Original Article

In Vitro Evaluation of Cytotoxicity in Novel Nano-Filled Bulk-Fill Flowable Dental Composites

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Cite as: Shreshtha M, Pradeep S, Dona S, Priscilla ASD , Vigneshwar T. In Vitro Evaluation of Cytotoxicity in Novel Nano-Filled Bulk-Fill Flowable Dental Composites. Rwanda J Med Health Sci. 2026;9(2): 426-434. https://dx.doi.org/10.4314/rjmhs.v9i2.13.

Abstract

Background
Nano-filled bulk-fill flowable composites have gained widespread use in restorative dentistry owing to their ease of manipulation, reduced polymerisation shrinkage, and improved physical and esthetic properties. Despite these advantages, the potential leaching of unreacted monomers or residual nanoparticles remains a concern, as such release may compromise the biological safety of these materials. Therefore, the present study aimed to assess the cytotoxic potential of a nano-filled bulk-fill flowable composite on 3T3-L1 fibroblast cells using the MTT assay. This evaluation was undertaken to elucidate the material's biosafety profile and its suitability for clinical application.

Methods
The in vitro cytotoxicity assessment was performed in accordance with ISO 10993-5 guidelines. The nano-filled light-cured composite was finely ground, and serial extract concentrations of 25, 50, and 75 mg/mL were prepared in culture medium. 3T3-L1 fibroblasts were exposed to these extracts for 48 hours, after which cell viability was quantified using the MTT assay by measuring absorbance at 570 nm. Morphological alterations in fibroblasts following exposure were examined using phase-contrast microscopy. Statistical analysis was conducted using one-way ANOVA followed by post-hoc testing, with significance set at $p < 0.05$.

Results
Mean cell viability was over 90 per cent at all of the concentrations (96.1 per cent, 94.3 per cent, 91.9 per cent), and was not significantly different at any of the controls ($p > 0.05$). Cells were attached with normal spindle morphology.

Conclusion
The tested nano-filled bulk-fill composite was found to be highly biocompatible and exhibit low cytotoxic effects, confirming that it can be used in clinical restorative cases.
Rwanda J Med Health Sci 2026;9(2):426-434

Keywords: 3T3-L1 fibroblasts, bulk-fill composite, cytotoxicity, health risk, nanofillers, MTT assay, product innovation, safety net

Introduction

Resin-based composites have become the material of choice for direct restorative procedures owing to their favourable aesthetic properties, ease of clinical manipulation, and predictable adhesion to enamel and dentine.[1,2] The development of bulk-fill flowable composites has further advanced restorative dentistry by enabling placement in increments of up to 4–5 mm while maintaining an adequate depth of cure.[3,4] These improvements have been achieved through modifications in resin chemistry, including optimised monomer systems, highly efficient photoinitiators, and increased translucency, all of which contribute to enhanced polymerisation. In addition, the incorporation of nanotechnology has facilitated the development of composites containing nanometre-sized fillers that exhibit superior mechanical properties, improved wear resistance, enhanced surface smoothness, and a higher degree of conversion than conventional microfilled and hybrid resin composites.[5–12]

Despite these technological advances, the biological safety of resin-based restorative materials remains an important consideration. Under clinical conditions, polymerisation is seldom complete, allowing residual monomers such as bisphenol A-glycidyl methacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), hydroxyethyl methacrylate (HEMA), and urethane dimethacrylate (UDMA) to leach from the polymer matrix into the surrounding oral environment.[13–15] Previous in vitro studies have demonstrated that these eluates can adversely affect periodontal and oral soft tissue cells, particularly human gingival fibroblasts, by reducing cell viability, inducing oxidative stress, promoting inflammatory responses, and activating apoptotic pathways.[13–17] Furthermore, the extent of these biological effects has been reported to depend on several factors, including the resin matrix composition, filler characteristics, degree of monomer conversion, and the amount and duration of residual monomer release.[16,17]

Although the cytotoxic potential of conventional resin-based composites has been extensively investigated, comparatively limited evidence is available regarding the biological performance of recently introduced nanofiller-based hybrid bulk-fill flowable composites. Moreover, studies directly comparing their cytocompatibility with that of conventional flowable composites on human gingival fibroblasts remain scarce.
Nanotechnology has enhanced the performance of resin-based composites, concerns regarding their biological interactions remain. Following polymer degradation or the release of residual constituents, nano-sized fillers and other leachable components may interact with adjacent cells, leading to oxidative stress through increased reactive oxygen species (ROS) generation under certain conditions. However, these effects depend on factors such as filler composition, particle characteristics, and exposure conditions. Despite their increasing clinical use, evidence regarding the cytocompatibility of nanofiller-based hybrid bulk-fill flowable composites, particularly on periodontal cells, remains limited, warranting further investigation.[18]

