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Comparison of the Effect of Two Groups of Resin Cements (Panavia F2 and Rely X Plus) and Zinc Phosphate Cement (Harvard) On the Induction of IL-6 by Category L-929 Mouse Fibroblast

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ABSTRACT

***Introduction**: Resin cement, despite its biocompatibility, has been extensively applied in restorative dentistry during recent years. The resin matrix comprises one or more 'light' co-monomer systems (e.g. HEMA) and 'heavy' monomer systems (including Bis-GMA) to decrease the monomers' viscosity and to increase the bonding strength to dentine. Acrylates, mainly methacrylates, have been revealed to cause cytotoxic impacts. **Objective:** This investigation contrasted the impact of resin cement (Rely X Plus and Panavia F2) and zinc phosphate cement (Harvard) on the initiation of IL-6 by category L-929 mouse fibroblast. **Method:** One resin cement (Panavia F2), one resin ionomer cement (Rely X Plus) and Harvard cement (Zinc Phosphate) were tested. The cement was prepared in hollow glass tubes (inner diameter of 5 mm, the height of 2mm) and 10 samples were dedicated to each group. The solution obtained from fibroblast cell located in a 6-well plate and after placing the samples into the sink plate, RPMI-1640 medium, 10% FBS, and the antibiotics streptomycin and penicillin were added to cultured cells. The culture plate was incubated in the CO2 incubator and was studied after 24 hours. Finally, the effect of tested cement on the induction of IL-6 was evaluated using the ELISA test method. The statistical analysis was performed using one-way ANOVA. **Results:** IL-6 Production was considerably different among the investigated groups (P<0.001). Harvard cement caused IL-1b releasing much more than the resin cement. **Conclusion:** Considering the limitation of this study, Harvard cement might be considered to have more cytotoxic potencies in comparison with the other tested materials.*

Keywords: *Resin cement, Resin ionomer cement, Zinc phosphate cement, Interleukin-6, L929 mouse fibroblast category.*

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INTRODUCTION

The last notable choice in a series of steps is the suitable determination of a luting agent that necessitates meticulous execution. It will be an indicator of the long-term success of fixed restorations. 100 years ago, this choice was easy with the accessibility of zinc phosphate cement as the only luting agent. Nowadays, various types of luting agents are accessible. A cautious technique is necessary for restorations of metal,

high-strength and low-strength ceramics, porcelain fused to metal, and partial or full coverage. The choice of suitable cement should be according to the information of physical and biological characteristics, and also other attributes of luting agents and restorative materials [1].

Resin cement is getting popular in the dental profession for several reasons. Most of the restorations could be cemented only with the resin cement. In most of the cases, resin cement is utilized in combination with enamel and dentin bonding materials and, therefore, is qualified to the micro-mechanical attachment to both structures utilizing the bonding agent. They can also bond to suitably treat fitted surfaces of restorations [2].

It has been long indicated that various ingredients of resinous materials can be released in an adjoining aqueous phase [3-5]. In this way, when implemented to a wet surface, such as dentin, uncured free monomers released from resin-based materials may distribute across dentinal tubules to reach the pulpal space [6]. Numerous investigations have revealed that released monomers lead to chemical injury to cultured cells [7, 8]. Besides, many *in vivo* investigations have revealed that uncured resin elements that reach the pulpal space result in the inflammatory response and tissue disorganization [9, 10].

The biocompatibility of dental materials has prevalently been examined utilizing human gingival fibroblasts. Their relative merits are that they can be simply recovered from patients and can be fast-growing in the normal culture media. Furthermore, they indicate a high sensitivity in cytotoxicity examinations. L-929 mouse fibroblasts and 3T3 mouse fibroblasts are other cell lines that have been extensively utilized. The selection of cell type is also dependent on the sort of biological endpoint utilized in the cytotoxicity test.

