REVIEW

Receptor technology – cell binding to P-15: a new method of regenerating bone quickly and safely-preliminary histomorphometrical and mechanical results in sinus floor augmentations

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Abstract. Modern implantology involves the application and optimization of bone engineering biomaterials and scaffolds to achieve predictability in quality and quantity of the regeneration result and to avoid the high morbidity factor of the present gold standard. In this respect, acceleration of (woven) bone formation and completeness of the regeneration result seems to be an reasonable attempt by multiplication of the whole cascades by duplicating all phases of cell binding, migration, proliferation and differentiation. Collagen I is an extracellular matrix protein with multiple main binding domains for osteogenic progenitor cells and therefore plays a crucial role in osteogenesis. PepGen P15 is the first man engineered collagen I binding domain for potential osteoblasts and is able to multiply the complete regeneration cascade. The article explains the principles of micromolecular receptor enginering and its application in sinus floor augmentations as a preliminary report. It presents the first clinical and histomorphometrical results of this new technology in sinus floor elevations. The future potential of individual bone regeneration will be discussed. (Keio J Med 53 (3): 166–171, September 2004)

Key words: biomimetic environment, osteogenic differentiation, receptor engineering

Introduction

Today, the goal of all dental and orthodontic surgery is complete oral rehabilitation. Sufficient bone volume is a particularly important factor in the long-term success rate of dental implant treatment. In many cases current surgical techniques in combination with the use of autogenous bone or bone grafting materials make it possible to provide sufficient bone for an implant placement. However, due to its high morbidity the gold standard has increasingly been regarded as critical. In addition, bone grafting and bone regeneration materials have evolved on a micromolecular basis in recent years and could offer effective alternatives in the future. In all cases the bone-inducing cell, the osteoblast, is the center of interest.

Osteoblasts develop from inactive mesenchymal progenitor cells by differentiation. The differentiation process proceeds in combination with a variety of genetic and hormonal factors. Although most of these substances are known (Babx 1, Cbfa 1, Osf 2, Dlx 5, Ihh, BMP, leptin)[1] the full interplay and mutual influences of the complex mechanisms and cascades have not yet been thoroughly studied. For this reason it is not surprising that experiments on influencing bone growth with single factors have run into difficulties. Clinically relevant results can often be obtained only by increasing the concentration by a factor of several thousand. On one hand, this unphysiological concentration increase interferes with the stoechiometry of the biochemical processes. The results must also be viewed with scepticism because of their unpredictabil-

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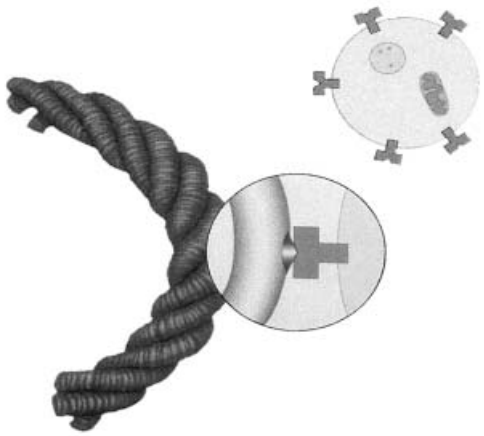


Fig. 1 Cell surface receptor contacts P15 binding domain in human collagen I initiating the natural osteogenic differentiation process.

ity, particularly with reference to the effectiveness of the applied highly concentrated substances. Their decomposition and intermediate products are in some cases still biologically active. In contrast to the above, cell binding is scientifically documented as the precursor of the differentiation cascade of osteoblasts.

