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An overview to biocompatibility of resin based restorative materials

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ABSTRACT

Nowadays, resin based materials find a wide range of use in dentistry due to their aesthetic properties, mechanical durability and cost advantages. Dentistry materials;They can have an effect because they are in direct contact with various tissues such as gums, tongue, lips and cheeks, in addition to periodontium, pulp, dentin and enamel. It is important that resin materials produced with new technologies to be used in restorative dentistry not only have mechanical, physical, functional and aesthetic properties, but also be carefully evaluated in terms of biological compatibility. The purpose of this review is to review the basic concepts and methods related to biocompatibility, to present data from studies on the cytotoxicity of resin-based materials, and finally to make recommendations for clinical applications.

Keywords:Biocompatibility, cytotoxicity, resin based dental materials, residual monomer

1. INTRODUCTION

Today, resin-based materials are widely used in dentistry due to their aesthetic properties, mechanical durability, and cost advantages.[1] Light-curing resin-based restorative materials are becoming widespread by undergoing innovations in every field of dentistry. The materials in this group include resin-based composites, resin cements, resin-modified glass ionomer cements, compomers, ormocers, fissure sealants, and dentin bonding agents. The goal of dental treatment is to achieve effective, yet safe, and long-lasting results for the benefit of patients. In order to achieve the desired physical and biological properties of resin-based restorative materials, it is critical to ensure effective polymerization.[2]

Dental materials can produce effects because they are in direct contact with various tissues, such as the gingiva, tongue, lips, and cheeks, in addition to periodontium, pulp, dentin, and enamel. Such contact can lead to allergic, toxic, mutagenic, carcinogenic, or inflammatory reactions. It is important that resin materials produced with new technologies to be used in restorative dentistry not only have mechanical, physical, functional, and aesthetic properties but also be carefully evaluated in terms of biological compatibility.[3]

The biocompatibility criteria of materials used in dentistry are as follows:

- Should not be harmful to pulp and soft tissues.
- They should not contain toxic substances that cause a systemic response by being released into the circulatory system and absorbed by diffusion.
- It must not contain potentially allergenic agents that may cause an allergic response.
- It should not have carcinogenic potential.[4]

Therefore, it is necessary to evaluate the potential harmful effects of these materials on oral tissues before they enter widespread clinical use.[5] Assessing the biocompatibility of materials is a step to ensure the safety of patients and the healthcare team, and this process involves various biological and physical property tests and risk-benefit analysis. It is of great importance that the analyses clarify any interaction of components released from materials with living tissues.[6]

Biocompatibility is a dynamic process that can change depending on time and conditions. In parallel with the changes that occur in the body over time, such as disease or aging, corrosion, load, occlusion, fatigue, or nutritional changes can be observed in materials. Therefore, it should be kept in mind that the initial biological response may change over time.[7]

Tests to assess the biocompatibility of materials, which in the past were usually performed on humans, now require a new material to be evaluated through extensive testing prior to human use. To determine whether a new material is biologically acceptable, a variety of standardized test methods have been applied. The biological properties of materials are usually started to be examined with simple in vitro testing methods using cell cultures. Animal experiments that are more expensive and time-consuming can come after these investigations. Following the

successful results of these tests, more extensive studies, such as utilization tests, should be performed.[8]

2. BIOCOMPATIBILITY OF RESIN-CONTAINING RESTORATIVE MATERIALS

It has been reported that resin-based materials may cause allergic reactions, apoptotic reactions, local immunologic effects, systemic estrogenic and carcinogenic effects, postoperative sensitivity, and long-term pulpal inflammation.[9]

In light-polymerized composite resins, the polymerization rate varies between 40% and 70%, and approximately 10% of the monomers do not participate in double bond formation and are released from the restoration as residual monomers. To overcome the negative effects of residual resin monomers released from resin materials, a number of studies have been conducted focusing on the biocompatibility of these materials. In addition to these monomers, initiators and fillers also have an impact on the biocompatibility of resin materials. The degree of conversion after polymerization, the release of free monomers, and the degradation of the resin matrix causes various degrees of cytotoxicity.[10]

Most of the composite resins available on the market used in treatments are not antibacterial because they contain inert inorganic fillers and organic monomers. Studies have reported that residual monomers released from composite resins may promote the growth and proliferation of microorganisms with the potential to cause caries.[11]

