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PATRICIO RUNNACLES

**EFEITOS DA LUZ DE UM FOTOPOLIMERIZADOR LED NA TEMPERATURA
PULPAR BASAL *IN VIVO* EM PRÉ-MOLARES HUMANOS E SUA
CORRELAÇÃO COM AS CARACTERÍSTICAS ANATÔMICAS DENTAIS *EX
VIVO***

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VIVO***

Tese apresentada como pré-requisito para obtenção do título de Doutor na Universidade Estadual de Ponta Grossa, no Curso de Doutorado em Odontologia - Área de concentração: Dentística Restauradora. Linha de Pesquisa: Propriedades Físico-químicas e Biológicas dos Materiais.

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Dedico este trabalho a toda a
minha família. Em especial a minha
esposa e filhas. Minha fonte de
inspiração, alegria e energia.

*A mí querida bubu que se fue
y me hubiera gustado mucho
compartir este momento, hasta
siempre...*

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RESUMO

RUNNACLES, P. **Efeitos da luz de um fotopolimerizador LED na temperatura pulpar basal *in vivo* em pré-molares humanos e sua correlação com as características anatômicas dentais *ex vivo*.** [Tese – Doutorado em Odontologia – Área de Concentração Dentística Restauradora - Universidade Estadual de Ponta Grossa; 2014].

Objetivo: Este estudo *in vivo* avaliou a temperatura basal da polpa em pré-molares hígidos superiores em humanos e o aumento da temperatura induzida pela luz emitida por um fotopolimerizador à base de Diodo Emissor de Luz (LED) *polywave* de alta potência; além disso, a possível correlação entre a variação de temperatura (ΔT) e o volume da coroa e da câmara pulpar *ex vivo*. **Material e métodos:** após aprovação da Comissão de Ética da Universidade Estadual de Ponta Grossa (UEPG) (protocolo #255,945), pré-molares direito e esquerdo de 8 pacientes voluntários (n=15), que necessitavam de extrações por motivos ortodônticos, receberam anestesia infiltrativa e intraligamentar e isolamento absoluto. Um pequeno acesso oclusal com exposição pulpar foi realizado usando alta-rotação, sob spray de ar e água constante, até atingir uma mínima exposição da polpa. Inseriu-se uma sonda estéril de um termopar *wireless* (Temperature Data Acquisition - Thermes WFI, Physitemp) diretamente no interior da câmara pulpar. Após a estabilização da temperatura basal da polpa, a face vestibular foi exposta à luz de um fotopolimerizador LED *polywave* (Bluephase 20i, Ivoclar Vivadent), usando modos de exposição selecionados: 10s em baixa potência (10s/L), 10s em alta potência (10s/H), 5s em potência turbo (5s/T) e 60s em alta potência (60s/H), respeitando um intervalo de 7 min entre cada exposição. A temperatura pulpar foi registrada a cada 0,2 s. Os valores absolutos e a variação da temperatura pulpar após exposição foram submetidos a análise estatística fator único (ANOVA) com medidas repetidas seguida pelo teste de Bonferroni ($\alpha=0,05$). A correlação entre as densidades de energia aplicadas e os valores das variações de aumento de temperatura foi estabelecida por uma análise de regressão linear. Após as exodontias realizadas de acordo com os planos de tratamento, os elementos dentários (n=11) foram armazenados em Timol 0,1% e as dimensões méso-distal da face oclusal (MD), cérvico-oclusal (CO), e vestibulo-lingual (VL) da coroa dos dentes foram medidas com um paquímetro digital. Os volumes coronário e da câmara pulpar foram determinados por meio de microtomografia computadorizada (μ CT) dos espécimes. **Resultados:** foi encontrada uma temperatura basal média consistente abaixo da temperatura corporal, de 35°C ($\pm 0,7^{\circ}\text{C}$). Todos os modos de ativação do LED promoveram aumento significativo de temperatura na câmara pulpar. Foi observada uma correlação positiva entre densidade de energia e variação térmica. Não houve correlação dos volumes coronal e da câmara pulpar com as variações térmicas, mas foi encontrada uma correlação negativa entre a medida VL das coroas e o ΔT no modo de exposição 10s/L.

Palavras-chave: Dente Pré-molar, Polpa Dentária, Temperatura Corporal, Cura Luminosa de Adesivos Dentários.

ABSTRACT

RUNNACLES P. **Effects of lighth emitted by a LED light curing unit on the *in vivo* baseline pulp temperature of intact human premolars and their correlation within *ex vivo* dental anatomic characteristics.** [Tese – Doutorado em Odontologia – Área de Concentração Dentística Restauradora - Universidade Estadual de Ponta Grossa; 2014].

Objective: This *in vivo* study evaluated the baseline pulp temperature in human intact upper premolars and the pulp temperature rise induced by light emitted by a high power polywave Light Emitting Diode (LED) light curing unit (LCU); also, the possible correlation between the change in temperature (ΔT) and the volume of the crown and the pulp chamber *ex vivo*. **Material and methods:** after approval of the local Ethics Committee was obtained (protocol #255,945), upper right and left first premolars requiring extraction for orthodontic reasons from 8 volunteers (n=15) received infiltrative and intraligamental anesthesia and were isolated using rubber dam. A small, occlusal preparation was made using high-speed hand piece, under constant air-water spray, until a minute pulp exposure was attained. The sterile probe from a wireless, NIST-traceable, temperature acquisition system (Temperature Data Acquisition - Thermes WFI, Physitemp) was inserted directly into the coronal pulp. Once the baseline pulp temperature was stable, the buccal surface was exposed to polywave light from a LED LCU (Bluephase 20i, Ivoclar Vivadent) using selected EMs: 10-s either in low (10-s/L) or High (10-s/H); 5-s-Turbo (5-s/T); and 60-s-High (60-s/H) intensities, allowing a 7-min span between each exposure. Peak PT values and PT increases from baseline (ΔT) after exposure were subjected to 1-Way, repeated measures ANOVAs, and Bonferroni's post-hoc tests ($\alpha=0.05$). Linear regression analysis was performed to establish the relationship between applied radiant exposure and ΔT . Linear regression analysis was performed to establish the relationship between applied radiant exposure and ΔT . the teeth were extracted according to treatment plan. The crown and pulp volumes were measured using micro-tomography scanner, and pulp chamber / crown volume ratio was determined. Afterwards, premolar buccal-lingual, cervico-occlusal, mesio-distal dimensions as well as between cusp distances were measured using a digital caliper. **Results:** consistent temperature below body temperature, resulting in an average core temperature of 35 ° C (+ 0.7 ° C) was found. All modes of activation of the LED promoted significant temperature rise in the pulp chamber. A positive correlation between energy density and thermal variation was observed. No correlation was observed between coronal volume, the pulp chamber with temperature changes. A significant negative correlation was noted between pulp temperature changes when 10-s/L was used and VL.

Keywords: Dental pulp, Pulp temperature, Premolar, LED dental curing lights, Premolar volume, Micro ct.

LISTA DE ABREVIATURAS E SIGLAS

3D	Tridimensional
ANOVA	Análise de variância
BL	Buco-lingual
cal	Caloria
cm	Centímetro
cm ² /s	Centímetro quadrado por segundo
CO	Cérvico-oclusal
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
COEP	Comissão de Ética em Pesquisa
EMs	Exposure modes
J/cm ²	Joule por centímetro quadrado
KV	Quilovolt
LCU	Light curing unit
LED	Diodo Emissor de Luz / Light Emitting Diode
μA	Microampere
μCT	Microtomografia computadorizada
μg	Micrograma
μm	Micrômetro
MD	Mésio-distal
mg	Miligrama
min	Minuto
ml	Mililitro
mm	Milímetro
mW/cm ²	miliwatt por centímetro quadrado
n	Número amostral
NIST	National Institute of Standards and Technology
nm	Nanômetro
PT	Pulp temperature
RC	Resin composite
RT	Room temperature
r ²	Coeficiente de determinação
s	Segundo
SD	Standart deviation
s/H	Segundos em alta potência
s/L	Segundos em baixa potência
s/T	Segundos em potência turbo
TA	Temperatura ambiente
TCLE	Termo de consentimento livre e esclarecido
TP	Temperatura pulpar
UEPG	Universidade Estadual de Ponta Grossa
VL	Vestíbulo-lingual
vs	Versus

LISTA DE SÍMBOLOS

α	Alfa (nível de significância)
ΔT	Variação de temperatura
$^{\circ}\text{C}$	Grau(s) Celsius
%	Porcentagem
$\pm$	Mais ou menos
$=$	Igual
$<$	Menor
τ	Constante de tempo
p	Significância estatística
$\approx$	Aproximadamente
#	Número

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1. INTRODUÇÃO

Na prática diária do consultório, com o intuito de remover os sinais da doença cárie e restabelecer a forma, estética e função do elemento dental, cria-se o risco de causar danos à polpa, por meio de procedimentos clínicos corriqueiros que podem aumentar a temperatura intra-pulpar, como durante os desgastes seletivos de estrutura dentária por fricção com brocas (Zach, Cohen¹ 1962, O'Leary et al.² 2013, Öztürk et al.³ 2004), pela reação exotérmica da polimerização de resinas compostas (Hussey et al.⁴ 1995) ou da auto polimerização de resinas acrílicas durante a confecção de coroas provisórias (Grajower et al.⁵ 1979, Castelnuovo, Tjan⁶ 1997), bem como pela utilização de laser (Anić et al.⁷ 1996, Martins et al.⁸ 2006, Lee et al.⁹ 2013) ou diodos emissores de luz (LED) (Al-Qudah et al.¹⁰ 2007, Asmussen, Peutzfeldt¹¹ 2005, Gomes et al.¹² 2013). Até mesmo procedimentos considerados mais simples, como a secagem do dente com spray de ar aquecido (Galan et al.¹³ 1991), polimento de restaurações com pontas de borracha (Mank et al.¹⁴ 2011) ou ainda clareamento associado à luz (Hahn et al.¹⁵ 2013, Klaric et al.¹⁶ 2013), são capazes de promover aquecimento dos dentes e da polpa dental como consequência. Além disso, os dentes ainda são expostos ao calor na rotina diária dos pacientes, como o calor transmitido pelo amálgama ou incrustações metálicas durante a ingestão de alimentos, uma vez que esses materiais possuem alta condutibilidade, difusão térmica e elétrica (Spierings et al.¹⁷ 1986).

Este aumento da temperatura, quando é transmitido e atinge a polpa dental, pode colocar em risco a homeostase do tecido (Castelnuovo, Tjan⁶ 1997, Millen et al.¹⁸ 2007, Baroudi et al.¹⁹ 2009), ocasionando desde dor pós-operatória até zonas de necrose (Nyborg, Brännström²⁰ 1968) em casos mais graves, quando o estímulo térmico é muito acentuado ou aplicado por muito tempo, pode até levar à necrose pulpar (Zach, Cohen²¹ 1965). Diante disso, é crucial que sejam determinados os limites de aumento de temperatura que o tecido pulpar consegue suportar sem sofrer danos teciduais irreversíveis. Com base no artigo de Zach e Cohen de 1965 (Zach, Cohen²¹ 1965), existe um consenso na literatura de considerar um aumento de até 5,5 °C como uma margem segura onde às inflamações pulpares decorrentes deste incremento térmico são totalmente reversíveis. O clássico estudo *in vivo* feito em macacos *Rhesus* em 1965 avaliou a

injúria térmica à polpa através de cortes histológicos realizados entre sete dias e três meses após a indução de calor por uma fonte metálica externa, acoplada ao esmalte vestibular dos dentes. Apenas os cortes dos espécimes que tiveram registrado aumento de temperatura até 5,5 °C obtiveram total remissão das inflamações adquiridas pelo excesso de calor intrapulpar. Progressivamente, os elementos dentários submetidos a temperaturas acima de 16°C sofreram 100% de necrose pulpar (Zach, Cohen²¹ 1965). Pautadas nessa margem de segurança, muitas metodologias foram empregadas e descritas na literatura para investigar o calor gerado e transmitido à polpa dentária pelos inúmeros procedimentos e materiais (Castelnuovo, Tjan⁶ 1997, Asmussen, Peutzfeldt¹¹ 2005, Klaric et al.¹⁶ 2013, Millen et al.¹⁸ 2007, Baldissara et al.²² 1997). Outras metodologias foram realizadas com o intuito de simular o potencial de dissipação desta energia térmica pelo complexo dos ligamentos periodontais, esmalte, dentina e polpa dental (Spierings et al.¹⁷ 1986, Jakubinek et al.²³ 2008, Preiskorn et al.²⁴ 2003). Também o uso de termografia infravermelha foi utilizada para verificar a emissão e excursão do calor durante restaurações de resina composta em dentes *in vivo* em pacientes (Hussey et al.⁴ 1995).

Em seu estudo, Zach e Cohen (Zach, Cohen²¹ 1965) fizeram a correlação de seus resultados entre *Macaca Rhesus* e os seres humanos, tornando-se referência para todos os estudos de temperatura e injúrias térmicas aos dentes, já citados em mais de 950 artigos. No entanto, não existe registro de qual seria a temperatura basal da polpa dental humana, deixando uma lacuna importante na interpretação de qual temperatura basal (inicial) a ser utilizada. Uma vez que esta informação não é especificada na literatura, alguns autores utilizaram como ponto de partida uma temperatura entre 33 - 34°C (Park et al.²⁵ 2010), entre 34 -35°C (Daronch et al.²⁶ 2007), 36,9°C (Preiskorn et al.²⁴ 2003), porém nenhum deles explica a origem do dado. Na maioria dos estudos, os autores utilizaram temperatura inicial de 37°C (Kodonas et al.²⁷ 2009, Kodonas et al.²⁸ 2009) (Millen et al.¹⁸ 2007, Baldissara et al.²² 1997, Yazici et al.²⁹ 2006) assumindo que esta seja a mesma da temperatura basal corpórea, enquanto outros autores utilizaram a temperatura ambiente (Leprince et al.³⁰ 2010). Somando a margem segura de aumento de temperatura de 5,5 °C (Zach, Cohen²¹ 1965) com a temperatura basal da polpa, tem-se o limite seguro que não deve ser ultrapassado pelo aumento de temperatura, podendo este

ser 40,5°C (Hargreaves et al.³¹ 2002) ou 42,5°C (Amano et al.³² 2006) (Kitamura et al.³³ 2005) conforme a temperatura basal previamente estipulada. Após a evidência de que em torno 15% dos espécimes dentários de macacos *Rhesus* sofreram necrose pulpar quando houve um aumento da temperatura pulpar (TP) maior que 5,5 °C por um estímulo de calor externo (Zach, Cohen²¹ 1965), tornou-se evidente o cuidado necessário que deve ser levado em consideração durante os procedimentos clínicos.

Um potencial agressor à homeostase pulpar utilizado todos os dias na clínica odontológica é a fonte de luz azul que desencadeia a reação de polimerização dos materiais resinosos. Os fotopolimerizadores LED que apresentavam em torno de 30-60 mW/cm² na primeira ou 140-600 mW/cm² na segunda geração (Asmussen, Peutzfeldt¹¹ 2005, Rueggeberg³⁴ 2011), tornaram-se cada vez mais potentes. Embora os fotopolimerizadores mais utilizados nos consultórios varie entre 800 a 1200 mW/cm². Atualmente os novos fotopolimerizadores podem depositar sobre o substrato uma grande intensidade de luz (2.200 mW/cm²) em muito pouco tempo (5s). Quando atinge o elemento dental, a luz causa uma excitação da matéria pela vibração de suas moléculas. Esta energia cinética se transforma então em energia térmica que uma vez captada pelo esmalte será transmitida para a dentina chegando até a polpa dental (Rueggeberg³⁵ 1999). Desta forma, é razoável presumir que quanto maior a intensidade de luz maior a temperatura.

A maioria dos estudos *in vitro* concorda que existe um aumento da temperatura no assoalho e na câmara pulpar decorrente da utilização de fontes de luz nos mais variados materiais restauradores fotopolimerizados (Martins et al.⁸ 2006, Asmussen, Peutzfeldt¹¹ 2005, Gomes et al.¹² 2013, Baroudi et al.¹⁹ 2009, Nammour et al.³⁶ 2010, Santini et al.³⁷ 2008, Randolph et al.³⁸ 2014). Vários estudos *in vitro* relatam um aumento significativo da temperatura no interior da câmara pulpar de dentes extraídos, variando entre 1,5 e 23,2 °C, durante a exposição à luz emitida por fontes de LED's (Baroudi et al.¹⁹ 2009, Park et al.²⁵ 2010, Kodonas et al.²⁷ 2009, Kodonas et al.²⁸ 2009, Yazici et al.²⁹ 2006, Leprince et al.³⁰ 2010, Eldeniz et al.³⁹ 2005, Oberholzer et al.⁴⁰ 2012). Esta variação no aumento da temperatura depende do tipo da emitância radiante e das características do dente (Millen et al.¹⁸ 2007, Baroudi et al.¹⁹ 2009, Park et al.²⁵ 2010, Yazici et al.²⁹ 2006, Leprince et al.³⁰ 2010, Eldeniz et al.³⁹ 2005, Oberholzer et al.⁴⁰ 2012, Baik et al.⁴¹ 2001, He et al.⁴² 2012,

Ebenezar et al.⁴³ 2010). Porém, existe um consenso de que a utilização de algumas fontes de luz LED pode resultar em elevação da temperatura da câmara pulpar para valores maiores que o recomendado como seguro, 5,5 °C, sendo qualquer aumento a partir daí considerado prejudicial para a polpa (Zach, Cohen²¹ 1965). Apesar das evidências de que fontes de luz de alta intensidade podem aumentar a temperatura da câmara pulpar, deve-se considerar que as condições *in vitro* não reproduzem as complexidades de um cenário *in vivo*. Um fator essencial no controle dessa temperatura interna da polpa é o papel desempenhado pela microcirculação pulpar, que parece ser fundamental para dissipar o calor transmitido à câmara pulpar (Daronch et al.²⁶ 2007, Kodonas et al.²⁷ 2009, Kodonas et al.²⁸ 2009).

A polpa dental é um tecido altamente vascularizado e contém o principal sistema de regulação para distribuição de calor nos dentes, capaz de dissipar o calor transferido por estímulos térmicos externos ao complexo dentina/polpa (Kodonas et al.²⁸ 2009, Raab⁴⁴ 1992). Mesmo estudos *in vitro* que simulam o fluxo da polpa (Kodonas et al.²⁷ 2009, Kodonas et al.²⁸ 2009) não foram capazes de reproduzir as mudanças dinâmicas no fluxo do fluido pulpar quando ocorrem mudanças de temperatura neste tecido, que são reguladas por um complexo sistema químico (Brännström, Johnson⁴⁵ 1970). Desta forma, deve-se supor que o sistema de regulação *in vivo* da polpa possa ser mais eficaz na dissipação de calor externo do que seria uma simulação da condição do fluxo pulpar. Torna-se muito complexo o estudo do aumento de temperatura pulpar quando dissociado de estudos *in vivo*. No entanto, não há na literatura nenhum estudo *in vivo* que tenha avaliado o aumento da temperatura intrapulpar durante e após a exposição de dentes hígidos com um fotopolimerizado LED de alta potência, como fonte de energia.

