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Review

Genotoxicity Induced by Dental Materials: A Comprehensive Review

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Abstract. *Genotoxicity is the capacity of an agent to produce damage in the DNA molecule. Considering the strong evidence for a relationship between genetic damage and carcinogenesis, evaluation of genotoxicity induced by dental materials is necessary for elucidating the true health risks to patients and professionals. The purpose of this article was to provide a comprehensive review of genotoxicity induced by dental materials. All published data showed some evidence of genotoxicity, especially related to dental bleaching, restorative materials and endodontic compounds. Certainly, such information will be added to that already established for regulatory purposes as a safe way to promote oral healthcare and prevent oral carcinogenesis.*

Genotoxicity is the ability of an agent to induce DNA damage. This means that in order for a chemical agent to be considered genotoxic, it needs to interact with genetic material. Currently, several chemical agents are categorized as genotoxic in the scientific literature. It is assumed that the human genome is continuously being damaged by different chemical substances. Nevertheless, eukaryotic cells are biological units highly specialized at neutralizing genotoxic insults through promoting DNA repair. A xenobiotic-metabolizing system and DNA repair machinery are critical for ensuring the integrity of the human genome (1). However, if any genetic damage is not efficiently repaired, a permanent lesion in the genetic apparatus may arise after cell replication, a phenomenon known as mutagenicity.

There are several methodologies established by the scientific community capable of detecting genetic damage and mutations in a wide range of end-points, such as: DNA strand breaks, point mutations, chromosome translocations, chromosomal loss or interference with spindle cell apparatus (2). Such methodologies are recognized by international regulatory agencies as the battery of tests required for validation of chemical agents that are released into the global market. This information is very important in clarifying the potential human health risks induced by such recent chemicals.

In recent decades, a plethora of dental materials has been introduced into the market. Many of them have been improved due to the current demand of clinical performance in the oral cavity. It is important to stress that many of these materials remain in the oral cavity for long periods, *i.e.* months or years (3). Additionally, dental clinicians manipulate these materials continuously in clinical practice. In this way, a risk assessment with regard to genotoxicity and mutagenicity of such materials is fundamental to ensuring the safety of people who are continually exposed to them. This means that all dental materials must be scrutinized in the light of genotoxicity, since it has been established that genetic damage is intimately linked to chronic degenerative diseases, such as cancer (4).

Thus, the objective of this comprehensive review was to report the results on genotoxicity induced by dental materials. Certainly, such information is not only important in order to validate them as being safe in clinical practice, but also in detecting possible lack of information that should be further investigated by researchers in new studies.

A comprehensive literature search for studies on 'DNA damage, genetic damage, genotoxicity and dental materials was performed between 2000 and 2017. In brief, a search of PubMed, MEDLINE, Embase and Google Scholar for a variety of articles (all publications until January 2017) was

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carried out using the key words above. Case reports and articles not written in English were excluded from the review. All other articles were identified and included in this review.

Dental Bleaching Agents

It seems obvious that dental bleaching agents should be able to induce genetic damage because these compounds generally contain hydrogen peroxide. Hydrogen peroxide is a potent oxidizing agent in eukaryotic cells (5). Oxidative stress induces genetic damage and mutations in living cells (5). There are some studies investigating the genotoxic potential of dental bleaching agents. Our own studies have demonstrated that six commercial dental bleaching agents (Clarigel Gold - Dentsply; Whitespeed - Discus Dental; Nite White - Discus Dental; Magic Bleaching - Vigodent; Whiteness HP - FGM and Lase Peroxide - DMC) were genotoxic using mouse lymphoma cells or Chinese ovary hamster cells *in vitro* (6, 7). Toothpastes containing whitening products were also found to be genotoxic using human gingival cells *in vitro* (8). *In vivo* studies have verified previous *in vitro* results. For example, in human oral mucosa cells exposed to bleaching treatment, the micronucleus frequency increased (9). Nevertheless, two bleaching sessions with 35% hydrogen peroxide at a one-week interval did not induce mutagenicity (no incidence of micronucleated cells) (10). The authors speculated that in-office bleaching did not induce DNA damage in gingival and lip tissue during the bleaching period (10). However, it is important to stress that oxidative DNA lesions induced by hydrogen peroxide are repaired by DNA repair systems. The base excision repair pathway is the most important cellular protection mechanism responding to oxidative DNA damage, being responsible for protecting cells and organisms from mutagenesis and carcinogenesis (11). Certainly, this explains the results found. Although few studies have been conducted so far, it is clear that dental bleaching agents can be genotoxic. Therefore, the use of tooth whitening products should be undertaken cautiously.

