PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
DOUTORADO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO EM MATERIAIS DENTÁRIOS

LUCIANA BORGES RETAMOSO

AVALIAÇÃO DA CITOTOXICIDADE, LIBERAÇÃO DE MONÔMERO RESIDUAL, SORÇÃO E SOLUBILIDADE EM ÁGUA DE RESINAS COMPOSTAS

Prof. Dr. Hugo Mitsuo Silva Oshima
Orientador

Porto Alegre
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Tese apresentada como parte dos requisitos obrigatórios para a obtenção do título de Doutor na área de Materiais Dentários pelo Programa de Pós-Graduação da Faculdade de Odontologia da Pontifícia Universidade Católica do Rio Grande do Sul.

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Prof. Dr. Paulo Afonso Burmann

À Deus,

Inesgotável fonte de inspiração.

Aos meus pais, Getulio Rocha Retamoso e Magdale Borges Retamoso

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DEDICO

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"Segue sempre teu coração,

A Preocupação olha em volta,

A Saudade olha para trás,

A descrença olha para baixo,

A Fé olha para cima

A Esperança olha para a frente".

(Autor desconhecido)

RESUMO

RETAMOSO, L. B. AVALIAÇÃO DA CITOTOXICIDADE, LIBERAÇÃO DE MONÔMERO RESIDUAL, SORÇÃO E SOLUBILIDADE EM ÁGUA DE RESINAS COMPOSTAS Orientador: Prof. Dr. Hugo Mitsuo Silva Oshima. Porto Alegre, PUCRS, Faculdade de Odontologia – Tese (Doutorado em Materiais Dentários), 2011.

O objetivo deste trabalho foi avaliar a toxicidade de resinas compostas utilizadas em Odontologia por meio do teste de citotoxicidade "in vitro", bem como determinar a sorção e solubilidade em água e a liberação de monômero residual destes materiais. Desta forma, foram montados 3 grupos de acordo com a classificação das resinas: resina nanoparticulada (Supreme), nanohíbrida (Esthet-X) e microhíbrida de partículas finas (4seasons). Um único incremento de resina foi inserido em uma matriz de teflon de 3mm de diâmetro e 2mm de espessura e foram imediatamente polimerizados. Cada grupo foi subdivido em 2 de acordo com a fonte de luz utilizada para polimerização das resinas compostas (luz halógena e LED) (n=10). A mensuração da sorção e solubilidade em água foi obtida pela pesagem, em balança de precisão, antes a após imersão em água e em dessecador. A liberação de monômero residual foi realizada por espectrofotometria por ultravioleta após 24, 48, 72 e 168 horas. O ensaio de citotoxicidade foi realizado por meio de cultura de fibroblastos (linhagem NIH/3T3) em meio D-MEM completo. Após obtenção de confluência de 80%, a suspensão foi adicionada sobre as placas de 24 poços, contendo os corpos de prova, sendo incubados em estufa a 37ºC, por 24, 48, 72 e 168 horas. Após esse período, a viabilidade celular foi verificada pelo teste do MTT. Os valores para cada teste foram tabulados e analisados estatisticamente. Os resultados demonstraram que a fonte de luz utilizada não influenciou a sorção e solubilidade em água. Entretanto a liberação de monômero residual e a citotoxicidade foram influenciadas pela fonte de luz, com a fotopolimerização com LED reduzindo a liberação de monômero e consequentemente, a citotoxicidade. O tempo interferiu apenas na liberação de monômero, com pico após 3 dias. Concluiu-se que todas as resinas estudadas demonstram alteração após imersão em água, diferentes níveis de liberação de monômero residual e citotoxicidade. Além disso, pôde-se afirmar que as resinas compostas fotopolimerizadas por LED apresentam menor liberação de monômero residual e citotoxicidade.

Palavras-chave: Citotoxicidade. Resinas Compostas. Sorção de água. Solubilidade. Monômero residual.

ABSTRACT

RETAMOSO, L. B. Evaluation of cytotoxicity, monomers releasing, water sorption and solubility of composite resins. Orientador: Prof. Dr. Hugo Mitsuo Silva Oshima. Porto Alegre, PUCRS, Faculdade de Odontologia – Tese (Doutorado em Materiais Dentários), 2011.

This study aimed to evaluate the toxicity of composite resins through an "in vitro" cytotoxicity test, as well as, to determine the water sorption, solubility and released monomers. The samples were divided into 3 groups: nanofiller composite resin (Supreme), nanohybrid composite resin (Esthet-X) e microhybrid composite resin (4seasons). Only one resin composite increment was placed into teflon molds (3mm diameter and 2mm high) and was photopolymerized. Each material was divided into 2 subgroups according curing light unit used to photopolymerized composites. Water sorption and solubility measurements were obtained by means of weighting the samples before and after water immersion and desiccation. To quantify the residual monomers released from composites, using ultraviolet spectrophotometry (UV). The cytotoxicity assay was performed by fibroblast culture (NIH/3T3 line) in complete D-MEM. With a confluence of 80% the suspension was added on the plaques of 24 wells with the samples and incubated at 37°C for 24, 48, 72 and 168 hours. The cell viability was quantified by MTT assay. The values were statistically analyzed and the results revealed that light curing unit did not influence water sorption and solubility. On the other hand, monomers release and cytotoxicity were influenced by photopolymerization. The different periods evaluated interfered only for leaching monomers, with maximal concentration at the 3-day period. We concluded that all composites demonstrated modification after water immersion, different ranges of monomers releasing and cytotoxicity. Thus, the monomers release and cytotoxicity decreased with composite resin were photopolymerized by LED.

Key words: Cytotoxicity. Composite resins. Water sorption. Solubility. Monomer release.

LISTA DE ILUSTRAÇÕES

LISTA DE SIGLAS E ABREVIATURAS

et al. et alli - e outros

ºC - graus Celsius

LED - light emittig diode, luz emissora de diodo

Bis-EMA - ethoxylated bisphenol A dimethacrylate, bisfenol A etoxilado

Bis-GMA - bisphenol-A glycol dimethacrylate, bisfenol-A glicidil metacrilato

TEGDMA - triethyleneglycol dimethacrylate, trietilenoglicol dimetacrilato

UDMA - urethane dimethacrylate, uretano dimetacrilato

mm - milímetro

nm - nanômetro

mJ/cm^2 - mili joule por centímetro quadrado

mW/cm^2 - mili watt por centímetro quadrado

µg - micro grama

h - hora

$\mu g/m^3$ - micro grama por metro cúbico

v - volume

$mJcm^2$ - mili joule centímetro quadrado

p - nível de significância

N - newton

mg/L - miligrama por litro

µL - micro litro

TPP - Tissue Culture Labware

D-MEM - Dulbecco's Modified Eagle Media

ATCC - American Type Culture Collection

s - segundos

MTT - 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium Bromide, 3-(4,5-dimetiltiazol-2yl) -2,5-difenil brometo de tetrazolina

PUCRS - Pontifícia Universidade Católica do Rio Grande do Sul

USA - United States of America

LISTA DE SÍMBOLOS

% por cento

® marca registrada

< menor que

> maior que

Ba bário

Al alumínio

CO_2 dióxido de carbono, gás carbônico

SUMÁRIO

1. INTRODUÇÃO GERAL

Define-se biocompatibilidade como a qualidade de um material em não causar injúrias ou efeito tóxico sobre os sistemas biológicos (Kao et al., 2007, Freitas et al., 2009). Para Wataha, 2000, a determinação da biocompatibilidade é um processo complexo que envolve testes "*in vitro*" e "*in vivo*".

A citotoxicidade das resinas compostas está principalmente associada a quantidade de monômeros liberada ao meio bucal (Wada et al., 2004; Al- Hiyasat et al., 2005). Alguns processos podem acarretar aumento da liberação de monômero e consequente redução da biocompatibilidade destes materiais, dentre os quais se destacam a sorção de água, a solubilidade em água e a liberação de monômero residual propriamente dita.

A sorção de água das resinas compostas pode reduzir suas propriedades mecânicas (El-Hadary e Drummond, 2000), pois a água absorvida é capaz de causar descolagem da matriz resinosa ou degradação hidrolítica da carga (Söderholm et al., 1984). Este processo ocorre quando as moléculas de água se difundem no material, iniciando uma degradação química (Braden e Clarke, 1984) com consequente aumento de peso do material.

A solubilidade é também uma degradação hidrolítica e resulta na separação da cadeia de polímero por ação da água (Ferracane, 1994), formando subprodutos. Esses subprodutos são liberados ao meio bucal, levando à redução do peso das resinas compostas.

Ambos os processos afetam as propriedades mecânicas (Ferracane, 1994) e possivelmente a citotoxicidade dos compósitos resinosos.

A quantidade de monômero residual depende do grau de conversão de monômero em polímero e de acordo com Hofmann et al., 2002, sempre está associado à

conjunção de vários fatores, incluindo os aparelhos fotopolimerizadores. Entre as fontes utilizadas para ativação da polimerização, destaca-se a energia na forma de luz halógena (LH) e o diodo emissor de luz (LED). A luz halógena é, ainda hoje, a fonte luminosa mais utilizada para fotopolimerizar os compósitos. As lâmpadas emitem uma luz azul de espectro de 400 -500 nm. As vantagens estão relacionadas ao seu baixo custo e sua fácil manutenção. Contudo, a LH apresenta limitações, como a diminuição gradual da produção de energia e do longo tempo de exposição.

O LED emite luz com espectro de 470 - 650nm e algumas resinas compostas demonstraram propriedades mecânicas similares quando polimerizadas com LED em baixo tempo de exposição quando comparado a LH (Hubbezoglu et al., 2007).

Considerando a importância da biocompatibilidade dos materiais restauradores utilizados nos mais diferentes procedimentos terapêuticos odontológicos, esta pesquisa apresenta como objetivo avaliar a citotoxicidade *in vitro* das resinas compostas e os fenômenos que nela podem interferir tais como: sorção de água, solubilidade em água e liberação de monômero residual, variando a fonte de luz.

2. OBJETIVOS

2.1. GERAL

Avaliar a citotoxicidade *in vitro* das resinas compostas e fenômenos correlatos como a sorção e solubilidade em água e a liberação de monômero residual.

2.2. ESPECÍFICOS

2.2.1. Avaliar a citotoxicidade *in vitro* de diferentes resinas compostas fotopolimerizadas por luz halógena e LED.

2.2.2. Avaliar a sorção e solubilidade em água de diferentes resinas compostas fotopolimerizadas por luz halógena e LED.

2.2.3. Avaliar a liberação de monômero residual de diferentes resinas compostas fotopolimerizadas por luz halógena e LED.