All local cells are in a three-dimensional extracellular matrix interconnected via collagen fibers. Cells move over them by haptotaxis. In this environment cell-to-cell and cell-to-matrix interactions are mediated by highly specific receptors.[2]

A short (766-GTPGPQGIAGQRGVV-780) sequence of collagen 1 α 1 – forming the major component of the organic components of bone at 85% – has been identified as a cell binding domain for mesenchymal progenitor cells.[3] The binding of osteoblast progenitor cells at this domain initiates their proliferation and differentiation (Fig. 1). The connection of the extracellular matrix (ECM) is initiated here by integrin receptors on the cellular membrane. The integrins transfer the information via an ''outside-in signaling'' process, which represents a transmembrane hierarchy of molecular responses such as receptor clustering and recruiting of signal and cytoskeleton proteins to focal adhesion.[4]

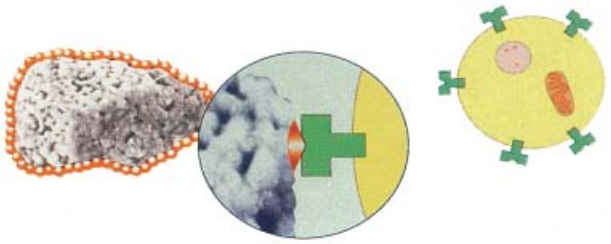
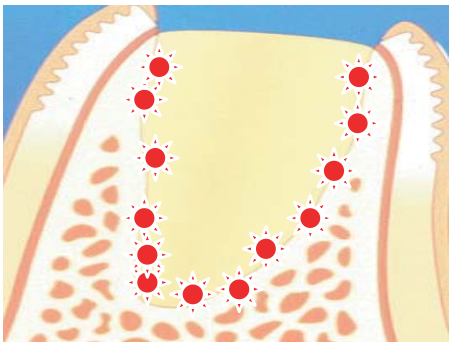


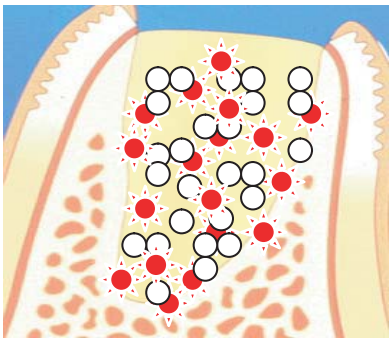
Fig. 2 Recombinant P15 binding domain on PepGen P 15 substrate simulate a collagenous biomimetic environment for the cells. Cell surface integrin receptor contacts Peppen P15 particle and the natural osteogenic differentiation process is triggered in the same way.

This amino acid sequence (P-15) can be produced in any desired quantity by synthetic processes. Since it is irreversibly bound to a carrier (Osteograf/N), it can be a substitute for the physiological collagen. A binding to this synthetically manufactured analogue (PepGen P-15®, Dentsply FRIADENT, Mannheim, Germany) triggers the same differentiation cascade of target cells as the binding to the corresponding domains of the collagen (biomimetics) (Fig. 2).

This cellular response could be demonstrated in various studies. Contrary to the use of conventional, biologically inert grafting materials, significantly higher cell proliferation rates have been observed on materials coated with P-15.[5,6] They allow for immediate activation of all osteoblast progenitor cells, while physiologically a collagen network forms first. This is the prerequisite for a multifocal formation of new bone, where unlike the classical defect healing process the bone is not organized exclusively starting from the vital bone walls (Fig. 3). The result is accelerated formation of new, highly vascularized bone of very high quality.



two-dimensional "classical" defect healing



three-dimensional, multifocal defect healing after filling the defect with PepGen P-15

Fig. 3 Various methods of defect healing with the use of micromolecular receptors.

Clinical Results

The receptor technology for bone remodeling in the form of the grafting material PepGen P-15® (DENTSPLY Friadent, Mannheim, Germany) has been available since 1999. Originally developed as a material for use in periodontics,[7,8] in 2000 the indications were extended to include all other intra-oral rehabilitations.[9] Today this material is widely used in the most various indications. To fill large defects PepGen P-15® is mostly mixed with other bone grafting materials. This means there are still enough receptor cells for cell attachment but the total material cost is reduced.