Dental Restorative Materials and Related Compounds

Dental resins are widely used in restorative dentistry, prosthetics, and orthodontics. Polymers and monomers in such materials are released into the oral cavity due to mechanical abrasion and chemical activity of salivary enzymes (12). Moreover, polymerization is always incomplete and usually leaves a considerable fraction of free monomers, which in turn are released into the oral cavity causing harmful effects on the oral tissues (12). After searching the literature, we found many studies investigating the genotoxic potential of dental polymers, the majority showing genotoxicity under several end-points and assays. Kleinsasser *et al.* published the first studies on such materials. They investigated *bis*phenol A glycidyl methacrylate (BISGMA) and triethylene glycol dimethacrylate (TEGMA) using human salivary glands and lymphocytes *in vitro*. The results showed that both BISGMA and TEGMA induced DNA damage as depicted by comet assay results (13, 14). Since the comet assay detects a whole spectrum of DNA lesions, such as single- and double-strand breaks in DNA, DNA adducts and incomplete repair sites, such findings are biologically important and should be considered when using these materials. Others have demonstrated similar findings using human lymphocytes, gingival fibroblasts, chinese hamster cells, oral cancer cells, murine macrophages and human keratinocytes, and V79 fibroblasts by comet assay and micronucleus test (15-21). These studies confirm that such materials pose risks of genotoxicity and mutagenicity in eukaryotic cells.

Rats exposed to methyl methacrylate presented mutagenicity as a result of an increased number of micronucleated cells in bone marrow (22). *In vitro* studies revealed that methyl methacrylate also induces DNA strand breaks (16). Results obtained from murine macrophages *in vitro* revealed that BISGMA exhibited genotoxicity in a dose-related fashion as a result of increasing numbers of DNA strand breaks and micronuclei formation (23). When people were occupationally exposed to methyl methacrylate, superoxide dismutase activity, malondealdehyde, and glutathione levels were significantly higher in blood samples of dental technicians when compared to matched controls (24). Analogous products, such as 2,2-*bis*[4-(acryloxypropoxy)phenyl]propane also increased micronuclei frequency in human gingival fibroblasts *in vitro* (25). The use of antioxidants such as melatonin, *N*-acetylcysteine and ascorbic acid reduced the genotoxic effects of methacrylate monomers (26, 27). Such data suggest that genotoxic and mutagenic effects induced by methyl methacrylate could arise *via* oxidative stress.

The Somatic Mutation and Recombination Test (SMART) in *Drosophila melanogaster* has been applied to analyze genotoxicity expressed as homologous mitotic recombination, point and chromosomal mutation. The mechanistic basis underlying the genotoxicity of urethane dimethacrylate (UDMA) and TEGDMA is related to homologous recombination and gene/chromosomal mutation (28). BISGMA and 2-hydroxyethyl methacrylate (HEMA) had no statistically significant effect on genetic apparatus (29, 30). Conversely, both compounds exhibited genotoxicity in V79 cells detected by comet assay and micronucleus test (15, 30). Again, comet assay showed that UDMA is a genotoxic agent in Chinese hamster cells and human lymphocytes *in vitro*, respectively (17, 31). According to some authors, the genotoxicity induced by TEGMA, UDMA and HEMA is

mediated by oxidative stress in eukaryotic cells (30, 31). Therefore, further studies investigating the role of reactive species formation as well as the activity of antioxidant enzymes, such as superoxide dismutase, catalase and glutathiones, are necessary in order to understand the genotoxic effects induced by these polymers at cellular and molecular levels.

