Effect of combined therapeutical methods on healing of intra-bony defects in regenerative periodontal surgery

Ph.D. Thesis

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1. Introduction

The ultimate goal of any periodontal therapy is the control of active inflammation, the arrest of disease progression and the reconstitution of the lost periodontal structures. It has been proven that conventional periodontal therapy did not succeed in providing predictable periodontal wound healing and tissue regeneration.

True periodontal regeneration means healing after periodontal treatment that results in the regain of lost supporting tissues including new acellular cementum attached to the underlying dentin surface, a new periodontal ligament with functionally oriented collagen fibres inserting into the new cementum and new alveolar bone attached to the periodontal ligament.

Studies and research has shown, that periodontal attachment and support structures may be predictably regenerated.[1-3] Not only their regeneration, but their evolution is also associated. Periodontal ligament, cementum and alveolar bone have their own potency for regeneration, and this can be encouraged by special conditions. After periodontal surgery four types of cells have the tendency to repopulate the previously diseased tooth root surface: epithelial cells, gingival connective tissue, alveolar bone and periodontal ligament cells. Epithelial cells migrates first along the cleaned root surface preventing the new attachment, cells of gingival connective tissue may attach too, but without formation of new cementum and periodontal ligament. This fibrous attachment may result in dentinal resorption and breakdown. The presence of slow healing bone may result ankylosis with the root without the protection of new cementum and new periodontal ligament.[4-6]

Classical methods to help the formation of new connective tissue, or to fill osseous defects have not resulted the regeneration of natural periodontal tissues.[7,8] Without a method, which allows the selective cellular repopulation of the root surface, the support system of bone, periodontal ligament and cementum cannot be renewed. Cementum and periodontal ligament cells can become established on the cleaned root surface if this surface is isolated from other tissues during the healing period.[9-11] This isolation during the initial healing allows the reestablishment of the support tissues.

The exclusion of epithelial and gingival connective tissue cells from the healing area by the use of a physical barrier may allow and guide periodontal ligament cells to

repopulate the detached root surface.[11] This observation provided the basis for the clinical application of the treatment principle termed „guided tissue regeneration" (GTR).

Guided tissue regeneration is not a procedure for the treatment of periodontitis, but rather a technique for regenerating defects which have developed as a result of periodontitis. Therefore, appropiate periodontal treatment should always be completed before GTR is initiated. Only well motivated patients with excellent oral hygiene can be a candidate for any kind of regenerative surgery.

Results showed that considerable but varying amounts of new connective tissue attachment had formed on the treated teeth and frequently, bone formation was incomplete. The varying results were ascribed to factors such as the amount of remaining periodontal ligament, the morphology of the treated defect, technical difficulties regarding membrane placement, gingival recession, membrane exposure and bacterial contamination of the membrane and the wound during healing.

Based on the biologic concept of GTR[4-6,11] and on reports about the formation of a new attachment apparatus in histologic specimens from human biopsies harvested following GTR treatment[1,2,10], it was suggested that clinical signs of probing attachment gain and bone fill can be accepted as evidence of periodontal regeneration in the evaluation of GTR procedures.[12]

Various types of bioabsorbable and non-bioabsorbable barrier materials were used in regenerative therapy of intrabony periodontal defects. Several studies have utilized non-resorbable membranes of expanded polytetrafluoroethylene (e-PTFE) especially developed for periodontal regeneration (Gore Tex Periodontal Material®). Membranes of e-PTFE have been used successfully in animal experiments and in clinical studies. Natural or synthetic bioabsorbable barrier materials for GTR have been introduced in order to avoid a second surgery for membrane removal. (Resolut®, Bio- Gide Perio®).

Regenerative methods based on barrier technique lack predictability, sufficient degree of efficacy and ease of use.

To eliminate these problems, the use of polypeptide growth factors, polypeptide differentiation factors and other biomolecules have been suggested.

Another way to address periodontal regeneration is to mimic the processes that take place during the development of the nascent root and the periodontal tissues.[13]

Morphological studies have shown that the cells of Hertwig's epithelial root sheath, which is an extension of the enamel forming dental organ, have a secretory phase during which enamel related matrix proteins are secreted and temporarily deposited onto the root surface providing an initial and essential step the formation of acellular cementum.[14] The cells close to the root surface seem to carry the message not only to form acellular cementum but also an associated periodontal ligament and alveolar bone.[15-18]

The discovery of the enamel matrix layer between the peripheral dentin and the developing cementum and its function provided the fundamental concept for enamel matrix derivative-supported tissue engineering in regenerative periodontal therapy.[19]

The use of bone grafts in regenerative periodontal therapy is based on the assumption that the promotion of bone regrowth may also induce cells in the bone to produce a new cementum layer with inserting collagen fibres on previously periodontitis-involved root surfaces. Grafting procedures often results in healing with a long junctional epithelium rather than a new connective tissue attachment.[20,21] A study suggests that the ingrowth of periodontal ligament tissue inhibited bone formation and the new cementum on the root surface and cementum-like substance observed around the implanted bone particles were formed by periodontal ligament cells. It appeared that the key cells in periodontal regeneration are periodontal ligament cells rather than bone cells.[22]

The final evidence that the progenitor cells for new attachment formation are residing in the periodontal ligament was provided in studies in which titanium dental implants were placed in contact with retained root tips whose periodontal ligament served as a source for cells which could populate the implant surface during healing preventing osseointegration.[23,24] These results prove that the progenitor cells for periodontal attachment formation reside in the periodontal ligament and not in the alveolar bone as previously assumed.[25]

In many clinical trials and animal experiments, the flap approach was combined with the placement of bone grafts or implant materials into the curetted bony defects with the aim of stimulating periodontal regeneration. The conception behind the use of bone grafts or alloplastic materials is the assumption that both the regrowth of alveolar bone and the formation of new attachment would be stimulated because these materials may either contain bone forming cells (osteogenesis), or serve as a scaffold for bone

formation (osteoconduction), or because the matrix of the bone grafts contains bone-inducting substances (osteoinduction).[26]

Autogenous grafts (autografts) may retain some cell viability and are considered to promote bone healing mainly through osteogenesis and osteoconduction. Results from studies indicate that the treatment of periodontal osseous defects with intraoral bone grafts may result in periodontal regeneration, but not predictably.[7,27] At the same time, due to the morbidity associated with the donor site and that root resorption sometimes results, extraoral autogenous grafts, as iliac crest marrow are not used in regenerative periodontal therapy today.

Allogenic grafts (allografts) were utilized in attempts to stimulate bone formation in intrabony defects in order to avoid the additional surgical insult associated with the use of autogenous grafts. Mineralized freeze-dried bone grafts (FDBA) and decalcified freeze-dried bone grafts (DFDBA) were used in several investigations. The controversial results regarding the effect of DFDBA on the regeneration of periodontal intraosseous defects along with great differences in the osteoinductive potential of commercially available DFDBA and the risk for disease transmission have raised concern about the clinical applicability of this material. In EU countries, the commercially available DFDBA is not granted a CE mark permitting distribution of the material within the community.[28]

Early studies examined the use of xenogeneic bone grafts (xenografts) in regenerative periodontal surgery and demonstrated no difference between the amount of clinical gain of attachment and bone fill obtained after use of one xenograft and intraoral autogenous bone graft. Similar histologic features were also demonstrated.[22,29] New processing and purification methods make it possible to remove all organic components from a bovine bone source and leave a non-organic bone matrix in an unchanged inorganic form. (Bio-Oss®, Bon-Apatite®). Implantation of Bio-Oss® can result in pocket reduction, attachment gain and bone fill in periodontal defects to the same extent as that of DFDBA.[30]

Alloplastic materials are synthetic, inorganic bone graft substitutes which are claimed to promote bone healing through osteoconduction (hydroxyapatite, beta-tricalcium phosphate, polymers, bio-active glasses). Studies using beta-tricalcium phosphate (Synthograft®, Cerasorb®) report varied results, leaving many open questions. At the

1996 American Academy of Periodontology World Workshop, it was concluded that synthetic graft materials function primarily as defect fillers.[28]

The biologic rationale behind the use of bone grafts or alloplastic materials in periodontal regenerative surgery is the assumption that the use of such materials may stimulate not only the regrowth of alveolar bone but also the formation of new attachment. This assumption is in conflict with current knowledge about the biology of periodontal wound healing, that repopulation of the detached root surface with cells from periodontal ligament is the prerequisite for new attachment formation.[28]

Biomodification of the root surface with enamel matrix proteins (Emdogain®) during surgery and following demineralization with EDTA has been introduced to encourage periodontal regeneration. The biologic concept is that the application of enamel matrix proteins may promote periodontal regeneration because it mimics events that took place during the development of the periodontal tissues.[14]

Proliferation and migration of periodontal ligament cells and synthesis of extracellular matrix as well as differentiation of cementoblasts and osteoblasts is a prerequisite for obtaining periodontal regeneration. Growth factor (GF) is a general term to denote a class of polypeptide hormones that stimulate a vide variety of cellular events such as proliferation, chemotaxis, differentiation and production of extracellular matrix proteins.[31] Therefore it is conceivable that growth factors may represent a potential aid in attempts to regenerate the periodontium.

Membrane materials must possess certain characteristics in order to be efficient. Among those it is important that the membrane is capable of keeping its shape and integral features, thereby maintaining the space created adjacent to the root surface. The collapse of the barrier may be prevented by means of implantation of a biomaterial into the defect to support the membrane. The biomaterial to be used for this purpose must not interfere with the process of periodontal regeneration and ideally it may also promote bone regeneration.

The type of bone graft, the volume and configuration of the defects may be important factors which might influence the clinical results. The bone replacement graft provides for regeneration through conductive or inductive processes and growth factors by inductive or cell-stimulating mechanisms. The conductive graft acts as a scaffold to support new tissue growth and can be replaced by the host tissue. The inductive process

involves the graft or growth factor stimulating the host tissues to regenerate lost tissues.[32] While this materials intended to promote bone formation may play an important role in periodontal regeneration, their combination with other agents capable of enhancing other relevant cell mediated phenomena in periodontal wound healing (enamel matrix derivatives, growth factors, differentiation factors of the platelet-rich plasma), have the potential to optimize the outcome of periodontal regeneration.

Combining osseous grafting with the wound healing promoted by enamel matrix proteins has the potential to result in a synergistic effect of both materials. This assumption is based on the fact that two distinct wound-healing principles may be applied together in one clinical situation. While a graft material has the ability to exert osteoconductive and/or osteoinductive effects besides maintaining defect space, EMPs can work at the periodontal ligament level to promote the formation of new cementum and the development of a new functional attachment unit.[33]

Although the use of a barrier membrane enhances our ability to regenerate the periodontium, its efficacy is limited to certain periodontal defects. Periodontal regeneration is unpredictable in circumferential, one- or two-wall intraosseous defects and in advanced furcation defects. This past decade, research has focused on two main approaches involving the use of biological mediators to selectively enhance cellular repopulation of the periodontal wound. The first approach involves the use of protein preparations and growth factors to regenerate tissues through the principle of biomimicry. Biomimetics is the science of mimicking natural processes or tissues, with the expectation that the regeneration cascade will proceed spontaneously. Enamel matrix derivative (EMD), platelet-rich plasma (PRP) preparation-fibrin glue, and growth factors such as platelet-derived growth factor (PDGF) purportedly function in this fashion. The second approach involves the use of growth and differentiation factors to enhance periodontal regeneration. Several of these growth factors and derivatives are present in bone and teeth and they have been shown to have in vitro effects on various types of cells within periodontium.[34]

2. Objectives and studies

2.1. Aims

General and main objectives of the present clinical investigations are the evaluation of the effect on healing of new combination techniques using different materials and adjuvants in regenerative surgery of intrabony periodontal defects. (Table 1.)
Combination of several types of membranes, bone substitutes, growth factors and other modalities like enamel matrix derivatives, root conditioning materials used in various combinations has been studied within the confines of regenerative periodontal surgery with diverse and dissimilar results and conclusions.
The questions examined in the present studies are the following:

1. a. Whether a synthetic graft material (a ß-TCP) with positive maxillo-facial reference is applicable in periodontal regenerative surgery;
   b. Are the clinical outcomes more favourable as in the same method with a natural bone mineral (NBM); (Study I.)
2. If important growth factors-containing autologous platelet-rich plasma enhances the clinical regenerative effect of natural bone mineral and guided tissue regeneration with a non-resorbable membrane (e-PTFE); (Study II.)
3. If platelet-rich plasma enhances the regenerative effect of natural bone bone mineral (NBM) and guided tissue regeneration with a resorbable membrane (collagen); (Study III.)
4. Whether the platelet-rich plasma's growth factors may influence positively the effect on periodontal healing of the synthetic bone graft and a non-resorbable polytetrafluoroethylene membrane; (Study IV.)
5. Is able the platelet-rich plasma (PRP) as a growth factors containing adjuvant to promote the effect of an another protein-mediated regenerative material (EMD) in regenerative surgery of intrabony periodontal defects; (Study V.)
6. Is the platelet-rich plasma capable to enhance the regenerative effect of natural bone mineral as a simple bone substitute material with regenerative potential in periodontal surgery; (Study VI.)

2.2. Studies

Study I.: The purpose of this study was to compare the healing of deep intrabony defects following treatment with an enamel matrix protein derivative (EMD) combined with either a natural bone mineral (NBM) or ß–tricalcium phosphate (ß-TCP).

Study II.: The aim of this study was to clinically evaluate the effect of PRP (platelet-rich plasma) on the healing of deep intrabony defects treated with NBM and GTR by means of a non-resorbable e-PTFE membrane.

Study III.: The aim of this study was to clinically compare treatment outcomes of deep intrabony defects treated either with PRP + NBM + GTR or NBM + GTR using a bioresorbable collage membrane.

Study IV.: The purpose of this study was to clinically evaluate the effect of PRP on the healing of intrabony defects treated with ß-tricalcium phosphate (ß-TCP) and GTR using a non-bioresorbable e-PTFE membrane.

Study V.: The goal of this study was to compare clinically the treatment of deep intrabony defects with either EMD + NBM + PRP or EMD + NBM.

Study VI.: This study explores the capacity of PRP to enhance the regenerative effect of a natural bone mineral.

Table 1. The studies

	Test	Control
Study I.	EMD + NBM	EMD + ß-TCP
Study II.	PRP + NBM + GTR	NBM + GTR
Study III.	PRP + NBM + GTRres.	NBM + GTRres.
Study IV.	PRP + ß-TCP + GTR	ß-TCP + GTR
Study V.	EMD + NBM + PRP	EMD + NBM
Study VI.	PRP + NBM	NBM

3. Background

3.1. General background

3.1.1. Expanded polytetrafluoroethylene membranes

The first devices for isolation, designed for guided tissue regeneration, was the expanded polytetrafluoroethylene (e-PTFE). e-PTFE barriers were used in several animal experiments and clinical studies. At the coronal border the membrane has a collar with an open microstructure portion which allows the ingrowth of connective tissue, preventing the apical migration of epithelium. The other part of barrier is occlusive in order to prevent the gingival tissues outside the barrier from interfering with the healing process at the root surface.[35]

The microstructure of expanded polytetrafluoroethylene can be adjusted to permit cellular infiltration and collagen penetration. The process produces a microstructure consisting of solid nodes interconnected by fine, highly-oriented fibrils. The inhibition of apical migration of gingival epithelium in early phase of healing by the open microstructure portion of barrier occurs through a phenomenon known as contact inhibition. The epithelium recognizes the tissue attached to the open microstructure as non-foreign and does not quickly migrate beyond it. The partially occlusive portion of membrane serves as barrier between the gingival connective tissue and the root surface. The space created over the defect allows cells from the remaining periodontal ligament to selectively repopulate the root surface. The occlusive portion of device allows also the incorporation by surrounding tissues and may retard the apical migration of epithelium during the later phase of periodontal healing.[36]

Factors, which may account for differences in the success of regenerative therapy may include the dimensions and morphology of osseous lesions, differences in dental plaque control and gingival inflammation, histological factors and the extent of bacterial contamination. Machtei et al.[37] have shown that histological examination of the inner surface of retrieved polytetrafluoroethylene membranes often demonstrated enlarged fibroblasts and associated matrix components. The number of fibroblasts on the inner surface of the membranes correlated with reduction of defect dimensions. This finding

suggests that histological analysis of retrieved membranes might be an useful predictor of future regenerative changes in the furcation defects. Other study shows no correlation between connective tissue structures and any of the outcome variables and suggests that large portions of the membrane surface free of deposits were previously occupied by connective tissue structures which have been detached during the reentry procedure, so the „membrane integration score" could underestimate the extent of connective tissue infiltration.[38]

Zucchelli et al.[39] demonstrated the importance of the integration of connective tissue in the outer surface of the barrier membrane on the clinical outcome of GTR surgical procedures. The amount of integrated connective tissue on the external layer of the resorbable barrier material was positively correlated with the amount of attachment gain and negatively with the increase of recession. The results of the study indicate that the process of connective tissue integration could be an important factor in preventing the the occurence of gingival recession and plaque colonization over barrier material. Connective tissue integration on the membrane material seems to depend on the biocompatibility and structure characteristics of the membrane. Factors related to the surgical technique may affect too the connective tissue and membrane integration: the correct positioning of the barrier, membrane stability during the healing, precise flap adaptation insures maximal contact area between the flap and barrier available for connective tissue integration.

Selvig et al.[40] reported fibroblast-like cells as predominant cell type observed in mid-portion of the investigated e-PTFE membrane surfaces, in one specimen structures which were interpreted as blood vessels, fibrous structures suggestive of collegen fibres, but distinction between collagen structures and fibrin was not possible at all specimen. Inflammatory cells were seen associated with connective tissue structures as well as with bacterial deposits. There did not seem to be a systematic difference in the nature and distribution of the adherent structures on the inner and outer surfaces of the membrane.

3.1.2. Collagen membranes

Due to the need for a second surgery to retrieve non-absorbable membranes, a demand for bioabsorbable membranes at least with comparable clinical outcomes became apparent.

There are basically three types of bioresorbable membranes: polyglycoside synthetic polymers (polylactic acid, polylactate/polygalactide copolymers), collagen and calcium sulphate.

Collagen membranes are of porcine and bovine origin and consist of either type I collagen or a combination of type I and type III collagen. Collagen membranes are degraded by collagenases and subsequently by gelatinases and peptidase.

