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PREFABRICATED 3D ALLOGENIC BONE BLOCK IN CONJUNCTION WITH STEM CELL-CONTAINING SUBEPITHELIAL CONNECTIVE TISSUE GRAFT FOR HORIZONTAL ALVEOLAR BONE AUGMENTATION: A CASE REPORT AS PROOF OF CLINICAL STUDY PRINCIPLES

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SO far there are no long-term data showing the superiority of any particular bone augmentation technique in conjunction with dental implant therapy. Dental implants require sufficient bone to be adequately stabilized. For some patients implant treatment would not be an option without horizontal or vertical bone augmentation. A variety of materials and surgical techniques are available for bone augmentation [1]. For horizontal alveolar bone defects, only bone grafts using autologous [2] and allogeneic bone blocks [3] have been described. Besides the «classic» autologous bone block, it is also possible to use allogeneic bone grafts with a stabilizing stem cell-containing subepithelial connective tissue graft. We have therefore used a «frame technique» with a demineralized allogeneic bone (Osteograft™ block) for the indication of preimplantological missing alveolar bone in the form of a proof of clinical study principles. The target parameter was its clinical effectiveness under three aspects:

- wound healing supported by the stem cell-containing subepithelial connective tissue graft;
- stability of the contouring effect and achievement of a stable implant site when implantation were performed as a «second approach»;
- standardization along the operational procedures with avoidance of secondary morbidity.

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Stem cell-containing subepithelial connective tissue graft. During mammalian tooth development, the oral epithelium invaginates into the underlying neural crest-derived mesenchyme. The ecto-mesenchymal cells are derived from the dorsal-most aspect of the neural tube and contribute to local tissues, including the alveolar bone [4]. From this aspect, it has been of great interest to identify a progenitor pool in dental tissues and investigate its regenerative potential for alveolar bone defects.

Two groups, including us [5], gained experiences with the isolation, cultivation, and characterization of palatal-derived stem cells (paldSCs), overview see Widera et al. [6].

Based on this key discovery we aim to develop a regenerative cell therapy for substitution of preimplantological missing alveolar bone using palatal-derived ecto-mesenchymal stem cells. Palate is a highly regenerative and richly innervated craniofacial tissue. This capability for rapid regeneration may be explained by the potential presence of at least one stem cell type within these tissues. Our interdisciplinary research group [5,7] isolated these so called palatal-derived stem cells (paldSCs) by minimally invasive periodontal surgery and cultured as dentaspheres under serum-free conditions in the presence of FGF-2 and EGF. In 2007 our research group [8] first describe the characterization and neuronal differentiation capacity of stem cells derived from inflamed periodontal tissues and from palate. Characterization of paldSCs by RT-PCR and flow cytometry revealed expression of several stem cell markers, such as nestin and Sox2 as well as STRO-1 and CD146 (results not shown). Cultivation of paldSCs in neuronal differentiation media indicated the high neuronal differentiation capacity of these stem cells from periodontal tissue. Cells adopted a neuronal morphology and expressed a variety of neuronal markers, such as β -III-tubulin, Map2, GAD67, neurofilament-L (NF-L), neurofilament-M (NF-M), neurofilament-H (NF-H), and synaptophysin. Further differentiation studies revealed that paldSCs exhibit an osteogenic differentiation capacity suggesting that these cells might be a suitable cellular tool for bone regeneration purposes.

Thus, we looked for a novel source of stem cells from the oral cavity being highly suited for regenerative purposes. To prove for the putative bone regeneration capacity of paldSCs in an in vivo [5] experimental setting

an athymic rat model was conducted. The results of our animal studies demonstrate also that isolating palDSCs from patients undergoing open flap minimally invasive periodontal surgery seems to be a simple and reliable stem cell resource to regenerate alveolar bone tissue elements at different levels. Several recent studies conducted by other research groups using the same protocol for DPSCs [9] that we developed for human palDSCs, found that neuronally differentiated murine DPSCs are immature, expressing only L-type calcium, but not neuron-specific sodium or potassium, channels. Ideally, the protocols used by Varga and colleagues [10] to transdifferentiate murine DPSCs to neuronal progenitors should be further enhanced by using the present knowledge obtained in induced pluripotent stem cell and direct reprogramming research. The differences in the neuronal phenotypes of human versus rodent palDSCs are certainly due in part to species differences, but the available data also suggest that the efficacy of currently available differentiation protocols has to be improved to obtain cell populations that are suitable for regenerative therapeutic purposes. All these cells have in common that they form neurosphere-like clusters and proliferate in serum-free culture in the presence of fibroblast growth factor-2 (FGF-2) and epidermal growth factor (EGF).

