Comparison of cytotoxicity between bulk-fill resins and conventional composite resins and the factors affecting toxicity

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IJASR 2022
VOLUME 5
ISSUE 1 JANUARY – FEBRUARY **ISSN: 2581-7876**

Abstract: Objective: Bulk-fill resin-based composites are gradually being used in dental treatment now, but only limited data are available on their biocompatibility. The aim of this review is to analyze and compare the cytotoxicity of bulk-fill resins with that of conventional composite resins. At the same time, the mechanisms of the cytotoxic effects were analyzed from the factors of filler, monomer, and initiator.

Methods: The narrative review approach was performed. The literature search was conducted using PubMed, Web of science, and Ebsco, and non-English articles were excluded.

Results and conclusion: The cytotoxicity of the resin was evaluated based on different biological endpoints, i.e., cell morphology, cell membrane effect, cellular metabolism, and cell growth ability. Evaluation of cytotoxicity assays can also be categorized into direct contact method, indirect contact method, and extract method according to the mode of contact. According to the results of the above cytotoxicity evaluation methods, the cytotoxicity of most bulk-fill resins at the recommended curing depth is comparable to that of conventional resins. In this review, factors such as fillers, monomers, and initiators were also discussed to analyse the mechanism of cytotoxicity.

Keywords: Bulk-fill resin, Composite Resin, Cytotoxicity, Biocompatibility

Introduction

Light-curing composite resins are one of the most commonly used materials in dental treatment, such as cavity filling, fissure sealing, bonding, and other restorative dental treatments. However, conventional resins have substantial limitations in that they can only be placed in 2mm increments to obtain a sufficient degree of conversion [1]. This method not only involves more operational steps but also has larger polymerization shrinkage, resulting in poorly fitting edges and microleakage [2]. Bulk-fill resin can increase the depth of cure to more than 4mm by adding new fillers, monomers, or photoinitiators [3]. It simplifies the clinical procedure and improves the effect of restorative treatment.

In a systematic review and meta-analysis [4], follow-up results between 1 and 10 years after filling with bulk-fill resins and conventional resins showed that bulk-fill resins showed slightly lower microhardness, but they were superior to conventional resins in terms of volume shrinkage, polymerization stress, cusp deflection, and marginal quality. Therefore, bulk-fill resins have shown similar or better performance in clinical trials compared to conventional resins and can reduce the operative time of posterior filling restorations [5], with higher patient satisfaction.

An important prerequisite for a material that can be applied safely in clinical practice is good biocompatibility. In this paper, the recent studies on the cytotoxicity of bulk-fill resins were reviewed, and the mechanisms affecting the cytotoxic effects were analysed from the factors such as fillers, monomers, and photoinitiators.

1. Cytotoxicity of bulk-fill resins

The main causes of cytotoxicity of composite resins are as follows: ①residual monomers [such as 2-Hydroxyethyl methacrylate (HEMA), triethylene glycol dimethacrylate (TEGDMA), bisphenol-A-glycidyl-methacrylate (Bis-GMA), *etc.*]. The release of residual monomers because the degree of conversion cannot reach 100% during resins polymerization reactions. ② Degradation products. The resin polymers will release degradation products over time. These residual monomers and degradation products can enter the oral mucosa, periodontal tissues, dentin and affect the pulp tissue and periapical tissues through the dentinal tubule. Besides directly damaging the above-mentioned tissues, they can also cause sensitivity and pain after filling by releasing free radicals. In vitro cytotoxicity studies have confirmed the significant cytotoxicity of these monomers.

According to ISO 10993-5:2009, the cytotoxicity evaluation of resins can be classified according to different biological endpoints or different modes of exposure.

1.1 Classification based on biological endpoints

The cytotoxicity of the resin was evaluated based on different biological endpoints, *i.e.*, cell morphology, cell membrane effect, cell metabolism ability, and cell growth ability, respectively.

1.1.1 Evaluation of cell morphology

The evaluation of cell morphological changes is the most intuitive method for evaluating the cytotoxicity of biomaterials, which is usually used as an auxiliary or supplement to other methods for qualitative determination. In this method, the morphological changes of cells before and after exposure to the material tested are observed by microscopy, including changes in size, shape, and nuclei, as well as the percentage of apoptotic and dead cells. Studies have shown that the reduction in cell viability and the change in cell morphology are in parallel [6, 7], which means that the change in cell morphology can reflect the cytotoxicity of the material.

