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# Permeability of Human Tooth Surfaces Studied *In Vitro* by Electrochemical Impedance Spectroscopy

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**Abstract**

Electrochemical Faradaic impedance spectroscopy was applied to evaluate dependence of the electrical resistance on human teeth. The experiments were performed using iodide anions as a redox probe to model permeability of teeth for fluoride upon an iontophoresis process. Tooth molars were used – as these are teeth most affected by tooth decay processes *in vivo*. Teeth compared included sound molars – with no evidence of pit and fissure decay, teeth with pits and fissures regarded 'clinically' as showing signs of decay, and teeth with crowns removed to present exposed dentin surfaces. A difference of more than an order of magnitude in electrical resistance was observed between sound molars and those regarded as showing evidence of tooth decay processes. Sound dentin, as expected from structural considerations demonstrated further significant reductions in measured resistance. Importantly, the difference in tooth resistance measured between carious and sound molars was shown to be much more representative of their structural integrity than comparison of digitally processed images of the teeth. The results support the utility of electrochemical Faradaic impedance spectroscopy for the development of understanding on how tooth electrical resistance may vary according to structural changes. This understanding may be useful to continued refinements in the use of electrical resistance measures as caries diagnostics and support generically the potential for iontophoretic processes in in-office fluoride treatments of teeth.

## 1. Introduction

Electrochemical methods have been previously applied to analyze the extent of damage in human teeth because of caries, as well to estimate permeability of teeth for various drugs [1]. The methods are usually based on the measurements of a current passing through a tooth [2] or an electrical resistance value [3] and include cyclic voltammetry [2], potentiodynamic techniques [2] and electrochemical impedance spectroscopy (EIS) [1,4-7]. Impedance spectroscopy is a very powerful tool for the analysis of interfacial properties and internal conductivity of electroactive materials upon different biological events occurring at their surfaces and in pores of 3D-structures. Impedance measurements provide detailed information on capacitance/resistance changes occurring at conductive or semiconductive surfaces. Thus, impedance spectroscopy is becoming an attractive electrochemical tool to characterize biomaterial films associated with electronic elements, allowing understanding of biological events at the respective surfaces. Faradaic impedance spectroscopy allows analysis of kinetics and mechanisms of reactions at the electrode surfaces providing important information for the development of the methods protecting the electroconducting materials from unwanted changes and degradation. EIS, also frequently used in various biosensors [8], is the most powerful technique to evaluate the electrical features of teeth and how these may change with structural variation. Commercial electronic caries detectors (CarieScanPro) use EIS for diagnostic purposes [9]. Improved understanding of how structural features contribute to electric properties of teeth may assist in refining the diagnostic and therapeutic applications of voltametric techniques, including the penetration of fluoride which can be applied through in-office iontophoresis [10-17]. While various versions of EIS spectroscopy have been extensively applied for characterization of human teeth *in vitro* [1,4-7], they were usually performed in the absence of redox probes and with little correlation to the teeth surface properties. Direct application of EIS to the analysis of $F^-$ transport into teeth is difficult since this anion cannot be used as a redox probe for the Faradaic impedance spectroscopy. In the present paper we examined application of iodide anion ($I^-$) as a model anion for $F^-$ upon EIS analysis of teeth with different extent of damage. Iodide anion has the advantage of being redox active, thus allowing direct application of the Faradaic impedance spectroscopy to the analysis of its penetration into teeth. $I^-$ is also useful due to the fact that its low reactivity with tooth mineral permits its transport through tooth structures without confounding adsorption loss due to variable tooth structure surface areas. In addition to impedance measures we also analyzed images of teeth with different damage to correlate quantitatively the teeth permeability for $I^-$ with the extent of the damage observed on the external teeth surfaces. Overall, the present work is complementary to the already extensive impedimetric analysis of human teeth [1,4-7] with the specific emphasis on teeth permeability for $I^-$ anions mimicking physiologically important $F^-$ and providing correlation between the electrical properties of the teeth and their visualized surface structures.

## 2. Experimental Section

*2.1. Chemicals.* Sodium iodide 99.5% ACS Reagent was purchased from Sigma Aldrich. Sodium phosphate (dibasic anhydrous) was purchased from Fisher Chemicals. Ultrapure water (18.2 MΩ cm) from NANOpure Diamond (Barnstead) source was used in all of the experiments.