In a one-year GTR study comparing the use of bioresorbable membranes, e-PTFE membranes or surgical debridement alone, significant gains in clinical attachment level were observed in all three groups.[41] There was no difference in clinical attachment level gain between the two membrane groups, which was significantly better than the surgical debridement control group. These findings which indicate that GTR procedures are equally effective using resorbable and nonresorbable membranes has been confirmed by other investigators.[42-44]

Since a non-absorbable membrane is required to stay in place for 4 to 6 weeks before being surgically removed, one may question the effectiveness of the collagen membrane in enhancing regeneration due to its relatively fast degradation rate. It was demonstrated that the period of time in which a collagen membrane remains intact is sufficient for preventing apical migration of epithelium during early periodontal wound healing, since the critical time for epithelial proliferation occurs within the first 14 days.[45,46]

The benefits of utilizing collagen barriers include promoting wound healing through clot stabilization, wound stability and haemostasis. They have been proven to significantly enhance periodontal regeneration in various animal studies and human clinical trials, however none of these studies has shown a complete regeneration.[47]

3.1.3. Enamel matrix derivatives

Acellular cementum is the most important tissue for the insertion of collagen fibres and plays thereby the largest role in attaching the tooth to the alveolar socket.[14] Studies of Slavkin and Boyde[48] and Slavkin[49] have shown that proteins, which are secreted during the tooth development by the Hertwig's root sheath, play a crucial role in the formation of acellular root cementum. These proteins referred to as enamel matrix proteins constitute the largest part of the enamel matrix.[14,50] They consist of a whole family of proteins, from which 90 % are amelogenins, and the remaining 10% consist of prolin-rich non-amelogenins, tuftelin, and other serum proteins.[50] It has been shown that the chemical structure of amelogenin remained more or less constant during evolution, even among the individual animal species, exhibiting only slight differences.[50] In a series of animal experiments on root development in rats, monkeys and pigs, it was immunohistologically demonstrated that the concentration of amelogenin rises dramatically during tooth development.[14] In addition a close connection between acellular cementum and amelogenin exists. These results have also been confirmed in investigations of human teeth, whereby some histological sections showed a thin layer of highly-mineralized enamel between dentin and root cement. This observation permits the assumption that the attachment of enamel matrix must occur on the dentin surface before the emergence of acellular cementum.[14] On the basis of animal studies the enamel matrix derivative (EMD) from the tooth pouches of not erupted teeth from young pigs were isolated, purified and lyophylisated. Since EMD are extreme hydrophobic, they were brought by means of a propylene glycol alginate (PGA) carrier into soluble form before their use in regenerative periodontal therapy.[51] A recent study has identified enamel matrix proteins and proteolytic enzymes present in EMD and compared them with those extracted from developing porcine enamel itself.[52] The results have shown that while developing enamel contained amelogenins, albumin, amelin, and enamelin, EMD contained only amelogenins. Thus, at the time being it may be assumed that the main component of EMD are amelogenins.[50-52]

Most data from controlled clinical studies indicate that the additional application of EMD in the context of surgical therapy of deep intrabony periodontal defects may lead to significantly higher gains of clinical attachment and defect fill compared to open flap debridement.[13,53-62] Comparative studies reported similar results after treatment of

intrabony defects with EMD or GTR, whereby the type of the GTR barrier (non-bioabsorbable or bioabsorbable) did not play a role.[54,58-61,63,64] The clinical results are comparable to those after GTR therapy. Experimental and clinical studies have indicated that the extent of the regeneration is determined by the available space under the mucoperiostal flap.[65,66] A collapse of the mucoperiostal flap may limit the area needed for the regeneration process and may thus affect the result of the therapy. In order to avoid these disadvantages, combination therapies between EMD and GTR and/or EMD and bone substitutes were tested. Observations from animal-histological and human-histological studies have demonstrated periodontal regeneration after treatment of intrabony defects with some of these combinations. In a prospective, controlled, clinical study the treatment of intrabony defects was evaluated following treatment with EMD, GTR, combination of EMD + GTR and open flap surgery.[59] The results have shown that all 3 regenerative procedures resulted in a significantly higher improvement of the clinical parameters compared to the conventional flap surgery; whereby the combination of EMD + GTR led to no additional improvement. Comparable results were also reported by others.[67,68]

Several studies have evaluated the effect of a combination of EMD and various types of bone grafts in the treatment of intrabony defects. Data from human histologic studies indicate that a combination of EMD and a natural bone mineral or bioactive glass may indeed result in formation of root cementum, periodontal ligament and mineralization around the graft particles.[69,70] On the other hand, the application of a natural bone mineral alone, resulted also in periodontal regeneration.[69] Data from controled clinical studies comparing treatment of intrabony defects with EMD alone or a combination of EMD and different types of bone substitutes seem to indicate that the combination of EMD and DFDBA or a natural bone mineral may additionally enhance the hard and soft tissue parameters compared to treatment with EMD.[71-74] Furthermore, clinical studies comparing treatment with a combination of EMD and a bone graft to bone graft alone did not demonstrate any advantage of the combination approach.[75-77] Thus, it may be speculated that the type of the bone substitute and the volume and configuration of the defects are also important factors which might influence the clinical results.

3.1.4. Growth factors and platelet-rich plasma

The expression of various growth and differentiation factors (GDFs) following bone and soft tissue injury during periodontal disease may regulate the repair and regenerative process. The objective for GDF administration in the treatment of periodontitis is to enhance the normal wound healing response, which may be of insufficient magnitude to promote the optimal regeneration of all attachment structures.[78]

The strategy is to amplify and accelerate the effects of growth factors contained in platelets, which are the universal initiators of almost all wound healing. In the organizing stage of a clot, growth factors play a major role in healing and osseous regeneration phenomena.[79] Autologous platelet-rich plasma (PRP) is nontoxic and non-immunoreactive, accelerates existing wound-healing pathways. PRP also can modulate and upregulate any growth factor's function in the presence of a second or third growth factor. This specific feature separates growth factors of PRP origin from recombinant growth factors, which are single growth factors that focus only on a single regeneration pathway.[80]

Platelet-rich plasma (PRP) is an autologous source of platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-beta/TGF-ß), that is obtained by sequestering and concentrating platelets by gradient density centrifugation.

The platelet-rich plasma (PRP) was introduced in the beginning of 1960's.[81,82] The aim of application was the management of thrombocytopenia in transfusion medicine. Later the use of PRP had become a common method for anticoagulation in cardiovascular-, neural-, orthopaedic- and general surgery leading to a better postoperative outcome.[80] Nowadays the PRP is widespreadly used to modify autologous fibrin sealants by adding it to boost the intrinsic characteristics of fibrin glues (through the addition of these platelet growth factors).[83]

In 1998, Marx et al. proposed the local application of autologous PRP to enhance the maturation of bone grafts.[80,84] The rationale for the local application of PRP in bone surgery is the release of growth factors present in the platelet. They include platelet-derived growth factor (PDGF), transforming growth factor-ß (TGF-ß), insulin-like growth factor-I (IGF-I) which are involved in reparative processes including osteogenesis.[85,86] There is local release of different growth factors from the α- and dens granules of platelets after activation. These growth factors activate the osteoblasts,

chondroblasts, fibroblasts and osteoprogenitor cells. They increase the extracellular matrix formation and haemopoesis.

PDGF seems to have numerous positive effects on wound healing, including mitogenesis, angiogenesis and up-regulation of other growth factors and cells. PDGF is the primary growth factor in platelets. It is the first growth factor in the wound and leads toward revascularization, collagen synthesis and bone regeneration.[87,88] TGF-ß represents a growth factor mechanism that not only can initiate bone regeneration but also can sustain long-term healing, bone remodelling of a maturing bone graft.[89]

Some studies investigate the capacity of platelets to initiate angiogenesis and collagen synthesis in animal experiments. Thrombin-released platelets produced angiogenesis, collagen synthesis was elevated.[90]

In vivo studies over many years have demonstrated that growth factors stimulate bone formation and bone healing due to their potent effects on bone cell metabolism.[91] In vivo several growth factors exhibited chemotactic effects towards human osteoblasts. PDGF demonstrates potent chemotactic as well as mitogenic effects on periodontal ligament (PDL) fibroblastic cells.[92]

It is proved that growth factors added to bone grafts and synthetic bone substitutes lead to better results in bone regeneration shortening the healing period.[93-98] Animal studies on local application of growth factors in orthopaedic, craniofacial, and periodontal surgery[99-102] have demonstrated that the effect depends on the dose.

The origin of growth factor applied to activate the healing processes can be recombinantly produced[103] or released from the added PRP. The advantages of PRP origin growth factors: cheap, autologous, contains every factor which have got role in wound healing. The disadvantages: difficult to control the effects of it and there can be technical problems with the PRP preparation. The advantages of recombinantly produced growth factors: clean, separated factor production, disadvantage: expensive and there is no physiologic interaction between the applied factors.

There are more methods to produce PRP. The best way is the plasmapheresis which produce huge amount of thrombocyte concentrate (versatile centrifugation method).[104]

The most simple „chairside" method is 2-step centrifugation procedure with venous blood.

3.1.5. Bone grafts

Although periodontal regeneration may be attempted with various graft materials used alone or with membranes only, combination of the two may also be indicated. Bone grafts and bone substitutes used in regenerative therapy are derived from bone or nonosseous materials.

Bio-Oss® is one of the most investigated graft materials, a natural bone mineral (NBM) made from bovine bone via a proprietary extraction procedure. The resultant bone mineral matrix has been reported to be highly similar to the mineral matrix of human bone[105]. The material is used mainly to fill bone defects in periodontal and maxillofacial surgery. Bio-Oss® by its trabecular architecture (pore size 300-1500 μm) and high porosity (70-75%)[106], promotes the invasion of blood vessels and bone cells[107] and thereby induces revitalization and ossification of the defect. This structure acts as a guiding scaffold for the formation of new bone. Osteoblasts form a layer on the Bio-Oss® mesh and osteoid and finally lamellar bone covers the materials trabeculae and thereby thickens the scaffold structure.[107]

The ability of this natural bone mineral (NBM) to enhance bone regeneration has been evaluated in several animal and human clinical studies. Some of the studies use the expression of bovine porous bone mineral (BPBM) or anorganic bovine bone mineral (ABBM).

The use of Bio-Oss® alone and in conjunction with GTR has been compared histologically. The results presented in this study, clearly demonstrate that the porous bone mineral matrix of the Bio-Oss® has the capacity to stimulate new bone and cementum formation and that this capacity is increased when the natural bone mineral was used in combination with a collagen membrane.[108]

Cerasorb® is a less investigated and applied synthetic graft material in the territory of periodontology, a beta-tricalcium phosphate (ß-TCP) used more in maxillofacial surgery before. Animal studies investigating the characteristics of alpha- and beta-tricalcium phosphate degradation, reported 80-90% of alpha-TCP granules and nearly 90-95% of beta-TCP granules resorbtion, respectively 95-97% of both type of TCP granules degradation after 86 weeks. Residual ceramic can be found within the newly formed trabeculae, which show a functional orientation. The ß-TCP shows an

accelerated degradation mode and has an optimal reactivity with the surrounding tissues.[109,110]

Studies have shown, both grafting materials (Bio-Oss®, Cerasorb®) are able to carry the platelet-rich plasma in PRP-related regenerative surgery of intrabony defects. In the combination of pure-phase ß-TCP and platelet-rich plasma, the use of non-resorbable membranes and suture materials are recommended.[111] The growth factors from platelet-rich plasma have chemotactic effect on macrophages and granulocytes which are responsible for the degradation of resorbable materials too. An excess of this cells in wound can sustain an inflammation which leads to early resorption of the beta-tricalcium phosphate.[112]

Combination of graft materials with enamel matrix derivatives may also be indicated[71,77], not only with membranes. In both cases, the main reason for the combination of methods could be the defect-morphology.

3.2. Background of the studies

Regenerative periodontal therapy aims to reform tooth's supporting tissues (i.e. root cementum, periodontal ligament and alveolar bone) which have been lost following periodontitis or trauma.[113]

3.2.1. Study I.

The use of enamel matrix proteins (EMD) for conditioning the detached root surfaces has been introduced as a biological modality for achieving periodontal regeneration.[14] Results from in vitro studies have shown that EMD modulates behavior of a variety of dental and non-dental cell types in different ways: it upregulates cAMP levels and induces the synthesis and secretion of TGF-ß and IL-6 in cultured periodontal ligament cells and gingival fibroblasts, modulates matrix synthesis, stimulates proliferation of periodontal ligament fibroblasts and of pre-osteoblasts and differentiation of immature osteoblasts.[114-120] EMD acts also as a cytostatic agent on cultured epithelial cells and thus, it may influence periodontal wound healing by inhibiting or at least slowing down

epithelial regrowth.[121] Treatment of different types of periodontal defects resulted both in animals and humans, in the formation of a new periodontal ligament, of new cementum with perpendicularly oriented collagen fibers and of new alveolar bone.[19,122-128] In intrabony periodontal defects, open flap debridement (OFD) followed by the additional application of EMD may lead to significantly higher CAL gain and defect fill compared to OFD alone.[13,53-55,57-59] On the other hand, due to its semi-fluid consistency, EMD possesses a limited space-making potential which in turn, may comport the risk of a flap collapse following its application.[125]

Clinical data have indicated that a combination of EMD to a bone substitute may help to overcome this shortcoming, especially in deep intrabony defects.[71-73,129] It was suggested that in this way, epithelial downgrowth may be inhibited and, at the same time, an indirect release of growth factors achieved. The use of a bone substitute may in turn, prevent a collapse of the flap, thus enhancing wound stability and providing space for the regeneration process.[65] One very well documented bone substitute is a bovine derived natural bone mineral (NBM).[30,69,108,130-136] The material is clinically well tolerated and since all organic components are removed, it does not elicit any allergic reactions.[30,69,108,130-136] It bears a close resemblance to human cancellous bone, shows an excellent osteoconductivity and is very well integrated into bone tissue.[69,108,131,132,136] Findings from human histological studies have shown that filling of intrabony defects with NBM alone may result in periodontal regeneration.[69,108] Results from controlled clinical studies have indicated that in intrabony defects, regenerative periodontal surgery using the combination of EMD and NBM may result in higher hard tissue fill than treatment with EMD alone.[71-73] Observations from human histologic case reports have provided clear evidence of cementum, periodontal ligament and bone formation following treatment with EMD + NBM.[69] It should however be pointed out, that NBM has a very slow resorption rate and thus, the material remains for an unknown time period in the defects. [108,131,132,136] For this reason, it would be of interest to combine EMD to a bone substitute which is completely resorbed and integrated into bone tissue. One such material, widely employed in dental and periodontal surgery is ß-tricalcium phosphate (ß-TCP) a biocompatible, osteointegrative and osteoconductive material which is fully resorbable.[137] The material is well tolerated and until now, no adverse effects such as allergic reactions have been reported.[137-144] The histological analysis

following filling of bone defects with this ß-TCP has also provided evidence of bone formation around the particles, thus pointing to the good biocompatibility and osteoconductivity properties of the material.[138] Although in periodontal defects ß-TCP did not show predictable regeneration of root cementum and periodontal ligament, results from human clinical studies have indicated that treatment of intrabony defects with ß-TCP may result in significant probing depth reductions and clinical attachment level gains.[139-145] Thus, based of the available data, it may be of both biological and clinical interest, to evaluate the combination of EMD and ß-TCP as a treatment option for periodontal defects. In this way, ß-TCP may serve as a fully resorbable placeholder, in the same time allowing EMD to enhance periodontal regeneration. However, according to the best of our knowledge, at the time being there are no data from clinical studies evaluating the healing of intrabony periodontal defects following treatment with EMD + ß-TCP.

Therefore, the aim of the present controlled clinical study was to evaluate the healing of deep intrabony defects following treatment with EMD + ß-TCP and to compare this treatment approach to that of EMD + NBM.

3.2.2. Studies II. and III.

Human histologic data have confirmed that periodontal surgical therapy involving defect fill with either a natural bone mineral (NBM) in combination with an enamel - matrix protein derivative or a collagen membrane may promote a regenerative type of healing characterized by formation of cementum, periodontal ligament and bone.[69,108,132,136] Controlled clinical trials have demonstrated that treatment of intrabony defects with a combination of NBM and collagen membrane may lead to significantly higher CAL gains and osseous fill than access flap surgery alone.[133-135,146]

The application of polypeptide growth factors have been shown to influence the proliferation and differentiation of cells that involved in periodontal wound healing.[147-152] Histologic results from animal studies have demonstrated periodontal regeneration following short-term application of polypeptide growth factors like platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF).[148-152] Data from controlled clinical studies have indicated that regenerative periodontal surgery and application of a

combination of recombinant human (rh) platelet-derived growth factor-BB (PDGF-BB) and rh insulin-like growth factor-I (IGF-I) or rhPDGF-BB on a ß-tricalcium phosphate (TCP) vehicle resulted in significantly higher improvements in terms of defect fill and gain of clinical attachment (CAL) compared with controls (i.e. application of the vehicle alone.[153,154] Platelet-rich plasma (PRP), which is an autologous volume of plasma with a four- to fivefold-increased platelet concentration above baseline, has been shown to contain several growth factors.[80] The application of PRP may enhance wound healing and shorten the healing period.[155] PRP has also been proposed to increase the rate of bone deposition and bone volume in combination with bone grafts during bone augmentation procedures.[156] It has also been suggested that the application of the PRP in dentistry may have successful results and beneficial outcomes in the tissue regeneration, bone repair and general wound healing.[155] The positive effect of PRP on bone healing could be attributed to the angiogenetic, proliferative and differentiating effects on osteoblasts of the high concentrations of TGF-ß and PDGF contained in PRP.[100] A beneficial effect of PRP on bone formation following bone augmentation procedures has already been reported in several clinical trials.[79,80,157,158] Recently, a combination of PRP with different types of grafting materials and barrier membranes has also been used in regenerative periodontal therapy.[159-164] Data from a case report series have suggested that treatment of deep intrabony periodontal defects with PRP combined with a bone allograft and GTR may result in significant clinical improvements compared to baseline values.[159] Findings from controlled clinical studies comparing treatment of deep intrabony defects with a combination of an anorganic bovine bone mineral (NBM), PRP and GTR with either open flap debridement (OFD), GTR or NBM alone have shown significantly higher CAL gains and defect fill following the combination approach.[160,161,163] However, to our knowledge, there is no evidence to evaluate whether a combination of PRP, NBM and GTR may additionally enhance the clinical outcomes compared to treatment with NBM and GTR.

Therefore, the present controlled clinical studies aimed to investigate the possible additional effect of PRP on the treatment of deep intrabony defects comparing the use of PRP + NBM + GTR to NBM + GTR, using a non-resorbable membrane (Study II.), and a resorbable barrier (Study III.) respectively.

3.2.3. Study IV.

Findings from animal studies have provided histologic evidence of periodontal regeneration following short-term application of polypeptide growth factors like platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF).[148-152,165] Platelet-rich plasma (PRP), an autologous volume of plasma with a high platelet concentration, has been shown to contain a high concentration of several growth factors.[80] In the last years, the effectivness of PRP in combination with different types of grafting materials with or without barrier membranes (GTR) has been evaluated in regenerative periodontal therapy demonstrating statistically significant clinical improvements compared to baseline values.[159-164,166-169] However, at the time being, the reported data on the beneficial effect of PRP are still conflicting. Studies comparing treatment of intrabony defects with PRP combined with different types of bone substitutes, with or without GTR to either open flap debridement (OFD), GTR or bone substitute alone have shown significantly higher CAL gains and defect fill following the combination approach.[160-164] On the other hand, very recent controled clinical studies have failed to show statistically significant differences when comparing treatment with PRP, bone substitutes and GTR to bone substitutes and GTR.[166-169] In most studies the used graft material was either an allograft, an anorganic bovine bone mineral (ABBM) or porous hydroxylapatite.[159-164,166,167] Another material, widely employed in dental and periodontal surgery is ß-tricalcium phosphate (ß-TCP) a purified, microcrystaline porous form of calcium phosphate with a Ca/PO4 ratio similar to natural bone. Histological studies in animals and humans have demonstrating that ß-TCP is biocompatible, may be incorporated into host bone, remodelled and eventually replaced by the host bone.[109,110,137,138,170,171] ß-TCP is well tolerated and until now, no adverse effects such as allergic reactions have been reported.[109,110,137-144,170-172] Therefore, it is of biological and clinical interest to use ß-TCP as bone substitute in conjunction with PRP. It might be hypothesized that ß-TCP may resorb, thus allowing the newly formed periodontal tissues to fill the available space. Histologic findings from animal studies have provided evidence that space-provision has a significant effect on bone regeneration following GTR.[173,174] This implies that from a biological point of view another reason for using a graft biomaterial such as ß-TCP in conjunction with GTR is to ensure space-provision by preventing a collapse of the mucoperiosteal flap. Very

recent findings from a controlled clinical study have suggested that treatment of intrabony defects with PRP, ß-TCP and GTR might reduce the occurrence of postoperative membrane exposures and accelerate bone density gain over a short time period (i.e. up to 6 months) although no differences were found at 12 months.[168] Results from another controlled clinical study comparing treatment of intrabony defects with ß-TCP, ß-TCP + PRP and ß-TCP + GTR have failed to show statistically significant differences between the 3 groups.[169] However, the data from controlled clinical studies evaluating the possible additional effect of PRP upon the healing of deep intrabony defects treated with ß-TCP and GTR are still limited.

Therefore, the aim of the present controlled clinical study was to evaluate the effect of PRP on the treatment of deep intrabony defects treated with ß-TCP and GTR.

3.2.4. Study V.

In human intrabony defects, periodontal regeneration has been demonstrated following treatment with an enamel matrix protein derivative (EMD), guided tissue regeneration (GTR), or certain combination modalities such as EMD + a natural bone mineral (NBM) or NBM + GTR.[1,10,69,108,124-128,132,136,175] Recent clinical research has attempted to develop new techniques consisting of minimally invasive surgery or the use various combinations of biologic active factors, bone substitutes/bone grafts with or without barrier membranes to improve additionally the outcome of regenerative therapy.[71-73,160-162,166,168,176-178]

Results from controlled clinical studies have indicated that treatment of deep intrabony defects with a combination of EMD + NBM may lead to higher CAL gains and osseous fill than treatment with EMD alone.[71-73]

Polypeptide growth factors (PGFs) have been shown to play an important role in the growth and differentiation of cells involved in periodontal wound healing.[147-152,165] Platelet-rich plasma (PRP) is an autologous volume of plasma with a 4- to 5-fold increased platelet concentration above baseline, and it is a proven source of growth factors.[80] The positive impact of PRP on bone healing could be attributed to the angiogenetic, proliferative and differentiating effects on osteoblasts of TGF-ß and PDGF that are present in PRP in high concentrations.[100] In the last years, PRP combined

with different types of grafting materials and barrier membranes has also been used in regenerative periodontal therapy.[159-164,166,168,179] It was also suggested that the use of PRP in combination with a bone graft/bone substitute may enhance the clinical management of the graft material and serve also as a membrane barrier.[160-162]

Although the combination EMD + NBM has been shown to result in higher clinical improvements compared to treatment with EMD alone.[71-73] The question arises whether the results may be further improved with the use of PRP. It may be speculated that the EMD application onto the root surface could stimulate migration of periodontal ligament (PDL) fibroblasts and promote formation of cementum with inserting collagen fibres. A subsequent defect fill with a combination of NBM + PRP might lead to an increase of growth factors in the wound area while, in the same time, PRP might act as a barrier membrane inhibiting epithelial cell proliferation and improving wound stability.

