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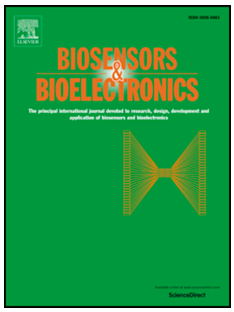

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PII: S0956-5663(19)31002-4

DOI: https://doi.org/10.1016/j.bios.2019.111923

Reference: BIOS 111923

To appear in: *Biosensors and Bioelectronics*

Received Date: 7 October 2019

Revised Date: 20 November 2019

Accepted Date: 25 November 2019

Please cite this article as: Wasilewski, T., Kamysz, W., Gębicki, J., Bioelectronic tongue: Current status and perspectives, *Biosensors and Bioelectronics* (2019), doi: https://doi.org/10.1016/j.bios.2019.111923.

CRediT author statement

DECLARATION OF THE AUTHORS' CONTRIBUTIONS

Publication: Tomasz Wasilewski, Jacek Gębicki, Wojciech Kamysz, **Bioelectronic Tongue: Current Status and Perspectives**, Biosensors and Bioelectronics.

Tomasz Wasilewski: Writing, original manuscript preparation, review and editing; **Wojciech Kamysz:** Supervision; **Jacek Gębicki:** Financial support, supervision.

BIOELECTRONIC TONGUES

- BIOMIMETIC SENSORS

- MICRO/NANO TECHNOLOGIES

- APPLICATIONS

- PERSPECTIVES

Bioelectronic Tongue: Current Status and Perspectives

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Abstract

In the course of evolution, nature has endowed humans with systems for the recognition of a wide range of tastes with a sensitivity and selectivity which are indispensable for the evaluation of edibility and flavour attributes. Inspiration by a biological sense of taste has become a basis for the design of instruments, operation principles and parameters enabling to mimic the unique properties of their biological precursors. In response to the demand for fast, sensitive and selective techniques of flavouring analysis, devices belonging to the group of bioelectronic tongues (B-ETs) have been designed. They combine achievements of chemometric analysis employed for many years in electronic tongues (ETs), with unique properties of bio-inspired materials, such as natural taste receptors (TRs) regarding receptor/ligand affinity. Investigations of the efficiency of the prototype devices create new application possibilities and suggest successful implementation in real applications. With advances in the field of biotechnology, microfluidics and nanotechnologies, many exciting developments have been made in the design of B-ETs in the last five years or so. The presented characteristics of the recent design solutions, application possibilities, critical evaluation of potentialities and limitations as well as the outline of further development prospects related to B-ETs should contribute to the systematisation and expansion of our knowledge.

Keywords: biosensor; electronic tongue; bioelectronic tongue; taste sensor; biomimetic sensor; microfluidics; nanomaterials

1. Introduction

Evolution has endowed living organisms with astonishing, multidimensional recognition systems; this potential goes beyond the artificial sensors which have been designed so far (Wang and Liu, 2015). Mimicking the unique properties of biological senses of taste has become an inspiration for the construction of the devices for the analysis of odours and flavourings (Son and Park, 2018). Integration of bio-inspired sensing materials (L. Lu et al., 2017; Wasilewski et al., 2018) – with micro/nanotechnology (Wang, 2013), aimed at the elaboration of the biosensor arrays with unique parameters – has attracted much interest of many scientific teams in the last decade (Wasilewski et al., 2017). Considerable progress in the combination of different transduction technologies with biomaterials and data processing using suitable chemometric tools (Cetó et al., 2016) has resulted in new concepts of next generation devices, which are capable of the analysis of compounds with an efficiency similar to that of their biological counterparts. The combination of biosensors with chemometric tools such as ANNs or PCA may represent an alternative to conventional biosensing methods. On the one hand, we have the specificity and selectivity of biosensors, and on the other, the use of ANNs modelling to derive meaning from complex or imprecise data, for example, in multivariate calibration or pattern recognition (Caruso, 2016).

The extensive field of electronic sense applications (Patel, 2014) has led to progress in the techniques of compound analysis, especially as far as the elaboration of new biomaterials for the

receptor elements of the biosensors is concerned (Wasilewski et al., 2018). Devices employing biosensors are a true alternative to classic techniques and their ongoing development allows for the acquisition of valuable chemical information from a specific matrix by using appropriate multivariate statistical qualitative/quantitative data processing-modelling techniques, aiming to overcome the drawbacks such as the occurrence of nonidealities, interference effects and collinearity between the measured variables and noise issues (Dias et al., 2017). Despite significant progress in the field of biosensors, there is an increasing demand for devices matching their biological references developed during the evolution process. The need for the improvement of the current designs is linked to the utilisation of the unique properties of the biosensors in real applications, where superior metrological parameters, elimination of sample preparation steps, miniaturisation, mobility, short response time and low price play the key role. Moreover, the development of chemical biosensors for the construction of arrays is focused on the improvement of sensitivity, selectivity and specificity with respect to analytes through the generation of the signals analogous to those in the biological senses. The elimination of interferences and matrix effects during the analysis of real samples are another challenge associated with these devices. Inspiration by the biological systems allows for overcoming some of the aforementioned inconveniences by the implementation of electronic tongues (Etongues, ETs) (Dias et al., 2017), where a sensor matrix provides a unique response to various analytes after data processing, thus enabling qualitative and quantitative analysis (Wang and Liu, 2019; Wasilewski et al., 2019). To improve the performance of ETs and broaden the application fields, biosensors have recently been incorporated into arrays, leading to the development of bioelectronic tongues (Bio-artificial tongues, B-Etongues, Bio-ETs, B-ETs), a term coined by Tønning et al., 2005. Utilising materials mimicking biological sensing systems to develop B-ET systems is a current trends (Wang and Liu, 2015), preceded by a history of intensive development.

The milestones in the history of (bio)sensors development are shown in Fig. 1.

Fig. 1. Milestones in the development of sensors/electronic tongues and biosensors/bioelectronic tongues.

According to a generally accepted definition, B-ETs are instruments consisting of a single biosensor or an array of biosensors capable of providing a wide and holistic response of the analysed species coupled with a chemometric tool able to interpret and extract meaningful data from complex readings. Due to the complexity of certain biosensors, even systems consisting of a single biosensor can be referred to as B-ETs (Cetó et al., 2016). The B-ETs, as one of the variants of ETs, are characterised by a high sensitivity owing to the application of a biological receptor element on the surface of the secondary transducer (Ha et al., 2015). A crucial step in the development of B-ETs is the proper selection of the biomaterial to act as the recognition element; it enables an identification of particular analytes, regardless of physical properties and complexity of the sample matrix. To be considered as a B-ET, the sensor's recognition component should be of biological origin and designed to work under well-defined conditions, in terms of selective and specific analytes in certain types of samples (Cetó et al., 2016).