Studies have reported that chemically and light-cured resin materials generally cause moderate cytotoxic reactions in vitro at 24-72 hours of contact, while cytotoxicity decreases significantly after 24-48 hours, especially in the presence of the dentin barrier. It is known that light-cured resins are less cytotoxic than chemically cured resins, but this effect is largely dependent on the light source used and the type of resin system. Three days after putting chemical or light-curing resin in cavities with 0.5 mm of dentin, usage tests show that there is low to moderate pulpal inflammation. On the other hand, there is almost no pulpal reaction when a sealer or bonding agent is used.[12]

During the polymerization process of resin-containing fissure sealants, residual monomers such as Bis-DMA and Bis-GMA may be released into saliva. Although a disadvantage due to the estrogenicity of BPA and DMA in fissure sealants has been mentioned, it has also been reported that this effect is negligible.[13]

Many studies show that a significant number of organic compounds remain as unbound residual monomers in the polymerized material. Increasing the size and number of fillers in the material content also leads to a decrease in the amount of residual monomer released. It has also been reported that these monomers cause inflammation in the tissue and inhibit dentin mineralization when applied in direct contact with pulp tissue. Hydrophobic monomers (Bis-GMA, UDMA) in adhesive systems show more cytotoxic effects than hydrophilic monomers (HEMA, TEGDMA). Hydrophilic monomers can move in dentinal fluids and carry hydrophobic monomers in dentinal tubules, causing cytotoxic effects in the pulp. The toxic effect of hydrophilic and hydrophobic groups together is more than the toxic effect they produce alone.[14-16]

Composite resins exhibit cytotoxic properties before and immediately after polymerization. However, their cytotoxicity decreases significantly after polymerization. After curing, oxygen-inhibition areas on composite resin surfaces contain unpolymerized monomers. If this surface is not removed after polymerization, the composite may show higher cytotoxicity.[17]

It has been reported that especially unpolymerized monomers that separate from the resin material can affect the course of the pulpal reaction. Therefore, it is recommended to use total-etch adhesive systems in superficial cavities and self-etch adhesive systems in young, deep, and permeable cavities for a successful post-op restoration. Self-etch adhesives are better for biocompatibility because their acid content doesn't completely destroy the smear plugs that stop unpolymerized monomers from getting into the pulp. This means that they can be used more often. Results showing that resin-based materials cause systemic toxicity have not yet been obtained.[9]

The monomer-rich oxygen inhibition layer formed on the surface leads to decreased durability and long-term discoloration of the restoration surface. In order to prevent the formation of the oxygen inhibition layer, methods such as performing the polymerization in an argon-rich environment, isolating the restoration surface by applying glycerin, or preventing its contact with oxygen by applying transparent tape should be applied. The use of finishing and polishing agents is critical to removing residual monomers, preventing plaque accumulation, and extending the clinical life of the restoration.[18]

In the long term, monomers can be released into the oral environment as a result of the degradation of the material. The degradation of the polymer structure is caused by saliva components, chewing forces, temperature changes, and microorganisms.[19]

3. BIOCOMPATIBILITY EVALUATIONS

3.1. Biocompatibility and Cytotoxicity

Biocompatibility is defined as the ability of a material to trigger appropriate biological responses at the tissue level without causing systemic and local toxicity, allergic reactions, mutagenic effects, or carcinogenic effects. A material does not necessarily have to be completely inert to be considered biocompatible, but that corresponds to the definition of a tolerable biomaterial. Biocompatibility is more than a static concept;it is a continuous state. There is a constant state of interaction between the complex biological system and the material;both the material can affect the biological environment and the biological system can affect the material.

A material that is biocompatible at first may become incompatible with changes in environmental conditions over time.[20] This is because as the body changes due to factors such as disease or aging, the material may deteriorate due to corrosion or fatigue, or the force interactions on the material may change due to factors such as occlusion or nutritional changes.[21]

Apart from the term biocompatible, biomaterials used in dentistry can also be referred to as biotolerant, bioinert, and bioactive. Biotolerant materials represent materials that are separated from bone tissue by a layer of fibrous tissue;polymethyl methacrylate, stainless steel, and cobalt alloys are examples in this category. Bioinert materials are materials that have the ability to form chemical bonds with bone tissue;materials such as titanium, zirconium, aluminum

oxide (alumina), and carbon are examples of this class. Bioactive materials are materials that are in direct contact with bone and tissue without chemical reactions;examples of this group include calcium phosphate, hydroxyapatite, calcium carbonate, and glass ceramics.[22]