However, at the time being, no data from controlled clinical studies are available evaluating the healing of deep intrabony defects following treatment with a combination of EMD + NBM + PRP.

Therefore, the aim of the present prospective, randomized, controlled clinical study was to compare the treatment of deep intrabony defects with EMD + NBM + PRP to EMD + NBM.

3.2.5. Study VI.

Platelet-rich plasma, besides its various applications in the field of medicine, has been used successfully in grafting of the maxillary sinus to accommodate dental implants and in guided bone regeneration procedures. Only a small number of studies have tested the efficacy of a combination of PRP and a bone graft, respectively PRP, guided tissue regeneration and bone substitutes in the treatment of periodontal intrabony defects.

In a human histologic study has been shown that the porous bovine bone mineral has the capacity to stimulate substantial new bone and cementum formation and that this capacity is further increased when the graft is used with a slowly resorbing collagen membrane.[108]

A randomized clinical trial demonstrated, that after six months the addition of a high concentration of autologous platelets to a bovine derived xenograft to treat intrabony defects significantly improved their clinical periodontal response.[163]
Recently another study has shown that the treatment with a combination of PRP and bovine porous bone mineral (NBM) led to a significantly favourable clinical improvement in periodontal intraosseous defects compared to using the xenograft alone. [180]

In contrast, a study which investigated the influence of platelet-rich plasma used as an adjunct to Bio-Oss® for the repair of bone defects adjacent to titanium dental implants has shown that when PRP is used, may be decreased the periimplant bone healing.[181]
The aim of this study to compare the healing of intrabony defects treated with a combination of platelet-rich plasma and a natural bone mineral (NBM) to NBM alone.

4. Materials and methods

4.1. Patient population

All patients were included in the studies after having signed an informed consent. The studies were performed in accordance with the Helsinki Declaration of 1975, as revised in 2000. All patients were treated at the Department of Periodontology, Semmelweis University Budapest, Hungary by the same surgeon (FD).
All patients received initial periodontal therapy consisting of oral hygiene instructions, supra - and subgingival scaling and root planing under local anaesthesia performed 2 to 3 months prior to the beginning of the study. Thus, the data reported at baseline, represent the clinical situation following initial therapy. The patients were consecutively enrolled in the study when following inclusion criteria were met: 1) no systemic diseases which could influence the outcome of the therapy, 2) a good level of oral hygiene (PI<1), 3) compliance with the maintenance program, 4) presence of one intrabony defect with a probing depth of at least 6 mm and an intrabony component of at least 3 mm as detected on the radiographs and measured at bone sounding, 5) no intrabony defects extending into a furcation area, 6) no teeth presenting furcation involvements. The following clinical parameters were assessed one week prior and one

year after the surgical procedure using the same periodontal probe[§]: plaque index (PI), gingival index (GI), bleeding on probing (BOP), (probing) pocket depth (PPD/PD), gingival recession (GR), and clinical attachment level (CAL). The measurements were made at six sites per tooth: mesiovestibular (mv), midvestibular (v), distovestibular (dv), mesiolingual (ml), midlingual (l), distolingual (dl) by a calibrated investigator who was not the same as the surgeon. The examiner was not aware, in any of the cases, of the type of treatment rendered.

The cemento-enamel junction (CEJ) was used as the reference point. In cases where the CEJ was not visible, a restoration margin was used for these measurements. The studies reports only measurements at the same deepest point of the selected defect. If two sites within a defect exhibited the same PD and CAL, it was decided by a toss of coin which site should be included in the analysis.

Pre- and postoperative radiographs were taken with the long cone parallel technique.

The patients were non-smokers or they smoked less than 10 cigarettes/day and were considered non-smokers.

4.2. Intra-examiner reproducibility

Five patients, not enrolled in the study, each showing at least 4 teeth (single and multi rooted) with probing depths ≥ 6 mm on at least one aspect of each tooth, were used to calibrate the examiner. The examiner evaluated the patients on 2 separate occasions, 48 hours apart. Calibration was accepted if more than 90% of the recordings could be reproduced within 1.0 mm difference.

4.3. Randomization

Using a randomized block approach, the defects were randomly assigned before surgery to the 2 treatment groups. Blocking to control for the effects of the prognostic variables, INTRA and CAL was used to decrease outcome variability. INTRA (the distance from the alveolar bone crest to the bottom of the defect) was estimated before surgery based on radiographs and transgingival bone sounding recordings.

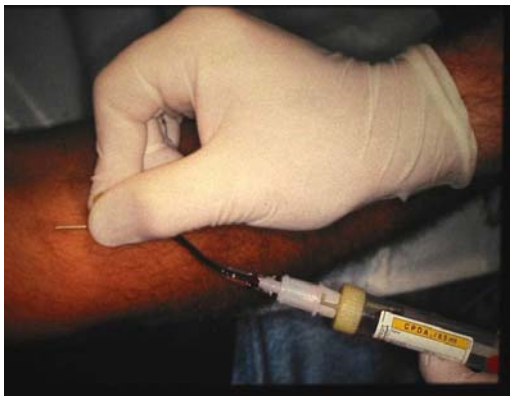
[§]UNC 15, Hu-Friedy, Chicago, IL, USA.

4.4. Platelet-rich plasma preparation (Studies II.-VI.)

The PRP preparation was performed by using a standardized kit immediately prior to operation.[¶] The employed system consists of a standard laboratory centrifuge with eight monovettes, a shaker and a kit with disposable material. One monovette was filled with 8.5 ml solution (7 ml blood and 1 ml citrate-phosphate-dextrose-adenine [CPDA] solution for anticoagulation). The first spin was performed at 2400 r.p.m. for 10 min. This procedure divided the blood into three basic components; red blood cells (RBC), platelet rich plasma (PRP) and platelet poor plasma (PPP). The red blood cell layer formed at the lowest level, the PRP layer in the middle and the PPP layer at the top. PRP and PPP were collected in a second monovette. Then, a second spin was performed at 3600 r.p.m. for 15 min. The platelet pellet concentrated at the bottom of the monovette, whereas the PPP on top. The PPP was removed so that only the PRP pellet remained in the monovette. (Fig. 1.) After re-suspending the platelet pellet within the remaining volume of plasma with the shaker, a 0.4 ml volume of PRP was produced. It was previously demonstrated that PRP volumes prepared with this technique contain a mean platelet count value of 2520 ± 834 x 10^3/µl and high mean concentration values of growth factors (i.e. 295 ng/µl PDGF-AB and 500 ng/µl TGF-ß1).[182]

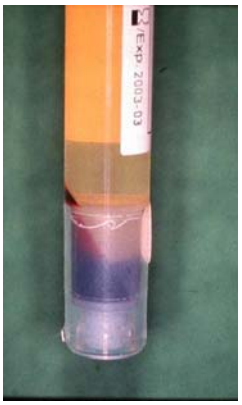
[¶] Curasan PRP kit, Curasan AG, Kleinostheim, Germany.

Fig1. Preparation of the platelet – rich plasma

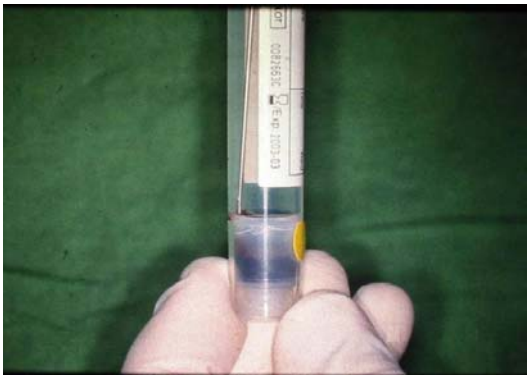


1/a. Drawing up the peripheral blood

1/b. The blood after the first centrifugation



1/c. The blood after the second centrifugation



1/d. The „pellet" and rest of plasma

4.5. Surgical procedures

4.5.1. Study I.

24 patients (16 females and 8 males), (aged from 34 to 67 years) suffering from advanced periodontal disease were included in this parallel design study (i.e. 12 patients in each group). Following local anaesthesia and intracrevicular incisions full thickness mucoperiosteal flaps were raised vestibularly and orally. Vertical releasing incisions were performed if seemed necessary for a better access to the surgical site or to achieve

a better closure. All granulation tissue was removed from the defects and the roots were thoroughly scaled and planed by means of hand and ultrasonic instruments. After defect debridement, in both groups, the root surfaces adjacent to the defects were conditioned for 2 min. with ETDA gel (pH 6.7)[||] to remove smear layer. Subsequently, the defects and the adjacent mucoperiosteal flaps were thoroughly rinsed with sterile saline to remove all EDTA remnants. (valid for studies I. and V.)

During surgery the following measurements were made: distance from the cemento-enamel junction to the bottom of the defect (CEJ-BD), distance from the CEJ to the most coronal extension of the alveolar bone crest (CEJ-BC). The intrabony component (INTRA) of the defects was defined as (CEJ-BD) – (CEJ-BC). (valid for all studies)

Following root conditioning, EMD[†] was applied onto the root surfaces and into the defects with a sterile syringe. The remaining EMD was then mixed with either NBM[#] or ß-TCP[##]. The defects were completely filled with the mixture of either EMD + NBM or EMD + ß-TCP. (Fig. 2.) Finally, the flaps were repositioned coronally and closed with vertical or horizontal mattress sutures.

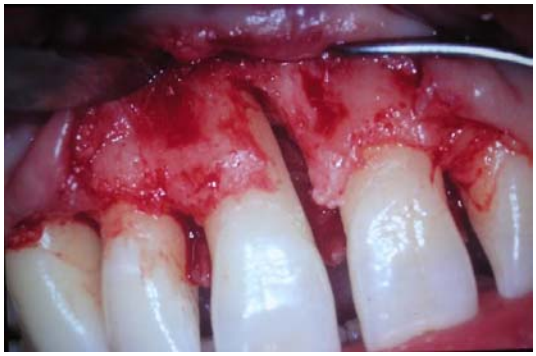
[||]PrefGel™, Straumann, Waldenburg, Switzerland

[†]Emdogain®, Straumann, Waldenburg, Switzerland

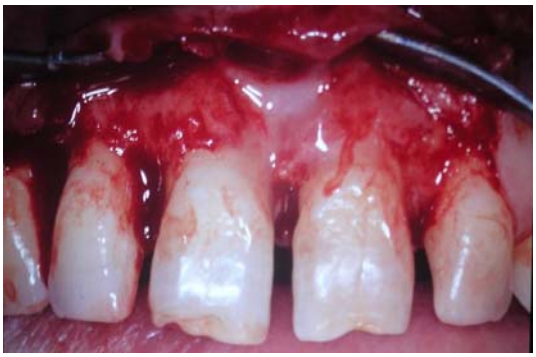
[#]Bio-Oss®, Geistlich, Wolhusen, Switzerland

[##] Cerasorb®, Curasan Pharma, Kleinostheim, Germany

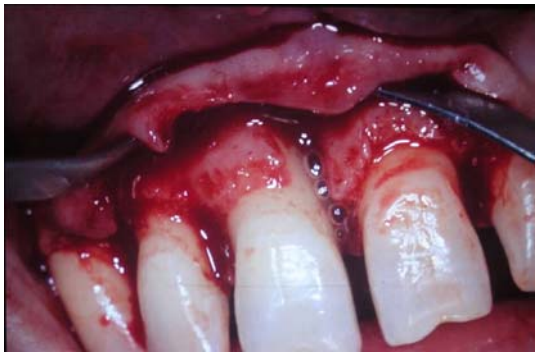
Fig.2.



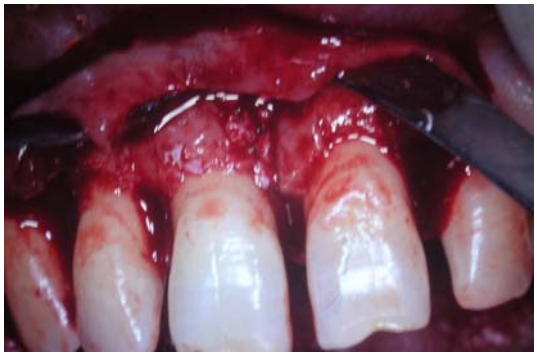
2/a. Deep intrabony defect



2/b. Conditioning with EDTA



2/c. Application of the EMD



2/d. Bio-Oss + EMD in defect

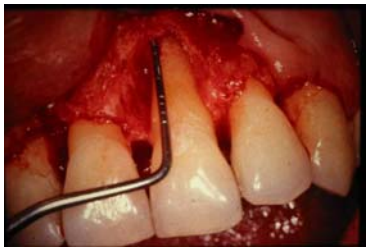
4.5.2. Study II.

Twenty-four patients (14 females and 10 males) with an age ranged from 26 to 55 years, were included in this study. There were no differences in the age distribution between the two groups. The study had a parallel design and 12 patients were recruited in each group (i.e. test and control group). Flap preparation and removal of the granulation tissue: see Study I. For a better access to the surgical site, the flap was in most cases extended 1 or 2 teeth mesially or distally. No conditioning of the root surfaces was performed.

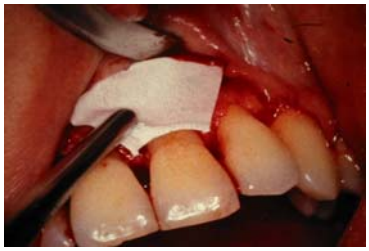
In the test group (PRP + NBM + GTR), at the time of application, PRP was firstly activated after combination with an equal volume of a sterile saline solution containing 10% calcium chloride and 100 U/ml sterile bovine thrombin. Within a few seconds, the

PRP displayed a sticky consistency. (valid for studies II.-VI.) Afterwards, NBM[#] granules (particle size 0.25 to 1.0 mm) were mixed with the coagulated PRP. Care was taken not to overfill the defects. Following grafting, a non-bioresorbable e-PTFE membrane[*] was trimmed and adapted over the entire defect so as to cover 2-3 mm of the surrounding alveolar bone and to ensure stability of the wound and of the graft material. The membranes were fixed to the same and /or neighbouring teeth by means of sling sutures[**]. (Fig. 3.)

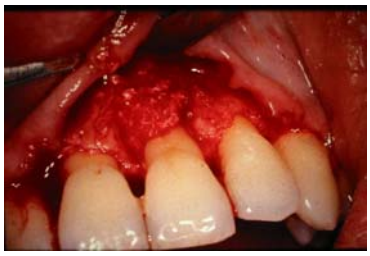
Fig.3.



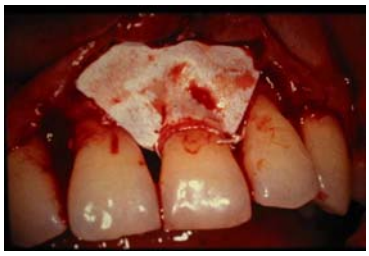
3/a. The defect



3/b. Checking the membrane



3/c. NBM + PRP placed in defect



3/d. Membrane fixed over implanted material

The same surgical protocol was also used for the control sites (NBM + GTR) with the exception of using PRP. Finally, the flaps were repositioned coronally and closed with vertical or horizontal mattress sutures[**]. The non-bioresorbable e-PTFE membranes were removed after 6 weeks. (Fig. 4.)

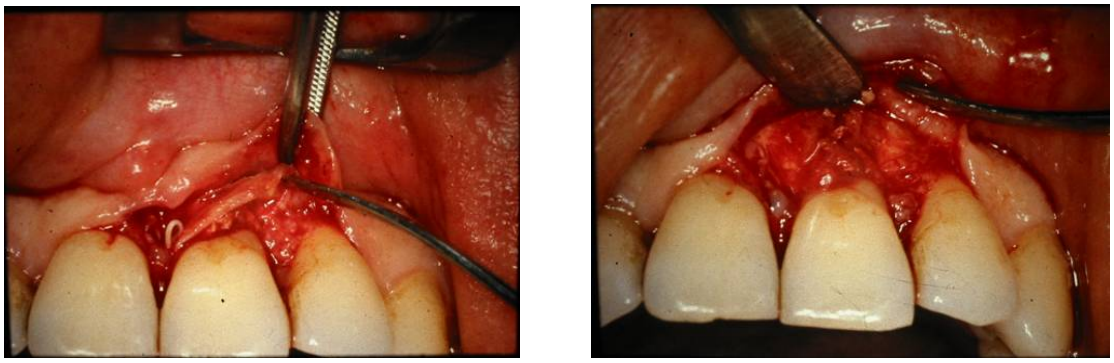
[#] Bio-Oss®, Geistlich AG, Wolhusen, Switzerland.

[*] W.L. Gore & Associates, Inc., Flagstaff, AZ, USA.

[**] 5-0, W.L. Gore & Associates, Inc., Flagstaff, AZ, USA.

[**] 5-0, W.L. Gore & Associates, Inc., Flagstaff, AZ, USA.

Fig.4.



4/a-b. Membrane removal after 6 weeks

4.5.3. Study III.

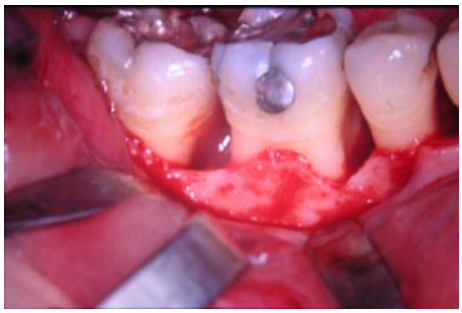
Thirty patients (16 females and 14 males; aged from 28 to 56 years), suffering from advanced periodontal disease, were included in this parallel design study (i.e. 15 patients in each group). Flap preparation and cleaning of the defect and root surface: see Study I. No conditioning of the root surfaces was performed.

Afterwards, the natural bone mineral (NBM) granules (particle size 0.25 – 1.0 mm, Bio-Oss®, were mixed with the coagulated PRP. Care was taken not to overfill the defects. Following grafting, a bioresorbable collagen membrane of porcine origin[‡] was trimmed and adapted over the entire defect so as to cover 2–3 mm of the surrounding alveolar bone and to ensure stability of the wound and of the graft material. No sutures or pins were used for membrane fixation or stabilization. (Fig. 5.) The same surgical protocol was also used for NBM + GTR sites, without using PRP.

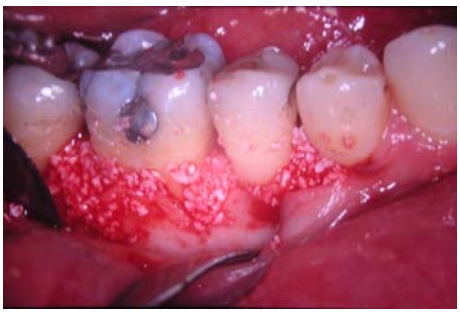
Finally, the flaps were re-positioned coronally and closed with vertical or horizontal mattress sutures.

[‡] Bio-Gide Perio®, Geistlich, Wolhusen, Switzerland

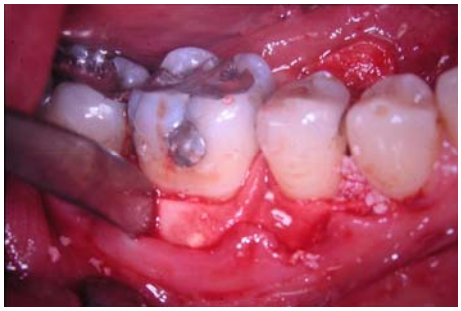
Fig.5.



