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Mutagenic potentials of dental cements as detected by the *Salmonella*/microsome test

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Abstract

The potential mutagenicity of a zinc phosphate (Poscal®), a polycarboxylate (Aqualox®) and glass ionomer cements with (Argion®) and without (Meron®) silver reinforcement were characterized by employing the Ames *Salmonella*/microsome test. The materials were eluted in dimethyl sulphoxide or physiologic saline and the aliquots were used either immediately or after an incubation period of 24 h at 37°C. Mutagenic effects of the materials were tested on *Salmonella typhimurium* strains TA 98, TA 100, TA 102 and TA 1535 using the standard plate incorporation assay, and in the presence or absence of S9 fraction from rat liver. Poscal® and Aqualox® elicited mutagenic effects on *S. typhimurium* TA 98 and TA 1535, whereas Meron® exhibited mutagenic effects on *S. typhimurium* TA 98. No mutagenic effects were detected for Argion®. The type of solvent, dose of the material and incubation as well as the interactions between these factors exhibited varying degrees of influences on the mutagenic activities of the cements ($P<0.05$ and $P<0.1$). We conclude that zinc phosphate, polycarboxylate, and glass ionomer cements may have possible mutagenic activities.

Keywords: Dental cements; Mutagenicity; Ames test; *Salmonella typhimurium*

1. Introduction

Concerns on the clinical performance of dental restorative materials have led to research on their biocompatibility [1–6] but, overall, there is still a scarcity of evidence on the genotoxic and mutagenic potentials of dental cements [7,8]. At present, there are several types of commercially available dental cements having a variety of chemical compositions. Of these, resin cements are used in combination with dentin bonding agents to improve the biomechanical properties of the tooth-restorative interface. Because the composition of dentin bonding agents may lead to adverse tissue reactions in the pulp, the mutagenic potentials of dentin bonding agents have been a topic of research interest so far [9–11]. In contrast, zinc phosphate, polycarboxylate, and glass ionomer cements are applied solely on the tooth structure, and the chemical constituents of these cements may also elicit mutagenic effects in the long run [8,9]. In essence, leachable substances from cements may cause short-term adverse pulpal effects, and even gain access to the periodontal tissue through numerous pathways [12].

The basic composition of zinc phosphate cements is zinc oxide (90–98%) and the principal modifier is magnesium oxide, usually in a concentration up to 10%. The liquid consists of 50% phosphoric acid, with incorporation of small quantities of aluminum and zinc. The composition of polycarboxylate cements is similar to zinc phosphate cements, principally zinc oxide and magnesium oxide. The liquid is an aqueous solution of polyacrylic acid or a copolymer of acrylic acid with unsaturated carboxylic acids. Glass ionomer cements are also composed of zinc oxide and polyacrylic acids, but zinc oxide is enriched with glass particles capable of ion release to tooth structure. Glass ionomer cements have also been amended with silver to improve its mechanical properties for use as load-bearing restorations [13].

Despite the extensive use of these cements for restorative purposes, there is a potentially narrow gap between their unsurpassed benefits and adverse effects in

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the context of long-term biocompatibility. With regard to the clinical significance of potential mutagenic effects of dental cements, the purpose of this study was to explore the mutagenic potentials of a zinc phosphate, a polycarboxylate, and glass ionomer cements with and without silver reinforcement by the Ames *Salmonella*/microsome mutagenicity test, a bacterial mutation assay, which is frequently used for mutagenicity testing due to its high sensitivity (83%) and recognized validity [14].

2. Materials and methods

2.1. Chemicals and positive mutagens

D-biotin, glucose-6-phosphate, β-nicotinamide adenine dinucleotide phosphate (NADP), L-histidine-HCl monohydrate, phenobarbital, 3-methylcholanthrene, crystal violet, tetracycline, and sodium chloride were purchased from Sigma Chemicals (Deisenhofen, Germany), ampicillin trihydrate and dimethyl sulphoxide (DMSO) was from Fluca (Neu-Ulm, Germany), oxoid agar, oxoid nutrient broth no. 2 from Oxoid Ltd. (England), and magnesium chloride, potassium phosphate, potassium chloride, citric acid monohydrate, sodium ammonium phosphate, sodium hydrogen phosphate and sodium dihydrogen phosphate were obtained from Merck (Darmstadt, Germany).

