PRGF for periodontal regeneration: A clinical pilot study

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INTRODUCTION

Periodontal disease is defined as an inflammatory destruction of the attachment apparatus of a tooth leading to loss of attachment between the root and the bone caused by periodontal pathogenic bacteria. In chronic periodontitis, a significant amount of alveolar bone can be resorbed leading to tooth mobility and ultimately to tooth loss (Graves et al. 2011, Bartold et al. 2010).

The treatment of periodontal disease always begins with a reduction and elimination of the inflammation. Once this is accomplished through debridement of the diseased root surface as well as patient oral hygiene instructions, the regenerative part of the treatment could be initiated when indicated. Many different techniques and materials have been described in the literature throughout the last decades resulting more or less predictable in periodontal regeneration. Beginning in the 1980th with the technique of "guided tissue regeneration" (GTR) using non-resorbable or resorbable barrier membranes, or beginning in the 1990th the use of enamelum matrix derivates (EMD) with or without bone grafting materials. Numerous investigators have described clinical and histological success with the GTR technique (Cortellini et al. 2007) as well as with the use of EMD (Koop et al. 2012). Platelet-rich-growth-factors (PRGF), a modification of fibrin glue made from autologous blood, is being used to deliver growth factors in high concentration to sites requiring osseous grafting. Growth factors released from the platelets include platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-ß), platelet-derived epidermal growth factor (PDGF), platelet-derived angiogenesis factor, insulin-like growth factor 1 (IGF-1), and platelet factor 4. These factors signal the local mesenchymal and epithelial cells to migrate, divide, and increase collagen and matrix synthesis (Anitua 2001, 2012a, Sanchez et al. 2003).

The use of PRGF in guided bone regeneration (GBR) is very well described (Anitua 2001, Anitua et al. 2008, Intini 2009). Furthermore, clinical and histological investigations showed positive results for the use of PRGF in sinus floor elevation (Anitua et al. 2012b).

In–vitro-studies demonstrated an increased human gingival fibroblast proliferation, migration and cell adhesion on collagen matrix, stimulated autocrine expression of growth factors and hyaluronic acid (e.g. Anitua et al. (2012c).

However, the clinical effect and benefit of the use of PRGF in periodontal regeneration is poorly understood and not well investigated. Furthermore, to our knowledge no histological studies have been performed in order to investigate the efficiency of PRGF in periodontal regeneration. Therefore, we decided to evaluate the effects of PRGF in periodontal regenerative therapies in a small, preliminary study performed in a private periodontal practice (Fachpraxis für Parodontologie und Implantologie (FPI-Hamburg), Dr. Ö. Solakoglu, Brunsberg 2, 22529 Hamburg).

In this clinical and histopathological pilot study in human specimens the effects and efficiency of PRGF (3rd phase) in periodontal regeneration will be investigated. Following the protocols of existing histological studies of guided tissue regeneration (GTR) in humans (e.g. Nyman et al. 1982, Stahl & Froum 1991) and the application of enamel matrix proteins (e.g. Bosshardt et al. 2005, Sculean et al. 2005) in periodontology, PRGF will be applied according to a standard clinical protocol to non-conservable teeth. After extraction, different histological investigations will be carried out in order to demonstrate probable periodontal repair or regeneration.

In this publication, we report our initial clinical findings and histopathological results of 4 teeth being treated according to our standard protocol described below.

MATERIAL AND METHODS

After written consent of the patients was obtained, a thorough periodontal examination evaluating the following parameters was carried out

- Pocket probing depth (PPD)
- Furcation involvement (FI)
- Gingival recession
- Clinical attachment level
- Tooth mobility
- Radiographic bone loss (horizontally and vertically)
- Vitality of the tooth

Following this initial examination a non-surgical periodontal therapy including deep scaling and root planning with oral hygiene instructions was performed by the same investigator.

Four to six weeks following initial therapy a reevaluation was initiated including the formulation of the prognosis of the individual teeth.

Furthermore, hopeless teeth were identified using the following criteria shown in table 1.

Following a thorough periodontal reevaluation, a surgical periodontal therapy with the goal of periodontal regeneration was carried out using the following protocol, shown in table 2, for teeth being included in this study according to the above mentioned criteria in table 1.