5/a. Intrabony defects



5/b. Bio-Oss + PRP in defects



5/c. Coverage with the collagen membrane

4.5.4. Study IV.

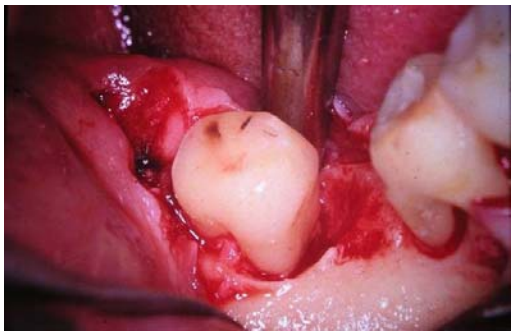
Twenty-eight patients (16 females and 12 males) with an age ranged from 28 to 58 years, were included in this study. There were no differences in the age distribution between the two groups. The study had a parallel design and 14 patients were recruited in each group (i.e. test and control group). Flap preparation and defect cleaning were performed (See: Study I.). The root surfaces were not conditioned. The intrabony component (INTRA) of the defects was defined as (CEJ-BD) – (CEJ-BC). The width of the intrabony defect was measured on the x-rays as distance from the coronal margin of the bony pocket to the root surface perpendicular to the tooth axis.

In the test group (PRP + ß-TCP + GTR), at the time of application, PRP was firstly activated after combination with an equal volume of a sterile saline solution containing 10% calcium chloride and 100 U/ml sterile bovine thrombin. Afterwards, ß-TCP granules (particle size 0.25 to 1.0 mm) were mixed with the coagulated PRP. Care was taken not to overfill the defects. Following grafting, a non-bioresorbable e-PTFE

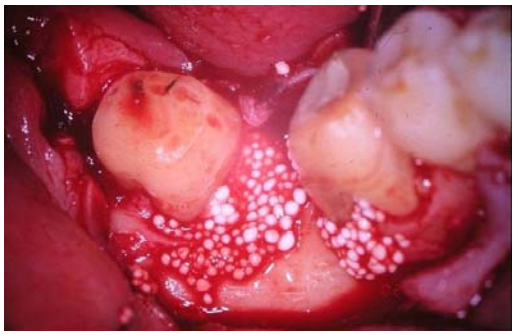
membrane was trimmed and adapted over the entire defect so as to cover 2-3 mm of the surrounding alveolar bone and to ensure stability of the wound and of the graft material. (Fig. 6.)

The membranes were fixed to the same and/or neighbouring teeth by means of sling sutures. The same surgical protocol was also used for the control sites (ß-TCP + GTR) with the exception of using PRP. Finally, the flaps were repositioned coronally and closed with vertical or horizontal mattress sutures. The non-bioresorbable e-PTFE membranes were removed after 6 weeks. (Fig. 7.)

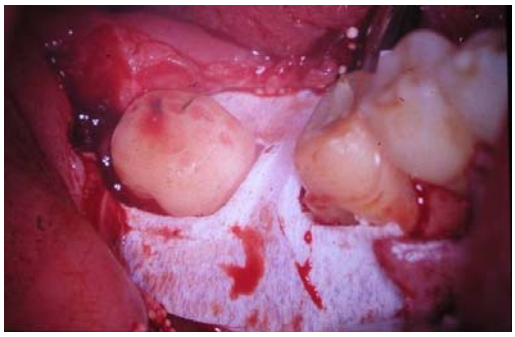
Fig.6.



6/a. The intrabony defect

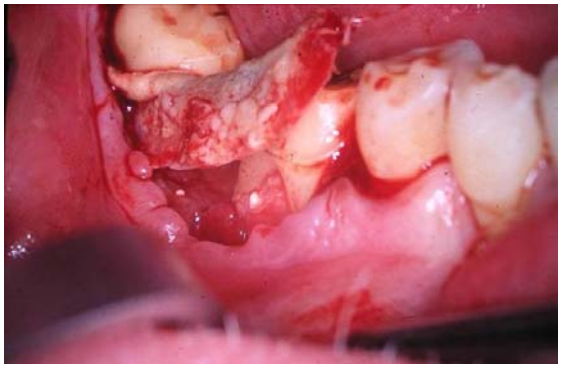


6/b. PRP + beta-TCP in defect

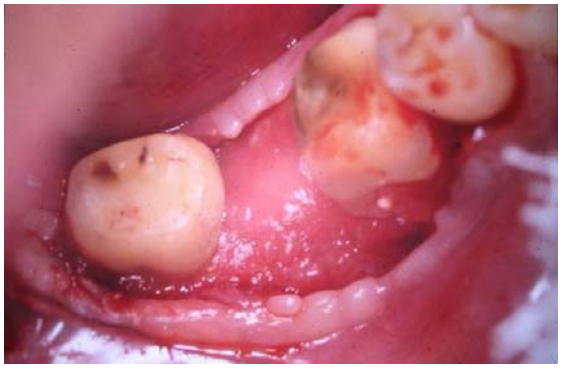


6/c. Coverage with e-PTFE barrier

Fig.7.



7/a. Membrane removal after 6 weeks



7/b. The „re-entry" after 6 weeks

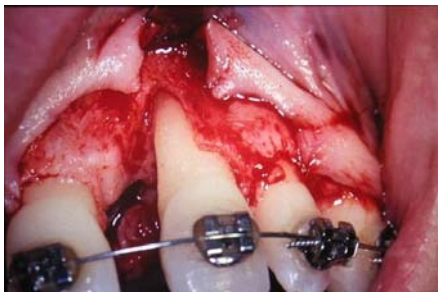
4.5.5. Study V.

26 patients (14 females and 12 males), (aged from 32 to 56 years), were included in this parallel design study (i.e. 13 patients in each group). Following local anaesthesia, intracrevicular incisions were performed extending to the neighbouring teeth. Then, full thickness mucoperiosteal flaps were raised vestibularly and orally. All granulation tissue was removed from the defects and the roots were thoroughly scaled and planed by means of hand and ultrasonic instruments.

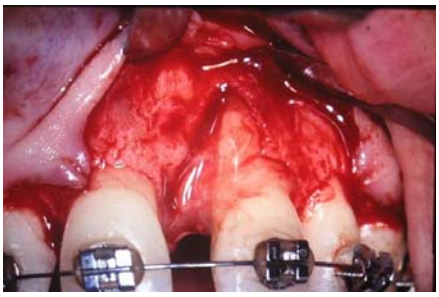
After defect debridement, in both groups the root surfaces adjacent to the defects were conditioned for 2 min. with ETDA gel (pH 6.7) (PrefGel®, previously BIORA, Sweden now Straumann, Switzerland) in order to remove the smear layer. The defects and the adjacent mucoperiosteal flaps were then thoroughly rinsed with sterile saline in order to remove all EDTA residues.

In the EMD + NBM + PRP group, EMD (Emdogain®) was first applied on the root surfaces, immediately followed by defect fill with NBM + PRP. (Fig. 8.)

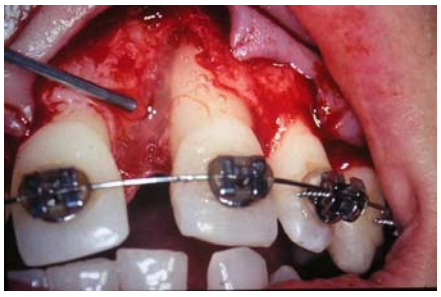
Fig.8.



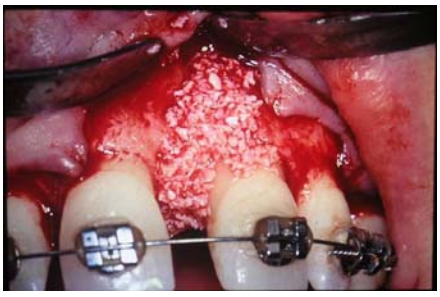
8/a. The defect



8/b. Conditioning with EDTA



8/c. Application of Emdogain



8/d. NBM + PRP in the defect

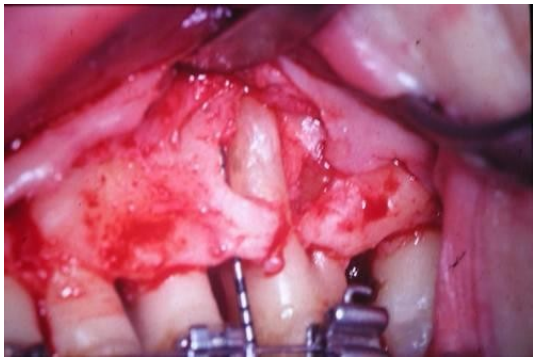
At the time of the application, coagulation of PRP was achieved by its combination with an equal volume of a sterile saline solution containing 10% calcium chloride and 100 U/ml of sterile bovine thrombin. Afterwards, the natural bone mineral (NBM) granules (particle size 0.25 to 1.0 mm, Bio-Oss®, were mixed with the coagulated PRP. In the defects treated with EMD + NBM, EMD was first applied onto the root surfaces and then the defects were filled with NBM. In both groups, care was taken not to overfill the defects. Finally, the flaps were repositioned coronally and closed with vertical or horizontal mattress sutures.

4.5.6. Study VI.

Thirty patients (21 females and 9 males) with an age ranged from 28 to 65 years, were included in this study. There were no differences in the age distribution between the two groups. The study had a parallel design and 15 patients were recruited in each group (i.e. test and control group). Flap preparation, removal of the granulation tissue and cleaning of root surface were performed (see: Study I.). For a better access to the surgical site or to achieve a better closure, the flap was in most cases extended 1 or 2 teeth mesially or/and distally. No conditioning of the root surfaces was performed. During surgery the following measurements were made: distance from the cemento-enamel junction to the bottom of the defect (CEJ-BD), (Fig. 9.), distance from the CEJ to the most coronal extension of the alveolar bone crest (CEJ-BC). The intrabony component (INTRA) of the defects was defined as (CEJ-BD) – (CEJ-BC).

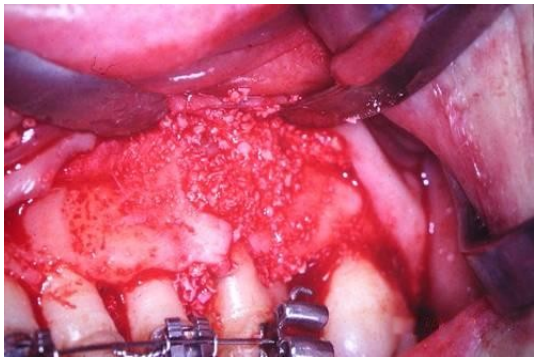
Afterwards, NBM granules (particle size 0.25 to 1.0 mm) were mixed with the coagulated PRP. Care was taken not to overfill the defects. (Fig.10.) The same surgical protocol was also used for the control sites (NBM) with the exception of using PRP. Finally, the flaps were repositioned coronally and closed with vertical or horizontal mattress sutures.

Fig.9.



Registration of the CEJ-BD

Fig10.



Bio-Oss + PRP in the defect

4.6. Postoperative care

All patients received antibiotics for one week (3x 500 mg amoxicillin /day). The postoperative care consisted of 0.2% chlorhexidine rinses twice a day for 4 weeks. Sutures were removed 14 days after the surgery. Recall appointments were scheduled weekly during the first 6 months after surgery and once per month following the rest of the observation period of 1 year. The recall appointments consisted mainly of reinforcement of oral hygiene measures and professional supragingival tooth cleaning.

4.7. Statistical analysis

The statistical analysis was performed using a commercially available software program[§§]. The primary outcome variable was the CAL. In the calculations the deepest site per tooth was included. For the statistical evaluation of the changes from baseline to one year the paired *t*-test was used. For the comparisons between the groups the unpaired *t*-test was used. The alpha error was set at 0.05. The power of the study, given at least 1 mm as a significant difference between the groups, was calculated to be 0.70.

[§§] SPSS®, for Windows, Chicago 2003, IL, USA.

5. Results

5.1. Study I. (EMD + NBM vs. EMD + ß-TCP)

The postoperative healing was uneventful in all cases. No complications such as allergic reactions, abscesses or infections were observed throughout the study period. A reentry procedure was performed in one case treated with ß-TCP and indicated a complete fill of the intrabony component. (Fig. 11.)

There were no differences in the gender distribution between the groups (i.e. 8 females and 4 males in the each of the two groups).

Table 2. illustrates for both groups the mean PI, GI and BOP. GI and BOP improved statistically significantly compared to baseline, but no statistically significant differences were found between the 2 groups.

Table 2.

Mean (±SD) Plaque, Gingival and Bleeding Scores at Baseline and the 1-Year Examination

	EMD + NBM	EMD + ß-TCP
Plaque index scores		
Baseline	0.3 ± 0.2	0.4 ± 0.2
12 months	0.4 ± 0.2	0.4 ± 0.3
Gingival index scores		
Baseline	1.0 ± 0.3	1.1 ± 0.2
12 months	0.5 ± 0.2	0.4 ± 0.3
Bleeding scores		
Baseline	34%	32%
12 months	11%	12%

The distribution and configuration of the defects are shown in Table 3.

Table 3.

Distribution and configuration of treated defects

	EMD + NBM (N= 12)	EMD + ß-TCP (N= 12)
1 – 2 wall	10	9
2 wall	2	3

The depth of the intrabony component as measured during surgery is presented in Table 4. There were no differences in the depth of the intrabony component between the two groups.

Table 4.

Baseline defect characteristics expressed in mm (mean ± SD)

Treatment	PD (mm)	CAL (mm)	CEJ-BD (mm)	CEJ-BC (mm)	INTRA(mm)
EMD + NBM (N=12)	7.9 ± 1.0	8.8 ± 1.1	10.2 ± 1.2	6.1 ± 1.1	4.1 ± 1.0
EMD + ß-TCP (N=12)	7.8 ± 1.2	8.8 ± 1.2	10.1 ± 1.1	6.1 ± 1.2	4.0 ± 0.9

At baseline, the mean PD was 7.9 ± 1.0 mm in the EMD + NBM group and 7.8 ± 1.2 mm in the EMD + ß-TCP group. No statistically significant difference was found. At one year the mean PD was 3.2 ± 0.6 mm in the EMD + NMB group and 3.2 ± 0.9 mm in the EMD + ß-TCP group (Table 5.). Thus, the PD decreased significantly in both groups compared to the baseline data ($p < 0.001$). No significant difference between the groups was found.

Table 5.

Clinical parameters at baseline and 1 year (N = 12 for each group)

	Baseline	1 Year	Difference	Significance
Probing depth				
EMD + NBM	7.9 ± 1.0	3.2 ± 0.6	4.8 ± 0.9	p<0.001
EMD + ß-TCP	7.8 ± 1.2	3.2 ± 0.9	4.6 ± 0.8	p<0.001
			NS	
Clinical attachment level				
EMD + NBM	8.8 ± 1.1	4.5 ± 0.6	4.3 ± 0.8	p<0.001
EMD + ß-TCP	8.8 ± 1.2	4.7 ± 1.2	4.1 ± 0.8	p<0.001
			NS	

At baseline the mean CAL was 8.8 ± 1.1 mm in the EMD + NBM group and 8.8 ± 1.2 mm in the EMD + ß-TCP group. No statistically significant difference was found between the groups. At one year the mean CAL was 4.5 ± 0.6 mm in the EMD + NBM group and 4.7 ± 1.2 mm in the EMD + ß-TCP group (Table 5.). Mean CAL gain was 4.3 ± 0.8 mm in the EMD + NBM group and 4.1 ± 0.8 mm in the EMD + ß-TCP group ($p < 0.001$). In both groups the CAL improved significantly compared to baseline but no statistically significant difference was observed between the two groups.

The frequency distribution of CAL gain for both treatment groups is shown in Table 6.

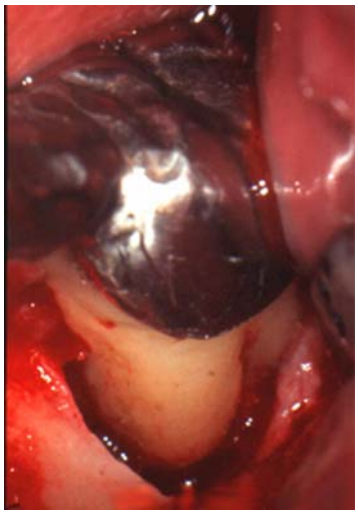
Table 6.

Frequency distribution of CAL gain (N = 12 for each group)

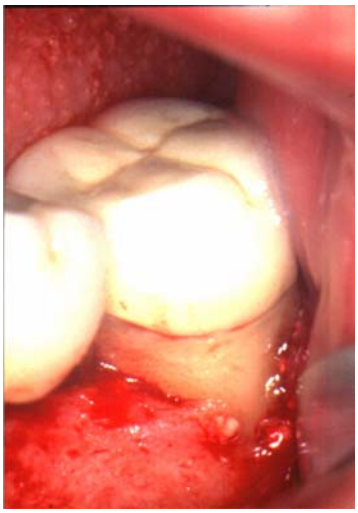
CAL gain (mm)	EMD + NBM		EMD + ß-TCP	
	N°	%	N°	%
3	3	25	3	25
4	2	17	4	33
5	7	58	5	42

In both groups all sites gained at least 3 mm of CAL. CAL gains of 4 or 5 mm were measured in the great majority of the cases (75%), irrespective of treatment modality. (Fig.12.)

Fig.11.

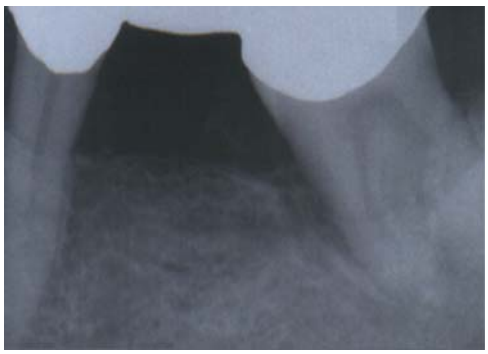


11/a. The defect

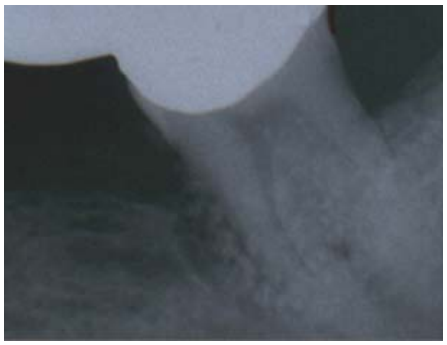


11/b. Re-entry after one year

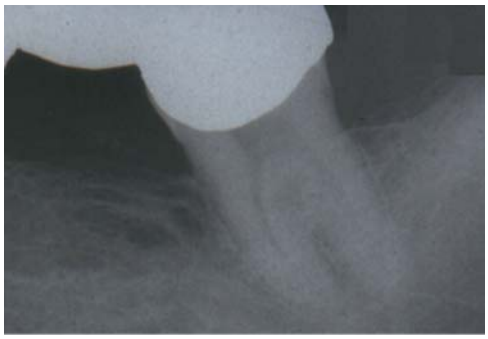
Fig.12. Surgery with ß-TCP + EMD



12/a. Preoperative X-ray picture



12/b. One day control



12/c. One year control

5.2. Study II. (PRP + NBM + GTR vs. NBM + GTR)

The postoperative healing was uneventful in all cases. Minor exposure of the coronal portion of the e-PTFE membrane occurred in the fifth week in 4 cases treated with NBM + PRP + GTR and in 5 cases treated with NBM + GTR. No other complications such as allergic reactions, abscesses or infections were observed throughout the study period. There were no differences in the gender distribution between the groups (i.e. 7 females and 5 males in each of the two groups). Table 7. illustrates for both groups the mean PI, GI and BOP. GI and BOP improved statistically significantly compared to baseline, but no statistically significant differences were found between the 2 groups.

Table 7.

Mean (±SD) Plaque, Gingival and Bleeding Scores at Baseline and the 1-Year Examination

	PRP + NBM + GTR	NBM + GTR	P value
Plaque index scores			
Baseline	0.4 ± 0.2	0.4 ± 0.2	NS
12 months	0.4 ± 0.2	0.5 ± 0.3	NS
P value	NS	NS	
Gingival index scores			
Baseline	0.9 ± 0.4	1.0 ± 0.4	NS
12 months	0.6 ± 0.2	0.5 ± 0.3	NS
P value	$p<0.05$	$p<0.05$	
Bleeding scores			
Baseline	41%	42%	NS
12 months	15%	14%	NS
P value	$p<0.05$	$p<0.05$	

NS = not statistically significant. No significant differences between the PRP + NBM + GTR and NBM + GTR groups were found.

The defects displayed a comparable distribution and configuration in the 2 groups (Table 8.).

The depth of the intrabony component as measured during surgery is presented in Table 9. There were no differences in the depth of the intrabony component between the two groups.

