Clinical and Histologic Evaluation of Human Intrabony Defects Treated with an Er:YAG Laser. A Case Report Study

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The aim of the present report was to clinically and histologically investigate the healing of advanced intrabony defects in humans following access flap surgery with root surface and defect debridement using an Er:YAG laser. Six patients, each of whom displayed one advanced intrabony defect around teeth or roots scheduled for extraction, were included in the study. The defects were treated with access flap surgery followed by root surface and defect debridement with an Er:YAG Laser (KEY2® KaVo, Biberach, Germany) (160 mJ, 10 pulses/s). The following clinical parameters were recorded at baseline and at 6 months: probing pocket depth (PD), gingival recession (GR) and clinical attachment level (CAL). Healing was uneventful in all cases. Six months after surgery, the teeth or roots were extracted together with some of their surrounding soft and hard tissues and processed for histologic evaluation. Clinical examination revealed PD reduction and a CAL gain in all six cases. The histologic evaluation revealed that healing was predominantly characterized by formation of a LJE along the instrumented root surface. Formation of a new connective tissue attachment (i.e. new cementum with inserting collagen fibers) was observed in two out of the six specimens. New connective tissue attachment was also accompanied by bone regeneration in one of these two specimens. There was no evidence of laser induced thermal side effects in any of the evaluated specimens. Within its limits, the present study failed to show predictable periodontal regeneration in advanced human intrabony defects following access flap surgery with root surface and defect debridement using an Er:YAG laser.

Key words: surgical periodontal treatment, Er:YAG laser, human histology, intrabony defects, long junctional epithelium

INTRODUCTION

A major goal of periodontal treatment is to resolve inflammation and thereby arrest disease progression (Caffesse et al, 1995). Ideally, periodontal therapy not only includes arresting the disease but also the regeneration of the tissues which have been lost due to disease. This includes *de novo* formation of connective tissue attachment and the regrowth of alveolar bone (Caton and Greenstein, 1993). The results from controlled clinical studies have shown that non-surgical (i.e., scaling and root planing using hand instruments (SRP)) and various types of conventional surgical treatment may lead to a clinically important and statistically significant PD reduction and CAL gain (Isidor and Karring, 1986; Kaldahl et al, 1996; Ramfjord et al, 1987). However, histologic studies demonstrated that healing following non-surgical and any type of conventional surgical periodontal therapy is mainly characterized by formation of a LJE along the instrumented root surfaces and no predictable regeneration of attachment apparatus (Bowers et al, 1989; Caton and Greenstein, 1993; Caton et al, 1980; Sculean et al, 2000, 2003). In this context, the formation of a smear layer after both mechanical SRP and ultrasonic instrumentation has been reported to be detrimental

to periodontal tissue healing as it may inhibit reattachment of cells to the root surface (Blomlöf and Lindskog, 1995; Polson et al, 1984). However, additional root surface conditioning with various substances such as ethylenediaminetetraacetic acid gel (EDTA) at neutral pH, citric- and orthophosphoric acids has been shown to be effective in removing the smear layer and exposing the collagenous matrix of dentin (Blomlöf and Lindskog, 1995; Blomlöf et al, 1996; Polson et al, 1984).
In addition to these conventional tools, the use of different lasers has been reported as an alternative therapy for root surface debridement (Aoki et al, 1994; Israel et al, 1997). Close attention has been paid to the clinical applicability of the Er:YAG laser with a wavelength of 2.94 μm in the near infrared spectrum. Because of the high absorption of its emission wavelength by water, this laser system provides a capability to effectively remove calculus from periodontally diseased root surfaces without causing thermal side effects to the adjacent tissue (Aoki et al, 1994; Eberhard et al, 2003; Schwarz et al, 2003d). Another important observation was the lack of a smear layer formation on the root surface after Er:YAG laser instrumentation (Schwarz et al, 2003d; Theodoro et al, 2003). However, histologic and SEM examination showed that under *in vitro* conditions the Er:YAG laser ablated not only the calculus, but also a certain amount of the superficial portion of the underlying cementum (Aoki et al, 1994; Israel et al, 1997; Schwarz et al, 2003d). In contrast, recently published studies reported a lack of cementum removal when laser instrumentation was performed under *in vivo* conditions (Eberhard et al, 2003; Schwarz et al, 2003d). Furthermore, the results from controlled clinical trials and case reports have indicated that non-surgical periodontal treatment with an Er:YAG laser leads to significant CAL gain, (Schwarz et al, 2001, 2003b; Watanabe et al, 1996) even over a 2-year period (Schwarz et al, 2003c).
Preliminary clinical results have also demonstrated that treatment of deep intrabony periodontal defects with the use of an Er:YAG laser and the application of an enamel matrix protein derivative (EMD) may lead to a clinically important and statistically significant gain of clinical attachment, although these results were not superior compared to the group of intrabony defects treated by SRP + EDTA + EMD (Schwarz et al, 2003e). Thus, all the available data taken together seem to indicate that the Er:YAG laser may possess some qualities which could enhance periodontal healing. However, it is currently unknown to what extent the use of an Er:YAG laser may influence the healing when used in conjunction with periodontal surgery. Therefore, the aim of the present series of case reports was to clinically and histologically investigate the healing of advanced intrabony defects in humans following access flap surgery with root surface and defect debridement using an Er:YAG laser.

