CHAPTER 1

GENERAL INTRODUCTION

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The increasingly ageing population worldwide has raised the prevalence of jaw atrophy and thus the need for a sufficient treatment. Maxillary (as well as mandibular) atrophy is caused by a prolonged edentulous state after loss of natural teeth. Dental implants can be installed to restore the missing teeth, but this requires sufficient alveolar bone volume. If this requirement is not met, the alveolar bone height can be increased with a specific surgical procedure, i.e. the maxillary sinus floor elevation procedure (MSFE), which was first performed by Tatum [1], but published by Boyne [2]. During the last decades, MSFE has become 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 [3, 4]. The most common donor sites for autologous bone are the anterior iliac crest and mandibular bone. Harvesting autologous bone has several disadvantages, such as donor site morbidity and limited availability of bone [5].

Alternatives for the use of autologous bone have been developed and evaluated. This resulted in the introduction and use of bone substitutes such as allograft (e.g. demineralized freeze-dried human bone, DemBone®), xenograft (e.g. demineralized bovine bone, Bio-Oss®), and purely synthetic grafting materials that are accepted and now commonly used for standard clinical dental and oral surgery procedures [6]. 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. The characteristics of the synthetic grafts are important for successful bone ingrowth; whereas HA is non-resorbable, β-TCP resorbs relatively fast. BCP combines these properties, and the additional high surface area and porosity of the particles facilitates attachment, proliferation and osteogenic differentiation of progenitor cells [7, 8]. Using synthetic grafting materials eliminates the need for a second operation site as well as potential additional complications.

Within the concept of bone tissue engineering, the sinus floor elevation model is unique by allowing histological examination of biopsies removed prior to implant insertion; we could demonstrate that synthetic bone substitutes show low bone ingrowth rates compared to autogenous bone graft due to the solely osteoconductive properties [4, 9]. Therefore, additional 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, either or not combined with a carrier, has been shown to stimulate osteogenic differentiation of undifferentiated cells, and to induce healing of critical size defects in several animal studies [10-13]. An *in vitro* study with goat adipose stem cells (ASCs) showed that the use of BMP-2 in a physiological, ng-range concentration and a short period of time can be very beneficial for tissue engineering purposes

using ASCs [14]. Human adipose tissue provides an easily accessible, expendable source of clinically relevant numbers of MSCs (ASCs), 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.

The MSFE procedure is an established model to investigate oral bone tissue engineering approaches, and fits perfectly in a one-step surgical procedure [15]. For an extensive review on the feasibility of this procedure we refer to chapter 1 of this thesis. This thesis concentrates on the optimization of several important steps (as illustrated in Figure 1) i.e. the use of ASCs for bone regeneration in MSFE, as well as the evaluation of the osteoconductivity of different calcium phosphate carriers.

Within this research project the following scientific questions were addressed:

1. Does the MSFE procedure using adipose stem cells fits within the one-step surgical procedure model?
2. Does a short treatment of human ASCs with BMP-2 affect osteogenic differentiation after seeding on calcium phosphate carriers?
3. Are the growth factor and cytokine expression profiles of human ASCs dependent on BMP-2 treatment and/or osteogenic differentiation?
4. Is there a difference in the volume and quality of bone after MSFE with BCP 60/40 and deproteinized bovine bone?
5. Does the ratio HA/β-TCP within a BCP carrier affect the rate of new bone ingrowth after MSFE?

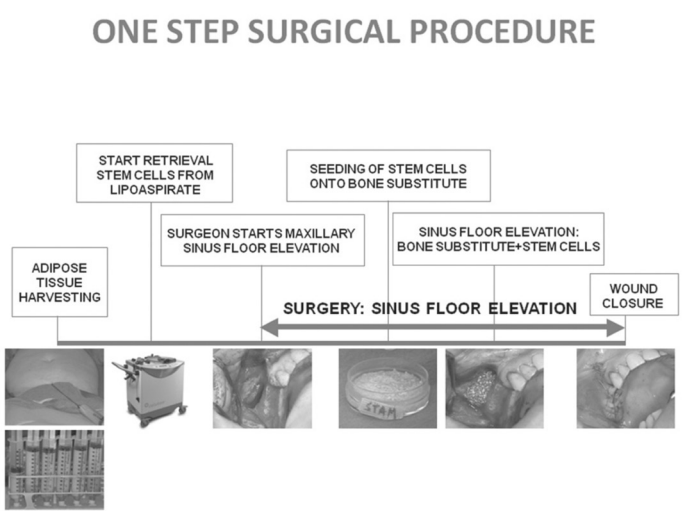

Figure 1. Scheme of the different steps in the one-step surgical procedure

In chapter 2 a review is provided on whether the human MSFE procedure could be applied as a model for bone regeneration enabling the application of one-step surgical procedures.

