

Electric impedance measurements at six different anatomic locations of macroscopically normal human oral mucosa

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We have used an electric impedance technique to explore the properties of the oral mucosa at various sites in the normal mouth. Investigations were performed on 26 healthy non-smoking subjects at 12 test areas, representing a range of mucosal types. Electric impedance spectra were measured in the frequency range 1 kHz to 1 MHz at five depth penetration settings of the instrument, and four indices were calculated for each depth. Statistically significant differences in the indices were found between most of these locations but not between contralateral sites at a similar position. The differences between some areas were considerably greater, and the differences between contralateral sites were smaller, than those encountered in the skin. Our results suggest that the choice of site for investigation of the oral cavity is more critical than with experimentation on the skin and that cognizance of this fact makes the oral cavity readily available for studies by the impedance method. □ *Bioengineering; dielectric properties; impedance spectroscopy; non-invasive method; oral surgery*

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Irritation reactions and diseases of the oral mucosa are usually assessed by inspection and palpation, which inevitably are influenced by observer variation. In the skin objective quantification of reactions has been made possible by non-invasive bioengineering techniques, such as laser Doppler flowmetry (LDF), transepidermal water loss (TEWL), ultrasound, and electric impedance (1-4), but there is a paucity of suitable methods for the oral cavity. LDF is based on the Doppler principle for detection of particle movement; in this case light is scattered by the erythrocytes in the blood stream, and the Doppler shift due to the degree of perfusion is measured. TEWL instruments measure the passive diffusion of water through the stratum corneum by sensing the moist gradient in a short 'chimney' held against the skin surface and converting the result to water mass per unit area and hour. Ultrasound measurements are based on reflection of ultrasonic waves between anatomic structures of different acoustic impedances. Electric impedance should be regarded as a spectroscopic technique in which the measured magnitude and phase angle are frequency-dependent, and the spectra will contain information about cellular shape and structure, the integrity of cell membranes, the relative proportions of intra- and extra-cellular space, ionic composition, and so forth. Techniques based on resistance and capacitance (5) rely on special cases of electric impedance, which are to some extent correlated with moisture or dryness. Friction (6) has also been proposed for measuring mucosal moisture changes. LDF has been used for comparing blood flow between the oral mucosa and the skin of the Rhesus monkey (7),

and ultrasound scanning for diagnosing oral soft-tissue lesions (8). Effective non-invasive methods for weak reactions have, however, not yet been developed.

Nilsson et al. (9) described a non-invasive method based on electric impedance, which they used on the mucous membrane after the experimental application of various dental materials and liquid substances; they found that the electric impedance technology was more sensitive than traditional visual observation of mucosal reactions. An improvement of this technique for the skin was found particularly useful for changes in the invisible or nearly invisible range (10). Recently, we introduced a set of four impedance indices that emphasize different aspects of impedance, and our experiments on the skin indicated that these were capable of differentiating between the reaction patterns produced by different irritants, as verified by histologic examination (11). Since the oral mucosa shows considerable structural variation from place to place, such as in the degree of keratinization, the aim of the present study has been to map electric impedance properties over a range of sites in the normal mouth.

Materials and methods

Test subjects

The study was performed on 26 healthy volunteers (17 women and 9 men) with macroscopically normal oral mucosa, recruited among the staff and students of the Karolinska Institute School of Dentistry. They were non-smokers without known skin disease or allergy, and