Two examples from a continuing study that is investigating the application of PepGen P-15® in sinus floor augmentation are described here. They not only demonstrate histologically and histomorphometrically the advantages of using the synthetic receptor but also show the primary stability of the implants that can be placed in the newly grafted bone. The histological results described in the literature are in some cases not coherent and are contradictory, with many different and unknown variables generally making it difficult to compare the results of the study.

In a study on bone regeneration conducted by Wheeler *et al.* in 2000,[10] between 11% and 19% newly formed bone was found regardless of the grafting material used (hip bone, intra-oral bone, alloplastic material (Interpore 200)). Surprisingly, this study demonstrated that the histological results with the use of autogenous bone were in some cases worse than with purely alloplastic materials.

The grafted sinus floor had the greatest volume of bone growth from the primary woven bone formation during the implant and prosthetic loading phase. The woven bone is successively converted to lamellar bone. According to the study conducted by Sartori *et al.* in 2003[11] the bone growth suddenly increases by a factor of almost three after the first year of prosthetic loading and then continues to increase slowly until the tenth year. The prerequisite for this bone growth is the comprehensive and qualitatively high-value primary bone formation (formation of woven bone) in the first six to twelve months. Again this depends on the individual regeneration potential and bioactivity of the grafting material used, the number of bone-forming cells and the number of collagen binding sites in the grafting material.

In our ongoing study, the primary sinus floor augmentation was filled with a mixture of PepGen P-15® (30%) to ''improve the quality and accelerate the regeneration'' and BioOss (0.5–1.0 gr., 70%) as a spacer in a two-stage process. The implants were placed after three, six or twelve months depending on the height of the alveolar ridge. The bone cylinders retrieved with the trephine drill (Brasseler Co., Lemgo, Germany; diameter 3.1 mm) during implant placement was histologically and histomorphometrically examined in the laboratory.

Case 1

The 65-year-old patient presented in our practice, where the examination showed a cyst in region 26 and extreme bone atrophy in the left lateral maxillary. The residual alveolar height (RAH) was 0.5 mm. The patient desired a fixed denture but refused an autogenous bone transplant.

The pre-operative orthopantomograph clearly shows the poor initial situation in the left lateral maxillary. As a result of the cystic process only a very thin cortical lamella remains adjacent to the maxillary sinus; the regenerative potential of the reduced alveolar ridge is poor. The sinus floor was vertically augmentated with the classical ''window technique'' with vertical grafting in the region of the cystic lesion. The cortical line separating the osseous lamella and the synthetic grafting material can be clearly seen.

As a result of the poor initial situation an extended healing period of 12 months was decided. The material removed with the trephine drill during implant placement was initially fixed in an ascending alcohol series, then embedded in TECHNOVIT 7200, and subsequently stained with toluidine blue for the histomorphometry. The histomorphometric measurement result showed of 49.03% by volume formation of new bone in the regeneration material. The histomorphometric evaluation was performed by the University of Freiburg, Germany. Two sections of the biopsy were fabricated and the percentual volumes of bone and bone substitute material was determined and mean values of the two results were taken.

