Translational Studies on Grafting Materials in Alveolar cleft repair

Summary

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The comprehensive objective of our studies was to conduct a methodological analysis of the various grafting materials available in alveolar bone grafting in order to improve the overall treatment outcomes of alveolar cleft repair in children with cleft lip and palate.

Chapter 1 The introduction describes the clinical perspectives of cleft lip and palate and the epidemiology. Cleft lip and palate is a congenital malformation that can be manifested either as syndromic or non-syndromic deformity. There is a regional and ethnic predilection for increased prevalence of this malformation among newborns in different parts of the world. However, the exact pathology and mechanism for developing this malformation remains unclear. The malformation results in a structural deficiency in the upper jaw significantly affecting the formation of a continuous dental arch leading to missing teeth, and difficulties in speaking and swallowing. This has a great impact on the patient's quality of life from birth into adulthood. Our aim was to investigate novel options to repair the skeletal deformity as a result of cleft and palate in a biologically reliable and feasible animal model.

In **Chapter 2** a comprehensive literature search on various grafting materials currently used for alveolar cleft repair was conducted. In order to assess the success of each grafting intervention, a clinical outcome was defined to evaluate the success of bone grafting. The percentage of bone filling of the cleft defect after grafting based on three-dimensional imaging modalities, which have been widely used for surgical assessment and planning in recent years, was used as the clinical outcome for the included studies. The search was performed electronically on MEDLINE, EMBASE, SCOPUS, Web of Science, and grey literature was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A total of 25 studies were included in the meta-analysis to compare the volumetric bone filling percentage. After stratifying the intervention groups, a pooled analysis of 25 studies using autogenous bone revealed a statistically significant reduction in cleft volume equivalent to 62.0% bone fill (95% CI), in contrast to 10 studies using tissue-engineered material with a bone filling percentage of 68.7% (95% CI). This demonstrates that there was no significant difference between autogenous bone grafts and novel tissue-engineered materials with regard to cleft filling capability. This was a noteworthy finding and indicated an increased efficacy of bone substitute materials as an effective option to autogenous bone grafts, which have long been considered the gold standard for grafting procedures.

In **Chapter 3** we compared the three-dimensional morphometric microstructure in human cadaveric bone specimens taken from various commonly utilized donor sites for autogenous bone grafting. The morphometric analysis was conducted using μCT, which generates

high-resolution datasets of bone structures and calcifications making this modality versatile for microarchitecture analysis and quantification of bone. Six bone specimens were obtained from various anatomical sites from 10 human dentate cadavers. The specimens were scanned and volumetrically reconstructed. We utilized analytical software to analyze the bone mineral density and structural morphometric analysis using bone indices: relative bone volume, surface density, trabecular thicknesses, and trabecular separation for each group. Morphometric analysis revealed statistically significant differences in the bone mineral density and relative bone volume index in the calvaria, mandibular ramus, mandibular symphysis groups when compared to those in the iliac crest and maxillary tuberosity, suggesting higher bone quality in the former groups than in the latter; tibial specimens expressed variable results. These findings further support previous studies suggesting that autogenous bone from different donor sites expresses variable bone quality and structural characteristics, with variable levels of success and healing outcome.

In **Chapter 4** we investigated whether xenogenic dentin particles inserted into the marrow space of rabbit tibia, a space where there is no solid bone tissue initially, would contribute to new bone formation. We knew from previous interventional studies that dentin possesses bone-forming properties and may possibly also be used as a bone augmentation material prior to implant placement. This phenomenon is seen in cases of dentoalveolar ankylosis, in which exposed dentin results in osseous replacement and formation of new bone. To test this hypothesis, dentin chips from human teeth were inserted into tibias of ten New Zealand White rabbits and histological processing was performed after 6 months. Bone formation was seen to a larger extent on dentin grafts located close to the native tibial bone wall and there was only minor inflammation. This was suggestive that dentin promotes new bone formation when located close to native cortical bone and may have a potential as a bone augmentation material.