3. ARTIGO 1

Water sorption and solubility of composite resin photopolymerized with different light source curing unit

Retamoso LB[1], Guimarães CLF[2], Scheid PA[3], Mota EG[4], Oshima HMS[5]

ABSTRACT

Water sorption and solubility are a process that water could cause hydrolytic degradation and reduce their mechanical properties. The aim of this in vitro study was evaluate the water sorption and solubility of different composite resins polymerized with two different light source curing units. Sixty samples were randomly divided into 3 groups according to the resin: nonofiller composite resin (Supreme), nanohybrid composite resin (Esthet-X) and microhybrid composite resin (4seasons). One half of the samples were polymerized for 40 seconds by a halogen light source and the other half was polymerized for 20 seconds by a LED light source (n=10). Water sorption and solubility measurements were obtained by means of weighting the samples before and after water immersion and desiccation. The results were submitted to statistical analysis (two-way ANOVA/Tukey) and demonstrated that water sorption and solubility were different for tested materials ($P<0.05$) and similar for light source curing units ($P>0.05$). Supreme presented the hightest values for water sorption and solubility, with statistical difference to 4seasons and Esthet-X, which were similar between then ($P>0.05$). We concluded that water sorption and solubility it also appeared to depend on material used and not depend on light source curing unit. And a nanofiller resin, Supreme, is the material tested more influenced by water.

INTRODUCTION

Water sorption by dental materials could reduce their mechanical properties (El-Hadary and Drummond , 2000), because the water absorbed could cause matrix debonding or hydrolytic degradation of the fillers (Söderholm et al., 1984). This is a diffusion process

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where water molecules diffuse into material, starting chemical degradation and forming products (Braden M, Clarke, 1984). This process results in increased weight.

Solubility is a hydrolytic degradation and results of separating polymer chains in the resin for water action (Ferracane, 1994) forming products. The degradation products were released from the material and may influence the dimensional composite, resulting in decrease of weight.

Water sorption and solubility affect the clinical performance of dental materials. The release of components can cause toxicity and color alterations, resulting in aesthetic problems to restorations. (Ferracane 1994).

Light cured composite resins are widely used in restorative dentistry. Polymerization of these materials leads to a crosslink of the monomers, forming polymer. Theoretically, a 100% conversion of monomer to polymer is possible, but as much as 25% to 50% of the methacrylate monomer double-bonds actually remain inactive in the polymer (Imazato et al., 2001). According to Hofmann et al., 2002; the degree of conversion of monomers in polymers is always proportionally associated with some factors, including light curing units.

Among the luminous energy used for polymerization of composite resins stand out the halogen light (HL) and light emitting diode (LED). QHL is the luminous source most frequently used in dentistry. The lamps emit a blue light with spectral range around 400-500 nm. The advantages are related with their low cost and easy maintenance. However, HL presented limitation, such as gradual decrease of energy output and relatively long exposure time. LED emit light with spectral range around 470-650 nm and some composite resins demonstrated similar mechanical properties curing with LED in lower exposure time compared to HL (Hubbezoglu et al., 2007).

The aim of this research was to test the null hypothesis that when different light sources were used to polymerize composite resins with different chemical composition there is no differences between water sorption and solubility.

MATERIALS AND METHODS

For these research, we used three different commercially available composite resin: Filtek Supreme XT® (Nanofiller composite resin, 3M/ESPE, St. Paul, MN, USA), Esthet-X® (Nanohybrid composite resin, Dentsply, Milford, USA) and 4Seasons® (Fine particle microhybrid composite resin, Ivoclar Vivadent, Schaan, Liechtenstein), according to Square 1.

Square 1. Composites resin characteristics and composition.			
Material	Composition	Filler vol. (%)	Filler wt. (%)
Filtek Supreme XT® (A2 enamel)	Bis-EMA, Bis-GMA, TEGDMA, UDMA, non-agglomerated/non-aggregated 20 nm nanosilica filler, agglomerated zirconia/silica nanocluster	59.5	82
Esthet-X® (A2 enamel)	Urethane modified Bis-GMA dimethacrylate, photoinitiators, stabilizers, barium boron fluoroalumino silicate glass, amorphous silica	60	77
4seasons® (A2)	Bis-GMA, TEGDMA, Barium aluminum fluoride glass Silicon dioxide	63-65	75-78

Twenty samples of each composite were placed into the teflon molds (3 mm in diameter and 2 mm in height), which were sandwiched between two glass slides. To ensure that the adhesive paste would be well distributed within the mold, a 5-N force was applied for 30 seconds.

One half of each of the 20 samples of three composite resin was polymerized for 40 seconds by a HL light source (Optilight Plus, Gnatus, Ribeirão Preto, SP, Brazil) with an 5 cm diameter light tip. The other half was polymerized for 20 seconds by a LED light source (Radii-cal, SDI, Bayswater, Australia) with an 5 cm diameter light tip. The times were different because, it is important to standardize the total energy irradiated. The energy is calculated as the product of the output of the curing unit and the time of irradiation, and it may be termed energy density (mJ/cm^2).

The outputs of the light tips emitted by a HL and LED were calibrated by a digital curing radiometer (Demetron, Danburry, Conn). The values were 16000 mW/cm^2 for HL and 16000 for LED.

HL: 400 mW/cm^2 X 40 s – 16000 $mJcm^2$

LED: 800 mW/cm^2 X 20s – 16000 mJcm^2

The water sorption and solubility measurements were realized according to Toledano et al., 2003. Ten disc specimens were used for each material. The diameter and the thickness of the specimens were measured and the volume (v) calculated.

The discs were conditioned in a desiccator for 3 days, containing calcium sulfate, at 37ºC until a constant weight had been achieved (w0). Then, the samples were placed in a glass vial containing 10 ml of distilled water. The vials were wrapped in aluminum foil to exclude light and placed in an incubator at 37ºC and at intervals removed, blot dried and weighed, then returned to water; this was continued until the weight change during 1 week became less than 0.32 μg (constant weight - w1).

Finally, the specimens were removed from the water and replaced in a desiccator, containing calcium sulfate, at 37ºC until a constant weight had been achieved. It was subsequently dried by placing it into a vacuum oven (25 in. of mercury) at 60ºC for 24 h and then reweighed for the last time (w2). These steps were carried out to evaluate water sorption (WS) and water solubility (WSL), in μg/cm^3.

WS= w1 – w2/V

WSL= w2 – w0/V

Where;

w0 is the sample weight before immersion

w1 is the sample weight after immersion

w2 is the sample weight after immersion and desiccation

STATISTICAL ANALYSIS

Data were analyzed using the Statistical Package for the Social Sciences 13.0 for Windows (SPSS, Inc., Chicago, IL, USA). To verify normality and homogeneity, Kolmogorov-Smirnov and Levene tests were used, respectively, with significance level of 5%.

With normal and homogeneous variables, two-way ANOVA (fixed factors: composite resin and curing unit) and Tukey HSD tests were used to identify intergroup differences, with a significance level of 5%.

RESULTS

1. Water Sorption

The results showed that water sorption was different for tested materials ($P<0.05$) and similar for light source ($P\geq0.05$). Esthet-X presented the lowest water sorption values and Supreme showed the highest values, with a significant difference between them. The results demonstrated that 4seasons showed intermodal values, without statistically difference for Esthet-X when HL were used to light cure ($P\geq0.05$). When LED was used, 4seasons showed different values than Supreme ($P<0.05$) but similar to Esthet-X. These results are summarized in Table 1.

Table 1:Water sorption mean and standard deviation (SD) in different composite resins and curing units

Composite Resin	Curing Unit	Mean (SD)
Supreme	Halogen Light	2,16 (0,69) a
Esthet-X	Halogen Light	0,95 (0,38) b
4seasons	Halogen Light	1,51 (0,58) b
Supreme	LED	3,14 (1,11) a
Esthet-X	LED	1,40 (0,55) b
4seasons	LED	1,47 (0,24) b

Same letters indicated no statistical difference for Tukey HSD

2. Water Solubility

The results showed that water solubility was different for tested materials ($P<0.05$) and similar for light source ($P\geq0.05$). 4seasons and Esthet-X, without statistical difference between them ($P\geq0.05$) presented the lowest values for water solubility. Supreme showed the highest values, with statistical difference for Esthet-X and 4seasons ($P<0.05$). These results are summarized in Table 2.

Table 2: Water solubility mean and standard deviation (SD) in different composite resins and curing units

Composite Resin	Curing Unit	Mean (SD)
Supreme	Halogen Light	1,36 (0,58) a
Esthet-X	Halogen Light	0,11 (0,07) b
4seasons	Halogen Light	0,14 (0,03) b
Supreme	LED	1,63 (0,39) a
Esthet-X	LED	0,62 (0,05) b
4seasons	LED	0,49 (0,04) b

Same letters indicated no statistical difference for Tukey HSD

DISCUSSION

The results of the present study partially accepted the hypothesis when different light sources were used to polymerize composite resins with different chemical composition there no differences between water sorption and solubility. The differences between compositions of composite resin result in different values.

The properties of composite materials depends organic matrix, inorganic filler particles and coupling agent. Water sorption is a diffusion process that occurs in the organic resin matrixes (Toledano et al., 2003). So, to composites with same organic matrixes are expecting similar water sorption values (Zui and Arai, 1986). Dental composites used in this study don´t have a similar organic matrix and they did not show similar results. Filtek Supreme had higher values of sorption and solubility than the others. Thus, according to Helvatjoglou et al., 1991, water sorption is also influenced by filler content.

The nanofilled composite presented the higher water sorption and solubility. This results contrasts with other study realized by Berger et al. 2009. The authors compare sorption and solubility of 3 resin based filling (Filtek Supreme, Renamel Microfill and Esthet X). In the present study, the tested composite resins had similar water sorption characteristics (except Filtek Supreme). A previous study found similar results in relation to this study. Silva et al. (2008) analyzed the correlation between the degree of conversion, solubility and salivary sorption of a hybrid (P60) and a nanofilled composite (Filtek Supreme) with similar polymeric matrices. Filtek Supreme presented higher solubility and salivary sorption than P60 and the authors attributed to the filler particle systems.

The nanofiller composite resin presented the highest water sorption and solubility. This result can be explained by altering the size of the particle fillers from micro to nano scales (Xia et al., 2008). With smaller particles, more particles were immersion in organic matrix, increasing the total surface area of matrix-filler interface. In accordance with Kalachandra and Wilson, 1992 the greater accumulated of water occur at the matrix-filler interface, where a greater surface area in fillers results allowed more water to accumulate.

Current literature suggests that the main reason for composite resin degradation in the oral environment is the hydrolysis of the silane, the coupling agent in the interface between fillers and the matrix (Söderholm et al., 1984; Nihei et al., 2008).

The water absorbed by dental composites is in contact with a silica surface. This preocess breaks siloxane bonds and form silanol groups, which facilitate particles debonding (Oysaed and Ruyter, 1986).