The cytotoxicity of most bulk-fill resins at a curing depth of 4 mm is comparable to that of conventional resins at the recommended curing depth of 2 mm [6-8], except for Beautifil bulk flowable (BBF, Shofu, Japan) and Beautifil bulk restorative (BBR, Shofu, Japan). In some studies, mouse fibroblasts (L929) [6, 7] and human dental pulp stem cells (hDPSCs) [8] were exposed to extracts of multiple cured resins for 24-72 h. The results showed that the cells mentioned above exposed to the extracts of BBF and BBR exhibited more cellular morphological changes of being smaller, rounder, with concentrated and fragmented nuclei, compared to other bulk-fill resins and conventional resins. This indicated that the toxicity of these two bulk-fill resins is greater. The reason is that they contain pre-reacted glass ionomer (PRG) fillers [6], which release fluoride and other ions after curing. Although it can inhibit acid production by bacteria in plaque and promote enamel remineralization, fluoride has been shown to play an important role in cytotoxicity, including causing cell damage, cell cycle arrest, mitochondrial dysfunction, DNA damage, and endoplasmic reticulum stress [9]. Other bulk-fill resins without PRG fillers showed similar cytotoxicity to conventional composite resins.

1.1.2 Evaluation of cell membrane effects

Evaluation of cell membrane effects is another effective method to reflect the cytotoxicity of resin materials, which is reflected by the change of cell membrane permeability. Increased cell membrane permeability and compromised lysosomal membrane integrity can result in the release of some enzymes from organelles when resin materials cause cell damage. The degree of cell damage can be reflected by the neutral red uptake assay (NRU assay) and the lactate dehydrogenase release assay (LDH assay). In particular, since the neutral red uptake assay reflects the integrity of the cellular lysosomal membrane, and the toxic effect of the composite resin on lysosomes precedes the toxicity to mitochondria. The NRU assay is more sensitive than other cytotoxicity tests that reflect cellular mitochondrial damage, such as the MTT assay [10]. Nascimento *et al.* [10] observed by this method that the activity of cells was reduced when L929 was exposed to 11 resins for 72 hours and 7 days, with the bulk-fill resin Opus Bulk Fill Flow (Opus, FGM, Brazil) and Filtek Bulk Fill (FBF, 3MESPE, Germany) showing a significant reduction in cell activity compared to the conventional resins. However, there was no significant difference in cytotoxicity between other bulk-fill resins and conventional resins. On the other hand, Haugen *et al.* [3]observed differences in the effects of the composite resin on different cells by LDH assay. In the study, the bulk-fill resin FBF had the greatest cytotoxicity to sensitive osteoblasts, but the toxicity of another bulk-fill resin, SureFil ® SDR Flow (SDR, Dentsply,

Germany), was slightly less than that of the conventional resin. In addition, the cytotoxic effects of these three materials on epithelial cells and fibroblasts were low and not statistically different.

1.1.3 Evaluation of cell metabolic activity

The degree of cell injury can be reflected by changes in the biological metabolism or the synthetic function of cells, which is the aspect of cellular metabolic activity to evaluate the cytotoxicity of resin materials. Cell metabolic activity can be detected using the method of tetrazolium salt compounds, which are degraded to coloured products by the effect of mitochondrial dehydrogenases. When there are more metabolically active cells, the coloured degradation products increase, so this method can be used to detect the cytotoxicity of materials. Among them, three methods, Thiazolyl Blue Tetrazolium Bromide assay (MTT assay) [10-12], water-soluble tetrazolium salt assay (WST assay) [8], and 3-(4,5-dime-thylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium(MTS method) [6], showed slight cytotoxicity or no cytotoxicity for bulk-fill resins and conventional resins at the curing depth recommended by the manufacturer. However, not all bulk-fill resins could achieve proper polymerization at the recommended curing depth [10]. For example, the bulk-fill resin Opus failed to polymerize completely at 4 mm, so it had large cytotoxicity. The study also proved that the MTT assay is more sensitive than the LDH assay, but the MTT assay is unstable and the results of repeated tests are quite different.

