UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

LETICIA FERREIRA DE FREITAS BRIANEZZI

**Effect of Er, Cr: YSGG laser irradiation in the physics properties of
adhesive systems applied over the dentin**

**Efeito da irradiação do laser de Er,Cr:YSGG nas propriedades físicas
de sistemas adesivos aplicados sobre a dentina**

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Tese apresentada a Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Doutor em Ciências no Programa de Ciências Odontológicas Aplicadas, na área de concentração Dentística.

Orientador: Prof. Dr. Sérgio Kiyoshi Ishikiriyama

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*“Sonho que se sonha só
É só um sonho que se sonha só
Mas sonho que se sonha junto é realidade”*

Raul Seixas

ABSTRACT

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Effect of Er, Cr: YSGG laser irradiation in the physical properties of adhesive systems applied over the dentin

The analysis of some properties is necessary to better understand the mechanism by which an increase of bond strength values occurs when the dentin / adhesive complex is irradiated with laser. Thus, this study evaluated some physical properties of dentin bonding system [DBS] applied on dentin that were irradiated with Er: YSGG laser. The present study was a 2-level factor: treatment in 2 sub-levels (without laser [C] and Er: YSGG [L] laser) and DBS in 4 sub-levels: 3-step total etch Adper[™] Scotchbond[™] Multi-Purpose [MP], total etch 2-step Adper[™] SingleBond 2 [SB], 2-step self-etch Clearfil[™] SE Bond [CSE], 1-step self-etch Adper[™] Single Bond Universal [SU]. The response variables were water sorption [WS] and water solubility [WSB] of DBS in dentin (n = 10) that were measured by gain and mass loss. The degree of conversion [DC] "in situ" (n = 3) was evaluated by Micro-Raman spectroscopy obtaining through the ratio of the polymerized / unpolymerized sample spectra. Infrared thermography analyzed the superficial temperature [TS] and the ceiling of the pulp chamber temperature [TC] during irradiation through a thermographic camera (n = 8). In addition, the characteristics of the resulting hybrid layer (n = 3) were also evaluated by scanning electronic microscopy (SEM). The bond strength (n = 10) was measured by the microtensile [MS] test with an area of approximately 0.64mm² of irradiated dentin and submitted to aging for 1 year. The data were analyzed according to the distribution and homogeneity of the values, and then, WS/WSB, DC and MS were submitted to ANOVA 2 criteria and TS / TC tests submitted to ANOVA 1 criterion, followed by Tukey test (p < 0.05). For the WS/WSB, the group L presented smaller values, whereas for the SB, L presented higher values than the non-irradiated group. In DC, a significant difference was noted only for the DBS factor. In infrared thermography it was observed that L promoted a high increase of TS, but with low propagation to pulp chamber to TC. For the SEM, the conventional DBSs presented a great depth of adhesives penetration into the dentine than the self-etching systems, especially when irradiated with laser. For MS, the lowest values for L were observed, except for SUL. In the fracture analysis, it was observed that the most prevalent failure mode for all groups

was adhesive and mixed. It was concluded that the irradiation with the Er, Cr: YSGG laser with the parameters used in the present study does not present clinical benefits, since it undermined most of the tested properties

Key words: Lasers. Dentin. Dentin Bonding Systems

RESUMO

RESUMO

A análise de algumas propriedades é necessária para melhor compreender o mecanismo pelo qual o aumento nos valores de resistência de união acontece quando o complexo dentina/adesivo é irradiado com laser. Dessa forma, este estudo avaliou algumas propriedades físicas de sistemas adesivos [SA] aplicados sobre a dentina que foram irradiados com laser de Er:YSGG. O presente estudo foi fatorial em 2 níveis: tratamento em 2 subníveis (sem laser [C] e com laser de Er:YSGG[L]) e sistema adesivo em 4 subníveis: convencional de 3 passos Adper™ Scotchbond™ Multi-Purpose [MP], convencional de 2 passos Adper™ SingleBond 2 [SB], autocondicionante de 2 passos Clearfil™ SE Bond [CSE], autocondicionante de 1 passo Adper™ Single Bond Universal [SU]. As variáveis de respostas foram a sorção [SO] e solubilidade [SB] em dentina de sistemas adesivos (n=10) que foram mensuradas pelo ganho e perda de massa. O grau de conversão [GC] “*in situ*” (n=3) foi avaliado por meio da espectroscopia Micro-Raman obtendo através da relação dos espectros de amostras polimerizadas/ não polimerizadas. A termografia infravermelho analisou a temperatura superficial [TS] e do teto câmara pulpar [TC] através de uma câmera termográfica (n=8). Além disso, também foram avaliadas as características da camada híbrida (n=3) por meio da microscopia eletrônica de varredura [MEV]. A Resistência de união [RA] (n=10) foi mensurada pelo teste de microtração com área de aproximadamente 0,64mm² e submetida ao envelhecimento 1 ano. Os dados foram analisados, de acordo com a distribuição e homogeneidade dos valores, e então, SO, SB, GC e RA foram submetidos ao teste ANOVA 2 critérios e TS/TC submetidos ao teste ANOVA 1 critério, seguido de teste Tukey (p<0,05). Para a SO/SB, o grupo L apresentou menores valores significativos, já para a SB o L apresentou valores maiores que o grupo não irradiado. No GC, notou-se uma diferença significativa apenas para o fator SA. Na termografia infravermelho observou que o L promoveu um aumento de alta TS, mas com baixa propagação no TC. Para o MEV, os SAs convencionais apresentaram grande profundidade de penetração dos adesivos na dentina do que os sistemas autocondicionantes, especialmente quando irradiados com laser. Para RA, foram observados os menores valores para o L com exceção do SUL. Na análise de fratura observou que o modo de falha mais predominante para

todos os grupos foi adesivo e misto. Concluiu-se que a irradiação com o laser Er, Cr: YSGG com os parâmetros do utilizados no presente estudo não apresentou benefícios clínicos, visto que prejudicou a maioria das propriedades testadas.

Palavras-chave: Lasers. Dentina. Adesivos Dentinários.

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LIST OF ACRONYMS

DBS	Dentin bonding systems
HEMA	2- Hydroxyethyl methacrylate
Nd:YLF	Neodymium-doped yttrium lithium fluoride
SDBSs	Simplified dentin bonding systems
Er, Cr: YSSG	Erbium, chromium: yttrium, scandium, gallium, garnet laser
MP	Scotch Bond Multipurpose
SB	Single Bond 2
CSE	Clear fill SE Bond
SU	Single Bond Universal
SEM	Scanning Electronic Microscopy
BisGMA	Bisphenol A Glycidyl Methacrylate
MDP	Methacryloyloxydecyl dihydrogen phosphate
TS	Superface Temperature
TPC	Pulpar Chamber Temperature
LED	Light emitting diode
HCL	Hydrochloric acid
ANOVA	Analysis of variance
CNPQ	National Council for Scientific and Technological Development
FAPESP	São Paulo Research Foundation
IPEN/CNEN-SP	Nuclear and Energy Research Institute of Sao Paulo
DC	Degree of conversion
MS	Microtensile Strength
FA	Failure Analysis
MPA	MegaPascal
ISO	International Organization of Standardization
mJ	MiliJoules
Hz	Hertz
W	Watts
J/cm ²	Joules / square centimeter

LIST OF SIMBOLS

Δ	Variation
$\pm$	Standard deviation
$\cong$	Approximately
$^{\circ}\text{C}$	Celsius degree
$^{\circ}\text{F}$	Fahrenheit degree
$\mu\text{g}/\text{mm}^3$	Microgram per cubic milimeter
μm	Micrometers

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1 INTRODUCTION

1 INTRODUCTION

Besides the search for minimally invasive dentistry in the treatment of dental caries, this philosophy has been used in aesthetic restorations. This is possible due to restorative materials evolution being one of the main: dentin bonding systems (DBS). Current DBS allows clinicians to do restorations without the need of a retentive cavity and because of this, DBS has been considered a revolutionary material for dentistry since they provide immediate bond strength (SUTIL AND SUSIN 2017).

Three different categories of DBS can presently be employed in resin bonding procedures: the etch-and-rinse, the self-etch and the more recently, the universal. Etch-and-rinse adhesives use phosphoric acid and primers containing hydrophilic monomers and solvents to displace the unbounded water and prepare the collagen scaffold for infiltration of the hydrophobic resin (PASHLEY et al 1989; SUTIL AND SUSIN 2017, BRESCHI et al., 2018). Self-etch adhesives use acidic primers containing hydrophilic monomers and solvents and, in another bottle, hydrophobic resin. They aim to preserve the smear layer by incorporating it into the adhesive layer (WATANABE et al., 1990; SUTIL AND SUSIN 2017, BRESCHI et al., 2018). New universal or multi-mode adhesives use acidic primers containing hydrophilic monomers, solvent, hydrophobic resin and other components in a single-step and it can be used in etch-and-rinse or self-etch protocol (LUQUE-MARTINEZ et al., 2014; SUTIL AND SUSIN 2017, BRESCHI et al., 2018).

