MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2022; 28: e932191
DOI: 10.12659/MSM.932191

Received: 2021.03.17
Accepted: 2021.09.15
Available online: 2021.10.07
Published: 2022.01.05

Effect of Twinlight Laser on the Attachment of Human Gingival Fibroblasts to the Root Surface In Vitro

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Financial support: This study was supported by the Department of Finance of Hebei Province (No. 2019061441-2)
Conflict of interest: None declared

Background: This study aimed to compare the effectiveness of subgingival scaling and root planing with the Twinlight laser, Er: YAG laser, and hand instrumentation on the removal of endotoxin and attachment of human gingival fibroblasts (HGFs) to cementum surfaces in vitro.

Material/Methods: Single-rooted teeth extracted for periodontal disease were collected and divided into 3 groups: group A, root planing with Gracey curet no. 5/6; group B, irradiation with Er: YAG laser; group C, irradiation with Er: YAG laser and Nd: YAG laser. Endotoxins were determined by the limulus amebocyte lysate test. Cell attachment and proliferation of HGFs on root specimens were evaluated by cell counting kit-8 assay. The root surface and cell morphology were observed by scanning electron microscope.

Results: A flat root surface with scratches was found in group A, Group B had a homogeneous rough morphology without carbonization, and group C had a non-homogeneous rough morphology with ablation. The endotoxin concentration was highest in group A ($P<0.05$) and lowest in group C ($P>0.05$). HGFs cultured in group B showed significantly increased adhesion and proliferation compared with groups A and C ($P<0.05$). HGFs in group B were well attached, covered densely by pseudopodia. HGFs in group A were round with poor extension and short pseudopodia, while the cells in the group C were in narrow, triangular, or polygonal shapes.

Conclusions: Twinlight laser-assisted periodontal treatment effectively improved the biocompatibility of root surface and promoted the attachment and proliferation of fibroblasts by removing calculus and reducing the concentration of endotoxins.

Keywords: Chronic Periodontitis • Fibroblasts • Laser Therapy • Er: YAG • Twinlight Laser

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/932191

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Background

Periodontitis is a chronic inflammatory disease of dental supporting tissue. As the main initiating factor of periodontal diseases, plaque biofilm can cause inflammation and immune responses and lead to the development of gingivitis, periodontal pockets, and attachment loss [1]. Endotoxin is the main component of the outer membrane of most gram-negative bacteria and has a direct cytotoxic effect [2]. Endotoxins inhibit the expansion and destruction of periodontal ligament fibers and play a key role in the occurrence and development of periodontal disease [3]. Scaling and root planing (SRP), as the conventional mechanical method, is the most commonly used therapy in nonsurgical periodontal treatment, and its effectiveness has been confirmed. However, some special anatomical sites, such as deep periodontal pockets (pocket depth >5 mm) and furcation involvement, are difficult to access. It is also difficult to completely remove bacterial deposits and toxins from root surfaces and periodontal pockets, which results in the poor formation of new attachments.

In recent years, an increasing number of researchers have been studying the role of lasers in periodontology [4]. Lasers with different wavelengths have been investigated for periodontal debridement, including the erbium: yttrium aluminum garnet (Er: YAG), 2940 nm; neodymium: yttrium aluminum garnet (Nd: YAG), 1064 nm; diode, 635 to 980 nm; and carbon dioxide (CO_2), 9600 nm and 10 600 nm [5]. It is reported that the Er: YAG laser can effectively ablate hard dental tissues [6]. The water molecules and hydroxyapatite particles in the target areas absorb a large amount of energy during Er: YAG laser irradiation. This energy accumulation leads to a rise in temperature and an instant microburst of water molecules [7]. The Nd: YAG laser can be easily absorbed in the hemoglobin and melanin, and it has excellent bactericidal, coagulating, and physiotherapy effects [8]. Based on these findings, the Er: YAG and Nd: YAG lasers are considered potential treatments of periodontal diseases. The current literature regarding lasers in periodontal therapy is contradictory. Many researchers reported the biocompatibility of the Er: YAG laser and its effectiveness during root debridement [9]. However, there is still no consistent evidence supporting the routine use of the Er: YAG laser in the treatment of periodontitis, either as an alternative monotherapy or adjunctive to traditional scaling and root planing. To date, it is believed that the Nd: YAG laser should be employed as an adjunct to traditional scaling and root planing rather than as a monotherapy. Although it has an excellent antimicrobial effect, there is no evidence that the Nd: YAG laser used as a monotherapy or adjunctive therapy for scaling and root planing is capable of "sterilizing" a periodontal pocket [6].

Because of the anatomical structure of periodontal tissues, a combination of the Nd: YAG and Er: YAG lasers could be a better choice to deal simultaneously with the problems of soft and hard tissues in patients with chronic periodontitis. Thus, a protocol for minimally invasive, efficient, and safe treatment that combines both types of high-power lasers was developed. Grzech-Leśniak et al [10] reported that the combination of Nd: YAG and Er: YAG lasers additionally improved the microbiological and clinical outcomes of nonsurgical periodontal therapy in patients with moderate to severe chronic periodontitis. It was found that the combined laser therapy could further diminish pathogenic microorganisms and improve the clinical outcomes of nonsurgical periodontal therapy, especially for patients with deep pocket depths (>4 mm [10] or >7 mm [11]). The Er: YAG laser was used for root debridement and the Nd: YAG laser was used for removing pocket epithelium and for purposes of detoxification. These researchers suggest that the combined irradiation of the Nd: YAG and Er: YAG lasers may be beneficial on a short-term basis, particularly when treating inaccessible areas, such as where there is furcation involvement and deep periodontal pockets.

The Twinlight laser clinical protocol, using the Nd: YAG and Er: YAG lasers in combination, suggests that the direction of the Nd: YAG laser should be to the periodontal pocket wall and the direction of Er: YAG laser should be to the root surface. However, the Nd: YAG laser will inevitably also irradiate the root surface when irradiating the periodontal pocket. Whether the irradiation of the Nd: YAG laser will affect periodontal adhesion in clinical operation is unknown. Therefore, in this study, the root surfaces from teeth with periodontitis were treated with either the Twinlight laser, Er: YAG laser, or hand instruments. We sought to identify a superior periodontal treatment that would obtain more regenerated periodontal attachments and provide new information for laser treatment of periodontitis by detecting the concentration of bacterial endotoxins and observing the surface morphology and attachment of human gingival fibroblasts (HGFs).

Material and Methods

Ethical Approval

This study was approved by the Ethics Committee of the School and Hospital of Stomatology, Department of Oral and Maxillofacial Surgery, Hebei Medical University, China (no. [2019]034). All participating patients read and signed an informed consent form before tooth extraction and gingival tissue collection.