At baseline, the mean PD was 8.6 ± 1.7 mm in the PRP + NBM + GTR group and 8.8 ± 1.7 mm in the NBM + GTR group. No statistically significant difference was found. At one year the mean PD was 3.1 ± 1.3 mm in the PRP + NBM + GTR group and 3.1 ± 1.0 mm in the NBM + GTR group (Table 10.). Thus, the PD decreased significantly in both groups compared to the baseline data ($p < 0.001$). No significant difference between the groups was found.

At baseline the mean GR was 1.8 ± 1.3 mm in the PRP + NBM + GTR group and 1.7 ± 1.1 mm in the NBM + GTR group, with no statistically significant difference (Table 10). At one year the mean GR measured 2.9 ± 1.2 mm in the NBM + PRP + GTR group and 2.8 ± 1.7 mm in the NBM + GTR group. The increase in GR was statistically significant for both groups ($p < 0.01$), but no difference between the groups was observed.

At baseline the mean CAL was 10.3 ± 1.4 mm in the PRP + NBM + GTR group and 10.4 ± 2.6 mm in the NBM + GTR group. No statistically significant difference was found between the groups. At one year the mean CAL was 5.7 ± 1.6 mm in the PRP + NBM + GTR group and 5.9 ± 1.8 mm in the NBM + GTR group (Table 10.). Mean CAL gain was 4.7 ± 1.1 mm in the PRP + NBM + GTR group and 4.6 ± 0.8 mm in the PRP + NBM group. In both groups the CAL improved significantly compared to baseline ($p < 0.001$) but no statistically significant difference was observed between the two groups.

Table 8.

Distribution and configuration of treated defects

	PRP + NBM + GTR (N= 12)	NBM + GTR (N= 12)
Maxilla	5	5
Mandible	7	7
Anterior teeth	4	4
Premolars	3	4
Molars	5	4
1 – 2 wall	10	9
2 wall	2	3

Table 9.

Baseline defect characteristics expressed in mm (mean ± SD)

Treatment	PD(mm)	CAL(mm)	CEJ-BD(mm)	CEJ-BC(mm)	INTRA(mm)
PRP + NBM + GTR (N=12)	8.6 ± 1.7	10.3 ± 1.4	11.5 ± 1.3	6.4 ± 1.1	5.1 ± 1.2
NBM + GTR (N=12)	8.8 ± 1.7	10.4 ± 2.6	11.8 ± 1.2	6.6 ± 1.4	5.2 ± 1.4
P value	NS	NS	NS	NS	NS

NS = not statistically significant. No significant differences between the PRP + NBM + GTR and NBM + GTR groups were found.

Table 10.

Clinical parameters at baseline and 1 year (N = 12 for each group)

	Baseline	1 Year	Difference	P value
Probing depth				
PRP + NBM + GTR	8.6 ± 1.7	3.1 ± 1.7	5.5 ± 1.2	p<0.001
NBM + GTR	8.8 ± 1.7	3.1 ± 1.0	5.7 ± 1.2	p<0.001
P value			NS	
Gingival recession				
PRP + NBM + GTR	1.8 ± 1.3	2.9 ± 1.2	1.2 ± 1.1	p<0.01
NBM + GTR	1.7 ± 1.1	2.8 ± 1.7	1.2 ± 0.9	p<0.01
P value			NS	
Clinical attachment level				
PRP + NBM + GTR	10.3 ± 1.4	5.7 ± 1.6	4.7 ± 1.1	p<0.001
NBM + GTR	10.4 ± 2.6	5.9 ± 1.8	4.6 ± 0.8	p<0.001
P value			NS	

NS = not statistically significant. No significant differences between the PRP + NBM + GTR and NBM + GTR groups were found.

The frequency distribution of CAL gain for both treatment groups is shown in Table 11.

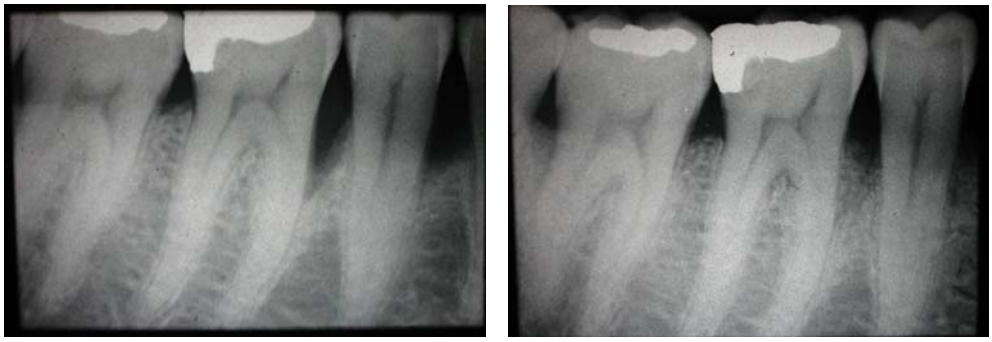
Table 11.

Frequency distribution of CAL gain (N = 12 for each group)

CAL gain (mm)	PRP + NBM + GTR		NBM + GTR	
	N°	%	N°	%
3	2	18	1	9
4	4	32	4	32
5	2	18	6	50
6	4	32	1	9

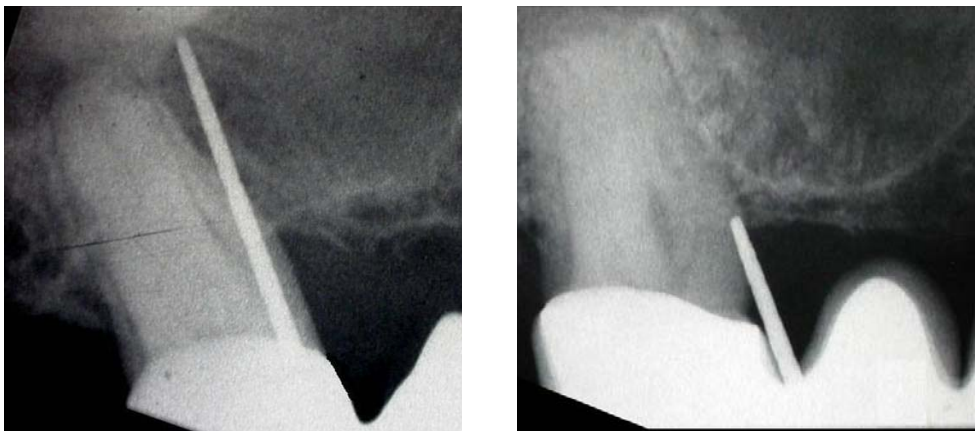
In both groups all sites gained at least 3 mm of CAL. CAL gains of ≥ 4 mm were measured in 83% (i.e. in 10 out of 12 defects) of the cases treated with PRP + NBM + GTR and in 92% (i.e. in 11 out of 12 defects) treated with NBM + GTR. (Fig.13.- 14.)

Fig.13. Surgery with PRP + NBM + GTR



13/a. Preoperative picture **13/b. After 12 months**

Fig.14. Surgery with NBM + GTR



14/a. Preoperative picture **14/b. After 12 months**

5.3. Study III. (PRP + NBM + GTRres vs. NBM + GTRres)

The post-operative healing was uneventful in all cases. No complications such as allergic reactions, abscesses or infections were observed throughout the study period. Membrane exposure occurred at three sites in the PRP + NBM + GTRres group and at four sites in the NBM + GTRres group. The exposed parts of the membranes disintegrated without any side effects. (GTRres means that in this study the used barrier was a resorbable membrane.)

There were no differences in the gender distribution between the groups (i.e. nine females and six males in the PRP + NBM + GTR and 10 females and five males in the NBM + GTR group). Table 12. illustrates for both groups the mean PI, GI and BOP. GI and BOP improved statistically significantly compared with baseline, but no statistically significant differences were found between the two groups.

Table 12.

Mean (± SD) Plaque, Gingival and Bleeding Scores at the treated sites at Baseline and the 1-Year Examination

	PRP+ NBM + GTR (N=15)	NBM + GTR (N=15)
Plaque index scores		
Baseline	0.8 ± 0.3	0.7 ± 0.6
12 months	0.7 ± 0.5	0.8 ± 0.5
Gingival index scores		
Baseline	1.1 ± 0.5	1.2 ± 0.5
12 months	0.5 ± 0.4	0.6 ± 0.4
Bleeding scores		
Baseline	52%	54%
12 months	18%	19%

GTR: guided tissue regeneration; NBM: natural bone mineral; PRP: platelet-rich plasma.

The defects displayed a comparable distribution and configuration in the two groups (Table 13.). The depth of the intrabony component as measured during surgery is presented in Table 14.

Table 13.

Distribution and configuration of treated defects

	PRP + NBM + GTR (N=15)	NBM + GTR (N=15)
Maxilla	8	7
Mandible	7	8
Anterior teeth	6	7
Premolars	3	3
Molars	6	5
1 – 2 wall	9	10
2 wall	4	3
3 wall	2	2

GTR: guided tissue regeneration; NBM: natural bone mineral; PRP: platelet-rich plasma

Table 14.

Baseline defect characteristics expressed in mm (mean ± SD)

Treatment	CEJ-BD (mm)	CEJ-BC (mm)	INTRA (mm)
PRP + NBM + GTR (N=15)	12.5 ± 2.2	7.0 ± 2.0	5.2 ± 2.1
NBM + GTR (N=15)	12.5 ± 2.4	7.2 ± 2.2	5.3 ± 2.2

CEJ–BD: cemento-enamel junction to the bottom of the defect; CEJ–BC: distance from the CEJ to the most coronal extension of the alveolar bone crest; GTR: guided tissue regeneration; INTRA: distance from the alveolar bone crest to the bottom of the defect; NBM: natural bone mineral; PRP: platelet-rich plasma.

There were no differences in the depth of the intrabony component between the two groups. At baseline, the mean PD was similar in the two groups and no statistically significant difference was found. At 1 year, the mean PD was decreased significantly in both groups compared with the baseline data ($p<0.001$). The mean PD reduction was

5.5 ± 1.3 mm in the PRP + NBM + GTR group and 5.5 ± 1.7 mm in the NBM + GTR group. No significant difference between the groups was found (Table 15.).

At baseline, the mean GR was similar between the two groups, with no statistically significant difference (Table 15.).

At 1 year, the mean GR increase was 1.1 ± 0.7 mm in the PRP + NBM + GTR group and 1.3 ± 0.8 mm in the NBM + GTR group. The increase in GR was statistically significant for both groups ($p<0.01$), but no difference between the groups was observed. No statistically significant difference was also found between the groups regarding the baseline mean value of CAL (Table 15.). The mean CAL gain was 4.5 ± 1.1 mm in the PRP + NBM + GTR group and 4.6 ± 1.1 mm in the NBM + GTR group ($p<0.001$). In both groups, the CAL improved significantly compared with baseline but no statistically significant difference was observed between the two groups.

Table 15.

Clinical parameters at baseline and 1 year expressed in mm (N=15 for each group)

	Baseline	1 Year	Difference	P value
Probing depth				
PRP + NBM + GTR	8.9 ± 2.3	3.4 ± 2.0	5.5 ± 1.3	p<0.001
NBM + GTR	8.9 ± 2.5	3.4 ± 1.0	5.5 ± 1.7	p<0.001
P value			NS	
Gingival recession				
PRP + NBM + GTR	1.9 ± 0.7	3.0 ± 1.1	1.1 ± 0.7	p<0.01
NBM + GTR	1.9 ± 1.2	3.2 ± 1.5	1.3 ± 0.8	p<0.01
P value			NS	
Clinical attachment level				
PRP + NBM + GTR	10.9 ± 2.2	6.4 ± 1.8	4.5 ± 1.1	p<0.001
NBM + GTR	11.1 ± 2.5	6.5 ± 2.3	4.6 ± 1.1	p<0.001
P value			NS	

GTR: guided tissue regeneration; NBM: natural bone mineral; PRP: platelet-rich plasma.

The frequency distribution of CAL gain for both treatment groups is shown in Table 16.

Table 16.

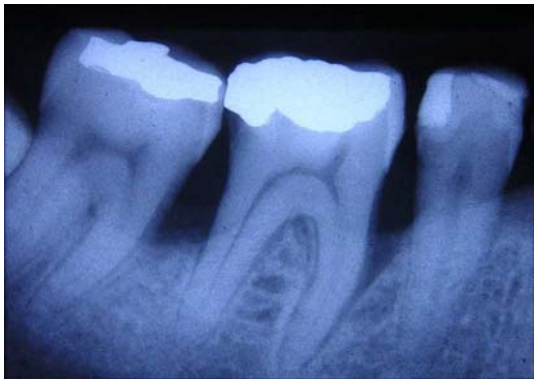
Frequency distribution of CAL gain expressed in mm (N=15 for each group)

CAL gain (mm)	PRP + NBM + GTR		NBM + GTR	
	N°	%	N°	%
3	3	20	2	13
4	4	27	6	40
5	6	40	3	20
6	1	6	4	27
7	1	6	0	0

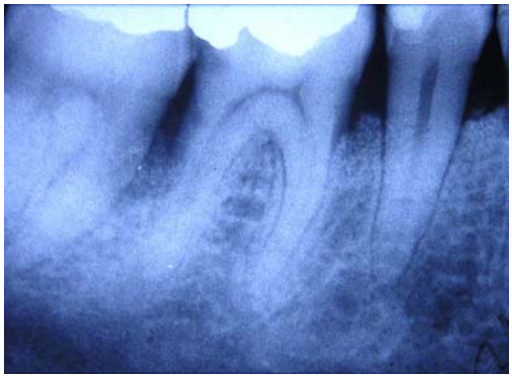
CAL: clinical attachment level; GTR: guided tissue regeneration; NBM: natural bone mineral; PRP: platelet-rich plasma.

In both groups, all sites gained at least 3 mm of CAL. CAL gains of ≥ 4 mm were measured in 80% (i.e. in 12 out of 15 defects) of the cases treated with PRP + NBM + GTR and in 87% (i.e. in 13 out of 15 defects) treated with NBM + GTR. (Fig. 15.)

Fig.15. Surgery with PRP + NBM + GTR (with collagen membrane)



15/a Preoperative picture



15/b. One year postoperative control

5.4. Study IV. (PRP + ß-TCP + GTR vs. ß-TCP + GTR)

The postoperative healing was uneventful in all cases. Minor exposure of the coronal portion of the e-PTFE membrane occurred in the fourth, fifth and sixth week in a total of 7 cases treated with PRP + ß-TCP + GTR and in 9 cases treated with ß-TCP + GTR. In order to prevent bacterial infection, additionally to chlorhexidine rinses, chlorhexidine gel was applied twice daily onto the exposed parts of the membranes until their removal. No other complications such as allergic reactions, abscesses or infections were observed throughout the study period. There were no differences in the gender distribution between the groups (i.e. 8 females and 6 males in the PRP + ß-TCP + GTR group and 7 females and 7 males in the each ß-TCP + GTR). Table 17. illustrates for both groups the mean PI, GI and BOP. GI and BOP improved statistically significantly compared to baseline, but no statistically significant differences were found between the two groups.

Table 17.

Mean (±SD) Plaque Index (PI), Gingival Index (GI) and Bleeding on Probing (BOP) Scores at Baseline and the 1-Year Examination

	PRP + ß-TCP + GTR (N= 14)	ß-TCP + GTR (N= 14)	P value
Plaque index scores			
Baseline	0.7 ± 0.2	0.8 ± 0.1	NS
12 months	0.6 ± 0.3	0.7 ± 0.2	NS
P value	NS	NS	
Gingival index scores			
Baseline	1.1 ± 0.2	1.0 ± 0.3	NS
12 months	0.6 ± 0.2	0.5 ± 0.4	NS
P value	$p<0.05$	$p<0.05$	
Bleeding scores			
Baseline	38%	40%	NS
12 months	11%	12%	NS
P value	$p<0.05$	$p<0.05$	

NS = not statistically significant. No significant differences between the PRP + ß-TCP + GTR and ß-TCP + GTR groups were found.

The defects displayed a comparable distribution and configuration in the 2 groups (Table 18.).

Table 18.

Distribution and configuration of treated defects

	PRP + ß-TCP + GTR (N= 14)	ß-TCP + GTR (N= 14)
Maxilla	7	9
Mandible	7	5
Anterior teeth	6	7
Premolars	2	3
Molars	5	4
1 – 2 wall	3	3
2 wall	9	8
3 wall	2	3

The depth of the intrabony component as measured during surgery is presented in Table 19. The width of the intrabony defects measured 3.2 ± 1.4 mm in the test group and 3.4 ± 1.0 mm in the control one. There were neither differences in the depth nor in the width of the intrabony component between the two groups.

At baseline, the mean PD was 9.1 ± 0.6 mm in the PRP + ß-TCP + GTR group and 9.0 ± 0.8 mm in the ß-TCP + GTR group. No statistically significant difference was found. At one year the mean PD was 3.3 ± 0.5 mm in the PRP + ß-TCP + GTR group and 3.6 ± 0.9 mm in the ß-TCP + GTR group (Table 20.). Thus, the PD decreased significantly in both groups compared to the baseline data ($p < 0.001$). No significant difference between the groups was found.

At baseline the mean GR was 1.0 ± 1.0 mm in the PRP + ß-TCP + GTR group and 0.9 ± 0.8 mm in the ß-TCP + GTR group, with no statistically significant difference (Table 20.). At one year the mean GR measured 2.4 ± 0.9 mm in the PRP + ß-TCP + GTR group and 2.4 ± 1.0 mm in the ß-TCP + GTR group. The increase in GR was statistically significant for both groups ($p < 0.01$), but no difference between the groups was observed.

Table 19.

Baseline defect characteristics expressed in mm (mean ± SD)

Treatment	PD(mm)	CAL(mm)	CEJ-BD(mm)	CEJ-BC(mm)	INTRA(mm)
PRP + ß-TCP + GTR (N=14)	9.1 ± 1.7	10.1 ± 1.3	11.1 ± 1.2	5.8 ± 1.3	5.3 ± 1.2
ß-TCP +GTR (N=14)	9.0 ± 0.8	9.9 ± 1.0	10.8 ± 1.3	5.6 ± 1.2	5.2 ± 1.3
P value	NS	NS	NS	NS	NS

NS = not statistically significant. No significant differences between the PRP + ß-TCP + GTR and ß-TCP + GTR groups were found.

At baseline the mean CAL was 10.1 ± 1.3 mm in the PRP + ß-TCP + GTR group and 9.9 ± 1.0 mm in the ß-TCP + GTR group. No statistically significant difference was found between the groups. At one year the mean CAL was 5.7 ± 1.1 mm in the PRP + ß -TCP + GTR group and 5.9 ± 1.2 mm in the ß-TCP + GTR group (Table 20.). Mean CAL gain was 4.1 ± 0.7 mm in the PRP + ß-TCP + GTR group and 3.9 ± 0.9 mm in the PRP + ß-TCP group. In both groups the CAL improved significantly compared to baseline ($p < 0.001$) but no statistically significant difference was observed between the two groups. In both groups all sites gained at least 3 mm of CAL (Table 21.). CAL gains over 4 mm were measured in 86% (i.e. in 12 out of 14 defects) of the cases treated with PRP + ß-TCP + GTR and in 64% (i.e. in 9 out of 14 defects) treated with ß-TCP + GTR (Fig.16.). A further analysis of the data by comparing the groups with or without the inclusion of 1-2 wall defects failed also to reveal statistically significant differences.

Table 20.

Clinical parameters at baseline and 1 year (N = 14 for each group)

	Baseline	1 Year	Difference	P value
Probing depth				
PRP + ß-TCP + GTR	9.1 ± 0.6	3.3 ± 0.5	5.8 ± 0.6	p<0.001
ß-TCP + GTR	9.0 ± 0.8	3.6 ± 0.9	5.4 ± 0.7	p<0.001
P value			NS	
Gingival recession				
PRP + ß-TCP + GTR	1.0 ± 1.0	2.4 ± 0.9	1.4 ± 0.8	p<0.01
.ß-TCP + GTR	0.9 ± 0.8	2.4 ± 1.0	1.5 ± 0.7	p<0.01
P value			NS	
Clinical attachment level				
PRP + ß-TCP + GTR	10.1 ± 1.3	5.7 ± 1.1	4.1 ± 0.7	p<0.001
ß-TCP + GTR	9.9 ± 1.0	5.9 ± 1.2	3.9 ± 0.9	p<0.001
P value			NS	

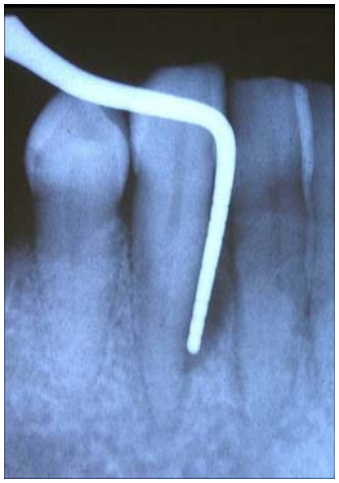
NS = not statistically significant. No significant differences between the PRP + ß-TCP + GTR and ß-TCP + GTR groups were found.