Várias características do comportamento físico de um elemento dentário frente à transmissão do calor já são conhecidas, como a baixa condutividade e difusividade térmica da dentina e do esmalte (Hargreaves et al.³¹ 2002). Além disso, os comportamentos químicos já se encontram em fase de estudo, como o choque térmico induzindo mediadores inflamatórios em células da polpa humana (Eberhard et al.⁴⁶ 2005). Neste contexto, as diferenças anatômicas poderiam também influenciar de alguma forma os efeitos dos estímulos térmicos no aumento da temperatura pulpar. Uma vez que o tamanho da coroa dental pode resultar em paredes de dentina mais espessas ou que o maior volume pulpar poderia resultar

em maior fluxo pulpar, as relações volumétricas entre tecidos duros e câmara pulpar poderiam influenciar no aumento da temperatura pulpar frente ao estímulo térmico ou acelerar a sua dissipação. Entretanto, não há na literatura nenhum estudo que tenha avaliado esta relação entre as dimensões da coroa dental, volume da câmara pulpar, e aumento *in vivo* da temperatura pulpar durante exposição de dente à luz do aparelho de fotopolimerização.

2. PROPOSIÇÃO

2.1 Proposição geral

2.1.1 – Determinar as mudanças de temperatura intrapulpar *in vivo* de pré-molares humanos, ocasionadas por um fotopolimerizador LED *polywave* de alta potência .

2.2 Proposição específica

2.2.1 Constatar a real temperatura basal pulpar *in vivo* de pré-molares superiores anestesiados em humanos.

2.2.2 Verificar em tempo real se existem mudanças da temperatura intrapulpar de pré-molares hígidos durante fotopolimerização com LED *polywave* de alta potência.

2.2.3 Verificar se algum dos diferentes modos de exposição do fotopolimerizador provoca aumento na temperatura pulpar acima do valor considerado crítico (5,5 °C).

2.2.4 Correlacionar os resultados de variação térmica *in vivo* com as dimensões anatômicas da coroa dos pré-molares, o volume coronal e da câmara pulpar.

3. MATERIAL E MÉTODOS

3.1 – Discriminação dos experimentos constantes neste trabalho

Os experimentos deste trabalho foram realizados em três etapas:

A primeira etapa foi um estudo observacional *in vivo* em pacientes com avaliação clínica: Experimento 1. Avaliação direta tempo-dependente da temperatura pulpar humana *in vivo* (Capítulo 1: *Direct measurement of time-dependent anesthetized in vivo human pulp temperature*).

A segunda etapa consistiu em um estudo *in vivo* intervencional em desenho experimental em séries de tempo: Experimento 2: Aumento da temperatura pulpar humana *in vivo* durante exposição a um fotopolimerizador LED polywave de alta potência (Capítulo 2: *In vivo temperature rise in anesthetized human pulp during exposure to polywave LED light curing unit*).

Na terceira etapa foi feita uma análise com os espécimes *ex vivos* em laboratório: Experimento 3. Correlação entre as dimensões de pré-molares hígidos, a idade do paciente, e o aumento da temperatura da polpa *in vivo* durante a exposição à luz de um fotopolimerizador LED polywave de alta potência. (Capítulo 3: *Correlation between intact premolar dimensions, patient's age, and in vivo increase in pulp temperature during exposure to light from of a high power LED curing unit*).

3.1.1 Seleção de pacientes

Para as intervenções, foram selecionados pacientes encaminhados por cirurgiões-dentistas participantes dos cursos de especialização em Ortodontia da cidade de Ponta Grossa (PR), com indicação de exodontia de primeiros pré-molares, como parte do plano de tratamento ortodôntico em andamento. A pesquisa foi realizada após a aprovação pela Comissão de Ética em Pesquisa em Seres Humanos (COEP) da Universidade Estadual de Ponta Grossa (UEPG) com protocolo numero #255,945.

Foram utilizados os primeiros pré-molares hígidos superiores direito e esquerdo. Oito voluntários foram recrutados com a idade variando de 12 a 30 anos

totalizando quinze dentes avaliados (n=15), um dente foi retirado da amostra por possuir restauração protética prévia. Todos os pacientes apresentaram as cartas solicitando as respectivas extrações dentárias, assinadas pelos ortodontistas.

3.1.2 - Critérios de inclusão

Foram estabelecidos os seguintes critérios de inclusão: pacientes com indicação de exodontia de pré-molares superiores por motivos ortodônticos, com elementos dentários hígidos e periodonto saudável e íntegro, que apresentassem saúde sistêmica compatível com os procedimentos cirúrgicos necessários; pacientes com requerimento das exodontias e autorização dos pais e/ou responsáveis de acordo com o termo de consentimento livre e esclarecido (TCLE - Anexo 1), os quais foram assinados e, foram informados sobre todos os procedimentos e riscos envolvidos ao serem submetidos à pesquisa e posterior cirurgia de exodontia.

3.1.3 - Critérios de exclusão

Os seguintes critérios de exclusão foram estabelecidos para o presente estudo: pacientes que não concordassem em aderir ao TCLE ou apresentassem doença cárie e/ou com restaurações diretas e indiretas realizadas previamente nos dentes em questão, com algum tipo de doença periodontal, perda de inserção óssea ou do periodonto, que foram submetidos a procedimentos estéticos de clareamento dental ou recontorno cosmético, apresentando algum tipo de sobrecarga oclusal, posição na arcada muito alterada, má formação de algum dos tecidos dentários (esmalte, dentina, cimento e polpa) e aqueles pacientes com saúde sistêmica comprometida impedindo os procedimentos cirúrgicos necessários e pacientes com necessidades especiais não cooperadores.

3.1.4 - Avaliação da temperatura pulpar

Os pacientes receberam anestesia local infiltrativa e intraligamentar de mepivacaína 2% com epinefrina 1:100.000 (DFL, Rio de Janeiro, RJ, Brasil) aproximadamente um anestube (1,8 ml) em média por elemento dentário. Foi realizado isolamento absoluto com dique de borracha e realizado um acesso

coronário (elementos 14 ou 24) de pequeno diâmetro em formato circular (Figura 1a) com alta rotação refrigerada por spray de ar e água utilizando uma ponta diamantada esférica 1014 (KG Sorensen, Cotia, SP, Brasil) até quase exposição do corno vestibular da polpa assim determinando apenas uma pequena entrada de aproximadamente 0,3 mm diâmetro (Figura 1b). Em seguida, foi feita uma pequena fenda na cúspide vestibular com uma ponta diamantada 3200 (KG Sorensen, Cotia, SP, Brasil), com o intuito de melhor acomodar a sonda do termopar.

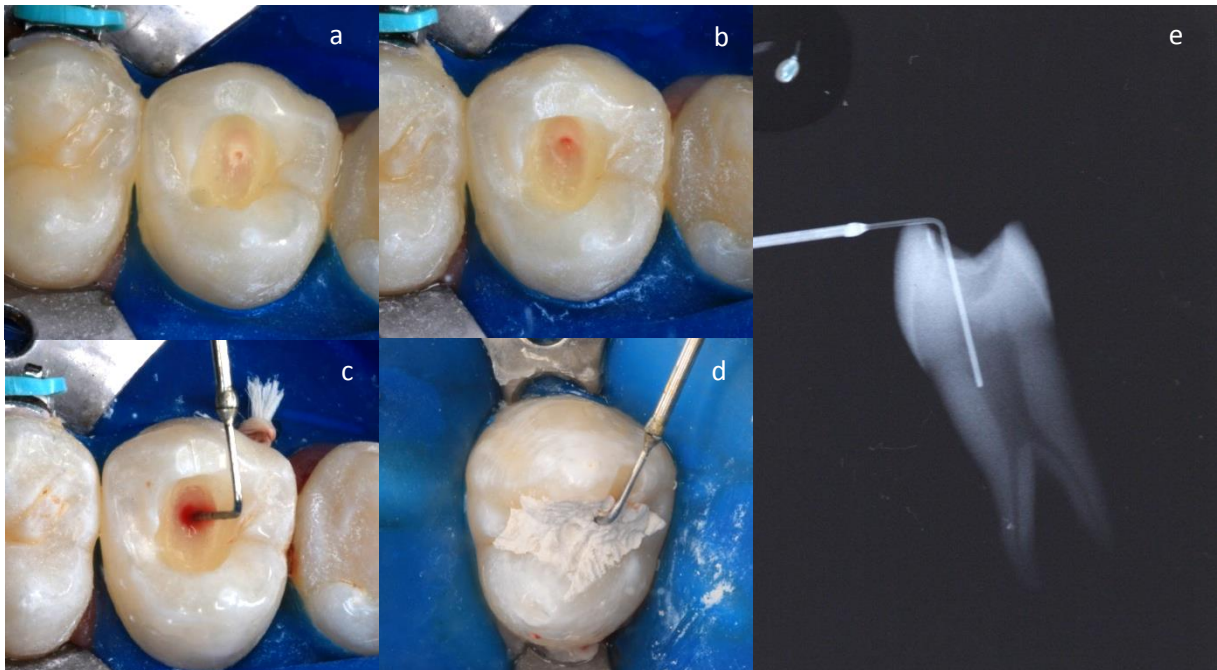


Figura 1 - 1a- pré-molar com acesso oclusal próximo à exposição, 1b- exposição concluída com sonda exploradora, 1c- inserção da sonda do termopar no interior da câmara pulpar, 1d- obturação do acesso com material restaurador provisório e 1e- radiografia periapical após a extração dentária ilustrando a provável posição da sonda do termopar no interior da câmara pulpar.

Para a análise da temperatura pulpar, utilizou-se um sistema de aquisição de temperatura sem fio (Data Acquisition - Thermes WFI; Physitemp, Clifton, NJ, EUA) que apresentava uma sonda termopar (tipo T NIST-traceable; Physitemp). A sonda ficava imersa no interior de um frasco estéril contendo soro fisiológico previamente ao início das análises (Figura 2). Quando a coleta de dados da temperatura era iniciada, a sonda então era inserida na polpa entrando aproximadamente 4 mm em seu interior próximo à parede vestibular da câmara pulpar (Figura 1c). Após inserção e estabilização da sonda no interior da polpa, um incremento de material restaurador provisório (Cavitec, CaiTHEC, SC, BRASIL) foi inserido no interior da cavidade

oclusal a fim de evitar uma possível troca de calor entre a polpa dental e o meio externo (Figura 1d). Quando a temperatura pulpar atingia um platô de estabilidade térmico, geralmente após 25 min, era registrada então a temperatura basal da polpa. Durante todo o teste uma sonda sobressalente ficou submersa em um recipiente com soro fisiológico, como controle da temperatura ambiente.

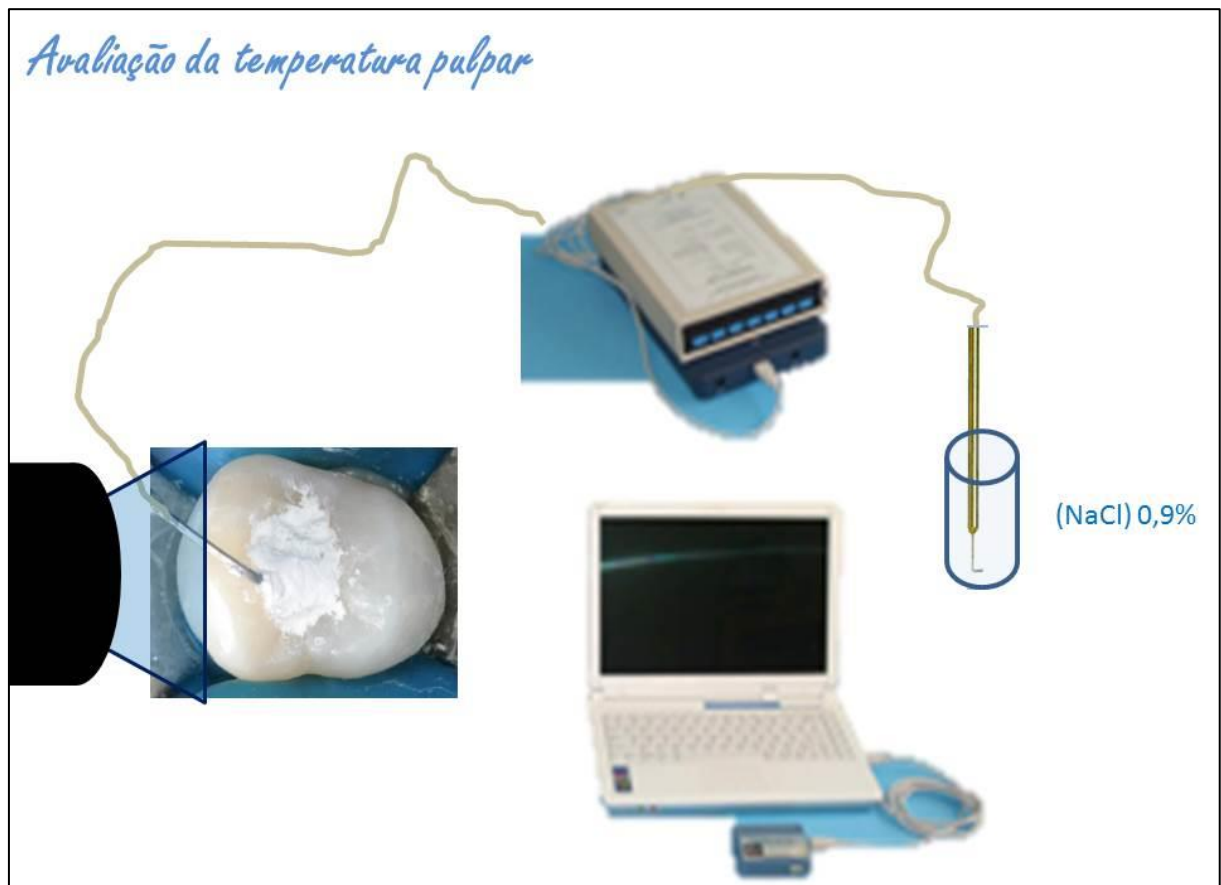


Figura 2 – Disposição do sistema de mensuração de temperatura (Data Acquisition–ThermesWFI; Physitemp, Clifton, NJ, EUA). Esquema mostrando uma sonda imersa em solução salina como controle da temperatura ambiente e a outra inserida no pré-molar em avaliação. Simulação da ponta do fotopolimerizador e luz sobre o dente.

Em seguida, a ponta da unidade de luz LED *polywave* de alta potência (Bluephase 20i, Ivoclar Vivadent, Liechtenstein) foi colocada junto à parede vestibular e ativada seguindo o protocolo descrito a seguir. Foram randomicamente sorteados e utilizados os seguintes modos de exposição do fotopolimerizador (Bluephase 20i, Ivoclar Vivadent, Liechtenstein): 10 s na baixa potência (656 mW/cm^2) (10-s/L), 10 s na alta potência (1244 mW/cm^2) (10-s/H), 5 s na potência Turbo (2204 mW/cm^2) (5-s/T) e 60 s na alta potência (1244 mW/cm^2) (60-s/H) e o operador não foi avisado quanto ao modo de ativação que utilizaria. Foi esperado

um intervalo de 7 min entre cada fotoativação para verificar o retorno à temperatura basal. Os valores de temperatura foram captados pelo termopar em tempo real a cada 0,2 s. Ao final da análise os dentes receberam um reforço da anestesia prévia e foram extraídos com a utilização de periótomo e forceps. Os dentes extraídos foram limpos e armazenados em solução de Timol a 0,1% para desinfecção (Farret et al.⁴⁷ 2010).



Figura 3 - Aparelho de fotopolimerização LED Polywave Bluephase 20i (Ivoclar Vivadent, Liechtenstein).

3.1.5 - Análise da constante de tempo do sistema de aquisição de temperatura

Para determinar o tempo de resposta da sonda termopar, três sondas foram conectadas ao sistema: uma sonda foi imersa em água a temperatura ambiente (TA) de ($\approx 25,5$ °C) em um becker, e outra foi imersa em água a 60 °C, em uma cuba de temperatura regulada eletronicamente (SL-155/22, Solab, Piracicaba, SP, Brasil). Uma sonda adicional foi colocada em TA, e de maneira intermitente foi imersa entre uma e outra. Em tempo real, os dados da temperatura das três sondas foram

captados e armazenados pelo termopar a cada 0,2 s durante 10 min. Neste tempo, uma sonda foi removida da TA e imediatamente submersa em água a 60 °C, onde permaneceu por 10 min. Após isso, a sonda foi removida da água quente e novamente foi colocada no banho TA por 10 min. Este procedimento foi repetido 8 vezes ($n=8$). Para cada sonda movimentada, um gráfico de temperatura versus tempo foi gerado (Figura 4), e o tempo médio corresponde a 63,2% do total do aumento da temperatura (constante de tempo - τ) foi determinado para todos os movimentos das sondas, sendo os valores comparados usando o student's t-test (Statistics 19, SPSS Inc, IBM Company, Armonk, NY, EUA). Nenhuma diferença estatística ($\alpha=0,05$) foi encontrada para τ entre a direção de troca das temperaturas ($p=0,1760$), então as médias dos 16 movimentos medidos e o desvio padrão foram calculados.