Sample Preparation and Group Allocation

A total of 50 teeth were freshly extracted from patients with stages III and IV periodontitis [12] and 3 healthy premolars

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were removed for orthodontic reasons. Inclusion criteria were as follows: (1) single-rooted teeth that had visible calculus and were free of caries; (2) patient had no history of scaling or root planing in the previous 6 months; (3) patient had no history of systemic diseases; and (4) patient did not smoke or consume alcohol. Immediately after tooth extraction, the teeth were washed with physiological saline to remove the attached soft tissue and immersed in phosphate-buffered saline (PBS) solution at 4°C.

After the subgingival plaque and calculus were removed with an ultrasonic scaler (P-tip, Electro Medical Systems, Switzerland) for 1 min, the samples were randomly divided into 3 groups. In group A (n=27), root surfaces were treated with manual instruments (Gracey curette, Hu-Friedy, Chicago, IL, USA). The working side of the Gracey curette no. 5/6 was placed about 1 to 2 mm on the calculus at 80° against the tooth surface until the root surface was smooth. In group B (n=27), root surfaces were irradiated with the Er: YAG laser as follows: MSP; 50 mJ/pulse; 15 Hz; 0.75 W; water 4, Air 4, 25 J/cm^2. In group C (n=27), using the Twinlight laser, root surfaces were irradiated with the Er: YAG laser as follows: MSP; 50 mJ/pulse; 15 Hz; 0.75 W; water 4, air 4, 25 J/cm^2. Next, they were irradiated with the Nd: YAG laser as follows: MSP, 15 Hz, 1.50 W.

With water cooling, the crown was cut off at 2 mm below the cementoenamel junction to 5 mm above the apical part of the root with an emery excircle cutting piece. After all treatments, the root slices were washed and soaked in PBS 3 times, each time at 20°C for 5 min. A total of 99 fragments (4×4×1 mm) with periodontitis and 6 fragments with healthy premolars were prepared from the root surfaces. Among them, 42 samples were used for quantitative detection of endotoxins, 9 samples were used for scanning electron microscopy (SEM) observation of root surfaces, 9 samples were used for HGF adhesion detection, and 45 samples were used for an HGF proliferation study. All samples were placed in the same glass plate filled with PBS solution, and the grouping of sample processing was randomly assigned. All the above operations were completed by one experienced and calibrated operator who did not partake in the allocation, examination, or statistical analysis and was supervised by a senior therapist. The single-blind experimental design was used for the experiment.

Laser Irradiation

A Twinlight laser (Lightwalker AT, Fotona, Slovenia) with Er: YAG and Nd: YAG laser equipment was used. The Er: YAG laser (emission wavelength 2940 nm) was selected. A periodontal handpiece (H-14) with a spot size of 0.5 mm was used and was moved continuously in contact with the root surface at an angle of 10° to 15° and a distance of 1.0 cm. The root surface was irradiated with the Er: YAG laser for 10 s each time, and continuous water spray was used for cooling. The Nd: YAG laser (wavelength 1064 nm) was used to irradiate the root surface at an angle of 30° with optic fiber (spot size of 300 μm). The Nd: YAG laser worked for 30 s each time, and the handpiece was continuously moved to cover the entire sample surface.

Endotoxin Quantitation

An orthodontic group was used as a negative control for the experiment and a periodontitis group was used as a positive control. The samples were divided into the following 5 groups: orthodontic group, periodontitis group, group A, group B, and group C, with 6 samples per group. In the orthodontic group, the periodontal ligament and residual soft tissue were removed. In the periodontitis group, residual soft tissue and plaque were removed with a new toothbrush and treated for 20 s per tooth. The procedures used in groups A, B, and C were as described above.

The treated samples were placed in depyrogenated EP tubes, and 1 mL of endotoxin test reagent was added to each tube. Then the samples were incubated in a 37°C water bath for 1 h and heated at 70°C for 10 min to inactivate the protein. Centrifugation was done at 3500 rpm at -4°C for 15 min, and the supernatant was discarded. The endotoxin concentration of each sample was determined by the limulus amebocyte lysate test according to the manufacturer's protocol. The optical density (OD) value was read by a microplate reader at a wavelength of 545 nm. The endotoxin concentration of each group was calculated with a standard curve.

Cell Culture

The gingival tissues used in this experiment were collected from healthy donors who underwent lower third molar extraction in the Department of Oral and Maxillofacial Surgery, Hospital of Stomatology Hebei Medical University. The collected tissues were washed 3 times in PBS containing antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin; Gibco). To cultivate the HGFs, the gingival tissues were cut into small pieces (1×1×1 mm^3) and cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) with antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin; Gibco) and 20% fetal bovine serum (FBS, Gibco) in 5% CO_2 at 37°C. The culture medium was changed every 2 days. When the primary cells covered 80% of the bottle bottom, the cells were passaged at a ratio of 1: 2 or 1: 3. The HGFs in passage 4 to 7 were used in this study. An inverted microscope (Olympus, Japan) was used to identify the cell morphology.

Cell Adhesion

Samples were put into a 24-well plate and the experiments were conducted in triplicate. The cell suspension was prepared

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and 1×10^5 HGFs were inoculated on each sample. The control group was inoculated with the same amount of cells, and the blank control group was established with a cell-free medium. After 2 h of cell culture, the liquid on the surface of the samples was removed with sterile filter paper. The samples were transferred to a new 24-well plate and washed with PBS buffer twice. An amount of 400 μL of DMEM and 40 μL of cell counting kit-8 (CCK-8) solution was added to each well. After 2 h of inoculation at 37°C, 100 μL of supernatant from each well was collected, and a spectrophotometer was used to determine the OD values at 450 nm. The relative cell adhesion rate was calculated as previously reported [13].

Cell Proliferation

Samples were put into a 24-well plate, and the experiments were conducted in triplicate. Single cell suspensions of HGFs at 1×10^5 cells/mL were obtained by cell trypsinization and then seeded on the specimens. Then the cells were cultured in DMEM containing 10% FBS and antibiotics for 5 days. The specimens were taken out at the same time every day and washed 3 times with PBS. CCK-8 analysis was used to evaluate cell viability [14].

Scanning Electron Microscopy

The specimens for SEM were fixed with 2.5% glutaraldehyde and dehydrated with graded ethanol solutions (50%, 70%, 80%, 90%, 95%, and 100%). After vacuum drying and sputter coating with gold, the HGFs inoculated on the root surfaces were observed by SEM (S-3500N, Hitachi, Japan). Micrographs of cell attachment on the specimens were taken from the sample surfaces at 400× to 2500× magnification.

