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Cytotoxicity of three light-cured resin cements on 3T3 fibroblasts

Citotoxicidade de três cimentos resinosos fotopolimerizáveis sobre fibroblastos 3T3

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Resumo

Introdução: Cimentos resinosos fotopolimerizáveis são materiais de eleição para a cimentação de facetas laminadas. Devem ser biocompatíveis oferencendo riscos mínimos ao uso clínico em pacientes. **Objetivo:** O objetivo desse trabalho foi avaliar *in vitro* a citotoxicidade de três cimentos resinosos: Variolink II (Ivoclar Vivadent), Allcem Veneer, (FGM) e Rely X Veneer (3M ESPE). **Material e método:** Vinte e quatro corpos de prova de cada cimento foram confeccionados em matrizes metálicas padronizadas e inseridos em placa de cultura de células de noventa e seis poços contendo fibroblastos da linhagem 3T3. As células foram cultivadas em meio de cultivo celular RPMI 1640 com 5% de soro fetal bovino, com 0,1% de penicilina/estreptomicina em estufa a 37°C, em atmosfera úmida com 5% de CO2. O grau de citotoxicidade de cada cimento foi avaliado após os tempos de contato de 24h, 48h e 72h através do método MTT (3-(4,5-dimetiltiazol-2yl)-2,5- difenil brometo de tetrazolina), que avalia a viabilidade celular pela função mitocondrial. Após os tempos estabelecidos, as amostras foram removidas, tratadas e levadas ao espectofotômetro de microplaca para leitura da absorbância em 570nm. **Resultado:** O cimento Variolink apresentou em 24h viabilidade de 72,24% (±6,80), em 48h de 83,92% (± 5,26) e de 92,77% (±5,59) em 72h. Allcem Veneer apresentou viabilidade de 70,46% (± 12,91) em 24h; 85,03% (± 21,4) em 48h e 70,46% (± 12,91) em 72h. O RelyX Veneer demonstrou viabilidade de 5,06% (± 0,88) em 24h; 5,84% (± 1,18) em 48h e 6,99% (± 1,34) em 72h. **Conclusão:** Estes resultados demonstraram que o cimento Rely-X se apresentou significativamente mais citotóxico nas condições testadas.

Descritores: Facetas dentárias; cimentos de resina; citotoxicidade; resinas compostas; sobrevivência celular.

Abstract

Introduction: Light-cured resin cements are the first choice for the cementation of laminate veneers. Ideally, they should be biocompatible and offer minimum risks to patients. **Objective:** The aim of this study was to evaluate, *in vitro*, the cytotoxicity of three resin cements: Variolink II, Ivoclar Vivadent (C1), Allcem Veneer, FGM (C2), and Rely X Veneer, 3M ESPE (C3). **Material and method:** Twenty four samples of each of the cements were fabricated in a standardized metal mold, light activated, and transferred to a 96-well cell plate with culture of fibroblasts. After 24, 48, and 72h of incubation, cytotoxicity was assessed and cell viability was calculated by the methyl-thiazol-tetrazolium (MTT) colorimetric assay. Absorbance was measured at 570 nm using a microplate spectrophotometer. **Result:** The following results were found: Variolink II presented viability of 72.24% (SD 6.80) after 24h, 83.92% (SD 5.26) after 48h, and 92.77% (SD 5.59) after 72h; Allcem Veneer exhibited viability of 70.46% (SD 12.91) after 24h, 85.03% (SD 21.4) after 48h, and 70.46% (SD 12.91) after 72h; Rely X Veneer showed viability of 5.06% (SD 0.88) after 24h, 5.84% (SD 1.18) after 48h, and 6.99% (SD 1.34) after 72h. **Conclusion:** Under these testing conditions, Rely X Veneer presented significantly higher cytotoxicity compared with those of the other light-cured resin cements assessed.

Descriptors: Dental veneers; resin cements; cytotoxicity; composite resins; cell survival.

