Biocompatibility of Dental Adhesives

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Abstract

The clinical success of dental materials is dependent not only on physical and chemical properties, but also on biological reliability. Materials containing resin, which are used in restorative dentistry, have the potential to create local or systemic reactions on tissues such as the pulp, gingiva and oral mucosa. When adhesive resins are applied directly on the pulp, infection or necrosis generally occurs. This is because the organic matrix of the adhesives contains components which may have a cytotoxic effect. These components have also been reported to cause allergic skin problems in the patient or in dentistry personnel. In recent years, resin monomers have been reported to lead to the formation of tumours in the saliva glands and toxicity in renal cells.

With some clinical precautions it is possible to reduce the direct biological risks which could be caused by materials containing resin. The selection of adhesive materials taking into consideration the monomer content and the cytotoxic pH range, is a factor which could reduce the possibility of cytotoxicity.

Keywords: Dental adhesives; Biocompatibility; Toxicity

Introduction

With increasing awareness in society of dental health and aesthetics, there have been increased efforts to meet expectations in the field of restorative dentistry. Adhesive materials which are resistant to chewing forces, can be more strongly attached to the tooth and which help to protect healthy tooth structure have come to the fore in previous studies. With the development of adhesive systems, there has been a reduction in problems such as reducing cavity size, postoperative sensitivity which negatively affects clinical success, edge discoloration and secondary decay [1-3].

Adhesive systems are based on the principle of replacing the inorganic structure in dental tissues with resin monomers in adhesive agents. The aim of the use of dental adhesive is to provide retention of the restorations containing resin through micromechanic binding occurring between the adhesive resin and dental hard tissues. Microleakage associated with retention loss, discoloration on the cavity margins, secondary decay and postoperative sensitivity are among the reasons for clinical failure of restorations. Through micromechanic adhesion of dental adhesives to enamel and dentin, composite resin is bound with co-polymerisation of double bonds in the oxygen inhibition layer. Micromechanical adhesion is a diffusion process in which resin binds to the collagen network in dentin and cavities occurring in the enamel. The diffusion capacity of adhesive monomers depends on the solubility concentrations, the time given for penetration, the diffusion coefficient of the monomer and the affinity to the opposite substrata [1-5].

Adhesive systems were classified in the past according to the production process and the relationship with the smear layer, whereas currently adhesive systems are classified as total-etch or self-etch adhesive systems according to the clinical application stages and interaction with dental tissues. In total-etch systems, in which the aim is removal of the smear layer, a bond is formed based on diffusion and micromechanic adhesion by forming areas of demineralisation on the surface dentin. In self-etch systems, demineralisation is formed in the enamel and dentin, aiming to modify the smear layer. Through monomer infiltration and polymerisation, chemical bonding to the calcium in the surface dentin is achieved in self-etch systems. Self-etch adhesives, which may be one or two stage, are named as weak, moderate or strong according to the acidity [5-7].

In the selection of materials to be used in restorative treatment, besides the mechanical and physical properties, prevention of bacteria penetration and biocompatibility with the pulp and other live tissues is expected. Biocompatibility of dental materials is defined as there being no or very few harmful effects on oral tissues. However, the number of materials that will not
have a negative effect on live tissues is negligible. The degree of biocompatibility of a material is associated with the patient, the function of the material and the conditions of placement. While changes in the material used occur related to fatigue, corrosion or occlusion, disease in the body and ageing also incur change. As a result of these alterations, there may be a change in the biological response initially given to the material. For example, if the patient is diabetic or a smoker, the response of the soft tissues to the material may be affected or acidic fluid consumption can change the corrosion properties of dental alloys and tissue response [8-10].

Other factors affecting biocompatibility are the surface properties of the dental materials and the interaction with the environment. Some materials lay the ground for the formation of gum and periodontal problems by toxins of gram negative bacteria increasing the attachment of dental plaque. Whether a material is biocompatible cannot be decided without defining its placement and function. In dentistry there is a question of direct or indirect contact of the dental material with the tooth and surrounding tissues. As a result of this contact, localised, short-term reactions (inflammation, allergy or toxic effect) can occur in the tissues adjacent to the area where the material is applied. According to the properties of the material and the amount used, these reactions can last longer and can therefore lead to permanent damage in the pulp [11-17].

It is possible to examine the risks against the dentin-pulp complex in the form of those which originate from microbiological leakage (indirect biological risk) or from the toxic properties of the materials (direct biological risk). Some researchers have considered that the harmful effects to which pulp is exposed are not associated with the chemical content of restorations containing resin, but with the penetration of micro-organisms as a result of polymerisation shrinkage. The amount of shrinkage of resins is affected by the form of the cavity preparation in particular, and the ratio of non-bonded surface to bonded surface, which is known as the C-factor. With care given to technical sensitivities at the stage of application of adhesive resins, it is possible to reduce indirect biological risks [18-21].