1.1.4 Evaluation of cell growth ability

It mainly refers to the measurement of cell proliferation after exposure to the tested materials, and mainly includes clone formation assays. Tsuchiya *et al.* [13] Indicated that clone formation assay is the most sensitive method for in vitro cytotoxicity evaluation. There is no study on the comparison of clone formation tests between bulk-filled resins and conventional resins, suggesting that this may be a direction for future research.

1.2 Classification based on contact modes

Evaluation of cytotoxicity assays is categorized into direct contact method, indirect contact method, and extracts method according to the mode of contact. An article [14] compared the sensitivity of these three methods and found that the extract method was poorly correlated with the other two methods and the least sensitive. However, the result of Lim *et al.* [15] showed the consistency of the three test models for resin cytotoxicity assay. The bulk-fill resins evaluated did not cause excessive cytotoxicity at a depth of cure of 4 mm [15].

However, the toxicity of the materials is not fully reflected by these commonly used methods, because the filling materials are separated from the pulp cells in vivo by dentin. The correlation between the cellular response shown by these methods and the response of the pulp cells practically in vivo is low. Therefore, Hume *et al.* [16] Proposed the dentin barrier method, which can simulate the situation that the toxic substances in the material act on the pulp cells through the dentin tubules after contact with the dentin. This method has been widely used to study the cytotoxicity of dental materials in recent years. It has been shown that the cytotoxicity of zinc oxide eugenol hydromorphone is much higher than that of animal experiments when measured by existing in vitro cytotoxicity assays. In contrast, the cytotoxicity obtained by using the dentin barrier method was the same as that observed in clinical applications.

In addition, the cells of the traditional test methods are cultured in monolayers. Recent research [17] has established three-dimensional dentin/pulp tissue mimics as an advanced assessment tool for cytotoxicity of dental materials (DentCytoTool). It can better simulate the growth environment of cells in the oral cavity and increase the clinical relevance of the experiment.

1.3 Reasons for differences in the results of cytotoxicity evaluation

The results of the cytotoxicity tests are related to the cells used, and there are significant differences in the results of toxic effects of different cell lines on the same resin. Human dental pulp fibroblasts (hDPFs) are more sensitive to the cytotoxicity tests than human dental pulp stem cells (hDPSCs) [18]. Human-derived cell lines are more sensitive to the assay of cytotoxicity than animal cell lines, such as L929 [19]. Since L929 is derived from mouse connective tissue, which is different in species from human cells. Moreover, there are some factors such as abnormal karyotype, genetic material changes, and so on, which lead to the difference between the toxic reaction ability and that of

normal cells in vivo. However, some studies have used human primary pulp cells [20] and primary gingival fibroblasts [21] to compare with L929 cells in toxicity tests, and the results were not significantly different. In general, a higher sensitivity indicates a more accurate response of such cells to the cytotoxicity of the material being tested. When evaluating the cytotoxicity of materials, different assays, as well as different cells, can be combined to simulate the cell growth environment in vivo if possible. This can reduce the experimental error and improve the correlation between the experiment and clinical treatment.

To some extent, the cytotoxicity of the resin is also related to the fluidity and viscosity of the resin. The high-viscosity type of bulk-fill resin has higher inorganic filler content and poor flowability, but it has better mechanical properties and lower cytotoxicity after curing. In contrast, the low-viscosity type of bulk-fill resin has a lower filler content, poorer mechanical properties, and higher cytotoxicity, but has better flowability.

According to the results of the above cytotoxicity evaluation methods, the cytotoxicity of most bulk-fill resins at the recommended curing depth is comparable to that of conventional resins, except for resins containing pre-reacted glass ionomer fillers (such as BBF, BBR, *etc.*) and some low-viscosity type bulk-fill resins (such as Opus, FBF, *etc.*), both of which are more toxic. However, the slight differences in the specific toxicity ranking may be due to the differences in the methods and evaluation criteria used in the different tests. A comparison of bulk-filled resins and conventional resins for cytotoxicity is shown in the accompanying table.

