Translational Research of oral Neural Crest-Derived Stem Cells (oNCSCs) in Regenerative Dentistry

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Introduction: Regeneration of dental tissues aims to utilize tissue engineering techniques to restore lost dental tissues including periodontal ligament and alveolar bone. As the oral cavity represents a complex system consisting of teeth, bone, soft tissues and sensory nerves, regenerative dentistry relies on the use of stem cells with relatively high developmental potential. Thus, other cellular sources such as those readily available in the postnatal cranio-facial area and particularly in oral structures offer a much better and realistic alternative as cellular regenerative sources. Direct methods of assessing native NCSCs and their trafficking properties is a big unmet need required to conclusively elucidate mechanisms of NCSC trafficking during pathological dental states. Detection of local-delivered NCSCs that remain in an undifferentiated state compared to their differentiated progeny also presents a significant challenge. As our understanding of the mechanisms of NCSC trafficking grows, the ability to enhance homing to periodontal lesions and to alveolar bone tissues around dental implants through engineered approaches should significantly reduce the number of cells required to achieve a therapeutic effect, and presumably provide better outcomes for patients.

oral Neural Crest-Derived Stem Cells (oNCSCs)

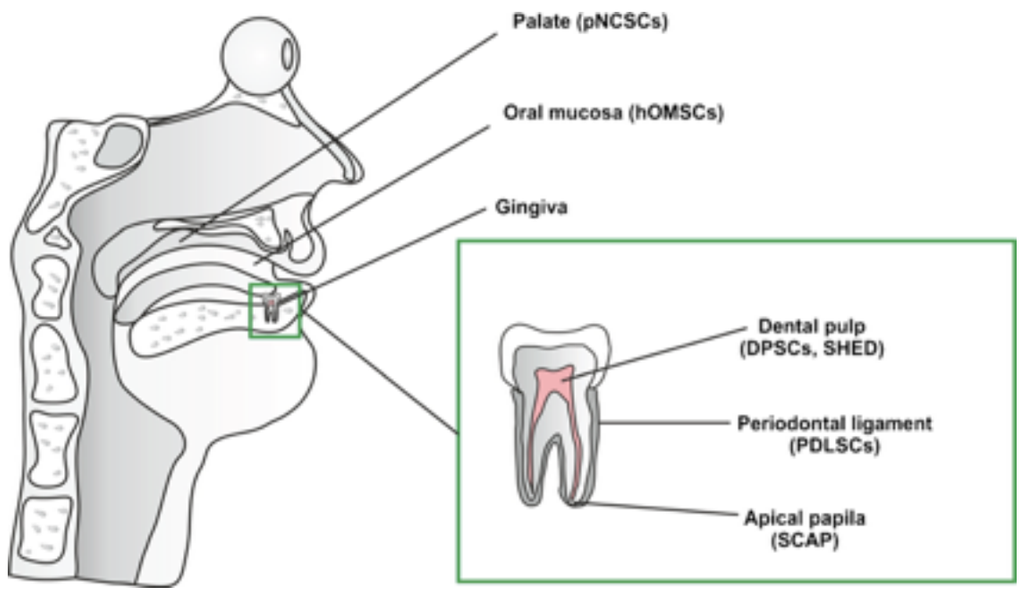

Fig. 1: Neural crest-derived human stem cells within the oral cavity (Grimm et al., Current Oral Health Reports 2015)

Material and methods: The secondary palate is a highly regenerative and heavily innervated tissue, which develops embryonically under direct contribution of neural crest cells. Is has been demonstrated that the human periodontium and the human palate contain Neural Crest-Derived Stem Cells (NCSCs), see Fig. 2, 3.

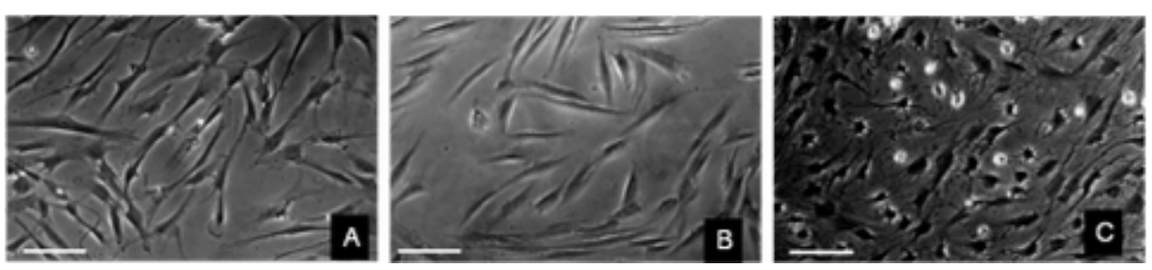

Fig. 3: Morphology of human periodontium-derived (PDL)-NCSCs cultures viewed under a phase contrast microscope.
(A) When cells were subjected to our perviously published 3-step neurogenic differentiation protocol (Király et al. Neurochem Int. 2009), after the first phase of neuroinduction cells changed morphology and became shorter and higher (B) After eight days the majority of PDL NCSCs cells display multipolar neuronal morhology, C) confluent oNCSCs after 3 weeks.

