



# The effects of using erbium, chromium-doped:yttrium-scandium-gallium-garnet laser on the surface modification, bacterial decontamination, and cell adhesion on zirconia discs: an *in vitro* study

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## Abstract

The use of zirconia for implants and abutments has become more prevalent in implant dentistry as an alternative to the commonly used titanium implants, and peri-implant disease can still affect them. The erbium, chromium-doped:yttrium-scandium-gallium-garnet (Er, Cr:YSGG) laser has emerged as a promising treatment modality. The purposes of this *in vitro* study were to (1) determine the effects of the laser on the surface roughness of zirconia discs; (2) determine the extent of removal of a single species biofilm, *E. coli*, on the zirconia discs after applying the laser; (3) determine the amount of cell adhesion and proliferation utilizing fibroblasts on zirconia discs after treatment with the laser. All treatments will be compared with the commonly used ultrasonic instrumentation and hand scalers. For the first aim, gross examination revealed noticeable surface damage on the discs when using ultrasonic and scalers but not for the laser group. For surface roughness, the mean roughness was  $Pa = 0.623 \pm 0.185 \mu\text{m}$ ,  $0.762 \pm 0.421 \mu\text{m}$ ,  $0.740 \pm 0.214 \mu\text{m}$ , and  $0.724 \pm 0.168 \mu\text{m}$  for control discs, and discs treated with either the Er,Cr:YSGG laser, ultrasonic instrumentation, and hand scalers respectively. There was no statistical significance among the groups ( $p = 0.628$ ). For bacteria decontamination, there was a statistical significance among the groups ( $p < 0.0001$ ). Statistical significance was seen between the control group and each of the three treatment groups, favoring the treatment groups ( $p < 0.0001$ ). Statistical significance was seen when comparing ultrasonic instrumentation and hand scalers ( $p = 0.000$ ) as well as when comparing the Er,Cr:YSGG laser to hand scalers ( $p = 0.007$ ), favoring both the ultrasonic instrumentation and Er,Cr:YSGG laser. No significance between the Er,Cr:YSGG laser group and the ultrasonic instrumentation group was noted ( $p = 0.374$ ). When comparing the cell attachment following treatment in each of the three groups and also without treatment (control), there was a statistical significance among the groups ( $p < 0.0001$ ) in terms of total cell count, favoring the control and the laser groups. Further evaluations with SEM showed differences in cell morphology indicating more adherent cells on Er,Cr:YSGG laser-treated surfaces. In conclusion, gross examination of the discs show clear surface changes when using ultrasonic instrumentation and hand scalers compared to the Er,Cr:YSGG laser group. The Er,Cr:YSGG laser was able to effectively ablate bacteria from zirconia disc. Fibroblast attachment on the surfaces of the zirconia discs shows more adherence when treated with Er,Cr:YSGG laser.

**Key words** Peri-implantitis · Peri-implant mucositis · Zirconia · Dental implants · Dental laser · Er,Cr:YSGG laser

Charles M. Pham and Chia-Yu Chen contributed equally to this work.

One sentence summary: Using the Er,Cr:YSGG laser for treatment of peri-implant disease does not cause gross surface damage, can effectively ablate bacteria, and allows for fibroblast adherence.

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## Introduction

The use of endosseous dental implants has been a widely accepted treatment modality for patients since their introduction in 1977 [1]. Complications have become more frequent since their implementation due to the prevalence of peri-implant disease such as peri-implant mucositis and peri-implantitis. Recently, the World Workshop on the Classification of Periodontal and Peri-Implant Diseases and

Conditions defined peri-implant disease and conditions to give us a definitive definition and description of peri-implant health, peri-implant mucositis, and peri-implantitis based on a review of the literature [2]. Peri-implant mucositis has been reported to occur in approximately 80% of patients, and peri-implantitis occurs in between 28 to 56% of patients [3]. This represents an important aspect of implant dentistry that requires multiple treatment modalities in order to ensure long-term success of dental implants.

Treatment of dental implants must be considered in a different manner than natural teeth due to the use of threads on the implant that engage into the alveolar bone for osseointegration that makes it difficult to fully decontaminate due to access. Furthermore, the abutment of the implant represents another site of potential biofilm accumulation that must be addressed when treating peri-implant diseases. This makes the treatment of dental implants more complicated. Multiple treatment protocols have been described for maintenance of implants or treatment of peri-implant inflammatory diseases [4].