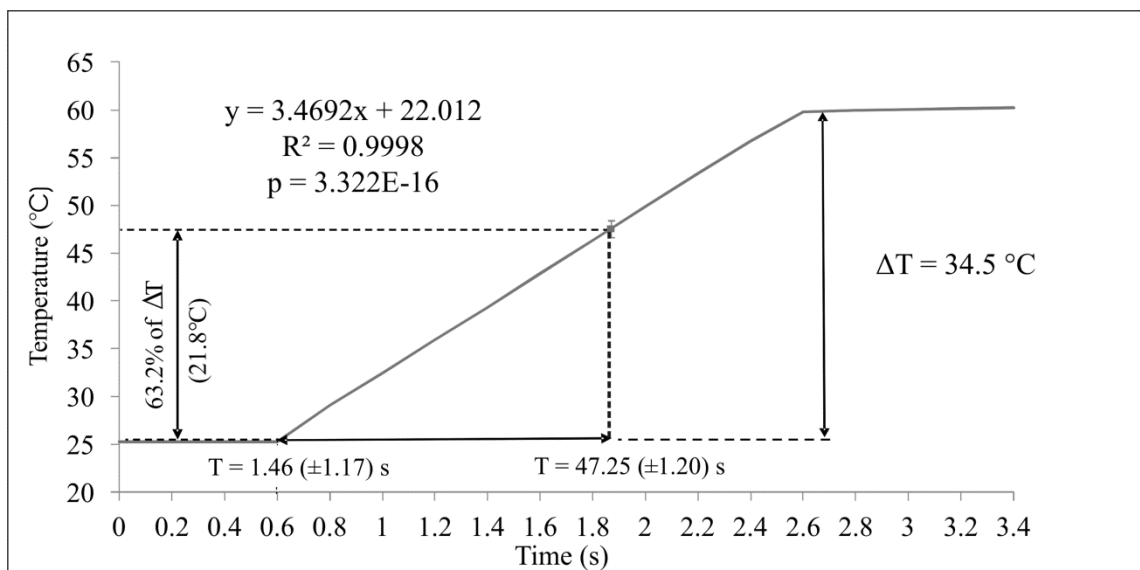


Figura 4 - Análise do gráfico da temperatura versus tempo que indica a constante de aquecimento. Para validar o tempo de resposta da sonda do termopar.

3.1.6 - Medição da emitância radiante e cálculo energia radiante do fotopolimerizador

A radiação espectral dos diferentes modos de exposição foi analisada cinco vezes para cada modo, utilizando um spectroradiômetro de laboratório (USB 2000, Ocean Optics, Dunedin, FL, USA) e uma esfera integrada de 6 polegadas

(Labsphere, North Sutton, NH, USA), previamente calibrado usando uma fonte de luz rastreável NIST. A extremidade da ponta do fotopolimerizador foi posicionada na entrada da esfera integrada, de modo que toda a luz emitida a partir da unidade fosse capturada. A radiância espectral durante cada modo de exposição foi capturada utilizando o software (Spectra- Suite v2.0.146, Ocean Optics) entre 350-550 nm, que também forneceu um valor total de energia emitida para essa faixa de comprimento de onda. A área de emissão óptica da extremidade distal do guia de luz foi calculada, e este valor foi dividido entre o valor de radiância espectral integrada para obter a radiância total da luz de cura para cada modo de exposição. Este valor foi então multiplicado pela duração da exposição à luz para obter o valor da energia radiante aplicada em cada superfície do dente para cada modo de exposição de luz (J / cm^2).

3.2 - Avaliação das dimensões coronárias

Os pré-molares tiveram suas dimensões calculadas com o auxílio de um paquímetro digital (Starrett 727-6/150; Starrett, São Paulo, Brasil). Foram determinadas as dimensões méso-distal da face oclusal (MD), cérvico-oclusal (CO), e vestibulo-lingual (VL) da coroa dos dentes.

3.2.1- Avaliação do volume coronário e da câmara pulpar em microtomografia computadorizada

Para análise dos volumes coronários e da câmara pulpar dos elementos dentários através de microtomografia computadorizada (μCT) no Laboratório de Análise de Minerais e Rochas (LAMIR) da Universidade Federal do Paraná (UFPR), os dentes foram restaurados apenas com a resina composta Herculite XRV (Kerr Corporation, Orange, CA, EUA) recompondo aproximadamente as mesmas dimensões oclusais. Os espécimes foram montados numa base giratória própria do equipamento (Figura 6a) e a base fixada dentro do scanner (Figura 6b). Os dentes então foram lidos por um scanner μCT (Figura 5, Skyscan 1172, Bruker-micro CT, Kontich, Bélgica). A leitura foi realizada com uma resolução com pixel isotrópico de $25,7 \mu\text{m}$ de tamanho, 100 KV, 100 μA , filtro de cobre + alumínio. Foram gerados aproximadamente 650 cortes tomográficos (Figura 7) por espécime, obtidos após um

giro de 180° de rotação no plano axial, longo eixo do elemento dentário, com uma rotação de 0,7°, com exposição de 1 segundo entre uma imagem e outra. Os resultados foram analisados pelo *scan* software (CTAn 1.11.1.0, Bruker-microCT, Kontich, Bélgica). Após as dimensões definidas pelo CTAn utilizamos o programa (CTVoxBruker-micro CT, Kontich, Bélgica) para editar imagens tridimensionais (Figura 8) e reconstrução em fatias, mostrando cortes internos e as interrelações dos tecidos dentários (Figura 9).



Figura 5 - Scanner μ CT Skyscan 1172, Bruker-micro CT, Kontich, Bélgica. Onde os espécimes foram avaliados resultando em aproximadamente 650 cortes para cada elemento dentário, que foram reconstituídos pelo software do aparelho e assim foi calculado o volume da coroa e da câmara pulpar de cada elemento.

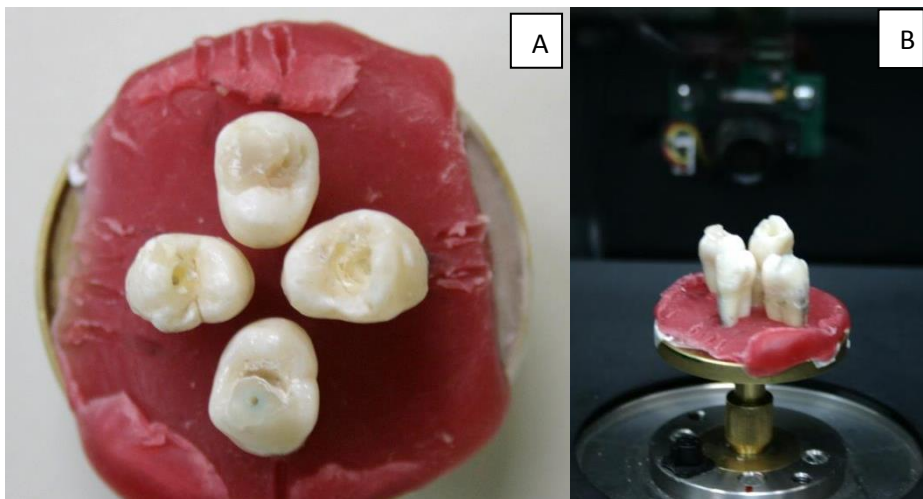


Figura 6 – A- Espécimes montados na base giratória do scanner, B- Base giratória instalada no micro CT.

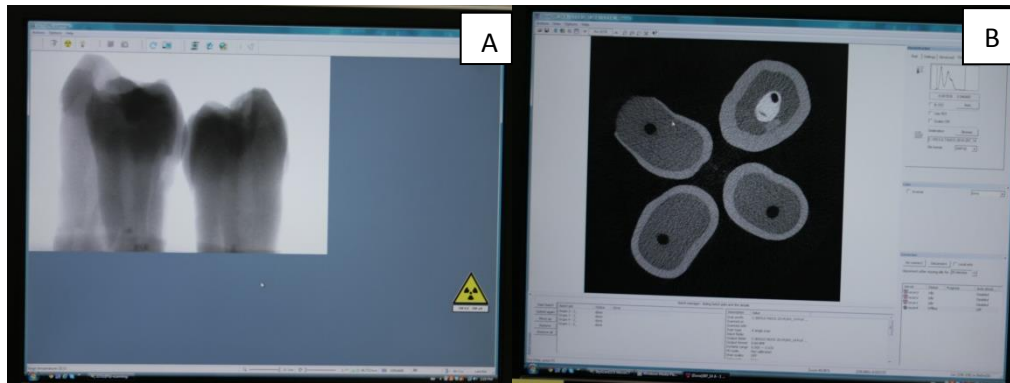


Figura 7 – A- Imagem no monitor da posição dos elementos dentários dentro do scanner, B- Cortes axiais dos pré-molares.



Figura 8 - Face mesial do dente 24 em um modelo 3D

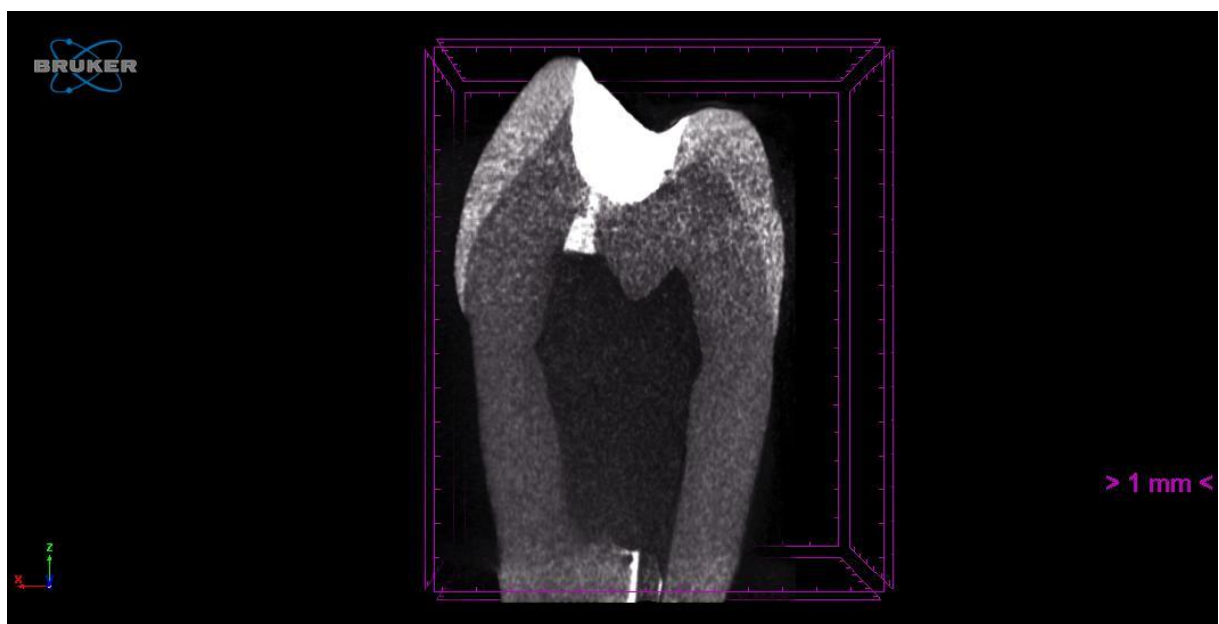


Figura 9 - Vista mesial de um corte μ CT, mostrando as características anatômicas do dente evidenciando a interrelação da polpa e da coroa do pré-molar, através da escala milimetrada é possível se ter uma idéia da espessura dos tecidos duros e da polpa.

4. ARTIGOS

4.1 – Capítulo 1 (Experimento 1)



Direct measurement of time-dependent anesthetized *in vivo* human pulp temperature

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KEY WORDS: dental pulp, organ temperature, body temperature, dental high-speed technique, premolar, LED dental curing lights

ABSTRACT

Objectives. Human intrapulpal tooth temperature is considered to be similar to that of the body ($\approx 37^{\circ}\text{C}$), although the actual temperature has never been measured. This study evaluated the *in vivo*, human, basal, coronal intrapulpal temperature of anesthetized upper first premolars.

Methods. After approval of the local Ethics Committee was obtained (protocol #255,945), upper right and left first premolars requiring extraction for orthodontic reasons from 8 volunteers, ranging from 12 to 30 years old, received infiltrative and intraligamental anesthesia. The teeth ($n=15$) were isolated using rubber dam and a small, occlusal preparation was made using high-speed handpiece, under constant air-water spray, until a minute pulp exposure was attained. The sterile probe from a wireless, NIST-traceable, temperature acquisition system (Thermes WFI) was inserted directly into the coronal pulp. Once the probe was properly positioned and stable, real-time temperature data were continuously acquired for approximately 25 min. Data ($^{\circ}\text{C}$) were subjected to 2-tailed, paired t-test ($\alpha=0.05$), and the 95% confidence intervals for the initial and 25-min mean temperatures were also determined.

Results. The initial pulp temperature value ($31.8 \pm 1.5^{\circ}\text{C}$) was significantly lower than after 25-min ($35.3 \pm 0.7^{\circ}\text{C}$) ($p<0.05$). The 95% confidence interval for the initial temperature ranged from 31.0 to 32.6°C and from 35.0 to 35.7°C after 25 minutes. A slow, gradual temperature increase was observed after probe insertion until the pulp temperature reached a plateau, usually after 15 min.

Significance. Consistent coronal, human, *in vivo* temperature values were observed and were slightly, but significantly below that of body core temperature.

Keywords: dental pulp, organ temperature, body temperature, dental high-speed technique, premolar, LED dental curing lights

1. Introduction

Pulp health integrity is crucial to any restorative treatment, and is a clinical challenge, because teeth are subjected to trauma by heat generated during restorative procedures, such as from use of high and low speed handpieces[1], restorative materials with exothermic setting[2], restoration finishing and polishing [3], as well as from application of high power light emitting diode (LED) curing units and laser sources to polymerize resin-based materials [4]. For these reasons, the consequences of heat, or other external stimuli, on pulp chamber temperature have been investigated [4,5].

Initial *in vivo* effects of heat on pulp temperature (PT) and its biological consequences were performed by Zach and Cohen in 1965 [6]. In that study, a 5.5°C PT increase in *rhesus* monkeys, from application of a hot metal source to the facial enamel surface, induced necrosis in 15% of evaluated pulps. Because of the high expense of animal testing, *in vitro* techniques using extracted teeth were developed to evaluate pulp chamber temperature change under simulated clinical conditions, while applying various external heat sources [5,7,8]. In these reports, the pulp chamber temperature, prior to application of temperature-raising effects, was pre-set at 37°C (human body temperature) [5,7,9], or at room temperature [4], or was not mentioned in the study [10,11]. Others simulated the influence of pulpal blood flow *in vitro*, to evaluate how this parameter might dissipate internal tooth heat generated by exposure to dental light curing units [5,12,13]. Conversely, these studies set the initial, baseline pulp chamber fluid temperature between 33°C and 34°C [13], or between 34°C and 35°C [12]. Using a finite element model, along with *in vitro* simulation of restorative procedures, a simulated temperature excursion at the pulp-dentin junction during photocuring of a dental restoration was calculated [14]. In that study, the authors observed that some exposure protocols may cause potentially dangerous PT increase, mainly when using high intensity lights, or when curing a thin resin layer. Although such studies provide important information regarding the effects of external heat sources on the temperature increase in *ex vivo* human tooth pulps, it is evident that accurate data on the thermal effects of such heat sources will depend on the actual *in vivo* temperature of human pulp tissue. However, no literature concerning *in vivo* baseline temperature of the human pulp can be found.

The purpose of this *in vivo* study was to develop a method to measure the baseline PT of locally anesthetized, human upper premolars. The research

hypotheses were that (1) consistent and reproducible intrapulpal temperature values can be obtained using a well-controlled measurement system, and that (2) the *in vivo* temperature of locally anesthetized human pulp tissue is similar to that of the human body (the baseline temperature from which subsequent temperature rise values could be measured).

2. Materials and methods

2.1. Study design

The study design was approved by the Ethics Committee at State University of Ponta Grossa (protocol #255,945). Eight volunteers, ranging from 12 to 30 years, requiring extraction of upper right and left first premolars for orthodontic reasons were selected from the Orthodontic specialization program in Ponta Grossa, Brazil, were recruited in February, 2013, and attended to between March and April, 2013. Patient inclusion criteria included: (1) treatment plans indicating premolar extractions for orthodontic reasons, (2) the presence of healthy, intact, non-carious, and non-restored, fully erupted treatment teeth, and (3) patients with well-controlled health conditions that allowed all procedures involved in the research to be performed with minimal risk. Exclusion criteria included (1) those patients who did not agree to volunteer for the study, (2) patients not meeting all of the inclusion criteria.

2.2. Intrapulpal temperature measurement

After written consents were obtained, each study volunteer received local, infiltrative and intraligamental anesthesia, using approximately 1.8 ml of 2 % Mepivacaine Hydrochloride (36 mg) with 1:100,000 epinephrine (18 µg) (Mepiadre, Nova DFL Industria e Comercio, Rio de Janeiro, RJ, Brazil), and the teeth (n = 15) were isolated using rubber dam, one at a time. As there is no previous human study to base sample size on, the present study was used as its own pilot. A small, occlusal preparation was made, using a round diamond bur (#1015, KG Sorensen, Cotia, SP, Brazil) in a high-speed handpiece, under air-water spray, until the preparation floor was near the buccal pulp horn (Fig. 1a). Then, a small, pencil-shaped diamond bur (#2134, KG Sorensen) was used to produce a minute pulp exposure, with no pulp bleeding (Fig. 1b). Care was taken to ensure that the same water flow and air pressure were used for each tooth, as well as the same time for each preparation. A wireless, NIST-traceable, temperature acquisition system

(Temperature Data Acquisition - Thermes WFI, Physitemp, Clifton, NJ, USA) was used to measure PT. Two probes were connected to that system and both were immersed in a 0.9% sterile saline solution at room temperature (RT), while the tooth was prepared. After pulp exposure, one probe was removed from water storage, inserted into the pulp chamber, and positioned to remain stable while PT was recorded in real-time (Fig. 1c). A small groove was created on the buccal cusp, close to the cusp tip (Fig. 1d), to allow the probe to rest on the cusp tip and ensure that the 1-cm long probe tip penetrated approximately 4 mm into the pulp chamber: in a similar position for all teeth (Fig. 1e). The other probe remained in the RT saline solution (approximately 22.0°C), as a reference. Room temperature was stable and controlled by air conditioning: 22°C. The occlusal preparation was then filled with a provisional restorative material (Cavitec, CaiTHEC Ltda, Rio do Sul, SC, Brazil) to minimize heat loss from the internal cut tooth surfaces (Fig. 1d). After probe stability was confirmed, real-time temperature data were continuously acquired every 0.2 s, for approximately 25 min, during which a stable PT was reached. This procedure was followed to avoid any possible effect of tooth cooling as a result of the occlusal surface endodontic access opening, resulting from water and air spray of the handpiece, which could be a potential confounding variable. At the end of data acquisition, the probe was carefully removed, and a small amount of additional interim restorative material was placed to cover the hole left from probe removal. The same process was then performed on the contralateral tooth, using a freshly autoclaved probe. When temperature measurements for both teeth were completed, each tooth was atraumatically extracted, as treatment planned. Following extraction, each probe was reinserted into the pulp chamber of its corresponding extracted tooth, and proximal surface X-rays were taken with the probe in position (Fig. 1e), to confirm probe placement during temperature acquisition. No form of bias was introduced in the study, because each patient was treated in the same manner. Results were strictly observational, with no imposed external variable applied.

