TÜBİTAK

Original Article

Turk J Med Sci
2012; 42 (Sup.1): 1157-1165
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E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-1201-60

Genotoxic effects of banding procedure with different orthodontic cements on human oral mucosa cells

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Aim: To assess the genotoxic and cytotoxic effects of banding procedure with 5 different orthodontic cements on human oral buccal epithelium cells.

Materials and methods: Fifty healthy volunteers (mean age: 14.54 ± 2.37 years) were randomly divided into 5 groups of 10. Preformed stainless steel molar bands (3M Unitek) were cemented to the upper and lower first molar teeth with 5 different orthodontic cements (Durelon, GC Fuji Ortho Band LC Paste Pak, Meron, Ultra Band Lok, and 3M Unitek Multi Cure) in each group. Genotoxic effects of the banding procedure for a 1-month period were evaluated using the micronucleus test (MNT). To monitor cytotoxic effects, binucleated cells (BNs), karyolysis (KL), and karyorrhexis (KR) were also evaluated in this setting.

Results: Analysis of micronuclei in buccal epithelial cells revealed a significant increase in chromosomal damage in all groups ($P < 0.01$). Significant differences were found in the number of BNs in the groups receiving treatment with Meron, Ultra Band Lok, Durelon, and 3M Multi Cure ($P < 0.01$). Banding with GC Fuji and Durelon significantly elevated KL frequencies ($P < 0.05$). Durelon had a significant effect on KR ($P < 0.01$).

Conclusion: Band cementation with conventional glass ionomer cement (Meron) showed the least genotoxic effects. The highest cytotoxic effects on human oral buccal epithelium cells were found in the polycarboxylate cement (Durelon) group.

Key words: Biocompatibility, cytotoxicity, genotoxicity, micronucleus test

Introduction

Several luting systems are routinely used in dentistry for the cementation of prosthetic restorations and orthodontic appliances. Conventional glass ionomer cements (GICs) are the most widespread materials since their introduction by Wilson and Kent, due to their ability to chemically adhere to mineralized tissue and metal (1,2).

Due to variations in chemical composition and setting reaction, products have been categorized as resin-modified GICs (RMGICs) or modified composites, and these have been used for cementing orthodontic bands (3,4). RMGICs are dual-setting: upon mixing, the acid–base reaction occurs and the light-initiated free-radical polymerization of resin also occurs (5). Polyacid-modified composite resins are composite materials consisting of partially silanized ion-leachable glass embedded in a light-activated polymeric matrix (6).

Orthodontic materials have to contact or interact with body tissue and fluids over extended periods. Orthodontic brackets, wires, composites, and cements have compounds known to have allergic, cytotoxic, mutagenic, and/or carcinogenic potential

Received: 24.01.2012 – Accepted: 29.03.2012
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(7). Evaluation of the cytotoxicity, genotoxicity, and biocompatibility of orthodontic material is as important as the physiological or mechanical properties of these materials.

Triethylene-glycoldimethacrylate (TEGDMA), urethane dimethacrylate (UDMA), 2-hydroxyethyl-methacrylate (HEMA), bisphenol A-diglycidyl dimethacrylate (Bis-GMA), and methyl methacrylate (MMA) are released from orthodontic resin-based adhesives. Releasing of these ions and their diffusion through oral tissue have genotoxic and cytotoxic effects (4,8–11). Results of in vivo and in vitro experiments were controversial about effects of glass ionomer cements on DNA breakage due to leachable resin components (12,13).

In genetics, genotoxicity describes a deleterious action on a cell's genetic material affecting its integrity. Genotoxic substances are known to be potentially mutagenic or carcinogenic. Today there are several methods used for evaluating the mutagenic potential of physical and chemical agents, such as metaphase chromosomal aberrations, micronuclei (MN), sister chromatid exchanges, host cell reactivation, and comet assay (14–17). The micronucleus test (MNT) is a very reliable method for chromosomal aberration assay and has been used to detect genotoxicity of resin monomers and resin materials (18). MN are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosome fragments or intact whole chromosomes lagging behind at the anaphase stage of cell division. The sensitivity of the MNT is increased by recording degenerative nuclear alterations, such as karyorrhexis (KR), pyknosis karyolysis (KL), condensed chromatin, and binucleated cells (BNs) in addition to the MN (19,20).