Analyzing stem cell-supported alveolar bone regeneration-A Powerful Tool for the Evaluation and Optimization of Strategies for Engineering Tissues using 3D imaging. The predominant application of *stem cell-supported alveolar bone regeneration* to date has been the nondestructive histological and 3D imaging analysis of trabecular bone. Our research work is motivated by the desire to better understand the precise relationship between trabecular alveolar bone architecture of the upper and lower alveolar bone and mechanical function and how alterations in this relationship are manifest in preimplantological situations (Fig. 1). DVT

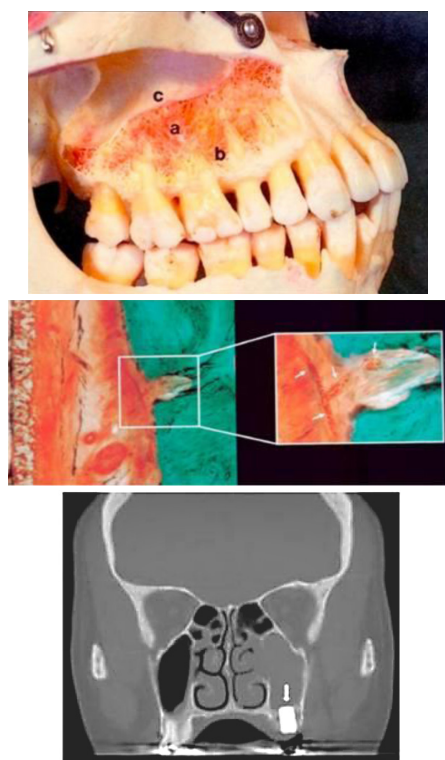


Fig. 1. Bone structure of Maxilla with teeth

imaging facilitated 3-D measurements of bone morphology parameters such as trabecular thickness, spacing, and density, as well as the connectivity of the trabecular network. Although 2-D histological analysis can provide estimates of some of these parameters, DVT analysis is more efficient, yields more quantitative information, and has the major advantage of being nondestructive. Thus, DVT systems have become an important tool in a variety of preclinical testing studies and have stimulated a rapidly growing number of new applications.

Bone Growth and Repair

The use of allogeneic bone grafts with a stabilizing *stem cell-containing subepithelial connective tissue graft* is not limited to mature, fully mineralized bone. This method has been used by two research groups, including us, as a quantitative outcome measure for investigations of bone growth and repair in animal experiments [5, 11]. Although not fully mineralized, newly formed bone within a growth plate provide adequate histological signs from surrounding soft tissues (Fig. 2). It is important to note, however, that

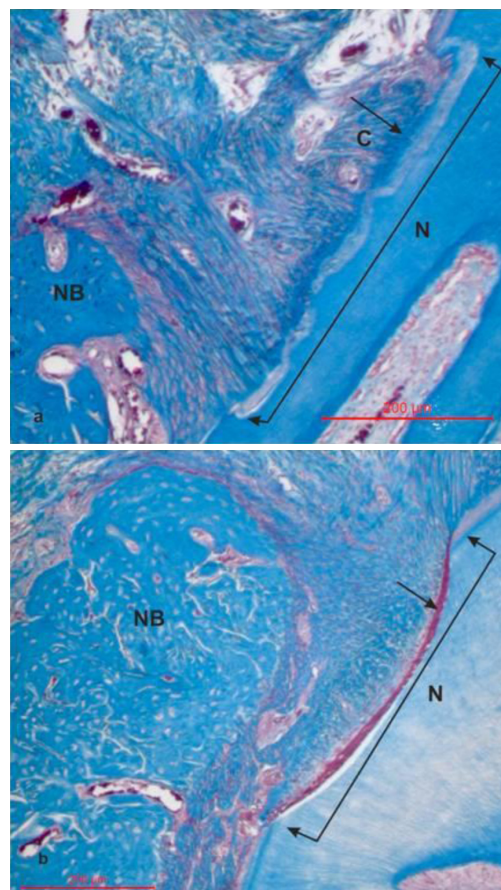


Fig. 2. Newly formed bone within a growth plate provide adequate histological signs from surrounding soft tissues (histology taken from the experiment)

additional histological analysis of specimens may be necessary to distinguish the regions of bone and adjacent soft tissue. As an example, we will use comparison of DVT images with the histological sections from the implant placement site in our *proof of principle* study to better understand and parametrically analyze the clinical procedure (Fig. 3). The overall conclusion is the application of a connective tissue

