Detection of Lead in Human Teeth by Exposure to Aqueous Sulfide Solutions

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Abstract

A recent study has shown that the presence of lead (Pb) as well as other base metals in esthetic restorative materials, especially dental cements, is detectable by color shifts induced by exposure of hardened specimens to a 0.1% (w/v) aqueous solution of sodium sulfide, Na₂S. The present study was initiated to determine the applicability of this simple exposure test to the detection of Pb in human teeth. Extracted whole teeth as well as sectioned, thin specimens were exposed first to either a 0.01% or a 0.001% (w/v) aqueous solution of lead nitrate, Pb (NO₃)₂, at 37°C for 24 h. After rinsing with distilled H₂O and a subsequent 24 h exposure to the 0.1% Na₂S solution at 37°C, the tooth specimens were examined visually and by a dental color analyzer for color changes. The latter color measurements were evaluated by the Lab system of Hunter, and the color difference, ΔE , was determined. Neither control specimens exposed to distilled H₂O only or to 0.1% Na₂S only exhibited any significant change in appearance after 24 h of storage at 37°C. However, specimens exposed first to the Pb (NO₃)₂ solutions showed discernible ΔE values after exposure to the Na₂S solution. ΔE was greatest for specimens exposed to the more concentrated Pb (NO₃)₂ solution. Most of the discoloration in both thin and intact tooth specimens was confined to the outermost layers of the tooth structure. For the intact specimens, the greatest degree of discoloration occurred in the cementum, the most permeable part of the tooth structure.

Introduction

Previous studies dealing with the occurrence of Pb in human teeth have revealed that this element is widely prevalent as a minor contaminant^[1,2]. Dentin has about three times the Pb content found in enamel. The outer portion of the enamel exhibits the highest concentrations of this metal. It has also been found that the Pb content is significantly greater in older teeth, e. g. 50 years, $\cong$ 550 ppm, than in younger teeth.

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In addition to the biological implications of these findings, there is the possibility of gradual tooth discoloration occurring with age due to the formation of dark-colored lead salts, e. g. lead sulfide (PbS). This potential for discoloration of tooth structure is further augmented in oral environments that generate relatively high concentrations of sulfide ion or related chemical species due to putrefaction (e. g. from diets comprising sulfur-containing foods).

In previous studies it was shown that Pb and other base metal contaminants present in esthetic dental restorative materials, especially dental cements, were readily detectable by exposure to an atmosphere of H_2S or to a dilute (0.1% w/v) aqueous solution of sodium sulfide, $\text{Na}_2\text{S}^{[3]}$.

The present preliminary study was initiated in order to determine if the technique of using 0.1% Na_2S solution for the detection of metal contaminants in cement and composite materials was also applicable as a method for detection of Pb or other base metals in contaminated teeth.

Materials and Methods

The chemicals used in this study were sodium sulfide, $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (ACS grade, Alfa Products, Danvers, MA), lead nitrate, $\text{Pb}(\text{NO}_3)_2$ (J. T. Baker Chemical Co., Phillipsburg, NJ), and freshly extracted human permanent teeth, from subjects approximately 20-40 years old.

I. Preparation of Specimens

A) Sectioned Tooth Specimens

By means of a water-cooled diamond disc (Slicer MC-501D, Maruto Co., Tokyo, Japan), some of the tooth specimens were sectioned into thin pieces, approximately 1.0 mm thick.

Individual sectioned specimens were then immersed in the following aqueous media for 24 h at 37°C : (1) distilled water (primary control), (2) 0.1% Na_2S at pH 9 (secondary control), (3) 0.01% $\text{Pb}(\text{NO}_3)_2$ and (4) 0.001% $\text{Pb}(\text{NO}_3)_2$ · (% are w/v).

After removal from solutions (3) and (4), the Pb-contaminated specimens were rinsed for 1 min under a stream of distilled water, dried with air for 20s, and immediately immersed in (2). After 24 h of immersion at 37°C , the specimens were removed, rinsed with distilled water for 1 min and dried with a blast of air for 20 s.

B) Intact Tooth Specimens

Individual intact tooth specimens were immersed in the following aqueous media for 24 h at 37°C : (1) 0.1% Na_2S at pH 9 (control), (2) 0.01% $\text{Pb}(\text{NO}_3)_2$. After immersion in solution (2), the Pb-contaminated intact tooth was rinsed for 1 min in a stream of distilled water, dried with air for 20 s, and immediately immersed in (1). After immersion at 37°C , the intact tooth specimens were removed, rinsed with distilled water for 1 min and dried with a blast of air for 20 s.

