ORIGINAL ARTICLE

Release phenomena and toxicity in polymer-based dental restorative materials

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Abstract. In this work, the origin of the release phenomena occuring in resin-based dental restorative materials is demonstrated using confocal fluorescence microscopy and tapping mode atomic force microscopy techniques. The surface structure (microcavities, protruded filler particles, grain boundaries, and cracks) produced by water environment on the surface of composite resins and resin-modified glass-ionomeric cements (RMGICs) are shown. The water absorption and the subsequent weight decrease, induced by leaching process, have been measured by gravimetric analysis. The different toxicity effects induced by the component release are described. (www.actabiomedica.it)

Key words: Dental restorative materials, toxicity of visible light-cured materials, confocal fluorescence microscopy, atomic force microscopy, dimensional variation

Introduction

Over the years an enormous range of polymeric materials (composite resins, compomers and resin-modified glass-ionomeric cements, RMGICs) used in restorative dentistry have been made and their properties have been studied. From the beginning, these materials have undergone a substantial development which shows no sign of abating. Their use in dentistry is chiefly restrictedto restorative filling materials, adhesives, and fissure sealants which employ bifunctional-methacrylates in their monomer phase. The proliperation of these chemically sophisticated (polymer-based restorative) materials has involved the study of a wide range of material properties,some of which are basic properties, and others that are specific to dentistry. Nonetheless, it is stillfair to say that their composition, chemistry, and toxic effects on patients have reached ultimateresults. Toxicity effect is mainly due to the release of components in the oral environment from thepolymeric material surface involved in the curing reaction. Although continuous improvementsha-

ve been reached (1-6), restoring in absence of tooth/restoration interfacial voids and porosity isdifficult to achieve. Any free surface, produced by voids and porosity, is involved in leaching processes. Free surfaces of tooth-restoration interfacial microgaps are due to marginal seal failures,induce microleakage of oral fluids and recurrent caries (7-18), and affect short- and long-termadhesion (interface bond strength, masticatory load distribution). These microgaps are affected by several factors including C-factor (cavity factor) as well as structural characteristics of the dentin and its conditioning methods (10, 14-18).

Another type of interfacial void causes nanoleakage (19-29). A current trend in dentin bonding involves the formation of a hybrid layer interdiffusion zone which permits the micromechanical bonding that is necessary for the dentin to interlock to the restoration. In this case porosity is produced at adhesive-hybrid layer, dentin-hybrid layer interface, and in the same hybrid layer. Incomplete resin infiltration into the collagen fibril meshwork, integrity of the collagen fibrils,structure of the hybrid layer resulting from chemical treatment

(acid etching), elution of inadequately polymerized resin, and removal of retained water, are factors affecting the interfacial (micro- and nano) porosity.

The polymerization contraction stress seems to be the primary cause in the development of the interfacial stress leading to the failure of the marginal seal (microgaps) and interfacial porosity formation (it plays a key role in the interfacial microgap formation). Different approaches have been attempted in order to alleviate the major shortcomings of dental composite resins, namely the polymerization contraction. An approach consists in enhancing the hydrophilicity of the commonly used hydrophobic resins. For this purpose, adhesive monomers containing hydrophilic groups are often incorporated in the base monomers. However, the addition of adhesive monomers shows an adverse effect, since the hydrophilic sites promote the absorption of water and hydrolytical instability (hydrolysis of the hydrophilic monomers and leaching of the resin), thus decreasing the interfacial micromechanical bond strength.

The other approach consists in the improvement of the synthesis of low-contraction resins that could substitute the currently used resins, namely bis-phenol glycidyl methacrylate (Bis-GMA), urethane dimethacrylate (UDMA), tri-ethylen glycol di-methacrylate (TEGDMA), etc. (30, 31). A new area of restorative materials in the early stage of research, the "low shrinkage materials", is now growing. However the risk that novel materials pose to dental tissue in terms of bio-functionality and bio-compatibility has not yet been assessed. However, whatever free surfaces of polymer-based material is considered, release phenomena in the oral environment occur as a rule. Hence the continuing growth of regulatory requirements with respect to toxicity, that adds an extra dimension to the development of dental materials is necessary.

