International Journal of Dentistry

Interactions between Oral Tissues and Extenal Light and Matters

Guest Editors: S. Nammour, H. S. Loh, R. De Moore, and C. P. Eduardo

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Editorial Board

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Editorial

Interactions between Oral Tissues and External Light and Matters

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We are currently in an era where the international scientific publication is highly valued, acting as a strong parameter for evaluation mechanism in ranking universities in the competitive scientific world. In this context, writing the editorial of this special issue gives us a great responsibility.

After a cautious revision of the several articles submitted for publication in this special issue by the Editorial Board of this journal, the accepted papers translate the commitment of the authors to the scientific research.

This special issue is a sample of the current research efforts addressing issues related to the interaction between the light and the oral tissues, highlighting subjects such as the treatment of pathologies associated with high intensity laser for better healing and welding, the use of high intensity laser treatment for cosmetic dentistry, evaluation of adhesive systems and dentin hypersensitivity, the detection and treatment decision of caries lesions with laser fluorescence, the treatment of root perforation using high intensity laser, and also the low level laser therapy for improving wound healing.

We hope that the readers can enjoy this special issue and improve their knowledge about the light interaction with the hard and soft oral tissues.

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Research Article

The Ablation Properties of CO_2 Laser Irradiating to Absorption Media: An In Vitro Study

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This study aimed to compare histological affected zone of tissue samples irradiated by defocused CO_2 laser at 1, 2, and 3W continuous wave with and without absorption media. The in vitro experiment was conducted in 70 tissue blocks. The samples were randomly allocated into 7 groups: 10 samples each group, namely, the groups irradiated with 1, 2, and 3W, defocused CO_2 laser for 5 seconds, the groups irradiated with 1, 2, and 3W, defocused CO_2 laser to the absorption media, and the media alone group as a control. Then the samples were stained with Masson's trichrome and measured the affected borders under light microscope at 10 $\times$ 10 magnification. There was no histological alteration in the groups irradiated with the defocused CO_2 laser to the absorption media while the groups without using the absorption media showed the tissue alteration by photoablation.

1. Introduction

The uses of CO_2 Laser in oral soft tissue surgery for benign soft tissue lesions [1–4] and potentially malignant disorders [5–8] were widely reported. These studies showed clearly the advantages of CO_2 laser in terms of precise and haemostatic ablation and proving less postoperative pain, swelling, and scare formation. The favorable healing of oral soft tissue after CO_2 laser surgery was explained by the mechanism of healing with less inflammatory reaction and fewer myofibroblasts compared with scalpel excision [9]. The immunohistological study by Zeinoun et al. [10] also found that the myofibroblast response and activity were slower and lack of contractile compared with the scalpel wound. By comparison with other type of laser such as diode laser and Nd-YAG laser, the CO_2 laser showed the narrow area of lateral-thermal damage [11, 12] leading the shorter period of healing and the less wound contraction.

In 2004, Sharon-Buller and Sela [13] reported the technique of using CO_2 laser irradiating transparent gel, acting as energy absorption, resulting in immediate pain relief in patients with oral ulcer. This, as the authors referred to be a nonablative photoreaction, differed from other laser-applications which were stated in the review [4]. However, the histological ablation properties of this technique have not been explored. Therefore, this study aimed to compare histologically affected borders of the tissue samples irradiated by defocused CO_2 laser at 1, 2, and 3W with and without transparent gel covering the tissue surface.

2. Materials and Methods

The laboratory experiment was conducted in 70 tissue blocks of $1 \times 1 \times 1$ cm ventral mucosa of the fresh pig tongues. The samples were randomly allocated into 7 groups, 10 samples each group as follows:

group 1: 1W defocused CO_2 laser continuous wave irradiating the tissue for 5 seconds,

group 2: 1W defocused CO_2 laser continuous wave irradiating the absorption media on tissue surface for 5 seconds,

group 3: 2W defocused CO_2 continuous wave laser irradiating the tissue for 5 seconds,

group 4: 2W defocused CO_2 continuous wave laser irradiating the absorption media on tissue surface for 5 seconds,

group 5: 3W defocused CO_2 continuous wave laser irradiating the tissue for 5 seconds,

group 6: 3W defocused CO_2 laser continuous wave irradiating the absorption media on tissue surface for 5 seconds,

group 7: Applying absorption media on tissue surface for 5 seconds.

2.1. The Sample Preparation. The samples were prepared based on the standard tissue block preparation for gross and histological study into the effect of high-intensity laser as used in the other studies [14, 15]. The fresh pig tongues were frozen in 4°C immediately after sacrificed and undertaken in the experiment within 24 hours. This can avoid the cell autolysis [16].

2.2. The Absorption Media. Based on Sharon-Buller and Sela [13] study, the absorption media must be transparent and mainly composed of water which highly absorbs CO_2 laser. They used Elmex gel, high fluoride concentration gel as the media. We used Sore mouth gel, 20% bezocaine, because this was a transparent gel recommended to be used intraorally.

2.3. The CO_2 Laser Machine and Its Irradiation. The 10.6-micron CO_2 laser (Smart pulse CO_2, Model: SNJ-1000, Korea) with adjustable power from 1 to 25W and 0.3 mm focal spot-diameter with articulated arm optical delivery was used in this experiment. The regimens were 1, 2, and 3W continuous wave at 2-time defocal length and 5-second irradiation with and without absorption media (Figure 1). The actual powers of the settings were measured by using optical power meter (THORLAB inc model D3MM). These were the same amount of powers which were on the surface of the samples. The measurement of the actual powers and theirs calculated fluences were shown in Table 1.

2.4. The Experimental Methods

(1) The samples were sutured with 3-0 black silk at both margins for locating the central point and placed on the customized apparatus. The ventral mucosa was used for the experiment.

(2) The samples were randomly allocated into 7 groups as follows: Groups 1, 3, and 5 were irradiated with defocused CO_2 laser for 5 seconds at 1, 2, and 3W, respectively.

Groups 2, 4, and 5 were applied with absorption media gel on the surfaces using the template; 5 mm diameter and 1 mm thickness, and then irradiated with defocused CO_2 laser for 5 seconds at 1, 2, and 3W, respectively.

Group 7 was applied with absorption media gel on the surfaces using the template, 5 mm diameter and 1 mm thickness for 5 seconds.

(3) All samples were strained with Masson'trichrome and then inspected under light microscope at 10×10 magnification.

2.5. Histological Measurement. According to the Masson's trichrome stain, the affected collagen by laser was indicated in red band [14, 17]. The borders of histological changes (Figure 2), namely, depth of vaporization (DV), depth of vertically affected border (DB), and width of horizontally affected border (WB) were measured in micron. The measurements were undertaken by 2 inspectors under double-blind randomized controlled trial. The before and after calibrations were conducted.

2.6. Data Analysis. The normality test was calculated using Shapiro-Wilk test. The data was described using descriptive statistics and compared with the groups using ANOVA and Tukey test multiple comparison. In case, the data was not in normal distribution, Kruskal Wallis would be applied.

3. Results

The data was in normal distribution. The intraclass correlation coefficient at 0.8 showed the P value being less than 0.001. Therefore, parametric statistics was used for analysis. There was no histological affected area in the groups irradiated with the defocused CO_2 laser to the absorption media while the histological changes were found in the groups irradiated with CO_2 laser directly (Figure 3 and Table 2).

The comparison of the measurements was shown in Table 3. The group irradiated with 3W defocused CO_2 laser had statistically larger depth of vaporization than the groups irradiated with the defocused 1 and 2W defocused CO_2 (P value = 0.001 and 0.013). The mean differences were 762.48 microns (95% CI = 287.26 to 1,237.70) and 588.71 microns (95% CI = 112.89 to 1,063.33), respectively. The depth of affected border of the 3W defocused CO_2 group was larger than the 1 and 2W defocused CO_2 groups (P value = 0.001 and 0.016). The mean differences were 410.10 microns (95% CI = 157.95 to 662.26) and 302.16 microns (95% CI = 50.00 to 554.32), respectively. The width of affected border of the 1W defocused CO_2 group was narrower than the 2 and 3W defocused CO_2 groups (P value < 0.001). The mean differences were −218. 17(95% CI = −142.94 to −293.40) and −247.93 microns (95% CI = −323.15 to −172.70), respectively.

4. Discussion

There was no detection of histological alteration of the all samples in the groups irradiated with defocused CO_2 laser to the absorption media. These were inspected under light microscope at 10×10 magnification. Owing to the fact

Table 1: The actual powers of CO_2 laser measured by the optical power meter.

Regimes	Mean Power (mW)	Standard deviation	95% confident interval (mW)	Calculated fluence (J/cm^2)
1 Watt	20.5	8.87	16.35 to 24.65	146.43
2 Watt	233.5	25.40	221.61 to 245.39	1,667.86
3 Watt	642.5	42.41	622.65 to 662.35	4,589.29
1 Watt with laser absorption media	0	0	0	0
2 Watt with laser absorption media	15.5	9.99	10.83 to 20.17	110.71
3 Watt with laser absorption media	118	25.87	105.89 to 130.11	842.86

*Spot area: 0.0007 cm^2.

Table 2: The histologically affected borders by the groups.

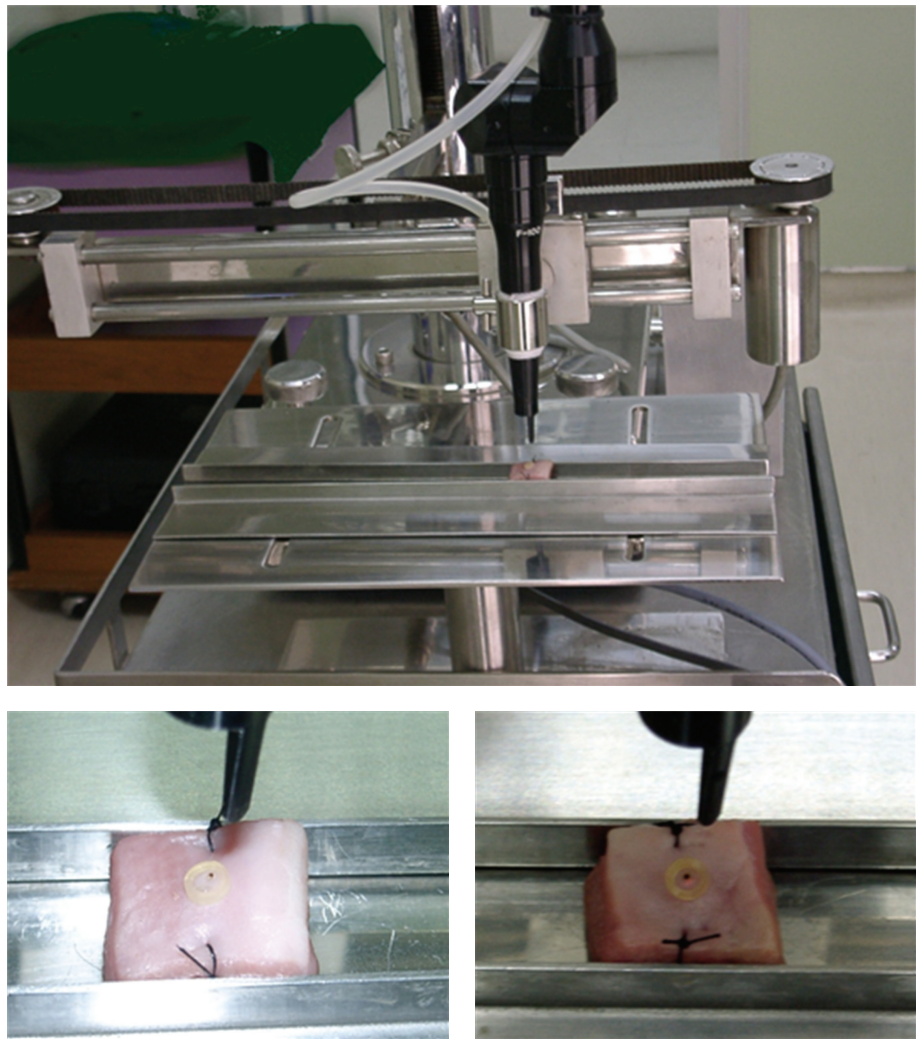
Group	Depth of vaporization (DV)			Depth of vertically affected border (DB)			Width of horizontally affected border (WB)		
	mean	SD	95% CI	mean	SD	95% CI	mean	SD	95% CI
1	218.56	195.39	78.79 to 358.34	287.81	132.95	192.70 to 382.91	369.15	60.76	325.68 to 412.61
2	0	0	0	0	0	0	0	0	0
3	392.92	207.13	244.76 to 541.10	395.75	163.88	278.52 to 512.98	587.32	79.39	530.52 to 644.11
4	0	0	0	0	0	0	0	0	0
5	981.04	685.53	490.64 to 1471.44	697.91	332.58	460 to 935.82	617.07	61.75	572.9 to 661.25
6	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0

SD : standard deviation, 95% CI = 95% confident interval.
Group 1: 1 W defocused CO_2 laser irradiation.
Group 2: 1 W defocused CO_2 laser irradiation with absorption media.
Group 3: 2 W defocused CO_2 laser irradiation.
Group 4: 2 Wt defocused CO_2 laser irradiation with absorption media.
Group 5: 3 W defocused CO_2 laser irradiation.
Group 6: 3 W defocused CO_2 laser irradiation with absorption media.
Group 7: Absorption media alone.

Table 3: The comparisons of the differences of histologically affected borders by the groups.

Affected border	Group	Compared group	Mean difference	95% CI of the differences	P value
DV	1 Watt	2 Watt	−174.37	−649.58 to 300.85	0.639
		3 Watt	−762.48	−1237.70 to −287.26	0.001*
	2 Watt	1 Watt	174.37	−300.85 to 649.58	0.639
		3 Watt	−588.71	−1063.33 to −112.89	0.013*
	3 Watt	1 Watt	762.48	287.26 to 1237.79	0.001*
		2 Watt	588.71	112.89 to 1063.33	0.013*
DB	1 Watt	2 Watt	−107.94	−360.09 to 144.21	0.546
		3 Watt	−410.10	−662.26 to −157.95	0.001*
	2 Watt	1 Watt	107.94	−144.21 to 360.09	0.546
		3 Watt	−302.16	−554.32 to −50.01	0.016*
	3 Watt	1 Watt	410.10	157.95 to 662.26	0.001*
		2 Watt	302.16	50.01 to 554.32	0.016*
WB	1 Watt	2 Watt	−218.17	−293.40 to −142.94	<0.001*
		3 Watt	−247.93	−323.15 to −172.70	<0.001*
	2 Watt	1 Watt	218.17	142.94 to 293.40	<0.001*
		3 Watt	−29.76	−104.98 to 45.47	0.595
	3 Watt	1 Watt	247.93	172.70 to 323.15	<0.001*
		2 Watt	29.76	−45.47 to 104.98	0.595

*: P-value < 0.05.
Depth of vaporization: DV.
Depth of vertically affected border: DB.
Width of horizontally affected border: WB.



(a) Laser irradiating through the absorption media (b) Laser irradiation directly to the tissue block

Figure 1: Laser irradiating to the samples.

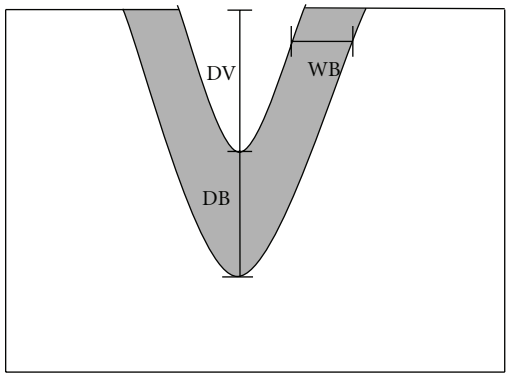


Figure 2: The measurements of the histologically affected borders. Depth of vaporization: DV. Depth of vertically affected border: DB. Width of horizontally affected border: WB.

that the actual laser power could not be detected by the optical power meter in the setting of defocused 1W CO_2 laser irradiation, the regime of either 2W or 3W defocused CO_2, of which laser power detected, is recommended for clinical application.

It was noticed that the 3W defocused CO_2 laser irradiating to the media as used in this research was able to transfer the higher power than the 1W defocused CO_2 laser irradiating directly without providing the ablative effect. It can be hypothesized that using this method the temperature of the tissue was not raised to the coagulative level of 50 to 60°C [18, 19]. Therefore, the clinical effect of this technique on pain control and wound healing reported by Sharon-Buller and Sela [13] tended to be related to low intensity laser inducing biomodulation [20].

In terms of application of defocused CO_2 laser for tissue vaporization, the group irradiated with 3W defocused CO_2 laser had larger depth of vaporization and depth of vertically affected borders than the others, while the group irradiated with 1W defocused CO_2 had less width of horizontal affected area than the others.

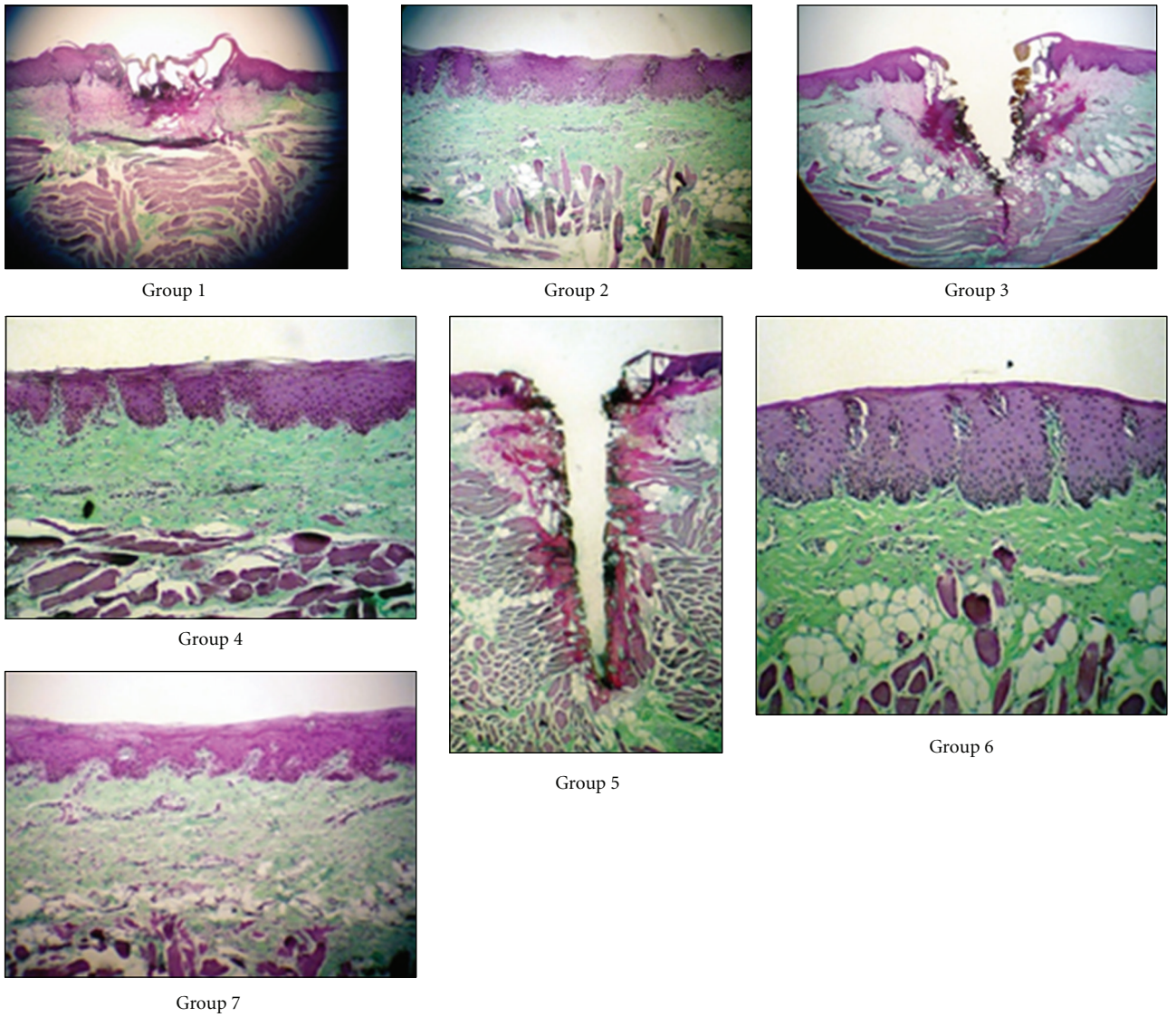


Figure 3: Histological finding of the groups at 10×10 magnification. Group 1: 1W defocused CO_2 laser irradiation. Group 2: 1W defocused CO_2 laser irradiation with absorption media. Group 3: 2W defocused CO_2 laser irradiation. Group 4: 2Wt defocused CO_2 laser irradiation with absorption media. Group 5: 3W defocused CO_2 laser irradiation. Group 6: 3W defocused CO_2 laser irradiation with absorption media. Group 7: Absorption media alone.