*2.2. Teeth preparation.* Human teeth were collected by dentists and periodontists in the Cincinnati region as part of a Procter and Gamble longstanding program of tooth preservation. Teeth are stored under refrigeration in saturated thymol solutions until preparation for testing. Second molars were selected from the tooth repository and separated into three groups: molars with pit and fissure regions showing no evidence of previous carious activity – sound to both visual examination and tactile probing – labeled as NF; molars with pits and fissures showing clear evidence of decay through visual means (no probing – as this could change the natural permeability) labeled as group CF; and molars with sound pits and fissures for later cross sectioning. In the third group – labeled as D for these studies, a diamond saw was used to section to enamel crown off the tooth below the dental-enamel junctions and fissure depth – but above the pulp region of the tooth. These samples represented teeth with exposed dentin to the oral environment. Selected teeth were mounted and protected with a polymer cylinder that allows only the crown (or surface with the removed crown) to face an external electrolyte solution, serving also as a reservoir for the internal conductivity. The top part of the sample included a screw to wire the tooth to the potentiostat, converting the tooth to the working electrode, Scheme 1. The reservoir allowed introducing the conductive buffer that penetrates into the roots to provide conductivity for the internal surface of the tooth. The teeth were kept refrigerated at 4 ºC, inside a buffer-moisturized box to avoid the dryness and cracks in the samples.

*2.3. Electrochemical Measurements.* Electrochemical measurements were performed with an ECO Chemie Autolab PSTAT 10 electrochemical analyzer using the software package FRA 4.9

(Frequency Response Analyzer) for the Faradaic impedance measurements. The measurements were performed with a three-electrode system in a standard cell (ECO Chemie), using a wired tooth as a working electrode, a Metrohm Ag|AgCl|KCl 3 M as a reference electrode, and a Metrohm Pt wire as a counter electrode. The measurements were carried out at room temperature (23 ± 2 °C). The Faradaic impedance spectra were recorded while applying a bias potential of 0.42 V *vs*. Ag/AgCl, and using a 10 mV alternative voltage in the frequency range 500 kHz – 10 Hz. The tooth was filled in the top part of the support with 600 µL of phosphate buffer 50 mM, pH 7.3. The electrochemical cell was filled with 10 mL of a phosphate buffer 50 mM, pH 7.3, solution containing 10 mM NaI as a redox probe. The impedance spectra were plotted in the form of complex plane diagrams (Nyquist plots). The experimental impedance data were analyzed by the Kramers-Kroenig procedure to confirm true frequency-dependence impedance. The experimental impedance spectra were fitted to equivalent circuits using commercial software (ZView version 2.1b, Scribner Associates, Inc.).

*2.4. Microscopy pictures and their analysis.* The pictures were taken with a MiScope®-MP digital microscope from Zarbeco Co. (Randolph, NJ). Analysis of microscopic images was performed using ImageJ (Java-based image processing freeware program developed at the National Institutes of Health) [18]. The analysis included 27 images of C-teeth (taken from 9 sample teeth) and 27 images of F-teeth (taken from 7 sample teeth). The initial color images were converted into 16 bit black/white images. A threshold of grey level was applied to separate "black" and "white" pixels on the teeth surfaces based on the analysis of the image grey level histograms. Then pixels with the grey level greater than the threshold were converted into black areas and pixels below the threshold were set as white areas, thus converting the teeth images into contrast images with absolutely white and black domains. The ratio of the white and black pixels was used to quantify the damage on the teeth surfaces. The average white-to-black ratio values (based on the analysis of 27 images) were normalized dividing them by the maximum light exposure value, 255 pixels, to achieve the relative number of pixels that represent healthy (white) or damaged (black) domains.

## 3. Results and Discussion

The conductive interface of the working electrode was embedded into a tooth body, thus being protected by the tooth from the direct contacting with the external electrolyte solution, Scheme 1. In this situation we expected to have a high impedance value created by the isolating tooth structure. In order to evaluate the contribution of different parts of the tooth, we started experiments with the teeth without the enamel crown, which itself was expected to be the most highly dense and electrically isolating part of the teeth. A tooth without a crown (sample D) exhibits a porous internal structure to the electrolyte solution, Figure 1, inset. We applied iodide anion, $I^-$, as a redox probe for the Faradaic impedance spectroscopy [19] assuming similarity between $I^-$ and $F^-$ anions but with the advantage that iodide shows low adsorptivity with tooth mineral, hydroxyapatite. Cyclic voltammetry experiments performed on a bare graphite electrode have demonstrated $E_{1/2}$ of ca. 0.42 V for the quasi-reversible redox process of the iodide oxidation, thus this value was selected as the bias potential for the impedance measurements. The obtained typical Faradaic impedance spectrum, Figure 1, shows the dominating mass-transport process (Warburg impedance) in a broad frequency range, while the electron transfer resistance, $R_{et}$, has a relatively small value of ca. 2 kΩ. Thus, the internal porous structure of the tooth (the dentin and pulp) does not create a big barrier for the redox probe penetration and electrochemical process. The obtained value of the $R_{et}$ was considered as a background resistance given by the teeth in case there is no enamel at all.

