Review

Electronic Nose and Tongue for Assessing Human Microbiota

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Abstract: The technological developments of recent times have allowed the use of innovative approaches to support the diagnosis of various diseases. Many of such clinical conditions are often associated with metabolic unbalance, in turn producing an alteration of the gut microbiota even during asymptomatic stages. As such, studies regarding the microbiota composition in biological fluids obtained by humans are continuously growing, and the methodologies for their investigation are rapidly changing, making it less invasive and more affordable. To this extent, Electronic Nose and Electronic Tongue tools are gaining importance in the relevant field, making them a useful alternative—or support—to traditional analytical methods. In light of this, the present manuscript seeks to investigate the development and use of such tools in the gut microbiota assessment according to the current literature. Significant gaps are still present, particularly concerning the Electronic Tongue systems, however the current evidence highlights the strong potential such tools own to enter the daily clinical practice, with significant advancement concerning the patients' acceptability and cost saving for healthcare providers.

1. Introduction

There has been a continuously growing interest in the scientific and, more at large, in the technological world over the past years about the creation of machines, tools and, to a larger extent, infrastructures, able to resemble the five senses, on which the human perception relies on, with alternate fortune. Focusing in particular on some specific sensory channels, special attention should be paid to chemical ones, that is to say the sensory modalities with whom the human being interacts with the surrounding environment thanks to chemically driven phenomena. In more depth, the human chemical senses, including olfactory and taste ones, rely on arrays of receptors situated in the nasal mucosa and in the tongue, respectively, which also act as transducers, being able to convert the chemical stimuli into an electrical signal, a system of transport of the obtained signal and a first line center of elaboration [1,2]. This implies that, in order to replicate the biological reality, the technology mentioned above must have an equivalent device corresponding, at least functionally, to each step of the process described above. In particular, the receptors and transducers, i.e., the sensing portion of the pathway, are actually replaced by an array of sensors (or biosensors), which vary in type and materials according to the kind of the chemical stimuli they must respond to. On the other hand, when it comes to the signal transportation system, it is matched by a series of amplifiers and, eventually, an A/D converter, which ultimately allows it to have a signal that is analyzable on a computer and has the smallest amount of noise possible. Finally, the center of elaboration of this complex system is replaced by computational algorithms, nowadays matched by Machine and Deep Learning tools, as well as other Artificial Intelligence algorithms. Overall, among those, Artificial Neural Networks are probably the most popular ones in this regard [3–6].

Through such devices, we are able to distinguish typical fingerprints due to the chemical compounds composing a gas mixture or a liquid solution. This ability is known to be very useful in many sectors, including the food industry [7], but also environmental protection [8] or safety appliances [9]. However, some of the most promising applications of artificial sensing technology in the research framework concern the biomedical field, and are also conceptually close to the basic idea of artificial senses, due to them somewhat mimicking the biological senses on which such technologies rely on, as previously argued. To this extent, the detection or characterization of certain chemical compounds in biological fluids, gases, as well as liquids, could help us to study the presence of biological processes in the human body, particularly those eventually related to the presence of any state of disease or unbalance with respect to the homeostasis of an organism [10]. Focusing in particular on pathological conditions, artificial sensing systems are therefore used, with this respect, for helping the detection of several clinically relevant disturbances, including respiratory disorders [11], cardiovascular diseases [12], neurodegenerative conditions [13], cancer [14], and a plethora of diseases of other kinds.

However, there are a number of conditions, particularly those somewhat related to such a metabolic unbalance, that could be indirectly controlled through some end products brought to the human body. As such, microorganisms such as bacteria, protists or microscopical fungi, eventually located in the human organism in either physiological or pathological conditions, could represent an interesting biomarker to be investigated in order to collect useful information about the pathological state an individual has been experiencing [15]. To this extent, a particular interest is growing concerning the analysis of the human microbiota, one the most important parts of which resides in the human intestine and is referred to as the gut microbiota. This specific assessment is of particular interest not only as gut microbiota is somewhat involved in the pathophysiology of multiple diseases (e.g., cancer, neurodegenerative conditions associated with aging, etc. [16,17]), but also since it affects the organism's physiology, e.g., its metabolism [18]. Nowadays, the gold standard tests to analyze the composition of the human microbiota involve various techniques, among which some such as spectroscopy and gene sequencing are widely used [19–21]. The application of those techniques, when compared to the use of E-nose and E-tongue technologies, is quite complex and expensive, therefore they are deemed not particularly convenient in naturalistic frameworks or they are thought to be poorly affordable when attempting to perform such investigations in the poorest countries of the world. Under such premises, the present review aims at showing the potential of electronic nose and tongue devices as a valid, more affordable and widespread alternative to the techniques most currently used for the detection of the microorganisms present in the human body. If confirmed to be viable, such approaches could form the basis for a large-scale investigation of the human microbiota, to be expanded, with the help of innovative Data Science methods and tools, within the wider framework of the ever more popular and emerging "p4 (predictive, preventative, personalized, participatory) medicine" [22]. Also, the dissemination of the good results and potential eventually brought by both E-nose and E-tongue tools could play an important part in the raising awareness devoted to the scientific community and eventually expanding to the clinical community, and could help to enhance their use beyond their current remit to date.

