

Article

# Retrieval of prefabricated zirconia crowns with Er,Cr:YSGG laser from primary and permanent molars

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**Abstract:** (1) Background: Prefabricated zirconia crowns are used to restore teeth in children. The purpose of this study was to evaluate the removal of these crowns with the erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser; (2) Methods: Twelve primary and 13 permanent teeth were prepared and prefabricated zirconia crowns were passively fitted and cemented with two resin modified glass-ionomer cements. Surface areas of prepared teeth and crowns were calculated. Crowns were removed using two laser settings: 4.5 Watts, 15 Hertz, 20 water/ 20 air, and 5 Watts, 15 Hertz, 50 water/50 air. The retrieval time and temperature changes were tested recorded. Data were analyzed using ANOVA with Tuckey's adjusted post hoc pairwise comparison t-test; (3) Results: The average time for crown removal was: 3 minutes, 47.7 seconds for permanent; and 2 minutes 5 seconds for primary teeth. The mean temperature changes were 2.48°C (SD=1.43) for permanent; and 3.14°C (SD=1.88) for primary teeth. The time to debond was significantly positively correlated with tooth inner surface area and volume, outer crown volume, and the cement volume; (4) Conclusions: Use of the Er,Cr:YSGG laser is an effective, safe and non-invasive method to remove prefabricated zirconia crowns cemented with RMGI cements from permanent and primary teeth.

**Keywords:** debonding; Er,Cr:YSGG laser, primary teeth, zirconia crown

## 1. Introduction

Dental caries continues to be the most common chronic childhood disease [1]. It can greatly affect child's well-being; lead to infection, poor nutrition, missed school and negatively influence learning and decrease overall quality of life. Clinical dentistry has evolved to treat this disease by removing the caries and restoring teeth with fillings or crowns.

The stainless-steel crown (SSC) has long been considered the "gold standard" to restore teeth with caries, cervical demineralization, developmental defects, and to temporarily restore permanent molars treated with a root canal in the growing patient [2]. Multiple longitudinal studies have demonstrated the superiority of SSCs over amalgam restorations for primary molars with multi-surface involvement [3,4]. Increasing demand by parents for esthetic restorations for their children disagrees with SSCs and has led to implementation of zirconia crowns to the pediatric dentist's armamentarium. Zirconia is a crystalline dioxide of zirconium with mechanical properties similar to

those of metals, and color similar to teeth. Ready-made zirconia crowns are available for primary incisors, molars and permanent molars [5]. Prefabricated zirconia crowns share the same indications as SSCs. While SSCs have better retention, zirconia crowns allow for better gingival health and less plaque accumulation, in addition to vastly better esthetics [6,7]. Zirconia crowns require more circumferential tooth reduction (minimum 1.5-2mm) for passive fit making cementation critical for their longevity [8]. This in turn, presents a challenge when zirconia crowns have to be removed [9]. Removal can be accomplished by sectioning using rotary instruments, which is time consuming and stressful for the patient and clinician [10-12]. Sectioning with high-speed burs can lead to iatrogenic tooth damage as it can be difficult to differentiate between the tooth and the tooth-colored materials [13].

Erbium lasers have been explored for removal of translucent crowns. The light admitted by erbium lasers is transmitted through the translucent material and selectively absorbed by water molecules and residual monomers in the resin and glass ionomer cements leading to ablation of the cement which results in reduced bond strengths and hydrodynamic ejection [14]. This strategy has been successfully applied for debonding of ceramic orthodontic brackets from teeth [13,15]. Temperature changes in the pulp chamber during laser irradiation should remain within tolerable range to not adversely affect the vitality of the pulp [11]. The time required to remove lithium disilicate crowns with high-speed burs takes approximately 6 minutes compared to laser assisted removal that can be done in 60-90 seconds [16]. Crowns removed by sectioning are destroyed, while laser debonded crowns remain undamaged and can be recemented.

