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REVIEW ARTICLE

Biocompatibility Evaluation of Resin-Based Restorative Materials: A Review

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Abstract

Biocompatibility is described as an appropriate biological response of a biomaterial in a living organism. It is known that biomaterials are not inert and the materials should be tested before they are allowed to be used in clinical practice. Various test methods have been developed and protocols have been determined for this purpose. Resin-based restorative materials are extensively used in dentistry due to the increased aesthetic demands of patients and the ease of use in clinical practice. As the restorative materials function in the mouth for long years, concerns about the biocompatibility of resin-based restorative materials become more important. Regarding the importance of this issue, the purpose of this review was to evaluate the local and systemic potential toxicity of resin-based restorative materials, toxicity test methods, and the mechanism of the cytotoxicity in living tissues.

Key words: Biocompatibility; Cytotoxicity; Dental restorative material; Photopolymerization; Resin monomer

Definition of biocompatibility and test methods

Biocompatibility is described as an ability of a biomaterial to perform its desired property without any adverse reactions in the beneficiary of the material.[1] When the material is placed in the living organism, interactions occur with the complex biologic system of the host which results in a biological response. Biocompatibility is a dynamic process and the biological response may change over time depending on the interactions between the host, material and the function of the material.[2,3] Dental materials are considered as biomaterials and they are expected to be nontoxic in living tissues. The materials are strictly tested by regulatory agencies before they are allowed to be used in clinical practise. The test methodologies are specified as in vitro, animal and usage tests.[2]

1- In vitro tests: In vitro tests are conducted outside of a living organism in laboratory conditions. ISO 10993 series for medical devices and 7045 series, which are specialized for devices used in dentistry have been developed for standardization of in vitro tests. These series include the biological evaluation of materials, the classification and description of test methods for biocompatibility evaluation in different aspects. The disadvantage of in vitro tests is their disputable relevance to the final usage of the material in a biological system.[4,5] In vitro cytotoxicity assays measure viability, plating efficiency or metabolic activity of the cells. Several tests such as lactate dehydrogenase (LDH) assay, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-1) assay, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) assay, neutral red uptake, trypsin blue test are used to assess the biocompatibility of dental materials.[4,6] However, these tests are in the format of end-point tests that continuous monitoring of the cell response is not possible. Recently, real time assay systems are devised for dynamic measurement of cell growth and viability.[6] These systems allow the measurement through many time points and create line graph that reflect the biological status of the cells. It is reported that real time cell assay systems help to obtain more realistic results compared to single end-point values of conventional cytotoxicity tests.[7]

2- Animal tests: If the results obtained through in vitro tests meet the material requirements, more extensive research is performed on experimental animals. Animal tests enable the observation of more complex reactions between the recipient and the tested material. However, it is difficult to control variables, time consuming, expensive and ethically

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controversial.[3,5,8]

3- Usage tests: In the final stage, the material determined to be biocompatible as a result of laboratory and animal experiments are used on volunteers. The usage tests are accepted as gold standard and give decisive result for investigation of the biocompatibility of the material.[4,5] Parameters such as pulpal and periodontal reactions, gingiva and oral mucosa irritations are evaluated in usage tests for dental materials.[9]

Biocompatibility evaluation of resin-based restorative materials

Resin-based restorative materials consist of an organic polymerizable matrix, filler materials, molecules initiating the polymerization reaction and silane coupling agents.[10] Mainly used components of the organic matrix are methacylate resin monomers such as bisphenol-A-glycidyldimethacrylate (Bis-GMA), hydroxyethyl methacrylate (HEMA) triethyleneglycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA).[11]

The photopolymerization of resin-based restorative materials is initiated by reaction of free radicals with methacrylate monomers and results in the generation of a highly cross-linked polymer structure.[12] As the photopolymerization proceeds, the viscosity of the cross-linked polymer network becomes so high which restricts the reaction of monomer molecules. Thus, the photopolymerization can not be completed even in optimal conditions. The ratio of double bonds that join the polymer network to the initial amount, expressed in % is defined as the degree of conversion.[13] The conversion of monomer to polymer in resin-based restorative materials varies between 43% to 75% and this ratio decreases to 35% in the presence of an oxygen inhibition layer.[14] Consistent with this result, studies have reported that acute release of monomers occurs in the first 24 hours.[15,16] Unreacted monomers eluted from resin-based restorative materials have been considered as a reason of hypersensitivity, allergic reactions, local and systemic toxic effects.[17] Therefore, it is critical to maximize the degree of conversion in order to obtain a more biocompatible restorative material.[18] It has been reported that the degree of conversion further increased to nearly 95% when the oxygen inhibition layer was removed by finishing and polishing techniques.[19]

