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Poster Presentations

P-144

Non-toxic concentrations of graphene oxide and its derivatives on ovarian stromal cells showed angiopoietic properties on chick embryo chorioallantoic membrane; An approach for augmented angiogenesis in tissue engineering and tissue transplantation

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Background: Assembling an artificial ovary is new fertility treatment and preservation tactic that mimic the function of the natural human ovary. Its goals are producing oocytes and releasing steroid hormones by the help of biocompatible materials which should minimize inflammation and enhance neo-angiogenesis. A successful angiogenesis process is essential for follicle development, ovarian tissue and artificial ovary transplantation. Graphene is a two-dimensional nanomaterial, mostly used in the form of graphene oxide (GO). It is an anti-apoptotic agent due to the trapping of vascular endothelial growth factor. In the recent years GO and its derivatives are in spotlight for their angiogenic properties and vasculogenesis.

Objective: We aimed to determine which GO derivatives and with which concentrations can better enhance angiogenesis on chick embryo chorioallantoic membrane.

Materials and Methods: Human ovarian cells were harvested. GO and its derivatives including lysine-treated GO (GL), arginine-treated GO (GA), carboxylated GO (GC) and polyethylene glycol (PEG) functionalized GO (Gpeg) were prepared. A mixture of ovarian cells was treated with the concentrations of 50-10000 ng/ml (based on published papers) of the mentioned materials for 48 h. The process was repeated for 8 days in 96 wells plate. As, concentrations close together, led to the same results, so at the next step, the cells were treated with higher concentrations of the mentioned materials (100-220000 ng/ml, based on a pilot study), for 8 days, in 48 wells plate. Since the cumulus cells (CCs) are the most important cells act in the follicle development, so CCs were treated with 100-220000 ng/ml of mentioned materials for 8 day in 48 wells plate, to find which kinds of nano materials and which concentrations are nontoxic for both ovarian and CCs. The MTT assay was used for cell viability. Then, the angiopoietic properties were assessed by chick embryo assay. Non treated cells were used as the control.

Results: The MTT assay after 48 h treatment with 50-10000 ng/ml of mentioned materials showed that 50-400 ng/ml of GO, all concentrations of GC, 200-10000 ng/ml of GL, 900-8000 ng/ml of GA and 50-1600 ng/ml of Gpeg caused higher cell proliferation. MTT assay after 8 days treatment with 50-10000 ng/ml of mentioned materials showed that 50-700 ng/ml of GO, 50-2000 ng/ml of GC, 50-8000 ng/ml of GL, and GA and 50-2000 ng/ml of Gpeg led to higher cell viability. MTT on CCs showed that 100, 300, 900, 2700, and 8100 ng/ml of GO and 100, 300, and 900 ng/ml of GA had higher cell proliferation. About GC, 100, 300, 900, and 2700 ng/ml had the same results as control. All concentration of Gpeg and GL led to lower proliferation, compare to control. So, 100, 300, 900, 2700, and 8100 ng/ml of GO, 100, 300, and 900 ng/ml of GA, 100, and 300 ng/ml of GC and 100 ng/ml of

GL and Gpeg were used for chick embryo assay. We concluded that 900, and 2700 ng/ml of GO, 100, and 300 ng/ml of GA and 100 ng/ml of GC, and Gpeg could enhance angiogenesis on chick embryo chorioallantoic membrane. GL was not so beneficial, also it caused embryo death in some eggs.

Conclusion: GO and its derivatives can act as an angiogenic agents, so it is proposed that they can be used in ovarian tissue transplantation and ovarian tissue engineering to enhance angiogenesis.

Key words: *Graphene oxide, Angiogenesis, Tissue Engineering, PEG..*