INTRODUCTION

Special interest with regards to the biocompatibility of dental materials has brought increasing attention to both dental professionals and patients. The current demand for cosmetic dentistry is associated with the desire for a harmonious and esthetic smile, combining beauty and function. In this context, veneers appear as ceramic restorations of high aesthetic standard, biocompatibility, and color stability[1,2]. Ideally, the cementation of veneers require biocompatible dental materials that do not present any possibility of cytotoxic effects or allergic reaction on oral tissues[3,4]. Resin cements have been widely used in those clinical situations, presenting some advantages such as excellent mechanical and handling properties, good esthetics, and ability to successfully bind to enamel. Nevertheless, some

disadvantages have been reported, such as thickening of cement film at the margins and possible micro-leakage due to polymerization shrinkage[5]. Therefore, this interface between veneers and dental structure should be regarded as a critical issue to be addressed. There is a close 25μ-thick contact between the resin cement and the oral environment, which may cause local irritation and risk to patients[6-8]. Resin cements are composed of a polymeric organic matrix based on methacrylate monomers, filler particles, and bonding and other chemical agents, which are responsible for the polymerization reaction[7,9]. This composition is similar to that of composite resins and, although their toxic effects are described in many investigations[3,4,6,10], few studies have evaluated the toxicity of resin cements[1,5]. Among the several resin cements available in the market, the light-cured ones are the first choice for the cementation of veneers due to their higher color stability and working time[5,6]. However, the polymerization reaction involves a free radical reaction in which the material is transformed from a viscous state to a rigid one. During this process, the terminal aliphatic C=C bonds are broken and converted to primary C-C covalent bonds. *In vitro* demonstrations found in the specific scientific literature show that monomer conversion is not complete and that at the end of the reaction part of the monomers are released from the material into the adjacent tissues, generating local and systemic adverse effects[3,4,10,11]. Gupta et al.[10], in 2012, conducted a literature review on the cytotoxic effect of composite resins. *In vitro* and *in vivo* studies have clearly identified that some components released from the material are toxic. Moreover, according to literature, the release phenomenon begins during the polymerization reaction and can last up to 21 days. This process can lead to material degradation over time[10]. The amount of components released and the extension of the polymerization reaction are related[12]. Previous studies have found that the resin monomers A- glycidyl methacrylate (Bis-GMA), triethilene glycol dimetacrylate (TEGDMA), and urethane dimethacrylate (UDMA) present in the organic matrix are potentially toxic[13,14].

The aim of the present *in vitro* study was to evaluate, in 3T3 fibroblasts, the cytotoxicity of three different light-cured resin cements used in the luting of laminate veneers for three different periods of time.

In vitro cytotoxicity tests are reproducible, cost-effective, ideal to predict clinical outcomes, and suitable for the evaluation of basic biological properties of dental materials[6,15,16]. The null hypothesis of this study is that the tested cements present no cytotoxic effects on oral tissues.

MATERIAL AND METHOD

Sample Preparation

Three of the most commonly used light-cured resin cements were tested in this study and are described in Table 1. Twenty-four sample disks, 2mm thick and 3mm in diameter, of each of the uncured cements were inserted into metallic mold to be light-cured. Before polymerization, a transparent plastic matrix strip (TDV) was placed on the top of the molds to avoid the formation of an oxygen-inhibited superficial layer, and the molds were compressed between two glass slides to remove excess material and bubbles and to obtain a flat surface. Subsequently, the samples were cured from one side for 40s using a LED photopolymerizer (Radii-Cal-SDI, Australia, 2014) operating in standard mode and emitting 760 mw/cm^2 irradiance, as measured by the incorporated radiometer. After polymerization, the cements were carefully removed from the mold to be disinfected with UV radiation for 40 min on each side and subjected to the cytotoxicity assay.