The positive mutagens sodium azide (NaN_3), 2-aminoflourene and benzanthracene were purchased from Sigma Chemicals (Deisenhofen, Germany), and daunomicina was purchased from Deva Holding (Istanbul, Turkey). Sodium azide was used on *S. typhimurium* TA 100 and TA 1535 strains in the absence of a metabolically active microsomal fraction from rat liver (S9). Daunomicina was used on *S. typhimurium* TA 98 and TA102 strains, benzanthracene was used on *S. typhimurium* TA 1535 in the presence of S9, and 2-aminoflourene was used on *S.* TA 98, TA 100 and TA 102 [15].

2.2. Tester strains

S. typhimurium TA 98, TA 100, TA 102 and TA 1535 were kindly provided by Dr. Bruce Ames (University of California, Berkeley, CA, USA).

Table 1
Mutagenicity of DMSO and physiologic saline eluates of Poscal® in Ames *Salmonella* assay in the absence of S9 fraction

Solvent	Incubation period	Dose (μl/plate)	Revertants per plate ± standard deviation ($n=3$)			
			TA 98	TA 100	TA 102	TA 1535
DMSO	Control	0	26±5	121±14	206±21	16±4
		25	26±10	109±2	207±14	18±4
	Fresh	50	20±3	103±13	212±17	22±9
		75	27±0.6	111±16	203±24	18±3
		100	30±6	116±10	172±12	16±4
	Control	0	19±4	92±3	171±15	10±2
		25	25±5	101±13	146±9	11±2
	24 h	50	30±5	89±20	135±20	14±3
		75	28±2	100±10	123±16	11±2
		100	41±5[a]	104±6	144±10	11±4
Physiologic saline	Control	0	28±10	116±18	197±18	14±2
		25	31±14	115±9	174±33	25±4[b]
	Fresh	50	19±0	97±9	237±22	18±2
		75	25±6	97±15	182±65	18±5
		100	33±11	122±3	90±70	18±6
	Control	0	27±9	99±13	182±6	10±3
		25	26±7	98±12	136±21	13±2
	24 h	50	24±8	91±10	138±29	11±1
		75	29±3	88±1	139±9	8±3
		100	23±8	89±19	128±14	12±3
Positive mutagens						
Sodium azide		1.5 μg/plate	—	559±104	—	789±88
Daunomicina		6 μg/plate	163±71	—	1501±172	—

The test compounds (100 mg for Poscal, Argion and Meron, 400 mg for Aqualox) were extracted in 2 ml DMSO or physiologic saline and extracts were used freshly or incubated for 24 h at 37°C and various aliquots were then tested for mutagenicity in the standard plate incorporation assay. The numbers of mutant colonies (revertants) per plate are mean values (±SD) from triplicates obtained in one experiment. Positive controls were as follows: 6 μg Daunomicina (TA 98, TA 102) and 1.5 μg NaN_3 per plate (TA 100, TA 1535) in the absence of S9.

[a] Mutagenicity.

[b] Weak mutagenicity.

2.3. Preparation of S9 fraction

Sprague–Dawley male rats were used for the preparation of liver S9 fraction. The animals were cared for according to the policies and principles established by the Animal Welfare Act and the NIH Guide for Care and Use of Laboratory Animals (publication #86-23). 3-Methylcholanthrene and phenobarbital were used for the induction of rat liver enzymes. 3-Methylcholanthrene was diluted in corn oil (125 mg/kg body weight) and injected intraperitoneally to each rat 5 days before sacrifice. In addition, phenobarbital was included into the drinking water (0.1% g/l) and administered for 5 days before sacrifice. During this period, the animals were kept in rooms illuminated from 07:00 to 19:00 h (12 h light/12 h dark cycle), maintained at 21–23°C, and had full access to pellet food and water ad libitum.

Preparation of the liver S9 fraction was based on the procedure described by Garner et al. [16]. Phenobarbital and 3-methylcholanthrene was used as inducers for S9 fraction in this test. Consequently, two types of cytochromes (cyt p-450 and cyt p-448) were activated by using these two chemicals [17]. The protein content of S9 fraction was found to be 12 mg/ml. During pilot tests, the S9 fraction was tested on *S. typhimurium* with 2-aminoflourene, which is a positive mutagen in the presence of S9. The number of revertant colonies obtained with 2-aminoflourene were 25–30 times higher than that of the control group and therefore, the protein content of S9 was sufficient for the metabolic activation system. Accordingly, the mutagenicity tests were undertaken using the S9 fraction with 12 mg/ml protein content.