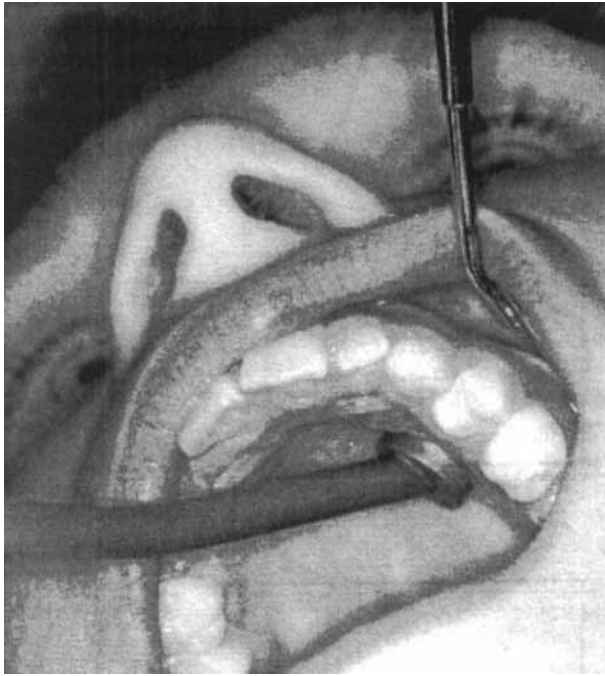


Fig. 1. The oral probe of the electric impedance spectrometer placed in the premolar area of the hard palate.

they had refrained from eating or drinking for at least 1 h before the start of the measurement. Their ages ranged from 20 to 37 years (mean, 26 years). The study was approved by the Huddinge University Ethical Committee for Human Research, and the measurements were carried out between April and May 1994.

Evaluation method

Electric impedance was measured using a SCIM electric impedance spectrometer (SCIM Medical AB, Kinna, Sweden), equipped with a specially designed intraoral probe (Fig. 1). The instrument records the magnitude and phase spectra of impedance at 31 logarithmically distributed frequencies between 1 kHz and 1 MHz in a volume under the probe. This volume can be selected to include contributions, stepwise, at five different depths (10, 12). The depth settings cannot be calibrated in millimeters because the contributions are dependent on the dielectric properties of the layers penetrated by the probing field. It is reasonable to assume that depth setting 1 would reflect mainly the properties of the upper epithelium and that depth setting 5 would reflect the superimposed properties of all layers down to and including the lamina propria, and perhaps even deeper, depending on the thickness of the upper layers. Impedance spectra were recorded at all five depth settings at each test site.

To extract information from the impedance spectra,

four indices were used, representing changes with frequency among the four major (but not independent) aspects of electric impedance (11,13).

The indices are as follows: magnitude index, $MIX = \text{abs}(Z_{20 \text{ kHz}}) / \text{abs}(Z_{500 \text{ kHz}})$; phase index, $PIX = \text{arg}(Z_{20 \text{ kHz}}) - \text{arg}(Z_{500 \text{ kHz}})$; real part index, $RIX = \text{Re}(Z_{20 \text{ kHz}}) / \text{abs}(Z_{500 \text{ kHz}})$; and imaginary part index, $IMIX = \text{Im}(Z_{20 \text{ kHz}}) / \text{abs}(Z_{500 \text{ kHz}})$, where $\text{abs}(Z_i)$ is the magnitude (modulus) of the complex electric impedance at the frequency i , $\text{arg}(Z_i)$ is the argument (phase angle) in degrees, and $\text{Re}(Z_i)$ is the real part and $\text{Im}(Z_i)$ the imaginary part of the complex electric impedance. The magnitudes and phase angles were obtained from the instrument, and the real and imaginary parts calculated by means of the following equations:

$$(1) \quad \text{Re}(Z_i) = \text{abs}(Z_i) * \cos[\text{arg}(Z_i)], \text{ and}$$

$$(2) \quad \text{Im}(Z_i) = \text{abs}(Z_i) * \sin[\text{arg}(Z_i)].$$

RIX reflects changes mainly in conductivity, whereas IMIX reflects mainly reactance changes, which are of a capacitive nature. MIX mirrors changes along the length of the vector describing the impedance in complex space, which is emphasized if the real and imaginary parts change in the same direction and proportion; PIX will be emphasized if the real and imaginary parts change in different directions and/or in different proportions.

Test procedure

Before the measurements were made, the subjects were asked to rinse their mouths with water, and additional rinsing was performed before attachment of the probe at the next test site and when dryness developed during the procedure. All measurements were made by the same investigator (L. Rundquist).

Readings were taken from the following test areas on both right and left sides: in the premolar area of the hard palate, in the premolar area of the floor of the mouth, in the occlusion plane of the anterior buccal mucosa, in the occlusion plane of the posterior buccal mucosa, on the anterior one-third of the dorsal surface of the tongue, and on the vermilion border of the lower lip.