In **Chapter 5** we further conducted an interventional testing of dentin as potential grafting in combination with ß-TCP, which is widely used for bone grafting in clinical practice, in the robust calvarial critical-size defects in ten New Zealand White rabbits. The purpose of this study was to evaluate bone healing in calvarial defects using two bone graft substitute materials; ß-TCP/HA versus composite non-demineralized xenogenic dentin with ß-TCP/HA mixture. We analyzed the osteogenesis and healing patterns of the defects at eight weeks after grafting using µCTƒ. Quantitative analysis of bone mineral density (BMD) and bone volume fraction of the new bone formation (BVTV) in the defects were also evaluated. We showed that defects filled with composite non-demineralized xenogenic dentin with biphasic ß-TCP expressed higher percentage bone volume fraction (BV/TV) than defects filled with ß-TCP only, suggesting a higher ratio of new bone formation. Histological analysis showed a significant percentage increase in bone formation and residual graft in the

composite dentin/ß-TCP group after eight weeks. This suggests that composite xenogenic dentin with ß-TCP shows improved osteogenesis when compared to biphasic ß-TCP alone.

Chapter 6 describes a novel animal model in New Zealand White rabbits for creating alveolar cleft defects with a properly simulated clinical defect environment for tissue-engineered bone-substitute materials testing without compromising the health of the animal. Cleft creation surgery was aimed at creating a complete alveolar cleft with a wide bone defect with an epithelial lining (oral mucosa) overlying the cleft defect. The defect was surgically created using simple methodology without the need for intubation, and the defects were allow to heal for a eight-week period. Clinical examination and imaging analysis eight weeks after cleft creation surgery revealed the establishment of a wide skeletal defect extending to the nasal mucosa simulating alveolar clefts in all of the sixteen rabbits. The model allows the simulation of the cleft site environment in a biological model and can be used to evaluate various bone grafting materials with regard to efficacy of osteogenesis and healing potenital without compromising the health of the animal.

In **Chapter 7** we conducted an interventional testing to evaluate the bone regeneration pattern and quantify bone formation after grafting the expimental alveolar clefts defects model in rabbits using composite xenogenic dentin and ß-TCP in comparison to ß-TCP alone. The unilateral alveolar cleft defects were created in sixteen New Zealand White rabbits according to the previously described methodology. Eight defects each were filled with ß-TCP, and composite xenogenic dentin with ß-TCP respectively. Bone regeneration of the healed defects was compared 8 weeks after intervention. Quantification of bone formation was analyzed using micro-computed tomography (µCT) and histomorphometric analysis. µCT and histomorphometric analysis revealed that defects filled with composite dentin/ß-TCP showed statistically higher bone volume fraction, bone mineral density and percentage residual graft volume compared to ß-TCP alone. We also noted an improved surgical handling of the composite dentin/ß-TCP graft. In this chapter we showed that composite xenogenic Dentin/ß-TCP putty expresses enhanced bone regeneration compared to ß-TCP alone in the reconstruction of rabbit alveolar cleft defects.

In **Chapter 8** the above findings were discussed in a comprehensive context. The majority of the alveolar cleft defects are being treated with autogenous bone, still being the gold standard in bone grafting, yet multiple types of donor bone were utilized. On the other hand, there has been an increasing application of tissue-engineered materials and various bone substitute materials in clinical practice in recent years. Recent interventional reports in the literature suggest improved clinical outcomes associated with these novel bone substitute materials. They may well be part of the daily clinical practice in the near future. Thus, there will be an increased need to perform interventional testings in reliable

animal models to evaluate healing and efficacy of these materials, and to optimize their physical and biological characteristics, such as graft resorption rate, osteoconductivity, and osteoinductivity.

Bone grafting techniques seem to be in a dynamic phase of development. With the recent technological advances in the field of biomaterials, and the enormous improvement in the diagnostic and treatment modalities, we should expect a vast increase in the amount of bone grafting replacement biomaterials with the hope to overcome the shortcomings of conventional grafting techniques, mainly the limited donor supply, the need for an extra surgical procedure, risk of wound infection, blood loss, and increased pain and discomfort for the patients. Bone grafting is one of the most widely used routine procedures in clinical practice, in dental implantology, traumatology, oncology, craniofacial surgery, and cosmetic surgery. Alveolar bone grafting represents only one aspect of skeletal defects in addition to several other developmental anomalies, traumatic conditions, oncologic resections, infections, and pathological conditions leading to defects in the bony skeleton ultimately requiring bone grafting procedures. Optimizing the grafting modality in each of these conditions is the responsibility of scientists and health care professional involved in scientific research on grafting materials.