

Preliminary impedance spectroscopy study for carious lesions detection

*Original*

Preliminary impedance spectroscopy study for carious lesions detection / Sannino, I.; Lombardo, L.; Angelini, E.; Parvis, M.; Arpaia, P.; Grassini, S.. - ELETTRONICO. - (2022), pp. 1-6. ( 17th IEEE International Symposium on Medical Measurements and Applications, MeMeA 2022 Messina, Italy 22-24 June 2022) [10.1109/MeMeA54994.2022.9856542].

*Availability:*

This version is available at: 11583/2971814 since: 2022-09-28T14:34:27Z

*Publisher:*

Institute of Electrical and Electronics Engineers Inc.

*Published*

DOI:10.1109/MeMeA54994.2022.9856542

*Terms of use:*

This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

*Publisher copyright*

IEEE postprint/Author's Accepted Manuscript

©2022 IEEE. Personal use of this material is permitted. Permission from IEEE must be obtained for all other uses, in any current or future media, including reprinting/republishing this material for advertising or promotional purposes, creating new collecting works, for resale or lists, or reuse of any copyrighted component of this work in other works.

(Article begins on next page)

© 2022 IEEE. Personal use of this material is permitted. Permission from IEEE must be obtained for all other uses, in any current or future media, including reprinting/republishing this material for advertising or promotional purposes, creating new collective works, for resale or redistribution to servers or lists, or reuse of any copyrighted component of this work in other works.

# Preliminary impedance spectroscopy study for carious lesions detection

Isabella Sannino

Department of Applied Science and Technology, Politecnico di Torino, Turin, Italy

Luca Lombardo

Department of Electronics and Telecommunication, Politecnico di Torino, Turin, Italy

Emma Angelini

Department of Applied Science and Technology, Politecnico di Torino, Turin, Italy

Marco Parvis

Department of Electronics and Telecommunication, Politecnico di Torino, Turin, Italy

Pasquale Arpaia

Department of Electrical Engineering and Information Technology (DIETI), University of Naples Federico II, Naples, Italy

Sabrina Grassini

Department of Applied Science and Technology, Politecnico di Torino, Turin, Italy

DOI: 10.1109/MeMeA54994.2022.9856542

# Preliminary impedance spectroscopy study for carious lesions detection

Isabella Sannino  
*Department of Applied Science  
and Technology  
Politecnico di Torino*  
Turin, Italy  
isabella.sannino@polito.it

Luca Lombardo  
*Department of Electronics  
and Telecommunication  
Politecnico di Torino*  
Turin, Italy  
luca.lombardo@polito.it

Emma Angelini  
*Department of Applied Science  
and Technology  
Politecnico di Torino*  
Turin, Italy  
emma.angelini@polito.it

Marco Parvis  
*Department of Electronics  
and Telecommunication  
Politecnico di Torino*  
Turin, Italy  
marco.parvis@polito.it

Pasquale Arpaia  
*Department of Electrical Engineering  
and Information Technology (DIETI)  
University of Naples Federico II*  
Naples, Italy  
pasquale.arpaia@unina.it

Sabrina Grassini  
*Department of Applied Science  
and Technology  
Politecnico di Torino*  
Turin, Italy  
sabrina.grassini@polito.it

**Abstract**—This preliminary study proposes the use of impedance spectroscopy as additional diagnostic method in clinical practice to assess carious lesions. The carious process leads to a characteristic loss of mineral and a subsequent increased porosity, which results in a higher liquid content than sound tissue. The absorbed liquid contains different ions coming from the oral environment which, together with the increased porosity, contribute to change the impedance of the tissue. Impedance measurement is able to detect such tissue modifications and, therefore, it can be a suitable approach for assessing the presence and the status of carious processes on teeth. Moreover, compared to other diagnostic techniques it is more promising, also for the development of in-vivo measurements, owing to its safety, reliability, simplicity, rapid response, cost-effective, robust, and adequate detection limit. This study compares impedance spectroscopy measurements collected by using two different types of probes for monitoring teeth with and without carious lesions. The authors used a Ni-Cr wire electrode with a diameter of 0.5 mm, and a hydrogel agar probe with a diameter of 5 mm. Impedance measurements were carried out in-vitro by means of the Ivium-n-Stat potentiostat with a two-electrode setup, on the occlusal surfaces of teeth with and without carious lesions; then, the impedance spectra were recorded and analyzed. The preliminary results highlight that both experimental probes allow detecting a shift in impedance phase spectra, which happens at different frequencies and can be correlated to healthy teeth and the carious lesions, respectively.