Biocompatibility refers to the safety of a material, while toxicity refers to the material's ability to cause potential damage to biological systems by chemical means. The term cytotoxicity describes cellular damage, while apoptosis refers to programmed cell death. If a material causes changes in the DNA structure, it is called genotoxic;if these changes are passed on to the next generation, the material is described as mutagenic. This can lead to permanent changes in the genetic material and potentially cause genetic disorders or diseases.[23]

Before every new dental material is introduced to the market, it should be investigated for biological risks, and its biocompatibility should be evaluated by various methods before clinical use. The main principle of biocompatibility testing is to determine in advance the type and number of potential reactions between a material and a biological system and the type and amount of structural and functional changes in the living organism. Testing programs include a hierarchical order in which various procedures are applied in a hierarchical sequence. Recently, a procedure has been adopted by a number of standardization bodies and certain international organizations that includes in vitro (primary) tests, animal experiments (secondary tests), and clinical studies in humans (use tests).[3]

3.2. In Vitro (Primary) Tests

Initial experimental tests were applied to determine the cytotoxic properties of materials. In in vitro tests, the biological reactions that occur as a result of contact with the tested materials placed on or in various cells or tissues outside the living organism are examined in the laboratory environment. In-vitro tests have many advantages over other tests;they are relatively less costly, simple to perform, experimentally manageable, reproducible, and standardized compared to other types of tests. In addition, they do not pose ethical and legal problems, such as the use of animals and humans for testing. One of the disadvantages is that they do not accurately reflect the in vivo situation. Materials that show success in these tests move to the next stage to be evaluated by animal experiments and clinical use test methods.[3,24]

In-vitro biocompatibility tests are performed in special test tubes outside a living organism using a wide range of cultures of cells and cell components. These biological systems consist of mammalian cells, cell organelles, tissues, bacteria, or specific enzymes. However, it is important to note that when materials are found to pose a risk in terms of biocompatibility under in vitro conditions, this does not necessarily mean that the same materials will show toxic effects under in vivo conditions.[25]

Cell culture tests, agar diffusion tests, filter diffusion tests, and dentin barrier tests are among the commonly used test methods for in vitro cytotoxicity evaluation of dental materials.

3.2.1. Cell culture test method: The working principle of this test method is based on the production and survival of tissue fragments mechanically separated from living tissues in appropriate nutrient solutions (animal embryonic extracts, serum and plasma amino acids and minerals, sugar salts, vitamins, and antibiotics) by providing in vitro conditions and the determination of potential cytotoxicity using a dose-response curve following the placement of the material into these cells.[26]

In cell culture studies, three different types of cell cultures are used:primary cell cultures, diploid cell cultures, and continuous cell cultures. Primary cell cultures are cultures that are taken from living tissues and cultured for more than 24 hours without in vitro cell proliferation. Pulp and gingival fibroblasts (GF) are a typical example of primary cell cultures. After the first passage of primary cell cultures, the transfer from one culture medium to another is called subculture. Diploid cell cultures are obtained from subculturing primary cultures. Compared to primary cell cultures, diploid cell cultures are more homogeneous, standardizable, and reproducible, and they have the advantage that they continue to reflect at least 75% of the karyotype of the tissue from which they are derived. Continuous cells (MDPC-23 mouse odontoblast cells, WI-38 human embryonic lung cells, L-929 mouse fibroblast cells, BALB/3T3 mouse embryo fibroblasts, HeLa human epithelial cells, ECV-304 human endothelial cells) are examples of transformed primary cells and have the ability to proliferate indefinitely. In the establishment of cell cultures, 95% humidity, 37 ˚C temperature and 5% CO2 are generally preferred and some antioxidative substances and antibiotics are added to the culture. Although the results obtained provide information about the potential cytotoxicity of the material, it is important that the available data should not be directly related to clinical conditions. Because immunologic and biologic reactions in the living organism may affect cytotoxicity.[3,8,27]