Next sample teeth for the EIS analysis were so-called carious fissures (sample CF). These teeth represent samples demonstrating significant decalcification damage in enamel – possibly lending to increases in tooth permeability and decreases in tooth resistance. These are shown in Figure 2, left inset. The teeth images were digitally processed to obtain high contrast pictures for the quantitative analysis of the visual damage extent on the crown surfaces, Figure 2, right inset. The obtained typical Faradaic impedance spectrum, Figure 2, shows the electron transfer resistance of ca. 27 kΩ, which is more than an order of magnitude higher than $R_{et}$ for the teeth with the crown removed. This result confirms the expected large contribution to the resistance from the densely organized enamel on the crown – even under circumstances of extensive visual caries activity. It should be noted that the impedance spectra were not perfectly fitted when the classical Randles and Ershler model was applied [20-22]. We analyzed the impedance spectra applying a modified Randles-Ershler equivalent circuit with a constant phase element (CPE) instead of an interfacial double-layer capacitance ($C_{dl}$), Scheme 2. The fitting of the experimental spectra with the equivalent electronic circuit demonstrated small deviation of the CPE from the pure capacitance, which originates from the space-distributed capacitance typical for many modified electrodes [8,23]. The extent of the CPE deviation from $C_{dl}$ could be estimated using the value of parameter n in Equation 1 [8,19]:

$$\text{Eq. 1:} \qquad CPE = A^{-1} (j\omega)^{-n}$$

CPE is converted into pure $C_{dl}$ when parameter n is 1, while the fitting of our experimental spectra resulted in the values of ca. 0.90-0.95 corresponding to almost capacitive meaning of the CPE.

In the final experiments we studied almost undamaged teeth with only small natural fissures on the surfaces designated NF, Figure 3, left inset. The teeth images were also digitally processed to derive the quantitative extent of dark areas on the surfaces, Figure 3, right inset. The typical Faradaic impedance spectrum for this kind of teeth (sample NF), Figure 3, shows very large $R_{et}$ value of ca. 350 kΩ, which dominates over the whole frequency range (no Warburg domain is observed). More than an order of magnitude difference comparing with the caries-affected teeth was observed in the values of the $R_{et}$ for the undamaged teeth. This was attributed to the absence of – or certainly a thinning of the electrically isolated enamel layer in the carries cavities, thus resulting in the dramatic decrease of their electrical resistance, Figure 4(A). Analysis of the digitally processed images of the caries-affected teeth, Figure 2, right inset, and the undamaged teeth, Figure 3, right inset, shows a relatively small difference between them, Figure 4(B). The difference in the cavities observed in the pictures is very small, even showing some overlapping, that can result in difficult diagnostic differentiation through visual means but importantly the difference measured by the Faradaic impedance spectroscopy is one order of magnitude.

## 4. Conclusions

The electrical resistance of teeth is important to diagnosis methods for caries, particularly pit and fissure caries as well as in assessing and refining office based procedures such as fluoride iontophoresis. Obviously, the application of a voltage to drive the fluoride anions or any other ion into the tooth body is going to be very sensitive to the conductance that the tooth presents. The present study has demonstrated that the tooth dentin and pulp is highly conductive for $I^-$ anions used as the redox probe. The existence of caries in fissures produced decreases the enamel layer resistance by more than an order of magnitude comparing with the undamaged teeth while still retaining significant increases in resistance as compared with dentin and pulp internal structures. These observations suggest that the detection of caries though electrical resistance may correlate with efficiency in potential delivery through processes like iontophoresis.