**Index Terms**—Impedance spectroscopy, caries detection, non-invasive measurements, biomedical measurements.

## I. INTRODUCTION

Dental caries may be defined as a tooth damage that can occur when decay-causing bacteria, within the bacterial biofilm (dental plaque), come into contact with sugars and starches from foods and drinks, they form an acid. This acid

can attack different parts of the tooth, including the tooth's surface or enamel, the dentine immediately underneath the enamel, and the cementum, the thin layer which covers the root, causing minerals lost and leading, over time, to a small hole in the tooth, called cavity [1]. If the dental caries is not treated it can cause pain, anxiety, stress, functional limitations, social handicap and disfigurement by tooth loss, infection and even death.

Dental caries is considered one of the most prevalent chronic disease whose outcome is the dental decay. According to the Global Burden of Disease Study 2017 [2], oral diseases affect nearly 3.5 billion people, hence, the dental caries which afflict 2.4 billion people, is considered the most important global oral health burden.

People of all ages can get tooth decay once they have teeth, from childhood through the senior years; indeed, untreated caries in primary teeth affect more than 530 million people worldwide [3].

Dental caries is a chronic disease whose evolution is slow in most people. In early tooth decay, there are not usually any symptoms. As tooth decay advances, usually when the carious lesion reaches the dentine, it may provoke a toothache or tooth sensitivity [4].

Tooth decay can be found during a regular dental check-up, by accurate visual inspection of clean teeth made by trained examiner [5]. In order to reduce the operator-dependent variability, a standardized visual detection procedure with a clinical scoring system, International Caries Detection and Assessment System (ICDAS), is commonly used for caries detection and caries status assessment, not only in clinical practice but also in dental education, epidemiology, and re-

search [6].

During the clinical detection of caries, sharp dental explorers are often used on diagnosis of fissure caries, causing damage to the enamel already weakened due to the carious attack [7] [8]. Dental caries detection and classification are complex tasks, hence, additional diagnostic methods are required in clinical practice to assess lesions that are invisible at the visual examination, especially if located on the proximal tooth surfaces [9].

The assessment of the dynamic process of caries development, is crucial to properly manage the dental diseases, to establish the prognosis and any preventive intervention. In particular, non-cavitated caries lesions, which are more widespread in developed countries than the cavitated ones, are particularly difficult to detect since a non-cavitated lesion is not visible to the unaided eye. However, false positives and false negatives may occur in the early detection of a lesion mainly due to not so effective detect methods.

Since the assessment of dental caries is strongly operator-dependent, clinical tools are very useful to help make such decisions. The optimal caries detection method should be minimally-invasive, accurate, reliable, easy to apply, suitable for all types of teeth surfaces, which enable targeted and cost-effective management of dental caries in order to improve patients compliance [10].

Several new technologies are nowadays employed by dental practitioners to reduce inter-subjects variability, increase the sensitivity and assess the early carious lesions. Among them, laser or light fluorescence are suitable methods for the quantitative assessment of early enamel lesions in visually inaccessible areas thanks to the change in the light characteristic due to the difference in fluorescence observed between healthy and demineralized enamel [11]. Other devices exploit Near-Infrared Light Transillumination (NILT); these techniques are suitable for enamel caries detection and monitoring by using different wavelengths. Nevertheless, they cannot precisely determine the depth of the lesion in dentin [12].

Some of the most promising approaches are based on Electrical Conductance Measurements (ECM) and Impedance Spectroscopy (IS) [13]. In particular, Impedance Spectroscopy is a fast and non-invasive technique already consolidated in different applications and scientific areas for electrical impedance measurements, such as characterisation of protective coatings and biomaterials, [14] [15] including dental alloys [16], physiological molecules monitoring and drug delivery [17].

The use of impedance spectroscopy techniques in medical field is very promising also for the development of *in-vivo* measurements, representing a safe alternative to ionizing radiography to detect caries, therefore, suitable for young patients and pregnant women [18]. Moreover, this technique could bring the additional advantage to enable the use of portable and low-cost instrumentation [19].