The genotoxicity of three glass ionomer cements used in dentistry, manufactured by American (Vitrebond), Japanese (Fuji I), and European (Ketac Cem) companies were examined with human peripheral lymphocytes in the presence or absence of metabolic activation. Vitrebond resulted in direct genotoxicity (32). Others detected genotoxicity using Chinese ovary hamster cells from the powder of Ketac Molar *in vitro* (33). In the same way, the liquid from Vitrebond at 0.1% dilution caused an increase of DNA injury (34, 35). Vitrebond also led to a genotoxic effect *in vitro* using Mammalian Cell Gene Mutation Test (HPRT Test) with Chinese ovary hamster cells as well as in the bacterial umu-test with Salmonella typhimurium (36). Eluates derived from resin modified glass ionomer cements commercially available caused severe genotoxic effects by increasing the frequencies of sister chromatid exchanges and chromosomal aberrations in peripheral blood lymphocytes *in vitro* (37, 38).

Dental restorative materials were biomonitored by comet assay using peripheral blood cells from young individuals. No significant difference was observed between amalgam and composite fillings. Interestingly, the association between dental fillings and DNA damage was increased by the number of fillings and by exposure time (39). Oral mucosa cells from humans of both genders showed that amalgams and resin-based composite fillings induced genotoxic damage in human oral mucosa cells by comet assay and micronucleus test (40-42). Conversely, others did not show any evidence of genotoxicity of dental composite resins or amalgam in human peripheral leukocytes as depicted by sister chromatid exchange and chromosomal aberration tests (43, 44). At least in part such conflicting results could be attributable to the different designs employed to determine genotoxicity induced by dental fillings because these studies did not evaluate the direct effect of dental restorative materials on genetic material. Further research on their possible genotoxicity is welcomed, since dental composite resins remain in intimate contact with oral tissue over a long period (42).

Aqueous extract from dental composite resins were evaluated by homologous mitotic recombination, point and chromosomal mutation effects in *D. melanogaster*. The results revealed that none of the tested eluates exhibited any signs of genotoxicity (28). Three flowable (Filtek Supreme XT Flow, Tetric EvoFlow, Gradia Direct Flo) and three non-flowable (Filtek Z250, Tetric EvoCeram, Gradia Direct Posterior) dental composite materials were assessed for genotoxicity using the comet assay in human peripheral blood leukocytes *in vitro*. The tested materials did not show genotoxic effects after exposure of leucocytes for 1 h (45).

The polymerization initiators for resins cured benzoyl peroxide, dimethyl-*p*-toluidine, and 1-allyl-2-thiourea showed genotoxic activity in the bioluminescent bacterial genotoxicity test (46). DNA damage was detected after exposure of human lymphocytes to dental primers. However, no significant increase in DNA damage was observed when cells were exposed to the dental primers (47). A significant increase in the number of chromatid breaks in human lymphocytes *in vitro* was observed for adhesives Adper Single Bond 2, Excite, and OptiBond Solo Plus using chromosomal aberration analysis (48-51). SMART in *D. melanogaster* was applied for this purpose as well. Adper Single Bond Plus induced statistically significant increases in the frequency of total spots at the highest concentration tested, while Prime&Bond 2.1 was positive for genotoxicity at all concentrations tested (28).

Finally, camphorquinone increased DNA damage in primary human gingival fibroblasts at all concentrations as a result of intracellular oxidative stress and subsequent down-regulation of glutathione level (52, 53).