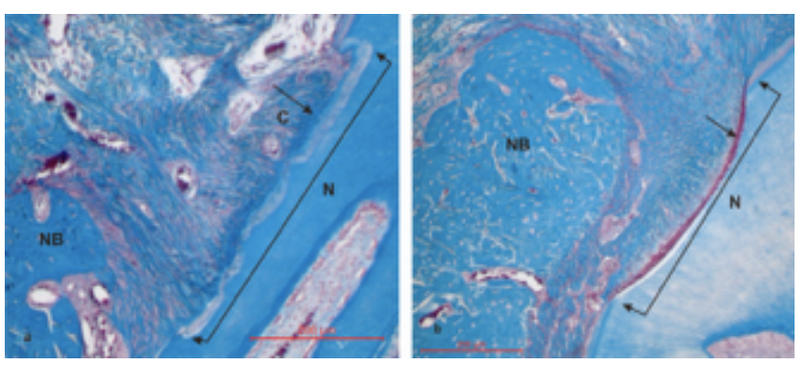

Fig. 4: Overview of a histologic section after transplantation of human periodontium-derived (PDL) NCSCs from a test site (rat model). Note the formation of new bone and the periodontal-like ligament (Alcian blue staining), Grimm et al. J Periodontol Reg Dent 2011

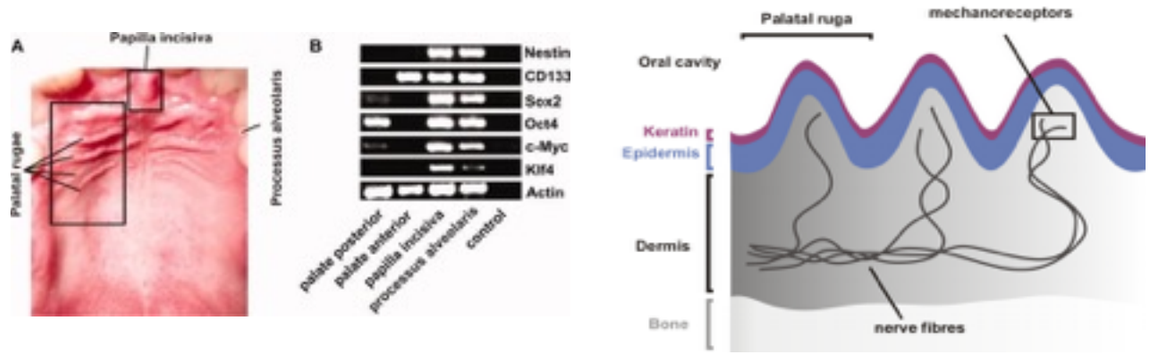

Fig. 2: Analysis of the expression pattern of cultured palatal-derived NC-SCs (D. Widera, Neural crest-derived Stem Cells as a Tool in Regenerative Dentistry, booklet, II. Symposion on Regenerative Medicine, Stavropol 2015)

Results: oNCSC-like cells formed sphere structures even after serial passages and expressed the NCC-related genes, p75, sox2, and nestin. These results demonstrate that the sphere-forming cells are multipotent oNCSCs (Widera et. al, 2009 Stem Cell), see Fig. 4. The purpose of the *„Proof of Concept Study“* is to evaluate the potential of Adult Human Palatinum as a Novel source of Neural crest-derived stem cells in combination with allogenic human bone for periodontal regeneration and alveolar bone augmentation.

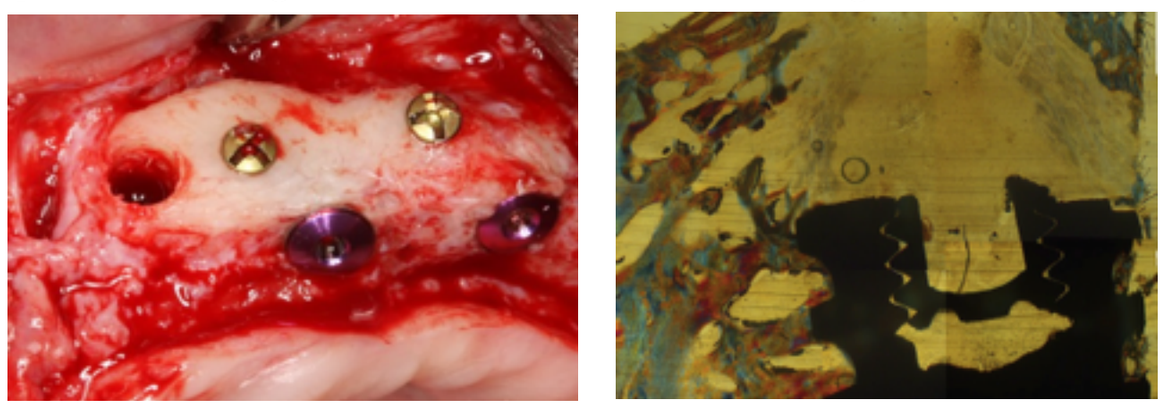

Fig. 5: Transplantation of autologous stem cell-rich tissue, *„Proof of Concept Study“:* Clinical view after opening the surgical site (left picture).
Histological Specimens (right picture) were embedded into Technovit 9100 (Heraeus Kulzer, Hanau, Germany). 80µm thick sections were cut through the specimens using a saw microtome (Leica 1600, Leitz, Wetzlar, Germany). The section showed a fully regenerated alveolar bone with typically trabecular bone structure around the dental implant.

Conclusion: This *proof-of-concept* clinical report used an evidence-based approach for the Transplantation of autologous stem cell-rich tissue for improving the vertical and the lateral alveolar bone augmentation in critical size alveolar defects (see Fig. 5). The number of potential therapeutic applications and their efficacy will continue to grow as the fundamental biology that is responsible for the oNCSC regenerative properties and homing responses continues to be elucidated.