The origin of the release phenomena from free surfaces is due to the degree of the polymerization conversion that is significantly lower than 100% in the bulk volume, but it is by far lower at the tooth/material interface or on the polymer free surface produced by addition reaction. In this case, owing to inhibition of the polymerization reaction produced by air oxygen and/or oxygen absorbed on the free surfaces, the degree of conversion decreases up to 20%. Accordingly, the larger the material surface the larger the toxicity effect.

In this work the monomers usually used in polymer-based restorative materials obtained by addition reaction are reviewed; informations concerning the origin of tooth-restoration microgap formation are given; experimental evidence of release phenomena studied by Confocal Fluorescent Microscopy and Tapping Mode Atomic Force Microscopy is shown, and the different toxicity effects produced by the component release are reported.

Polymeric materials

Among the available monomers, the most frequently encountered are Bowen's resin and urethane dimethacrylate. However, due to the high viscosity of these monomers, diluents are added to allow them to incorporate more filler and to flow sufficiently at mouth temperature for packing into the tooth cavity. Fig. 1 shows some dental composite monomers.

Organic components of GICs and RMGICs, that are also used as lining materials, are mainly polyacrylic acids, photocurable monomers, tipically hydroxyethylmethacrylate (HEMA), and possible additional photopolymerizable monomers, such as Bis-GMA.

The polymer matrix binds the ingredients together (fillers, activator-initiator system, pigments), but at the same time is responsible for the inherent shrinkage, heat produced by the polymerization reaction, and toxicity effects.

Bis-glycidyl methacrylate (Bis-GMA) or Bowen's resin exhibits methacrylate end groups which can be polymerized like the autopolymerizing acrylic monomers when chemically or by visible light activated (468 nm wave length halogen or plasma light, or L.E.D.). It shows several advantages over methyl methacrylate, such as the molecular weight of 512 compared with 100 for MMA, a larger molecule which less readily diffuses, lower thermal expansion, lower polymerization contraction, and lower volatility.

Together with these advantages, the problem of high viscosity caused by the bulky phenyl rings in its chains and considerable hydrogen bonding between molecules are present. The inability to work with a material with such a high viscosity at room temperature (120 Pas) has resulted in the addition of lower vi-

scosity monomers such as TEGDMA, EGDMA, etc. To overcome the disadvantages of using diluents with the original bis-GMA monomers, alternative systems with reduced viscosity have been developed (bis-MA, bis-EMA, bis-PMA, etc., see Fig. 1). Among these, the hydroxy group elimination results in the reduction of hydrolytic degradation. Aliphatic dimethacrylates, such as urethane dimethacrylate monomers, have been used as substitutes of Bis-GMA. Due to the absence of phenyl groups in the polymer chains, their flexibility and toughness is significantly higher compared to Bowen's resin.

Reduced stiffness and increased polymerization contraction are the undesirable consequences. In order to reduce the polymerization contraction, ring opening systems which expand on polymerization have been also suggested, but at the present time they are far from ideal with respect to optical and aesthetic characteristics.
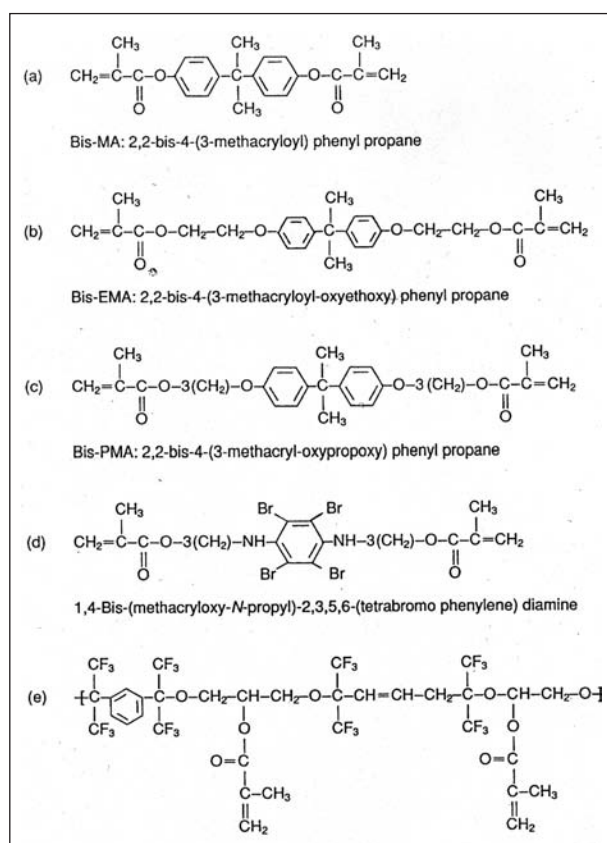