Assessment of all 12 test sites was normally completed within 15 min. The instrument is controlled by a computer program that measures automatically and stores the results in a database within a few seconds after the probe has made good contact with the mucosa.

Statistics

Student's unpaired t test was used for comparisons between different locations in the oral cavity; the distributions were accepted as normal. The following significance scale was used: * for $0.01 < P \leq 0.05$, ** for $0.001 < P \leq 0.01$, and *** for $P \leq 0.001$.

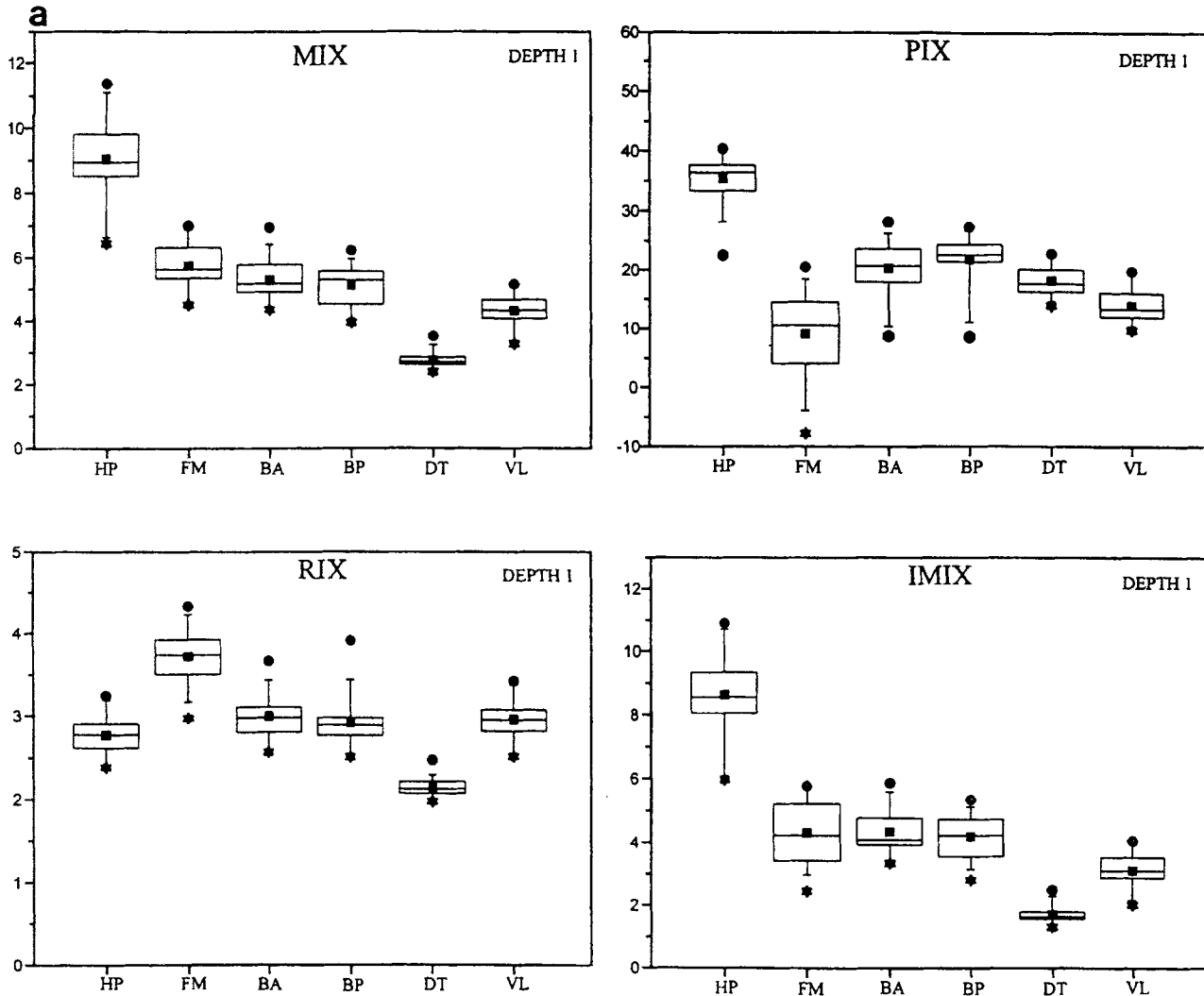


Fig. 2. Box charts for depth 1 (2a) and depth 5 (2b) of the electric impedance indices magnitude index (MIX), phase index (PIX), real part index (RIX), and imaginary part index (IMIX) at different oral locations. HP = hard palate; FM = floor of the mouth; BA = buccal mucosa anterior; BP = buccal mucosa posterior; DT = dorsal surface of tongue; and VL = vermillion border of lower lip. The bottom of the box marks the 25th percentile, the median line the 50th percentile, and the top of the box the 75th percentile. The square symbol in the box marks the mean. The bottom of the vertical line marks the 5th percentile and the top the 95th. The bottom symbol shows the minimum value and the top symbol the maximum value.

Results

The values of the impedance indices showed no dramatic differences at different depth settings. Consequently, only results from the minimal and maximal depth settings are presented (depths 1 and 5). The differences between the left and right side of the same anatomic location were negligible, the relative difference being less than 3% for all indices. The differences obtained from repeated probe settings on the same site were even less, whereas a weak barely visible response induced by sodium lauryl sulfate would typically yield a deviation from base line of 40% for MIX, 70% for PIX, 20% for RIX, and 70% for IMIX (unpublished data).

The mean values for the two sides were therefore used. The results (Fig. 2) showed that the greatest differences in electric impedance exist between the hard palate and the dorsal surface of the tongue, and the least between the anterior and posterior parts of the buccal mucosa, at both depths 1 and 5.

Significant differences ($P \leq 0.001$) were found between most of the studied anatomic areas. However, no such differences were shown for MIX, PIX, or IMIX between the anterior and posterior buccal mucosa, for RIX between the lower lip and the posterior buccal mucosa, or for IMIX between the floor of the mouth and the anterior buccal mucosa. Apart from a few exceptions, notably in the PIX index between the floor