Genotoxicity and cytotoxicity of different dental materials have been identified in several investigations (1,12–18,21,22). There have been no studies investigating the genotoxic effects of banding procedures. The aim of this study was to assess the frequencies of micronucleated cells in the oral mucosa after band cementation with 5 different orthodontic cements. To monitor cytotoxic effects, BNs, KL, and KR were also evaluated in this setting.

Materials and methods

The procedures used in the present study were in accordance with the guidelines of the Helsinki Declaration on human experimentation. The study protocol was approved by the ethics committee of İnönü University (07/06/2011; no. 2011/73). All human subjects were fully informed and gave written, informed consent.

Fifty healthy volunteers (17 boys and 33 girls) with a mean age of 14.54 ± 2.37 years (range: 10.7–18.3 years) were selected from consecutive patients referring for orthodontic treatment to the Department of Orthodontics, İnönü University, who fulfilled the following inclusion criteria:

- Nonsmokers
- Nondrinkers
- Good oral hygiene
- No decays
- No fillings
- No use of oral antiseptic solutions

The subjects were randomly divided into 5 groups of 10. All patients underwent lateral and frontal cephalometric X-rays and panoramic dental radiography. To assess the genotoxicity and cytotoxicity of the banding procedure, the present study was started 3 months after X-ray exposure.

Buccal epithelium cell samples were collected 4 times from each patient. First samples were collected at the beginning of the study and accepted as the control (T1). Separation elastic rings (Dentaurum, Pforzheim, Germany) were then inserted for banding. After 3 days, the separation elastics were removed and second samples were taken (T2). 3M preformed stainless steel molar bands (3M Unitek, Monrovia, CA, USA) were cemented to the upper and lower first molar teeth with different orthodontic cements in each group. The cements used in this study are shown in Table 1. The cements were mixed and allowed to set, or they were light-polymerized in strict compliance with the manufacturer's recommendations. The third sample collection was performed 1 week after molar band insertion (T3). The final records (T4) were taken 1 month later.

Table 1. Materials used in this study.

Brand	Type	Component	Chemical composition	Manufacturer	Lot number
Durelon	Polycarboxylate cement	Powder	Zinc oxide, stannous fluoride, tin dioxide Water, polyacrylic acid	3M ESPE, Seefeld, Germany	374808
		Liquid			388421
GC Fuji Ortho Band LC Paste Pak	Resin-modified glass ionomer	Automix cartridge	Paste A: Fluoroaluminosilicate glass, dimethacrylate, silicon dioxide, urethane dimethacrylate (UDMA) Paste B: Polyacrylic acid, distilled water, silicon dioxide, polybasic carboxylic acid, initiator	GC Corporation, Tokyo, Japan	0807281
Meron	Glass ionomer luting cement	Powder	Mixture of silicate fillers, polyacrylic acid, tartaric acid, and initiators	VOCO GmbH, Cuxhaven, Germany	0950487
		Liquid			0946388
Ultra Band Lok	Polyacid-modified composite	Single Paste	Glass frit (PNOC), amorphous silica, bisphenol A-diglycidyl methacrylate (Bis-GMA), sodium fluoride	Reliance Ortho Prod., Itasca, IL, USA	0904670
3M Unitek Multi Cure	Resin-modified glass ionomer	Powder	Silane-treated glass, potassium persulfate Polycarboxylic acid copolymer, water, 2-hydroxyethylmethacrylate (HEMA)	3M Unitek, Monrovia, CA, USA	9FT
		Liquid			9JK

Sample collection: Before cell collection, the participants rinsed their mouths twice with tap water. Epithelial cells were collected from buccal mucosa, scraping the middle part of the inner cheeks with sterile cement spatulas. The samples were smeared onto clean microscope glass slides. From each sample, 3 to 5 slides were prepared. All slides were air-dried and immediately fixed in methanol and glacial acetic acid (3:1). The slides were stained with acridine orange (Sigma Chemical Co., St. Louis, MO, USA) for 10 min. Acridine orange was dissolved in bidistilled water (0.01%).

Evaluation of the slides: The slides were examined under a light microscope (Olympus Optical Co., Tokyo, Japan). A total of 2000 cells from each set of slides were scored. MN and nuclear abnormality identifications were based essentially on the criteria of Tolbert et al. (20).

Statistical analysis: The data were analyzed using SPSS for Windows 14.0 (SPSS Inc., Chicago, IL, USA). The descriptive measurements are presented as mean and standard deviation or as median, minimum, and maximum as appropriate.

