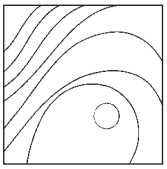
Evaluation of Different Dental Lasers' Ability to Congeal Pooled Blood: An In Vitro Study



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Most dental lasers claim that they can aid in hemostasis during oral and periodontal surgery. To date, there are no studies that compare different lasers' ability to congeal pooled blood. The aim of the study was to see if there was a difference in dental lasers' ability to congeal pooled human blood in vitro. Whole blood was collected from donors, with 0.5 mL (premolar socket volume for all tests) aliquoted into microcentrifuge tubes. Different dental lasers (810-nm diode, 940-nm diode, 1,064-nm Nd:YAG, 2,790-nm Er,Cr:YSGG, 2,940-nm Er:YAG, and 10,600-nm CO_2) were applied to the whole blood for 0, 15, 30, and 45 seconds. The sample tubes were centrifuged, and the supernatant color was scored to assess the degree of congealing. Additional samples of blood were tested for time needed for maximum congealing and temperature change. Analysis of supernatant colors showed that there were significant differences in the degrees of congealing for the 810-nm diode, 940-nm diode, and 2,790-nm Er,Cr:YSGG lasers when compared to the 1,064-nm Nd:YAG, 2,940-nm Er:YAG, and 10,600-nm CO_2 lasers, but not within those groupings. Additionally, the 1,064-nm Nd:YAG laser increased the temperature of the blood samples more than the other lasers and had a shorter time for maximum congealing. There were differences in the dental lasers' ability to congeal pooled human blood in an in vitro model. Nd:YAG, Er:YAG, and 10,600-nm CO_2 lasers were able to achieve a greater degree of congealing at an earlier time point. The Nd:YAG laser produced the most heat and was the fastest to complete coagulation. Int J Periodontics Restorative Dent 2020;40:e147–e154. doi: 10.11607/prd.4773

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The use of dental lasers has increased in recent years and provides the potential for improved treatment outcomes.[1–3] There are a wide variety of dental lasers currently in use; however, the most commonly used are diode, Nd:YAG, Er,Cr:YSGG, Er:YAG, and 10,600-nm carbon dioxide (CO_2) lasers. Each type of laser has its own unique properties based on the wavelengths emitted and the energy produced by those wavelengths. The most common elements within tissues that absorb laser energy (chromophores) are hemoglobin, melanin, hydroxyapatite, and water. Lasers that produce shorter wavelengths tend to be absorbed more by melanin and hemoglobin, whereas longer wavelengths tend to be absorbed by hydroxyapatite and water.[4] Additionally, lasers differ in the depth to which they penetrate fluids and tissues.[4] Shorter wavelengths penetrate tissues more deeply.[5]

It is often necessary to enhance and stabilize the blood clot in a tooth extraction socket, either as a standalone procedure or in conjunction with socket grafting. The blood clot is an amalgamation of platelets, neutrophils, and red blood cells that are contained in a fibrin network.[6] The clot recruits inflammatory cells and is later replaced by granulation tissue and subsequently by mature connective tissue. Lasers could aid in initiating early phases of blood clotting more rapidly.

Table 1 Laser Settings Used for Congealing Blood

	Laser	Wattage	Hz	Tip diameter × length	Pulse duration
Group 1	810-nm diode	3.0	Continuous	400 μm × 9 mm	CW
Group 2	940-nm diode	2.5	Continuous	400 μm e4 tip × 9 mm	CW
Group 3	1,064-nm Nd:YAG	3.6	20	360 μ fiber	550 μsec
Group 4	2,780-nm Er,Cr:YSGG	0.75	50	500 μm MZ5 tip × 9 mm	60 μsec
Group 5	2,940-nm Er:YAG	1.8	20	Varian 600/μm × 14 tip	1,000 μsec VLP
Group 6	10,600-nm CO_2	2.0	20	LS9005-07 tip	25 msec

CW = continuous wave; msec = millisecond; μsec = microsecond.