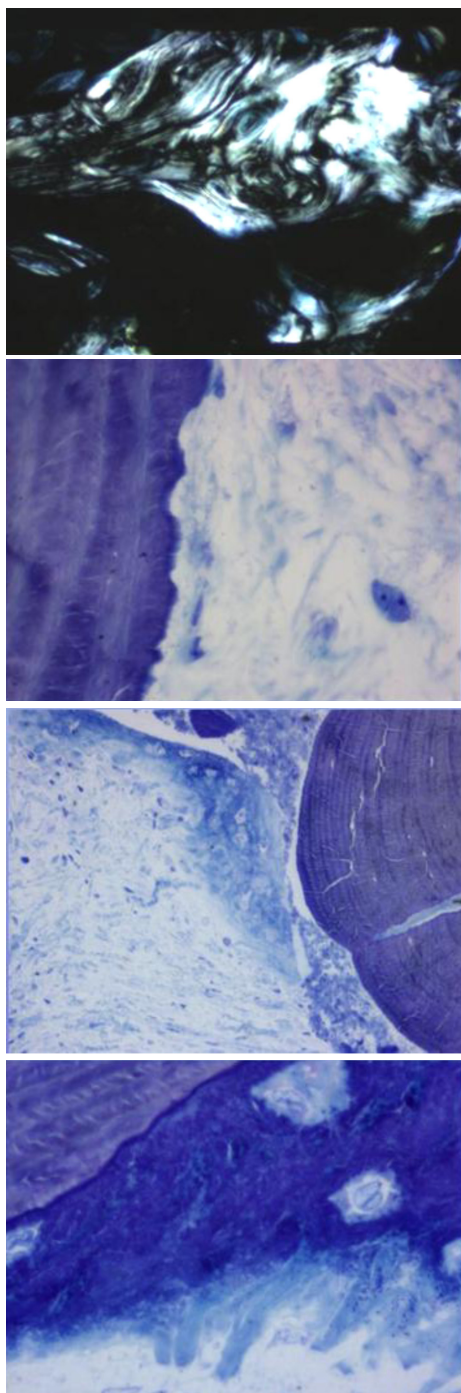


Fig. 3. Human histological sections from the implant placement site in our *proof of principle* study to better understand and parametrically analyze the clinical procedure using Osteograft™ block ex regio 46, see case presentation

graft placed at the buccal aspect of the bony wall at implants installed immediately after tooth extraction yielded a preservation of the hard tissues, and the peri-implant mucosa was significantly thicker and more coronally positioned at the test compared with the control sites.

Case report. A 48-year old patient presents with a partial edentulous lower jaw and the desire of a fixed implant-supported prosthesis in our study center. Assessing the real bone situation using DVT imaging of the lower jaw we can see a much worse bone site than expected in comparison with the

clinical picture. An average bone width of 2–3 mm does not allow immediate implantation. Further, the entire mandibula ridge must be augmented laterally with allogenic bone blocks. We decided to use a stem cell-containing subepithelial connective tissue graft and allogenic human bone. As a carrier of our stem cell containing soft tissue from the palate we have used a sterile, high-safety (donor selection, virus testing, chemical cleaning, processing and sterilization) allograft bone product, derived of human donor bone (OsteoGraft™ block). The high biologic regeneration capability of this allogenic bone block results in a predictable clinical outcome.

Properties of OsteoGraft™ block

- Preserved biomechanical properties
- Sterile without antigenic effects
- Storable at room temperature for 5 years
- Osteoconductive properties sup-porting natural and controlled tissue remodeling.