The repeated measures ANOVA (RANOVA) test was used to test the differences in degenerative nuclear alterations (MN, BNs, KL, and KR) in the different periods within groups, and Bonferroni multiple comparison tests were used to compare all pairs of groups. The Kruskal–Wallis H and Conover's multiple comparison tests were used to evaluate the significance of differences (T4–T1) among groups. The level of significance was set at $P < 0.05$.

Results

Comparison of the frequency of MN, BNs, KL, and KR at different time points within groups can be seen in Table 2. When we compared the MN rates and degenerative nuclear alterations between different time points within groups, statistically significant differences were found. Analysis of MN in buccal epithelial cells revealed a significant increase in chromosomal damage between T1 and T4 in all groups ($P < 0.01$). Statistically significant differences were found in the number of BNs between T1 and T4 in the groups treated with Meron, Ultra Band Lok, Durelon, and 3M Multi Cure ($P < 0.01$). Banding with

Table 2. Comparison of the genotoxic and cytotoxic effects of the cements at different time points within groups.

	Group	T1 Mean ± SD	T2 Mean ± SD	T3 Mean ± SD	T4 Mean ± SD	RANOVA	Bonferroni					
							T1–T2	T1–T3	T1–T4	T2–T3	T2–T4	T3–T4
MN	GC Fuji	2.50 ± 1.17	2.70 ± 1.25	3.50 ± 1.58	6.80 ± 1.22	***			***		***	***
	Meron	3.50 ± 1.54	3.00 ± 1.94	3.60 ± 1.83	6.00 ± 2.30	***			***			***
	Band Lok	2.10 ± 1.37	2.50 ± 1.50	4.90 ± 2.99	7.10 ± 2.13	**			**		**	
	Durelon	1.60 ± 1.17	2.20 ± 1.35	5.60 ± 2.98	7.70 ± 1.94	***		***	***		***	
	3M Multi Cure	1.20 ± 1.22	1.20 ± 0.78	5.50 ± 2.32	6.80 ± 2.97	**		**	**	**	**	
BN	GC Fuji	8.10 ± 3.14	9.30 ± 2.86	11.10 ± 3.14	11.20 ± 3.22	NS						
	Meron	7.30 ± 2.00	9.90 ± 4.01	13.30 ± 4.64	11.90 ± 2.84	***		***	***			
	Band Lok	5.00 ± 1.76	7.40 ± 2.71	12.50 ± 4.55	11.30 ± 2.98	***	***	***	***	***	***	
	Durelon	4.10 ± 2.18	9.20 ± 2.48	9.00 ± 2.70	12.10 ± 2.13	***	***	***	***			
	3M Multi Cure	5.60 ± 2.71	7.90 ± 3.44	8.70 ± 2.35	12.70 ± 3.36	**			**		**	
KL	GC Fuji	0.70 ± 0.76	0.20 ± 0.42	0.70 ± 0.82	1.90 ± 1.10	*			*		*	*
	Meron	0.70 ± 0.82	0.60 ± 0.96	0.90 ± 0.99	1.50 ± 1.26	NS						
	Band Lok	0.10 ± 0.31	0.40 ± 0.84	1.50 ± 1.35	0.80 ± 1.03	NS						
	Durelon	0.00 ± 0.00	1.20 ± 1.22	1.70 ± 1.49	2.40 ± 1.43	**		**	**			
	3M Multi Cure	0.20 ± 0.42	0.80 ± 1.03	1.50 ± 1.43	1.90 ± 1.37	**					**	**
KR	GC Fuji	1.00 ± 0.94	0.50 ± 0.70	0.20 ± 0.42	1.60 ± 1.26	NS						
	Meron	1.20 ± 1.22	0.60 ± 0.51	0.80 ± 1.03	1.10 ± 0.99	NS						
	Band Lok	0.60 ± 0.51	0.00 ± 0.00	1.30 ± 1.33	1.70 ± 1.63	***	***					
	Durelon	0.30 ± 0.67	0.90 ± 0.73	1.70 ± 1.41	2.20 ± 0.91	**			**		**	
	3M Multi Cure	0.40 ± 0.84	1.10 ± 0.99	1.10 ± 0.73	2.10 ± 1.72	NS						

SD: Standard deviation, MN: micronuclei, BN: binucleated cells, KL: karyolysis, KR: karyorrhexis.
RANOVA: Repeated measures ANOVA, *P < 0.05, **P < 0.01, ***P < 0.001.