After postoperative healing of 3 to 6 months one tooth was removed atraumatically and a socket preservation procedure was carried out using a resorbable bone grafting material and PRGF (Puros®, PRGF).

Following tooth extraction, a histopathologic evaluation was carried out at the University of Bonn using the method established by Prof. Donath. For details see Frentzen et al. (2003). In short, the specimen was embedded undecalcified in an ultraviolet light activated polymethylmethacrylate (Technovit 7200®, Heraeus-Kulzer) after dehydration. After polymerization, parallel sections along the longitudinal axis were cut in a microsawing machine and the desired final thickness (10-20 µm) obtained using a microgrinding system (Exakt, Norderstedt, Germany). For staining, toluidine blue stain was applied.

RESULTS

Clinical cases

In order to perform histopathological investigations the study tooth needed to be extracted and analyzed according to the protocol. However, a number of teeth that were treated according to the study protocol and were determined for later extraction and histopathological analysis demonstrated a remarkable degree of periodontal regeneration which was diagnosed clinically (probing pocket depth), as well as radiografically. Therefore, those teeth were not extracted and analyzed, but left in the patient's mouth.

Case 1

This illustrated case represents a tooth that was determined to be hopeless according to the evaluation criteria in the Material & Methods section in this study. This particular tooth number 14 demonstrated in the beginning of the treatment the following pathologies (Fig. 1-2):

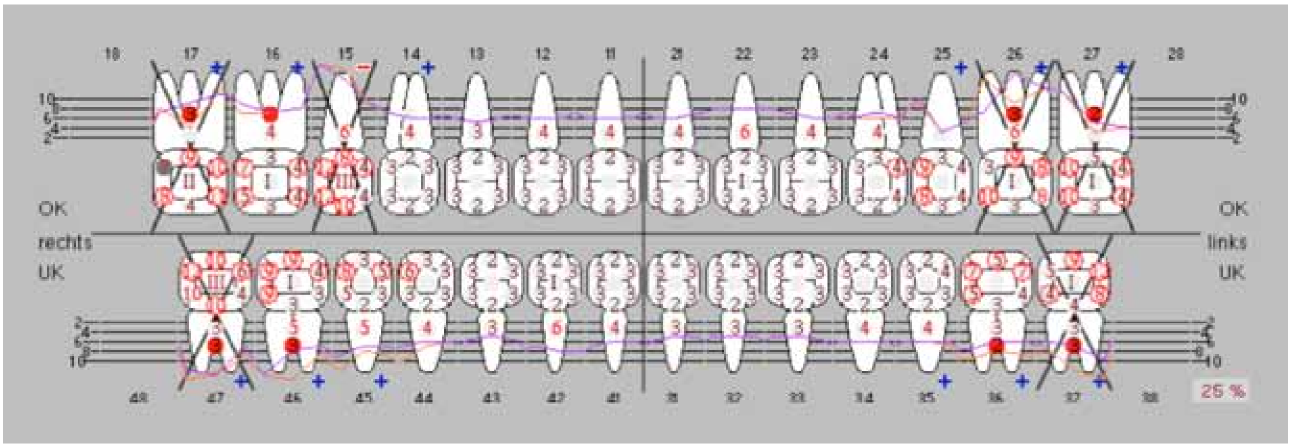

Fig. 1
Initial periodontal charting October 13th, 2010

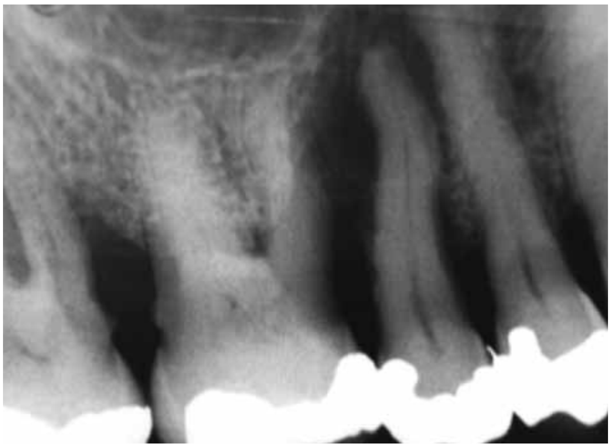

Fig. 2
Initial periapical radiograph of teeth # 14-16. Tooth # 15 demonstrated an extreme radiolucency in the distal and periapical aspects. A perio-endo lesion was radiographically confirmed.