Despite the technological development, the visual and radiographic examinations remain the most common choices, among dental practitioners, owing to their reliability, sim-

ilarity, and adequate validity, although they are unable to detect caries lesions at a very early stage [20]. One of such techniques is the bitewing radiography (BWR), regardless of such limitations, it assesses caries extensions into dentin in relation to the dental pulp [21].

Impedance Spectroscopy, as other X-ray-free diagnostic methods, may fit the urgent need to reduce the potential health risk of ionizing radiations exposure, since the electrical measure detects the variation of electrical resistance or impedance due to the change in teeth structure or composition. The carious process leads to a characteristic loss of mineral and a subsequent increased porosity, which results in a higher liquid content than sound tissue. The absorbed liquid contains different ions coming from the oral environment which, together with the increased porosity, contribute to change the impedance of the tissue [22].

Impedance Spectroscopy is also employed to evaluate root canal length, as well as to characterize the enamel and dentin structures owing to the appreciable higher conductivity of the dentine than the enamel. Moreover, impedance measurements have been employed to investigate microleakage between tooth and filling materials [23] [24] and to investigate the effect of smear layer [25] and dentine conditioners [26].

Nowadays, some devices which exploit alternating current impedance spectroscopy technique, are available on the market, such as CarieScan Pro™ (Orangedental, Biberach, Germany) that is one of the most popular. This device is made of a bundle of wires, which makes the measurement process not straight forward in small teeth area [27] [28].

Caries process may be stopped or reversed, resulting in conservation of the damaged tissue or in a restoration of the demineralized dental tissue. Therefore, it is crucial to take action as early as possible to preserve damaged tissue and to avoid further invasive interventions [29] [30]. The impedance measurements may represent a valuable solution to early caries detection allowing to personalize the treatments and improve patients compliance.

This work deals with the use of Impedance Spectroscopy as a non-invasive method for caries detection, using two different probes, a Ni-Cr wire electrode with a diameter of 0.5 mm, and a hydrogel agar probe with a diameter of 5 mm. Indeed, hydrogels are widely used in impedance measurements in different scientific fields, since they represent a simple and affordable way to prepare reliable, easy to handle, and personalized electrodes [31] [32].

This work is organized as follows: the preliminary study and the measurement procedure is described in section II together with the description of sample preparation: II-A, and the experimental set up: II-B. Meanwhile, the results are presented and discussed in section III.

## II. MATERIALS AND METHODS

### A. Teeth Preparation

In this preliminary study, decayed and healthy teeth are used: two un-erupted human third molars and two decayed canines, extracted within one year for periodontal reasons. All

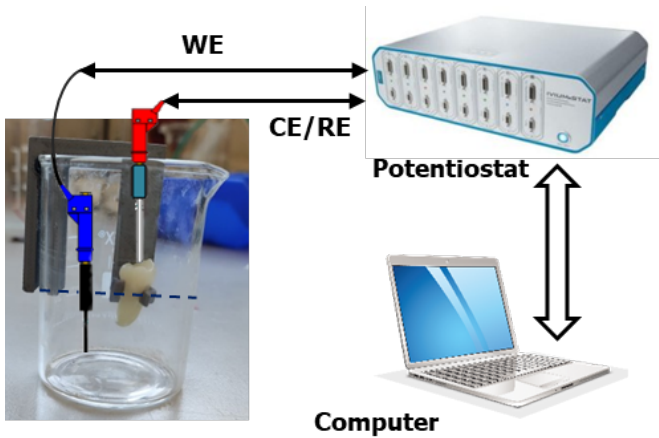


Fig. 1. Impedance measurements experimental setup: the measuring cell include a tooth, placed in 3D-printed PLA support, partially immersed in the Ringer's solution; the working electrode on the tooth surface while the counter electrode is in the solution; a computer controlled Potentiostat is connected to the two electrodes.

teeth were collected with informed consent in the Department of Cariology and Operative Dentistry, University of Turin (Italy). The ethical committee of the University of Turin approved the study protocol (DS\_00071\_2018).