GC Fuji and Durelon significantly elevated the KL frequencies ($P < 0.05$). Only the group treated with Durelon showed a significant effect on KR between T1 and T4 ($P < 0.01$).

Table 3 gives the median, maximum, and minimum value of differences between T4 and T1 time points. A statistically insignificant increase was detected in the number of MN with GC Fuji compared to Meron ($P > 0.05$). The increase in the number of MN with Ultra Band Lok, Durelon, and 3M Multi Cure was statistically significant compared to Meron ($P < 0.05$). Durelon and 3M Multi Cure caused statistically significant increases in the frequency of BNs compared to GC Fuji ($P < 0.05$) and in KR compared to Meron ($P < 0.05$).

Discussion

The MNT is an alternative to the chromosomal aberration assay (23). Micronucleus assay in exfoliated buccal mucosa cells has been used systemically in genetic biomonitoring of individuals exposed to several genotoxic chemicals, such as tobacco products, pesticides, and alcohol (24).

Some monomers might lead to serious DNA damage in mammalian cell systems as indicated by the induction of genotoxic effects, (25) but these mechanisms of reactions are not completely elucidated yet. Bakopoulo et al. (1) indicated that different types of dental cement differed extensively in their genotoxic and cytotoxic potential and their ability to affect chromosomal integrity, cell-cycle progression, DNA replication, and repair in normal cultured human lymphocytes. The authors stated that their results could not be directly extrapolated to the clinical situation. Furthermore, the main target of the present study was to investigate genotoxic and cytotoxic effects of the banding procedure with different orthodontic cements in a real clinical situation.

When we compared the increase in the number of MN within groups, the MNT showed that the banding procedure may have had genotoxic effects in the present study. Although insertion of separating elastics did not affect the number of MN, band cementation with tested materials increased the formation of the numbers of MN. Pithon et al. (26) revealed that separating elastics are considered to be biocompatible, as in accordance with our study. However, Durelon and 3M Multi Cure had a significant effect on the MN frequency within the first week after cementation, whereas GC Fuji and Meron showed this effect 1 week after band cementation.

It has been shown that monomers of dental resins are able to influence the normal cell cycle, probably as a result of DNA damage (27). Bakopoulo et al. (1) observed that RMGICs caused extensive genotoxic effects by significantly increasing the frequencies of sister chromatid exchange and chromosomal aberrations, whereas the GICs displayed only minor cytogenetic effects. Schweikl et al. (22) analyzed various components, including dental resin-based materials, for the formation of MN in mammalian cells to identify genotoxic potencies of the chemicals. In that in vitro study they found that HEMA, GMA, and TEGDMA elevated the MN number significantly, whereas Bis-GMA and UDMA slightly elevated it. In accordance with Schweikl et al. (22), GC Fuji containing UDMA in its chemical composition caused an insignificant increase in the frequency of MN compared to Meron in the present study. 3M Multi Cure containing HEMA was also found to be more genotoxic than conventional GICs (i.e. Meron). Band-Lok contains Bis-GMA and caused more genotoxic effects when compared to GC Fuji and Meron. Schweikl et al. (22) directly used Bis-GMA on mammalian cells in vitro and found that Bis-GMA and UDMA slightly elevated the MN number. We used the compound type commercially available. This controversial increased Bis-GMA efficiency in our study may have resulted from interactive relations between Bis-GMA and the other chemical materials included.

The differences between T4 and T1 in terms of BNs were significant in all groups except GC Fuji. Monitoring the frequency of KL and KR showed a statistically significant increase in the Durelon-applied group. Hanks et al. (28) showed that different zinc-containing cements had highly cytotoxic effects. Schmalz (29) also documented that zinc phosphate cements caused toxic reactions in mouse fibroblast cultures. Several in vitro studies evaluated the cytotoxicity of GICs on cultured cells and supported the idea that leachable components of the dental

Table 3. Median, maximum, and minimum values of differences between T4 and T1 time points.

