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Effect of Graphene Oxide on mammalian spermatozoa membrane lipid remodeling

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Introduction

Capacitation is the final step in spermatozoa's preparation to fecundate the oocyte. This process occurs after ejaculation and can be controlled in vitro. One of the changes that take place during capacitation is the membrane's remodeling. It consists of the relocation of cholesterol to the apical part of the head and its depletion, which is dependent on oxysterols formation and extracellular cholesterol acceptors (like BSA or MBCD) (1). Furthermore, since they have no transcription system and are therefore little able to adapt to strange environments, spermatozoa can be used to analyze in vitro the reproductive toxicity of new materials, like Graphene Oxide (GO) which has been attracting so much attention due to it's unique quality and the possible application in medicine (2). It has been shown that exposure of spermatozoa to different concentrations of GO (0,5; 1; 5; 10 µg/mL) increase capacitation and In vitro fertilization rates. To better understand the mechanism of action of GO, greater attention has been paid to the changes occurring in the membrane(3).

Methods

FRAP — Sperm incubated in capacitating medium with GO (0,5; 1; 5 µg/mL) for 2h. Stained with the lipophilic probe DilC12. Then accessed a confocal microscope to study the membrane fluidity

Cholesterol Staining with Filipin III — Sperm incubated in capacitating medium with GO (0,5; 1; 5 µg/mL) for 2h. Stained with the cholesterol probe Filipin III and visualized with a confocal microscope to observe the distribution of cholesterol and analyzed by flow cytometry to prove the depletion of cholesterol from the membrane

DSC — Sperm incubated in capacitating medium with either BSA, MBCD or GO (1 µg/mL) for 2h. Following which membrane was isolated and used for differential scanning calorimetric analysis at a rate of 10ºC/min.

Results

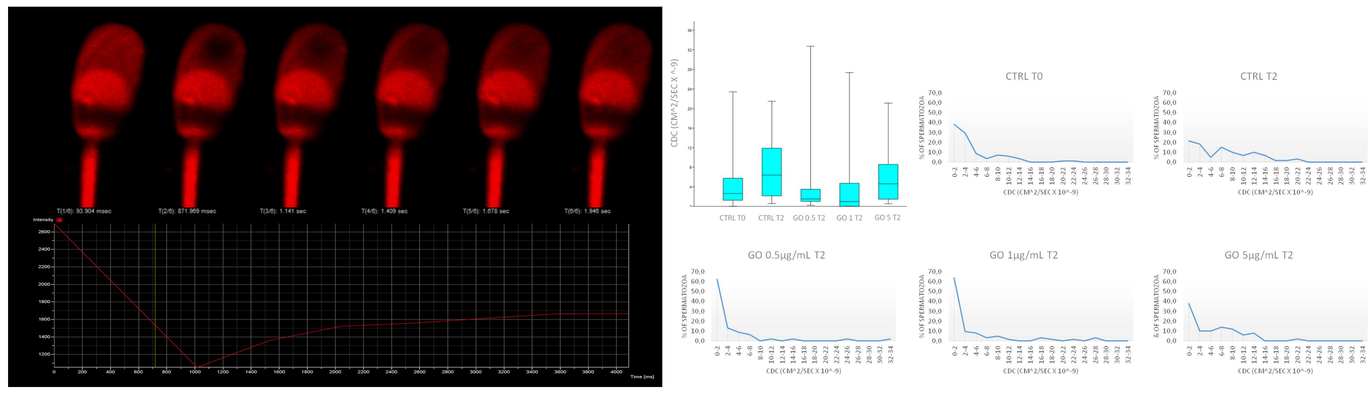

Figure 1. Membrane fluidity test by Fluorescence Recovery After Photobleaching (FRAP) assay of spermatozoa stained with DilC12 using a confocal microscopy.