Before dental materials are presented for clinical use, they must be evaluated for biocompatibility and successfully pass different toxicity tests. Biocompatibility tests should identify harmful components within the material and their effects, determine the dose of chemicals released and observe the response to this dose. Within the structure of dental adhesives, there are acrylic resin monomers (acrylates, methacrylates), starters (amines, camphorquinone), inhibitors (butylhydroxytoluene, monomethyl ether hydroquinone), solvents (water, ethanol, acetone), filler particles and some special substances (glutaraldehyde, MDPB monomer).

Some of these components are easily separated from resins through degradation, and form free radicals which are related to cytotoxicity or show a toxic effect by not being able to be polymerised. Although MDPB (12-methacryloyloxy-dodecyl-pyridinium bromide), which can be a part of the structure of adhesive resins, is a monomer with a bactericide effect, it has been reported to have a toxic effect on human cells at high concentrations (>250 μg/mL-1). The direct biological risk originating from the components in question depends on several factors, including the easy freeing of polymer from the structure, a resolution which will increase diffusion, a dose which will incur a biological reaction and chemical properties [21-27].

Some components which are expressed from dental materials, may lead to the synthesis of a series of proteins or inflammatory reactions. Previous studies made on this subject have determined that allergic responses may form to silver, tin, copper, chrome, nickel, mercury or organic components of composite resins. An increase of mercury expressed from amalgam fillings in bodily fluids such as saliva and blood or accumulation in different tissues is known to lead to toxicity related to oxidative tissue damage. During the hardening of the surface of materials containing resin, the structure of the material can be easily impaired due to full polymerisation not occurring when there is contact of the surface with air.

Insufficient polymerisation of resins has been correlated with the formation of the oxygen inhibition layer and direct application of adhesive resins onto the pulp has been claimed to prevent the transformation of monomers to polymers. As a result of degradation and erosion which occur with heat light and mechanical and chemical effects, monomers are eventually expressed from adhesive resins. Esterases originating in human saliva, water and other solvents in the dissolving of the polymer network caused by the formation of oligomers and monomers, lead to a softening of the organic matrix and easier penetration of the solvents. It has been determined that as a result of insufficient polymerisation with the effect of oral fluids, Bis-GMA (bisfenol-A glycidyl methacrylate), HEMA (Hidroxyethyl methacrylate), UDMA (urethane dimethacrylate) ve TEGDMA (triethylene glycol dimethacrylate) monomers are expressed from the organic matrix [28-33].

The potential for materials containing resin to form a cytotoxic effect on pulp tissue depends on the incomplete polymerisation and dissolution of free monomers with oral fluids or consumed dietary products within the first 24 hours. It has been stated that high concentrations of monomers exposed during or after polymerisation eventually lead to immunosuppression. However, it has been emphasised that this effect is lower than the effect of mercury chloride or methyl-mercury chloride. In biocompatibility tests made using humans and animals in recent years, it has been determined that adhesive resins cause reactions ranging from mild inflammation of the pulp to severe apoptotic cell death. In an in-vivo study made on L929 mouse fibroblasts, the cytotoxicity of Bis-GMA, HEMA, UDMA, TEGDMA and their combinations was evaluated and it was reported that after 24-72 hours exposure, the mitochondrial activities of the fibroblasts changed and...
caused expression of inflammatory mediators in the pulp tissue [12,13,28,30,33-37].

Free monomers with a cytotoxic effect are also excellent surfaces for cariogenic bacteria. It has been determined that they promote the proliferation of cariogenic micro-organisms such as L acidophilus and S sobrinus of EGDMA (ethylene glycol dimethacrylate) and TEGDMA. The growth of S mutans and S salivarius has been said to be stimulated by TEGDMA and the formation of secondary decay under restorative materials containing resin has been explained in this way. In brief, free monomers cause the proliferation of micro-organisms that play a role in the development of decay and trigger cellular and molecular mechanisms that lead to changes in the pulp, causing local and systemic allergic reactions [26, 38-40].