2. How Does an E-Nose Work?

Among the various kinds of artificial senses that could eventually be useful to investigate the biological matrices associated with human beings, and aimed at studying the presence and eventual composition of the human gut microbiota, chemical senses are the most widely investigated methods. In fact, they enable the assessment of the (bio-)chemical properties of such biological matrices. Therefore, E-nose and E-Tongue tools could probably represent the most suitable approach to the problem.

Overall, as mentioned above, E-nose tools are replacing more traditional analytical equipment in recent times, ideally providing benefits to those aspects where gas chro-

matography (GC) systems, mass spectrometers (MS), ion mobility spectrometers (IMS) and optical systems display more issues. In fact, such approaches include an excellent stability of the signals, counterbalanced by their high complexity, aside from where E-noses can be indeed more competitive [23]. Specifically, throughout the years, and even more in the last decades, multiple approaches have been used to design E-nose tools however, to date, the "gas-sensors electronic nose", which could be defined as "the classical approach", is still the most common as it is the one that best resembles human olfaction [24]. This approach consists of an array of sensors, which are supposed to be the equivalent of biological olfactory receptors, capable of detecting and, with proper signal processing, somewhat distinguish the chemical gaseous substances eventually present within a given sample or a defined environment (Figure 1). The biggest drawback this type of E-nose presents is that the individual sensors cannot be manufactured with high enough specificity to fully replicate the human olfactory neurons. Indeed, it should be stated that biological olfactory receptors are incredibly selective; a quality that requires the artificially manufactured receptor to have an irreversible interaction with the target chemical gas detected [25,26]. To go into depth on this specific argument, in fact, it should be specified that the human receptors have a relatively short life (generally one or two months), although on the other hand, artificial sensors need to be able to work for a much longer period, otherwise the costs of this kind of technology would be too high. Indeed, it is worth stating that the affordability of their choice with respect to more traditional methods is probably one of the main characteristics for which they can be considered a valid alternative to gold-standard methods [27].

The approach just mentioned can vary according to the type of sensors included. The ones most commonly employed in such a similar tool include the MOS (Metal Oxide Sensors), also referred to elsewhere as "chemiresistors", and being composed of n-type metal oxides. Such large-scale adoption of those devices is significantly fostered by their low cost, if compared to other devices, allowing their significant spread into the market and also enabling their use within large sensor arrays featuring devices with complementary characteristics [28,29]. Overall, their structure is composed of various grains of metal oxide and contains, therefore, lots of pores where the air full of O_2 enters, making the latter accumulate on the surface of the grains. This way, the transportation of electrons through the n-type metal can occur as the O_2 acts like an acceptor, given its nature as an oxidizing gas and not a reducing one. Consequently, at the interface with the surrounding ambient containing O_2, a depletion layer originates and expands according to the entity of the previously mentioned oxidation-reduction reactions [30]. Overall, this sequence of events leads to an increase in the energy needed for an electron to enter the conduction band and, as a direct consequence, a drop in conductivity and a rise in the electrical resistance occurs. Into this framework, the adsorption of O_2 creates a physisorption effect, which is a type of adsorption where no chemical bonds are formed, however only Van Der Waals interactions occur; that is to say weak bonds that could increase selectivity [31]. In this situation, the layer of grains is put between two electrodes, which are used to measure the modulation of the electrical resistance associated with the voltage and the current generated.

Instead of using electrodes, the layer of grains can also be used as the metal gate of a MOSFET, using Pt as the metal and SiO_2 as the substrate. It has been proved that silicon carbide, instead of simple silicon as the substrate, allows us to obtain MOSFETs correctly operating at higher temperatures [32]. Indeed, temperature is a very important factor for the proper functioning and operation of any kind of sensor. When used to detect volatile organic compounds (VOCs), this kind of technology goes through a reducing phase, which is caused by the reactions eventually occurring between the adsorbed oxygen and the VOCs themselves, which reduces the surface density of the former and therefore inhibits the receptive effect of O_2. This varies the resistance of the layer of grains and, thus, the current through it and the voltage. The range of operation for MOS is quite large, since they usually work at 300–500 °C, a temperature at which reactions are reversible, and there is no chemiadsorbed water generated to prevent other reactions with VOCs from occurring. High temperatures, as in the case of silicon carbide, also allow the detection of compounds

like hydrocarbons not normally found by the sensors otherwise [33], unless using totally different, and often much more expensive, technologies (e.g., in photo-ionization detectors [34]). This is the main reason why this kind of device usually includes a heater to reach an adequate working temperature.

Instead of metal oxides, conducting polymers such as polyacetylene, polypyrrole, polyaniline and others can be used, as they go through a similar process to the one described above [35]. However, compared to metal-oxide based sensors, they generally have a lower conductivity, and lower gas selectivity and sensitivity. Another significant difference is that they act as p-type conductors. In order to improve their performance in gas sensing, especially by increasing their conductivity, they have to be treated through a chemical doping process. In more detail, such a process mainly consists of the application of acids or redox reactions so that the backbone can be deprived of electrons, making them positively charged and reaching a high conducting level, up to a value between 1 and 105 S/cm [36].

