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***In vitro* effects of periodontopathic bacteria on the proliferation, stemness, and osteogenic potential of human mesenchymal stem cells**

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Introduction:

Periodontitis is a bacterial biofilm induced inflammatory disease that results in the destruction of the periodontal components including the destruction of the PDL, cementum, gingiva, and alveolar bone. Achieving complete periodontal regeneration by conventional methods has shown some limitations with certain extensive cases. That is what made concern to be directed towards the use of stem cells in such cases. This study mainly concentrates on the immortalized human mesenchymal stem cells (hMSCs), and their ability to withstand culturing and incubation with the bacteria that are involved in periodontitis, namely, *Actinobacillus actinomycetemcomitans*, and *Eikenella corrodens*, in order to elucidate their effects on the behavior of the hMSCs regarding their stemness, degree of osteogenic differentiation, and proliferation capacity.

Methodology and Results:

For the first time the periodontal pathogens, namely the *Actinobacillus actinomycetemcomitans*, and the *Eikenella corrodens* were managed to be grown in the normal cell culture medium (DMEM). After stopping the growth of the bacteria with gentamycin, bacteria were incubated for two months with the immortalized hTERT hMSCs with the ratios of 1:1 and 1:100 resulting in five different cell-bacteria experiments (AA1, AA100, EC1, EC100, and control). Bacterial induction on the MSCs' proliferation was determined by cell counting applying the Cellometer and ki-67 via FACS analysis. The results suggested that the higher bacterial numbers (ratios), the higher the proliferation rate of the MSCs. During and after the two months of bacterial incubation, the MSCs were checked for the effect of bacterial induction in terms of stemness by applying immunohistochemistry for the stem cell markers (CD44, CD29, CD166, and CD105). Furthermore, real time PCR determined the relative expression ratios of the stemness markers *c-myc*, and *klf4* genes, and their effect on the relative expression of the osteogenic *col1*, and *runx2* genes. Results that were obtained from the immunohistochemistry and the real time PCR showed that the MSCs in all of the different cell experiments still preserved their stemness after bacterial incubation. To confirm these previous results, MSCs in the different cell experiments were directed to the osteogenic differentiation lineage. The cells were divided into two major groups. One group was osteodifferentiated with the presence of bacteria (O-AA1+, O-AA100+, O-EC1+, and O-EC100+), while the other was osteodifferentiated without the presence of bacteria (O-AA1-, O-AA100-, O-EC1-, O-EC100-, and O-control). The osteodifferentiation was confirmed by alkaline phosphatase staining, and immunohistochemistry using antibodies against osteopontin. Cells in all of the nine different experiments demonstrated positive reactions with both of the alkaline phosphatase staining and the immunohistochemistry. Further, the degree of osteogenic induction was determined using the real time PCR for the *runx2* and *col1*. MSCs in all of the nine different experiments showed up-regulation of both the *runx2* and *col1* genes confirming the osteodifferentiation.

Discussion:

Regarding the effects of the periodontopathic bacterial incubation on the proliferation, degree of stemness, and osteogenic differentiation of the hMSCs, it can be stated that periodontal pathogenic induction can be used as an enhancing factor that can increase the hMSCs proliferation, and can improve and enhance their stemness potential, and osteogenic differentiation.

Keywords:

Periodontitis; immortalized human mesenchymal stem cells; periodontopathic bacteria.