The volume of each aqueous medium in A and B was 5.0 ml (in vials sealed with Teflon-lined screw caps).

II. Measurement of Color Difference

After immersion in the 0.1% Na_2S solution and drying with a blast of air for 20 s, both the sectioned and intact tooth specimens were immediately subjected to visual and colorimetric examination using 10 sheets of round filter paper as a white

background.

Colorimetric examination of these specimens was performed using a dental color analyzer (Dental Color Analyzer N-S 100, J. Morita Co., Tokyo, Japan). The method of color measurement was the same as that employed by the Lab System of ADAMS and the color difference, ΔE , was determined by the Japanese Industrial Standard Z 8730.

Values for the Lab System are defined as follows: "L" is the light index. The higher the value, the greater the lightness. The values "a" and "b" are chromatic indices. A plus sign for the "a" axis indicates the red color zone and a minus sign the green color zone. A plus sign for the "b" axis signifies the yellow color zone and a minus sign the blue color zone. If the coordinates of Lab values obtained from the before-after observations are assumed to be (L_1 , a_1 , b_1) and (L_2 , a_2 , b_2), then differences in these values are (ΔL , Δa , Δb), and ΔE can be calculated from the following expression:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

III. Elementary Analysis of Sectioned Specimens

An electron microprobe analyzer, EMA-SM 7 (Shimazu Co., Tokyo, Japan) was employed to detect and measure heavy metal elements (Cu, Fe, Pb, Ag, and Hg) present in each tooth specimen. Conditions used for line analysis were as follows: accelerating voltage-20 kV, sample current- 1×10^{-8} A, beam spot diameter-1 μm .

Results and Discussion

The results of this study are shown in Figs. 1-15 and Tables 1-6. Figures 7-12 and Tables 1-6 were obtained using a dental color analyzer, and Figs. 13-15 were obtained by electron microprobe line analysis. Neither the primary control specimens (exposed to distilled water only) nor the secondary control specimens (exposed to 0.1% Na_2S solution only) exhibited any significant change in appearance of the enamel part of the specimen after storage at 37°C for 24 h (Figs. 1-4, 7-10, Tables 1-4). The negative results obtained with the secondary controls implied that these untreated tooth specimens either had low content of Pb or other base metals or that the availability of any heavy metal ions for reaction with sulfide ion (S^{2-}) was restricted by their location in the apatitic tooth structure.

The specimens that were exposed first to the $\text{Pb}(\text{NO}_3)_2$ solutions immediately showed discernible color differences (ΔE) after exposure to the Na_2S solution. ΔE was greatest in the specimens exposed to the more concentrated $\text{Pb}(\text{NO}_3)_2$ solution, 0.01%. Most of the dark discoloration in both thin and intact tooth specimens was confined to the outermost layers of the tooth structure. In the case of the intact tooth, which was first exposed to the 0.01% $\text{Pb}(\text{NO}_3)_2$ solution, the discoloration that occurred after immersion in the Na_2S solution was greatest ($\Delta E = 39.3$) in the cementum area, although slight discoloration ($\Delta E = 13.8$) also occurred in the enamel (Figs. 5 and 11, Table 5). Cross-sectioning of the darkened cementum

revealed that most of the interior portion was unchanged but that discoloration also occurred in the dentin layer of this part of the tooth (Fig. 5)

Furthermore, in the case of the intact tooth which was exposed to 0.1% Na₂S solution only at 37°C for 24 h, brown discoloration ($\Delta E = 33.3$) occurred in the cementum area with only very slight discoloration ($\Delta E = 4.3$) in the enamel area (Figs. 6 and 12, Table 6). The results of elementary analysis of the sectioned tooth specimens (Figs. 13-15) indicated that most of the specimens contained significant amounts of Cu (0.00-0.20 wt%) and Fe (0.00-0.35 wt%) and only minor amounts of Ag and Pb.

Therefore, these results suggest the possibility that the discoloration of the intact tooth, which was exposed to the 0.1% Na₂S solution only, might be mainly caused by the formation of dark-colored sulfides of contaminating heavy metals (Cu, Fe, Ag, and Pb) already present in the tooth structure.

In earlier studies^[3,4], it was noted that for Pb-contaminated esthetic dental materials, the rate and severity of discoloration was greatest for dental cements. Only one composite, the microfilled Isopast (commercially available in the market), which has a comparatively high water absorption and may be more easily penetrated by S²⁻, showed severe discoloration. The greater discoloration observed in the relatively porous cementum region of the tooth would seem to parallel these findings, i. e. the rate and severity of color formation is related to the permeability of the Pb-contaminated specimen to S²⁻ or related sulfur-containing chemical species^[3,4]. Further work is now in progress to expand the scope of these findings to other metal contaminants as well as other types of dental material.

