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***Abstract*—This work deals with the design and development of a non invasive though rather effective solution to detect carious lesions. The main aim of the study is to develop a simple automatic approach for the assessment of the tooth demineralization, which represents the earlier stage of the dynamic carious process, by means of impedance spectroscopy. In particular, impedance measurements were carried out on 50 extracted human teeth. Teeth were demineralized in-vitro according to a validated protocol; then, a morphological analysis of the tooth surface was performed by scanning electron microscopy to confirm enamel demineralization. The proposed approach tries to take advantage of the change of impedance phase due to the demineralization process. Data analysis confirmed that the best frequency for discriminating between demineralized and non-demineralized teeth is about 15 Hz, and this parameter can be used for building up an automatic classifier based on Multilayer Perceptron (MLP) topology. Impedance data were processed by using a modified single neuron, which allows classifying demineralized and sound teeth with an error rate of about 7%, estimating therefore the presence of carious lesions in progress. The presented work can be considered as a feasibility study with a final goal to conceive a simple and low-cost measurement system to identify caries at an early stage**

***Index Terms*—Impedance spectroscopy, multilayer perceptron, tooth demineralisation, tooth lesion classifiers, carious lesions**

I. Introduction

Dental caries is one of the most common oral microbial disease affecting people worldwide [1]. It is the leading cause of oral pain and tooth loss, and it significantly impacts on people's quality of life also due to its correlated infections, anxiety, stress, functional constraints and face disfigurement by tooth loss [2]. It represents a serious health problem, since people are susceptible to the disease throughout their lifetime [3]. Dental caries, also referred as 'tooth decay', is characterized by the progressive destruction of dental tissues through endogenous bacteria located on tooth natural surface in the biofilm (dental plaque). The oral plaque bacteria, by fermenting dietary carbohydrates, produce weak organic acids which demineralize tooth tissues. The acids propagate into the enamel, dentin or cementun and destroy the mineral crystals, mainly composed by carbonated hydroxyapatite, leading thus to tooth demineralization. The expression 'dental caries' or 'caries' can describe both the carious lesion (cavitated or non-cavitated) and caries process itself [4] [5]. At its early stage, the caries is non-cavitated and limited to the enamel surface, early visible lesions, identified as white spots, are visible when the carious lesion progresses, indeed, the mineral concentrations in that spot are generally lower than the surrounding health enamel [6]. If demineralisation advances on the tooth surface, a cavity occurs and grows as a function of time [7].

The demineralization takes place in whichever form and position on the teeth in the mouth. Hence, dental caries can be classified in several categories according to different parameters. They can vary from the incipient caries on the outer enamel surface, which generally are non-cavitated, to the subsurface enamel layer demineralization with pit and fissure caries, or secondary caries, to the lesions that involve a deeper part of the tooth such as enamel caries, dentinal caries, root caries. The caries process is similar for all types of caries and teeth and it always starts with the demineralization. However, human body has its ows mechanism to repair dental caries lesions. Indeed, demineralization can be stopped and even reserved by calcium and phosphate, together with fluoride and other minerals present in human saliva. This process, named remineralization, together with demineralization occur numerous times daily with final outcome the cavitation or the reversal of dental caries [8]–[10].

Dental caries process can be stopped and potentially reversed in its early stages, therefore is crucial to detect them at a very early stage in order to plan proper therapeutic procedures with a tissue-preserving approach and avoid the progression till the tooth loss. The detection of the dynamic process of carious lesions, is decisive to properly manage the prognosis and any preventive intervention, and to assess the efficacy of therapeutic procedure. Regardless all the technological progress, the gold standards for the early detection of dental carious lesions, in clinical practice, remain the employ of visual and visual–tactile tools, such as sharp explorers and dental radiographs [11]. These clinical diagnostic methods are operator dependent and not risk-free; moreover, they are characterized by poor sensitivity and specificity for the early detection of carious lesions [12] [13]. Thus, to support dentists in caries lesion detection and diagnosis the usage of AI, especially deep learning, is growing with promising results [14]. However, the proposed studies are mainly based on images, such as bitewing radiography, cone-beam computed tomography, panoramic radiography [15], or less common clinical techniques like near-infrared light transillumination [16] or optical coherence tomography [17]. The accuracy of deep learning for classifying carious lesion varies greatly according to the used techniques, i.e. it ranges between 68.0% to 78.0% for near-infrared transillumination images, from 87.6% to 95.4% for bitewing radiographs, and it can reach 95.2% with optical coherence tomography images [18].