Erbium family lasers include two lasers with close basic properties except slight differences in wavelength range, pulse duration and energy: 1. Erbium-doped-yttrium aluminum garnet (Er:YAG) (2940 nm); and 2. Erbium, chromium: yttrium-scandium-gallium-garnet (Er,Cr:YSGG) (2780 nm). Technique of using Er:YAG laser has been validated by removing safely and efficiently lithium disilicate crowns from zirconia and titanium implant abutments [17,18]. Recent studies have proven wide range of functions for erbium family lasers, like oral pathogens control [19-24], soft tissue de-epithelialization [25], bone decortication [26], and fiber post retrieval [27].

The Er,Cr:YSGG laser (Waterlase iPlus, Biolase, USA) is an all-tissue laser with ability to treat hard tissue and soft tissue. It is more effective than Er:YAG and high-power diode lasers in surface treatment of glass fiber post [28] and can be used to remove lithium disilicate crowns with minimal damage to the crown and tooth [16]. Facilitating crown removal with laser as a non-invasive alternative to rotary instruments could improve clinical practice considering that pre-formed zirconia crowns are becoming more common in the pediatric population. To date, no studies of this type have been conducted on primary teeth.

The aim of this in vitro study was to analyze the feasibility of use of Er,Cr:YSGG laser for retrieval of prefabricated zirconia crowns cemented with resin-modified glass ionomer (RMGI) cements from primary and permanent molars while also establishing most effective and least aggressive laser settings. Second aim was to explore if correlations exist between the time required to remove a crown and the surface area of the crown, abutment tooth and the cement volume. Third aim was to assess temperature changes in the pulpal chamber during laser irradiation at two different settings. Additionally, it was of interest to examine how laser irradiation affects structure of the crown and tooth surface and if previously performed debonding has an effect on the adhesion properties of the consequent recementation.

## 2. Materials and Methods

Teeth were stored in isotonic saline solution and evaluated for the amount of remaining non-carious tooth structure. Teeth with fractured crowns, gross caries, or previous restorations were excluded from the study.

Twelve primary (G1, N=12) and 13 permanent teeth (G2, N=13) were prepared following the manufacturer's instructions with a 1-2mm of occlusal reduction and 20-30% overall clinical crown reduction. Prepared surfaces of all teeth were scanned using computer assisted digital (CAD)

technology and files were imported into Meshmixer© software (Autodesk, Inc) to calculate tooth surface area (mm<sup>2</sup>) and the tooth volume (mm<sup>3</sup>). (Figure 1A) Prefabricated zirconia crowns (Nusmile®, Houston, USA) were selected for the most intimate passive fit, dried and cemented on teeth following manufacturer's guidelines. Finger pressure was utilized to properly seat and stabilize crowns for approximately 20 seconds. Crowns were first flash cured for 5-10 seconds with a (800-1200 mW/cm<sup>2</sup>) curing light and then for an additional 10 seconds on facial, lingual and occlusal surfaces mimicking the clinical situation where interproximal sites are not accessible. Following cementation, the crowns were scanned again using CAD technology and the outside crown surface area (mm<sup>2</sup>) including marginal cement filling the space between passively fitted crown and the tooth and the outside volume (mm<sup>3</sup>) were calculated the by the Meshmixer© software. (Figure 1B) All teeth were stored in moist containers for 24-48 hours before retrieval was initiated. For each debonding experiment, the same experimental steps were repeated for each group.

First experiment for primary (G1-BC1, N=12) and permanent (G2-BC1, N=13) teeth was performed using BioCem Universal Active Cement resin modified glass ionomer (RMGI) cement (BioCem, NuSmile®, USA). Following debonding, the crowns and teeth were cleaned of residual cement and crowns were recemented back with BioCem cement on primary (G1-BC2, N=12) and permanent (G2-BC2, N=13) teeth. The group of 12 permanent teeth, (G2-RX1, N=12) was recemented a third time with another RMGI cement (RelyX. 3M ESPE, St. Paul, Minn., USA).

### Laser Settings

This study utilized the Er,Cr: YSGG (Biolase® Waterlase iPlus) laser with two different settings. First experiment to retrieve crowns from primary (G1-BC1) and permanent (G2-BC1) teeth was performed using settings: 4.5 Watts, 15 Hertz, 20 water and 20 air with the Turbo MX9 handpiece (WaterlaseiPlus, Biolase, USA).