Endodontic Compounds

Endodontic compounds have been extensively investigated for genotoxicity. A good number of studies has been published using different end-points such as DNA damage, mutations and cell death. All studies were conducted *in vitro*. Chromosomal aberrations in SHE cells were induced by treatment with ethylene diaminetetra-acetic acid (EDTA), formocresol (a mixture of formalin and tricresol), sodium arsenite, *p*-chlorophenol, *p*-phenolsulfonic acid, sodium hypochlorite, erythrosine B, prilocaine hydrochloride, procaine hydrochloride, and sodium arsenite (54). The genotoxic potential of formocresol is reported in the literature with conflicting results, despite the underlying mechanisms of genotoxicity induced by formaldelyde (the main component of formocresol) being DNA interstrand crosslinks. For example, Chinese hamster cells were treated with formocresol and two types of calcium hydroxide paste. The results showed that formocresol significantly increased DNA damage (55). Others have demonstrated absence of genotoxic effects induced by formocresol, *p*-monoclorofenol and calcium hydroxide in the same cellular type *in vitro* (56-58). Calcium hydroxide pastes caused DNA damage as depicted by comet assay as a result of oxidative stress (59). Formocresol, *p*-monochlorophenol, and calcium hydroxide were not able to modulate alkylation-induced genotoxicity or oxidative DNA damage *in vitro* (60). Consequently, it is possible that such endodontic materials do not interfere with DNA repair systems.

Chloramphenicol, *p*-chlorophenol, *p*-phenolsulfonic acid, sodium hypochlorite, and tetracycline hydrochloride exhibited a negative result for chromosomal aberrations *in vitro* (54). A recent study published by Pires *et al*. also reported that iodoform pastes did not induce DNA damage in human peripheral lymphocytes *in vitro* (59). The zinc oxide eugenol-based sealers (Canals, Canals-N, and Tubilseal) were found to cause limited genotoxicity in mammalian cells (61). Assessment of three dyes used for disclosing dental plaque showed chromosomal aberrations induced by basic fuchsin, but not by acid fuchsin and erythrosine B. Three local anesthetics, lidocaine hydrochloride, prilocaine hydrochloride, and procaine hydrochloride, were negative in chromosomal aberration testing (54). Chlorhexidine caused DNA damage *in vitro* (59, 62, 63) but also presented genotoxic potential in terms of total mutations per wing in the *D. melanogaster* wing-spot test (64). Others have demonstrated that chlorhexidine does not induce genetic damage *in vitro* (57, 65). Nevertheless, chlorhexidine is potent at causing genotoxic damage in several tissues and organs, such as liver, kidney and oral mucosa cells *in vivo* (66-69). When associated with calcium hydroxide, chlorhexidine exerts both anti-oxidant and pro-oxidant activities through scavenging superoxide radicals by the xanthine/xanthine oxidase reaction and induction of reactive species production including hydrogen peroxide and superoxide radicals, respectively (70).

Taking into consideration that mineral trioxide aggregate is in contact with periodontal tissues, bone and pulp tissues, it is important to determine its putative genotoxic effects. After searching the literature, the consensus seems that mineral trioxide presents good biocompatibility because no studies have shown genotoxicity to be induced by mineral trioxide aggregate in mammalian cells. For example, neither mineral trioxide aggregate nor Portland cement produced genotoxic effects *in vitro* (71-78). This finding was confirmed when human peripheral blood cells were exposed to mineral trioxide aggregate and Portland cements, the evaluation of genotoxicity being conducted by comet assay (79, 80). Mineral trioxide aggregate cements based on calcium silicate were not found to be potentially genotoxic (55). Nevertheless, calcium-enriched mixtures were genotoxic at concentrations of 15.6 and 250 μg/ml; however, this was less than that of mineral trioxide aggregate (73). Resin sealer based on mineral trioxide aggregate, such as AH Plus and Fillapex MTA sealer, induced micronucleus formation *in vitro* (81, 82). Resin-based sealers (Topseal, AH 26, and AH Plus) also caused a dose-dependent increase in genotoxicity, but no such effect was seen with the calcium hydroxide-based sealer (Sealapex) (83, 84). Conversely, neither genotoxicity nor mutagenicity was revealed for AH Plus using primary human periodontal ligament fibroblasts (85, 86). Other root canal sealers such as Epiphany Sealant and RealSeal Root Canal Sealant, in both polymerized and unpolymerized form, did not induce DNA damage in human peripheral lymphocytes (87). Camargo *et al*. (88) and Bin *et al*. (81) demonstrated that epoxy resin-based sealer (AH Plus), mineral trioxide aggregate (Filipex), a single methacrylate-based sealer (EndoRez), and a silicone-based sealer (RoekoSeal) were genotoxic in Chinese hamster fibroblasts (v79) cells *in vitro*. Camargo *et al*. also confirmed that resin-based sealers increased the micronucleus frequency, and Acroseal delayed the cell cycle in G_2 phase using the same *in vitro* cellular test system (88).