Recently, the use of zirconia (zirconia-dioxide,  $ZrO_2$ ) has been used more widely as a substitute for the more traditionally used titanium implants as well as a material for the abutment in the final restoration. Transmucosal zirconia implant abutments have been shown to have safe long-term functional and esthetic results as well as favorable soft and hard tissue reaction [5]. It has also been shown to be favorable for use as an abutment due to having a low bacterial adherence after evaluation of zirconia discs that have been left in the oral cavity for 24 h [6]. Zirconia implants have been shown to be successful when used to support dental prostheses. A recent systematic review found that the overall survival rate of zirconia implants after 1 year of function was 92% [7]. It has been shown to have a high level of acceptance in terms of treatment modality for patients and has similar clinical results in terms of bleeding on probing, probing depths, and attachment levels compared to natural teeth [8]. With the more widespread use of zirconia in implant dentistry, it becomes more necessary to evaluate treatment modalities for when they are affected with peri-implant diseases either at the abutment or fixture level.

The use of dental lasers has been reported for nonsurgical treatment of periodontal diseases [9, 10]. Furthermore, the use of lasers has shown promising results around dental implants as well [11]. The erbium, chromium-doped:yttrium, scandium, gallium, and garnet (Er,Cr:YSGG) laser has a wavelength of 2780 nm. The laser energy allows for microablation of tooth structure, bone, or soft tissue. It has been used for surgical treatment of peri-implant disease as well as nonsurgical use [12–14]. This has led to promising results on the management of this complicated ailment for dental implants.

The efficacy of the Er, Cr: YSGG laser on decontamination and surface changes is correlated with parameters such as

power (watts), frequency (Hz), pulse duration, diameter of the tips, and distance between the tips to the irradiated surfaces [15]. A recent *in vitro* study showed that use of both conical and side firing tips at 1.5 W/30 Hz did not seem to damage the titanium implant surface [16]. Another study showed that when 2.5 W/25 Hz was used, oral biofilm was effectively removed from contaminated titanium; however, this study did not report whether there were surface changes [17].

There is a paucity of reports on the management of peri-implant disease around zirconia; therefore, this *in vitro* study hopes to (1) determine the effects of using the Er,Cr:YSGG laser on the surface modification of zirconia discs; (2) determine the extent of removal of a single species biofilm, *E. coli*, on the zirconia discs; and (3) determine the amount of cell adhesion and proliferation as well as examine the morphology of fibroblasts on zirconia discs after treatment. All treatments will be compared with the commonly used ultrasonic instrumentation and hand scalers.

## Materials and methods

### Zirconia disc preparation

Yttria-stabilized zirconia (YTZP) discs, sintered and polished, were obtained from a dental laboratory (Cusp Dental Research Inc, MA, USA). Each disc measured 10 mm in diameter and 2 mm in thickness. Prior to any treatment, discs were ultrasonically cleaned and degreased in 0.5% sodiumdodecyl sulfate (SDS; Sigma, MO, USA), deionized water, acetone (Sigma, MO, USA) and ethanol (Sigma, MO, USA), sequentially, for 20 min in each solvent, and then autoclaved at 121 °C for 20 min.

### Surface treatment

For each aim, the discs were divided and treated the same way:

- 1) Control group (no treatment)
- 2) Laser-treated group: discs were treated with the Er,Cr:YSGG laser (Waterlase iPlus, Biolase, Irvine, CA, USA) with a wavelength of 2780 nm in short pulse “H” mode (60 $\mu$ s, pulse rate 30 Hz) via an end firing tip with a spot size of 1 mm. The power setting was 1.5 W with air/water of 40%/50% to simulate the recommended clinical setting for treatment of peri-implant disease using this laser. The laser tip, attached to a handle, was slowly moved along the zirconia surface while keeping a distance of about 0.5 mm between the tip and the surface.
- 3) Ultrasonic instrumentation–treated group: discs were treated with cavitron (Cavitron Plus Ultrasonic Scaler,

Dentsply Sirona, York, PA, USA) instrumentation with 20 strokes of the instrument on each disc.

- 4) Hand instrumentation–treated group: discs were treated with stainless steel periodontal curettes (Younger Good 7-8 Curette Hu-Friedy, Chicago, IL, USA) with 20 strokes of the instrument on each disc.