Consequently, an early accurate and precise diagnosis of the carious lesion is extremely desirable for dental practitioners. In addition, the detection method should be noninvasive, painless, reliable with high sensitivity, and suitable for all kind of tooth surfaces, aiming to preserve dental tissues and overall dental health, as well as permit specific and cost-effective management of dental caries to improve patients compliance.

Impedance Spectroscopy meets these requirements, since it is a fast and non-invasive technique already validated in several applications and scientific areas for electrical impedance measurements, such as characterization of protective coatings and biomaterials, [19] including dental alloys [20], physiological molecules monitoring, and drug delivery [21].

In dentistry, the use of impedance spectroscopy has been widely employed to determine root canal length and to characterize the structure of dentin and enamel, by exploiting the dentine conductivity, which is appreciably higher than the enamel one. Thus, impedance spectroscopy measurements have been applied to evaluate the effect of dentine conditioner [22] and smear layer on dentine impedance, along with the challenging identification between tooth and filling materials [23] [24], but also for carious lesions detection with fluidic probes [25] or carbonated fiber electrode [26].

The application of impedance spectroscopy on carious lesions detection is based on the characteristic demineralization of the carious process, which increase the porosity due to the tooth structure. The more porous structure, compared to sound tooth tissues, is wherefore filled by ions from the oral environment. Electrical measurements can assess this variation by detecting the consequent impedance decreasing [27] [28].

Consequently, the use of impedance measurements in dentistry, may fit the urgent need to reduce the potential health risk of ionizing radiations exposure, and it has a high potential for in-vivo application. Furthermore, another valuable advantage of this technique is the possible implementation on portable and low-cost instrumentation [29]. Among all the medical devices available on the market, the most popular one, which exploits alternating current impedance spectroscopy technique is CarieScan Pro™ (Orangedental, Biberach, Germany).

Unfortunately, the device probe is composed by a bundle of wires, which makes difficult to perform repeated measurements on restricted surfaces [30] [31].

This work deals with the application of Impedance Spectroscopy as a non-invasive technique for detection of demineralization, the earlier stage of carious process. The employed device includes two wire electrodes with a diameter of 0.1 mm as probe. Impedance measurements were performed both on sound and in-vitro demineralized teeth. Data were processed and a classification algorithm was developed to automatically discriminate demineralized and non-demineralized teeth.

II. Methods

A. Sample Preparation and Demineralization Procedure

The limited availability of human teeth and the impossibility to perform in-vivo experiments is the main reason of the development of in-vitro studies. In the last decades, scientific literature widely exploited in-vitro demineralization test to investigate essential processes related to mineral loss and carious advancements [32].

The possibility to use human teeth enables experimental systems to mimic natural caries process. Nevertheless, the dynamic process of dental carious is not easily replicable since the enamel surface is continuously subject to cycles of demineralization and remineralizaion in the oral environment [8].

Moreover, this kind of approach cannot take into account all the environmental factors that affect the enamel demineralization, and despite the use of human teeth is advisable for the study, they present a high inter- and intra-variability, due to several factors, including teeth age.

However, the main benefits of models and in-vitro tests are the possibility to reproduce any kind of carious lesion, e.g, white spot lesion, secondary caries restorations or caries around orthodontic brackets as well as root and dentine caries, in a shorter period of time compared to the natural caries process [33] [34].