Statistical Analysis

SPSS version 21.0 (IBM Corp, Armonk, NY, USA) was used for the statistical analysis. The data are described as $\bar{\chi}\pm s$. One-way analysis of variance (ANOVA) was used for comparison among groups, and multiple comparisons were performed by the Student-Newman-Keuls method. Repeated measures ANOVA was used to analyze repeated observations. $P<0.05$ was considered to be statistically significant.

Results

Morphology of Sample Surface

The root surfaces that were scraped by the Gracey curette (group A) were smooth and flat, with scratches and scattered calculus coated by a smear layer. There were signs of cementum ablation, and no dentin tubules were exposed (**Figure 1A, 1B**). The surfaces irradiated by the Er: YAG laser (group B) were rough and homogeneous, with no obvious fusion, carbonization, scratches, crack, or smear layer. Granular protrusions were found in the cementum, and no molten mineral was deposited (**Figure 1C, 1D**). After the combined irradiation (group C), the root surfaces showed a non-homogeneous morphology and were composed of different degrees of tooth hard-tissue ablation and a small amount of smear layer. The dentin tubules were partially fused or closed, without any carbonization (**Figure 1E, 1F**).

Quantitative Detection of Endotoxins

There were no statistical differences in endotoxin concentrations between all samples in the orthodontic group and periodontitis group ($P>0.05$). Compared with that of the periodontitis group, the endotoxin concentration of each treatment group decreased significantly ($P<0.05$). Among the 3 experimental groups, the endotoxin concentration in group A was the highest and was significantly different from that in groups B and C ($P<0.05$). The concentration of endotoxin in group C was the lowest, and there was no significant difference between group C and group B ($P>0.05$). Differences in endotoxin concentrations compared before and after each treatment showed no statistical difference between groups B and C ($P>0.05$) and were all statistically significantly different from that of group A ($P<0.05$) (**Tables 1, 2; Figure 2**).

Cell Culture

After 7 to 10 days of culture, a small number of cells emerged from the center of the tissue block and, at around 14 days, they covered the bottom of the culture bottle (**Figure 3A**). After subculture of passages 2 to 3, the cells were arranged in a radial or swirling pattern, showing a typical fibroblast morphology (**Figure 3B**). The cells were a long spindle-shape or star-shape, and the nuclei were round or oval. Cytoplasmic processes and nucleoli were clearly visible. Immunocytochemical staining showed vimentin-positive cytoplasm and cytokeratin-negative cytoplasm in the cells (**Figure 3C, 3D**). The growth curve of HGFs was close to the S-type, which indicated that HGFs grew well and according to the characteristics of culture and proliferation in vitro (**Figure 4**). HGFs in passages 4 to 7 were used in the experiments.

Cell Adhesion

The relative adhesion rates (%) of each group after 2 h of culture are shown in **Table 3**. The differences among the 3 experimental groups were significant ($P<0.05$). Multiple comparisons between the groups showed that the relative adhesion rate of group B was significantly higher than that of groups A and C (**Figure 5**).

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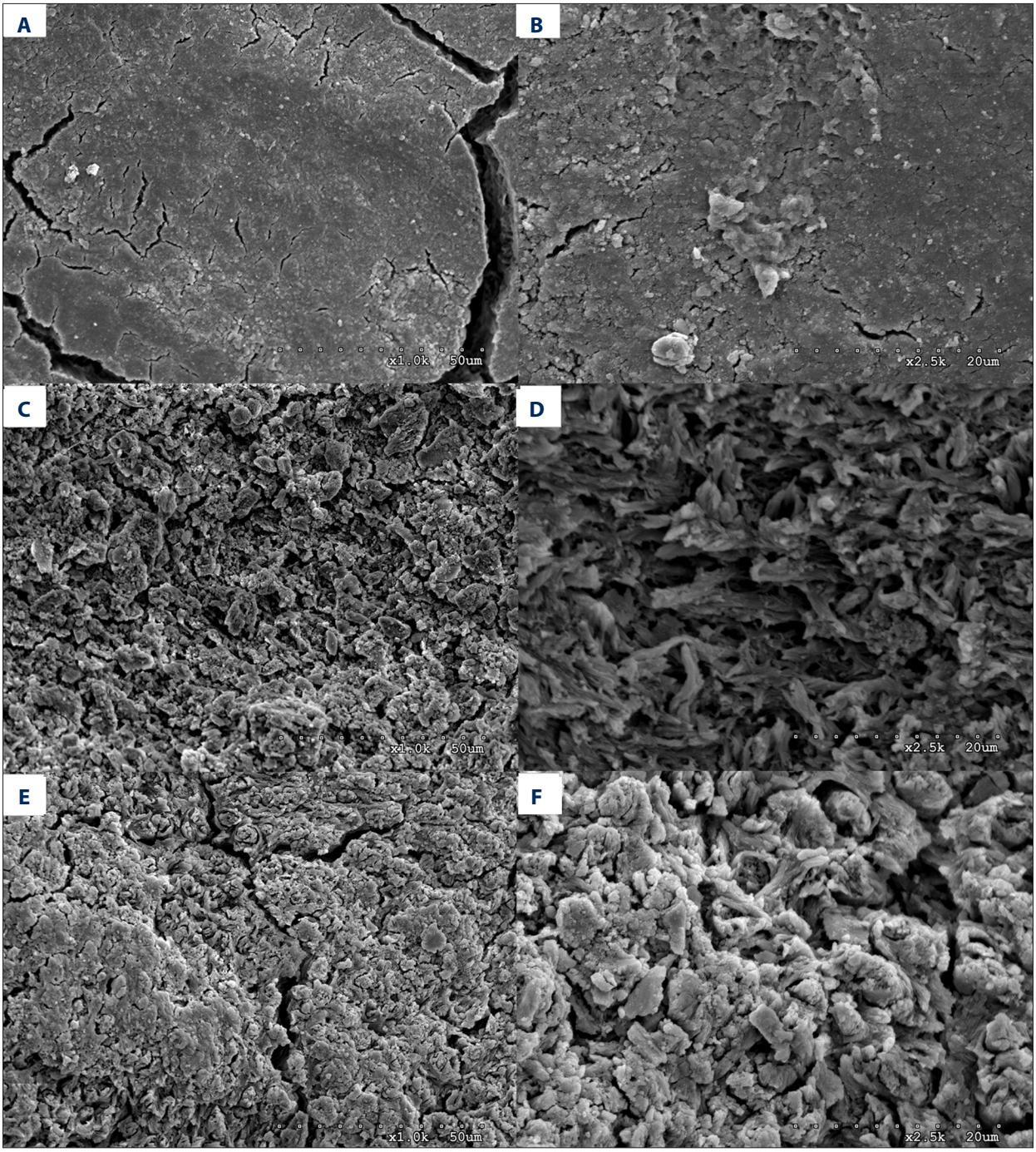

Figure 1. (**A, B**) The root surface scraped by the Gracey curette (group A) was smooth and flat (scanning electron microscope [SEM], 1000×, 2500×). (**C, D**) The surface irradiated by the Er: YAG laser (group B) was rough and homogeneous (SEM, 1000×, 2500×). (**E, F**) The surface irradiated by the Er: YAG and Nd: YAG lasers (group C) showed a non-homogeneous morphology (SEM, 1000×, 2500×).