These findings may be useful in clinical applications. It is well known, and verified here, that external appearance of teeth is a poor diagnostic predictor of caries status in pits and fissures. Tactile diagnosis – though slightly more reliable is known to be damaging to teeth and may ensure need for a restoration (filling) and can be counterproductive to institution of topical remineralization strategies. Current laser optical methods, such as Diagnodent [24], cannot assess the suitability of lesions for concentrated iontophoretic therapy. As an integrated alternative, one may consider the use of electrical resistance measures of teeth to complement laser image diagnosed caries to simultaneously assess the susceptibility of advanced lesion areas (not easily seen optically) to focused iontophoretic therapies with fluorides or antimicrobials. Commercial electronic caries detectors (CarieScanPro) use impedance measurements for diagnostic purposes providing an accuracy level of 94.8% in detecting caries and healthy tooth structure and reducing the need for potentially harmful X-rays imaging [9,25]. Extending this approach to the analysis and optimization of iontophoresis will greatly benefit the teeth therapy and improve the health care. Such a non-invasive approach to fissure caries treatment combines established diagnostic (Diagnodent or CarieScanPro) and fluoride therapies in a defined way, being the most advanced technology in dentistry. Although the experimental design used in the present work and the measurements performed *in vitro* are different from those in clinical practice, the results obtained prove that the impedance spectroscopy is the powerful tool not only for the analysis of human teeth, but also for establishing novel therapeutic approaches with the use of iontophoresis. This finding may also have potential implications in designing commercial instrumentation for teeth treatments improving the health care.

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## 5. References


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**Scheme and Figure Captions:**

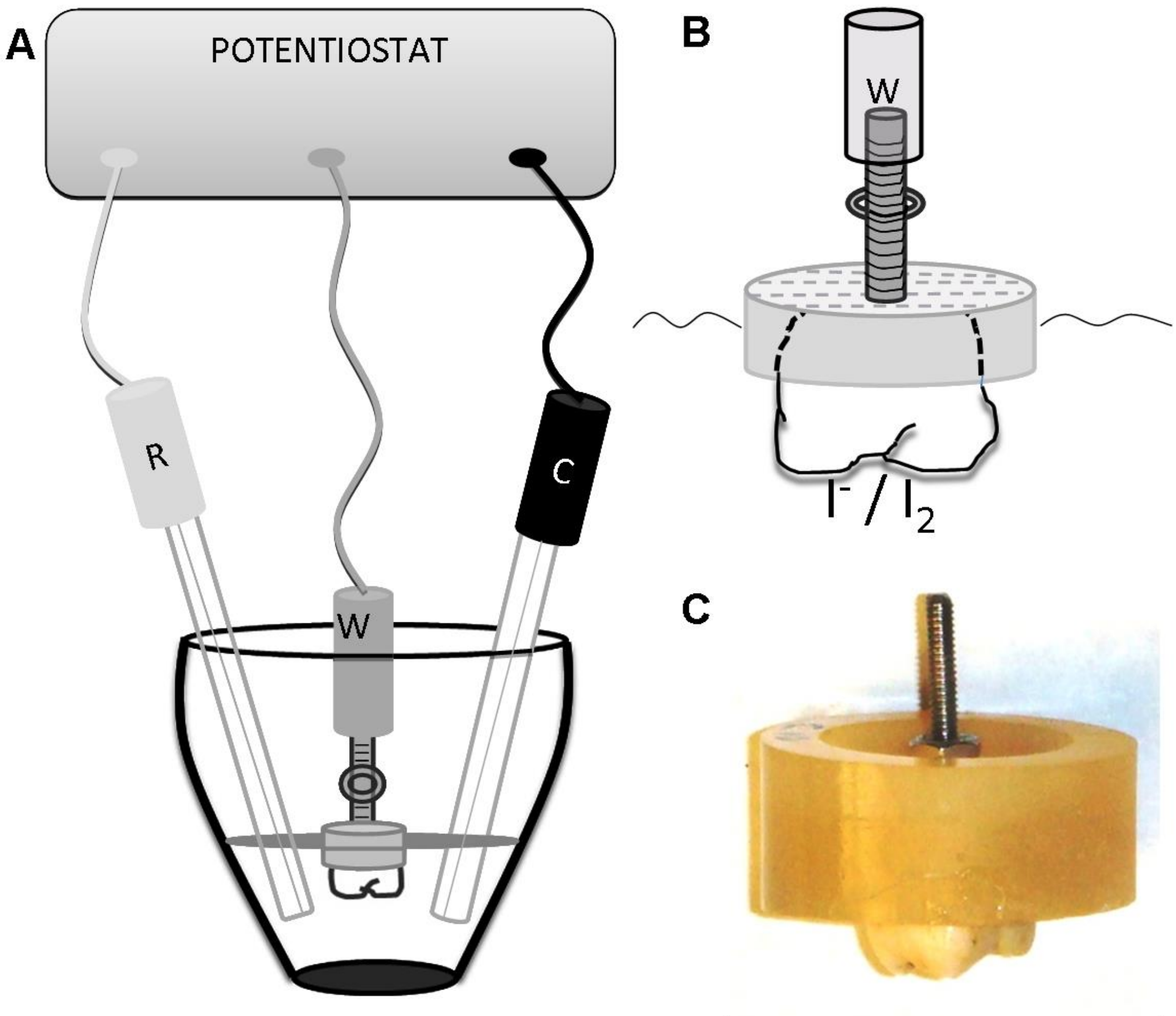
**Scheme 1.** (A) The setup for the electrochemical measurements using a tooth as a working electrode. (B) Electrical wiring scheme for a tooth. (C) Photo image of the tooth wiring.