Pure calcium silicate-based cements, modified calcium silicate-based cements and three resin-based calcium silicate cements showed no genotoxicity by comet assay in human osteoblast cells (87). GuttaFlow, Epiphany, Diaket, IRM, SuperEBA and Hermetic were tested on human peripheral blood lymphocytes using the comet assay and chromosomal aberration analysis; the results were considered positive for genotoxicity (89).

Orthodontic Devices

Since orthodontic devices remain in the oral cavity for long periods, several studies have struggled to investigate the outcomes induced by the release of toxic metal ions from orthodontic alloys. Such information is important for biocompatibility of fixed appliances. No genetic damage was detected *in vitro* for eluates obtained from orthodontic brackets (90-93).

When patients were treated using stainless steel orthodontic brackets and nickel-titanium or stainless steel arch wires, oral mucosa cells were evaluated by micronucleus assay. Again, no significant difference was found in micronucleus frequency following orthodontic therapy (94, 95). Dental casting alloys (Co-Cr-Mo and Ni-Cr) commonly used in fixed and removable prosthodontic appliances that are in contact with the oral epithelium *in vivo* showed genetic damage in those wearing metal appliances (96). Others have demonstrated mutagenicity in buccal mucosa cells induced by orthodontic devices in patients submitted to orthodontic therapy; however, such mutations have been attributed to acute exposure only (97). Further studies are necessary to elucidate this issue.

Dental Implants

Titanium (Ti) is currently the most widely used material for manufacturing dental implants. Changes in the surface of commercial pure Ti can determine the functional response of cells, and is therefore a critical factor for the success of the implant. Some studies showed that the untreated titanium surface causes DNA damage, chomosomal aberration and micronuclei, contributing, therefore, to genotoxicity. Such

findings were attributed to increased surface roughness and changes in titanium oxide layer thickness (98). Nevertheless, Ti alloy (Ti-6Al-4V) was neither cytotoxic nor genotoxic *in vitro* (98, 99). *In vivo* studies revealed no significant differences in micronucleus frequency from patients with dental implants when compared to matched controls (101-103). The frequency of sister chromatid exchange was found to be significantly higher in patients treated with nickel-chromium intermaxillary fixation devices than those treated with Ti miniplates (104).

Conclusion

In this review, we present published results reporting genotoxicity of dental materials. Some materials, such as dental bleaching agents, restorative materials and endodontic compounds, have been successfully investigated for genotoxicity *in vitro*. To the best of our knowledge, few studies have been conducted *in vivo*, especially murine experimental models. Moreover, some materials such as orthodontic devices or dental implants, have been poorly investigated so far, and results are still controversial. Further studies using different end-points and focusing on the role of oxidative stress and DNA repair machinery using *in vivo* test systems are fundamental for elucidating the human health risks induced by these materials. Therefore, this area needs further investigation for the safety of both professionals and patients in order to promote oral health and prevent diseases such as cancer, since these materials remain in the oral cavity for long periods of time.

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