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Table 1. Endotoxin concentration of each group.

Group	n	Endotoxin concentrations (EU/mL)	F	P
Group Orthodontic	6	0.72±0.12[&]		
Group Periodontitis	18	12.75±1.63[#]		
A	6	3.84±1.44[#&]	176.292	<0.001
B	6	1.15±0.58[&*]		
C	6	0.85±0.32[&*]		

[#] Statistically significant vs the orthodontic group, $P<0.05$; [&] statistically significant vs the periodontitis group, $P<0.05$; * statistically significant vs group A, $P<0.05$.

Table 2. Endotoxin difference between each treatment group.

Group	Difference (EU/mL)	F	P
A	8.09±1.96[&]		
B	11.67±1.91[#]	17.633	<0.001
C	12.45±1.20[#]		

[#] Statistically significant vs group A, $P<0.05$; [&] comparison between groups, $P<0.05$.

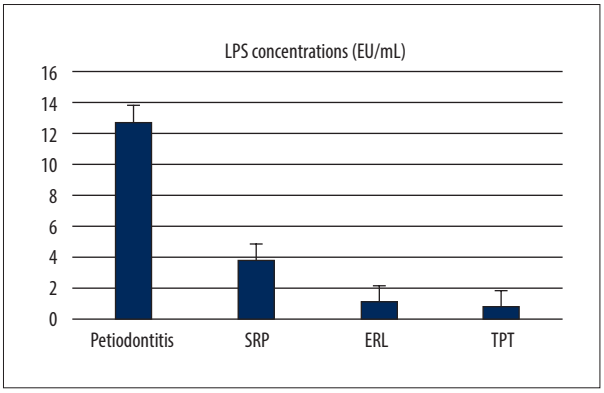

Figure 2. Concentrations of endotoxin in the cementum after treatment. Statistically significant vs the control group, $P<0.05$ * Statistically significant vs group A, $P<0.05$

Cell Proliferation

The CCK-8 method was used to detect cell proliferation, and the average absorbance was expressed as x±s (**Table 4**). The number of cells increased at first and then decreased slowly, being the fastest on the third day and reaching a peak on the fifth day. On the first day, the OD value of group A was significantly lower than that of groups B and C, and no significant difference between the Er: YAG laser debridement group and the Twinlight laser debridement group was found ($P>0.05$). No significant differences in pairwise analysis was found at any other time ($P<0.05$) (**Figure 6**).

Microscopy of Cells Cultured on the Sample Surface

In group A, HGFs were round and flat and had a reduced extracellular matrix. The cells were sparse and poorly stretched, and the pseudopodia were few and short (**Figure 7**). In group B, the cells adhered well and grew densely. The cells extended outward, and the pseudopodia were evenly distributed around the cells. The pseudopodia were intertwined into a network and tightly attached to the surface of the sample (**Figure 8**). The cell body was full and fully extended, and the sample surface was covered by the extracellular matrix of the HGFs. The number of HGFs in group C was lower than that in group B in most visual fields under the microscope, and the pseudopodia in group C were fewer and shorter than those in group B. The cell extension was weaker in group C, and the shapes of the HGFs were narrow triangles or polygons in the distant pores (**Figure 9**).

Discussion

Periodontitis is one of the most common chronic inflammatory diseases in the world. It is mainly caused by bacterial plaque and calculus that are attached to the root surfaces of teeth, resulting in attachment loss of the periodontal ligament. Therefore, the priority of periodontal therapy is to remove the pathogenic microorganisms that form dental plaque and calculus on root surfaces to promote the healing and regeneration of damaged periodontal tissue. The cementum plays an important regulatory role in periodontal regeneration and is necessary for the formation of new attachments [15]. Therefore,

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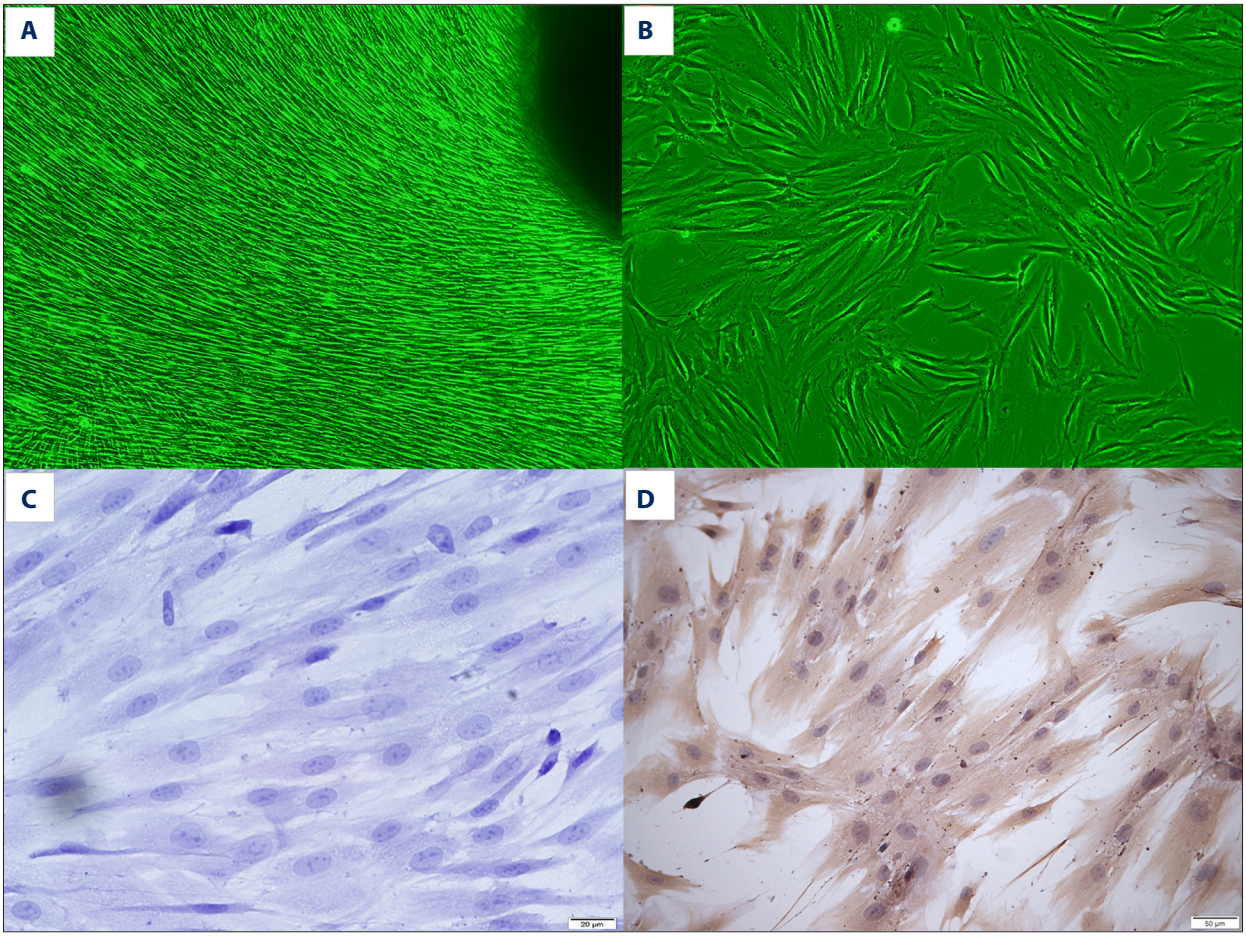