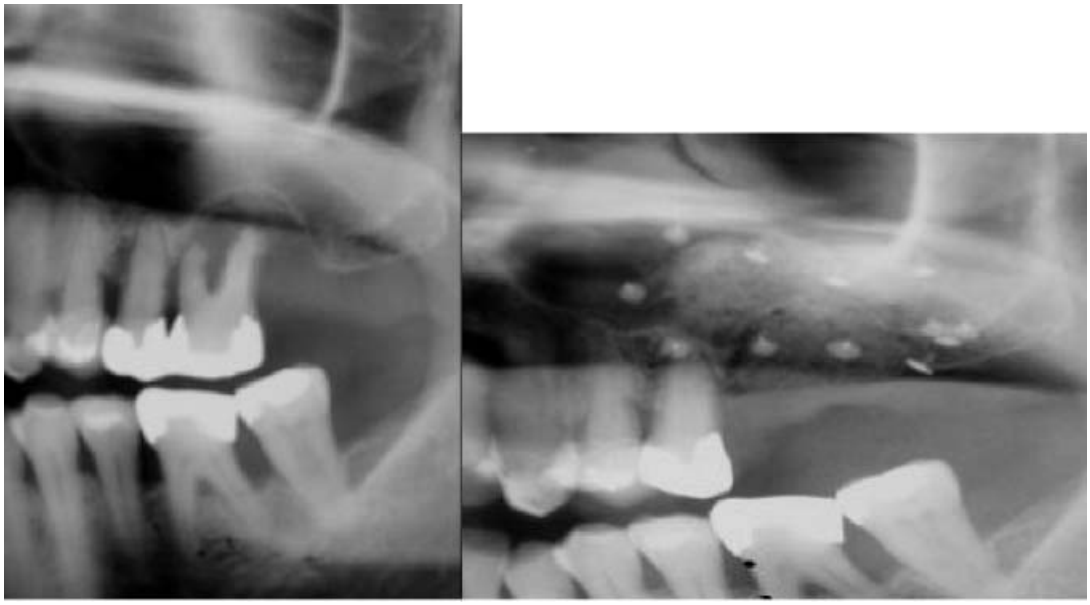
The histological view of the newly formed woven bone shows not only complete vascularization of the regeneration material but also almost complete conversion of the filler material BioOss. The ossification centers are in the immediate vicinity of the dark Osteograf N particles.

Case 2

The 37-year-old patient had a primary sinus floor augmentation and immediate implant placement in region 24 in the left maxilla. The residual alveolar ridge height in the grafted region was 1–4 mm.

Figures 8 to 10 show the procedure for sinus floor elevation. The greater residual alveolar ridge height compared to case 1 allowed a primary healing period of six months.

The implant was placed analogously to case 1. Figure



Figs. 4 and 5 Pre-operative and post-operative orthopantomograph after removal of teeth, cystectomy and simultaneous sinus floor elevation with PepGen P-15® and BioOss.

10 shows the bone core harvested from region 27. The thin and paler region as residual alveolar ridge can be clearly seen in the top section without a microscope, while the large lower segment is newly formed bone.

The XiVE implants (DENTSPLY Friadent, Mannheim, Germany) were threaded in place by machine in region 26 (diameter 5.5, L 13) and region 27 (diameter 5.5, L 13) and the insertion torque was measured simultaneously to assess the final primary stability (FRIOS UNIT E, DENTSPLY Friadent, Mannheim, Germany) Slightly different torque measurements were achieved for the two implant positions, although both of them were well above the normal values in the area of sinus floor elevation. Implant in area 26 reached a top

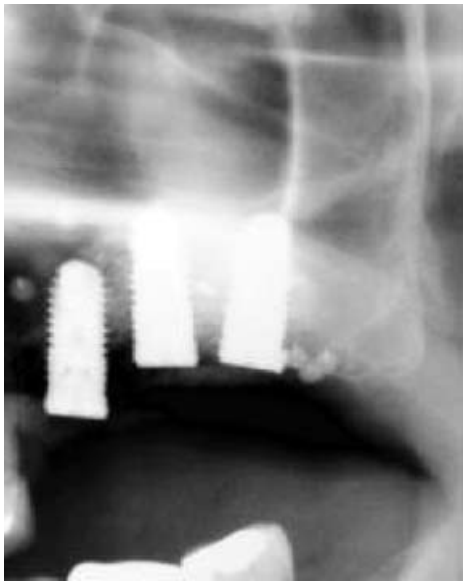


Fig. 6 Sinus lift and subsequent implant placement after 12 months healing period with no signs of the cortical line. The loss of volume of the grafting material appears to be clinically minor.