Different light sources did not affect values in this study. Some other proprieties of composite resin such as degree of conversion (Cunha et al., 2009), microhardness (Franco et al., 2007), compressive strength (Silva and Dias, 2009) have the same result where compared different light sources. LED-lights and HL did not differ in relation to sorption and solubility in different composite resin, when total energy is the same.

CONCLUSION

We concluded that water sorption and solubility are influenced by composite resin used and not depend on light source curing unit. The nanoparticle resin, Supreme, is the material tested more influenced by water.

REFERENCES

1. El-Hadary A & Drummond JL (2000) Comparative study of water sorption, solubility, and tensile bond strength of two soft lining materials. Journal of Prosthetic Dentistry **83(3)** 356-61.
2. Söderholm KJ, Zigan M, Ragan M, Fischlschweiger W & Bergman M. (1984) Hydrolytic degradation of dental composites. Journal of Dental Research **63(10)** 1248-54.
3. Braden M & Clarke RL (1984) Water absorption characteristics of dental microfine composite filling materials. Biomaterials **5(6)** 369-72.
4. Ferracane JL (1994) Elution of leachable components from composites. Journal of Oral Rehabilitation **21(4)** 441-52.
5. Imazato S, McCabe JF, Tarumi H, Ehara A & Ebisu (2001) Degree of conversion of composites measured by DTA and FTIR Dental Materials **17(2)** 178-183.
6. Hofmann N, Hugo B & Klaiber B (2002) Effect of irradiation type (LED or QTH) on photoactivated composite shrinkage strain kinetics, temperature rise, and hardness. European Journal of Oral Science **110(6)** 471–479.
7. Hubbezoglu I, Bolayir G, Dogan OM, Dogan A, Ozer A & Bek B (2007) Microhardness Evaluation of Resin Composites Polymerized by Three Different Light Souces. Dental Material Journal **26(6)** 845-53.

8. Toledano M, Osorio R, Osorio E, Fuentes V, Prati C & García-Godoy F (2003) Sorption and solubility of resin-based restorative dental materials. Journal of Dentistry **31(1)** 43–50.
9. Zui S & Arai K (1986) A study on visible light-cured composite resins. The influence of long-term water immersion on physical and mechanical properties. Dental Materials Journal **5** 602-15.
10. Helvatjoglou MA, Papadogianis Y, Koliniotou E & Kubias S (1991) Surface hardness of light-cured and self-cured composite resins. Journal of Prosthetic Dentistry **65(2)** 215-20.
11. Berger SB, Palialo ARM, Cavalli V & Giannini M (2009) Characterization of water sorption, solubility and filler particles of light-cured composite resins. Brazilian Dental Journal **20(4)** 314-318.
12. Silva EM, Almeida GS, Poskus LT & Guimaraes JGA (2008) Relationship between the degree of conversion, solubility and salivary sorption of a hybrid and a nanofilled resin composite. Journal of Applied Oral Science **16(2)** 161-6.
13. Xia Y, Zhang F, Xie H & Gu N (2008) Nanoparticle-reinforced resin-based dental composites. Journal of Dentistry **36(6)** 450-455.
14. Kalachandra S & Wilson TW (1992) Water sorption and mechanical properties of light-cured proprietary composite tooth restorative materials. Biomaterials **13(2)** 105-109.
15. Nihei T, Dabanoglu A, Teranaka T, Kurata S, Ohashi K, Kondo Y, Yoshino N, Hickel R & Kunzelmann KH (2008) Three-body-wear resistance of the experimental composites containing filler treated with hydrophobic silane coupling agents. Dental Materials **24(6)** 760-764.
16. Oysaed H & Ruyter IE (1986) Composites for use in posterior teeth: mechanical properties tested under dry and wet conditions. Journal of Biomedical Materials Research **20(2)** 261-71.
17. Cunha LG, Alonso RC, Neves AC, de Goes MF, Ferracane JL & Sinhoreti MA (2009) Degree of conversion and contraction stress development of a resin composite irradiated using halogen and LED at two C-factor leves. Operative Dentistry **34(1)** 24-31.
18. Franco EB, dos Santos PA & Mondelli RF (2007) The effect of different light-curing units on tensile strength and microhardness of a composite resin. Journal Applied of Oral Science **15(6)** 470-74.

19. Silva CM & Dias KRHC (2009) Compressive Strength of Esthetic Restorative Materials Polymerized with Quartz-Tungsten-Halogen Light and Blue Led. Brazilian Dental Journal 2**0(1)** 54-7.

4. ARTIGO 2

In vitro cytotoxicity of composite resin photopolymerized with different light source curing unit

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ABSTRACT

Polymerization efficiency of composite materials used in dentistry may be influenced by inherent factors in the material and curing light unit. The aim of this in vitro study was evaluate the citotoxicity of different composite resins polymerized with two different light source curing units. Samples were randomly divided into 3 groups according to the resin: nonofiller composite resin (Supreme), nonohybrid composite resin (Esthet-X) and microhybrid composite resin (4seasons). One half of the samples were polymerized for 40 seconds by a halogen light (HL) source and the other half was polymerized for 20 seconds by a LED light source (n=4). NIH/3T3 cells were plated in a 96-well and maintained in a humidified incubator for 24 hours at 37ºC. The incubation medium was replaced by the immersed medium in which the samples were stored for 24, 48, 72 and 168 hours. Then, cells were incubated in contact with eluates for 24 hours. The cell mitochondrial activity was evaluated by the methyl tetrazolium test (MTT). The data were statistically analyzed by three-way analysis of variance (ANOVA) and Tukey HSD tests. The results demonstrated that cytotoxicity were similar for times ($P>0.05$), different for tested materials ($P<0.05$) and light source curing units ($P<0.05$). All resins presented decrease in cell viability when compared to control ($P<0.05$). The polymerization with LED decreased the cytotoxicity for Esthet-X ($P<0.05$). We concluded that cytotoxicity was not influenced by times, with all resins presented different ranges of cytotoxic effects. The curing light unit influenced the cytotoxicity of composites, with resin photopolymerized by LED increasing cell viability of composites in relation to HL.

INTRODUCTION

Light cured composite resins are widely used in restorative dentistry The light causes camphorquinone activation, which produces free radicals in combination with amines (Mills et al., 1999). Polymerization starts and continues when light intensity is enough to support camphorquinone in stimulated state (Caughman et al.,1995).

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Polymerization efficiency of composite materials used in dentistry may be influenced by factors inherent in the material (Gioka et al., 2005) and curing light unit.

With regardless the curing light unit, the polymerization capacity of which is directly related to the light power as well as irradiation time. If the resin material is adequately polymerized, a higher degree of conversion and lower unreacted monomers is expected.

Polymerization of these materials leads to a crosslink of the monomers, forming polymer. According to Hofmann et al., 2002, the degree of conversion of monomers in polymers is always proportionally associated with some factors, including light curing units. Theoretically, a 100% conversion of monomer to polymer is possible, but as much as 25% to 50% of the methacrylate monomer actually remains inactive in the polymer (Imazato et al., 2001).

When a composite material is immersed in water or saliva, some of the components, such as unreacted monomers (Bis-GMA and TEGDMA) (Örtengren et al., 2001) and filler particles (Söderholm, 1983) are leached out of the material, it is defined as solubility. These products can be released into salivary fluids, contact the mucosa tissues and it is associated to a variety of cytotoxic responses observed in tissues (Gioka et al., 2005; Freitas et al., 2009).

The aim of this research was to test the null hypothesis that when different light sources (Halogen Light and Light Emitting Diode) are used to polymerized composite resins with different chemical composition there are no differences between cytotoxicity. A further aim was to evaluate cytotoxicity of composites in different periods.

2. MATERIAL AND METHODS

2.1 Materials

Three different dental composites were tested in the research: Filtek Supreme XT® (Nanofiller, 3M/ESPE, St. Paul, MN, USA), Esthet-X® (Nanohybrid, Dentsply, Milford, USA) and 4Seasons® (Fine particle microhybrid, Ivoclar Vivadent, Schaan, Liechtenstein), according to Square 1.

Square 1: Composite resins characteristics and composition			
Material	Composition	Filler vol. (%)	Filler wt. (%)
Filtek Supreme XT® (A2 enamel)	Bis-EMA, Bis-GMA, TEGDMA, UDMA, non-agglomerated/non-aggregated 20 nm nanosilica filler, agglomerated zirconia/silica nanocluster	59.5	82
Esthet-X® (A2 enamel)	Urethane modified Bis-GMA dimethacrylate, photoinitiators, stabilizers, barium boron fluoroalumino silicate glass, amorphous silica	60	77
4Seasons® (A2 enamel)	BIS-GMA, UDMA, TEGDMA, Barium glass filler, silanized ytterbium trifluoride, mixed oxide, silanized Ba-Al-fluorosilicate glass, silanized, highly dispersed silicone dioxide	63-65	75-77

Eigth samples of each composite were placed into teflon molds (3 mm in diameter and 2 mm in depth), which were sandwiched between two glass slides. To ensure that the adhesive paste would be well distributed within the mold, a 5-N force was applied for 30 seconds.

One half of each of the 8 samples of three composite resin was polymerized for 40 seconds by a HL light source (Optilight Plus, Gnatus, Ribeirão Preto, SP, Brazil) with an 5 cm diameter light tip. The other half was polymerized for 20 seconds by a LED light source (Radii-cal, SDI, Bayswater, Australia) with an 5 cm diameter light tip. The times were different because, it is important to standardize the total energy irradiated. The energy is calculated as the product of the output of the curing unit and the time of irradiation, and it may be termed energy density ($mJcm^2$).

The outputs of the light tips emitted by a HL and LED were calibrated by a digital curing radiometer (Demetron, Danburry, Conn). The values were 16000 $mJcm^2$ for HL and 16000 for LED.

HL: 400 mW/cm^2 X 40 s – 16000 mJcm^2

LED: 800 mW/cm^2 X 20 s – 16000 mJcm^2

All specimens were prepared and handled under aseptic conditions to limit the influence of biologic contamination on the cell culture tests.

2.2 Preparation of liquid extracts of materials

The extraction methodology is according to ISO 10993 part 5 – Tests for in vitro toxicity. We used 24 well microplates (TPP®, Switzerland), where the specimens assessed were placed in contact with 400µL of DMEM medium for 24, 48, 72 and 168 hours incubation times respectively. After, the culture medium containing material extracts was sterile filtered for use on the cell cultures.

2.3 Cell Line, culture conditions and cellular densities

Fibroblast NIH/3T3 cell line was obtained from ATCC (ATCC® Number: CRL-1658TM) and maintained in Dulbecco's Modified Eagle's Medium (DMEM, Gibco®, EUA) supplemented with 10% fetal bovine serum and 0,1% gentamicin, 1% penicillin/streptomycin (Gibco®, EUA) at 37°C in a humidified atmosphere of 95% air, 5% CO_2.

The cells were harvested and diluted to a density of 2x10^4 cells/well in DMEM medium. The cell suspension was shaken and then 200µL aliquots were added to each well of 96 well culture microplates (TPP®).