Extraction of teeth is a common procedure in dentistry, and effective management of bleeding is crucial for optimal healing. After successful removal of a tooth, the extraction socket will begin to fill with blood. Formation and stabilization of the blood clot typically is completed in the first 24 hours[7] but may be impaired or interrupted in a patient for a variety of reasons.[8–11] Complications after the formation and stabilization of the blood clot, such as dry socket, can also lead to impaired healing and postoperative complications due to lack of blood clot stability. Excessive washing away of the blood clot has been considered a risk factor for poor healing and infection.[12] There are a variety of modalities for achieving better hemostasis after an extraction: injection of local anesthesia containing vasoconstrictor, gauze pressure, topical hemostatic agents such as topical thrombin and tranexamic acid, and localized cautery.[9]

Most laser manufacturers claim that their device can influence coagulation of blood in a surgical field. The mechanisms through which this is achieved are tissue absorption of the laser energy and an increase in heat in a limited area.[13,14] Tissue absorption specifically deals with laser radiation being absorbed by specific elements (hemoglobin, melanin, fibrinogen [by diode and Nd:YAG lasers], and water [Erbium and CO_2 lasers], etc) and altering these elements. Furthermore, at 60°C, proteins begin to denature. Denaturation of proteins involves the unfolding of the proteins' structure, causing aggregation.[15] The combination of these two mechanisms causes sealing of the ends of vessels and congealing of pooled blood. The coagulative ability of lasers is an appealing attribute for practitioners when performing surgical procedures. The photothermal effect of lasers is what enhances coagulation through the heating and constriction of blood vessels as well as the congealing of pooled blood.[4] There are several methods for measuring the degree of coagulation or congealing of blood. Photo-optical measuring (used in this study) consists of assessing the change in the color of supernatant and pellicle of whole blood after centrifugation,[16] an approach that was utilized by Lüthje and Ogilvie[17] and Jia et al.[18]

Materials and Methods

Six dental lasers were used in this experiment: 810-nm and 940-nm diode lasers (uninitiated tip); 1,064-nm Nd:YAG laser; 2,790-nm Er,Cr:YSGG laser; 2,940-nm Er:YAG laser; and 10,600-nm CO_2 laser (see Acknowledgements for the lasers' product and manufacturer specifications). The parameters recorded for each laser were wavelength (nm), output power (W), pulse duration (none [continuous wave], milliseconds, or microseconds), energy per pulse (mJ), and pulse repetition rate (Hz). Each laser was set to their respective manufacturer's settings for coagulation/hemostasis (Table 1). An external power meter was used to verify within 0.1 W proper power output from each laser prior to use (Powermax PM30, Coherent). Total applied energy was calculated. For all laser procedures, proper safety procedures and protocols were used in accordance with the American National Standard for the Safe Use of Lasers (ANSI Z136.1-2007).

Two separate 10-mL volumes of whole blood were drawn from systemically healthy young adult volunteers who read and signed

informed consent as per the Colorado Multiple Institutional Review Board (COMIRB #17-0211). All blood was considered to be equal, as donors were of roughly the same age and in good health. To simulate a mandibular premolar extraction-site volume,[18] 0.5 mL of blood was aliquoted into 30 modified microcentrifuge tubes (2.0 mL polypropylene Microcentrifuge Tube, BiochromCorp). Blood draws were completed at two separate time points, and each draw was randomized to laser used and exposure time. The blood samples were then treated with one of the dental lasers in a random sequence allocation. Using the manufacturer's specifications for coagulation/hemostasis, each laser was applied to the microcentrifuge tubes for five different periods of time for each of the six different lasers tested: The laser energy was applied for 0, 15, 30, or 45 seconds as determined by a preliminary study, and a fifth application measured the time for maximum coagulation/congealing. The delivery tip of each dental laser was inserted by one author (K.J.L.) into the blood, moving in a slow up-and-down motion to congeal the blood. A second 940-nm diode–laser group was run as a duplicate, with similar settings.

Once all samples had been treated with the different lasers in turn, as well as the untreated controls, the microcentrifuge tubes were carefully transferred and centrifuged (80-2 Centrifuge, Costway) at 150 g for 7 minutes[17] to separate supernatant liquid from congealed blood. The microcentrifuge tubes were then transferred to a standardized photography station and photographed.

