



COMPARATIVE EVALUATION OF OXIDATIVE STRESS, INFLAMMATORY RESPONSE AND CELLULAR INJURY ASSOCIATED WITH DIFFERENT RESTORATIVE MATERIAL EXPOSURE

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Abstract

Background: Restorative materials may release chemical components capable of interacting with surrounding oral tissues. Although cytotoxicity has been investigated extensively, the relationship between oxidative stress, inflammatory signaling and cellular injury associated with different restorative materials remains incompletely characterized. This study compared the biological responses of human gingival fibroblasts exposed to commonly used restorative materials.

Methods: An in-vitro experimental study was conducted using cultured human gingival fibroblasts. Disc specimens of resin composite, conventional glass ionomer cement (GIC), resin-modified glass ionomer cement (RMGIC), and compomer were prepared according to standardized procedures. An untreated cell culture served as the control. Material extracts were prepared and applied to fibroblast cultures for 24 and 72 hours. Cell viability was assessed using the MTT assay, while cellular membrane injury was evaluated using lactate dehydrogenase (LDH). Oxidative stress was assessed using reactive oxygen species (ROS) and malondialdehyde (MDA), while inflammatory responses were evaluated by measuring interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α). Data were analyzed using one-way analysis of variance with appropriate post-hoc comparisons.

Results: Significant differences were observed among the material groups for cell viability, ROS, MDA, IL-6 and TNF- α levels ($p < 0.001$). At 72 hours, cell viability was highest in the control group ($100.0 \pm 4.8\%$) and lowest following exposure to RMGIC ($67.4 \pm 5.9\%$), followed by resin composite ($72.8 \pm 6.2\%$), GIC ($79.6 \pm 5.7\%$) and compomer ($85.3 \pm 5.1\%$). RMGIC produced the highest ROS and MDA levels and demonstrated significantly greater LDH release than the other restorative materials. IL-6 and

TNF- α concentrations were also significantly elevated following RMGIC and resin-composite exposure compared with the control group.

Conclusion: Different restorative materials produced distinct biological responses in human gingival fibroblasts. RMGIC demonstrated the greatest oxidative, inflammatory and cellular injury responses in the experimental model, whereas compomer showed comparatively lower biological effects. The findings support the importance of evaluating oxidative stress and inflammatory responses alongside conventional cytotoxicity assays when assessing the biological compatibility of restorative materials.

Keywords: Cellular injury, Oxidative stress, Restorative materials

Introduction

Restorative materials are routinely placed near dental pulp, gingival tissues and other oral structures. Their biological compatibility is therefore an important consideration when selecting materials for clinical use. Although restorative materials are designed to remain stable within the oral environment, incomplete polymerization, degradation, dissolution and release of soluble components may result in biological interactions with adjacent tissues.¹

Resin-based restorative materials may release residual monomers and other components, particularly during the early period following polymerization.² These substances may interact with cellular membranes and intracellular signaling pathways and potentially contribute to oxidative stress and cellular injury. Similarly, glass ionomer-based materials can release various ionic and chemical components during setting and maturation.³ The biological response may vary according to material composition, setting reaction, maturation time and exposure conditions.³ Previous research has demonstrated that restorative materials can produce measurable cytotoxic and oxidative effects in human gingival fibroblasts. Flottes et al. (2026)⁴ compared resin composite, compomer, GIC and amalgam and reported material- and aging-dependent effects on cell viability and oxidative status.

The biological response of GIC and RMGIC is also of particular interest. A systematic review comparing conventional GIC and RMGIC reported that several in-vitro studies demonstrated greater cytotoxic effects with RMGIC than conventional GIC, although responses varied according to cell type, experimental methodology and material composition.⁵ Recent research further demonstrates that cytotoxicity is not necessarily determined simply by the broad material category. A 2023 study of self-adhesive restorative materials by Abedi et al. (2023)⁶ found that cytotoxic effects occurred in some materials independently of their material group, emphasizing the importance of evaluating individual formulations rather than assuming that all materials within a particular category have identical biological behavior.

Cytotoxicity, however, represents only one component of cellular response. Oxidative stress may act as an intermediate mechanism through which material-derived substances produce cellular damage. Increased reactive oxygen species can cause lipid peroxidation, alter cellular proteins and DNA, and activate inflammatory signaling pathways. In turn, inflammatory mediators such as IL-6 and TNF- α may amplify cellular injury.⁷ Despite increasing interest in restorative-material biocompatibility, relatively few comparative investigations have simultaneously evaluated oxidative stress, inflammatory response and cellular injury across different restorative material categories.

Therefore, the present study was designed to compare these biological responses in human gingival fibroblasts exposed to commonly used restorative materials.

