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Electrical impedance – a new parameter for oral mucosal irritation tests

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The potential for dental materials to irritate human oral mucosal membranes was assessed by an electrical impedance technique. A small electrode at the site of irritation on the inside of the cheek and a large electrode on the outside of the cheek were used. Skin impedance was reduced by inundation with ECG-gel. An irritation index was formed by calculating the quotient between the impedance absolute value at 20 kHz and the impedance value at 1 MHz. Electrical impedance technology was found to be more sensitive than traditional visual registration of mucosal reactions. Two series of experiments were performed in which special appliances were constructed to ensure duplicate measurements on the small area of exposed buccal mucosa. Liquid samples elicited differentiated fast response, which was insignificantly influenced by mechanical factors. The mechanical irritation induced by merely the shape of a solid specimen prevents detection of slight mucosal irritation from potentially leaking substances.

1. Introduction

Biological evaluation of dental materials recommended by international and national organizations [1–3] includes oral mucous membrane irritation tests. These tests are designed to assess the response of the tissues to materials intended for temporary or permanent use adjacent to the oral mucosa. Implantation of test materials in the hamster cheek pouch has been recommended for testing hard dental materials [3]. For testing liquid or semi-solid materials, intimate contact with the palatal mucosa in the guinea pig or rat has been recommended [1, 2]. The response of oral mucous membrane to topical exposure to selected liquid dental materials has been studied on the tongue of mongrel dogs [4].

The intention of the animal test is to predict adverse toxicological reactions of medical and dental materials and devices. Many of the animal models have been criticized for lack of scientific validity and also on ethical grounds. It would therefore be justified to test the irritation effects directly on the human oral mucosa by non-invasive techniques. Electrical impedance is such a technique, which has been applied in studies on skin diseases and skin moisturization [5–7]. The aim of this paper is to evaluate whether electrical impedance is an appropriate parameter for biological testing of solid and liquid dental materials on human oral mucosa.

2. Materials and methods

2.1. Test subjects

The solid materials test series involved 20 healthy volunteers, 18 women and 2 men, 25–55 years old. Criteria for exclusions were known skin diseases or suspected allergies of any kind.

In the test series with liquids 10 healthy volunteers, all female, age range as above, were chosen by the same criteria.

The test subjects were recruited from the personnel at the dental school or the neighbouring university hospital and all subjects enrolled in this research signed an Informed Consent Form approved by the Karolinska Institute Ethical Committee for Human Research. The study protocol has been found acceptable by them on 28 August and 2 October 1989. The performance of this study according to the protocol is described below.

2.2. Materials tested

Solids: dental porcelain (Duceram, Ducera) dental composite resin (Concise, 3M), glass-polyalkenoate cement (Chem-fil, DeTrey Dentsply), cold-curing acrylic (Swedon, Svedia Dental), zinc-oxide-eugenol cement (IRM, DeTrey Dentsply). Samples were prepared 24 h before application.

Liquids: physiological saline (NaCl 0.9%), phosphoric acid (H_3PO_4 37.5%), sodium lauryl sulphate (SLS 1.0%).

2.3. Test samples

Each solid material was tested on five subjects, with a dental porcelain control on the contralateral tooth. The positions (left or right) of the test sample and

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control sample were randomized. The samples were carefully shaped as cylinders with rounded edges, height 2 mm, diameter 5 mm. Each cylinder had a stainless steel core and base and was cemented to the first upper molar teeth (16 and 26) by using a minimal amount of glass-polyalkenoate cement applied to the base, as shown in Fig. 1a. The cylinder also served as guide for the forked electrode holder shown in Fig. 1c and d. Each solid sample was borne by the subjects for three weeks.

The liquid samples were absorbed on cotton pellets. Each pellet was placed in the well of an applicator ensuring close but gentle contact with the buccal mucosa as illustrated in Fig. 1b. The back of the applicator provided a means of reproducible positioning, with the teeth as reference points. The buccal side exposed was selected at random. Physiological saline and phosphoric acid were applied for 5 min, sodium lauryl sulphate for 10 min.

