CHAPTER 8

GENERAL DISCUSSION

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The developing fields of bone tissue engineering and regenerative medicine have identified skeletal defects, such as insufficient jaw bone for oral implant placement, as attractive translational targets. Bone tissue engineering comprises cells, biomaterials (preferably biodegradable), environmental stimuli and biologics to trigger differentiation of stem cells. The ultimate goal of this thesis is to achieve regeneration of functional bone tissue during and after sinus floor elevation, and therefore an evaluation of the applied stem cells is needed, as well as an evaluation of the properties of calcium phosphate scaffolds involved.

This thesis describes the functionality of ASCs for bone tissue engineering and proposes a novel concept of a one-step surgical procedure for human sinus floor elevation using calcium phosphate scaffolds. In previous studies we evaluated goat and human ASCs for use in spinal interbody fusion; it was shown that goat ASCs could be applied in a one-step procedure as part of a spinal fusion model [1]. In line with these studies the feasibility of their human ASC counterparts for their use in a one-step surgical procedure for human maxillary sinus floor elevation was evaluated, addressing attachment time to scaffolds, and optimal treatment with differentiation-enhancing growth factors.

Next, the effects of the secretome of osteogenically stimulated ASC after seeding on calcium phosphate carriers were also evaluated. Furthermore the known methods for evaluating bone ingrowth within a biopsy were refined, which provided better insights in the processes of new bone formation. This improved method allowed us to compare new bone formation when different bone graft materials were used in sinus floor elevation. The before mentioned validations occurred in different phases and on different levels throughout this study, and together provide the framework for successful implantation strategies.

In chapter 2 the feasibility of the human MSFE model for bone tissue engineering studies was discussed. In chapter 3, the short treatment of hASCs with BMP-2 in a low dose was evaluated. This was prompted by two considerations: (1) induction times should be really short in order to fit within the one-step surgical concept; and (2) the use of BMP-2 has been extensively debated recently, since it could be damaging to cells and tissues when administered in higher dosages *in vitro* as well as locally *in vivo* [2, 3]. Our ex vivo stimulation and physiological dosaging in the ng-range ensure patient safety in our MSFE model by reducing the body exposure to BMP-2 to a minimum.

As a start, the properties of hASCs, and the culturing conditions for hASC attachment to several calcium phosphate carriers were thoroughly investigated and optimized. Aspects like cell attachment time, influence of temperature on cell attachment, time of BMP-2 treatment of hASCs, and a surface (topography, chemistry) effect on proliferation and osteogenic differentiation of ASCs were evaluated. The authors found that hASCs attached rapidly to the surface of different calcium phosphate carriers, independent of BMP-2 pre-treatment. Interestingly it was concluded that subjection of hASCs to a low dose of BMP-2 for 15 minutes was sufficient to stimulate the expression of osteogenic genes and to inhibit expression of an adipogenic marker *in vitro* for at least 21 days. The exact mechanism for such a prolonged effect is yet unknown.

When hASCs were seeded on biphasic calcium phosphate (BCP) and β-tricalcium phosphates (TCP) scaffolds, a slight difference in osteogenic gene expression levels was observed on the two scaffold types. However, the most striking finding was the strong osteogenesis-promoting effect of the surface topography of the biphasic calcium phosphate (BCP) and β-tricalcium phosphates (TCP) scaffolds compared to cells cultured on plastic. The strongest osteogenic differentiation was found when combining BMP-2 induction followed by seeding on a calcium phosphate scaffold.

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To evaluate whether the secretome of osteogenically stimulated hASCs seeded on BCP and TCP scaffolds may affect the wound repair process in parallel with their osteogenic differentiation, *in vitro* studies of the dynamic changes of the ASC secretome were performed. Therefore, in chapter 4, the effects of hASCs after seeding *in vitro* on BCP and TCP were tested; the dynamic changes of the angiogenic secretome of the hASCs during the osteogenic differentiation process were investigated. Surprisingly, no correlation was found between the degree of differentiation and the secreted growth factor expression pattern of hASCs. This suggests that the growth factors produced by hASCs do not seem to work in an autocrine/paracrine fashion to stimulate osteogenic differentiation *in vitro*, yet the substrate still does have a strong effect on the differentiation of hASCs. An alternative explanation may be that our *in vitro* study conditions lack interaction with environmental factors present *in vivo* (e.g. iterative interactions with local cells). Previous research also showed that MSCs and/or their secretome in particular could be applied in a therapeutic way; they are able to migrate to injured tissues and, in some cases, even able to replace damaged tissue [4-10].