Many studies report that bonding at enamel is well established (ABUNA et al., 2016; BRESCHI et al., 2018), but in dentin there are still many problems that may compromise the bonding in short and long term (SPENCER et al., 2010; TJADERHANE et al., 2015; SUTIL AND SUSIN 2017, BRESCHI et al., 2018). Etch-and-rinse adhesives in dentin are more sensitive. This material requires a subtly moistened dentin after acid etching, being susceptible to operator error that can affect the condition of the substrate, leading to the collapse of the collagen network. Thus, self-etch adhesives appeared in an attempt to minimize this sensitivity and to reduce the clinical time (WATANABE et al., 1990; WANG AND SPENCER et al., 2005). Universal or multi-mode dental adhesives were developed mainly with regard to dentin,

aiming to simplify the clinical steps, to reduce the sensitivity of clinical techniques and to increase the bonding and long-term clinical success (SUTIL; SUSIN 2017).

However, regardless the advances in dental materials, the hybrid layer created on the variable and dynamic organic dentin phase is not perfect, and may fail over time, inducing marginal discolorations, marginal leakage and subsequent loss of retention of the composite restoration. The success of dentin bonding depends mainly of the qualitative permeation of DBS into the substrate and also the physical-mechanical properties/quality of the polymer (BRESCHI et al., 2018).

The minimally invasive dentistry concept aims to limit the cavity preparation to caries removal, although this limit is still unclear. The immediate bond strengths to caries-affected dentin are commonly 20-50% lower than to sound dentin, and even lower with caries-infected dentin (CARVALHO et al., 2012, PERDIGÃO et al., 2010). Lower mineral content allows phosphoric acid or acidic monomers to demineralize the matrix more deeply than in normal dentin, resulting in even more residual water in exposed collagen matrix (ITO et al., 2005). The hybrid layer tends to be much thicker and poorly infiltrated by resin, regardless of the bonding system used (PERDIGÃO et al., 2010). These aspects should be considered because affects directly the strength and durability of the dentin-resin interface.

Another scenario that may interfere with dentin adhesion is the presence of solvents and water into the DBS itself, that should ideally be removed before curing because it can reduce the monomer conversion and negatively affect the mechanical properties of the adhesive (MJOR et al., 2002). By other side, water is needed to maintain dentin collagen scaffold expansion for the resin monomer infiltration, however, moisture excess may cause a phase separation between hydrophobic and hydrophilic monomers, resulting in irregular resin infiltration with blisters and voids at the interface. The consequences of the poor resin infiltration and conversion are the reduced durability of the interface, increased enzymatic degradation of the exposed collagen, and the hydrolysis of the poorly polymerized adhesive (TOLEDANO et al., 2001, TJADERHANE et al., 2015; BRESCHI et al., 2018).

Dentin wet bonding requires primers or primer/adhesives that contain hydrophilic and ionic monomers to ensure proper hybridization of the exposed collagen

matrix. 2-Hydroxyethyl methacrylate (HEMA) has many advantages that make it the most commonly used hydrophilic adhesive monomer: it is a small monomer, well solvable in water, ethanol and/or acetone, it acts as a solvent for hydrophobic monomers and is relatively biocompatible in polymerized form. An extensive review listed 62 commercially available adhesives, out of which 48 (77%) contained HEMA. Hydrophilicity, however, is a two-edged sword, as for example, HEMA absorbs water both in the cured and uncured states, which may inhibit polymerization, reduce mechanical properties and lead to the hydrolytic degradation of polymerized adhesive (VAN LANDUYT et al., 2007; TJADERHANE et al., 2015).

In attempts to reduce the hydrolytic degradation of adhesive, less hydrophilic HEMA-free adhesives have been created, however, the solvation effect of HEMA is also lost. When other solvents, such as acetone or ethanol, evaporate, water tends to separate adhesive components, making these adhesives prone to phase separation (VAN LANDUYT et al., 2007). The resulting water blisters may lower the immediate bond strength. The studies examining the durability of bond strength with HEMA-free adhesives are limited and the results are conflicting, but generally, loss of bond strength seems to occur. Extended evaporation (air drying) of the adhesive layer may reduce the blister formation (VAN MEERBEEK et al., 2005).

Another attempt to improve the performance of DBS evolves the laser technology (light amplification by stimulated emission of radiation). In Operative dentistry, laser is researched to caries prevention (GERALDO-MARTINS et al., 2014) to remove both sound and decayed tooth tissues, dentin sensivity treatment, conditioning of dental substrate - replacing phosphoric acid, on DBS previous cured - to improve the bonding and sometimes to cure composite resin (KINOSHITA et al., 2003; HOSSAIN et al., 1999).

The first attempts to use laser at operative dentistry presented unsatisfactory results because it was used to prepare the cavity (AMARAL et al., 2008) or even as conditioning of enamel and dentin - replacing phosphoric acid previous DBS (ODA et al. 2001) which caused a decrease in values of bond strength (ODA et al., 2001; KINOSHITA et al., 2003; AMARAL et al.2008).

The technique reported by Gonçalves et al. (1999) demonstrated significant results in dentin bond strength values when Nd:YLF laser was irradiated over the “bond” of etch-and-rinse system applied in dentin, but prior to curing. These authors attributed this performance to the creation of a new substrate composed of recrystallized hydroxyapatite after being melted in the presence of resin monomers, resulting in a substrate that is physically more resistant (GONÇALVES et al., 1999). Other studies investigated this same technique using Nd:YAG laser and the results was promising in dentin due to the higher values of bond strength (FRANKE et al., 2006; MARIMOTO et al., 2013).

In the attempt to find an alternative of a laser with better cost benefit, Maenoso et al. (2015) investigated the effect of diode laser using the same technique and obtained similar results for simplified DBS (MAENOSONO et al., 2015). In addition to the role of the laser’s interaction with dentin, the authors also emphasized the evaporation of solvents as an advantage of laser use, reducing the bond’s susceptibility to degradation over time (MAENOSONO et al., 2015). The hypothesis for such results was that the heat assisted in the evaporation of the solvents, whose the effect was more significant in the simplified adhesives, which present alcohol in the composition. Brianezzi et al. (2017) using the same laser and parameter that MAENOSONO et al. (2015) observed a significant increase in the degree of conversion of all tested simplified dentin bonding systems, with no impact on water sorption and solubility. The authors related that heating caused by the laser could have aided in the movement of the molecules, which would justify the increase of the degree of conversion in this study and improvement of the microtensile strength of the other studies (BRIANEZZI et al., 2017).

The other laser that could exacerbate these previously mentioned effects is the Er, Cr: YSSG laser because it presents the maximum absorption peak (2780nm) coincident with hydroxyapatite and water absorption peak (HOKE et al., 1990; SEKA et al., 1996; VISURI et al., 1996), and this interaction could aid in the evaporation of water that is present in dentin/DBS. Er, Cr: YSSG laser was widely used for the preparation and conditioning of cavities with unfavorable results, however, the irradiation with this laser on DBS applied in dentin before curing has not been investigated yet.

Water is needed to maintain dentin collagen scaffold expansion for the resin monomer infiltration, however, excess moisture may cause a phase separation between hydrophobic and hydrophilic monomers, resulting in irregular resin infiltration with blisters and voids at the interface (TOLEDANO et al., 2001; TJADERHANE et al., 2015; BRESCHI et al., 2018). Thus, the water should ideally be removed before polymerization because it can reduce the monomer conversion and negatively affect the mechanical properties of the adhesive (MJOR et al., 2002). The hypothesis is that the Er, Cr:YSGG laser, due to high interaction with water, could better aid in the removal of residual water from the DBSs and substrate more efficiently than the other types of laser. These strategies to reduce the DBS susceptibility to hydrolytic degradation are desirable, as most of their failure is attributed to this limitation (TJADERHANE et al., 2015).

Thus, the aim of the present thesis was to evaluate the effect of irradiation with Er, Cr: YSGG laser on different DBS applied on human dentin before polymerization, over some important physical properties: water sorption/solubility on dentin, degree of conversion and microtensile strength.

The present study also sought to evaluate the elevation of temperature during irradiation (DBS and pulp chamber) and the morphological aspects of hybrid layer resulting of this irradiation. The results were described in 1 of the articles.

2 ARTICLES

2 ARTICLES

2.1 ARTICLE

The article presented in this thesis was written according to the Dental Materials instructions and guidelines for article submission.

Infrared thermographic and morphological analysis of dentin bonding systems irradiated with Er,Cr: YSSG laser.

Abstract

Objective: The aim of this study was to evaluate the temperature increasing and propagation during irradiation of dentin bonding systems (DBS) already applied on dentin substrate with Er,Cr:YSSG laser and the morphology of hybrid layer after irradiation procedure.

Methods: For thermography analysis, sixteen teeth were prepared and randomly divided into four groups according to the DBS factor: 3-step total-etch Adper™Scotchbond™ Multi-Purpose [MP], 2-step total-etch Adper™ SingleBond 2 [SB], 2-step self-etch Clearfil™ SE Bond [CSE], 1-step self-etch Adper™ Single Bond Universal [SU] irradiated with Er,Cr:YSSG laser (2780 nm). For scanning electron microscopy (SEM) analysis, the laser factor were added: irradiated [L] or not with Er,Cr:YSSG laser [C], and twenty-four teeth were divided into eight groups (n=3). A thermographic camera was used to perform the in vitro experiment, in which the laser was used to irradiate the teeth/DBS samples and the infrared images were captured instantly and analyzed by specific software. Two standardized points were chosen (surface and pulp chamber) on the infrared images to register the maximum temperature reached. In addition, a qualitative analysis of the hybrid layer through SEM images analysis was performed. Data from thermography was analyzed by one-way ANOVA and Tukey tests ($p<0.05$).

