

Graphene oxide interacts with sperm membrane and affects their acquisition of fertilizing ability

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Over the last few years, graphene oxide (GO), the most widely investigated water-soluble functionalized analogue of graphene, has been widely used in regenerative medicine and biotechnology. Data on toxicity are conflicting. For instance, it has been found that these materials could have time, dose- and dimension-dependent cytotoxic effects on important cellular models such as erythrocytes and fibroblasts [1], whereas other studies demonstrated no toxicity of GO on fibroblasts [2].

Due to the lack and controversy of works evaluating the toxicity of GO on spermatozoa [3,4], we carried out an experimental set up to assess the effects of GO on mature mammalian spermatozoa. In particular, we studied the interaction between GO and boar sperm membranes during capacitation (i.e. the process that leads the spermatozoa to gain their fertilizing ability).

To this aim, we exposed male germ cells at different concentrations of GO (50, 10, 5, 1 and 0.5 µg/mL) for 4h in a validated in vitro system able to promote the capacitation. At 1h intervals, we assessed the percentage of spermatozoa showing structural damages at the acrosomes (Figure 1) and the membrane fluidity by using a confocal microscopy-based Fluorescence Recovery After Photobleaching method (Figure 2). Finally, we performed an in vitro fertilization experiment (IVF) to verify the effect of GO on fertilizing ability of spermatozoa (Figure

3). As it is evident from the results obtained, GO promotes a concentration-dependent increase in loss of acrosomes and interacts with membranes decreasing their fluidity on decreasing the concentration of GO.

Very interestingly, GO seems to negatively affect the IVF outcomes, although a possible positive effect on fertility can be monitored for one of the less concentrated sample concentrations (1 µg/mL GO).

References

- [1] Liao, K.H. et al. *ACS Appl. Mater. Interfaces* 3, 2607-2615 (2011).
- [2] V. Ettorre et al. *Carbon* 102, 291-298 (2016).
- [3] Akhavan, O. et al. *Carbon N. Y.* 95, 309-317 (2015).
- [4] Hashemi, E. et al. *RSC Adv.* 4, 27213 (2014).

Figures

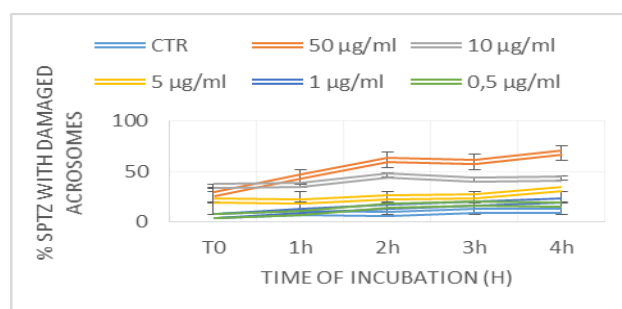


Figure 1. Effect of GO on acrosome integrity

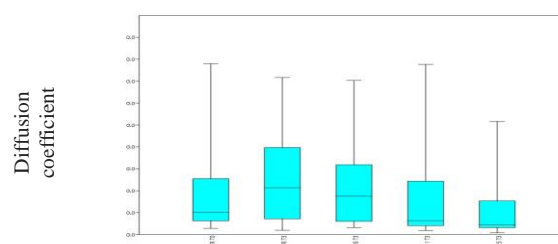


Figure 2. Effect of GO on membrane fluidity

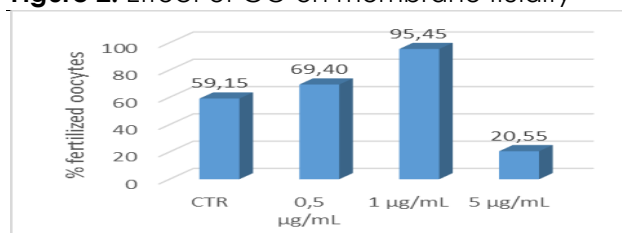


Figure 3. Effect of GO on IVF outcome