- Probing pocket depth up to 12mm
- Mobility degree III
- Vitality test negative
- Perioendo-Lesion
- Gingival Recession of 6mm

On February 22nd 2011, after initial periodontal therapy as well as root canal treatment and occlusal stabilization of the tooth was carried out, the regenerative periodontal surgery according to the protocol material & methods (points n-w) was carried out using EDTA for the removal of the smear layer and PRGF in conjunction with an allograft material and the PRGF fibrin membrane. The intraoperative view can be seen in fig. 3-4).

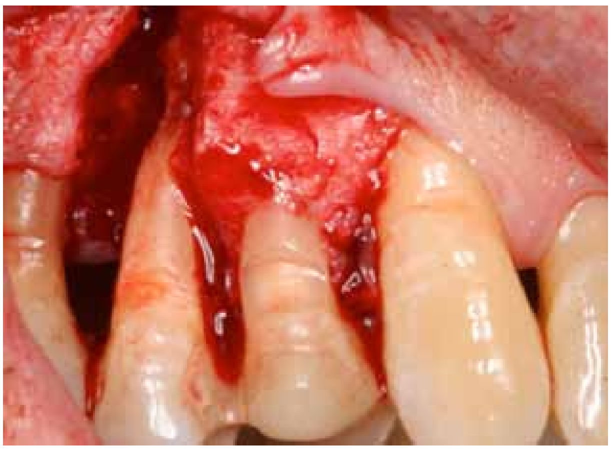

Fig. 3
Clinical intraoperative view of tooth # 15. The radiographic findings of extremely advanced periodontal bone loss approaching 100% of the root length were confirmed. Furthermore, the occlusal splinting can be seen.

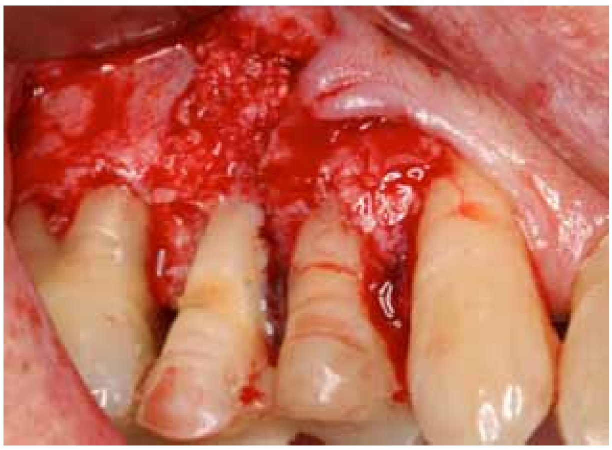

Fig. 4
Intraoperative view of tooth #15 after completion of bone grafting procedure using PRGF and a resorbable bone grafting material.

According to the protocol the tooth was radiographically reevaluated after 6 month postoperatively, prior to the scheduled removal and histopathological analysis. The postoperative radiograph is shown in fig. 5.

Due to this remarkable periodontal regeneration and the extreme patient satisfaction it was decided to leave this tooth in place and start a recall program for the patient for periodontal maintenance. The latest postoperative periodontal charting performed on May 8th 2012 is shown in fig. 6.

Fig. 5

Postoperative radiograph 6 months after bone grafting procedure and completion of endodontic treatment. A significant bone fill on the distal and periapical aspects of tooth # 15 were confirmed.