Results: In all DBSs, Er,Cr:YSSG laser promoted a high superficial temperature increase (dentin/DBS) with significant difference among the groups, but the propagation was low, with no difference between the adhesives on the ceiling of the pulp chamber. For SEM, total-etch DBSs had greater penetration depth of resin tags into dentin than self-etch systems, especially when irradiated with laser.

Significance: Er,Cr:YSSG laser can be considered a tool for heating adhesive systems in a localized way, with the advantage of the low propagation of heat towards the pulp.

Key words: Laser. Dentin-Bonding Agents. Thermography.

1. Introduction

The bond in enamel substrate is very stable, however, bonding in dentin has been the target of many studies due to the complex characteristics of this substrate. Due to the presence of water, the process of hydrolysis of the adhesive polymeric matrix can compromise bonding in dentin in short and long-term. [1]

The hydrolysis of the adhesive happens because the dentin bonding system (DBS) are composed of solvents, such as ethanol, water and acetone, to help the penetration of the hydrophilic resinous monomers into collagen fibrils, besides the water that may be present in the dentin substrate itself or in the composition of self-etching DBS to allow ionization of the monomers. [2,3]

These solvents and unbound water should evaporate from the substrate prior to the adhesive polymerization, [3,4] however, this evaporation is not complete because the monomer/water ratio increases as the process progresses, decreasing the water vapor pressure and, consequently, decreasing the capacity of the solvents and water to evaporate totally.[5] The unbound water will prevent the suitable polymerization of the DBS, leaving open channels for the monomers to solubilize and initiate the hydrolytic degradation.[3,6]

Some methods have been studied to assist the removal of this unwanted residual water prior to curing to improve bonding: preheating the DBS, [13] heating the DBS with heated air [14], increasing the evaporation time and also laser as a form of heating. [28] Some authors have found promising results when applying different types of lasers on DBSs. [7,8,9,19] Gonçalves et al. [7] attributed the performance of laser due the creation of a new substrate composed of recrystallized hydroxyapatite after being melted in the presence of resin monomers. Some studies [10,12,13,14] have reported that the increase of temperature aid in the evaporation of the solvent on DBS as well as improvement of the mechanical properties. Brianezzi et al. [11] related that temperature increase promoted by the laser irradiation would increase the degree of conversion and improve the microtensile bond strength related by other studies.

According to the action mechanism of the laser, the increase in the temperature promoted by the irradiation depends mainly of wavelength and interaction with substrate/material. Despite studies have been reported the use of different types of laser to improve adhesion process, Neodymium laser (1064nm) is most commonly used.[15,16] However, Er,Cr:YSSG laser that presents wavelength of 2780nm has not

been investigated in this association of irradiation over uncured DBS applied on the dentin, but only for cavity preparation [17] or surface treatment prior to the DBS application [18] with unsatisfactory results. Due the proximity of Er,Cr:YSSG laser wavelength with the absorption wavelength peak of hydroxyapatite and water,[19,20,21] a high interaction with substrate and water occurs with a consequently great temperature increase. This could better aid in the evaporation of unbound water and solvent that is present on dentin and DBS.

Although the increase of the temperature is desired due to the benefits reported by the different studies, the concern with effects of thermal increase with the pulp must exist. Thus, the objective of this in vitro study was to evaluate the temperature increasing promoted by Er,Cr:YSSG laser irradiation on uncured DBS applied over human dentin and the heat propagation towards pulp chamber (infrared thermographic (IT)); and a comparative analysis of the resulting hybrid layer morphology after the irradiation (SEM).

2. Materials and methods

This study was previously approved by the Ethics Committee of Research on Human Beings at Bauru School of Dentistry, University of São Paulo (n. 49813315.5.0000.5417) (Attachment 1).

2.1. Experimental Design

The Infrared Thermography involved one factor: dentin bonding system (DBS) in four levels: Adper™Scotchbond™ Multi-Purpose [MP] (3M ESPE, St Paul, MN, EUA); Adper™ SingleBond 2 [SB] (3M ESPE, St Paul, MN, EUA); Clearfil™ SE Bond [CSE] (Kuraray Co. Ltda., Osaka, Japan) and Adper™ Single Bond Universal [SU] (3M ESPE, St Paul, MN, EUA). The quantitative response variables were ΔTS (superficial variation) and ΔTPC (pulp chamber roof variation) (°C). The complementary morphological analysis (SEM) were performed with and without irradiation with erbium, chromium-doped yttrium, scandium, gallium and garnet (Er,Cr:YSSG -2790 nm) laser (Biolase water lplus, Irvine, CA, USA).

The null hypotheses were: 1- there was no difference between the DBSs superficial temperature increasing; 2- there was no difference between pulp chamber temperature according DBSs groups; (3) there was no difference in the morphology of hybrid layer considering different DBSs and laser Irradiation.

The classification, composition and application technique of materials are described in table 1.

Table 1 - Commercially available dentin bonding system used in this study, their classification, composition and application technique according manufactures.

Dentin Bonding System	Classification	Composition	Application technique
Adper™ Scotchbond™ Multipurpose	Three-step etch-and-rinse system	Primer: HEMA**, water, copolymer of polyalcenoic acid. Bond: HEMA**, Big-GMA*** and camphorquinone	<ol style="list-style-type: none"> 1. Etch dentin surface with phosphoric acid 37% for 15s 2. Rinse for 15s 3. Gently dry with absorbent paper 4. Apply primer with a microbrush 5. Gently air-dry for 5s 6. Apply adhesive with a microbrush and remove excess
Adper™ Single Bond 2	Two-step Etch-and-rinse system	HEMA**; Bis-GMA***, ethanol; silane treated silica filler; glycerol 1,3 dimethacrylates; diuretanedimethacrylate and	<ol style="list-style-type: none"> 1. Etch dentin surface with phosphoric acid 37% for 15s 2. Rinse for 15s 3. Gently dry with absorbent paper

		copolymer of polyacrylic and polyitaconic acids.	<ol style="list-style-type: none"> 4. Apply adhesive with a microbrush and remove excess 5. Gently air-dry for 5s
Clearfil SE™ Bond	Two-step self-etch system	<p>Primer: MDP*; HEMA**; hydrophilic aliphatic dimethacrylate; dl-Camphorquinone; N,N-Diethanol-p-toluidine; Water.</p> <p>Bond: MDP*; HEMA**; Bis-GMA***; hydrophobic aliphatic dimethacrylate; dl-Camphorquinone; N,N-Diethanol-p-toluidine; colloidal silica.</p>	<ol style="list-style-type: none"> 1. Apply primer with a microbrush for 20s 2. Gently dry-air for 5s 3. Apply bond with a microbrush and remove excess
Adper™ Scotchbond™ Universal	Universal system (used as one-step self-etch system with wet-technique)	MDP*; HEMA**; Bis-GMA***; silica treated silane; ethanol; decamethylenedimethacrylate; water; 1,10-decanediol dimethacrylate; copolymer of polyacrylic and polyitaconic acids; Camphorquinone; N,N-dimethylbenzocaine; methacrylate 2-dimethylmonoethyl; methyl ethyl ketone.	<ol style="list-style-type: none"> 1. Apply bond actively for 20s 2. Gently air-dry for 5s

*MDP – 10-Methacryloyloxydecyl dihydrogen phosphate

**HEMA - 2-hydroxyethyl methacrylate

***BISGMA - bisphenol-A glycidyl methacrylate

2.2. Infrared Thermography (IT)

2.2.1. Sample preparation

For analysis of the surface temperature variation ΔTS (ΔT = Maximum temperature – initial temperature) and the pulp chamber temperature variation ΔTPC , 16 human third-molars were selected. All teeth were sectioned transversely in occlusal third, and after in longitudinal mesio-distal axis (Figure 1). Eight halves were used for each DBS group (n=8). The distance between the pulp chamber and occlusal surface were standardized in 3mm, through additional occlusal surface wear (Arotec, Cotia, São Paulo, Brazil).

After the samples sectioning and cleaning in ultrasound device, all samples were kept in a humid environment under refrigeration until analysis.

The thermographic camera (ThermaCam FLIR SC3000 Systems, Boston, USA) was positioned perpendicular to the longitudinal axis of the sample (Figure 1), with the pulp chamber facing the camera. The DBS were applied according to the manufacturer's instructions on the occlusal surface and prior to their photopolymerization they were irradiated with laser. For MP and CSE the laser irradiation was performed over the primer component.

The laser tip was positioned perpendicularly at 3 mm from occlusal surface of the sample for automatic zigzag scanning/irradiation of the entire surface (BioPDI, XY Table, São Carlos, SP, Brazil) (Figure 1). The irradiation parameters are described in table 2. The irradiation initiation and the continuous recording of the temperature during surface scanning were synchronized, but the thermal measurement ended 15s after the end of the laser irradiation, aiming to obtain a characteristic curve of the temperature decline as a function of time.

Table 2 - Parameters used for irradiation

Parameter	Value
Energy per pulse (output)	6.25 mJ
Frequency	10 Hz
Power	0.0625 W
Time	30s
Area	36mm ²
Energy density	5.20 J/cm ²
Tip diameter	800 µm

Figure 1. A- Schematic drawing of the tooth sectioning with occlusal surface 3mm from pulp chamber; B- Position of the sample with the pulp chamber facing the thermographic camera and the laser tip positioned 3mm from occlusal surface ready for irradiation.