Figure 1. Monomers of composite resins

Interfacial microgaps

The polymerization contraction of the above-mentioned monomers is a cause of concern (Fig. 2). Due to the polymerization contraction, microgaps appear at tooth/cavity interface. This only occurs when the tooth-restoration adhesive force is lower than the cohesive force of tooth (enamel or dentin) tissue or restoration.

The microgaps increase in width owing to the leaching process induced by percolation of the oral fluids, which reduce low polymerized interfacial layer. Microgaps around the cavity margins remain possible sites where the percolation of oral fluids and the introduction of bacteria (bacterial colonization) can occur. These gaps have been measured; they are particularly problematic at the junction between the dentin of the cervical margin and the composite resin. Since the bond to enamel is stronger than that to dentin it results in the polymer pulling away from the weaker dentin bond as the restorative material shrinks on curing. This creates a space at the cervical margin of large proportion with respect to bacteria cell dimensions. Even worse, the light-activated dental composites contract towards the light source and therefore away from the cervical margin on setting. The development of recurrent caries, discoloration, pulpal irritation and thermal sensitivity are thus increased. The use of the acid etch technique for enamel together with a bon-
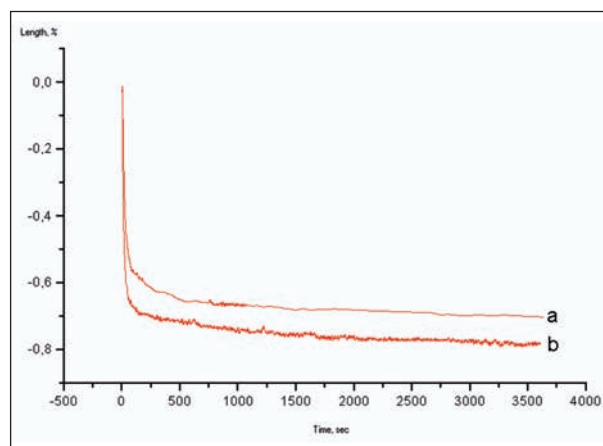

Figure 2. Polymerization contraction of composite (Venus) by laser beam scanning apparatus. Curves a) and b) are referred to two different specimens

ding agent for dentin minimises these undesirable effects, when incremental packing is used.

Hydrolytic degradation

All materials show absorption of oral fluids. Fig. 3 indicates that the water uptake mainly occurs in the first days immediately after immersion, subsequently a long-term erosion process takes place. Resin-modified glass-ionomeric cements (RMGICs) exhibit a more rapid and larger water uptake compared with composite resins (32-36).

Due to the water absorption, RMGICs release several components (organic molecules, silicates, fluoride, calcium, aluminum, and other ions) into the oral environment (37-42) promoting a protective action mainly due to the fluoride and calcium ions (absorption to dentin, antibacterial properties (37, 38), etc.), and/or a detrimental action (inflammatory actions of soft tissues). Accordingly, the mechanical properties can also change (34, 43-48).