**Figure 1.** Typical Faradaic impedance spectrum of a tooth with a crown being removed. Inset: Picture of a tooth without the crown showing the ivory part. The experiment was performed in the presence of 50 mM phosphate buffer, pH 7.3, using 10 mM NaI as a redox probe upon application of a bias potential of 0.42 V and an alternative voltage of 10 mV in the frequency range of 500 kHz – 10 Hz.

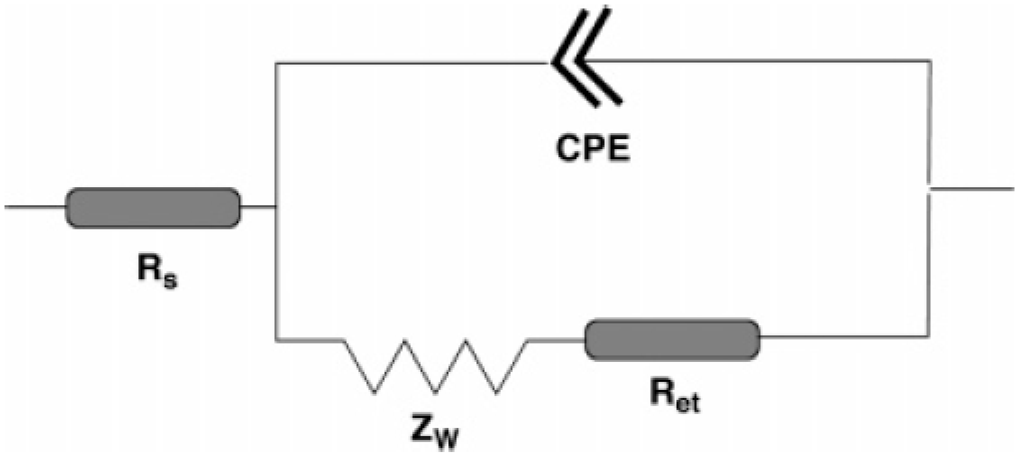
**Figure 2.** Typical Faradaic impedance spectrum of a caries-infected tooth. The insets show the picture of a tooth with caries (left) and the digitally processed image (right). The experiment was performed in the presence of 50 mM phosphate buffer, pH 7.3, using 10 mM NaI as a redox probe upon application of a bias potential of 0.42 V and an alternative voltage of 10 mV in the frequency range of 500 kHz – 10 Hz.

**Figure 3.** Typical Faradaic impedance spectrum of a clean tooth. The insets show the picture of a tooth with some fissures (left) and the digitally processed image (right). The experiment was performed in the presence of 50 mM phosphate buffer, pH 7.3, using 10 mM NaI as a redox probe upon application of a bias potential of 0.42 V and an alternative voltage of 10 mV in the frequency range of 500 kHz – 10 Hz.

**Figure 4.** (A) Comparison of electron transfer resistances ($R_{et}$) for the teeth with caries and clean teeth derived from the Faradaic impedance spectra. (B) Comparison of visual damage on the surfaces of carries-affected teeth and clean teeth derived from the digitally processed images. The higher % of the white areas corresponded to the smaller extent of the surface damage.