Figure 3. (**A**) The primary culture cells of human gingival fibroblasts (HGFs) (inverted microscope, 100×). (**B**) The fourth generation of culture cells of HGFs (inverted microscope, 100×). (**C**) Immunocytochemical staining showing cytoplasm of cultured HGFs was negative for cytokeratin (inverted microscope, 400×). (**D**) Immunocytochemical staining showing cytoplasm of cultured HGFs was positive for vimentin (inverted microscope, 200×).

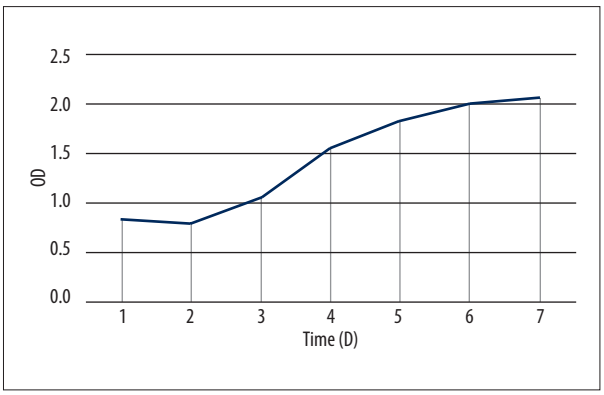

Figure 4. The cell growth curve of human gingival fibroblasts.

the first step to obtaining periodontal tissue regeneration is eliminating the bacterial deposits on the root surface, which enables the root surface to have good biocompatibility after periodontal therapy.

Table 3. Relative adhesion rate of human gingival fibroblasts (%) (n=3).

Group	$\bar{x}\pm s$
A	29.13±1.56
B	35.10±1.08*
C	32.43±0.57*#

* Statistically significant vs group A, $P<0.05$; # statistically significant vs group B, $P<0.05$.

Lasers have been used in dentistry since their introduction in 1960. The Er: YAG laser, which has a wavelength of 2940 nm, appears to be a good choice for ablation of dental hard tissues and bone [16]. Several in vitro studies have suggested favorable biocompatibility of periodontitis-affected root specimens from human teeth following Er: YAG-mediated scaling [17].The 1064-nm wavelength of the Nd: YAG laser exhibits high absorption in pigmented soft tissues and hemoglobin and is thereby effective

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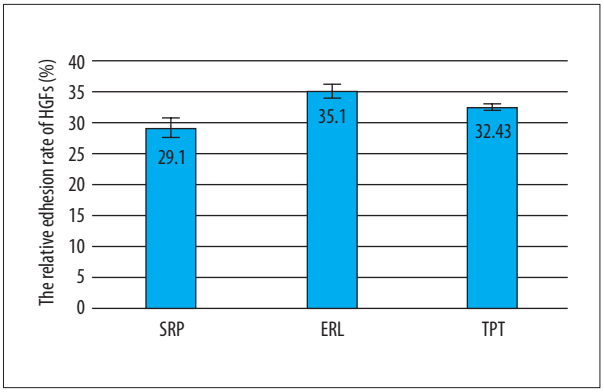

Figure 5. The relative adhesion rate of human gingival fibroblasts in each group (%).

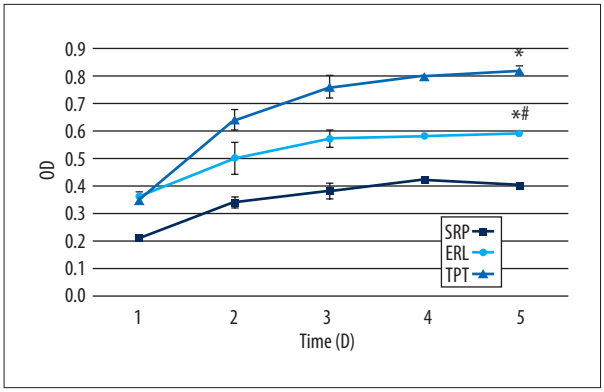

Figure 6. The proliferation of human gingival fibroblast cells in each group. * Statistically significant vs group A, $P<0.05$; # Statistically significant vs group B, $P<0.05$.

Table 4. Optical density value of each group at different times ($\bar{\chi}\pm s$).

Group	Time				
	1	2	3	4	5
A	0.21±0.01	0.34±0.02	0.38±0.03	0.42±0.01	0.40±0.01
B	0.35±0.02*	0.64±0.06*	0.76±0.03*	0.80±0.01*	0.82±0.01*
C	0.36±0.01*	0.50±0.04*#	0.57±0.04*#	0.58±0.01*#	0.59±0.02*#

* Statistically significant vs group A, $P<0.05$; # statistically significant vs group B, $P<0.05$.

in coagulation and hemostasis during soft-tissue surgical procedures [18]. It is this proclivity for soft-tissue applications that has resulted in promotion of the Nd: YAG laser for treatment of periodontal diseases. Both the Er: YAG and Nd: YAG lasers are suitable for periodontal therapy. Therefore, the Twinlight laser was developed and with soft and hard tissue lasers, it can be effectively applied to soft and hard tissue, giving it high clinical efficacy. The manufacturer recommended protocol for the Twinlight laser is as follows: "Laser treatment is performed in 3 steps, including decontamination of the periodontal pocket (Nd: YAG), removal of the pocket epithelium and the content of the periodontal pocket, scaling of the root surfaces (Er: YAG), and stabilization of the blood clot inside the periodontal pocket (Nd: YAG)". However, the Nd: YAG laser will inevitably irradiate the root surface when irradiating the periodontal pocket. It is unknown whether the irradiation of the Nd: YAG laser will affect periodontal adhesion in clinical operation. To address this question, we designed the present in vitro experiment.