2.4. Test materials and sample preparation

One zinc phosphate cement (Poscal®; lot: 540550785; powder/liquid ratio: 2:1), one polycarboxylate cement (Aqualox®; lot: 90740; powder/liquid ratio: 1:2), a silver-reinforced glass ionomer cement (Argion®; lot: 87527; powder/liquid ratio: 7:1), and a glass ionomer cement (Meron®; lot: 92613; powder/liquid ratio: 3:1) were used in this study. All materials were kindly provided from Voco GmbH, Cuxhaven, Germany. The cements were mixed according to the recommendations of the manufacturer and crushed into powder form in a ceramic mortar upon setting (approximately 6 min). 0.1 g of Poscal®, Meron® and Argion®, and 0.4 g of Aqualox® was eluted in 2 ml of DMSO or physiologic

Table 2
Mutagenicity of DMSO and physiologic saline eluates of Poscal® in Ames *Salmonella* assay in the presence of S9 fraction

Solvent	Incubation period	Dose (μl/plate)	Revertants per plate ± standard deviation ($n=3$)			
			TA 98	TA 100	TA 102	TA 1535
DMSO	Control	0	40±10	98±4	230±5	17±1
		25	23±6[a]	98±16	184±39	13±3
	Fresh	50	28±12[a]	95±9	243±22	12±4
		75	40±7	99±1	237±38	18±9
		100	38±16	93±1	276±67	16±1
	Control	0	26±6	100±7	230±5	17±1
		25	31±3	90±11	259±56	17±6
	24 h	50	30±4	86±13	223±29	9±3
		75	25±4	89±15	220±30	13±6
		100	23±4	84±3	328±28	16±4
Physiologic saline	Control	0	26±6	99±5	230±5	17±1
		25	33±6	110±8	183±51	15±1
	Fresh	50	26±3	95±14	179±17	17±3
		75	24±5	105±5	242±8	16±6
		100	29±9	85±15	257±37	20±2
	Control	0	26±6	99±5	230±5	17±1
		25	28±4	106±16	279±32	13±5
	24 h	50	29±6	111±35	243±33	14±1
		75	28±8	112±14	293±41	12±3
		100	21±12	102±18	283±20	14±8
Positive mutagens						
2-Aminoflourene		10 μg/plate	1006±120	534±56	481±84	—
Benzantracen		2 μg/plate	—	—	—	35±6

The test compounds (100 mg for Poscal, Argion and Meron 400 mg for Aqualox) were extracted in 2 ml DMSO or physiologic saline and extracts were used freshly or incubated for 24 h at 37°C and various aliquots were then tested for mutagenicity in the standard plate incorporation assay. The numbers of mutant colonies (revertants) per plate are mean values (±SD) from triplicates obtained in one experiment. Positive controls were as follows: 10 μg 2-aminoflourene (TA 98, TA 100, TA 102) and 2 μg benzanthracene (TA 1535) per plate in the presence of S9.

[a] The correlation between solvent, dose, and incubation period is significant in comparison with the control group ($P<0.1$).

saline (0.9% NaCl) and the aliquots were used for mutagenicity tests either immediately (fresh) or after 24 h incubation in glass vials at 37°C and in 60–90% humidity [10]. For each material, the minimum and maximum quantities of aliquots from DMSO or physiologic saline were 10 and 200 μl, respectively.

2.5. Cytotoxicity testing

The amounts of test materials used in the mutation assays were selected in the cytotoxicity assay. The rationale behind this test was to determine whether the test doses of the materials would have any cytotoxic effect. 0.1 ml of a suitable dilution of an overnight bacterial culture was added to 2-ml top agar along with different concentrations of the tested chemicals. The top agar was poured onto nutrient agar plates and assessment of cytotoxicity was performed after 24 h incubation at 37°C [18].

2.6. Mutagenicity tests

The *Salmonella* mutagenicity assay was carried out according to method described by Maron and Ames [15]. Oxoid nutrient broth no. 2 was used for overnight culture. For plate incorporation assays, 0.1 ml of bacterial tester strain, 0.25 ml of S9 mix if appropriate, and the sample to be tested was added to 2 ml of molten top agar. The contents were mixed and poured on agar plates. After 72 h of incubation, revertant colonies were counted [19]. At least 3 plates were used for each dose and each experiment was repeated two or three times. The strains were checked routinely for ampicillin resistance, ultraviolet-light sensitivity, crystal-violet sensitivity, histidine requirement and spontaneous reversion rate. The materials were stored at −80°C.