In order to improve the metrological parameters of the sensors and to expand their potential capabilities, various B-ETs have been designed (Table 2). Phenomena correlated with high selectivity/specificity of the tastant and taste receptors (TRs) interactions are applied and simulated in B-ETs based on natural and artificial receptors. Implementation of the nanostructures, *e.g.* carbon nanotubes (CNTs), nanowires, *etc.,* has significantly accelerated the progress in micro- and nanodevices useful in the construction of B-ETs (Ha et al., 2015). Owing to the utilisation of the nanostructures, B-ETs possess a unique advantage over classic ETs by mimicking specific operation of their biological counterparts; they revolutionise application possibilities of the biosensor arrays in real fields (Parmin et al., 2019). Implementation of the nano-supporting materials and appropriate tools for data processing leads to the application of the biosensors and their arrays in the food, pharmaceutical, diagnostic industries, *etc*.

The progress in the development of B-ETs creates new application possibilities for these devices and can result in their future successful commercialisation. Moreover, gustatory-inspired biosensors can constitute a convenient platform for the study of the taste perception and recognition mechanisms by discovering specific relations between TRs and flavourings. The microflow devices and implementation of the achievements of nanotechnology offer a high potential regarding the construction of the new-generation B-ETs, providing new applications and an understanding of the *in vitro* mechanisms underlying the phenomenon of taste signals cascade generation as well as interpretation of complex intracellular interactions accompanying taste perception. Understanding the joint chemical space of tastants and of inter-species differences (*i.e.* human *vs* rodents) in chemosensory signal detection may be value added. Enhancers of receptor responses will not only provide more sensitive biosensors but may also be used as taste modulating agents.

Despite the relatively short history of B-ETs, there are devices which have been successfully tested on real samples. Examples of biosensors and B-ETs prototypes have shown that their general operation principle fulfils the expectations. However, their successful commercialisation requires the improvement of their design and the signal processing methods. The main challenges related to the design of biomimetic systems include:

- Development of simple-to-operate and reproducible techniques of transducer modification with natural and synthetic receptors;
- Using chemometrics to solve biosensors' interferences, matrix effects, drift and cross-sensitivity as well as calibration problems;
- Improvement of stability and reproducibility of the biological elements;
- Development of data processing systems allowing real time monitoring;
- Development of portable devices (sensors-on-chip);
- Limitation of interference due to non-specific absorption of ligands;
- Amplification of generated signals and improvement of the S/N ratio;
- Development of biosensors with human-like activity, mimicking operation of the brain and the tongue;
- Limitation of non-specific binding on exposed surfaces of transducers;
- Development of multiplexed biosensor arrays and signal processing methods;
- Simplification of the design and cost reduction.

There is currently no technique that would fulfil all the requirements. The devices belonging to the group of bioelectronic senses, owing to the operation principle inspired by neurophysiology, constitute a promising direction of chemical analysis development. Further progress in biomimetic systems is possible thanks to the discovery of structural and functional aspects of processing of stimuli in living organisms as well as monitoring of the parameters analogous to those of biological senses. This paper presents the trends, a critical evaluation of the state of the art, achievements and prototype devices regarding biomimetic-based biosensors and biosensor arrays.

2. Biological and Artificial Taste Sensing Systems

Since the presentation of the first electronic tongue by Vlasov (Fig. 1) (Vlasov et al., 2008), a number of papers and applications involving this device has significantly increased (Cetó et al., 2016). The operation principle of this instrument relies on the use of a sensor array cross-reacting to the analytes in a liquid sample. Application of sensors with a broad response range is a standard approach to the ET design, highly sensitive and selective biosensors providing an increasingly common solution implemented in B-ETs (del Valle, 2016). Taking advantage of a specific biorecognition element, based on biological components of the sense of taste, during construction of

the sensor array, substantially broadened the spectrum of potential applications of these devices (Cetó et al., 2016, 2015; Ha et al., 2015). In some respects, the principle of ET and B-ET operation is based on the biological sense of taste which, together with odour and visual information, enable mammals to make a decision as to whether a particular item is dangerous to eat or if there are potential dangers. Taste evaluation involves taste buds located in the papillae of tongue epithelium (Barretto et al., 2015). Recent studies have revealed that the integration of odour sensations with taste information could occur at the level of gustatory receptor cells (Malik et al., 2019). Sweet, sour, bitter, salty and umami tastes can be sensed because of signal generation by 10,000 taste buds, each with 50-100 tightly packed TR cells (Ha et al., 2015). The plasma membrane of these TR cells contains specific taste detectors able to perceive a wide variety of tasting molecules dissolved in saliva (Fig. 2). Salty and sour tastes directly activate ion channels, whereas bitter, umami and sweet tastes are elicited by GPCRs. Lately, new taste qualities have been debated, including fat taste (mediated by GPR40 and GPR120), metallic, kokumi taste related to mouthfulness, thickness and long-tasting savoury sensations. Moreover, other trigeminal stimuli, such as menthol or eucalyptol, are also responsible for cooling sensations (Duffy et al., 2017; Freund and Lee, 2018). TR cells can be categorised into three major classes (termed types I, II, and type III cells) based on ultrastructural features, protein markers and a distinct function. The type I cells and type III receptor cells are involved in salt and sour taste detection, respectively. Types II receptor cells express taste detector for umami, sweet and bitter compounds (Briand and Salles, 2016). The top part of these cells contains TRs, which can involve ionic channels (for example, salty TRs reacting to sodium and potassium cations, sour TRs sensitive to organic and inorganic acids) or metabotropic receptors (sweet TRs reacting to monosaccharides, bitter TRs sensitive to alkaloids and inorganic salts, umami TRs) (Peng et al., 2015). Of these five modalities, sweet and umami tastes are mediated by TAS1Rs (T1Rs), while bitter taste – by TAS2Rs (T2Rs). Distinctive extracellular regions for TAS1Rs and transmembrane domains for TAS2Rs are the predicted regions of ligand binding (Di Pizio et al., 2019; Shichida et al., 2013). The recently collected evidence has indicated that TAS1Rs and TAS2Rs are not only limited to the oral cavity. Moreover, some bitterants are pharmacologically promiscuous, with the hERG potassium channel, cytochrome P450 enzymes and carbonic anhydrases as common off-targets (Lee et al., 2017). TRs belong to the family of seven transmembrane G-protein coupled receptors (GPCRs), which share structural features and transduction mechanisms (Foster et al., 2014). The unique binding properties of gustatory receptors provide a unique selectivity to tastant molecules of the human tongue. Therefore, human gustatory receptor-based biosensors have an incomparable advantage to mimic the unique responses of the human tongue in terms of selectivity and sensitivity. For the five basic tastes, their signal pathways are shown in Fig. 2.