For composite resins and compomers, the absorption of oral fluids involves a release process mainly related to the low-polymerized molecules. Water and saliva uptake can relieve areas of stress concentration and beneficially act in counteracting the polymeriza-

Figure 3. Water absorption of resin-based glass-ionomeric cements by gravimetric analysis. Curve B, Vitremer; D, Fuji II; F, Fuji Lining; H, Vitrebond. The tangent to a curve point gives the velocity of the process (absorption in the first days, or long-term erosion)

tion contraction. Unfortunately water uptake shows considerable deleterious effects on the mechanical properties. Both the yield stress and the fracture toughness have been shown to decrease by up to 30%. Attempts have been made to explain the process of water absorption and the related phenomena in terms of the diffusion process described by the Fick's law (33-37). The solution of Fick's equation is an exponential expression, which includes the basic parameter of the diffusion process, the diffusion coefficient (D). The assumption that the absorbed water obeys Fick's law implies that the specimen is homogeneous, and the partial solubility or dissolution occuring during the absorption can be neglected. The D values of RMGICs calculated within the framework of Fickian diffusion range from 10^{-11} to 10^{-12} m^2/s. This suggests that the deterioration of these restorative materials would occur in a short time; on the contrary, the mechanical properties show long-term stability after an initial decrease. Therefore the assumption that the hydrolytic degradation is due to the uptake of water, which uniformly diffuses into the bulk material, has not been proved.

Recently, using confocal fluorescent microscopy (49), it has been shown that the water absorption process mainly involves the specimen surface and limits itself to the low polymerized surface layer. Fig. 4 shows the hydrolytic degradation produced by water absorption related to two representative areas of a RMGIC surface (Vitremer). Figures 4a and 4c show porosity and filler aggregates after a short immersion in water (1-2 days). Fig.s 4b and 4d show the same areas after 45 days of immersion in water. The loss of image resolution and the decrease in volume of the surface bubbles indicated that the absorption of water and the swelling of the matrix were occuring. The same details, reported in Figures 4b and 4d, periodically observed for 500 days, showed no further changes.

The thickness of the removed surface layer can be evaluated by the diameter of the surface bubbles removed by erosion. Usually, bubbles and filler aggregates of 20 μm in diameter or lower were completely removed when located immediately below the surface. Consequently the thickness of the removed surface layer can be considered to be about 20 μm. Some grains and cracks appeared after long water storage.
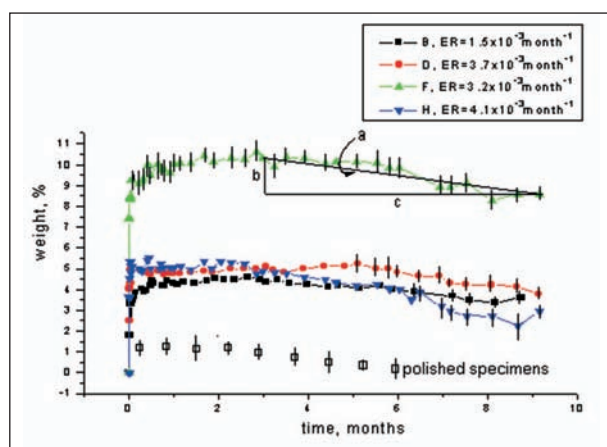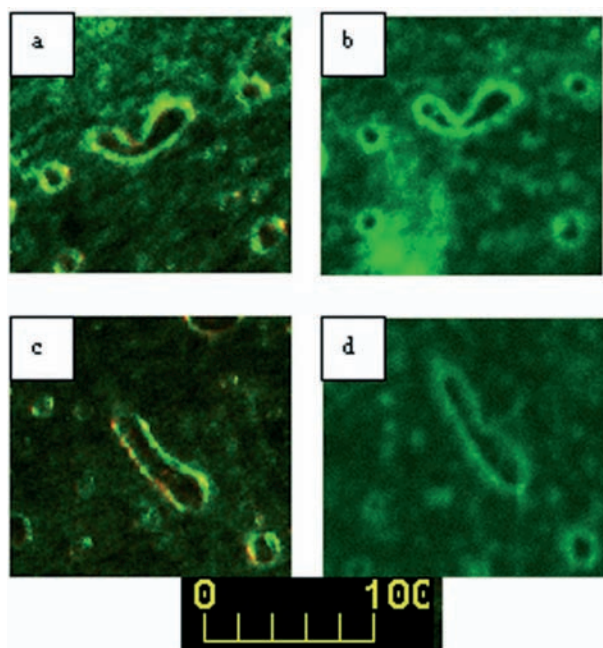
Figure 4. Water uptake observed by confocal fluorescent microscopy in a RMGIC (Vitremer). a) and c) show porosity and filler aggregates after a short immersion (1-2 days) in water; b) and d) show the same areas after 45 days. For longer immersion, no further changes are observed
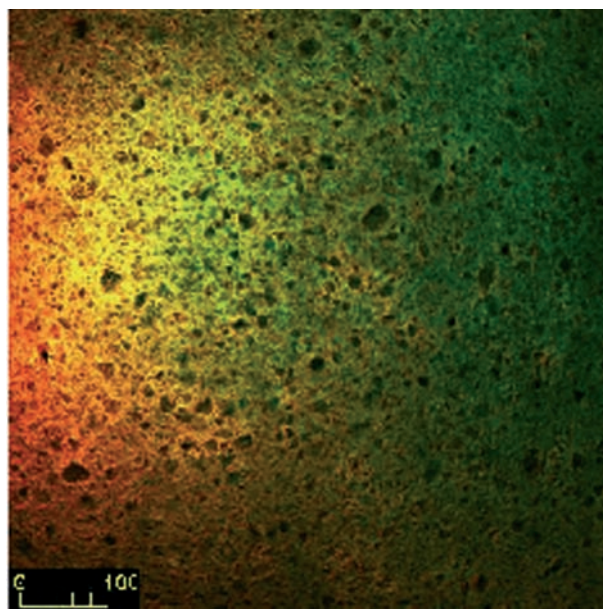