In a 4-year study conducted on 296 patients, 23% of patients showed an allergic reaction to gold, 9% to palladium, 6% to mercury and 8% to one or more materials containing resin. In another study, eczema-like skin symptoms were seen in 27% of dentistry personnel and in 12% of patients and it was concluded that 2% of these originated from resin materials. In a similar study, 7% of dental practitioners were determined with skin symptoms as a reaction to materials containing resin. It can be said that high concentrations of the materials in question cause damage to the cell structure and over time, may affect the functions of some organs. For example, a material which displays acute systemic toxicity within 24 hours, may show subacute toxicity at the end of a period such as 3 months and in a longer period, chronic systemic toxicity [40-42].

Other factors affecting the cytotoxicity of adhesive resins are the preparation of the cavity, the thickness of the remaining dentin and the amount of polymerisation. The dentin layer assists in the reduction of the cytotoxicity of the material by absorbing free monomers. Following placement of the adhesives to the deep dentin, the main cause of inflammation or necrosis seen in the pulp is thought to be bacterial microleakage via the dentin tubules. The direct biological risks which may be formed by materials containing resin are related to the permeability of the dentin which allows inward diffusion of chemical and bacterial products and outward flow of fluid to the pulp. Diffusion is affected by heat and the chemical structure of molecules. In cases where there is no outward flow of dentin fluid or inward diffusion of bacteria and degradation products of the material, several reactions may occur in the pulp. Although dentin tissue behaves as a diluent of diffuse substances, the amount of diffusion is in reverse proportion to the dentin thickness [19-21].

Dentin permeability may vary due to calcium in the tubules or phosphate sedimentation causing sclerotic dentin formation. In these types of cases where dentin permeability is reduced or there is sufficient dentin thickness, adhesives cannot cause inflammation in the pulp. However, when dentin tubules are dense and wide in diameter, permeability increases in cavities close to the pulp and consequently, the pulp tissue is exposed to the toxic effects of the restorative materials. Therefore, when opening the cavity, removing dentin tissue from an unnecessary site should be avoided. In cases where the dentin is acidified, there is increased potential for non-polymerised monomers to form a biological risk associated with increased permeability. Some previous studies have reported that the application of total-etch adhesive to deep cavities caused chronic inflammation and a granulomatous reaction in human pulp. Therefore, it is suggested that total-etch adhesives are used in surface cavities. The use of self-etch adhesive systems is recommended in cavities which are deep and have increased permeability thereby leaving the packing and smear layer which limit the diffusion of monomers to the pulp [6,7,11,43,44].

Potential biological risks which may originate from materials containing resin can be reduced with some clinical precautions. For example, the pressure of dentin fluid, with a flow direction from the pulp outwards and which prevents the entry of substances, is reduced with the effect of local anaesthesia. When cleaning the cavity, protection of the hypermineralised dentin layer makes diffusion of monomers more difficult. Attention to these details during cavity preparation, and selection of the adhesive system according to the clinical status, will reduce the direct biological risks. In some studies, adhesive materials being tested for cytotoxicity were placed over the dentin barrier and it was reported that the presence of a dentin layer of 8500m was sufficient for protection of the pulp and adhesive systems with a low pH did not cause damage to the pulp. It was also shown that polymerisation of the materials did not stimulate cytotoxicity [19,23,43,45].

Kuşdemir et al. [11] and Vajrabhaya et al. [46] reported that the toxic effects of adhesive materials reduced with an increase in dental thickness. This result suggests that monomers which can reach the pulp via dentin tubules can be diluted with dentin fluid related to increased dentin thickness and thus their cytotoxicity can be reduced [11,46].

In the polymerisation of dental adhesive or composite resins with a light source, it is expected that the use of a strong light source will increase the rate of polymerisation and thereby, the transformation of monomers to polymers. However, rather than increasing the polymerisation of resin, due to the excessive heat formed, it has been seen that the dentin fluid moves towards the pulp and allows non-polymerised monomer particles to reach the pulp. If the amount of light is insufficient, the transformation of monomers to polymers is limited. In a study which used traditional light sources, the transformation rate in polymerised adhesives was determined to be 70% at most and 25%-50% of double bonds of methacrylate monomers remained without entering any reaction. In another study, the dentin fluid which accumulated on the cavity floor after polishing with acid was reported to negatively affect polymerisation of primer or adhesive resins. Taking the view that good polymerisation will result in low biological risk, it is thought that fine adhesive agents and the use of an LED (blue-
emitting diodes) light source which is strong but does not over-heat will create more rapid polymerisation and increase the rate of transformation of monomers to polymers [19,44,47].

**Discussion**

Before presenting dental materials for use, they must be tested in respect of safety and the effects on the dentin-pulp complex. Studies have intensified on the subject of increasing the biocompatibility of adhesive materials. In the evaluation of biocompatibility, a series of primary, secondary and usage tests have been envisaged and a set of standards have been issued by some institutions. These standards encompass regulations allowing protection of the subjects, research procedures and institutional inspection. Primary tests comprise cell toxicity tests, systemic toxicity tests, mutagenic tests and other in-vitro tests. Secondary tests comprise allergy, mucosal irritation and inflammation tests applied to animals. Usage tests are the equivalent of clinical tests [48-50].