Fig. 2. Examples of natural stimuli and taste detectors involved in the recognition of the five primary taste qualities. All these taste GPCRs use a common transduction pathway with a Gβγ-activated phospholipase C and a transient receptor potential cation channel subfamily M member 5 **(A,B)**. T1R1, T1R2 and T1R3 are often co-expressed and responses to both sweet and umami stimuli can be detected in the same cells. The sweet receptor family includes the metabotropic glutamate receptors (mGluR), the calcium-sensing receptor (CaSR) and the metabotropic GABAB receptor (GABABR) **(B)**. Class C GPCRs share structural features including a Venus flytrap (VFT) domain, connected to the heptahelical transmembrane domain by a short cysteine-rich domain. The polycystic kidney disease 2-like 1 (PKD2L1) ion channel, which is co-expressed with PKD1L3, has been demonstrated to be necessary for the detection of sour compounds **(B).** The epithelial Na channel (ENaC) has three subunits and is thought to transduce salty taste in rodents **(C)**. The glucose transporter type 4 (GLUT4) transports glucose by facilitative diffusion, whereas sodium/glucose cotransporter 1 (SGLT1) is Na dependent **(D)**. One or both of these transporters are hypothesised to be part of an alternative glucose-sensing pathway similar to the one used in pancreatic β cells and reproduced with permission from (Roper and Chaudhari, 2017).

The proximate stimulus for sour taste is intracellular acidification rather than extracellular protons. The influx of protons through the channel generates a small depolarising current and, furthermore, the accumulation of protons inside the cell. As a result, depolarisation of the type III sour sensing cells such that they reach the threshold for action potential initiation. For sour sensations, several

membrane ion channels have been proposed as transducers, including ENaCs, hyperpolarisation activated cyclic nucleotide gated channels and acid sensing ion channels (ASICs) (Roper and Chaudhari, 2017). The detection of bitter, sweet and umami compounds involves a common transduction mechanism. GPCR signalling desensitises rapidly as a consequence of receptor phosphorylation. The binding of the tastants to their receptors leads to the dissociation of a heterotrimeric G protein including the Gα subunit, named α-gustducin. Gα subunits were originally proposed to activate cAMP signalling, but the current view is that their primarily function is to control Gβγ subunits. cAMP also seems to have a longer term role by maintaining signalling proteins in a responsive state through protein kinase A activation. The dissociation of the Gβγ interacts with a phospholipase C-β2 (PLC-β2), leading to an inositol 1,4,5-triphosphate (IP3) increase which opens ion channels on the endoplasmic reticulum, thus generating a release of calcium. Then, the increase in intracellular calcium activates the opening of a transient potential ion channel, TRPMP5, generating a sodium influx and the depolarisation of TR cells (Behrens and Meyerhof, 2013; Briand and Salles, 2016).

The receptor-tastant interaction generates transduction of an intracellular signal in TR cells, which reaches brain *via* secondary higher-order neurons, where it is processed in order to trigger taste sensations (Herzog et al., 2019). Flavour is a multimodal perception including gustatory, oral somatosensory and retronasal olfactory signals in the mouth in the presence of stimuli. It results from the integration of these sensations, mainly in the brain. As far as taste is concerned, it activates an area of the anterior insula which represents the identity and intensity of tastes, and a part of the orbitofrontal and anterior cingulate cortex representing the reward value of taste (Briand and Salles, 2016). In the taste signalling cascade, TR cells play a key role in taste perception; initiation of their activity in the taste buds or in the taste nerves produces taste sensations. Taste coding is a specific tastant-receptor interaction involving a complex series of stimulation and potential generations (Lee et al., 2017). TR cells and taste tissues can be stimulated by many ligands originating from different flavourings (Baldwin et al., 2014). Moreover, sensory neurons can also reveal taste recognition properties *via* reaction to biochemical signals transmitted by neurotransducers (Koga and Bradley, 2000). The potential of tissues and cells as receptor elements of the taste biosensors is influenced by the improvement of biosensors' metrological parameters as far as sensitivity and selectivity/specificity are concerned.

A comparison of the operation principle of the electronic senses of taste (ET and B-ET) based on the biological sense of taste is shown in Fig. 3.

Fig. 3. Schematic comparison of the structure and the operation principle of electronic, bioelectronic and human tongues.

The biosensors mimicking biological mechanisms in a receptor part, implemented also in the form of biosensor arrays, can react to selected substances in different ways. These are further subjected to suitable signal processing and data analysis, which make it imaginable to obtain the holistic information about a sample. Secondary transducers are used to convert biological signals into ones which are analytically useful. Most of the reported B-ETs employ biosensors, essentially based on voltammetric/amperometric, potentiometric and piezoelectric transduction, with maximum contribution of voltammetric transducers (Matysik, 2017). For example, by using amperometric enzyme biosensors, direct conversion of a redox reaction into current can be directly observed as an analytical signal (Al-Issa et al., 2015; del Valle, 2016). In potentiometric sensors, signal generation from the potential value created by diffusion of ions across a membrane is measured (Gutiérrez et al., 2007). Coupling appropriate biosensors with chemometric tools enables the deconvolution of complex analytical signals from mixtures of similar matrix composition. In the case of biosensors, this particularly supports the determination of group specificity substrates – resolution of mixtures of compounds, for which the biosensor presents a slightly differentiated response or, alternatively,

estimation of global indexes (del Valle, 2016). The mentioned chemometric tools are conventional pattern recognition techniques, in essence of linear nature, which can be somehow limited if the sensors considered have clear nonlinear behaviour. Typical chemometric procedures to develop the multivariate response model are the methods of pattern recognition (PARC), partial least squares (PLSs), principal component analysis (PCA) and multivariate analysis as artificial neural networks (ANNs). Multidimensional data sets obtained from the biosensor array can be processed using qualitative and quantitative methods with the further evaluation of model performance, extensively presented in the literature (Cetó et al., 2016; Lavine and Workman, 2013). Various physical and chemical effects like adsorption of sample components, temperature deviations, surface chemical reactions, *etc.* may lead to substantial deviation in sensors' readings (Wasilewski et al., 2019). For example, a single sensor standardization approach (similar to single wavelength standardization recommended in spectroscopy) can significantly improve the extent of calibration lifetime (Panchuk et al., 2016). The lifetime, stability, reproducibility, and calibration requirements of the biosensor are influenced significantly by the selected bioreceptors (Morales and Halpern, 2018). Reproducibility is one of the parameters for the evaluation of biosensor array performance, intensive efforts have been made to develop successful immobilization strategies in order to assure greater sensitivity and stability of biosensors (Batool et al., 2019; X. Wu et al., 2017). The type of immobilization method mainly affects the activity and the stability of enzymatic biosensors and drastically influences factors such as accuracy of measurements, sensor-to-sensor reproducibility and operational lifetime (Unnikrishnan et al., 2013; Zheng et al., 2011).