Fifty human undamaged front incisors, extracted due to parodontic reasons, were collected for this study. All the 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 were cleaned, disinfected and soft tissue debris and bone fragments were removed. The selected teeth had complete root formation present with no dental

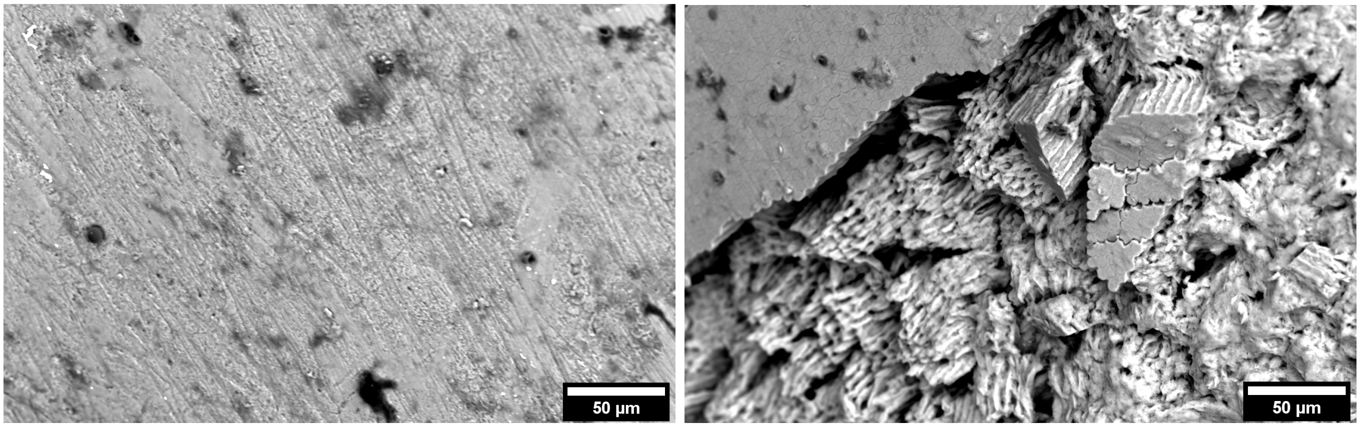

Fig. 1. Scanning electron microscope (SEM) images of sound enamel on the left and demineralized enamel surface on the right.

fillings or sealants on their surface, or evident cracks. Then, the extracted teeth were stored in hermetically sealed vials containing 0.5% w/v sodium hypochlorite (NaClO) solution at 4 °C, in order to avoid dehydration.

Each tooth was sectioned, and the anatomical root cut with a low-speed saw under constant water irrigation. All samples were cleaned and dried with an airflow, and then a selected window of 3 mm x 3 mm on the enamel surface was isolated, while the rest of the tooth surface was covered with a protective organic coating. The selected window was the central section of the third medium of coronal vestibular face, in order to have a surface as flat as possible.

Each sample was immersed in a demineralization solution and incubated at 38 °C for 96 h without stirring. After demineralization, all samples were flushed with distilled water and stored in hermetically sealed vials containing distilled water at room temperature. The demineralization protocol was validated in previous studies [35]. The demineralisation solution was prepared with 0.05 mol/L acetate buffer (pH 5.0), 1.28 mmol/L of Ca ($CaCl_2$), and 0.74 mmol/L Pi (KH_2PO_4).

All the measurements were performed before demineralisation and immediately after taking out the samples from the solution in order to monitor any change in tooth structure.

Furthermore, a morphological characterisation of the demineralized teeth has been performed by scanning electron microscopy (Phenom XL G2 Desktop SEM, Thermo Fisher Scientific), in order to confirm enamel demineralization. SEM observations have been performed in low vacuum conditions without any surface or dehydration treatment.