The supernatant liquid was visually examined for color vs the apical clot. Based on preliminary studies, a more appropriate novel scale (Fig 1) was used to visually assess the color of the supernatant liquid. The photographs of the treated microcentrifuge tube were scored (K.J.L.) at the time of the experiment and then labeled and saved for future data analysis. These steps were replicated nine times for each donor's blood.

While the control (0 seconds), 15-, 30-, and 45-second treatment groups were being centrifuged, the remaining test tubes were tested for maximum congealing. Each laser was applied to test tubes in the same, slow up-and-down motion until there was a noticeable change in the surface appearance and there was a tactile change in the consistency of the blood. The time needed to experience this change was recorded. This test was done in triplicate.

Additionally, some of the blood from each draw was used for temperature analysis. The thermocoupler used for analysis was calibrated using boiling water and ice. A baseline temperature reading was taken in the middle of the microcentrifuge tube and recorded. Each laser was then applied to the microcentrifuge tubes in the same up-and-down motion for 30 seconds. Each laser was set to the manufacturer's hemostasis settings as described above. Temperature was measured at the bottom, middle, and top portions of the microcentrifuge tubes.

Photographs of all samples were given unique identification numbers and were randomized for scoring analysis. In order to assess interexaminer reliability, 10% of the photographs were randomly analyzed in duplicate. Scoring analysis was completed by seven co-residents under a standardized viewing setup. Scorers were calibrated prior to analysis to ensure they were analyzing only the supernatant liquid and using the Losin-Metzger scale correctly. Scorers viewed each image for approximately 20 seconds and recorded a score of 1 to 5 based on the Losin-Metzger (L-M) scale (Fig 1).

Statistical Analysis

Supernatant scores, maximum congealing time, and temperature analysis were compiled and analyzed using Microsoft Excel (Microsoft) and Prism 8 software (GraphPad). L-M scores were uploaded to Microsoft Excel and unrandomized. For each sample, descriptive statistics were calculated; this was repeated for each time point. To test if there was an overall difference in the median scores at 30 seconds, Kruskal-Wallis tests were completed. Kruskal-Wallis tests were performed for each laser to analyze if there was a difference in L-M scores as a function of time. In order to compare the degree of congealing between one laser and another after 30 seconds of application of laser energy, individual Mann-Whitney tests were performed. In total, 15 individual Mann-Whitney tests were performed each with $\alpha = .05$ to test for significance. Descriptive statistics were also completed for temperature analysis.

