Leaching of additives and degradation products from cold-cured orthodontic resins

Henning Lygre, Knut N. Klepp, Einar Solheim and Nils Roar Gjerdet
Department of Dental Biomaterials and Department of Orthodontics and Facial Orthopedics, School of Dentistry, and Department of Clinical Biology, Section of Oral Pharmacology, School of Medicine, University of Bergen, Bergen, Norway

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Unstimulated saliva was collected from orthodontic patients and subjected to combined gas-chromatography and gas-chromatography/mass-spectrometry analyses. Saliva was collected before insertion of removable orthodontic appliances made of cold-cured resins. Saliva was then collected 1–2 months after insertion of the appliances and 1 week after they had been removed. Phenyl benzoate (PB) and phenyl salicylate (PS) were identified in pooled saliva samples from patients wearing the appliances. Biphenyl and 2-methoxy-4-hydroxy-benzophenone in addition to PB and PS were identified in a study with in vitro specimens made of orthodontic resin. The leaching of compounds from these test specimens processed by a powdering technique and a pre-mix technique was compared. □ *Chemical analysis; cold-cured resins; saliva*

Henning Lygre, Department of Dental Biomaterials, School of Dentistry, Årstadveien 17, N-5009 Bergen, Norway

A large proportion of children in Western societies receive orthodontic treatment. Thirty-five per cent of Norwegian children are treated by orthodontists (1). The patients are exposed to various metals, polymers, and other materials. Appliances including resins are used for various periods in most cases; in addition an unknown percentage of children are treated with removable appliances by general practitioners. The so-called cold-cured resins facilitate a rapid and easy method for manufacture of removable orthodontic appliances (2).

The frequency of adverse reactions among Norwegian children in general public dentistry (3–18 years old) has been assessed to be 1:2600 (3), and among Norwegian orthodontic patients 1:100 (4). Adverse reactions have been reported in patients with removable orthodontic appliances made from resins (4). Organic compounds leaching from cold-cured resins have been demonstrated in vitro (5, 6) and in vivo (7). Hitherto methyl methacrylate monomer (MMA) is the only organic compound that has been separated, identified, and quantified in saliva from patients with orthodontic appliances made of cold-cured resins (7).

The aim of the present study was to apply a combined gas-chromatography and gas-chromatography/mass-spectrometry technique to separate and identify organic additives and degradation products leaching from orthodontic cold-cured resins both in vivo and in vitro. Furthermore, we aimed to compare the leaching of organic compounds from test specimens of cold-cured orthodontic resins processed by the commonly used powdering technique and a pre-mix technique.

Materials and methods

Laboratory specimens

Twenty test specimens from Orthodontic Resin (batch 090188 powder, batch 0510589A liquid, L. D. Caulk Co., Division of Dentsply International Inc., Milford, Del., USA) were made in dental stone molds

(Vel-mix), with a diameter of 50 mm and a depth of 3 mm. Ten specimens were made by a pre-mix technique using 1 part (weight) liquid and 2.5 parts powder, which were mixed before being poured. Another 10 specimens were processed in accordance with the powdering technique. Preliminary tests showed that the powder to liquid ratio with this method showed greater variation than with the pre-mix technique. With the powdering technique the stone molds were saturated in water, and the polymer powder was layered into the molds, infiltrated with monomer liquid, and successively built up to a thickness of 3 mm. For both techniques, curing was performed in a pressure vessel containing water at 40°C and air pressure of 0.2 MPa for 5 min. Samples were also taken from the monomer liquid and the powder and analyzed separately. The free surface of test specimens was examined by scanning electron microscopy (SEM).

Patient material

The patients included in this study were treated at the Department of Orthodontics and Facial Orthopedics, School of Dentistry, University of Bergen. Patients from 6 to 12 years old participated. A questionnaire on demographic, special, and general anamnestic information was collected for each patient. Saliva samples were collected before insertion of removable orthodontic appliances (group A, $n = 43$), during the period of active use 1–2 months later (group B, $n = 34$), and 1 week after the appliances had been removed (group C, $n = 8$). The patients used removable cold-cured appliances made of resin (Orthodontic Resin) processed by the powdering technique.