STUDY DESIGN

Patient Selection

Six patients (3 women, 3 men, mean age 45 years) with one advanced intrabony periodontal defect each around teeth (in total 6 roots) previously scheduled for extraction were selected for treatment. All patients volunteered and received verbal and written information about the purpose and possible risks of the study. Written informed consent was obtained prior to the start of the study. The study protocol was approved by the ethical committee of the Semmelweis University of Medicine, Budapest, Hungary. Criteria for inclusion in the study were: 1) no systemic diseases that could influence the outcome of the therapy; 2) no periodontal root surface treatment within the last 12 months; and 3) a good level of oral hygiene (Plaque Index < 1) (Löe, 1967).

Clinical Management

All patients received oral hygiene instructions and full-mouth laser scaling under local anesthesia 2 months prior to the surgical procedure. All operative procedures were performed under local anesthesia.
Following intracrevicular incisions, full thickness mucoperiosteal flaps were raised buccally and lingually. Notches serving as landmarks for the histologic measurements were prepared on the root surfaces at the most apical part of the defects using a small round bur (diameter: 2 mm). Thus, any periodontal ligament tissue which might be present on the root surface coronal from the apical notch should be considered *de novo* formed connective

Table 1 Defect configuration and clinical measurements at Baseline (0) and after 6 months (6)

Defect	Walls	INTRA	PD(0)	PD(6)	GR(0)	GR(6)	CAL(0)	CAL(6)
1	1–2	4	10	5	3	5	13	10
2	1	4	7	4	3	4	10	8
3	1–2	3	8	5	2	4	10	9
4	1–2	5	10	6	2	4	12	10
5	1–2	4	9	6	4	5	13	11
6	2	5	8	5	3	4	12	9
Mean ± SD			8.7±1.1	5.2±0.7	2.8±0.7	4.3±0.5	11.7±1.2	9.5±1.0

Walls = type of defect (2-wall, etc.)
INTRA = intrabony component

tissue attachment. Root surface and defect debridement was performed with an Er:YAG laser device (KEY2®, KaVo, Biberach, Germany) emitting a pulsed infrared radiation at a wavelength of 2.94 μm without any other mechanical instrumentation. Laser parameters were set at 160 mJ/pulse and 10 pulses/s (Eberhard et al, 2003; Schwarz et al, 2001, 2003b, 2003c, 2003e); pulse energy at the tip (size 0.5 x 1.65 mm) was approximately 120 mJ/pulse. The laser beam was guided onto the root surfaces under water irrigation with a specially designed periodontal handpiece (2056, KaVo, Biberach, Germany) and a chisel-shaped glass fiber tip. The treatment was performed from coronal to apical in parallel paths with an inclination of the fiber tip of 15 to 20 degrees (Folwaczny et al, 2001) to the root surface. The instrumentation was carried out until the operator felt that the root surfaces and defects were adequately debrided. The amount of time needed for root surface and defect debridement was, on average, 10 minutes. All treatments were performed by the same experienced operator.