2.3. Statistical analysis

Normal distribution of continuous variables was tested by the Shapiro-Wilk test. PT at the time of initial probe insertion and after 25-min in place (expressed in degree Celsius - °C) were subjected to 2-tailed, paired t-tests (pre-set alpha=0.05). The 95 % confidence intervals of population means obtained at these two evaluation

periods were also calculated. All statistical analyses were performed using personal statistical software (Statistics 19, SPSS Inc, IBM Company, Armonk, NY, USA)

2.4. Temperature acquisition system response time

To determine the response time (time constant) of the temperature acquisition instrument, three probes were connected to the system: one probe was immersed in distilled water at RT ($\approx 25.5^{\circ}\text{C}$), and other was immersed in distilled water at 60°C . An additional probe was placed in the RT water, and was intermittently moved from that fluid to the higher-temperature one. Real-time temperature data were continuously acquired every 0.2 s from the three probes for 10 min. During this time, one probe was removed from the RT water, and immediately immersed in water at 60°C , where the probe was left for 10 min. Afterwards, the probe was removed from the hot water and returned to RT water again, for 10 min. This procedure was repeated 8 times ($n=8$). For each probe movement, a temperature vs time plot was developed, and the average time corresponding to 63.2% of the total temperature increase (time constant - τ) was determined for all probe movements, and was compared using a student's t-test. No statistical difference (pre-set $\alpha = 0.05$) was found for τ between direction of temperature change ($p=0.1760$), so the overall average of the 16 measurements, and its standard deviation, was determined.

3. Results

3.1. Intrapulpal temperature

No study volunteers were excluded. Initial and 25-min PTs, as well as their 95% confidence intervals are presented in Table 1, while Fig. 2 shows an example of the 25-min long, real time PT profile analysis from the moment of probe insertion into the pulp chamber. The other probe remained in the sterile saline water, and acted as a reference. A rapid, small temperature drop was noted when the probe was removed from the sterile saline, was transferred through room air, and then inserted into the pulp chamber. Immediately after probe insertion, temperature values increased to approximately $31.8 \pm 1.5^{\circ}\text{C}$: the "initial" temperature measurement. Following this event, a slow, gradual temperature increase was observed until the PT reached a plateau at approximately $35.3 \pm 0.7^{\circ}\text{C}$, which was significantly higher than the initial temperature ($p<0.05$). The 95% confidence interval for the initial probe

insertion temperature ranged from 31.0 to 32.6°C, and from 35.0 to 35.7°C after 25 min.

3.2. Temperature acquisition system time constant

The mean system time constant is displayed in Fig. 3. For the ΔT of 25.5°C to 60°C, the average τ was 1.46 s (time required for temperature to reach 63.2% of ΔT); it took 1.46 s for the data acquisition device to record a 21.8°C increase in temperature. The system demonstrated an immediate, linear response to temperature change, and not a classical “sigmoidal profile” seen when other temperature measurement instruments are used. The slope of the regression line between the two temperature extremes can also be used to determine the time required for the system to provide a 1-degree Centigrade temperature change: only 0.07 s.

4. Discussion

In the current study, a probe from a wireless, NIST-traceable, temperature acquisition system was inserted directly into the pulp to provide a real time PT analysis. It is important to note that only 0.07 s was needed for the measurement instrumentation to demonstrate a 1 °C change. Thus, the time-based PT changes observed can be attributed exclusively to pulp physiologic responses and to thermal properties of tooth, rather than to characteristics of the temperature data acquisition system itself. This aspect helps substantiate the current methodology as a reliable tool to assess *in vivo* intrapulpal temperature. Thus, the first hypothesis was accepted. In this regard, consistent baseline, plateau temperature values were observed, having a very low standard deviation ($\pm 0.7^\circ\text{C}$), with a confidence interval ranging from 35.0 to 35.7°C

In contrast to the assumption that anesthetized human pulp has the same temperature as the human body core (approximately 37°C)[5,7], lower temperature values ($35.3^\circ\text{C} \pm 0.7^\circ$) with a temperature range lower than that observed in the axilla: 36.2 to 37.5°C [15] were observed within the pulp, after 25 min of stabilization. Thus, the second research hypothesis was rejected. Interestingly, such a finding corroborates the assumption made by Spierings et al. [16], who assumed the baseline PT as being 35.2°C, using a finite element method, and helps explain why temperature values at the external pulpal surface of preparations or the external

temperature of some teeth range from 28 to 30°C [17,18]. In this context, it must be emphasized that vasoconstriction promoted by epinephrine might have contributed to these lower values. As previously demonstrated, vasoconstriction promoted by the anesthetic solution with epinephrine results in reduced pulp blood flow and volume [19-21]. More specifically, 20 min later after epinephrine is administered in infiltrative anesthesia, pulp blood flow and volume reach approximately between 60% to 80% of its original values, depending on the amount and type of vasoconstrictor as well as the characteristics of every pulp [20-22]. Once PT is closely related to blood flow [23], lower pulp blood volume eventually leads to a lower PT values in comparison to those values observed in a pulp with a regular blood flow and volume as a consequence. However, only further studies comprising the use of anesthetic solution without epinephrine can confirm such assumptions.

The comparison of current data with any other study evaluating *in vivo* baseline PT is not possible, because no such study could be found. Unfortunately, baseline PT prior to external heat application on the tooth facial surface was not reported by Zach and Cohen [6], who observed that an intrapulpal temperature increase of 5.5°C resulted in irreversible pulpal necrosis in 15% of the evaluated *rhesus* monkeys. Based on that evidence, and assuming that PT was similar to that of the human body core (approximately 37°C), other studies confirmed that the increase in PT above 42°C or 43°C led to short-term, heat-induced pulp cell degradation [24,25]. As a consequence, other authors assumed those temperature values as critical for pulp viability [14,26]. However, based on the current findings, along with the Zach and Cohen evidence, it is possible to speculate that some damage may be expected when the PT reaches approximately 40°C or 41°C: values lower than those considered critical in other studies [14,26]. Only further investigation addressing this issue can confirm such speculation.

Significantly lower PT was observed immediately upon probe insertion into the pulp chamber following tooth preparation. A similar temperature drop was also observed in another *in vivo* study evaluating the temperature of a preparation pulpal floor [18]. Conversely, *in vitro* studies reported significant temperature increase within the pulp chamber, ranging from 5.9°C to 11.7°C, during cavity preparation, because of the changes in the air pressure and water flow from the high-speed handpiece[27,28]. In those studies, no pulpal microcirculation was simulated and a decrease in temperature was only noted when the highest water flow rate was used

along with the high-speed handpiece[27]. In addition, it is noteworthy that intraligamental anesthesia using anesthetic solution with vasoconstrictor was administered in the current study, to ensure patient comfort. Thus, vasoconstriction occurred at the root apex, within the periodontal ligament, and within the pulp tissue itself [19,20]. Blood microcirculation compensates for temperature changes caused by external thermal stimuli to the dentine-pulp complex [5,29]. Thus, the association of compromised pulp ability to modulate PT due to lower blood flow rates, influenced by vasoconstriction, and effective air-water spray during cavity preparation, may help explain the lower initial PT values observed in the current study.

After tooth preparation, approximately 20 min was required for the pulp to reach a stable, baseline value, after a slow, gradual increase. Usually, a restorative procedure involving application of adhesive systems and resin composites is started immediately after tooth preparation. As a consequence, the temperature of both tooth structure and pulpal tissues at the time of light-curing may be lower than the baseline values recorded after a long data acquisition time. In this regard, although some *in vitro* studies have shown significant increase in *in vitro* pulp chamber temperature as a result of application of a dental light curing unit to the tooth, ranging from 1.5°C to more than 4°C [4,9], clinicians may expect that both tooth and pulp tissues will be cool prior to the moment of the first light exposure. Therefore, the cooling effects of air-water spray during tooth preparation may partly compensate for the heating effects of high power light curing units. It should be mentioned, however, that a deep preparation was made on the occlusal surface, to expose the pulp, leaving a thin dentin layer between the preparation floor and the pulp. Because dentin has a low thermal conductivity [30], the influence of any thermal stimulus on PT depends on other variables, such as thickness of remaining dentin and preparation depth [9]. Thus, it is possible that the cooling effect of air-water spray during cavity preparation using high-speed handpieces was more pronounced in this condition.

It should be noticed that the abovementioned assumptions cannot be extrapolated to clinical situations where tooth preparation involving the use of high-speed handpieces with air-water cooling and restorative procedures capable of generating heat are performed in different clinical appointments. As an example, indirect restorative procedures such as the seating of ceramic indirect restorations or laminates do not include the cooling effects on the pulp caused by the use of these handpieces. As a consequence, such teeth will be more prone to the deleterious

effects of heating caused by the light emitted by the LED LCU than will be the teeth that were subjected to a cavity preparation using high-speed handpieces with air-water cooling immediately before the restorative procedure. In this scenario, clinicians may attempt to cool the tooth and pulp down before such restorative procedures are performed by applying air-water spray or wet cotton prior to the beginning of such procedures, or even air blow the prepared tooth during the exposure to light emitted by the LED LCU. Curiously, previous tissue cooling has usually been performed in some types surgical procedures in other tissues, such as human heart and brain [31,32]. Conversely, care must be taken not to cool the teeth down to extremely low temperature values, once such a low temperature may result in lower monomer conversion and compromise the mechanical properties of bonding agents and resin composites as a consequence [33,34]. Further studies are required to confirm the effectiveness of these cooling procedures on preventing pulp overheating.

Most study volunteers consisted of young patients: predominantly 14 years old. Previous studies demonstrate that human body temperature may be lower in the elderly [35], so lower baseline PT values would be expected in that group. In addition, all volunteers were evaluated in the afternoon, when body temperature reaches the highest values [36]. Literature demonstrates that body temperature can decrease to values lower than 37°C early in the morning [37]. Thus, clinicians may expect even lower PT when restoring a tooth during that period of the day.

Based on the current findings, it is advisable that upcoming *in vitro* studies aiming to evaluate the effects of thermal *stimuli* on changes in pulp chamber temperature should establish methods to simulate PT values ranging from 35.0°C to 35.7°C, which are significantly lower than those observed for human body. In this regard, future *in vivo* studies will test the effect of a dental light curing unit, using different exposure times and output modes on intrapulpal temperature rise. In addition, exposures to similar teeth having facial Class V preparations are planned to test the effect of tooth structure loss on *in-vivo* temperature rise during light-curing.

5. Conclusion

Within the limitations imposed in this study, it is concluded that *in vivo* coronal human pulp presents consistent temperature values, which are significantly below that of body temperature, depending on time of measurement after tooth preparation.

Acknowledgments

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Conflict of interest

The authors declare that they have no conflict of interest.

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LEGENDS

Fig. 1: 1a - Deep occlusal preparation made to produce a minor pulp exposure. 1b - Pulp exposure after use of a pencil-shaped diamond bur. 1c - Probe insertion into the pulp chamber. 1d - Cavity sealing with provisional restorative material. 1e - X-ray analysis after tooth extraction to confirm if the probe was properly positioned into the pulp chamber.

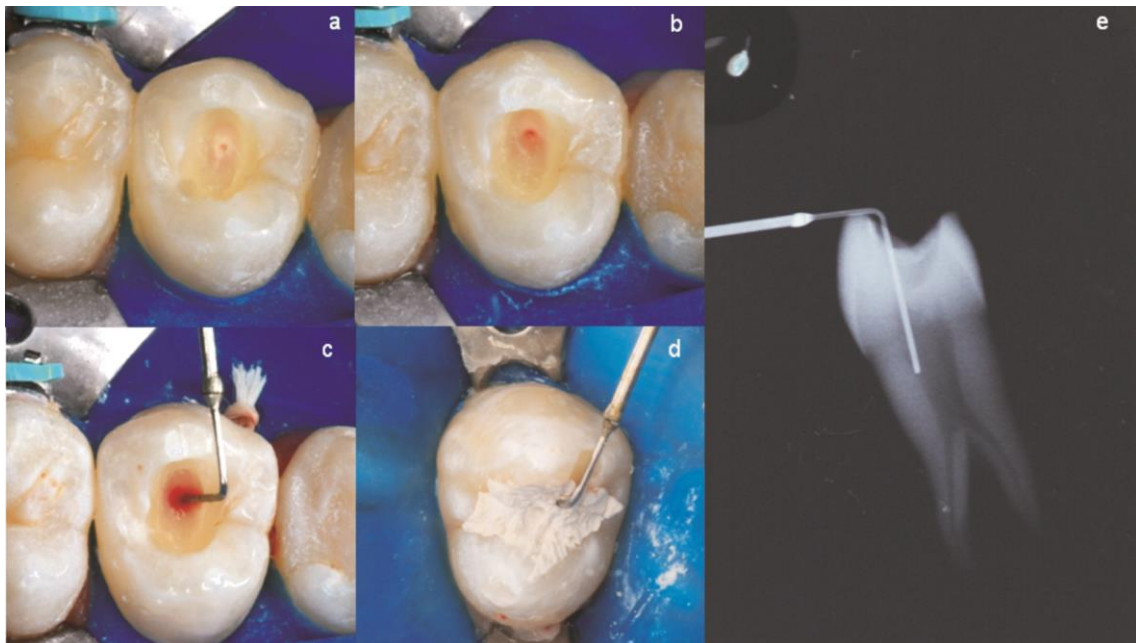
Fig. 2: Real time profile example of PT recording from the first moment the probe was immersed in sterile saline water, removed, and inserted into the pulp chamber, and allowed to remain there for 25 min.

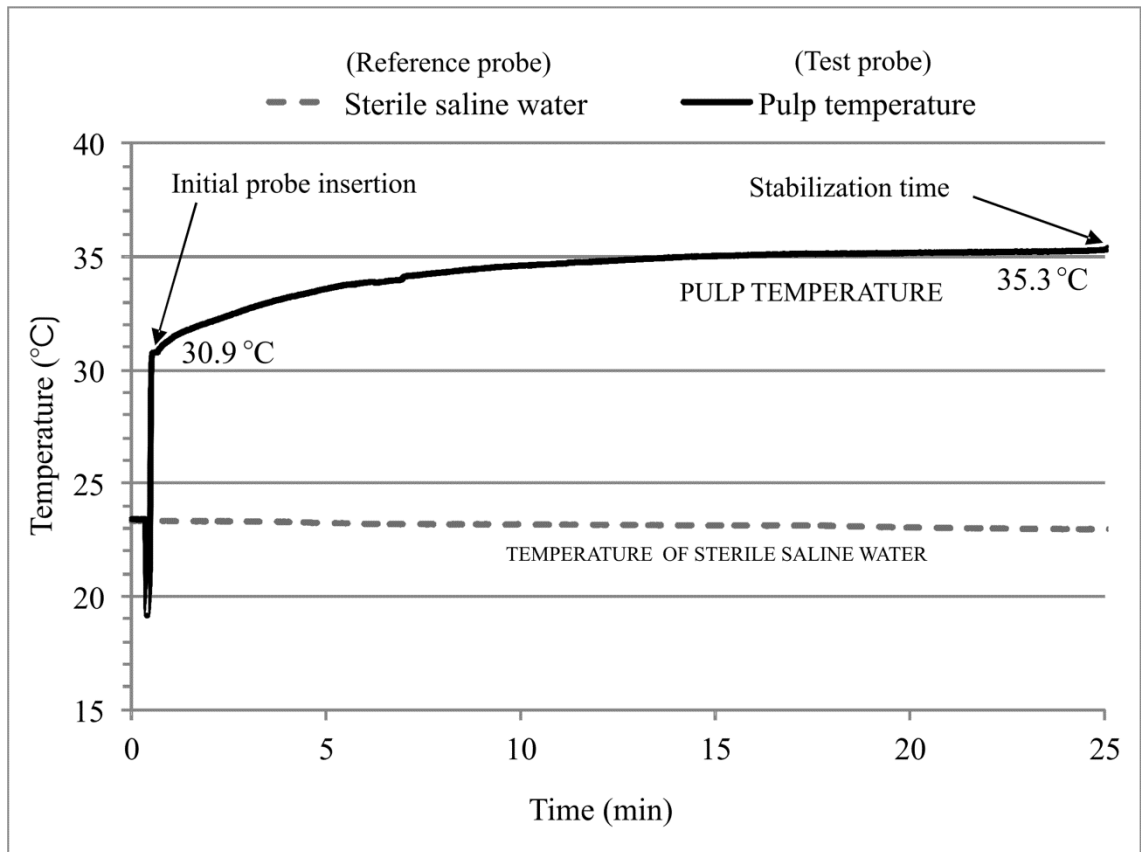
Fig. 3: Plot of the mean temperature vs time relationship used to calculate thermal time constant of the temperature data acquisition system. The time point corresponding to 63.2 % of temperature change between 25.5°C and 60.0°C corresponded to the time constant for the temperature measurement system.

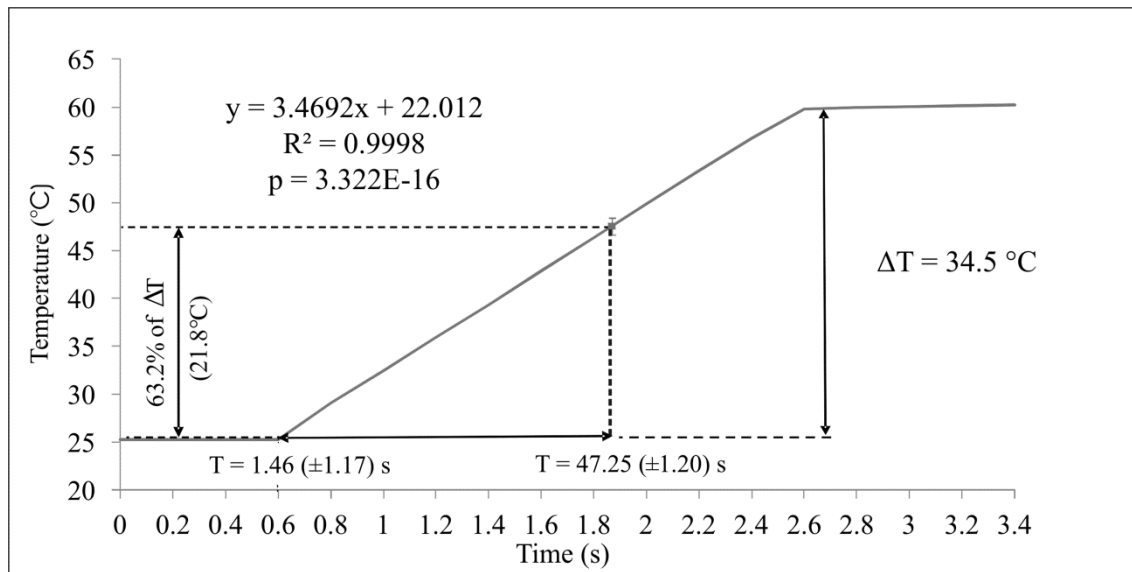
Table 1 - Initial and 25-min baseline *in vivo* PT (standard deviation)

	Pulp temperature (°C)	95% Confidence Level
Initial PT	31.8 (1.5) B	31.0 - 32.6
Baseline PT	35.3 (0.7) A	35.0 - 35.7

Means followed by different letters are significantly different (pre-set alpha = .05).







