Original Article

Effect of Bacterial Lipopolysaccharide Contamination on Gutta Percha- versus Resilon-Induced Human Monocyte Cell Line Toxicity

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Abstract

Objectives: Cytotoxic effects of obturation materials were tested in presence and absence of endotoxin on human monocytes in vitro.

Materials and Methods: Human monocytes from THP-1 cell line were cultured. Three millimeters from the tip of each Resilon and gutta percha points were cut and directly placed at the bottom of the culture wells. Cultured cells were exposed to gutta percha (groups G1 and G2) and Resilon (R1 and R2). Ten μg/ml bacterial lipopolysaccharide (LPS) was added to the culture wells in groups G1 and R1. Positive control included the bacterial LPS without the root canal filling material and the negative control contained the cells in culture medium only. Viability of cells was tested in all groups after 24, 48, and 72 hours using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay for at least 3 times to obtain reproducible results. Optical density values were read and the data were analyzed using three-way ANOVA and post hoc statistical test.

Results: The results showed that cells in G2 had the lowest rate of viability at 24 hours, but the lowest rate of viable cells was recorded in G1 at 48 and 72 hours. The effect of LPS treatment was not statistically significant. Resilon groups showed cell viability values higher than those of gutta percha groups, although statistically non-significant (P=0.105). Cell viability values were lower in gutta percha than Resilon groups when LPS-treated and LPS-untreated groups were compared independently at each time point.

Conclusion: It could be concluded that none of the tested root canal filling materials had toxic effects on cultured human monocyte cells whether in presence or absence of LPS contamination.

Keywords: Gutta percha; Root canal filling material; Immunologic cytotoxicity; Monocyte

INTRODUCTION

Biocompatibility of root canal filling materials is of particular importance particularly when an inadvertent intimate contact occurs between the extruded root canal filling material and the adjacent periapical tissue cells [1, 2]. On the other hand, a substantial number of teeth requiring root canal treatment have necrotic pulps in which contamination with bacterial toxins is a common finding.

It has been well established that gram-negative anaerobic species are the predominant etiologic bacteria in endodontic infections [3]. These bacteria have LPS in their outer cell membrane as their virulence factor [4]. LPS, also called endotoxin, stimulates the inflammatory

mediators, activates the complement system and macrophages and causes host cell toxicity and bone resorption after being released during bacterial cell proliferation or death [5, 6]. It has also been demonstrated that filled root canals allow leakage of LPS in vitro [7].

Root canal fillings are currently performed by a semisolid material as a core in conjunction with various types of sealers. Gutta percha is considered the most widely used core material; but recently, Resilon (LLC, Madison, Connecticut) was introduced in endodontics to bring bonding technology into the root fillings. Although the literature is rich on the physical properties of Resilon-filled root canals, studies concerning its biological properties are infrequent. The aim of this study was to compare the cytotoxic effects of these two materials in presence or absence of *Escherichia coli* endotoxin on human monocytes in vitro.

MATERIALS AND METHODS

THP-1 human monocyte cell line was obtained from the Cell Bank of Pasteur Institute, Tehran, Iran and cultured in a laboratory setting in 25 mm^2 culture flasks which contained RPMI 1640 (Gibco BRL, Grand Island, NY), supplemented with penicillin (100 U/ml) (Gibco BRL, Grand Island, NY), streptomycin (100 µg/ml) (Gibco BRL, Grand Island, NY), L-glutamine (2mM) (Sigma Chemical Co., St. Louis, MO), and 10% fetal bovine serum (FBS) (Gibco BRL, Grand Island, NY) at 37 ° C with 5% CO2.

The cells were stored in their best growth state in nitrogen tanks so that 3-5 million cells in 90% FBS and 10% dimethyl sulfoxide were stored at -20◦C for 1 hour and then at -70◦C for another 24 hours and eventually preserved in liquid nitrogen at -137◦C. The viability of cells was assessed by Trypan blue dye staining method. In case more than 90% viable cells were obtained, 105 cells per well were seeded into flat-bottom 96-well culture plates (Nunc-Immuno-Plate Maxisorp, Nunc, Denmark) for evaluation.