In the next three chapters the effect of bone substitutes, and peri-operative techniques during sinus floor elevation, on the ingrowth of new bone was investigated. Firstly in chapter 5 two different scaffolds, biphasic calcium phosphate (BCP) and deproteinized bovine bone (DBA) were compared within a split-mouth model for MSFE. The authors found that after six months there were no differences regarding bone and graft volume, however BCP showed a more active bone remodeling process. In this study experiments with the histological evaluation technique as well as the micro-Ct analysis were executed. In the following studies these techniques were applied in conjunction with other study parameters.

In chapter 6 it was evaluated whether a collagenous membrane, placed over the maxillary sinus cavity after the elevation procedure, would result in enhanced bone ingrowth. The authors showed that the use of a resorbable collagen barrier did not increase mineralized bone volume, and actually decreased the amount of osteoid, indicating a lower activity of osteoblasts, or a lesser amount of active osteoblasts in the sites. This difference in results might be explained by our extensive and multifactorial methods (1mm-regions of interests, micro-CT, histomorphometry and clinical x-ray data), yet more human studies with a larger number of patients need to be done to confirm our data.

In chapter 7 the performance of a new biphasic calcium phosphate (BCP 20/80) Was evaluated In contrast to previous bone biopsy evaluating studies, in which parameters were averaged over the whole biopsy or over two regions only (native bone and graft area) [11, 12], our refined techniques resulted in a better and more detailed insight in the processes involved in

bone (re)generation in MSFE. Although not significant, there was a clear trend in both the μ-CT and the histomorphometrical analyses 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 scaffold in MSFE performs at least equal, but most likely better in bone augmentation when compared to the BCP 6/40 standard.

SUMMARY AND PERSPECTIVE

In summary, the authors proposed and performed preparatory studies to implement and optimize a novel bone tissue engineering approach for maxillary sinus floor elevation performed in patients with insufficient jaw bone height to allow dental implant placement. In this thesis, we provide strong indications that ASCs may contribute to the (re)generation of bone tissue after a MSFE procedure using bone substitutes, and that their application may well be performed in the one-step surgical concept postulated by our group. In fact, in a parallel project, a phase I clinical trial has been performed which confirmed this statement; it was found that the one-step surgical concept was feasible, safe, and that there was an additive effect of the stem cells (significant on some parameters; clear trend on other parameters) with respect to new bone formation when compared to BCP/TCP scaffolds alone.

Our experimental data suggest that the efficacy of ASC-mediated bone formation may be increased by performing a short (15 min) BMP-2 stimulation of the freshly isolated ASCs prior to *in vivo* implantation. However, the latter approach needs further validation in *in vivo* bone formation studies, e.g. in a calvaria defect model and/or goat large bone defect models. We postulate that the inclusion of the BMP-stimulation step may be a next-generation approach to further increase adipose stem cell-mediated bone formation efficacy.

The BMP-2 stimulation may also compensate for the likely suboptimal (too low) loading mechanical loading conditions in our current MSFE model. This aspect of non-optimal loading condition, which likely results in an underestimation of the full potential of the ASC-supplementation since mechanical stimuli are an important stimulatory factor for ASC bone formation-promoting actions, should be given more attention. In this respect, an earlier dental implant placement than the currently used 6-month time frame may have beneficial effects since it is expected that the dental implants will transduce the loading much more efficiently to the bone augmentation area.

The authors conclude 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. The outcome of these and future studies will have pivotal implications for bone tissue engineering models in other fields such as orthopaedic surgery; predictions can be made for outcomes regarding spinal fusion surgeries and knee

defect reconstructions [13]. Also for craniofacial surgeries the MFSE model can be useful; for frontal sinus reconstructions, cranioplastic reconstructions, mandibular reconstructions and nasal septum corrections, the process of culturing ASCs will not be necessary and the outcome can be predicted without taking biopsies after surgery [14]. Still additional clinical research needs to be done to evaluate the outcome of a one-step surgical model in larger defects, as well as defects that are not completely surrounded by a natural bone cavity.

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