Conclusions

- 1) The greatest discoloration in both sectioned and intact teeth was observed for specimens that were immersed in 0.01% Pb (NO₃)₂ solution.
- 2) In the case of intact tooth specimens that were first exposed to 0.01% Pb (NO₃)₂ solution, the greatest degree of discoloration ($\Delta E = 39.3$) occurred in the cementum, the most permeable portion of the tooth structure.
- 3) In the case of the intact tooth which was exposed to 0.1% Na₂S solution only, easily discernible discoloration ($\Delta E = 33.3$) occurred in the cementum portion, probably due to the formation of dark-colored sulfides of heavy metals (e. g. Cu, Fe, Ag, Pb) contained in natural teeth as contaminants.

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Table 1 Results of discoloration 1 | (Thin Specimens)

	Before			After			E
	L	a	b	L	a	b	
Primary Control	63.2	-1.5	-22.1	62.1	-1.0	21.9	1.2
S. D.	1.25	3.56	0.71	1.20	2.68	0.15	
Secondary Control	62.3	-2.6	-19.9	59.9	-1.8	-21.0	2.8
S. D.	1.13	0.92	0.64	2.39	1.69	0.55	
0.001% Pb (NO ₃) ₂	57.9	-0.5	-18.4	43.0	2.0	-18.6	15.1
S. D.	1.10	1.02	0.86	1.12	1.05	0.66	
0.01% Pb (NO ₃) ₂	60.7	-1.1	-19.5	12.9	3.6	-8.9	49.2
S. D.	1.10	0.61	0.25	1.18	0.40	2.48	

1 | : Maxillary right incisorTable 2 Results of discoloration 3 | (Thin Specimens)

	Before			After			E
	L	a	b	L	a	b	
Primary Control	67.0	3.2	-25.4	64.4	4.2	-25.6	2.8
S. D.	2.02	1.25	2.06	0.85	0.66	1.46	
Secondary Control	59.2	1.0	-22.1	56.8	0.6	-21.7	2.5
S. D.	2.61	2.93	2.41	3.27	2.42	1.89	
0.001% Pb (NO ₃) ₂	60.4	-0.5	-24.5	43.4	2.5	-21.3	17.6
S. D.	2.29	1.36	0.80	2.21	0.61	1.35	
0.01% Pb (NO ₃) ₂	61.0	-0.8	-23.1	10.2	1.5	-8.7	52.9
S. D.	1.85	2.17	1.14	2.15	1.50	1.10	

3 | : Maxillary right cuspidTable 3 Results of discoloration 4 | (Thin Specimens)

	Before			After			E
	L	a	b	L	a	b	
Primary Control	59.4	3.6	-26.3	58.3	2.5	-26.2	1.6
S. D.	3.90	0.52	0.84	3.42	0.93	1.17	
Secondary Control	61.0	2.9	-23.7	58.5	-2.1	-24.5	5.6
S. D.	6.78	4.71	1.32	6.19	3.51	0.79	
0.001% Pb (NO ₃) ₂	58.6	-0.4	-23.1	48.1	0.3	-18.9	11.3
S. D.	4.60	0.99	1.53	4.71	1.75	1.99	
0.01% Pb (NO ₃) ₂	57.3	-2.6	-23.3	9.2	0.6	-7.5	50.7
S. D.	3.12	3.21	1.69	2.45	0.59	1.22	

4 | : Maxillary left premolar

Table 4 Results of discoloration 8 (Thin Specimens)

	Before			After			E
	L	a	b	L	a	b	
Primary Control	60.0	3.4	−29.7	56.0	0.2	−30.4	5.4
S. D.	2.61	2.14	0.40	3.01	3.91	1.01	
Secondary Control	60.0	3.5	−30.9	57.0	3.4	−29.9	3.2
S. D.	3.01	2.78	1.36	3.95	1.44	2.51	
0.001% Pb (NO ₃) ₂	58.4	1.0	−30.5	31.8	6.9	−23.2	27.8
S. D.	3.34	2.45	1.19	4.91	2.74	2.17	
0.01% Pb (NO ₃) ₂	56.1	4.5	−27.8	9.9	5.0	−10.1	49.9
S. D.	4.12	1.83	0.87	1.53	1.39	1.66	