With respect to root surfaces, the Er: YAG laser-exposed samples (group B) was rough and homogeneous, with no obvious fusion, carbonization, scratches, crack, or smear layer. Granular protrusions were found in the cementum, and no molten mineral was deposited. This result was similar to that of previous studies, which reported no apparent heat-induced damage, such as crazing, melting, or carbonization, of the surface [19]. After the combined irradiation (group C), the root surface showed a non-homogeneous morphology, and was composed of different degrees of tooth hard tissue ablation and a small amount of smear layer. The dentin tubules were partially fused or closed, without any carbonization. Therefore, the Nd: YAG laser has been shown to induce undesirable changes on the root surface [20].

Endotoxin is found in the subgingival plaque and calculus that is attached to the root surface in periodontitis. The infiltration of endotoxins into the cementum is one of the most destructive periodontal pathogenic factors and directly inhibits the growth of fibroblasts in tissue culture [21]. Previous studies proved that the removal of infected cementum promotes tissue healing [22]. However, some studies suggest that the attachment of endotoxins to the surface of the cementum is loose and removable [23]. Furthermore, excessive root planing to remove endotoxins can lead to dentin hypersensitivity or pulp injury and affect the new attachment of fibroblasts [24]. Therefore, the concept of excessive root planing for endotoxin removal has been questioned in various studies [25]. To remove all bacterial deposits, subgingival instruments will inevitably remove excessive cementum, resulting in scratches and peeling on the root surface in the same direction as hand instruments. Consequently, the results of SEM micrographs showed that the root surfaces scraped by the Gracey curette

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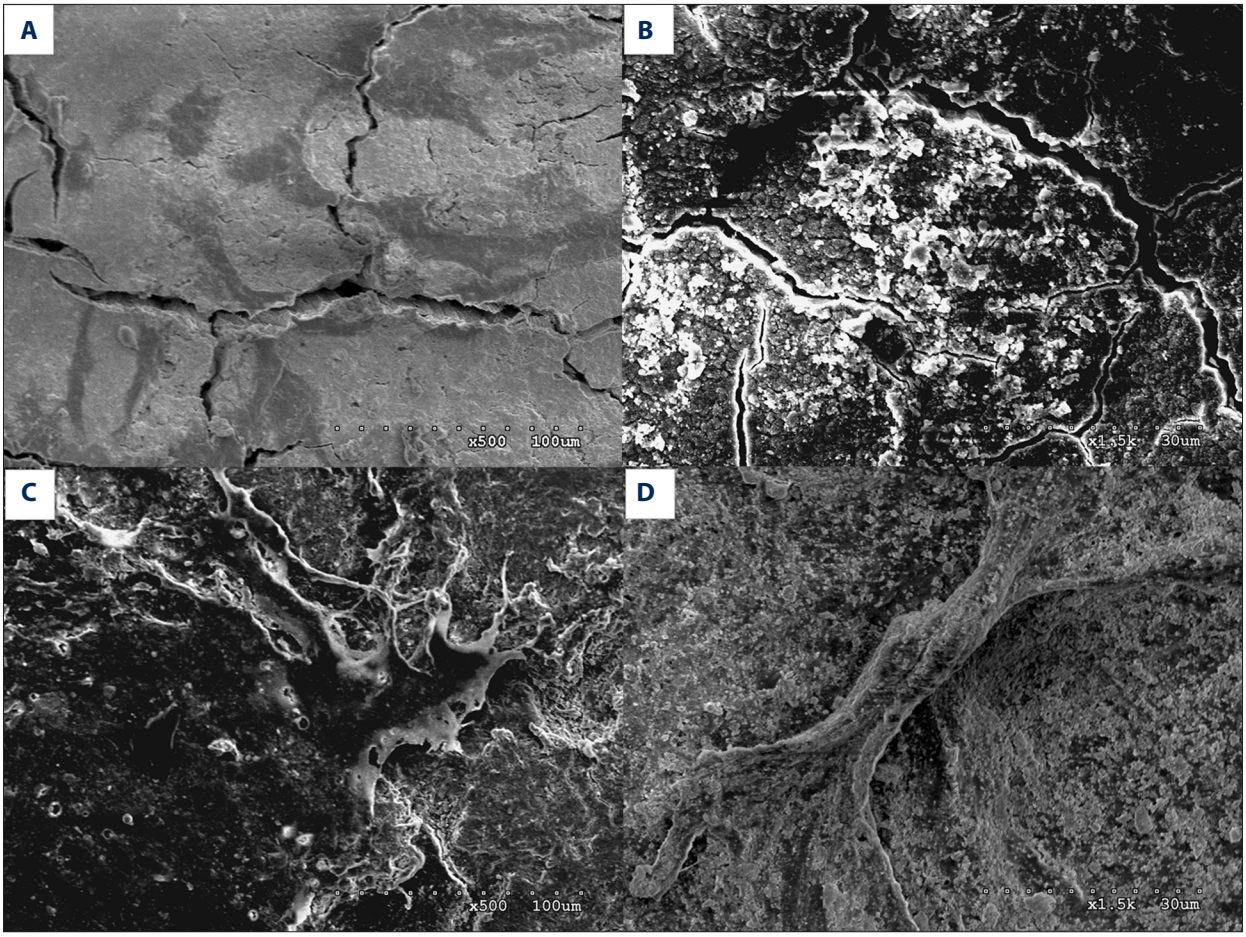

Figure 7. In group A, the cells were round and flat with the less extracellular matrix. (**A-D**) Scanning electron microscope (SEM) at 24 h, 500×; 24 h, 1500×; 48 h, 500×; and 48 h, 1500×.

(group A) were smooth and flat, with scratches and scattered calculus. In the present study, patches of dental calculus could be observed in group A but not in group B and group C. The result of the endotoxin quantitative test was consistent with the SEM observation. Among the 3 experimental groups, the endotoxin concentration in group A was the highest and was significantly different from that in groups B and C. Similarly, compared with group A, groups B and C showed rougher root surfaces with no dental plaques. The rougher root surface obtained by minimally invasive laser treatment contains less endotoxin, so it is unnecessary to use hand instruments for excessive debridement.

