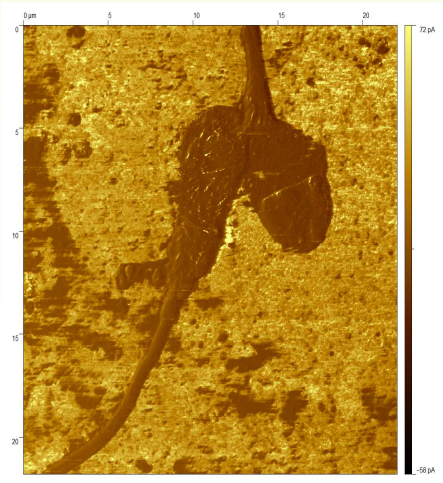

Fig. 4: Severe defects of sperm heads after 30 minutes heating at 50.0 C.

Conclusion

The AFM images clearly highlight many details of normal spermatozoa and spermatozoa damaged by heating. This technique could be an important tool in the research of oxidative stress and understanding its effect on male infertility.

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