2.2.2. Thermographic Analysis

This analysis was performed using a thermographic camera at an initial temperature that was set at $T_{\text{initial}}=20^{\circ}\text{C}$. The thermal camera captures images in the infrared spectrum and generates different shades of gray or colors like the images of a continuous film. These images demonstrated heat dissipation and temperature

variation (ΔT) inside dental tissues (Figure 2). This camera is equipped with a detector of the type Quantum Well Infrared Photodetector refrigerated to 70K, able to register temperatures from -20°C to 2000°C , with variation of $\pm 1^{\circ}\text{C}$.

In each second a temperature was recorded and the one used was the maximum temperature reached. For the quantitative analysis, two standardized points were selected: one located on the occlusal flattened surface, 3 mm below the laser tip (Spot1: $\Delta T_{\text{S-occlusal}} = \text{maximum-initial temperature}$), which was considered for determining the surface temperature of the DBS/hybrid layer, and the second point 3mm below the first point corresponding to the roof of the pulp chamber (Spot2: $\Delta T_{\text{PC}} = \text{maximum-initial temperature}$).

Figure 2. Sequence of images obtained from thermographic camera showing heat dissipation during laser irradiation. Different colors represent different temperatures, the exact maximum temperature value was determined by computer software

2.3. *Scanning Electron Microscopy (SEM)*

2.3.1. Sample Preparation

For SEM analysis, 24 teeth divided in 8 groups according the DBS and laser irradiation factors (n= 3) were used. The teeth had the roots sectioned, and the crowns had the occlusal surface flattened with #320 and #600 grit. The energy density was standardized for irradiation. After the application of DBS and irradiation of the laser, the DBSs were light cured and the composite resin (Filtek™ Z250 (3M ESPE, St Paul, USA)) was inserted by incremental technique. The restored teeth were immersed in deionized water and kept in an oven at 37 °C.

After the storage period, the specimens were sectioned in longitudinal slices (1.0 mm ± 0.1mm) with a serial cut machine (Isomet™ Low Speed Saw®, Buehler, Lake Bluff, USA) and diamond blade (Exttec Dia. Wafer blade 5 "x.015x1 / 2, Exttec Corp-Einfeld, USA) at low speed and under water cooling. One slice of each tooth was randomly selected for morphological analysis.

The selected slices were stored in deionized water and then immersed in 18% hydrochloric acid solution (HCl 5n) for 30s, to remove the superficial smear layer, washed for 30s with deionized water, followed by immersion in 5% sodium hypochlorite for 15 minutes to remove all collagen not infiltrated by DBS and subsequent washing for 30 seconds. The samples remained 12 hours drying at room temperature and then mounted on aluminum stubs and metallized with gold-palladium (Dentron Vacuum - Desk IV Moorestown / NJ - USA) prior to SEM observation (JSM-T220A, JOEL, Inc, Tokyo, Japan).

In the SEM, the representative specimen of each group was qualitatively analyzed in the dentin of the adhesive interface with a standardized magnification of 1000x and 1500x.

3. Statistical Analysis

Data of temperature variation was calculated and analyzed statistically with Statistica software (Statsoft®, Tulsa, OK, USA). As the assumptions of normal distribution were satisfied, data was subjected to one-way ANOVA ($p < 0.05$) followed by Tukey's test ($p < 0.05$).

4. Results

4.1. Infrared Thermography

Infrared thermography had two response variable: ΔTS and ΔTPC . It was observed a significant difference only for the ΔTS (Table 3). It noted that MP presented higher values of ΔT followed by SB ($p = 0.0004$) > CSE ($p = 0.0003$) = SU ($p = 0.011$).

Table 3. Mean and standard deviation of ΔTS (°C)

DBS	ΔTS (°C)
MP	$68,86 \pm 15,46$ a
SB	$57,8 \pm 4,05$ b
CSE	$34,66 \pm 14,06$ c
SU	$42,07 \pm 5,73$ c

* Different lowercase letters show differences between rows

For ΔTPC , all groups were similar $MP=SU=SB=CSE$. (Table 4).

Table 4. Mean and standard deviation of Δ TPC ($^{\circ}\text{C}$)

DBS	Δ TPC
MP	4.27 ± 1.0 a
SB	3.7 ± 1.5 a
CSE	3.6 ± 0.64 a
SU	4.0 ± 0.52 a

* Different lowercase letters show differences between rows

4.2. Scanning Electron Microscopy (SEM)

It was not observed morphological differences between MP and MPL group, although the irradiation was performed after primer (Figure 3). For SBL, it was possible to observe longer tags than SB (Figure 4). For CSEL, it was observed a quantitative

increase of tags regarding CSE, however with shorter tags characteristic of self-etch DBS (Figure 5). For SU no morphological difference could be evidenced by the laser irradiation (Figure 6).

Figure 3. SEM images of adhesive-dentin interface with Adper™ Scotchbond™ Multi-Purpose: A- MP without laser irradiation; B- MPL with laser irradiation. B-It was observed the total etch pattern with longer tags in greater quantity. It was not observed difference between MP and MPL.

Figure 4. SEM images of adhesive-dentin interface with Adper™ SingleBond 2. A-It was also observed a pattern for total-etch DBS. B-Furthermore, it is possible to observe longer tags promoted by laser irradiation(SBL) than control (SB).

Figure 5. SEM images of adhesive-dentin interface with Clearfil™ SE Bond.A- I was observed a pattern of self-etch DBS with shorter tags and smaller quantity than total-etch DBS.B- In addition, it was observed a quantitative increase and thicker tags for SEL.

Figure 6. SEM images of adhesive-dentin interface with Adper™ Single Bond Universal. A-It was observed short tags and in smaller number than CSE.B- For this group no morphological difference could be evidenced by the laser irradiation

5. Discussion

Heating of DBS can improve some properties such as monomer solution, viscosity and degree of conversion.[22] Some studies showed that laser, which is also a suggested method for heating, can also promote increase of microtensile bond strength.[7,8,9,10] According Brianezzi et al. [11], the increase of temperature by laser irradiation could have aided in the movement of the molecules, which would justify the increase of the degree of conversion of DBS, however, it is unknown at what temperature the DBS/dentin reach during the irradiation process.

All DBSs had an increase in superficial temperature, being that MP adhesive had reached the higher temperature followed SB>SE= SU, thus first null hypothesis was denied. About ΔTS , it is important to consider two factors: DBS and dentin substrate. For the first, the literature demonstrated that temperature of DBS around 54 - 60°C enhanced maximal polymerization rate. [22] Although this parameter was not evaluated in the present study, it was observed that the values within this range were achieved mainly for self-etch DBS considering at room temperature ($\cong 60^\circ\text{C}$). Besides, it is important to analyze if the higher temperature increase achieved by the other groups could affect negatively adhesive components or the dentin substrate.

Another important aspect is interaction of laser with the DBS composition. The higher values of temperature achieved by MP and SB, when compared with CSE and SU, could be justified due to the greater presence of residual water in the conditioned substrate with phosphoric acid that requires the wet technique to avoid the collapse of collagen fibrils, as the Er, Cr: YSGG laser shows affinity with water.

SU and SE presented lower temperature increase. Besides both have water in the composition, they are applied over dry substrate, and therefore the total water quantity was lower than MP and SB. Probably the remaining water in the substrate is more significant than intrinsic DBS water and meanwhile they heated less.

For the second factor, Schaller et al. [24] demonstrated that heating up the dentin to about 86°C (360 K) affects only residual water present in the pores of dentin substrate without altering the molecular structure of collagen. Furthermore, values up to about 146°C (420K) leads degradation of collagen phase. In the present study, the higher temperature was achieved by MP (around $\cong 100^\circ\text{C}$) considering the ambient temperature, thus the laser irradiation with these parameters probably did not reach the degradation of collagen phase.

Although the heating of the DBS improves some properties, for clinical aspect there is a great concern with the propagation of heat to the pulp organ. For pulp chamber ceiling, all groups also presented an increase in temperature due to the propagation of heat generated by laser irradiation in the occlusal surface, but there was no significant difference between all groups, thus the second null hypothesis was accepted. Zach and Cohen [25] reported pulp irreversibility in 15% of the teeth of rhesus monkeys with a temperature elevation of 5.6°C, 60% for an elevation of 11°C, and 100% for a temperature elevation of 16.6°C, showing a potential histopathological alteration in the pulp tissue when the temperature exceeds 5.6°C. Regardless of the high temperatures reached by the DBS irradiated by this type of laser, the heat propagation was low and within the limits established for clinical use without pulp involvement.