Attached table Literature review of cytotoxicity comparison between bulk-fill resins and conventional resins

authors	title	test materials	experimental design	observation content	period	cells type	results	other items	other experimental results
Toh WS, Yap AU, Lim SY	In Vitro Biocompatibility of Contemporary Bulk-fill Composites	standard composites: ZFR/ZFF bulk-fill PRG RBCs: BBR/BBF bulk-fill non-PRG RBCs: SDR/EXP/TNC	Extracts of 2mm, 4mm thickness specimens, diluted at 1:1, 1:2, and 1:10 concentrations	①Cell Morphology ②MTS assay	24h	L929	①The cytotoxicity of PRG bulk-fill resins was greater than that of non-PRG. ②At 4-mm thickness, undiluted extracts of bulk-fill non-PRG RBCs had significantly higher cell viability than the standard composites.		
Demirel G, Gur G, Demirsoy FF, Altuntas EG, Yener-Ilce B, Kilicarslan MA	Cytotoxic effects of contemporary bulk-fill dental composites: A real-time cell analysis	conventional flowable RBCs: CMF conventional paste-like RBCs: CME flowable bulk-fill RBCs: VBF/FBF/SDR/XTB/BBF paste-like bulk-fill RBCs: TEC/FBR/QXF/XTF/BBR	4mm specimens in direct contact	①Cell Morphology ②Cytotoxicity assay with iCELLigence system	24, 48 and 72 h	L929	①PRG containing bulk-fill composites were severely toxic at all time points 24, 48 and 72 h. ②None of the tested composites demonstrated high cell viability (>70%) at 48 and 72 h. ③Flowable and paste-like composites of the same brand exhibited similar cytotoxic properties.		
Lee S-M, Kim S-Y, Kim J-H	Depth-Dependent Cellular Response from Dental Bulk-Fill Resins in Human Dental Pulp Stem Cells	conventional composite resin: ZFF bulk-fill composite resins: SDR/VBF/BBF	Extracts of 2mm, 4mm, 6mm thickness specimens	①WST assay ②Images of live and dead cells	24h	hDPSCs	All bulk-fill resins except BBF revealed cytotoxicity from 4 to 6 or 2 to 4 mm, while ZFF was cytotoxic at over 2 mm.	①Depth of Cure (scraping test and Vickers hardness profile methodology) ②Differentiation capability	①Depth of cure was detected from 3.55 to 4.02 mm in the bulk-fill resins (vs. ~2.25 mm in conventional resin), and 80% hardness compared with that of a fully polymerized top surface was determined from 4.2 to 6 mm in the bulk-fill resin (vs. 2.4 mm in conventional resin). ②Antidifferentiation was revealed at a depth of 4-6 mm in the bulk-fill resin.
Nascimento AS, Lima DB, Fook MVL	Physicomechanical characterization and biological evaluation of bulk-fill composite resin	conventional resins: Z350/Z350F bulk-fill resins: ABF/FBFF/FBFS/SDR/TEF/AF/XTF/XTB/Opus	extracts of the diluted resins to obtain 1:2 and 1:10 concentrations	①MTT assay ②Neutral Red test	72h and 7d	L929	①The bulk-fill resins analyzed in this study exhibited low and/ or no cytotoxicity to L929 cells, except for Opus, which showed moderate cytotoxicity according to the MTT assay. ②When the NR test was used, results were not satisfactory for all composites.	①degree of conversion (FTIR) ②Vickers hardness (SEM and AFM) ③surface morphology ④organic filler (EDS)	The resins presented acceptable values for microhardness, degree of conversion, and surface morphology.
Haugen HJ, Marovic D, Par M, Thieu MKL, Reseland JE, Johnsen GF	Bulk Fill Composites Have Similar Performance to Conventional Dental Composites	conventional composite material: TEC bulk fill composite materials: SDR/FBF	Extract obtained from estimated daily salivary flow rate	①Cell morphology ②LDH assay	24h	①A549 cells ②HGF ③Primary human osteoblasts (hOB)	Conditioned media accumulated for 24 h from FBF and TEC were cytotoxic to primary human osteoblasts.	①Depth of Cure (scraping test) ②Degree of Conversion (FTIR) ③Polymerisation Shrinkage (μCT-scans) ④Filler Mass Fraction (thermogravimetric analysis apparatus) ⑤Morphology of Filler (SEM) ⑥Flexural Strength and Flexural Modulus (three-point bending test) ⑦Vickers Hardness (indentation test) ⑧Surface Topography Parameters (abrasion test) ⑨Monomer Elution (HPLC)	Compared to the conventional composite, the tested bulk fill materials performed equally or better in most of the tests, except for their hardness, elastic modulus, and biocompatibility with osteoblasts.
Gonçalves F, Campos LMP, Rodrigues-Jùnior EC, et al	A comparative study of bulk-fill composites: degree of conversion, post-gel shrinkage and cytotoxicity	conventional composites: Z350/ZFF bulk-fill composites: AB/EP/SF/FBF/FBFF/VBF	Extracts of 4mm thickness specimens	MTT assay	24h	HGF	All materials, including bulk-fill and conventional composites, were classified as non-toxic, with cell viability higher than 70%.	①Degree of conversion (FTIR) ②Volumetric shrinkage (the strain gauge method)	①Bulk-fill composites exhibited volumetric shrinkage similar to or lower (1.4 to 0.4%) than that of conventional composites (1.7–2.1 %). ②Only four of the bulk-fill composites were able to sustain a homogeneous conversion at the 4-mm depth.
Marigo L, Spagnuolo G, Malara F, et al	Relation between conversion degree and cytotoxicity of a flowable bulk-fill and three conventional flowable resin-composites	conventional flowable RBCs: VDF/XTE/HRI bulk fill flowable RBC: SDR	Extracts of 2mm and 4mm thickness specimens	MTT assay	24h	hDPSCs	All tested materials show light cytotoxic effects	①Degree of conversion (FTIR) ②Monomers Elution (HPLC) ③Vickers Hardness (indentation test)	①DC values and the hardness change in function of thickness and type of material ②The monomers amount leached from each specimen is influenced by thickness but it is always very low.