Leaching of specimens in Ringer solution and ethanol

The test specimens were placed in separate glass vessels with 50 ml of liquid solvent. The liquid was either Ringer solution (1 l = 40.5 g NaCl, 89 g KCl, 1.125 g $CaCl_2$, distilled water, pH 6.0) or redistilled ethanol. The vessels were subjected to agitation (100 rpm) at 37°C, for 7 days for the Ringer solution and for 20 h for the ethanol. To extract organic compounds from Ringer solution, 1 ml of distilled ethylacetate (868, Merck, E. Merck, Darmstadt, Germany) with an internal standard of diethylphthalate (2 μg/ml) (822323, Merck-Schuchardt, Schuchardt, Hohenbrunn bei München, Germany) was added to the solution, mixed with 5 ml of distilled ethylacetate in a separatory funnel and extracted (8). Extraction was then done twice with 3 ml ethylacetate. The extracts were transferred to screw-capped glass vials (2783/2 Heigar & Co. A/S, Oslo, Norway) and evaporated to 100–200 μl at 60°C. After cooling, the evaporated samples were transferred to sample vials (02-MTV, Vials PK A 100 Cromacol Limited, London, UK) with inner vials (2-CV, Vials PK A 100, Cromacol).

Evaporation of the ethanol during the 20-h leaching period was compensated for by addition of ethanol up to the original volume of 50 ml. One milliliter of distilled ethylacetate with an internal standard of diethylphthalate (2 μg/ml) was added to 1 ml of ethanol. The samples were transferred to screw-capped glass vials and evaporated to 100–200 μl at 60°C. All glassware used in this study was rinsed in distilled ethylacetate and heated at 100°C for at least 12 h before use.

Saliva sampling and processing

Unstimulated saliva was collected from each patient for 5 min, while keeping her/his mouth closed. B samples were obtained without the appliance in the mouth, immediately after it had been removed from the mouth. The saliva samples were collected in glass beakers (C SB-134, Kebo, Oslo, Norway) covered by aluminum foil and kept at −20°C until analyzed.

To extract organic compounds from saliva (1 ml), distilled ethylacetate (1 ml) with internal standard of diethylphthalate (2 μg/ml) was added. The extraction was done three times, as previously described (9). A recovery test was performed on saliva and on Ringer solution with known added amounts of phenyl benzoate and phenyl sa-

licylate. The calculated recovery was 80–110%.

Analytic procedure

A gas chromatograph (GC) (Perkin Elmer Autosystem Gas Chromatograph, Perkin-Elmer Corp., Norwalk, Conn., USA) was used. The instrument was equipped with a flame ionization detector and a fused silica column of 25 m × 0.32 mm internal diameter and a film thickness of 0.52 μm (Ultra 2 WCOT, Hewlett-Packard Co., Avondale, Pa., USA). The temperature program was from 150°C to 200°C at 5°C/min; the injector temperature was 200°C, and the detector temperature 250°C. Splitless injection was used. Peak areas and retention times were recorded by an integrator (PE, Model 1020 GC Plus Upgrade, S100–0310).

A gas-chromatography/mass-spectrometry (GC/MS) system (Hewlett Packard 5970 MSD) with an autosampler (Hewlett Packard 7673) was used to verify the identity of the compounds separated by GC. The fused silica columns used were the same as those described for GC. The quantitative detection limit of dibutylphthalate (DBP) was 0.1 μg/ml (9). The quantitative detection limits of dicyclohexylphthalate (DCHP), phenyl benzoate (PB), and phenyl salicylate (PS) were 0.5 μg/ml, 0.5 μg/ml, and 0.1 μg/ml, respectively. Precautions were taken to prevent contamination from the needle/septum system during analyses (9).