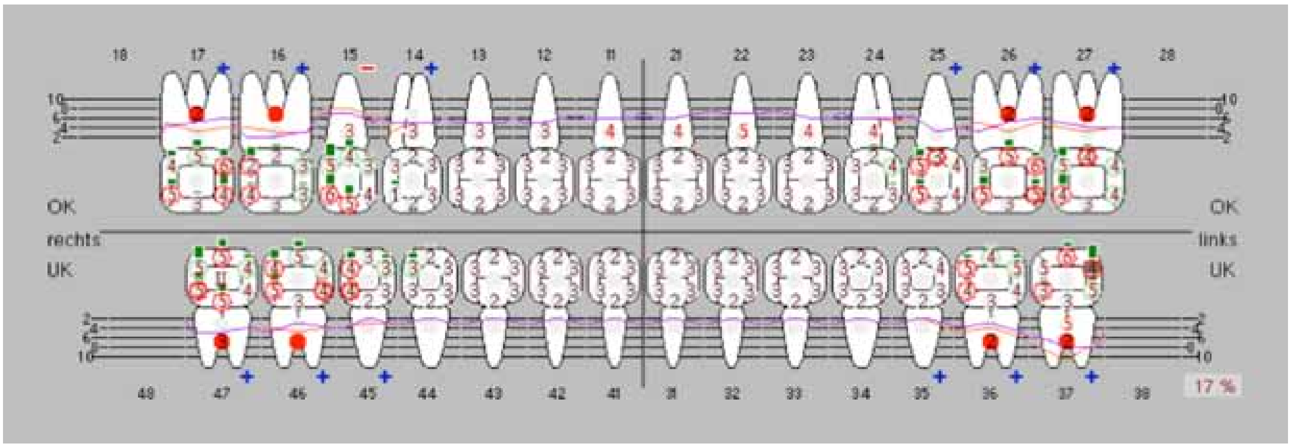

Fig. 6

Periodontal charting from May 8th, 2012. The PPD and the BOP are significantly reduced approximately 1,5 years after initiation of periodontal therapy and all teeth are still in place.

Cases 2 and 3

Two more teeth (cases 2 and 3, not shown in this publication) that were initially determined hopeless showed also an excellent result after the regenerative periodontal therapy and were therefore not extracted. The patients function on those stabilized teeth very well and are extremely satisfied with the result. They are currently undergoing periodontal maintenance care.

Case 4

The following tooth of a different patient in this pilot investigation was treated according to the protocol and investigated histopathologically. The initial radiograph as well as the clinical view are shown below (Figs. 7 -8) demonstrating a significant amount of vertical infrabony defects, as well as radiolucencies in the furcation area.

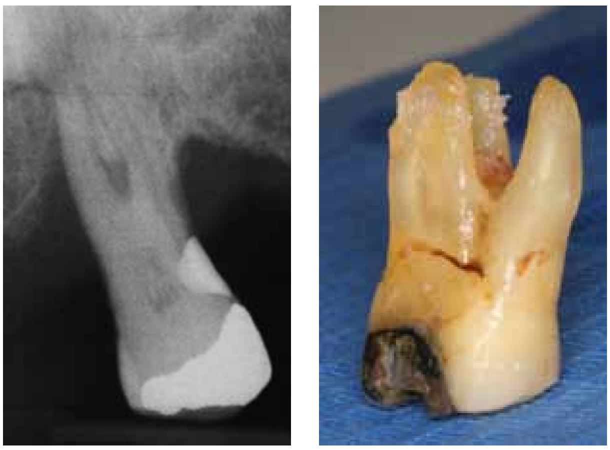

Fig. 7-8

Radiographic and clinical view of the study tooth of Case # 4. This tooth # 17 was considered hopeless and was extracted for histological anaylsis according to the study protocol 6 months following surgical procedure using PRGF and a resorbable bone grafting material.

Clinically, this maxillary molar tooth 16 demonstrated advanced pocket probing depth up to 12mm, a furcation involvement degree III as well as mobility of degree II. Intraoperatively, the clinical and radiographic findings were confirmed and the above mentioned protocol was carried out using PRGF as the regenerative agent.

However, 6 month following this surgical procedure, this particular tooth did not demonstrate a significant amount of attachment gain clinically or gain of alveolar bone radiographically and was extracted according to the protocol for histopathological analysis.

Histological evaluation

The tooth showed normal crown, pulp and root canal structures (Figs. 9,10). In the coronal third, a circumferential defect penetrating into cementum and dentin was found partly filled by a bony tissue (Fig. 9). On one side, coronally of the defect, cancellous bone encountered the root surface (Fig. 9). The spongy trabecules were covered by osteoblasts, resorption lacunae and focally osteoclasts (Fig. 11). More apically, it was covered by junctional epithelium. Contralaterally, the defect appeared triangular with the bony peak penetrating into the dentin (Fig. 12). A connection with the pulp could not be verified.