2 Factors affecting the cytotoxic effect

2.1 Filler

Bulk-filled resins increase the depth of cure by reducing the filler content or increasing the filler size to reduce light scattering and increase light transmittance. Bulk-fill resin SDR [3, 22] and SonicFill (SF, Kerr, USA) [23] employ the method of increasing the filler size with irregular filler particles of about 10-20μm in diameter to reduce light scattering and improve the degree of conversion. For the size of the filler particles, the degree of conversion is lower at wavelengths smaller than the incident blue light (400-480 nm) and closer to the output wavelength of the curing unit [24]. It is related to the scattering effect of the penetrating light in the process of activation with light. Once the filler sizes are increased over 500 nm, the degree of conversion can be improved, resulting in an increased depth of cure.

On the other hand, the filler content is negatively correlated with the light transmission [25]. For example, FBF reduces the content of filler, so as to increase the depth of cure [3]. At the same time, however, it uses different sizes of spherical high refractive index silylated zirconia/silica filler particles instead of granular glass fillers [3, 23]. Although it can improve mechanical properties, such as bending strength and fracture toughness [26], the high refractive index of zirconia leads to a decrease in light transmittance [23], which makes the degree of conversion and depth of cure of FBF lower than that of SDR, and the cytotoxicity is correspondingly higher.

It is noteworthy that decreasing the filler content will bring about an increase in the toxicity of the resin. Since inorganic fillers can cause less toxicity than resin matrix [19, 24], decreasing the filler content will correspondingly increase the matrix content, which leads to a corresponding increase in unreacted monomers and degradation products. It has been proved that the reduction of filler content causes a more obvious toxic response in cells [27] and inflammation in tissues [28], which is associated with mitochondrial dysregulation caused by increased release of reactive oxygen species (ROS) [29]. Materials with low filler content will release more degradation products bis-hydroxy-propoxy phenyl propane (BisHPPP, derivative of bis-GMA), methacrylic acid (MA), and unreacted monomer triethylene glycol dimethacrylate (TEGDMA) [29] after being exposed to esterase [30]. Furthermore, TEGDMA has been proved to be closely related to inflammation, inhibition of cell proliferation and differentiation, induction of apoptosis [31], and DNA damage.