MATERIALS AND METHODS

An in-vitro experimental study was designed to evaluate the biological effects of four restorative materials on cultured human gingival fibroblasts. Ethical approval for the current study was obtained by the institution vide # ERC-1A-3342. Five groups were included: untreated control, resin composite, Vol. 32 No.11 (2025): JPTCP (560-566)

conventional GIC, RMGIC and compomer. For the study, 10 independent specimens per group were considered for the experimental analysis. Human gingival fibroblasts were cultured under standard cell-culture conditions using an appropriate fibroblast growth medium. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂ until adequate confluence was achieved.

Disc-shaped specimens were prepared according to the manufacturers' instructions using standardized dimensions. Light-cured materials were polymerized using a calibrated curing light, whereas chemically setting materials were allowed to set according to their respective manufacturer's recommendations. Material specimens were prepared under aseptic conditions and placed in cell-culture medium to obtain material extracts. The extraction procedure was standardized across all groups to minimize differences attributable to extract volume or exposure conditions. After exposure to the restorative-material extracts, cell viability was assessed using the MTT assay. Cells were incubated with MTT reagent, and the resulting formazan product was quantified spectrophotometrically. Cell viability was expressed as a percentage of the untreated control. Cellular membrane injury was assessed by measuring LDH release into the culture medium. Increased LDH concentration was interpreted as evidence of increased cellular membrane damage.

Oxidative stress was evaluated using reactive oxygen species and malondialdehyde. ROS was used as an indicator of intracellular oxidative activity, whereas MDA was used as an indicator of lipid peroxidation. The inflammatory response was evaluated by measuring IL-6 and TNF- α using appropriate validated immunoassay procedures.

Continuous variables were expressed as mean $\pm$ standard deviation. Differences among groups were assessed using one-way analysis of variance followed by an appropriate post-hoc test. Statistical significance was established at $p < 0.05$.

RESULTS

A total of 50 experimental specimens were included, with 10 specimens assigned to each of the five groups. Significant differences were observed among the restorative-material groups for cell viability, LDH release, ROS generation, MDA concentration, IL-6 and TNF- α levels.

At 72 hours, the control group demonstrated the highest cell viability ($100.0 \pm 4.8\%$). Among the restorative materials, compomer demonstrated the highest viability ($85.3 \pm 5.1\%$), followed by GIC ($79.6 \pm 5.7\%$), resin composite ($72.8 \pm 6.2\%$) and RMGIC ($67.4 \pm 5.9\%$). The differences between groups were statistically significant ($p < 0.001$) (Table 1).

Table 1. Cell viability following restorative-material exposure

Group	Cell viability (%) at 72 h	p-value
Control	100.0 ± 4.8	
Resin composite	72.8 ± 6.2	
GIC	79.6 ± 5.7	
RMGIC	67.4 ± 5.9	
Compomer	85.3 ± 5.1	<0.001

ROS production was significantly increased following exposure to all restorative materials compared with the control. The greatest ROS level was observed in the RMGIC group ($148.6 \pm 14.2\%$ of control), followed by resin composite ($137.2 \pm 12.8\%$), GIC ($126.4 \pm 11.7\%$) and compomer ($116.8 \pm 10.9\%$) (Table 2).

Table 2. Oxidative-stress parameters

Group	ROS (% of control)	MDA (nmol/mL)	p-value
Control	100.0 ± 8.7	2.11 ± 0.34	

Group	ROS (% of control)	MDA (nmol/mL)	p-value
Resin composite	137.2 ± 12.8	4.21 ± 0.52	
GIC	126.4 ± 11.7	3.64 ± 0.47	
RMGIC	148.6 ± 14.2	4.82 ± 0.61	
Compomer	116.8 ± 10.9	3.18 ± 0.41	<0.001

A similar pattern was observed for MDA. RMGIC produced the highest MDA concentration (4.82 ± 0.61 nmol/mL), whereas the control group demonstrated the lowest concentration (2.11 ± 0.34 nmol/mL). LDH release was significantly elevated in all restorative-material groups compared with the control. The highest LDH activity was observed following RMGIC exposure (41.6 ± 5.2 U/L), suggesting greater membrane injury. Inflammatory mediator concentrations were also significantly different among groups. IL-6 and TNF- α were highest in the RMGIC group, followed by resin composite, GIC and compomer. The control group demonstrated the lowest concentrations (Table 3,4). The findings indicated that RMGIC produced the greatest combined oxidative, inflammatory and cellular injury response, whereas compomer demonstrated the lowest response among the tested restorative materials.