In chapter 3 the effect of short treatment of human ASCs with BMP-2 after seeding on a calcium phosphate carrier is evaluated.

In chapter 4 the gene expression profiles of growth factors expressed by differentiating human ASCs are evaluated.

In chapter 5 the gain of mineralized bone was compared between deproteinized bovine bone allograft (DBA) and biphasic calcium phosphate (BCP) after MSFE, using a split-mouth design.

In chapter 6 it we explored whether a collagenous barrier membrane covering the lateral window after MSFE using β-TCP affects bone formation.

In chapter 7 we evaluated the performance of a new biphasic calcium phosphate (BCP 20/80) using Micro-CT and histomorphometrical analysis in a novel approach.

Finally, in chapter 8 the main conclusions of this thesis are discussed and placed in a broader perspective. In addition, based on the results of our studies using calcium phosphate scaffolds and ASCs, we emphasize that our novel concept of a one-step surgical procedure for human MSFE might be applicable in other surgical disciplines as well.

REFERENCES

1. Tatum H, Jr. Maxillary and sinus implant reconstructions. Dental clinics of North America. 1986;30(2):207-29.
2. Boyne PJ, James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. Journal of oral surgery (American Dental Association : 1965). 1980;38(8):613-6.
3. Browaeys H, Bouvry P, De Bruyn H. A literature review on biomaterials in sinus augmentation procedures. Clinical implant dentistry and related research. 2007;9(3):166-77.
4. Klijn RJ, Meijer GJ, Bronkhorst EM, Jansen JA. A meta-analysis of histomorphometric results and graft healing time of various biomaterials compared to autologous bone used as sinus floor augmentation material in humans. Tissue engineering Part B, Reviews. 2010;16(5):493-507.
5. Raghoebar GM, Louwerse C, Kalk WW, Vissink A. Morbidity of chin bone harvesting. Clinical oral implants research. 2001;12(5):503-7.
6. Zijderveld SA, Schulten EA, Aartman IH, ten Bruggenkate CM. Long-term changes in graft height after maxillary sinus floor elevation with different grafting materials: radiographic evaluation with a minimum follow-up of 4.5 years. Clinical oral implants research. 2009;20(7):691-700.
7. Guha AK, Singh S, Kumaresan R, Nayar S, Sinha A. Mesenchymal cell response to nanosized biphasic calcium phosphate composites. Colloids and surfaces B, Biointerfaces. 2009;73(1):146-51.
8. Saldana L, Sanchez-Salcedo S, Izquierdo-Barba I, Bensiamar F, Munuera L, Vallet-Regi M, et al. Calcium phosphate-based particles influence osteogenic maturation of human mesenchymal stem cells. Acta biomaterialia. 2009;5(4):1294-305.
9. Zijderveld SA, Zerbo IR, van den Bergh JP, Schulten EA, ten Bruggenkate CM. Maxillary sinus floor augmentation using a beta-tricalcium phosphate (Cerasorb) alone compared to autogenous bone grafts. The International journal of oral & maxillofacial implants. 2005;20(3):432-40.
10. Kim CS, Choi SH, Choi BK, Chai JK, Park JB, Kim CK, et al. The effect of recombinant human bone morphogenetic protein-4 on the osteoblastic differentiation of mouse calvarial cells affected by Porphyromonas gingivalis. Journal of periodontology. 2002;73(10):1126-32.
11. Cowan CM, Aalami OO, Shi YY, Chou YF, Mari C, Thomas R, et al. Bone morphogenetic protein 2 and retinoic acid accelerate in vivo bone formation, osteoclast recruitment, and bone turnover. Tissue engineering. 2005;11(3-4):645-58.
12. Cowan CM, Shi YY, Aalami OO, Chou YF, Mari C, Thomas R, et al. Adipose-derived adult stromal cells heal critical-size mouse calvarial defects. Nature biotechnology. 2004;22(5):560-7.
13. Xia L, Xu Y, Wei J, Zeng D, Ye D, Liu C, et al. Maxillary sinus floor elevation using a tissue-engineered bone with rhBMP-2-loaded porous calcium phosphate cement scaffold and bone marrow stromal cells in rabbits. Cells, tissues, organs. 2011;194(6):481-93.
14. Knippenberg M, Helder MN, Zandieh Doulabi B, Wuisman PI, Klein-Nulend J. Osteogenesis versus chondrogenesis by BMP-2 and BMP-7 in adipose stem cells. Biochemical and biophysical research communications. 2006;342(3):902-8.
15. Helder MN, Knippenberg M, Klein-Nulend J, Wuisman PI. Stem cells from adipose tissue allow challenging new concepts for regenerative medicine. Tissue engineering. 2007;13(8):1799-808.