Four independent cultures were used to each treatment time. Each microplate was incubated 24h at the conditions described previously to cellular adherence and identified as follow: 24, 48, 72 and 168 hours exposure time.

2.4 MTT reduction assay

The MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay were performed to assess the viability/proliferation of the cells. The MTT assay is based on inhibition by chemical injury of the reduction of soluble yellow MTT tetrazolium salt to a

blue insoluble MTT formazan product by mitochondrial succinic dehydrogenase (Liu et al., 1997).

After adherence, the cells were rinsed with DPBS and then 100µL aliquots of the extracts as were added to each well, followed by incubation of plus 24h period. After the exposure period, cells were rinsed again and then 90µL of pre-warmed DMEM medium followed by 10µL MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) 5 mg/mL in PBS] was added to each well. The incubation time was 4h at 37°C.

At the end of this period, 100µL/well of Dimetil Sulfoxide (DMSO) was added to solubilize the purple formazan crystals produced. Optical densities (OD) were measured at 570 nm in an ELISA reader and cell viability was calculated according to the following formula

$$\text{Cell Viability (\%)} = \frac{\text{OD of test group}}{\text{OD of control= group}} \times 100$$

STATISTICAL ANALYSIS

Data were analyzed using the Statistical Package for the Social Sciences 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). To verify normality and homogeneity, Kolmogorov-Smirnov and Levene tests were used, respectively, with significance level of 5%.

With normal and homogeneous variables, three-way ANOVA (fixed factors: composite resin, curing unit and time) and Tukey HSD tests were used to identify intergroup differences, with a significance level of 5%.

RESULTS

The results indicated that cytotoxicity of composites resin was influenced by resin type ($P<0.05$) and light curing unit ($P<0.05$). However, cell viability was similar ($P>0.05$) in different evaluated periods.

After 24 hours, the results showed that all tested materials presented cytotoxicity effect in 3T3 fibroblasts cells, demonstrating statically significance difference for control ($P<0.05$). The results indicated statistical difference for tested materials ($P<0.05$) and light

source (P<0.05). When composites were photopolymerized by LED, Esthet-X presented the highest viability values (P<0.05), followed by Supreme and 4-seasons, without statistical difference between them (P>0.05). The polymerization with HQL indicated that Esthet-X presented the highest cytotoxicity effect (P<0.05). 4seasons showed intermodal values, without statistically difference for Supreme (P>0.05) (Table 1).

Table 1. Cell viability percentages by MTT Assay after 24 hours

Groups	Light Curing Unit	n	Mean*	SD**
Control	_____	4	100 A	0
Supreme	LED	4	38,23 E	7,28
Supreme	HL	4	37,92 E	4,08
4-seasons	LED	4	34,2 C,D,E	14,73
4-seasons	HL	4	17,24 D,E	7,67
Esthet-X	LED	4	56,19 C	4,21
Esthet-X	HL	4	9,86 B	3,99

* Means of the same letter indicated no difference (ANOVA/Tukey)
** SD indicates standard deviation

After 48 hours, cell viability was different for tested materials (P<0.05) and light source (P<0.05). 4seasons and Supreme, without statistical difference between them (P≥0.05) presented the highest values for cell viability, similar to control (P<0.05). Esthet-X showed the lowest values (P<0.05). Esthet-X photopolymerized by LED source presented superior values to cell viability than HL (P<0.05), according to Table 2.

Table 2. Cell viability percentages by MTT Assay after 48 hours

Groups	Light Curing Unit	n	Mean*	SD**
Control	_____	4	100 A	0
Supreme	LED	4	45,41 A,B	11,24
Supreme	HL	4	34,81 B,C	10,72
4-seasons	LED	4	67,58 A	10,92
4-seasons	HL	4	46,66 A,B	9,25
Esthet-X	LED	4	25,84 C	4,54
Esthet-X	HL	4	4,46 D	1,15

* Means of the same letter indicated no difference (ANOVA/Tukey)
** SD indicates standard deviation

After 72 hours, all tested materials presented cytotoxicity effect in 3T3 fibroblasts cells, demonstrating statically significance difference for control (P<0.05). Only light curing unit (P<0.05) influenced the cell viability, with HL decreased cell viability than

LED (P<0.05). Esthet-X polymerized by HL presented the lowest values (P<0.05), followed by Supreme and 4season, without difference between them (P>0.05). On the other hand, the LED polymerization resulted in similar viability for all composites (P>0.05). These results are summarized by Table 3.

Table 3. Cell viability percentages by MTT Assay after 72 hours

Groups	Light Curing Unit	N	Mean*	SD**
Control	_____	4	100 A	0
Supreme	LED	4	32,68 B	9,99
Supreme	HL	4	35,88 B	7,95
4-seasons	LED	4	45,43 B	13,01
4-seasons	HL	4	30,59 B	11,67
Esthet-X	LED	4	51,83 B	11,46
Esthet-X	HL	4	2,99 C	0,74

* Means of the same letter indicated no difference (ANOVA/Tukey)
** SD indicates standard deviation

After 168 hours, all tested materials presented cytotoxicity effect in 3T3 fibroblasts cells, demonstrating statically significance difference for control (P<0.05). Resin type (P<0.05) and light curing unit (P<0.05) influenced the cell viability. Esthet-X presented the highest values (P<0.05), followed by Supreme and 4season, without difference between them (P>0.05), using LED curing unit. On the other hand, the HQL polymerization resulted in greater viability for Supreme (P<0.05). 4seasons showed intermodal values (P<0.05) and Esthet-X the smaller 3T3 cell viability (P<0.05), according to Table 4.

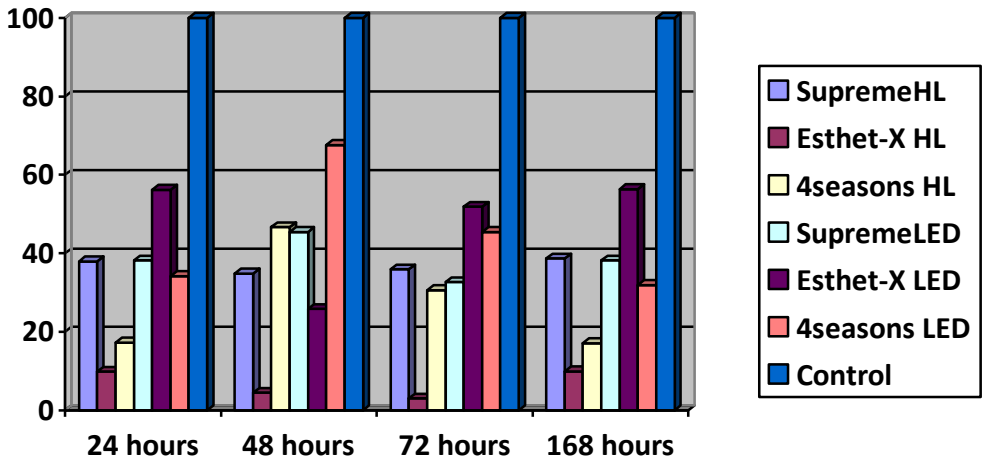
Table 4. Cell viability percentages by MTT Assay after 168 hours

Groups	Light Curing Unit	n	Mean*	SD**
Control	_____	4	100 A	0
Supreme	LED	4	38,18 C,E	4,51
Supreme	HL	4	38,66 C,E	8,53
4-seasons	LED	4	31,94 D,E	8,63
4-seasons	HL	4	17,05 D	7,34
Esthet-X	LED	4	56,38 C	5,43
Esthet-X	HL	4	9,91 B	4,17

* Means of the same letter indicated no difference (ANOVA/Tukey)
** SD indicates standard deviation

After all periods, the plates were analyzed on an inverted light microscope (Axiovent 25, Carl Zeiss SMT, Thornwood, NY) with a 10X objective, and photomicrographs were obtained. The photomicrographs revealed that control group exhibited increases in the number of cells, confluent growth, and fusiform cells, typical of normal fibroblast development (Figure 1A). This was different from tested composites (Figure 1B-1G), which presented inhibition of cell proliferation and growth, with significant alterations indicated by the presence of more round cells, mostly with darkened and granular aspects, suggesting lysis with cell death.

Graphic 1: Percentage of cell viability according different times



DISCUSSION

The results of the present study rejected the hypothesis when different light sources were used to polymerize composite resins with different chemical composition there are no differences between cell viability.

The mechanical properties of composite materials depends organic matrix, inorganic filler particles and coupling agent. However, composite toxicity is associated to monomers released from organic matrix (Hanks et al., 1991). Dental composites used in this study don't have a similar organic matrix and they did not show similar results. Matrix of Esthet-X is essentially formed by urethane modified Bis-GMA, 4-seasons by Bis-GMA,

TEGDMA and UDMA, and Supreme formed by Bis-GMA, Bis-EMA, TEGDMA and UDMA.

Esthet-X had lower values of cell viability ($P<0.05$) than the others, when photopolymerized by HL in all tested periods. We suggest that this difference could be explained by the absence of TEGDMA. Accoding Malkoc et al., 2010 TEGDMA, a co-monomer, has an important function because it decreases the viscosity of the Bis-GMA, thus allowing increased filler content and decreased Bis-GMA percentage. Current literature suggests that the presence of bisphenol A is associated to high indices of toxicity (Ratanasathien et al., 1995; Issa et al., 2004; Vitral et al., 2010).

Our results corroborates with Carvalho et al., 2010, that evaluated the residual monomers in orthodontics composites using a light-emitting diode (LED) or a halogen light, and compared the residual monomers in different areas of the composite. LED leaves less residual monomer than does the halogen light, with the same energy density, consequently more cell viability.

However, Ak et al., 2010, advocated that residual monomers increased when composite resins were photopolymerized by LED. We suggest that this difference is associated to different energy density used (irradiation time X output of the curing).

The cytotoxicity of Esthet-X decreased when photopolymerized by LED than HL. The level of crosslinking of composites irradiated with LED is higher than HL. This is accompanied by more degree of cure (Jagdish et al., 2009), less leached monomer (Archegas et al., 2009) and less pronounced toxic effects using HL.

The LED efficiency is related to light power of at least 300 mW per square centimeter (Shortall and Harrington, 1996), a narrow spectral range with a peak around 450-470 nm, which matches the optimum absorption wavelength for the activation of the camphorquinone photoinitiator (Mills et al., 1999).

Beriat et al., 2010 analyzed cytotoxicity in L-929 mouse fibroblasts of different composites using HL and LED until 72 hours and concluded that there was no interpretable pattern of cytotoxicity among the restorative materials. However, composites polymerized with LED demonstrated less cytotoxicity in short periods. Our study evaluated until 7 days and obtained no differences among different periods. But, we suggest that in longer periods, should be decrease in cytotoxicity effects. Bis-GMA, TEGDMA and UDMA were detectable for all tested composites until 28 days. But high performance liquid

chromatography (HPLC) analysis demonstrated maximal concentration at the 7-day period (Archegas et al., 2009).