5. Conclusion

Histological changes were found in the groups irradiated with 1, 2, and 3 W defocused CO_2 laser continuous wave for 5 seconds. The group irradiated with 3 watts CO_2 laser continuous wave had larger depth of vaporization and depth of vertically affected border than the others, while the group irradiated with 1 watt had less width of horizontal affected area than the others. The 2 and 3 W defocused CO_2laser continuous waves irradiating for 5 seconds through the absorption media, transparent high-water content gel, can deliver the energy to the surface of tissue without causing ablation.

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Research Article

Er:YAG Laser and Fractured Incisor Restorations: An *In Vitro* Study

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Introduction. The aim of this study was to analyse the effects of an Er:YAG laser on enamel and dentine in cases of dental restorations involving fractured teeth, utilizing the dental fragment. *Materials and Methods*. Seventy-two freshly extracted bovine incisors were fractured at the coronal level by using a hammer applied with a standardized method, and the fragment was reattached by using three different methods: Er:YAG laser, orthophosphoric acid, and laser plus acid. The different groups were evaluated by a test realized with the dynamometer to know the force required to successfully detach the reattached fragment and by a microinfiltration test by using a 0.5% methylene blue solution followed by the optic microscope observation. *Results*. The compression test showed only a slight difference between the three groups, without any statistical significance. The infiltration test used to evaluate the marginal seal between the fracture fragment and the tooth demonstrated that etching with Er:YAG laser alone or in combination with orthophosphoric acid gives better results than orthophosphoric acid alone, with a highly significant statistical result. *Discussion*. Reattaching a tooth fragment represents a clinically proven methodology, in terms of achieving resistance to detachment, and the aim of this work was to demonstrate the advantages of Er:YAG laser on this procedure. *Conclusion*. This "*in vitro*" study confirms that Er:YAG laser can be employed in dental traumatology to restore frontal teeth after coronal fracture.

1. Introduction

Dental traumatology is a multidisciplinary branch of dentistry that requires a number of specific skills where, in cases of emergency, decisions have to be made within a limited timeframe and with effects that are only possible to be evaluated at a later date [1]. The technique of tooth fragment reattachment should be adapted, both in cases of simple coronal fracture (enamel and superficial dentine) as well as in complicated coronal fracture (deep dentine with pulp exposure) [2].

While in the first case the fragment may even be reattached immediately, in complicated coronal fractures the main concern should be the protection of the pulp and not necessarily the fragment, which should be kept hydrated in the fridge in a container marked with the patient's full name and the date of the trauma. The solution in the container should be changed at regular intervals and the seal checked, since in some cases, fragments may be stored for some months before being reattached.

The field of adhesive dentistry was born in 1955 by Buonocore with the description of the utilisation of orthophosphoric acid and composite resin in order to obtain restorations with high bond strength and reduced microleakage [3, 4]. In 1990 laser technology was introduced in conservative dentistry by Hibst and Keller, who described the possibility to use an Er:YAG laser as an alternative to conventional instruments such as the turbine and micromotor [5, 6]. Widespread interest in employing this new technology is related to its significant number of advantages, as described in several scientific studies. In fact, Er:YAG laser technology allows for efficient ablation of hard dental tissues, thanks to the affinity of its wavelength to water and hydroxyapatite, without the risk of micro- and macro-fractures which have

been observed by using conventional rotating instruments [7–9]. The dentin surface treated by laser appears clean, without a smear layer and with the tubules open and clear [10].

Thermal elevation in the pulp, recorded during Er:YAG laser irradiation, is less to that recorded by using turbine and micromotor in the same conditions of air/water spray [11, 12]. This wavelength has an antimicrobial decontamination effect on the treated tissues, which destroys both aerobic and anaerobic bacteria [13]. The most interesting aspects of this new technology are related to the goals of the modern conservative dentistry: "minimally invasive dentistry" and "adhesive dentistry." Er:YAG lasers can reach spot dimensions smaller than 1 mm, which enables the possibility to make a selective ablation of the affected dentin while preserving the sound tissue in order to realize very limited restorations [14].

Several in vitro studies demonstrated that the preparation of enamel and dentine by Er:YAG laser followed by orthophosphoric acid etching enhances the effectiveness in terms of reduced microleakage and increased bond strength [15]. Several authors have proposed the utilisation of laser technology also for the restoration of the frontal teeth fractured by traumatic events [16].

The aims of this in vitro study were to test the usefulness of the Er:YAG laser in the treatment of tooth fractures, by evaluating strength and microleakage of restorations obtained by bonding the broken fragment directly to the tooth.

2. Materials and Methods

Ninety-six bovine incisors were extracted by removal of the periodontal ligament with a sharp blade and subsequently carefully cleansed with sodium hypochlorite in 2% distilled water solution (Amukine 20 mL/1000 mL of distilled water). Samples were stored in a fridge at 4°C in a physiological solution, changed once per week. The teeth, removed from the container with anatomical pincers, were individually positioned in a tool table vice clamped at belt level with two felt pads. A 100 g weight hammer was employed to fracture the dental crowns, using one or two clean blows (Figure 1).

Each fractured tooth was replaced with its own fragment immersed in a container filled with 0.9% physiological solution. Fracture procedure produced more than one fragment in twenty-four teeth, resulting in exclusion from the study. The remaining seventy-two teeth (consisting of one single fracture fragment) were randomly subdivided into two groups of thirty-six teeth each (group 1 and group 2). Each group was subdivided into subgroups A-B-C, consisting of twelve dental elements.

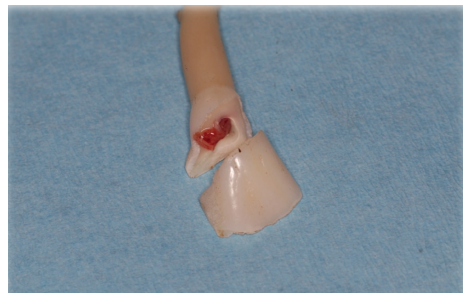

Figure 1: Fracture produced in the bovine tooth by hammer.

2.1. Groups 1A and 2A (Laser Etching)

(a) Laser etching was realized with an Er:YAG laser (Fidelis Plus III, Fotona, Slovenia) with the following parameters: 150 mJ, 10 Hz, 1.5 W, VSP (100 μsec) pulse duration, 29.9 J/cm^2 fluence, with water/air spray and an R02 handpiece (angle of 90° with an 0.80 mm spot at a distance of 1.2 mm). The procedure was made on both the fracture fragment and the tooth. In order to check the energy output from the handpiece, even though the articulated arm delivering system has a negligible loss of energy, a power meter (Ophir Nova II, thermal head F150A, Israel) was used.

(b) The fragments and teeth were dried with air spray for 15 s and then treated with applying adhesive (Prime & Bond NT Dual Cure, DENTSPLY Caulk, Milford, CT, USA) in a single step with a single-use brush, both on the tooth and the fragment.

(c) After leaving the tooth and fragment for 15 s, they were both photopolymerized by lamp (3 M DENTSPLY De Trey, Konstanz, Germany) for 20 s.

(d) A thin layer of Estelite Flow Quick composite (Tokuyama Dental Corp., Japan) was applied to the tooth.

(e) The fragment was positioned to the tooth by hand and maintained in this position during polymerization with the halogen lamp (3 M DENTSPLY) for 30 s on the vestibular face and 30 s on the palatal face.

(f) The restored teeth were replaced in physiological solution inside proper containers.

2.2. Groups 1B and 2B (Acid Etching)

(a) The fractured surfaces of twenty-four teeth with their own fracture fragments were treated by the application of orthophosphoric acid gel at 37% concentration for 30 s, (both tooth and fragment) and then rinsed with water spray for 15 s.

The steps (b), (c), (d), (e), and (f) were the same as described for the groups 1A and 2A.

2.3. Groups 1C and 2C (Acid and Laser Etching)

(a) The fractured surfaces of twenty-four teeth with their respective fracture fragments were firstly treated with an Er:YAG laser (Fotona) with the parameters previously described. Fragments and teeth were dried with an air spray for 15 s and etched with orthophosphoric acid gel at a concentration of 37% for 30 s (both tooth and fragment).

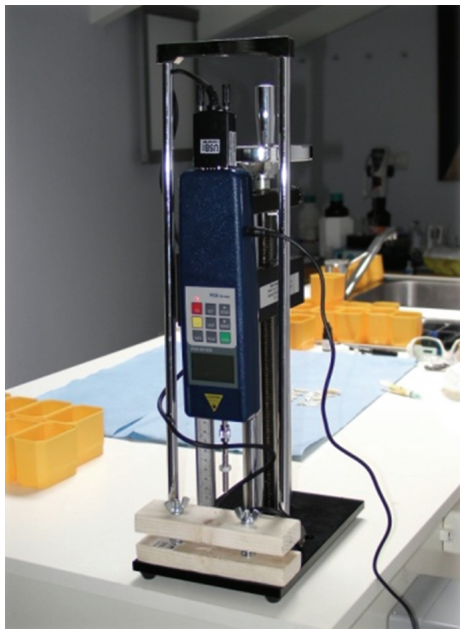

Figure 2: Dynamometer used to make the compression tests.

The steps (b), (c), (d), (e), and (f) were the same as described for the groups 1A, 2A, 1B, and 2B.

Compression Test. The teeth of group 1 were used to measure the force required to successfully detach the reattached fragment, using a dynamometer (PCE series SH 500, PCE Group, Lucca, Italy) with a resolution of 0.1 N, with ±0.5% accuracy, mounted on a SLJ50 manual stand made by the same company.

The procedure was performed as follows.

(a) Each tooth was clamped, at neck level, in a wooden vice mounted at the base of the stand.

(b) The dynamometer was brought into contact with the crown and a gradually increasing compressive force was applied until the detachment of the tooth fragment (Figure 2).

(c) The procedure was recorded on a chart using appropriate software applied to the above-mentioned dynamometer (software for PCE SH500, PCE Group, Lucca, Italy).

3. Statistical Analysis

Microleakage. The teeth of group 2 were used to analyse the microleakage as follows.

(1) The radicular apex was hermetically sealed with wax.

(2) Each tooth was waterproofed up to 0.5 mm from the edge of the fracture, through the application of two coats of transparent nail varnish (nitrocellulose dimethyl acetone MPH air, Rimini, Italy) applied with a drying interval of 10 min.

Figure 3: Tooth cut in 2 parts in the vestibule-lingual direction in order to obtain 2 symmetrical fragments.

Table 1: Criteria used by the two blind operators to assign the scores for infiltration evaluation.

Extent of dye recorded	Code
Absence of penetration	0
Limited penetration on the enamel portion of the wall	1
Penetration which also involves the dentine portion of the walls without affecting the roof of the pulp chamber	2
Penetration which reaches as far as the roof of the pulp chamber and affects it	3

Table 2: Forces recorded by the traction test to obtain the detachment of the fragments.

Sample	Group A (laser)	Group B (acid)	Group C (laser + acid)
1	500	1266	292
2	546	436	336
3	626	336	796
4	614	860	836
5	456	932	166
6	586	716	607
7	1036	606	315
8	460	616	1115
9	322	422	490
10	620	646	779
11	484	436	323
12	638	486	736

(3) Each sample was immersed in a 0.5% methylene blue solution for 12 h.

(4) Every sample was rinsed with tap water and then replaced in a container with physiological solution.

(5) After about 1 h, the teeth were removed, dried firstly with absorbent paper and then with an air spray.

(6) The previously applied transparent varnish was removed with acetone, and any remaining trace of varnish was eliminated with a rubber tip.

(7) The root was eliminated by using a diamond disk approximately 4 mm from the amelocemental junction, and the remaining part of the tooth was cut in

Table 3: Statistical analysis of the forces in the traction test.

	Group A (laser)	Group B (acid)	Group C (laser + acid)
Mean	598,33	648,166	565,583
Standard deviation (SD)	182,62	270,46	288,19
Sample size (N)	12	12	12
Std. Error of mean (SEM)	52,717	78,074	83,193
Lower 95% conf. limit	482,80	476,33	382,48
Upper 95% conf. limit	714,86	820,01	748,69
Minimum	322,00	336,00	166,00
Median (50th percentile)	600,00	611,00	551,50
Maximum	1036,00	1286,00	1115,00
Normality test KS	0,2527	0,1699	0,2038
Normality test P value	0,0331	>0,10	>0,10
Passed normality test?	No	Yes	Yes

Table 4: Results obtained by the infiltration test.

Sample	Group A (laser)		Group B (acid)		Group C (laser + acid)	
	Vestibular	Palatal	Vestibular	Palatal	Vestibular	Palatal
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	3	3	0	0
4	0	0	3	3	0	0
5	0	0	3	3	0	0
6	0	0	0	0	0	0
7	0	0	3	3	0	0
8	0	0	3	3	0	1
9	0	0	0	0	0	0
10	0	0	0	0	3	3
11	0	1	3	3	0	0
12	0	0	3	3	0	0

2 parts in the vestibule-lingual direction in order to obtain 2 symmetrical fragments (Figure 3).

(8) Each fragment was examined with an optical microscope (Novex zoom Stereo RZ, Euromex Microscopen, the Netherlands) in order to evaluate the penetration of methylene blue using a scale as described in the ISO technique. Two different operators blindly conducted the examination, and the criteria of the scores are described in Table 1.

Statistical analysis was performed with the Chi-squared test, normally used to compare the tallies or counts of categorical responses between two (or more) independent groups, and a one-way analysis of variance (ANOVA) test, used in cases where there are more than two groups; statistical significance was achieved for $P > 0.05$.

4. Results

Forces (N) required for the detachment of teeth fragments are shown in Table 2.

Statistical analysis of the fracture forces under compression for each subgroup of group 1 (1A-1B-1C) did not reveal any statistically significant differences ($P = 0.7227$) (Table 3). The results of the infiltration are showed in the Table 4.

The comparison of the three different etching methods, considering microleakage in terms of low degree (degrees 0-1) and high degree (degrees 2-3) of infiltration showed a highly significant result ($P < 0.0001$) with 0 high-degree infiltration samples for the laser etching (group 1A), 14 high-degree infiltration samples for group 1B, and 2 high-degree infiltration samples for group 1C.

5. Discussion

Reattaching a tooth fragment represents a clinically proven methodology, in terms of achieving resistance to detachment; the aim of this work was to measure, in quantitative terms, the adherence of the fracture fragment to the tooth as well as the seal in the interface zone by an infiltration test. In the literature, there is no similar protocol describing the use of laser etching as applied to tooth fragment reattachment techniques. The infiltration test used demonstrated that etching with an Er:YAG laser alone or with orthophosphoric

acid gave better results than by etching with orthophosphoric acid alone, in a statistically significant manner. All samples demonstrated a degree of marginal infiltration even if

(1) in the samples etched with the Er:YAG laser alone, the majority of the samples do not show evidence of dye infiltration (23/24 observed with optical microscope);

(2) at the level of the adhesion area of the samples etched with the Er:YAG laser and orthophosphoric acid, an absence of infiltration was noted in 21 out of 24 samples;

(3) in samples etched with orthophosphoric acid alone, only 10 out of 24 samples showed no marginal infiltration, while 14 out of 24 samples showed evidence of infiltration which reached the pulp chamber (degree 3).

Results of the infiltration test showed that the use of etching with Er:YAG laser gave a highly significant result compared to the use of orthophosphoric acid, and furthermore, that the use of the Er:YAG laser alone compared to Er:YAG laser and orthophosphoric acid demonstrated evidence of less infiltration, even if not by a statistically significant extent.

It was decided to use, in order to assess the adherence comparing the sample groups, a compression test instead of the flexural test normally utilised in these types of studies. The reason is that this "in vitro" situation is more similar to the mechanical forces applied to incisors during "in vivo" mastication.

The results showed only a slight difference between the three differently etched subgroups in group 1. Group B, etched with acid only, had the best values, as reported in Table 4, for maximum (1286 N), minimum (336 N), and average (611 N) while Group C (laser + acid) had the worst values for maximum (1115 N), minimum (166 N), and average (551 N).

The differences between all the groups were not statistically significant ($P < 0.1$) and this means that the use of an Er:YAG laser combined with acid etching gives the same bond strength as the acid-only etched teeth.

The result of this work also holds significance in regard to the controversial role of orthophosphoric acid etching when using lasers for cavity preparation. Many authors support the necessity of orthophosphoric acid etching also after Er:YAG irradiation [17] while others have demonstrated the efficacy of laser preparation alone in terms of adhesion [18].

In fact, there is no evidence of any significant difference for conditioning enamel and dentine between using the Er:YAG laser alone or in combination with orthophosphoric acid and, according to these results, Er:YAG laser should be the first choice for conditioning enamel and dentine.

6. Conclusion

The Er:YAG laser may be used in conservative dentistry as an alternative to conventional instruments and in association with orthophosphoric acid, with several advantages, such better strength bond [19], reduced microleakage [20], and also lower discomfort and higher patient satisfaction [21].

This "in vitro" study, even if considered as preliminary due to the limited number of samples, confirms that it can be employed also in dental traumatology, to restore frontal teeth after coronal fracture, with the advantage of improved adhesion of the dental fragment to the tooth, in particular by decreasing microleakage.

In fact, all the microinfiltration tests made on bovine-extracted samples demonstrated a statistically significant difference between the laser-treated and non-laser-treated groups. The compression test did not show significant differences between the sample groups, indicating that Er:YAG laser does not reduce the adhesion of composite resin when compared to the traditional instruments.

Regarding the methodology of this study, it would be interesting also to analyse samples with SEM in order to see, both in the teeth and fragments, the ultrastructural differences by using different preparation and etching techniques.

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Research Article

Use of ICDAS-II, Fluorescence-Based Methods, and Radiography in Detection and Treatment Decision of Occlusal Caries Lesions: An In Vitro Study

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Aim. To use visual inspection (ICDAS-II), laser fluorescence (LF), fluorescence based camera (FC) and radiographic examination (BW) for detection of caries and for treatment decision. *Methods*. The occlusal sites of 84 extracted permanent teeth were examined using all methods and treatment decisions (preventive or operative care) were recorded based on each method independently. For validation of the findings, fissures were opened with rotating instruments and clinical depth was determined as gold standard. Correlations (r_s), sensitivity, specificity and AUC were calculated. McNemar test was used to show whether different methods led to significant changes in treatment decisions. *Results*. Highest correlation was found between ICDAS-II and FC (r_s 0.84), ICDAS-II and gold standard (0.82) and FC and gold standard (0.81). ICDAS-II provided the highest performance (AUC 1.0), followed by FC (0.95) and LF (0.88). The greatest difference was found for treatment planning of dentine lesions, where the use of FC (cut-offs according to the literature) had the greatest agreement between operative treatment and dentine lesions, followed by use of ICDAS-II. *Conclusion*. ICDAS-II may have high potential for detection and treatment planning, and other devices, especially the fluorescence camera, can add substantial information to the visual examination, enabling examiners plan treatment more accurately.

1. Introduction

Apart from thorough detection and diagnosis of dental caries, compiling a treatment plan is another important task of a dentist. The initial assessment of hard tooth tissue is normally visual and, depending on indication and availability, X-rays are used for further detection and treatment planning.

The visual classification system International Caries Detection and Assessment System (ICDAS-II) was developed to provide clinicians, epidemiologists, and researchers with an evidence-based method for standardized data collection in different settings and better comparison between studies [1]. Reproducibility and accuracy of ICDAS-II have already shown to be promising for occlusal caries detection [2]. ICDAS criteria have the potential to aid treatment planning [3]. Depending on the visual assessment, activity of a lesion, and patient's risk status, the preferred care options might be tilted towards preventive or operative treatment.