2.7. Statistical analysis

The data obtained from the experiments were analyzed by three-factor factorial experiment in one way classification design fixed model with significance levels sets at $P<0.05$ and $P<0.1$ and further evaluated by LSD and Dunnett C analyses to determine the groups leading to significant differences and resulting in mutagenic effects [20]. During the statistical analyses, the influence of solvents (DMSO and physiologic saline), incubation (fresh or 24 h), dose-dependent

Table 3
Mutagenicity of DMSO and physiologic saline eluates of Aqualox® in Ames *Salmonella* assay in the absence of S9 fraction

Solvent	Incubation period	Dose (μl/plate)	Revertants per plate ± standard deviation ($n=3$)			
			TA 98	TA 100	TA 102	TA 1535
DMSO	Control	0	25±0	78±24	215±16	14±5
		12.5	24±6	81±11	254±23	13±4
	Fresh	25	29±4	71±2	193±93	8±1
		50	25±5	88±10	228±32	11±3
		100	28±4	84±23	256±9	12±1
	Control	0	29±3	70±12	182±6	14±2
		12.5	34±6	88±5	226±19	13±4
	24 h	25	30±3	73±7	210±26	10±3
		50	36±3	88±10	219±44	17±3
		100	28±6	93±10	205±16	9±4
Physiologic saline	Control	0	38±19	93±8	188±5	14±5
		12.5	21±6	103±13	225±63[a]	10±1
	Fresh	25	15±3	101±5	296±100	10±3
		50	19±3	101±9	245±43[a]	11±3
		100	20±15	92±15	260±26[a]	13±4
	Control	0	27±6	96±7	171±15	14±2
		12.5	25±9	93±2	191±12	10±3
	24 h	25	27±5	97±13	214±18[a]	14±7
		50	22±6	112±13	232±9[a]	13±1
		100	27±4	107±4	138±30[a]	13±1
Positive mutagens						
Sodium azide		1.5 μg/plate	—	657±248	—	2146±914
Daunomicina		6 μg/plate	220±73	—	1500±172	

The test compounds (100 mg for Poscal, Argion and Meron, 400 mg for Aqualox) were extracted in 2 ml DMSO or physiologic saline and extracts were used freshly or incubated for 24 h at 37°C and various aliquots were then tested for mutagenicity in the standard plate incorporation assay. The numbers of mutant colonies (revertants) per plate are mean values (±SD) from triplicates obtained in one experiment. Positive controls were as follows: 6 μg Daunomicina (TA 98, TA 102) and 1.5 μg NaN_3 per plate (TA 100, TA 1535) in the absence of S9.

[a] The correlation between solvent, dose, and incubation period is significant in comparison with the control group ($P<0.05$).

effects (5 different doses; 0–200 μl), and correlations between these parameters (Binary or Triple Interaction) were compared. This implies that $2 \times 2 \times 5 \times 3$ combinations are compared one by one or together by the statistical method. The differences between the revertant colonies of the test groups and the control group were tested with Student's *t*-test at 95% confidence level. Doses higher than the mean of the control group and consequent mutagenic condition were defined as "mutagenic", whereas an increase in dose approaching to, but not reaching a two-fold increase was defined as "weak mutagenic" [19].

3. Results

3.1. Mutagenic potentials of cements

As a sequel of cytotoxicity tests, it was found that none of the test doses of the materials exhibited cytotoxic effects, even for increased doses (0.4 g/2 ml Aqualox). All test materials led to varying degrees of mutagenicity on different *Salmonella* strains, but did not have any effect on *S. typhimurium* TA 100. The 100 μl/plate dose of Poscal® eluted in DMSO and incubated for 24 h at 37°C elicited mutagenic effects on *S. typhimurium* TA 98 in the absence of S9 fraction. 25 μl/plate dose of fresh aliquots of this material eluted in physiologic saline yielded weak mutagenic effects on *S. typhimurium* TA 1535 (Tables 1 and 2). In the presence of S9 fraction, 100 μl/plate of fresh Aqualox® eluted in both DMSO and physiologic saline elicited weak mutagenic effects on *S. typhimurium* TA 1535. Dose-dependent mutagenic effects were detected for the material eluted in DMSO and incubated for 24 h, and 12.5 and 25 μl/plate doses eluted in physiologic saline were mutagenic on *S. typhimurium* TA 98 (Tables 3 and 4). Mutagenic activities were not detected in any test conditions for Argion® (Tables 5 and 6). 25, 50 and 100 μl/plate doses of fresh Meron® eluted in DMSO exhibited mutagenic effects on *S. typhimurium* TA 98 in the absence of S9 fraction (Tables 7 and 8).