It is noted that the highest value of ΔT occurred in occlusal surface for all groups, but this difference did not occur in the pulp chamber ceiling. The hypothesis is that as Er,Cr:YSSG laser exhibits affinity with hydroxyapatite and water, during irradiation occur an absorption of the laser energy by the hydroxyl ion from hydroxyapatite and by the water constituting the surface.[20] As this energy is absorbed, mainly in the surface, less energy that propagates in the substrate, and therefore the difference found in the surface was decreasing toward the pulp. This interaction is important because promotes greater superficial heating and it promotes less heat propagation into the dental structure and periodontium. [26] This high interaction with the substrate allowed the use of extremely low power (0.0625 W), compared to other studies with other types of laser.[7,8,9,10]

Regarding the morphological characteristics of the hybrid layer, the results of the present study are in accordance with the literature. It was observed a significant difference between total-etch and self-etch DBS when the qualitative/quantitative of resin tags was compared. Both conventional DBS presented larger and more numerous resin tags when compared with self-etch ones. The self-etch DBS presented shorter and fewer resin tags, and the 1step self-etch presented almost total absence of tags. [27,28]

The increase of temperature is important to promote the evaporation of solvents and reduce the viscosity of the DBS [28]. For MP and SB DBS, whose temperature increase was significantly higher, probably a higher thermal effect in the SB occurred because the evaporation of the water that it may have been optimized due to the presence of ethanol in the DBS. This higher evaporation of the water in the SB may have been responsible for the greater infiltration of this DBS in the substrate (longer tags), despite the lower ΔT s. In the self-etching DBS, these differences were not evident due to the characteristics such as: lower formation of tags due to the absence of previous acid conditioning.

6.Conclusion

According to the findings in this study, the Er,Cr:YSSG laser irradiation is effective to heat the different DBS, with low heat propagation within dentin structure, regardless of DBS used. The temperature increasing promoted by this laser is also effective to promoted morphological changes in the hybrid layer in some DBS. The effect of the heat on physical properties promoted by this laser in some DBS needs further investigation.

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2.2 ARTICLE

The article presented in this thesis was written according to the Dental Materials instructions and guidelines for article submission.

Effect of Er, Cr: YSGG laser irradiation in the physics properties of different dentin bonding systems

Abstract

Objective: The aim of this study was to evaluate the effect of Er, Cr: YSGG laser irradiation in the physics properties of the different dentin bonding systems (DBS) applied in dentin substrate.

Methods: this study presented two variation factors: laser, divided into two levels (Er, Cr: YSGG [L] and Control - not irritated [C]) and DBS divided into four levels (Adper™ Scotchbond Multipurpose [MP], Adper™ Single Bond 2 [SB], Clearfil™ SE Bond [CSE], Scotchbond™ Universal Adhesive [SU]). The response variables were: water sorption/solubility ($\mu\text{g}/\text{mm}^3$) [WS/WSB], degree of conversion (%) [DC], microtensile bond strength (MPa) [MS]. After application of DBS over dentin substrate and before curing, in the L groups the irradiation was performed over the primer on MP and CSE and after primer/adhesive on SB and SU. After 7 days, the specimens were cut to obtain squared-shape blocks for WS/WSB, slices for one-year DC and sticks for one-year MS. The data was analyzed by two-way ANOVA, followed by Tukey test for multiple comparisons ($p < 0.05$).

Results: All irradiated groups presented lower WS/WSB than respective control groups. For DC, it was noted a significant difference only for DBS factor. Laser irradiation promoted a significant drop in MS values, except for SU-L group.

Significance: The use of the Er, Cr: YSGG laser was not able to improve the properties analyzed, including impairing the properties of most DBS.

Keywords: Dentin Bonding Agents. Lasers. Dentin

1.Introduction

The heterogeneity of dentin limited the adhesive bonding procedures; the same water required to keep the collagen network open for resin penetration [1,2] limited the degree of conversion. Furthermore, this water promoted hydrolytic degradation of collagen matrix and dentin bonding system (DBS), compromising the durability of the bond strength. [3,4,5] Biodegradation of resin dentin interfaces also increases bacterial microleakage [6] leading to secondary caries.

To improve the performance of the different DBS, some methods are being investigated to try to eliminate this water/solvent present in the hybrid layer after the application of the DBS, such as heating the DBS with heated air, [12] increasing the evaporation time and also different types of laser as a form of heating.[11] Some studies [12,13,14] have reported that the increase of temperature aid in the evaporation of the solvent of DBS and improve the bonding strength.

Lasers have been used to improve the bond strength of DBS, specially simplificated DBS. The method described by Gonçalves et al. [7] have been showed promising results for diode and Nd: YAG laser. In these methods the DBS is applied on dentin and irradiation occur previously polymerization. [7,8,9,10] Some studies have shown that the laser could recrystallize the hydroxyapatite, [7] help evaporation of the solvent due to heating and improve the microtensile bond strength using this method. [7,8,9] Other studies related that this solvent evaporation by laser can promote increase in degree of conversion that could justify many favorable results from reports. [8, 11, 22]

As the heat promoted by laser depends of the interaction between laser wavelength and substrate, among other factors, Erbium, Chromium doped Yttrium Scandium Gallium Garnet (Er, Cr: YSGG) laser could be the best assist to remove this water/solvent off the collagen network after penetration of DBS due the greater interaction with water. Its wavelength of the 2780nm is very close to the absorption peak of water and hydroxyapatite thus promoting a higher temperature increase that could better aid in the evaporation process of water/solvent from hybrid layer.

Thus, the objective of this in vitro study was to evaluate the effect of irradiation with Er, Cr: YSGG laser over DBS in the physics properties: water sorption (WS) and water solubility (WSB), degree of conversion (DC) and microtensile bond strength (MS).

2. Materials and method

This study was previously approved by the Ethics Committee of Research on Human Beings at Bauru School of Dentistry, University of São Paulo (n. 49813315.5.0000.5417) (Attachment 1).

2.1. *Experimental Design*

This study involved two factors: treatment in two levels (irradiated[L] or not irradiated [C] with Er, Cr: YSGG laser- WaterLase IPlus, Biolase, Irvine, CA, USA) and dentin bonding system in four levels (Adper™Scotchbond™ Multi-Purpose [MP] - 3M ESPE, St Paul, MN, EUA, Adper™ SingleBond 2 [SB] -3M ESPE, St Paul, MN, EUA, Clearfil™ SE Bond [CSE] - Kuraray Co. Ltda., Osaka, Japan and Adper™ Single Bond Universal [SU] -3M ESPE, St Paul, MN, EUA). The quantitative response variables were: water sorption/solubility (WS/WSB) ($\mu\text{g}/\text{mm}^3$), degree of conversion (DC) (%) and microtensile bond strength (MS) (MPa).

The null hypotheses were: 1- there was no difference between the DBSs irradiated or not with laser in the water sorption/ water solubility properties, 2- there was no difference between the DBSs irradiated or not with laser in the degree of conversion, 3- there was no difference between the DBSs irradiated or not with laser in microtensile bond strength values.

Composition of materials used are described in the table 1.

Table 1 - Commercially available systems, their classification, compositions and application technique according manufacturer.

Dentin Bonding	Classification	Composition	Application technique
Adper™ Scotchbond Multipurpose (MP)	Three-step etch-and-rinse system	Primer: HEMA**, water, copolymer of polyacenoic acid. Bond: HEMA**, Big-GMA*** and camphorquinone	Etch dentin surface with phosphoric acid 37% for 15s Rinse for 15s Gently dry with absorbent paper Apply primer with a microbrush Gently air-dry for 5s Apply adhesive with a microbrush and remove excess
Adper™ Single Bond 2 (SB)	Two-step Etch-and-rinse system	HEMA**; Bis-GMA***, ethanol; silane treated silica filler; glycerol 1,3 dimethacrylates; diuretanedimethacrylate and copolymer of polyacrylic and polyitaconic acids.	Etch dentin surface with phosphoric acid 37% for 15s Rinse for 15s Gently dry with absorbent paper Apply adhesive with a microbrush and remove excess Gently air-dry for 5s
Clearfil SE™ Bond (CSE)	Two-step self-etch system	Primer: MDP*; HEMA**; hydrophilic aliphatic dimethacrylate; dl-Camphorquinone; N,N-Diethanol-p-toluidine; Water. Bond: MDP*; HEMA**; Big-GMA***; hydrophobic aliphatic dimethacrylate; dl-Camphorquinone; N,N-Diethanol-p-toluidine; colloidal silica.	Apply primer with a microbrush for 20s Gently dry-air for 5s Apply bond with a microbrush and remove excess
Scotchbond™ Universal (SU)	Universal system (used as one-step self-etch system with wet-technique)	MDP*; HEMA**; Bis-GMA***; silica treated silane; ethanol; decamethylenedimethacrylate; water; 1,10-decanediol dimethacrylate; copolymer of polyacrylic and polyitaconic acids; Camphorquinone; N,N-dimethylbenzocaine; methacrylate 2-dimethylmonoethyl; methyl ethyl ketone.	Apply bond actively for 20s Gently air-dry for 5s

2.2. Water sorption and water solubility

2.2.1. Sample preparation

This test was performance according ISO 4049 as reference with some modifications (dimensions, sample type and substrate because of the interaction of laser with water and hydroxyapatite). Square-shaped blocks of dentin (6,0x 6,0x 1,0mm) were obtained, weighed and randomized before the application of DBS that was performed according manufacturer instructions (table1). For treatment with laser, after the DBSs application over the dentin blocks, they were irradiated with Er, Cr: YSGG laser with parameters described in table 2. The laser tip was positioned perpendicularly at 3 mm from specimen for automatic zigzag scanning (BioPDI, XY Table, São Carlos, SP, Brazil). For MP and CSE groups the laser irradiation was performed after primer and for SB e SU after primer/bond. After the treatments and before DBS curing, all the specimens were air-drying for 20s from a distance of 10 cm to help the evaporation of solvents. After all the specimens were light cured with LED curing unit (Blue Star 2 light, Microdont, São Paulo, SP, Brazil) covering the entire specimen surface at a power density of 1000mW/cm² for 20s.