### Surface characterization

After treatment of each disc on both sides following the assigned group protocol ( $n=5$  surfaces per group), the disc surfaces were analyzed by three different methods. (1) Gross visual examination of the discs was completed to determine the effects of each instrument on the discs. (2) Scanning electron microscopy (SEM) (Zeiss Ultra55 field emission scanning electron microscope; ZEISS, Oberkochen, Germany) analysis was completed to examine any noticeable change in surface texture. (3) A surface contact profilometer (DetakXT Profilometer, Bruker, Billerica, MA, USA) was utilized to calculate the average surface roughness (Pa in  $\mu\text{m}$ ) for each disc. Measurements were made 5 times at random on each disc surface, moving for a distance of 1 mm, and at 3 mg of stylus force. Measurements of surface roughness were averaged together for each disc for quantitative analysis.

### Bacteria culture

To investigate the efficacy of different surface decontamination treatments, discs ( $n=12$  per group) were inoculated with green fluorescent protein (GFP)–expressing *Escherichia coli* (ATCC 25922GFP) for 24 h. In specific, *E. coli* culture was grown overnight in a shaker at 28 °C for 24 h in the Lysogeny broth (LB) until OD600 reached 0.4–0.5. Subsequently, bacterial concentration was adjusted to  $2 \times 10^6/\text{mL}$  with LB. In a 24-well plate, discs were placed into each well and immersed in 1 mL *E. coli* containing LB for 24 h into each well. Four discs per group were used. At the end of the incubation, non-adherent bacteria were rinsed off with phosphate buffered saline (PBS) and each disc was treated according to the assigned group protocol (control, Er, Cr:YSGG, ultrasonic instrumentation and hand scalers) as described previously. Following treatments, the discs were rinsed with PBS, fixed in 4% paraformaldehyde (PFA) for 10 min and then washed twice with PBS before being mounted for fluorescent microscopy. A fluorescence microscope (Revolve, ECHO, CA, USA) with  $\times 10$  objective was used to examine the discs. Fluorescent photographs were taken with a 3.2 MP monochrome SCMOS camera at 3 random sites on each disc. A cell count was completed using ImageJ (National Institutes of Health, Bethesda, MD, USA) to examine the effectiveness of removal of the bacteria from each of the discs. These values were averaged for each group for comparison.

### Fibroblast cell adhesion

For the third aim, 5 discs were used for each group for quantitative analysis with a cell count using luminescent cell viability kit (CellTiter-Glo Luminescent Cell Viability Assay, Promega, Madison, WI, USA) and 3 discs for each group were used for SEM qualitative analysis (total of  $n=8$  per group). Discs were initially treated according to the assigned group protocols and autoclaved prior to cell incubation. Mouse fibroblast NIH3T3 cells were maintained in DMEM medium (Thermo Fisher Scientific) supplemented with 10% FBS at 37 °C in 5% CO<sub>2</sub> and 95% atmospheric air. Cells were seeded on the 4 types of treatment surfaces at 50k cells/cm<sup>2</sup> in 1 mL of culture medium in each well of a 24-well plate for 72 h. At the end of the incubation period, unbound cells were washed off with the culture medium and specimens were transferred to a new well where a 400- $\mu\text{L}$  mixture of culture medium and CellTiterGlo solution at 1:1 ratio was added. Cell lysis was induced by 2-min vigorous shaking with an orbital shaker, and the plate was left to stabilize for 10 min before luminescent reading. Three discs from each group were fixed with 4% PFA, and then SEM analysis was completed to examine the morphology of the attached fibroblasts on the discs.

### Statistical analyses

One-way analysis of variance (ANOVA) was conducted to compare measurements between the control and test groups, and the Tukey HSD post hoc test was applied to assess differences that were statistically significant. Data that did not show normal distribution after testing for normality was analyzed using the Kruskal-Wallis non-parametric test and post hoc Dunn's multiple comparison test. The significance level adopted was 5% for all tests.