The calculation of the amount of pro-inflammatory mediators in cell culture supernatants of the cells treated with the resin-based materials is a sensitive and effective technique that may reveal a direct biochemical link between the parameters determined *in vitro* and clinical influences such as inflammation *in vivo*. In recent years, some investigations have focused on the impact of dental materials and their ingredients on the inflammatory markers. For instance, Noda et al. noted that sublethal treatment with triethylene glycol dimethacrylate (TEGDMA) and Hydroxyethyl methacrylate (HEMA) for two weeks results in the modification of tumor necrosis factor-alpha (TNF-α) secretion by THP-1 monocytes. In another assessment, Schmalz et al. noted that the key molecules in the occurrence of inflammation (such as PGE2 or IL-6 and IL-8 were released from human oral tissue culture models after exposure to compounds of dental materials. In another investigation, it has been determined that the amount of Interleukin-1 beta (IL-1β) released from 3D tissue-engineered human oral mucosal models significantly enhanced in case of exposure to high TEGDMA-with experimental composite resins.

Considering the potential harms related to the resin cement and their inflammation influences toward pulp cells, further investigations are needed to test their biocompatibility. The goal of the current investigation was to contrast the influence of two groups of zinc phosphate cement (Harvard) and resin types of cement (Panavia F2 and Rely X Plus) on the induction of IL-6 on rat fibroblast L-929.

MATERIALS AND METHODS

An experimental study was designed. The data collection was according to observed outcomes and completed forms.

L-929 fibroblast cell line preparation

The L929 fibroblast cell line of rat gingiva was obtained from Iran Pasteur Institute and initially passaged on culture flasks.

Confluent cells were detached with trypsin EDTA solution (Gibo, Scotland) and seeded in 6 well plates (5×10^5 cells/mL) containing RPMI-1640 medium. The cells were cultured in the flask.

To verify cell viability before cytotoxicity examinations, cells were primarily immersed in trypan blue and observed using a light microscope (magnification of ×40). The cytotoxicity evaluations were performed when over 90% of cells were viable.

The original culture medium without exposure to cement discs was considered as the negative control group, while those exposed to

hypochlorite solution served as the positive group.

Sample preparation for cytotoxicity test

Table 1 shows the commercially available adhesive resin cement, resin ionomer, and zinc phosphate types of cement tested in this investigation.

Table 1. Types of cement and their properties.

Type of cement	Setting mechanism	Manufacturer	Cement
Adhesive Resin	Dual cure	Kurary, Japan	Panavia F2
Resin Ionomer	Self-cure	3M, USA	Rely X Plus
Zinc phosphate	Chemical (acid-base)	Hoffman, Germany	Harvard

A total of 30 disk-shaped specimens, 2 mm in thickness and 5 mm in diameter were prepared from each material using sterilized hollow glass mold. The negative control comprised of cells immersed in plates with empty glass discs (without any cement). The positive control was the cells immersed in a sodium hypochlorite solution and were all exposed to the dye.

Method

The molds were filled with uncured material. Then, they were sealed with a mylar strip to protect the resin cement against the oxygen inhibition zone. The resin cement was polymerized by a halogen light-curing unit (Demetron_Kerr, Danbury, CT, USA) with an output irradiance of 550 mW/cm^2 with an exposure time of 40 seconds according to the vendor's recommendations merely from one side of the disc.

Cured samples were detached from the glass molds and immediately immersed in light-proof glass bottles containing fibroblast solution, RPMI_1640 culture medium (Gibco, Scotland), streptomycin-penicillin antibiotics and FBS 10% solutions (Gibco, Scotland) while the discs were thoroughly floated in the solution. Samples were deposited in a CO_2 incubator at 37 °C (CO_2: 5%; T: 37 °C; W>90%) for 24 hours. Then, IL-6 of the samples were measured by an ELISA kit (Bendermed, Austria) and using an ELISA reader.

Statistical analysis

The data were statistically analyzed by SPSS v. 13. The effect of various types of cement on the induction of IL-6 was contrasted utilizing one-way ANOVA ($P<0.05$).