Clinical Measurements

The following clinical parameters were assessed prior to surgical treatment and before biopsy removal using the same type of manual periodontal probe (PCP 12, Hu-Friedy Co., Chicago, Illinois, USA): PD, GR and CAL. All measurements were made by one blinded and previously calibrated investigator at six aspects per tooth: mesio-vestibular (mv), mid-vestibular (v), disto-vestibular (dv), mesio-oral (mo), mid-oral (o) and disto-oral (do). In cases where the cemento-enamel junction (CEJ) was not clearly distinguishable, the margin of a restoration was used for the measurements. During surgery, the following measurements were made: distance from the CEJ to the bottom of the defect (CEJ–BD) and 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) minus (CEJ–BC). The study reports only measurements at the same deepest point of the selected defect.

Biopsy Removal and Histological Preparation

Following local anesthesia, paramarginal incisions were performed and full-thickness mucoperiosteal flaps were raised. The teeth or roots were then removed together with their surrounding soft and hard tissues. After postsurgical healing, patients were provided complete prosthodontic rehabilitation. Immediately upon removal, the biopsies were fixed in 10% buffered formalin, decalcified in EDTA, dehydrated, and fixed in paraffin. Mesiodistal serial sections were cut parallel to the long-axis of the teeth with the micrometer set at 8 μm. Sections representing the central part of the defect were selected for histologic evaluation and stained with hematoxylin-eosin.

RESULTS

The configuration and depth of the intrabony component as measured during surgery is presented in Table 1. The postoperative healing was uneventful

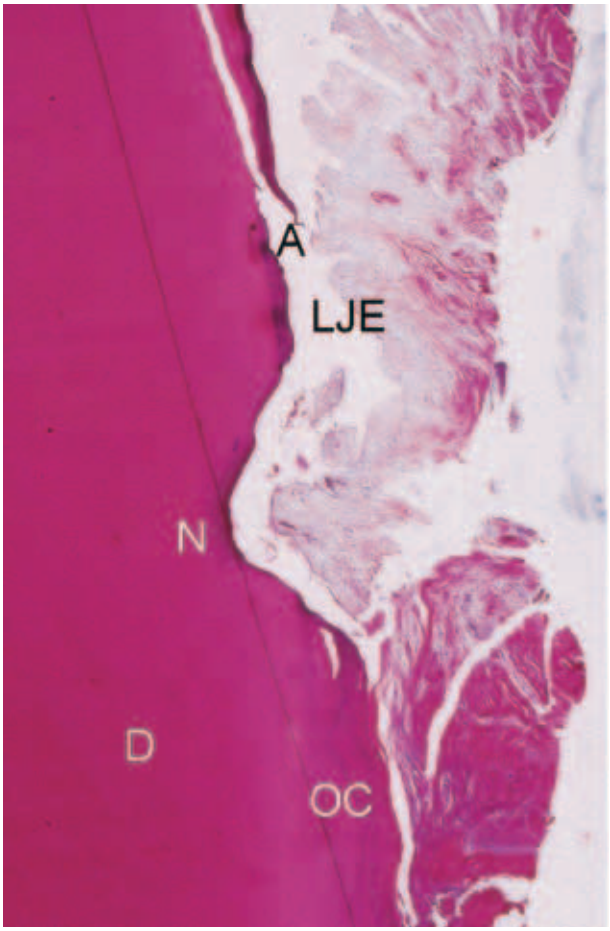

Fig. 1 Following access flap surgery with root surface and defect debridement using an Er:YAG laser histologic view revealed formation of a LJE (LJE) along the instrumented root surface. A=artifact; D=dentin; N=notch, OC=old cementum (hematoxylin-eosin stain; original magnification x 25).