Direct contact testing is the application of materials or components directly onto cells in culture for short periods of time (> 24 hours). In a direct contact test, the material is in physical contact with the culture medium or cells. Since water-soluble materials will dissolve well in the culture medium, very good contact between the material and the cells is ensured. In the extract contact test method, after the material is kept in a liquid solvent, the soluble components of the material are brought into contact with the cells and their cytotoxicity is examined. This suspension of the components dissolved from the material with the liquid is called "extraction liquid". Serum-free medium, serum-containing medium, physiological salt solution or other suitable solvents can be used as solvent.[28]

Traditional cell culture studies are based on the establishment of monolayer, two-dimensional (2D) cultures by placing cells on various planar surfaces to provide mechanical support or suspending them in a thin layer of liquid media. 2D cell cultures are easily applicable for cell-based screening studies and have proven to be a convenient and effective tool for discovering drug candidate molecules. However, the 2D surface does not reflect the in vivo cell properties more accurately, as the cells do not lay flat and form a multilayered structure. With the creation of 3D in vitro cell-based systems with multilayered cell clusters, cell tests that are more like living tissues are giving more accurate results and can better mimic the specificity of in vivo tissues.[29]

3.2.2. Agar diffusion test method: This method, which is one of the common barrier tests used in cytotoxicity evaluations, is easy and inexpensive to apply. When assessing cellular activity, trypan blue, which can stain dead cells, or

neutral red dye, which accumulates in the lysosomal matrix of living cells and is released when the membrane is damaged, is used. The response of the material in the cell is examined by evaluating the decolorization and lysis of the cells. If the test material or its components cannot dissolve or diffuse in agar, these materials cannot be evaluated because they cannot show any effect on the cells.[30,31]

3.2.3. Filter diffusion test method: Since many materials frequently used for restorative treatment do not come into direct contact with the cells, it is thought that it would be more appropriate to add a filter between the material and the cell culture in order to obtain a more objective result. In the filter diffusion test, which is one of the indirect contact tests, cellulose acetate with a pore size of 0.45 µm is used as a barrier agent. In order to evaluate the cytotoxicity of the material, primary cells (fibroblasts and epithelial cells) are placed on one surface, and the material to be evaluated is placed on the other surface in the filter diffusion test. The components released from the test material must diffuse through the pores on the filter. Evaluation is performed by measuring the decolorized area formed in the cultured cells or by examining the dye intensity.[8,32]

3.2.4. Dentin barrier test method: Dentin barrier tests are a complementary method to cytotoxicity tests. Dentin tissue functions as a barrier to protect the pulp against dental materials applied to the tooth. In this method, which was developed to mimic this property of dentin structure, the diffusibility of the tested material is measured. In this test method, which mimics in vivo conditions more than other tests, human dentin tissue or dentin samples obtained from bovine teeth are used as a barrier between the components released from dental materials and the target cell. Although the use of human dentin seems to be more appropriate in terms of mimicking in vivo conditions, the use of bovine dentin is more advantageous because it differs less in permeability compared to human dentin and can be obtained in desired amounts.[3,25,33]

3.3. Cytotoxicity Assessment Methods

In order to evaluate the cytotoxic effect of dental materials or components released from the material, some biological markers such as cell viability and death, cell membrane permeability, cell organelles, protein, DNA, RNA synthesis, and cell division are examined.

MTT and neutral red are widely used to evaluate the cytotoxicity of materials. XTT, Acid Phosphatase, Resazurin, LDH, Kenacid Blue, and Sulforhodamine B are known as other cytotoxicity stains.

Four different test methods are used for the evaluation of cytotoxicity.

3.3.1. Tests to assess vitality: These tests allow colorimetric or fluorescent measurements and are used to determine the proportion of viable cells in culture. These methods are limited to measuring membrane permeability and cannot measure sublethal cell changes. Diacetyl fluorescent or neutral red dye taken into cells with intact membrane integrity or dyes such as trypan blue, erythrosine, or naphthalene black that enter the cell structure with disrupted membrane integrity are used and examined spectrophotometrically.[34]

3.3.2. Tests to assess life: These are very fast and easy-to-apply methods in which only dead cells are evaluated, which can be seen in the first few days or after the cells are exposed to toxic effects. However, the toxic effects to which cells are exposed may be reversible or may be seen in the long term. Therefore, long-term tests are more useful in determining the viability rate.[3]

3.3.3. Tests to assess cell proliferation: This method includes 3H-thymidine and bromodeoxyuridine immunohistochemical techniques, which are applied when there are few samples. To assess cell proliferation of material components, a growth curve obtained by counting cells in culture after a few days is used.[28]

3.3.4. Tests to assess cell metabolism: Some of the ways to check how metabolically active cells are the colorimetric MTT, XTT, LDH, MTS, WST-1, and alamar blue tests. These give quick results and are used to see what kind of long-term damage will be done.