SEM images were acquired using an acceleration voltage of 15 kV working at distance of about 8.5 mm. Fig. 1 shows a SEM image of a sound enamel (on the left) which presents a smooth surface with some pits and scratches. After demineralization (Fig. 1 on the right) the enamel surface exhibits the loss of enamel prism core but retained periphery; residual hydroxyapatite crystallises show a rod-like morphology in the lesion on the enamel surface.

B. Impedance Measurements and Data Acquisition

Impedance measurements were performed both on sound and demineralized teeth. In order to ensure an appropriate measurement procedure without any tooth damage, a 3D-printed PLA holder was specifically designed in order to fix the tooth sample inside the measurement cell.

The sample was soaked in a physiological saline solution (0.9% w/v sodium chloride - NaCl - solution) to avoid dehydration and guarantee a good sample visibility during the measurement. The impedance measurements were performed using an Ivium-n-Stat potentiostat (Ivium Technologies BV, Netherlands), with a two-electrode configuration which includes two platinum electrodes with a diameter of 0.1 mm. The working electrode was positioned on the selected polish free section of the central dental crown, while the counter electrode was dipped into the physiological saline solution. Impedance measurements were acquired using a sinusoidal stimulus of 10 mV, in the frequency range from 10^{-1} Hz to 10^4 Hz, collecting 5 points per frequency decade.

In the selected enamel area, different measurements on different points were recorded and processed with Ivium software. The frequency response of impedance magnitude and phase were recorded on the different teeth and compared. Fig. 2 and Fig 3 show the recorded impedance on two teeth before and after demineralization (96 h of immersion). Different traces correspond to different points on the same tooth. As can be seen, all spectra show a capacitive-like behaviour at low frequencies which becomes resistive approaching higher frequencies. The resistive behaviour is related to the solution resistance, and in a certain extent, to the tooth interface. While, at lower frequencies the capacitive behaviour is due to the double layer capacitance settled at the interface between the tooth surface and the probe. In particular, Fig. 2 refers to a tooth which was demineralized after the treatment, while Fig. 3 shows the spectra collected on a tooth which didn't experienced any demineralization nevertheless it was subjected to the same treatment. It is clearly visible that the demineralized tooth exhibits a remarkable phase shift due to the surface demineralization, while no significant change is visible in the

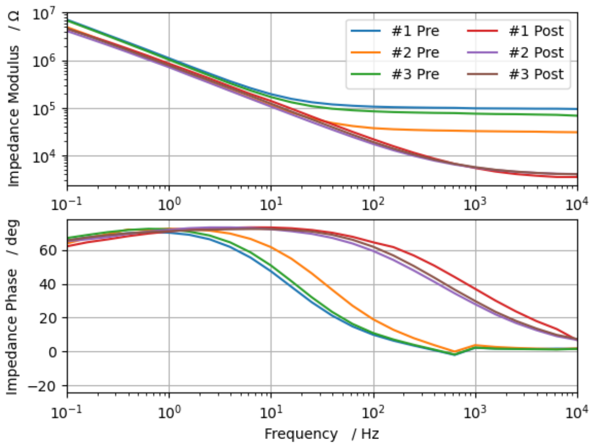

Fig. 2. Impedance spectra collected on a demineralized tooth. Spectra were collected on three different areas on the tooth surface, before and after demineralization. A phase shift of about one decade due to the demineralization is clearly visible.

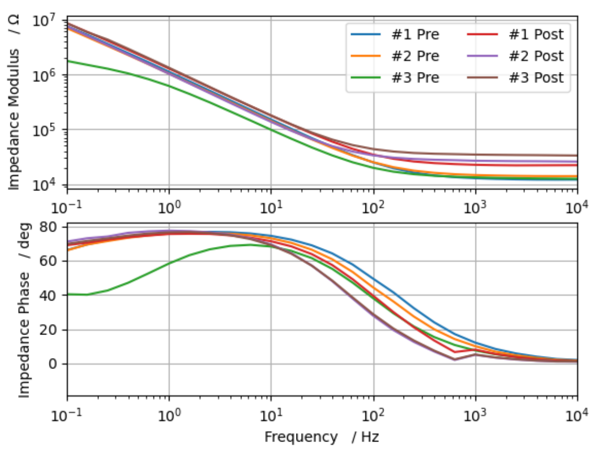

Fig. 3. Impedance spectra collected on a non-demineralized tooth. Spectra were collected on three different areas on the tooth surface, before and after the demineralization treatment. However, the measurement area didn't exhibit any demineralization and, therefore, no significant phase shift is visible in the spectra.

other tooth.

