Graphene Oxide Promotes Epithelial Mesenchymal Transition In Ovine Amniotic Epithelial Stem Cells Affecting Their Immunomodulatory Properties

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Introduction:

Regenerative medicine is focusing the attention on immune engineering strategies taking advantage of biomaterials to influence stem cells fate and to stimulate the production of immune modulatory factors to be employed in cells free treatment (1). The Graphene Oxide (GO) was proved to be able to affect stem cells behaviour and to modulate their immune response (2). According to this evidence, the present project was designed to produce secretome with immune modulatory potential by combining GO and/or LPS with Ovine Amniotic Epithelial Stem Cells (oAECs), which exhibited teno-regenerative and high immunomodulatory properties *in vitro* and *in vivo* (3), to be used in regenerative medicine applied to tendon regeneration.

Methodology:

AECs isolated from the amniotic membrane of ovine fetuses (4) were seeded in standard condition (AEC) or with 25µM of progesterone (AEC+P4) (4) or on GO functionalized cover slides (GO). At 70% of confluence, the cells were treated with 1 µg/ml LPS (4) for 1h (AEC+LPS; AEC+P4+LPS, GO+LPS), and incubated for 24h in the Serum Free media. At the end of the experiment, the Epithelial Mesenchymal Transition (EMT) process was assessed within the different tested group by analysing Cytokeratin 8 (CYTO8) and Vimentin (VIM), epithelial and mesenchymal markers respectively, protein expressions and by evaluating the involvement of pSMAD2 and SMAD2/3 pathways. The collected conditioned media (CMs) were used to analyse the profile expression of 40 immunomodulatory cytokines by Inflammation Antibody Array Membrane.

Results:

The results demonstrated that GO alone or with LPS (GO+LPS) induces AEC morphology changes shifting the protein patterns expression toward the mesenchymal phenotype, as showed by negativity to CYTO8 and positivity to VIM. This data suggests the GO accelerated EMT process in AEC and AEC+LPS. In contrast, P4 was able to maintain the epithelial phenotype with higher CYTO8 expression in AEC (AEC+P4) (4) also after inflammatory stimulus (AEC+P4+LPS). Moreover, the upregulation of pSMAD2/SMAD2 proteins ratio was observed in GO group and especially in GO+LPS samples compared to those present in AEC, AEC+LPS, AEC+P4 and AEC+P4+LPS ($p<0.001$) supporting the EMT transition GO-dependent. Furthermore, preliminary inflammatory array assay results on cells CMs suggested that GO was able to modify the cytokines expression profile in cells secretome, by reducing in particular the anti-inflammatory IL10, IL11, IL13 TIMP2 (tissue metalloproteinase inhibitor 2) and CXCL9 (recruiter of leukocytes) chemokine release highly induced in AEC after LPS treatment.

Conclusions:

These preliminary data demonstrated that GO accelerates the EMT process in AEC altering their immune response and affecting the release of immune factors. In order to employ these secretomes in regenerative medicine, more experiments are needed to deepen the knowledge of the link between EMT and immunomodulation and to evaluate their biological effects on immune cells.

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Key words: Immune regenerative strategies, AECs, secretome, Graphene Oxide, Tendon regeneration