These are the reasons why systematic in-vitro biocompatibility testing is still a must. The MTT colorimetric assay is a dependable indicator of the cytotoxicity of the materials because it measures the activity of the mitochondrial enzymes.[19] The well-established fibroblast cell lines like L929, NIH-3T3 and 3T3-L1 are close models of oral soft-tissue behaviour and thus most commonly used at the preliminary stage of analysis.[20,21]

Based on this, the current study aimed to evaluate the cytotoxic effects of a nano-filled bulk-fill flowable composite on the 3T3-L1 fibroblast cells by the MTT assay method with the intent of elucidating its bio-safety and clinical applicability.

Methods

Study design and setting

This in-vitro experimental research was conducted in January 2025 to assess the cytotoxic potential of a nano-filled bulk-fill flowable composite using the MTT colorimetric assay on 3T3-L1 mouse embryonic fibroblast cells. The investigation took place in the Department of Conservative Dentistry and Endodontics, in collaboration with the Institutional Central Research Laboratory. The entire methodology followed the specifications outlined in ISO 10993-5 for biological evaluation of medical materials and ISO 10993-12 for the preparation and handling of samples.

Study population and eligibility criteria

The assay utilised 3T3-L1 mouse embryonic fibroblast cells (ATCC® CL-173™, American Type Culture Collection, USA). Only cells from passages 3 to 10 exhibiting a characteristic spindle-like morphology and a viability greater than 95% were included in the analysis. Cultures were excluded if they showed any sign of contamination, altered morphology, or over-passaging.

Sample size and sampling procedures

The research design had one untreated control and three experimental concentrations, 25, 50, and 75 mg/mL, which were tested in triplicate to increase credibility. The number of samples taken was in line with the provisions of the ISO 10993-5 of in-vitro cytotoxicity testing. Wells were randomly selected so that no bias could occur when testing.

Data collection instruments, procedures and quality control

The test material was a nano-filled bulk-fill flowable composite (the brand was chosen due to the principles of confidentiality). Its material was worked out in aseptic conditions and cured with the help of LED light-curing unit (450470 nm, 1000 mW/cm 2) for 20 s. The material was then finely ground by a sterile porcelain mortar and pestle after it was cured.

To obtain the extracts, 0.2 g/mL of the powdered composite was immersed in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 1 % penicillin-streptomycin. The suspension was incubated for 48 hr at 37 °C in a humidified atmosphere containing 5% CO2, then passed through a 0.22 µm syringe filter to eliminate residues. The filtered extract was diluted with complete culture medium to obtain working concentrations of 25, 50, and 75 mg/mL. Individual cell cultures were exposed to each concentration separately, whereas untreated cells maintained in complete culture medium served as the negative control. All experimental and control groups were incubated under identical conditions before cell viability was determined using the MTT assay.

3T3-L1 cells were maintained in DMEM enriched with 10 % FBS and 1 % penicillin-streptomycin at 37 °C under 5 % CO_2 in a humidified incubator. Once cell confluence reached 80–90 %, subculturing was carried out using 0.25 % trypsin-EDTA in which following cell seeding, the culture medium was replaced after 24 h to remove non-adherent cells and residual trypsin. Then the medium was renewed every 48-72 h, or as required, until the desired cell confluence was achieved. A suspension c ontaining 1×10^4 cells was dispensed into each well of a 96-well plate and cultured for 48 h to permit adequate cell attachment. The existing culture medium was then removed from all wells and replaced with 100 µL of composite extract at concentrations of 25, 50, or 75 mg/mL for the respective treatment groups. Wells assigned to the control group received 100 µL of fresh complete culture medium without the composite extract. Following treatment, all plates were maintained under identical incubation conditions until cell viability was evaluated using the MTT assay.

After a 48-hour exposure period, 20 mL of MTT reagent (5 mg/mL in phosphate-buffered saline) was added to each well and incubated for 4 h to permit formazan formation.

After that, the medium was disposed and 150 mL of dimethyl sulfoxide (DMSO) solution was added to dissolve formazan crystals. The absorbance of the samples and the standard was measured at 570 nm with the microplate spectrophotometer.