III. Classification Algorithm

The principal aim of this work is to develop a simple automatic approach for the assessment of the demineralization of teeth by performing impedance measurements on the teeth surface. The proposed approach tries to take advantage of the change of phase due to the demineralization process. Thus, an automatic classifier was developed by employing a modified single neuron as shown in Fig. 4

In order to assess the best parameters for the classifiers, preliminary data analysis was carried out on the acquired spectra. It is clear that the phase shift due to the tooth

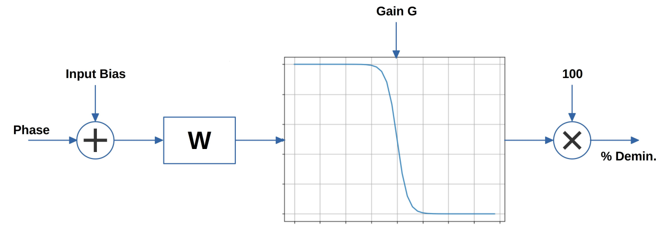

Fig. 4. The employed classifier using an inverted sigmoid as decision function.

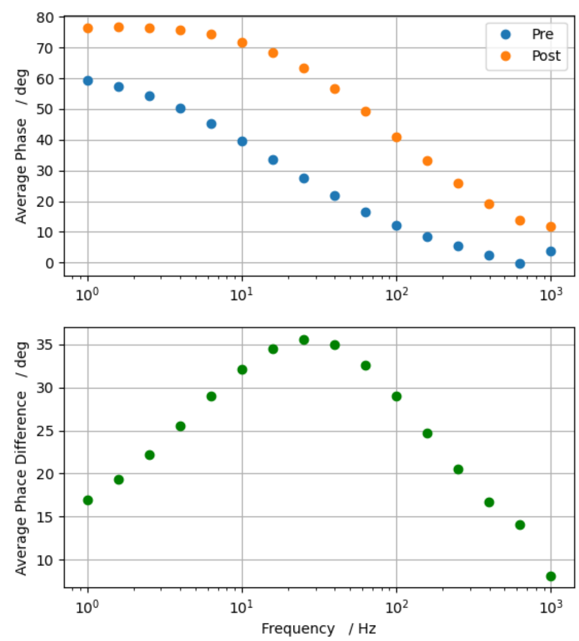

Fig. 5. First plot shows the average phase of all samples, where blue dots represent measurements carried out before the demineralization, while orange dots post-demineralization. The bottom plot shows average phase difference before and after demineralization for each frequency. The frequency spans in the range 1 Hz to 1 kHz. The highest value of average phase difference is achieved at a frequency of about 15 Hz.

demineralizzation is the main parameter that should be taken into account.

A total of about 110 spectra were processed. In particular, about 70 spectra refer to demineralized teeth while other 40 to non-demineralized ones. The first analysis was carried out by computing the average phase difference before and after demineralization for different values of frequency from about 1 Hz to 10 kHz. The results, shown in Fig. 5 (on top), highlight a remarkable phase change considering the measurement before and after treatment. Then, it was computed the average phase difference between the pre- and post-treatment samples with the aim of finding the best frequency value for assessing the demineralization of the teeth. The average phase difference is reported in Fig. 5 (at the bottom). It is evident how the higher the phase difference, the simpler and more effective it will be to discriminate between demineralized and non demineralized teeth. Therefore, the best frequency for assessing demineralization was taken as 15 Hz.

