

GENERAL SUMMARY

ADIPOSE STEM CELLS FOR BONE TISSUE ENGINEERING IN A
HUMAN MAXILLARY SINUS FLOOR ELEVATION MODEL:
STUDIES TOWARDS CLINICAL APPLICATION



GENERAL SUMMARY

The increasingly ageing population worldwide has raised the prevalence of jaw atrophy and thus the need for a sufficient treatment. Maxillary atrophy is caused by a prolonged edentulous state after loss of natural teeth. To restore the missing maxillary teeth with prosthetic devices, dental implants can be installed if the alveolar bone volume is sufficient. If this requirement is not met, the maxillary alveolar bone height can be increased by means of a specific surgical procedure, i.e. the maxillary sinus floor elevation procedure (MSFE). For decades MSFE has been a standard pre-implant surgical procedure to increase alveolar bone height in the posterior maxilla.

Autologous bone is still considered the golden standard as graft material for bone augmentation procedures in general and for MSFE in particular, since the graft contains osteoblasts and osteoprogenitor cells, thereby providing osteoconductive and osteoinductive properties necessary for allowing the migration and subsequent differentiation of progenitor cells. Harvesting autologous bone (from the anterior iliac crest or mandible) has several disadvantages, such as donor site morbidity and limited availability of bone. Alternatives for the use of autologous bone have been developed and evaluated. This resulted in the introduction and use of bone substitutes such as allografts, xenografts and purely synthetic grafting materials that are accepted and now commonly used for standard clinical dental and oral surgery procedures. Synthetic bone substitutes such as β -tricalcium phosphate (β -TCP, e.g. Ceros[®]), hydroxyapatite (HA), and biphasic calcium phosphate (BCP, mixtures of HA/ β -TCP, e.g. Straumann BoneCeramic[®]) are interesting alternatives to use in MSFE because they are available in unlimited quantity, have an infinite half-life and may therefore be used as off-the-shelf products. Using synthetic grafting materials eliminates the need for a second surgical procedure as well as potential additional complications.

Growth factors and/or osteoblast precursor cells are required to provide the osteoinductive potential of the tissue-engineering construct. One specific growth factor is bone morphogenetic protein-2 (BMP-2), which is a potent osteoinductive molecule that has been shown to stimulate osteogenic differentiation of undifferentiated cells. An *in vitro* study with goat adipose stem cells (ASCs) showed that the use of BMP-2 in a physiological, nanogram-range concentration and a short period of time can be very beneficial for tissue engineering purposes using ASCs.

In this thesis progenitor cells were used isolated from human adipose tissue. This tissue provides an easily accessible, expendable source of clinically relevant numbers of mesenchymal stem cells, thereby allowing innovative one-step regenerative treatment strategies. This new concept overcomes the problems currently encountered with cellular therapies: need for *in vitro* expansion, high costs, and repeated surgeries. Moreover, when using non-induced, minimally manipulated cells, many regulatory hurdles can be avoided, thereby accelerating clinical introduction.

Within the concept of bone tissue engineering, the maxillary sinus floor elevation model is unique by allowing histological examination of biopsies that have been retrieved prior to implant insertion. Therefore, this thesis evaluates and describes the functionality of human ASCs

for bone tissue engineering and proposes a novel concept of a one-step surgical procedure for human maxillary sinus floor elevation using calcium phosphate scaffolds.

In **chapter 2** the authors reviewed whether the human MSFE procedure could be applied as a model for bone regeneration enabling the application of one-step surgical procedures. It was concluded that the use of freshly isolated ASCs in a one-step surgical procedure is a feasible and innovative cellular basis for bone tissue engineering, and that the MSFE model is well-suited for monitoring the ingrowth of new bone and understanding the mechanisms behind the bone remodeling process.

In **chapter 3** the effect of short treatment of human ASCs with BMP-2 after seeding on a calcium phosphate carrier was evaluated. It was investigated whether short (15 minutes) incubation with BMP-2 suffices to trigger osteogenic differentiation of hASCs seeded on calcium phosphate carriers. The authors found that hASC attachment to the different scaffolds was similar, and unaffected by BMP-2. BMP-2 stimulated gene expression of the osteogenic markers CBFA1, collagen-1, osteonectin, and osteocalcin in hASCs seeded on BCP and β -TCP. Down regulation of osteopontin expression by BMP-2 was seen in BCP-seeded cells only. BMP-2 treatment inhibited expression of the adipogenic marker PPAR- γ . Thus, 15 minutes BMP-2 pre-incubation of hASCs seeded on BCP/ β -TCP scaffolds had a long-lasting stimulating effect on osteogenic differentiation *in vitro*.