The second experiment for primary (G1-BC2) and permanent (G2-BC2) teeth was performed using the same RMGI cement and slightly higher laser settings: 5 Watts, 15 Hertz, 50 water and 50 air with the Turbo MX9 handpiece. Higher setting was also combined with a lighter tapping forces or digital manipulation applied for a crown removal. These settings were chosen based on other studies [29], and our observations [17,18] with the goal of achieving efficient retrieval time at low settings while avoiding temperature increases.

The third experiment included only permanent teeth (G2-RX3) using the same laser setting as in a second experiment: 5 Watts, 15 Hertz, 50 water and 50 air and a different RMGI cement.

### Laser Debonding Procedure

The crowns were irradiated on the buccal, lingual and occlusal surfaces for 30 seconds in continuous motion of the handpiece in a back and forth motion 4-5 mm from the crown surface. The interproximal surfaces were not irradiated directly in order to mimic adjacent teeth being present in the mouth. To test if the crown could be removed, the crowns were examined with digital palpation for ease of removal and a crown tapping instrument was used on the buccal and lingual margins. If crown could not be successfully removed, the tooth was subjected to additional 30 second intervals of laser irradiation followed with the attempt of crown removal after each of the intervals until the crown could be retrieved. The irradiation time was measured in 30 second intervals.

### Pulpal Temperature

Before initiating crown removal, a hole (3-4 mm diameter) was drilled through the furcation (Figure 1C) of each tooth to enable insertion of a temperature probe (Sper Scientific® 800008) into the pulpal chamber. (Figure 1D) Pulpal temperatures were recorded at the baseline and throughout the entirety of the procedure in 30-second intervals.

### Scanning Electron Microscopy Analysis

The surfaces of teeth and crowns of one primary and two permanent teeth following first and second laser irradiation experiments were examined under scanning electron microscope (SEM) (JEOL 6610LV, JEOL, Japan) to examine structural integrity and possible surface damage to the crown and

tooth due to laser irradiation. Following successful debonding the underlying intaglio surface and the cameo surface of the crown and the cameo surface of the tooth were inspected and analyzed.

### Statistical Methods

Differences in average debond time and average increase in temperature between primary and permanent teeth were assessed using equal and unequal variance t-tests as appropriate. Repeated measures ANOVA with Tukey's adjusted post hoc pairwise comparisons was used to determine differences in the debond time across the primary and permanent teeth groups debonding attempts. Pearson's correlations were used to determine the association between the crown metrics and the time necessary to debond the crown. Multiple linear regression was used to determine the relationship between time to debond and the cement volume and the ratio of the outer to inner surface area.

### 3. Results

A total of 12 permanent and 12 primary teeth were utilized in this study. Permanent teeth were debonded a total of three times and primary twice. Summary of the volume and surface area metrics for the crowns are given in Table 1.

#### Debonding Time

The average time for crown removal using Er,Cr:YSGG laser for permanent molars was 3 minutes (min) and 54.2 seconds (sec). The average time for primary molar crown removal was 2 minutes and 5 seconds. Permanent molars took, on average, 1 minute and 49.2 seconds longer than primary teeth, which was statistically significant ( $p < 0.0001$ ).

Due to the high correlations among the crown metrics, they could not all be considered for the overall models for time to debond. The cement volume and the ratio of inner to outer surface area were selected as the most informative and utilized for the analysis. The pairwise correlation for these two variables was low ( $r = 0.04$ ,  $p\text{-value} = 0.7513$ ).

#### Primary teeth

Since the average debond time was the same for both debond attempts with primary teeth (G1-BC1, G1-BC2), it was not statistically significant ( $p\text{-value} = 1.00$ ). Time to debond crowns from the primary teeth was only significantly correlated with the cement volume ( $r = 0.62$ ,  $0.63$  for the two debond attempts respectively). Correlations are given in Table 2. The ratio of the outer to inner surface area was not significantly associated with the debond time for the primary teeth for either the first ( $p\text{-value} = 0.6345$ ) or the second ( $p\text{-value} = 0.6055$ ) debond attempts. (Figure 2A) The cement volume was marginally significantly associated with the debond time for primary teeth at both debond attempts. (Figure 2B) For the first attempt, a 1-mm<sup>3</sup> increase in cement volume had an estimated .85 second increase in the time to debond ( $p\text{-value} = 0.0572$ ). For the second attempt, a 1mm<sup>3</sup> increase in cement volume was associated with a 0.72 second increase in the time to debond. Complete results are given in Table 3..