The filler content is relevant to the mechanical properties of the material [32]. Reducing the filler content will reduce its mechanical properties, such as microhardness [4] and elastic modulus [33]. Therefore, the mechanical properties, biocompatibility, and depth of cure of the materials are considered in order to obtain a suitable formulation.

2.2 Monomer

The bulk-fill resin changes the matrix composition by adding new monomers with relatively high molecular masses, such as urethane dimethacrylate (UDMA), aromatic UDMA (AUDMA), and 1,12-dodecanedioldimethacrylate (DDDMA) [2], resulting in increased light transmission and thus increased depth of cure.

Bulk-fill resin SDR has a high degree of conversion because it contains a patented modified UDMA that chemically embeds polymerization modifiers into the resin backbone [34]. As a result, it is able to control the polymerization kinetics, improve conversion rates and reduce polymerization shrinkage [35]. Bisphenol-A- glycidyl-methacrylate (Bis-GMA), a matrix commonly used in conventional resins, is a highly viscous monomer that contains side hydroxyl groups and a rigid aromatic ring of bisphenol A, which negatively affects the degree of conversion [2]. While many bulk-fill resins are UDMA-based materials combining different monomers. The intermolecular hydrogen bond formed by imino groups in UDMA is weaker, which makes its viscosity lower than that of Bis-GMA. In the polymerization process, the activity and migration rate of monomers are reduced, which significantly improves the degree of conversion [36] and mechanical properties [37], without increasing polymerization shrinkage, and with less cytotoxicity [38].

According to previous studies [39], the performance of bulk-fill resins and conventional resins in leaching monomer is equivalent. However, Pongprueksa *et al.* [40] Found that monomer release after 2 mm increment filling of conventional resins was lower than that of bulk-fill resins. This is due to the slightly lower conversion of the bulk-filled resins at 4 mm, resulting in higher monomer release than the conventional resins. Nevertheless, in a toxicity

assay in vitro, cytotoxicity and genotoxicity of exposure to extracts were lower than exposure to single components of the composite resin [41], such as monomers, initiators, and additives, respectively. The antagonistic effect of various components in the extract [42], and the components in it can combine with saliva by protein [43], so the toxicity will be reduced. It suggests that the toxicity of the material in the actual situation is lower than that shown in the in vitro tests, especially the single component tests.

2.3 Other factors

Apart from changing the type of monomer and filler, the depth of cure can be increased by adding new photoinitiators. For example, Tetric Evoceram Bulk Fill (TEC, Ivoclar-Vivadent, Liechtenstein) adds a new photoinitiator, dibenzoylgermanium derivative Ivocerin, which generates free reactive groups during the reaction, thus increasing the efficiency of the polymerization reaction [44]. Compared with traditional photoinitiators, Ivocerin has a higher degree of conversion [45, 46] and colour stability [47] which is considered to be an effective photoinitiator to replace Camphoroquinone (CQ) [48]. In vitro studies have also shown that Ivocerin has low cytotoxicity and no mutagenic effects [49, 50].

In addition, the mismatch of refractive index between filler and monomer will scatter the irradiation light at the interface between resin and filler, which will lead to a lower conversion rate and greater cytotoxicity [22]. The selection of the color of the composite resin also affects the toxicity, and the composite material with a darker color has higher cytotoxicity [51].

3 Conclusion

With the rapid development of composite resin materials, more and more studies are focusing on biocompatibility while exploring the mechanical properties of the resins. In this paper, different detection methods were used to prove that the cytotoxicity of bulk-fill resins was similar to that of conventional resins, and the factors affecting the toxicity of bulk-fill resin were discussed. Although there are many reports on cytotoxicity and genotoxicity of bulk-fill resins in vitro, there is still a lack of research on implantation and sensitization. Therefore, future studies can perhaps concentrate on these aspects, so as to comprehensively evaluate the biocompatibility of materials and provide a guarantee for better clinical application of bulk-fill resins. Predictably, after the improvement of physical properties and mechanical properties, bulk-fill resins may be used as a substitute for conventional resins.

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