Apart from purely visual and visual-tactile caries diagnosis, there are several other methods for the detection of dental caries on occlusal surfaces. This includes radiography, laser or light fluorescence-based methods, and electrical impedance measurements. It is well known that fluorescence-based methods make use of the phenomenon that carious lesions fluoresce more strongly than sound tissues when excited by light at specific wavelengths. The devices DIAGNOdent and DIAGNOdent pen (KaVo, Biberach, Germany) function on the same principle: they emit red light at 655 nm that causes fluorescence of bacterial metabolites in infected dentine [6]. The fluorescence emitted from the

tooth is measured and translated into a numerical scale from 0 to 99. The VistaProof fluorescence camera and the recently devised VistaCam iX (both: Dürr Dental, Bietigheim-Bissingen, Germany) have LEDs that emit high-energy blue-violet light at 405 nm onto the tooth surface. This wavelength stimulates porphyrins produced by caries-related bacteria to emit red light, containing less energy. Sound enamel, in contrast, sends out green light. This fluorescence is recorded by the camera, transferred to a computer, and processed with special software (DBSWIN, Dürr). The result is a digital image that shows lesions in different colors with respective numerical values between 0 and 4, predicting the extent and depth of caries [5, 7–9].

Radiographic examination is quite commonly used in caries detection and generally it is possible to detect approximal lesions earlier than with visual diagnosis alone [10]. However, the validity of detecting enamel caries is low for the occlusal surfaces [11].

To date, only a few studies exist which look at the impact of using visual inspection, radiographic examination, or laser fluorescence devices in treatment decision [12, 13]. No study to date has looked at the impact of the use of the new VistaCam iX fluorescence device in treatment decisions. Thus the aim of this study was to evaluate the performance of visual ICDAS-II, laser fluorescence, fluorescence-based camera, and radiographic (BW) examinations for occlusal caries detection and their ability to make treatment decisions when used as a single method or as a combination of two methods.

2. Material and Methods

2.1. Sample Selection and Visual Examination. Eighty-four permanent posterior teeth without occlusal restorations were available for the study. The teeth were collected in a dental practice and informed consent was obtained for the use of teeth for scientific purposes. The teeth were stored in a thymol-water solution immediately after extraction. Within 24 h, they were cleaned thoroughly and then stored in water. The occlusal surfaces were photographed digitally (Leica Zoomsystem Z6 APO/QWin Standard V 3.4.0 software, Leica Microsystems, Wetzlar, Germany). One site within the pit and fissure system of each tooth was marked on black and white images of the tooth surface for ease of relocation.

All sites were visually examined by two investigators (doctoral student calibrated by an experienced investigator) using the International Caries Detection and Assessment System (ICDAS-II) [14] and a consensus score for each site was achieved. The chosen sites were recorded as:

0 = sound ($n = 13$);

1 = first visible sign of noncavitated lesion seen only when the tooth is dried;

2 = visible noncavitated lesion seen when wet and dry;

3 = microcavitation in enamel;

4 = noncavitated lesion extending into dentine seen as an undermining shadow;

5 = small cavitated lesion with visible dentine: less than 50% of surface;

6 = large cavitated lesions with visible dentine in more than 50% of the surface.

2.2. Examination of Occlusal Surface with the DIAGNOdent Laser Fluorescence Device (LF) and the VistaCam iX Fluorescence-Based Camera (FC). Prior to the evaluations, the examiners were trained in using the devices according to the manufacturer's recommendations. The DIAGNOdent laser fluorescence device (LF) was first calibrated using a ceramic standard as suggested by the manufacturer. Prior to the laser fluorescence readings, the device was zeroed using an obviously sound enamel spot on the tooth (zero value). This overcomes inherent differences in tooth color. Using tip A, the laser fluorescence device was moved along the surface of the investigation site and the peak value was recorded (possible reading values 0–99). Using the VistaCam iX fluorescence-based camera (FC), images of all occlusal tooth surfaces were taken.For each tooth, the distance spacer was used in order to achieve optimum results with the caries filter. The optical head of the camera was placed on the occlusal surface of the tooth and the control ring was pressed in order to freeze the image taken by the camera. The saved images were analyzed by the camera software (DBSWIN, Dürr Dental) with possible reading values from 0–4. Detailed information on the scales of both LF and FC is given in Tables 1 and 4.

For both fluorescence methods, the sites were assessed independently from each other. Intraexaminer reproducibility for both devices was assessed by repeating the measurements on 1/3 of the investigation sites ($n = 28$) within 1 day.

2.3. Examination of Bitewing Radiographs (BW). Digital BW radiographs were taken of all teeth using the Gendex dental X-ray machine (Soredex, Helsinki, Finland) at 65 kV, 6.5 mA, and exposure time of 0.12 seconds. All radiographs were processed by the Digora Optime image scanner (Soredex, Helsinki, Finland). The teeth were placed in rows (3 teeth in each row) in suitable molds (A-R X-ray model, frasaco GmbH, Tettnang, Germany).

The digital bitewings were then viewed on an 18 in. TFT (thin-film transistor) color monitor (FlexScan L 768, EIZO, Avnet Technology Solutions GmbH, Nettetal, Germany) by the investigators and a consensus score for each site was achieved. The sites were recorded using the following scores:

0 = no radiolucency,

1 = radiolucency in the outer half of enamel,

2 = radiolucency in the inner half of enamel, up to the enamel-dentine junction,

3 = radiolucency in the outer half of dentine,

4 = radiolucency in the inner half of dentine.

2.4. Determination of Treatment Decision. For all sites a consensus treatment decision was made by the examiners using the following scores: 0 = no treatment/use of fluorides,

Table 1: Cross-tables showing the relationship between the different methods and the gold standard.

	Gold standard (clinical lesion depth)					
	0	1	2	3	4	Total
ICDAS-II scores						
0 (sound)	13	0	0	0	0	13
1-2 (enamel lesion)	0	13	16	8	4	41
3–6 (dentine lesion)	0	0	2	9	19	30
Total	13	13	18	17	23	84
LF scores*						
0–7 (sound)	12	9	10	2	1	34
8–24 (enamel lesion)	1	2	6	9	6	24
25–99 (dentine lesion)	0	2	2	6	16	26
Total	13	13	18	17	23	84
FC scores**						
0–0.9 (sound)	1	0	0	0	0	1
>0.9–2 (enamel lesion)	12	13	18	16	13	72
>2 (dentine lesion)	0	0	0	1	6	7
Total	13	13	18	17	19	80
FC scores***						
0–1.2 (sound)	13	8	4	0	0	25
1.3–1.4 (enamel lesion)	0	4	5	2	0	11
>1.4 (dentine lesion)	0	1	9	15	19	44
Total	13	13	18	17	19	80
Bitewing scores						
0 (sound)	12	12	16	16	13	69
1-2 (enamel lesion)	0	0	0	0	0	0
3-4 (dentine lesion)	1	1	2	1	10	15
Total	13	13	18	17	23	84

*Cutoffs according to the literature [4].
**Cutoffs according to the manufacturer's recommendation.
***Cutoffs according to the literature [5].

1 = sealant application, 2 = round bur and sealant application, and 3 = restoration (e.g., composite, amalgam, and glass ionomer). For further analysis the treatment decisions were collapsed into preventive care (pc: score 0-1) and operative care (oc: score 2-3).

2.5. Determination of Lesions' Depth (Gold Standard). To determine the depth of the lesions, all tooth surfaces were opened with rotating instruments. The end point of the excavation was reached when there was no longer any sign of caries [15]. In the process, the lesions' depths were divided up into the following categories: sound tooth surface (score 0), caries in the enamel (clinical score 1), caries down to the enamel-dentine junction (clinical score 2), and caries in the first third of dentine (clinical score 3), caries down to the second third of dentine or near pulp, or pulp already effected (clinical score 4).

All the sites were investigated by two examiners and a consensus clinical score was derived for each investigation site.

2.6. Data Management and Statistical Evaluation. All findings were recorded on data collection forms and later transferred to an Excel table. The statistical analysis was performed using MedCalc, Version 11.3.4.0. Intraexaminer reproducibility was calculated using the intraclass correlation coefficient (ICC). The relationship between all the systems was assessed using the Spearman rank correlation.

The consensus clinical scores were used to calculate sensitivity and specificity at the D_1 and D_3 diagnostic threshold. At the D_1 diagnostic threshold all clinical scores 1–4 were classed as caries, for the D_3 diagnostic threshold clinical scores 3 and 4 were classed as caries only. Using these sensitivity and specificity values ROC (receiver operating characteristic) analyses were carried out for each method. Sensitivity and specificity values for the LF measurements were obtained at the cut-off values according to the literature [4]. For the FC, sensitivity and specificity values were used firstly at the cut-off values according to the manufacturer and secondly at the cut-off values which were determined according to the literature [5]. The performance of each method (AUC) was interpreted by using the following classification: 0.50–0.60 fail, 0.60–0.70 poor, 0.70–0.80 fair, 0.80–0.90 good, and 0.90–1.0 excellent [16, 17].

A nonparametric test (the McNemar test) was used to show whether different methods or combinations of

Table 2: Spearman's correlation coefficients between different methods.

	Spearman's correlation coefficient (r_s)			
	LF	FC	BW	Gold standard
ICDAS-II	0.66**	0.84**	0.36**	0.82**
LF	—	0.72**	0.27*	0.69**
FC	—	—	0.26*	0.81**
BW	—	—	—	0.22*

*Correlation significant at the 0.05 level (two tailed).
**Correlation significant at the 0.01 level (two tailed).

methods lead to significant changes in treatment decisions ($\alpha = 0.05$).

3. Results

A total of 84 occlusal sites on posterior teeth were examined (52 molar and 32 premolar teeth). The distribution of the sites according the ICDAS-II criteria was code 0 = 13, code 1 = 15, code 2 = 26, code 3 = 12, code 4 = 2, code 5 = 12, and code 6 = 4.

While using the FC device, 4 investigation sites could not be assessed due to technical problems. Thus, further statistical calculation for the FC was performed using 80 sites. Intraclass correlation coefficients (ICC) for intraexaminer reproducibility were 0.98 for the LF device and 0.97 for the FC.

The distribution of the sites according to the different methods cross-tabulated with the gold standard scores is presented in Table 1. All methods assessed nearly all sound tooth surfaces correctly, except for FC when the cut-off values according to the manufacturer were used.

Spearman correlation coefficients between all the methods are presented in Table 2. All correlations were significant at the 0.01 level or at the 0.05 level, respectively (two tailed). The highest correlation was found between ICDAS-II and FC measurements followed by the correlation between ICDAS-II and the gold standard. The lowest correlation was found between the findings of bitewing radiography and the gold standard.

Sensitivity, specificity, and the areas under the ROC curves (AUC) at D1 and D3 diagnostic thresholds are presented in Table 3. The use of ICDAS-II, LF, and FC demonstrated good to excellent diagnostic performances (AUC between 0.88 and 1.0), while the performance of bitewing radiography for detection of occlusal lesions was shown to be weak (AUC 0.56 and 0.59, resp.). At the D1 diagnostic level specificity for the FC was low using the cut-off suggested by the manufacturer. When other cutoffs [5] were used, specificity increased to 100%. The same findings were observed for the sensitivity of the FC at D3 diagnostic level.

Table 4 presents the distribution of the sites according to the different methods cross-tabulated with the treatment decision. Table 5 shows whether the treatment decisions changed significantly from preventive care to operative care when different methods were used for treatment planning. For example, when treatment decisions with ICDAS-II were cross-tabulated with treatment decisions after bitewing assessment, operative rather than preventive treatment was chosen for significantly more sites ($n = 19$ and $P = 0.003$). Similar findings were observed for treatment decisions involving ICDAS-II versus FC with regular cutoffs ($P < 0.001$), LF versus FC ($P < 0.001$), or LF versus BW ($P = 0.019$). When the FC findings were classified according to the cutoffs in the literature [5], the treatment decisions shifted significantly toward the operative approach ($P < 0.001$).

In Table 6 the treatment decision after combining each method with the visual system ICDAS-II is cross-tabulated with the treatment decision of the method alone. It can be seen that the best agreement was found when the treatment decision was based on a combination of ICDAS-II and FC (cutoffs set by the manufacturer) compared to ICDAS-II-based decisions alone, followed by a combination of ICDAS-II and bitewing radiography.

In Table 7 the results of the treatment decision according to different methods and the gold standard are cross-tabulated. It can be seen that non-operative care for sound surfaces was suggested with each method, indicating high specificity of the treatment decision. With the FC the decision for operative treatment was made for 10 surfaces with caries only in the enamel, when cutoffs according to the literature were used. The corresponding numbers for the other methods were much lower (between 0 to 4 surfaces with enamel lesion). On the other hand, almost all of the clinically dentine lesions were planned to be treated operatively (34/36).

4. Discussion

Ideal management of a caries lesion includes not only the use of criteria for extent of lesion but also treatment planning which should express the results of lesion assessment in terms of background lever care, preventive treatment options, and operative treatment options [18]. The specific treatment options recommended for specific lesions and patients will depend upon a variety of factors, such as lesion activity and monitoring lesion behavior over time [18]. Of course, these factors cannot be considered in an in vitro study such as this, but only factors such as lesion depth and extent, color, and the tooth surface where the lesion is assessed.

In the present study, occlusal lesions were determined using various currently common methods of caries detection and treatment options recommended on the basis of these examinations. In the process, methods of fluorescence and laser fluorescence were given particular attention. The results show that the visual method and the fluorescence camera exhibited the highest correlation to the gold standard (Table 2). By contrast, caries detection using bitewing radiographs showed the lowest correlation, as well as the lowest diagnostic quality with regard to the detection of occlusal lesions (Table 3).

Modern dentistry now offers many options for preventive treatment (e.g., the use of fluorides or other remineralizing agents, fissure sealing, caries infiltration, etc.). In order to make such decisions, however, we need to carefully detect

Table 3: The area under the ROC curve (AUC), sensitivity (SE), and specificity (SP) for each method at the D_1 and D_3 diagnostic thresholds.

D_1 diagnostic threshold	ICDAS-II	LF	FC		BW
AUC (95% CI)	1.0 (0.96–1.0)	0.88 (0.79–0.94)	0.95 (0.88–0.99)		0.56 (0.44–0.67)
SE (%)	100	69.0*	100**	82.1***	19.7
SP (%)	100	92.3*	7.7**	100***	92.3
D_3 diagnostic threshold	ICDAS-II	LF	FC		BW
AUC (95% CI)	0.92 (0.84–0.97)	0.88 (0.79–0.94)	0.93 (0.85–0.98)		0.59 (0.48–0.67)
SE (%)	70.0	52.5*	19.44**	94.4***	27.5
SP (%)	95.5	90.9*	100**	77.3***	90.9

CI: confidence interval.
*Cutoffs according to the literature [4].
**Cutoffs according to the manufacturer's recommendation.
***Cutoffs according to the literature [5].

Table 4: Cross tables showing the relationship between the different methods and the treatment decisions.

	Preventive care	Operative care	Total
ICDAS-II scores			
0 (sound)	13	0	13
1-2 (enamel lesion)	41	0	41
3–6 (dentine lesion)	0	30	30
Total	54	30	84
LF scores*			
0–7 (sound)	34	0	34
8–24 (enamel lesion)	24	0	24
25–99 (dentine lesion)	0	26	26
Total	58	26	84
FC scores**			
0–0.9 (sound)	1	0	1
>0.9–2 (enamel lesion)	72	0	72
>2 (dentine lesion)	0	7	7
Total	73	7	80
FC scores***			
0–1.2 (sound)	25	0	25
1.3-1.4 (enamel lesion)	11	0	11
>1.4 (dentine lesion)	0	44	44
Total	36	44	80
Bitewing scores			
0 (sound)	69	0	69
1-2 (enamel lesion)	0	0	0
3-4 (dentine lesion)	0	15	15
Total	69	15	84

*Cutoffs according to the literature [4].
**Cutoffs according to the manufacturer's recommendation.
***Cutoffs according to the literature [5].

Table 5: Cross tables showing the relationship between the treatment decisions when different methods were used.

Treatment decision after using	Number of teeth related to the treatment decision							
	LF*		FC**		FC***		BW	
ICDAS-II	pc	oc	pc	oc	pc	oc	pc	oc
pc	45	9	54	0	34	20[a]	50	4
oc	13	17	19[a]	7	2	24	19[b]	11
LF*			pc	oc	pc	oc	pc	oc
pc			57	1	34	24[a]	54	3
oc			16[a]	6	2	20	15[c]	11
FC**					pc	oc	pc	oc
pc					36	37[a]	65	8
oc					0	7	3	4
FC***							pc	oc
pc							33	3
oc							35[a]	9

pc: preventive care, oc: operative care.
*Cutoffs according to the literature [4].
**Cutoffs according to the manufacturer's recommendation.
***Cutoffs according to the literature [5].
Within columns, significant differences are represented by different superscript letters (the McNemar test: a: $P < 0.001$/b: $P = 0.003$/c: $P = 0.019$).

healthy hard tooth tissue as well as initial lesions so that they too can be given preventive therapy. The visual ICDAS-II and apparatus-based (LF and FC) methods used here have shown their potential for discovering and differentiating a range of carious lesions in a variety of studies [2, 5, 19].

The present study investigated whether each of these means of detection alone would be capable of indicating an adequate treatment recommendation with regard to a preventive or operative treatment option when the recommended or already published cutoffs were used to differentiate healthy teeth, enamel lesions, and dentine caries. Since the reproducibility of the fluorescence systems in particular is very high, it should be assumed that the detection of the lesions, and thus the therapy decision would be possible after using the method several times (monitoring) as well as if the attending dentist should change. In this study, the ICC values for the LF and FC were high for intraexaminer reproducibility and the figures were comparable to studies already published [5, 20]. The reproducibility of ICDAS-II of one of the reference examiners in this study was the subject of other studies, where intraexaminer kappa values were in a range of 0.82–0.93 [2, 21, 22]. In the present study,

Table 6: Cross tables showing the relationship between the treatment decisions when each method was combined with the visual inspection.

Treatment decision after using	Number of teeth related to the treatment decision							
	ICDAS-II + LF*		ICDAS-II + FC**		ICDAS-II + FC***		ICDAS-II + BW	
ICDAS-II	pc	oc	pc	oc	pc	oc	pc	oc
pc	45	9[a]	54	0	34	20[b]	50	4
oc	0	30	0	30	0	30	0	30
LF*	pc	oc						
pc	45	13[b]						
oc	0	26						
FC**			pc	oc				
pc			54	19[b]				
oc			0	7				
FC***					pc	oc		
pc					34	2		
oc					0	44		
BW							pc	oc
pc							50	19[b]
oc							0	15

pc: preventive care, oc: operative care.
*Cutoffs according to the literature [4].
**Cutoffs according to the manufacturer's recommendation.
***Cutoffs according to the literature [5].
Within columns, significant differences are represented by different superscript letters (the McNemar test: a: $P = 0.004$/b: $P < 0.001$).

Table 7: Cross tables showing the relationship between the treatment decision according to different methods and the gold standard.

Treatment decision after	Gold standard (clinical lesion depth)			
	0 (sound)	1-2 (enamel lesion)	3-4 (dentine lesion)	Total
ICDAS-II				
pc	13	29	12	54
oc	0	2	28	30
Total	13	31	40	84
LF*				
pc	13	27	18	58
oc	0	4	22	26
Total	13	31	40	84
FC**				
pc	13	31	29	73
oc	0	0	7	7
Total	13	31	36	80
FC***				
pc	13	21	2	36
oc	0	10	34	44
Total	13	31	36	80
Bitewing				
pc	12	28	29	69
oc	1	3	11	15
Total	13	31	40	84

pc: preventive care, oc: operative care.
*Cutoffs according to the literature [4].
**Cutoffs according to the manufacturer's recommendation.
***Cutoffs according to the literature [5].

a consensus decision was made for the ICDAS-II, and hence no reproducibility values were calculated.