#### Permanent teeth

For permanent molars, there were significant differences in the debonding times based on the three experiments ( $p\text{-value} < 0.0001$ ). First experiment (G2-BC1/Debond 1) was the fastest, with an average of 2:34.60, and was marginally significantly faster than second experiment (G2-BC2/Debond 2) (average difference= 78.38 seconds; adjusted  $p\text{-value} = 0.0513$ ). Third experiment (G2-RX/ Debond 3) required the longest time (average=307.5 seconds) and took significantly more time than Debond 1 (average difference: 152.81 seconds, adjusted  $p\text{-value} = 0.0002$ ). Debond 3 was also longer than Debond 2, but this difference was not statistically significant (average difference=74.4 seconds, adjusted  $p\text{-value} = 0.0732$ ). All further analyses were performed by debond attempt due to the varying conditions of the attempt.

The time to debond crowns off permanent molars was significantly, positively correlated with the inner surface area ( $r = 0.63$ ), inner volume ( $r = 0.70$ ), outer volume ( $r = 0.73$ ), and the cement volume

( $r=0.56$ ). Although they were not statistically significant, it was also negatively correlated with the ratio of the outer to the inner surface area ( $r=-0.44$ ), and the ratio of the volume ( $r=-0.30$ ). (Figure 2C) This indicates that crowns that are larger relative to the abutment are faster to debond. A similar pattern was seen for the second experiment (Debond 2), with significant positive correlations between the time to debond and: total crown volume ( $r=0.61$ ), inner surface area ( $r=0.77$ ), outer surface area ( $r=0.76$ ), inner ( $r=0.76$ ) and outer ( $r=0.73$ ) volume. Again, the debond time was negatively correlated with the ratio of the inner to outer surface area ( $r=-0.41$ ) and volume ( $r=-0.37$ ) but these were not statistically significant. The time for the third experiment, Debond 3, which utilized a different cement, was not significantly correlated with any of the measures, but the strongest correlation was with the cement volume ( $r=0.38$ ). Correlations are given in Table 2.

Cement volume was associated with a significant increase in the time to debond permanent crowns for the first debond experiment settings (G2-BC1). (Figure 2D) For a 1mm<sup>3</sup> increase in the volume of the cement, the debond time required an additional 1.2 seconds ( $p$ -value=0.0369). There was marginal evidence of a significant decrease in first permanent molar debond time by an estimated 16.7 seconds for a 1-unit increase in the ratio ( $p$ -value=0.0853). Cement volume was not significantly associated with permanent molar debond time for the second (G2-BC2)( $p$ -value=0.4150) or third (G2-RX)( $p$ -value=0.2485) debond attempts. There was marginal evidence of a decrease in second debond time (Debond 2) based on the ratio, with an estimated 13.23 second decrease for a 1-unit increase in the ratio ( $p$ -value=0.1948). Complete results are given in Table 3.

### Temperature

The mean temperature changes were 2.48°C (SD=1.43) for permanent teeth and 3.14°C (SD=1.88) for primary teeth. Although the primary teeth had greater temperature change, the difference was not statistically significant ( $p=0.1219$ ). Data on temperature changes are given in Table 4.