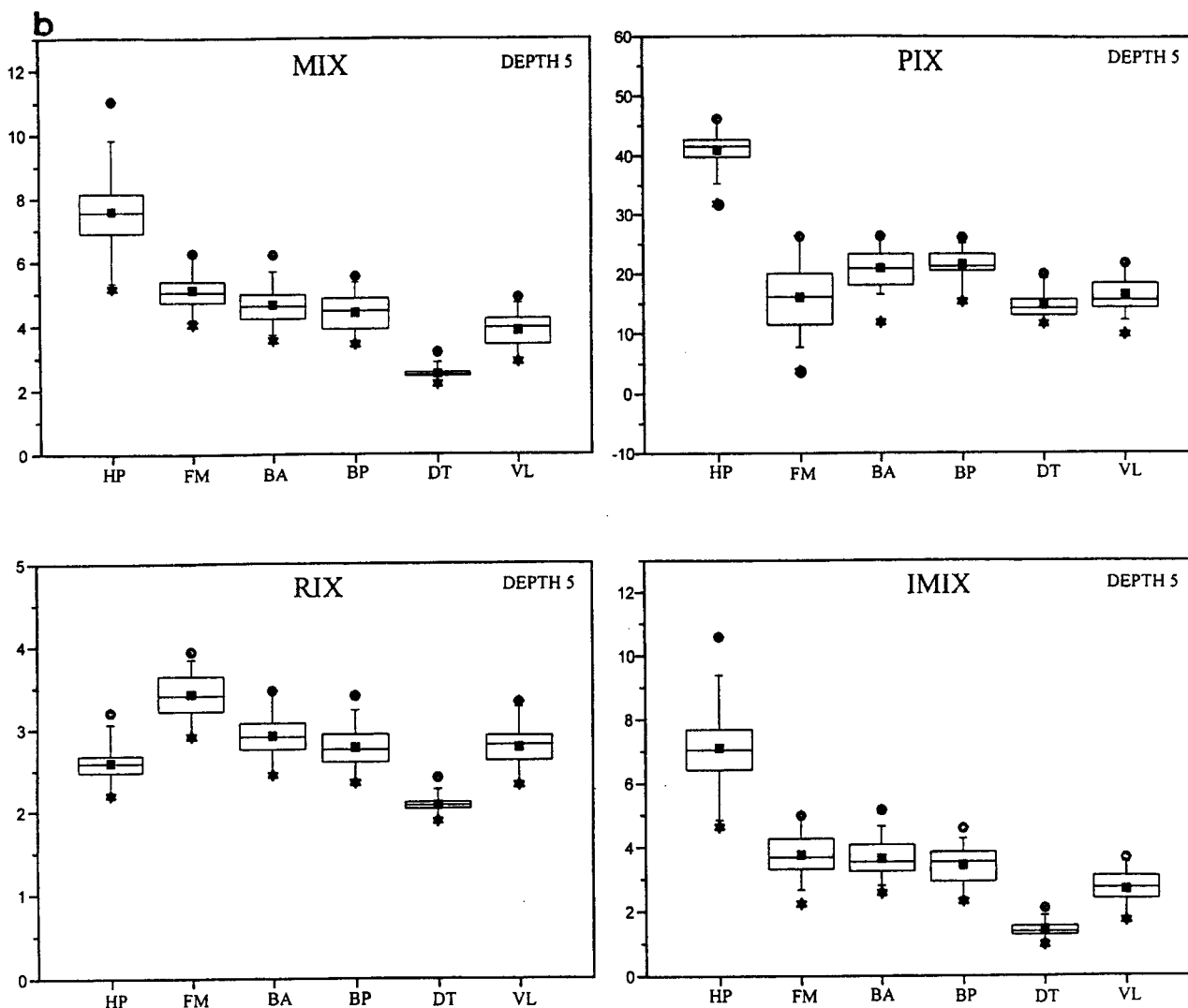


Fig. 2. (continued).

of the mouth and the tongue and lip, respectively, no significant differences were observed between results at the two depth settings.

Discussion

Until now, the mapping of weak oral mucosal reactions or invisible or barely visible oral diseases in an objective manner has been hindered by the lack of satisfactory methods. However, Nilsson et al. (9) proposed an electric impedance technique as a candidate for this purpose, since their device had been able to detect irritant reactions in the buccal mucosa below the visual threshold. The present technology is improved over that used by Nilsson et al. The oral mucosa varies considerably in structure at different places, and collection of base-line data from normal subjects, as in

this study, is essential before experimental work involving mucosal reactions can be properly planned or diseases studied.

The differences in electric impedance at various sites in the oral mucosa are much greater than those in the skin (early skin study using laboratory equipment and only one index (14), and a more extensive study using the same instrument and indices as in a study in preparation by Nicander et al.) due to the considerable structural variations that occur. Three main types of mucosa can be recognized: 1) the oral lining mucosa, which is non-keratinized on, for example, the buccal surfaces and floor of the mouth but keratinized on the lips; 2) the keratinized masticatory mucosa, present on, for example, the hard palate; and 3) the specialized mucosa, which is both keratinized and non-keratinized and is found on the dorsal surface of the tongue (15). The major factor behind the differences in electric

