6 Summary

Implant-borne tooth restorations have become a standard of care in modern dentistry. Ridge augmentation procedures have clearly widened the scope of implant treatment. There has also been an ongoing effort to enhance and accelerate osseointegration of dental implants by optimizing their surface design.

Designing implant surfaces which elicit excellent cell and tissue responses requires a fundamental understanding of the processes involved in tissue integration of endosseous implant materials at a molecular level. Roughened titanium surfaces like the titanium plasma-sprayed (TPS) surfaces have been preferred for the endosseous area of dental implants in order to increase the total surface area available for osseous apposition. In recent years, there has been the tendency to replace titanium plasma-sprayed surfaces by microroughened, sandblasted and/or acid-etched surfaces in order to accelerate osseointegration. Another approach to improve osseous integration of dental implants has been the utilisation of calcium phosphate coated implants, since these coatings have been found to accelerate initial stabilization of implants by enhancing bony ingrowth and stimulating osseous apposition to the implant surface. Plasma-sprayed hydroxyapatite (HA) has been most commonly used for coating dental implants fabricated from titanium or titanium alloy. However, a drawback of plasma-spraying is the thermal instability it subjects the HA powder to. The plasma-sprayed HA coatings may have different crystallinities. This is directly related to their dissolution, this being higher for less crystallization. Thus, heat treatments are often applied to increase the crystallinity. This, however, may result in stresses between the coating and the underlying titanium alloy due to the mismatch in thermal expansion coefficient. Therefore, novel

calcium titanium and calcium titanium zirconia orthophosphates have been developed which are suitable for plasma-spraying onto titanium substrata and have a thermal expansion coefficient similar to that of titanium and titanium alloy. Thus heat-treatment of the sprayed coatings can be applied to increase the crystallinity without creating stresses between the coating and the underlying titanium substrata.

The quantitative evaluation of the gene and protein expression of osteogenic markers by osteoblasts grown on different biomaterials has been proven to be valuable for assessing the osteogenic capacity of candidate implant materials. Techniques to quantitatively relate the expression of bone-related mRNAs to their respective proteins as a measure of phenotypic differentiation have recently been established.

In the work presented here, this methodology was used to investigate the effect of a range of endosseous implant materials on osteoblastic cell differentiation. Thus, the effect of five novel bioactive calcium titanium and calcium titanium zirconium orthophosphates (CTP-S1, CTP-S2, CTP-S3, CTZP-S1 and CTZP-S2) on the osteoblastic phenotype of human bone-derived cells (HBDC) was examined and the observations were compared to those for cells on implant materials already clinically used, i.e. plasma-sprayed HA-coated titanium (HA), a titanium plasma-sprayed surface (Ti-TPS) and a sandblasted and acid-etched titanium surface (Ti-DPS).

Surfaces of CTP-S1 and CTP-S3 had the most effect on osteoblastic differentiation, as a greater expression of an array of osteogenic markers was induced than by cells grown on Ti-DPS and HA. This suggested that these novel implant materials may possess a higher potency to enhance osteogenesis. Consequently, CTP-S1 and CTP-S3 appear to be promising bioceramics for producing calcium phosphate coatings on titanium substrata. HA-coated titanium

stimulated osteoblastic differentiation to a greater extent than Ti-DPS and Ti-TPS Furthermore, Ti-DPS surfaces induced greater osteoblast proliferation and differentiation than Ti-TPS.

Among the various techniques to reconstruct or enlarge a deficient alveolar ridge, guided bone regeneration (GBR) has become a predictable and well-documented surgical approach. In addition, sinus floor elevation techniques have become a well established pre-implantology procedure for alveolar ridge augmentation of the posterior maxilla. Although autogenous bone grafts are unequivocally accepted as the standard of care, bone substitute materials are being extensively studied in order to avoid the harvesting procedure of autogenous bone. The reasons most frequently cited for using an alternative bone grafting material are donor site morbidity and insufficient volume of (intraorally) harvested autogenous bone. The use of synthetic bone graft materials eliminates the risk of virus or prion contamination associated with the use of allografts. Apart from requirements such as clinical manageability and safety, an ideal bone replacement material should serve as a temporary scaffold for bone remodeling and thus resorb rapidly while undergoing complete remodeling and substitution by newly formed bone in view of placing dental implants in such augmented sites. Compared to the synthetic bone substitute materials which are currently clinically used, there is a considerable need for more rapidly biodegrading bone substitute materials. As a result, there has been an ongoing search for synthetic, biodegradable bone substitute materials which degrade rapidly, but still stimulate osteogenesis at the same time. This real need has led researchers at the Federal Institute of Materials Research and Testing in the FRG to formulate a range of novel, bioactive, rapidly resorbable calcium-alkali-orthophosphate materials.