In the periodontal healing response, fibroblasts in gingival tissue synthesize collagen fibers connecting the gingiva and cementum, which play a key role in new tissue formation [26]. At present, fibroblast attachment is the main index to evaluate the biocompatibility of the tooth root surface [27]. The characteristics of HGFs are similar to those of periodontal ligament cells, namely HGFs can exert osteogenic potential under certain stimulation, and their proliferative activity is better than that of periodontal ligament cells [28]. Therefore, observing the morphology and proliferation of HGFs on the root surface was used to detect the biocompatibility of the root surface after different periodontal treatments. The results of the CCK-8 assay showed a consistent growth tendency of HGFs on the specimens and cell slides. The HGFs grew rapidly on days 1 to 3 and entered a plateau phase on days 4 to 5 owing to the limitation of growth space. The absorbance of group A decreased, while the absorbance of the other 2 groups still increased on the fifth day. We speculated that the reason may have been be due to the presence of a smear layer and residual endotoxins leading to the decrease of vital cells. Meanwhile, the cell growth in groups B and C was affected only by the growth space, showing a decreased growth rate with no reduction in cell number. This result confirmed that endotoxins were not conducive to the adhesion of HGFs on the root surface.

Some studies have suggested that cell morphology is an indicator to measure the adhesion of cells to different surfaces. Flat cells are firmly attached to the root surface through a large number of cell matrix and pseudopodia, while round cells are

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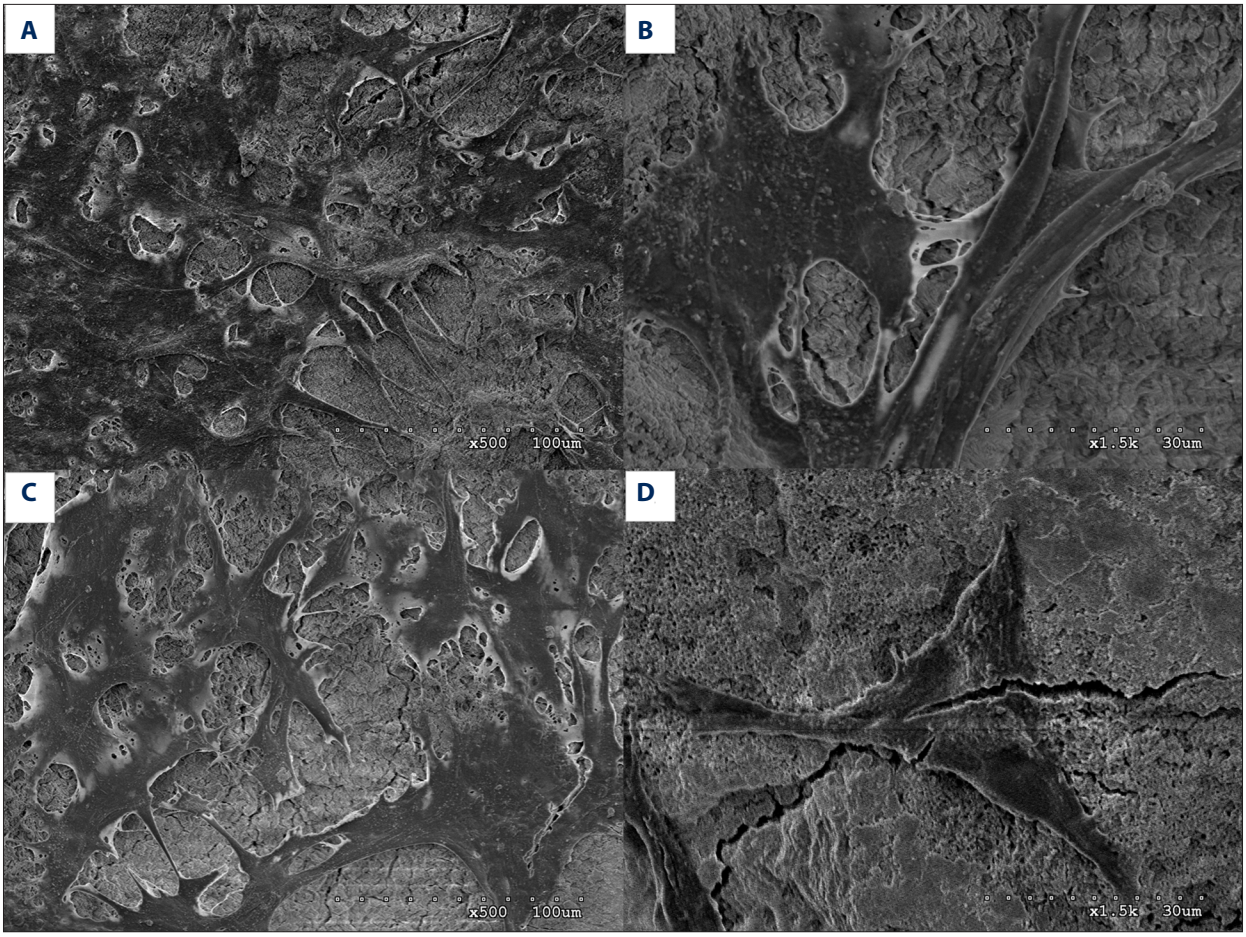

Figure 8. In group B (Er: YAG laser), the cells adhered well and grew densely. (**A-D**) Scanning electron microscope (SEM) at 24 h, 500×; 24 h, 1500×; 48 h, 500×; and 48 h, 1500×.

considered to have poor adhesion or to be recently obtained through mitosis division [29]. In the present study, the HGFs on specimens irradiated by the Er: YAG laser (group B) were in a long spindle shape with abundant cytoplasm, many protrusions, and pseudopodia. This was similar to the morphology of the HGFs cultured on glass slides under an inverted microscope. However, the HGFs in group A had irregular shapes and grew slowly. After 48 h of co-culture, round or oval cells were found on the root surfaces in group A. The number of HGFs and the density of pseudopodia in group C were significantly less than that in group B. This trend was consistent with the adhesion rate of HGFs on the 3 different specimens. The 2-h cell adhesion rate of group B was significantly higher than that of the other 2 groups, and the cell adhesion rate of group C was significantly higher than that of group A. Combined with the SEM results of root surface morphology, we speculated that the factors influencing the cell adhesion rate and cell morphology were as follows: (1) A small amount of calculus and endotoxins attached to the surface was found in group A, but no similar phenomenon was found in group B and group C. These infectious residues had adverse effects on early cell adhesion; and (2) the samples in group B showed the highest surface roughness and surface energy without any melting carbonization. This characteristic could have attracted more collagen and fibronectin and thus improve the adhesion of the HGFs. Group A showed a smooth surface; however, the cells were arranged in parallel on the smooth surface, and the mechanical adhesion was poor. This is consistent with the research by Dunn et al [30], who found that HGFs can form actin terminal fibers and produce collagen. This enables HGFs a better mechanical interlocking performance on the rough surface with grooves and better resistance to lateral shear forces. It used to be widely accepted that the smooth surface is more suitable to HGF growth [31]; however, recently, an increasing number of researchers have suggested that the rough surface is more conducive to cell adhesion [32]. The results of the present study showed that the HGFs on the rough surface irradiated by the Er: YAG laser proliferated better than did the cells on the smooth surface (group A and group B). In group C, the root surface showed a non-homogeneous rough morphology, which was composed of different levels of hard tissue ablation and a small amount of smear layer. The irradiation of the Nd: YAG laser on root surfaces causes