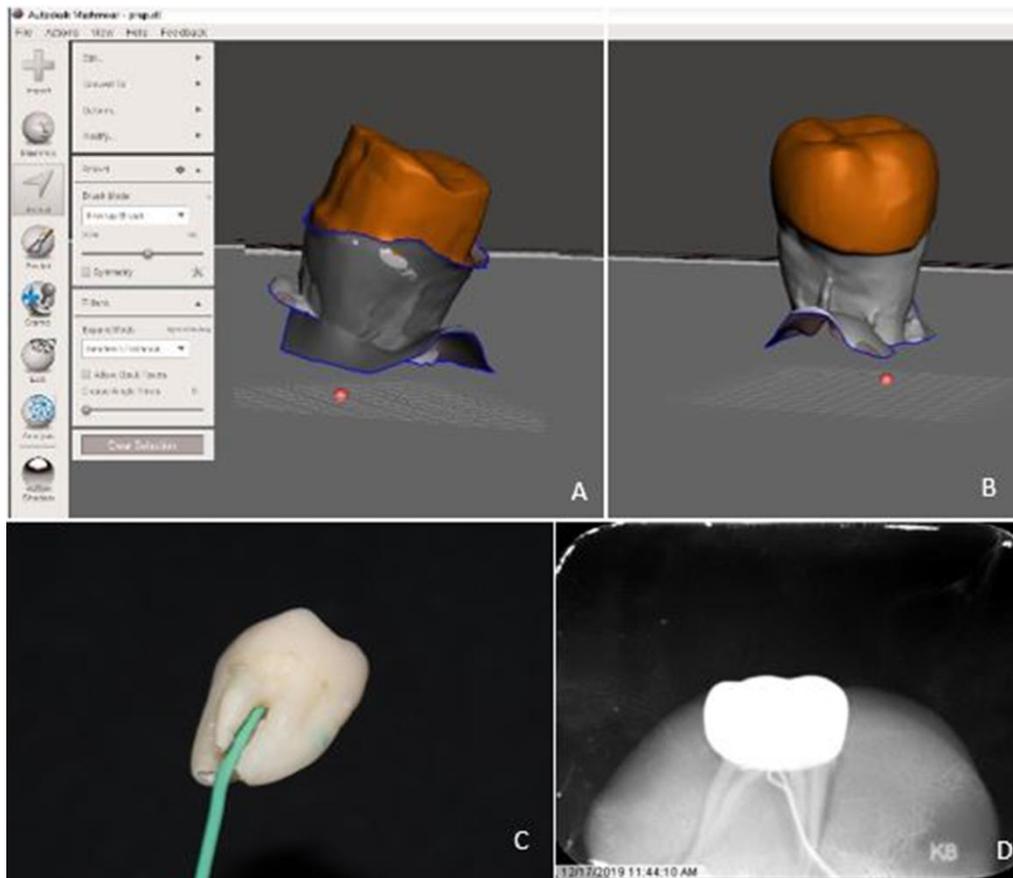
### Clinical and Scanning Electron Microscopy (SEM) Examination

After debonding, each crown and tooth were examined to analyze the adherence of cement to the dentin or crown. The cement appeared to stay attached either to the intaglio surface of the crown (Figure 3 A) or tooth surface. (Figure 3B) Neither teeth nor crowns exhibited structural changes or damage suggestive of photoablation or thermal ablation. No carbonization was observed on the tooth or crown. Slight partial ablation of the cement caused by Er,Cr:YSGG laser irradiation was occasionally observed on tooth surface (Figure 3C) and intaglio surface of the crown (Figure 3 D) with no visible cracks or fractures on the SEM analysis.