However, among the main drawbacks experienced by both MOS and conducting polymer-based sensors are significant drift and lack of repeatability. Such issues can be solved by changing the approach in the construction of E-nose systems, for example using optical-based tools, such as those applying non-dispersive infrared detection approaches [37]. Their principle of operation relies on the modification of optical parameters of the system in response to the interaction with volatile molecules. For example, the tool described by Esfahani [38] is based on the molecular absorption of Infrared and the consequent fall in the detected signal, in turn to be related to molecular groups. Since different chemicals absorb specific infrared frequencies, the number of photons absorbed is directly proportional to the power of the photon beam from the emitter, thus related to the concentration of the gas detected. Beyond being low-cost, those sensors were also displayed to have good sensitivity for major compounds of the human breath, including some related to pathological conditions, e.g., methane, acetone, ethanol.
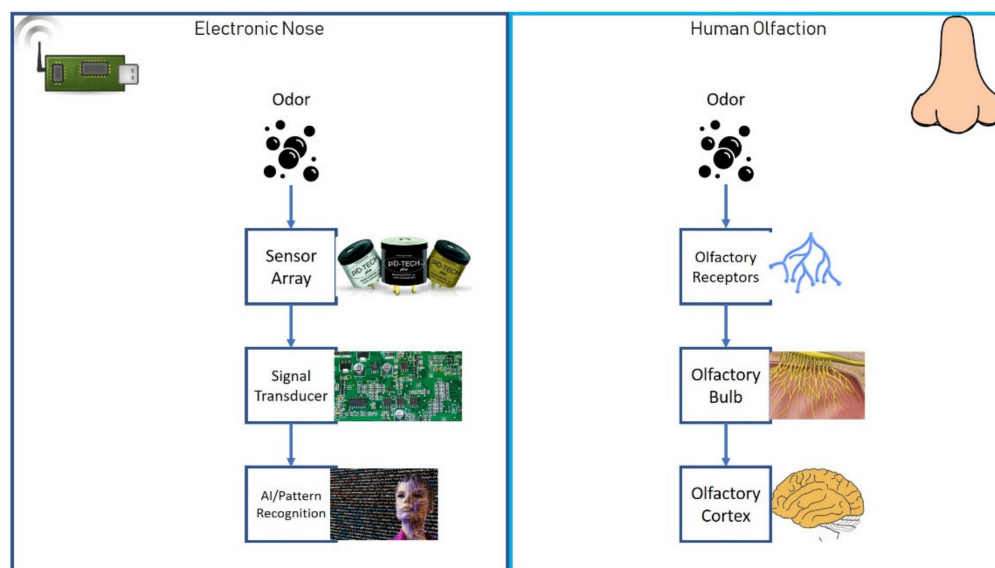

Figure 1. Comparison between electronic nose and human olfaction functioning.

In order to make the E-nose system operate properly, it is essential to carefully select the sensing element. As such, nanomaterials, and nanoparticles in particular, are considered among the preferred elements for this purpose, thanks to their interesting surface properties, their tunability from the physical–chemical point of view, and their excellent stability. Furthermore, their doping with metal elements can even improve the electrical and optical properties of sensing elements, as well as their sensitivity for various gases [39]. Finally, the reduced size for such materials allows the packing of multiple arrays within a limited space, as happens biologically in the human (or animal) nose, while at the same

time providing a broader interaction site with the odorous compounds [40]. When it comes to miniaturization, interesting materials, like graphene, find their place in the E-nose technology, with the graphene field-effect transistor (GFET) often employed as gas sensors, with very low power consumption, good selectivity, miniaturization, and low cost, especially with some design adaptations [41]. Finally, the choice for the most suitable technology to be employed should be performed, according to the application to which the E-nose is applied, with different approaches better responding to the one or the other requirements.

3. E-Nose Use in the Analysis of the Composition of Human Microbiota

The technology the E-Nose relies on, is a non-invasive alternative to support the diagnosis of pathologies based on the changes of the human microbiota, especially in the case of that hosted by the human gut. The interest in this kind of biomarker has been growing in recent times, therefore many of the publications in this specific sector dated to the last decades. More specifically, wrapping up the actual literature on the topic, a very important application of the E-Nose for this purpose was made by Arasaradnam et al. [42]. In this work, the authors tried to use the E-nose technology to support the diagnosis of Inflammatory Bowel Disease (IBD), the etiology of which involves variations in the presence and concentration of bacteria living in the gastrointestinal tract [43]. From the clinical point of view, the term IBD refers to two different conditions: Chron's Disease (CD) and Ulcerative Colitis (UC). In this regard, the aim of the study was to analyze the efficacy by an E-nose tool in detecting such diseases compared with the gold standard method of Field Asymmetric Ion Mobility Spectroscopy (FAIMS). The sixty-two patients involved were divided into three groups: those with CD, those with UC and the control group. A second split was applied to separate the subjects affected by IBD in relapse from those in remission. Concerning the E-nose tool, the researchers used a system composed of an array of 18 metal oxide sensors to analyze urine samples from such individuals. By Principal Component Analysis (PCA), they were able to downscale the components of the data vectors down to three, obtaining charts clearly separating the data from the different groups with only a few overlaps [44]. As highlighted, the significant differences between the profiles of the patients' VOCs demonstrate the importance to be paid to the analysis of the composition of the gut microbiota in the diagnosis of IBD. The comparison with the results of the FAIMS confirmed the efficacy of the E-Nose, which was deemed to be able to detect multiple compounds thanks to the modulation of the electric resistance of the metal oxide sensors.