A high-performance liquid chromatography (HPLC) system (Hewlett Packard 1050) with a detector diode array (1040) and a HPLC/MS system (Vestec Model 201) with thermospray were applied for the control analyses. The column was packed with C-18 on 3-μg particles. A mobile phase, 70% methanol in 0.1 M ammonium acetate, was pumped at a flow rate of 1 ml/min.

Control analyses were performed with benzoyl peroxide and extracts from leached specimens on HPLC, HPLC/MS, and GC at various injection temperatures, to exclude the possibility that the compound PB might be formed from the decomposition of benzoyl peroxide as an analytical artefact by the high temperature in the GC column.

Scanning electron microscopy

Samples from the surface of test specimens that had been subjected to leaching were mounted on aluminum stubs. They were sputter-coated with approximately 25 nm of Au-Pd. Samples were then examined by SEM (Jeol JSM 6400 Scanning Microscope).

Presentation of results and statistics

The amounts of PB and PS were calculated in μg/cm^2 and μg/ml, on the basis of the mean of two duplicate analyses related to a standard curve. The other compounds were qualitatively analyzed. For identification of compounds in saliva, samples were pooled and subjected to GC/MS. Identification of organic compounds by GC/MS was con-

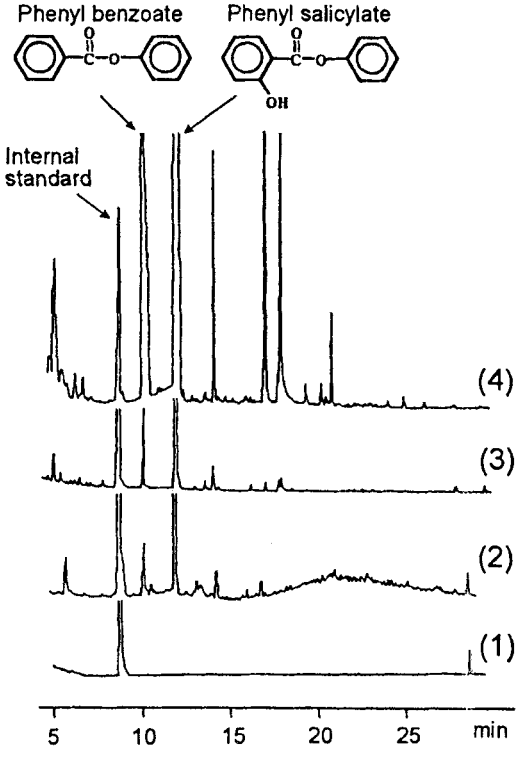

Fig. 1. Gas-chromatographic (GC) chromatograms from saliva before insertion of orthodontic appliances (1) and after insertion of appliances (2). In addition, GC chromatograms from the in vitro study with Ringer solution (3) and ethanol (4).

Table 1. Data from questionnaire and in vivo gas chromatography (GC)-GC/mass spectrometry (MS) study

Patients	Group A ($n = 43$) (20, 23)*	Group B ($n = 34$) (17, 17)*	Group C ($n = 8$) (6, 2)*
Subjective symptoms after wearing the orthodontic appliances	—	1 (Itching)	—
Mucosal reactions, in accordance with Lindquist et al. (10)	0	12	0
No. of peaks in chromatograms			
Median	4	8	5
Range	(1–16)	(5–27)	(3–10)

* Number of boys, girls.

firmed by standard solutions of the compounds.

The number of peaks in the chromatograms from salivary samples was counted by the chromatography software, indicating the number of organic compounds in the samples which could be separated at the GC. The Mann–Whitney two-sample test was used to test for statistical significance. A significance level of $p < 0.05$ was chosen.

