การยึดเกาะของเซลล์เอ็นยึดปริทันต์มนุษย์บนผิวรากฟันที่ได้รับการขูดด้วยเครื่องขูดอัลตราโซนิกส์และฉายเลเซอร์ Er,Cr:YSGG

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บทคัดย่อ

วัตถุประสงค์: การรักษาโรคปริทันต์โดยไม่อาศัยการทำศัลยกรรมปริทันต์ยังคงให้ผลการรักษาที่จำกัด ปัจจุบันมีการศึกษาที่แสดงถึงผลดีของการใช้เลเซอร์ในทางทันตกรรมมากขึ้น การใช้เลเซอร์ร่วมในการรักษาจึงอาจเพิ่มผลสำเร็จในรักษาโรคปริทันต์ได้ งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลทางห้องปฏิบัติการของการใช้เลเซอร์ Er,Cr:YSGG ในการปรับสภาพผิวรากฟันที่เป็นโรคปริทันต์อักเสบ หลังผ่านการขูดหินน้ำลายและเกลารากฟันด้วยเครื่องขูดอัลตราโซนิกส์ ต่อการยึดเกาะของเซลล์เอ็นยึดปริทันต์มนุษย์ที่เลี้ยงในห้องปฏิบัติการ

วัสดุอุปกรณ์และวิธีการ: เตรียมผิวรากฟันตัวอย่าง จากฟันที่ถอนด้วยโรคปริทันต์อักเสบรุนแรง และฟันปกติที่ถอนด้วยเหตุผลทางทันตกรรมจัดฟัน แบ่งเป็น 5 กลุ่ม คือ กลุ่มควบคุมลบ เตรียมจากฟันเป็นโรคปริทันต์ กลุ่มที่เป็นโรคปริทันต์ผ่านการขูดหินน้ำลายและเกลารากฟันด้วยเครื่องขูดอัลตราโซนิกส์เพียงอย่างเดียว กลุ่มที่ผ่านการขูดหินน้ำลายและเกลารากฟันด้วยเครื่องขูดอัลตราโซนิกส์ และได้รับเลเซอร์ Er,Cr:YSGG ที่ความถี่ 30 เฮิรตซ์ อีกกลุ่มหนึ่งใช้ความถี่ 50 เฮิรตซ์ตามลำดับ และกลุ่มควบคุมบวกเตรียมจากฟันปกติ จากนั้นเลี้ยงเซลล์เอ็นยึดปริทันต์มนุษย์ให้เกิดการยึดเกาะกับผิวรากฟันแต่ละกลุ่ม จนวันที่ 5 จึงทำการเปรียบเทียบจำนวนเซลล์ที่ยึดเกาะโดยการตรวจสอบความมีชีวิตของเซลล์บนผิวรากฟัน ใช้จำนวนตัวอย่างกลุ่มละ 25 ชิ้น

ผลการทดลอง: ผิวรากฟันที่ได้รับเลเซอร์มีจำนวนเซลล์ยึดเกาะมากกว่ากลุ่มที่ผ่านการขูดหินน้ำลายและเกลารากฟันด้วยเครื่องขูดอัลตราโซนิกส์เพียงอย่างเดียวและกลุ่มที่เป็นโรคปริทันต์ พบว่ากลุ่มที่ใช้เครื่องขูดอัลตราโซนิกส์เพียงอย่างเดียว มีจำนวนเซลล์น้อยกว่ากลุ่มฟันปกติอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) ขณะที่กลุ่มที่ได้รับเลเซอร์ที่ความถี่ 50 เฮิรตซ์ มีจำนวนเซลล์ยึดเกาะจำนวนมาก ไม่แตกต่างจากกลุ่มฟันปกติอย่างมีนัยสำคัญทางสถิติ

สรุปผล: การใช้เลเซอร์ Er,Cr:YSGG สามารถปรับสภาพผิวรากฟัน ช่วยให้เกิดสภาวะที่เหมาะสมและมีความเข้ากันได้ทางชีวภาพ จึงเอื้อต่อการยึดเกาะของเซลล์เอ็นยึดปริทันต์มนุษย์

