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 were known skin diseases or suspected allergies of any kind.

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 (H₃PO₄ 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
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.

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 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.