In-vitro cell culture methods are tests which are quick and easy to apply, low-cost, controllable and repeatable. However, their conformity to in-vitro conditions is a matter of debate. The reason for this is that in living organisms the first cellular response to the material used is given by the immune system. While in-vitro tests and animal experiments have been found insufficient in the replication of the clinical environment, there are ethical and legal restrictions to clinical studies. The most important of these restrictions is the principle of not causing harm to living organisms.

In recent years, in-vitro tests and animal experiments have been very widely used together. In studies which have evaluated biocompatibility, some materials which have been thought to be clinically useful have shown unsuccessful performance at the stage of in-vitro tests or animal experiments and have been witnessed to be the complete opposite. Furthermore, some researchers have questioned the benefit of methods other than usage tests. Although the current prominent view is that clinical tests should be given greater priority, the reality is that some materials become available on the market without having undergone sufficient clinical testing [39,48,49,51].

The greater part of knowledge obtained related to the biological risks of materials containing resin has come from in-vitro studies. In methods testing in-vitro cytotoxicity, non-polymersed samples of adhesive materials are placed directly into the culture environment and evaluation is made of parameters such as cell vitality and morphology, enzyme activity, cell metabolism and membrane integrity. To identify the rate of living cells in the culture, different stains (trypana blue, erythrosine, naphthalene black, diacetyl fluorescent or neutral red) are used which can enter the cell structure. In the evaluation of metabolic disturbances, MTT, LDH and Alamar blue tests are primarily used [51-53].

In biocompatibility research made related to adhesive resins, it has been reported that monomers can affect cytotoxicity in different ways. One of these studies reported that Bisfenol-A caused side effects on the ovaries and the fertility of mice. When Bis GMA and HEMA are used together, the cytotoxic effect has been stated to be increased. In studies which have questioned the potential of specific resin components such as HEMA and TEGDMA to create apoptosis or necrosis, it has been reported that HEMA could be rapidly diffused along the dentin, cell growth was inhibited and the cycle was disrupted. Vesicles or odema were observed to have formed in the oral mucosa and lips with HEMA, which can cause contact dermatitis and delayed over-sensitivity.

Due to hydrophilic properties, TEGDMA can penetrate membranes and enter intra-cellular molecular reactions by mixing with oral tissues and it thus stimulates mitochondrial damage, prevents cell growth and total polar lipid synthesis, causes large DNA deletions (genotoxicity) in breast cells and it has been emphasised that it is 2-5 times more toxic than HEMA for lung cells. In addition, dermatitis and some allergic reactions (bronchospasm, urticaria etc) have been seen on the face and different body areas following treatment with resins containing TEGDMA [15,30,36,40-42,54-57].

In a study by Li et al. [17] evaluating the cytotoxicity of 5 different adhesive materials (Super-Bond, Clearfil SE Bond, G-Bond, Single Bond, and Adper Easy One) on human periodontal ligament cells, the cytotoxicity of the adhesive materials was reported to be affected by different concentrations and times of application. Cal et al. [24] tested the biocompatibility of 5 different dental adhesives (Admira Bond, Adper Single Bond Plus, Clearfil SE Bond, Clearfil S3 Bond and Hellobond) and it was determined that all the materials showed a severe cytotoxic effect on human gingival fibroblast cells within the first 24 hours.

Sun et al. [16] tested single-stage self-etch dental adhesives (Adper Easy One, iBond, Clearfil S3 Bond and G-Bond) on human periodontal ligament fibroblast cell culture and reported that they caused a reduction in cell vitality and morphological changes. In the light of recent studies, there is increasing interest in the pulp irritation created by the acidic and resin monomer components expressed from dental adhesives.

**Conclusion**

Care must be shown to some sensitive points to be able to protect against the potential cytotoxic effects of adhesive resins. Some of the precautions which can be taken are that a risk-benefit analysis should be made when selecting a new material for use, the use of adhesive resins as direct capping material should be avoided, in deep dentin cavities, a base material should be placed between the restoration and the dentin to prevent harmful effects of the resin on the pulp, there should be interventions which will increase polymerisation, precautions should be taken to protect the gingiva and oral mucosa of the patient and to avoid skin contact with these types of materials.

**Conflict of Interest**

The authors have no conflicts of interest relevant to this article.

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References