4.2 – Capítulo 2 (Experimento 2)

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***In vivo* temperature rise in anesthetized human pulp during exposure to polywave LED light curing unit**

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ABSTRACT

Objectives. This *in vivo* study evaluated pulp temperature (PT) rise in human premolars during exposure to a light curing unit (LCU) using selected exposure modes (EMs).

Methods. After local Ethics Committee approval, intact first upper premolars, requiring extraction for orthodontic reasons, from 8 volunteers, received infiltrative and intraligamental anesthesia. The teeth (n=15) were isolated using rubber dam and a minute pulp exposure was attained. A sterile probe from a wireless, NIST-traceable, temperature acquisition system was inserted directly into the coronal pulp chamber, and real time PT (°C) was continuously monitored while the buccal surface was exposed to polywave light from a LED LCU (Bluephase 20i, Ivoclar Vivadent) using selected EMs: 10-s either in low (10-s/L) or High (10-s/H); 5-s-Turbo (5-s/T); and 60-s-High (60-s/H) intensities, allowing a 7-min span between each exposure. Peak PT values and PT increases from baseline (ΔT) after exposure were subjected to 1-Way, repeated measures ANOVAs, and Bonferroni's post-hoc tests ($\alpha=0.05$). Linear regression analysis was performed to establish the relationship between applied radiant exposure and ΔT .

Results. All EMs produced higher peak PT than the baseline temperature ($p<0.001$). The 60-s/H mode generated the highest peak PT and ΔT ($p<0.001$), with some teeth exhibiting ΔT higher than 5.5°C. A significant, positive relationship between applied radiant exposure and ΔT ($r^2=0.916$; $p<0.001$) was noted.

Significance. Exposing intact, *in vivo* anesthetized human upper premolars to a polywave LED LCU increases PT, and depending on EM and the tooth, PT increase can be higher than the critical ΔT , thought to be associated with pulpal necrosis.

Keywords: Light-curing of dental resins, Body temperature/radiation effects, Dental pulp/radiation effects, dental pulp cavity, body temperature changes, humans

1. Introduction

Recently, new light emitting diode (LED) light curing units (LCUs), with radiant emittance values exceeding $2,000 \text{ mW/cm}^2$, have become commercially available, purportedly allowing clinicians to save time during in-office bleaching treatment or restorative procedures [1,2]. As a consequence, several *in vitro* studies report a significant temperature increase within the pulp chamber of extracted teeth, ranging from 1.5 to 23.2 °C, during exposure to light emitted by such LCUs [2-9]. Although this temperature range depends on the LCU type, radiant emittance, and characteristics of the tooth [2,4,5,7-13], there is a consensus that the use of some LED LCUs can result in a pulp chamber temperature rise to values higher than the threshold temperature increase of 5.5 °C, considered harmful for the pulp [14].

Despite the evidences that high-intensity LCUs can increase pulp chamber temperature, one must consider that *in vitro* conditions do not reproduce the complexities of an *in vivo* scenario. For example, most *in vitro* studies did not simulate the influence of the dental pulp. This tissue is highly vascularized and contains the main regulatory system for heat distribution in teeth, capable of dissipating the heat transferred by external thermal stimuli to the dentin/pulp complex [6,15]. Even *in vitro* studies that simulate pulp flow [3,6] were not capable of reproducing the dynamic changes in pulp fluid flow, when temperature changes in this tissue occur [16]. Indeed, because any external thermal stimuli can change the fluid movement either inward or outward from the pulp depending on the stimuli, it is important to consider that the actual *in vivo* pulp regulatory system may be more effective in dissipating external heat than would be a simulated pulp flow condition. However, no information is available in the literature regarding the *in vivo* temperature increase within the human pulp when teeth are exposed to light from a high intensity LED LCU.

The purpose of this *in vivo* study was to evaluate pulp temperature (PT) increase of anesthetized, vital, unrestored, human upper premolars during exposure to an LED-based, dental light curing unit, applying varying values of radiant exposure. The tested alternative hypotheses were that (1) all exposure modes (EM) of the LCU produce a significant PT increase over that of the baseline temperature values; (2) none of the applied EMs can produce a PT increase higher than the potentially harmful threshold temperature increase of 5.5 °C; and (3) there is a direct, positive relationship between applied radiant exposure and the PT increase.

2. Material and methods

2.1. *In vivo* measurement of pulp temperature increase

This study was approved by the Ethics Committee at State University of Ponta Grossa (protocol #255,945). Eight volunteers, ranging from 12 to 30 years, requiring extraction of upper right and left first premolars (n=15) for orthodontic reasons were selected from the Orthodontics specialization Program in Ponta Grossa, Brazil, were recruited in February, 2013, and attended to between March and April, 2013. Patient inclusion criteria included: (1) treatment plans indicating premolar extractions for orthodontic reasons, (2) the presence of healthy, intact, non-carious, and non-restored, fully erupted treatment teeth, and (3) patients with well-controlled health conditions that allowed all procedures involved in the research to be performed with minimal risk. Exclusion criteria included (1) those patients who did not agree to volunteer for the study, (2) patients not meeting all of the inclusion criteria.

A single tooth at a time from a volunteer was treated by receiving infiltrative and intraligamental anesthesia, using approximately 1.8 ml of 2 % Mepivacaine Hydrochloride (36 mg) with 1:100,000 Epinephrine (18 µg) (Mepiadre, Nova DFL Industria e Comercio, Rio de Janeiro, RJ, Brazil), after which the tooth was isolated using rubber dam. A small, occlusal preparation was made in the center of the tooth using a round diamond bur (#1015, KG Sorensen, Cotia, SP, Brazil) in a high speed handpiece, providing air-water spray, until the preparation pulpal floor was near the buccal pulp horn. Then, a small, pencil-shaped diamond bur (#2134, KG Sorensen) was used to produce a minute pulp exposure, with no pulp bleeding. Care was taken to ensure that same water flow and air pressure were used for each tooth, as well as the same time spent for each preparation. A wireless, NIST-traceable, temperature acquisition system (Temperature Data Acquisition - Thermes WFI, Physitemp, Clifton, NJ, USA) was used to measure PT. Two calibrated temperature probes were connected to that system and both were immersed in a room temperature, 0.9 % sterile saline solution, while tooth preparation was performed. After pulp exposure, one probe was removed from the saline solution and inserted into the pulp chamber through the occlusal access opening and was positioned to remain stable, while PT was measured. A small groove was created on the buccal cusp, close to the cusp tip, to allow the probe to rest on the cusp tip incline and ensure that the 1-cm long probe tip penetrated approximately 4 mm into the pulp chamber: in a similar position for all

teeth. The other probe was kept in the saline solution at room temperature (approximately 22.0 °C), as a reference. The room temperature was stable, and controlled by air conditioning set to approximately 22 °C. The preparation was then filled with provisional restorative material (Cavitec, CaiTHECLtda, SC, Brazil), to minimize heat loss from the tooth through the occlusal preparation walls and pulp access. After probe stability was confirmed, real-time temperature data were continuously acquired every 0.2 s, until a stable PT was reached: between 15 and 25 min. The LCU tip was placed against the buccal tooth surface and in a similar position with respect to the curing unit body, and the tooth was sequentially exposed to the radiant output from a polywave LED LCU (bluephase® 20i, Ivoclar Vivadent, Schaan, Liechtenstein) in the following EMs: 10-s in low intensity (10-s/L); 10-s in high intensity (10-s/H); 5-s in Turbo intensity (5-s/T); and 60-s in high intensity (60-s/H). A 7-min time span between each exposure was allowed for the PT to return to baseline levels. The sequence of EMs was randomly determined and the operator was not aware of which mode was being used. The time into the data acquisition when each light mode was applied was recorded, so that a time-based overlay of light activation and temperature could be made. At the end of the temperature data acquisition, the probe was carefully removed from the tooth, which was then atraumatically extracted as treatment planned. The probe was then reinserted into the pulp chamber of the extracted tooth and X-ray images were obtained from the proximal side, with the probe in position as it was intraorally, to confirm that the probe was properly inserted into the pulp chamber during temperature measurement.

2.2. Time constant analysis of the temperature acquisition system

To determine the response time (time constant) of the temperature acquisition instrument, three probes were connected to the system: one probe was immersed in water at RT (≈ 25.5 °C) in a Beaker, and another was immersed in water at 60 °C, in a heated circulating water bath with electronic temperature control (SL-155/22, Solab, Piracicaba, SP, Brazil). An additional probe was placed in the RT water, and was intermittently moved from that fluid to the higher-temperature one. Real-time temperature data were continuously acquired every 0.2 s from the three probes for 10 min. During this time, one probe was removed from the RT water, and immediately immersed in water at 60 °C, where the probe was left for 10 min.

Afterwards, the probe was removed from the hot water and returned to RT water again, for 10 min. This procedure was repeated 8 times ($n=8$). For each probe movement, a temperature vs time plot was developed, and the average time corresponding to 63.2% of the total temperature increase (time constant - τ) was determined for all probe movements, and was compared using a student's t-test (Statistics 19, SPSS Inc, IBM Company, Armonk, NY, USA). No statistical difference (pre-set alpha 0.05) was found for τ between direction of temperature change ($p = 0.1760$), so the overall average of the 16 measurements and its standard deviation were determined.

2.3. Radiant emittance measurement and radiant exposure calculation of the LCU

The spectral power of the different EMs was recorded five times each, using a laboratory grade spectro radiometer (USB 2000, Ocean Optics, Dunedin, FL, USA) and a 6-in integrating sphere (Labsphere, North Sutton, NH, USA), previously calibrated using a NIST-traceable light source. The LCU tip end was positioned at the entrance of the integrating sphere, so all light emitted from the unit was captured. Wavelength-based, spectral power emission during each EM was recorded using software (Spectra- Suite v2.0.146, Ocean Optics) between 350 to 550 nm, which also provided a total emitted power value for that wavelength range. The optical emitting area of the distal end of the light guide was calculated, and this value was divided into the integrated spectral power value to derive the total radiant exitance from the curing light for each EM. This value was then multiplied by the light exposure duration to derive the value of radiant exposure applied to each tooth surface for each light output mode (J/cm^2).

2.4. Statistical Analyses

Peak PT ($^{\circ}\text{C}$) and the PT increase during exposure to the LCU over that of the pre-exposure baseline value (ΔT) were subjected to a one-way, repeated measures ANOVA, followed by the Bonferroni's post-hoc test. Linear regression analysis was performed to examine the relationship between applied radiant exposure level and ΔT . The total spectral power and radiant exposure delivered by the evaluated EMs

were compared using a 1-way ANOVA followed by Tukey's post-hoc test at a pre-set alpha of 0.05. Post-hoc power analysis was performed for the statistical analyses of peak PT values, ΔT , emitted spectral power, and radiant exposure. All statistical analyses were performed using a personal statistical software (Statistics 19, SPSS Inc, IBM Company).

3. Results

3.1. *In vivo* pulp chamber temperature increase from curing light exposure

For the number of evaluated teeth ($n=15$), the *in vivo* study was adequately powered for EM factor, for both peak PT values and ΔT values (over 99.0%; $\alpha = 0.05$). Significant PT rise was observed during each EM in comparison to the baseline PT ($p < 0.001$) (Table 1). Exposure to 60-s/H resulted in the highest peak PT and highest ΔT as well ($p < 0.001$), with some pulp chambers exhibiting PT increase higher than 5.5 °C. Ten-s/L produced the lowest ΔT ($p < 0.001$). No significant difference in peak PT was observed between the 10-s/H and 5-s/T groups, which in turn, produced significantly higher peak PT and ΔT than did the 10-s/L mode ($p < 0.001$). Peak PT and ΔT values of teeth exposed to 10-s/H and 5-s/T EMs were significantly lower than those observed during exposure to 60-s/H ($p < 0.001$).

In the time/temperature profiles of PT increase during exposure to the LED LCU using the different EMs (Fig. 1), a fast increase in PT was observed after light initiation, while the magnitude of the PT increase was greater as higher radiant exposure levels were delivered. A short time interval of approximately 1 s between the moment when curing light was turned on and PT started to increase was observed in all EMs. When the LCU shut off, the PT still increased for a few seconds, and then slowly decreased to the pre-exposure baseline value, taking approximately 4 to 5 min to do so. The PT increase after the light shut off was more pronounced when the 10-s/H (Figs. 1c and 1d) and 5-s/T (Figs. 1e and 1f) EMs were used than for 10-s/L (Figs. 1a and 1b) and 60-s/H conditions (Figs. 1g and 1h). Indeed, using the 60-s/H mode, a very short time elapsed prior to observing the peak temperature after the light turned off (Figs. 1g and 1h).

3.2. Time constant analysis of the temperature data acquisition system

For the ΔT range of 25.5 °C to 60 °C, the average τ was 1.46 s (time required for temperature to reach 63.2% of ΔT); it took 1.46 s for the data acquisition device to record a 21.83 °C increase in temperature. The system demonstrated an immediate, linear response to temperature change, and not a classical “sigmoidal profile” seen using other temperature measurement instruments. The slope of the regression line between the two temperature extremes can also be used to determine the time required for the system to indicate a 1-degree Centigrade temperature change; only 0.070 s. On the other hand, the time required for the PT to rise during the exposure to light ranged from 15.5 s to 61.2 s (Table 2).

3.3. Polywave LED radiant emittance and radiant exposure values

For the number of radiant exposure replications ($n=5$), the method was adequately powered for EM factor, for both LCU radiant emittance and radiant exposure (over 99.0 %; $\alpha = 0.05$). Radiant emittance values observed in the High intensity EM were approximately 1.9 times higher than those observed in Low intensity mode ($p < 0.0001$) (Table 1), while values using the Turbo mode were 1.8 times higher than those in High intensity mode ($p < 0.0001$), and 3.4 times higher than those observed using the Low intensity mode ($p < 0.0001$). The 10-s/L EM delivered the lowest radiant exposure value among all evaluated EMs ($p < 0.0001$), while the 60-s/H mode provided the highest ($p < 0.0001$). The radiant exposure delivered using 10-s/H and 5-s/T was higher than that delivered by the 10-s/L mode ($p < 0.0001$), but lower than that delivered using the 60-s/H mode ($p < 0.0001$). The radiant exposure delivered using 10-s/H was slightly higher than that provided by the 5-s/T mode ($p < 0.0001$) (Table 1). A significant, positive relationship between applied radiant exposure and ΔT ($r^2 = 0.916$; $p < 0.001$) was detected using linear regression analysis (Fig. 2). In other words, ΔT increased linearly as higher radiant exposure was delivered to the tooth.

4. Discussion

All LCU EMs produced significant PT rise values (both peak PT and ΔT), thus the first alternative hypothesis was accepted. Although previous studies report a maximum ΔT of 23.2 °C within the pulp chamber during exposure to blue light using simulated, *in vitro*, in-office bleaching or restorative procedures [2-7,10-12], a low ΔT of between 0.5 and 4.8 °C was observed during exposure to the different EMs. Such a discrepancy between *in vitro* and *in vivo* variation in ΔT may be attributed to the thermal behavior of teeth: a complex heat conduction process associated with physiological protective mechanisms, such as dentinal fluid and blood flow attempting to protect against temperature change [15,17-19]. In other words, in addition to the relatively low thermal conductivity and diffusivity of enamel and dentin [19], the thermal properties of the tooth are also closely dependent on the fluid volume within dentin tubules and blood perfusion rate [15]: aspects functioning as a heat sink under thermal change. The lack of such pulpal physiological protective mechanisms in *in vitro* studies [2-7,10-12] demonstrates the important impact that such features have on the *in-vivo* thermoregulation of pulpal soft tissue.

All EMs generated significant ΔT values, but only the delivery of the highest radiant exposure value of 74.6 J/cm² caused ΔT values close to the 5.5 °C threshold values previously observed in the Zack and Cohen [14] study (Table 1). Indeed, although the overall average value for that EM was 4.8 °C, the ΔT values in some teeth did exceed 5.5 °C (Fig. 1). Therefore, the second alternative hypothesis was rejected. This finding means that exposure to high intensity light over long periods of time, such as 60 s, might be harmful for the pulp in some patients, assuming the temperature thresholds from Zach and Cohen's work apply to human teeth under clinical conditions. Moreover, it is important to note that intact facial surfaces of premolars were exposed to LED light in this study. In this scenario, an enamel/dentin barrier of approximately 3-mm thick is interposed between pulp chamber and curing unit tip (based on X-ray analysis of the extracted teeth used in the current study), producing approximately 90% to 95% attenuation of the curing light output reaching the pulp [20,21]. However, when a deep preparation is involved in a restorative procedure, lower light attenuation is expected, because of the thinner dentin barrier remaining [20,21]. As a consequence, the expected ΔT values could be predominantly higher than the threshold temperature increase of 5.5 °C. Thus, the

current findings can only be extrapolated to the clinical situation, such as in-office bleaching procedures or when ceramic laminates or direct composite lamination procedures are applied to intact teeth. In addition, the previously determined temperature threshold for potential pulpal damage was performed on monkeys [14], and not on humans. Only correlation of recorded *in vivo* temperature values during LED light exposure on deep cavity preparations with observed histological changes in humans can prove the clinical validity of this temperature rise value.

The linear regression analysis revealed a high, positive relationship ($r^2 = 0.9163$) between applied radiant density energy and PT increase, so the third alternative hypothesis was accepted. In other words, the results demonstrated that the higher radiant density energy delivered to intact teeth, the higher will be ΔT in pulp chamber tissue. In this regard, it is important to note that the delivery of radiant exposure values above 80 J/cm^2 will eventually result in a pulp temperature increase over the threshold temperature increase of 5.5°C . This finding is of some concern, because some new LED LCUs are capable of delivering high radiant exposure in short exposure periods, and clinicians usually expose teeth to powerful LED light for a long period of time during bleaching procedures or restorative approaches involving ceramic laminates or direct composite lamination.