Long-term monomer release of resin-based restorative materials

Dental restorations are in interaction with the oral environment dynamically. As resin-based restorative materials are expected to function for long years, they may degrade and components of the materials release into the oral environment in time. The reasons for degradation could be chewing forces, microorganisms, temperature changes, enzymes or saliva.[20] Mastication: While functioning, resin-based restorative materials are exposed to mechanical stress constantly. This situation results in wear on restoration surface and the release of components from the material.[20] Saliva: The main ingredient of the saliva is water. Since dental resins are polar molecules, water molecules easily penetrate the polymer network and ease the release of unreacted monomers.[21] In addition saliva, pH can vary from alkaline to acid and may cause chemical deterioration to dental restorations.[20] Microorganisms: Lactic acid produced by bacterias promotes the hydrolisis of the restoration. In addition, oral biofilm formation may also cause degradation, thus revealing different components from the restoration.[20,22] Enzymes: Some form of enzymes that are present in saliva and dentinal fluid are responsible for the breakdown of the linkages. The endopeptidases comprise matrix metalloproteinases and cysteine cathepsins are capable of hydrolytic degradation of hybrid layer.[23] In vitro studies confirmed the long-term elution of monomers for 1, 3, and 12 months.[16,24,25] It was also claimed that monomer elution is expected to be increased by the degradation and wear in the oral environment. Biocompatibility studies are mainly focused on the release of unreacted monomers in short-term period; whereas biodegradation of restorative materials and metabolic by-products also play a crucial role on potential toxicity in living tissues.[26] The long-term effects of unreacted monomers on biocompatibility are still unclear. Long-term chronic exposure and systemic adverse effects must also be considered when assessing the potential toxicity of the eluted compounds.[24]

Release of formaldehyde

Small amounts of formaldehyde may be released from dental polymers as a result of oxidation of unreacted methacrylate groups or degradation of the oxygen inhibition surface layer[27]. Oysaed et al.[27] reported that using mylar strips during polymerization or finishing the restoration surface using sandpapers caused a significant decrease in the release of formaldehyde. The amount of formaldehyde was still detectable even after 115 days although the concentrations were below toxic levels. Formaldehyde could also be released as a metabolic by-product of TEGDMA.[26] But, it has been shown that TEGDMA-metabolites in organs have not reached toxic levels in guinea pigs.[28] Another in vitro study confirmed that the levels of formaldehyde do not cause toxic effects in human pulmonary cells.[26]

Release of Bisphenol-A

Bisphenol A (BPA) is a synthetic chemical generally found in polycarbonate plastics and epoxy resins. Free BPA is absorbed through the skin, oral mucosa, respiratory epithelium or gastrointestinal system. Various epidemiological studies have reported an association between BPA exposure and obesity, asthma and neuro-behavioural disorders in children.[29–31] The tolerable daily intake is advised by governmental regulatory agencies. European Food Safety Authority (EFSA) derived a reference dose of 0,004 mg BPA/kg[32], The United States Environmental Protection Agency (USEPA) determined the tolerable daily intake as 0,05 mg BPA/kg.[33] BPA is not included as a substance in dental materials, whereas BPA derivatives such as Bis-DMA and Bis-GMA are used in their structures.[34] However, it has been shown that Bis-DMA has been hydrolyzed to BPA through salivary esterases. The chemical structure of Bis-GMA prevents hydrolysis at ester linkages and it is not affected by enzymatic hydrolysis.[35,36] Salivary BPA concentration decreased over time with different concentrations across in vivo studies;[34,37–39] the highest exposure (385 ng/mL) was measured 10 minutes after placement and lowest exposure (0,25 ng/mL) measured 1 week after placement.[37] It was observed that BPA levels in saliva returned to pretreatment levels in 8 hours[40] to 1 month[34] after placement. BPA concentration in urine is another indicator to measure systemic exposure. Urinary BPA level was increased in the range of 43%[40] and 354%[41] 24 hours after the placement in vivo studies. However, the concentrations were similar to pretreatment levels 1 month after treatment.[42–44] Maserejian et al.[45] reported there was no association between placement of resin-based restorative materials and neuropsychological, behavioral or physical development in pediatric patients over 5 years. In conclu-

sion,[40,46,47]

- Photopolymerizable restorative materials should be used as an alternative to self-curing restorative materials and Bis-GMA should be preferred instead of Bis-DMA in resin composition of materials.
- Restorations should be placed with a rubber dam to reduce elution in saliva and more than four treatments per appointment should not be performed.
- Finishing and polishing procedures should be applied to remove oxygen inhibition layer.
- Gargling for 30 seconds after placement of restoration is suggested for dilution of BPA concentration.