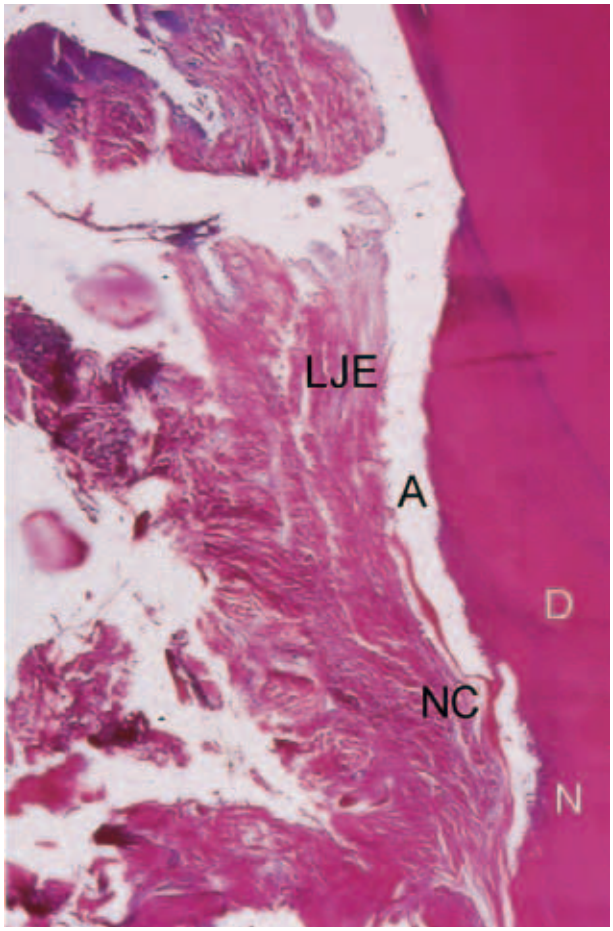

Fig. 2 A minute amount of reparative cementum was observed only occasionally, and was limited to the most apical part of the defect. A=artifact; D=dentin; N=notch; NC=new cementum (hematoxylin-eosin stain; original magnification x 25).

in all cases. No complications such as abscesses or infections were observed throughout the study period. Furthermore, there were no adverse effects of Er:YAG laser treatment on tooth vitality or tooth sensitivity. The clinical results at 6 months revealed PD reduction and a CAL gain in all treated defects (Table 1). The histologic evaluation showed that in four out of the six specimens, healing was predominantly characterized through formation of a LJE along the instrumented root surface (Figs. 1–2). A minute amount of reparative cementum was observed only occasionally, and was limited to the most apical part of the defect (Figs. 2–3).

However, in two out of the six specimens, the histologic assessment revealed the formation of new connective tissue attachment (i.e. cementum with inserting collagen fibers) coronally to the notch on the root surface (Fig. 4). In one of these two specimens the new attachment was also accompanied by new bone (Fig. 5a). The newly formed cementum was connected to the newly formed bone (Fig. 5b). Artifacts (splits between LJE or regenerated cementum and the root surface) were observed in all biopsies (Figs. 1–6). The Er:YAG laser produced homogeneous and nearly smooth root surfaces without visible traces of the fiber tip in use. Loss of cementum was generally non-existent or minimal (Figs. 1–5). Histologically, no thermal damage, such as carbonization, melting or cracking, was observed.