E-nose systems were seen to be particularly useful for the very early detection of necrotizing enterocolitis by analyzing fecal Volatile Organic Compounds in preterm newborns. A Cyranose 320 E-nose (Smiths Detections, Pasadena, CA, USA) equipped with 32 polymer sensors was employed to this extent, with data collected from 128 infants analyzed by basic statistics. According to the authors, fecal VOC profiles of infants with necrotizing enterocolitis could be discriminated from controls, within a time window of 2–3 days before the clinical onset, making the Cyranose 320 and, to a broader extent, the E-nose approach potentially useful for the early prediction of such disease in at-risk individuals [45], and potentially applicable in all those scenarios where an imbalance of the gut microbiota due to pathological conditions or sepsis is present [46–51].

One of the first studies, which reported the use of an E-nose to diagnose conditions related to the gastrointestinal tract, aimed to find an innovative, non-invasive way to detect colorectal cancer (CRC), alternative to the gold-standard classical methods [52]. The group led by de Meij harnessed a chemical gas analyzer containing an array of 32 polymer sensors, the electrical resistance of which could be modulated by the interaction with the chemical compounds of the VOCs coming from fecal samples. In that specific experimental scenario, this device was connected to a fecal container collecting fecal gas escaping from a hole made by a needle. The system can be synthesized as a closed loop, as the container of the E-nose was pierced and connected to the other container by an identical tube. This choice had the aim of preventing the headspace to be diluted by the entrance of ambient air,

possibly affecting the good quality of the sample collected. The subjects of the experiment described included healthy individuals, who composed the control group, and people who were prescribed a colonoscopy, which actually represents the gold standard test for the CRC. In order to understand the viability of using the E-nose technology for this purpose, the results of the e-nose were compared to the ones of the Fecal Immunochemical Tests (FIT), a methodology which is quite commonly used nowadays. After the application of the PCA, an independent *t*-test to evaluate the capability of the electronic device to discriminate data between the groups was carried out. In addition, a non-specified test was performed to calculate the probability of having a type 1 error, which could have been caused by the lack of information about the medical history of the subjects, and a ROC curve was finally plotted. From the investigation conducted, the results showed for the E-nose had a sensitivity of 85% and a specificity of 87%, thus suggesting the electronic nose is a very effective alternative to the currently used tests, which could be either more complex or more expensive [52].

Still on the same topic, Westenbrink and colleagues [53] developed an E-nose made up of 13 electrochemical and optical sensors, selected from commercially available ones. Urine samples were analyzed through the E-nose from this cohort with CRC, a cohort of individuals with IBD, and controls. Through Linear Discriminant Analysis and K-nearest neighbor, CRC were distinguished from IBD with 78% sensitivity and 79% specificity.

Another interesting approach was presented by Zonta and colleagues that, based on chemoresistive sensors (metal oxides and sulfides) attempted at distinguishing stool samples of healthy subjects from those of patients with CRC at different stages [54]. There, 20 sensors were tested in different arrays composed of given units each, in order to understand which combination was capable of distinguishing between such samples. As such, tin- and titanium-oxide sensors were seen to have a good sensitivity in terms of their responses to healthy subjects with respect to affected patients' stool. In the small cohort tested, the device evaluated just provided a 5% classification error rate, with just one false positive person, whereas false negatives were absent according to the authors.

Several years later, the same group investigated the performances of a device based on five semiconductor-based nanostructured gas sensors in identifying CRC presence by fecal volatile compounds against colonoscopy, used as the gold-standard diagnostic technique for the disease considered. The gas sensors composing the device, named SCENT A1, are based on chemoresistive nanostructured semiconductors (iron and samarium oxide, titanium, tantalum and vanadium oxides, tin and titanium oxide at 20%, indium oxide, tin and titanium oxide at 25% with gold nanoparticles). In this study, 398 samples were processed and then analyzed by Machine Learning, specifically the Support Vector Machine (SVM), with performances over 80% in terms of sensitivity and specificity (84.1 and 82.4%, respectively), against a positive predictive value of 72% and a negative predictive value of 91% [55].

Quite recently, CRC was the target disease within the investigation by Tyagi and colleagues, performed using the portable E-nose PEN3, followed by GC-TOF-MS to profile the urinary metabolome of the disease mentioned above [56]. The PEN3 E-nose (Airsense Analytics GmbH, Schwerin, Germany) is a portable tool combining a gas sampling unit and a sensor array, the latter consisting of 10 different thick film metal oxide sensors, operating between 250 and 550 °C. In the investigation retrieved, PEN3 and GC-TOF-MS demonstrated high accuracy for the separation of CRC and non-cancer individuals, with a 0.81 AUC.

Other notable applications of the E-nose in the detection of gastrointestinal (GI) diseases linked to the changes of the gut microbiota include studies regarding irritable bowel syndrome (IBS) and infectious diarrhea (ID) [57]. The former condition usually causes lots of problems in diagnostics as a result of its clinical similarity to the already discussed IBD. Shepherd et al. [58] coupled a metal oxide sensor device to gas chromatography in order to discriminate individuals affected by IBS from those with IBD. The difference from the previously described studies was that the device used here contained a single sensor

instead of an array of sensors. However, by analyzing the modulation of the electrical resistance of the sensor, the authors of the presented study found a fairly good discrimination capability, with a sensitivity and a specificity of 76% and 88%, respectively. These results prove the great potential of an electronic nose containing multiple metal oxide sensors even in a complex task like that represented by the discrimination between IBD and IBS. Furthermore, the E-nose performances could also be further improved in the discrimination of IBS from healthy subjects, and were not particularly high according to the conclusions drawn by the authors of the study [58]. In general, when possible, an overall consensus was reached in the literature, as the preference towards the use of urine samples rather than fecal ones was highlighted due to the fact they are easily obtained. In fact, results from the literature suggest that the use of urine samples should lead to the same results as fecal samples do, given that VOCs produced by gut microbiota fermentation processes are able to cross the intestinal barrier and can therefore be found in the human urine, as well [59]. Therefore with comparable results, the access to urine samples, which much easier than with fecal ones, is usually preferred.