8 : Maxillary left molarTable 5 Results of discoloration 4 [Successive immersion of intact tooth in 0.01% Pb (NO₃)₂ solution (24 hours) ; then in 0.1% NaS solution (24 hours)]

	Before			After			E
	L	a	b	L	a	b	
Enamel Region of Tooth	54.2	4.5	−25.7	48.0	4.9	−13.4	13.8
Cementum Region of Tooth	60.5	6.5	−23.3	22.9	0.8	−13.5	39.3

4: Maxillary left premolarTable 6 Results of discoloration 8 [Intact tooth immersed in 0.1% Na₂S solution (24 hours)]

	Before			After			E
	L	a	b	L	a	b	
Enamel Region of Tooth	58.9	7.1	−35.5	57.1	4.0	−33.1	4.3
Cementum Region of Tooth	56.7	8.5	−30.5	26.5	3.5	−17.5	33.3

8 : Mandibular left molar

- + Immersed in distilled water (24 hours):
 - Immersed in 0.1% Na_2S solution (24 hours):
 - ** Successive immersion in 0.001% $\text{Pb}(\text{NO}_3)_2$ solution (24 hours); then in 0.1% Na_2S solution (24 hours)
 - Successive immersion in 0.01% $\text{Pb}(\text{NO}_3)_2$ solution (24 hours); then in 0.1% Na_2S solution (24 hours)
- temp. = 37°C

Fig. 1 Effect of immersion in Na_2S solution on Pb-contaminated thin specimens of extracted tooth (1)

- + Immersed in distilled water (24 hours):
 - Immersed in 0.1% Na_2S solution (24 hours):
 - ** Successive immersion in 0.001% $\text{Pb}(\text{NO}_3)_2$ solution (24 hours); then in 0.1% Na_2S solution (24 hours)
 - Successive immersion in 0.01% $\text{Pb}(\text{NO}_3)_2$ solution (24 hours); then in 0.1% Na_2S solution (24 hours)
- temp. = 37°C

Fig. 2 Effect of immersion in Na_2S solution on Pb-contaminated thin specimens of extracted tooth (3)

- + Immersed in distilled water (24 hours):
 - Immersed in 0.1% Na_2S solution (24 hours):
 - ** Successive immersion in 0.001% $\text{Pb}(\text{NO}_3)_2$ solution (24 hours); then in 0.1% Na_2S solution (24 hours)
 - Successive immersion in 0.01% $\text{Pb}(\text{NO}_3)_2$ solution (24 hours); then in 0.1% Na_2S solution (24 hours)
- temp. = 37°C

Fig. 3 Effect of immersion in Na_2S solution on Pb-contaminated thin specimens of extracted tooth (4 |)

- + Immersed in distilled water (24 hours):
 - Immersed in 0.1% Na_2S solution (24 hours):
 - ** Successive immersion in 0.001% $\text{Pb}(\text{NO}_3)_2$ solution (24 hours); then in 0.1% Na_2S solution (24 hours)
 - Successive immersion in 0.01% $\text{Pb}(\text{NO}_3)_2$ solution (24 hours)
- temp. = 37°C

Fig. 4 Effect of immersion in Na_2S solution on Pb-contaminated thin specimens of extracted tooth (8 |)

Successive immersion in 0.01% $\text{Pb}(\text{NO}_3)_2$ solution (24 hours) then in 0.1% Na_2S solution (24 hours)
temp. = 37°C

Fig. 5 Effect of immersion in Na_2S solution on Pb-contaminated intact tooth structure (4)

Successive immersion in 0.1% Na_2S solution (24 hours)
temp. = 37°C

Fig. 6 Effect of immersion in Na_2S solution on intact tooth structure (8)

Fig. 7 Effect of immersion in Na₂S solution on Pb-contaminated thin specimens of extracted tooth

Fig. 8 Effect of immersion in Na₂S solution on Pb-contaminated thin specimens of extracted tooth

Fig. 9 Effect of immersion in Na_2S solution on Pb-contaminated thin specimens of extracted tooth

Fig. 10 Effect of immersion in Na_2S solution on Pb-contaminated thin specimens of extracted tooth

Fig. 11 Effect of immersion in Na_2S solution
on Pb-contaminated intact tooth

Fig. 12 Effect of immersion in Na_2S solution
on intact tooth

Fig. 13 Electron microprobe analysis of thin specimen of extracted tooth

Fig. 14 Electron microprobe analysis of thin specimen of extracted tooth

Fig. 15 Electron microprobe analysis of thin specimen of extracted tooth