RESULTS

The mean values and the standard deviations of data are shown in Table 2. The one-way ANOVA demonstrated that the cement type had a key influence on the Interleukin-6. The IL-6 concentration in 3 groups and the negative control group were significantly different ($P<0.001$). Table 2 summarizes the result of this study. In this study, it was found that the highest concentration of IL-6 was associated with Harvard cement ($p<0.001$) while Panavia F2 had the least impact. The lowest concentration of IL-6 was related to Rely X Plus and Panavia F2. No considerable difference was observed between the 2 types of cement and negative control.

DISCUSSION

The current research determined the impact of resin (Rely X Plus and Panavia F2) and zinc phosphate (Harvard) types of cement on the induction of IL-6 by utilizing mouse fibroblast category L-929. In this study, it was found that the highest concentration of IL-6 was associated with Harvard cement while Panavia F2 had the least impact. The lowest concentration of IL-6 was associated with Rely X Plus and Panavia F2, and no considerable difference was observed between them and negative control.

The research resources are not available for directly comparing the results. We compared the results of the related investigations. In 2006, Souza et al. [11] investigated the influence of glass ionomer cement on cell cultures and subcutaneous tissues in the rat. They showed that these types of cement cause some evidence of a moderate to a severe inflammatory response in cells and tissues after 1 week. This effect is proportional to the number of toxic substances released from these products. The amount of cytotoxicity also significantly enhanced with the progression of time.

Interestingly, in this investigation, Rely X cement demonstrated some evidence of inflammatory response. Pereira et al [12] stated that the systems of total etching or self-etching when they are directly associated with the dentin, have no significant effect on the induction of inflammatory cytokine production. The current study also contrasted the negative control group and Panavia F2 (System Self Etch) and demonstrated no significant difference in the amount of IL-6 produced.

In an investigation by Ulker et al. [3], the cytotoxicity of several resin types of cement (including BisCem, Bistite II DC, Rely X Unicem Clicker, MaxCem, and Panavia F 2.0) was examined by utilizing 3D pulp cell cultures. Based on their findings, the survival of cells was comparable to that of MaxCem and negative control, and all other tested materials were cytotoxic for the 3D cell cultures. Kong et al. [10] determined the cytotoxic effects of resin types of cement (Panavia F, Super-Bond C&B, and Chemiace II) after polymerization on cultured human dental pulp cells. All investigated types of cement induced slight cytotoxicity. They reported that there is a significant difference between the Super-Bond C&B and the Panavia F groups. Nonetheless, the cytotoxic influences of Chemiace II and Super-Bond C&B was not declared to be significantly different. They implied that the cytotoxicity on human pulp cells was related to the sorts of cement and the concentration of the elution. According to the findings, Super-Bond C&B was the least cytotoxic material.

In this investigation, the lowest concentration of IL-6 was associated with Rely X Plus and Panavia F2 and resin types of cement were contrasted with Harvard zinc phosphate cement. Also, in this research, we utilized a fibroblast cell culture that is an accurate test.

Trubiani O, et al. [13] examined the cell growth, phenotypic characteristics, as well as IL-6 and IL-8 secretion in expanded *ex vivo* human dental pulp mesenchymal stem cells (DP-MSCs) after treatment with HEMA. In their investigation, HEMA demonstrated cytotoxicity, repressed cell growth, and resulted in morphological modifications in cultured DP-MSCs. Besides, the soluble mediators of inflammation (including IL-6 and IL-8 cytokines) were up-regulated in treated samples. The direct utilization of HEMA potentially results in inflammation, leading to a toxic response and cell injury in DP-MSCs. In the current investigation, the resin types of cement up-regulated the soluble mediators of inflammation (including IL-6 cytokines).

CONCLUSION

We revealed that Harvard cement is possibly the least biocompatible cement which should be utilized with caution. Panavia F2 was shown to be the most biocompatible long-term cement exhibiting the least amount of interleukin-6 after 24 hours compared to Rely X Plus and Harvard. Yet, authors believe that further robust investigations are needed to study these types of cement.

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Conflict of interest

No conflict of interest is declared.

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Ethical clearance

This article was taken from laboratory research.

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