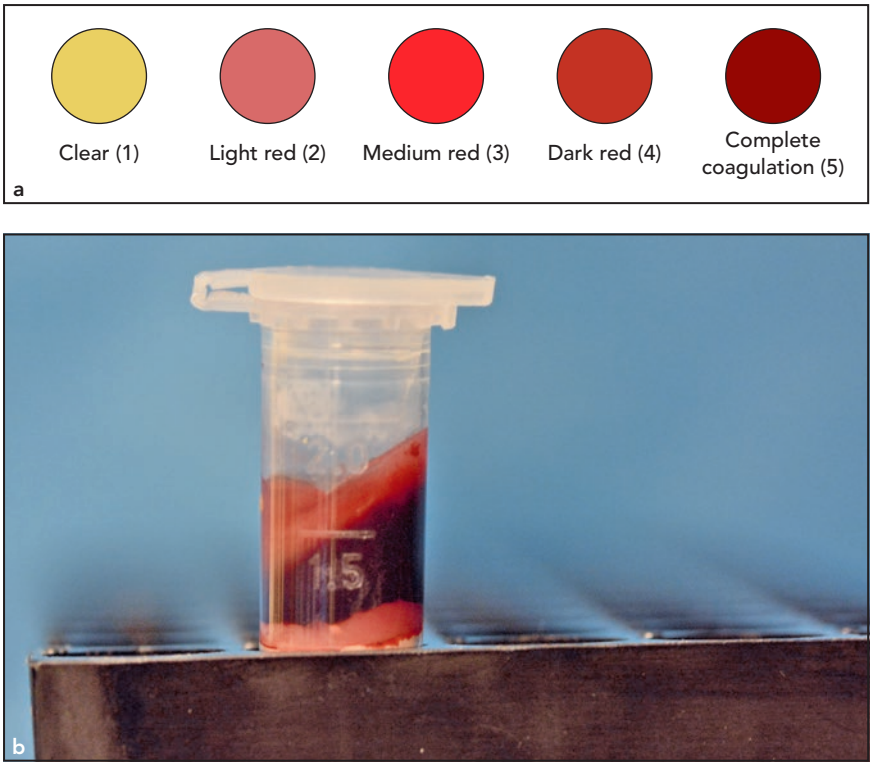

Fig 1 (a) *The Losin-Metzger scale for determining degree of congealing of blood based on color of the supernatant liquid.* (b) *This example blood sample would receive a Losin-Metzger score of 2* (light red).

Table 2 Degree of Blood Congealing as a Function of Time

	P
D810	.0094
D940	< .0001
Nd:YAG	< .0001
Er,Cr:YSGG	.02
Er:YAG	.0032
CO_2	< .0001

See Table 1 for wavelengths.
All *P* values are statistically significant (< .05) and were calculated using Kruskal-Wallis test.

The mean scores for temperature analysis were analyzed using an ordinary one-way analysis of variance (ANOVA) with α = .05 to test for significance. Time for maximum congealing was evaluated in a similar way: completing descriptive statistics and then an ordinary one-way ANOVA with a α = .05. Interexaminer reliability was calculated using Fleiss' kappa analysis.

Results

A total of 250 samples of blood were analyzed. The degree of congealing tended to vary in relation to time and type of laser used (Fig 2). There was a statistically significant difference (SSD) in the degree of congealing as exposure time increased for each of the lasers (Table 2).

Based on clinical observations during the study in terms of visual and tactile appearance of the blood as it was congealing, the 30-second time point was used for comparison of congealing among lasers. Mann-Whitney *U* tests showed that there were differences among lasers' congealing ability at 30 seconds. These results are summarized in Table 3. In short, the 10,600-nm CO_2, Er:YAG, and Nd:YAG lasers did not differ significantly from each other in terms of degree of coagulation, but they did differ significantly from the 810-nm diode (D810), 940-nm diode (D940), and Er,Cr:YSGG lasers.

Temperature measurements after 30 seconds of laser application were similar between the bottom, middle, and top of the microcentrifuge tubes. The mean temperatures were 43°C, 43°C, 53°C, 32°C, 33°C, and 31°C for the D810, D940, Nd:YAG, Er,Cr:YSGG, Er:YAG, and CO_2 lasers, respectively (Fig 3). One-way ANOVA showed an SSD (P < .0001) among the lasers in terms of temperature.

Time for Maximum Congealing Analysis

The times for which there were appreciable tactile and visual changes indicating complete congealing of the blood sample were different among the lasers. The mean

times for complete congealing were 115, 106, 39, 173, 153, and 139 seconds for the D810, D940, Nd:YAG, Er,Cr:YSGG, Er:YAG, and CO_2 lasers, respectively (Fig 4). One-way ANOVA showed that there were SSDs in the mean amount of time needed for complete congealing among the different lasers ($P < .0001$), with the Nd:YAG laser having the shortest maximum congealing time.

The calculated total amount of energy applied to each sample is listed in the Table 4. When comparing the total amount of energy delivered at the 30-second time point, there are clearly differences among the lasers: The Er,Cr:YSGG laser had considerably less energy delivered than the Nd:YAG and diode lasers (22.5 J vs 108.0 J).

The two duplicate 940-nm diode laser tests almost exactly matched for all parameters evaluated.

Using Fleiss' interexaminer reliability equation, a Kappa value of 0.48 was obtained. This suggested moderate interexaminer agreement.