The analysis of AUC, sensitivities, and specificities at different diagnostic thresholds (Table 3) showed that ICDAS-II had excellent AUC as well as high sensitivity and specificity. Initial in-vitro studies using ICDAS-II for detection of occlusal lesions [2, 7] showed lower overall performance of ICDAS-II (AUC between 0.73–0.88). Later studies by some authors of the present study clearly showed an increase of ICDAS-II performance, sensitivity, and specificity values [15, 22] which might be explained by the enhanced training in this system. Comparing the performance of the fluorescence systems with other studies, it can be shown that the reported figures stay constant. For example, the performance of laser fluorescence measurements was reported to be between 0.66–0.86 in different studies and under different conditions [7, 20, 23]. The performance of the newly introduced VistaCam iX fluorescence camera has not yet been the subject of many studies. Jablonski-Momeni et al. [5] calculated AUC between 0.87 and 0.92 for 2 examiners at the D1 and D3 diagnostic threshold. The authors showed that there was no significant difference to the performance of the already well-known VistaProof. The performance of this device ranged between 0.75 and 0.96 in different studies [5, 7, 24] and would thus seem to be a useful tool for the detection of occlusal lesions. However, the good performance of the apparatus-based procedure must be seen in a somewhat differentiated way: different cut-off points were predefined by the manufacturer at the launch of the VistaCam iX camera. These cut-off points were intended to facilitate differentiation of the caries stages from sound to dentine caries with the help of the numerical scale. According to this, values ≥ 1.0 should be considered proof of the beginning of enamel caries, while dentine caries supposedly produced values ≥ 2.0. Previous studies [5] of the optimum sensitivity and specificity have shown that this cut-off point should be set at 1.4/1.5 to detect dentine lesions appropriately, which means that lesions >1.5 can be expected to indicate dentine caries. A comparable cut-off point (>1.4) for the determination of dentine caries was found in other studies using the VistaProof [9, 24, 25]. In the present study, treatment decisions were determined using the cut-off points according to the manufacturer and according to the literature [5], respectively. It could be seen that sensitivity values at the D1 and D3-level were higher when cutoffs according to the literature [5] were used (Table 3). When these cutoffs were used for treatment planning, almost all of the clinically dentine lesions were planned to be treated operatively (34/36, Table 7). However, it should be taken into account that 4 investigation sites could not be assessed due to technical problems while using the FC for caries detection.

The use of BW for occlusal caries detection showed low sensitivity (Table 3). Other authors had reported higher values of sensitivity but similar specificity values when BW radiographs were evaluated for occlusal caries detection [26, 27]. Looking at the correlation coefficients found in our study (Table 2) it can be observed that BW had the weakest correlation compared to all other methods and to the gold standard, respectively. When BW was used for treatment decisions, more than 70% (29/40, Table 7) of clinically dentine lesions were planned to be treated in a preventive manner. Usually studies on using radiography and treatment decisions tend to use BW for approximal caries detection rather than for occlusal lesions [28]. Diniz et al. [13] suggested in a recently published study that the ICDAS examination shows better performance than radiographic examination for occlusal caries detection. These findings were confirmed by the present study.

In vitro studies usually establish the validity of a detection system by using a gold standard against which the sensitivity and specificity of the diagnostic method can be calculated. A common gold standard used for occlusal caries lesions is the histological evaluation of hard tissue sections, with large variations in the methodology. Teeth are hemisected at the place to be examined, or hard tissue sections are prepared whose thickness can vary considerably [29]. In order to be close to clinical conditions we determined the depth of the lesions by opening the tooth surfaces with rotating instruments. This method might show some shortcomings, and in order to validate whether the caries was removed in total sectioning of the excavated teeth could probably have been an additional option.

The results of our study show that preventive care for sound surfaces was suggested with each detection method, indicating high specificity of the treatment decision (Table 7). Almost all enamel lesions which were found clinically were planned to be treated using preventive treatment options. The greatest difference was found for treatment planning of dentine lesions, where the use of FC (with cut-offs according to the literature) had the greatest agreement between operative treatment and dentine lesions, followed by use of ICDAS-II. This information may suggest that the use of FC is the primary criterion for evaluating the condition of the dental surface before the establishment of a treatment plan, followed by ICDAS-II. But it should be taken into account that use of detection tools should always be seen as an adjunct to a primary visual detection of dental caries and thus it should rather be the other way round: that a tooth surface is first observed by a visual detection method and then as a second step, another device can be used for further detection. Treatment options can be suggested as a consequence of the examinations.

Ie and Verdonschot [30] observed that although carious lesions can be observed by visual examination, caries preventive strategies will not be initiated until very late. A solution to this problem would be to detect caries lesions at an early stage of development, for example, by using systems like ICDAS-II. In the study by Diniz et al. [13] ICDAS has proved to be reliable for early caries detection, but there was no strong correlation with histology. In the present study the findings (correlation to gold standard, performance, sensitivity, and specificity values) indicate that ICDAS-II may have a high potential for detection and treatment planning, and that other devices, especially the fluorescence camera, could add substantial information to the visual examination, enabling examiners to plan treatment more accurately.

Of course, issues like caries activity and patient-based information cannot be taken into account for treatment decisions within the limitations of an in vitro study.

Conflict of Interests

The authors declare that they have no conflict of interest.

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Research Article

Management of Root Perforations Using MTA with or without Er:YAG Laser Irradiation: An In Vitro Study

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The aim of this in vitro study is to compare the microleakage of a root perforation sealed with MTA (mineral trioxide aggregate) (group M) to that sealed with MTA following Er:YAG laser irradiation (group ML). Forty-two recently extracted human monoroot teeth were used. Two cavities were prepared on each root surface. Randomly, on each root, the exposed dentine of one cavity was irradiated prior to MTA filling using an Er:YAG laser with the following settings: 200 mJ/pulses under an air water spray, 10 Hz, pulse duration of 50 μsec, and 0.7 mm beam diameter. All cavities were then sealed with MTA. submitted to thermocycling and immersed in 2% methylene blue dye solution for 12 h. The penetration of methylene blue in the microleakage of cavity was observed and recorded. The mean value dye penetration in cavities sealed with MTA following Er:YAG laser irradiation (23.91 ± 14.63%) was lower than that of unlased cavities sealed only with MTA (25.17 ± 17.53%). No significant difference was noted. The use of an Er:YAG laser beam for dentinal conditioning prior to MTA filling of perforated roots did not decrease significantly the microleakage of MTA sealing when compared to the conventional use of MTA filling.

1. Introduction

Root perforations connect root canal spaces with periodontal tissues. The connection may occur as a result of iatrogenic causes during root canal treatment (at the level of the floor of the cameral cavity or at different levels of the root) or during prosthetic treatment for postcanal penetration. It may be also inducted by external root resorption or by the caries process. Prognoses for perforated roots depend on the time lapsed before the perforation is sealed, the localization and size of the perforation, and the quality of the sealing material used.

Mineral trioxide aggregate (MTA) has been used in a variety of surgical and nonsurgical endodontic applications [1]. Several studies have demonstrated that MTA offers good-quality sealing of dentine [2–7]. Unlike other commonly used materials such as glass ionomer and reinforced zinc oxide-eugenol cementpagebreak (Super-EBA; Harry J; Bosworth Company, Skokie, IL, USA), MTA is a biocompatible material and allows bone formation [8–11]. MTA is commonly accepted as the best choice for root perforation treatment.

On the other hand, Raldi et al. [12] demonstrated that the surface of dentin irradiated by an Er:YAG laser has better cell adhesion than MTA surfaces and unlased dentinal surfaces. Furthermore, Baraba et al. [13] showed that an Er:YAG laser, used under specific irradiation conditions, is more efficient than mechanical drills for enamel and dentin ablation. Delmé et al. [14] demonstrated that the use of acid etching is mandatory to obtain good adhesion and retention with resin composites.

The purpose of this study was to evaluate the capacity of the Er:YAG laser to improve the quality of MTA sealing and to compare the microleakage of roots sealed using MTA versus tose sealed by MTA assisted by an Er:YAG laser.

2. Materials and Methods

2.1. Root Cavities Preparation. Forty-two recently extracted human mono-roots were used (mainly canines and second premolars), stored in distilled water at 4°C. Two cavities were prepared on the opposite sides of the cervical part of each root, using a standard diamond bur of 1.7 mm diameter and 2 mm depth with a stopper (Meissinger 828G017, Hager-Meisinger Gmbh, Neuss Germany) (Figure 1). The bottom of the cavities did not have any connection with root canals.

2.2. Laser Irradiation Parameters. The laser apparatus was an Er:YAG laser (wavelength: 2940 nm; Fidelis Plus II, Fotona, Slovania). Laser settings were 200 mJ, 10 Hz, SSP mode (50 μsec), energy density per pulse: 44,1 J/cm^2, water spray: 10 mL/min, air: 20 mL/min, noncontact hand piece (model R 02-C), beam diameter of 0.7 mm, and distance to target: 7–9 mm.

On each root, one of the cavities was randomly selected. Only the surface of the cavity was irradiated superficially in one passage. A total number of 39 teeth were used for sealing measurements, and three teeth were used for SEM views (group ML).

2.3. Preparation of MTA. One commercial brand of gray MTA (Proroot; Dentsply Maillefer, Ballaigues, Switzerland) was used. The cement was prepared according to the manufacturer recommendations. MTA was placed in each cavity. Light pressure was applied to the MTA using wet cotton pellets. The surface was burnished with a B-3 condenser/ball burnisher (Analytic, Synbron Endo, Orange, USA) in order to improve the marginal sealing. The roots were stored in moist conditions at 37°C for one week.

Before MTA sealing of the cavities, the dentine of the drilled and unlased cavities was left without any complementary treatment (group M). The smear layer was left on the dentinal surface.

2.4. Thermocycling. One week after MTA sealing of the cavities and before thermocycling, the root surfaces were polished by means of sof-lex Pop-on discs (medium, 3 M, ESPE, USA) under cold water, with the aim of avoiding an eventual excess of MTA overhanging the border of the cavities. Next, the roots were thermocycled for 1000 cycles, for 24 h, from 5°C to 55°C (Willytec Thermocycler V 2.9, Westerham, Germany).

2.5. Microleakage Measurements. Two coats of an acid-resistant marine varnish (Alkydurethan varnish, Trimetal, Belgium) were applied, leaving a 0.5 mm border around the edge of each cavity in order to protect the rest of the tooth from the dye solution. The roots were immersed in 2% methylene blue solution for 12 h. Great care was taken to keep the apices of the roots out of the dye solution (the coronal parts of the teeth were held in wax to prevent the root apices from being immersed). The teeth were rinsed and brushed (Oral B, Braun).

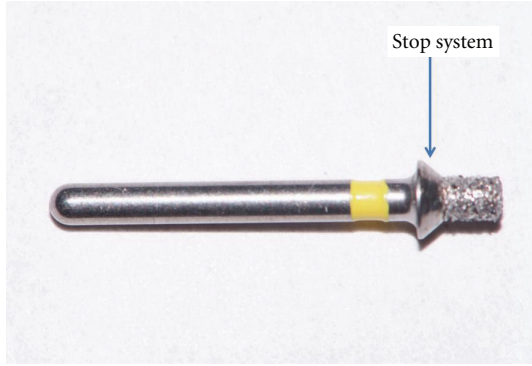

Figure 1: View of the diamond bur used for cavity preparations. Arrow shows the stop system.

Each root was sliced longitudinally in order to allow observation of both cavities. An average of three slices were collected from each root. All specimens were examined blindly by three examiners. Penetration of the dye was measured in millimeters using Visilog 5.3 analysis software (Noesis vision, St Laurent, PQ, Canada). For each cavity, the deepest penetration of the methylene blue dye was recorded (D value) after agreement of the examiners. The measurements were calibrated by reference to the total length of the diamond bur (2 mm). The percentage of dye penetration was calculated by dividing the deepest penetration of the dye by the total depth of the cavity (2 mm) (D/2 mm $\times$ 100 = % of dye penetration). Percentages of dye penetration in all cavities were used for statistical analysis (t-Student). A normality test, using the Kolmogorov and Smirnov method, was performed and followed by a test for the qualitative paired data.

The comparison of means and SD of microleakage was expressed in percentage of dye penetration.

2.6. SEM Analysis. Three more monoroots were used for SEM analysis (Hitachi S-4700-II FESEM, Tokyo, Japan). The aspect of the drilled dentine without any complementary treatment was compared to the aspect of the drilled and Er:YAG-conditioned dentine.

3. Results

The mean and standard deviation value of the percentage of dye penetration in the unlased cavities filled with MTA (group M) was 25.17 ± 17.53% (minimum value = 10; maximum value = 73). The mean value and standard deviation of the percentage of dye penetration in the group of cavities filled with MTA following Er:YAG laser irradiation (group ML) was 23.91 ± 14.63% (minimum value = 10; maximum value = 58) (Figure 2). The mean values of microleakage and dye penetration resulting from the use of an Er:YAG laser to precondition dentine were the lower of the two groups (Figure 2). All groups passed normality tests using the Kolmogorov and Smirnov method.

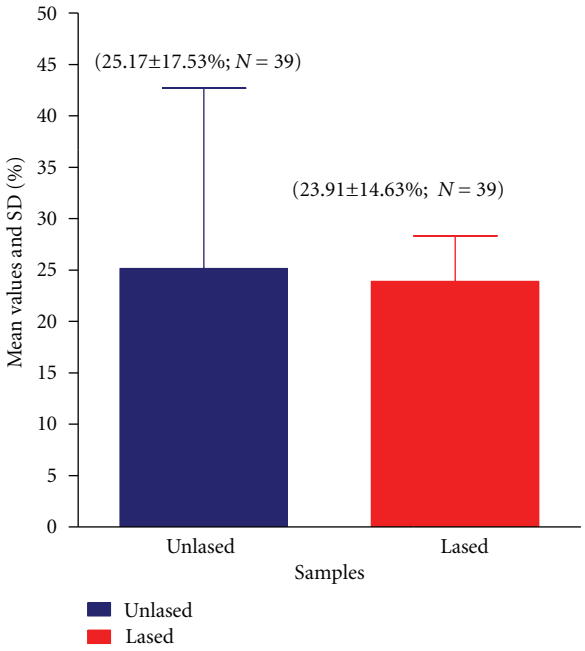

Figure 2: Mean values and standard deviation of the dye penetration (microleakages) in cavities filled with MTA. Lased: mean of Dye penetration in the Er:YAG lased dentine cavities (group ML); Unlased: mean of dye penetration in the unlased dentin cavities filled with MTA (group M).

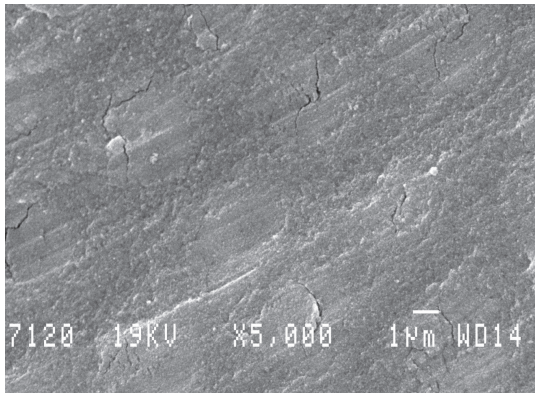

Figure 3: View of the dentine drilled without any complementary treatment exhibited a smear layer covering the tubules (group M). Magnification: 5000x. Scale bar = 1 μm.

The difference between the percentage of dye penetration in cavities sealed with MTA (group M) that in those sealed with MTA following Er:YAG laser irradiation (group ML) was not statistically significant (paired and two-tailed t-test; $P = 0.2519$; $t = 1.235$ with 8 degrees of freedom; 95% confidence interval).

The dentine drilled without any complementary treatment exhibited a smear layer covering the tubules and the total surface of the exposed dentine (Figure 3). On the other hand, on the dentine conditioned by Er:YAG laser, the Er:YAG laser had removed the smear layer (Figure 4). The tubules were totally opened. The Er:YAg laser produced a selective and preferential ablation of intertubular dentine, while the peritubular dentine (higher mineralization) was less ablated (Figure 4).

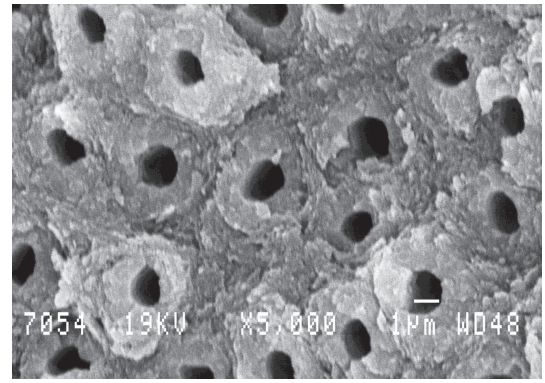

Figure 4: View of the dentine conditioned by Er:YAG laser (group ML), the Er:YAG laser had removed the smear layer. The tubules are totally opened. The Er:YAG laser produced a selective and preferential ablation of intertubular dentine, while the peritubular dentine (higher mineralization) was less ablated. Magnification: 5000x. Scale bar = 1 μm.

4. Discussion

The literature mentions several methods for the evaluation of root canal fillings. However, methylene blue is not the best choice for the measurement of the quality of sealing of root canal fillings. In our study, we evaluated the microleakage in the marginal area between the MTA and dentine. We used this dye because of its common use in several articles for the evaluation of microleakage of dentinal fillings [15–17].

Several studies showed that the use of an Er:YAG laser for cavity preparation has many advantages. Er:YAG laser (2940 nm) conditioning of perforated dentine under an air-water spray is noncontact and decontaminating [18]. The irradiation of root surfaces can remove bacterial endotoxin. Furthermore, according to our results and other studies, an Er:YAG laser beam is able to remove the dentinal smear layer and open the dentinal tubules [19, 20]. On the other hand, the conditioning of dentin by Er:YAG laser irradiation can favor cells adhesion [13]. For these reasons, we selected the Er:YAG laser for the dentinal conditioning of root perforation treatment.

The properties of MTA were studied and described. Garthner and Dorn [21] proposed the ideal characteristics of MTA: simple manipulation, radio-opacity, stability in three dimensions and in a moist environment for a long period, nonresorbability, quality adhesion to dentin, and biocompatibility with desmodontal cells.

Fournier and Bouter [9] recommended the consideration of three properties: cytotoxicity of filling material, long-term sealing strength, and simplicity of manipulation.

MTA contains a hydrophilic powder of tricalcic silicate, iron, tricalcic aluminate, tricalcic oxide, silice oxide particles, and a bismuth oxide for radio-opacity 9. Manufacturers

produce two kinds of MTA (white or gray) [22]. Both offer good cellular biocompatibility and good sealing to prevent leakages [22–26].

Several studies explained that the biocompatibility of the MTA may be due to the release of calcium hydroxide in water [3, 10, 26, 27]. Other studies reported that MTA is well tolerated by periodontal tissues [28, 29]. Torabinejad et al. [3] showed the fibrous formation in contact with MTA through histological studies. Pitt Ford et al. [30] and Thomson et al. [31] observed that the MTA sealing of root perforations induces cement formation. Because of these qualities of tightness, MTA is mainly indicated for retroapical fillings [30], apexification [32], and root perforations [1, 5–7, 33]. The prognosis for a perforated root depends on the time elapsed before the sealing of the perforation, the localization and size of the perforation, and the sealing quality of the material used [34].

In accordance with the previous articles, our results demonstrate a higher sealing (in mean) of MTA to Er:YAG laser-conditioned dentine [35, 36] versus unlased MTA-sealed dentine. However, the statistical analysis did not show any significant difference. Our SEM study shows that Er:YAG laser conditioning of dentine removes the dentinal smear layer and has a less ablative effect on peritubular dentine, which has higher mineral content. The more effective sealing of MTA to Er:YAG-lased dentine may be due to the interaction between MTA components and the exposed mineral content of Er:YAG-lased dentine (chemical effects). A second explanation may be the increase of microretention in lased dentin and dentinal opened tubules (physical effect) (Figure 4).

An intimate contact between MTA and the dentine is required, but in the literature, the necessity to remove or not the smear layer prior to MTA application to exposed dentine is not clear. There is a necessity to have a standard clinical procedure mentioning the necessity or not to remove the smear layer. For this reason, we did not remove the dentinal smear layer from the exposed dentin prior to the application of MTA.

5. Conclusion

The use of an Er:YAG laser beam for dentinal conditioning prior to MTA filling of perforated roots or localized root crack treatments did not decrease significantly the microleakage of MTA sealing when compared to the conventional use of MTA filling.

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Research Article

In Vitro Wound Healing Improvement by Low-Level Laser Therapy Application in Cultured Gingival Fibroblasts

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The aim of this study was to determine adequate energy doses using specific parameters of LLLT to produce biostimulatory effects on human gingival fibroblast culture. Cells (3×10^4 cells/cm^2) were seeded on 24-well acrylic plates using plain DMEM supplemented with 10% fetal bovine serum. After 48-hour incubation with 5% CO_2 at 37°C, cells were irradiated with a InGaAsP diode laser prototype (LASERTable; 780 ± 3 nm; 40 mW) with energy doses of 0.5, 1.5, 3, 5, and 7 J/cm^2. Cells were irradiated every 24 h totalizing 3 applications. Twenty-four hours after the last irradiation, cell metabolism was evaluated by the MTT assay and the two most effective doses (0.5 and 3 J/cm^2) were selected to evaluate the cell number (trypan blue assay) and the cell migration capacity (wound healing assay; transwell migration assay). Data were analyzed by the Kruskal-Wallis and Mann-Whitney nonparametric tests with statistical significance of 5%. Irradiation of the fibroblasts with 0.5 and 3 J/cm^2 resulted in significant increase in cell metabolism compared with the nonrradiated group ($P < 0.05$). Both energy doses promoted significant increase in the cell number as well as in cell migration ($P < 0.05$). These results demonstrate that, under the tested conditions, LLLT promoted biostimulation of fibroblasts in vitro.