The classification of the biosensors used in B-ETs can be accomplished by several criteria, usually as presented in this review, by the biochemical recognition element.

2.1. Tissues-Based Bioelectronic Tongues

The application of the biological sense elements as active parts of the biosensors enables the acquisition of information about bioactive analytes with a high sensitivity, selectivity and specificity (Mehrotra, 2016). In the sense of taste systems, the receptor elements (composition of TRs) are well preserved in tissues (*e.g.* taste epithelium), which allows the simulation of *in vivo* processes. Tissues of living organisms immobilised on fitting transducer platforms generate signals in response to specific stimuli on account of interaction with the active element. Potential utilisation of tissues in the biosensors for B-ETs is associated with proper production and immobilisation on the transducer surface. As compared to the cultured cells, the intact tissues can be easily obtained with the fairly preserved taste-coding network structure. Tissues for the biosensors can be produced either from genetically modified cells *via* direct modification or by isolation from taste organs (Karami et al., 2019). Preparation of taste epithelium usually consists in the collection of a tissue section from intact taste buds in order to maintain cells' functionality. As compared to the cultured cells, intact tissue can easily be obtained with a well-preserved primary structure (Liu et al., 2013c). Recent developments in tissue engineering have shown the application of PVDF (poly(vinylidenefluoride-trifluoroethylene)) scaffolds to preserve the contractility of cardiomyocytes and promote cell–cell communication (Gouveia et al., 2017). It creates a flexible support for cell contraction and the aligned microfibres deposited on top of the nanofilm create conditions for cell alignment and electrical stimulation of the seeded cells. Changes in the potential of the tissue-based biosensor are usually recorded using microelectrode arrays (MEAs). The MEA chips can serve for a multi-channel recording of taste-induced signal for temporal-spatial analysis. In addition, they have the benefit of detecting signals of many cells synchronously, which is convenient for the comparative analysis of the recorded information in parallel. *In vitro* detection of cell potential with respect to salty taste can be monitored with the taste buds in epithelium and MEAs as the secondary transducer of the B-ET (Liu et al., 2013c). The developed tissue-MEA based B-ET provides an effective and reliable platform for recognising and distinguishing key salt components (Fig.3). Multi-channel results indicated two non-overlapping functional populations of TR cells in the taste buds mediating the salt sensing.

Furthermore, the multi-channel analysis provides the information concerning salt sensing pathways. Based on a 36-channel MEA as the platform monitoring the signals generated from taste epithelium, it was presented as a fast and reliable system for detection of bitter taste (Liu et al., 2013a). Responses of the B-ET reveal a dose-dependent behaviour in amplitudes and firing rates in stimulations of the same bitterness at different concentrations. It should be indicated that the authors obtained successful recording 11 times out of 21 experiments with the protocol of intact taste stimuli. In conclusion, factors such as sensor sensitivity and tissue stability should be addressed as crucial points in the biosensor design. The well tissue-electrode hybrid junction can only guarantee the potential recording. Studies on the genetic engineering of cells and MEAs provide an attractive approach of specific and selective responses to different tastes. The intact tissues containing type II cells of TR cells can be also considered as the sensing elements for the bitter-sensing biosensors. A well-preserved functionality of the taste epithelium and recording of generated signals using a proper transducer can provide promising approach to the measurement of sweet (natural substances and sweeteners) (Zhang et al., 2014), bitter (sucrose octaacetate, denatonium benzoate, quercetin) (Wei et al., 2017) and pungent (capsaicin and gingerol) substances (Qiao et al., 2015). Capsaicin can also be used as a TRPV1 channel activator to study the antagonistic effects of capsazepine, AMG 517, loureirin B, aconitine, tetrahydropalmatine and anandamide through the prepared TB tissue biosensors (Xiao et al., 2019). TB tissue of Sprague-Dawley (SD) rats expressing TRPV1 can be used as a sensing component to prepare a TB tissues-based biosensor. It is a step towards an explanation of the role of, among other things, amide groups in allosteric regulation of cannabinoids receptors and the analgesic mechanism. This can be reflected in investigation of nociceptive signals and throws new light on the construction of lead compounds and molecular structure optimisation in the development of analgesic drugs for TRPV1 (transient receptor potential vanilloid 1) channels.

Biological tissues, as multicellular structures of different cell types, interact with each other to provide specific biological functions. Apposite *in vitro* co-culture is necessary to explain internal mechanisms occurring in different biological tissues, including cell physiology, intercellular signalling pathways, geometry and composition of extracellular matrix (ECM) (Goers et al., 2014). For instance, a co-culture of neurons with tooth tissues in a biomimetic microflow system allowed the explanation of teeth innervation (Pagella et al., 2014). The neurons in sensory nerves can also reveal the ability to identify the information about taste by reaction to biochemical signals transmitted *via* neurotransmitters (L. Lu et al., 2017). Transmission of the signal between nerve and TR cells occurs with paracrine neurotransmitters and it is possible only if they are at a convenient distance. The first experimental observation of the intercellular transmission of taste signals from primary TR cells to neurons using calcium ion influx imaging was reported by Yun *et al.* (Yun et al., 2018). A highly sensitive, microfluidic tongue extracellular matrix-based platform simulating signal transmission from tastant-treated TR cells to adjacent neuronal cells was presented (J. S. Lee et al., 2018). Microfluidic studies have shown that the phenotypic characteristics and the functionality of primary taste cells for taste sensing can be improved by incorporating tissue-derived matrix components in the microfluidic systems, implying the potential usefulness of microfluidic devices as high-throughput standardised taste sensor platforms. However, the discrepancy in the optimal ECM concentration between the culture conditions (2D bulk versus 3D microscale culture) was observed and should be further elucidated. Utilisation of the ECM enables the simulation of signal transmission between taste cells and adjacent neuronal cells, thus paved the way for *in vitro* modelling of taste sensing and related mechanisms (J. S. Lee et al., 2018). The ECM-based system recreates the tongue's microenvironment and significantly improves the functionality of taste cells for sensing tastant molecules by enhancing cellular adhesion and gustatory gene expression compared with conventional collagen-based systems. In another work, Ivanova et al., 2017, established a more systematic approach for the

adhesion of NIH/3T3 fibroblasts on natural ECM components such as vitronectin and laminin for the analysis of adhesion properties of biomimicking materials. By using this approach, additional and more specialised live-cell biosensors can be developed. ECMs has been demonstrated as a powerful approach towards the immobilisation of viable cells on rigid, hostile surfaces and a helpful tool in electronics-integrated functional tissue models (Gupta et al., 2019).