Table 2 - Parameters used for irradiation

Parameter	Value
Energy per pulse (output)	6.25 mJ
Frequency	10 Hz
Power	0.0625 W
Time	30s
Area	36mm ²
Energy density	5.20 J/cm ²
Tip diameter	800 µm

2.2.2. Water Sorption and Water Solubility Analysis

The samples were stored in desiccators at 37°C [Cuber with silica gel (Mesh Blue 2-4 mm, Synth, São Paulo, SP, Brazil)]. Test had three phases for obtaining M1, M2 and M3.

M1: mass of the specimen after the first desiccation. After the treatments, the specimens were weighed daily in an analytical balance (GR-202, A & D ENGINEERING, INC., San Jose, USA), until to obtain a constant mass (oscilation of 0.002g).

M2: mass of specimen after immersion in water. The specimens were stored in distilled water at 37°C and were weighed daily until to obtain a constant mass (oscilation of 0.002g). Before the weighing each specimen was dried with three absorbent paper.

M3: mass of the specimen after a new desiccation. After M2, the specimens were subjected to a further desiccation process on silica and a new weighing were carried out to obtaining M3, observing the oscillation limit of 0.0002 g.

The values of WS/WSB, in micrograms per cubic millimeter ($\mu\text{g} / \text{mm}^3$), were calculated using the following equations:

$$(\text{Sorption}) \left| WS = \frac{m_2 - m_1}{V} \right. (\text{Solubility}) \left| WSB = \frac{m_1 - m_0}{V} \right.$$

The volume (V) in mm^3 of each specimen was determined by the formula of $V = x^2 \cdot h$, where x^2 corresponds to the working area and h is the mean value of height (thickness) that was measured at three different points (Digimatic Calibrator, Mitutoyo Sul Americana, Rio de Janeiro, Brazil).

2.3. Degree of conversion

2.3.1. Sample preparation

For Raman spectroscopy, 24 teeth had the crown sectioned, then the area of 36mm^2 for irradiation was delimited in the exposed dentin with white nail enamel (Risque, Barueri, SP, Brazil) and the treatments were performed as described in the previous test. After application of the DBS and irradiation, the composite resin (Filtek™ Z250, 3M ESPE, St Paul, USA) was inserted by the incremental technique with consecutive deposition of two 1.5mm thick increments. The curing of each increment was performed with the same LED for 20s. After application of the resin, the teeth were immersed in deionized water and kept in an oven at 37 ° C.

After 1 year, the specimens were sectioned in the long axis on the serial cutting machine (Isomet™ Low Speed Saw®, Buehler, Lake Bluff, USA) to obtain slices with 1.0mm thickness. Additional wear was performed when necessary to obtain standardized slices of average thickness of 1.0 mm ± 0.1mm.

2.3.2. Degree of Conversion Analysis

The Raman spectra of the specimens were recorded on a Micro Raman spectrometer (CRAIC Micro Raman, model PV 20/30, San Dimas, CA, USA) with excitation at 830nm, coupled to a microscope with 40x magnification. The Micro-Raman spectrophotometer was first calibrated to zero and then to the coefficient values using a silicon sample. The spectra were recorded on the hybrid layer of each specimen with a Raman camera. Uncured DBS spectra (R) were taken as reference. The ratio of the double bond content of the monomer to the polymer was calculated according to the following formula:

$$DC = (1 - \frac{R_{cured}}{R_{uncured}}) \times 100$$

• R is the ratio between the aliphatic and aromatic peaks at 1638 centimeters-1 and 1610 centimeter-1 in cured and uncured adhesives.

2.4. *Microtensile Bond Strength (MS)*

2.4.1. Sample preparation

For microtensile strength test the sample was prepared in the same way of degree of conversion. However, the sample were sectioned further longitudinally to obtain square sticks with approximately 0.64mm² area (n=10).

2.4.2. Microtensile Bond Strength Analysis

This test was performed only after 1 year of water storage using the universal testing machine Instron 3342 (Illinois Tool Works, Norwood, IL, USA). The cross-sectional area of each specimen was measured with a digital caliper (Digimatic Caliper Absolute, Mitutoyo Corp, Kawasaki, Japan) and the values were inserted into the onboard BlueHill software (BlueHill® Materials Testing Software, Norwood, IL, USA). Then, the sticks were fixed individually with a cyanoacrylate-based adhesive (Loctite Super Bonder Gel Control, Henkel Ltda, São Paulo, SP, Brazil) into the machine's dispositive (JIG 1 Microtensile, Article 31 Odeme, Santa Catarina, Brazil). The adhesive interface was positioned perpendicular to the tensile forces generated by the testing machine. The tension (N) was applied at a constant speed of 0.5 mm/min, with maximum load of 500 N, and the force required to break the stick was registered.

2.4.3. Failure mode analysis (FM)

The segments of the fractured sample were evaluated to define the type of the failure with a portable digital microscope (Dino Lite Microscope Plus, AnMo Electronics Corp, New Taipei City, Taiwan) at 40x magnification and classified by failure modes: Adhesive (A); Cohesive in Dentin (CD); Cohesive in Resin (CR) and Mixed (M). The percentage of each type of failure was obtained.

3. Statistical Analysis

Data was calculated and analyzed statistically with Statistica software (Statsoft®, Tulsa, OK, USA). The assumptions of normal distribution and of equality of variances were checked for all the variables using Kolmogorov-Smirnov and Levene test, respectively. As the assumptions were satisfied, data was subjected to two-way ANOVA ($p < 0.05$) followed by Tukey's test ($p < 0.05$) for individual comparisons.

4. Results

For WS, no difference was observed for DBS, only treatment. Groups irradiated by laser presented lower values than non-irradiated.

Table 3. Mean and standard deviation of the Water Sorption ($\mu\text{g}/\text{mm}^3$)

Treatment	MP	SB	CSE	SU
Control	123,44 $\pm$ 15,4 Aa	124,87 $\pm$ 8,0 Aa	130,25 $\pm$ 28,5 Aa	136,0 $\pm$ 22,6 Aa
Laser	119,94 $\pm$ 23,9 Ba	101,24 $\pm$ 13,7 Ba	103,41 $\pm$ 9,5 Ba	116,0 $\pm$ 12,0 Ba

* Upper case letters show difference between lines

** Lowercase letters show difference between columns

For WSB, it was observed significant results for the two factors, as well as the interaction between them. In the control groups, significant differences between DBSs were found ($\text{CSE} > \text{SU} = \text{SB} = \text{MP}$). Laser irradiation promoted a significant decrease in WSB values for all groups. In the irradiated groups, significant differences between DBSs also occurred ($\text{CSE-L} > \text{SU-L} = \text{SB-L} = \text{MP-L}$).

Table 4. Mean and standard deviation of Water Solubility ($\mu\text{g}/\text{mm}^3$)

Treatment	MP	SB	CSE	SU
Control	-1,49 $\pm$ 5,28 Aab	-6,92 $\pm$ 4,31Ab	12,39 $\pm$ 13,51 Aa	-7,67 $\pm$ 5,03 Ab
Laser	-47,8 $\pm$ 17,1 Bd	-33,68 $\pm$ 5,25 Bcd	-12,13 $\pm$ 5,7 Bb	-31,97 $\pm$ 5,50 Bc

* Upper case letters show difference between lines

** Lowercase letters show difference between columns

For DC, It was noted a significant difference only for DBS factor. Laser irradiation did not promote any significant difference in DC (Table 5). SU presented higher values of degree of conversion ($\text{SU} > \text{MP} > \text{CSE} = \text{SB}$).

Table 5. Mean and standard deviation of Degree of Conversion (%)

Treatment	MP	SB	CSE	SU
Control	52,0 $\pm$ 7,6 Ab	35,8 $\pm$ 0,8Ac	38,3 $\pm$ 1,65Ac	72,37 $\pm$ 0,51Aa
Laser	57,2 $\pm$ 2,0Ab	35,4 $\pm$ 0,83Ac	38,0 $\pm$ 3,0Ac	73,2 $\pm$ 1,52Aa

*Upper case letters show difference between lines

** Lowercase letters show difference between columns

For MS, it was observed significant differences for the two factors and interaction between them. In the control groups, no significant differences between DBSs could be noted. It was observed lowest values of MS for groups irradiated with laser than not irradiated with exception of SU-L (SU-L > CSE-L = SB-L = MP-L).

Table 6. Mean and standard deviation of Microtensile Strength (MPa)

Treatment	MP	SB	CSE	SU
Control	24,3 ± 14,0 Aabce	35,6 ± 13,0 Aab	29,6 ± 9,8Aabc	29,5 ± 9,8Aabc
Laser	9,8 ± 8,8 Be	17,7±13,9 Bec	20,8 ±10,8 Bcbe	38,4 ± 9,5 Ba

*Upper case letters show difference between lines

** Lowercase letters show difference between columns

For FM, the most predominant failure mode for all groups was adhesive. Description of the distribution is presented in table 7.