1. Introduction

Tissue healing involves an intense activity of diverse cell types, such as epithelial and endothelial cells, as well as fibroblasts which play a key role in this process [1]. Fibroblasts secrete multiple growth factors during wound reepitelialization and participate actively in the formation of granulation tissue and the synthesis of a complex extracellular matrix after reepitelialization [1]. All these processes directly involve the proliferation and migration capacity to these cells [1]. The use of low-level laser therapy (LLLT) has been proposed to promote biostimulation of fibroblasts and accelerate the healing process [2].

Previous studies have evaluated the effect of LLLT on the proliferation and migration of human gingival fibroblasts as well as other cellular effects and responses, such as protein production and growth factor expression [2–6]. Nevertheless, there is a shortage of studies investigating irradiation parameters capable of promoting biostimulatory effects on fibroblasts in order to establish an ideal irradiation protocol for these cells [7]. Therefore, the aim of this study was to determine the most adequate energy doses using specific parameters of LLLT to produce biostimulatory effects on human gingival fibroblast cultures in an in vitro wound healing model.

2. Material and Methods

2.1. Gingival Fibroblast Cell Culture. All experiments were performed using human gingival fibroblast cell culture (continuous cell line; Ethics Committee 64/99-Piracicaba

Dental School, UNICAMP, Brazil). The fibroblast cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA), with 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L glutamine (Gibco, Grand Island, NY, USA) in an humidified incubator with 5% CO_2 and 95% air at 37°C (Isotemp; Fisher Scientific, Pittsburgh, PA, USA) [8]. The cells were subcultured every 2 days in the incubator under the conditions described above until an adequate number of cells were obtained for the study. The cells (3×10^4 cells/cm^2) were then seeded on sterile 24-well acrylic plates using plain DMEM supplemented with 10% FBS for 48 h.

2.2. LLLT on Fibroblast Culture. The LLLT device used in this study was a near infrared indium gallium arsenide phosphide (InGaAsP) diode laser prototype (LASERTable; 780 ± 3 nm wavelength, 0.04 W maximum power output), which was specifically designed to provide a uniform irradiation of each well (2 cm^2) in which cultured cells are seeded [8, 9]. The power loss through the acrylic plate was calculated using a potentiometer (Coherent LM-2 VIS High-Sensitivity Optical Sensor, USA), which was placed inside the culture plate. After this measure, the power loss of the plate was determined as 5%. After that, the power of all diodes was checked and standardized. Therefore, a final power of 0.025 W reached the cultured cells. This standardization was performed as previously described in the literature [8, 9]. For the evaluation of cell metabolism, the radiation originated from the LASERTable was delivered on the base of each 24-well plate with energy doses of 0.5, 1.5, 3, 5, and 7 J/cm^2, and irradiation times of 40, 120, 240, 400, and 560 s, respectively. The laser light reached the cells on the bottom of each well with a final power of 0.025 W because of the loss of optical power in each well due to the interposition of the acrylic plate. The cells were irradiated every 24 h totalizing 3 applications during 3 consecutive days. The cells assigned to control groups received the same treatment as that of the experimental groups. The 24-well plates containing the control cells were maintained at the LASERTable for the same irradiation times used in the respective irradiated groups, though without activating the laser source (sham irradiation) [8, 9]. Twenty-four hours after the last irradiation (active or sham), the metabolic activity of the cells was evaluated using the MTT assay (described below). Based on cell metabolism results, the two most effective irradiation doses were selected to evaluate the cell number (trypan blue assay), cell migration capacity by using the wound healing assay (qualitative analysis) and the transwell migration assay (quantitative analysis), as described below.

2.3. Analysis of Cell Metabolism (MTT Assay). Cell metabolism was evaluated using the methyltetrazolium (MTT) assay [8–10]. This method determines the activity of succinic dehydrogenase (SDH) enzyme, which is a measure of cellular (mitochondrial) respiration and can be considered as the metabolic rate of cells.

Each well with the fibroblasts received 900 μL of DMEM plus 100 μL of MTT solution (5 mg/mL sterile PBS). The cells were incubated at 37°C for 4 h. Thereafter, the culture medium (DMEM; Sigma Chemical Co., St. Louis, MO, USA) with the MTT solution were aspirated and replaced by 700 μL of acidified isopropanol solution (0.04 N HCl) in each well to dissolve the violet formazan crystals resulting from the cleavage of the MTT salt ring by the SDH enzyme present in the mitochondria of viable cells, producing a homogenous bluish solution. Three 100 μL aliquots of each well were transferred to a 96-well plate (Costar Corp., Cambridge, MA, USA). Cell metabolism was evaluated by spectrophotometry as being proportional to the absorbance measured at 570 nm wavelength with an ELISA plate reader (Thermo Plate, Nanshan District, Shenzhen, China) [8, 9]. The values obtained from the three aliquots were averaged to provide a single value. The absorbance was expressed in numerical values, which were subjected to statistical analysis to determine the effect of LLLT on the mitochondrial activity of the cells.

2.4. Viable Cell Counting (Trypan Blue Assay). Trypan blue assay was used to evaluate the number of cells in the culture after LLLT application. This test provides a direct assessment of the total number of viable cells in the samples as the trypan blue dye can penetrate only porous, permeable membranes of lethally damaged (dead) cells, which is clearly detectable under optical microscopy [11]. The LLLT protocol was undertaken as previously described using energy doses of 0.5 and 3 J/cm^2. Cell counting was performed in the experimental and control groups 24 h after the last irradiation (active or sham). The DMEM in contact with the cells was aspirated and replaced by 0.12% trypsin (Invitrogen, Carlsbad, CA, USA), which remained in contact with the cells for 10 min to promote their detachment from the acrylic substrate. Then, 50 μL aliquots of this cell suspension were added to 50 μL of 0.04% trypan blue dye (Sigma Aldrich Corp., St. Louis, MO, USA), and the resulting solution was maintained at room temperature for 2 min so that the trypan blue dye could pass through the cytoplasmic membrane of the nonviable cells, changing their color into blue. Ten microliters of the solution were taken to a hemocytometer and examined with an inverted light microscope (Nikon Eclipse TS 100, Nikon Corporation, Tokyo, Japan) to determine the number of total cells and nonviable cells. The number of viable cells was calculated by deducting the number of nonviable cells from the number of total cells [8]. The number of cells obtained in the counting corresponded to $n \times 10^4$ cells per milliliter of suspension.

2.5. Cell Migration

2.5.1. Wound Healing Assay. The wound healing assay was used because it is a classic method of evaluation in vitro tissue healing assays [12, 13]. After 48 h of cell culture, a sterile 5 mL pipette tip was used to make a straight scratch on the monolayer of cells attached to the acrylic substrate, simulating a wound. Formation of the in vitro wound

was confirmed under an inverted microscope (TS 100, Nikon, Tokyo, Japan). The LLLT protocol was undertaken as previously described using energy doses of 0.5 and 3 J/cm^2. Twenty-four hours after the last irradiation, the cells were fixed in 1.5% glutaraldehyde for 1 h, stained with 0.1% violet crystal for 15 min, and washed twice with distilled water. Wound repopulation was assessed with a light microscope (Olympus BX51, Miami, FL, USA) equipped with a digital camera (Olympus C5060, Miami, FL, USA).

2.5.2. Transwell Migration Assay. The capacity of human gingival fibroblasts to migrate through a cell permeable membrane was assessed using 6.5 mm-diameter transwell chambers (Corning Costar, Cambridge, MA, USA) with polycarbonate membrane inserts (8 μm pore size) [14]. The chambers were placed in 24-well plates containing 1 mL of plain DMEM per well. The cells were seeded onto the upper compartment of the chamber (1.5×10^4 cells/cm^2) and incubated at 37°C for 48 h. After this period, the LLLT protocol was undertaken as previously described using energy doses of 0.5 and 3 J/cm^2. Twenty-four hours after the last irradiation (active or sham), the cells that had migrated through the membrane to the lower compartment of the chamber were fixed in 1.5% glutaraldehyde for 1 h, incubated with 0.1% violet crystal dye for 15 min, and washed twice with distilled water. After the last wash, the stained cells were viewed under a light microscope (Olympus BX51, Miami, FL, USA) equipped with a digital camera (Olympus C5060, Miami, FL, USA) and photomicrographs from three randomly chosen fields were taken at ×10 magnification for counting the number of migrated cells using the image-analysis J 1.45S software (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). Two samples of each group were evaluated and the experiment was performed in triplicate.

2.6. Analysis of Migrated Cells by Scanning Electron Microscopy (SEM). Part of the specimens used in the transwell migration assay was also used for the analysis of the cells by SEM. Twenty-four hours after the last irradiation (active or sham), the culture medium was aspirated and the transwell inserts were fixed in 1 mL of 2.5% glutaraldehyde in PBS for 2 h. Then, the glutaraldehyde solution was aspirated and the cells adhered to the transwell inserts were washed with PBS and distilled water two consecutive times (5 min each) and then dehydrated in a series of increasing ethanol concentrations (30, 50 and 70%, one time for 30 min each; 95 and 100%, two times for 60 min each) and covered 3 times with 200 μL of 1,1,1,3,3,3-hexamethyldisilazane (HMDS; Sigma Aldrich Corp., St. Louis, USA) [8]. The transwell inserts were stored in a desiccator for 24 h, sputter-coated with gold, and the morphology of the surface-adhered cells was examined with a scanning electron microscope (JMS-T33A scanning microscope, JEOL, Tokyo, Japan).

2.7. Statistical Analysis. Data from MTT, Trypan blue and Transwell assay had a nonnormal distribution (Kolmogorov-Smirnov, $P < 0.05$) and were analyzed by the Kruskal-Wallis and Mann-Whitney nonparametric tests. A significance level of 5% was set for all analyses.

Table 1: Succinate dehydrogenase enzyme (SDH) production by human gingival fibroblasts detected by the MTT assay according to the energy dose used in the low-level laser therapy.

Energy dose (J/cm^2)	MTT (%)
0 (control)	100 (96–104) C*
0.5	111 (110–113) B
1.5	94 (92–97) D
3	117 (113–119) A
5	95 (81–108) CD
7	92 (91–96) D

Values expressed as medians of SDH production (P25–P75) ($n = 12$). *Same letters indicate no statistically significant difference (Mann-Whitney, $P > 0.05$).

Table 2: Number of viable cells (%) detected by the trypan blue assay, according to the energy doses used in the low-level laser therapy.

Energy dose (J/cm^2)	Number of viable cells (%)
0 (control)	100 (95–104) B*
0.5	133 (112–175) A
3	168 (149–181) A

Values expressed as medians of SDH production (P25–P75) ($n = 8$). *Same letters indicate no statistically significant difference (Mann-Whitney, $P > 0.05$).

3. Results

3.1. Analysis of Cell Metabolism (MTT Assay). Data from SDH production by human gingival fibroblast cultures (MTT assay) after LLLT, according to the energy dose are presented in Table 1.

Regarding the energy dose of 5 J/cm^2 no statistically significant difference between the irradiated group and the nonirradiated control group was observed ($P > 0.05$). Conversely, irradiation of the fibroblast cultures with doses of 0.5 J/cm^2 and 3 J/cm^2 resulted in 11% and 17% increases in cell metabolism, respectively, differing significantly from the control group ($P < 0.05$). The cells irradiated with 1.5 J/cm^2 and 7 J/cm^2 presented the lowest metabolic rate compared with the nonirradiated control group (6% and 8% decrease, resp., $P < 0.05$).

3.2. Viable Cell Counting (Trypan Blue Assay). The number of viable cells (%) after LLLT application, according to the energy dose, is presented in Table 2.

Comparison among the energy doses revealed that irradiation of the human gingival fibroblast cultures with 0.5 J/cm^2 and 3 J/cm^2 increased the number of viable cells by 31% and 66%, respectively, differing significantly from the control ($P < 0.05$), but without statistically significant difference between each other ($P > 0.05$).

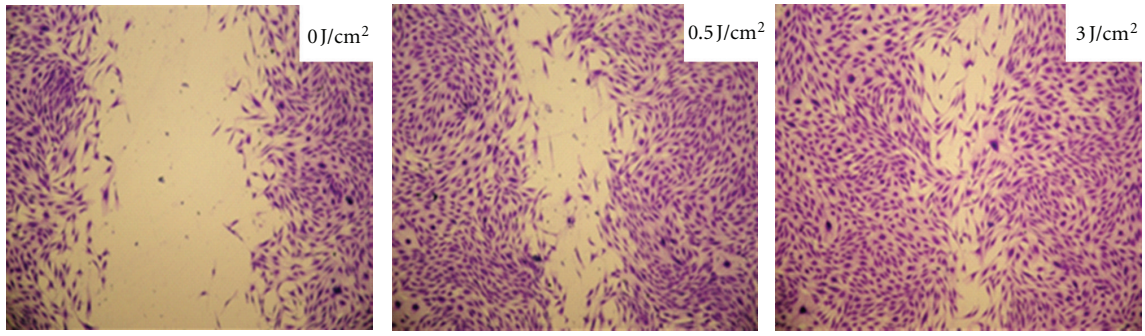

Figure 1: Photomicrographs showing human gingival fibroblast cultures seeded in 24-well plates after LLLT. The control group exhibits a large cell-free area on acrylic surface. The group irradiated with 0.5 J/cm^2 exhibits cell proliferation and migration, with consequent reduction of the "in vitro wound" size. The group irradiated with 3.0 J/cm^2 presented more intense cell proliferation and migration, resulting in almost complete closure of the "in vitro wound."

Table 3: Cell migration (%) by the transwell assay, according to the energy dose used in the low-level laser therapy.

Energy dose (J/cm^2)	Cell migration (%)
0 (control)	100 (91–107) B*
0.5	118 (109–123) A
3	120 (116–122) A

Values expressed as medians of SDH production (P25–P75) ($n = 6$). *Same letters indicate no statistically significant difference (Mann-Whitney, $P > 0.05$).

3.3. Fibroblast Migration

3.3.1. Wound Healing Assay. The analysis of the monolayer of human gingival fibroblasts after irradiation of the "in vitro wound" showed more intense cell migration, with consequent better coverage of the substrate (wound repopulation) (Figure 1).

3.3.2. Transwell Assay. Data from the transwell assay after LLLT, according to the energy dose are, presented in Table 3.

Comparison among the energy doses revealed that irradiation of the human gingival fibroblast cultures with 0.5 J/cm^2 and 3 J/cm^2 increased cell migration by 16% and 18%, respectively, differing significantly from the control ($P < 0.05$), but without statistically significant difference between each other ($P > 0.05$).

3.4. Analysis of Migrated Cells by Scanning Electron Microscopy (SEM). The SEM analysis of the transwell inserts, which complemented the viable cell counting by the trypan blue assay, revealed that the fibroblasts were capable of migrating through the transwell membrane. The cells obtained from human gengiva did not change their morphology after been submitted to LLLT (Figure 2).

4. Discussion

Different LLLT modalities have been used for diverse treatments in the health fields. In Dentistry, LLLT has been widely investigated and indicated for accelerating the healing process, especially in the treatment of ulcerative oral mucosa lesions [15, 16].

Several in vitro studies have evaluated the effect of LLLT on healing [7, 17]. Nevertheless, current research involving irradiation of cell cultures has not yet established the irradiation patterns specific for the different cell lines. Establishing the ideal irradiation parameters and techniques is mandatory for the development of sequential studies that can determine the potential biostimulatory effect of LLLT on oral mucosa cells, such as keratinocytes and fibroblasts, which are directly involved in the local healing process.

In the present study, the metabolic activity of human gingival fibroblast cultures after LLLT with different energy doses was evaluated to determine the adequate doses to produce biostimulatory effects on these cells in vitro. The results for SDH production showed that the 0.5 and 3 J/cm^2 doses increased cell metabolism. Therefore, these two most effective irradiation doses were selected to evaluate the number of viable cells as well as the cell migration capacity. The increase of SDH production after irradiation of gingival fibroblasts has also been observed by Damante et al. [18], using a similar laser prototype to the one used in the present study. In the same way as in the present study, the SDH production results also served as guide for subsequent experiments that evaluated the expression of growth factors by cultured fibroblasts.

In the present study, a significant increase in the number of viable cells that presented normal morphological characteristics (SEM analysis) was observed after LLLT using doses of 0.5 and 3 J/cm^2. These results confirm those of previous laboratory investigations in which LLLT with the same wavelength as that of the present study (780 nm) increased the proliferation of gingival fibroblasts [19, 20]. Kreisler et al. [2] also reported increase of fibroblast cell culture in vitro after direct and consecutive low level laser irradiations. The mechanism by which LLLT can promote biostimulation and induce proliferation of different cell types remains a controversial subject [20, 21]. Some authors [21, 22] claim that this mechanism is derived from light absorption by the enzyme cytochrome c oxidase in the cells, which participates in the cascade of oxidative respiration.

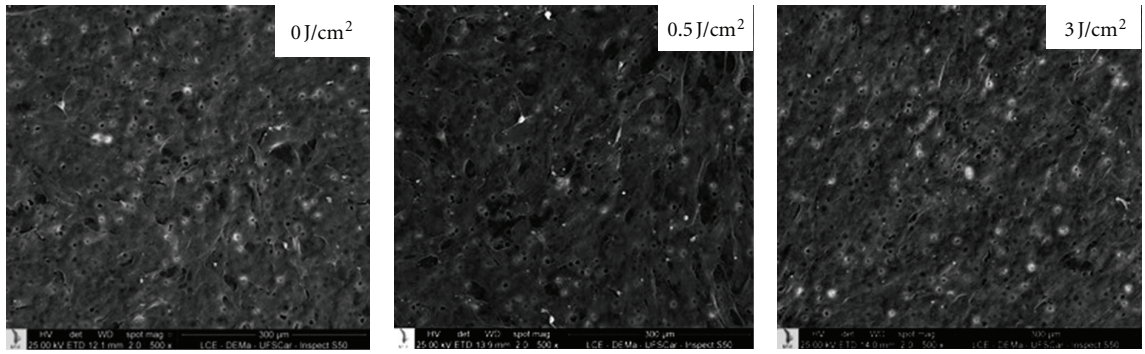

Figure 2: SEM micrograph showing cells with normal morphology that migrated through the transwell membrane. SEM ×500.

Eells et al. [23] demonstrated the increase in the production of this enzyme after different LLLT application of cell cultures. It has also been suggested that the mechanism of cell proliferation induced by LLLT might be derived from the activation of singling pathways, such as the MAPK and PI3K/Akt pathways, which control both cell proliferation and regulation of gene expression [21, 24].

Fibroblast cell migration and proliferation are essential events for tissue healing and are directly related with its success [1, 3]. In the present study, the effect of LLLT on the capacity of gingival fibroblast migration, using two energy doses capable of increasing cell metabolism (0.5 and 3 J/cm^2), was evaluated qualitatively, by the wound healing assay, and quantitatively, by the transwell migration assay. Both methodologies demonstrated that LLLT was able to increase the migration capacity of fibroblasts and the quantitative analysis of the results revealed no significant difference between the energy doses. These results are in accordance with those of previous investigations [7, 17], but studies using the transwell migration method to evaluate the LLLT on cell cultures are still scarce. This methodology is relevant because it measures the number of cells that can pass through the transwell membrane inserts, demonstrating their migration capacity after stimulation by LLLT.

Diverse mechanisms are involved in cell migration during tissue healing, including expression and secretion of growth factors [1]. Previous studies demonstrated that LLLT may cause positive effects on cells by increasing growth factor expression, which could be a form of action of specific laser parameters on cell migration [2, 25]. A recent study of our research group demonstrated that LLLT had a biostimulatory effect on epithelial cells in vitro by increasing their metabolic activity, number of viable cells and expression of growth factors [8]. In the present paper, the biostimulation of human gingival fibroblast cultures by LLLT with consequent increase in the number of viable cells and cell migration capacity demonstrates the efficacy of specific laser parameters and irradiation technique on the healing process. In addition, the obtained results are supportive to those of previous in vivo studies in which acceleration of the healing process was observed after LLLT [15, 16, 26], but the limitations of an in vitro experiment should be considered.

In conclusion, the findings of the present study demonstrated that the preset laser parameters in combination with the sequential irradiation technique caused biostimulation, proliferation, and migration of human gingival fibroblast cultures. These encouraging laboratory outcomes should guide forthcoming studies involving tissue irradiation with laser and its effects on in vivo tissue healing.