คำสำคัญ: เลเซอร์ในทางทันตกรรม Er,Cr:YSGG การรักษาโรคปริทันต์โดยไม่อาศัยการทำศัลยกรรมปริทันต์ การปรับสภาพผิวรากฟัน การขูดหินน้ำลายและเกลารากฟันด้วยเครื่องอัลตร้าโซนิกส์

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PDL Fibroblasts Attachment to Root Surfaces Treated by Ultrasonic Debridement and Er,Cr:YSGG Laser

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Abstract

Background/objectives: Nonsurgical periodontal therapy is clinically limited in success outcome. Several studies of dental laser have been previously published the clinical improvement especially when it was used in adjunct with conventional scaling and root planing. This in vitro study was performed to evaluate the effects of Er:Cr:YSGG laser for adjunctive using with ultrasonic root debridement on the attachment of human periodontal ligament fibroblasts to periodontal disease root surfaces.

Materials and methods: Root surface specimens were prepared from extracted hopeless periodontal disease teeth and healthy premolars extracted for orthodontic reason. Root specimens were divided into five groups as different treated conditions: untreated periodontal disease group, untreated healthy group, periodontal disease treated by ultrasonic debridement only group, ultrasonic debridement followed by 30 Hz Er,Cr:YSGG laser irradiation and another group with 50 Hz laser irradiation. Twenty-five specimens in each group were cultured with human periodontal ligament fibroblasts. The attached cells were compared by cell viability assay after 5 days of culture.

Results: Cell viability test revealed higher attached cells in Er,Cr:YSGG laser irradiation groups than ultrasonic debridement only and untreated periodontal disease group. Ultrasonic debridement only group had significantly lower attached cells compare to untreated healthy group ($p < 0.05$). 50Hz laser irradiation group yielded the highest attached cells close to healthy group as the statistical test showed no significantly different between these two groups.

Conclusion: The adjunctive use of Er,Cr:YSGG laser on previously diseased root surface may enhanced surface biocompatibility, therefore, facilitate in cell attachment.

Keywords: Er,Cr:YSGG, Laser, Periodontitis, Root treatment, Ultrasonic debridement

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Introduction

The major purpose of periodontal treatment is to control inflammatory process caused by bacterial infection. Bacteria adhere to the root surface inside periodontal pocket in the form of biofilms, colonies of microbes encased by self-produced matrix, which are impossible to eliminate by patient's self-care or any kind of chemical agents [1]. Only mechanical removal of subgingival deposit by dental professionals can disrupt dental plaque biofilms, therefore, the gold standard of nonsurgical periodontal therapy is still scaling and root planing concurrently with oral hygiene instruction until now [2]. Scaling and root planing can be achieved by hand instrumentation with various types of instrument designs to facilitate this operation. It is still considered as time consuming procedure which required high skill of the operator especially in the deep periodontal pocket and furcation area of multiple root molar [3]. The precisely movement of operator's hands accompany with properly controlled force must be used to remove calculus. To overcome these obstacles, power-driven scaler such as ultrasonic scaler has been invented [4]. The vibration of scaler tip in high frequency can remove adhered deposits from tooth structure efficiently. Furthermore, ultrasonic scaler also generates cavitation [5] and acoustic microstreaming [6] which add cleansing effects to this device. New generation ultrasonic scaler has optimized frequency and amplitude to be safely used subgingivally with subgingival tips. Clinical studies showed similar treatment outcomes between hand and ultrasonic scaler [7]. However, in the deep periodontal pocket and complicated multiple root molar, the approaching of mechanical instrument is sometimes impossible. Another limitation is mechanical scaling and root planing leaves smear layer which can inhibits cell reattachment [8].

Dental laser has been introduced in many fields of dentistry. Several studies of Erbium family laser reported bactericidal, detoxification, calculus removal without the formation of smear layer [9, 10]. Er,Cr:YSGG is the latest laser of Erbium family. Er,Cr:YSGG laser is suitable to use in periodontal treatment due to highly absorb in both hard and soft tissue which are the main components of the periodontium. Er,Cr:YSGG with appropriate energy setting could be use for calculus removal without alterations to the root structure [11, 12]. Irradiated root surface by Er,Cr:YSGG laser became more favorable for cell attachment. [13]. Er,Cr:YSGG can be promising treatment option. Nevertheless, the laser device consists of laser handpiece with the delicate optical tip which may difficult to use for calculus removal in clinical situation due to lack of tactile sensitivity to detect calculus. In case of heavy calculus deposit, the lasing time must be increase and this would jeopardize surrounding tissue.