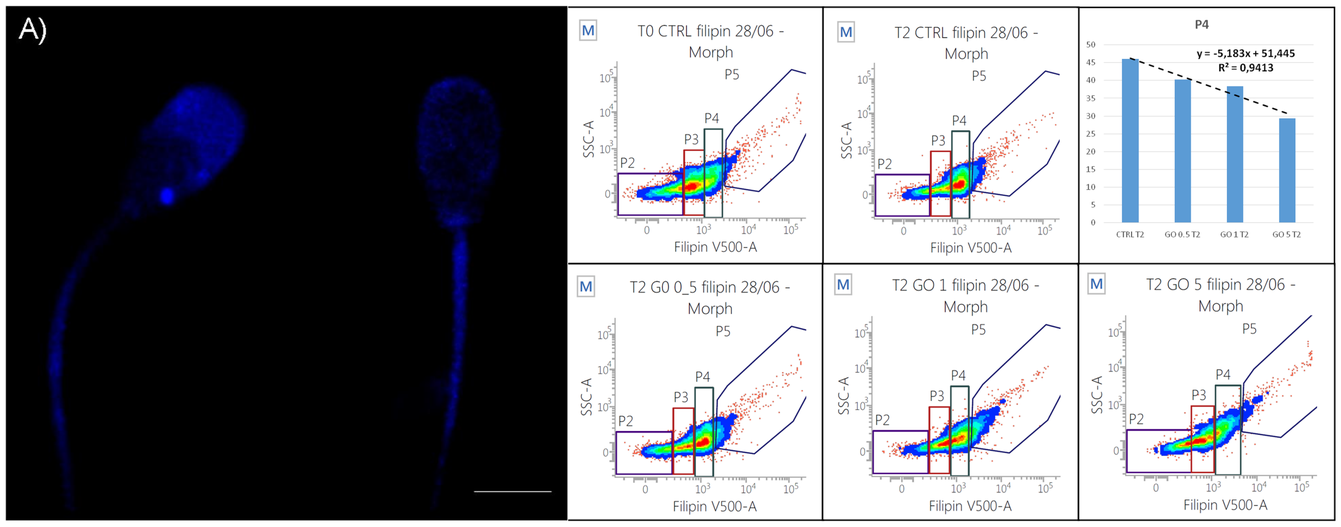

Figure 2. Cholesterol staining with Filipin III observed by confocal microscopy(A) and Flow cytometry. P2-P5 denote the different sperm subpopulation found in each sample, on the basis of filipin III emission and SSC value.

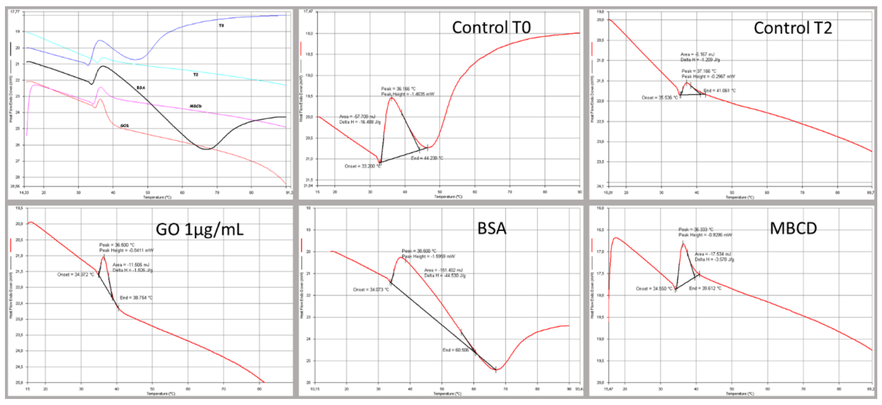

Figure 3: Differential Scanning Calorimetric analysis of the membrane response to a temperature scan. Comparison between Graphene Oxide and other molecules known to remove cholesterol (BSA and MBCD).

References

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Conclusions

Graphene Oxide is interacting with the spermatozoa membrane, altering the fluidity of the membrane (as evidenced by the FRAP analysis). Furthermore, flow cytometry shows proof of a decrease in cholesterol levels, in a concentration dependent manner. The comparison between GO and other cholesterol depletory molecules (BSA and MBCD) by DSC indicate a similar effect in the membrane.