In the work presented here, the osteogenic potential of a range of these novel ceramic bone substitute materials was assessed by evaluating their effect on osteoblastic cell differentiation.

The results demonstrated that several of these materials (GB9, GB9N, GB14, R1 and R1+SiO_2) are able to induce gene and protein expression of an array of osteogenic markers characteristic for the osteoblastic phenotype and thus possess the potency to enhance osteogenesis. Moreover, these materials induced greater expression of these osteogenic markers compared to tricalcium phosphate, while exhibiting a higher biodegradability, thus rendering them promising bone substitute materials. These results are clinically very significant, as tricalcium phosphate has received considerable attention as a synthetic bone graft material for alveolar ridge augmentation and sinus floor elevation procedures in implant dentistry. An additional attractive feature of these novel calcium alkali orthophosphates is that they can be used for fabricating three-dimensional scaffolds with various pore sizes for tissue engineering purposes.

However, the underlying mechanisms by which these rapidly resorbable bone substitute materials induce enhanced osteoblastic differentiation are not fully understood. To obtain a fundamental understanding of the stimulatory effect of these bioactive ceramics on osteogenesis, the atomic and molecular phenomena occurring at the material surface and their effects on the reaction and signaling pathways of cells and tissues must be elucidated. With bioactive ceramics, solution-mediated surface reactions take place after immersion in biological fluids. These reactions include dissolution, reprecipitation and ion-exchange phenomena in combination with protein adsorption. There is support for the view that the enhanced cellular and tissue responses to bioactive ceramics are related to enhanced fibronectin adsorption at

their surfaces. Thus, to decipher the complexity of the reactions at the bioactive ceramic-bone interface, it is logical to first analyze the surface transformation and protein adsorption events and then study the osteoblast responses to these bioactive surfaces. This involves attachment of the cell to the biomaterial surface, followed by intracellular signaling which regulates osteoblast proliferation and differentiation, and ultimately leads to the establishment of the osteogenic phenotype at the bone-bioactive ceramic interface. Adequate surface analysis techniques and methodologies to study these signaling pathways have been established only recently. Consequently, further exploration of the material dependent effects reported here will involve the study of solution-mediated surface reactions and the subsequent cell adhesion mechanisms and intracellular signal transduction events which eventually lead to the enhanced osteoblastic differentiation observed with these materials.

The effect of bioactive bone substitute materials interacting with bone tissue on these signaling pathways in osteoblast function and differentiation is currently not understood. Once these factors are identified and studied, it should be possible to alter biomaterial molecular components and surface characteristics in ways that promote optimal cell adhesion, proliferation and differentiation and thus to create bone substitute materials whose surface chemistry preferentially boosts the osteogenic cascade, leading to more expeditious and enhanced bone formation in combination with rapid biodegradation of the material. The knowledge generated in this way will facilitate, in its turn, the creation of a novel generation of biomaterials in which the surface chemistry can be engineered so as to elicit a specific biological response resulting in the enhancement of osteogenesis and bone regeneration. This way, a totally new concept would be introduced to biomaterials research in implant dentistry. Rather than following an empirical approach by implanting new materials of

which the tissue response is characterized, the knowledge about the molecular mechanisms of tissue integration can then be used to strategically design biomaterials with the goal to elicit the desired tissue responses. Additional, novel approaches for optimizing the surface characteristics of endosseous implant materials may include the use of RGD peptides or ion implantation techniques.

Equally important will be the correlation between *in vitro* data with *in vivo* phenomena, by focusing on the detection of the osteogenic markers in the tissue surrounding the present bone substitute materials subsequent to implantation by using novel molecular techniques in hard tissue histology. This implies first correlating quantitative gene and protein expression of the osteogenic markers *in vitro* with quantitative histomorphometric evaluation of the amount of bone formed after biomaterials implantation. This is in addition to determining the decrease in particle size. And second quantifying the expression of these markers in histologic sections obtained from *in vivo* experiments is critical to comparing the expression of the various markers *in vitro* and *in vivo.* These recent advances in histologic techniques facilitate characterizing the tissue response at the bone-biomaterial interface *in vivo* at a molecular level and, thus, can contribute significantly to enhancing our understanding of tissue integration of endosseous implant materials.