Results

Pooled saliva samples from 12 patients undergoing active treatment (group B) showed the presence of PB and PS with selected ion monitoring (SIM) in GC/MS (Fig. 1). The amount of PS (2.9, 0.9, and 1.5 μg/ml) in samples from group B was estimated from the GC chromatograms in three patients (Patients 18, 21, and 22); the amount of PB was below the quantitative detection limit for individual subjects but was detected. None of the substances were

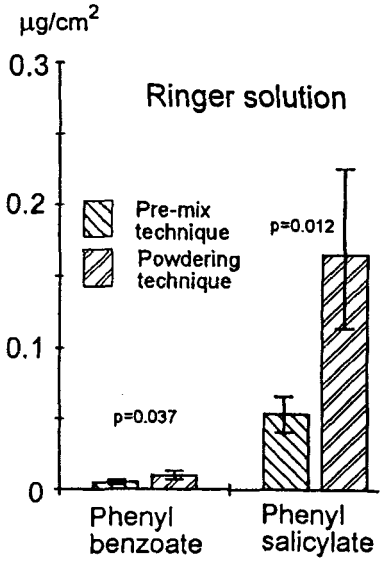

Fig. 2. Comparison of leaching with a pre-mix technique and the powdering technique. Specimens leached for 7 days in Ringer solution. Median values (bars) and quartiles (vertical lines) of leaching phenyl benzoate and phenyl salicylate from test specimens ($n = 5$) made of orthodontic resin are indicated. P values are given.

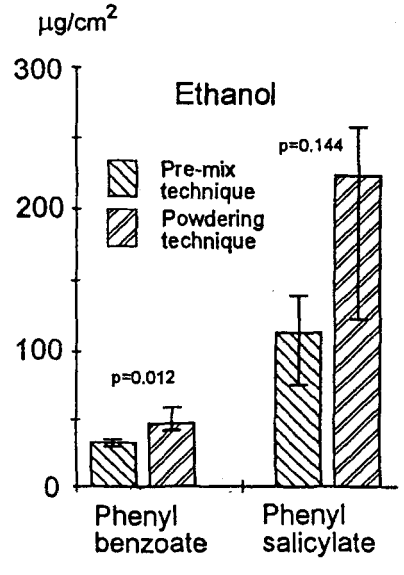

Fig. 3. Comparison of leaching with a pre-mix technique and the powdering technique. Specimens leached for 20 h in ethanol. See also legend to Fig. 2.

found either before start of treatment (group A) (Fig. 1) or after termination of the treatment (group C). The median value for DBP and DCHP was below the quantitative detection limit in groups A, B, and C. In thirty-five per cent of the patients mucosal reactions were found beneath the appliances, in accordance with the criteria of Lindquist et al. (10) (Table 1).

The compounds PB and PS were quantified in vitro with test specimens leached in Ringer solution (Fig. 2) and ethanol (Fig. 3). Separate GC analyses of the orthodontic resin liquid showed a high content of PS. Biphenyl was identified in both Ringer solution and ethanol. In addition, 2-hydroxy-4-methoxy-benzophenone and biphenyl were identified in the ethanol solvent.

The number of peaks recorded in chromatograms from saliva in group B was significantly higher than for group A ($p = 0.001$), and group C ($p = 0.004$) (Table 1).

Scanning electron micrographs from the surface of test specimens showed polymer beads, more pronounced with a pre-mix technique (Fig. 4A) than with the powdering technique (Fig. 4B)

The total amount of leached organic compounds from test specimens ($n = 5$) processed with the powdering technique and a pre-mix technique was compared by measuring the total height of peaks in the chromatograms. The difference was statistically significant ($p = 0.022$). The amounts of both PB and PS were higher with the powdering technique than with the pre-mix technique (Figs. 2 and 3), except for PS in ethanol (Fig. 3).

Discussion

It is well known from in vitro tests that cold-cured (chemically activated) resins release more MMA, methacrylic acid (MA), and benzoic acid (BA) than do heat- and microwave-cured types (11). In a previous report the content of DBP in saliva from patients with new heat-processed dentures was quantified. In some patients it was also possible to identify PB in saliva (9).