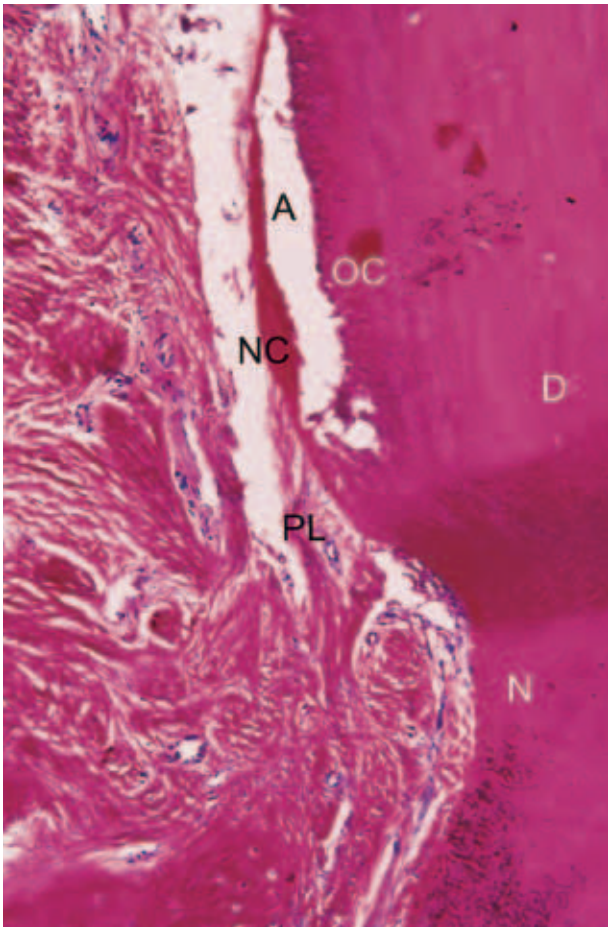

Fig. 3 Higher magnification of notch area demonstrated some formation of cementum with inserting periodontal ligament fibers. A=artifact; D=dentin; N=notch; NC=new cementum; OC=old cementum; PL=new periodontal ligament (hematoxylin-eosin stain; original magnification x 50).

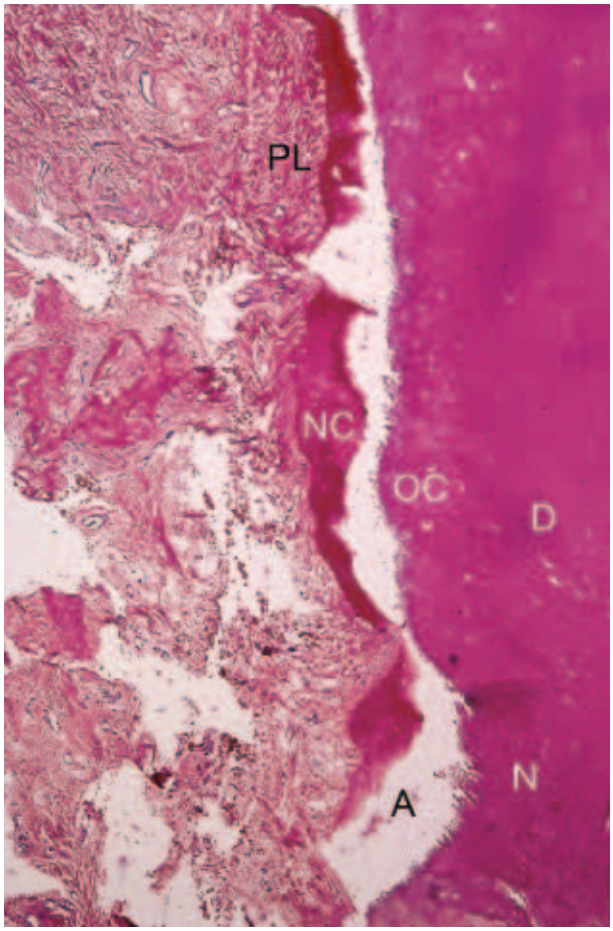

Fig. 4 Cementum with inserting collagen fibers has formed on the root surface in the demarcated area. There were no signs of thermal damage, such as carbonization, melting or cracking. A=artifact; D=dentin; N=notch; NC=new cementum; OC=old cementum; PL=new periodontal ligament (hematoxylin-eosin stain; original magnification x 25).

DISCUSSION

The results of the present series of case reports have shown that surgical treatment of advanced intrabony periodontal defects followed by root surface and defect debridement using an Er:YAG laser led to clinical improvements as indicated by PD reductions and CAL gains. The observation that the use of an Er:YAG laser for root surface and defect debridement neither led to postoperative complications nor to impaired clinical healing indicates that this type of laser may not have any detrimental effect when employed in conjunction with periodontal surgery. In this context, it needs to be pointed out that this series of case reports are the first data evaluating the use of an Er:YAG laser for the treatment of advanced intrabony periodontal defects. However, to a certain extent, the present clinical findings corroborate the results from recent controlled clinical studies evaluating conventional access flap surgery (Camargo et al, 2000; Cortellini et al, 1996; Sculean et al, 2001).