After extraction, the teeth, examined and classified by an expert dentist, were cleaned and soft tissue debris and bone fragments were removed. Then, the extracted teeth were stored in hermetically sealed vials containing 0.5% w/v sodium hypochlorite ( $\text{NaClO}$ ) solution at  $4^\circ\text{C}$ , with the aim to avoid dehydration. All measurements were performed promptly after taking out the specimens from the hypochlorite solution and externally drying with tissue paper. A dedicated 3D-printed PLA support, fixed into the measuring cell, was used as teeth sample-holder, to guarantee the proper measurements execution without damage the tooth.

In order to prevent drying of wet samples and offer good sample visibility during the measurement, almost two-thirds to three-quarters of the tooth root was soaked in Ringer's solution. Among the many different available fluids which simulates the inner environment of a living body, the authors decided to employ the Ringer's solution. This fluid is isotonic with both the blood and artificial saliva, and it simulates very well the oral environment and the aggressiveness of body fluids. Therefore, it is an optimal choice in many in-vitro tests and in the characterisation of the interaction between biomaterials and physiological environments [33] [34]. A great benefit of immersion of soaking the samples in artificial body fluids is the possibility to easily and quickly perform experiments without involving animals in the initial stages of research.

### B. Impedance Measurements

Impedance measurements were carried out in-vitro, on the occlusal surfaces of both healthy teeth and with carious lesion previously visually examined, using the Ivium-n-Stat

potentiostat (Ivium Technologies BV, Netherlands) with a two-electrode setup. Then data obtained with the different methods were processed using the Iviumsoft software.

The first measurements configuration includes two Ni-Cr wire electrodes with a diameter of 0.5 mm; the counter-electrode was dipped in the Ringer's solution, while the working electrode was positioned on the coronal access cavity of the tooth.

In the second measurement setup, two different probes were used as electrodes. A Ni-Cr wire is used as counter electrode, while the other Ni-Cr wire (the working electrode) was embedded with a hydrogel hemisphere of agar with a diameter of 5 mm. The agar hydrogel was used to improve the electric contact with tooth surface and to make the measurement setup more stable. The hydrogel probe was realized by mixing 10 g of dehydrated agar powder (Sigma-Aldrich) in  $100\text{ cm}^3$  of distilled water. Then, the solution was brought to the boil while stirring, until the powder was fully dissolved. Finally, the solution was placed in a special mold and allowed to cool until the solidification.

A schematic representation of the experimental setup, used during both the measurement configurations, is shown in Fig. 1. All impedance measurements were carried out in the frequency range from  $10^{-1}$  Hz to  $10^4$  Hz, using a sinusoidal stimulus of 10 mV, collecting 5 points per frequency decade.

Thereupon, all the teeth samples were analyzed, and consecutive impedance spectroscopy measurements were carried out on the tooth surfaces in different points; subsequently, values at each investigation site were recorded and processed.

## III. RESULTS AND DISCUSSION

Four teeth were analyzed in this study: two without carious lesions and two with carious lesions. All measurements were performed first using a Ni-Cr wire as working electrode and then using the agar hydrogel setup. The impedance measurements carried out with a Ni-Cr wire electrode are presented as Bode diagrams in Fig. 2, where multiple impedance spectra were collected on distinct parts of different teeth, both healthy and decayed.

All spectra show a capacitive-like behaviour at low frequencies and a resistive behaviour at higher frequencies. The former can be associated to the double layer capacitance established at the interface between the probe and the tooth surface. The latter is related partly to the solution resistance and, in a certain extent, to the tooth interface. As far as carious teeth are concerned, the phase reaches its maximum at about  $-70^\circ$  and then decreases to about  $-50^\circ$  in the last part of the spectrum. In the case of healthy teeth, the phase maximum reaches about  $-60^\circ$  and then decreases reaching values close to  $-40^\circ$ .

Measurements carried out using the hydrogel agar probe are reported in Fig. 3. As it can be seen, in this case lower values are measured for the impedance magnitude. Moreover, phase values remain close to  $0^\circ$  for a longer part of the spectrum and, comparing with measurements presented in Fig. 2, these spectra appear less noisy.