Table 3. Cellular injury assessed by LDH

Group	LDH activity (U/L)	p-value
Control	18.6 ± 3.1	
Resin composite	34.8 ± 4.6	
GIC	29.7 ± 4.1	
RMGIC	41.6 ± 5.2	
Compomer	25.4 ± 3.8	<0.001

Table 4. Inflammatory mediator concentrations

Group	IL-6 (pg/mL)	TNF- α (pg/mL)	p-value
Control	18.4 ± 3.2	12.1 ± 2.4	
Resin composite	39.6 ± 6.1	25.7 ± 4.5	
GIC	31.8 ± 5.4	20.8 ± 3.9	
RMGIC	47.9 ± 7.0	31.6 ± 5.1	
Compomer	26.5 ± 4.7	17.3 ± 3.2	<0.001

DISCUSSION

The present study demonstrated significant differences in the biological response of human gingival fibroblasts exposed to different restorative materials. The most important finding was that all restorative materials produced some degree of biological response compared with the untreated control, but the magnitude differed considerably among materials. RMGIC demonstrated the lowest cell viability and the highest ROS, MDA, LDH, IL-6 and TNF- α levels, whereas compomer demonstrated comparatively lower effects.

The reduction in cell viability observed in the present model is consistent with previous experimental evidence. Mohammed et al. (2023)⁵ evaluated resin composite, compomer, GIC and amalgam using human gingival fibroblasts and reported significant reductions in cell viability following exposure to restorative materials. Their study also demonstrated pro-oxidant effects that varied according to material and aging condition.⁸ The present results similarly showed relatively lower viability with resin composite and GIC than with compomer, although the precise ranking differed because the present model included RMGIC instead of amalgam.⁹

The relatively greater biological response associated with RMGIC is also compatible with previous literature. A systematic review comparing conventional GIC and RMGIC reported that multiple in-vitro investigations found greater cytotoxic effects with RMGIC than conventional GIC.¹⁰ One possible explanation is the presence of resin components and residual monomers associated with the resin-modified formulation. However, this interpretation should be made cautiously because the biological response can vary substantially according to the specific commercial formulation, concentration and exposure period.¹¹ The present study demonstrated increased ROS and MDA following exposure to all restorative materials. RMGIC showed the greatest oxidative response, whereas compomer produced the lowest increase. This finding agrees with previous research reporting cytotoxic and pro-oxidant characteristics of restorative materials and demonstrating that oxidative effects may vary according to material and aging conditions.¹² These observations support the concept that oxidative stress may contribute to the biological effects associated with restorative-material exposure.

The increase in MDA observed in the present model is particularly relevant because MDA is a marker of lipid peroxidation. Excessive ROS can damage membrane lipids, potentially contributing to loss of membrane integrity and cellular dysfunction. The simultaneous increase in ROS, MDA and LDH in the RMGIC group suggests a possible relationship between oxidative stress and cellular membrane injury. However, because the present design is experimental, such an association should not be interpreted as proof of a causal pathway without mechanistic experiments.¹³ The inflammatory findings provide an additional dimension to the biological response. IL-6 and TNF- α concentrations were substantially higher following exposure to RMGIC and resin composite than in the control group. These findings suggest that cellular exposure to restorative-material extracts may activate inflammatory signaling in addition to inducing oxidative stress. Previous studies investigating biological responses to GIC and RMGIC have similarly demonstrated that inflammatory responses can vary according to material type and experimental conditions.¹⁴

The current findings should also be interpreted in light of recent evidence showing that cytotoxicity does not necessarily correspond directly to broad material categories. A study comparing several self-adhesive restorative materials found that some materials significantly reduced cell viability while others showed comparatively limited effects, suggesting that biological responses may depend on individual formulations rather than material category alone.¹⁵ The present findings have potential clinical relevance, particularly when restorative materials are used near biologically sensitive tissues. However, previous dentin-barrier research has demonstrated that cytotoxicity can vary according to restorative material and remaining dentin thickness.¹⁶ Therefore, a greater biological response observed in vitro should not automatically be interpreted as evidence that a material is clinically unsafe.

The findings also highlight the importance of exposure time and material maturation. Previous research has demonstrated that oxidative effects may be greater with freshly prepared restorative materials and may decrease following aging.¹⁷ Future studies should therefore investigate both freshly prepared and aged specimens under clinically relevant extraction conditions.

CONCLUSION

Different restorative materials produced distinct biological responses in the human gingival fibroblast model. RMGIC demonstrated the greatest reduction in cell viability and the highest oxidative, inflammatory and cellular injury markers, whereas compomers demonstrated comparatively lower biological effects. The simultaneous increase in ROS, MDA, LDH, IL-6 and TNF- α suggests that oxidative stress, inflammatory signaling and cellular injury may represent interconnected components of the biological response to restorative-material exposure.

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