TR cells do not fully cover a pre-cultured neuronal network. For this reason, the neuron cells are directly exposed to culture medium and affected by tastants (Peng et al., 2015). The application of the agarose gel (used as a skin cover on the neuron) is an efficient way to suppress a direct stimulation of neurons by tastants in a co-culture of neuron-gustatory cells. Agarose gel has been widely used for the separation of biomolecules due to the well-defined mesh size. The diffusion of small molecules through agarose gel depends on molecular size, pore size and gel-molecule interactions (Tokita, 2016). Implementation of the agarose gel skin results in an effective increase in selectivity of the stimulation of the TR cells by delaying tastants diffusion to neurons. It has been demonstrated that the agarose gel skin is a simple, fast and effective means to increase the signal selectivity of cellular responses in the co-culture of multiple types of cells (Le-Kim et al., 2019). The co-culture of different cells with neural networks is especially promising due to multilevel neuron interaction (Kaboré et al., 2018). Hence, the tissues and nerves of living organisms are considered to be promising candidates for the receptor elements of taste biosensors (Wu et al., 2015), and the attempts of co-culturing of neurons and TR cells mimicking the biological tongue are an indispensable step in the investigation of an intercellular response. The co-culture systems mimicking the biological tongues are essential to investigate intrinsic cellular responses to tastants and to develop new generation of B-ETs (J. S. Lee et al., 2018; Yun et al., 2018). An example of the isolated epithelium-based B-ET is shown in Fig. 4.

Fig. 4. Diagram of tissue-based B-ET (A) with the pattern of 36 channel MEA (B) and intact taste epithelium to record extracellular potentials from taste buds. Reproduced with permission from (Liu et al., 2013c).

2.2. Cells-Based Bioelectronic Tongues

The unique properties of biological systems are an inspiration to the implementation of their components as the receptor elements of chemical biosensors. A particular interest in TR cells is partially associated with their high affinity to the recognition of a wide spectrum of chemical compounds in the arrays of complex composition. The progress in the production process, amplification and processing of the signals from the components mimicking biological taste systems has led to the development of biosensors based on TR cells (Wu et al., 2014). Based on their histological appearance, the TR cells types can be divided into: type I (dark), type II (light) and type III (intermediate), type IV (basal) and type V (marginal) cells (Witt and Reutter, 2015). Basal cells with small microvilli (hair-like structures) at their apex are usually gustatory receptor cells acting as taste sensation elements for taste qualities. Differentiated marginal cells are possibly involved in the formation of the taste pore. Cells of type I, II and III are elongated and form the sensory epithelium of the bud. The narrow neck of type I cells interweaves around type II cells. In contrast, type II cells have a short and straight neck and a plasma membrane thicker than that of type I cells. Their microvilli project deeper within the taste pit than those of type I cells. Tight junctions seal the elements of the taste bud from the bud pit, so only the tips of the cells are exposed to tastants (Lee et al., 2017). Different TRs are expressed in different subsets of TR cells, which exhibit various properties and functions of taste sensation (Barretto et al., 2015). For example, it has been revealed that ATP is released from the type II cells of TR (Kinnamon and Finger, 2013) *via* special hemichannels in response to electric and chemical stimulation (Ohla et al., 2019). Release of ATP plays a key role in intercellular communication along the path of taste information transmission from the taste buds to

the central nervous system (Wong, 2018). It is still the subject of ongoing discussion whether multiple TAS2Rs are co-expressed in the same TR cells and whether or not mammals can discriminate between properties of various bitter tastes (Di Pizio et al., 2019). It is speculated that a single human taste receptor (hTAS2R) should respond to a wide range of bitter compounds because a number of compounds identified by humans as bitter is much higher than the number of hTAS2Rs. It has also been demonstrated that some hTAS2Rs differentiate between a broad spectrum of bitter tastes with a high selectivity (J. Chen et al., 2019). However, some hTAS2Rs show much lower specificity, especially hTAS2R38, which displays the affinity exclusively with the bitter compounds containing a thiourea group (N-C=S) in their structure (Qin et al., 2019; Tran et al., 2018), whereas T2R16 reacts to β-glucopyranoside (Kuhn and Meyerhof, 2013). In contrast to previous studies using either germ cell-based or *in vivo* B-ETs, cell-based B-ET presented by Qin *et al.* has high specificity on the receptor level, which explains the sophisticated bindings between bitter receptors and ligands (Fig. 5-1). The superiority of this B-ET is due to sole receptor expressed Caco-2 cells. Accordingly, irregular specificity between other hTAS2Rs should be taken into account when they are applied as the receptor elements in biosensors. Human bitter receptor cells (*e.g.* T2R38) are also promising recognition elements to detect specifically compounds by using Caco-2 cells, by endogenous expressing and interdigitating with an impedance sensor as a transducer. Qin et al., 2019 have developed a unique bio-inspired cell-based B-ET for the high-specificity detection of N-C=S-containing compounds, which can be used for pharmaceutical studies and drug development. Since isothiocyanates (agonists of T2R38) have shown desirable benefits as a cancer chemo-preventive drug (Liu et al., 2018), B-ETs can be a promising tool in finding more T2R38 agonists, which can be helpful in lung and esophageal cancers. Moreover, G-protein coupled receptors can combine with each other to form more complex structures to account for the selectivity towards more bitter tastes detection (Geppetti et al., 2015). GPCRs can interact with each other to form homomers or heteromers. Homomers involve interactions with the same receptor type while heteromers involve interactions between two different GPCRs. These receptor–receptor interactions modulate not only the binding, but also the signalling and trafficking properties of individual receptors. Opioid receptor heteromerisation (either with members of the same family) has been extensively investigated with the objective of identifying novel therapeutic targets (Fujita et al., 2014).

