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Graphene Oxide drives capacitation dependent membrane remodeling in mammalian spermatozoa

J. Simões[1], M. Ramal Sanchez[1], A. Di Stefano[2], A. Fontana[2], P. Lanuti[3], E. Ercolino[3], M. Marchisio[3], L. Botto[4], P. Palestini[4], G. Capacchietti[1], L. Valbonetti[1], V. Russo[1], N. Bernabò[1], B. Barboni[1]

[1] Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, 64100 Teramo, Italy; 2 Department of Pharmacy, University "G.d'Annunzio"; Chieti-Pescara, 66100, Chieti, Italy; 3 Cytomorphology Laboratory, Center on Aging Science and Translational Medicine (CeSI-MeT), University "G.d'Annunzio" Chieti-Pescara, Italy. [4] School of Medicine and Surgery, University of Milano

Introduction

Spermatozoa are highly differentiated cell that suffer through several stages of preparation before being able to fecundate the oocyte. Capacitation is the final step in of this process and takes place in the female tract or *in vitro*. One of the key changes occurring 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, bicarbonate signaling and extracellular cholesterol acceptors (like BSA or MBCD) (1).

Furthermore, sperm cells are poorly adaptable to new environments but very sensitive to them because they have no transcription mechanism and rely strongly on extracellular signals for their function. Therefore, spermatozoa are very useful models for toxicity studies of new materials. One such example is Graphene Oxide (GO) which has been attracting the worlds attention since its discovery in 2005 due to the incredible qualities (like strength and conductibility) and amazing range of possible application, including many in the medical field(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

Membrane Fluidity

- Membrane fluidity was measured by the FRAP technique using the lipophilic probe DilC12.

Cholesterol/Phospholipids ratio

- Lipids were extracted from spermatozoa and analyzed by HPTLC

Membrane potential

- Membrane potential was assessed by flow cytometry using the fluorescent probe Bis-oxonol

Calcium intake

- Intracellular calcium concentration was analyzed by flow cytometry using the calcium sensitive probe FURA 2-AM

References

1. Boerke, A. et al. Biol. Reprod 2013, 88, 21
2. Liu, S. B. et al. ACS Nano 2011, 5, 6971−6980
3. Bernabò, N. et al. Carbon. (In press)

Results

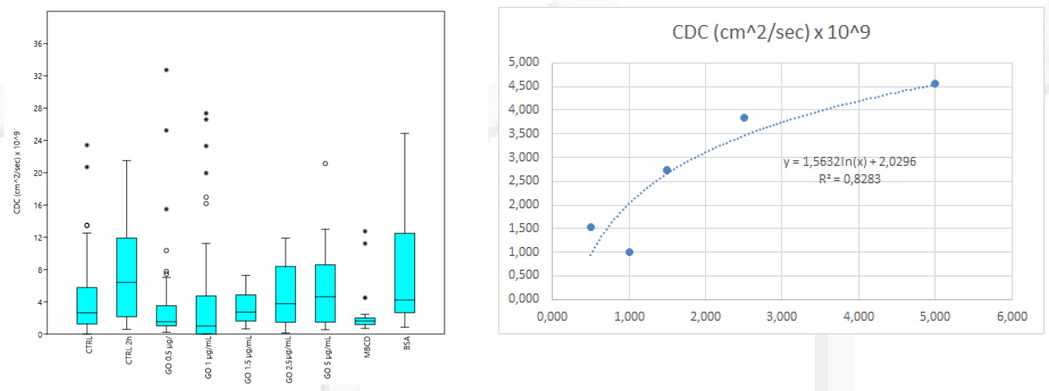

Figure 1: Membrane fluidity test by Fluorescence Recovery After Photobleaching (FRAP) assay of spermatozoa stained with DilC12 using a confocal microscopy.

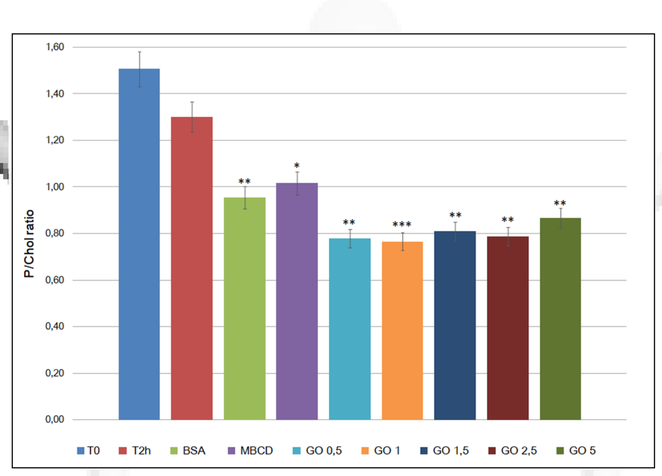

Figure 2: Cholesterol/Phospholipids ratio measured by HPTLC after 2 hours of incubation in conditions favouring capacitation with GO added to the medium.

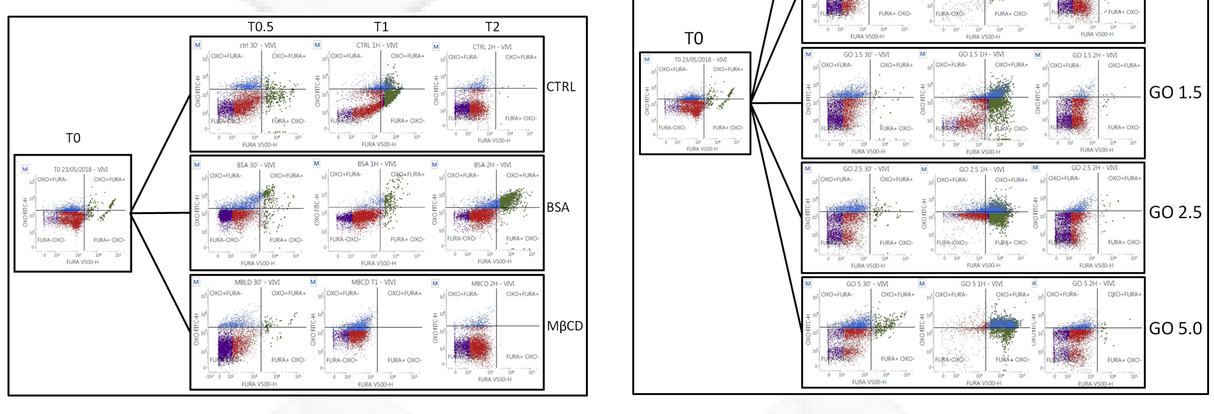

Figure 3: Flow cytometry analysis of spermatozoa stained with FURA 2-AM for calcium, Bis-oxonol for membrane depolarization and 7AAD for cell viability.

Conclusions

Graphene Oxide is interacting with the spermatozoa membrane in a way that resembles other cholesterol accepting molecules (BSA and MBCD), in particular at a concentration of GO of 1µg/mL there is a very demarked decrease in total cholesterol content as measured by HPLC. Furthermore, we can observe an increase in the calcium intake at 1h of incubation which is likely related to the opening of ion channels in the membrane. Whether this is due to a direct interaction between GO and the channels or if is a side effect of the decrease in cholesterol is still unclear. However, the results obtained so far confirm the belief that GO works as a cholesterol depleting molecule that favors capacitation.