Both limitations lead to concept of combination treatment; ultrasonic debridement follow by Er,Cr:YSGG irradiation. There are not enough data to evaluate this method especially in cell attachment experiment. The aim of this study was to investigate the in vitro effects of Er:Cr:YSGG laser and its frequency of irradiation in adjunctive using with ultrasonic root debridement on the attachment of cultured human periodontal ligament (HPDL) fibroblasts.

Materials and methods

Specimen Preparation

The extracted hopeless severe periodontitis teeth and healthy premolars extracted by orthodontic reason were collected and used in this study. All teeth must have no caries, fillings, fracture and endodontic treatment. Root surface specimens were prepared by trephine bur No.4/5 (4 mm inner diameter), cut approximately 4 mm below cemento-enamel junction in mesio-distal direction. Cylinder shape of root surfaces was acquired. Diamond disk was used for trimming until specimens had 2 mm height (Fig. 1). Root surface specimens were cleaned and kept in normal saline solution at 4°C until the time of treatment.

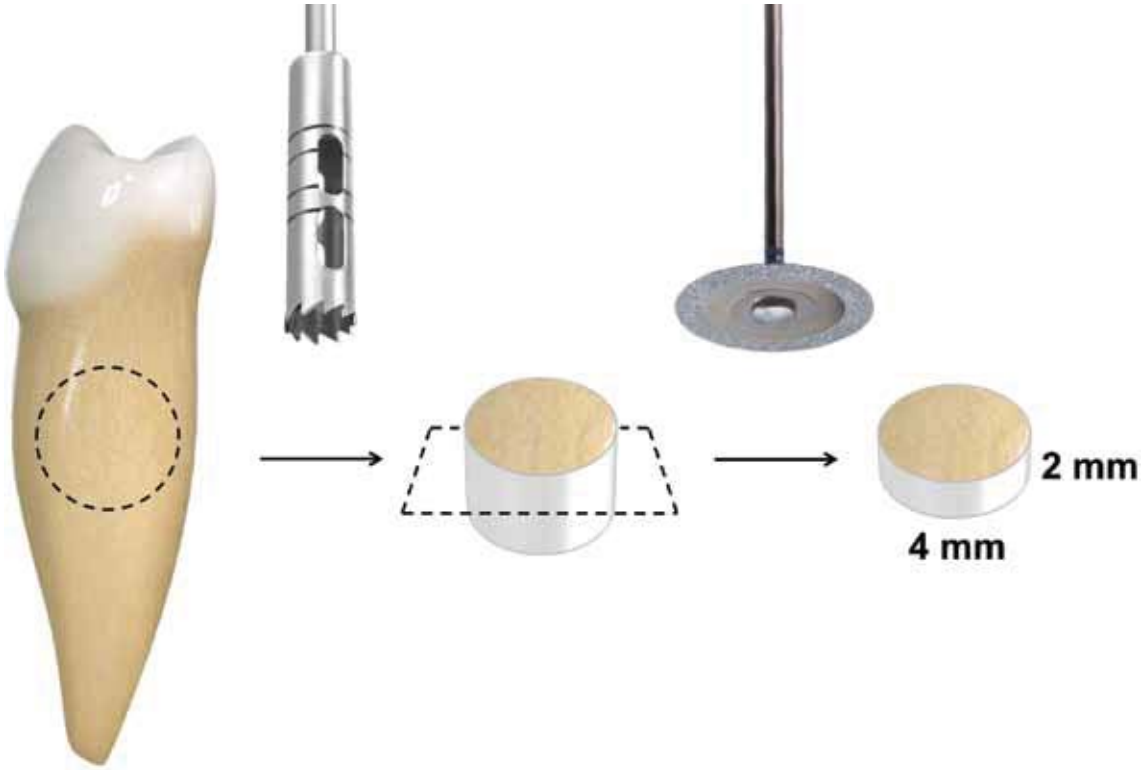


Figure 1. Specimen preparation in this study.