Cell-based biosensors, comprising living cells expressing natural receptors coupled with secondary transducers for monitoring intracellular signals, enable a more precise mimicking of the natural sensory and neurotransmission systems. A bottleneck in the development of the sensors based on TR cells is the acquisition of sufficiently functional cells and optimisation of the transducer coupling techniques. It seems likely to guarantee the ability to respond to particular taste stimuli and ensure an appropriate conversion of chemical signals into measurable cell and molecular responses. The main approaches to the acquisition of functional TR cells can be divided into two categories as follows: (*i*) isolation of primary TR cells directly from animal taste buds, which contain native TRs reacting to the chemical signals present in flavour substances; (*ii*) bioengineered mammalian cell lines, heterologically expressed with functional TR, which undergo co-expression with molecules (*e.g.* gustducin*)* – indispensable for intracellular taste transduction (Chikazoe et al., 2019). The TR cells are usually directly isolated from the tongue epithelium of the SD rats (Bigiani et al., 1997). Immobilisation on the transducer is usually preceded by deposition of a polymer layer (*e.g.* poly-L-ornithine and laminin mixture) to improve the biocompatibility and effectiveness of cell immobilisation (Chen et al., 2011). Moreover, it has been shown that accurate immobilisation enhances performance parameters of biosensors, including response time, a better correlation to target concentration and stability of the response with respect to time (Gupta et al., 2019). Properly isolated cells of primary receptors can be cultured on the surface of light-addressable potentiometric

(LAPs) sensors, allowing the detection of a response of single receptor cells under various tastant stimulations (C. Wu et al., 2017). Recently, a bionic *in* vitro cell-based B-ET has been developed for bitter and umami detection (Wei et al., 2019) with MEA as secondary transducer. For the first time, the cardiomyocytes have been proposed as a biorecognition element and provided a promising way in taste detection and pharmaceutical studies. The taste-based biosensors are more and more frequently made using bioengineered cell lines, expressed by defined types of TRs of mammalian cells. HEK293T cells are successfully cultured and reformed as the TR cells which are employed as the receptor element for bitter and sweet tastes (Bassoli et al., 2014). Biologically modified HEK-293 cells, reformed by expression with hT2R4 receptor, were also used to construct a LAPS biochip with a movable focused laser illuminating the desired single cell. It was pointed out that both chemical receptors could respond to their natural target molecules in a dose-dependent manner within a certain concentration range (Du et al., 2014b).

It has been shown that the type II cells relay the taste signals to surrounding TB cells by ATP release in a cell membrane voltage-dependent manner, which plays a substantial role in taste signal transduction (Kinnamon and Finger, 2013; Wong, 2018). An ATP-sensitive aptamer-modified LAPS surface for bitter stimulation was used to develop a TB cell-based biosensor (Du et al., 2018). Dual extracellular recording was applied to distinguish variations in cell membrane potentials as well as ATP release of a single TB cell. In addition, microelectrode array (MEA) was applied as a useful platform for monitoring the cell response to different types of taste sensations and TC chips for the taste transduction mechanism (Liu et al., 2013a, 2013c; W. Zhang et al., 2017). A hybrid B-ET for the detection of sour taste and the mechanisms of its coding, based on MEA and TR cells was explored to detect sour tastant from TR cells during and after stimuli, which also functioned as a switch (W. Zhang et al., 2017). They suggested that acid-sensing ion channels should contribute to the On response, and polycystic kidney disease protein-like (PKDL) channels coding the offset stimulations, respectively. In addition, the response was attenuated after it has been already stimulated, which might be due to the inactivation or desensitisation properties of the channels. The designed MEA and TR cell-based B-ET provided a promising platform to recognize taste stimulations and help study taste chemosensory and the coding mechanism in a non-invasive manner (Fig. 5-2). It is also possible to discriminate between a mixture of tastes with electrochemical impedance analysis using the cells with sweet and bitter receptors Hui *et al.* (Hui et al., 2012, 2014). Impedance data from this type of biosensor based on TR cells are processed using bistable stochastic resonance (SR) for calculating the signal-to-noise ratio. Taste receptors expressed within TR cells play an important part in tastants detection. When tastant molecules bind to specific receptor of TR cells, the chemicals (such as Ca^{2+}, K^{+}, neurotransmitters, *etc*.) are released. An electrochemical working station revealed specific reaction to particular mixtures of bitter and sweet tastes, offering a useful way in the research on the gustatory mechanism. Recently, a cell-based reusable sensor platform technology using histamine type 1 receptors for drug effect screening has been demonstrated (Pham Ba et al., 2017). Another proposed concept involved an electrolyte-semiconductor (ES) structure in the LAPS system for biological sensing and imaging purposes (Du et al., 2018; Wu et al., 2019). The utilisation of mammalian male reproductive cells as the receptor element of a sensor provided sensitive and selective detection of bitter taste (Hu et al., 2016). By using calcium and probenecid (bitter receptor blocker). Hu *et al.* confirmed that T2R activation contributed to the cell impedance responses of this germ cell-based biosensor. Apart from the application of single cells for the construction of a B-ET, there is also an approach taking advantage of the entire animals, together with the brain-machine interface (BMI), as the receptor element of biosensors (Qin et al., 2017, 2016). In this type of bioelectronic systems, a rodent gustatory system is considered as a black box transforming the chemical input (*e.g.* bitter stimulation) into an electric output (*e.g.* extra-cellular potentials of a single

neuron or neural networks in gustatory cortex); it further utilises these neural electrophysiological signals to decode the quality and intensity of stimulation. Recent studies in molecular biology have shown that TRs is not only expressed in taste cells, but also in the airways and the gastro intestinal (GI) tract (Ekstrand et al., 2017). The cell-based biosensors would offer an efficient way of studying the chemosensation in the GI systems, which have a potential for the treatment of diabetes and obesity (Fournel et al., 2016). The method of spatial manipulation of highly sensitive mammalian cells and guide them directly to the target electrodes in a bioelectronic sensor was presented by Pedraza et al., 2015. The developed cell-based MEAs glucose sensor uses the multi-parametric sensing ability of pancreatic islet cells for the treatment of diabetes.