## Results

### Surface modification

After treatment with each group, gross examination revealed noticeable surface damage on the discs when using ultrasonic and scalers but no noticeable damage with the laser group (Fig. 1a, upper row). When compared to the control, the Er,Cr:YSGG laser–treated discs showed similar appearance after treatment. SEM analyses show minimal changes between groups (Fig. 1a, lower row). For surface roughness, the mean roughness were Pa=  $0.623 \pm 0.185 \mu\text{m}$ ,  $0.762 \pm 0.421 \mu\text{m}$ ,  $0.740 \pm 0.214 \mu\text{m}$ , and  $0.724 \pm 0.168 \mu\text{m}$  for control discs, and discs treated with either the Er,Cr:YSGG laser, ultrasonic instrumentation, and hand scalers respectively

**Table 1** Results for surface roughness, bacterial count and number of attached fibroblasts after application of different surface treatments

	Surface roughness (Pa, $\mu\text{m}$ )	Bacterial count	Fibroblast count
Control	0.623 $\pm$ 0.185	9070.333 $\pm$ 3619.705	552322.395 $\pm$ 51742.897
Er, Cr: YSGG	0.762 $\pm$ 0.421	78.750 $\pm$ 8.776	475621.803 $\pm$ 87744.661
Cavitron	0.740 $\pm$ 0.214	73.00 $\pm$ 5.627	615029.457 $\pm$ 138841.954
Hand scaler	0.724 $\pm$ 0.168	935.853 $\pm$ 501.732	659870.766 $\pm$ 153276.387
	$p=0.628$	$p<0.0001$	$p<0.0001$

(Table 1 and Fig. 1b). There was no statistical significance among the groups ( $p=0.628$ ).

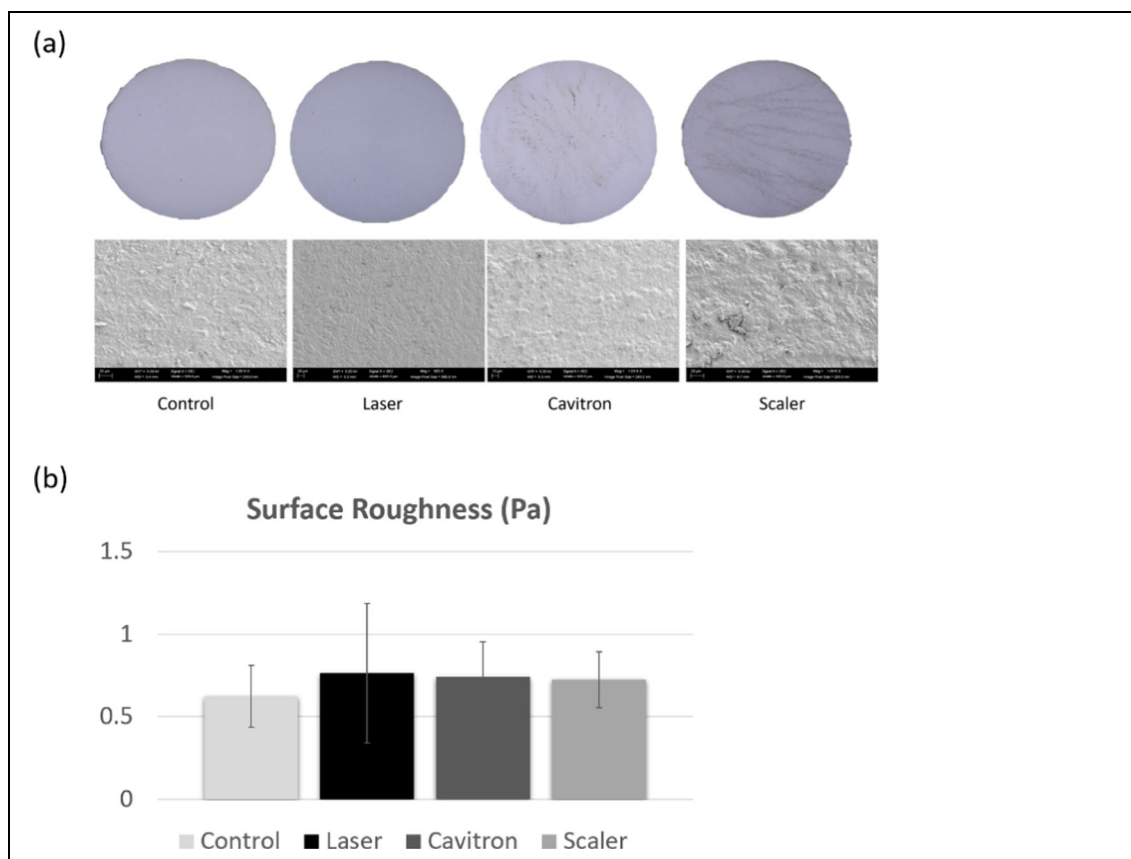
### Bacteria decontamination

For bacteria decontamination, there was a statistical significance among the groups ( $p < 0.0001$ ), and we can see the noticeable difference when examining the discs under fluorescence microscopy (Fig. 2a). Statistical significance in the number of bacteria after performing a cell count was seen between the control group and each of the three treatment groups, favoring the treatment groups ( $p < 0.0001$ ). Post hoc Dunn's multiple comparison test revealed statistical