A total of 125 root surface specimens were divided into 5 groups as different treated conditions (Table 1).

Table 1. Fives groups of root surface specimens.

Perio	Untreated periodontal disease group
Ultra	Periodontal disease treated by ultrasonic debridement only
Laser30	Periodontal disease treated by ultrasonic debridement follow by laser irradiation 1.5 W, 40% water, 20% air, H-mode, frequency 30 Hz
Laser50	Periodontal disease treated by ultrasonic debridement follow by laser irradiation 1.5 W, 40% water, 20% air, H-mode, frequency 50 Hz
Normal	Untreated healthy group

Ultrasonic Debridement

Root surface specimens in Ultra, Laser30 and Laser50 groups were debrided by piezoelectric ultrasonic scaler (P5 Newtron® XS, Satelec, France) with micro curette insert No. H3. Ultrasonic debridement was done at the power level of 7-8 until the root surface specimens were visually clean.

Er,Cr:YSGG Laser Irradiation

Laser30 and Laser50 groups were irradiated by Er,Cr:YSGG laser (WaterLase iPlus™, Biolase, USA) with the settings for periodontitis treatment i.e. 1.5 Watt (W) of energy, 40% water, 20% air, H-mode, 30 and 50 Hertz (Hz) of frequency respectively. The RFPT-5 tip (radial firing perio tip) was used for laser irradiation with 1 mm distance away from root surface. According to clinical usage of this tip, the laser tip was placed parallel to the surface of root specimen, lasing thoroughly in left-right and up-down directions for 30 seconds. Specimens were rinsed with distilled water.

All specimens were autoclaved in phosphate buffered saline (PBS) at 121°C for 15 minutes before cell culture experiment.

HPDL Fibroblasts Culture

Human periodontal ligament (HPDL) fibroblasts used in this experiment were obtained from extracted premolars of 16 years old healthy female subject by the objective of orthodontic treatment. Periodontal ligament tissues were dissected from middle part of the root. Tissue fragments were placed in culture medium consist of DMEM, 10% FBS, L-glutamine and Antibiotics until cells reached confluency. Cells were trypsinized and the fourth passage was used for this experiment. This study protocol was approved by the Ethics Committee in Human Research at the Faculty of Dentistry, Srinakharinwirot University.

To compare the number of attached cell on the root specimens of each group. HPDL fibroblasts were cultured with root specimens in 6-well plates. HPDL fibroblasts were seeded at the concentration of 2.0x105 cells/ml. After allowed

cells attachment for 24 hours, specimens were transferred to 24-well plates. Attached cells on the root surface specimens were continued incubation. The cultured medium was changed on the third day. On the fifth day, cell viability assay was performed to compare the number of attached cells. This study utilized WST-8 assay (CCK-8, Dojindo, Japan) which is colorimetric assay. Water-soluble tetrazolium salts were reduced by hydrogenase enzymes from the viable cells. End-products were yellow color of water-soluble formazan which represented the number of the living cells. Root specimens were rinsed with PBS to remove unattached cells. Specimens were moved to 96-well plates with 100 μL of cultured medium and 10 μL of WST-8 solution. After 4 hours of reactions, the solutions of each well were pipetted to new 96-well plates. The absorbances of colored solutions were measured as optical density (OD) by microplate reader at the 450 nm of wavelength. The measurements were repeated 3 times. The experiment procedure was shown in figure 2.