The present histologic evaluation showed that in all specimens, healing was predominantly characterized by formation of a LJE along the instrumented root surface. Formation of a minute amount of cementum with inserting collagen fibers was found only occasionally, and was limited to the most apical part of the defects. These observations are in agreement with findings from previous histologic studies which have shown that healing following non-surgical therapy, as well as con-

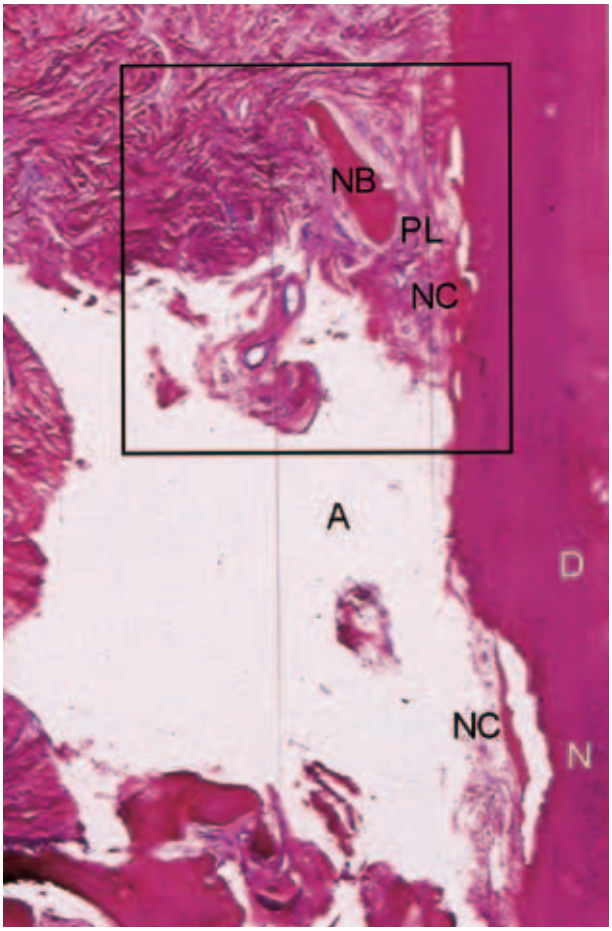

Fig. 5a Histologic view of a specimen showing periodontal regeneration. A=artifact; D=dentin; N=notch; NB=new bone; NC=new cementum; PL=new periodontal ligament (hematoxylin-eosin stain; original magnification x 25).

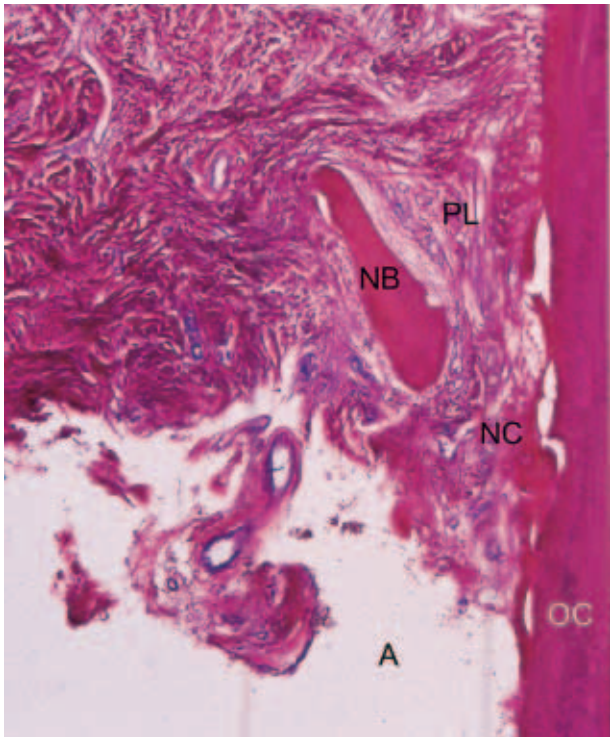

Fig. 5b Higher magnification of the regenerated area shown in Fig. 5a demonstrated formation of cementum and of alveolar bone. A=artifact; D=dentin; N=notch; NB=new bone; NC=new cementum; PL=new periodontal ligament (hematoxylin-eosin stain; original magnification x 50).