Curiously, the 5-s/T and 10-s/H EMs developed similar ΔT and peak PT values in pulp chamber tissue, although the 5-s/T mode had almost twice the radiant emittance of 10-s/H mode (Table 1). However, it should not be overlooked that the exposure period using the 5-s/T mode was half that in 10-s/H mode, so both EMs delivered almost the same radiant exposure. In contrast to the current results, some authors [22] observed, in a simulated condition, that a lower temperature increase can be obtained for the same total delivery of radiant exposure if light exposure is performed using a lower radiant emittance over a long time period. Nevertheless, according to the same authors, that effect was more evident when the LCU was applied to a deep preparation having less remaining dentin thickness. Therefore, it is possible that the presence of a thick dentin/enamel wall, such as that in the intact teeth used in the present study, between the curing unit tip and pulp chamber might have masked this correlation between exposure duration, irradiance, and temperature rise. Thus, in such clinical conditions, it is reasonable to assume that ΔT is closely related to the amount of delivered radiant exposure rather than to radiant emittance alone.

In the current study, a delay of approximately 1 s was observed between the moment when the curing light turned on and the moment when PT started to increase, regardless of the EM (Fig. 1). After the LCU shut off, a further increase in ΔT , as well as in peak PT, were observed for approximately 10 s after teeth were exposed to the 10-s/L and 10-s/H EMs, and for 15 s when the 5-s/T EM was used. Conversely, the highest PT rise values in the 60-s/H EM were observed right after the LCU shut off, after which the PT slowly decreased gradually until it reached the pre-exposure baseline value ($\approx 35.3^\circ\text{C}$), requiring approximately 5 min to do so. These findings corroborate those from Linsuwanont et al. (2007)[17] and may be attributed to the relatively low thermal diffusivity of dentin ($\approx 1.87 \times 10^{-3} \text{ cm}^2/\text{s}$) and enamel ($\approx 4.79 \times 10^{-3} \text{ cm}^2/\text{s}$) [23]. As blue light strikes the enamel surface, part of the light energy is reflected, part is converted into thermal energy, while the remaining portion passes through to the substrates below [24]. The enamel surface thus has the highest initial temperature rise and can be regarded as a heat source [24]. The thermal energy from enamel dissipates inward toward the dentin and pulp chamber, as well as outward to the ambient environment [25]. Due to its low thermal diffusivity, dentin has not only the ability to transfer thermal energy but also the capability to store it [25]. Thus, thermal energy dissipates gradually towards the pulp, resulting in a sustained higher temperature in the pulp chamber, even after the curing light has shut off. This thermal behavior of dentin deserves some concern when incremental layers of resin composite are sequentially placed in a preparation followed by individual exposure to the curing light. In that clinical situation, it is reasonable to assume that multiple exposures to an LCU during a short time period could result in higher thermal energy storage in dentin, so any drop in PT would only be expected at the end of the complete restorative procedure.

The effect of temperature rise resulting from exposure to the metal temperature-measuring probe itself during an experimental run needs to be addressed. Although the amount of this contribution cannot be separated as its own factor, the rate at which temperature of the probe decreased when performing the system time constant analysis can be of assistance. During that analysis, when the probe was surrounded totally by water, the rate of temperature change was extremely linear with respect to time, and it took only 0.070 s to register a 1°C temperature change. Because the rate of temperature decrease in an exposed tooth when the curing light was turned off was much lower than the response rate of the system and also

because further increase was observed in pulp temperature for 10 s after light shut off when teeth were exposed to the 10-s/L and 10-s/H EMs, and for 15 s when the 5-s/T EM (Fig. 2), it is thought that the temperature values recorded in the present work represent mostly the effect of heating the surrounding environment rather than from that of the light-exposed metal probe.

Intraligamental anesthesia using anesthetic solution with vasoconstrictor was used to ensure that the volunteers felt no pain during the analysis. Thus, vasoconstriction may have occurred at the root apex, along the periodontal ligament, and within the pulp chamber, as previously demonstrated by others [26-28]. As mentioned previously, blood microcirculation is responsible for dissipating the temperature change caused by external thermal stimuli to the dentin/pulp complex [6,15], so lower blood flow rates resulting from vasoconstriction may have impaired the cooling effects of pulp flow during and after exposure to the curing light. Therefore, clinicians may expect lower ΔT values when only infiltrative anesthesia is performed without vasoconstrictor.

The volunteer selection was based on patients demonstrating the need for tooth extraction as part of their approved orthodontic treatment, so most volunteers consisted of young patients: approximately 14 years. It is well known that young patients present higher pulp blood flow and larger pulp chamber volumes than do older patients [29-31]. Thus, pulp response to thermal *stimuli* may vary according to the patient age. In addition, the current study was only performed on upper intact premolars, so differences in ΔT may be expected in teeth with smaller crown sizes and different thickness of buccal enamel/dentin walls, such as upper and lower incisors, which might allow more light to pass through and reach the pulp chamber.

Future work will investigate the effect of tooth removal on intrapulpal temperature rise by using controlled sized Class V preparations. In addition, the examination of histological analysis of control teeth exposed to similar LCU conditions, but whose pulp chambers were never violated for temperature measurement, will be performed to examine early signs of cellular inflammatory reactions.

5. Conclusions

Based on the limitations imposed in the current study, the following conclusions can be made: (1) exposing intact upper premolars to an LED-based, polywave LCU developed significant ΔT and peak PT in all evaluated EMs; (2) overall, none of the EMs led to higher ΔT than the threshold temperature increase of 5.5 °C, although some teeth exposed to 60-s/H EM exhibited PT increase higher than 5.5 °C; (3) a direct, positive relationship was observed between radiant exposure delivered to the tooth and ΔT , when intact teeth were exposed to the curing light.

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Conflict of interest

The authors declare that they have no conflict of interest.

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LEGENDS

Fig. 1: Examples of real time temperature increase in the pulp chamber during the exposure to light in 10-s/L (a and b), 10s/H (c and d), 5-s/T (e and f), and 60-s/H (g and h) EMs. The shaded areas represent the time interval when the teeth were exposed to LED curing light.

Fig. 2: Regression analysis plot of ΔT above the pre-exposure, baseline temperature ($^{\circ}\text{C}$) within the pulp vs. applied radiant exposure.

Table 1 – Mean (SD) radiant emittance, radiant exposure, and *in vivo* maximum temperature values and temperature increase within the pulp chamber (ΔT) above baseline value

Exposure Duration / Curing Mode	Radiant Emittance (mW/cm ²)	Radiant Exposure (J/cm ²)	Max Pulp Temp (°C)	ΔT Above Baseline (°C)
Baseline	-	-	35.3 (0.7) D	-
10-s/L	656 (12) C	6.56 (0.12) D	35.8 (0.7) C	0.5 (0.2) C
10-s/H	1244 (9) B	12.44 (0.09) B	36.3 (0.7) B	1.0 (0.3) B
5-s/T	2204 (35) A	11.02 (0.18) C	36.2 (0.7).B	1.0 (0.3) B
60-s/H	1244 (9) B	74.64 (0.55) A	40.1 (1.2). A	4.8 (1.0) A

Means followed by different upper case letters (within column) are significantly different based on a one-way repeated measures ANOVA followed by the Bonferroni's post-hoc test for temperature values within pulp and ΔT above baseline, and a one-way ANOVA followed by Tukey's post-hoc test for radiant emittance and radiant exposure (pre-set alpha = 0.05).

s/L = seconds/low s/H = seconds/High s/T = seconds/Turbo

Table 2 - Comparison between time required for peak pulp temperature rise and temperature measurement system response time

Exposure Duration / Curing Mode	ΔT (°C)	Measured time (s) for temperature rise observed (SD)	Measurement system response time (s)
Labtest	21.83	-	1.46
-	1	-	0.066
10-s/L	0.49	15.5 (4.4)	0.032
10-s/H	1.04	15.6 (5.4)	0.069
5-s/T	0.95	12.2 (6.9)	0.063
60-s/H	4.81	61.2 (0.7)	0.321
s/L = seconds/low s/H = seconds/High s/T = seconds/Turbo			

Figure 1

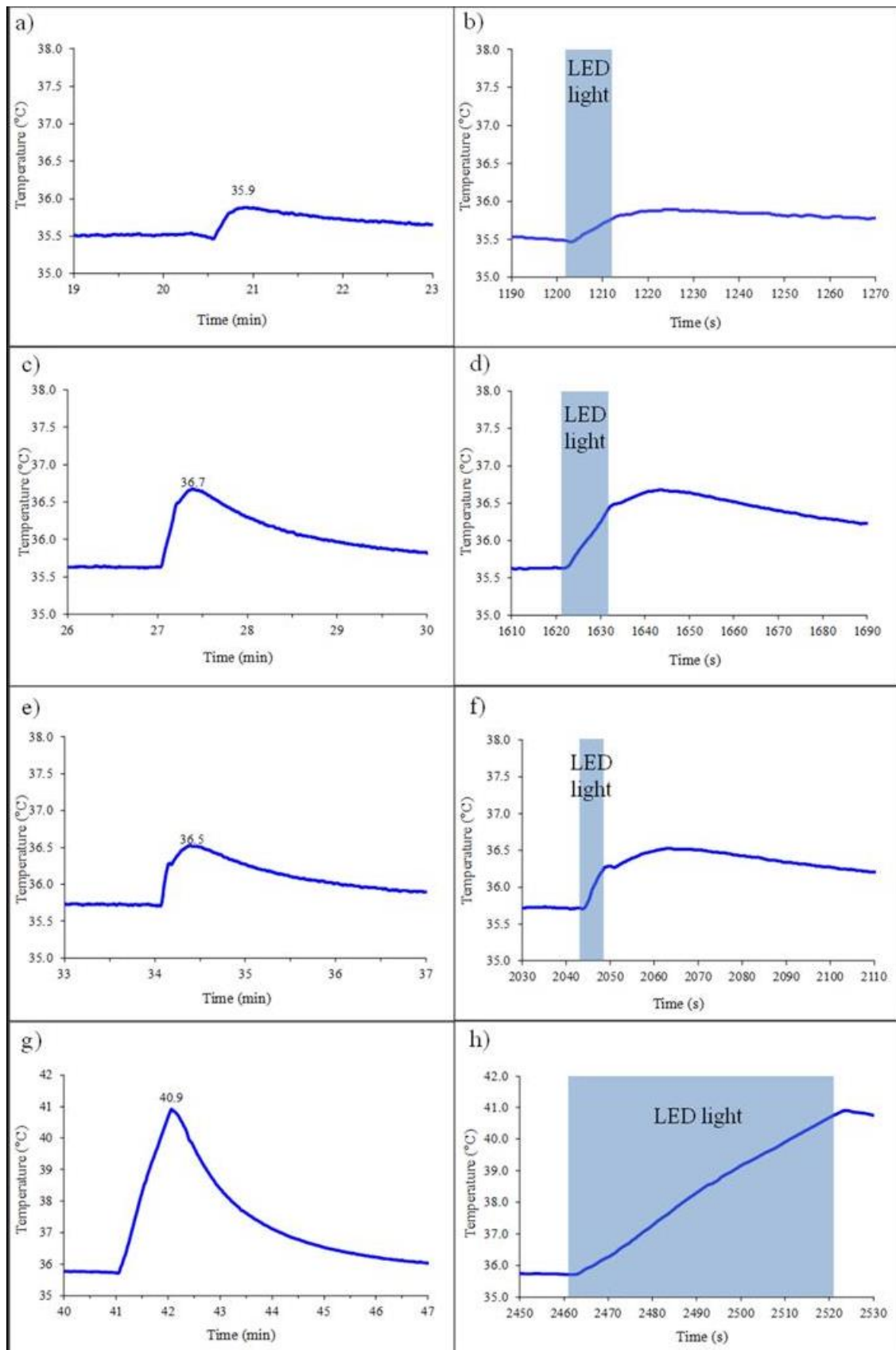
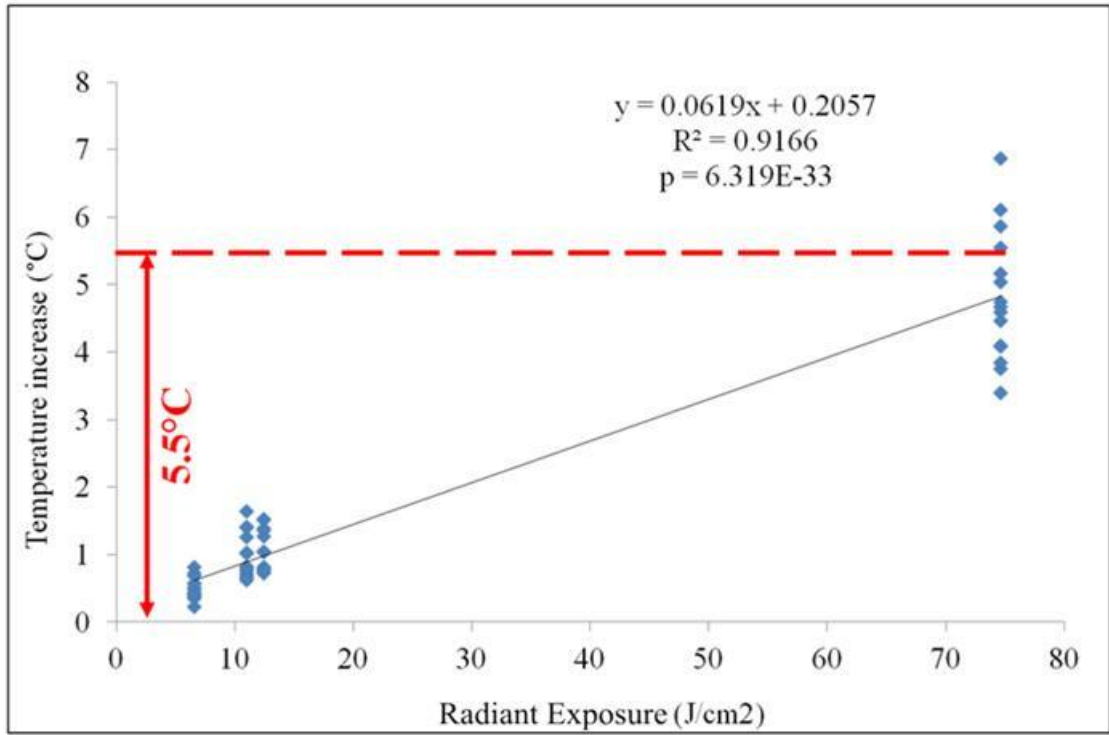


Figure 2



4.3 – Capítulo 3 (Experimento 3)

Correlation between intact premolar dimensions, patient's age, and *in vivo* increase in pulp temperature during exposure to light from of a high power LED curing unit

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Abstract

The aim of this study was to evaluate the correlation between the dimensions of premolar crown, patient's age, and *in vivo* changes of the pulp temperature (PT) when exposed to light emitted by a high power polywave LED light curing unit (LCU). The study was previously approved by the Ethics Committee of the Estate University of Ponta Grossa (protocol #255.945). The teeth (n=11) were isolated using rubber dam and a minute pulp exposure was attained using a high-speed handpiece, under constant air-water spray. A sterile probe from a wireless, NIST-traceable, temperature acquisition system was inserted directly into the coronal pulp chamber, and real time PT (°C) was continuously monitored while the buccal tooth surfaces were exposed to light from a polywave LED LCU (Bluephase 20i, Ivoclar Vivadent) in the following EMs: 10-s in low intensity (10-s/L); 10-s in high intensity (10-s/H); 5-s in Turbo intensity (5-s/T); and 60-s in high intensity (60-s/H). A 7-min time span was given between each exposure. After PT analysis was accomplished, the teeth were extracted according to treatment plan. The crown and pulp volumes were measured using micro-tomography scanner, and pulp chamber / crown volume ratio was determined. Afterwards, premolar buccal-lingual, cervico-occlusal, mesio-distal dimensions as well as between cusp distances were measured using a digital caliper. The relationship between the premolar measured dimensions, patients' age, and the range in PT increase was calculated by Pearson's Correlation test. The range in PT rise was neither related to the range in most crown dimensions nor to the range in patients' age. A significant negative correlation ($p=0.041$) was observed between buccal-lingual distance and PT increase.

Key-words: temperature, dental pulp, light curing units.

Introduction

Patient's demand for aesthetical and more conservative treatment has grown considerably in the last decades, and resin composite (RC) has become largely used by clinicians to respond to the high patient's expectations. Once the long-term success of restorative procedures with RC is highly dependent on its optimal polymerization, the quality of light curing units (LCUs) is crucial to ensure that high degree of conversion of polymer-based materials is achieved during any restorative procedure[1]. In this regard, new light emitting diode (LED) LCUs with irradiance values exceeding $2,000 \text{ mW/cm}^2$ have become commercially available, purportedly allowing clinicians to save time during in-office restorative procedures or bleaching treatment[2,3].

Although the use of such powerful LED LCUs have resulted in optimally polymerized resin composite (RC) after a quite short-time exposure to curing light[2,4], several *in vitro* studies have shown a significant temperature increase ranging from 1.5 to 23.2 °C within the pulp chamber of extracted teeth, during exposure to light emitted by such LCUs [3,5-11]. Despite the evidences that this range depends on the LCU type, irradiance, and characteristics of the tooth [3,6,7,9-15], there is a consensus that the use of some LED LCUs can result in a pulp chamber temperature rise to values higher than the threshold temperature increase of 5.5 °C, considered harmful for the pulp.[16]

In vivo, human teeth have a complex protective mechanism to dissipate the heat generated by external stimuli, such as those caused by hot food and beverages, or by some restorative procedures, such as use of high- and low-speed handpieces[17], restorative materials with exothermic setting[18], finishing and polishing procedures[19], as well as from application of light emitted by high power LED LCUs and laser sources to polymerize resin-based materials[10]. In this context, a complex heat conduction process associated with physiological protective mechanisms, such as dentinal fluid and blood flow attempting to protect against temperature change [20-23], function as a heat sink under thermal change, along with the relatively low thermal conductivity and diffusivity of enamel and dentin[23]. In this regard, the thickness of remaining dentin has an important role in attenuating the heat transmitted to the pulp during the tooth exposure to light from the LCU[24]. Besides, once pulp blood flow is influenced by patient's age[25]and pulp volume, it

should be expected that pulp capacity to protect the tooth against external thermal stimuli would be related to patient's age. Therefore, it is reasonable to assume that variations in crown and pulp chamber dimensions and patient's age would have significant influence on *in vivo* pulp temperature (PT) increase during the exposure to light from a high power LED LCU. However, no information is available on the literature regarding the relationship between tooth crown and pulp chamber dimensions, patient's age and the *in vivo* temperature increase within the pulp.

The aim of the current study was to evaluate the effect of the intrinsic dimensional variations in intact upper premolar as well as patient's age on the *in vivo* PT increase. The research hypotheses were that: (1) there is a negative correlation between premolar crown dimensions and PT increase caused by exposure to light from LED LCU with selected exposure modes (EMs); (2) there is a negative correlation between pulp chamber volume, patient's age, and PT increase regardless of EMs.