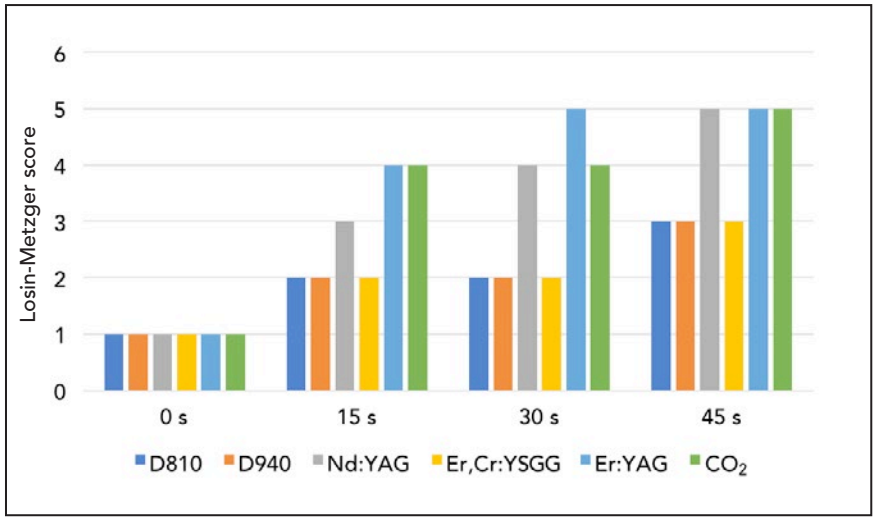

Fig 2 *Results of the degree of congealing for each laser and each exposure time. See Table 1 for wavelengths.*

Table 3 Comparison of Different Lasers' Ability to Congeal Blood

	P
CO_2 vs D810	.0016*
CO_2 vs D940	.0023*
CO_2 vs Er,Cr:YSGG	.0059*
CO_2 vs Er:YAG	.6247
CO_2 vs Nd:YAG	.443
D810 vs D940	.6954
D810 vs Er,Cr:YSGG	.9782
D810 vs Er:YAG	.0132*
D810 vs Nd:YAG	.0054*
D940 vs Er,Cr:YSGG	.6954
D940 vs Er:YAG	.0264*
D940 vs Nd:YAG	.0109*
Er,Cr:YSSG vs Er:YAG	.0124*
Er,Cr:YSSG vs Nd:YAG	.0218*
Er:YAG vs Nd:YAG	.2821

See Table 1 for wavelengths.

Discussion

Proper wound healing is an important aspect in the outcomes of periodontal therapy. Extraction of compromised teeth is a common therapy in periodontics. After successful extraction, the first step in healing is formation of a blood clot. The process of forming and stabilizing a blood clot is very complex. The addition of materials or interventions have been shown to improve coagulation after an extraction.[8–10,20,21] Lasers of various wavelengths have been proposed as enhancing clot formation and obviating the need for membranes, sutures, or other materials, thereby saving time. The results from the degree of congealing aspect of this experiment indicate that there are differences among the lasers tested. Not all available dental laser wavelengths were evaluated in this study (eg, 980-nm or 1,064-nm diode lasers and 9,300-nm CO_2 lasers, etc.). The ones that were available at the authors' facility were used. Therefore, the results of this study may apply only to those devices from

Table 4 Calculated Amount of Energy Delivered by Each Laser

	D810 (3.0 W)	D940 (2.5 W)	Nd:YAG (3.6 W)	Er,Cr:YSGG (0.75 W)	Er:YAG (1.8 W)	CO_2 (2 W)
0 s	0	0	0	0	0	0
15 s	45	37.5	54	11.25	27	30
30 s	90	75	108	22.5	54	60
45 s	135	112.5	162	33.75	81	90

Measurements are given in Joules. See Table 1 for wavelengths.

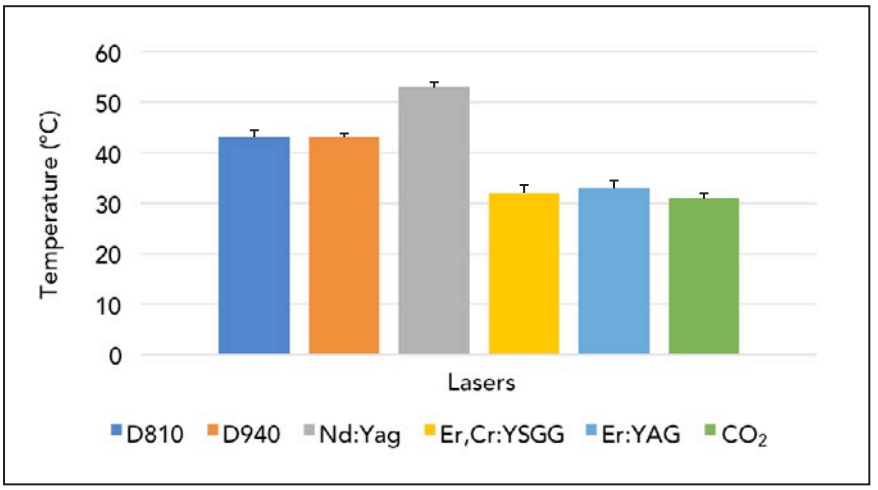

Fig 3 *Mean temperatures of blood samples at the middle of the tube after 30-second laser application.*