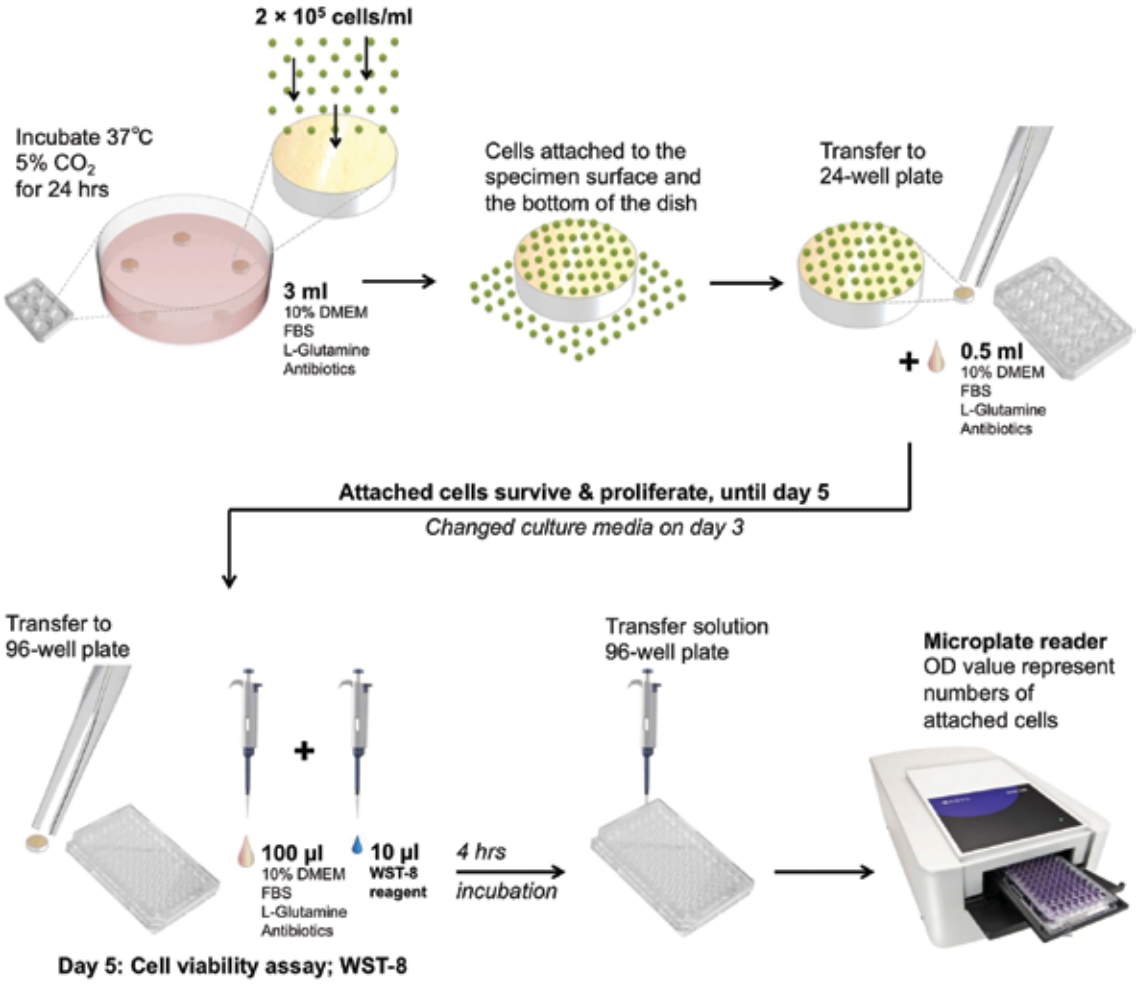


Figure 2. Experiment procedure for cell viability test on root surface specimens.