Acknowledgments

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Clinical Study

Treatment of Dentine Hypersensitivity by Diode Laser: A Clinical Study

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Introduction. Dentine hypersensitivity (DH) is characterized by pain after stimuli that usually provoke no symptoms. This study compared the effectiveness of GaAlAs diode laser alone and with topical sodium fluoride gel (NaF). *Materials and Methods*. The study was conducted on 10 patients (8 F/2 M, age 25–60) and 115 teeth with DH assessed by air and tactile stimuli measured by Numeric Rating Scale (NRS). Teeth were randomly divided into G1 (34 teeth) treated by 1.25% NaF; G2 (33 teeth) lased at 0.5 W PW (T on 100 m and T off 100 ms), fluence 62.2 J/cm^2 in defocused mode with a 320 μ fiber. Each tooth received three 1′ applications; G3 (48 teeth) received NaF gel plus laser at same G2 parameters. NRS was checked at each control. *Results*. Significant pain reduction was showed. The NRS reduction percentages were calculated, and there was a concrete decrease of DH above all in G3 than G2 and G1. *Conclusion*. Diode laser is a useful device for DH treatment if used alone and mainly if used with NaF gel.

1. Introduction

Dentine hypersensitivity (DH) is an abnormal response of the exposed vital dentine to thermal, chemical, or tactile stimuli. The prevalence of DH has been reported ranging from 4 to 57% in many studies in the literature, depending on the population samples studied [1, 2]. In patients affected by periodontitis, DH prevalence was even higher ranging between 60 to 98% [3]. However, DH prevalence is likely to increase in next years since more adults keep their teeth into later life. This condition may affect patients at any age, and both genders are equally affected [4, 5].

Pain of dentinal origin is sharp, localized, and of short duration. Although different theories have been proposed for the mechanism involved in DH etiology, recent studies gave support to Brannstrom's hydrodynamic theory [6], according to this a stimulus applied to open tubules dentin increases the flow of dentinal tubular fluid, with mechanical deformation of the nerves located into the inner ends of the tubules or in the outer layers of the pulp [7]. Type A delta fibers are supposed to be responsible for dentinal sensitivity being probably activated by the hydrodynamic process [8].

The most common factors involved in DH are abrasion, caused by inadequate intensity tooth brushing; abfraction, caused by teeth flexion due to abnormal occlusal forces; parafunctions or occlusal disequilibrium; erosion, secondary to the presence of acids in the oral cavity, as in bulimia nervosa or gastroesophageal reflux; anatomic predisposition due to structural deficiency of the enamel-cement junction; cavity preparations in vital teeth that expose dentine or badly controlled dentinal acid conditioning [8–10].

Orchardson and Gillam showed that DH affects above all the vestibule-cervical area of teeth [4]. Cervical DH has probably a multifactorial etiology, and more than a cause is related to this painful manifestation. Therefore, several treatments must be associated to reduce DH to satisfactory levels. According to Garone-Filho [10] the abfraction, caused by occlusal overload, is the most common etiological factor related to DH. So an occlusal adjustment should be always associated to the treatment of DH.

Furthermore, according to Pashley [11], there are two types of dentinal permeability: intratubular, into the dentinal tubules, and intertubular, between the tubules in dentinal

matrix. The sensitive dentine is permeable through its thickness; any treatment that reduces dentinal permeability must reduce dentinal sensitivity. The greatest dentine diffusion capacity allows the best interaction with the desensitizing agent. In fact, occlusion of the exposed dentinal tubules may decrease dentinal sensitivity level [9–14]. However, the DH sometimes persists despite of the effective sealing of the tubules, so indicating that further mechanisms are involved in nerves activation instead of or in addition to the hydrodynamic mechanism. Many authors suggested the hypothesis concerning the release of neuropeptides from the activated nervous terminations and, subsequently, the induction of a neurogenic inflammation. This hypothesis should signify that the symptoms of DH could become self-sustainable up to a certain point [8, 14].

Another great problem related to DH is its evaluation, since pain is a highly subjective sensation. Nevertheless, it is possible to classify the DH according to Matsumoto's criteria. In this classification, three degrees of DH are recognized: grade 1 mild discomfort/pain, grade 2 moderate pain, and grade 3 characterized by intense and unbearable pain [15].

Through literature examination emerges that there is no therapy that can always reduce pain at satisfactory levels, even with the combination of different protocols. According to Landry and Voyer [16], there is not an ideal desensitizing agent but any kind of treatment for DH should be effective from the first application and must satisfy these parameters established by Grossman since 1934 [17]: (1) not irritating pulp, nor causing pain, (2) easy application, (3) long-lasting effect, (4) not discoloring or staining teeth, (5) not irritating soft tissues or periodontal ligament, (6) low cost.

Every treatment, that reduces dentinal permeability, diminishes dentinal sensitivity. The occlusion of dentinal tubules leads to the reduction of dentinal permeability so decreasing the degree of DH [11]. According to the hydrodynamic theory, the effectiveness of dentine desensitization agents is directly related to their capacity of promoting the sealing of the dentinal canaliculi [12].

Conventional therapies for DH are based on the local application of desensitizing agents, either professionally or at home. The most frequently used agents can be classified as protein precipitants [18], tubule-occluding agents [19, 20], tubule sealants [21]. The sodium fluoride gel (NaF), which belongs to the tubule-occluding agents family, is the most commonly used agent [4, 22–25]. Its mechanism relies on the mechanical occlusion that is accomplished by precipitation of insoluble calcium fluoride crystals within the tubules without adhesion. For this reason, it cannot resist to the stresses of the oral environment and its action decreases with time [4, 23].

In the last fifteen years, the introduction of lasers gave further possibilities to DH therapy [22, 26–29]. Laser photobiomodulating action in dental pulp was reported by many authors as in Villa et al. [30], with histological studies of dental pulp of mice, after laser irradiation in teeth with exposed dentine. In this study, the authors registered a large quantity of tertiary dentine production in lased teeth, that caused the physiological obliteration of tubules, while the nonirradiated control showed intense inflammatory process that, in some cases, evolved into necrosis. Focusing on the role of laser in DH therapy, it is possible to show that its action is twofold. By one side, the low-level power lasers [14, 31], also called "soft lasers," act directly on nerve transmission, with a depolarization process that prevents the diffusion of pain to SNC; however, their effectiveness seems poorer in higher degrees of DH. By the other side, high-power lasers such as: diode 980 nm and 808 nm, KTP 532 nm, Nd: YAG 1064 nm, CO_2 10600 nm, Er, Cr: YSGG 2780 nm, and Er: YAG 2940 nm act on DH provoking a melting effect with crystallization of dentine inorganic component and the coagulation of fluids contained into the dentinal tubules. Among these "high power" devices, diode lasers are the most studied and the ones that gave the best results in several clinical protocols even in high-grade DH cases.

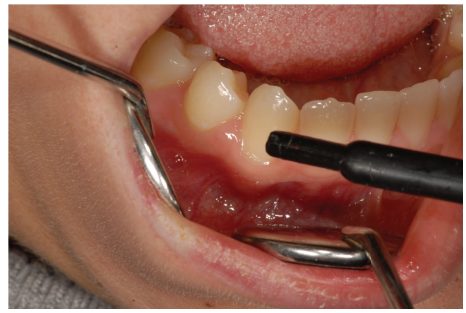

Figure 1: Air stimulus application.

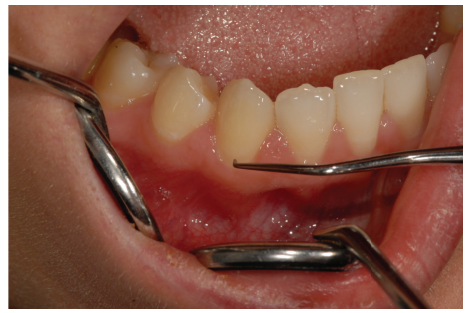

Figure 2: Tactile stimulus application.

The aim of this study is to assess the efficacy of a diode GaAlAs laser alone and in combination with topical sodium fluoride gel (NaF) in the treatment of DH in order to evaluate the possibilities of this device in the management of this painful condition.

2. Materials and Methods

The study was conducted on 10 patients (8 females and 2 males; aged from 25 to 60 years) and in a total of 115 teeth with DH assessed by mean of both air (Figure 1) and tactile (Figure 2) stimuli measured by the Numeric Rating Scale (NRS).

The inclusion criteria for patient enrollment were based on: the absence of local (e.g., cavities, fractures) and/or systemic pathologies, on the absence of contraindications to

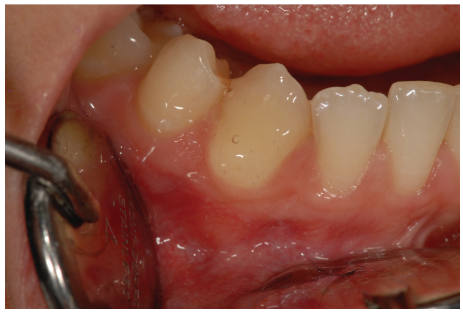

Figure 3: NaF gel application (Group 1).

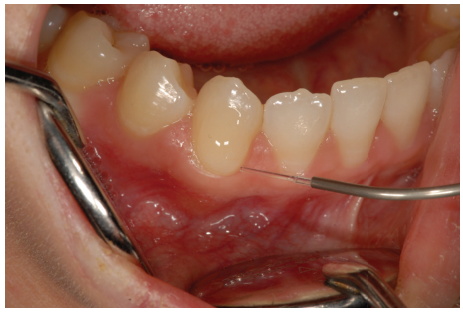

Figure 4: GaAlAs laser application (Group 2).

the proposed therapies (e.g., allergies to desensitizing agents) and on the presence of teeth with DH evaluated by pain response to both air and tactile stimuli that were registered by NRS scale (from 0 to 10, where 0 meant the absence of pain and 10 represented an unbearable pain and discomfort felt by the patients in their life); at last no desensitizing therapy had to be previously performed, nor analgesic drugs had to be recently assumed.

Before any treatment, all the patients received a hygiene professional program with oral hygiene instructions and the teeth vitality of all sites was assessed.

For each patient, the sensitive sites were randomly divided into three groups:

(i) Group 1 (G1) (34 teeth) treated with 1.25% NaF applied for 60 seconds on tooth surface (Figure 3);

(ii) Group 2 (G2) (33 teeth) lased by a GaAlAs laser (DoctorSmile, Lambda S.p.A., Brindole (Vi), Italy, 980 nm) with these parameters: 0.5 W in PW (T on 100 ms and T off 100 ms) and fluence of 62.2 J/cm^2 in no contact mode and using a fiber of 320-micron diameter. Each site received 3 applications of 1 minute each (Figure 4) once a week for three weeks;

(iii) Group 3 (G3) (48 teeth) treated using both NaF gel and diode laser at the same parameters of G2. The NaF gel was left on tooth surface for 60 seconds before the irradiation; in this way, the laser system could favor the permanence of desensitizer for a longer time than when it was used alone (Figure 5).

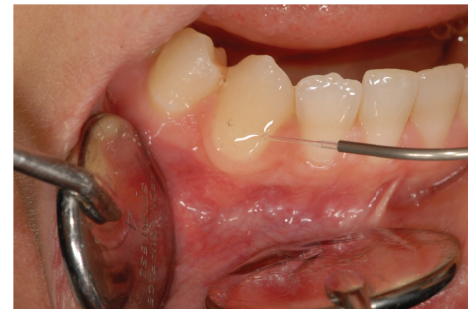

Figure 5: GaAlAs laser application + NaF gel application.

Patients' response to cold air blast was assessed by a short blast of 1-second duration at a distance of 0.5 cm for each tooth. Both air and tactile stimuli evaluations were performed before and after every treatment session, for a total of 3 treatment sessions at a distance of about one week each other.

The obtained results have been statistically analyzed through the Graphpad Prism 5.0 software.

3. Results

All the groups registered significant improvements of discomfort. A reduction of DH occurred during the treatment sessions, and the positive values were maintained after 1 month (Tables 1, 2, and 3).

Comparing the three regimens, a higher decrease of DH was registered in G3, followed by G2 and G1, respectively, whose results seem to be almost superimposable. The NRS reduction percentage was valued for each group between the first pretreatment and the third posttreatment session (Immediate−/−). The values were divided depending on the kind of stimulation. For the air stimulus, the reduction percentage was, respectively, 10.19% (I) for G1; 22.35% (I) for G2; 25.04% (I) for G3. Furthermore, the tactile stimulus took down: 4.13% (I) for G1; 6.77% (I) for G2; 9.96% (I) for G3.

Regarding to the statistical analyses, the data relating to the probe test (Figure 6) were subjected to the Kruskal-Wallis's test which demonstrated the reliability of the study ($P < 0.0001$). The comparative Dunn's test showed a statistically significant difference in G3 ($P < 0.001$), and in G1 ($P < 0.01$). In G2 the obtained improvement were lesser statistically significant ($P < 0.1$).

The results obtained with the cold blast (Figure 7) air were always analyzed through the Kruskal-Wallis's test which demonstrated the reliability of the study ($P < 0.0001$) and the comparative Dunn's test showed a statistically significant difference in each treated group ($P < 0.001$).

The improvement obtained in the samples of the probe test was not statistically significative ($P > 0.05$) instead of the samples of the cold blast air in which the improvements from the first treatment to the last one were superimposable.

Table 1: Chart of NRS pretreatment, posttreatment, and at 1-month control values of the G1 (only NaF gel).

Preair evaluation	Postair evaluation	Air control 1 month	Preprobe evaluation	Postprobe evaluation	Probe control 1 month
4	7	0	3	1	0
5.5	0	0	6.5	0	0
5.5	0	0	3	0	0
10	2	0	6	0	0
10	0	0	10	0	0
5.5	0	0	5	1	0
3	0	0	5	1	0
3	0	0	3	0	0
4	0	0	0	0	0
3	0	1	0	0	0
1	0	2	0	0	0
1	0	0	0	0	0
1	0	2	1	0	0
2	0	0	0	0	0
2	0	0	0	0	0
2	0	0	0	0	0
1	0	0	0	0	0
0	0	2	6	0	0
0	0	0	6	0	0
7	1	0	7	0	0
4	1	1	0	0	0
1	0	0	0	0	0
1	0	0	0	0	0
1	0	0	0	0	0
1	0	0	0	0	0
0	0	0	2.5	0	0
0	0	0	4	0	0
2	0	0	0	0	0
3	0	0	0	0	0
10	0	0	0	0	0
10	2	0	4.5	0	0
5	0	0	0	0	0
5	0	0	0	0	0
5	0	0	0	0	0

4. Discussion

Through literature examination, it is clarified that the ideal treatment for DH does not exist, even in case of combination of different protocols.

Conventional therapies for the treatment of DH comprehend the topical use of desensitizing agents, either professionally or at home such as protein precipitants [18], tubule-occluding agents [19, 20], tubule sealants [21], and, recently, lasers [22, 26–29].

Several studies [32–34] describe a synergistic action of lasers in association with desensitizing agents. In fact, the laser system can favor the permanence of the desensitizer for longer time than when they are used alone. For this reason, if laser device is used in addition to a conventional desensitizing agent, the latter remains above the tooth surface for 60 seconds before the irradiation.

Focusing on the effectiveness of the sole diode laser, this was investigated by several authors. Matsumoto et al. [35] showed an 85% improvement in teeth treated with laser; Aun et al. [36] reported success in laser-irradiated teeth in 98% of their cases; Yamaguchi et al. [37] noticed an effective improvement index of 60% in the group treated with laser compared to the 22.2% of the control nonlased group; Kumazaki et al. [38] showed an improvement of 69.2% in the group treated with laser compared to 20% in the placebo group. Gerschman et al. [39], in a double-blind study, found significant values in the laser-treated group. In fact, sensitivity to thermal stimuli was reduced by 67%, whereas the placebo group had a reduction of 17%, sensitivity to

Table 2: Chart of NRS pretreatment, posttreatment, and at 1-month control values of the G2 (only Diode laser).

Preair evaluation	Postair evaluation	Air control 1 month	Preprobe evaluation	Postprobe evaluation	Probe control 1 month
6	0	1	5	0	0
2	0	0	0	0	0
3	0	0	2.5	0	0
4	0	0	1.5	1	0
8	4	0	3	0	0
8	4	0	3	0	0
3	0	0	0	0	0
1	0	0	0	0	0
1	0	0	3.5	0	0
4	3	1	0	0	0
5	1	0	0	0	0
4	0	1	0	0	0
4	1	3	0	0	0
2	0	0	0	0	0
5	2	1	2	0	0
2	2	2	0	1	2
1	0	0	0	0	0
2	0	0	0	0	0
5	0	0	0	0	0
7	0	0	3	0	0
1	0	0	0	0	0
1	0	0	0	0	0
1	0	0	0	0	0
3	0	0	3	0	0
3	0	2	3	0	0
2	0	0	0	0	0
6.5	0	0	0	0	0
8	3.5	0	0	0	0
5	3	0	0	0	0
5	0	0	0	0	0
5	2	0	0	0	0
5	0	0	0	0	0
5	3	0	0	0	0

tactile stimuli was reduced by 65%, while the placebo group showed a reduction of 21%. Another study carried out by Brugnera et al. [40] showed the immediate analgesic effect using a diode laser.

In this study, significant improvements in pain and discomfort were always registered after the session treatments (I) even if in no case the percentages of pain reduction arrived to the high values registered in the literature.

As a first laser showed the best immediate results alone and in combination with gel, since the percentages of pain reduction in G2 and G3 were more than twice than G1 values. In our sample, the best results were obtained by the association of laser and NaF gel therapy (G3). This group registered the highest I reduction, in particular for air blast stimulation. It is probable that the better performance of combined treatment was due to the higher NaF gel adhesion to the dentinal tubules when combined with laser energy. In the laser group, G2, the immediate pain reduction was very high especially at air stimulation (22.35% I); in the same group, the improvement at tactile stimulation was poorer after the treatment (6.77% I). The lower reduction values were registered in the sole gel group, G1, by both stimulations, in the immediate period.

Even in consideration of the short sample analyzed, it is possible to speculate that the laser-induced superficial melting permits to keep longer the tubules occlusion by NaF gel emphasizing the reduction of DH-related pain.

5. Conclusion

According to these results, the GaAlAs laser showed a very high capability to improve immediately the DH-related pain, both alone and even better in combination with NaF gel. On the other hand, the sole gel results, even if positive, cannot

Table 3: Chart of NRS pretreatment, posttreatment, and at 1-month control values of the G3 (NaF gel + Diode laser).

Preair evaluation	Postair evaluation	Air control 1 month	Preprobe evaluation	Postprobe evaluation	Probe control 1 month
7	0	0	4	0	0
7	0	4	1	0	0
8	8	8	9	7	4
2	0	0	7	0	0
2	0	0	0	0	0
8	7	5.5	9	0	0
5	0	3	5	0	0
9	4	6	9	0	0
7	0	7	7	3	0
4	5	3	8	0	2
4	2	3	0	0	0
7	0	0	9	0	0
7	0	0	7	0	0
10	1	3	10	2	0
6	0	0	3	0	0
8	0	0	8	0	0
10	10	0	10	1	0
5	1	0	3.5	0	0
3	0	0	1.5	0	0
3	0	0	3	0	0
9	0	0	9	0	0
10	5	0	1.5	0	0
10	7	0	3	0	0
5	1	2	0	0	0
6	2	0	0	0	0
10	0.5	0.5	2	0	0
10	1	0	2	0	0
10	1	0	2	0	0
2	0	0	0	0	0
3	2	0	2	0	0
3	2	0	4	1	0
4	0	0	4	1	0
2	0	0	0.5	0	0
5	0	0	0	0	0
9	0	2	0	0	0
8	1	0	8	0	0
1	0	0	0	0	0
3	0	0	0	0	0
3	0	0	0	0	0
1	0	0	1	0	0
10	0	0	0	0	0
10	0	0	0	0	0
0	0	0	2.5	0	0
5	0	0	0	0	0
5	4	0	0	0	0
10	4	0	0	0	0
8	3	0	0	0	0
5	0	0	0	0	0

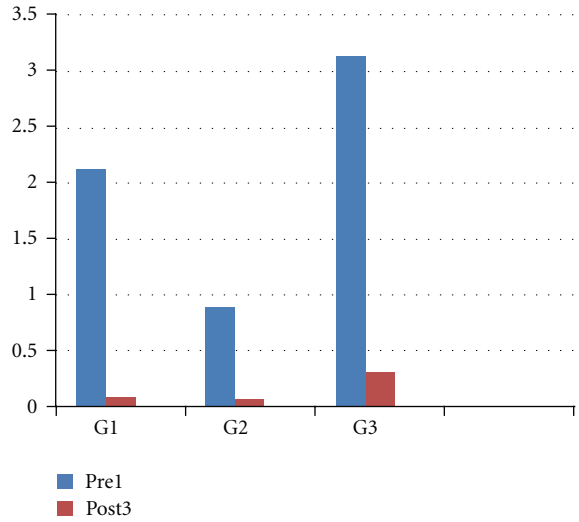

Figure 6: Illustrative representation of the improvements to the tactile stimuli from the first treatment to the third one.