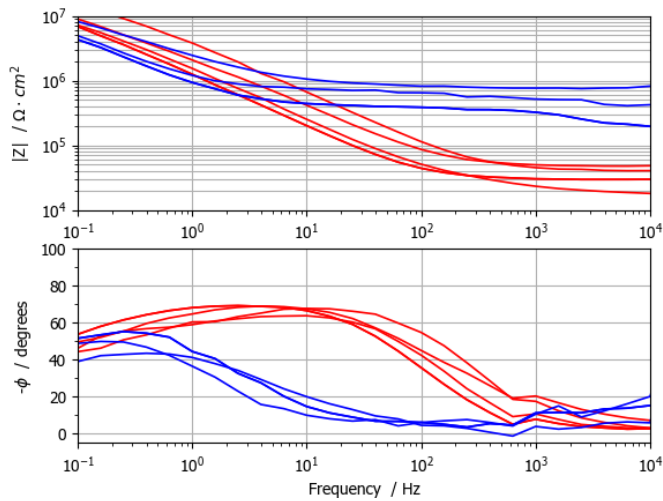


Fig. 2. Red lines: measurements collected on multiple points of different decayed teeth. Blue lines: measurements collected on multiple points of different sound teeth. Measurements performed using Ni-Cr wire electrodes.

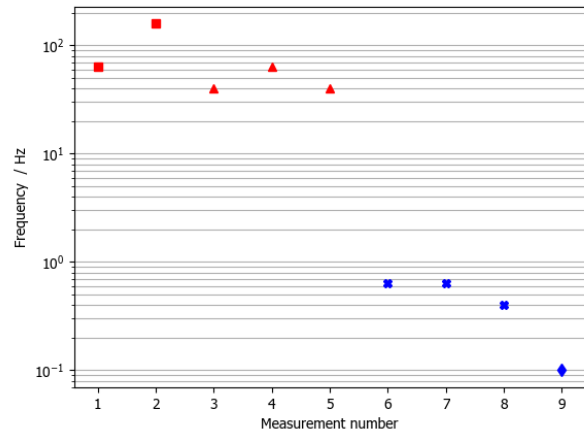


Fig. 4. Frequency at which the impedance phase reaches  $-45^\circ$  both for carious teeth (in red) and for sound teeth (in blue) previously shown in Fig. 2. Measurements performed using Ni-Cr electrodes.

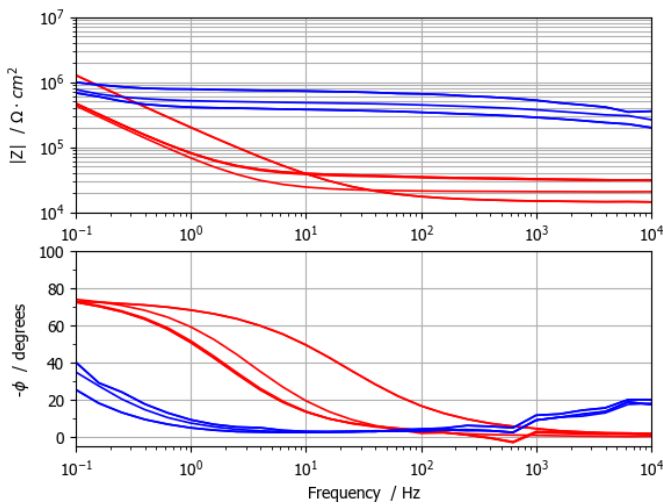


Fig. 3. Red lines: measurements collected on multiple points of different decayed teeth. Blue lines: measurements collected on multiple points of different healthy teeth. Measurements performed using the agar probe.

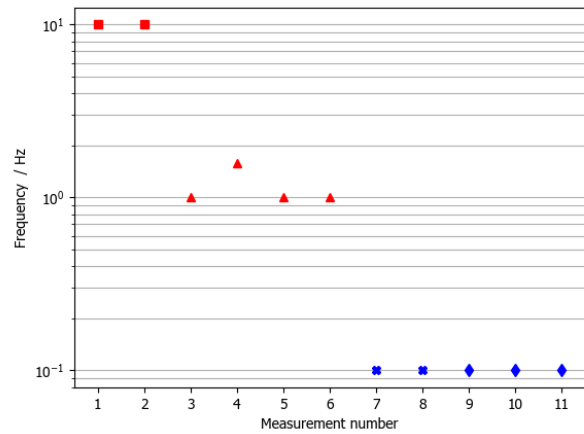


Fig. 5. Frequency at which the impedance phase reaches  $-45^\circ$  both for carious teeth (in red) and for sound teeth (in blue) shown in the previous Bode diagram Fig. 3. Measurements performed using the agar probe.