Surgical Procedures. We decided to use a non-removable zirconia bridge on two implants. The residual ridge areas have been augmented by bone-blocks finally congruently adapted to the spongy bone base and screwed with osteosynthesis screws using a 3D copying machine (Fig. 4). An approximately 10×6 mm subepithelial connective tissue graft (SCTG) has been harvested from the palate in the second premolar to second molar region as the source of ecto-mesenchymal stem cells. The SCTG trimmed precisely to adapt to the recipient site.

Conclusion. Quantitative tools such as DVT, 3D copying machines for congruently adapting the bone block to the spongy bone base, and standardized histological analysis are needed for tissue engineering to evolve beyond a qualitative, observational field and accelerate the clinical realization of regenerative technologies. As faster, higher-resolution DVT systems, and 3D copying machines become available for both *ex vivo* and clinical studies and the development of improved standardized histological hard tissue analysis methods to be extended to nonmineralized tissues, additional novel applications related to tissue engineering are sure to emerge.

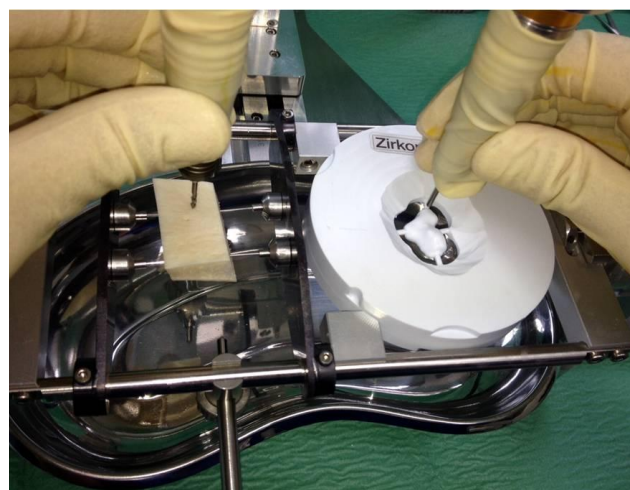


Fig. 4. 3D copying machine to congruently adapting the Osteograft™ block to the spongy bone base

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For three-dimensional reconstruction of defects of the alveolar ridge to conduct Summary. In the article the analysis of existing technologies for new sources of stem cells mouth for maxillofacial surgery and dental implantology. The authors provide the results of their research on laboratory animals have shown that the most simple and reliable source of stem cell resources for the regeneration of the bone of the alveolar process of the jaws can serve subepithelial the soft palate. The ability of stem cells from this area to the differentiation can be used when planning and implementing interventions in the maxillofacial region to ensure stability of the contour effect and dental implants.

Key words: stem cells, implantology, differentiation

ПРИМЕНЕНИЕ ГОТОВЫХ ТРЁХМЕРНЫХ АЛЛОГЕННЫХ КОСТНЫХ БЛОКОВ В СОЧЕТАНИИ С СОДЕРЖАЩИМИ СТЕВЛОВЫЕ КЛЕТКИ СУБЭПИТЕЛИАЛЬНЫМИ СОЕДИНИТЕЛЬНЫМИ ТРАНСПЛАНТАТАМИ ДЛЯ ГОРИЗОНТАЛЬНОГО НАРАЩИВАНИЯ АЛЬВЕОЛЯРНОЙ КОСТИ: ПРИМЕР ИСПОЛЬЗОВАНИЯ РАЗРАБОТАННЫХ ПРИНЦИПОВ ЛЕЧЕНИЯ В КЛИНИКЕ

В.-Д. ГРИММ, М. ПЛЁГЕР, И. ШАУ, М. А. ВУКОВИЧ, Е. В. ЩЕТИНИН, А. Б. АККАЛАЕВ, А. В. АРУТЮНОВ, С. В. СИРАК

В статье проводится анализ существующих на сегодняшний день технологий получения новых источников стволовых клеток ротовой полости для челюстно-лицевой хирургии и дентальной имплантологии. Авторами приведены результаты собственных исследований на лабораторных животных, которые показали, что наиболее простым и надежным источником стволовых клеточных ресурсов для регенерации костной ткани альвеолярных отростков челюстей могут служить субэпителиальные ткани мягкого нёба. Способность стволовых клеток из данной области к дифференциации может быть использована при планировании и осуществлении оперативных вмешательств на челюстно-лицевой области для обеспечения стабильности контурного эффекта и дентальных имплантатов.

Ключевые слова: стволовые клетки, имплантология, дифференциация