Table 21.

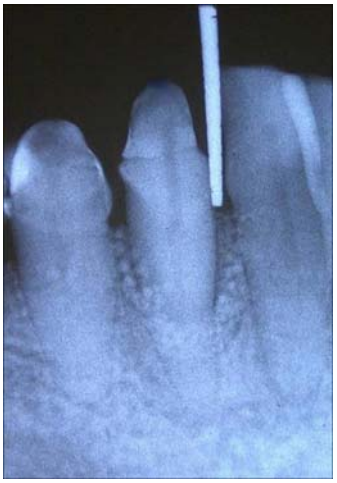
Frequency distribution of CAL gain (N = 14 for each group)

CAL gain (mm)	PRP + ß-TCP + GTR		ß-TCP + GTR	
	N°	%	N°	%
3	2	14	5	36
4	9	65	6	43
5	2	14	2	14
6	1	7	1	7

Fig.16. Surgery with PRP + ß-TCP + GTR



16/a. Preoperative picture



16/b. 12 months control

5.5. Study V. (EMD + NBM + PRP vs. EMD + NBM)

All patients completed the study. The postoperative healing was uneventful in all cases. No complications such as allergic reactions, abscesses or infections were observed throughout the entire study period. A slight wound dehiscence, however without exposure of the graft particles, occurred in the third week at two sites in the EMD + NBM + PRP and at three sites in the EMD + NBM group. All dehiscences epithelialized within a few days without any side effects.

There were no differences in the gender distribution between the groups (i.e. 7 females and 6 males) in each of the two groups.

Table 22. illustrates for both groups the mean PI, GI and BOP. GI and BOP improved statistically significantly compared to baseline, but no statistically significant differences were found between the two groups.

Table 22.

Mean (± SD) Plaque, Gingival and Bleeding Scores at the treated sites at Baseline and the 1- Year Examination

	EMD + NBM + PRP (N= 13)	EMD + NBM (N= 13)
Plaque index scores		
Baseline	0.7 ± 0.3	0.9 ± 0.1
12 months	0.7 ± 0.2	0.6 ± 0.3
Gingival index scores		
Baseline	1.3 ± 0.2	1.4 ± 0.3
12 months	0.7 ± 0.3	0.8 ± 0.2
Bleeding scores		
Baseline	58%	60%
12 months	19%	18%

The defects displayed a comparable distribution and configuration in the two groups (Table 23.).

Table 23.

Distribution and configuration of treated defects

	EMD + NBM + PRP (N= 13)	EMD + NBM (N= 13)
Maxilla	9	8
Mandible	4	5
Anterior teeth	6	5
Premolars	4	4
Molars	3	4
1 – 2 wall	7	8
2 wall	6	5

The depth of the intrabony component as measured during surgery is presented in Table 24. There were no differences in the depth of the intrabony component between the two groups.

Table 24.

Baseline defect characteristics expressed in mm (mean ± SD)

Treatment	CEJ-BD (mm)	CEJ-BC (mm)	INTRA (mm)
EMD + NBM + PRP (N=13)	12.2 ± 1.2	7.1 ± 1.1	5.1 ± 1.1
EMD + NBM (N=13)	12.1 ± 1.3	6.9 ± 1.2	5.2 ± 1.2

At baseline, the mean PD was similar in the two groups and no statistically significant difference was found. At one year the mean PD was decreased significantly in both groups compared to the baseline data ($p < 0.001$). Mean PD reduction was 5.8 ± 1.8 mm in the EMD + NBM + PRP group and 5.9 ± 1.3 mm in the EMD + NBM group. No statistically significant difference between the groups was found (Table 25.).

At baseline the mean GR was similar between the two groups, with no statistically significant difference (Table 25.). At one year the mean GR increase was 1.0 ± 1.0 mm in the EMD + NBM + PRP group and 0.9 ± 1.3 mm in the EMD + NBM group. The increase in GR was statistically significant for both groups ($p < 0.01$), but no difference between the groups was observed.

No statistically significant difference was also found between the groups regarding the baseline mean value of CAL (Table 25.). Mean CAL gain was 4.8 ± 1.3 mm in the EMD + NBM + PRP group and 5.0 ± 0.9 mm in the EMD + NBM group ($p < 0.001$). In both groups the CAL improved significantly compared to baseline but no statistically significant difference was observed between the two groups.

Table 25.

Clinical parameters at baseline and 1 year expressed in mm (N = 13 for each group)

	Baseline	1 Year	Difference	Significance
Probing depth				
EMD + NBM + PRP	8.8 ± 1.9	3.1 ± 0.9	5.8 ± 1.8	p<0.001
EMD + NBM	8.8 ± 2.0	2.8 ± 1.6	5.9 ± 1.3	p<0.001
			NS	
Gingival recession				
EMD + NBM + PRP	1.9 ± 1.4	2.9 ± 1.5	1.0 ± 1.0	p<0.01
EMD + NBM	1.8 ± 1.4	2.7 ± 1.5	0.9 ± 1.3	p<0.01
			NS	
Clinical attachment level				
EMD + NBM + PRP	10.8 ± 2.0	6.0 ± 1.5	4.8 ± 1.3	p<0.001
EMD + NBM	10.5 ± 1.6	5.5 ± 1.4	5.0 ± 0.9	p<0.001
			NS	

The frequency distribution of CAL gain for both treatment groups is shown in Table 26.

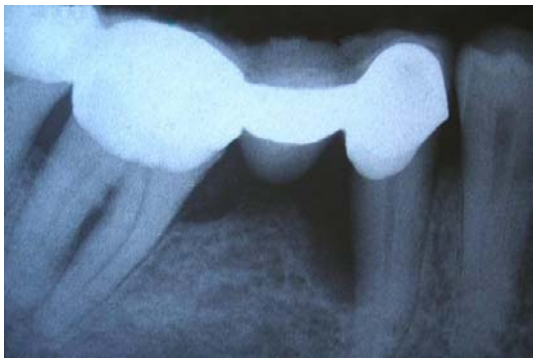
Table 26.

Frequency distribution of CAL gain expressed in mm (N = 13 for each group)

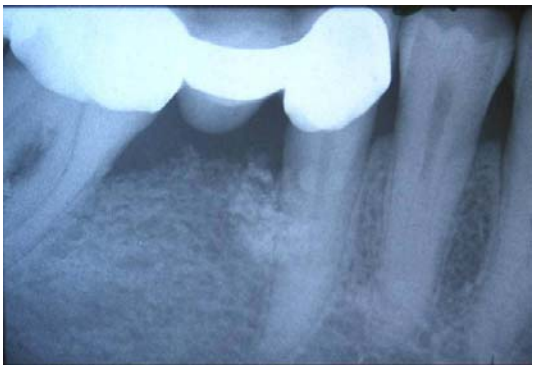
CAL gain (mm)	EMD + NBM + PRP		EMD + NBM	
	N°	%	N°	%
3	3	23	0	0
4	2	15	4	31
5	5	39	6	46
6	1	8	2	15
7	2	15	1	8

In both groups all sites gained at least 3 mm of CAL. CAL gains of ≥ 4 mm were measured in 77% (i.e. in 10 out of 13 defects) of the cases treated with EMD + NBM + PRP and in 100% (i.e. in 13 out of 13 defects) treated with EMD + NBM. (Fig. 17.-18.)

Fig.17. Case from the test group (EMD + NBM + PRP)

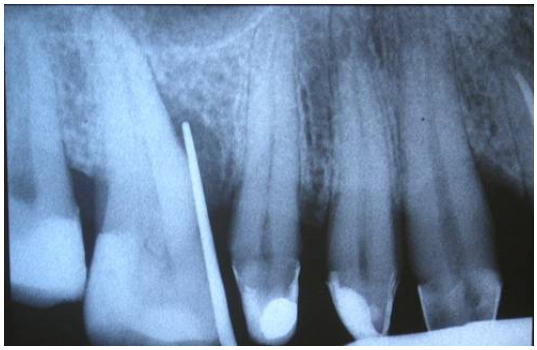


17/a. X-ray picture at baseline

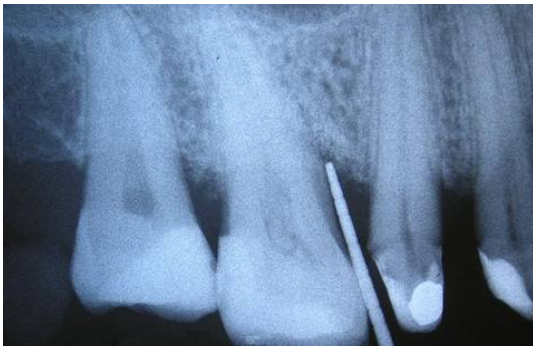


17/b. X-ray after 1 year

Fig.18. Case from the control group (EMD + NBM)



18/a. X-ray picture at baseline



18/b. X-ray after 1 year

5.6. Study VI. (PRP + NBM vs. NBM)

The postoperative healing was uneventful in all cases. No complications such as allergic reactions, abscesses or infections were observed throughout the study period. There were minor differences in the gender distribution between the groups (i.e. 5 males in test group and 4 males in control group). Table 27. illustrates for both groups the mean PI, GI and BOP. GI and BOP improved statistically significantly compared to baseline, but no statistically significant differences were found between the two groups.

Table 27.

Mean (± SD) Plaque, Gingival and Bleeding Scores at Baseline and at the 1 – Year Examination

	PRP + NBM (N=15)	NBM (N=15)
Plaque index scores		
Baseline	0.8 ± 0.1	0.7 ± 0.1
12 months	0.7 ± 0.2	0.6 ± 0.2
Gingival index scores		
Baseline	1.2 ± 0.2	1.3 ± 0.3
12 months	0.6 ± 0.1	0.7 ± 0.4
Bleeding scores		
Baseline	43%	42%
12 months	15%	13%

The defects displayed a comparable distribution and configuration in the two groups (Table 28.).

Table 28.

Distribution and configuration of treated defects

	PRP + NBM	NBM
1 – 2 wall	9	9
2 wall	6	6

The depth of the intrabony component as measured during surgery is presented in Table 29. There were no differences in the depth of the intrabony component between the two groups.

Table 29.

Baseline defect characteristics expressed in mm (mean ± SD)

Treatment	PD(mm)	CAL(mm)	CEJ-BD(mm)	CEJ-BC(mm)	INTRA(mm)
PRP + NBM (N=15)	8.6 ± 1.8	10.2 ± 2.0	11.0 ± 1.8	5.9 ± 1.7	5.1 ± 1.7
NBM (N=15)	8.5 ± 2.0	9.3 ± 2.7	10.7 ± 2.2	5.8 ± 2.0	4.9 ± 2.1

At baseline, the mean PD was 8.6 ± 1.8 mm in the PRP + NBM group and 8.5 ± 2.0 mm in the NBM group. No statistically significant difference was found. At one year the mean PD was 3.4 ± 1.4 mm in the PRP + NBM group and 3.1 ± 1.3 mm in the NBM group (Table 30.). Thus, the PD decreased significantly in both groups compared to the baseline data ($p < 0.001$) . No significant difference between the groups was found.

At baseline the mean GR was 1.6 ± 1.5 mm in the PRP + NBM group and 0.8 ± 0.8 mm in the NBM group, with no statistically significant difference (Table 30.). At one year the mean GR measured 2.1 ± 1.8 mm in the NBM + PRP group and 1.7 ± 1.5 mm in the NBM group. The changes in GR were statistically not significant in both groups ($p < 0.01$) and only minor differences between the groups were observed.

At baseline the mean CAL was 10.2 ± 2.0 mm in the PRP + NBM group and 9.3 ± 2.7 mm in the NBM group. No statistically significant difference was found between the groups. At one year the mean CAL was 5.5 ± 1.9 mm in the PRP + NBM group and 4.9 ± 2.0 mm in the NBM group (Table 30.). Mean CAL gain was 4.7 ± 1.9 mm in the PRP + NBM group and 4.4 ± 1.7 mm in the NBM group. In both groups the CAL improved significantly compared to baseline ($p < 0.001$) but no statistically significant difference was observed between the two groups.

Table 30.

Clinical parameters at baseline and 1 year (N=15 for each group)

	Baseline	1 Year	Difference	Significance
Probing depth				
PRP + NBM	8.6 ± 1.8	3.4 ± 1.4	5.2 ± 1.8	p<0.001
NBM	8.5 ± 2.0	3.1 ± 1.3	5.3 ± 2.0	p<0.001
			NS	
Clinical attachment level				
PRP + NBM	10.2 ± 2.0	5.5 ± 1.9	4.7 ± 1.9	p<0.001
NBM	9.3 ± 2.7	4.9 ± 2.0	4.4 ± 1.7	p<0.001
			NS	

The frequency distribution of CAL gain for both treatment groups is shown in Table 31.

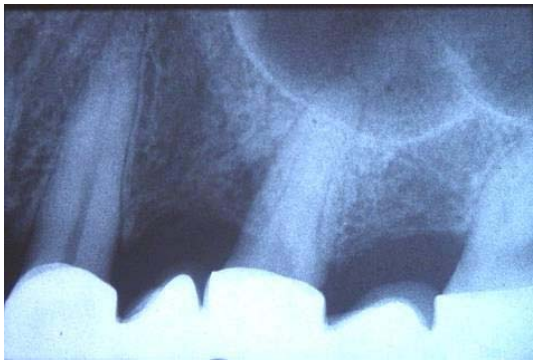
Table 31.

Frequency distribution of CAL gain (N=15 for each group)

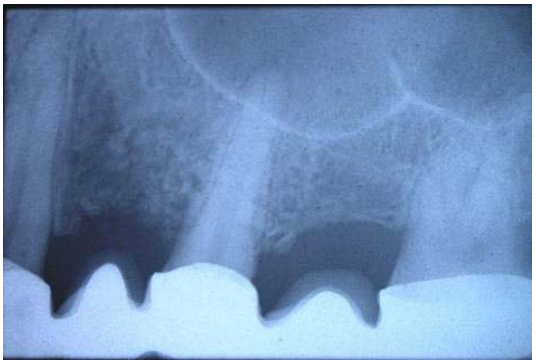
CAL gain (mm)	PRP+NBM		NBM	
	N°	%	N°	%
1	0	0	1	6.67
2	3	20	1	6.67
3	1	6.67	3	20
4	3	20	1	6.67
5	2	13.33	6	40
6	4	26.67	2	13.33
8	2	13.33	1	6.67

CAL gains of ≥ 4 mm were measured in 73 % (i.e. in 11 out of 15 defects) of the cases treated with PRP + NBM and in 66 % (i.e. in 10 out of 15 defects) treated with NBM. (Fig. 19.-20.)

Fig.19. Case from the test group (PRP + NBM)

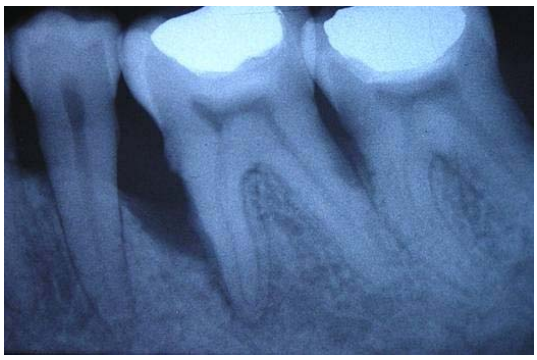


19/a. X-ray picture at baseline

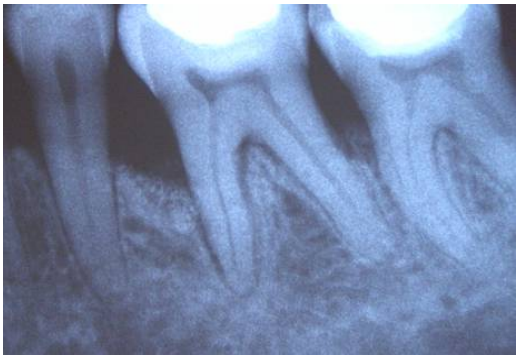


19/b. X-ray after 1 year

Fig.20. Case from the control group (NBM)



20/a. X-ray picture at baseline



20/b. X-ray after 1 year

6. Discussion

6.1. Discussion of the studies

6.1.1. Study I.

The results of this study have demonstrated that treatment of deep intrabony defects with either the combination of EMD + NBM or EMD + ß-TCP may lead to significant PD reduction and CAL gain compared to baseline values. No statistically and clinically significant differences in any of the investigated parameters were observed between the two treatment modalities. The lack of adverse reactions such as allergies, abscesses, or rejection of the implanted material indicates that both combination techniques were well

tolerated. These findings are in agreement with the results of previous studies which have failed to show that any of the used materials elicits any allergic or foreign body reactions[13,19, 30, 53-55, 57-59, 69, 71-73, 108, 122-136, 138-144]. However, it should be pointed out that based on the present results no conclusions can be drawn regarding the efficacy of EMD alone or the two types of bone substitutes alone in the treatment of intrabony defects.

Moreover, it should also be realized that the study does not have enough statistical power to rule out the possibility of a difference between the two groups. Further studies, with a much higher number of defects would be needed to detect an eventual difference between the treatments.[183]

The observation that treatment with EMD + NBM yields excellent clinical results in terms of PD reduction and CAL gain is in line with findings from previous clinical studies.[71-73,129] Results from controlled clinical studies comparing treatment with EMD + NBM to EMD alone have demonstrated that the combination treatment may indeed result in significantly higher CAL gain and defect fill than treatment with EMD alone.[71-73] The obtained results were explained by the space maintenance property of NBM which might have prevented flap collapse and subsequent increase of gingival recession.[71-73] In the three mentioned studies mean CAL gain following treatment with EMD + NBM measured 3.1 mm, 3.4 mm and 5.8 mm, respectively. The corresponding values for treatment with EMD alone were 1.7 mm, 2.9 mm and 4.9 mm. Slight differences in the reported results may be explained by differences in the initial depth of the defects (i. e. the deeper the defect the greater the gain in CAL), but also by the employed surgical technique.[62,66,184-186] Data from previous clinical studies strongly suggest that the supracrestal preservation of soft tissues, may have a significant influence upon postoperative soft tissue recession and subsequent CAL gain.[62,184-186]

When interpreting the clinical results obtained with the combination of EMD + NBM it needs to be mentioned that recent observations from a human histological study have indicated that this treatment may indeed promote formation of cementum, periodontal ligament and bone.[69] However, it should be kept in mind that data from controlled clinical studies comparing regenerative treatment with EMD + NBM to NBM alone have failed to demonstrate a significant difference between the two procedures.[76, 77] Thus, it is still unclear to what extent the combination of EMD + NMB may additionally improve the treatment outcome compared to defect fill with NBM alone.

The significant PD reductions and CAL gains measured in the EMD + ß-TCP group seem to indicate that also this combination technique may lead to favourable clinical results. This view is further supported by the fact that no significant differences in any of the investigated clinical parameters were found between the two investigated procedures. However, caution should be exercised when interpreting the significance of the clinical results obtained with this treatment modality. Since histology is the only valid method to visualize the investment of new periodontal ligament fibres to the root, it is important to emphasize that the clinical results obtained in the EMD + ß-TCP group need to be supported by histological evidence[113]. It is still unclear to what an extent the CAL gain obtained after this treatment represents a real periodontal regeneration rather than a defect fill without a new connective tissue attachment. Therefore, although it cannot be excluded that clinically no differences in terms of PD reduction and CAL gain were detected, histological differences may still be present between the two regenerative procedures. Further studies are thus required to evaluate the type of periodontal healing that occurs in human intrabony periodontal defects following treatment with EMD + ß-TCP.

In conclusion, within its limits Study I. has shown that at one year after surgery, both investigated therapies resulted in significant PD reductions and CAL gains.