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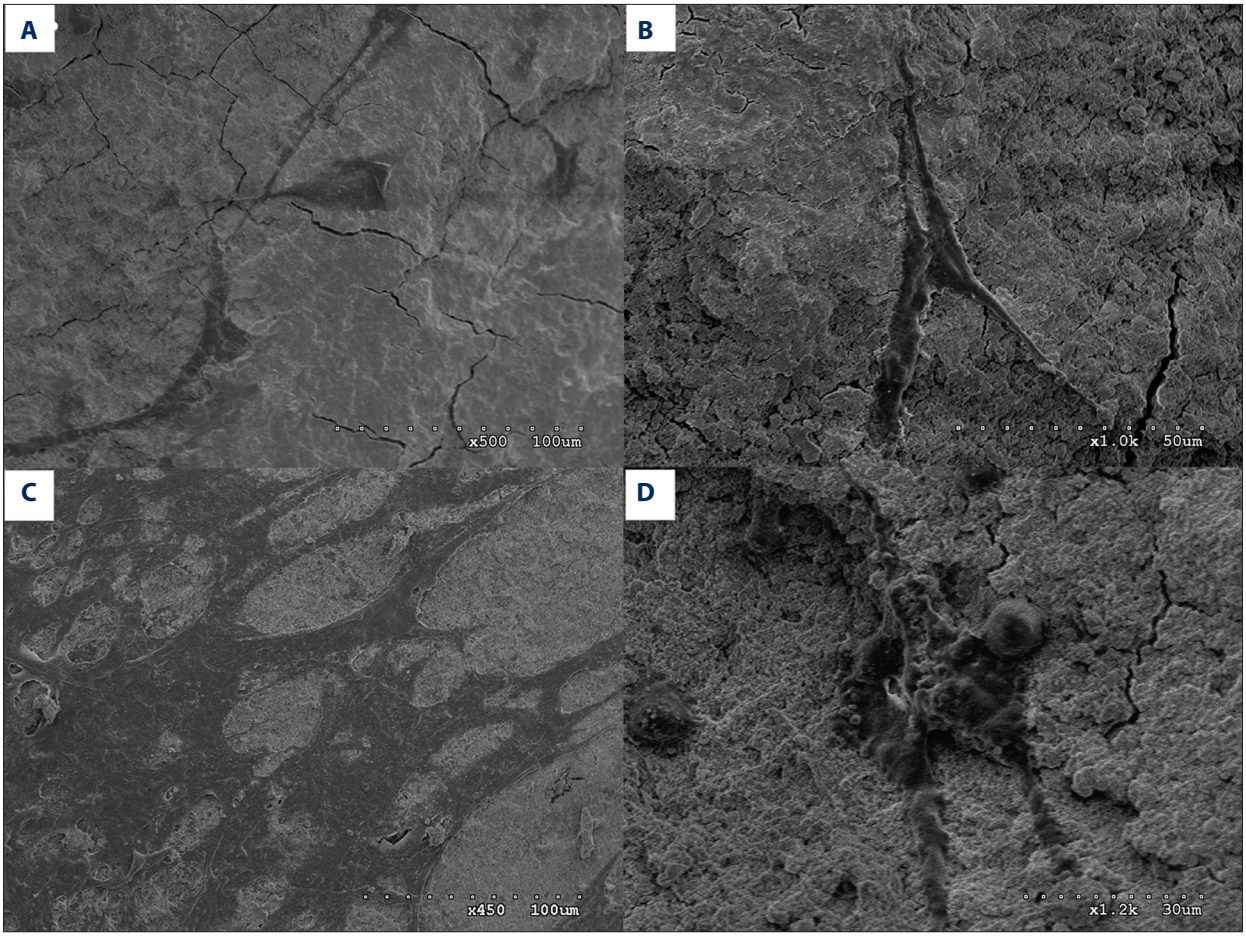

Figure 9. In group C (Er: YAG laser and Nd: YAG laser), the cell extension was weaker, (**A-D**) Scanning electron microscope (SEM) at 24 h, 500×; 24 h, 1500×; 48 h, 500×; and 48 h, 1500×.

adverse reactions, such as the thermal damage of dental hard tissue and thermal denaturation of matrix protein [33]. In previous studies, root surface alterations such as pit and crater formation and melting of root mineral surface were created by the Nd: YAG laser when it was used at the higher energy setting and longer exposure time, whereas the lower energy setting and shorter exposure time produced a relatively smooth surface with considerably fewer modifications [34]. Therefore, in the present experiment, a low-energy Nd: YAG laser and shorter exposure time was used to irradiate the root surface. However, we still found that it had some alterations and adverse effects on the root surface that was irradiated with the Er: YAG laser. Because of the melting of the root mineral surface, the HGFs in group C had fewer and thinner pseudopodia. At high magnification, the cells had narrow triangular or polygonal shapes, which indicated that the melting of root surfaces after Nd: YAG laser irradiation may decrease biocompatibility and reduce the adhesion of HGFs. Therefore, we concluded that the combined irradiation of the Er: YAG and Nd: YAG lasers to the root surface to prevent root surface damage had no benefit in enhancing periodontal attachment. In contrast, the Nd: YAG laser decreased the biocompatibility of periodontitis-affected root specimens by excessive thermal accumulation. Consequently, it is particularly important in the clinical setting to make sure that the Nd: YAG laser fiber is facing toward the periodontal pocket wall instead of the root surface.

However, since this experiment was conducted only in vitro, evidence from well-designed in vivo studies and clinical trials is needed. We need to apply the Er: YAG and Nd: YAG lasers in soft and hard tissues, respectively, to further explore their relative biocompatibility and periodontal attachment.

Conclusions

Compared with manual instruments, Twinlight laser-assisted periodontal therapy was shown to have superior biocompatibility with the root surface. It achieved better fibroblast attachment and proliferation by removing calculus and reducing endotoxins. Finally, the Er: YAG laser was found to be more suitable for hard tissue treatment than was the Nd: YAG laser.

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Declaration of Figures' Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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