Material and Methods

***In vivo* measurement of pulp temperature increase**

This study was approved by the Ethics Committee at State University of Ponta Grossa (protocol #255,945). Eight volunteers, ranging from 12 to 30 years, requiring extraction of upper right and left first premolars (n=11) for orthodontic reasons were selected from the Orthodontic specialization program in Ponta Grossa, Brazil, were recruited in February, 2013, and attended to between March and April, 2013. Patient inclusion criteria included: (1) treatment plans indicating premolar extractions for orthodontic reasons, (2) the presence of healthy, intact, non-carious, and non-restored, fully erupted treatment teeth, and (3) patients with well-controlled health conditions that allowed all procedures involved in the research to be performed with minimal risk. Exclusion criteria included (1) those patients who did not agree to volunteer for the study, (2) patients not meeting all of the inclusion criteria.

A single tooth at a time from a volunteer was treated by receiving infiltrative and intraligamental anesthesia using 2% Mepivacaine Hydrocloride with Epinephrine, using approximately 1.8 ml of 2 % Mepivacaine Hydrocloride (36 mg) with 1:100,000

epinephrine (18 µg) (Mepiadre, DFL Industria e Comercio, Rio de Janeiro, RJ, Brazil), after which the tooth was isolated using rubber dam. A small, occlusal preparation was made in the center of the tooth using a round diamond bur (#1014, KG Sorensen, Cotia, São Paulo, Brazil) in a high speed handpiece, using air-water spray, until the preparation pulpal floor was near the buccal pulp horn. Then, a small, pencil-shaped diamond bur (2134, KG Sorensen) produced a minute pulp exposure, with no pulp bleeding. Care was taken to ensure that same water flow and air pressure were used for each tooth, as well as the same time spent for each preparation. A wireless, NIST-traceable, temperature acquisition system (Temperature Data Acquisition - Thermes WFI, Physitemp, Clifton, NJ, USA) was used to measure PT. Two calibrated temperature probes were connected to that system and both were immersed in a room temperature, 0.9% sterile saline solution, while tooth preparation was performed.

After pulp exposure, one probe was removed from the water and inserted into the pulp chamber and was positioned to remain stable, while PT was measured. A small groove was created on the buccal cusp, close to the cusp tip, to allow the probe to rest on the cusp tip and ensure that the 1-cm long probe tip penetrated approximately 4 mm into the pulp chamber: in a similar position for all teeth. The other probe was kept in the saline solution at room temperature (approximately 22.0°C), as a reference. The room temperature was stable, and controlled by air conditioning set to approximately 22°C. The preparation was then filled with provisional restorative material (Cavitec, CaiTHECLtda, SC, Brazil), to minimize heat loss from the tooth through the preparation walls and pulp access. After probe stability was confirmed, real-time temperature data were continuously acquired every 0.2 s, until a stable PT was reached: between 15 and 20 min. The LCU tip was positioned against the buccal tooth surface in a similar manner with respect to the curing unit body, and the tooth was sequentially exposed to the radiant output from a polywave LED LCU (bluephase 20i, Ivoclar Vivadent, Ivoclar Vivadent, Schaan, Liechtenstein) in the following Exposure Modes (EMs): 10-s in low intensity (10-s/L); 10-s in high intensity (10-s/H); 5-s in Turbo intensity (5-s/T); and 60-s in high intensity (60-s/H). A 7-min time span between each exposure was allowed for the PT to return to baseline levels. The sequence of EMs was randomly determined and the operator was not aware of which mode was being used. The time into the data acquisition

when the light was delivered was recorded, so that an overlay of light activation and temperature could be made. At the end of the temperature data acquisition, the probe was carefully removed from the tooth, which was then atraumatically extracted as treatment planned. The probe was then reinserted into the pulp chamber of the extracted tooth and X-rays were taken from the proximal side, with the probe in position as it was intraorally, to confirm that the probe was properly inserted into the pulp chamber during temperature measurement.

Analysis of crown and pulp chamber volumes and premolar crown dimensions

The extracted teeth were cleaned with periodontal curettes to remove remaining soft tissues and were then kept in Timol solution 0,1%. The teeth were mounted in stubs and Micro Computed Tomography (μ CT) scans were performed using a μ CT scanner (Skyscan 1172, Bruker-microCT, Kontich, Belgium), in the following parameters: tube voltage, 100 kV; tube current, 100 μ A; isotropic pixel size of 25.7 μ m, 650 tomography cross-sections per tooth obtained after 180° rotation on the tooth long axis, on a step size of 0.7°. Based on the sliced image data, three-dimensional structures (Fig 1) were obtained using a scan software (CTAn 1.11.1.0, Bruker-microCT, Kontich, Belgium), and the volumes of crown and coronal pulp cavity were measured. Both volumes were measured a part from the root region, so a virtual section was drawn perpendicular to the tooth axis, at the cementum-enamel junction to split the crown from the root. Pulp/crown ratio was determined by dividing pulp volume by crown volume values. All measurements were carried out by the same examiner.

The premolar crown mesio-distal (MD), cervico-occlusal (CO), buco-lingual (BL) dimensions, as well as the distance between cusps were measured using a digital caliper (Starrett 727-6/150; Starrett, Sao Paulo, Brazil).

Statistical analysis

PT increase values after exposure to curing light, premolar crown and pulp dimensions, and patient's age along with standard deviations and coefficient of variations were determined. Pearson's correlation test was performed to evaluate any

possible correlation between temperature increase, crown and pulp volumes, crown/pulp ratios, the evaluated crown dimensions, and patients' ages.

Results

Correlation between tooth dimensions and pulp temperature increase

The means, standard deviations, and coefficient of variation of patients' ages, crown dimensions, crown and pulp volumes, as well as PT increase after exposure to different EMs are displayed in Table 1. PT increase exhibited coefficient of variation apparently higher than that shown by crown and pulp dimensions. Only the coefficient of variation observed in crown/pulp volume ratio was apparently similar to that observed on PT increase.

The results of Pearson's correlation test are displayed in Table 2. No significant correlation was observed between most evaluated crown / pulp dimensions and the PT increase, regardless of EMs. The only significant negative correlation was noted between the BL distance and PT increase in 10s-L ($p = 0.041$). Despite the lack of statistical significance, higher non-significant correlation was noted between BL distance and 10s-H (Pearson's correlation = -0.733) and 5s-T (Pearson's correlation = -0.531) than the correlation values observed in other comparisons. A positive significant correlation was observed between BL dimension and pulp volume ($p = 0.023$).

Discussion

No significant correlation was observed between most crown dimensions including crown volume and PT increase, and the range in PT rise for each EM. Therefore, the first hypothesis was rejected. Only a significant negative correlation was noted between the BL dimension and PT increase caused by 10s-L group ($p = 0.041$). Despite the lack of statistical significance, the same trend was observed between BL dimensions and PT increase promoted by 10s-H and 5s-T EMs. In other words, higher BL dimensions resulted in lower PT increase when energy density of approximately 12 J/cm^2 was delivered to the tooth. One reason for this result is the

possible relationship between the BL dimension and the thickness of buccal surface, as higher BL dimensions might result in thicker buccal surface. Once the LCU tip was positioned on the buccal surface, this surface acts as a barrier against the emitted light. For instance, a 3-mm thick enamel/dentin barrier interposed between pulp chamber and curing unit tip can promote 90% to 95% attenuation of the curing light output reaching the pulp [26,27]. Besides, dentin has relatively low thermal conductivity [28], so a thicker dentin layer can also protect pulp better from the heat generated on the enamel surface during exposure to light. This relationship between dentin thickness and the rise in pulp chamber temperature has been observed in other studies [24,29].

Conversely, a positive significant correlation between BL dimensions and pulp volume were also observed, so higher BL dimensions infers higher pulp volumes as well. Therefore, although higher BL dimensions might result in thicker buccal surface, it is not possible to confirm any clear linear relationship between BL dimensions and the thickness of the buccal surface since pulp chamber volume also increases. This evidence was strengthened by the high coefficient of variation (28.41%) observed in the crown/pulp ratio. Based on this finding, one could also attribute the negative significant correlation between BL dimensions and PT increase to the higher pulp volume in teeth with greater BL dimensions as well. However, this was not confirmed in the current study as a non significant weak correlation was observed between pulp volume and PT range regardless of the EM. Thus, the second hypothesis regarding the correlation between PT rise range and pulp chamber volume was rejected. Apparently, the range in BL thickness played a more important role in PT range than did pulp volume itself. For this reason, these findings cannot be extrapolated to a clinical scenario where a deep cavity is prepared on the buccal surface, where a thin dentin layer remains. In this scenario, the importance of pulp volume on PT control should not be discarded. Further *in vivo* studies are required to address this clinical situation. Besides, it should be noticed that infiltrative and intraligamental anesthesia using anesthetic solution was administered, so vasoconstriction occurred at the root apex, within the periodontal ligament, and within the pulp tissue itself.[30,31] Blood microcirculation compensates for temperature changes caused by external thermal stimuli to the dentine-pulp complex [8,21]. Thus, the association of compromised pulp ability to modulate PT due to lower blood flow rates may have contributed to the low

correlation between pulp volume and the range in PT increase observed in the current study.

In this study, a weak negative correlation between patient's age and PT increase was observed, so the second hypothesis regarding the correlation between patient's age and PT rise range was rejected. Conversely, it is well known that young patients present higher pulp blood flow rate than do older patients[32-34], so teeth from older patients would present higher PT increase as a consequence of decreased pulp flow once low pulp blood flow rate is related to higher increase in pulp chamber temperature, as previously demonstrated *in vitro*[3]. For this reason, differently from the findings obtained in the current study, a positive correlation should be expected. However, because of the inclusion criteria, patients' age ranged only from 13 to 14 year, with only two patients being 28 and 29 years old, respectively. As a consequence, due to the lack of wider range in patient's ages, this assumption could not be confirmed.

The current study aimed to correlate the range observed in PT increase and the range in crown and pulp volumes, dimensions of intact upper premolars. Therefore, the current results should not be extrapolated to comparisons between teeth exhibiting morphology and sizes that are significantly different from those observed in intact upper premolars. For instance, the lower crown/pulp ratios, dimensions, and volume of incisor crowns in comparison to those observed in intact upper premolars may do compromise the protective effects of such dimensions on PT, so higher PT increase is expected in these teeth.

Conclusions

Within the limitations imposed in this study, neither crown / pulp volumes, most selected measured crown dimensions of intact upper premolars, nor patient's age had any influence on the range in PT increase caused by exposure to light from a LED LCU.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Legends:

Figure 1: Illustrative 3D images obtained after μ CT scan. Tooth crown proximal view (1a) and pulp chamber view before pulp chamber volume was determined (1b and 1c).

Table 1: Mean, standard deviation, and coefficient of variation of Δt and tooth dimensions

		Statistic
Patient age	Mean	16
	Std. Deviation	6.3
	Coefficient of Variation	39.6 %
Crown volume	Mean	316.99 mm ³
	Std. Deviation	41.09
	Coefficient of Variation	12.96 %
Pulp volume	Mean	17.03 mm ³
	Std. Deviation	3.19
	Coefficient of Variation	18.74 %
Crown / pulp ratio	Mean	19.40
	Std. Deviation	5.51
	Coefficient of Variation	28.41 %
Buccal-lingual	Mean	9.32 mm
	Std. Deviation	0.53
	Coefficient of Variation	5.71 %
Between cup distance	Mean	6.66 mm
	Std. Deviation	0.43
	Coefficient of Variation	6.51 %
Cervico occlusal dimension	Mean	7.92 mm
	Std. Deviation	0.32
	Coefficient of Variation	4.00 %
Mesio-distal	Mean	6.68 mm
	Std. Deviation	0.51
	Coefficient of Variation	7.57 %
10s-L	Mean	0.53 °C
	Std. Deviation	0.16
	Coefficient of Variation	30.8 %
10s-H	Mean	1.05 °C
	Std. Deviation	0.31
	Coefficient of Variation	29.69 %
5s-T	Mean	0.94 °C
	Std. Deviation	0.24
	Coefficient of Variation	25.82 %
60s-H	Mean	4.57 °C
	Std. Deviation	0.96
	Coefficient of Variation	20.96 %

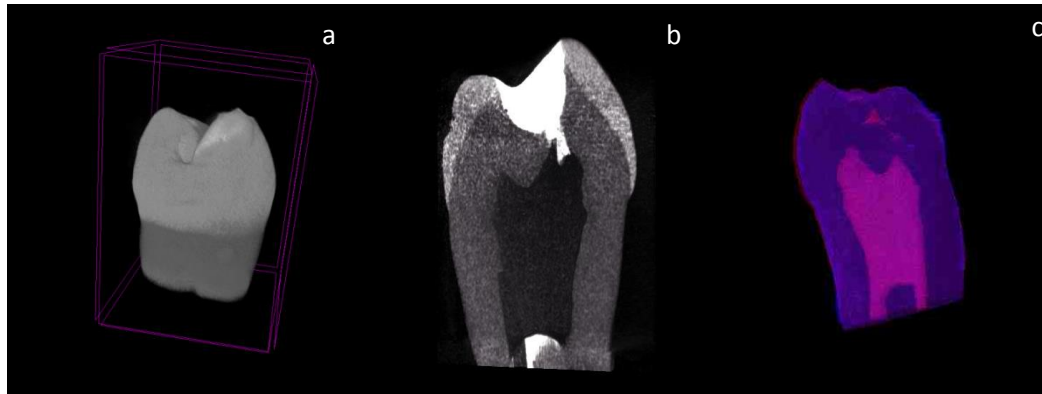
10s-L: ten-second exposure at Low intensity; 10s-H: ten-second exposure at High intensity; 5s-T: five-second exposure at Turbo intensity; 60s-H: sixty-second exposure at High intensity

Table 2: Pearson's correlation between Δt and tooth dimensions

		10s-L	10s-H	5s-T	60s-H	BL
Age	Pearson Correlation	-0.285	-0.187	-0.275	0.063	0.233
	Sig. (2-tailed)	0.396	0.582	0.414	0.853	0.614
Crown volume	Pearson Correlation	0.074	-0.068	0.009	-0.252	0.139
	Sig. (2-tailed)	0.829	0.844	0.98	0.454	0.766
Pulp volume	Pearson Correlation	0.122	0.218	0.389	0.48	.822*
	Sig. (2-tailed)	0.721	0.52	0.237	0.135	0.023
Crown / pulp ratio	Pearson Correlation	-0.073	-0.197	-0.316	-0.493	-0.614
	Sig. (2-tailed)	0.831	0.562	0.344	0.123	0.142
BL	Pearson Correlation	-.775*	-0.733	-0.531	0.172	1
	Sig. (2-tailed)	0.041	0.061	0.22	0.713	
Between cusp dimension	Pearson Correlation	0.331	0.289	0.621	0.457	-0.255
	Sig. (2-tailed)	0.469	0.529	0.137	0.303	0.581
Cervico-occlusal dimension	Pearson Correlation	0.121	0.323	0.187	0.259	-0.449
	Sig. (2-tailed)	0.797	0.48	0.688	0.575	0.313
Mesio-distal	Pearson Correlation	-0.698	-0.561	-0.597	0.074	.887**
	Sig. (2-tailed)	0.081	0.19	0.157	0.874	0.008

Pearson correlation values followed by asterisk indicates statistical significance at the 0.05 level (2-tailed). BL: buccal-lingual distance

Figure 1



5 – DISCUSSÃO

Na leitura da temperatura basal proposta no primeiro artigo, foi encontrada uma consistente temperatura de 35,3°C, verificada através de um platô estável que sempre se formava após 25 min da inserção da sonda no interior do elemento dentário, sempre bem abaixo de 37°C, valor este correspondente à temperatura corporal basal média. Uma alta acurácia foi percebida nesta metodologia uma vez que o desvio padrão ($\pm 0.7^\circ\text{C}$) foi sempre pequeno. O intervalo de confiança variou de 35,0 a 35,7°C, sendo esta temperatura encontrada inferior à temperatura sistêmica obtida pela axila, que varia de 36,2 a 37,5°C (Wunderlich, Reeve⁴⁸ 1869). Curiosamente, sempre após 3-4 min de preparo do acesso oclusal com ponta diamantada em alta rotação refrigerada com spray de ar e água, a temperatura média observada foi de 31,8°C, com um intervalo de confiança de 31-32,6°C ($\alpha=5\%$). Este é um dado bastante importante clinicamente, pois demonstra uma refrigeração prévia da polpa causada pelo preparo cavitário, o que poderia servir como uma proteção à polpa para posteriores injúrias térmicas. Esse mesmo procedimento de refrigeração prévia do tecido também é adotada em procedimentos cirúrgicos, como cirurgias cardíacas (Luehr et al.⁴⁹ 2014) e intracranianas (Wijayatilake et al.⁵⁰ 2012) como conduta protetiva.

O fotopolimerizador LED utilizado neste trabalho foi capaz de gerar aquecimento pulpar. Desta forma, a hipótese levantada no segundo artigo apresentado de que todos os modos de ativação da unidade de luz provocariam aquecimento foi aceita, uma vez que todos os grupos tiveram um aumento significativo da temperatura pulpar em relação aos valores basais. Os resultados obtidos no presente estudo não encontram pareio na literatura, uma vez que não existe até o presente momento outro trabalho *in vivo* que utiliza uma unidade fotopolimerizadora como fonte de energia. No entanto, podemos verificar certa correlação com diversos trabalhos *in vitro* (Gomes et al.¹² 2013, Porko, Hietala⁵¹ 2001, Hannig, Bott⁵² 1999, Hansen, Asmussen⁵³ 1993, Tjan, Dunn⁵⁴ 1988). Pode-se verificar que nos trabalhos *in vitro* o aumento da TP é bem maior que o encontrado neste trabalho quando comparamos as mesmas densidades de energia (6,56 – 12,44 J/cm²). Contudo, não há nenhum trabalho que tenha aplicado uma densidade de energia semelhante (74,64 J/cm²) em uma única aplicação *in vivo*. Ainda assim, o

ΔT registrado, 0,5 a 4,8°C de aumento da temperatura na câmara pulpar foi menor que o descrito nos estudos *in vitro* que utilizaram menores densidades de energia e que observaram maiores aumentos de temperatura. (Baroudi et al.¹⁹ 2009, Park et al.²⁵ 2010, Kodonas et al.²⁷ 2009, Yazici et al.²⁹ 2006, Eldeniz et al.³⁹ 2005).