The result of this study showed that light-cured composites have moderate to severe cytotoxicity (Jagdish et al., 2009; Ahrari et al., 2010). This issue may be explained by elution of residual unpolymerized monomers (Archegas et al., 2009), degree of cure (Jagdish et al., 2009) and others factors (such as the presence of activator, primer, and the solubility of the components) (Jagdish et al., 2009).

However, the results of the present in vitro study remain unclear, and further studies using different test methods are needed for composites. Research efforts should focus on assessing long-term biologic effects of composites.

CONCLUSIONS

We could conclude that:

1. All resins in different times presented different ranges of cytotoxic effects.

2. The curing light unit influenced the cytotoxicity of composites, with LED increasing cell viability of composites.

3. The cytotoxicity was not influenced by time.

REFERENCES

1. Mills R, Jandt K, Ashworth S. Dental composite depth of cure with halogen and blue light emitting diode technology. Br Dent J 1999; 186:388-391.

2. Caughman F, Rueggeberg F, Curtis J. Clinical guidelines for photocuring restorative resins. J Am Dent Assoc 1995; 126:1280-1286.

3. Gioka C, Bourauel C, Hiskia A, Kletsas D, Eliades T, Eliades G. Light-cured or chemically cured orthodontic adhesive resins? A selection based on the degree of cure, monomer leaching, and cytotoxicity. Am J Orthod Dentofacial Orthop 2005; 127:413–419; quiz 516.

4. Hofmann N, Hugo B, Klaiber B. Effect of irradiation type (LED or QTH) on photoactivated composite shrinkage strain kinetics, temperature rise, and hardness. Eur J Oral Sci 2002; 110: 471–479.

5. Imazato S, McCabe JF, Tarumi H, Ehara A, Ebisu S. Degree of conversion of composites measured by DTA and FTIR Dent Mater 2001; 17:178-183.

6. Örtengren U, Wellendorf H, Karlsson S, Ruyter IE. Water sorption and solubility of dental composites and identification of monomers released in an aqueous environment. J Oral Rehabil 2001; 28:1006-1115.

7. Söderholm KJ, Zigan M, Ragan M, Fischlschweiger W, Bergman M. Hydrolytic degradation of dental composites. J Dent Res 1984; 63:1248-54.

8. Freitas MPM, Oshima HMS, Menezes LM, Machado DC, Viezzer C. Cytotoxicity of Silver Solder Employed in Orthodontics. Angle Orthod 2009; 79:939-44.

9. Liu Y, Peterson DA, Kimura H, Schubert D. Mechanism of Cellular 3- (4,5-Dimethylthiazol-2-yl) -2,5-Diphenyltetrazolium Bromide (MTT) Reduction. J Neurochem 1997; 69:581- 593.

10. Hanks CT, Strawn SE, Wataha JC, Craig RG. Cytotoxic effects of resin components on cultured mammalian fibroblasts. J Dent Res 1991; 70:1450–1455

11. Malkoc S, Corekci B, Ulker HE, Yalçın M, Şengüne A. Cytotoxic effects of orthodontic composites. Angle Orthod 2010; 80:759–764.

12. Ratanasathien S, Wataha JC, Hanks CT, Dennison JB. Cytotoxic interactive effects of dentin bonding components on mouse fibroblasts. J Dent Res 1995; 74:1602–1606.

13. Issa Y, Watts DC, Brunton PA, Waters CM, Duxbury AJ. Resin composite monomers alter MTT and LDH activity of human gingival fibroblasts in vitro. Dental Mater 2004; 20: 12–20.

14. Vitral JCA, Fraga MR, Souza MA, Ferreira AP, Vitral RWF. In-vitro study of the cellular viability and nitric oxide production by J774 macrophages with ceramic, polycarbonate, and polyoxymethylene brackets. Am J Orthod Dentofacial Orthop 2010; 137:247-253.

15. Carvalho FAR, Almeida RC, Almeida MA, Cevidanes LHS, Leite MCAM. Efficiency of light-emitting diode and halogen units in reducing residual monomers. Am J Orthod Dentofacial Orthop 2010; 138:617-622.

16. Ak AT, Alpoz AR, Bayraktar O, Ertugrul F. Monomer Release from Resin Based Dental Materials Cured with LED and Halogen Lights. Eur J Dent 2010, 4:34-40.

17. Shortall AC, Harrington E. Guidelines for the selection, use, and maintenance of visible light activation units. Br Dent J 1996; 181:383-7.

18. Jagdish N, Padmanabhan S, Chitharanjanc AB, Revathi J, Palani G, Sambasivamf M, Sheriff K, Saravanamurali K. Cytotoxicity and Degree of Conversion of Orthodontic Adhesives. Angle Orthod 2009; 79:1133–1138.

19. Archegas LRP, Rached RN, Ignacio SA, Vasconcelos EC, Ramos DL, Souza EM. Identification and Quantification of Monomers Released from Dental Composites Using HPLC. Braz Arch Bio Techno 2009, 52:855-862.

20. Beriat NC, Ertan AA, Canay S, Gurpinar A, Onur, MA. Effect of polymerization Methods on the Cytotoxicity of Dental Composites. Eur J Dent 2010; 4:287-292.

21. Ahrari F, Afshari JT, Poosti M, Brook A. Cytotoxicity of orthodontic bonding adhesive resins on human oral fibroblasts. Eur J Orthod 2010; 32:688-92.

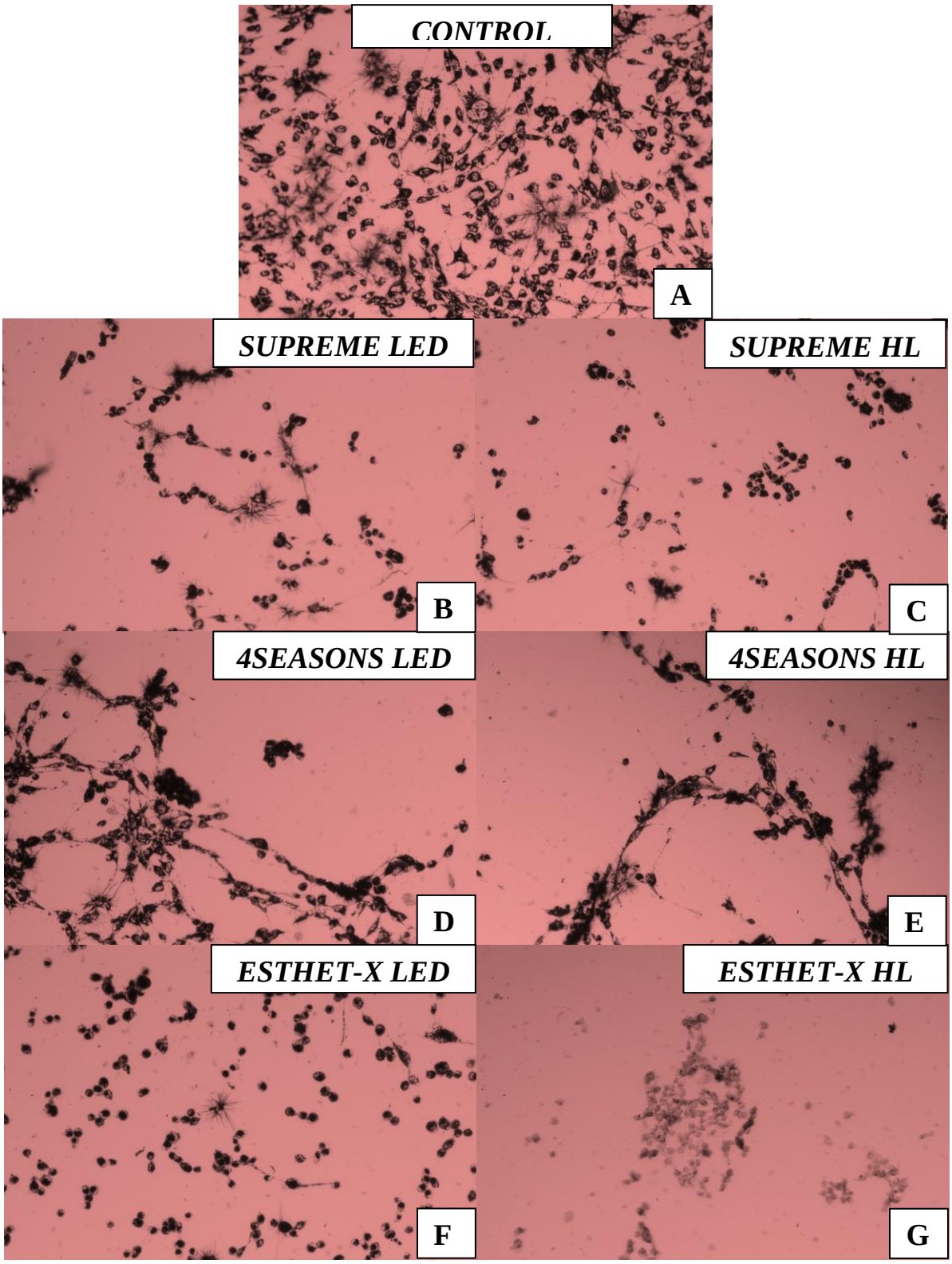


Figure 1: Photomicrographs of different resin after 168 hours. Note decreasing in cell number and growth inhibition. Thus, it observed presence of round cells, indicating cell death. A: Control, B: Supreme LED, C: Supreme HL, D: 4seasons LED, E: 4seasons HL, F: Esthet-X LED and G: Esthet-X HL.

5. ARTIGO 3

Monomers release of composite resin photopolymerized with different light source curing unit

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ABSTRACT

Insufficient polymerization with high residual monomers results in inferior mechanical and physical properties. The aim of this in vitro study was evaluate the efficacy of different light curing units to polymerize composite resins with different chemical composition. Only one increment of composite resin were plated in teflon molds (3 X 2 mm) and the samples were randomly divided into 3 groups according to the resin: nanofilled composite resin (Supreme), nanohybrid composite resin (Esthet-X) and microhybrid composite resin (4seasons). One half of the samples were polymerized for 40 seconds by a halogen light source and the other half was polymerized for 20 seconds by a LED light source (n=4). After, samples were immersed in methanol at 37°C for 24, 48, 72 and 168 hours and we used UV visible light spectroscopy to measure the amount of monomers released. The results were submitted to statistical analysis (three-way ANOVA/Tukey) and demonstrated that monomers release were different for tested materials ($P<0.05$), light curing units ($P<0.05$) and periods ($P<0.05$). We observed increasing of monomers releasing until the 72 hours and decreasing on the 168 hours for Supreme. Esthet-X indicated increasing in monomer releasing until 168 hours. 4seasons demonstrated the highest values after 24 hours, followed by decreasing after 48 and 72 hours and increasing after 168 hours. We concluded that monomers release decreased when composite resins were photopolymerized by LED. Thus, the lixiviation was influenced by chemical composition and periods.