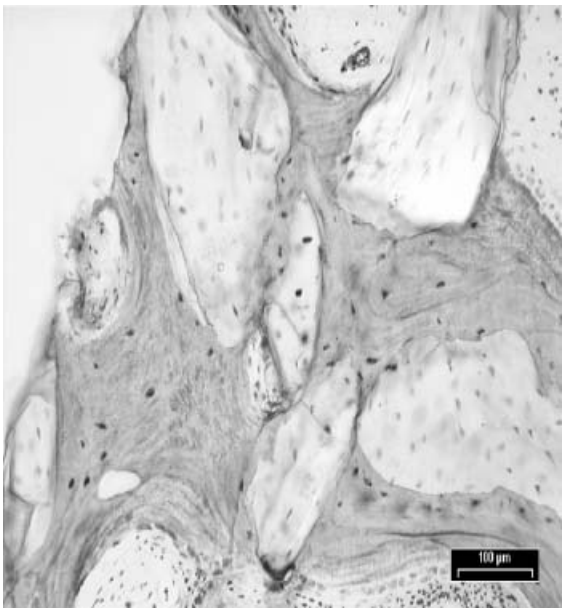


Fig. 7 Histological view of the trephined material with persistent new bone, complete vascularization and generally incorporated spacer (BioOss).

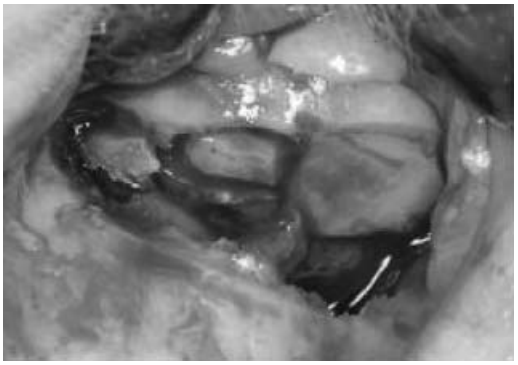


Fig. 8 View of the ''Underwood septa''.

torque value of 46.9 Ncm and implant in area 27 scored at 39.9 Ncm.

Discussion

Clinical results and scientific documentation have shown that predictable positive results can be achieved with augmentation using bone grafting materials of the most varied types and origins. Today, the desire for accelerated regeneration is at the center of scientific interest.

The first osteoconductive factor was discovered as early as 1965 and named the ''bone morphogenetic protein''.[12] In 1979 osteoconductive glycoproteins could be extracted from bone.[13] However, because the complex interactions of the effective mechanisms of all non-cellular growth factors are still not fully explained, bone can be induced only with greatly increased, unphysiological concentrations of these substances. The questions that arise as a result of the biochemical stoechiometry and the decomposition and intermediate products remain largely unanswered or have not been considered. For this reason, the only practical method so far is activation of mesenchymal stem cells, which are directly differentiated to osteoblasts. This is enabled by a peptide (P-15) bound to a carrier, which could be identified as a cell-binding domain of the collagen 1 α 1 in scientific studies. The biological potential that supports bone formation has been demonstrated by *in vitro* studies as well as in clinical applications. The receptor technology has proven to be a practical and cost-efficient method of accelerating bone regeneration. In clinical use, it is significant that the implants reach a sufficient primary stability in the ''synthetically'' grown bone. This primarily depends on the thickness of the residual alveolar ridge, and also on the strength and complete regeneration of the grafting material. The values measured were in some cases extremely high,

Fig. 9 Filling the new sinus with PepGen P-15® and BioOss mixture after immediate implant placement in region 24 and soft tissue management with FRIADENT EsthetiCap.