Cytotoxicity Assay

Effects of the materials on mitochondrial function were measured by colorimetric assay as described by Mosmann[17]. A total of 5 x 10^4 3T3-Swiss albino (ATCC^R CCl-92^{TM}) cells/ml were seeded in 96-well plates and were incubated in 5% CO_2 atmosphere at 100% humidity and 37 °C in RPMI 1640, penicillin (100 U/mL) and streptomycin (100 mg/mL), supplemented with 5% fetal bovine serum. The final volume of the medium in each well was 200 μL. After 24 hours of incubation, the culture medium was changed and the groups of samples were added to the wells in each experimental period tested (24h, 48h, and 72h). After insertion of the test specimens into the wells, the culture medium was not changed until the end of the assay. Cell viability was calculated as percentage of the control group and measured by the MTT assay based on the ability of a viable cell mitochondrial dehydrogenase to reduce the yellow MTT dye to insoluble blue formazan crystals. After adding the solvent, absorbance of the converted dye cells was measured using a microplate spectrophotometer at the wavelength of 570 nm. Cells cultured in culture medium were considered as 100% cell viability for 72h.

Statistical Analysis

Data were analyzed using analysis of variance (One-way ANOVA) followed by the Dunnett's test for multiple comparisons compared to cells cultured in the presence of culture medium - 100% of viability. Results were considered statistically significant when $p<0.05$, n = 6.

Table 1. Tested cements and the composition of their monomers according to the manufacturers

TRADE NAME	MANUFACTURER	COMPOSITION OF MONOMERS
VARIOLINK	VIVADENT IVOCLAR	BisGMA
		UDMA
		TEGDMA
RELY X VENEER	3M ESPE	BisGMA
		TEGDMA
ALLCEM VENEER	FGM	BisGMA
		BisEMA
		TEGDMA

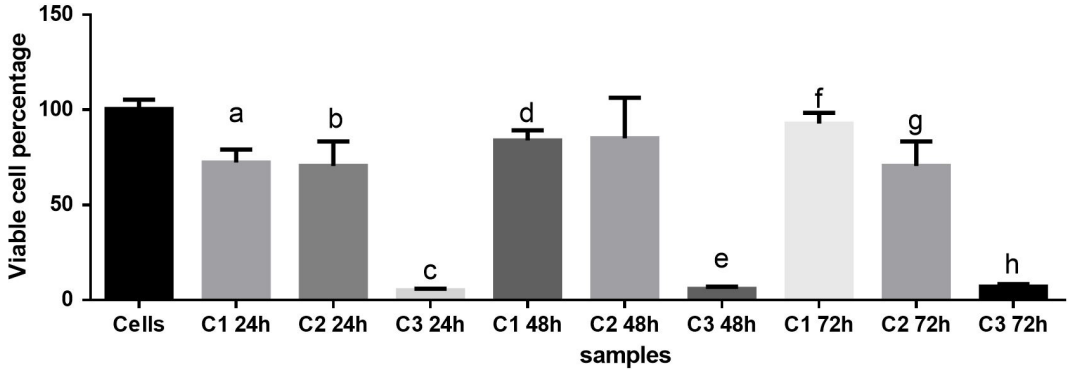

Figure 1. Cell viability. 3T3-Swiss albino (ATCCR CCL 92-TM) cells cultured in only culture medium (control group) for 72h and cells in contact with cements C1 (Variolink II), C2 (AllCem Veneer), and C3 (Relly X Veneer) at different times after 24, 48 and 72 hours (a,b,c,d,e,f,g,h), means $p<0.05$, One-way ANOVA, followed by the Dunnett's test for multiple comparisons, compared to cells cultured in the presence of culture medium, n=6.

RESULT

Cytotoxicity was correlated with setting time and cement type factors. The percentages of viable cells of each experimental group were compared with those of the negative control - 3T3 cells were grown in culture medium supplemented with 5% fetal bovine serum for 72 hours. The results of the present study showed that Variolink II presented viability of 72.24% (SD=6.80) after 24h, 83.92% (SD=5.26) after 48h, and 92.77% (SD=5.59) after 72h; AllCem Veneer exhibited viability of 70.46% (SD=12.91) after 24h, 85.03% (SD=21.4) after 48h, and 70.46% (SD=12.91) after 72h; Rely X Veneer showed 5.06% (SD=0.88) after 24h, 5.84% (SD=1.18) after 48h, and 6.99% (SD=1.34) after 72h. Values were considered statistically significant when $p<0.05$. Based on the results, Rely X Veneer proved to be the most cytotoxic cement at all contact times tested, followed by AllCem Veneer and Variolink II, respectively. Cell viability of the control and experimental groups at the three contact times (24, 48 and 72h) are shown in Figure 1.