6.1.2. Study II.

Study II. indicated that both regenerative approaches may lead to significant clinical improvements in terms of PD reduction and CAL gain although no statistically significant differences were found between the two treatment modalities. The implanted materials were well tolerated. These observations corroborated those from previous studies, which have failed to show that any of the used materials may elicit any allergic or foreign body reaction. The present results obtained in the test group (PRP + NBM + GTR) were comparable to those reported by others.[160-162] However, it has to be pointed out that in all currently available studies evaluating treatment of intrabony periodontal defects with bone substitutes + PRP + GTR, bioresorbable barriers were used.[160-162] These studies have shown that treatment of deep intrabony defects with PRP + NBM + GTR resulted in significantly higher PD reduction, CAL gain and defect fill compared

to treatment with either open flap debridement (OFD) alone or GTR alone.[160,161] In a comparative split-mouth study comparing treatment with PRP + NBM to NBM alone, statistically significantly higher CAL gain was obtained in the combination group (i.e. 3.15 mm in the PRP + NBM group versus 2.31 mm in the NBM group).[163] On the other hand, the available data on a comparison between treatment with PRP + NBM + GTR and PRP + NBM have failed to show significant differences between the two groups.[162] In that split-mouth study, 21 patients, each with one pair of intrabony defects, were randomly treated with either PRP + NBM + GTR or PRP + NBM. The clinical measurements revealed at 6 months following therapy, in the PRP + NBM + GTR group a mean CAL gain of 4.12 ± 0.78 mm on buccal and 4.16 ± 0.83 mm on lingual sites. In the PRP + NBM group mean CAL gain was 3.78 ± 0.72 mm on buccal and 3.84 ± 0.76 mm on lingual sites. No statistically significant differences were detected between the two groups.[162]

The present results obtained in the NBM + GTR group compared well to those obtained in previous controlled clinical studies which have shown that treatment of intrabony defects with NBM + GTR by means of bioresorbable collagen barriers may result in significantly higher PD reductions and CAL gains compared to OFD.[134,135,146] These studies have reported a mean CAL gain of 2.1 mm, 4.0 mm and 3.3 mm, respectively following treatment with NBM + GTR.[134,135,146]

Slight differences between the present results and those reported by others following treatment of intrabony defects with PRP + NBM + GTR or NBM + GTR might be explained by differences in the initial depth of the defects (i. e. the deeper the defect the greater the gain in CAL) and by the employed surgical technique.[187,188] Data from previous clinical studies suggested that the employed surgical technique including careful preservation of supracrestal soft tissues, might have a significant influence upon postoperative soft tissue recession and subsequent CAL gain. [186,188]

Furthermore, in the present study, in both groups all sites gained at least 3 mm of CAL. CAL gains of ≥ 4 mm were measured in 83% (i.e. in 10 out of 12 defects) of the cases treated with PRP + NBM + GTR and in 92% (i.e. in 11 out of 12 defects) treated with NBM + GTR. These results seemed to indicate that, at least from a clinical point of view, treatment with PRP + NBM + GTR did not add a significant benefit to treatment with NBM + GTR. When interpreting the healing type obtained with NBM + GTR, it

might be mentioned that human histologic studies have provided evidence of cementum, periodontal ligament and bone formation following this regenerative treatment modality.[108,132,136] Thus, the clinical results obtained with this treatment approach may represent both a clinical improvement, and also, at least to a certain extent, a regenerative type of healing. When interpreting the results obtained in the PRP + NBM + GTR group, it should be kept in mind that the precise mechanism of PRP on periodontal regeneration is still not well understood. It was suggested that PRP contains high concentrations of several growth factors such as PDGF and TGF-ß which may strongly modulate the regeneration process.[159-164,189,190] Data from in vitro studies have shown that PRP stimulated the proliferation of PDL and osteoblastic cells while, in the same time, epithelial cell proliferation was inhibited.[189,190] It was also speculated that due to its fibrinogen content, PRP reacts with thrombin and induces fibrin clot formation which in turn, is capable of upregulating collagen synthesis in the extracellular matrix and promotes a favourable scaffold for cellular migration and adhesion.[161] Other authors have suggested that following coagulation the PRP preparation exhibits a "sticky consistency" which may improve the clinical handling properties of the combination of PRP and the grafting material.[79,159-164] Another property which was attributed to PRP is its possible haemostatic activity which in turn, may enhance blood clot stabililty.[161,162] Since PRP preparation uses the patient's own blood, the risk of disease transmission is practically eliminated. However, from a clinician's point of view it is important to point to the practical aspects related to PRP preparation, which involves an additional step to the surgical procedure.

On the other hand, the results of the Study II. should be analyzed with caution. The treatment group NBM + GTR showed a remarkable CAL gain and probing pocket reduction resulting in optimal resolution of the periodontal problem. This minimizes the possibility for PRP showing any beneficial effect on the combined treatment results. Thus, the potential positive influence of the PRP might be masked from the significant high contribution of the other regenerative materials on the clinical outcomes.

Furthermore, it has to be pointed out that the frequent recall appointments associated with supragingival tooth cleaning may have also significantly influenced the clinical outcome. It has been extensively demonstrated that postoperative plaque control is one

of the key factors influencing periodontal healing following both conventional and regenerative periodontal therapy.[134, 135, 188, 191-193]

At present, according to our knowledge, no other data from controlled clinical studies are available comparing treatment with PRP + NBM + GTR to treatment with NBM + GTR which makes direct comparisons difficult. Additionally, the post-healing evaluation period was longer in this study (i.e. 1 year) compared to the other studies mentioned above (i.e. 6 months). Long-term evaluation is preferable as may indicate clinical stability of the obtained results. On the other hand, it should also be realized that the study did not have very high statistical power to rule out the possibility of a difference between the two groups.[183] Additional studies, with a much higher number of defects would be needed to detect an eventual difference between the treatments.

In conclusion, within its limits, Study II. has shown that at one year after regenerative therapy in 2 and 1-2 wall intrabony defects, excellent clinical results were obtained with bovine bone mineral (NBM) and GTR with a non-resorbable barrier, with or without the addition of PRP.

6.1.3. Study III.

The results Study III. have shown that treatment of deep intra-bony defects with both the combination of PRP + NBM + GTR and NBM + GTR may lead to significant PD reduction and CAL gain compared with baseline values. However, no statistically significant differences in any of the investigated parameters were found between the two treatment modalities. Both combination techniques were well tolerated. The present results obtained in the PRP + NBM + GTR group compare well with those reported by others.[160-162]

Results from a recent controlled clinical study have demonstrated that treatment of deep intra-bony defects with PRP + NBM + GTR resulted in a significantly higher PD reduction, CAL gain and defect fill than treatment with OFD alone.[161] In another controlled clinical study, treatment with PRP + NBM + GTR yielded significantly higher clinical improvements compared with treatment with GTR alone.[160] Moreover, in a comparative split-mouth study comparing treatment with PRP + NBM to NBM alone, statistically significantly higher CAL gain was obtained in the combination group (i.e.

3.15 mm in the PRP + NBM group versus 2.31 mm in the NBM group).[163] On the other hand, the data available on a comparison between treatment with PRP + NBM + GTR and PRP + NBM have failed to show significant differences between the two groups.[162] In that split-mouth study, 21 patients, each with one pair of intrabony defects, were randomly treated with either PRP + NBM + GTR or PRP + NBM. The clinical measurements revealed, at 6 months following therapy, a mean CAL gain of 4.12 ± 0.78 mm on buccal and 4.16 ± 0.83 mm on lingual sites in the PRP + NBM + GTR group. In the PRP + NBM group, the mean CAL gain was 3.78 ± 0.72 mm on buccal and 3.84 ± 0.76 mm on lingual sites. No statistically significant differences were detected between the two groups.[162]

The present results obtained in the NBM + GTR group compare well with those obtained in other controlled clinical studies, which have shown that treatment of intra-bony defects with NBM + GTR may result in significantly higher PD reductions and CAL gains compared with OFD.[134,135,146] These studies have reported a mean CAL gain of 2.1, 4.0 and 3.3 mm, respectively, following treatment with NBM + GTR.[134,135,146]

Slight differences between the present results and those reported by others following treatment of intrabony defects with PRP + NBM + GTR or NBM + GTR may be explained by differences in the initial depth of the defects. Based on the literature, in deeper defects, a greater CAL gain is achieved.[187,188] In this study, bony defects with a moderate depth (i.e. approximately 5 mm) were included. Also, data from previous clinical studies suggest that the surgical technique used, including careful preservation of supracrestal soft tissues, may have a significant influence upon post-operative soft tissue recession and subsequent CAL gain. [186,188]

Furthermore, in Study III., in both groups, all sites gained at least 3 mm of CAL. CAL gains of ≥ 4 mm were measured in 80% (i.e. in 12 out of 15 defects) of the cases treated with PRP + NBM + GTR and in 87% (i.e. in 13 out of 15 defects) treated with NBM + GTR. These results seem to indicate that, at least from a clinical point of view, treatment with PRP + NBM + GTR does not seem to confer a significant benefit to treatment with NBM + GTR. When interpreting the healing type obtained with NBM + GTR, it needs to be mentioned that human histologic studies have provided evidence of cementum, periodontal ligament and bone formation following this regenerative treatment modality.[108,132,136] Thus, the clinical results obtained following this treatment

approach may not only represent a clinical improvement but also, at least to a certain extent, a regenerative type of healing. Moreover, when interpreting the results obtained in the PRP + NBM + GTR group, it should be kept in mind that the precise mechanism of PRP on periodontal regeneration is still not well understood. It was suggested that PRP contains high concentrations of several growth factors such as PDGF and TGF-ß, which may strongly modulate the regeneration process.[159-162,189,190] Data from in vitro studies have shown that PRP stimulated the proliferation of PDL and osteoblastic cells while, at the same time, epithelial cell proliferation was inhibited.[189,190] It was also speculated that due to its fibrinogen content, PRP reacts with thrombin and induces fibrin clot formation, which in turn is capable of up-regulating collagen synthesis in the extracellular matrix and promotes a favourable scaffold for cellular migration and adhesion.[161] In this study, no blood parameters or growth factors concentration were evaluated. Of course, this entails the risk of a production of PRP volumes with low platelet/growth factors concentrations that can result in disappointing results. It has previously been shown that PRP volumes prepared with this technique contain an optimal platelet count.[182] Moreover, in a clinical practice setting, it appears difficult to evaluate the blood of every patient and subsequently decide whether an application of PRP is preferable or not. The rather limited results obtained in the PRP group may be explained by the use of the natural bone mineral, which does not contain any vital bone cells and thus, may be either not or only to a minor extent be influenced by PRP. On the other hand, it should be kept in mind that this assumption was recently questioned as it was shown that the addition of PRP to a bovine-derived xenograft may significantly improve the clinical outcomes in intra-bony defects.[163]

A lack of beneficial effects of PRP on periodontal and bone regeneration has also been reported by others.[179,194,195] Very recent findings of a randomized prospective clinical split-mouth study have failed to show a positive influence of autologous platelet concentrate (APC) on clinical and radiographic outcomes at 12 months following GTR therapy.[168,179] In the study referred to, the application of APC was evaluated in the treatment of deep infra-bony defects additional to a bioresorbable membrane combined with ß-TCP.[168,179] Furthermore, the study also included testing of the growth factor levels in the APCs used in their study and confirmed a high concentration of PDGF-AB, PDGF-BB, TGF-ß1 and IGF-I.[179]

Another property that was attributed to PRP is its possible haemostatic activity, which in turn may enhance blood-clot stabililty.[161,162] However, the PRP application involved the use of bovine thrombin, which is not an autologous material. Regarding this material, until now no disease transmission or immunogenic reactions have been reported. Also, the risk for coagulopathies is minimal due to low dose, high product purity and topical application of PRP.[100] Some authors have questioned the necessity of using thrombin[196], whereas others insist on using it.[100] According to the previous literature, PRP is expected to have an influence especially on the bone tissue.[80,100,156] On the other hand, it is well known that a real regeneration process can only be demonstrated histologically[197] and thus a surgical re-entry or bone sounding are of limited value when evaluating this issue. This may be especially true when a non-resorbable bone substitute such as NBM has been used to fill the defects.[69,108,136,160-162] Furthermore, it has been demonstrated previously that periodontal regeneration and defect fill will, in most cases, lead to CAL gain.[113,136]

Additionally, from a clinician's point of view, it is important to point to the practical aspects related to PRP preparation, which involves an additional step to the surgical procedure. Furthermore, at present, no data from human histologic material are available evaluating healing following treatment with PRP + NBM + GTR and thus, it is still unclear to what extent the use of PRP may enhance or impair the regeneration process compared with other, less complicated, treatment modalities such as NBM + GTR.

At present, according to our knowledge, no other data from controlled clinical studies are available comparing treatment with PRP + NBM + GTR with treatment with NBM + GTR, which makes direct comparisons difficult. Additionally, the post-healing evaluation period was longer in this study (i.e. 1 year) compared with the other studies mentioned above (i.e. 6 months). Long-term evaluation is preferable as it may indicate the clinical stability of the results obtained.

In conclusion, within its limits, Study III. has shown that (i) at one year after regenerative surgery with both PRP + NBM + GTR and NBM + GTR, in significant PD reductions and CAL gains were found, and (ii) the use of PRP has failed to improve the results obtained with NBM + GTR.

6.1.4. Study IV.

The results of this study have demonstrated that treatment of deep intrabony defects with either the combination of PRP + ß-TCP + GTR or ß-TCP + GTR may lead to statistically significant PD reduction and CAL gain compared to baseline values. Our postoperative observations are in line with observations from previous studies, which have failed to show that any of the used materials may elicit any allergic or foreign body reaction.[109,110,137-144,167-172] On the other hand, in both groups a relatively high number of membrane exposures was observed in the fourth, fifth and sixth week following surgery. This observation is in agreement with those from previous reports evaluating the effect of non-bioresorbable e-PTFE membranes for treatment of intrabony periodontal defects.[113] It was suggested that in such cases, membrane exposure may also be related to the implanted e-PTFE material which does not integrate into the tissues surrounding the defect.[113] Thus, when using non-bioresorbable e-PTFE membranes for periodontal regeneration, a late exposure (i.e. between week four and six) of the membrane material may often be expected.

In the present study, at one year following regenerative surgery, mean CAL gain measured 4.1 ± 0.7 mm in the PRP + ß-TCP + GTR group and 3.9 ± 0.9 mm in the PRP + ß-TCP group. In both groups all sites gained at least 3 mm of CAL. CAL gains of 4 mm were measured in 86% (i.e. in 12 out of 14 defects) of the cases treated with PRP + ß-TCP + GTR and in 79% (i.e. in 11 out of 14 defects) treated with ß-TCP + GTR. However, no statistically significant differences in any of the investigated parameters were observed between the two treatment modalities. These results compare well with those of two very recent randomized controlled clinical study evaluating the additional effect of PRP upon the healing of deep intrabony defects treated with either ß-TCP + GTR or ß-TCP.[168,169] In one study, at 12 months following regenerative surgery, in both groups, 88% of the sites displayed a CAL gain of 4 mm although no statistically significant differences between the two groups were found.[168] In other study 30 intrabony defects were treated in a total of 25 patients with ß-TCP, PRP + ß-TCP or PRP + ß-TCP + GTR.[169] At 12 months following therapy the relative attachment gain measured 2.4 mm, 2.1 mm and 2.5 mm without any statistically significant differences between the groups.

Slight differences between the present results and those previously mentioned may be explained by differences in the initial depth of the defects. It has been shown that in deeper defects, a greater CAL gain can be expected.[187,188] The present results are also in line with those from two other controled clinical studies employing a similar experimental design, but using as graft material NBM and GTR by means of either non-bioresorbable e-PTFE or collagen membranes.[166,167] In those studies, CAL gains of 4 mm were measured in 80% and 83% respectively, of the sites treated with PRP + NBM + GTR and in 92% and 87% respectively, of the sites treated with NBM + GTR. The results have indicated that, at least from a clinical point of view, treatment with PRP + NBM + GTR did not add a significant benefit to treatment with NBM + GTR. When interpreting the healing type obtained with NBM + GTR, it needs to be mentioned that human histologic studies have provided evidence of cementum, periodontal ligament and bone formation following this regenerative treatment modality.[108,132,136] Data from in vitro studies have shown that PRP stimulated the proliferation of PDL and osteoblastic cells while, in the same time, epithelial cell proliferation was inhibited.[189,190] It was also speculated that due to its fibrinogen content, PRP reacts with thrombin and induces fibrin clot formation which in turn, is capable of upregulating collagen synthesis in the extracellular matrix and promotes a favorable scaffold for cellular migration and adhesion.[161] Taken together, the present results, together with those from other controlled clinical studies using a comparable experimental design, suggest that the additional use of PRP may not yield additional improvements compared to treatment with either NBM + GTR or ß-TCP + GTR. When interpreting the results, it needs to be also pointed to the results of other studies which have shown that treatment of deep intrabony defects with PRP combined with different types of bone substitutes, with or without GTR may result in significantly higher clinical improvements than OFD alone, GTR alone or bone substitute alone.[160-164] In a comparative split-mouth study comparing treatment with PRP + NBM to NBM alone, statistically significantly higher CAL gain was obtained in the combination group (i.e. 3.15 mm in the PRP + NBM group versus 2.31 mm in the NBM group).[163] Findings of another controlled study comparing treatment with PRP + NBM + GTR to PRP + NBM

have failed to show significant differences between the two groups, thus questioning the need for GTR when using PRP.[162] In that split-mouth study, 21 patients, each with

one pair of intrabony defects, were randomly treated with either PRP + NBM + GTR or PRP + NBM. The clinical measurements revealed at 6 months following therapy, in the PRP + NBM + GTR group a mean CAL gain of 4.12 ± 0.78 mm on buccal and 4.16 ± 0.83 mm on lingual sites. In the PRP + NBM group mean CAL gain was 3.78 ± 0.72 mm on buccal and 3.84 ± 0.76 mm on lingual sites. No statistically significant differences were detected between the two groups.[162] Recent data have indicated that factors as the number of bony walls and defect width seem to significantly influence the treatment outcome.[188,198] In the present material, the width of the intrabony defects measured 3.2 ± 1.4 mm in the test group and 3.4 ± 1.0 mm in the control one, which is comparable to those reported in a recent controlled study evaluating the effect of recombinant human platelet derived growth factor and ß-TCP in the treatment of advanced intrabony defects.[154] Thus, it may be speculated that the use of a graft biomaterial such as ß-TCP in conjunction with GTR, could be advantageous by acting as a space maintaining scaffold and preventing the collapse of the soft tissues into the bony defect.

This view seems also to be supported by the present results where treatment with ß - TCP + GTR resulted in significant CAL gain and PD reduction, thus minimizing the possibility for demonstrating a possible additional effect of PRP. Furthermore, a comparison of the two groups with or without inclusion of 1-2 wall defects failed also to reveal statistically significant differences. Therefore, a potential positive influence of the PRP on the clinical outcomes, might have been masked by the excellent results obtained with the other regenerative materials (i.e. ß-TCP + GTR) used in the study.

However, when using different types of bone grafting materials for periodontal regeneration, it should be kept in mind that results from animal studies have demonstrated that an osteoconductive biomaterial may also obstruct the space, thus preventing periodontal regeneration.[174] Further preclinical and clinical research is warranted to systematically evaluate the effect of various types of biomaterials in providing space maintenance and promoting periodontal wound healing and regeneration and to distinguish these effects from the pure osteoconductive properties of the biomaterials.

The frequent recall appointments associated with supragingival tooth cleaning may have also significantly influenced the clinical outcome. It has been extensively demonstrated

that postoperative plaque control is one of the key factors influencing periodontal healing following both conventional and regenerative periodontal therapy.[188,191-193] Moreover, it should be also kept in mind that the study may not have the statistical power to rule out the possibility of a difference between the two groups.[183] Additional studies, with a much higher number of defects may be needed to detect an eventual difference between the treatments.

In conclusion, within its limits, Study IV. has shown that at one year after surgery, both therapies resulted in significant PD reductions and CAL gains.

6.1.5. Study V.

The results of Study V. have shown that regenerative periodontal surgery in deep intrabony defects with both combination approaches may lead to significant PD reduction and CAL gain compared to baseline values. No adverse reactions were observed throughout the entire study period of one year, which in turn indicates that the employed materials and their combinations were well tolerated. No statistically significant differences in any of the investigated parameters were found between the treatments. CAL gains of ≥ 4 mm were measured in 77% (i.e. in 10 out of 13 defects) of the cases treated with EMD + NBM + PRP and in 100% (i.e. in al 13 defects) treated with EMD + NBM. These results indicate that, at least from a clinical point of view, treatment with EMD + NBM + PRP does not add a significant benefit to treatment with EMD + NBM. However, when interpreting this findings, it has to be kept in mind that at the time being no other data evaluating the treatment of intrabony defects with EMD + NBM + PRP are available, and therefore, direct comparisons with other studies are not possible.