**Scheme 1.**




**Scheme 2.**

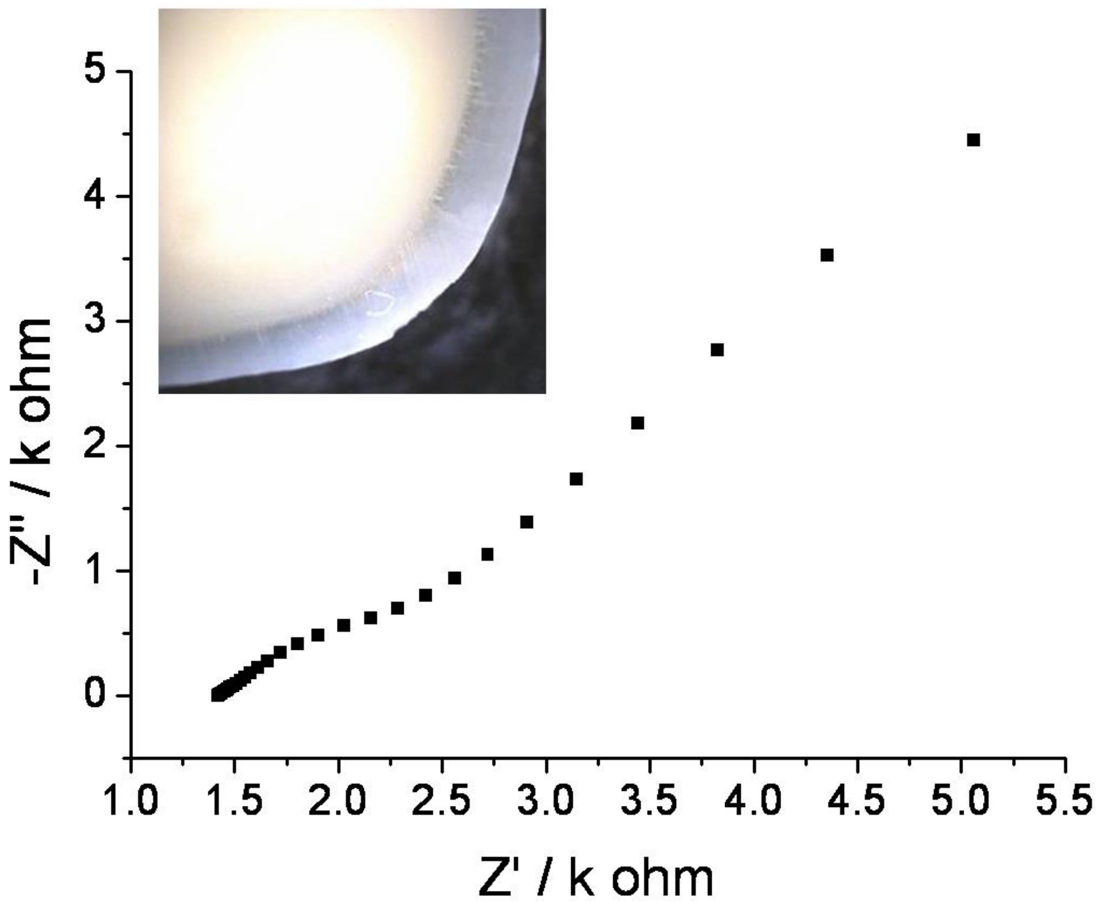