2.4. Measurements

Electrical impedance was measured with a system comprising standard laboratory instruments (Fig. 2). In all measurements the amplitude of the electrical source signal over the electrodes was 25 mV. Contact with the oral mucosa was provided by a small sintered AgCl electrode on one leg of a forked electrode holder as shown in Fig. 1c and d. For geometrical reasons, a two-electrode system was used. The second electrode, which was a standard Ag–AgCl ECG-electrode, was applied to the skin of the cheek after ample spread of ECG-gel.

An irritation index was achieved by calculating the quotient between the impedance absolute value at 20 kHz and the impedance absolute value at 1 MHz. The frequencies chosen for this irritation index to a certain extent compensate for geometrical artefacts introduced by, for example, variations in cheek thickness during the course of investigation. The results of the measurements are presented as per cent change in irritation index relative to a nominal (pre-application) value. A decrease in index reflects indirectly increased irritation, as manifested in oedema.

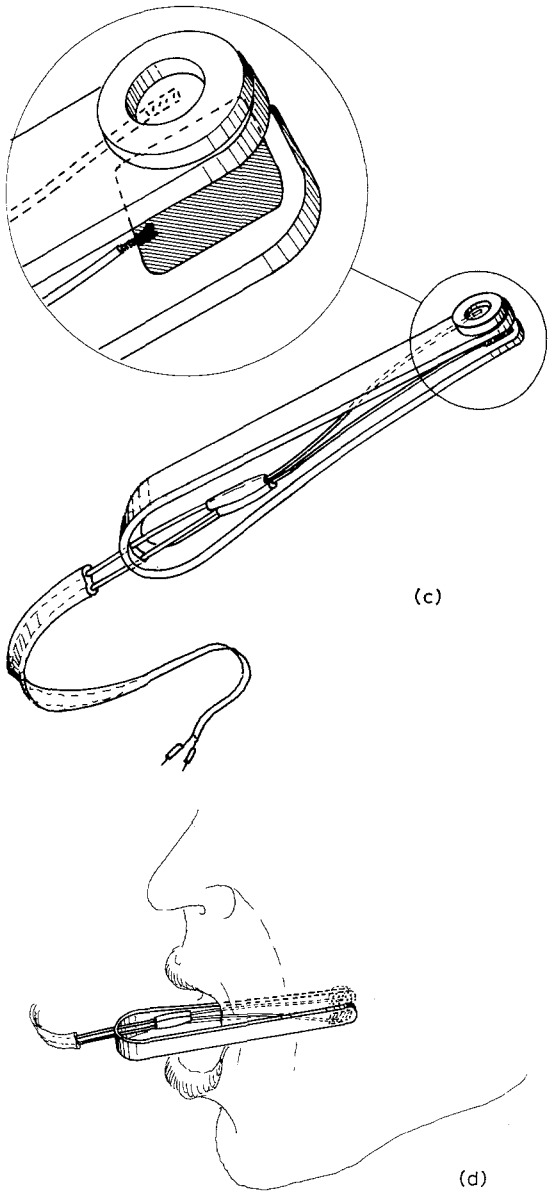
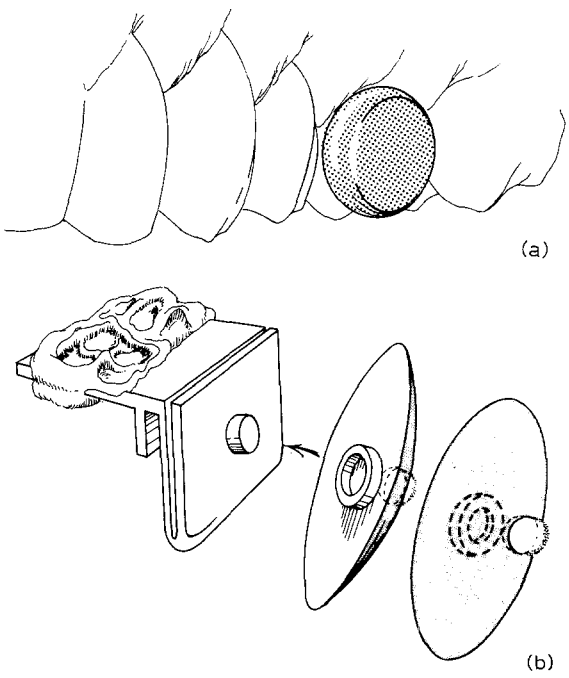


