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TITLE: Bone-forming Genes Expression of Osteoblasts Cultured on Polymeric Nanosctructured Membranes.

ABSTRACT BODY:

Objectives: To evaluate the effect of polymeric nanostructured membranes in osteoblasts differentiation.

Methods: Nanostructured membranes were produced by electrospinning and functionalized with SiO_2-NPs (NanoMyP). They were then doped with doxycycline by immersion in an aqueous doxycycline solution. The following groups were established: 1) Undoped membranes (HOOC-M), 2) SiO_2-NPs functionalized membranes (HOOC-Si-M), 3) SiO_2-NPs functionalized membranes and doped with doxycycline (Dox-HOOC-Si-M). Membranes were subjected to MG63 osteoblast-like cells culturing (ATCC, Manassas, VA, USA) during 48h. Differentiation was assessed by real-time quantitative polymerase chain reaction (RT-qPCR) and Field Emission Scanning Electron Microscopy. In the RT-qPCR; TGF-β1, Runx-2, ALP, OPG, RANKL and BMP-2 were studied. Three membranes of each group were subjected to each test and both tests were performed in triplicate. Mean comparisons were conducted by one-way ANOVA and Tukey tests ($p<0.05$).

Results: The RT-qPCR results are in the Table -means (nG of mRNA per nG of House Keeper gene) and standard deviations-. Letters indicate differences between membranes. All the studied genes were overexpressed in the Dox-HOOC-Si-M group, except RANKL which was downregulated, when compared with HOOC-M. The OPG/RANKL ratio, which expresses the bone-building activity of osteoblasts, was up-regulated in 28-fold change by the Dox-HOOC-Si-M when compared with HOOC-M group. The osteoblasts cultured on the modified membranes, showed an elongated spindle-shaped morphology, which has been associated with a more differentiated state.

Conclusions: The functionalization of the polymeric membranes with SiO2-NPs and Dox produced an increase of osteogenic gene expression on cells. Supported by Ministry of Economy and Competitiveness and European Regional Development Fund [MAT2017-85999P MINECO/AEI/FEDER/UE].

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