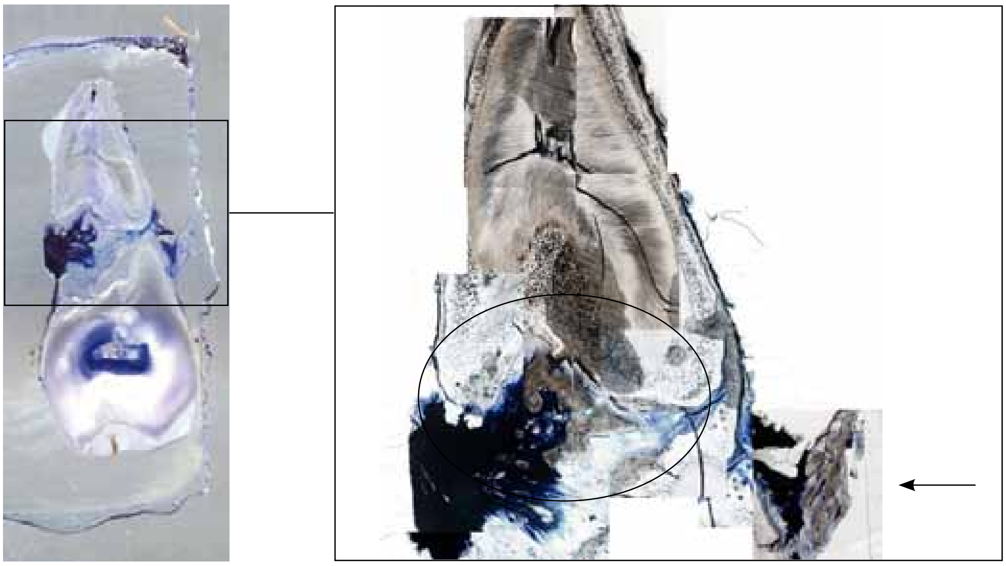

Fig. 9a-b

a: low magnification of a grinding section, box indicates higher magnification in b;

b: root and apical crown, defect with penetrating bone-like tissue (circle), cancellous bone near root surface (arrow); toluidine blue staining, x5.

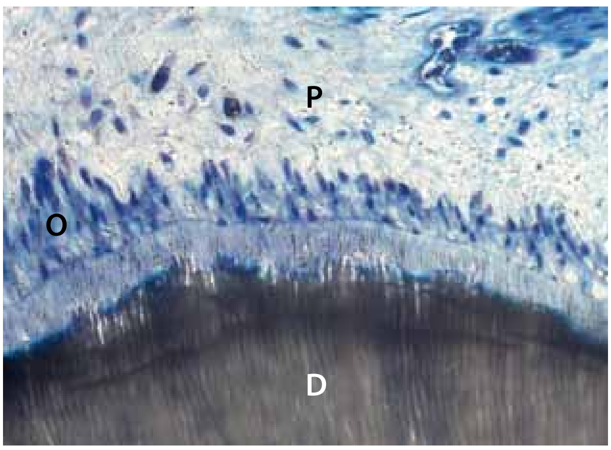

Fig. 10

Normal pulp (P) with odontoblasts (O), D = dentine; toluidine blue, x40.

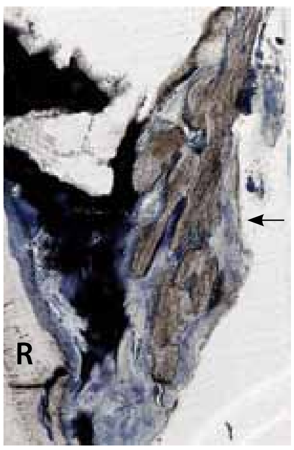

Fig. 11

Cancellous bone (arrow) bridging the periodontal space and encountering root surface (R); toluidine blue, x10

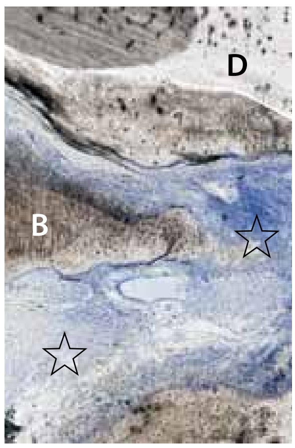

Fig. 12

Contralateral defect with penetrating bony peak (B) into connective tissue of the defect (stars), D = dentine; toluidine blue, x10

The defect was filled with collagenous connective tissue, granulation tissue with vessels and some infiltrations and epithelial rests of Malassez. In the center, bony tissue with osteoid seams and osteoblasts was observed (Fig. 13). From the surrounding connective tissue, fibers were penetrating the bone from its surface and running parallel through the bony matrix. Therefore, the bone appeared as a form of bundle bone (Fig. 14). Osteoid seams were also observed along the surface of the dentin covering the defect. Towards the former root surface, connective tissue connected the defect with the periodontal ligament. In this area, also infiltrations and a proliferating junctional epithelium could be found (Fig. 15).