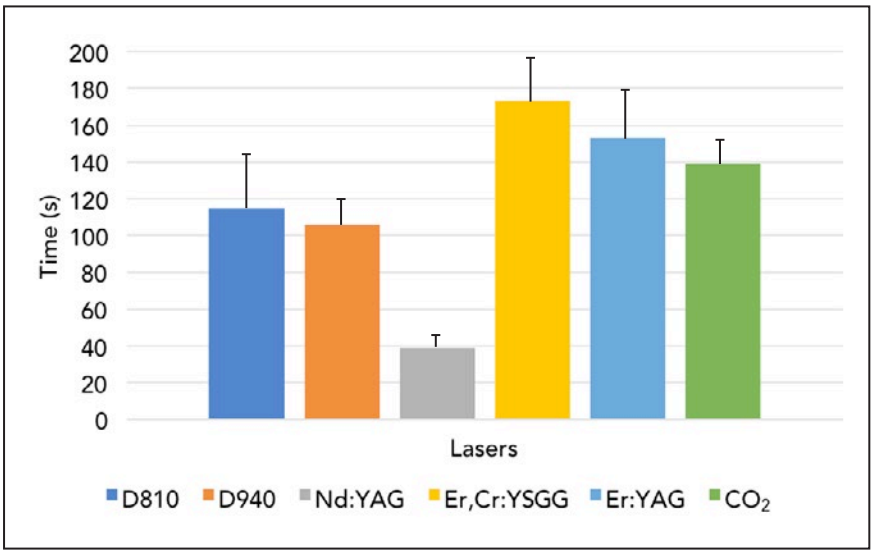

Fig 4 *Time needed to achieve maximum congealing based on visual and tactile feedback.*

specific manufacturers. In addition, the two diode lasers were used with uninitiated tips, and the two Erbium lasers and the 10,600-nm CO_2 laser were used without air or water sprays (so as not to blow away the blood). The D810, D940, and Er,Cr:YSGG lasers all had inferior degrees of congealing abilities when compared to the Nd:YAG, Er:YAG, and 10,600-nm CO_2 lasers, which were able to achieve a greater degree of congealing at an earlier time point. The differences were clinically and statistically significant. The clinical differences were based on the color, texture, and tactility of blood. The reason for the differences in ability to congeal pooled blood may be attributed to inherent laser properties, namely, energy produced and heat generated.[5,22] The times for maximum congealing for the Er:YAG and 10,600-nm CO_2 lasers were greater than that for the Nd:YAG, while all three lasers had similar degrees of congealing. This apparent discrepancy is due to the following: During the maximum congealing portion of the experiment, the 10,600-nm CO_2 and Er:YAG samples had a change in color; however, the tactile feel of the viscosity of the sample of the blood did not change as rapidly as with the Nd:YAG samples.

One of the limitations of this study is that it is an in vitro study. The lasers' effects on the blood vessels themselves have been eliminated using this model. The study used untreated whole blood to analyze the effects of dental lasers. However, the blood samples' ability to congeal was not affected by the clinical cellular and matrix components or

biologic events that would occur in an in vivo extraction. The cells, matrix elements, growth factors, and proteins that may influence coagulation in an extraction site were not available in this in vitro study, and all congealing was a result of the application of the dental lasers. The volume of human blood used mimicked the volume of a human mandibular premolar extraction socket.[18] Of interest and surprising to the authors, in the excess untreated blood, congealing did not occur even 1 to 2 hours after being drawn.