Estrogenic effects

BPA mimics estrogen-like activity by binding to the estrogen receptors at subtoxic concentrations.[48] It has been demonstrated that BPA and Bis-DMA significantly stimulated estrogenic activity while Bis-GMA did not have such effect.[36,49] The minimum concentration at which Bisphenol-A caused estrogenic activity was calculated as 0.1µmol/L and above 1 µmol/L for hydroxy-4-methoxy-benzophenone (HMBP), 2,2-dimethoxy-2-phenylacetophenone (DMPA) and 2,6-di-tetra-butyl-p-cresol (BHT).[49] Some researchers claimed that leachable concentrations from resin-based restorative materials were below the reported dose required for estrogenicity,[50,51] while other in vitro studies found evidences for estrogenic activity.[49,52]

Systemic toxicity

The systemic intake of components released from resin-based composites can be through oral mucosa epithelium, via dentinal tubules to pulp, absorption from respiratory system or gastrointestinal tract.[53–55] Potential systemic or reproductive toxic effects of resin monomers were investigated in various animal studies. The monomers did not reach to a dose that would cause systemic or reproductive toxic effects and were mostly excreted via different pathways.[28,56,57] In preclinical studies, acute oral toxicity is determined by the Lethal Dose50 (LD50), which is the calculated dose that kills 50% of the experimental population.[2] According to the European Union, the Regulation on Classification, Labelling and Packaging, the chemicals with LD50 of <2000mg/kg bw are necessitated to be labelled for acute oral toxicity.[58] None of LD50 values of dental monomer and comonomers were found to be above this value in animal studies. Therefore an acute oral toxicity can not be expected for resin-based restorative materials.[2]

Local toxicity

Substances leached from resin-based restorative materials may generate toxic effects in adjacent tissues such as gingiva, oral mucosa or alveolar bone.[2] Local toxicity is measured with a value of Toxic Concentration50 (TC50), the concentration that causes a reduction in cell metabolism or death by 50%).[2,3] Many in vitro studies have been conducted to determine cytotoxicity of resin-based restorative materials and contradictory results have been obtained.[19,59,60] The results show differences depending on the resin composition of the material, cell type or test methods. Human cell lines are found to be more sensitive to long-term incubation with composites than mammalian cell lines.[61] Human gingival cells and 3T3 fibroblast cells are reported to be less sensitive than human pulp cells in another study[62]. Nascimento at al.[60] revealed different results between the neutral red and MTT tests. Rajic et al.[63] found that cured forms of composites did not show any toxic effect, whereas uncured forms exhibited a certain level of toxicity. Completely curing is not always possible due to the existence of saliva or anatomical problems in clinical conditions. Therefore, biocompatibility should be tested in vitro and in vivo to clarify actual effects of the restorative materials.[19]

Cytotoxic effects on cell metabolism

Reactive oxygen species (ROS) are generated either by metabolic reactions of the cells or result from exposure to radiation, UV light or other environmental factors. ROS function in signaling pathways in low-moderate concentrations, but overproduction of ROS is linked to various diseases such as cancer, early aging, neurodegenerative disorders. The human body has a complex defense system including a variety of antioxidants that balances the cell-damaging effects of ROS. Glutathione is a thiol antioxidant that is capable of preventing damage to important cellular departments caused by ROS.[64,65] Studies have shown that concentrations of 0,1mM Bis-GMA,[66] 0.33 mM TEGDMA, 1.6mM HEMA and 0.1 mM UDMA deplete the intracellular glutathione levels and promote cell damage in a concentration-dependent manner in human gingival fibroblasts.[67] Furthermore, glutathione depletion caused by TEGDMA, HEMA and Bis-GMA is associated with subsequent increase of ROS, which may have a contribution to the toxicity of these monomers.[68–70] Antioxidants such as N-acetylcysteine, ascorbate, Trolox may have the potential to inhibit the detrimental effects of monomers.[71,72] HEMA and TEGDMA have been considered as reason of arrest at phases of the cell cycle which leads to growth retardation, cytotoxicity or apoptosis.[73] Apoptosis is a programmed physiological process of cell death, meanwhile necrosis is usually promoted by tissue inflammation associated with clinical symptoms.[74] Reichl et al.[75] reported that TEGDMA induces apoptosis; HEMA, Bis-GMA and UDMA mainly induced necrotic cell death. Another in vivo study presented that the number of apoptotic epithelium cells was decreased in patients with amalgam restorations with an aging time of 1 week while that of composite was increased.[76] The toxicity for the monomers was ranked as Bis-GMA> UDMA> TEGDMA> HEMA. It is claimed that the highest toxicity of Bis–GMA could be explained by the liposolubility of Bis–GMA since the phospholipid layer constitutes a major component of cell membrane.[77]