All the experiments were made in a laminar airflow hood to achieve sterility. Each test was performed thrice to be sure that there is consistency, and there were positive and negative control groups. Qualitative assessment of cell morphology was done using a phase-contrast inverted microscope (Olympus CKX41, 100X). The features that could be observed, including the rounding, shrinking, detachment, or membrane blebbing, were taken as signs of cytotoxic effects.

Data analysis

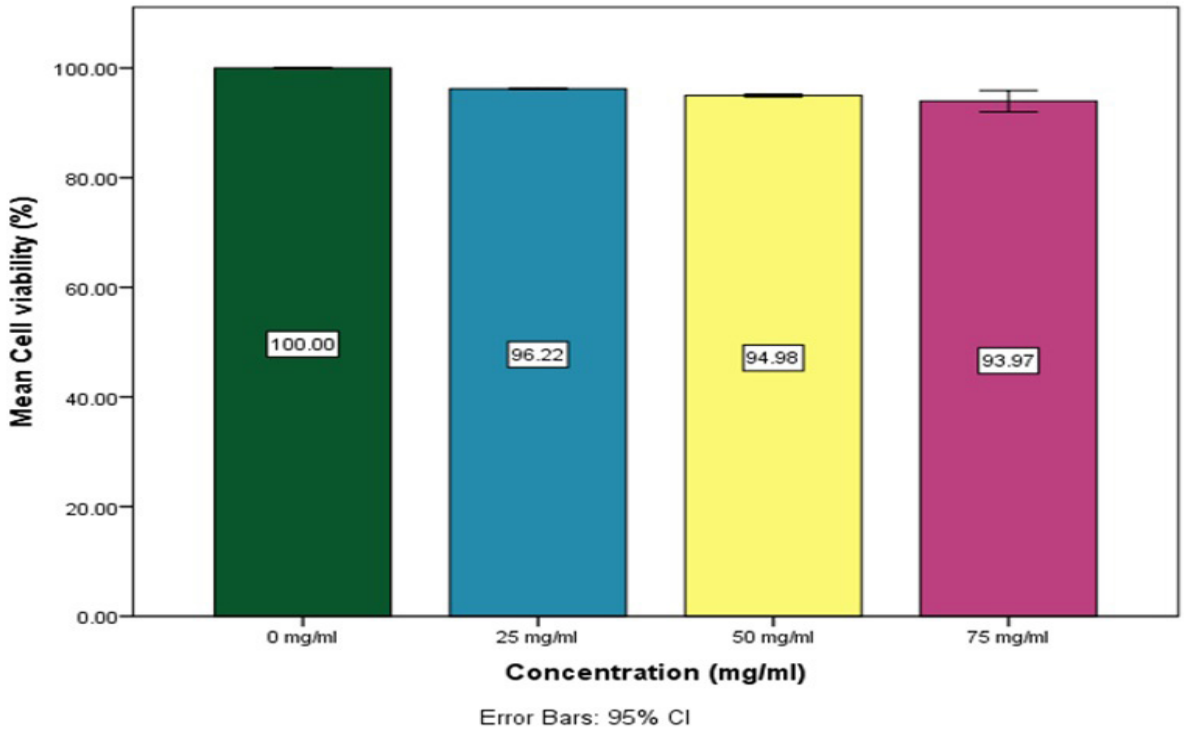
Results are presented as the mean ± standard deviation (SD). We used a one-way ANOVA followed by Tukey's post-hoc test to compare the means of cell viability (%) (measured via MTT assay at 570 nm) across three different extract concentrations (25,50, and 75 mg/mL). Statistical significance was set at $p<0.05$. All statistical calculations were performed using IBM SPSS Statistics Version 25.0.

Ethical considerations

The study protocol was reviewed and approved by the Institutional Review Board (IRB) and Scientific Review Board (SRB) of Saveetha Dental College (Approval No: SRB/SDC/ENDO-2302/24/271). Since the research exclusively utilised standardised cell lines, informed consent was not applicable. Written approval for the use of the laboratory facilities and material handling was obtained from the Institutional Central Research Laboratory, Saveetha Dental College and Hospitals.

Results

The MTT assay, performed after 48 hours of exposure to composite extracts at concentrations of 25, 50, and 75 mg/mL, demonstrated that the nano-filled bulk-fill flowable composite consistently maintained high levels of fibroblast viability across all groups. As presented in Table 1 and Graph 1, the control group exhibited 100% viability, while the experimental groups showed viability values of 96.28 ± 0.15% and 93.97 ± 0.57% at increasing concentrations.