Disadvantages (Table 1) of the cell-based biosensors have hampered their practical applications and are the main bottleneck of the TC-based biosensors. In the future, the research in this field should put an emphasis on developing new techniques to obtain functional TR cells and a convenient and efficient way for a combination with transducers. The trends connected with the cell-based biosensors are mainly focused on the bioengineered acquisition of cells in a cheaper and more efficient way, which accelerates the development of biomimetic chemical sensors. The technique of coupling cells and transducer is also a decisive factor influencing bioengineered cell-based biosensors. With the introduction of new techniques (such as tissue reengineering, 3D and 4D printing), growing cells onto 3D multifunctional platforms would become easier, leading to the development of a new generation of cell-based biosensors (Gupta et al., 2019). The future of B-ETs development is connected with the cell-based biosensors capable of fast, complex and high-throughput measurements. Moreover, the problems with the combination of the whole cells with the transducer platforms based on nanomaterials (related to dimensions, proper positioning, non-specificity and high noise) can be overcome by the application of natural receptor-expressing cell-derived nanovesicles mimicking the whole cell-like natural receptor-mediated intracellular transduction, extensively presented in Section 2.3.1.

Examples of cell-based B-ETs are shown in Fig. 5.

Fig. 5. Examples of cell-based biosensing instruments. 1) Schematic diagram showing cell-based bioinspired B-ET for iso-thiocyanates bitterness detection (A). Activation of the IP3/DAG pathway induces increased intracellular Ca2+ and membrane truffles (B), application of bitter compounds induces morphological and attachment changes as well as increases cell-electrode impedance (C). Copyright with permission from (Qin et al., 2019). 2) A biomimetic whole-cell based B-ET with MEA transduction: A switch for On- and Off- response of acid sensations. Acid-sensing ion channels and Polycystic-kidney disease-like channels (A) for acid sensing biosensor design (B) by extracellular electrophysiological recordings of taste cells. Copyright with permission from (W. Zhang et al., 2017).

2.3. Receptors-Based Bioelectronic Tongues

The progress in the research conducted on the mechanisms of smell and taste transduction, which occurred recently together, has had a significant impact on the development of the receptor-based biosensors for chemical sensing (Doty, 2015). There are about 100 taste receptors composed of a lipid bilayer in the taste buds of the human tongue. It has been reported that sweetness, bitterness and umami receptors are expressed in not only taste buds, but also digestive organs and kidneys. The coding of taste is the specific interaction of tastants with taste receptors or ion channels in cells, where a complex series of stimulation occurs and the potentials are generated (Liu et al., 2013b).

The preparation of primary cells results in killing animals and is impossible to control the acquisition of reproducible biorecognition elements. The TRs are usually extracted from the cells expressed with functional TRs. Mammalian cell lines or bacterial cells, which are heterologously expressed with the functional TRs, could solve these problems (Du et al., 2014a). Functional membrane receptors are used as the biosensor elements (Wu et al., 2014). As with the cell-based

biosensors, a key stage in the design of the receptor-based biosensors is the efficiency of acquisition of pure membrane receptors and their immobilisation on a transducer. The successful extraction of GCPRs is considered to be a critical point for the development of B-ETs. The successful extraction of GPCRs, considered to be a critical point in B-ETs development, involves purification and manipulation techniques relying either on a commercially produced silica-based column or on a centrifuge (often both) (Kumar and Plückthun, 2019). Although it has been known that magnetic beads can provide an elegant answer to these issues (Lorenzen et al., 2019). GPCRs are highly relevant in the medical field as drug targets (Hauser et al., 2017), appealing as a recognition elements in biosensors due to the wide variety of ligands they can detect (Song et al., 2014). In view of the crucial character of the functional TRs in biomimetic sensors, there is an urgent need for the optimisation of the purification techniques of transmembrane receptors and their immobilisation on the transducers (Wu et al., 2013). Among other things, it involves an appropriate modification of the transducers' surface in order to ensure effective immobilisation of the functional membrane receptors (Altintas, 2017). The simplest and most convenient solutions involve physical adsorption methods. However, they are characterised by low stability and reproducibility due to the problems with control of chemical and physical properties of the adsorbed molecules. It is the chemical modification of the transducer surface, *e.g.* covalent polymer coating or site-specific conjugation, which improves thermal, mechanical and solvolytic stability as well as reproducibility of the receptor-based biosensors (Kumar et al., 2018). Employing self-assembled monolayers (SAMs) can also provide the useful improvement of the effectiveness and stability of receptors immobilisation on the transducer (Giménez-Gómez et al., 2017). A combination of high stability of SAMs and high specificity of aptamers (nucleic acid ligands) constitutes an interesting alternative to classical immobilisation techniques (Singh et al., 2019).

The expression systems based on bacteria enable mass production of recombined TR proteins (Son et al., 2017). In the majority of cases, expressed receptors are collected and purified from lysed cells (*e.g. Escherichia coli*), followed by coupling with a secondary transducer. Recently, a new concept based on electrolyte-semiconductor (ES) structure has been suggested in the LAPS system for biological sensing and imaging purposes (Tu et al., 2018; Wu et al., 2019). This helps to maintain receptor activity and simplifies the B-ETs design (J. Wang et al., 2019). Mammalian TRs allow the recognition of various molecules responsible for taste sensations. For instance, human sweet TRs hT1R2-hT1R3 of class C GPCRs specifically bind with compounds such as aspartame, saccharin and sucrose. This enables discrimination between nourishing and non-nourishing forms of sweeteners (Koizumi et al., 2011). Implementation of indium tin oxide (ITO) as a semiconductor enabled simple, inexpensive and sensitive detection of the analytes (D.-W. Zhang et al., 2017). This concept has led to the design of a novel bioelectronic taste sensor based on bioengineered *E.coli* cells combined with the ITO-constructed electrochemical sensors – considerably simple, robust and inexpensive assay for detecting specific bitter substances (J. Wang et al., 2019) (Fig. 6-1). The proposed biosensor showed dose-dependent responses to denatonium (bittering agent in the prevention of accidental poisoning), while native *E. coli* did not response to the stimuli, indicating the communication of the hT2R4 receptor with denatonium controlling cellular metabolism. For the first time, *E. coli* cells expressed with taste receptors have been used as a sensitive element directly, with no additional steps such as cell/protein immobilisation, and protein extraction were collected.