Table 4
Mutagenicity of DMSO and physiologic saline eluates of Aqualox® in Ames *Salmonella* assay in the presence of S9 fraction

Solvent	Incubation period	Dose (μl/plate)	Revertants per plate ± standard deviation ($n = 3$)			
			TA 98	TA 100	TA 102	TA 1535
DMSO	Control	0	25 ± 5	73 ± 11	248 ± 58	13 ± 2
		12.5	15 ± 3	91 ± 25	199 ± 36	13 ± 4
	Fresh	25	23 ± 4	92 ± 19	316 ± 4	21 ± 1
		50	25 ± 5	103 ± 9[a]	280 ± 10	14 ± 3
		100	19 ± 7	97 ± 7[a]	294 ± 37	23 ± 8[b]
	Control	0	54 ± 14	111 ± 10	175 ± 14	13 ± 2
		12.5	70 ± 10[a,c]	158 ± 5[a]	183 ± 33	21 ± 1
	24 h	25	108 ± 10[a,c]	136 ± 21[a]	152 ± 36	18 ± 2
		50	110 ± 4[a,c]	114 ± 4	172 ± 19	16 ± 6
		100	124 ± 13[a,b]	105 ± 11	217 ± 18	13 ± 4
Physiologic saline	Control	0	25 ± 5	73 ± 11	248 ± 58	13 ± 2
		12.5	20 ± 3	116 ± 10[a]	187 ± 61	15 ± 2
	Fresh	25	22 ± 1	114 ± 17[a]	291 ± 1	21 ± 3
		50	22 ± 4	94 ± 13	272 ± 28	17 ± 5
		100	23 ± 4	63 ± 6	324 ± 18	25 ± 13[b]
	Control	0	53 ± 13	111 ± 10	154 ± 26	13 ± 2
		12.5	102 ± 49[a,c]	126 ± 9	184 ± 5	19 ± 5
	24 h	25	105 ± 5[a,c]	159 ± 19[a]	188 ± 12	20 ± 3
		50	72 ± 21	146 ± 7[a]	144 ± 27	15 ± 4
		100	62 ± 1	124 ± 10	165 ± 31	16 ± 6
Positive mutagens						
2-Aminoflourene		10 μg/plate	1130 ± 297	426 ± 166	331 ± 98	—
Benzantracen		2 μ/plate	—	—	—	35 ± 6

The test compounds (100 mg for Poscal, Argion and Meron 400 mg for Aqualox) were extracted in 2 ml DMSO or physiologic saline and extracts were used freshly or incubated for 24 h at 37°C and various aliquots were then tested for mutagenicity in the standard plate incorporation assay. The numbers of mutant colonies (revertants) per plate are mean values (±SD) from triplicates obtained in one experiment. Positive controls were as follows: 10 μg 2-aminoflourene (TA 98, TA 100, TA 102) and 2 μg benzanthracene (TA 1535) per plate in the presence of S9.

[a] The correlation between solvent, dose, and incubation period is significant in comparison with the control group ($P < 0.05$).

[b] Weak mutagenicity.

[c] Mutagenicity.

3.2. Effects of solvents, dose of the sample, and incubation on mutagenicity

Either in the presence or absence of S9 fraction, the mutagenic effects of the materials had strong correlations with the solvent used, applied dose, and the incubation procedure as well as the combination of these parameters. These correlations depended on the test material and exhibited various interactions on different strains, and revealed that the test conditions had a crucial effect on the mutagenic activity.

4. Discussion

In the present study, the mutagenic potentials of a zinc phosphate, a polycarboxylate, and glass ionomer cements with and without silver reinforcement were evaluated using the *Salmonella*/microsome gene mutation assay. The rationale behind the use of *S. typhimurium* TA 1535 was based on the findings of Prival and Zeiger [21]. Accordingly, it was possible to detect weak mutagenic potentials for Poscal® ($S9^-$, physiologic saline, fresh, 25 μl/plate) and Aqualox® ($S9^+$, DMSO, physiologic saline, fresh, 100 μl/plate) on *S. typhimurium* TA 1535 with this approach.

Besides zinc oxide as the main constituent, Poscal® includes magnesium oxide, silicone dioxide, calcium oxide, bismuth trioxide, barium oxide, and barium sulphate. Aqualox® is also mainly composed of zinc oxide and magnesium oxide. Zinc oxide is reported not to elicit mutagenic effects on *S. typhimurium* TA 98 and TA 100 and magnesium oxide does not yield mutagenic effects on *S. typhimurium* TA 97, TA 100, and TA 102 in the presence or absence of a metabolically active microsomal fraction from rat liver (S9 fraction) [22,23]. Likewise, calcium oxide and silicone dioxide has been reported not to exhibit mutagenic effects on *S. typhimurium* strains [23,24]. Accordingly, present findings on Poscal® are not at least related to zinc oxide, calcium oxide, and silicone dioxide content. Further analysis on these cements may reveal ingredients leading to mutagenic effects in the future.