INTRODUCTION

Restorative composite resins have in their composition monomers, inorganic filler particles, a coupling agent and initiators (Ferracane, 1994).

Most composites have camphorquinone has initiator. The light 468 nm wavelength causes camphorquinone activation at the highest degree, which produces free radicals in

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combination with amines (Filip and Vladimirov, 2006), initiating polymerization According to Imazato et al., 2001, a 100% conversion of monomer to polymer is possible, but as much as 25% to 50% of the methacrylate monomer actually remains inactive in the polymer. The unreacted monomers from organic matrix are leached by materials (Moon et al., 2004).

Insufficient polymerization with high residual monomers results in inferior physical properties. Moreover, the majority of unreacted components may be released within the first few days (Geurtsen, 1998).

One of the greatest concerns of the researchers has been the quality of polymerization, since the introduction of light-cured resin-based composites (Topcu et al., 2010). Halogen light is the most commonly curing unit used to polymerize composite resin (Filip and Vladimirov, 2006; Carvalho et al., 2010 Topcu et al., 2010). Their advantage is that this is a low cost technology (Retamoso et al., 2010), while their principal drawbacks is a decline in irradiance over time due to the aging of lamp and filter (Moon et al., 2004).

Mills et al., 1999 indicated an alternative curing unit such as light emitting diode (LED) to polymerize composite resins. LED curing units use less power and have a longer life and greater durability than conventional halogen lamps. They have a narrow spectral range with a peak around 450-470 nm (Stahl et al., 2000), which matches the optimum absorption wavelength for the activation of the camphorquinone initiator (Mills et al., 1999).

So, the aim of this study was evaluate the efficiency of photopolymerization of different curing units to decrease the release residual monomers from nanofilled, microhybrid and nanohybrid composites commercially available.

MATERIAL AND METHODS

1. Materials

Three different dental composites were tested in the research: Filtek Supreme XT® (Nanofiller composite, 3M/ESPE, St. Paul, MN, USA), 4Seasons® (Fine particle microhybrid, Ivoclar Vivadent, Schaan, Liechtenstein) and Esthet-X® (Nanohybrid, Dentsply, Milford, USA) according to Square 1.

Square 1: Composite resins characteristics and composition			
Material	Composition	Filler vol. (%)	Filler wt. (%)
Filtek Supreme XT® (A2 enamel)	Bis-EMA, Bis-GMA, TEGDMA, UDMA, non-agglomerated/non-aggregated 20 nm nanosilica filler, agglomerated zirconia/silica nanocluster	59.5	82
Esthet-X® (A2 enamel)	Urethane modified Bis-GMA dimethacrylate, photoinitiators, stabilizers, barium boron fluoroalumino silicate glass, amorphous silica	60	77
4Seasons® (A2 enamel)	BIS-GMA, UDMA, TEGDMA, Barium glass filler, silanized ytterbium trifluoride, mixed oxide, silanized Ba-Al-fluorosilicate glass, silanized, highly dispersed silicone dioxide	63-65	75-77

Eigth samples of each composite were placed into the teflon molds (3 mm in diameter and 2 mm in depth), which were sandwiched between two glass slides. To ensure that the adhesive paste would be well distributed within the mold, a 5-N force was applied for 30 seconds.

One half of each of the 8 samples of three composite resin was polymerized for 40 seconds by a HL light source (Optilight Plus, Gnatus, Ribeirão Preto, SP, Brazil) with an 5 cm diameter light tip. The other half was polymerized for 20 seconds by a LED light source (Radii-cal, SDI, Bayswater, Australia) with a 5 cm diameter light tip. The times were different because, it is important to standardize the total energy irradiated. The energy is calculated as the product of the output of the curing unit and the time of irradiation, and it may be termed energy density (mJ/cm^2).

The outputs of the light tips emitted by a QHL and LED were calibrated by a digital curing radiometer (Demetron, Danburry, Conn). The values were 16000 mJcm2 for HL and 16000 for LED.

HL: 400 mW/cm2 X 40 s – 16000 mJcm2

LED: 800 mW/cm2 X 20s – 16000 mJcm2

Immediately after polymerization, specimens were placed in contact with 10mL of methanol for 24, 48, 72 and 168 hours.

2. Evaluation of monomers released

1g of composite resin were dissolved in 10ml of chloroform (J. T. Baker Inc, Phillipsburg, NJ, USA), after, the solution were centrifuged (4000 rpm for 15 minutes) (Q222TM, Quimis Aparelhos Cientificos Ltda., São Paulo, Brazil) to separate the monomers and inorganic particle fillers. Particle filler were discarded and the supernatant (monomers and chloroform) were submitted to rotary evaporator (R-210/215, BUCHI Labortechnik Flawil, Switzerland). At 62ºC, the solvent evaporated and the monomers were immersed in methanol (J. T. Baker Inc, Phillipsburg, NJ, USA). We used methanol because it acts an inhibitor in this type of polymerization, while maintaining the samples characteristics for spectroscopy analysis.

After, standard solutions of composite monomers were prepared by dissolving the solution in varied concentrations (0.004 to 0.6 mg/mL).

The coefficients (R) obtained by a linear regression analysis for Filtek Supreme XT, Esthet-X and 4seasons were 0.9984, 0.9989 and 0.9997, respectively.

The analysis of the released monomers was carried out by UV spectrophotometer (UV/Vis spectrophotometer Aglient, Scientific Equipament Source, Ontario, Canada). The detection was performed at wavelength of 250 nm.

All the measurements were performed four times for each of the extracts.

3. Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). To verify normality and homogeneity, Kolmogorov-Smirnov and Levene tests were used, respectively, with significance level of 5%.

With normal and homogeneous variables, three-way ANOVA (fixed factors: composite resin, curing unit and periods) and Tukey HSD tests were used to identify intergroup differences, with a significance level of 5%.

RESULTS

The results indicated that monomer releasing of composites resin was influenced by resin type ($P<0.05$), light curing unit ($P<0.05$) and evaluated periods ($P<0.05$).

Analyzing the periods, we observed increasing of monomers releasing until the third day. On the seventh day, the values indicated decreasing in monomer release for Supreme. Esthet-X indicated increasing in monomer releasing until 168 hours. 4seasons demonstrated the highest values after 24 hours, followed by decreasing after 48 hours and 72 hours and increasing after 168 hours.

After 24 hours, the results indicated statistical difference only for tested materials ($P<0.05$). 4seasons presented the highest monomers releasing values ($P<0.05$), followed by Esthet-X and Supreme, with statistical difference between them ($P<0.05$).

After 48 hours, monomers releasing was different for tested materials ($P<0.05$) and light source ($P<0.05$). When materials are polymerized by halogen light, Esthet-X showed the highest values ($P<0.05$), followed by 4seasons and Supreme, with statistical difference between them ($P<0.05$). LED curing unit decreased Esthet-X monomers releasing, demonstrating the lowest values ($P<0.05$), followed by Supreme and 4seasons, without statistical difference between them ($P>0.05$).

After 72 hours, the results indicated statistical difference only for tested materials ($P<0.05$). Esthet-X HL and Supreme LED presented the highest monomers releasing values and 4seasons polymerized by HL, the highest values ($P<0.05$). Other composites presented similar monomer releasing ($P>0.05$).

After 168 hours, only resin type ($P<0.05$) influenced the monomers releasing. 4seasons presented the highest values ($P<0.05$), Esthet-X showed intermodal values ($P<0.05$) and Filtek Supreme indicated the lowest concentration of monomers releasing ($P<0.05$). All results were summarized in Table 1.

TABLE 1. Concentration of released monomers from dental composites after 24, 48, 72 and 168 h

COMPOSITES	24 HOURS	48 HOURS	72 HOURS	168 HOURS
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Supreme HL	0,4287 ± 0,22A,D,a	0,6085 ± 0,04A,a	0,8624 ± 0,19A,C,b	0,4022 ± 0,07A,a
Esthet-X HL	0,8706 ± 0,18B,a	0,9844 ± 0,24B,C,a	1,0691 ± 0,15A,a	1,4056 ± 0,07B,b
4-seasons HL	1,6132 ± 0,09C,a	0,9843 ± 0,08C,b,c	0,8879 ± 0,1A,C,b	1,5968 ± 0,35B,a
Supreme LED	0,4479 ± 0,09A,D,a	0,7901 ± 0,13A,C,a	1,0345 ± 0,18A,b	0,4821 ± 0,13A,a
Esthet-X LED	0,7104 ± 0,09B,D,a	0,6098 ± 0,09A,a	0,7757 ± 0,04C,a	0,8536 ± 0,17A,C,a
4-seasons LED	1,4195 ± 0,19C,a	0,9834 ± 0,09C,a,c	0,7151 ± 0,05B,C,b	1,3844 ± 0,29B,a

DISCUSSION

The results of the present study rejected the hypothesis when different light sources were used to polymerize composite resins with different chemical composition there no differences between monomer releasing.

Ferracane, 1994, stated that size and composition of monomers present in composites, solvent type and degree of conversion determine the quantity of leachable components.

The expected reduction in monomer releasing at increased storage times was shown only for Filtek Supreme. Esthet-X presented increasing over time and 4seasons demonstrated the highest values after 168 hours. These differences in monomer releasing among the different materials could be explained by differences in matrix composition. Matrix of Esthet-X is essentially formed by urethane modified Bis-GMA, 4-seasons by Bis-GMA, TEGDMA and UDMA, and Supreme formed by Bis-GMA, Bis-EMA, TEGDMA and UDMA.

Other studies (Tanaka et al., 1991) found that small monomers were extracted in considerably higher quantities than the large monomers. TEGDMA molecules, being smaller and having lower molecular weight, are leached out at a faster rate than the larger Bis-GMA molecules. This theory explains the increasing over time obtained by Esthet-X, with absence of TEGDMA.

Archegas et al., 2009, quantified the main residual monomers released from composites after 1, 7, 14 and 21 days. They concluded that most of the monomers demonstrated maximal concentration at the seventh day. On the other hand, Örtengren et

al., 2001 observed that maximal monomer concentration in the eluate was observed after 168 hours.

The solvent which a composite is immersed affect the monomers extraction. Laboratory studies have used different storage substances, as water, artificial saliva, alcohol, and acid or basic solvents (Filip and Vladimirov, 2006, Ferracane, 2006, Archegas et al., 2009). The rate and extent of elution appear to be greater in organic solvents, as compared with elution into pure water. This difference can be attributed to the greater ability of the organic solvent to penetrate and swell the polymer network, facilitating the liberation of unreacted monomers and promoting a stronger degradative effect (Ferracane, 1994).