Figure 1 (a) Solid material sample cemented on the first upper molar. Sample serves as guide for electrode holder shown in (c) and (d). (b) From the left: Position holder referred to first molars with guide for forked electrode holder and the applicator of liquid samples (centre). To the right: The buccal side of the applicator showing well and cotton pellet. (c) Details of the electrode holder showing the fitting to the guide seen in (a) and (b). (d) Forked electrode holder in position for electrical impedance measurements.



At low frequencies the total impedance of the involved tissue compartments of the cheek will be greatly affected by the condition of the skin and the quality of electrode contact with the skin. In preliminary tests, no difference could be observed above 10 kHz between impedance measured via skin contact on the cheek and measurement via a hypodermic needle "immersed" in the cheek from the outside. At high frequencies, above 500 kHz, alterations induced in the oral mucosa did not influence the impedance value to a great extent. The geometry of the electrical

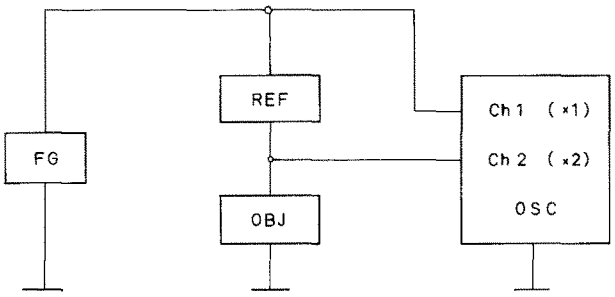


Figure 2 Principle of measurement. FG function generator (Hewlett Packard 3314A), REF decade resistance box, OBJ electrodes and test object, OSC oscilloscope (Gould 1604), Ch1 channel 1/trace 1, Ch2 channel 2/trace 2. At a chosen frequency REF is adjusted so that the peak-to-peak size of trace 1 equals that of trace 2, the amplification in channel 2 being twice that of channel 1. After adjustment the absolute value is read directly on REF. This method gives accurate results of absolute value (within 2%) but is not suitable for resolving impedance into its real and imaginary parts.

current path is quite critical and the impedance value at a high frequency may thus be used for normalization.

As is well known from the field of body composition analysis [8] the changes observed with electrical impedance methods at low or intermediate frequencies reflect changes in extracellular space, since the electrically insulating cell membranes confine the electrical current of these frequencies to this region. At higher frequencies the electrical current is also coupled capacitively through the cell membranes, and the measurement method then reflects electrical impedance of all electrolytes in the region defined by the electrode geometry. Objective signs of irritation are oedema, erythema and heat, and the connection to irritation is thus oedema on a cellular level. In the frequency range zero to a few megahertz the geometry of the electrical field generated from our electrodes may be regarded as a static case. At still higher frequencies, however, electrical properties of specific molecules would increasingly contribute and the electrical field pattern more difficult to predict.

In the solid materials test series, one impedance measurement was carried out a few hours before application (the nominal value) and consecutive measurements once every following workday of the week. During each of the second and third weeks measurements were taken twice. The test samples were removed after the last measurement in the third week. During the fourth week, measurements were performed twice to register post-irritation impedance values.

In the liquid materials series, impedance was measured before and immediately after application of the samples and then after 5, 15, 30, 60 and 120 min. The nominal (pre-application) value given is the average of three measurements taken at 5 min intervals.

3. Results

All solid materials tested showed the same characteristic impedance pattern. Because no differences could be detected between the responses of these small groups they were pooled together. In Fig. 3 the response of all freshly made solid materials as compared to the response of the reference (dental porcelain) is combined in one graph. The standard deviation of each point is given in Table I.

Solid materials induced a gradual lowering of the irritation index during the first week, with the maximum rate of change during the first two days. The index remained at the level attained after the first week during the test period and even some days thereafter.