ventional access flap surgery results mainly in formation of a LJE to the bottom of the defect and no predictable *de novo* formation of connective tissue attachment and regrowth of alveolar bone (Bowers et al, 1989; Caton and Greenstein, 1993; Caton et al, 1980; Sculean et al, 2003). However, two specimens showed the formation of new cementum along the instrumented root surface with inserting collagen fibers. In one of the specimens, the new attachment was also accompanied by new bone.

Until now, periodontal regeneration has been histologically observed only after a combination of surgical access and various additional procedures such as barrier membranes, or application of EMD (Bowers et al, 1989; Sculean et al, 1999a, 1999b, 2000; Yukna and Mellonig, 2000). There might be several explanations for the present findings. First, it is important to point out that diverse histologic reults reflect the biologic variability in wound healing. On the other hand, as mentioned above, the lack of a smear layer formation on the root surface after Er:YAG laser instrumentation might have facilitated reattachment of cells from the periodontal ligament. In this context, it is important to point to the results from previous studies which have shown that the surface structure of previously diseased roots after Er:YAG laser instrumentation seem to offer better conditions for the adherence of periodontal ligament

(PDL) fibroblasts than SRP with hand instruments (Rossa et al, 2002; Schwarz et al, 2003a). Nevertheless, the present series of cases failed to show predictable periodontal regeneration after this treatment modality.

Another important point is the lack of controls using conventional open flap debridement for comparison in each patient. However, it must be pointed out that the present case reports were set up as a proof of principle study, not as a comparative study. On the other hand, as mentioned above, recent histologic studies provide clear evidence that healing following non-surgical therapy, as well as conventional access flap surgery results mainly in formation of a LJE (Bowers et al, 1989; Caton and Greenstein, 1993; Caton et al, 1980; Sculean et al, 2003). Another important observation of the present study was the presence of histological artefacts in all specimens. The clinical significance of such artefacts is still controversial. While some authors consider the presence of splits between the newly formed and old cementum or dentin to be mainly a result of the decalcification process during the histological preparation (Listgarten, 1972), others have interpreted similar findings as a poor quality of the regenerated tissues (Nalbandian and Frank, 1980). Further research is needed in order to clarify this issue.

The present investigation also demonstrated that the Er:YAG laser produced homogeneous and nearly smooth root surfaces. A loss of cementum was generally non-existent or minimal. These findings are in agreement with results from recent studies which reported a lack of cementum removal after Er:YAG laser instrumentation under *in vivo* conditions (Eberhard et al, 2003; Schwarz et al, 2003d). The histologic evaluation showed that after SRP 73.2% of root dentin was completely denuded from cementum, while only a minimal cementum reduction was apparent after laser irradiation (Eberhard et al, 2003).

In contrast, previous studies showed that the Er:YAG laser ablated not only the calculus, but also the superficial portion of the underlying cementum when instrumentation was performed *in vitro* (Aoki et al, 1994; Folwaczny et al, 2001). This discrepancy might be explained by a diffusion of heat within the pocket, due to the presence of bleeding that occurs during laser instrumentation under *in vivo* conditions. Nevertheless, energy settings should be kept to a minimum in order to avoid a removal of underlying sound tissue. Recently, a subgingival calculus detection system with fluorescence induced by 655 nm InGaAsP diode laser radiation has been included in an Er:YAG laser device. Preliminary *in vitro* results have shown that 655 nm diode laser radiation induces significantly stronger fluorescence in subgingival calculus than in cementum, suggesting that calculus removal may be selectively performed (Folwaczny et al, 2002). The clinical relevance of this system still remains questionable. Another point of interest may be the evaluation of the relative cost-effectiveness of laser treatment when compared to other well documented treatment procedures used for periodontal flap surgery.

In conclusion, within its limits, the present study failed to show predictable periodontal regeneration in advanced human intrabony defects following access flap surgery with root surface and defect debridement using an Er:YAG laser.

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