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THE HUMAN DENTAL PULP AS A SOURCE FOR STEM CELLS WITH NEUROGENIC DIFFERENTIATION POTENTIAL

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Objective: Current available therapies are unable to adequately promote functional recovery in patients suffering from neurological disorders such as stroke, which are a major cause of death and permanent disability. As an alternative treatment, cell-based therapies are now emerging. Human neural stem cells are the most promising candidates to induce neuronal repair, but due to ethical and practical considerations, alternative sources of progenitor cells with neuronal differentiation potential are needed. The aim of the present study was to differentiate stem cells that were isolated from the human dental pulp (hDPSCs) towards functionally active neuronal cells *in vitro*.

Methods: hDPSCs were subjected to a two-step protocol. First, neurosphere-formation was used to acquire neuronal induction. Secondly, neuronal maturation was induced, based on cAMP and NT-3 signaling. The ultrastructural characteristics of intra-neurospheral hDPSCs and their microenvironment were determined by means of transmission electron microscopy (TEM). Neurogenic maturated hDPSCs (d-hDPSCs) were subjected to immunocytochemical, PCR, ultrastructural and electrophysiological analysis. An Enzyme-Linked Immunosorbent Assay (ELISA) was performed for VEGF, NGF, BDNF and GDNF in order to evaluate the secretome of hDPSCs before and after neurogenic differentiation.

Results: Based on the ultrastructural analysis it was shown that within the neurospheres, intercellular communication was promoted. Moreover, hDPSCs grew out of the neurospheres *in vitro* and established a neurogenic differentiated hDPSC culture (d-hDPSCs) which was characterized by the increased expression of the neuronal markers NeuN, MAP-2 and NCAM compared to the non-differentiated counterpart. Moreover, the secretion of BDNF, VEGF and NGF differed between d-hDPSCs and hDPSCSs. Ultrastructurally, d-hDPSCs acquired neuronal features including multiple intercommunicating cytoplasmic extensions and increased vesicular transport. Finally, patch clamp analysis demonstrated the functional activity of d-hDPSCs by the presence of TTX-sensitive voltage-gated sodium and TEA-sensitive potassium channels. A subset of d-hDPSCs was able to fire a single action potential.

Conclusions: Although promising results were achieved, establishing a completely functional d-hDPSC culture remains a challenge. The results in this study demonstrate that hDPSCs are capable of neuronal commitment with distinct features of neuronal cells.