The results from controlled clinical studies evaluating the effect of a combination of PRP with different types of bone substitutes and GTR in regenerative periodontal therapy are somewhat controversial. While some reports have shown significantly higher CAL gains and defect fill following the combination of bone substitutes, PRP and GTR,[160,161] others have failed to show a significant benefit of PRP.[166,168] Furthermore, the study design of some papers[160,161] includes too many different variables between the groups, which make impossible to draw any definitive

conclusions regarding the sole effect of PRP. In a controlled split-mouth study, comparing treatment with PRP + NBM to NBM alone, statistically significantly higher CAL gain was obtained in the combination group (i.e. 3.15 mm in the PRP + NBM group versus 2.31 mm in the NBM group).[163] On the other hand, the results of a recent controlled clinical study comparing treatment of intrabony defects with PRP + NBM + GTR to NBM + GTR have demonstrated excellent clinical outcomes after both combination approaches, but no statistically significant differences in any of the investigated parameters were found between the groups.[166] At one year after therapy, mean CAL gains measured 4.5 ± 1.1 mm in the PRP + NBM + GTR group and 4.6 ± 1.1 mm in the NBM + GTR, respectively. CAL gains of ≥ 4 mm were measured in 80% (i.e. in 12 out of 15 defects) of the cases treated with PRP + NBM + GTR and in 87% (i.e. in 13 out of 15 defects) treated with NBM + GTR.

The present results obtained in the EMD + NBM group are in line with those obtained in other controlled clinical studies, which have shown that treatment of intrabony defects with EMD + NBM may result in significantly higher CAL gains compared to treatment with EMD alone.[71-73] Results from a split-mouth study have indicated higher increase in gingival recession following treatment with EMD (i.e. 0.8 ± 0.8 mm) than following treatment with EMD + NBM (i.e. 0.3 ± 0.6 mm) while the re-entry demonstrated significantly higher bone fill in the EMD + NBM group (i.e. 4.0 ± 0.8 mm) compared to the EMD one (i.e. 3.1 ± 1.0 mm).[72] In a further controlled clinical trial, 60 deep intrabony defects in 60 patients with chronic periodontitis were treated with the simplified papilla preservation flap and defect fill with either EMD + NBM or EMD alone.[73] Both treatments resulted in clinically and statistically significant improvements in terms of CAL gain, PD reduction and radiographic bone fill when compared to baseline. However, treatment with EMD + NBM resulted in significantly higher CAL gain (5.3 ± 1.1 mm versus 4.3 ± 1.0 mm) and less increase in gingival recession (0.4 ± 0.6 mm versus 0.9 ± 0.5 mm) than treatment with EMD.

Slight differences between the present results obtained with EMD + NBM and those referred to, might be related to differences in the initial depth of the defects. It is well documented that in deeper defects, a greater CAL gain may be achieved.[66] Furthermore, in the present study, the application of EMD + NBM was slightly different compared to those referred to.[71-73] While in this study, EMD was first applied on the root surfaces

and subsequently followed by defect fill with NBM, in the referred studies, the defects were filled the mixture of EMD + NBM.

When interpreting the healing type obtained with EMD + NBM, it needs to be pointed to the results of a human histologic study which has demonstrated formation of cementum, periodontal ligament and bone formation following this regenerative treatment modality.[69] Thus, the clinical results obtained following this treatment approach may not only represent a clinical improvement, but also, at least to a certain extent, a regenerative type of healing.

There might be several explanations for the lack of difference between the two treatment groups. One aspect, may be related to the uncomplete understanding of the precise mechanism of PRP upon periodontal regeneration. It was suggested that PRP contains high concentrations of several growth factors such as PDGF and TGF-ß which may strongly modulate the regeneration process.[159-162,179,189,190] It was also speculated that due to its fibrinogen content, PRP reacts with thrombin and induces fibrin clot formation which in turn, is capable of upregulating collagen synthesis in the extracellular matrix and promotes a favorable scaffold for cellular migration and adhesion.[161] In the present study, no blood parameters or growth factors concentration were evaluated. This in turn, may comport the risk of a production of PRP volumes with low platelet/growth factors concentrations. On the other hand, it has been previously shown that PRP volumes prepared with this technique contain an optimal platelet count.[182] When addressing this issue, it should be also kept in mind that in a clinical practice setting it is difficult to evaluate the blood of every patient and subsequently decide, whether an application of PRP is preferable or not.

Furthermore, data from in vitro studies indicate that also EMD may influence periodontal wound healing by an indirect stimulatory effect on the release of growth factors during periodontal wound healing and by inhibiting or at least retarding epithelial downgrowth.[114,115,117,120,121] Since both PRP and EMD seem to have a stimulatory effect upon wound healing, it may be speculated that the stimulatory effect provided by only one of these two materials might be sufficient to create an optimal healing environment and thus, it questions the additional benefit of this combination.

On the other hand, it should be kept in mind that the lack of a difference between the two groups may also be related to the rather limited number of treated defects (e.g. 13

defects in each group) and therefore, the study may not have the statistical power to rule out the possibility of a difference between the two groups.[183] For superiority trials in the treatment of periodontal intrabony defects using regenerative materials, a sample size of approximately 30 persons per group has been estimated to be needed, considering a desirable difference between groups of 1.0 (± 1.3) mm CAL gain.[183] However, it needs to be pointed out, that from a practical point of view, it is very difficult to recruit for a mono-centre randomized controlled clinical trial such a large number of patients.

In conclusion, within its limits, Study V. has shown that i) at one year after regenerative surgery, both treatments resulted in statistically significant PD reductions and CAL gains, and ii) the use of PRP has failed to enhance the results obtained with EMD + NBM.

6.1.6. Study VI.

Study VI. indicated that both regenerative approaches may lead to significant clinical improvements in terms of PD reduction and CAL gain although no statistically significant differences were found between the two treatment modalities. No complications or adverse reactions were detected (see: Study I. and II.). The present results obtained in the test group (PRP + NBM) were comparable to those reported by others.[162] In a comparative split-mouth study comparing treatment with PRP + NBM to NBM alone, statistically significantly higher CAL gain was obtained in the combination group (i.e. 3.15 mm in the PRP + NBM group versus 2.31 mm in the NBM group).[163] A comparison between treatment with PRP + NBM + GTR and PRP + NBM have failed to show significant differences between the two groups.[162] In that split-mouth study, 21 patients, each with one pair of intrabony defects, were randomly treated with either PRP + NBM + GTR or PRP + NBM. The clinical measurements revealed at 6 months following therapy, in the PRP + NBM + GTR group a mean CAL gain of 4.12 ± 0.78 mm on buccal and 4.16 ± 0.83 mm on lingual sites. In the PRP + NBM group mean CAL gain was 3.78 ± 0.72 mm on buccal and 3.84 ± 0.76 mm on lingual sites. No statistically significant differences were detected between the two groups.[162]

The results obtained in the present study, a PD reduction from 8.6 ± 1.8 mm to 3.4 ± 1.4 mm in the test group and from 8.5 ± 2.0 mm to 3.1 ± 1.3 mm in the control group,

respectively a CAL gain of 4.7 ± 1.9 mm in the PRP + NBM group and a CAL gain of 4.4 ± 1.7 mm in the NBM group were not comparable with the previous cited clinical outcomes.

Differences between the present results and those reported by others following treatment of intrabony defects with PRP + NBM + GTR , PRP + NBM or NBM alone might be explained by differences in the initial depth of the defects (i. e. the deeper the defect the greater the gain in CAL) and by the employed surgical technique.[187, 188] Data from previous clinical studies suggested that the employed surgical technique including careful preservation of supracrestal soft tissues, might have a significant influence upon postoperative soft tissue recession and subsequent CAL gain. [186,188]

Furthermore, CAL gains of ≥ 4 mm were measured in 73 % (i.e. in Study VI., in 11 out of 15 defects) of the cases treated with PRP + NBM and in 66 % (i.e. in 10 out of 15 defects) treated with NBM. These results seemed to indicate that, at least from a clinical point of view, treatment with PRP + NBM did not add a significant benefit to treatment with NBM. When interpreting the healing type obtained with NBM + GTR, it might be mentioned that human histologic studies have provided evidence of cementum, periodontal ligament and bone formation following this regenerative treatment modality.[108,132] Thus, the clinical results obtained with this treatment approach may represent both a clinical improvement, and also, at least to a certain extent, a regenerative type of healing. On the other hand, the results of the present study should be analyzed with caution. The treatment group NBM showed a remarkable CAL gain and probing pocket reduction resulting in optimal resolution of the periodontal problem. This minimizes the possibility for PRP showing any beneficial effect on the combined treatment results. Thus, the potential positive influence of the PRP might be masked from the significant high contribution of the other regenerative materials on the clinical outcomes.

At present, according to our knowledge, only one other data from controlled clinical studies is available comparing treatment with PRP + NBM to treatment with NBM in regenerative surgery of human periodontal intrabony defects which makes direct comparison difficult. Additionally, the post-healing evaluation period was longer in this study (i.e. 1 year) compared to the other study mentioned above (i.e. 6 months). Long-term evaluation is preferable as may indicate clinical stability of the obtained results. On

the other hand, it should also be realized that the study did not have very high statistical power to rule out the possibility of a difference between the two groups.[183] Additional studies, with a much higher number of defects would be needed to detect an eventual difference between the treatments.

In conclusion, within its limits, Study VI. has shown that at one year after regenerative therapy in 2 and 1-2 wall intrabony defects, excellent clinical results were obtained with natural bone mineral (NBM) with or without the addition of PRP.

6.2. General discussion

Eleven periodontal regenerative methods were evaluated in six randomized controled clinical trials. 162 cases were treated in parallel studies which compared two methods within the frameworks of one trial. .76

The main regenerative line was assured by either mechanical (non-resorbable and bioresorbable membranes) or biological (enamel matrix proteins) barriers. In all experiments were used graft materials. The used bone substitutes were either a xenograft, a natural bone mineral or a synthetic graft, a type of tricalcium phosphate. The reason for application of graft materials was the severe morphological status of the intrabony defects on the one hand and the necessity of a vehicle for the investigated platelet-rich plasma on the other hand.

Main clinical outcome of the studies was the clinical attachment level (CAL), secondary was the pocket depth (PD), or probing pocket depth (PPD). Statistical cross-evaluations of the methods were not performed.

Analysing methods without addition of platelet-rich plasma we can conclude that both regenerative lines based on GTR or EMD were very successful irrespectively of the type of the bone substitute. Clinical attachment gain (CAL gain) was 4.6 ± 0.8 mm using NBM with non-resorbable and 4.6 ± 1.1 mm with resorbable membrane. Using ß-TCP + GTR the CAL gain was 3.9 ± 0.9 mm. Regarding the enamel matrix derivatives - mediated lines the clinical attachment level gain was in one of the NBM + EMD groups 4.3 ± 0.8 mm and 5.0 ± 0.9 mm in the other. Using ß-TCP + EMD, this value was 4.1 ± 0.8 mm.

Comparable were the rates of CAL gain in groups where platelet-rich plasma was added to bone grafts supposing to have a complementary regenerative potential which can give extra clinical benefits during the periodontal tissue engineering procedures. The clinical attachment gains using platelet-rich plasma (PRP), NBM and GTR with a non-resorbable and a bioresorbable (collagen) membrane were 4.7 ± 1.1 mm, respectively 4.5 ± 1.1 mm. The value of the main clinical outcome in PRP + ß-TCP + GTR group was 4.1 ± 0.7 mm and 4.8 ± 1.3 mm in the PRP+NBM group where enamel matrix proteins were used as main regenerative adjuvants (EMD).

Two groups (PRP + NBM and NBM), which destitutes of the classical mechanical and biological-chemical barriers (GTR, EMD) shows also high values of CAL gain: 4.7 ± 1.9 mm respectively 4.4 ±.1.7 mm.

Changes of probing pocket depth were also prominent. Following the same order of assignment, the decrease of PPD was 5.7 ± 1.2 mm in NBM + GTR and 5.5 ± 1.7 mm in NBM + GTR group using the collagen membrane. The ß-TCP + GTR group shows a 5.4 ± 0.7 mm decrease of probing depth. The NBM + EMD groups and the ß-TCP + EMD group showed the following rates of PPD decrease: 4.8 ± 0.9 mm, 5.9 ± 1.3 mm and 4.6 ± 0.8 mm. Both NBM + GTR groups and the ß-TCP + GTR group each of them involving the application of platelet-rich plasma presented the undermentioned values of PPD-decrease: 5.5 ± 1.2 mm, 5.5 ± 1.3 mm, 5.8 ± 0.6 mm. In the PRP + NBM + EMD group this value was 5.8 ± 1.8 mm.

In the PRP + NBM, and in the NBM groups not only the CAL gains but also the PPD reductions were remarkable: 5.2 ± 1.8 mm, respectively 5.3 ± 2.0 mm.

The evaluation of this data leads to the recognition of the fact that all of the above clinically analyzed regenerative methods are effective in regenerative surgery of deep intrabony periodontal defects. The choice of the periodontist between this methods depends on from many factors: the morphology and accessibility of the defect, the experience and medical concept of the surgeon, patient's approach to different types of materials, the financial and technical possibilities, the practical and scientific observations of the periodontal operator.

7. Conclusions

Analysing the results of each study and synthesizing the clinical outcomes of eleven regenerative methods (including ten combined modalities of periodontal tissue engineering), the following inferences may be concluded:

1. a. A synthetic graft material, a beta-tricalcium phosphate used with positive references in maxillo-facial surgery is successfully applicable in periodontal combined regenerative techniques; (Study I. and IV.)
   b. The clinical parameters obtained are not favourable as in case of using the same technique with a natural bone mineral; (Sudy I.)
2. Important growth factors-containing autologous platelet-rich plasma does not enhance the regenerative effect of a natural bone mineral and a non-resorbable membrane; (Study II.)
3. The platelet-rich plasma does not enhance the regenerative potential of a natural bone mineral and guided tissue regeneration by means of a bioresorbable membrane; (Study III.)
4. Platelet-rich plasma's growth factors do not have a positive influence on periodontal healing after regenerative surgery with a synthetic bone graft and a non-resorbable membrane; (Study IV.)
5. A growth factors-containing adjuvant, the autologous platelet-rich plasma has not even had the minimal promotional regenerative effect on an another protein-mediated regenerative material, on enamel matrix derivatives; (Study V.)
6. Clinical regenerative effect of a natural bone mineral was not increased by addition of platelet-rich plasma; (Study VI.)

Statistical evaluation of the main clinical variables bears record to significant improvement in each examination. This outcomes indicates that all of the analysed procedures allow periodontal regeneration and are capable of promoting the natural regenerative capacity of these tissues. In variant combined methods the effects of the used natural bone mineral and beta-tricalcium phosphate are comparable.

In five studies it was clinically proven that in periodontal applications the platelet-rich plasma has not additional benefit for healing. This especially applies to its combined use with enamel matrix derivatives.

The option of the periodontal surgeon between this methods depends mainly on the defect morphology, the medical concept of the physician, the technical possibilities and the clinical experience of the periodontal specialist.

8. Summary

Several methods are available to enhance the healing and regeneration of periodontal tissues after surgical therapy of intrabony defects. The main indications for the use of combined regenerative procedures are the extent and morphology of the osseous lesions. The six studies of the present dissertation focused on the clinical effect of different barrier techniques, bone substitutes, enamel matrix derivatives and one growth factors containing adjuvant used in various combinations on the healing of severe periodontal intrabony impairments.

Synthetic, xenogeneic and autologous materials were used in this randomized clinical studies. Mechanical barriers (polytetrafluoroethylene and collagen membranes) for GTR, biological barriers/enamel matrix proteins (EMD), synthetic (ß-TCP) and xenogeneic (NBM) bone grafts and autologous platelet-rich plasma (PRP) were combined in the test and control groups of the trials.

Main clinical variable was the clinical attachment level (CAL) and subsidiary the pocket depth (PD), estimated at baseline and after one year.

The summation of the results after the statistical analysis takes cognizance of the followings: a) each of the eleven regenerative methods evaluated (ten combined procedures) leads to significant CAL gain and PD decrease; b) using ß-TCP or NBM with EMD or with PRP + GTR and GTR's, the difference between the parameters of the test and control groups were not statistically significant; c) in four studies was confirmed that the addition of PRP to graft materials has not increased significantly the positive outcomes independent from type of barrier or graft; d) adding platelet-rich plasma to natural bone mineral no benefit was observed from the point of view of the clinical variables; e) the polypeptide proteins of the platelet-rich plasma does not enhance the clinical regenerative effect of enamel matrix proteins.

In conclusion, the option of the periodontal surgeon between this methods depends mainly on the defect morphology, the patient's approach to the different types of materials, the medical concept of the physician, technical possibilities and the clinical experience of the periodontist.

Összefoglaló

A parodontális szövetek gyógyulásának és regenerációjának elősegítésére számos módszer áll rendelkezésre az intraosszeális defektusok sebészi kezelése során. A kombinált regeneratív eljárások alkalmazásának fő javallata a csont-léziók kiterjedése és morfológiája.

Az értekezés hat tanulmánya különféle membránok, csontpótlóanyagok, zománc matrix derivátumok és egy növekedési faktorokat tartalmazó autológ adjuváns kombinációinak gyógyulásra történő klinikai hatását vizsgálja parodontális csontdefektusok esetében.

Szintetikus, xenogén és autológ anyagokat használtunk a vizsgálatok során. A tanulmányozott teszt és kontroll csoportokban az irányított szövetregenerációhoz (GTR) használt mechanikai barriereket (polytetrafluoroethylene és kollagén membránok), a biológiai barrierként használt zománc matrix proteineket (EMD), szintetikus (ß-TCP) és xenogén (NBM) csontpótlókat valamint vérlemezkében gazdag plazmát (PRP) kombináltunk.

A fő klinikai paraméter a klinikai tapadás-szint (CAL), másodlagos változó pedig a tasakmélység (PD/PPD) volt, melyek értékeit preoperatív, majd egy év után regisztráltuk.

Az eredmények összegzése és a statisztikai értékelések a következő megállapításokhoz vezettek: a) mind a tizenegy regeneratív módszer (köztük tíz kombinált) szignifikáns CAL növekedéshez és PD csökkenéshez vezetett; b) a teszt és a kontroll csoportokban regisztrált értékek közti különbség nem volt statisztikailag szignifikáns ß-TCP vagy NBM és EMD együttes alkalmazása, illetve PRP + GTR és GTR-el történő kombinált alkalmazásuk során; c) négy vizsgálat igazolta, hogy a PRP hozzáadása a graftokhoz nem növelte szignifikánsan a pozitív klinikai paraméterek értékeit, membrán és graft típustól függetlenül; d) a klinikai változók szempontjából nem járt előnnyel a vérlemezkében gazdag plazma hozzáadása a természetes csontásványhoz; e) a vérlemezkében gazdag plazma polypeptid fehérjéi nem növelték a zománc matrix proteinek regeneratív hatását.

Végeredményben, a parodontális sebész választását befolyásoló tényezők a fenti módszerek közül a következők: a defektus morfológiája, a páciens különböző típusú anyagokhoz való hozzáállása, a műtő fél orvosi koncepciója, a technikai lehetőségek és a parodontológus klinikai tapasztalata.

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10. Abbreviations

ABBM – anorganic bovine bone mineral
APC – autologous platelet concentrate
BC – bone crest
BD – bottom of the defect
BOP – bleeding on probing
BPBM – bovine porous bone mineral
CAL – clinical attachment level
cAMP – cyclic adenosine monophosphate
CEJ – cemento-enamel junction
CPDA – citrate-phosphate-dextrose-adenine
DFDBA – demineralized freeze-dried bone allograft
EDTA – ethylene diamine tetra-aecetic acid
EMD – enamel matrix derivative /Emdogain
EMP – enamel matrix protein
e-PTFE – expanded polytetrafluoroethylene
FDBA – freeze-dried bone allograft
GDFs – growth and differentiation factors
GF – growth factor
GI – gingival index (Löe-Silness)
GR – gingival recession
GTR – guided tissue regeneration
IGF – insulin-like growth factor
IL – interleukin
INTRA – intrabony component
NBM – natural bone mineral
NS – not statistically significant
OFD – open flap debridement
PD – probing depth
PDGF – platelet-derived growth factor
PDL – periodontal ligament
PDGFs – polypeptide growth factors
PI – plaque index (Silness-Löe)
PGA – propylene glycol alginate
PPD – probing pocket depth
PPP – platelet poor plasma
PRP – platelet-rich plasma
SD – standard deviation
TCP – tricalcium-phosphate
TGF – transforming growth factor

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12. Publications

12.1. Thesis-related publications

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