Table 7. Failure mode analysis (%)

Groups	A	CD	CR	M
MP	63%	8%	8%	4%
SB	75%	11%	7%	3%
CSE	81%	13%	2%	2%
SU	67%	8%	8%	4%
MP-L	68%	13%	0%	0%
SB-L	81%	5%	2%	0%
SE-L	94%	3%	0%	3%
SU-L	80%	12%	8%	0%

*Upper case letters show difference between lines

** Lowercase letters show difference between columns

5. Discussion

Lasers have been tested to improve the physicochemical interactions between the moist dentin substrate and the resin-based DBS. [4] Heating promoted by lasers

and dental literature [12,13,14] have been reported the increase of temperature aid in the evaporation of the solvents presented in DBS as well as improvement of mechanical properties. Furthermore, many authors [7,10] observed that neodymium and diode laser improved the bond strength of DBS irradiated prior to their curing. However, this situation has not yet been investigated with the Er, Cr: YSGG laser.

In this study, the Er, Cr: YSGG laser promoted a significant drop in the sorption (WS) of the different DBSs. Some studies have indicated that increasing WS may impact negatively the long-term stability of the polymer. [15] These smaller values of WS could have occurred due to the greater evaporation of the solvent promoted by the heating of the laser. These results are in agreement with other studies that related that longer periods of evaporation, heated air-stream or preheating of DBS can reduce the WS/WSB. [12, 13]

Furthermore, laser irradiation promoted a significant drop in the solubility of DBSs too. MP-L, SB-L and SU-L presented significant difference than respective control groups. Many factors could affect the solubility of DBS: presence of chemical groups capable of forming hydrogen bonds with water; crosslinking degree; presence of residual monomers; and crystallinity of the polymer. [16] Sideridou et al. [17] reported that methacrylate polymers exhibit a spatial heterogeneity in which some parts densely cross-linked and some parts are loosely cross-linked.

It can be observed that the WSB test presented negative results which is not a common finding in the literature. This may have happened due to modification in the test using DBS applied on the dentin due to the interaction of the laser with the substrate/DBS and not only DBS. For control groups, the negative results could be explained due to the presence of remaining water in the dentin. For laser groups, the hypothesis is that heating promoted by Er,Cr:YSGG laser could have been aided the solvent evaporation decreasing the WS/WSB, but at the same time could have affected the spatial heterogeneity of polymer resulting in a greater amount of the chemical groups capable of forming hydrogen bonds with water, allowing that part of the water obtained in M2 to be maintained at the desiccation end ($M3 > M1$). Since laser irradiation affected the WS/WSB of most DBSs, the first null hypothesis was denied.

The irradiation with Er,Cr:YSGG laser did not affect DC after 1 year. It was observed only significant differences among DBSs, with higher values for SU. Scientific studies showed that this DBS presents a new technology which allows them to be used as etch-and-rise or self-etching. Furthermore, these results could be justified due the

viscosity and composition of this DBS that presents silane, vitrebond copolymer and 10-methacryloyloxydecyl dihydrogen phosphate monomer (MDP) that could promote a higher stability of the polymer. [18] Besides the differences in the chemical composition, clinically it is possible to observe that this DBS is less viscous than the others. According Danroch et al., [19] lowest viscosity may confer an increase in the mobility of the radicals and reacted monomers, resulting in further curing and higher DC. In this way, the second null hypothesis was accepted.

For MS, it was observed significant differences between irradiated and not irradiated groups. Irradiated-groups were affected negatively, with exception SU-L that did not present a significant drop in the MS values. Drop in bond strength values over time are expected [reference] regardless of the type of adhesive, however the laser irradiation promoted a significantly greater drop. In this way, it is believed that although the heating promoted by the laser has assisted in the evaporation of the solvent, it may altered the spatial heterogeneity of the polymer as previously discussed. This phenomenon may have allowed the formed polymer to have a greater amount of the chemical groups capable of forming hydrogen bonds with water or greater amount of loosely cross-linked, that after 1-year resulted in greater degradation of the adhesive interface. It is observed that the SU-L group was not affected like the others and this may have occurred due to the higher values of DC obtained for this DBS.

Another factor that could also interfered in the MS test is the degradation of collagens fibrils promoted by the high temperature increase. Moreto et al. [21] reported that the Er,Cr:YSGG laser parameters used in his study promoted in a specific morphological pattern of dentin and collagen fibrils that negatively affected the bond strength, due the interaction of laser with the dental hard tissue. However, according to Schaller et al. [23], the degradation of collagen fibrils begins from values up to about 146°C (420K). According to data not yet published on this research group the temperature of this study did not reach this level, and therefore this degradation probably did not occur.

Since all laser irradiation presents a large variety of parameters for irradiation, other configurations should be tested since some parameters may achieve the ideal temperature to promote benefits to the physics properties of the DBSs.

6. Conclusion

Based on the results, Er, Cr: YSGG laser irradiation in an attempt to improve adhesion should be better investigated, since the irradiation parameters and strategy used in the present study compromised negatively the most physics properties of the most DBSs.

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3 DISCUSSION

3 DISCUSSION

Most procedures in dental clinic are adhesive procedures but the bonding adhesive systems with dentin is weak due its particularities. Due to this weak interaction, many researchers are looking for alternatives to improve its properties and achieve better marginal sealing and greater durability. Therefore, this study investigated the effects of the high-power laser technology at DBS properties when irradiated prior to its curing on dentin.

Er, Cr: YSGG laser irradiation promoted a high temperature variation and a significant decrease in WS/WSB values, mainly in total-etching DBS (MP and SB). According Schaller et al. (2004) heating up to about 86°C (360 K) affects only residual water present in the tubules of dentin substrate without altering the molecular structure of collagen. Furthermore, values up to about 146°C (420K) leads to degradation of collagen phase (SCHALLER et al., 2004). Temperature variation of MP 68,86°C and SB 57,8°C, considering the room temperature of 20°C and standard deviation would result in a MP 103,46°C and SB 81,85 °C in the worst scenario. Thus, in the present study the temperature increases probably did not promoted collagen phase degradation but could have assisted in the evaporation of residual water and solvents that could justify the decrease in the WS/WSB values.

The higher values of temperature for total-etching than self-etching DBS can be explained due to the greater presence of remaining water in the conditioned substrate with phosphoric acid that requires the wet technique to avoid the collapse of collagen fibrils. Probably Er, Cr: YSGG laser interacted with remaining/solvent water due to the proximity of laser wavelength (2780nm) to the water absorption peak. MP presents water its composition that justify the higher temperature values and lowest values of WS/WSB.

On the other hand, SB presents ethanol as solvent instead water in its composition which justifies a lower heating than MP. This ethanol was included in SB as a solvent to facilitate the penetration of resin monomers on the dentin surface (CARVALHO et al., 2003). The incorporation of the ethanol solvent may favor the adhesion to dentin by removing the remaining water. This happen because when a

specific amount of water is mixed with the ethanol solvent, the resulting mixture presents the same composition both in liquid and in gaseous state, called azeotrope. Thus, when the ethanol evaporates, water will evaporate together (YIU et al., 2005; LIDE; HAYNES, 2009). The ethanol associated with high temperature could have allowed a greater evaporation of the remaining water from dentin resulting in longer resin tags as seen in SEM analysis and also decrease in WS/WSB values.

Despite those "benefits" promoted by the laser irradiation, the temperature increase may also have promoted a change in the spatial heterogeneity of the polymer that allowed more water to accumulate therein and it may have promoted an exacerbated degradation of the adhesive interface after 1-year, mainly for MP-L and SB-L. Probably, this spatial change occurred due to the greater number of chemical groups caused by heating that allowed greater hydrogen bonding with water (NISHITANI et al., 2006). This would justify the lower values of bond strength after 1 - year for these groups, since the maximum temperature increase did not reach the collagen degradation temperature.

When studying the temperature increase of materials is a great concern to evaluate how much of this temperature reaches the pulp organ. In this aspect laser has a great advantage because it was observed that all groups of DBS had a higher temperature variation only in the surface region, but in the ceiling of the pulp chamber the temperature variation was significantly lower. According Colluzzi et al.(2004), laser energy can interact in one of four ways with the target tissue depending the optics properties at that tissue. This phenomenon can explain the different results for different lasers. *Transmission* on the laser energy directly through the tissue is the inverse of absorption and this effect is wavelengths-dependents. Whereas the water-containing tissue fluid readily absorb the erbium laser family and CO₂ laser wavelengths at the surface, less energy is transmitted adjacent tissue (COLUZZI et al., 2004; COLLUZZI et al., 2008). These phenomena can explain the results of the present study as little temperature increase occurred near the pulp chamber.

For self-etching DBS irradiated with Er, Cr: YSGG laser, a decrease in WS values also occurred. Superficial temperature at the worst scenario would reach 67,8°C for SU-L and 68,12°C for SE-L. These lowest values of temperature for self-etching DBS can be justify due to the less residual water in the substrate due to dry technique.

Although both DBS present water in their composition, the remaining water seems to be more important for interaction with the laser due to its greater amount. Besides the self-etching DBSs have presented lower temperature increase when compared with total etching DBSs, probably this temperature was enough to promote a decrease in the WS values.

Although the increase in temperature promoted reduction of the WS/WSB values of all the DBSs, there was no change in the DC values. The difference of the DC in the DBS tested only between the DBS, without influence of the laser. The higher values of DC were presented by the SU group and the chemical composition could justify this difference. The SU DBS group presents 10-MDP, silane and the vitrebond copolymer and probably these components are responsible for the higher DC values (YOSHIDA et al., 2012). Although the CSE has a similar temperature to the SU and also presents the 10-MDP, this DBS does not present silane and the vitrebond copolymer which seems to have influenced the results of DC.

