An evaluation of the application of the aperture scanning near-field optical microscopy for ultra-structures analysis of anomalous human spermatozoa

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Abstract

In the practice of the assisted reproductive technologies the morphology of spermatozoa is a fundamental parameter to evaluate men fertility. In the routine protocol such analysis is commonly carried out by a light microscopy. Peculiar region of the spermatozoon such as head, neck, midpiece, principal and terminal part of the flagellum are the main regions to be considered for identifying sperm defects [1].

A more detailed investigation and description of the organization and structures of these cellular regions is generally performed by transmission electron microscopy (TEM). Although this microscopy technique is a powerful tool for ultrastructures analysis of cell and tissue, the sample preparation is time-consuming and expensive, moreover this staining protocol might interfere with the cell structure and generate artifacts in the images. In this respect the scanning probe microscopies, which require a very simple sample preparation, offer several advantages in the investigation of cell structural organization. Among them the scanning near-field optical microscopy (SNOM) held interesting promising in the study of biological systems. SNOM can provide simultaneously topographical information and optical images of the sample with a resolution beyond the diffraction limit [2]. Moreover the technique enables to combine superficial information with inner cellular organization in a single acquisition [3,4]. Such ability is very interesting to analysis the organization of peculiar functional structures and organelles inside the cell with high spatial resolution.

In this work sperm cells are immobilized on poly-L-lysine coated coverslips, fixed according to a standard procedure and imaged by aperture SNOM in air. The images obtained on normal spermatozoa (Fig.1) are compared with those obtained on anomalous spermatozoa (see an example in Fig.2). A comparison with the structures observed in TEM images is also made, to understand the potentiality of the technique to analysis sperm cell structures without the need of specific sample preparation.

The analysis of the optical SNOM images demonstrates significant differences between optical features observed in normal and anomalous spermatozoa. Moreover the optical features detected in the SNOM transmission images exhibit good similarities with TEM images both for normal and cell defects detected in anomalous sperm cells. This analysis reveals the potentialities of the SNOM technique in the embryology field. This opens to the application of the technique to the analysis of spermatozoa to obtain high resolution information about morphological variations as result of cell defects or drug treatments, which might be relevant in the biomedical field.

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Figure 1: SNOM images of a normal spermatozoon as classified according the morphological parameters: SNOM Topography (A), SNOM Reflection (B) and SNOM Transmission (C).

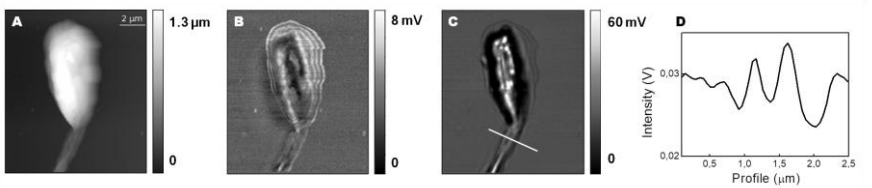

Figure 2: SNOM images of an anomalous sperm cell as classified according the morphological parameters: SNOM Topography (A), SNOM Reflection (B) and SNOM Transmission (C) and profile along the white line (D).