In general, the results obtained led to the consideration that the e-nose technology has been proved to be a tool of good efficacy for the identification of an individual's enterotype, that is representing the species of microorganisms living in someone's gastrointestinal tract, even for different purposes than that of pathological diagnosis. Indeed, as shown by Hosfield et al. [60], the information provided by the analysis of VOCs, about which type of bacteria colonizes a child's gut corresponds to the one delivered by the RNA sequencing approach. As such, the latter showed that the species of bacteria located in the children's gastrointestinal tract are mainly three: *Bacteroides*, *Prevotella* and *Ruminoccus*. By analyzing VOCs with an E-nose and dividing the patients into three groups according to the enterotype, the researchers were able to find a correlation between the signal output provided by the device sensors and the specific enterotypes characterizing an individual. The ability of the whole device to discriminate between enterotypes was investigated through the so-called Fast Frugal Tree algorithm [61], finding an accuracy of about 88% using only two sensors.

The ability of this new technology to discriminate between different kinds of bacteria and, sometimes, even microscopical fungi, such as yeast, makes it usable in various fields, in the biomedical world and beyond. Focusing on the domain of interest for our paper, the biomedical field, those include, for example, the detection of infections derived not only from the variation in the composition of the gut microbiota, but also from the presence of pathogens in other parts of the organism (e.g., in the upper respiratory tract and the urinary tract [62]). For instance, Pavlou et al. [63] successfully identified the presence or absence of *Mycobacterium tubercolosis* in 90% of the sputum samples of a cohort of patients enrolled, by using an electronic nose with an array of 14 conductive polymers. The samples not correctly classified by their algorithms came mainly from healthy subjects, thus the sensitivity associated to the E-nose test compared to microscopy and culturing systems of diagnosis was very high [63]. Similarly, good results were obtained in the past from the same kind of samples, that is to say sputum samples, in the detection of fungi (e.g., *Aspergillus fumigatus*, which is responsible for invasive pulmonary aspergillosis). This organism was detected with an accuracy of around 89%, confirming that the presence of different kinds of microorganisms can lead to a modified breathprint in the infected individuals [64].

Interestingly, E-nose tools could also be useful to detect changes in gut microbiota composition due to the supplementation of probiotics in particular conditions, such as propionic acidemia [65]. In a proof-of-concept study, a Cyranose 320, already mentioned above, was employed to analyze urine samples and putting it into relationship with probiotic supplementation. The E-nose tool was deemed useful in the monitoring of the disease progression, ultimately helping to validate interventions or treatments to control the existing condition.

Also, feeding composition can be investigated with an E-nose too. Through the Cyranose 320, a Dutch group was somewhat able to distinguish between breastfed and formula milk-fed preterm newborns through the analysis of fecal VOCs [66], although the study was carried out in a very small cohort of children (15 + 15). Unfortunately, the same E-nose tool was not sensitive enough to discriminate the gestational age and mode of delivery of pre-term infants based on their fecal VOC composition [67].

Finally, E-nose tools can be employed to investigate the influence of lifestyle factors in the composition of the microbiota. According to Bosch and colleagues [68], using Cyranose 320 for the assessment, age, gender, BMI, smoking habits, dietary preferences, co-morbidity and medication use all have unique effects on fecal VOC composition, which are detectable with a smart, inexpensive approach. Such a tool gives us the chance to possibly expand the application of such tools for diagnostic purposes.

4. Principles of Functioning of the E-Tongue

Aside from the sense of smell, the sense of taste also plays an important starring role in the wider framework of sensoriality, and in particular when it comes to the chemical senses. As such, the scientific knowledge concerning human taste is continuously gaining importance and achieving the same level of knowledge as that concerning human olfaction; however, its translation into an artificial sense encounters more issues if compared with that occurring with the artificial olfaction, or the so-called electronic nose [69–71] (Figure 2). As such, the technology of the electronic tongue should rely on different materials, as tasty substances can be perceived by the natural tongue of humans or animals just when these are dissolved into a liquid solution [72]. This means that the E-tongue should attempt to process liquid samples instead of gaseous ones, with noteworthy limitations mainly in terms of repeated measurements and related reliability and signal reproducibility. However, one main feature in common between the E-nose and the E-tongue is represented by the reliance those systems have on an array of not selective sensors. Indeed, it is widely accepted that taste sensors should be broadly selective, as the perception of the five basic tastes (bitter, sweet, sour, salty and umami) is strictly related to the concentration of the chemical compounds of a solution, however, at the same time does not derive from the single concentrations per se but rather from the combinations of these [73,74]. For instance, putting sucrose, a sugar commonly used in everyday life, into a solution of caffeine, sweet taste intensity, which is caused by the increase in sucrose concentration, rises, whereas the bitterness, caused by the presence of caffeine, drops, even when the concentration of the causing substance remains unvaried. This occurs since bitterness, as well as all the other taste sensations, depends on the combination of the concentrations of different substances and not on individual concentrations per se [75].