The secretome of stem cells strongly determines the outcome of tissue engineering strategies. It was investigated how the secretome of human adipose stem cells (hASCs) is affected by substrate, BMP-2 treatment, and degree of differentiation. Therefore, in **chapter 4** it was hypothesized that as differentiation progresses, hASCs produce increasingly more factors associated with processes such as angiogenesis and bone remodeling. The authors found that, compared to plastic, hASCs cultured on BCP showed ≥ 2 -fold higher expression of ~ 20 factors, amongst which cytokines such as IL-6, growth factors such as FGF7 and adhesion molecules such as VCAM1. However, expression of another ~ 50 genes was decreased ≥ 2 -fold on BCP compared to plastic, even though hASCs differentiate better on BCP than on plastic. BMP-2-treatment increased the expression of ~ 30 factors by hASCs seeded on BCP, while it decreased the expression of only PGF, PPARG and PTN. No clear association between the degree of osteogenic differentiation of hASCs and the pattern of trophic factor production was observed. Considering the observed lack of association between the degree of differentiation and the production of factors associated with angiogenesis and bone remodeling by hASCs, future bone regeneration studies should focus more on systematically orchestrating the secretome of stem cells, rather than on inducing osteogenic differentiation of stem cells only. Short incubation with BMP-2 may be a promising treatment to enhance both osteogenic differentiation and environmental modulation.

In **chapter 5** the gain of mineralized bone in human biopsies was compared between deproteinized bovine bone allograft (DBA) and BCP after MSFE, using a split-mouth design. The authors found that patients were prosthetically successfully restored. All but one of the implants survived, and peri-implant mucosa showed healthy appearance and stability. Bone volume, graft volume, degree of bone mineralization, and osteoclast and osteocyte numbers were similar, but BCP-grafted biopsies had relatively more osteoid than DBA-grafted biopsies.

The authors concluded that the BCP and DBA materials showed similar osteoconductive patterns and mineralized bone, although signs of more active bone formation and remodeling were observed in BCP- than in DBA-grafted biopsies.

In **chapter 6** the effect of a collagenous barrier membrane covering the lateral window in MSFE procedures on the bone formation in β -TCP was described. For decades this has been an important topic of debate, since the benefit of a collagenous barrier membrane has not been irrefutably proven. The authors found that comparable outcomes between the groups with and without a membrane were observed regarding osteoconduction rate, bone and graft volume, osteoclast number, and structural parameters of newly formed bone per region of interest. However, osteoid volume in grafted maxillary sinus floors without a membrane was significantly higher than with membrane. In conclusion, these findings demonstrate that the clinical application of a bioresorbable collagenous barrier membrane covering the lateral window in MSFE procedures using β -TCP was not beneficial for bone regeneration, and even decreased osteoid production, which might lead to diminished bone formation in the long run. Further research needs to be done to confirm these data.

Despite the successful use of biphasic calcium phosphate with a hydroxyapatite/tricalcium phosphate (HA/TCP) ratio of 60/40, the high percentage of HA may hamper efficient scaffold remodeling. Therefore, in **chapter 7**, it was hypothesized that the use of BCP 20/80 in a MSFE procedure will result in a higher quantity of bone and/or better bone quality in the grafted maxillary sinus compared to BCP 60/40. A comparative study between these two types of calcium phosphate bone substitutes has not previously been performed in a human model. The authors found that, although not significant, there is a clear tendency in the μ -CT and the histomorphometrical analysis towards more bone ingrowth in the 20/80 versus the 60/40 BCP variant. Osteoid volumes were comparable between both groups, while osteoclastic activity was significantly higher in the 60/40 group, indicating more balance towards bone formation in the BCP 20/80-treated patients. The authors concluded that the novel BCP 20/80 bone substitute performs at least equal, but most likely better in MSFE procedures compared to the BCP 60/40.

Finally, in **chapter 8** the main conclusions of the studies described in this thesis are discussed as well as the future implications of the findings. In short, the authors concluded that the use of freshly isolated and BMP-2-pretreated human ASCs in a one-step surgical procedure is a feasible and innovative cellular basis for bone tissue engineering, and that the MSFE model is well-suited for monitoring the ingrowth of new bone and understanding the mechanisms behind the bone remodeling process. The outcome of these and future studies will have pivotal implications for bone tissue engineering models in other fields, such as orthopedic surgery.