Pfeifer et al., 2009, analyzed the influence of monomer content on degree of conversion, flexural properties of BisGMA co-polymers. It were tested some formulations containing BisGMA, UDMA, TEGDMA and BisEMA after ethanol immersion. The authors concluded that composites BisGMA, TEGDMA and UDMA presented the best relation with degree of conversion and mechanical properties.

This in vitro study obtained decrease in monomer releasing with LED. Our results corroborates with Carvalho et al., 2010, that evaluated the residual monomers in orthodontics composites using a light-emitting diode (LED) or a halogen light, and compared the residual monomers in different areas of the composite. LED leaves less residual monomer than does the halogen light, with the same energy density.

However, Ak et al., 2010, advocated that monomers release increased when composite resins were photopolymerized by LED. We suggest that this difference is associated to methodology. Ak et al., 2010 used different energy density (irradiation time X output of the curing) and High Performance Liquid Chromatography (HPLC) was used to measure the amount of monomers released.

Filtek Supreme released more monomers when photopolymerized by halogen light than LED. The level of crosslinking of composites irradiated with LED is higher than HL. Because the efficiency is related to light power of at least 300 mW per square centimeter (Shortall and Harrington, 1996), a narrow spectral range with a peak around 450-470 nm, which matches the optimum absorption wavelength for the activation of the camphorquinone photoinitiator (Mills et al., 1999).

CONCLUSION

We could conclude that:

1. All resins in different periods presented different ranges of monomers release.

2. The curing light unit influenced the monomer release of composites, with LED decreasing the monomers lixiviation from composites.

3. The monomer release was influenced by time.

REFERENCES

1. Ferracane JL. Elution of leachable components from composites. Journal of Oral Rehabilitation 1994; 21:441-52.

2. Filip IA, Vladimirov SB. Residual Monomer in a Composite Resin After Light-Curing with Different Sources, Light Intensities and Spectra of Radiation Braz Dent J 2006; 17:34-38.

3. Imazato S, McCabe JF, Tarumi H, Ehara A, Ebisu S. Degree of conversion of composites measured by DTA and FTIR Dental Materials 2001; 17: 178-183.

4. Moon HJ, Lee YK, Lim BS, Kim CW. Effects of various light curing methods on the leachability of uncured substances and hardness of a composite resin. Journal of Oral Rehabilitation 2004; 31:258–264.

5. Geurtsen W. Substance released from dental resin composites and glass ionomer cements. Eur J Oral Sci 1998;106:685.

6. Topcu FT, Erdemir U, Sahinkesen G, Yildiz E, Uslan I, Acikel C. Evaluation of Microhardness, Surface Roughness, and Wear Behavior of Different Types of Resin Composites Polymerized With Two Different Light Sources. J Biomed Mater Res Part B: Appl Biomater 2010; 92B:470–478.

7. Carvalho FAR, Almeida RC, Almeida MA, Cevidanes LHS, Leite MCAM. Efficiency of light-emitting diode and halogen units in reducing residual monomers. Am J Orthod Dentofacial Orthop 2010;138:617-22.

8. Retamoso LB, Onofre NML, Hann L, Marchioro EM. Effect of light-curing units in shear bond strength of metallic brackets: an in vitro study. J Appl Oral Sci 2010;18:68-74.

9. Mills R, Jandt K, Ashworth S. Dental composite depth of cure with halogen and blue light emitting diode technology. Br Dent J 1999;186:388-391.

10. Stahl F, Ashworth SH, Jandt KD, Mills RW. Light-emitting diode (LED) polymerization of dental composites: Flexural properties and polymerization potential. Biomaterials 2000;21:1379–1385.

11. Tanaka K, Taira M, Shintani H, Wakasa K, Yamaki M. Residual monomers (TEGDMA and Bis-GMA) of a set visible-light-cured dental composite resin when immersed in water. J Oral Rehabil 1991; 18:353-362.

12. Archegas LRP, Rached RN, Ignacio SA, Vasconcelos EC, Ramos DL, Souza EM. Identification and Quantification of Monomers Released from Dental Composites Using HPLC. Braz Arch Bio. Techno 2009, 52:855-862.

13. Örtengren U, Wellendorf H, Karlsson S, Ruyter IE. Water sorption and solubility of dental composites and identification of monomers released in an aqueous environment. J. Oral Rehabil. 2001; 28:1006-1115.

14. Pfeifer CS, Silva LR, Kawano Y, Braga RR. Bis-GMA co-polymerizations: influence on conversion, flexural properties, fracture toughness and susceptibility to ethanol degradation of experimental composites. Dent Mater. 2009 Sep;25(9):1136-41. Epub 2009 Apr 23.

15. Ak AT, Alpoz AR, Bayraktar O, Ertugrul F. Monomer Release from Resin Based Dental Materials Cured with LED and Halogen Lights. Eur J Dent 2010, 4:34-40.

16. Shortall AC, Harrington E. Guidelines for the selection, use, and maintenance of visible light activation units. Br Dent J 1996;181:383-7.

6. DISCUSSÃO GERAL

As propriedades dos compósitos resinosos dependem da matriz orgânica, das partículas de carga e do agente de união. A sorção de água caracteriza-se como um processo de difusão, que ocorre dentro da matriz (Toledano et al., 2003). Desta forma, Zui e Arai, 1986 teorizaram que as resinas que apresentarem a mesma matriz orgânica, possivelmente apresentariam valores similares de sorção de água.

A maioria das resinas utilizada em Odontologia apresenta matriz semelhante, pois eram derivadas do monômero Bis-GMA. Entretanto, buscando melhoria nas propriedades físicas e mecânicas, estes materiais estão em constante modificação. Assim, diversos monômeros foram adicionados, dentre os quais se destacam: TEGDMA, UDMA, Bis-EMA, Bis-GMA modificado por uretano (Archegas et al., 2009).

Apesar dessa semelhança, pôde-se observar, nesta pesquisa, que a sorção e solubilidade em água foram diferentes para as resinas testadas. A Filtek Supreme apresentou os maiores valores para sorção e solubilidade em água. Teoriza-se que, este processo também é influenciada por outros fatores, como o conteúdo inorgânico (Helvatjoglou et al., 1991)

A alteração do tamanho das partículas de escalas micrométricas para manométricas elevou as propriedades mecânicas destes materiais. Porém, ocorreu também, um aumento geral na interface matriz/carga (Xia et al., 2008), com consequente elevação no acúmulo de água dentro destes materiais. Este fato pode ser justificado pela pesquisa de Kalachandra and Wilson, 1992, que demonstraram que é na interface matriz/carga o principal local de deposição da água.

Sabe-se ainda que, a principal causa da degradação das resinas em ambiente oral é a hidrólise do silano, agente responsável pela união das partículas de carga à matriz orgânica (Söderholm et al., 1984; Nihei et al., 2008). Quando a água penetra no material entra em contato com superfície de sílica, causando quebra da união facilitando a descolagem das partículas da matriz e consequente liberação no ambiente oral (Oysaed and Ruyter, 1986).

Com relação à toxicidade, os resultados indicaram que a Esthet-X apresentou maior citotoxicidade quando fotopolimerizada com luz halógena ($p<0,05$). Essa diferença pode ser justificada pela ausência do monômero TEGDMA na matriz orgânica. Uma pesquisa realizada por Malkoc et al., 2010 descreveu que este monômero diluente apresenta papel fundamental na química das resinas. A partir de sua adição, há redução na viscosidade e

porcentagem de Bis-GMA, além de aumento na incorporação de carga. Desta forma, mesmo a maior solubilidade do material evitará grande liberação de Bis-GMA, tendendo a reduzir o grau de toxicidade, pois está bem descrito na literatura que este monômero está altamente associado a altos índices de toxicidade (Ratanasathien et al., 1995; Issa et al., 2004; Vitral et al., 2010).

Observando a liberação de monômero residual, pôde-se observar que moléculas pequenas são lixiviadas com maior facilidade que as maiores (Tanaka et al., 1991. Desta forma, a molécula de TEGDMA seria lixiviada antes que Bis-GMA, pois apresenta menor tamanho e baixo peso molecular. Esta pode ser uma das teorias que explicam o aumento de liberação de monômero revelada pela Esthet-X, já que esta resina não apresenta TEGDMA em sua composição.

Archegas et al., 2009, quantificou o sprincipais monômeros liberados de resinas compostas restauradoras após 1, 7, 14 e 21 dias. Os autores concluíram que o pico de liberação ocorre em até 7 dias. Por outro lado, Örtengren et al., 2001 observou que a concentração máxima de monômeros ocorre após o sétimo dia.

Acredita-se que a composição química do material apresenta papel essencial no momento máximo de concentração de monômero residual. Além disso, o solvente utilizado na extração dos monômeros também é importante (Filip and Vladimirov, 2006, Ferracane, 2006, Archegas et al., 2009. Solventes orgânicos parecem demonstrar maior habilidade para penetração no polímero, aumento sua degradação e consequentemente, facilitando a lixiviação de monômeros não reagidos (Ferracane, 1994).

Analisando os resultados obtidos com relação à fonte de polimerização das resinas compostas, notou-se que o LED reduziu a citotoxicidade ($p<0,05$), entretanto, a sorção e solubilidade não foi influenciada.

Carvalho et al., 2010 avaliou a eficiência da fonte de luz na liberação de monômeros residual em diferentes áreas de compósitos ortodônticos. A polimerização com LED reduziu a liberação de monômero quando comparado à luz halógena. A área avaliada não foi influenciada pela fonte de luz. Por outro lado, no estudo de Ak et al., 2010, o uso do LED elevou o nível de monômero residual devido ao baixo grau de conversão de monômero. Sugere-se que esta diferença esteja associada à densidade de energia empregada na metodologia. No segundo, a densidade utilizada para o LED foi inferior à da luz halógena.

A melhor eficiência do LED obtida na presente pesquisa pode estar relacionada ao espectro de luz emitido, em torno de 450-470 nm, que coincide com o comprimento de onda de ótima absorção pela canforoquinona (Mills et al., 1999). A canforoquinona é normalmente o iniciador mais utilizado nas resinas.

O resultado da presente pesquisa demonstrou que os materiais testados apresentam moderada a severa toxicidade e leve sorção e solubilidade em água. Isto pode ser explicado pela liberação de monômeros não polimerizados, pelo grau de conversão de monômero em polímero e possivelmente a outros fatores como a presença de ativador.

Desta forma, outras pesquisas devem ser realizadas com o intuito de verificar a sorção e solubilidade em água, assim como a liberação de monômero residual e a biocompatibilidade dos compósitos resinosos em longo prazo.