There are many differences among dental lasers. They vary in physical properties, absorption characteristics, and wavelengths produced. These differences account for the variability in the way in which the lasers perform. Some lasers are primarily used for soft tissue procedures, as the wavelengths generated interact primarily with melanin and hemoglobin (diode and Nd:YAG lasers). Other lasers, like Er,Cr:YSGG, Er:YAG, and CO_2, have a higher affinity for water and hydroxyapatite, and the Erbium types are used primarily for hard tissue procedures; 10,600-nm CO_2 lasers are not safe to use on hard tissues. The penetration depth is different among lasers as well, with Nd:YAG able to penetrate up to 5 mm and diode lasers 2 to 3 mm. At a depth of 0.3 to 0.5 mm, Erbium and CO_2 lasers have a far smaller penetration. With all these differences, it is easy to imagine that there would be differences in lasers' ability to congeal pooled blood.[5]

There was a great deal of variability in the calculated amount of energy produced by each laser, ranging from 22.5 to 108.0 J at 30 seconds. The reason for this difference is that there was variation in the manufacturers' protocols in terms of power, repetition rate, pulsing, etc, recommended for hemostasis, congealing, and/or coagulation. No one protocol is appropriate for all dental lasers. Each laser's amount of power differed: 3.0, 2.5, 3.6, 0.75, 1.8, and 2.0 W were used for the D810, D940, Nd:YAG, Er,Cr:YSGG, Er:YAG, and 10,600-nm CO_2 lasers, respectively. It can be easy to think that fewer watts would yield less congealing; however, the Er:YAG and 10,600-nm CO_2 lasers produced less power than either of the diode lasers but were better at congealing. This shows that no single parameter is the key element of effectiveness.

Temperature differences were also considerable among the lasers. After application for 30 seconds, the Nd:YAG laser heated the blood to an average of 53°C while the 10,600-nm CO_2 laser only heated the blood to 31°C, with the other lasers falling somewhere in between. Temperature change is a contributing factor to congealing blood. Proteins in plasma have been shown to begin the early stages of denaturation between 43°C and 45°C. Denaturation results in changing of the proteins' shape,[23] and some authors have reported that coagulation occurs at 60°C.[24] While the D810, D940, and Nd:YAG lasers all approached temperatures where protein denaturation would occur, these higher temperatures did not necessarily equate to greater degrees of coagulation. The 10,600-nm CO_2 and Er:YAG lasers both had average temperatures less than what is usually needed for protein denaturation to occur and still had statistically significantly better congealing outcomes than lasers that produced higher temperatures. Clinically, excess heat would be of concern, as it may lead to tissue necrosis if not properly controlled.

While all dental lasers state that they can improve hemostasis, the results from this experiment suggest that some lasers may be superior to others in terms of ability to congeal blood at an earlier time point and to a greater extent. When considering the use of a dental laser as part of a surgical procedure, clinicians should consider in what way they want the laser to aid them, as each laser is proficient in its own ways (removal of hard vs soft tissues, etc). For aiding in the coagulation of blood after a dental extraction, clinicians should consider Nd:YAG, Er:YAG, and 10,600-nm CO_2 lasers based on the results of this study. Each laser's specific manufacturer's settings must be adhered to, and proper laser training is important.

Limitations of the current study include: all blood samples were considered equal even though they were from multiple donors, operator error (movements of the laser, transition from benchtop to centrifuge, timing of laser application), availability of whole blood, novel subjective scoring scale used, differences in coagulation/hemostasis/congealing settings (manufacturer's protocols), and benchtop study. Future studies could include repeating the study with blood from a single

donor, using digital photo-optical analysis for determining degree of coagulation, evaluating other laser wavelengths, or an in vivo extraction study.

Conclusions

The data from this in vitro experiment showed that different dental lasers have different abilities to congeal blood in an in vitro extraction socket model. The 10,600-nm CO_2, Er:YAG, and Nd:YAG lasers were able to congeal blood to a greater extent at an earlier time point than the D810, D940, and Er,Cr:YSGG lasers. Reasons for this can be attributed to the amount of heat and energy delivered; however, the process is likely more complicated, and the type and amount of laser energy must account for some of the difference in ability to rapidly congeal blood. The Nd:YAG laser produced the most heat and was the fastest to complete coagulation.

Acknowledgments

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