Each of the three liquid samples, on the other hand, induced clearly differentiated responses. The standard deviation of each point is given in Table II.

Changes in irritation index were already seen after minutes of exposure to liquid samples, as seen in Fig. 4. A maximum response was reached around 5 min after exposure to liquid irritants and the response diminished gradually over the 2 h observation period.

4. Discussion

After the introduction in 1972 of the currently recommended oral mucosal irritation test of dental

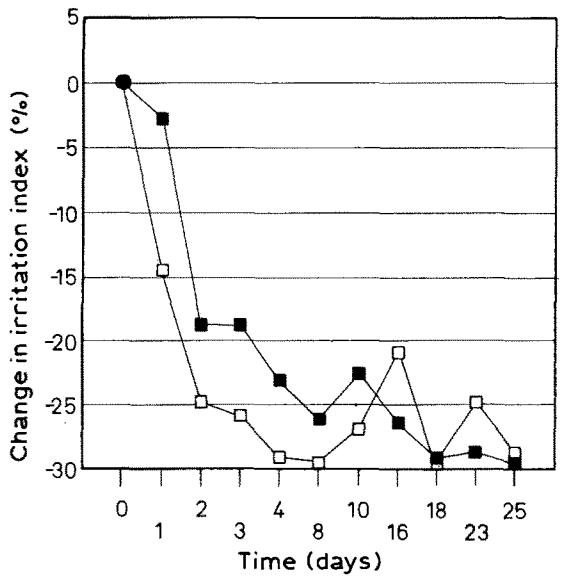


Figure 3 Change in irritation index during and after exposure to solid dental materials. Controls (■) and freshly made test materials (□). Nominal value (●) registered a few hours before cementation of test samples.

TABLE I Change in irritation index in per cent of nominal (pre-application) value during and after exposure to solid dental materials (Exposure from day 0 to day 21)

Days after application	Control material ($n = 20$) Mean	SD	Test material ($n = 20$) Mean	SD
1	− 2.8	40.7	− 14.5	35.7
2	− 18.7	18.4	− 24.8	16.7
3	− 18.8	25.5	− 25.9	16.6
4	− 23.1	24.1	− 29.1	19.8
8	− 26.2	19.4	− 29.5	18.4
10	− 22.5	37.8	− 26.9	17.8
16	− 26.5	27.6	− 20.9	26.9
18	− 29.2	19.4	− 30.0	22.7
23	− 28.7	18.4	− 24.8	29.7
25	− 29.6	15.2	− 28.8	15.7

TABLE II Change in irritation index in per cent of nominal (pre-application) value after exposure to liquid dental materials (Exposure for 5 min for NaCl and H_3PO_4, and 10 min for SLS, before first registration)

Minutes after application	NaCl ($n = 10$) Mean	SD	SLS ($n = 10$) Mean	SD	H_3PO_4 ($n = 10$) Mean	SD
0	− 10.1	16.0	− 13.3	13.6	− 28.4	15.5
5	− 4.7	14.2	− 15.0	17.4	− 29.2	22.9
15	− 1.6	16.6	− 14.4	16.2	− 16.0	29.7
30	− 5.8	12.1	− 13.3	10.6	− 23.8	21.7
60	− 3.7	10.2	− 8.2	16.4	− 14.6	24.3
120	− 9.0	12.5	− 7.5	15.2	− 10.7	28.6

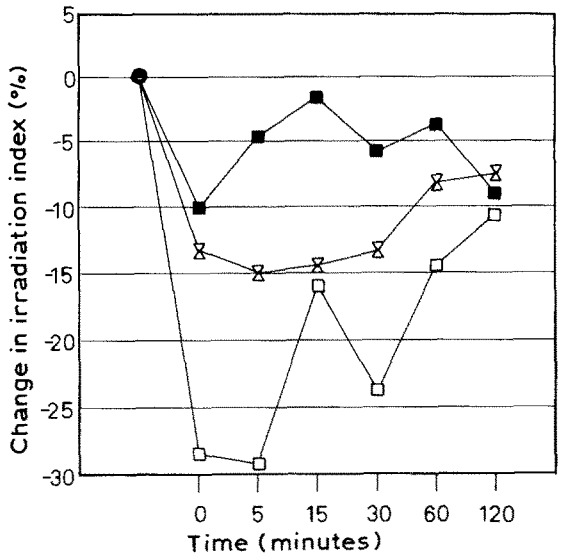


Figure 4 Change in irritation index after exposure to liquid dental materials NaCl (■), H_3PO_4 (□) and SLS (⧖) for 10 min. Nominal value (●) average of three registrations taken at 5 min intervals immediately before application of test samples.