The root canal filling materials used in this study were sterile size #20, 0.02 taper Resilon (LLC, Madison, Connecticut) and the same size gutta percha (Meta BioMed, Korea) points. Three millimeters from the tip of each point was cut and directly placed at the bottom of the culture wells. Cultured cells were exposed to gutta percha (groups G1 and G2) and Resilon (groups R1 and R2) segments. Root canal filling materials were contaminated with 10 µg/ml bacterial LPS (Escherichia coli; Sigma, St. Louis, MO, USA) in groups G1 and R1 while being exposed to the cultured cells. Positive control group included the bacterial LPS, but no obturation material and the negative control comprised of the cells in culture medium only.

Viability of cells was tested in all groups after 24, 48, and 72 hours. Each test was performed at least 3 times to obtain reproducible results. In this regard, four hours prior to completion of each time period, the test and control groups were subjected to sterile 5mg/ml MTT powder (Sigma Chemical Co, St Louis, MO, USA) dissolved in PBS solution, according to the manufacturer's instructions. The samples were then incubated at 37 ° C in 5% CO2 for another four hours. The plates were centrifuged at 2000 rpm for five minutes. The supernatant was discarded. Subsequently, isopropanol and 0.04% hydrochloric acid (100λ) were added. The mixture was agitated on a rotator for 45 minutes. The results were read by ELISA reader (Behring, Marburg, Germany) at a wavelength of 570nm considering 630nm wavelength as the reference. Data were analyzed using three-way ANOVA and Tukey's post hoc statistical test at 95% confidence level and SPSS 15.0 for Windows (SPSS Inc., Chicago, IL) was used as the statistical tool.

RESULTS

The mean and standard deviation for all groups, treatments and intervals are shown in Table 1.

Optical density (OD) values for cell viability showed an almost constant time-dependent decrease in all experimental and control groups. MTT assay results indicated the highest value for cell viability in negative control group at all three time points. At 24 hours, wells containing non-treated gutta percha segments (G2) indicated the lowest rate of cell viability. At 48 and 72 hours, the least number of viable cells were recorded in LPS-contaminated gutta percha group (G1).

The effect of three- and two-way interactions was not significant according to the statistical tests used. The P-value was calculated to be 0.995 for the three-way interactions. The interaction effect of time and the obturation materials was not significant either (P=0.914).

Also, the interaction between the obturation material and LPS treatment was not significant (P=0.942). The interaction effect of time and LPS treatment was not significant either (P=0.750).

There was a significant difference among gutta percha, Resilon and control groups (P=0.031). Tukey's multiple comparisons showed that although Resilon groups indicated more cytotoxic responses than gutta percha groups, the difference was not statistically significant (P=0.105).

Gutta percha groups showed significantly more cytotoxic responses in comparison to the control group (P=0.034). On the other hand, there was no significant difference between the cytotoxic responses of Resilon groups and control, irrespective of the LPS treatment (P=0.866). A significant decrease in cell viability was observed in all experimental groups (irrespective of the material tested or LPS contamination) at 72 hours (P<0.05).

Cell viability in groups with LPS treatment was lower than that in groups without LPS treatment at all time points except for G1 and G2 groups in which almost equal results were obtained only at 24 hours. Overall, the effect of LPS treatment was not significant (P=0.280). Resilon groups showed higher cell viability than gutta percha groups, although statistically non-significant (P>0.05).

Cell viability values were lower in gutta percha than Resilon groups when LPS-treated and LPS-untreated groups were compared independently at each time point.

DISCUSSION

Biocompatibility of endodontic materials is of particular interest, because incorporation of toxic ingredients may cause irritation or degeneration of host tissues [8].

