The effect of hypoxia on the ability of differentiated human dental pulp stem cells to promote endothelial cell proliferation and neurite outgrowth in vitro

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After a peripheral nerve transection, endogenous nerve repair is often incomplete and unsatisfactory thus posing a major clinical hurdle. Engineered neural tissue (EngNT) has emerged as novel tissue engineering strategy for the repair of critical gap peripheral nerve defects [1]. Previous studies have shown that EngNT can be created with differentiated human dental pulp stem cells (d-hDPSCs), which supported neuronal regeneration in vitro (Martens et al., 2014). However, when tested in a long-gap peripheral nerve repair model the results revealed fewer regenerated fibres compared to autograft but enhanced vascularisation compared to an empty tube [2]. This led to the hypothesis that the hypoxic environment in vivo altered the implanted cell phenotype such that angiogenesis, rather than neuronal regeneration, was promoted [2]. The aim of this study therefore was to explore the angiogenic and neurotrophic behaviours of human dental pulp stem cells (hDPSCs) and their differentiated progeny in vitro following exposure to different levels of oxygen..

hDPSCs and d-hDPSCs were cultured for 24 h under 1%, 3% and 16 % oxygen conditions after which conditioned media were collected. The effect of these discrete secretomes were assessed on human umbilical vein endothelial cells (HUVECs) and dissociated sensory neurons obtained from rat dorsal root ganglia (DRG). HUVECs were cultured in each secretome for 24, 48 and 72 h. Proliferation was quantified by counting the number of positive-Ki67 cells in the total population (stained with Hoechst), under a fluorescence microscope. DRG were extracted from the spines of Sprague Dawley rats (200-250g) and enzymatically digested using collagenase before seeding them into a well plate and incubating them for 48 h with each secretome. Following this, neurons were immunostained for anti – βIII tubulin to quantify neurite length using ImageJ.

The secretome produced from culturing hDPSCs and d-hDPSC in 3% O_2 increased the proliferation rate of endothelial cells compared to 1% and 16% O_2. At 3% O_2 HUVECs cultured in d-hDPSC secretome revealed significantly higher proliferation rates compared to cells cultured with hDPSCs secretome. Neurons cultured in 1% and 3% O_2 secretome had the shortest neurite lengths. Neurite length increased by approximately two fold in the 16% O_2 condition compared to 1% and 3%.

These results support the hypothesis that under low oxygen conditions hDPSCs and d-hDPSCs release increased levels of pro-angiogenic factors but decreased levels of neurotrophic factors compared to their behavior under standard ambient cell culture oxygen. This is an important consideration in the design of engineered tissues for implantation into a nerve repair environment, since adequate angiogenesis is required to support implanted cell survival, but neurotrophic factor release must also be maintained in implanted cells.

References

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