**Figure 1.**

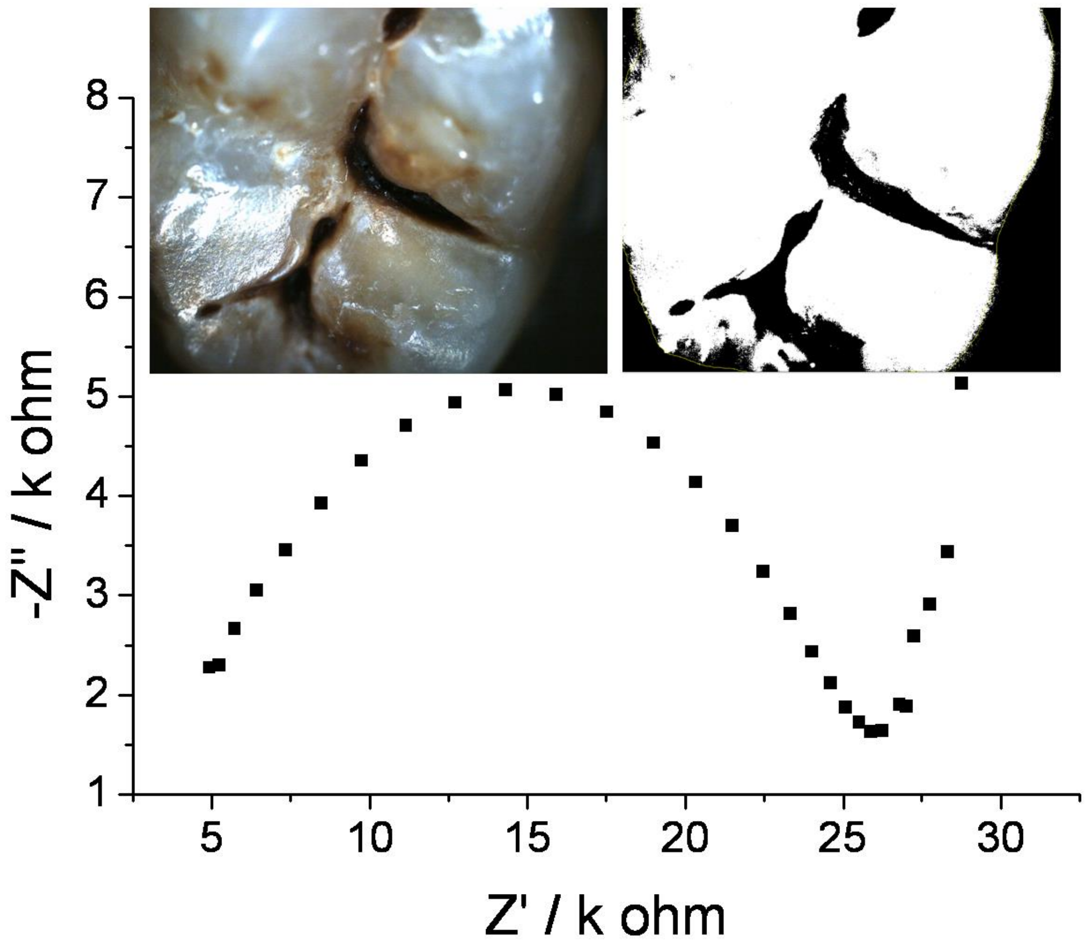
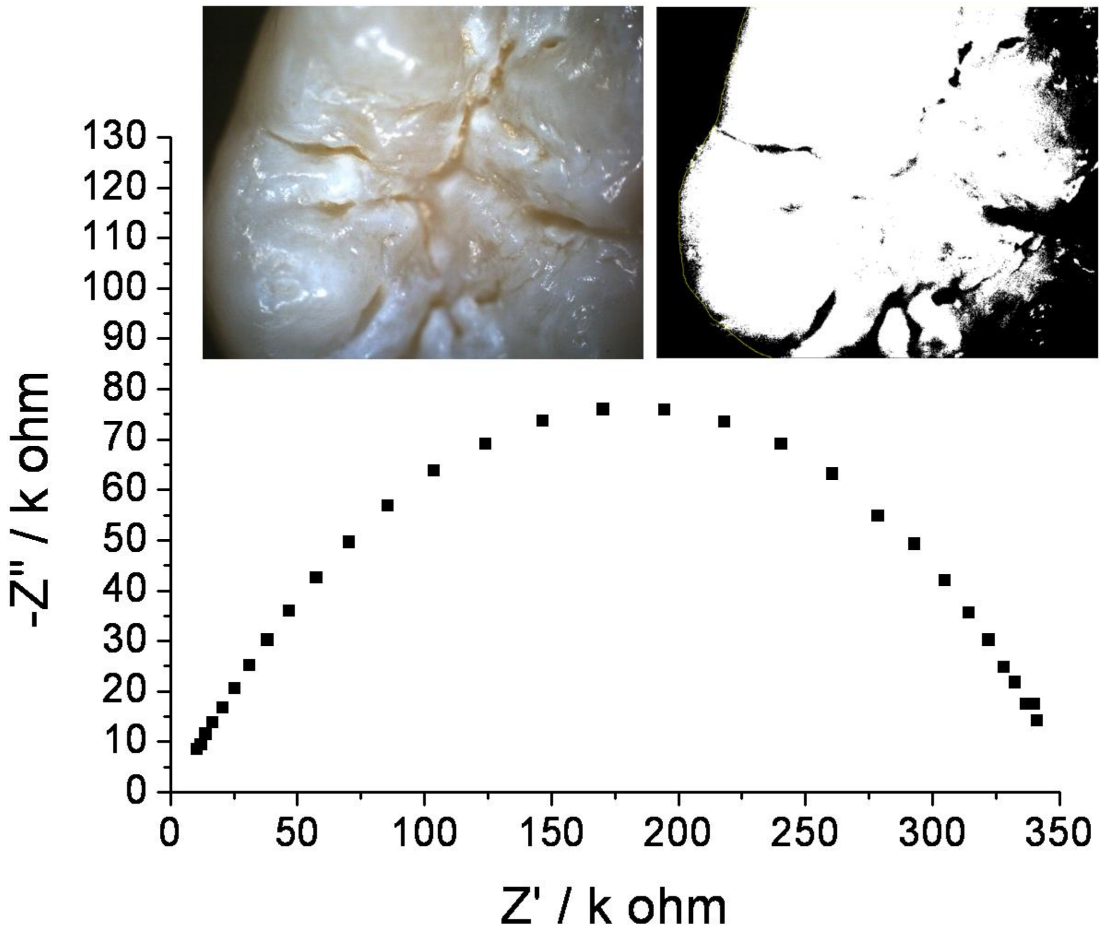