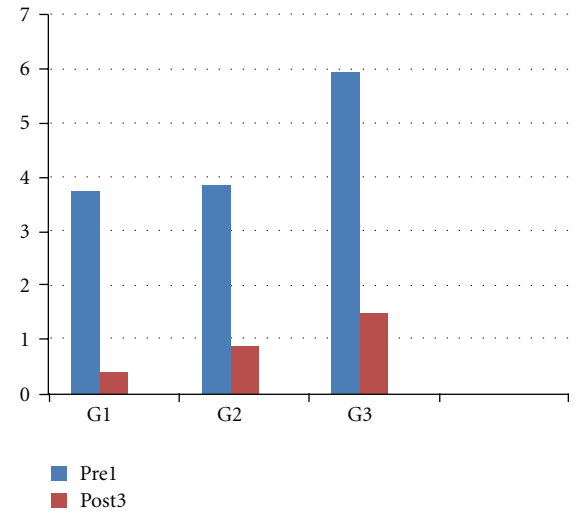

Figure 7: Illustrative representation of the improvement to the air stimuli from the first treatment to the third one.

equalize the performances of laser in the immediate. These results have to be confirmed by greater samples of patients and by longer follow-up periods (e.g., 3 and 6 months) to confirm or not the long-lasting action of the combined laser and gel therapy.

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Research Article

Laser Welding and Syncristallization Techniques Comparison: *In Vitro* Study

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Background. Laser welding was first reported in 1967 and for many years it has been used in dental laboratories with several advantages versus the conventional technique. Authors described, in previous works, the possibility of using also chair-side Nd : YAG laser device (Fotona Fidelis III, $\lambda = 1064$ nm) for welding metallic parts of prosthetic appliances directly in the dental office, extra- and also intra-orally. Syncristallisation is a soldering technique based on the creation of an electric arc between two electrodes and used to connect implants to bars intra-orally. *Aim*. The aim of this study was to compare two different laser welding devices with a soldering machine, all of these used in prosthetic dentistry. *Material and Methods*. In-lab Nd : YAG laser welding (group A = 12 samples), chair-side Nd : YAG laser welding (group B = 12 samples), and electrowelder (group C = 12 samples) were used. The tests were performed on 36 CrCoMo plates and the analysis consisted in evaluation, by microscopic observation, of the number of fissures in welded areas of groups A and B and in measurement of the welding strength in all the groups. The results were statistically analysed by means of one-way ANOVA and Tukey-Kramer multiple comparison tests. *Results*. The means and standard deviations for the number of fissures in welded areas were 8.12 ± 2.59 for group A and 5.20 ± 1.38 for group B. The difference was statistical significant ($P = 0.0023$ at the level 95%). On the other hand, the means and standard deviations for the traction tests were 1185.50 ± 288.56 N for group A, 896.41 ± 120.84 N for group B, and 283.58 ± 84.98 N for group C. The difference was statistical significant ($P = 0.01$ at the level 95%). *Conclusion*. The joint obtained by welding devices had a significant higher strength compared with that obtained by the electrowelder, and the comparison between the two laser devices used demonstrated that the chair-side Nd : YAG, even giving a lower strength to the joints, produced the lowest number of fissures in the welded area.

1. Introduction

In 1967, Gordon described the possibility of welding the metallic portions of dental prosthesis using a laser and this technique has been used since the 1970s in dental laboratories, rapidly demonstrating its advantages over traditional welding methods [1].

In fact, the procedure can be carried out directly on the master cast, thereby eliminating the risk of inaccuracies and distortions due to the duplication of the model [2]. Moreover, the heat source is a concentrated high-power light beam, so minimizing distortion problems in the prosthetic pieces [3]. The process allows the possibility of welding adjacent to acrylic resin or ceramic parts with neither physical (cracking) nor colour damage [4], thereby allowing a reduction of working time by eliminating the necessity to remake broken prosthetic or orthodontic appliances.

Laboratory tests have shown that laser-welded joints have a high, reproducible strength [5]. Laser welding technique has been used for many years in dental technician laboratory to manufacture prosthetics by connecting the different pieces and in repairing broken appliances. Unfortunately, there are more very important disadvantages such as costs and sizes of devices, and also the difficulty in the management of the parameters, which needs a long training period and makes this technique strictly operator dependent.

In previous works, authors have described the possibility of using the same Nd : YAG laser used in dental office for surgery interventions to weld metallic pieces of prosthetic and orthodontic appliances and due to fiberoptic delivery

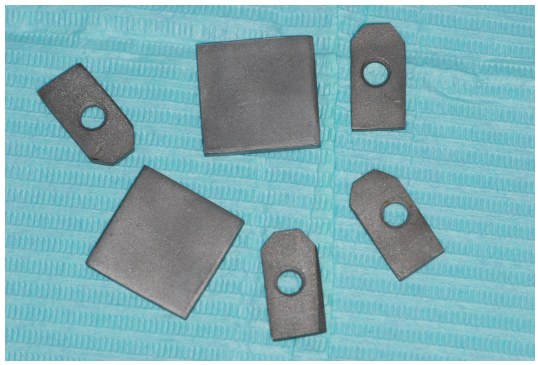


Figure 1: The metal plates used in the tests.

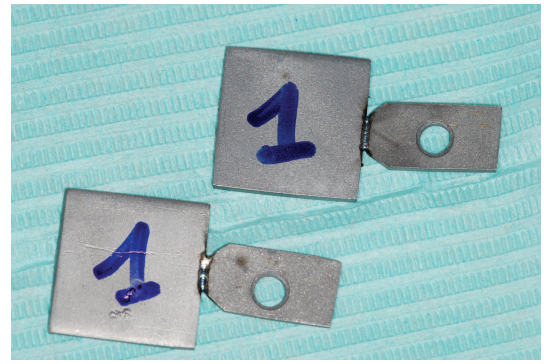


Figure 2: Plates of group A welded by in-lab Nd : YAG laser welding.

system of this device, they also proposed the possibility of direct intra-oral welding by dentists themselves [6].

A further different method, described for intra-oral welding, is based on the creation of an electric arc between two electrodes under an argon gas flux and it is called "syncristallisation" [7, 8]; unfortunately, there are more limits: it is not effective on every kind of metal and alloy, and it cannot be used on patients with pacemakers, it cannot work with filler metal, and some of the energy necessary for the welding process, which is concentrated between the two electrodes, spreads to the adjacent area (teeth, acrylic, ceramic, etc.).

The laser welding technique, as described before, is effective on all metals and alloys, can be applied either with or without filler metal and shielding gas, and, due to the extremely small spot size of the beam (0.6 mm), is able to limit the high temperature required to a very limited area.

Furthermore, it can be used on all patients and does not require a new and specific appliance, but utilizes an appliance currently available for oral treatments in the dental office.

The aim of this study is to compare the welding process obtained by three different devices: an "in-lab Nd : YAG laser welding," a "chair-side Nd : YAG laser welding," and a "Syncristallisation machine," by analysing the strength of the joints and by microscopic observation of the samples, in order to determine the more proper technique for clinical use.

2. Materials and Methods

Thirty-six plates of $20 \times 20 \times 1$ mm dimension were divided into three groups of twelve samples (1A, 1B, and 1C), and thirty-six CoCrMo plates of $8 \times 29 \times 1$ mm dimension were divided into three groups of twelve samples (2A, 2B, and 2C); on each plate of group 2 a hole of 3 mm diameter was performed (Figure 1).

Each plate of group 1A was welded to a plate of group 2A by In-lab Nd : YAG laser welding; the two parts were edge-to-edge connected (Figure 2).

The device used was Titec LASER 50 L (Orotig, Brescia, Italy) with these parameters: Wavelength 1064 nm, beam spot 1.8 mm, peak power 4.3 kW, working distance: 15 mm. Volt 270, energy/pulse 2.7 J, 4.0 Hz frequency, pulse duration 2.3 msec, output power 2.4 KW, fluence 1516 J/cm^2.

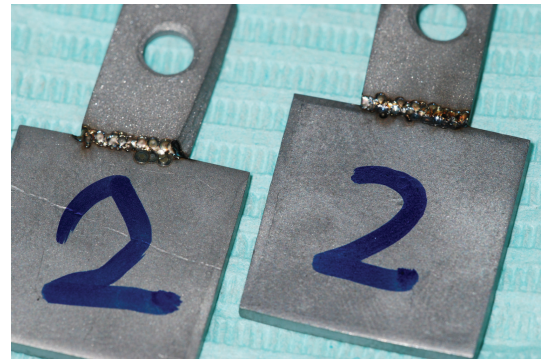


Figure 3: Plates of group B welded by chair-side Nd : YAG laser welding.

A single passage without metal filler was performed. Each plate of group 2A was welded to a plate of group 2B by chair-side Nd : YAG laser welding, the two parties were edge-to-edge connected (Figure 3).

The device used was Fidelis III Plus (Fotona, Ljubljana, Slovenia), with these parameters: Wavelength 1064 nm, output power 9.85 W, frequency 1 Hz, pulse duration 15 msec, spot diam 0.6 mm, working distance 40 mm, energy 9. 85 J, fluence 3300 J/cm^2.

Due to the optic fiber delivery system (900 μm diameter), a power meter was used to check if there was no loss of energy (Ophir Nova II, thermal head F150A, Israel).

A single passage without metal filler was performed. The parameters used were the "standard" for each device and the fluence values were very different, due to the smallest laser beam diameter and longer pulse duration of the chair-side Nd : YAG laser welding.

Each plate of group 3A was soldered with a plate of group 3B by an electrowelder (Figure 4) using the syncristallisation; in this case, due to the limit of this technique (creation of an electrical current through an electrode), it was not possible to connect the two parts in an edge-to-edge mode, so they were soldered one over the other with an overlapping of 3 mm.

The device used was VISION STRATEGICA (Newmed, Reggio Emilia, Italy) with these parameters: 25 V, 50 Hz, and 312 J.

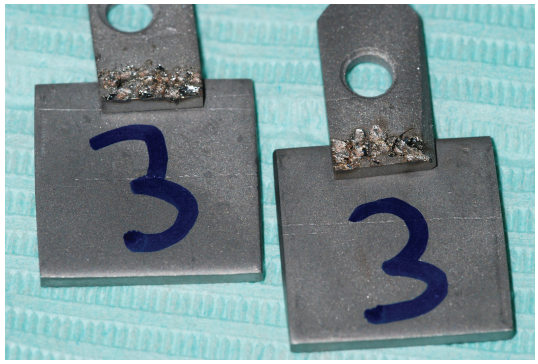


Figure 4: Plates of group C soldered by the electrowelder.

The soldering process was done by a series of points because with this technique it is not possible to use metal filler.

Twenty-four plates, twelve of group A and twelve of group B, were observed by two different operators with optical microscope (Novex zoom Stereo RZ, Euromex Microscopes, Netherland) in order to count the number of fissures present in each plate. The values were statistically analysed with Students *t*-test.

Then all the thirty-six welded plates, (twelve of the group A, twelve of the group B, and twelve of the group C), were connected to a dynamometer system (SBS-KW-300A, Steinberg, Berlin, GER) by means of a bar inserted in the hole.

Each plate was clamped, on the opposite side, in a wood vice mounted on the base of the stand. Then traction was applied until the two parts were broken. All the values of the traction tests of all groups were reported and statistically analysed using one-way (ANOVA) and Tukey-Kramer Multiple comparison test.

3. Results

The microscopic observation was limited to the plates of groups A and B in order to compare the number of fissures present (Figure 5).

In group A the highest score was 12 and the minimum was 4, the mean and standard deviation were 8.12 ± 2.59.

In group B the highest score was 8 and the minimum was 3, the mean and standard deviation were 5.2 ± 1.38 (Figure 6).

The *T* Student test showed that the difference between the means was significant ($P = 0.0023$ at the level 95%).

On the other hand, the traction tests on group A pointed out that the highest value (expressed in N) was 1708 and the minimum was 870, the mean and the standard deviation were 1185.5 ± 288.56 N.

In the traction tests on group B, the highest value (expressed in N) was 1077 and the minimum was 670, the mean and the standard deviation were 896.41 ± 120.84 N.

In the traction test on group C, the highest value (expressed in N) was 402 and the minimum was 172, the mean and the standard deviation were 283.58 ± 84.98 N.

The mean values and SD of traction tests in each group are reported in Figure 7.

ANOVA statistical tests showed that the difference between the means of traction tests were significant ($P = 0.01$ at the level 95%).

4. Discussion

While the comparison between the two laser devices was not difficult due to the similar welding process, the comparison between the results of laser welding and electrowelding was not easy, due to the great differences in procedures between the techniques.

However, because to date they are the only two ways to make an intra-oral welding, the effort to compare them, even with a lot of difficulties, was justified.

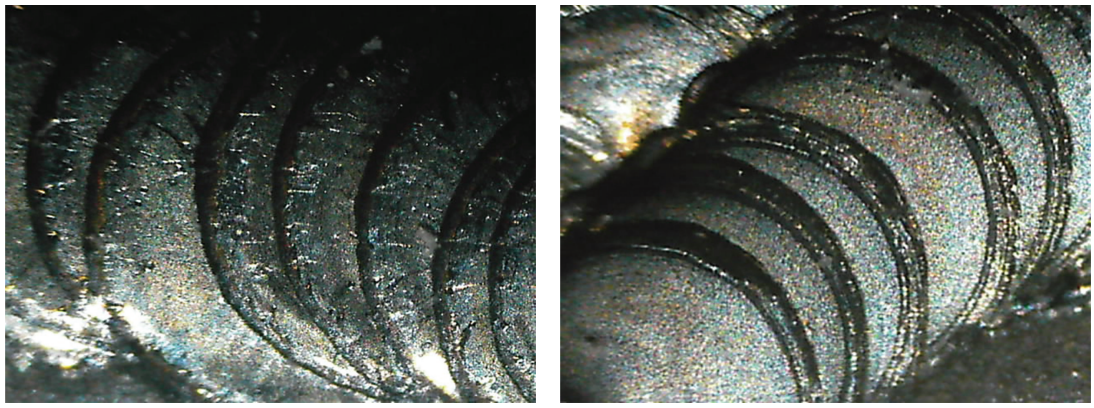
Laser technology is the most efficient method for applying thermal energy to small areas and, according to many Authors [9, 10], it is one of the best fusion-welding techniques for dissimilar metals. This depends on the possibility of modern laser appliances to focus the light beam on a very reduced focal point. This beam imparts energy into the metal causing it to heat up locally to a temperature above the liquid. So, the metal evaporates, a cavity is formed immediately under the heat source and a reservoir of melted metal is produced around it. As the heat source moves forward, the hole is filled with the melted metal from the reservoir and this solidifies to form the weld bead [11]. The best advantage is that the weld can usually be placed exactly where it is required, that is, at the point of workpiece abutment [12].

Hot cracking susceptibility during welding is usually evaluated when the strain or stress is changed during the process, but the use of a pulsed Nd : YAG laser, where power is continuously decreased with time, may control the rate of solidification and can effectively reduce hot cracking in alloys [13]. The cracks are generated during or after welding, and they are determined by the laser output, spot diameter, and laser beam diameter, [14]. This might explain the difference in the fissures numbers observed between group A and B samples.

Authors, in previous works, demonstrated by *in vitro* study on bovine jaws that during the welding process by Chair-side Nd : YAG laser welding, the temperature in the surrounding structures, in particular the pulp chamber, is very low and biologically harmless [15].

The so-called "syncristallisaton" technique was introduced in dentistry at the end of the 70s [16]. Despite the fact that many clinical cases are described in several works [17], there is a lack of *in vitro* studies on the physical mechanisms and the thermal elevations in the biological tissues. This makes it still very difficult to do a review of the literature.

The process of syncristallisation consists in an atoms movement resulting in the creation of a crystalloid structure in the area of junction [18]. The solder exploits the high temperature generated on the welding surface for a time of two thousands of a second and less. This is due to the resistance of the metals to the electric current flow and works by binding all those materials, such as titanium, surgical steel



(a) (b)

Figure 5: Microscopic vision of group A (a) and group B (b) laser welded plates.

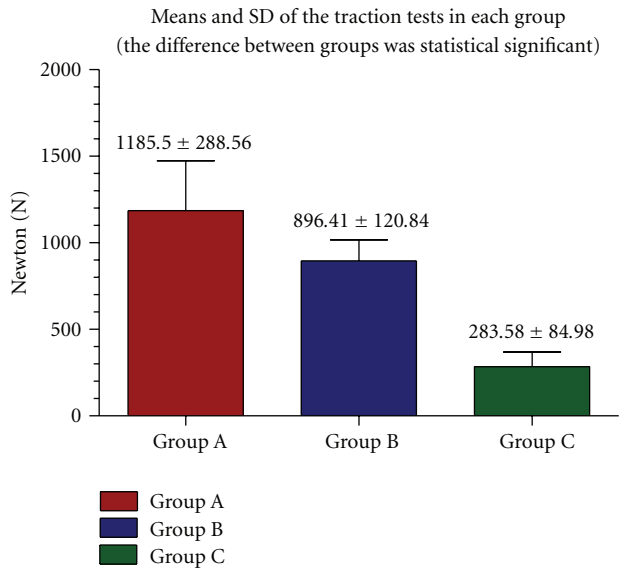


Figure 6: Mean and SD of the number of fissures in groups A and B.

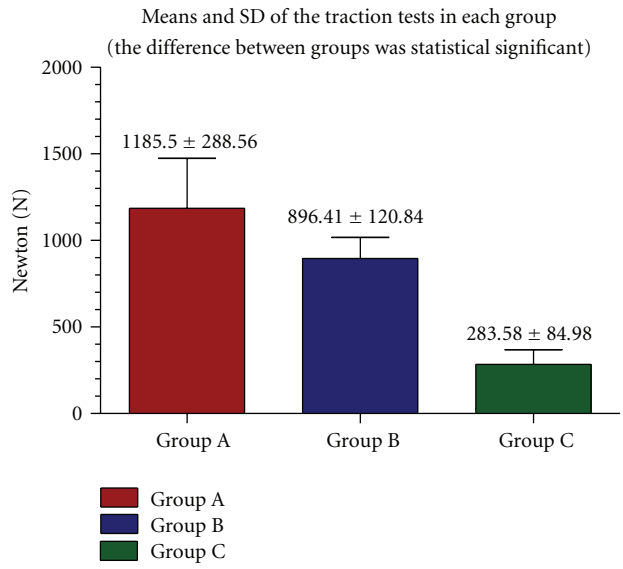


Figure 7: Mean and SD of traction tests in each group.

and nonnoble metal alloys, which are poor conductors of electricity [19].

Thanks to the very low conductibility of these metals and alloys and to the brevity of the exposure to the electric current, no tissue damage seems to result from this procedure [20] even if *in vitro* studies on the thermal elevation are very few.

Furthermore, unlike industrial solders that can operate only in the presence of argon and without oxygen in the atmosphere, the electrowelder used in dentistry works in the presence of oxygen, water, physiological oral fluids, and blood [21].

In this study the electrowelder seemed to give the lowest joint traction test values compared to the laser welding techniques, even if it is simpler, faster, and without parameters to adjust. Moreover, the technique of the syncristallisation has a great limitation consisting in the possibility to weld only with an overlapping of the two portions.

The best values in the mechanical tests were given by the plates welded by in-lab Nd: YAG laser welding even if, probably due to the higher energy delivered, the number of fissures noticed was higher than that observed in the plates welded by a chair-side Nd: YAG laser welding. Next studies about the application of these welding techniques will regard the thermal elevation comparison by *ex vivo* tests on implants in bovine jaws.

5. Conclusion

The use of the chair-side Nd : YAG laser welding may be considered as a good technique for dental applications in prosthetics, orthodontics, and implantology, even if further studies with different metals and alloys, and also *ex vivo* tests on bovine jaws will be necessary to confirm the results obtained by this work.