Figure 5. Microcavities observed by confocal fluorescent microscopy in RMGIC after removal of the low polymerized surface layer
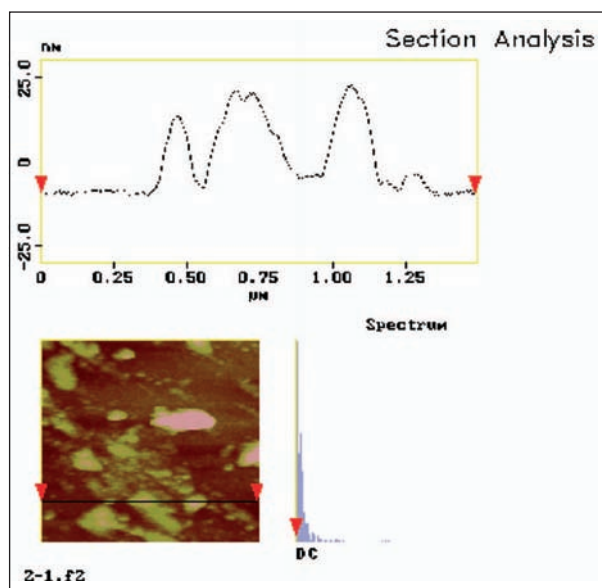

Figure 6. Protruded filler particles by Atomic Force Microscopy in RMGIC after removal of the low polymerized surface layer

Leaching

Usually, the absorbed water induces the leaching process, which, in turn, produces the release of free, residual unpolymerized monomers, low polymerized polymer molecules, filler particles, andfiller by-products. Fig.s 5 and 6 show the final view of RMGIC surfaces after the removal of the low polymerized layer. The etched surfaces are characterized by the cavity formation (Fig. 5) and protruded filler particles (Fig. 6). The view of Fig.s 5 and 6 does not change when materials are soaked in water for a long time (1-2 years). This fact indicates that the leaching phenomenon mainly involves the specimen surface.

From a biological viewpoint, the leaching components can induce toxic effects. In addition, the leaching leads to the possible rupture of the polymer/tooth interfacial bond increasing the tooth-restoration gap.

The polymer properties are also dependent on the degree of the polymerization conversion of the carbon-carbon double bonds in the methacrylate groups and on the degree of the cross-linking betwen bis-GMA molecules. The degree of the polymeriza-

tion conversion of Bis-GMA monomer has been shown to be approximately 55% for a chemically cured system and slightly less (48%) when UV radiation is used. The blue light can produce up to a 70% degree of conversion in the bulk material, when a diluent is used. In this case the increase of the degree of conversion is induced by a greater molecular mobility during curing. The improved degree of conversion produces stronger Bis-GMA/TEGDMA resins. Such a situation should be expected since the increased cross-link density indicates greater stiffness due to the reduced mobility of the polymer chains.