It has been reported that glass ionomer cements exhibit varying degrees of cytotoxicity, and this behavior has been attributed to the polyacrylic acid content

Table 5
Mutagenicity of DMSO and physiologic saline eluates of Argion® in Ames *Salmonella* assay in the absence of S9 fraction

Solvent	Incubationperiod	Dose (μl/plate)	Revertants per plate ± standard deviation ($n=3$)			
			TA 98	TA 100	TA 102	TA 1535[a]
DMSO	Control	0	21±4	82±11	206±21	10±3
		25	23±7	78±10	181±60	10±5
	Fresh	50	16±3	81±20	118±56	10±6
		75	10±5	90±3	201±39	10±6
		100	21±4	66±12[b]	183±11	11±7
	Control	0	26±9	103±9	171±15	12±7
		25	18±7	99±1	207±50	14±8
	24 h	50	19±5	86±4[b]	164±8	14±10
		75	21±6	84±10[b]	225±67	9±3
		100	20±6	87±10[b]	163±42	11±6
Physiologic saline	Control	0	21±4	112±5	206±21	10±3
		25	21±4	96±1[b]	183±31	12±6
	Fresh	50	19±5	104±13	233±35	13±8
		75	25±5	103±7	180±32	14±8
		100	20±4	96±14[b]	201±99	12±3
	Control	0	24±7	96±5	171±15	12±7
		25	24±4	82±1	209±15	14±8
	24 h	50	22±4	90±2	222±37	13±9
		75	28±10	101±6	212±21	16±10
		100	21±10	83±9	174±68	15±10
Positive mutagens						
Sodium azide		1.5 μg/plate	—	559±105	—	588±134
Daunomicina		6 μg/plate	174±90	—	1589±218	—

The test compounds (100 mg for Poscal, Argion and Meron, 400 mg for Aqualox) were extracted in 2 ml DMSO or physiologic saline and extracts were used freshly or incubated for 24 h at 37°C and various aliquots were then tested for mutagenicity in the standard plate incorporation assay. The numbers of mutant colonies (revertants) per plate are mean values (±SD) from triplicates obtained in one experiment. Positive controls were as follows: 6 μg Daunomicina (TA 98, TA 102) and 1.5 μg NaN_3 per plate (TA 100, TA 1535) in the absence of S9.

[a] $n=17$.

[b] The correlation between solvent, dose, and incubation period is significant in comparison with the control group ($P<0.05$)

Table 6
Mutagenicity of DMSO and physiologic saline eluates of Argion® in Ames *Salmonella* assay in the presence of S9 fraction

Solvent	Incubation period	Dose (μl/plate)	Revertants per plate ± standard deviation ($n=3$)			
			TA 98	TA 100	TA 102	TA 1535
DMSO	Control	0	47 ± 10	101 ± 13	230 ± 5	13 ± 2
		25	48 ± 1	95 ± 8	213 ± 20	11 ± 1
	Fresh	50	57 ± 14	115 ± 18	201 ± 3	18 ± 5
		75	53 ± 5	114 ± 11	223 ± 22	17 ± 1
		100	49 ± 6	110 ± 7	213 ± 14	11 ± 10
	Control	0	46 ± 5	98 ± 6	230 ± 5	13 ± 2
		25	49 ± 5	110 ± 2	213 ± 24	18 ± 10
	24 h	50	48 ± 7	107 ± 6	173 ± 17**	16 ± 4
		75	45 ± 6	115 ± 7	224 ± 13	12 ± 1
		100	52 ± 20	148 ± 20*	201 ± 49	17 ± 4
Physiologic saline	Control	0	38 ± 6	102 ± 5	230 ± 5	13 ± 2
		25	36 ± 12	104 ± 12	197 ± 6**	16 ± 2
	Fresh	50	41 ± 3	107 ± 3	204 ± 14	14 ± 2
		75	34 ± 4	98 ± 12	213 ± 27	16 ± 2
		100	28 ± 3	97 ± 15	213 ± 11	14 ± 3
	Control	0	35 ± 8	104 ± 5	230 ± 5	13 ± 2
		25	39 ± 4	98 ± 8	219 ± 36	19 ± 6
	24 h	50	33 ± 4	114 ± 6	183 ± 22**	17 ± 3
		75	39 ± 3	111 ± 8	281 ± 63**	17 ± 4
		100	37 ± 5	96 ± 12	297 ± 14**	17 ± 1
Positive mutagens						
2-Aminoflourene		10 μg/ plate	1338 ± 326	418 ± 93	481 ± 84	—
Benzantracen		2 μg/plate	—	—	—	35 ± 6