significance when comparing ultrasonic instrumentation and hand scalers ( $p=0.000$ ) as well as when comparing the Er,Cr:YSGG laser to hand scalers ( $p=0.007$ ), favoring both the ultrasonic instrumentation and Er,Cr:YSGG laser. The only two groups when compared that did not show statistical significance were the Er,Cr:YSGG laser group and the ultrasonic instrumentation group ( $p=0.374$ ) (Table 1 and Fig. 2b).

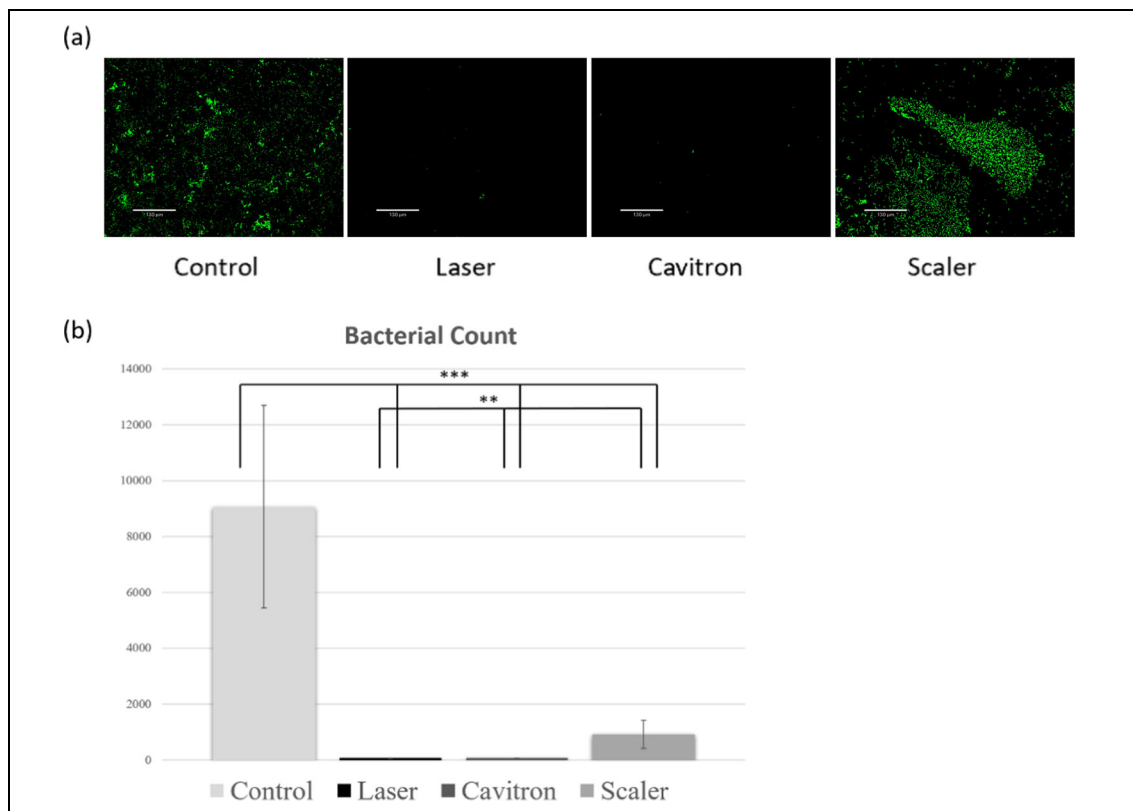
### Cell attachment

When comparing the cell attachment following treatment in each of the three groups and also without treatment (control), there was a statistical significance between the groups ( $p <$



**Fig. 1** Surface characterization following different treatments. **a** Upper row: gross examination of each disc after treatment. Lower row: SEM analysis of each disc after treatment at  $\times 1000$  magnification. **b** Surface

roughness after measurements with profilometer. There was no statistical significance among the groups



**Fig. 2** Remaining bacterial count following decontamination. **a** Examination of each disc under fluorescent microscopy at  $\times 10$  magnification. **b** Bacteria count after treatment. (\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.0001$ )

0.0001) in terms of total cell count (Table 1 and Fig. 3a). The control and laser groups had significantly more attached cells when compared to the two conventional treatment groups (cavitron and scaler). While there was no significant difference between the control and the laser groups ( $p = 0.903$ ), SEM analysis revealed differences in the mode of fibroblast attachment. Fibroblasts demonstrated a more elongated spindle-cell morphology on the laser group compared to the more rounded shapes on all the other surfaces. The fibroblasts on the laser-treated surface appeared flattened with filopodia extension, suggesting focal adhesion kinase-dependent migration and cell growth (Fig. 3b).