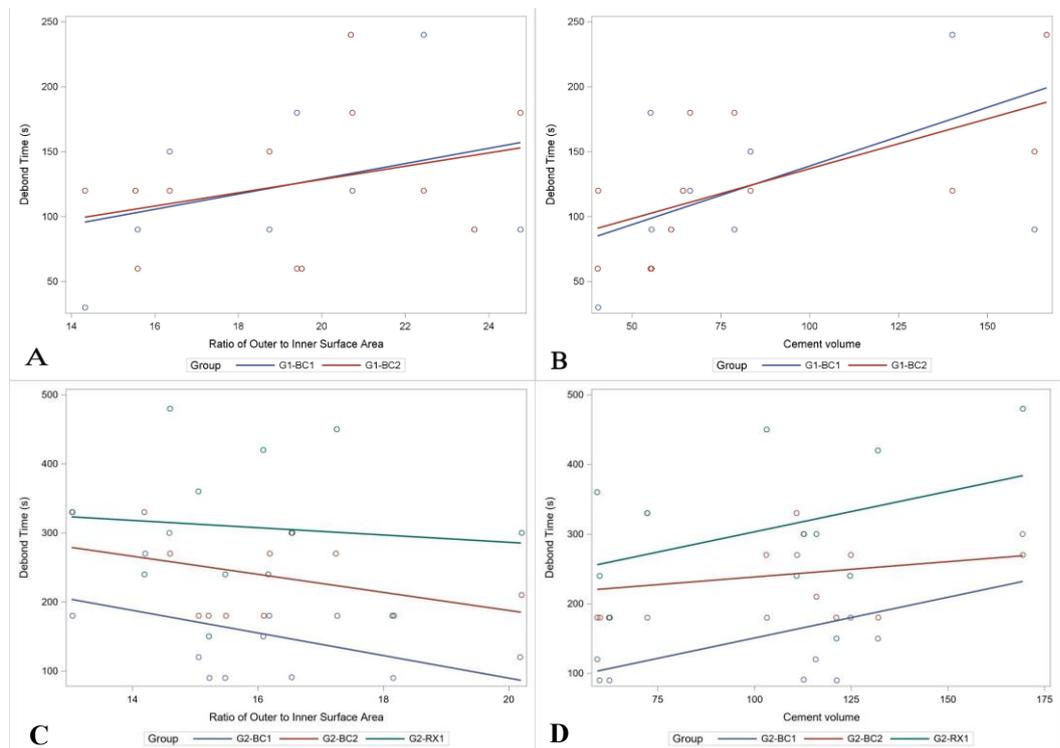
### 3.2. Figures, Tables

#### A Figure legends:

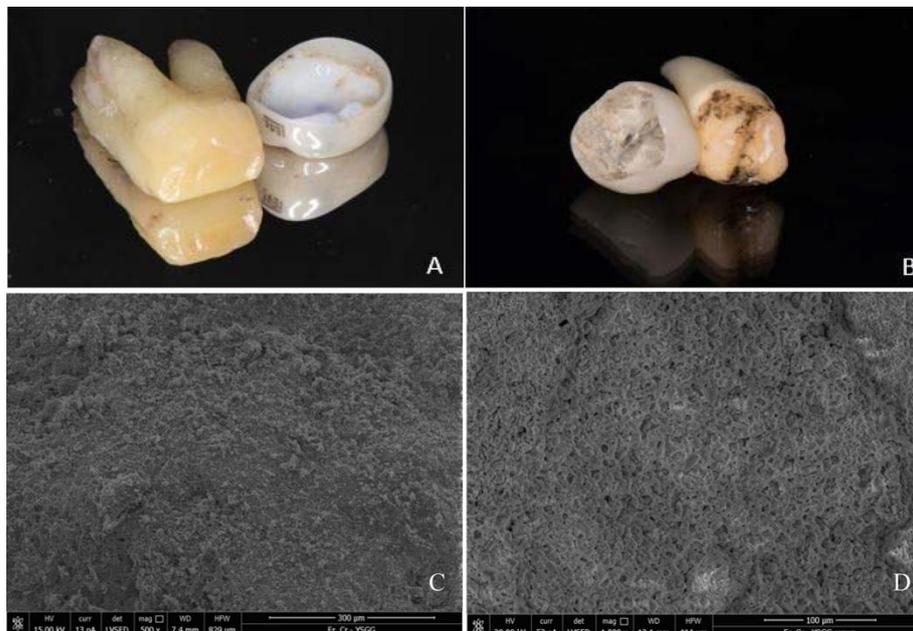
**Figure 1:** Prepared surfaces of teeth (A) and cameo surfaces of the crowns (B) were scanned to calculate tooth surface area and the tooth volume. To measure pulpal temperature changes, a 3 mm diameter hole was drilled through the furcation (C) to enable insertion of a temperature probe into the pulpal chamber (D).



**Figure 2:** Correlations of Debond time with Ratio of outer to inner surface area (A,C) and Cement volume (B,D) for primary (A,B) and permanent (C,D) teeth.



**Figure 3:** Clinical view of the tooth and crown following laser-debonding from primary (A) and permanent (B) tooth. Tooth surface (C) and Intaglio surface of the crown (D) appeared to be undamaged and covered by the residual cement on the SEM analysis.