The MTT test is the most widely used test method among biocompatibility evaluation methods because it can give fast and sensitive results, and even materials with very low toxicity can be evaluated. It makes it possible to examine the cytotoxicity of a large number of samples with fewer experimental steps. The colorimetric MTT test measures how active the dehydrogenase enzyme is by looking at how it changes yellow MTT into purple formazan crystals that can't be dissolved in water. A succinate dehydrogenase enzyme is found in the mitochondria of living cells in culture medium. It breaks down yellow tetrazolium salts into purple formazan crystals that can't be dissolved. If the dehydrogenase enzyme activity of the cell is affected due to the cytotoxic effect of the material, formazan crystals are not formed.[35]

3.4. Animal Experiments (Secondary)

The material to be tested is placed in some experimental animals (mice, rats, sheep, cats, dogs, and pigs) in order to examine the interactions that may occur between the material and the biological environment. In these experiments, it is important to control various variables such as the species, age, and sex of the animals, the way the animal is exposed to the material, the duration of contact, and the method by which the biological response will be evaluated. Some of the methods used in secondary tests are intraoral and intra-abdominal tests, inhalation tests, dominant lethal tests, irritation and sensitization tests, and intramuscular and intra-bone or subcutaneous implantation tests. Since a complex organism is used in this test method, the biological response of tissues is more meaningful than in vitro tests. However, the control of variables in in vivo testing is more difficult, and the complex ways in which the biological response occurs make it difficult to quantitatively evaluate the results obtained. Ethical debates and the growing importance of issues such as animal rights are gradually reducing the use of these tests. Another disadvantage of these tests is that they are time-consuming and expensive. There are also doubts about the similarity of the response in animals to the response in humans.[36,37]

3.5. Clinical Studies (Usage Tests)

Tests have the potential to reflect the clinical picture. This method, which is based on observing the response of a material that has been determined to be safe as a result of laboratory and animal experiments by using it on volunteer humans, provides more realistic results in terms of biocompatibility. This test method defines the situation that

may occur when the material is used in the clinic. Usage tests can be performed on animals or humans. When these tests are performed on humans, they are called "clinical trials." Tests of use can only be carried out after the material is ready for clinical use. The material can only be implanted in a human volunteer after all pre-clinical tests have been performed. These tests, which give very close information about the clinical use of the materials, have some disadvantages. These disadvantages include the complexity and expense of the tests, difficulties in controlling the experiment and evaluating the data obtained, and the need for long periods of time, such as months or years, when long-term effects are to be investigated. Many legal responsibilities that are not required in in vitro experiments and animal experiments arise in human experiments. These responsibilities include the approval of official institutions and informed consent from the patient. In dentistry, these tests, called pulp irritation tests, are performed by applying the material to be tested to the cavities opened in intact, caries-free teeth of humans or other suitable animals to be extracted for orthodontic reasons. The material to be tested is left on the teeth for a while, then the teeth are extracted and prepared for histologic examination. In histologic examination, acute or chronic inflammation and odontoblastic reactions in the pulp are examined. Periapical tissue damage that may occur due to the use of endodontic materials, which are widely used in dentistry, is tested in experimental animals. After the material to be tested is placed in the root canals of the teeth, a histologic examination is performed. Tests such as patch tests, prick tests, and radioallergosorbent tests are applied to determine the allergic potential of dental materials on humans. Allergic properties of materials in experimental animals can also be examined by skin sensitization tests before the material is used clinically.[24,31,38]

4. CYTOTOXICITY STUDIES ON RESIN-CONTAINING MATERIALS

In their experimental studies on rats, Bakır et al.[39] compared the local and systemic effects of existing pulp coating materials containing resin with traditional materials. Despite their high physical properties, low solubility, and ease of use, they reported that pulp coating materials polymerized with a light source may cause cytotoxic effects due to their resin monomer content.