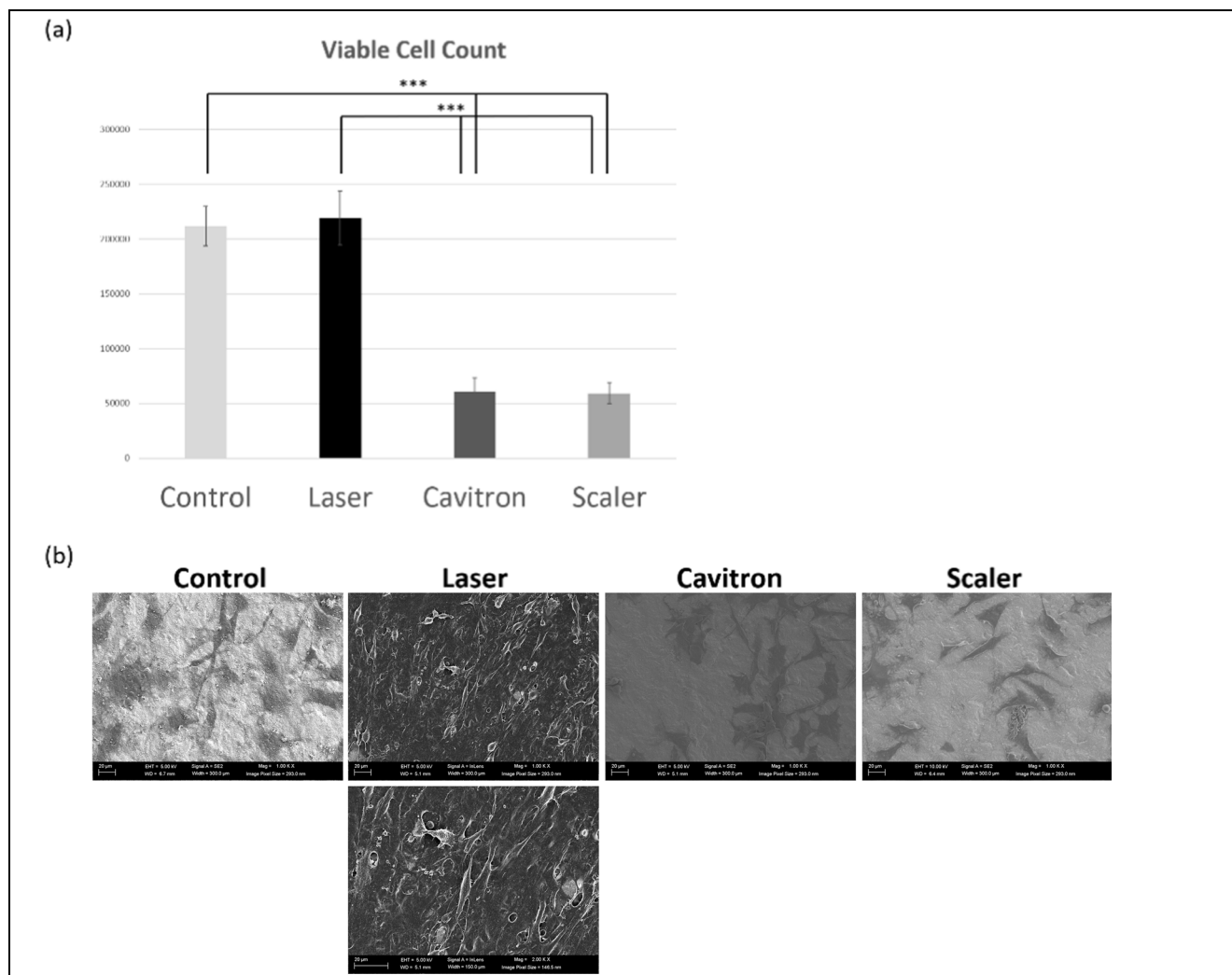
## Discussion

The Er,Cr:YSGG laser has been shown in an *in vitro* study to effectively ablate biofilm from titanium surfaces without causing measurable physical changes to the surface [18]. In contrast, another study shows that the Er,Cr:YSGG laser causes a decrease in surface roughness of zirconia with an increase of roughness in titanium [19]. The results of the present study indicate that using the Er,Cr:YSGG laser on zirconia surfaces did not cause noticeable surface changes to the discs and was able to effectively ablate bacteria. The

