Chemico-physical and biocompatibility characterization of GO-based collagene biomembranes

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Nowadays, promoting regeneration is a major goal in the field of bone regeneration and tissue engineering and different biomaterials have been employed to favor osteoblast adhesion and proliferation [1]. Among these, Derma® commercial biomembranes by Tecnoss are commonly used for bone regeneration and to reduce the inflammatory response around the implanted area. Derma® biomembranes are obtained from derma of porcine origin and are completely resorbable. Their strong consistency and resistance allow a perfect stabilization and a prolonged protection of underlying graft in large regeneration procedures, together with a strong barrier action to guide the growth of epithelium and preventing its invagination [2,3].
The aim of this study is to enhance the biomembrane performances, such as the wound healing properties of the raw material, introducing Graphene Oxide (GO) in the biomembrane scaffolds. In particular, the goal is to reduce the time needed for the complete bone regeneration. GO has emerged as an attractive species to be used as an additive for biomedical materials due to its good manipulability, ability to be dispersed in aqueous solutions and stability as a single layer material. Thanks to the biocompatibility at low concentrations and 2D nature, GO have recently captured the attention as cell culture substrate and, combined with other materials, to provide functional and biologically active surfaces [4]. GO was synthesized in our facilities using a modified Hummers method [5,6]. TEM, SEM and Raman measurements revealed that the GO produced is ranging from 2 to 5 layer thick.
A solution of GO in water at two concentrations (namely 2 and 10 μg/mL), ultrasonicated for 30 min and centrifugated at 5500 rpm for 15 min were added to pieces of square biomembranes (0.5x0.5 mm lateral dimensions). After the liquid evaporation (all night under hood), the pieces were placed in a 96 multiwell plate (see Figure 1) for the in vitro biocompatibily tests on primary human gingival fibroblasts (HGFs) and mesenchymal stem cells. In order to evaluate the ability of the samples to conditioning the medium, HGFs were seeded in a 96 multiwell plate on as received Derma and on the two different modified and cultured in Dulbecco's modified Eagle's medium DMEM (Euroclone, Pero, MI, Italy) + 10% Fetal Bovine Serum (FBS) for 1, 3 and 7 days. After each experimental time, an MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was carried out. HGFs viability was evaluated by MTT test at the established experimental points, showing no differences of HGFs viability in GO-coated biomembranes and pristine biomembranes. This initial evaluation lead us to suppose that the modified biomembranes do not condition the medium, and that the GO-coated biomembranes are not toxic on HGFs.
In order to evaluate the effect of the GO in the induction of differentiation of human mesenchymal stem cells towards osteoblastic lineage [7], pristine biomembranes and GO-coated biomembranes were investigated using a LDH test. Preliminary results show no toxicity of the GO-modified material and experiments are on going in order to evaluate their differentiating activity.
In order to study the surface and volume morphology of the GO-modified biomembranes, SEM, TEM, AFM and ToF measurements will be performed. Thermogravimetric analysis (TGA) will be carried out to quantify the amount of GO covering the biomembranes. XPS experiments will also be performed in order to study the physical and chemical composition of the surface of the hybrid material.

References

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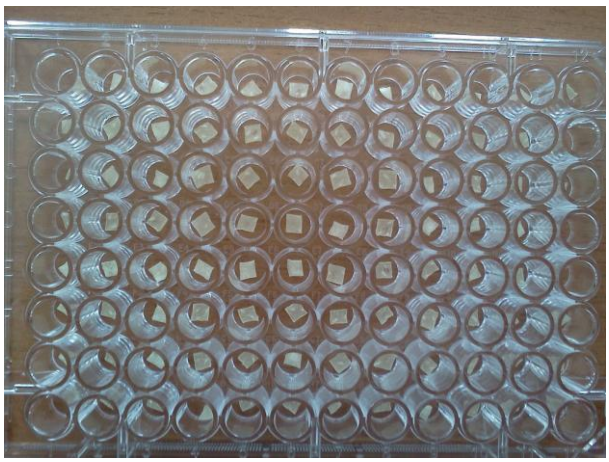

Figure 1: GO modified Derma biomembranes in a 96 multiwell plate