Focusing more on the technological part of the E-tongue tools, it should be stated that the most common type of sensors for an E-tongue include potentiometric devices. Those particular sensors rely on an ion-selective membrane, which generates an electric potential [76]. These kinds of sensors are also alternatively named as ion-selective electrodes (ISEs). More specifically, their signal consists of the voltage or electromotive force calculated between the obtained potential and a reference electrode. Concerning the selectivity of the sensor, it usually varies according to the composition of the membrane, which changes the permeability to certain particles [77]. This principle of functioning is somewhat similar to the one of the cellular membrane. For particular applications, customized sensors are commonly designed. Examples for that include a sensor based on a lipid polymer membrane that was realized and published by Wu et al. [78]. Overall, the selectivity of a potentiometric sensor can be described by the Nikolsky–Eisenman equation:

$$E = E^\circ + \frac{RT}{Z_i F} \ln \left[a_i + \sum_j K_{ij} \left(a_j \right)^{z_i / z_j} \right]$$

where:

E is the measured voltage between the sensor and the reference electrode;

E° is the standard potential of the membrane-based electrode;

R is the constant of ideal gases;

T is the absolute temperature;

Z_i and Z_j are the valences, respectively, of the species the concentration of whom has to be calculated and of another ion present in the solution and interfering with the species;

F is the Faraday constant;

a_i and a_j are the activities, respectively, of the primary ion and the interfering one;

K_{ij} is the selectivity constant.

From the equation, it comes to the eye that the response of the sensor has a logarithmic dependence from the activity of the ions contained in the solution analyzed. In order for this relation to be applicable, the sensors generally need to be poorly selective. This statement is due to the fact that, otherwise, when the solution contains lots of ions, which generate a response from the sensors, the Nikolsky–Eisenman equation is not valid [79]. Usually, the response of the ISEs is Nernstian, therefore it can be assumed that the Nernst equation can be applied to derive the concentration of a certain analyte from the voltage measured by the sensing part of the device.

Overall, ISEs are normally classified by the material composing the membrane. They can be polymeric membrane-based sensors [80], glass electrodes, e.g., sensor with membranes made of chalcogenide glass [81], and precipitate-based membranes sensors, which have membranes made of compounds such as silver halides [82] or metal sulfides [83]. Overall, the polymer membrane-based devices are the most used tools, mainly as a result of their high chemical versatility, characteristics particularly appreciated when it comes to the choice for such devices. As an alternative to simple ISEs, a potentiometric sensor can also be made of ion-sensitive field-effect transistors (ISFETs), which have the capacity of detecting the changes in the pH of a solution, as they are sensitive to the variations in the concentrations of either H^+ or OH^-. They have a very similar principle of functioning to the one of the MOSFET [84].
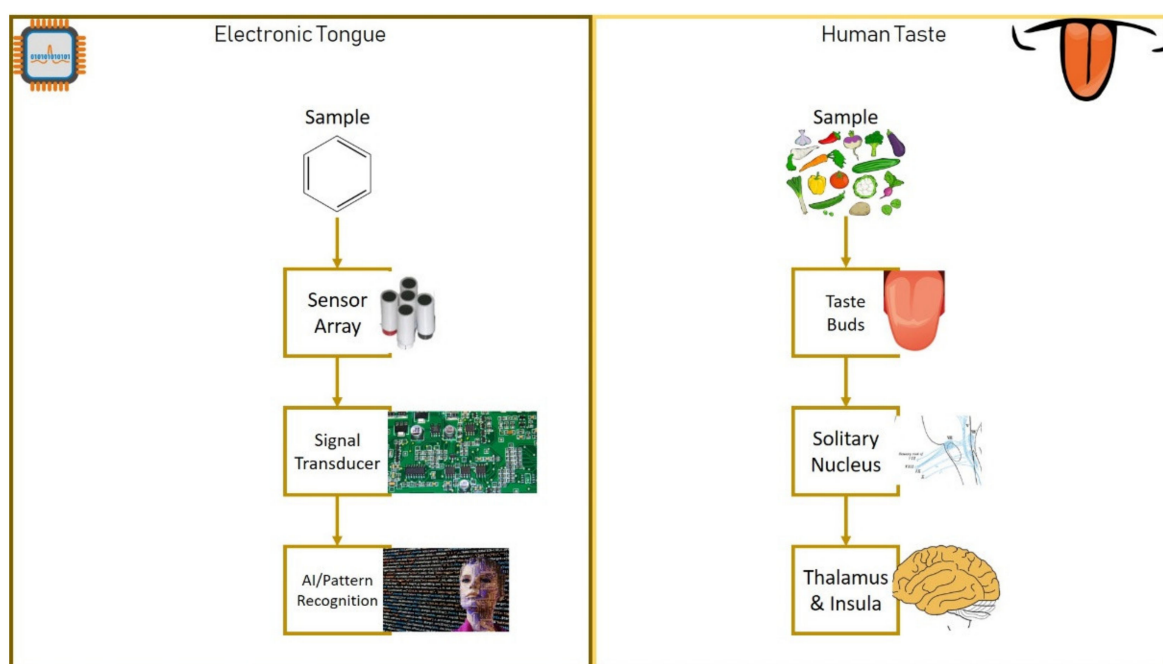

Figure 2. Comparison between electronic tongue and human taste functioning.

