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PARACRINE EFFECTS OF SECRETOME FROM STEM CELLS IN THE CULTIVATION OF KERATINOCYTES

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Paracrine factors, such as growth factors and cytokines, are released by stem cells, which are involved in the tissue regeneration process. Up to now, the secretome from human exfoliated deciduous teeth (SHED), has still not been evaluated with the objective to analyze their contribution on epithelial regeneration/repair. The aim of this study has been to investigate the in vitro paracrine effects of SHED in the culture of HaCaT cells. SHED were isolated, characterized and the SHED conditioned medium (SCM) was collected. Extracellular vesicles (EV) were isolated by sequential centrifugations at increasing speeds (300g for 10 min; 2000g for 20 min; 16500g for 20 min; 120000g for 99 min). The last pellet was resuspended in sterile PBS and ultracentrifuged again at 120000g for 99 min. Exosomes were characterized by transmission electron microscopy (TEM) and Zetasizer to determinate morphology and diameter. The amount of protein was measured by Bicinchoninic Acid Protein Assay. Various proportions of SCM and EV were tested. A total of 1,600 HaCat cells and SHED were seeded and cultured on a 96-well plate. Cell viability was evaluated by MTT and Live/Dead assay. To evaluate cell migration, the scratch test assay was carried out after 1 day. Finally, the cell viability of HaCaT cells treated with exosomes of FBS and SHED were compared. Results were expressed as mean ± standard deviation and statistical analysis of data was performed by independent samples t-test. Cells showed a proliferative peak between the fourth and sixth day. After 6 days of culture of HaCaT cells in various concentrations of SCM (50, 60, 70, 80, 90 and 100%), it was demonstrated that the best concentration of SCM for the culture was 50%. HaCat cells cultivated with SCM 50% showed higher viability (Abs. 0.179±0.05) than those grown with control medium (Abs. 0.135±0.03), with a significant difference ($p<0.001$). Extracellular vesicles mean size was 166.8nm, while TEM showed vesicles with a round shape. The total quantity of protein was 102,06µg. HaCat treated with 0,4µg/µl of EV showed a significant difference in cell viability compared with the control group (Abs. 0.51±0.04 and 0.29±0.01 respectively with $p<0.001$). In contrast, HaCat treated with 1,2 µg/µl of EV did not show a difference in cell viability. Migration test using SCM 50% showed a percentage gap closure of 89%, compared to 67% from the control group. In addition, the live/dead assay showed a visible increase in the number of HaCaT cells after its treatment with 50% SCM. Cell viability of HaCaT cells treated with exosomes of FBS and SHED did not show a significant difference (Abs. 0,52±0.03). The results suggest that SHED released factors have the ability to increase the migration and viability of the keratinocytes involved in the epithelial regeneration process, which means a faster healing process and shows a new perspective and free therapeutic cell strategy for the use of the SHED secretome in epithelial repair.