Also using the agar probe the experiments show a significant difference between sound and carious teeth. Teeth without caries show a longer resistive behaviour compared to decayed teeth. Actually, the behaviour change, from resistive to capacitive-like, occurs at different frequencies depending whether the impedance measurements are performed on sound or decayed teeth. For this reason, the frequency at which the phase reaches  $-45^\circ$  was used as threshold value to classify sound and carious teeth.

Fig. 4 reports the frequency value at which the phase reaches  $-45^\circ$  for different carious and non-carious teeth, measured with Ni-Cr wire electrodes. The same information is reported in Fig. 5, referred to measurements performed with the hydrogel agar probe.

As can be seen, in both cases this parameter is able to

discriminate samples in two different clusters. Carious teeth, represented by red symbols, group in the upper part of the graph, as they reach the  $-45^\circ$  value for the phase at higher frequencies. Conversely, sound teeth are in the lower part of the plot, as in this case the phase value remains close to  $0^\circ$  for a longer frequency range. It is worth to notice that in the case of the agar probe the  $-45^\circ$  value is not even reached, as can be seen in the spectra presented in Fig. 3. For these samples, the lowest measured frequency is reported (i.e.  $10^{-1}$  Hz).

This preliminary study shows the possibility to exploit impedance spectroscopy to monitor sound teeth and carious lesions, and that two different probes, a Ni-Cr wire electrodes with a diameter of 0.5 mm, and an agar probe, can be used to detect a variation in dental tissue electrical characteristics when a carious lesion occurs.

#### IV. CONCLUSIONS

The scientific technological breakthrough of the last century have deeply revolutionized dental diseases management and dental practice. Nevertheless, dental caries still represents the principal chronic disease affecting people. This preliminary study proposes the use of impedance spectroscopy as additional diagnostic method in clinical practice to assess carious lesions.

The preliminary results highlight that both the proposed experimental probes allow detecting a shift in impedance phase spectra, that can be correlated to sound teeth and teeth with carious lesions.

Future works will evaluate the proposed methodology in different solutions such as the artificial saliva, or using different probes. Moreover, other studies will include more samples with the purpose to assess the method accuracy by improving the statistics.

In addition, statistical analysis and more elaborate algorithms will be tested, above all machine learning tools or multivariate analysis [35]–[37]. Furthermore, once validated, the proposed method may be used also to assess the demineralization status of human dentine and a new probe may be developed.