Statistical Analysis

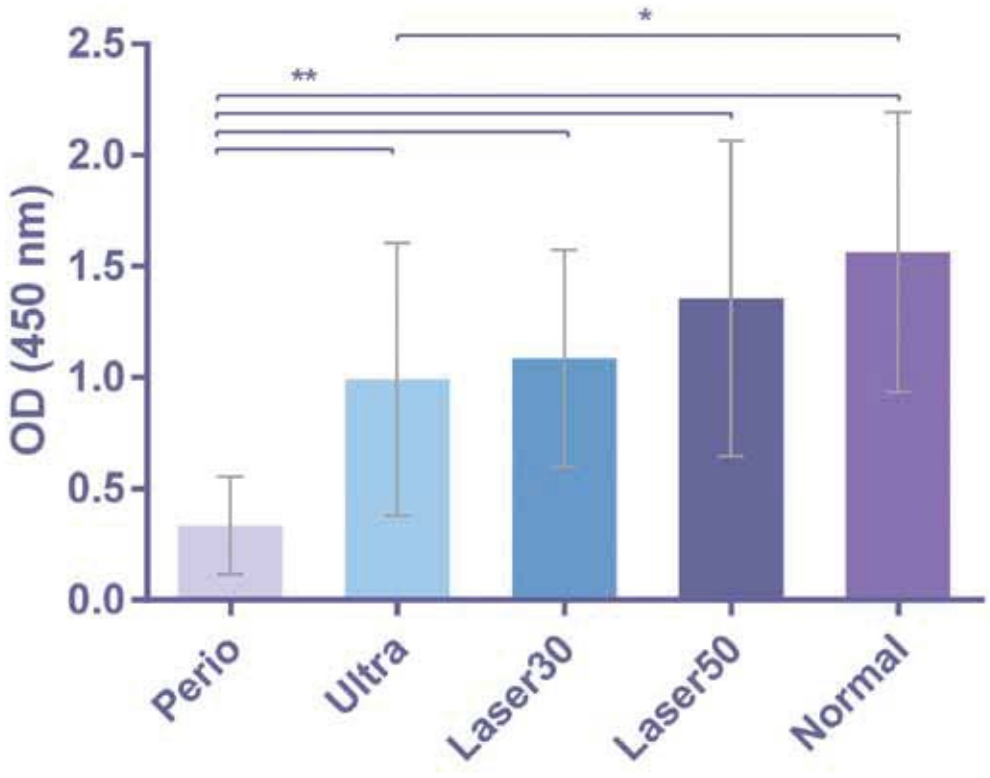
OD values of each group were calculated into mean and standard deviation. The differences between each group were analyzed by Kruskal-Wallis and Dunn's multiple comparisons test using statistical software.

Results

Mean and standard deviation of OD values which represented the number of viable cells attached on the root surface specimens were shown in table 2 and figure 3

Table 2. Mean and standard deviation values of OD which represented the number of viable cells attached on root surface specimens.

	Perio (N=25)	Ultra (N=25)	Laser30 (N=25)	Laser50 (N=25)	Normal (N=25)
OD (450 nm)	0.33 ± 0.22	0.99 ± 0.61	1.09 ± 0.49	1.36 ± 0.71	1.56 ± 0.63



*Statistically significant difference (Dunn's multiple comparisons test; $p < 0.05$)

**Statistically significant differences (Dunn's multiple comparisons test; $p < 0.01$)

Figure 3. Comparison of viable cells on root surface specimens between groups.

Among the treated groups, the Laser50 group exhibited the greatest number of attached cells. This was followed by the Laser30 and the Ultra group, respectively. However, the differences were not statistically significant. The mean OD of Laser50 group was noticeable close to the Normal group as the statistical test revealed no significantly different between these two groups. Ultra group was found to have the lowest attached cells in all treated groups and was statistically significant lower than the Normal group which served as positive control of this experiment ($p < 0.05$). Perio group, negative control of this experiment, showed the least number of attached cells ($p < 0.01$).

Discussion

Dental lasers have been continuously developed and improved. Er,Cr:YSGG laser is used for periodontal therapy due to the wavelength is highly absorbed in both soft and hard tissue of the periodontium. This study was performed to confirm the capability of human periodontal ligament fibroblasts in re-attach to periodontal disease root surface treated by ultrasonic debridement followed by Er,Cr:YSGG laser irradiation.

Root surface bio-modification has been extensively studied in the past. The attempt to modify previously diseased root surface has been introduced to enhance biocompatibility of the root surface. Various kinds of chemical agent such as tetracycline, citric acid, EDTA have been used in order to remove smear layer formed by mechanical scaling and root planing, eliminate bacterial contamination of the surface, expose collagen matrix in cementum. Although this treatment modality seemed to have no additional benefit in the treatment of chronic periodontitis [14], this concept emphasized the importance of root surface bio-modification.

The effect of laser on the root surface bio-modification reported positive in vitro outcomes especially in Erbium family. Irradiated root surface exhibited no smear layer, reduced bacterial endotoxin, without thermal damage [9]. In 2002 Schoop et al. observed more spindle-shape fibroblasts announced in root surface irradiated by Er:YAG [15]. Schwarz et al. (2003) reported higher number of periodontal ligament fibroblasts on previously diseased root surfaces treated by Er:YAG compare to ultrasonic and curette groups [16]. Feist et al. (2003) found that Er:YAG laser irradiation promoted faster adhesion and growth compared to root planing [17]. Crespi et al. (2006) compared Er:YAG treated versus ultrasonic scaler also found significantly higher cell density number in laser-treated specimens [18]. These findings might indicate the ability of Erbium family laser to create biocompatible root surface but there were no strong data support from the Er,Cr:YSGG laser.