Genotoxicity studies

The human genome is constantly being damaged by different chemical components and genome instability leads to the development of chronic degenerative diseases. Genetic damage is assessed in various methods such as detection of chromosomal or clastogenic changes, micronuclei formation, sister chromatid exchanges, base mutations.[78] The studies investigating genotoxic potentials of dental polymers confirmed that resin monomers induced DNA damage on human peripheral blood lymphocytes, gingival fibroblasts, macrophages.[79–81] Bis-GMA induced DNA strand breaks and micronucleated cells in a dose-related manner in murine macrophages in another study.[82]

Genotoxicity of composite and amalgam restorations were analyzed by using peripheral blood cells from individuals in some studies. Di Pietro et al.[83] revealed that restorative materials exhibited genotoxic effects increased by time and number of the fillings. Whereas, other studies did not present any evidence regarding genotoxicity of composite resins.[63,84,85] Further research about genotoxicity is required as neither of these

in vivo studies evaluated the restorative materials directly on genetic material.

Antimicrobial properties

Components of resin-based restorative materials are thought to have a contribution to bacterial growth. Hansel et al.[86] published that comonomers EGDMA and TEGDMA promote proliferation of cariogenic bacterias such as Streptococcus sobrinus and Lactobacillus acidophilus. Another study revealed that TEGDMA increased the proliferation of Streptococcus mutans and Streptococcus salivarius in pH-dependent manner.[87] This situation also contributes to the explanation of secondary caries developing under resin-containing restorations. Some researchers have denied the cytotoxic effects of resin monomers on pulp and blamed bacterial contamination. It has been believed that the space between restorative material/ adhesive and cavity walls creates an area for bacterial colonization and the acid production of the bacteria has an effect on the pulp.[88] The gap has been reduced below 1µm in new generation adhesive systems. However, even this distance is sufficient for the colonization of bacteria such as Lactobacillus, whose diameter is smaller than 0.1 micrometer.[10] Streptococcus mitis a bacterium that is the predominant species in soft tissue surfaces and saliva. It was reported that co-cultivation of Streptococcus mitis with human gingival fibroblasts caused a significant decrease in toxic effects of HEMA and the mortality of human gingival fibroblasts decreased after 48 and 72 hours.[89]

Conclusion

The release of free monomers from resin-based materials into the oral cavity occurs immediately after polymerization and in the long-term. An effective polymerization of the restorative material plays an important role in reducing residual monomer. The manufacturer's recommendations such as light source, light intensity, curing time should be followed during polymerization. Rubber dam should be used in order to prevent the monomers from joining the systemic circulation. After polymerization, the oxygen inhibition zone should be removed with finishing and polishing agents.

In vitro studies have shown that monomers released into the oral cavity have the potential to show cytotoxic effects. Results of in vitro and in vivo biocompatibility evalution of resin-based restorative materials vary across the studies. In vitro tests are often preferred because they are reproducible and easy to control variables. However, in vitro studies show more sensitivity to materials than in vivo studies as laboratory conditions can not completely mimic clinical conditions. Therefore, the most effective way to evaluate biocompatibility is the combined use of in vitro and in vivo tests.

Endodontics is a field of dentistry where relatively urgent applications are concentrated. For this reason, since the Covid-19 outbreak started, endodontists in particular have difficulty in delaying treatment. However, the full implementation of the recommended treatment approaches and measures may make it possible to overcome this pandemic period with the least damage.

Author Contributions

C.Ç and N.Ö conceived the ideas; C.Ç collected and analysed the data; C.Ç and N.Ö led the writing.

Conflict of Interest

The authors of the current article certify that they have no affiliations with any organization or entity with any financial interest.

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