Podemos destacar algumas características das metodologias aplicadas *in vitro* como a provável diferença dos resultados. Vários trabalhos deixaram os elementos em banho de imersão para manter a temperatura estável ou tentando assemelhar-se a condição real, porém utilizando temperatura inicial (basal) *in vitro* diferente, 37°C, (Baldissara et al.²² 1997, Kodonas et al.²⁷ 2009, Kodonas et al.²⁸ 2009, Millen et al.¹⁸ 2007, Yazici et al.²⁹ 2006) superior àquela observada no presente estudo (35,3°C) para um pré-molar superior anestesiado. Alguns trabalhos tentaram controlar variáveis reproduzindo condições encontradas na boca, porém sem reproduzir a microcirculação da polpa (Goodis et al.⁵⁵ 1989). Possivelmente, a microcirculação da polpa seja uma variável de grande influência no controle do calor transferido ao tecido pulpar (Kodonas et al.²⁷ 2009, Kodonas et al.²⁸ 2009). O complexo dentina/polpa tem mecanismos de defesa autorregulatórios que respondem de diferentes formas e níveis frente ao aumento de calor seja pelo fluido dos túbulos dentinários, fluxo pulpar (Raab⁴⁴ 1992, Lin et al.⁵⁶ 2011, Linsuwanont et al.⁵⁷ 2007, Goodis et al.⁵⁸ 2000, Lin⁵⁹ 2010) ou pela condutividade ou difusão térmica da dentina e do esmalte (Hargreaves et al.³¹ 2002, LIN⁵⁹ 2010).

A hipótese levantada no segundo artigo apresentado de que nenhum modo de ativação seria capaz de promover aquecimento maior que 5,5 °C foi rejeitada, pois apesar dos grupos 10s/L, 10s/H, 5s/T se manterem em patamares descritos como seguros, com aumento da temperatura menor que 5,5 °C (Zach, Cohen²¹ 1965), no grupo 60s/H, 33,33% das aplicações ultrapassaram o valor tido como seguro, podendo ocasionar possíveis danos a alguns dentes expostos. Existe uma divergência de interpretações na literatura de onde seria o limite seguro da temperatura, embora a maioria dos autores concorde com o aumento na ordem de 5,5 °C como seguro (Zach, Cohen²¹ 1965). Por causa dos resultados de Zach e Cohen (1965) (Zach, Cohen²¹ 1965), alguns autores colocam como limite seguro um aumento até 40,5°C (Hargreaves et al.³¹ 2002) enquanto outros como 42,4 °C (Amano et al.³² 2006, Kitamura et al.³³ 2005). A diferença entre os limiares de

tolerância determinados por diferentes autores pode ser atribuída a diferentes interpretações da temperatura basal estipulada. Deste modo, para aqueles autores que consideram a temperatura pulpar basal como sendo 37°C e ao se considerar os achados de Zach e Cohen (1965), o limiar de tolerância para a temperatura pulpar seria de 42,4°C. Por outro lado, para aqueles que consideram a temperatura basal como sendo 35,0°C, o limiar de tolerância pulpar seria de aproximadamente 40,5°C. Da mesma forma, para os que utilizaram em seus estudos entre 33 e 34°C, o limite poderia ser entre 38,5 e 39,5°C. Deste modo, com a interpretação da temperatura basal ser de 35,3°C, o limite seguro seria de 40,8°C. Assim, estudos adicionais em humanos são necessários para se determinar melhor o limiar de tolerância pulpar ao aumento de temperatura. Nos trabalhos de Baldissara et al. (1997) não verificaram nenhum tipo de alteração celular ou histológica nos cortes realizados entre 60 e 91 dias após o estímulo térmico com aumento de 8-9°C em pré-molares humanos in vivo (Baldissara et al.²² 1997), contrariamente aos achados de Zach e Cohen (1965). Nesse estudo, os resultados mostraram baixa susceptibilidade celular ao calor (Baldissara et al.²² 1997), quando foi utilizado um termoresistor para gerar calor acoplado a uma incrustação metálica acoplada à face oclusal de pré-molares.

No presente estudo, a exposição à luz foi realizada em apenas uma aplicação seguida de 7 min de intervalo. Possivelmente, em um típico ato clínico restaurador em que incrementos de resina são adicionados à cavidade e fotopolimerizados em sequência, as aplicações consecutivas de luz podem ter um intervalo menor, não permitindo o retorno à temperatura basal, e consequentemente registrar níveis maiores de temperatura. Somando o calor gerado pelas reações exotérmicas dos materiais resinosos como polimerização do sistema adesivo (Godoy et al.⁶⁰ 2007), o cenário poderia ser pior, principalmente em dentes com cavidades mais profundas, com aproximadamente 1 mm de remanescente dentinário (Ozturk et al.⁶¹ 2004). Todavia deve-se considerar que é recomendado para cavidades profundas o uso de forradores como o cimento de ionômero de vidro e o cimento de hidróxido de cálcio. Fazendo assim uma proteção à polpa.

Outra diferença encontrada entre os trabalhos *in vitro* e *in vivo* que pode alterar significativamente os resultados é o fato de vários trabalhos utilizarem metodologias distantes da realidade bucal, como por exemplo, o uso de fragmentos

de dentina (Tjan, Dunn⁵⁴ 1988, Ozturk et al.⁶¹ 2004). Com isso, diferentes metodologias impedem a possibilidade de uma reprodução fidedigna das mesmas condições de condutividade térmica (dentina $0,0015 \text{ cal cm sec}^{-1} \text{ cm}^{-2} \text{ }^{\circ}\text{C}^{-1}$ e esmalte $0,0022 \text{ cal cm sec}^{-1} \text{ cm}^{-2} \text{ }^{\circ}\text{C}^{-1}$) e difusividade térmica ($0,00183 \text{ cm}^2/\text{sec}^{-1}$ para a dentina e $0,00469 \text{ cm}^2/\text{sec}^{-1}$ e para o esmalte), encontradas em dentes hígidos (Hargreaves et al.³¹ 2002).

Ficou comprovada a relação positiva ($r^2 = 0,9163$) entre a densidade de energia depositada e o aumento da temperatura pulpar. As duas são diretamente proporcionais como já havia sido destacado na literatura (Asmussen, Peutzfeldt¹¹ 2005) e observado na análise de regressão da figura 2 (capítulo 2). A hipótese apresentada no segundo artigo da relação positiva entre densidade de energia depositada e aumento de temperatura foi aceita. Interessante destacar que os grupos 5s/T e o grupo 10s/H apresentaram resultados de aquecimento pulpar semelhante mesmo o primeiro com a metade do tempo de exposição e pior intensidade (potência) em relação ao segundo. É possível que a irradiância seja mais importante do que o tempo em contraste com os achados de outros autores (Jakubinek et al.²³ 2008). No entanto, de acordo com os mesmos autores, este efeito foi mais evidente quando a luz do LED foi aplicada em uma cavidade profunda tendo menor espessura de dentina remanescente. Portanto, é possível que a presença de uma parede de dentina/esmalte espessa entre a ponta da unidade de fotopolimerização e câmara pulpar tal como nos dentes intactos usados no presente estudo, pode ter mascarado esta relação entre a duração da exposição, irradiância, e o aumento da temperatura.

Nota-se que mesmo após a interrupção do estímulo luminoso, a temperatura continua a subir mantendo a inércia térmica por aproximadamente 10 s em média antes de iniciar o retorno aos valores da temperatura basal $35,3^{\circ}\text{C}$. Desta forma observa-se que a energia recebida pelos tecidos duros continua a irradiar sendo transmitida até a polpa dissipando o aumento da temperatura. Esta demora provavelmente seja atribuída à baixa difusividade térmica da dentina ($1,87 \times 10^{-3} \text{ cm}^2/\text{s}$) e do esmalte ($4,79 \times 10^{-3} \text{ cm}^2/\text{s}$) (Brown et al.⁶² 1970). Apenas no modo de exposição 60s/H (potência $1244 \text{ mW}/\text{cm}^2$ e densidade de energia $74,64 \text{ J}/\text{cm}^2$) a

queda da temperatura se inicia logo após a interrupção do estímulo com um período de aproximadamente 5 min até estabilizar novamente.

A anestesia local infiltrativa e intraligamentar com vaso constritor que foi ministrada para evitar desconfortos aos pacientes pode ter ocasionado uma redução no fluxo pulpar como demonstrado em outros estudos (Hashimoto et al.⁶³ 2014, Kim et al.⁶⁴ 1984). Com isso, o efeito de refrigeração da polpa talvez tenha sido diminuído, uma vez que o mesmo está relacionado ao fluxo pulpar (Kodonas et al.²⁸ 2009, Raab⁴⁴ 1992).

Os resultados de aumento de temperatura pulpar foram correlacionados, pelo teste de Pearson, com as dimensões aferidas das coroas dos pré-molares, bem como o volume avaliado pela microtomografia da câmara pulpar e da coroa. No entanto, não foram encontradas correlações positivas, na tentativa de estabelecer uma relação entre ΔT e as variações anatômicas nos elementos dentários estudados. Uma correlação fraca e negativa e sem significância entre a idade dos pacientes e o aumento de temperatura também foi observada. Deve-se salientar que devido aos critérios de inclusão e exclusão, os voluntários apresentavam idades muito semelhantes, sendo apenas dois voluntários com maior idade. Deste modo, tamanha proximidade na faixa etária avaliada não permite extrapolar os resultados para comparações que envolvam sujeitos com maior variação de idade. Por outro lado, foi observada uma correlação positiva significativa entre as dimensões VL e o aumento da temperatura pulpar. Possivelmente, isso pode ser atribuído à possível relação entre a dimensão VL e a espessura da face vestibular. Como consequência, maior dimensão VL resulta em maior espessura da face vestibular, a qual atenuaria com mais eficiência os efeitos da luz e do calor gerado pela luz do fotopolimerizador. Deve-se salientar que a sequência de estudos presente neste trabalho está relacionada à variação de temperatura pulpar em pré-molares íntegros. Desta forma, pode-se esperar estes resultados em situações clínicas como clareamento dental ou cimentação de facetas ou *onlays*, em que a luz atravessará uma estrutura que se assemelhe a estrutura de uma coroa íntegra. Mais estudos são necessários para saber o comportamento da polpa em cavidades mais profundas como em classe V, frente ao aquecimento oriundo de fotopolimerização com LED de alta potência, um equipamento de uso corriqueiro em nossos consultórios, mas que pode gerar um aquecimento do tecido pulpar e ocasionar eventuais danos.

6. CONSIDERAÇÕES FINAIS

Com a metodologia empregada neste estudo, podemos concluir que:

- A temperatura basal da polpa dental humana anestesiada de pré-molares *in vivo* é de 35,3°C e pode variar de 35 a 35,7°C.
- Todos os modos de exposição do LED testado produzem um aumento da temperatura pulpar significativo em comparação com os valores basais.
- O modo de exposição 60s/H foi capaz de promover um aumento da temperatura pulpar maior do que aquele considerado pela literatura como seguro à polpa, (5,5 °C), em 4/12 dos espécimes avaliados.
- O aumento da temperatura pulpar não apresentou correlações nem com o volume coronal nem com volume da câmara pulpar, assim como a maioria dos parâmetros avaliados.

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ANEXO A

Aprovação do projeto pela Comissão de Ética em Pesquisa da Universidade Estadual de
Ponta Grossa. COEP - UEPG

UNIVERSIDADE ESTADUAL DE
PONTA GROSSA - UEPG



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito da exposição da luz de um fotoativador LED de alta potência na temperatura intrapulpar in vivo e na resposta inflamatória pulpar.

Pesquisador: Cesar Augusto Galvão Araújo

Área Temática:

Versão: 1

CAAE: 14591513.9.0000.0105

Instituição Proponente: Universidade Estadual de Ponta Grossa

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 255.945

Data da Relatoria: 25/04/2013

Apresentação do Projeto:

Quinze sujeitos voluntários apresentando indicação de exodontia de pré-molares superiores higidos por razões relacionadas a tratamento ortodôntico serão avaliados na presente pesquisa. serão confeccionadas cavidades em algumas facas dos dentes para inserir cuidadosamente uma sonda termopar, a fim de medir a temperatura pulpar. Em tempo real, por 15 segundos previamente à ativação, durante a exposição à luz, e por mais 60 segundos após exposição à luz. Além da avaliação térmica, as mudanças de fluxo sanguíneo através de Laser Doppler. Assim como na análise de temperatura pulpar, os valores de fluxo sanguíneo basais serão utilizados como controle na comparação com a variação do fluxo após fotoativação (n=15).

Já para a análise histológica, serão considerados dois grupos, um grupo de dente onde não haverá exposição e outro grupo de dentes com exposição a luz do fotoativador (n=7).

Cortes histológicos na espessura de 5 µm serão obtidos, os quais serão corados com Hematoxilina e Eosina (HE) e analisados em microscopia óptica de luz, com o

objetivo de se realizar um estudo histomorfométrico com auxílio do programa ImagePro-Plus®.

Objetivo da Pesquisa:

Objetivo Primário:

A avaliação das possíveis alterações pulpares pela exposição à luz por uma unidade de fotoativação LED de alta potência.

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Objetivo Secundário:

1. Avaliar e quantificar de forma direta e em tempo real a alteração de temperatura pulpar *in vivo* durante a exposição de pré-molares expostos a um fotoativador LED de alta potência.
2. Avaliação por meio da Fluxometria de Laser Doppler (Moor Instruments, Wilmington, DE, EUA) as mudanças do fluxo sanguíneo *in vivo* ocasionadas pela exposição à luz do fotoativador.
3. Verificar através do microscopia óptica de luz as características histomorfométricas do tecido pulpar para se averiguar a possível presença de alterações inflamatórias pulpares.

Avaliação dos Riscos e Benefícios:**Riscos:**

Riscos para os sujeitos da pesquisa de contaminação ou dor existem por se tratarem de procedimentos que envolvem respostas biológicas e individuais, porém os mesmos serão atendidos seguindo os protocolos de anestesia local e analgesia sistêmica necessários e já estabelecidos por órgãos internacionais. Também será respeitada a cadeia asséptica. Todo instrumental e campo cirúrgico serão devidamente esterilizados na Universidade Estadual de Ponta Grossa por funcionários treinados seguindo os protocolos estabelecidos pela vigilância sanitária. Para todo e qualquer procedimento, os profissionais envolvidos utilizarão os Equipamentos de Proteção Individual apropriados, como avental cirúrgico, gorro, máscara, óculos de proteção, e luvas cirúrgicas esterilizadas.

Benefícios:

Atualmente, não há na literatura nenhuma informação a respeito da temperatura basal da polpa dental e dos efeitos da exposição à luz de fotoativadores LED de alta potência nesta temperatura e fluxo pulpares e suas consequências na saúde do tecido pulpar. Tais informações são fundamentais para o melhor entendimento dos mecanismos reguladores da pulpa dental bem como do risco que o uso de fotoativadores muito potentes pode representar à vitalidade do dente. Desta forma, todos os resultados gerados neste estudo serão divulgados na forma de artigos científicos contribuindo com informações relevantes e inéditas, uma vez que o projeto é de cunho original. Os pacientes voluntários na presente pesquisa receberão como maior benefício: orientações de saúde bucal, bem como será atendida sua necessidade de realizar extrações dentárias de forma gratuita.

Comentários e Considerações sobre a Pesquisa:

Projeto inovador e de grande relevância pois trará informações sobre temperatura basal da polpa dental e dos efeitos da exposição à luz de fotoativadores LED de alta potência nesta temperatura e fluxo pulpares e suas consequências na saúde do tecido pulpar.

Considerações sobre os Termos de apresentação obrigatória:

Os termos estão de acordo com as exigências legais.

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Recomendações:

Alterar no campo "desenho" o número de sujeitos voluntários.

Conclusões ou Pendências e Lista de Inadequações:

Sou de parecer favorável a execução da pesquisa.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

PONTA GROSSA, 25 de Abril de 2013

Assinado por:

P/ Ana Cristina Oltramari Toledo
(Coordenador)

Endereço: Av. Gen. Carlos Cavalcanti, nº 4748 - B. Maré 12

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ANEXO B

Termo de consentimento livre e esclarecido, fornecido aos pacientes.

TERMO DE CONSENTIMENTO ESCLARECIDO**Temperatura intra pulpar**

Universidade Estadual de Ponta Grossa (UEPG)

Doutorado em Odontologia – Área de Concentração Dentística Restauradora

Você ou seu (sua) filho (a) está convidado a participar de um projeto de pesquisa que está sendo feito com a aprovação da UEPG. As informações seguintes tem por objetivo lhe fornecer condições de informar-se sobre o estudo e decidir participar voluntariamente:

Este estudo ira verificar a temperatura, alterações biológicas e inflamatórias da polpa dental (“nervo”), quando exposta a unidade fotopolimerizadora (“luz azul usada para endurecer obturação”). Esta pesquisa será feita *in vivo* (dente na boca) depois estes dentes serão extraídos para avaliações. Serão utilizados somente dentes pré-molares com indicação de extração por motivos ortodônticos. Para realizar o estudo serão feitos procedimentos de desgaste (corte) em forma da letra V na parede do dente e assim exposto à luz e avaliado com o aparelho Doppler e em seguida restaurados com um cimento de ionômero de vidro na parte da manhã, à tarde serão feitas as extrações dos dentes para subseqüentes pesquisas. O estudo será composto de quatro fases: primeira sessão marcado previamente, segunda e terceira no mesmo dia (a ser agendada em comum acordo) e a ultima uma semana após. Na primeira, anamnese (avaliação médica) e molde da boca. Na segunda, confecção dos desgastes, avaliação com termômetro e Doppler, restauração com ionômero de vidro. Na terceira, extração dos dentes indicados. Na quarta, remoção dos pontos da cirurgia. Participando da pesquisa os Srs.(as) terão suas extrações realizadas gratuitamente e os pacientes serão acompanhados por um profissional experiente enquanto necessário e sem custo. Todos os procedimentos serão realizados com anestesia local, para que nenhum paciente tenha sensibilidade acentuada durante a pesquisa. Todos os dados pessoais serão mantidos em sigilo.

Você pode sair desta pesquisa a qualquer momento e não acarretará em prejuízo para buscar tratamento dentro da UEPG pelos meios comuns. Qualquer informação ou dúvida sobre o projeto ou procedimentos serão respondidas pelo Dr. Patricio Runnacles Fone: 0xx41- 9986-8202. Este projeto está em processo de aprovação pela Comissão de Ética em pesquisa da UEPG, para esclarecer qualquer duvida entre em contato com a Comissão de Ética pelo telefone 0xx42 3220-3108. Universidade Estadual de Ponta Grossa Av. Carlos Cavalcanti, 4748 – Uvaranas Bloco M - Sala 12 - Campus Universitário CEP: 84030-900 - Ponta Grossa - PR

Tendo lido esta declaração, concordo em participar (ou autorizar meu filho (a) a participar) deste projeto de pesquisa clinica na UEPG.

Assinatura do paciente ou responsável

/ /
data

Assinatura do pesquisador

/ /
data