The correlation between solvent, dose, and incubation period is significant in comparison with the control group (*$P<0.05$ and **$P<0.1$).
The test compounds (100 mg for Poscal, Argion and Meron 400 mg for Aqualox) were extracted in 2 ml DMSO or physiologic saline and extracts were used freshly or incubated for 24 h at 37°C and various aliquots were then tested for mutagenicity in the standard plate incorporation assay. The numbers of mutant colonies (revertants) per plate are mean values (±SD) from triplicates obtained in one experiment. Positive controls were as follows: 10 μg 2-aminoflourene (TA 98, TA 100, TA 102) and 2 μg benzanthracene (TA 1535) per plate in the presence of S9.

of the material [2,25]. Recently, Müller and collaborates [26] also demonstrated that both DMSO and Ham's F12 culture medium extracts of a glass ionomer cement, Vitrebond™, led to a clear genotoxic effect in the in vitro mammalian cell gene mutation test (HPRT Test) with CHO cells as well as in the bacterial umu-test with *S. typhimurium* TA 1535/pSK1002. In contrast, it has also been shown through Ames *Salmonella*/microsome test that glass ionomer cements do not elicit mutagenic effects [8,27]. Clearly, the literature on the biocompatibility of glass ionomer cements is still inconclusive and the reason probably lies beneath the differences in compositions of the materials tested. In the present study, although Argion® did not exhibit mutagenic effects on *S. typhimurium* strains, it seems that the incubated DMSO extract of the material might have a mutagenic potential on *S. typhimurium* TA 100 in the presence of S9 fraction (Table 6). Accordingly, the question arises whether the polyacryric acid content of Aqualox®, Meron®, and Argion® may be responsible for the mutagenic effects on *S. typhimurium* strains. It has been already demonstrated that acrylic acid, the monomer of polyacrylic acid, capable of being eluted both in water and inorganic solvents do not lead to mutagenic effects on *S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537 in the presence or absence of S9 fraction [28]. Overall, the main constituents of these cements probably do not have mutagenic potentials, and further research is indicated to elucidate the mutagenic potentials of their minor constituents.

Unlike other test materials, Aqualox® led to mutagenic effects in the presence of S9 fraction. This implies that a new metabolite or a product had a mutagenic potential. In addition, the fresh extracts of the material yielding mutagenic effects on *S. typhimurium* TA 1535 implies that, the fresh extract leads to base pair substitutions. 24 h incubated extracts of Aqualox® elicits frameshift mutations in *S. typhimurium* TA 98. Both Poscal® and Meron®, exhibiting no mutagenic potentials in the presence of S9 fraction reveals that the mutagenic effect of these cements are eliminated as a sequel of metabolic activity.

The Ames *Salmonella*/microsome test is capable of detecting 83% of the carcinogens as mutagenic, when the suggested protocol is followed [15]. This implies that the Ames' test is not able to detect all known carcinogens, and therefore, any battery of test should include both in vitro and in vivo test for evaluation of the mutagenic activity of chemicals [29]. Mutagenicity tests may occasionally exhibit false-positive results, and

Table 7
Mutagenicity of DMSO and physiologic saline eluates of Meron® in Ames *Salmonella* assay in the absence of S9 fraction

Solvent	Incubation period	Dose (μl/plate)	Revertants per plate ± standard deviation ($n=3$)			
			TA 98	TA 100	TA 102	TA 1535
DMSO	Control	0	20±4	100±16	183±25	14±5
		10	17±5	92±8	137±8	11±3
	Fresh	25	97±47[a,b]	136±26	160±20	12±1
		50	40±14[a]	102±10	155±10	12±4
		100	62±18[a,b]	125±26	185±36	11±2
	Control	0	19±2	83±10	216±29	14±2
		10	20±3	94±18	169±31	8±1
	24 h	25	21±2	72±19	155±66	5±4
		50	20±2	82±2	158±4	9±3
		100	20±4	99±8	117±71	11±8
Physiologic saline	Control	0	27±5	87±15	165±52	14±5
		25	26±9	87±4	161±22	7±3
	Fresh	50	29±5	78±9	193±36	6±1
		100	23±6	104±9	230±23[b]	9±2
		200	22±1	115±23	127±72	12±3
	Control	0	29±3	83±7	201±39	14±2
		25	35±9	94±19	195±39	8±2
	24 h	50	29±2	100±18	138±34[b]	8±1
		100	32±11	87±28	159±12	12±2
		200	24±7	114±31	167±36	13±5
Positive mutagens						
Sodium azide	1.5 μg/plate	—	649±66	—	2146±914	
Daunomicina	6 μg/plate	436±299	—	1514±176	—	