Group	GC (a)			Meron (b)			Band Lok (c)			Durelon (d)			3M (e)			Kruskal–Wallis	Conover									
	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max		ab	ac	ad	ae	bc	bd	be	cd	ce	de
MN T4-T1	4.00	2.00	7.00	2.50	−2.00	6.00	4.50	2.00	9.00	6.00	3.00	11.00	6.00	−1.00	10.00	*					*	*	*			
BN T4-T1	4.00	−3.00	8.00	5.00	0.00	9.00	7.00	2.00	9.00	7.50	2.00	12.00	8.00	1.00	13.00	*			*	*						
KL T4-T1	1.00	−1.00	13.00	1.00	−2.00	4.00	0.50	−1.00	3.00	2.00	0.00	5.00	1.00	0.00	4.00	NS										
KR T4-T1	1.00	−2.00	4.00	0.00	−3.00	3.00	0.00	−1.00	4.00	2.00	0.00	3.00	1.00	−1.00	5.00	*						*	*			

Med: Median, Min: minimum, Max: maximum, MN: micronuclei, BN: binucleated cells, KL: karyolysis, KR: karyorrhexis.

*P < 0.05, NS: not significant.

materials are responsible for adverse effects to cell culture (9,30). Lee et al. (21) investigated the cytotoxicity of resin monomers using the MTT assay. They stated that all experimental monomers exhibited a dose-dependent cytotoxic effect, and the ranking of the cytotoxicity was GMA > TEGDMA > HEMA. Costa et al. (9) examined the cytotoxic effects of GICs and RMGICs, and they found that all experimental materials were cytotoxic to the odontoblast cells; the GICs were the least cytotoxic. The increased cytotoxicity of the RMGICs has been mainly attributed to the release of the monomer HEMA, which is frequently added to their chemical composition because it acts as both a consolvent and a comonomer (1,14). In accordance with the literature, conventional GIC showed the least cytotoxic activity in the present study.

Methacrylate monomers, such as HEMA, UDMA, and Bis-GMA, are incorporated in the lipid bilayers of cell membranes that are solubilized by the unreacted monomers. These small, hydrophilic, and flexible monomers induced cell-cycle arrest, and DNA double-strand breaks may lead to chromosome fragmentation for MN formation (25). These genotoxic and cytotoxic effects can be explained as mediated at least in part by oxidative DNA damage.

Metal ions released from fixed orthodontic appliances such as stainless steel, cobalt, chrome, silver, and nickel can induce DNA damage in oral mucosa (31,32). In the present study, not only resin monomers but also stainless steel orthodontic molar bands might have influenced the frequency of MN. Stainless steel materials caused the least biological damage whereas titanium materials produced more cytotoxicity and genotoxicity (33).

Rapid release of residual monomer from orthodontic resins was observed in the first 24 h and this rate decreased with time. The epithelial cells of the oral mucosa undergo rapid turnover and regeneration, usually every 7 to 14 days. Thus, genotoxic effects of the banding procedure might not remain in the long term. Studies have shown that the changes induced by appliances are reversible (34). Fixed orthodontic treatment is not able to induce mutagenic or cytotoxic effects in oral mucosa cells during at least a 1-year follow-up period (35). These early cytotoxic and genotoxic effects after band application should be considered in genetically predisposed patients clinically.

Exfoliated oral mucosa cells were collected 3 months after X-ray exposure in the present study. Ionizing radiation is known to damage DNA (36). Thus, researchers evaluated the possible genotoxic effects of radiation exposure as measured by the formation of MN. Although Cerqueira et al. (19) found that radiation induced a genotoxic effect on epithelial gingival cells that increased the frequency of chromosomal damage and nuclear alterations, some studies showed that exposure to radiography did not induce MN in target buccal epithelium cells but did promote cytotoxicity after 10 days of exposure (36,37). MN frequency was found to increase shortly after radiotherapy, followed by a return to baseline 12 weeks later (38). Thus, buccal epithelium cell samples were collected 3 months after dental radiography taken in the present investigation.

The influences of tobacco smoke and alcohol consumption have also been considered as a relevant factor with MN (37). All individuals recruited to participate in this study were nonsmokers and were not alcohol users. None of them used oral antiseptic solutions. Release of monomers from dental resin composites and their diffusion through oral tissue have genotoxic and cytotoxic effects (8). To compare the data with accuracy, all patients included had no fillings and no caries.

Within the limitations of this study, the following conclusions may be drawn:

- Band cementation with all tested orthodontic cements has genotoxic effects.
- Cementation with a conventional GIC (Meron) showed the least genotoxic effects.
- Banding with tested orthodontic cements showed cytotoxicity.
- Polycarboxylate cement (Durelon) showed the highest cytotoxic effects.

However, the results of the present clinical study remain unclear and further studies using different test methods are needed for the banding procedure with tested cements. Research efforts should focus on assessing the long-term biological effects of band cementation.

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