Manaspon C et al.[40] examined the effects of different pulp coating materials on pulp stem cells and found that DyCal® and TheraCalTM LC were toxic to cells. Cell attachment, spreading, proliferation, and migration were compromised when cells were exposed to DyCal® or TheraCalTM LC. In contrast, ProRoot® MTA and BiodentineTM exhibited positive behavior in terms of biocompatibility and were reported to support cell activities towards regeneration potential.

The study by Kraus et al.[41] looked at how biocompatible dental resin monomers were in a lab setting. They found that BisGMA was the most toxic, followed by UDMA, TEGDMA, and finally HEMA.

Gonçalves et al.[42] examined the effects of different brands of conventional and bulk-fill composites on human fibroblasts and found that the best results were obtained in the bulk-fill composite with the highest filler content and the lowest monomer content, and polymerization of 4 mm thickness in bulk-fill composites did not cause adverse effects.

Bandarra et al.[43] evaluated the biocompatibility of glass ionomer cements, resin-modified glass ionomer cements, and resin cements by the MTT cytotoxicity evaluation method using 3T3 mouse fibroblasts and found that cell viability in the presence of glass ionomer cement was higher than that of resin-modified glass ionomer cement and resin cements.

In this research, Moussa et al.[44] found that adding an antibacterial monomer and cross-linker to a resin adhesive could make adhesive restorations last longer. However, these changes did not affect the cytotoxicity of the adhesive.

Brzovic Rajic et al.[45] evaluated the cytotoxicity and genotoxicity of six different dental nanocomposite materials, three conventional and three flowable composite resin materials, in human lymphocytes and concluded that while polymerized conventional composites showed no cytotoxic or genotoxic effects important for the clinical application of these materials, unpolymerized forms exhibited some level of cytotoxicity and genotoxicity, mainly due to the monomers in their composition.

When Gociu et al.[46] examined the biocompatibility of composite resins, they reported that Bis-GMA and TEGDMA' in the organic structure caused a decrease in cell viability by increasing reactive oxygen products.

Attik et al.[47] studied the effects of different resin materials on gingival fibroblasts and found that there was a link between the ratio of resin monomers and cytotoxicity, and that the amount of resin monomers was just as important as the ratio. They reported that TEGDMA significantly decreased vitality and was more effective on cytotoxicity compared to Bis-GMA.

In Taghizadehghalehjoughi et al.[48]'s study, in which they examined the cytotoxic effects of composite materials on gingival fibroblast cells, they concluded that factors such as the structure of the material, the filler ratio, monomer type, and filler content are effective as a whole in the cytotoxicity of a material;the presence of TEGDMA and EGDMA monomers are monomers that increase the potential toxicity of the material;and particles added to the filler content (Fluro aluminosilicate particles, iterum trifluoride particles, etc.) may affect cytotoxicity.

Bapat et al.[49] compared the cytotoxicity of conventional, resin-modified, and ceramic-modified glass ionomer cements on osteoblast cell culture and mouse fibroblasts and found that conventional glass ionomer cement showed a less cytotoxic effect.

Asdada et al.[50] evaluated the cytotoxicity and antidifferentiation effects of bulk fill composites against human dental pulp stem cells (hDPSCs) in three compartments corresponding to the depth (0-2, 2-4 and 4-6 mm) from the light source region and found that the cytotoxic effect increased with increasing depth. In order to protect the ability of dental pulp stem cells to survive and differentiate, they said that care should be taken when choosing bulk-fill resins, especially when fixing deep cavities.

Pagano et al.[51] tested how harmful different universal adhesives were to human fibroblast cells. They found that the extract method gave more accurate results and that the adhesives tested had different effects, with Optibond Solo Plus being the least harmful. FuturaBond M Plus had the most toxic effect.

Çelik et al.[52] (2019) examined the biocompatibility of composite resin, amalgam, compomer, and glass ionomer cement materials with a neutral rejection test. In the study, test specimens aged in artificial saliva for 7 days and 21 days immediately after the preparation of restorative materials were used, and finishing and polishing processes were applied to the specimens. All groups exhibited statistically significantly lower cell viability than the control group. However, the cell viability of the Dyraxt XP composite sample aged for 21 days was reported to be above 70%. It was also found that glass ionomer cement and composite resin reduced cell viability more than compomer and amalgam.