For MS, laser irradiation promoted a significant decrease in the MS values after 1-year as already discussed. The SU-L presented a different behavior since the MS values did not decrease after 1-year. This result probably also can be explained by the chemical composition. As seen in the DC results, the SU group presented a significant higher values when compared with the other groups and this higher DC maybe responsible for this behavior over time, without decrease in the MS values even with laser irradiation.

Zabeu et al. 2017 evaluated immediate bond strength of the same DBS and with the same laser and parameters. They observed that Er, Cr: YSGG laser did not decrease the immediate values of bond strength. So, it seems that the decrease in MS values take time to occur, probably because of greater number of chemical groups that allowed greater hydrogen bonding with water did not occur immediately.

The majority of works performed with Er, Cr:YSGG laser are under other conditions such as to do the cavity preparation(reference), in the conditioning of dentin to improve the adhesion quality of glass ionomer cements (GARBUI et al., 2013), to prevent enamel and dentin demineralization (RAMALHO et al., 2015). The strategy tested in the present study has not been investigated yet and justify this study.

The results of this study are contrary to previous studies that have suggested that other kind of laser can improve bond strength. This proves that the benefits promoted by laser irradiation are totally dependent on the type of laser and the erbium laser, despite the greater interaction with the dentin substrate, which resulted in a higher temperature increase, did not present the expected benefits for most stickers tested.

In relation to the tests used in the present study, WS/WSB test were carried out in a different manner. Normally, according ISO 4049, these tests use only pure DBS samples, but as laser presents interaction with substrate, in the present study they were performed in association with dentin substrate. In this way, a larger amount of DBS is used in the test of the ISO what is not used in clinical reality. Even tough, the results of WS of control groups are in accordance with the results found in the literature (WANG et al., 2016; BRIANEZZI et al., 2017). However, the values of WSB found in this study were much lower than in other studies (CHINELI et al., 2014; VALE et al., 2014; WANG et al., 2016; BRIANEZZI et al., 2017). These differences may have occurred due to the modifications made in the test due to the peculiarities of the study, such as the presence of dentin and less amount of DBS

Most of study evaluated DC through Raman immediate, after 24hrs 7 days or 6 months. In this study was evaluated after 1 year. The findings for microtensile strength after 1 year are according de literature. In this study media values for SU was 29,5 Mpa, Marchesi et al.(2014) stored in the same solution and found values 26.8 Mpa.

For morphological characteristics of the hybrid layer, the results of the present study are in accordance with the literature. It was observed a significant difference between total-etch and self-etch DBS when the presence/absence and a qualitative/quantitative analysis of resin tags was compared. Both 3 and 2-step total-etch DBS presented larger and more numerous resin tags when compared with self-etch ones. The self-etch DBS presented shorter and fewer resin tags, and the 1 step self-etch presented almost total absence of tags (LAFUENTE et al., 2012, REIS et al., 2009). The laser promoted some morphological differences due to the thermal increase, and the differences detected in the present study are in accordance with studies that evaluated the effect of temperature increase on the morphological characteristics of the hybrid layer (REIS et al., 2009)

Despite the limitations of the present in vitro study, the use of erbium laser, with the parameters tested, should be better investigated since it undermined the stability of adhesion in most DBS. However, more studies are required with different parameters.

4 FINAL CONSIDERATION

4 FINAL CONSIDERATION

The Er, Cr: YSGG laser can be considered an effective tool for localized heating due to high interaction with the dentin substrate. The heating promoted by laser improvement of some tested properties, however, promoted a greater degradation of the adhesion over time when compared to not-irradiated groups.

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ATTACHMENT

ATTACHMENT A – Approval by the Ethics Committee of Research on Human Beings at Bauru School of Dentistry

FACULDADE DE
ODONTOLOGIA DE BAURU-
USP

PARECER CONSUBSTANCIADO DO CEP**DADOS DA EMENDA**

Título da Pesquisa: Efeito da irradiação do laser de Er,Cr:YSGG nas propriedades físicas de sistemas adesivos aplicados sobre a dentina

Pesquisador: Leticia Ferreira de Freitas Brianezzi

Área Temática:

Versão: 3

CAAE: 49813315.5.0000.5417

Instituição Proponente: Universidade de São Paulo - Faculdade de Odontologia de Bauru

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.745.588

Apresentação do Projeto:

Justificamos algumas alterações que deverão ser realizadas no projeto "Efeito da irradiação do laser de diodo e Er,Cr:YSGG nas propriedades físicas de sistemas adesivos em dentina". As alterações foram devidamente preenchidas no projeto, que segue em anexo. O que encontra-se grifado em amarelo seria as alterações que gostaríamos de acrescentar e, em vermelho que gostaríamos de excluir.

1- A princípio, no projeto enviado ao Comitê de Ética em Pesquisa (CEP) da Faculdade de Odontologia de Bauru (FOB/USP), foi considerado a utilização de dois lasers (Diodo e Er, Cr:YSGG). No entanto, após a realização da termografia infravermelho (teste piloto) foi observado que o aumento da temperatura atingido do laser de diodo ($\pm 3^{\circ}\text{C}$) não foi suficiente para melhorar as propriedades físicas. De acordo com alguns trabalhos (Castro et al. 2013, Vale et al. 2014, Argolo et al. 2014) o aumento necessário para haver melhora das propriedades físicas é em torno de 40°C . Devido a isso optou-se por trabalhar somente com o laser Er, Cr: YSGG que apresentou um aumento em torno de 40°C sem causar danos a polpa e remover o laser de diodo.

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**FACULDADE DE
ODONTOLOGIA DE BAURU-
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Continuação do Parecer: 1.745.588

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_778999_E2.pdf	31/08/2016 12:24:48		Aceito
Folha de Rosto	folha_de_rosto.pdf	30/08/2016 19:05:37	Leticia Ferreira de Freitas Brianezzi	Aceito
Outros	justificativa_cep.pdf	30/08/2016 19:03:34	Leticia Ferreira de Freitas Brianezzi	Aceito
Declaração de Pesquisadores	compromisso.pdf	30/08/2016 18:47:37	Leticia Ferreira de Freitas Brianezzi	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	termo_cessao_dentes_cirurgiao_dentista_cep.pdf	30/08/2016 18:44:13	Leticia Ferreira de Freitas Brianezzi	Aceito
Declaração de Instituição e Infraestrutura	Declaracao_instituicao.pdf	30/08/2016 18:41:23	Leticia Ferreira de Freitas Brianezzi	Aceito
Orçamento	orcamento_cep.docx	19/08/2016 22:07:32	Leticia Ferreira de Freitas Brianezzi	Aceito
Projeto Detalhado / Brochura Investigador	projeto_cep.docx	19/08/2016 21:51:19	Leticia Ferreira de Freitas Brianezzi	Aceito
Declaração de Pesquisadores	DECLARACAO_COMPROMISSO_RESULTADOS.pdf	05/11/2015 13:41:35	Leticia Ferreira de Freitas Brianezzi	Aceito
Outros	OFICIO.pdf	05/11/2015 13:40:12	Leticia Ferreira de Freitas Brianezzi	Aceito
Outros	TERMO_DE_CESSAO_DE_DENTES.docx	28/09/2015 09:51:04	Leticia Ferreira de Freitas Brianezzi	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO.docx	28/09/2015 09:43:29	Leticia Ferreira de Freitas Brianezzi	Aceito
Orçamento	ORCAMENTO.docx	21/09/2015 22:58:06	Leticia Ferreira de Freitas Brianezzi	Aceito
Outros	QUESTIONARIO_PESQUISADOR.pdf	21/09/2015 22:56:09	Leticia Ferreira de Freitas Brianezzi	Aceito
Declaração de Instituição e Infraestrutura	DECLARACAO_INSTITUICAO.pdf	21/09/2015 22:52:39	Leticia Ferreira de Freitas Brianezzi	Aceito
Cronograma	CRONOGRAMA.docx	21/09/2015 22:12:28	Leticia Ferreira de Freitas Brianezzi	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

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ATTACHMENT B – Term of assignment of teeth by private practice **TERMO DE CESSÃO DE DENTES - Cirurgião-dentista**

Eu, Joberg de Souza Lima, cirurgião-dentista, inscrito no CRO 86.885, com consultório situado na Rua José Rebouças de Carvalho, 906- Vila São Felipe, cidade Avaré, SP CEP 18705-350, cedo o(s) 130 dente(s) permanente(s) para o desenvolvimento da pesquisa "Efeito da Irradiação do laser de Er,Cr:YSGG nas propriedades físicas de sistemas adesivos aplicados sobre a dentina", de autoria de Leticia Ferreira de Freitas Brianezzi, sob orientação do Prof. Dr. Prof. Dr. Sérgio Kiyoshi Ishikiriama, declarando que este(s) dente(s) foi(ram) extraído(s) por indicação terapêutica, cujos históricos fazem parte dos prontuários dos pacientes de quem se originam, arquivados sob minha responsabilidade. Estou ciente de que o(s) dente(s) será(ão) utilizado(s) para a referida pesquisa a qual deverá ter sido previamente aprovada por um Comitê de Ética em Pesquisa, sendo preservada minha identidade na divulgação do trabalho.

DECLARO, cumprir as exigências contidas na Resolução 466/12.

Avaré, 3 de junho de 2019.

Dr. Joberg de S. Lima
Cirurgião - Dentista
CROSP 86.885

Nome/Assinatura do cirurgião-dentista