**Table 1.** Summary Statistics for Permanent and Primary Crowns

	Mean, SD	
	Permanent Teeth (n=12)	Primary Teeth (n=12)
Crown Volume	201.76, 32.69	93.92, 43.83
Inner surface area	171.69, 28.16	88.77, 26.85
Outer surface area	270.77, 25.94	166.31, 43.43
Ratio surface area (outer:inner)	160.02, 19.03	193.12, 33.63
Inner volume	233.65, 67.20	76.45, 40.10
Outer volume	537.47, 108.75	254.16, 102.52
Ratio volume (outer:inner)	236.8, 36.21	379.89, 166.57
Cement volume	103.69, 34.01	84.64, 45.67

\*Volume in mm<sup>3</sup>, Surface Area in mm<sup>2</sup>

**Table 2.** Pairwise Correlations between Crown Metrics and Time to Debond

	Permanent Teeth			Primary Teeth	
	Debond 1	Debond 2	Debond 3	Debond 1	Debond 2
Crown Volume	0.433	<b>0.613</b>	0.070	0.328	0.201
Inner surface area	<b>0.626</b>	<b>0.766</b>	0.059	0.225	0.181
Outer surface area	0.506	<b>0.760</b>	0.008	0.573	0.408
Ratio surface area (outer:inner)	-0.439	-0.406	-0.096	0.298	0.310
Inner volume	<b>0.697</b>	<b>0.764</b>	0.219	-0.085	0.188
Outer volume	<b>0.731</b>	<b>0.725</b>	0.251	0.381	0.441
Ratio volume (outer:inner)	-0.303	-0.374	-0.091	0.505	0.164
Cement volume	0.561	0.244	0.377	<b>0.623</b>	<b>0.632</b>

\*values in bold are significantly different from 0, p-value<0.05

**Table 3.** Linear Regression Results for Relationship between Cement Volume, Ratio of Outer to Inner Surface area and Time to Debond

	Cement Volume		Ratio of Outer:Inner Surface area	
	B (SE)	P-value	B (SE)	P-value
Permanent				
<b>Debond 1:</b> 4.5w, <i>BioCem Cement</i>	1.19 (0.48)	0.0369	-16.71(8.65)	0.0853
<b>Debond 2:</b> 5.0w, <i>BioCem Cement</i>	0.45 (0.53)	0.4150	-13.23 (9.44)	0.1948
<b>Debond 3:</b> 5.0w, <i>RelyX Cement, no minimal tapping</i>	1.17 (0.95)	0.2485	-5.63 (16.90)	0.7468
Primary				
<b>Debond 1:</b> 4.5w, <i>BioCem Cement</i>	0.85 (0.39)	0.0572	2.60 (5.29)	0.6345
<b>Debond 2:</b> 5.0w, <i>BioCem Cement</i>	0.72 (0.32)	0.0524	2.34 (4.38)	0.6055

**Table 4.** Summary of Crown Temperature during Debonding

	Maximum			Average		
	Temperature	SD	Range	Delta	SD	Range
Permanent						
Debond 1	22.9	0.93	21.9-25.6	2.4	0.98	0.1-4.5
Debond 2	26.8	1.03	24.5-28.6	2.1	1.19	0.4-4.2
Debond 3	26.6	1.16	25.5-29.9	3.0	1.98	1.6-8.9
Primary						
Debond 1	26.1	2.82	22.1-30.7	4.0	2.17	1.0-8.1
Debond 2	26.7	1.49	24.4-29.9	2.4	1.17	1.1-4.9

#### 4. Discussion

Lasers implement traditional dental practice and can help reduce procedure time, improve treatment outcomes and increase patient acceptance. As a less invasive and a more efficient treatment option, laser-assisted crown removal presents a good alternative to rotary instrumentation making the procedure easier on the patient and less damaging to the tooth

structure and restoration [9]. The laser beam is partially absorbed by the remnant water in the resin monomer and then by both the water and inorganic matter present in the dentin. During the thermo-mechanical reaction between laser light and a chromophore in the luting cement, water micro explosions occur and result in reduction of the adhesive resin strength between the tooth and the crown [12,13,21-23].