Srivastava et al.[53] evaluated the cytotoxicity of nanocomposites, flowable composites, and composite materials on human lymphocyte cells. They found that all three materials showed cytotoxic effects, and flowable composites had a higher cytotoxic effect than composites and nanocomposites. They argued that the cytotoxic effect observed was due to HEMA and TEGDMA released from the structure of the materials.

Aydin et al.[54] in animal experimental studies in which they evaluated the biocompatibility of frequently used single clinical self-etch adhesive systems histopathologically, they observed that the reinforcement formed against the material from living cells decreased over time and increased fibrocollagen development. They argued that the decrease in initial inflammatory rates against all materials may be due to the release of residual monomer and surgical trauma.

Süsgün Yıldırım et al.[55] in their experimental study in which they qualitatively and quantitatively evaluated the cytotoxic potential of five different single-stage self-etch adhesives, in the first stage, they qualitatively evaluated the cytotoxic activities of the materials in monkey kidney epithelial cell culture medium by direct contact method. As a second stage, they quantitatively evaluated the cytotoxic potential of four different dilutions of the test materials (1%, 0.1%, 0.01%, 0.001%) on L929 mouse fibroblast cells in three different time periods (24 hours, 48 hours, 72 hours). They reported that all adhesives tested showed varying degrees of cytotoxicity, which increased statistically significantly with dose.

Güngör et al.[56] in their in vitro studies, they evaluated the effect of bioactive pulp capping materials on human dental pulp stem cells (hDPSC) in terms of cell viability and bioactivity through mineralization potential. In their study, primary hDPSCs were cultured with experimental nBG, Biodentine, TheraCal LC, and ProRoot mineral trioxide aggregate (MTA) extracts. Cell viability was measured for 1, 3, and 7 days by water-soluble tetrazolium salts (WST-1) assay. Alizarin red staining was used to detect the formation of mineralized nodules, and alkaline phosphatase (ALP) activity was measured by a photometric method. As a result, the cell viability of hDPSCs decreased in all groups except nBG, and the lowest cell viability was determined in TheraCal LC in all incubation periods. They reported that nBG and MTA showed significantly higher ALP activity than the control group.

5. APPROACHES TO IMPROVE BIOCOMPATIBILITY

Strengthening the covalent bonds with the resin to prevent monomer leakage may make cytotoxicity avoidable. Zwitterionic polymer contains both cationic and anionic functional groups and is neutral. In zwitterionic polymers, a new generation material, the near-perfect biocompatibility of biocides and the antimicrobial, bactericidal, and anticariogenic effects of cationic quaternary ammonium compounds seem promising in combined use.[57]

Beyond methodological differences, the cytotoxic effects of resin-based dental materials are due to the residual monomers released. Using low-intensity light, inadequate illumination time, moisture contamination, improper handling of the material, excessive distance between light and material, and neglecting the manufacturer's instructions may increase cytotoxic effects. Therefore, all necessary precautions should be taken in clinical applications to prevent monomer release and degradation.[58]

The biocompatibility of materials is important for studies that test the properties of new biomaterials. To do this, stem cells from pulp tissue are used. This tissue has different cell populations and is very important for dentin regeneration. Propolis-containing pulp coating material has been shown to be a good alternative to calcium-phosphate-containing products due to its positive effect on increasing the antioxidant defense capacity of stem cells.[59]

Fortilin is a multifunctional protein involved in various cellular processes. Its potential as a bioactive molecule that can be incorporated into dental materials is promising. Bio-GIC (GIC supplemented with chitosan, tricalcium phosphate, and recombinant fortilin from Fenneropenaeus merguiensis) shows improved calcium deposition comparable to Biodentine. It has been stated that Bio-GIC can be further developed as a bioactive material for dentin regeneration.[60]

CONCLUSION

The application of different materials containing low- or non-toxic agents for long-term oral use in dentistry is gaining importance for both patients and staff. New materials are being added every day thanks to advances in molecular biology and tissue engineering. Before new materials are put on the market and used in patient treatments, they must be evaluated for biological compatibility, because compatibility with living tissues is a determining factor in the clinical success of the material. Following the rules established by various national and international standardization organizations is essential for these studies. Only materials that give positive results in all stages of biocompatibility testing can be accepted to be placed on the market for use by patients. When the studies conducted in recent years on this subject are examined, it is important to investigate the biological properties due to the diversification and development of the materials offered for use.

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