In all cases, even when the largest degree of polymerization contraction is reached in the bulk material, about 20% or more of the original monomer concentration is not involved in the surface chemical reaction and, consequently, is available for the leaching process.

It can be supposed that different matrix sites participate to the leaching process with different kinetics. An immediate contribution is given by dissolution of the low polymerized interfacial tooh/material surface layer, while the long-term contribution is given by boundaries of grains and cracks (Fig. 7).

Toxicity

In spite of increasing interest in these materials, research concerning the biocompatibility of polymer-based dental materials are still controversial. In vivo (animal) studies showed that microleakage and subsequent bacterial invasion produce allergic reactions and detrimental biological effects on dental pulp (50). Frequently the results obtained with different experimental methods are contradictory and need an improvement of standardized test methods in vitro (51);although physical properties of resin composites are constantly being improved, studies have shownthat the use of these resins as restorative materials is occasionally associated with necrosis and irritation of the pulp, as well as of the periodontium.

I*n vitro* toxic reactions, produced only by component release have been shown (52).

The change in the chemical structure of the composite and the variation in the ratio of the filler and monomer produce a significant effect on the element
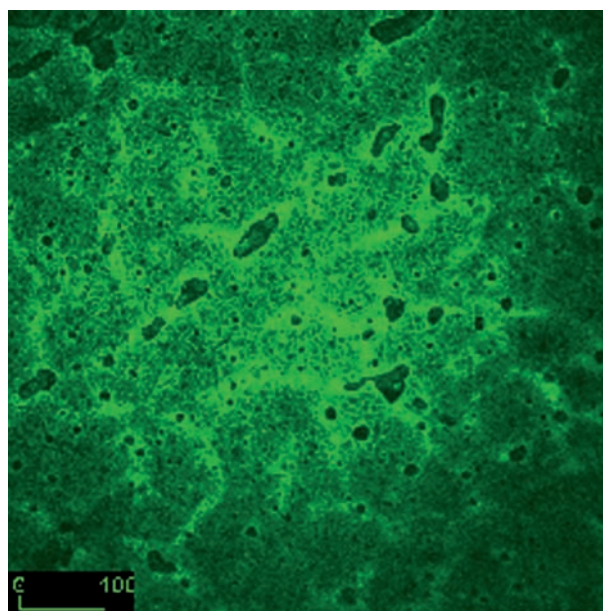

Figure 7. Grain boundaries and cracks in compomer (F2000) by confocal fluorescent microscopy

release and cytotoxicity level of the material,It has been reported (53) that the flowable materials of the traditional composites were more toxic than the standard ones.

It has been also shown that both Bis-GMA and TEGDMA have reproductive toxic effects in female mice when they were administreted at doses of 25 and 100 μg/kg intragastrically for 28 days (54). Some investigations have examined the cytotoxicity of these monomers on mammalian cell cultures using a wide range of assay techniques and variable levels of cytotoxicity have been reported. Such monomers are toxic to human gingival fibroblasts and Ha CaT Keratinocytes (55).Many factors affect the toxicity of the components leached from the resin. In general, the monomers are more cytotoxic after 24 h of aging and become less toxic with aging (56). The removal of theleachable components from polymerized composites using organic solvents completely decreased their cytotoxicity (57).

It has been widely demonstrated that dental composite monomers may cause a wide range ofadverse clinical effects, e.g. irritation to skin, eyes, or mucous membranes, and gastrointestinalcomplaints. In rabbit lung, the inhaled dental composite particles (< 10 μm) might even lead to chronic inflammation (58-60).

Conclusions

Confocal fluorescent microscopy and atomic force microscopy investigations regarding the visible light-cured polymer-based restorative materials indicate that release phenomena involve only the surface of the cured specimens. Consequently, the *in vitro* toxicity studies better reflect the usual clinical cases of restored teeth, when the experimental methods concern release phenomena of the surface of polymerized restorative materials. In fact the toxicity can radically change when the effect of a single component is analyzed.

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