**Figure 2.**


130
120
110
100
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80
70
60
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40
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20
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-Z'' / k ohm
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200
250
300
350
Z' / k ohm

**Figure 3.**

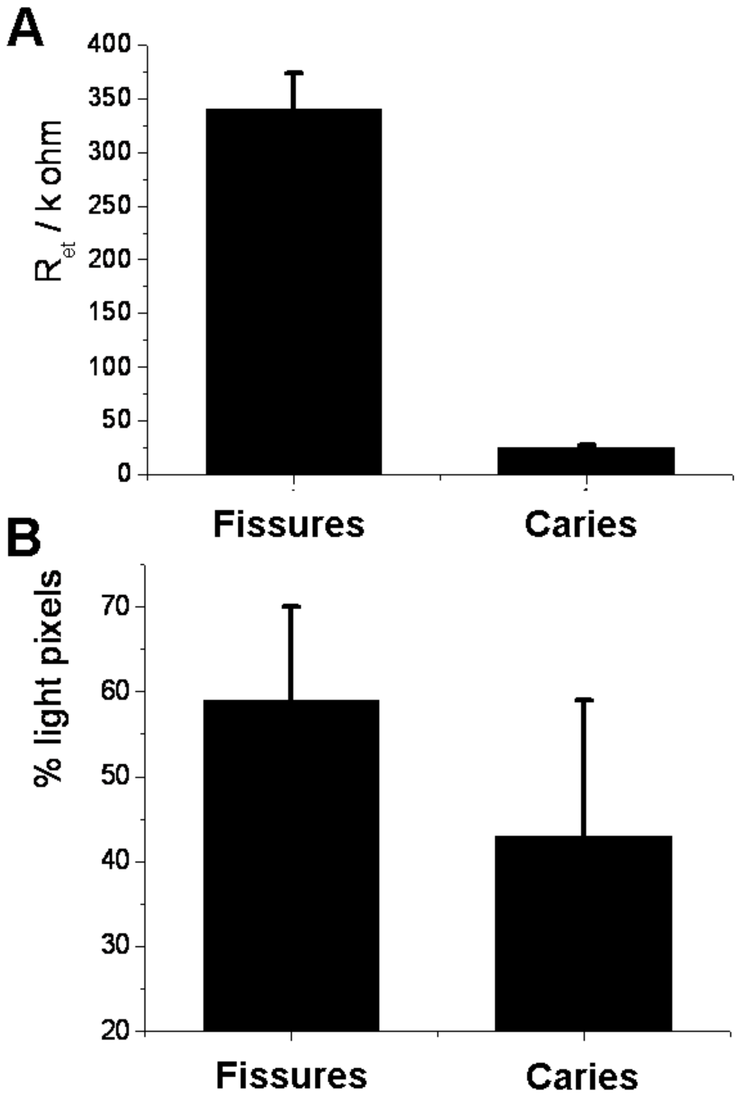
A
400
350
300
250
200
150
100
50
0
$R_{et}$ / k ohm
Fissures
Caries
B
70
60
50
40
30
20
% light pixels
Fissures
Caries

**Figure 4.**