5. E-Tongue in the Analysis of the Human Microbiota: Possibilities and Open Challenges

Nowadays, the applications of E-tongue devices in the medical and biomedical fields are still quite scarce. The main usage of this technology has been found in the food industry, and in particular for purposes of quality control of foods and packaging, to check for the shelf-life of particular foods and similar applications (e.g., [85]). Focusing in particular on the biomedical universe, and the argument of the present paper, there are some examples of the testing of biological fluids, such as urine and sweat [86–89]. However, those tests usually aim to detect substances, such as creatinine or other biochemical compounds, which are not strictly related to bacteria or microscopical fungi. For this reason, they do not really apply to the specific topic of this paper. Another use of such an approach in the biomedical research can be seen in both in vitro and in vivo studies, where this kind of technology can be harnessed in order to analyze eventually damaged tissues or to perform cytotoxicity tests. Beyond its usage for quality control of alimentary items, the E-Tongue technology is also largely employed in other subdomains of the food-related universe, including in the analysis of water samples [90], also for environmental purposes [91], and for testing the efficacy of taste masking of pharmaceuticals [92]. Nonetheless, the electronic tongue systems have been used also in the past years and decades to discriminate between microorganisms or to analyze cell cultures [93]. This was applied in the food industry, where the approach was prevalent, as stated above, and beyond that. Thus, in light of this information, in this paragraph some examples of this application related to the electronic tongue are reported in order to support a larger use of this technology in the analysis of microorganisms residing in the human body, either in normal physiological states or within pathological conditions.

First of all, a few recent studies explored the potential of the E-tongue systems in the clinical and (bio-)medical field. In this regard, Al Ramahi et al. [94] used a device composed of an array of seven ISFET sensors, complemented by an Ag/AgCl reference electrode. The goal of the study described was to successfully discriminate between different species of common bacteria. Such species included *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. In the protocol described, the microorganisms were cultivated and the bacterial isolates were then analyzed at three different times of the day. The results obtained by those scientists showed that the electronic tongue could distinguish between the three strains involved in the study. Furthermore, according to the authors, the electronic tongue system implemented was seen to be able to even detect the presence of certain bacteria before the stationary phase of their growth is reached. This fact is deemed to be particularly important, since it might support the idea that such a device could be used in the early diagnosis of diseases eventually caused by bacteria infections in humans [94].

The development of an E-tongue to detect the presence of urinary dysfunctions was performed by Lvova and colleagues [95] using different potentiometric chemical sensors. Creatinine levels were analyzed, showing promising results with respect to standard methods. On the other hand, the urines of individuals were correctly classified in 92.2% of cases thanks to partial least square regression discrimination analysis (PLS-DA) and feed forward back-propagation neural networks (FFBP NN).

A somewhat similar approach was adopted years later to detect bladder cancer from urine composition [96]. Several Machine Learning approaches were tested with such inputs, achieving an overall satisfactory 72% accuracy (71% sensitivity, 58% specificity). Such metrics were further increased to 76% accuracy (80% and 75% for sensitivity and specificity, respectively), when only older subjects were included in the analysis.

Furthermore, there have been some attempts aimed at the use of taste sensors to determine the presence or absence of certain microorganisms in the gut microbiota. For instance, De Vincentis et al. [97] recruited patients, who were diagnosed with symptomatic uncomplicated diverticular disease (SUDD), the pathogenesis of whom largely depends on the microbiota. According to the authors, the final aim of the study was to assess whether the rifaximin-based therapy was effective in changing the composition of the microbiota associated with the disease investigated. In the study, the authors used fecal samples

diluted in distilled water and then centrifugated. The electronic tongue implemented was made of a silver-based electrode, a gold counter electrode and a platinum reference. From the data obtained within the study, it could be concluded that the ability of the E-tongue to detect certain bacteria was almost optimal and no better results could be obtained by integrating the information delivered by the E-tongue with the one delivered by an E-nose eventually evaluated as a complementary tool to the E-tongue applied [97].

In addition, as briefly stated above, the E-tongue has been employed over the years to search for microbiologically-derived contamination in food and water thanks to its capability to find endotoxins coming from bacteria in liquid samples. This subject is also very important in the medical field, as water and aqueous solutions are widely used in medical treatments and therapies [98]. Their use in this particular sort of analysis could pave the way for a more in-depth employing of the electronic tongue in the investigation about the human gut microbiota, which is the pivotal topic of the present review article. Finally, the use of electronic tongue tools was also seen when it comes to detecting microscopical fungi eventually involved in diseases also affecting human beings, such as the already mentioned *Aspergillus* [99].