Er,Cr:YSGG laser with the 2,780 nm wavelength close to Er:YAG (2,940 nm) has been widely used for periodontal treatment at present day, but there are few studies in cell attachment on Er,Cr:YSGG irradiated surface. The available information about energy settings in clinical usage was come from the expertise more than experimental research. This study also used two different frequency settings to determine proper energy output for root surface modification.

The result of this study demonstrated that Er,Cr:YSGG laser was able to create biocompatibility root surface on previously diseased root. The adjunctive use of Er,Cr:YSGG at 1.5W,

40% water, 20% air, H-mode, 50 Hz of frequency showed higher attached cells compared to ultrasonic debridement only. However, there was no statistically significant difference. This trend was in agreement with the findings of Hakki et al. (2010) [13]. Although the mentioned study used conventional end-firing laser tip, this study used radial firing tip designed for periodontal treatment. With the 30 Hz frequency of laser, the number of attached cells was not significantly higher than ultrasonic debridement. This may because of the lower frequency, the higher laser energy ablate root surface specimen. The surfaces of this group were found to have distinctively irregular. This finding was similar to Galli et al. (2009) the laser produced rough and irregular surface which was less favorable for cell adhesion and growth [19].

Untreated periodontal disease group presented the least attached cells. The surfaces of this group were found to have residual calculus, roughness, and irregular. Although sterilization with autoclave was applied before cell culture, bacterial endotoxin might reside in the surface. These interferences could diminish cell attachment and growth in this group. For the untreated healthy group, the number of attached cells was the highest. This finding was in contrary with Hakki et al. (2010) which found that laser treated group exhibited higher attached cell than healthy group [13]. However, this discrepancy was not statistically significant in this study.

This study used WST-8 instead of conventional MTT assay for cell viability test. The advantages of this method are higher sensitivity because WST-8 can also detect dehydrogenase enzyme not only from mitochondria but also from the entire part of the cells. The WST-8 only needs one step procedure therefore, reduce experimental error. To accurately compare the number of attached cells on the specimen by chemical reagent, the surface area of each specimen must be equal. Root specimens in this study were prepared with trephine bur, resulted in equal circular surface area. According to the pilot study, specimens were prepared in rectangular shape which was impossible to cut equal in size.

Recently clinical studies of Er,Cr:YSGG laser [20-22] have positive treatment outcomes. The similar results of these studies were reduction of probing depth, attachment gain without relation to gingival recession. These findings might prove the potential of Er,Cr:YSGG to facilitate in the regeneration of periodontium. Based on Melcher's hypothesis [23], periodontal wound healing depends on the progenitor cells that migrate to root surface. True periodontal regeneration could be achieved when periodontal ligament cells attached to the root surface. These reasons explained the concept of deep pocket therapy (DPT) by Er,Cr:YSGG laser. The pocket lining epithelium and granulation tissues were ablated by the Er,Cr:YSGG laser with the radial firing tip. The outer epithelium was also removed to retard epithelial downgrowth allowed regeneration of periodontium. The finding of this study would imply that Er,Cr:YSGG could enhance cells attachment to root surface consequently, facilitate in periodontal regeneration.

Nonsurgical periodontal therapy could be accomplished using scaling and root planing but the treatment outcomes were limited. Mechanical debridement could not totally remove subgingival contaminants especially in complicated root morphology. Smear layer formation after debridement

also inhibited the attachment of cells to the root surface. Er,Cr:YSGG laser application may improve treatment results by detoxification, smear layer removal and modification of the root surface to increase biocompatibility for cells attachment and growth.

Further studies are required to understand cellular behavior on the irradiated surface such as confocal microscopy, scanning electron microscopy which might be helpful in evaluating the distinctive data trends even though there were no statistically significant differences in this experiment.

Conclusion

Within the limits of this experimental design, the results of this study indicated that the adjunctive use of Er,Cr:YSGG laser on previously diseased root surface may enhanced cell attachment compared to ultrasonic debridement only.

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