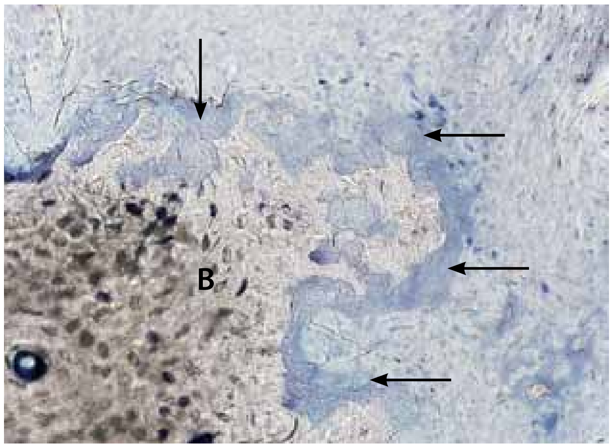

Fig. 13

Osteogenesis around penetrating bone (b) with osteoid (O) seams; toluidine blue, x20

DISCUSSION

In our mind it is important to distinguish between clinical and histological signs for successful periodontal regeneration. In several publications in humans only clinical and radiological signs for periodontal regeneration were evaluated (Carnevale & Kaldahl 2000, Cortelllini et al. 2007). According to these clinical findings the regenerative procedure was determined successful, no information of the amount of true periodontal regeneration versus periodontal repair or root resorption is available in these clinical studies.

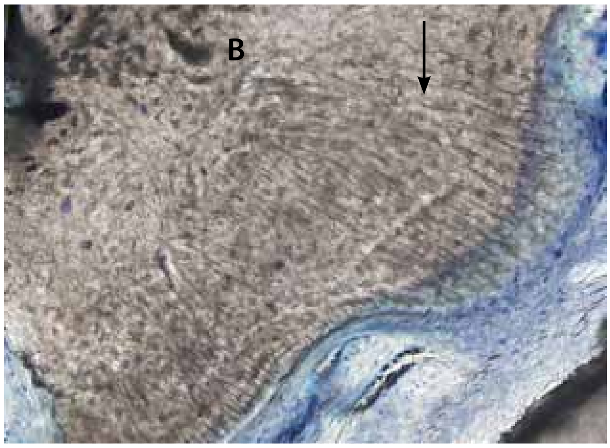

Fig. 14

Fibers (arrow) penetrating newly formed bone (B) in the defect; toluidine blue, x20

This "dilemma" was also experienced in our publication. Teeth that were initially considered hopeless and therefore determined for histopathological investigation turned out to be successfully regenerated using the described surgical protocol. Obviously, those teeth were left in the patient's mouth to their great benefit, however, a meaningful histopathological investigation looking into the potentially great effects oft he uses of PRGF in periodontal regeneration could have not been carried out on those teeth.

The circumferential defect found in the histological specimen (tooth 4) represents the pre-existing periodontal defect observed in this patient which was determined clinically as well as radiographically originating from a chronic inflammatory periodontal lesion. This lesion showed all clinical signs of advanced periodontal disease including pocket probing depth (PPD) of 12mm, bleeding on probing (BOP), furcation involvement of degree III as well as mobility.