Graph 1. 3T3-L1 cells MTT assay following 48 h incubation with nanofilled bulk-fill composite (075mg/mL). Mean ± SD (n=3); viability 90% and above at all concentrations.

Table 1. MTT test of 3T3-L1 cells on 48 h exposure to nanofilled bulk-fill flowable composite

Concentration (mg/ml)	Cell Viability % (Mean ± SD)
0	100
25	96.28 ± 0.15
50	94.98 ± 0.10
75	93.97 ± 0.57

They give results as mean ± SD (n=3), and the cell viability remained at or above 90% in all the concentrations.

Although a gradual reduction in viability was noted with increasing extract concentration, this decrease did not reach statistical significance ($p > 0.05$). One-way ANOVA revealed no significant difference at various concentrations compared to the control group ($p = 0.073$) (Table 2,3). These findings indicate that the composite extracts did not exert a cytotoxic effect at any of the tested concentrations, suggesting that the material is biocompatible and well-tolerated by fibroblasts under the conditions of the present study.

Table 2. One way ANOVA between all the groups

Variables	Sum of Squares	df	Mean Square	F	P value
Between Groups	0.635	2	0.318		
Within Groups	0.455	6	0.076	4.187	0.073
Total	1.091	8			

Table 3. Pairwise comparison of mean absorbance values among the control, 25 mg/mL, 50 mg/mL, and 75 mg/mL groups using Tukey's HSD post hoc test

Variables	Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	25 mg/ml	0.43333	0.26519	0.054	0.5841	2.2826
(0 Mg/ml)	50 mg/ml	0.76667	0.26519	0.070	1.4274	3.1259
	75 mg/ml	0.80004	0.26519	0.073	1.9508	3.6492
	0 mg/ml	-0.43333	0.26519	0.054	-0.5841	-2.2826
25 mg/ml	50 mg/ml	0.07333	0.22493	0.944	-0.6168	0.7635
	75 mg/ml	0.59667	0.22493	0.084	-0.0935	1.2868
	0 mg/ml	-0.76667	0.26519	0.07	-1.4274	-3.1259
50 mg/ml	25 mg/ml	-0.07333	0.22493	0.944	-0.7635	0.6168
	75 mg/ml	0.52333	0.22493	0.127	-0.1668	1.2135
	0 mg/ml	-0.80004	0.26519	0.073	-1.9508	-3.6492
75 mg/ml	25 mg/ml	-0.59667	0.22493	0.084	-1.2868	0.0935
	50 mg/ml	-0.52333	0.22493	0.127	-1.2135	0.1668

Note: The mean difference is significant at the 0.05 level.

Discussion

This paper aimed to evaluate the cytocompatibility of a nano-filled bulk-fill flowable composite on fibroblast cell cultures at various concentrations. The purpose was to verify whether the material meets the established biological safety criteria for use as a restorative material.

The present study demonstrated that the nanofiller-based hybrid bulk-fill flowable composite maintained fibroblast viability above 90% across all tested concentrations, indicating favourable cytocompatibility. Previous studies have similarly reported cell viability above the 70% threshold defined by ISO 10993-5 for resin-based restorative materials, although the observed biological response varies with material composition, cell type, extraction protocol, and exposure conditions.[22,23]

Variations in resin chemistry and filler characteristics may further influence the release of residual monomers and the subsequent cellular response.[10]

The consistently high cell viability observed in the present study provides additional evidence supporting the cytocompatibility of this recently introduced nanofiller-based hybrid bulk-fill flowable composite, for which biological data remain limited. Nevertheless, further in vitro and in vivo studies are required to validate these findings under clinically relevant conditions. The elevated cell viability suggests that the composite releases very few harmful substances during or after polymerisation.[24,25] These findings provide further evidence that the evaluated nanofiller-based hybrid bulk-fill flowable composite exhibits favourable biological compatibility with fibroblast cells under the tested conditions. Given the limited cytocompatibility data available for this recently introduced material, the present study expands the existing evidence and supports its biological safety, thereby contributing to its preclinical evaluation and potential clinical application. [24,25] Overall, the findings indicate that the evaluated nanofiller-based hybrid bulk-fill flowable composite exhibited favourable cytocompatibility under the conditions of this in vitro investigation. Unlike many previous studies that primarily evaluated conventional resin composites, the present study provides additional biological evidence for a recently introduced nanofiller-based hybrid bulk-fill flowable composite using a standardised ISO 10993-5 protocol and multiple extract concentrations. Therefore, the tested composite can be considered to be biologically safe within the parameters of this in vitro model.