disparities in the reported results could be due to the use of different laser settings. In this study, the settings used for the laser were the same settings recommended to treat peri-implantitis intraorally from the manufacturer (1.5 W, 30 Hz, 40%/50% air/water). This setting has also been used to treat peri-implantitis with successful outcomes in clinical studies [13, 14]. The decontamination effect of the Er,Cr:YSGG laser could be contributed to its wavelength being in the region of the major absorption peak for water; therefore, with the laser irradiation, water quickly expanded and evaporated, creating acoustic waves strong enough for abrupt disintegration of the bacterial cell wall [20]. A study by Gordon et al. has shown that Er,Cr:YSGG laser was able to achieve intratubular water expansion and collapse as deep as 1000  $\mu\text{m}$  in depth [21].

Interestingly, gross inspections of zirconia surfaces revealed damages caused by use of ultrasonic and hand scalers; however, no statistical difference in surface roughness was found when examined by a profilometer. This could be explained by the technical nature of the instruments. The debridement with ultrasonic and hand scalers was performed in a similar fashion to clinical settings (20 strokes per sample) in direct contact with the surface; the strokes left behind marks visible on the surface. These marks were larger in diameter than the profilometer tip and the measurements were





**Fig. 3** Fibroblast cell attachment following surface treatment. **a** Cell count of fibroblasts 72 h after seeding ( $*p < 0.05$ ;  $**p < 0.01$ ;  $***p < 0.0001$ ). **b** SEM analysis of each disc and fibroblasts at  $\times 1000$  magnification (upper row) and  $\times 2000$  for the laser-treated disc

performed in multiple spots, some without the strokes; therefore, it was not surprising that the profilometer failed to reveal statistical significance among the groups.

One important factor for dental implants is the mucosal seal around abutments to prevent biofilm accumulation. This is dependent on fibroblast attachment and the material of the abutment. After exposure of biofilm to implant and abutment surfaces, it was seen that an inflammatory lesion forms in the connective tissue that surrounds the implant and abutment [22]. The results of a canine study showed that the material used for the abutment portion of implants affects the location and quality of the attachment of the peri-implant mucosa and implant itself [23]. Fibroblasts are collagen fiber-producing cells that are seen in the connective tissue of peri-implant tissues, and most are seen directly next to implant abutment surfaces [24]. They are important to the soft tissue seal around implants and have been the subject of *in vitro* studies on attachment, spreading, and proliferation of fibroblasts [25]. The

characteristic of fibroblast morphology has also been studied in terms of attachment. Studies focused on morphology of fibroblasts that appeared more attached, elongated, and stretched out onto surfaces [26–28].

After treatment of peri-implant disease, it is important to ensure that soft tissue attachment is favorable to have an appropriate mucosal seal. The present study showed that the use of the Er,Cr:YSGG laser allowed more fibroblast attachment compared to the other treatment modalities. Furthermore, the cells appeared more spindle in shape and adherent on the surfaces treated with the laser. Such fibroblast morphology favors soft tissue attachment to zirconia and resembled what was observed on Laser-lok titanium surface morphologically which was shown to induce a direct connective tissue attachment in a human histological study [29–31]. To the best of the author's knowledge, our study is the first to evaluate fibroblast morphology after treatment of zirconia surfaces with a dental laser. We speculate that such result could be due to rise in

surface energy and hydrophilicity after application of the laser and could be subjects of future studies.

## Conclusion

Although gross examination of the discs shows clear surface changes when using ultrasonic instrumentation and hand scalers compared to the Er,Cr:YSGG laser group and control group, no difference in surface microroughness was seen. The Er,Cr:YSGG laser was able to effectively ablate bacteria from zirconia discs and was more effective than hand instrumentation. Fibroblast attachment on the surfaces of the zirconia discs shows more adherence when treated with Er,Cr:YSGG laser.

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