#### REFERENCES

- [1] R. H. Selwitz, A. I. Ismail, and N. B. Pitts, "Dental caries," *The Lancet*, vol. 369, no. 9555, pp. 51–59, 2007.
- [2] S. L. James, D. Abate, K. H. Abate, S. M. Abay, C. Abbafati, N. Abbasi, H. Abbastabar, F. Abd-Allah, J. Abdela, A. Abdelalim, *et al.*, "Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the global burden of disease study 2017," *The Lancet*, vol. 392, no. 10159, pp. 1789–1858, 2018.
- [3] N. Kassebaum, E. Bernabé, M. Dahiya, B. Bhandari, C. Murray, and W. Marcenes, "Global burden of untreated caries: a systematic review and metaregression," *Journal of dental research*, vol. 94, no. 5, pp. 650–658, 2015.
- [4] W. Thomson, "Dental caries experience in older people over time: what can the large cohort studies tell us?," *British dental journal*, vol. 196, no. 2, pp. 89–92, 2004.
- [5] M. T. Merchan and A. I. Ismail, "Measurement and distribution of dental caries," in *Burt and Eklund's Dentistry, Dental Practice, and the Community*, pp. 154–170. Elsevier, 2021.
- [6] A. I. Ismail, W. Sohn, M. Tellez, A. Amaya, A. Sen, H. Hasson, and N. B. Pitts, "The international caries detection and assessment system (icdas): an integrated system for measuring dental caries," *Community dentistry and oral epidemiology*, vol. 35, no. 3, pp. 170–178, 2007.
- [7] O. M. Yassin, "In vitro studies of the effect of a dental explorer on the formation of an artificial carious lesion," *ASDC journal of dentistry for children*, vol. 62, no. 2, pp. 111–117, 1995.
- [8] E. Beltrami, L. da Costa Silveira, and R. Perretto, "The effect of the exploratory probe on demineralized enamel," *Dens*, vol. 6, no. 1-2, pp. 1–4, 1990.
- [9] A. Ismail, "Visual and visuo-tactile detection of dental caries," *Journal of dental research*, vol. 83, no. 1\_suppl, pp. 56–66, 2004.
- [10] A. F. Zandoná and D. T. Zero, "Diagnostic tools for early caries detection," *The Journal of the American Dental Association*, vol. 137, no. 12, pp. 1675–1684, 2006.
- [11] A. A. Adeyemi, F. D. Jarad, G. N. Komarov, N. Pender, and S. M. Higham, "Assessing caries removal by undergraduate dental students using quantitative light-induced fluorescence," *Journal of Dental Education*, vol. 72, no. 11, pp. 1318–1323, 2008.
- [12] M. Abdelaziz, I. Krejci, T. Perneger, A. Feilzer, and L. Vazquez, "Near infrared transillumination compared with radiography to detect and monitor proximal caries: A clinical retrospective study," *Journal of dentistry*, vol. 70, pp. 40–45, 2018.
- [13] A. Jablonski-Momeni, M. Heinzl-Gutenbrunner, R. Haak, and F. Krause, "Use of ac impedance spectroscopy for monitoring sound teeth and incipient carious lesions," *Clinical oral investigations*, vol. 21, no. 8, pp. 2421–2427, 2017.
- [14] L. Es Sebar, L. Iannucci, C. Gori, A. Re, M. Parvis, E. Angelini, and S. Grassini, "In-situ multi-analytical study of ongoing corrosion processes on bronze artworks exposed outdoors," *Acta IMEKO*, vol. 10, no. 1, pp. 241–249, 2020.
- [15] C. Noè, L. Iannucci, S. Malburet, A. Graillot, M. Sangermano, and S. Grassini, "New uv-curable anticorrosion coatings from vegetable oils," *Macromolecular Materials and Engineering*, vol. 306, no. 6, 2021.
- [16] D. Sun, G. S. Frankel, W. A. Brantley, R. H. Heshmati, and W. M. Johnston, "Electrochemical impedance spectroscopy study of corrosion characteristics of palladium–silver dental alloys," *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, vol. 109, no. 11, pp. 1777–1786, 2021.
- [17] G. Annucci, P. Arpaia, U. Cesaro, O. Cuomo, M. Frosolone, S. Grassini, N. Moccaldi, and I. Sannino, "A customized bioimpedance meter for monitoring insulin bioavailability," in *2020 IEEE International Instrumentation and Measurement Technology Conference (I2MTC)*, pp. 1–5, IEEE, 2020.
- [18] M. Mohanraj, V. R. Prabhu, R. Senthil, *et al.*, "Diagnostic methods for early detection of dental caries-a review," *International Journal of Pedodontic Rehabilitation*, vol. 1, no. 1, p. 29, 2016.
- [19] L. E. Sebar, L. Iannucci, E. Angelini, S. Grassini, and M. Parvis, "Electrochemical impedance spectroscopy system based on a teensy board," *IEEE Transactions on Instrumentation and Measurement*, vol. 70, pp. 1–9, 2020.
- [20] F. Litzenburger, G. Schäfer, R. Hickel, J. Kühnisch, and K. Heck, "Comparison of novel and established caries diagnostic methods: a clinical study on occlusal surfaces," *BMC Oral Health*, vol. 21, no. 1, pp. 1–10, 2021.
- [21] F. Schwendicke, M. Tzschoppe, and S. Paris, "Radiographic caries detection: a systematic review and meta-analysis," *Journal of dentistry*, vol. 43, no. 8, pp. 924–933, 2015.
- [22] C. Longbottom and M.-C. Huysmans, "Electrical measurements for use in caries clinical trials," *Journal of Dental Research*, vol. 83, no. 1\_suppl, pp. 76–79, 2004.
- [23] M. Andrei, C. Pirvu, and I. Demetrescu, "Electrochemical impedance spectroscopy in understanding the influence of ultrasonic dental scaling on the dental structure–dental filling interface," *European journal of oral sciences*, vol. 122, no. 6, pp. 411–416, 2014.
- [24] N. Scotti, A. Comba, M. Cadenaro, L. Fontanive, L. Breschi, C. Monaco, and R. Scotti, "Effect of lithium disilicate veneers of different thickness on the degree of conversion and microhardness of a light-curing and a dual-curing cement," *The International journal of prosthodontics*, vol. 29, no. 4, pp. 384–388, 2016.
- [25] P. J. Carrigan, D. R. Morse, M. L. Furst, and I. H. Sinai, "A scanning electron microscopic evaluation of human dentinal tubules according to age and location," *Journal of endodontics*, vol. 10, no. 8, pp. 359–363, 1984.
- [26] N. Pradelle-Plasse, F. Wenger, and P. Colon, "Effect of conditioners on dentin permeability using an impedance method," *Journal of dentistry*, vol. 30, no. 5-6, pp. 251–257, 2002.
- [27] D. Mortensen, I. Hessing-Olsen, K. R. Ekstrand, and S. Twetman, "In-vivo performance of impedance spectroscopy, laser fluorescence, and bitewing radiographs for occlusal caries detection," *Quintessence Int*, vol. 49, no. 4, pp. 293–299, 2018.
- [28] D. Mortensen, K. Dannemand, S. Twetman, and M. K. Keller, "Detection of non-cavitated occlusal caries with impedance spectroscopy and laser fluorescence: an in vitro study," *The open dentistry journal*, vol. 8, p. 28, 2014.
- [29] F. Schwendicke, T. Walsh, M. Fontana, L. Bjørndal, J. E. Clarkson, T. Lamont, C. Levey, G. Gostemeyer, R. M. Santamaria, D. Ricketts, *et al.*, "Interventions for treating cavitated or dentine carious lesions," *The Cochrane Database of Systematic Reviews*, vol. 2018, no. 6, 2018.
- [30] A. Comba, T. Maravic, L. Valente, M. Girlando, S. R. Cunha, V. Checchi, S. Salgarello, F. R. Tay, N. Scotti, L. Breschi, *et al.*, "Effect of benzalkonium chloride on dentin bond strength and endogenous enzymatic activity," *Journal of Dentistry*, vol. 85, pp. 25–32, 2019.
- [31] A. W. Hassel, K. Fushimi, and M. Seo, "An agar-based silver–silver chloride reference electrode for use in micro-electrochemistry," *Electrochemistry communications*, vol. 1, no. 5, pp. 180–183, 1999.