materials in laboratory animals, proposed improvements have been mainly limited to techniques. Great attention has been paid to measures to retain the test samples in the hamster's cheek pouch by double-suturing or a retention collar [9, 10]. The more important task of evaluation of the mucosal response is in fact seriously hampered when, for example, discs are sewn via perforations directly to the inside of the hamster cheek pouch. It is also difficult to determine whether reactions in the palatal mucosa are in response to the irritant effect of the tested sample or the sample holders [11].

So far hundreds of animals have been killed in oral mucosal irritation tests of solid dental materials. The information obtained has not been useful in predicting the effect in humans [9, 12].

It was recognized by Piliers *et al.* in 1979 [12] that, "In view of the complexities regarding the translation of animal biocompatibility testing of alloys as presently conducted to a predictable situation for humans, the ultimate test currently appears to lie with the human subject". The results of the present study suggest that a system based on an electrical impedance technique meeting the requirements for an oral mucosal irritation test in humans of some kinds of dental materials can be designed. The relevance and the sensitivity of the method will be discussed in relation to the presented results.

The impedance method demonstrated changes in the human buccal tissue after exposure of solid and liquid dental materials, even in the absence of clinical signs of irritation. It is also interesting to note the difference in the time intervals for the appearance and duration of changes in impedance values after exposure to these two types of materials. The slower but long-lasting effect of the solid materials is interpreted as pure mechanical irritation. Although the test materials appear to induce a more rapid response than the porcelain control, the evidence is not strong because the standard deviations indicate that the difference is not significant. The more rapid change in impedance value and also the relatively fast return to normal values after exposure to liquid irritants appears to be caused by a direct chemical effect on the mucosa. It is interesting to note that the three liquid samples exerted different degrees of change in impedance as expressed in the irritation index.

The use of guides for test applicators and forked electrode holder ensured that the registering impedance electrodes correspond to the buccal area exposed to the test material and allowed repeated measurements at an identical location. A small electrode at the site of irritation on the inside of the cheek and a large electrode on the outside creates a conical field with highest electrical current density in the vicinity of the test site. Thus the measured impedance will be dominated by the information reflecting irritation in the mucosal membrane. In addition, because the equivalent electrode size, due to ample spread of ECG-gel, is large compared to the distance across the cheek, the impedance value is less sensitive to the exact spread of ECG-gel. However, the thickness of the cheek may vary from day to day on the same test person, and variations may occur on a short timescale due to muscle strain, etc.

The major artefact in the measurement system is, however, the impedance of the skin, which in the natural state is very high compared to moist tissue. Inundation with ECG-gel will reduce this parameter considerably, but the quality of the skin contact is still critical. The frequencies selected for the irritation index in this study tend to minimize this artefact while maintaining a high degree of sensitivity for the desired information.

In its present form the method is not yet applicable for individual diagnosis because the standard devi-

ation in each point is not negligible (Tables I, II). Averages of 10 or more test values are required to make any statistical statements. However, further development of the measurement system has virtually eliminated the variance encountered in this study (Ollmar [13]), indicating that a system can be designed to register irritation effects even in individual cases.

The impedance technique would also be an appropriate test for extracts of solid or semi-solid dental materials, in accordance with the outlines of profound new test guidelines. Of course, positive and negative control materials must be identified and standardization of test conditions must be confirmed. It is anticipated that new standards for mucosal irritation tests of medical materials and devices are to be proposed in the near future; introduction of the impedance technique as a non-invasive clinical test is therefore timely. Preliminary tests on skin reactions in irritation tests also indicate that the impedance technique is very sensitive in this application [14].

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