6. Conclusions

The present review aimed to investigate the current state of the art and innovative topic of artificial senses in the detection of gut microbiota within biological samples. As stated, in this approach, Electronic Nose (E-nose) and Electronic Tongue (E-tongue) tools, mimicking the chemical senses, are probably the most useful devices to be eventually applied to solve the problem. This is particularly true as a result of the intrinsic nature of the biological samples being analyzed, which include gaseous or liquid matrices coming from the human body. Some examples for that include breath samples, urine samples, fecal samples, etc. With respect to the current state of the art technology, including well-grounded tools for analyzing such biological samples, E-nose and E-tongue systems represent a significant innovation since their characteristics could be of interest for a wide range of end-users. More specifically, when compared with gold standard approaches for the analysis of biological samples, E-nose and E-tongue devices offer a drastic reduction in costs, higher flexibility, noteworthy portability, without particular drops in the accuracy of the results provided [100]. Such characteristics could potentially lead to the adoption of such tools for a large-scale employment within biological sample analysis. Both E-nose and E-tongue tools could be effectively used for broad population screenings that could be carried out even at the individual's premises or at small clinical centers, possibly not requiring a particularly burdensome economic effort. Moreover, their use could be easily extended to those regions or countries where the purchase of gold-standard machineries is not economically viable or convenient and where large-scale screenings leading to early diagnosis of some burdensome diseases are lacking and are particularly desirable, too. Overall, their use on a large-scale could be feasible since individuals with eventual warnings related to their health conditions could be referred to more classical approaches, using more well-grounded methodologies to investigate the eventual health condition in more detail. This would be translated into a significant money saving for hospitals and health systems, at large, without a dramatic loss in terms of healthcare service quality. At the same time, this approach will allow the referral of those individuals who require the gold-standard testing methodologies to such approaches, relieving the burden of waiting lists normally affecting public health systems in various countries. The use case scenario could be otherwise extended to even larger scales when E-nose and E-tongue tools could be adopted even at the General Practitioner ambulatory setting, allowing an even more capillary monitoring of the healthcare status of an individual, and enabling early detection of eventually existing conditions, at reasonably early stages [101].

However, one should also consider the drawbacks of such innovative tools in order to hypothesize their use in a totally effective way. As such, the main deficit concerning the development of true E-nose tools is represented by the lack of odor sensors. In fact,

traditional gas sensors are poorly selective, and they do not truly resemble the sensing part of the biological sense of smell, as they do not have a specific filter function for odorants like odor receptor cells do. In addition, despite recent technological developments, the sensitivity of most gas sensors is usually lower than the odor threshold of most odorous compounds. In addition, the lack of standardization of E-nose tools, of their sampling protocols, of the transport of their samples, of the storage conditions and of the analytical methodologies adopted actually limits the comparisons between different studies [102] and should be carefully tailored [103].

From another perspective, the signal coming from the E-nose systems should also be duly processed, by using multiple steps, including raw signal cleaning, interference reduction, and so forth until the choice for the most suitable algorithms to be applied within the Machine Learning/Deep Learning framework, pivotal to extract significant information from the E-nose tools [104]. Such constraints should be carefully considered when attempting to effectively apply such methodologies to the assessment of odorous compounds [23]. However, once we have fixed the eventual issues associated with such devices, gut microbiota is a particularly interesting biomarker to be investigated as its chemical composition or, better, a variation in it, is somewhat related to a number of clinical conditions, which are mainly those where a metabolic imbalance is associated, or other disorders without such characteristics.

Therefore, to understand how artificial chemical senses, notably E-nose and E-tongue, find use in the analysis of such matrices could be pivotal in paving the way for clinicians, researchers and scientists to foster the use of such devices in the analysis of similar biological matrices. According to the literature reviewed, E-nose systems see a much larger employment in the field of the characterization of the gut microbiota in biological samples drawn from human individuals. This is probably due to the deeper knowledge of such devices from the scientific community at large, and also to the early commercialization of electronic noses by a number of manufacturers throughout the globe. Such tools were seen to be somewhat effective in the characterization of the gut microbiota in biological samples, particularly when it comes to the analysis of the human breath. Those most commonly in use among electronic nose devices, from the point of view of the sensors, include those featuring an array of Metal Oxide Sensors (MOS), combining a good level of performances with considerably lower costs with respect to other sensors, and with a good degree of customizability upon the end-users' needs. The choice for those sensors is fully compliant with what was stated above in terms of money saving and possible transferability to a wide range of applications.

On the other hand, E-tongue systems, albeit being largely used to analyze liquid samples in a wide range of applications, mostly within the food industry, are still poorly employed in the characterization of the human gut microbiota. This fact could be due to different reasons, among which are the lack of availability of electronic tongue systems on the market, the lack of knowledge, from the clinical part, about the E-tongue tools implemented at least as a prototype level, and the drawbacks eventually carried out by some of the electronic tongue solutions. These issues might include the difficulty in carrying out repeated measures given the somewhat limited possibility for the sensors to be fully cleaned and responsive after the first analysis carried out on the target sample. However, the good results obtained by the Electronic tongue tools in different scenarios not strictly pertaining to the analysis of the human gut microbiota, but mainly relying on the quality assessment of food, wastewaters and environment-related liquid matrices, would suggest the feasibility of using such devices even within clinical and biomedical frameworks. This review has, among its main scopes, the aim of raising awareness about the important roles such tools could play in the relevant framework, and hopefully its reading by the scientific community would enhance the use of artificial senses in addressing the specific clinical question.

Future developments in the field would include the larger application of electronic noses, and the first attempts for electronic tongues, in the assessment of human gut micro-

biota. Furthermore, a more extensive research towards the improvement of the sensing materials actually employed in such applications is desirable. More knowledge is also needed when it comes to the methodologies for sensor output signal processing, including the application of innovative, up-to-date Machine Learning and/or Deep Learning algorithms that could help scientists detect eventual characteristics of interest for the signals produced by such devices [105,106].

In conclusion, both the electronic tongue and the electronic nose could represent very effective approaches supporting the gold standard tests for the evaluation of human microbiota composition and for the diagnosis of pathologies it is involved with. While the usefulness of the e-nose in Medicine and Biomedical Engineering has been proved multiple times, and the microbiota investigation is just one of the multiple fields of application for this technology, the E-tongue still needs to find its place in such fields. However, the examples and experimental results reported in similar scenarios show its great potential, being inexpensive and relatively simple in comparison to other techniques currently available.

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