Prefabricated zirconia crowns offer improved appearance in the pediatric population and can be removed from the tooth by erbium laser irradiation. Unlike the traditional SSCs, prefabricated zirconia crowns rely on a passive-fit cementation and not mechanical retention. The first two experiments on permanent teeth suggested that larger crowns relative to the size of the abutment tooth are faster to debond, reinforcing the importance of proper crown size on retention. This trend was not observed for the third experiment that utilized a different cement. Although inconclusive, this difference could be contributed to the properties of cements, such as water or monomer content. More research is needed to analyze the specific types of cements as they relate to debond times using the Er,Cr:YSGG laser. Greater cement amount was associated with a longer debonding time. Longer irradiation and more laser energy may possibly be required to ablate the water and monomer components in the larger cement volume which somewhat coincides with the poorer passive "crown-fit". Given that the crown and cement volumes were not standardized, more research is needed on this topic to further explore the differences.

The force of tapping versus using just digital manipulation for crown removal was not standardized in this study, but can also affect the crown retrieval time. It is difficult to predict at what point during the laser irradiation, the bond between the crown and tooth is loose enough to enable the crown removal. Thus, following incremental irradiation, attempts to remove the crown range from tapping on the crown margin to just being able to remove digitally the crown of the tooth. The crown may therefore be retrieved following irradiation by tapping or digital manipulation depending on the retentive features of the tooth and the extent and strength of the residual bond remaining. When stronger tapping forces are applied, the crown can be removed following shorter irradiation as mechanical force is used to break residual adhesive bonds rendering time shorter as evident from the results in the first set of experiments. Tapping forces on an extracted tooth can often exceed the force that can be tolerated in-vivo, especially on a pediatric patient. For sensitive patients unable to tolerate tapping forces or in the scenarios where tapping may cause iatrogenic damage to the tooth or restoration, the irradiation time can be extended by 1-2 minutes enabling the retrieval of the crown by only using gentle digital manipulation.

Laser irradiation can result in increased temperature in the pulpal chamber and surrounding tissues. For all experimental groups in this study, the temperature in the pulpal chamber remained within tolerable range during the laser irradiation. Small differences observed among the groups can be attributed to the proximity of the pulpal chamber to the irradiated surfaces and the setting of the laser. Primary teeth have larger pulpal chambers and thinner layer of dentin and thus the heat generated during irradiation may have stronger thermal effects on the pulp. Similar trends were observed in the smaller sized permanent molars. Irradiation at higher laser settings did not improve the debonding efficiency, but did result in higher temperature increases. Therefore, it may be safer to use lower laser settings on teeth with larger pulps and thinner dentinal barrier between the cemented crowns and the pulpal chamber. Longer irradiation time also did not lead to higher temperature increases and therefore additional irradiation time may be preferable to aggressive tapping forces or higher power laser settings [28]. Future research may be necessary to investigate the effect on pulpal temperature in anterior teeth with smaller crowns.

Irradiation with Er,Cr:YSGG laser doesn't seem to damage the retrieved prosthesis or the tooth structure rendering the retrieved restoration reusable when indicated. Following Er,Cr:YSGG irradiation, no structural surface damage to the tooth or crown was observed on SEM analysis.

These findings are consistent with previous Er:YAG findings for lithium disilicate crowns debonded from titanium and zirconia implant abutments [19,20].

This study is not without limitations. The same teeth were reused for second and third experiment making it difficult to determine the influence of repeated bonding and irradiating. Pulpal temperature was affected by the room and water temperature, which had slight daily variations. This study was performed on extracted teeth and therefore does not take into the account the challenges presented in clinical implementation of this technique.

Laser-assisted crown removal could be applied in clinical practice to remove prefabricated zirconia crowns when a tooth needs to be treated endodontically or the crowns need to be replaced with permanent restorations. Crowns may occasionally also not be optimally seated during the cementation process and this method provides reversible method to retrieve the crown and improve the crown seat and cementation.

**Supplementary Materials:** The following are available online at [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1), Figure S1: title, Table S1: title,

**Author Contributions:** For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, X.X. and Y.Y.; methodology, X.X.; software, X.X.; validation, X.X., Y.Y. and Z.Z.; formal analysis, X.X.; investigation, X.X.; resources, X.X.; data curation, X.X.; writing—original draft preparation, X.X.; writing—review and editing, X.X.; visualization, X.X.; supervision, X.X.; project administration, X.X.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript.”, please turn to the [CRediT taxonomy](#) for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

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