Subsequently, a modified single neuron classifier was developed so that given as input the phase at 15 Hz it is able to provide at its output a value between 0% and 100%, where 0% corresponds to a totally non-demineralized tooth and 100% a totally demineralized one. Therefore, a threshold of 50% was employed to discriminate between the two possible situations. The classifier was designed to employ an inverted sigmoid decision function. The key parameters which allow the classifier to properly operate are the Input Bias, the weighting Factor W and the Sigmoid Gain G. These parameters were set trying to minimize the error rate of the classifier according to the following considerations:

1) the bias was set at the average phase value of the demineralized teeth plus the demineralized phase standard deviation multiplied by 1.5, obtaining a value of 65.5°.

2) The sigmoid gain defines the smoothness of the decision function. In particular, setting an high gain gives a steep function, while a value lower than 1 provides a smoother sigmoid. The best results was obtained with a value of 2.

3) The weighting factor was set directly to 1 in order to avoid further changes in the input phase.

Then the available tooth measurements were processed by the classifier by using as input the single phase value at 15 Hz. The classification results are shown in Fig. 6. Here the demineralization state is reported as percentage of demineralization, being 100% the expected output for demineralized tooth and 0% the expected output for a non-demineralized one. About 100 teeth were processed by the classifier (about 75 demineralized and 25 non-demineralized, accordingly to the available spectra). As it is possible to see most of the teeth are correctly classified.

In total, the classifier failed on only 7 teeth over about 100 achieving an error rate of about 7%. In particular, 3 demineralized teeth were classified as non-demineralized, and 4 non-demineralized teeth were classified as demineralized.

IV. Conclusions

This work is aimed to investigate the possibility to discriminate between sound and demineralized teeth by using impedance spectroscopy measurements. The first set of measurements was performed on all the samples before the demineralization procedure, then on the same demineralized sample in order to prove that a change in dental tissue electrical characteristics occurs if the tissue is demineralized. Results are showed in Fig. 2 as Bode diagrams, where multiple impedance spectra were collected on distinct parts of the same tooth teeth, before and after the demineralization.

Fig. 2 and 3 point out that the change in the phase spectrum is related to demineralization. Moreover, data analysis confirmed that the best frequency for discriminating between demineralized and non-demineralized teeth is about 15 Hz, and this parameter can be used for building up an automatic classifier base on a single neuron. This behavior paves the way

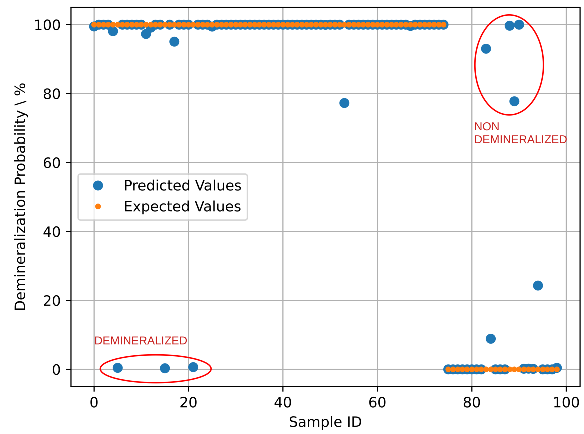

Fig. 6. The results of the employed classifier. Blue dots are the predicted values of the neural network on all teeth, while orange dots are the expected values. Classification errors are highlighted by red circles.

for implementing an easy and effective identification of carious teeth. In addition, working at a fixed frequency is a plus in this case as it is possible to conceive a simple and low cost system to identify caries at an early stage. The low frequency involved and the large phase difference can be acquired by a Teensy 3.6, which has embedded both the DAC, to generate the signal stimulus, and the ADC to measure the current. At the same time, the Teensy board has the computing capability to implement the classifier, thus, enabling the possibility to have a portable non-invasive device for the direct detection of carious processes.

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