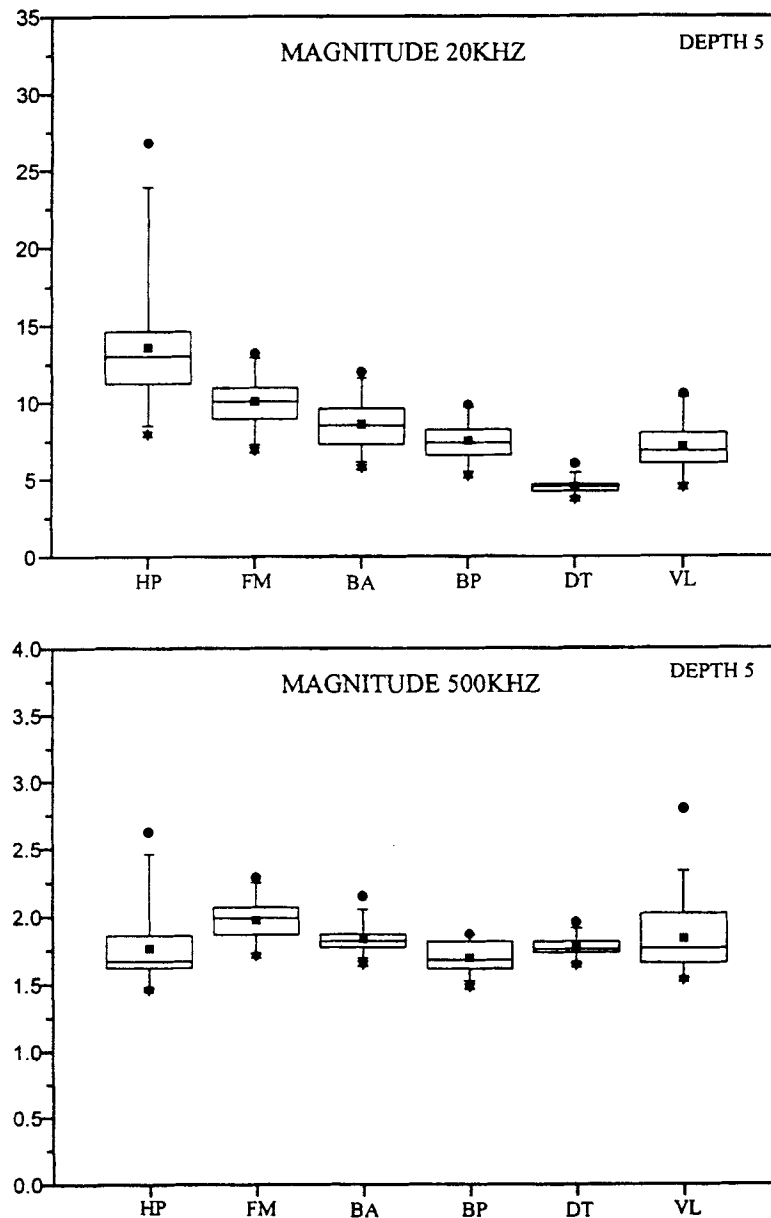


Fig. 3. Box chart for measured values of the magnitude at 20 kHz and 500 kHz. The symbols of the boxes are the same as in Fig. 2a and 2b.

impedance between the different types of mucosa seems to be the degree of keratinization. The most keratinized part, the hard palate, shows the highest electric impedance, whereas the specialized mucosa on the dorsal surface of the tongue gives the lowest value at 20 kHz. This is consistent with the fact (Fig. 3) that the measured magnitudes are dominated by the contribution from the most superficial layer of the mucosa at lower frequencies, whereas the higher frequencies are more readily transmitted by capacitive coupling through this layer and include more information about tissue properties below this level. Interestingly, the data from the

keratinized hard palate are somewhat similar to those of the normal skin, also heavily keratinized.

The four indices, derived from the measured magnitude and phase values at 20 kHz and at 500 kHz, represent changes with frequency in the four major aspects of electric impedance. Our studies on the skin indicate that these indices give optimum weighting over the frequency range spanned and entail a normalization, which reduces inter-individual and inter-site variations (11, 13). Our study of various irritative reactions in the skin (11) suggests the possibility of correlating histologic findings with patterns in the set of

indices—that is, a classification—while each index at the same time can be used to quantify the reaction or deviation from base line. We find this pattern recognition approach, starting with the biologic reality and establishing correlations with information in the impedance spectra, easier to justify than squeezing data into simplistic models of living tissues (13). A similar study, using induced reactions in the oral mucosa, including both electric impedance measurements and histologic evaluation of biopsy specimens is in progress.

As clearly there are significant differences in base-line level for different oral regions but not for contralateral (or buccal ipsilateral) sites in the same region, the latter should be the most suitable to use as controls.

We conclude by emphasizing the importance of using contralateral sites as controls in studies of mucous reactions or diseases, and this factor is more critical for studies of the oral cavity than of the skin. Our findings also indicate that the impedance method used may prove to be an effective tool for measuring, objectively and non-invasively, experimental or pathologic alteration of the oral mucosa, particularly for weak, invisible, or barely visible reactions.

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