7. CONCLUSÕES

A partir deste estudo, pôde-se concluir que**:**

1. Todas as resinas compostas testadas apresentam sorção e solubilidade em água, com a resina nanoparticulada, Supreme, demonstrando maior sorção e lixiviação de seus componentes;

2. Todas as resinas compostas testadas apresentam diferentes níveis de citotoxicidade e, a fotopolimerização com LED reduziu a toxicidade destes materiais;

3. Todas as resinas compostas testadas apresentam liberação de monômeros e, a fotopolimerização com LED reduziu a lixiviação de monômeros não reagidos.

8. REFERÊNCIAS

1. Kao CT, Ding SJ, Min Y, Hsu TC, Chou MY, Huang TH. The cytotoxicity of orthodontic metal bracket immersion media. Eur J Orthod 2007; 29: 198-203.
2. Freitas MPM, Oshima HMS, Menezes LM, Machado DC, Viezzer C. Cytotoxicity of Silver Solder Employed in Orthodontics. Angle Orthod 2009; 79:939-44.
3. Wataha JC. Biocompatibility of dental casting alloys: a review. J Prosthet Dent 2000; 83: 223-34.
4. Wada H, Tarumi H, Imazato S, Narimatsu M, Ebisu S. In vitro estrogenicity of resin composites. J Dent Res 2004; 83: 222-226.
5. Al-Hiyasat AS, Darmani H, Milhem MM. Cytotoxicity evaluation of dental resin composites and their flowable derivatives. Clin Oral Invest 2005; 9: 21-25.Kao et al., 2007
6. El-Hadary A, Drummond JL. Comparative study of water sorption, solubility, and tensile bond strength of two soft lining materials. Journal of Prosthetic Dentistry 2000;83: 356—61.
7. Braden M, Clarke RL. Water absorption characteristics of dental microfine composite filling materials. Biomaterials 1984;5:369—72. Ferracane JL. Elution of leachable components from composites. Journal of Oral Rehabilitation 1994; 21:441-52.
8. Hofmann N, Hugo B, Klaiber B. Effect of irradiation type (LED or QTH) on photoactivated composite shrinkage strain kinetics, temperature rise, and hardness. Eur J Oral Sci 2002; 110: 471–479.
9. Hubbezoglu I, Bolayir G, Dogan OM, Dogan A, Ozer A, Bek B. Microhardness Evaluation of Resin Composites Polymerized by Three Different Light Souces. Dental Mater J 2007; 26(6):845-53.
10. Toledano M, Osorio R, Osorio E, Fuentes V, Prati C, García-Godoy F. Sorption and solubility of resin-based restorative dental materials. Journal of Dentistry 2003; 31:43–50.
11. Archegas LRP, Rached RN, Ignacio SA, Vasconcelos EC, Ramos DL, Souza EM. Identification and Quantification of Monomers Released from Dental Composites Using HPLC. Braz Arch Bio. Techno 2009; 52:855-862.
12. Helvatjoglou MA, Papadogianis Y, Koliniotou E, Kubias S. Surface hardness of light-cured and self-cured composite resins. Journal of Prosthetic Dentistry 1991;65:215-20.

13. Xia Y, Zhang F, Xie H, Gu N. Nanoparticle-reinforced resin-based dental composites. Journal of Dentistry 2008; 36:450-455.
14. Kalachandra S, Wilson TW. Water sorption and mechanical properties of light-cured proprietary composite tooth restorative materials. Biomaterials. 1992; 13:105-109.
15. Söderholm KJ, Zigan M, Ragan M, Fischlschweiger W, Bergman M. Hydrolytic degradation of dental composites. Journal of Dental Research 1984;63:1248-54.
16. Nihei T, Dabanoglu A, Teranaka T, Kurata S, Ohashi K, Kondo Y, Yoshino N, Hickel R, Kunzelmann KH. Three-body-wear resistance of the experimental composites containing filler treated with hydrophobic silane coupling agents. Dental Materials 2008; 24:760-764.
17. Oysaed H, Ruyter IE. Composites for use in posterior teeth: mechanical properties tested under dry and wet conditions. Journal of Biomedical Materials Research 1986;20:261—71.
18. Ratanasathien S, Wataha J C, Hanks C T, Dennison J B 1995 Cytotoxic interactive effects of dentin bonding components on mouse fibroblasts. Journal of Dental Research 74: 1602–1606.
19. Issa Y, Watts DC, Brunton PA, Waters CM, Duxbury AJ. Resin composite monomers alter MTT and LDH activity of human gingival fibroblasts in vitro. Dental Mater 2004;20: 12–20.
20. Vitral JCA, Fraga MR, Souza MA, Ferreira AP, Vitral RWF. In-vitro study of the cellular viability and nitric oxide production by J774 macrophages with ceramic, polycarbonate, and polyoxymethylene brackets. Am J Orthod Dentofacial Orthop 2010;137:247-53.Carvalho FAR, Almeida RC, Almeida MA, Cevidanes LHS, Leite MCAM. Efficiency of light-emitting diode and halogen units in reducing residual monomers. Am J Orthod Dentofacial Orthop 2010;138:617-22.
21. Ak AT, Alpoz AR, Bayraktar O, Ertugrul F. Monomer Release from Resin Based Dental Materials Cured with LED and Halogen Lights. Eur J Dent 2010, 4:34-40.
22. Mills R, Jandt K, Ashworth S. Dental composite depth of cure with halogen and blue light emitting diode technology. Br Dent J 1999;186:388-391.

9. ANEXOS

Comissão Científica e de Ética
Faculdade da Odontologia da PUCRS

Porto Alegre 08 de abril de 2010

O Projeto de: Pesquisa

Protocolado sob nº:	0071/09
Intitulado:	Avaliação da toxicidade de materiais utilizados na clínica ortodôntica
Pesquisador Responsável:	Prof. Dr. Hugo Mitsuo Silva Oshima
Pesquisadores Associados	Luciana Borges Retamoso; Denise Cantarelli Machado; Maria Perpétua Mota Freitas
Nível:	Doutorado

Foi ***aprovado*** pela Comissão Científica e de Ética da Faculdade de Odontologia da PUCRS em *08 de abril de 2010.*

Este projeto deverá ser imediatamente encaminhado ao CEUA/PUCRS

Profa. Dra. Ana Maria Spohr
Presidente da Comissão Científica e de Ética da
Faculdade de Odontologia da PUCRS

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Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMITÊ DE ÉTICA PARA O USO DE ANIMAIS

Ofício 169/10 - CEUA

Porto Alegre, 22 de outubro de 2010.

Senhor Pesquisador:

O Comitê de Ética para o Uso de Animais, em resposta à submissão do projeto intitulado "Avaliação da toxidade de materiais utilizados na clínica ortodôntica" informa que o referido projeto de pesquisa não utiliza animais em sua metodologia e, por não se enquadrar nos critérios da Lei nº 11.794, de 08 de outubro de 2008 e no Regimento Interno do CEUA, será arquivado.

Atenciosamente,

Profa. Dra. Anamaria Gonçalves Feijó
Coordenadora do CEUA – PUCRS

Ilmo. Sr.
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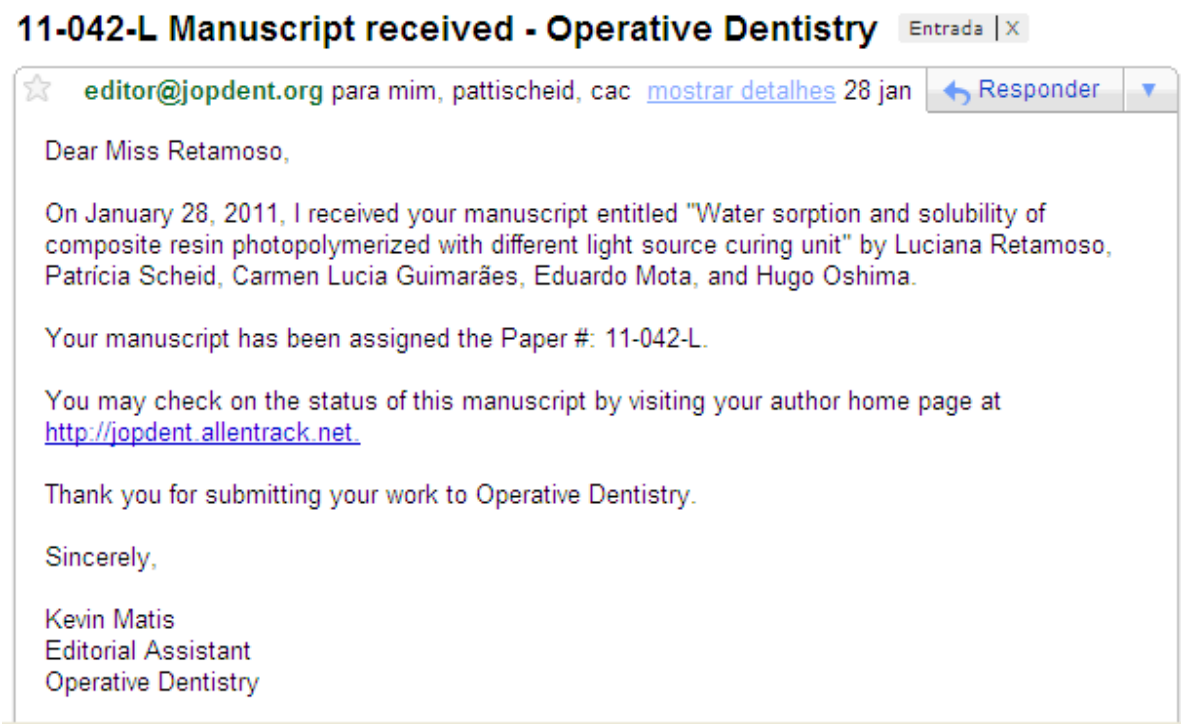
10. APÊNDICES



11-042-L Manuscript received - Operative Dentistry Entrada | X

editor@jopdent.org para mim, pattischeid, cac mostrar detalhes 28 jan Responder

Dear Miss Retamoso,

On January 28, 2011, I received your manuscript entitled "Water sorption and solubility of composite resin photopolymerized with different light source curing unit" by Luciana Retamoso, Patrícia Scheid, Carmen Lucia Guimarães, Eduardo Mota, and Hugo Oshima.

Your manuscript has been assigned the Paper #: 11-042-L.

You may check on the status of this manuscript by visiting your author home page at http://jopdent.allentrack.net.

Thank you for submitting your work to Operative Dentistry.

Sincerely,

Kevin Matis
Editorial Assistant
Operative Dentistry

European Journal of Oral Sciences - EOS-4965-MAN-11 Entrada | x

oral.sciences@odontologi.gu.se para mim mostrar detalhes 20 jan | Responder

20-Jan-2011

Dear Miss Retamoso:

Thank you for submitting your manuscript entitled "In vitro cytotoxicity of composite resin photopolymerized with different light source curing unit" to the European Journal of Oral Sciences. It has been successfully submitted online and is presently being given full consideration.

Your manuscript ID number is EOS-4965-MAN-11.

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European Journal of Oral Sciences