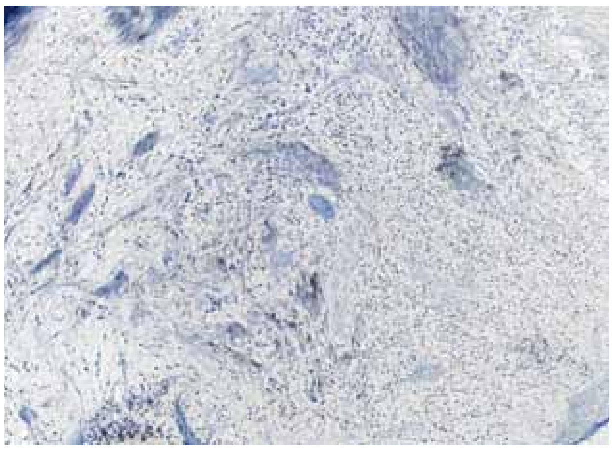

Fig. 15

Periodontal ligament near defect showing signs of inflammation; toluidine blue, x10

The signs of repair by formation of bony tissues are probably related to the effects of PRGF, since no other bone substitute or scaffold has been used during the surgical approach. On one side of the specimen the formation of a bony bridge was observed, probably connecting the alveolar bone with the root surface. This could be interpreted as a beginning ankylosis due to repair processes related to root resorption. The role of PRGF in this process is unclear, it could also be a result of the damage of the periodontal ligament (PDL) during the surgical approach. However, this cancellous bone showed signs of bone remodeling. Contralaterally, de novo formation of bone-like tissues with ongoing osteogenesis including osteoid formation and osteoblast activity was observed. It is possible that these cells originated from the PDL under the influence of PRGF. Physiologically, PDL cells with osteogenic capacity, may be promoted by growth factors in periodontal regeneration, which has been shown in multiple publications investigating the role of enamelum matrix derivates (EMD) in periodontal regeneration. It needs to be determined if the growth factors in PRGF like PDGF, TGF-ß1, FGF, bone sialoprotein or Vascular Endothelial Growth Factor (VEGF) have a similar potential for cell recruitment in periodontal regeneration.

According to morphological aspects, the newly formed tissue appeared as fibrous bone; however we cannot rule out cellular intrinsic fiber cementum, which has developed for example after the application of enamel matrix proteins along human tooth roots (Bosshardt et al. 2005). Similar effects have been shown for the use of PRGF in socket preservation where endogenous bone formation was induced after the application of PRGF. Furthermore, an increased bone density was found in sockets treated with PRGF compared to the control group (Anitua & Orive 2010). In a recent publication, the enhancement of bone regeneration and revascularization is also speculated to underly healing processes in the PRGF therapy of bisphosphonate-related osteonecrosis of the jaw (Mozzati et al. 2012).

After PRGF activation, different osteogenic growth factors and proteins can be released from platelets, like PDGF, TGF-ß1, IGF, bone sialoprotein. The release of VEGF promoting the osteogenesis and angiogenesis relationship may also play a role (Anitua et al. 2008). Cytokines may also be involved in wound healing processes as shown for the expression of anti-inflammatory interleukins after third molar extraction with the application of PRGF (Mozzati et al. 2010).

The histological analysis of the bony tissue located in the depth of the defect showed a bony structure resembling bundle bone as found normally along the periodontal alveolar border: fibers appearing like Sharpey's fibers penetrating from the surrounding connective tissue into the bone and running parallel rectangularly to the surface into the inner matrix, thus, forming a sort of bony attachment. This could be interpreted as an attempt of regenerating a normal periodontium with alveolar attachment in the region of the periodontal defect.

The advantages of the grinding section methods are that no decalcification is necessary, a good structural preservation is given as well as quick processing of the specimens. However, the disadvantages are that there are no special staining methods, e.g. immunohistochemical methods possible. Only standard staining methods can be obtained and only a few grinding sections are available. Therefore, the analysis of decalcified paraffine sections of teeth obtained from future cases will give us the possibility for more detailed investigations. Further histological and especially immunohistochemical studies will be necessary to characterize newly formed bone-like tissue observed in the grinding sections of this case in teeth from the next patients participating in this study.

These preliminary clinical findings clearly demonstrate a positive effect for the regeneration of the periodontal tissues using of PRGF according to this established protocol. An ongoing number of teeth that were initially determined hopeless gained a significant amount of periodontal attachment that was evaluated clinically as well as radiographically and led to the decision to leave these teeth in place. Therefore, a histopathological analysis could not be performed.

Further studies are needed to get more information on a histological level about the gold standard of periodontal regeneration in terms of surgical technique and regenerative agent used

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1997	Final exams : University of Hamburg. Approbation and dental licence
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