- [32] D. Butler, N. Goel, L. Goodnight, S. Tadigadapa, and A. Ebrahimi, "Detection of bacterial metabolism in lag-phase using impedance spectroscopy of agar-integrated 3d microelectrodes," *Biosensors and Bioelectronics*, vol. 129, pp. 269–276, 2019.
- [33] A. Sobczak-Kupiec, E. Olender, D. Malina, and B. Tyliczszak, "Effect of calcination parameters on behavior of bone hydroxyapatite in artificial saliva and its biosafety," *Materials Chemistry and Physics*, vol. 206, pp. 158–165, 2018.
- [34] C. M. Brett, I. Ioanimescu, and F. Trandafir, "Influence of the biological fluid on the corrosion of dental amalgam," *Corrosion Science*, vol. 46, no. 11, pp. 2803–2816, 2004.
- [35] L. Iannucci, "Chemometrics for data interpretation: Application of principal components analysis (pca) to multivariate spectroscopic measurements," *IEEE Instrumentation and Measurement Magazine*, vol. 24, no. 4, pp. 42–48, 2021.
- [36] N. Giaquinto, M. Scarpetta, M. Spadavecchia, and G. Andria, "Deep learning-based computer vision for real-time intravenous drip infusion monitoring," *IEEE Sensors Journal*, vol. 21, no. 13, pp. 14148–14154, 2021.
- [37] B. Lindholm-Sethson, J. Nyström, M. Malmsten, L. Ringstad, A. Nelson, and P. Geladi, "Electrochemical impedance spectroscopy in label-free biosensor applications: multivariate data analysis for an objective interpretation," *Analytical and bioanalytical chemistry*, vol. 398, no. 6, pp. 2341–2349, 2010.