DISCUSSION

It has been demonstrated that resin cements present toxic reaction in cell cultures by unreacted monomers released from the material during the polymerization reaction, which is related to thickness, light intensity, and photoactivation times of the material[8,12]. Is has also been demonstrated that comonomers, additives, and reaction products are released from polymerized cements[12,18]. According to Ferracane[15], 2013, cytotoxic effects are associated with incomplete polymerization reaction. The consequences of this process are degradation, opening of gaps, and failure of the laminate veneers[19]. It is known that the greater the amount of unreacted monomers contained in a cured cement, the higher the toxic effects[1]. Furthermore, it has been reported that the nature of the matrix monomers can significantly influence the release of toxic components[8].

The null hypothesis of the present study - tested cements present no cytotoxic effects on oral tissues - has to be rejected.

Our results showed that the tested cements presented varying degrees of cytotoxicity which decreased overtime and that Rely X Veneer was the most cytotoxic cement, but no significant differences were observed between AllCem Veneer and Variolink II. This behavior can be explained by the different compositions of the cements, mainly related to the nature of the matrix monomers. Rely X Veneer is composed of only two monomers: BisGMA and TEGDMA, whereas Variolink II and AllCem Veneer present an additional monomer type, UDMA and BisEMA, respectively. A current study has demonstrated that TEGDMA and BisGMA present severe cytotoxicity according to the following rank order: BisGMA>UDMA>TEGDMA>BisEMA[8]. Therefore, based on our previous results, it is possible to suggest that the presence of a third monomer in the veneer composition may decrease their cytotoxic effect. Moreover, UDMA and BisEMA, present in Variolink II and in Allcem Veneer, respectively, are low-weight monomers that allow greater reactivity in forming the polymer chain, which can be considered another possibility to explain the lower cytotoxicity of these cements.

In 2009, Schmid-Schwap et al.[3] tested several cements, including Variolink II and Rely X Unicem, on their self- and dual-cured presentation forms. The authors reported that Rely X was more cytotoxic than Variolink II, corroborating the findings of this study. Furthermore, dual-cured specimens showed lower cytotoxicity than chemically cured specimens[3]. The authors suggested that light activation could have interfered on their results by reducing the cytotoxic effects. This hypothesis was corroborated by Gupta et al.[10], who stated that cytotoxicity is inversely related to light activation and influenced by the type of the light curing unit. These authors also reported that any factor that limits or undermines the polymerization of cements, such as low light intensity, short light curing time, and longer distance between material surface and light source, may contribute to significantly increase their cytotoxic effects, and that fast curing using a high-power unit may be beneficial for composites to minimize the release of toxic substances[10].

According to Trumpaite-Vanagiene et al.[18], the first hours after polymerization are the most critical, with greater release of toxic

substances that decrease over time. Our results are in line with these observations, as we observed highest cytotoxicity within the first 24h, followed by a decrease after 48h. Nevertheless, in 2011, Mahasti et al.[20] tested Panavia F2 resin cement and Rely X Plus after 1h, 24h, and 1 week and found that cytotoxicity was directly proportional to time. In addition to the similar methodology used in their research, these different findings can be related to the use of different cements at different time intervals. Also, the colorimetric MTT assay used by the authors is fast, objective, and applies to all metabolically active cells[16,20]. Although it can be considered a valuable screening assay, additional *ex vivo* and clinical trials are necessary to confirm the validity of their results.

CONCLUSION

Based on the present *in vitro* study, we conclude that light-cured resin cements present different levels of cytotoxicity that decrease over time. Rely X Veneer was significantly more cytotoxic than the other cements assessed, which highlights the need of advising professionals about their possible toxic and undesirable reactions.

It is mandatory to follow the manufacturers' instructions of all materials prior to use. Laboratory assessments alone cannot be used to predict the clinical success of a resin cement. Well-controlled clinical trials are necessary to improve knowledge about the biocompatibility of materials.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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