The test compounds (100 mg for Poscal, Argion and Meron, 400 mg for Aqualox) were extracted in 2 ml DMSO or physiologic saline and extracts were used freshly or incubated for 24 h at 37°C and various aliquots were then tested for mutagenicity in the standard plate incorporation assay. The numbers of mutant colonies (revertants) per plate are mean values (±SD) from triplicates obtained in one experiment. Positive controls were as follows: 6 μg Daunomicina (TA 98, TA 102) and 1.5 μg NaN_3 per plate (TA 100, TA 1535) in the absence of S9.

[a] Mutagenic effect.

[b] The correlation between solvent, dose, and incubation period is significant in comparison with the control group ($P<0.05$).

Table 8
Mutagenicity of DMSO and physiologic saline eluates of Meron® in Ames *Salmonella* assay in the presence of S9 fraction

Solvent	Incubation period	Dose (μl/plate)	Revertants per plate ± standard deviation ($n=3$)			
			TA 98	TA 100	TA 102	TA 1535
DMSO	Control	0	29±5	103±8	243±6	17±1
		10	28±6	131±10	302±18	18±6
	Fresh	25	25±2[a]	105±22	265±37	15±5
		50	30±6	123±6	234±20	11±4
		100	25±6[a]	123±9	243±15	16±4
	Control	0	27±2	117±10	243±6	17±1
		10	29±1	119±12	224±48	21±3
	24 h	25	35±8	129±27	267±23	12±6
		50	29±10	125±7	346±35	12±6
		100	36±12	109±4	231±57	12±5
Physiologic saline	Control	0	29±5	103±8	243±6	17±1
		10	29±4	110±10	291±60	21±7
	Fresh	25	24±7	115±11	240±10	19±4
		50	30±3	118±5	253±58	18±7
		100	32±5	118±10	323±80	22±2
	Control	0	27±2	117±10	243±6	17±1
		10	26±6	119±1	307±40	18±4
	24 h	25	31±3	115±9	314±17	19±4
		50	24±4	112±7	339±38	13±1
		100	23±6	107±16	312±31	17±2
Positive mutagens						
2-Aminoflourene		10 μg/plate	1025±82	631±47	429±31	—
Benzantracen		2 μg/plate	—	—	—	35±6

The test compounds (100 mg for Poscal, Argion and Meron 400 mg for Aqualox) were extracted in 2 ml DMSO or physiologic saline and extracts were used freshly or incubated for 24 h at 37°C and various aliquots were then tested for mutagenicity in the standard plate incorporation assay. The numbers of mutant colonies (revertants) per plate are mean values (±SD) from triplicates obtained in one experiment. Positive controls were as follows: 10 μg 2-aminoflourene (TA 98, TA 100, TA 102) and 2 μg benzanthracene (TA 1535) per plate in the presence of S9.

[a] The correlation between solvent, dose, and incubation period is significant in comparison with the control group ($P<0.05$).

it is not possible to draw a conclusive statement based solely on a single study. Further, depending on the clinical performance of dental cements, one might be able to address the question as to whether there is any clinical evidence suggesting the mutagenicity of zinc oxide, polycarboxylate, and glass ionomer cements.

5. Conclusion

In this study, the mutagenic potential of a zinc phosphate, a polycarboxylate, and glass ionomer cements with and without silver reinforcement were explored by the Ames *Salmonella*/microsome test. The zinc phosphate and polycarboxylate cements elicited mutagenic effects on *S. typhimurium* TA 98 and TA 1535, the glass ionomer cement exhibited mutagenic effects on *S. typhimurium* TA 98, but mutagenic effects were not detected for the silver-reinforced glass ionomer cement. The highest mutagenic potential was found for Meron® followed by Aqualox® and Poscal®. Within the limits of this study, we conclude that zinc phosphate, polycarboxylate, and glass ionomer cements may have possible mutagenic effects.

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