In the present study PB, PS, 2-hydroxy-4-methoxy-benzophenone, and biphenyl were identified in vitro from test specimens made of an orthodontic resin. The quantity of PB and PS from test specimens leaching in ethanol may be taken as the maximum content of these substances in the cold-cured resin. In our study it was not possible to identify 2-hydroxy-4-methoxy-benzophenone from the liquid or powder of orthodontic resin. Thus the chemical similarity between the compounds benzoyl peroxide, PS, and 2-hydroxy-4-methoxy-benzophenone indicated

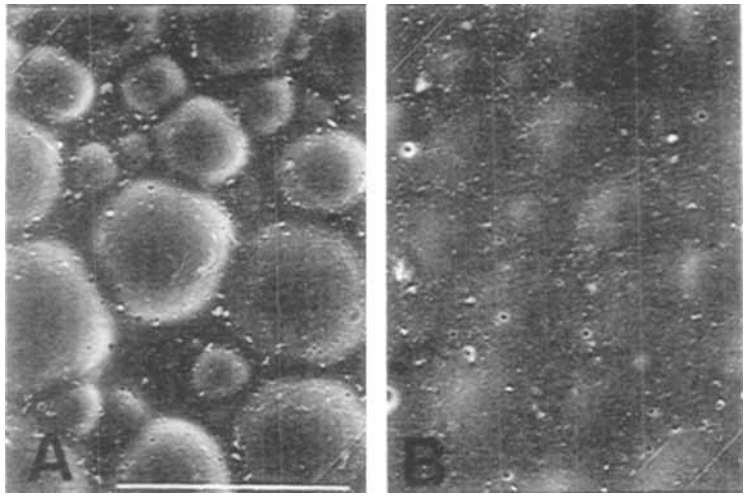

Fig. 4. Scanning electron micrographs from the surface of test specimens leached in Ringer solution. Specimen processed by the pre-mix technique (A). Specimen processed by the powdering technique (B). Horizontal bar = 100 μm.

that the latter could be a result of radical reaction. The present in vitro results showed that the total amount of organic compounds revealed by GC and the quantity of leaching PB and PS were significantly higher with the frequently applied powdering technique for processing of orthodontic appliances than with a procedure using a pre-mix technique. The results of the SEM of test specimens processed with the powdering technique indicated that the amount of monomer liquid was higher in the surface than with the pre-mix technique.

In saliva from three patients with active use of appliances (samples from group B) the amount of PS was quantified. One of these patients showed a mucosal reaction beneath the appliance (Table 1). As shown in our in vitro study, the amount of PB in vivo (saliva) was lower than the amount of PS. PS has been reported to cause allergy (12–14) when used as an additive in plastic polymers (15). No such remarks could be found for dental polymers. PB, probably a degradation product from the initiator benzoylperoxide, and 2-hydroxy-4-methoxybenzophenone, the latter used to improve color stability in plastics, have been identified as cytotoxic factors from a commercial bisphenol-A-glycidyl dimethacrylate (BIS-GMA)-containing dental composite in an in vitro study by Rathbun et al. (16). Phthalates have been found in conjunction with denture base materials (9), but the leaching was not quantified from the present resin product. However, PS, usually added to plastic as a light absorber, may exhibit plasticizer properties (17). The high quantities of PS in orthodontic resin could possibly replace the phthalates.

In a clinical situation, removable appliances containing leachable lipid-soluble organic compounds are placed directly against the oral mucosa. The leaching-out mechanism for these compounds, as for the MMA in orthodontic resin (6), would most probably be a slow process. Lipid-soluble small molecules are known to modulate membrane functions, probably by interaction with membrane lipids as a result of their hydrophobicity (18). The information about the influence of PB and PS on membrane lipids in cells is scarce. The present study, however, has shown that saliva from patients with cold-cured removable orthodontic appliances, made with the powdering technique, has a significant increase in number of organic compounds separated by GC. In these patients leached low-molecular compounds may possibly act as haptens in the induction of delayed hypersensitivity reactions.

In conclusion, the present study has shown that additives and degradation products are leached to saliva from cold-cured orthodontic appliances. It is possible to separate and identify these leaching compounds by a combined GC and GC/MS technique. The powdering technique for processing of cold-cured orthodontic resins seemed to give significantly higher quantities of leaching compounds in vitro than a pre-mix technique.

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