The outcomes of the present study parallel earlier investigations showing that bulk-fill composites generally cure more efficiently than traditional flowable materials. Their enhanced depth of polymerisation is attributed to improved optical properties and refined initiator chemistry, which allow curing light to travel further within the composite. As a result, the proportion of residual, unreacted monomers remaining after setting is markedly lower. This limitation of monomer release reduces the likelihood of adverse cellular responses. Taken together, these observations reinforce the view that bulk-fill systems provide both superior curing behaviour and a more favourable biological profile.[3,4,6] Ilie and Hickel [3] and Bakopoulou et al [14] stressed that an improved initiator system and good optical translucency can lead to enhanced mechanical stability and good biological behaviour. Similarly, Schweikl et al [10] noted that a complete process of polymerisation has genotoxic effects that can be reduced, which in turn prevents the possibility of causing harm to the surrounding tissues.

Mechanistically, the greater translucency and the even distribution of nano-scale fillers in these formulations facilitate better transmission of light, which forms a uniform polymer network throughout the material.[7,12] Chemically speaking, the nano-scale fillers that are being used in these formulations are inert and have low levels of solubility, which limits the number of unreacted monomers and thereby minimally oxidative and apoptotic stress on the exposed cells. [15,16]

Although this risk is seemingly minor when the fillers are properly encased in a cross-linked resin structure, such as by the use of isolated nanoparticles [26,27,28] isolated nanoparticles have been previously reported to induce reactive oxygen species and mitochondrial disruption.

In clinics, a high level of conversion and a stable filler-matrix bond are crucial for reducing residual monomer leakage and increasing the longevity of restorations.[29] Recently, the introduction of biofunctional and antibacterial nanocomposites—particularly those reinforced with zinc oxide and silver nanoparticles—has broadened the therapeutic potential of restorative materials by combining favourable mechanical properties with sustained antimicrobial activity.[30,31]

Nevertheless, the complex dynamics of the intraoral environment cannot be completely replicated under in vitro conditions. Factors such as salivary enzymes, fluctuating pH, and continuous microbial challenges may accelerate material degradation and influence long-term clinical performance. [32]

Long-term exposure models to examine oxidative stress responses, cytokine production, and gene expression patterns, as well as animal or clinical testing to verify the safety of functional conditions, should be part of future research.[33]

Limitations

Although the composite exhibited high cytocompatibility in the present study, these findings should be interpreted with caution. The investigation was constrained by its in vitro design, which does not fully replicate the complex biological and biochemical interactions of the oral environment. Although the 3T3 fibroblast cell line is a well-established model for in vitro cytocompatibility assessment, cellular responses to resin composite eluates may vary among different fibroblast cell lines because of inherent biological differences. Consequently, direct comparisons with studies employing L929 cells or primary human fibroblasts should be made with caution, and the influence of cell line selection on the observed cell viability cannot be excluded. In addition, the relatively short exposure period and the absence of artificial ageing or degradation protocols may limit the extrapolation of these findings to long-term clinical conditions. Future studies incorporating multiple cell lines and clinically relevant ageing models are warranted to provide a more comprehensive evaluation of the biological performance of this material. Furthermore, only one material formulation was evaluated, restricting the generalizability of the results to other nano-filled bulk-fill composites.[32]

Conclusion

Within the limitations of this in vitro study, the evaluated nanofiller-based hybrid bulk-fill flowable composite demonstrated favourable cytocompatibility with 3T3 fibroblast cells. These findings are consistent with previous studies using L929 and other fibroblast cell models, thereby extending the existing evidence to this recently introduced material. As cellular responses may vary with cell type and incubation duration, particularly following prolonged exposure, the findings should be interpreted within the conditions of the present study. Further studies incorporating multiple cell lines, longer incubation periods, ageing protocols, and in vivo models are required to confirm the long-term biological performance of this material.

Author Contributions

SM: Conceptualization, Methodology, Data Curation, Formal Analysis, Visualisation, Writing – original draft, Writing – review and editing; PS: Data collection; DS: Supervision, Critical review, and Final approval of the manuscript.

Funding

The study was not granted any targeted grant by either the public, commercial, or not-for-profit funding agencies.

Conflict of interest

Shreshtha Muskan and Pradeep S are associated with patent application No. 202541000312 concerning the composite resin evaluated in this study, which represents a potential competing interest. The other authors declare no conflicts of interest.

Generative AI declaration

No artificial intelligence (AI) tools were used in this study.

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