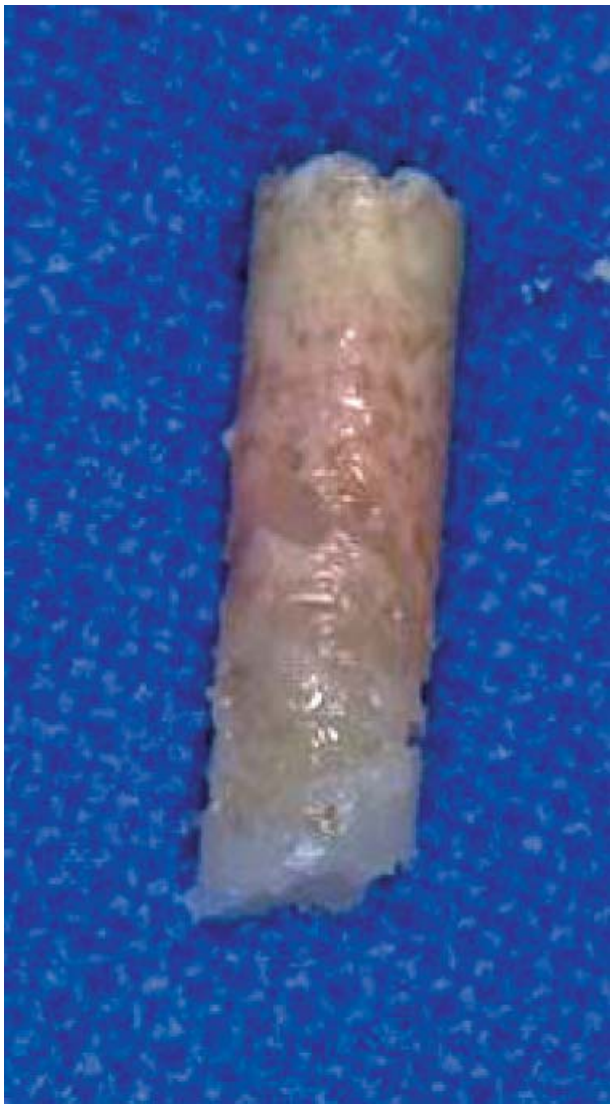


Fig. 10 Bone core from region 27 after six months healing time.

even in the weak site of the displaced sinus floor, and is comparable with the DI bone of the mandible. This is impressive, because the alveolar ridge had a residual vertical height of 0–1 mm. The torque achieved in region 27 is solely attributable to the synthetic grafting material. The long-term success rate in the grafted region is determined by the fastest possible and optimum attachment of this area to the vascularization of the substrate. The complete vascularization of the grafting material is an excellent sign of quality and an indication for the vitality of the woven bone and the functionality of the osteoblasts. Initial histological evaluations indicate that more bone is present in the regenerated material as a result of the multiplication of the bone regeneration induced by the PepGen P-15®, and the functional and force-oriented secondary integration of the implants can proceed much more effectively during the prosthetic loading phase. The constant volume is the immediate result of the cell potentiating effect and the complete vascularization of the grafted material. While with autogenous transplants a volume loss of 30–50% is recorded, with PepGen P-15® the volume loss is approximately 5–10% only. This is the result of the high inorganic proportion, the lower cellular conversion and the fibrin contraction during the maturation process.

Conclusion

Using the triggering mechanisms of a recombinant collagen binding domain,[14,15] complete bone regeneration cascades may be multiplied successfully leading to a faster and more predictable regeneration result in sinus floor augmentations.[16]

PepGen P15 simulates a biomimetic cell-attractive environment for osteogenic cells and controls the stoechiometric processes of the bone regeneration cascades. Clinically bone regeneration in sinus floor augmentations seems to be enhanced. The histologically examined biopsies show a complete vascularization and an increased number of osteogenic cells. Previous attempts in micromolecular bone regeneration focussed mainly on highly concentrated protein deliveries (mitogenes, morphogenes), vector techniques, mesenchymal stem cell therapy using various types of scaffolds with various success. In opposite to the above mentioned methods, receptor engineering seems to be a promising, less expensive and more practical treatment option for a more natural and stoechiometric correct bone regeneration.

Statistical results are still requested to verify the clinical benefits. This is the focus of our ongoing study in sinus floor augmentations which will be published in the next article.

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