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Research Article

An *In Vitro* Evaluation of Leakage of Two Etch and Rinse and Two Self-Etch Adhesives after Thermocycling

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Our experiment evaluated the microleakage in resin composite restorations bonded to dental tissues with different adhesive systems. 40 class V cavities were prepared on the facial and lingual surfaces of each tooth with coronal margins in enamel and apical margins in cementum (root dentin). The teeth were restored with Z100 resin composite bonded with different adhesive systems: Scotchbond Multipurpose (*SBMP*), a 3-step Etch and Rinse adhesive, Adper Scotchbond 1 XT (*SB1*), a 2-step Etch and Rinse adhesive, AdheSE One (*ADSE-1*), a 1-step Self-Etch adhesive, and AdheSE (*ADSE*), a 2-step Self-Etch adhesive. Teeth were thermocycled and immersed in 50% silver nitrate solution. When both interfaces were considered, SBMP has exhibited significantly less microleakage than other adhesive systems (resp., for SB1, ADSE-1 and ADSE, $P = 0.0007$, $P < 0.0001$ and $P < 0.0001$). When enamel and dentin interfaces were evaluated separately, (1) for the Self-Etch adhesives, microleakage was found greater at enamel than at dentin interfaces (for ADSE, $P = 0.024$ and for ADSE-1, $P < 0.0001$); (2) for the Etch and Rinse adhesive systems, there was no significant difference between enamel and dentin interfaces; (3) SBMP was found significantly better than other adhesives both at enamel and dentin interfaces. In our experiment Etch and Rinse adhesives remain better than Self-Etch adhesives at enamel interface. In addition, there was no statistical difference between 1-step (ADSE-1) and 2-step (ADSE) Self-Etch adhesives.

1. Introduction

Currently, resin composites are more often used for direct posterior teeth restorations since many advances were made in adhesion and adhesives long-term performances. Adhesives are necessary to prevent leakage on resin composite restorations while dental composites are not able to bond to dental tissues. However, clinical microleakage remains the major cause for composite restorations failures implying postoperative sensibility, margin colorations, secondary decay, or pulpal inflammation [1–5]. Therefore, manufacturers have proposed many different adhesives involving different adhesion strategies. These adhesive systems were well described by Van Meerbeek et al. [6–8]: the Etch and Rinse (ER) adhesive systems (in three or two clinical steps), the Self-Etch (SE) adhesive systems (in two or one clinical step(s)), and the glass ionomer adhesives [6–9]. In their *in vitro* studies, several authors have reported different dental adhesive systems' bonding performance [10–24]. Therefore, results from thermocycling experiments have already pointed statistical differences between the ER adhesion strategy and the SE adhesion strategy [19, 25–27].

Therefore, the purpose of this study was to evaluate bonding performance of different dental adhesives after thermocycling: 2 ER adhesives (Scotchbond Multipurpose, *SBMP*—3M ESPE AG, Seefeld, Germany—a 3-step ER adhesive and Adper Scotchbond 1 × T, *SB1* 3M Espe AG, Seefeld, Germany—a 2-step ER adhesive) and 2 SE adhesives (AdheSE, *ADSE*—Ivoclar Vivadent AG, Schaan, Liechtenstein—a 2-step SE adhesive and AdheSE One, *ADSE-1*—Ivoclar Vivadent AG, Schaan, Liechtenstein—a 1-step adhesive) were evaluated according to the microleakage that was observed.

2. Materials and Methods

Twenty recently extracted human third molars were randomly selected for this experiment. The teeth were stored in

Table 1: The different tested adhesive systems and their components.

Adhesive systems		
Name of the adhesive	Type of adhesives	Components
SBMP	ER, 3 steps	Phosphoric acid (35%) Primer = HEMA, polyalkenoic acid copolymer, water Bonding = HEMA, Bis-GMA, amines, photoinitiator
SB1	ER, 2 steps	Phosphoric acid (35%) Adhesive (primer + bonding) = dimethacrylates, HEMA, polyalkenoic acid copolymer, silanized silicium, alcohol, water, photo-initiator
ADSE	SE, 2 steps	Primer = dimethacrylate, phosphonic acid acrylate, initiators, stabilizers Bonding = HEMA, dimethacrylate, silicon dioxide, Initiators, stabilizers
ADSE-1	SE, 1 step	Derivates of bis-acrylamide, water, alcohol, bis-methacrylamide dihydrogen phosphate, amino acid acrylamide, hydroxyl alkyl methacrylamide, alkyl sulfonic acid acrylamide, highly dispersed silicon dioxide, initiators, stabilizers, and potassium fluoride

In this table, the tested adhesives are displayed according to the adhesion's strategy and the type of adhesives: Etch and Rinse (ER) adhesive systems (SBMP and SB1) and Self-Etch adhesive systems (ADSE, ADSE-1). SBMP: Scotchbond Multipurpose Plus (3 M ESPE AG, Dental products, Seefeld, Germany). SB1: Adper Scotchbond 1 XT (3 M Espe AG, Dental products, Seefeld, Germany). ADSE = AdheSE (Ivoclar Vivadent AG, Schaan, Liechtenstein). ADSE-1: AdheSE One (Ivoclar Vivadent AG, Schaan, Liechtenstein).

a refrigerated saline solution for a maximum of 3 months as recommended by the ISO norms (ISO. Guidance on testing of adhesion to tooth structure. International Organization for Standardization. TR 11405,1-4, Geneva, Switzerland, 1994). All patients and an appropriate Ethical Committee have approved the collection of extracted teeth. Two cavities were drilled on the facial and the lingual sides of each tooth. All the cavities ($n = 40$) were rectangular, standardized for dimensions and shape ($h \times w \times l = 2\,\text{mm} \times 2\,\text{mm} \times 3\,\text{mm}$) and were prepared with a cylindrical diamond bur (diameter = 0.9 mm) at the coronal-radicular junction: the margins were butt-jointed, half in the enamel and half in the root dentin. After that, the apices were fixed in an autopolymerizing resin (Paladur, Heraeus-Kulzer GmbH & Co. KG, Hanau, Germany).

The forty cavities were randomly assigned in 4 groups according to tested adhesive systems (Table 1):

(i) Scotchbond Multipurpose (SBMP) (3M ESPE AG, Dental products, Seefeld, Germany), a 3-step Etch and Rinse (ER) adhesive system;

(ii) Adper Scotchbond 1 × T (SB1) (3M ESPE AG, Dental products, Seefeld, Germany), a 2-step Etch and Rinse (ER) adhesive system;

(iii) AdhSE (ADSE) (Ivoclar Vivadent AG, Schaan, Liechtenstein), a 2-step Self-Etch (SE) adhesive system;

(iv) AdhSE One (ADSE-1) (Ivoclar Vivadent AG, Schaan, Liechtenstein), a 1-step Self-Etch (SE) adhesive system.

All the tested adhesives were used according to the manufacturer's instructions. Immediately after bonding procedures, the cavities were filled with two oblique increments of a microhybrid composite (Z100, 3M ESPE AG, Dental products, Seefeld, Germany). The photopolymerization was carried out for all materials with a halogen lamp (XL 3000, 3M ESPE AG, Dental products, Seefeld, Germany). Composite restorations were polished by means of diamond drills and disks (Hawe Neos Dental, Bioggio, Switzerland). The polishing was carried out under a spray of water. After that, all the specimens were immersed in a saline solution for twelve weeks (in a refrigerator at 5°C). Thereafter, the teeth were thermocycled for 800 cycles (5°C–55°C) for 22 hours. After thermocycling, the teeth were immersed in a 50% silver nitrate solution (for 6 hours) and in a 25% vitamin C solution for 10 minutes (pH about 2) [25, 26]. After immersion, three grooves (3 mm depth, 1 mm width) were drilled with a diamond bur in each restoration to obtain four surfaces of observation. The interfaces that occurred between the teeth and the filling was described in our previous studies [25, 26]. Briefly, the cylindrical diamond drill (0.9 mm diameter) was placed perpendicular to the composite restoration. Three grooves 3 mm deep and 1 mm wide were cut on each restoration: one at the mesial margin, one at the distal margin, and one right in the middle of the filling (Figure 1). These preparations yielded four evaluating surfaces for each composite restoration (Figure 2), for a total of 160 viewing surfaces for all tested adhesives. Each surface allowed one observation in enamel and one in dentin (lecture areas), for a total of 320 observations (160 in enamel and 160 in dentin).

Each section was examined by twofold magnification by means of an optic microscope (Carl Zeiss, SAS, Oberkochen, Germany). Each tooth was observed twice by the same operator (blinded test).

Arbitrarily, the evaluation of leakage was made with a 6-point severity scale (Figure 3, Table 2) [25].

We have postulated that higher scores of microleakage (scores 3, 4, and 5) after thermocycling would be responsible for clinical failure of the bonding (Figure 4).

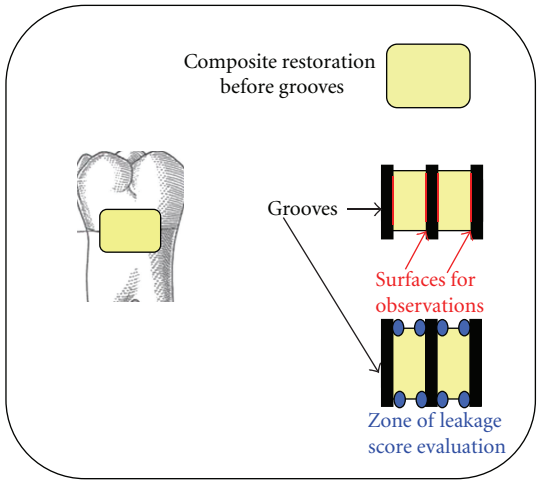

Figure 1: Diagram showing placement of the three grooves on each restoration to provide eight observation areas.

2.1. Statistical Analysis. Results are expressed as means ± standard deviations (± SDs). Microleakage scores were analyzed by means of generalized linear mixed models (GLMMs) assuming an ordinal logistic link function. Covariates in the model were (1) adhesive systems and (2) interface (enamel or dentin). The model also accounts for repeated measurements on the various teeth. All the results were considered to be significant at the 5% critical level ($P < 0.05$). Statistical calculations were made using the SAS 9.1 (version 8.2 for Windows) package.

3. Results

Microleakage mean score calculation for each tested adhesive system was analyzed by statistical model, which takes repeated evaluations into account (4 observations for each interface, enamel, or dentin). Therefore, all the observed scores of microleakage for each adhesive at enamel or at dentin interface are not displayed.

The mean scores of microleakage for all tested adhesive systems are shown in Table 3.

In our study, SBMP was significantly different from other adhesives: SBMP has shown a lower mean score of microleakage (0.30 ± 0.49) than other tested adhesives ($P = 0.0007$ for SB-1 and $P < 0.0001$ for the other tested adhesives).

Table 4 reports the statistical comparison between the mean scores of microleakage of the 4 tested adhesives.

As seen in Table 4, there was no statistical difference between SB1 and ADSE ($P = 0.0799$), neither between SB1 and ADSE-1 ($P = 0.072$) nor between ADSE and ADSE-1 ($P = 0.96$).

Table 5 shows the mean scores of microleakage for the 4 tested adhesives at enamel and dentin interfaces.

For ADSE and ADSE-1, the mean scores of microleakage were significantly lower at dentin than at enamel interfaces.

Table 2: The 6-point severity scale to evaluate the microleakage.

Scores	Signification
Score = 0	No leakage
Score = 1	Leakage up to the enamel-dentin junction or a depth of 0.5 mm on the radicular wall
Score = 2	Leakage up to the maximum half of the lateral wall (leakage depth ≤1 mm)
Score = 3	Leakage over half of the lateral wall (1 mm < leakage depth < 2 mm)
Score = 4	Subtotal leakage on the whole of the lateral wall (leakage depth = 2 mm)
Score = 5	Total leakage partly or entirely on the pulpal wall of the cavity (leakage depth >2 mm)

Table 3: Mean microleakage scores of the different tested adhesive systems.

ER adhesive systems		SE adhesive systems	
	Mean microleakage scores (± SDs)		Mean microleakage scores (± SDs)
SBMP	0.30 ± 0.49	ADSE	0.88 ± 0.82
SB1	0.64 ± 0.66	ADSE-1	0.84 ± 0.72

In this table, the tested adhesives are displayed according to the adhesion's strategy and type of adhesives: Etch and Rinse (ER) adhesive systems (SBMP and SB1) and Self-Etch adhesive systems (ADSE, ADSE-1). SBMP. Scotchbond Multipurpose; SB1. Scotchbond 1 XT; ADSE. AdheSE; ADSE-1. AdheSE One.

Table 4: Statistical differences among the tested adhesives and level of significance (P).

	SBMP	SB1	ADSE	ADSE-1
SBMP	—			
SB1	0.0007	—		
ADSE	<0.0001	0.0799	—	
ADSE-1	<0.0001	0.072	0.96	—

SBMP. Scotchbond Multipurpose; SB1. Scotchbond 1 XT; ADSE, AdheSE; ADSE-1. AdheSE One.

4. Discussion

For the past few years, composite has become current restorative material and today it often replaces amalgam restorations in posterior teeth [28–31]. Nevertheless, restorative composite is not able to bond to dental tissues. Therefore, the use of an adhesive system is always required. As result of numerous advances in adhesive technology and adhesion knowledge, there are many adhesive systems available on the market. To avoid confusing and incorrect uses of the adhesives, Professor Bart Van Meerbeek has proposed a classification according to different adhesion strategies and adhesives: the Etch and Rinse (ER) adhesive systems, the Self-Etch (SE) adhesive systems, and the glass ionomer adhesive systems [6, 7, 32]. The ER adhesives always involve the use of phosphoric acid, which permits demineralization of the dental tissues and, after rinsing, a complete elimination of the smear layer. Therefore, in the course of the ER adhesion

(a) (b)

Figure 2: Pictures of the composite restoration before and after grooves' preparation.

Table 5: Mean microleakage scores of the tested adhesives at the enamel and dentin interfaces.

Strategy of adhesion	Adhesive systems	Mean microleakage scores (± SD)		P
		Enamel	Dentin	
ER	SBMP	0.30 ± 0.52	0.30 ± 0.46	0.86
	SB1	0.68 ± 0.73	0.60 ± 0.59	0.79
SE	ADSE	1.03 ± 0.70	0.73 ± 0.91	0.024
	ADSE-1	1.20 ± 0.65	1.48 ± 0.60	<0.0001

In this table, the tested adhesives are displayed according to the adhesion's strategy and type of adhesives: Etch and Rinse (ER) adhesive systems (SBMP and SB1) and Self-Etch adhesive systems (ADSE, ADSE-1).
SBMP: Scotchbond Multipurpose; SB1: Scotchbond 1 XT; ADSE: AdheSE; ADSE-1: AdheSE One.

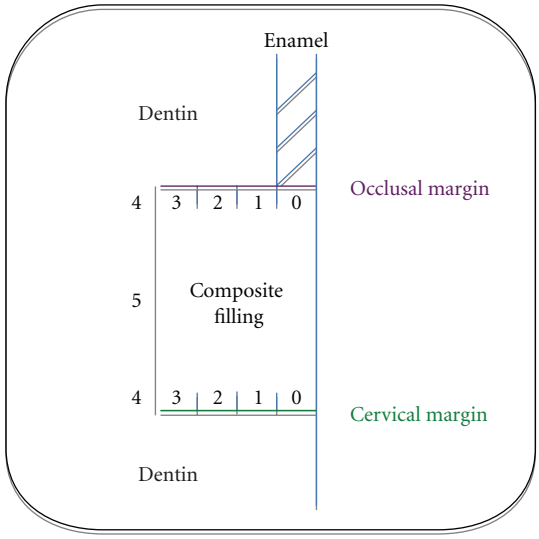

Figure 3: Diagram showing the 6-point evaluation scale for leakage.

Figure 4: Picture showing a 2 score of microleakage (left) and 4 score of microleakage (right).

strategy, the adhesive resin (bonding) is applied in a different clinical step: the demineralization and the hybridization of dental substrate appear consecutively. On the contrary, with the SE adhesives the demineralization and the impregnation of the adhesive into the enamel-dentin support appear simultaneously. The demineralization process results from the acidic monomers, which are components of the adhesive system. Therefore, the SE adhesive must not be rinsed. There are currently 4 different types of SE adhesives, which are indexed according to their pH value: the ultramild SE (pH about 2.5), the mild SE (pH about 2), the intermediary

strong SE (pH about 1.5), and the strong SE (pH < 1) [6–8, 32]. On the enamel, for both ER and SE adhesive systems, bonding to the tissue is essentially micromechanical. On the dentin, for the ER adhesives, the mechanisms of adhesion are mainly micro-mechanical because the phosphoric acid is a very strong acid (pH about 0.5). Phosphoric acid completely dissolves the mineral and so, the collagen fibers are totally exposed after etching. For the SE adhesives, the adhesion to the dentin is both micro-mechanical and chemical [6–8]: the self-etch monomers are often less acidic than phosphoric acid and then some minerals remain attached to the collagen fibers, permitting chemical links between dental substrate and functional groups of the adhesive monomers.

Laboratory experiments have permitted comparison between different bonding materials and have pointed statistical differences between different adhesive systems [10–24]. Currently, a lot of studies and reviews agree about the best performances of the ER adhesive systems at the enamel and also at the dentin interface for some 3-step adhesives [11, 16, 19, 32–37]. Concerning dentin interface, several authors admit that some SE adhesives, in particular mild and ultra-mild, are able to create chemical bonds with hydroxyapatite crystals within the dentinal tissue [7, 8, 32, 36–40]. Nevertheless, some authors suggest that these mild and ultra-mild SE adhesives have poor adhesion capacity to the enamel tissue: so, they recommend the use of phosphoric acid on the enamel surface before applying the SE adhesive [32, 34, 37, 41–43].

Currently, *in vitro* microleakage [11, 19] and mechanical tests [16, 35] often show the superiority of the 3-step ER adhesives. For several authors, these adhesive systems are always the "gold standard" [6, 7, 32, 36, 37, 44], in particular the Optibond FL (*Kerr, European Union Representative, Scafati (SA), Italy*) [5, 7, 45] and/or the Scotchbond Multipurpose Plus (SBMP) (*3 M Espe AG, Seefeld, Germany*) [5, 36, 44]. The results of our study are in agreement with the data from the literature: in our experiment, SBMP has shown the best results in terms of microleakage.

Nevertheless, for some authors, 2-step mild and ultra-mild SE adhesives can give comparable results than those obtained by some 2-step ER adhesives and also, by some 3-step ER adhesive systems [6–10, 32, 34, 36, 37]. In fact, our results have shown that ADSE (2-step SE) and SB1 (2-step ER) have statistically comparable mean scores of microleakage.

Concerning the 1-step SE adhesives, some *in vitro* studies have shown their poor performances [7, 34, 46]. Our results do not confirm this fact: there is no statistical difference between the mean scores of microleakage of ADSE (a 2-step SE) and its simplified clinical version, ADSE-1 (a 1-step SE). In addition, the mean scores of microleakage of these two mild SE adhesives (pH about 2) are lower at the dentin interface than at the enamel interface. These observations agree with data from the literature: ADSE and ADSE-1 are not efficient to create a sufficient micro-mechanical retention at the enamel surface [6, 7, 16, 34, 43]. Nevertheless, at the dentin surface, these mild SE adhesives are able to create a partial demineralization of this tissue to allow a micro-mechanical adhesion [6, 7]. In addition, some functional monomers of these SE adhesives might form chemical bonds with the calcium of the residual hydroxyapatite crystals linked to the collagen fibers [7, 32, 38–40]. The chemical bonds between ADSE functional monomers have not been clearly identified yet, but this adhesive has given good results in our study, like in the study of Bradna et al. [10].

5. Conclusion

In this study, confirming previous studies about marginal microleakage of the ER adhesive systems, SBMP, a 3-step ER adhesive, has significantly less microleakage comparing to other adhesive systems and can be considered like a reference adhesive. The parameters of this experiment (hydrolysis and thermocycling) have shown the good *in vitro* behaviour of SBMP. Therefore, we can expect that this ER adhesive will be clinically satisfying. In fact, this adhesive has been widely used for many years and their performances have seemed good. The 2-step ER adhesive that was tested in our study has shown a significantly greater mean score of microleakage than the tested 3-step ER adhesive system, but all the tested adhesives showed minimal leakage.

In the limits of our study, ADSE and ADSE-1 show poor microleakage, particularly on the dentin. Nevertheless, we suggest these mild SE adhesives can be used when the margins of the cavity are located on dentin and/or using phosphoric acid only on the enamel margin in order to optimize micro-mechanical interlocking.

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