Table 1. The mean and standard deviation values for all experimental groups (G1= LPS-contaminated gutta-percha, G2= non-treated gutta percha, R1= LPS-treated Resilon, R2= non-contaminated Resilon, Ctrl= control)

| Time | Group | LPS | | | |
| | | Yes | | No | |
		Mean	SD[1]	Mean	SD
24h	G1	1.2194	.42592	1.2242	.61105
	R1	1.5348	.34896	1.4408	.32628
	Ctrl	1.6621	.56052	1.5680	.43649
48h	G1	1.0350	.11629	.9344	.32303
	R1	1.4353	.12854	1.1649	.32443
	Ctrl	1.4201	.14450	1.1941	.60798
72h	G1	.4214	.20261	.3444	.24855
	R1	.5264	.03982	.4918	.04792
	Ctrl	.5544	.11901	.5402	.09641

Use of in vitro models offers the possibility of studying the effects of material components released on cultured cells [8]. It has been well established that potentially toxic components released from a variety of endodontic materials may enter the blood circulation and cause systemic effects [9]. Cytotoxicity assessment of dental materials can be performed either in vitro or in vivo. Use of cell lines is a common method for testing the cytotoxicity of dental materials because it is simple and yields reproducible and controllable results. In vitro testing also makes it possible to compare several materials using the same cell lines under the same conditions [10]. Macrophages are among the most important cells in inflammatory responses. In vitro evaluation of initiation of inflammatory and immune responses to dental biomaterials and their components, may predict biological responses in vivo [11, 12]. Adverse reactions of macrophages to components of some dental materials at specific concentrations have been reported [9, 13, 14].

The aim of this study was to evaluate the effects of sterile and LPS-contaminated Resilon on viability of human monocyte/macrophage cell line (i.e., THP-1) in comparison with gutta percha under similar conditions. Cytotoxicity evaluation in this study was performed at three time points of 24, 48, and 72 hours by the MTT assay in a triplicate fashion to obtain reproducible results. This technique is based on the reductive cleavage of yellow tetrazolium salt to a purple-colored formazan crystal by the dehydrogenase activity of intact mitochondria. Therefore, this conversion can only be seen in living cells [15-17].

Although gutta percha is considered inert, our findings as well as those of several other studies showed that it may elicit moderate levels of toxicity [18, 19]. Some authors [20, 21] have stated that such effect is attributable to the leakage of zinc ions. Gutta-percha may be well tolerated, but severe tissue injuries [22] as well as activation of complement components contributing to periapical inflammation

[23] have also been observed. Resilon was introduced as a core root canal filling material to endodontic market for use in conjunction with resin sealers especially Epiphany for root canal obturation to produce a so-called intra-radicular monoblock [24]. Superiority of Resilon-filled to conventionally filled root canals has been a matter of debate in terms of physical properties. On the other hand, few biological investigations have been conducted to evaluate the effects of Resilon on human and animal cells, tissues and organs. Studies about Resilon convey that although this material is well tolerated by the periapical tissues of sub-human primates [25], some concerns exist regarding the risk of alkaline or enzymatic hydrolysis [26-28]. Our findings are comparable with those of Key et al, [10] who showed that cytotoxicity of Resilon was equal to that of gutta percha, although cellular models differed. In addition, Chang et al. did not show noticeable cytotoxicity of gutta percha on human gingival fibroblasts during 24 hours of incubation [29]. Similar results were also obtained for human immune cells [30] and L929 fibroblasts derived from rats [31, 32]. As stated previously, cytotoxic effects of gutta percha points may be attributable to leakage of some leachable toxic components, such as zinc oxide. This is difficult to establish, however, because the material composition is often proprietary [29, 33].

CONCLUSION

It can be concluded that none of the root canal filling materials tested in the current study had toxic effects on cultured human monocyte cells whether in presence or absence of LPS.

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