2.3.1. Nanovesicles-Based Bioelectronic Tongues

The need for the integration of the functional components of biological senses systems with transducer platforms in nanoscale (*e.g.* Carbon Nanotubes- CN) results in progress in the development of miniaturised mammalian cells – nanovesicles, nanosomes and nanodiscs (Molinaro et al., 2018). Mammalian receptor cells usually have a diameter of 10 µm, so they cannot be coupled

with the transducers in nanoscale. Moreover, mammalian cells produce a number of signals regarded as interferences; they also generate high noise caused by cell metabolism. In order to monitor the signals generated by cells, it is necessary to control their number and position. Nanovesicles utilising the mammalian cell-derived components as the recognition elements can offer an alternative strategy as the mammalian cells provide an effective environment for the expression of fully functional class C GPCRs (Kwon et al., 2019). Production of class C GPCRs, especially dimeric GPCRs, using bacteria, is hindered due to their size and the complicated structure (Park et al., 2019). Nanovesicles using the components of mammalian receptor cells offer effective environment for the expression of fully-functional class C GPCRs (Yoshida et al., 2019). The use of a lipid membrane, immobilising hTRs, enables the construction of B-ETs capable of biological sensing of taste (Ahn et al., 2018; Kim et al., 2011; Song et al., 2014, 2013). The acquisition of suitable cells of TRs expression involves cytochalasin B, which destabilises cytoskeleton and propagates the formation of nanovesicles. The resulting structures adopt a spheroidal form of uniform diameter of about few hundred nm. Mammalian cells can be used for heterodimer expression of the TRs. This allows the construction of a stable cell line expressing hTAS1R3, and then also expressed hTAS1R2 in the hTAS1R3-expresed cell line (Song et al., 2014). Co-expressed TRs played the role of heterodimer receptors of human sense of smell. Immobilisation of nanovesicles on the SWNT-FET transducer provided the B-ET design with a specific response to sweet taste (Song et al., 2014). This also permitted the verification of dimer GPCR activity. Human TRs, such as heterodimeric class C GPCR composed of human TR type 1 member 1 (T1R1) and member 3 (T1R3) (umami TRs), due to their complicated structure, create many difficulties during implementation in the biosensors for the B-ETs construction. The B-ETs using nanovesicles with heterodimeric human T1Rs have been successfully demonstrated to have human-like performance for discriminating between sweet and umami tastes (Jang et al., 2016). With the application of the nanovesicles expressing hTR, composed of the class C GPCRs hTAS1R1 and hTAS1R3, separately immobilised on a multichannel of a graphene-based FET (GFET), a duplex B-ET mimicking human umami and sweet taste perception was successfully demonstrated (Fig. 6-2). The enhancing effect of cyclamate has been detected by using the nanovesicle-based umami taste sensor with the heterodimeric receptor. The presented technique can be a useful tool for the detection of tastes instead of the sensory evaluation and development of new artificial tastants in the food and beverage industry. The results confirmed that the nanovesicles maintain their original function, which is the signal transduction of the taste receptor cells, using photolithography and reactive-ion etching (RIE) processes to prepare graphene micropatterns (GMs) in the FET system – a reliable electrical contact can be preserved. Ahn *et al.* also expressed hTAS1R1 in the hTAS1R3-expressed cell line (Ahn et al., 2018). They used an umami ligand binding domain called Venus flytrap (VFT) originating from T1R1, instead of using the whole heterodimeric complex of receptors – immobilised on the same transduction platform. As compared to the previous B-ET model (Jang et al., 2016), the constructed B-ET system for umami taste was characterised by a simpler construction process and a higher real-time response sensitivity (*approx.* 1 nM) to monosodium L-glutamate.

The nanovesicles-based systems ensure successful integration with the support in nanoscale (with nanosupporting materials, *e.g.* CNs, graphene, *etc.*) owing to ionic transport *via* GPCR-mediated ion flux and suitable signal transduction. They also provide advantages resulting from stability, as they can be stored for a few months at low temperature with no risk of a significant drop in activity (Xu et al., 2019). In addition, their properties exhibit a low dependence on environmental factors, such as temperature, buffer conditions and time of analysis, which are relatively variable in case of the cell-based biosensors (Goh et al., 2017; Honary and Zahir, 2012). However, the isolation of nanovesicles from the TR cells enables incorporation in their structure of membrane proteins and

cytoplasmic components which, among other elements, are responsible for signal transduction. Thus, nanovesicles are characterised by properties similar to the TR cells. In addition, they exhibit some properties of protein materials, *e.g.* a possibility of large-scale production. The serious limitation of nanovesicles application to the construction of the biosensors is the lack of their regeneration for reuse due to the absence of an ion pump in their structure (Z. Chen et al., 2019). Moreover, they have limitations as biosensor recognition elements because they are biological materials, i.e. they do have a shelf life. Currently, more stable and reusable alternatives to nanovesicles, such as nanodiscs (Denisov and Sligar, 2017), are under development (M. Lee et al., 2018). It is speculated that a new generation of B-ETs will take advantage of particular domains in TRs, which are responsible for the binding of flavour molecules and taste sensations. The coupling of appropriate domains of the TRs, which selectively and specifically bind particular ligands (instead of the utilisation of a receptor complex) with proper transducer platforms, makes it possible to construct B-ETs with parameters similar to those of their biological counterparts (Ahn et al., 2018). B-ETs, which employ the N-terminal parts of class C GPCRs, possess a high potential as the tools for GPCRs structural investigations, taste standardisation and successful implementation in real applications. Sweet receptor-based B-ETs will play a great role in understanding the mechanisms of sweetness inhibition and the possible design of new sweetness inhibitors for pharmaceutical and food development industries.

Examples of receptor-based B-ETs are shown in Fig. 6.

Fig. 6. Examples of receptor-based biosensing devices. 1) A bioelectronic taste sensor based on hT2R4 efficiently expressed in E. coli combined with ITO-constructed electrochemical sensors (A) for a specific bitter compounds detection by capacitance-voltage measurements (B). Copyrighted with permission from (J. Wang et al., 2019). Production of nanovesicles expressing human taste receptors T1R1/T1R3 and T1R2/T1R3 (A) for the production of graphene-based FET duplex B-ET (B) used for umami/sweet detection using current-voltage recordings (C). Adapted with permission from (Jang et al., 2016).

2.4. Enzymes-Based Bioelectronic Tongues

Enzymes are specific macromolecular biological catalysts that accelerate chemical reactions through lowering activation energy. Enzymes play a key role in life processes as they catalyse biochemical reactions (Monteiro and Almeida, 2019). They usually bind with ligands due to their complementary shape. The use of enzymes as the receptor elements of biosensors depends on their effective immobilisation on the transducer surface. This immobilisation is ensured either directly or using an appropriate matrix: (i) covalent bonding, (ii) physical adsorption, (iii) entrapment, and (iv) affinity (Nguyen et al., 2019). It has been discovered that the association of MWCNT with poly(amino acid) for the modification of electrode represents a useful platform for immobilising of biomolecules such as xanthine oxidase enzyme. An example of the production process is shown in Fig. 7

One of the aims of immobilisation procedures is to establish direct communication between the electrodes and enzymes, the so-called direct electron transfer (DET) enabling the construction of the biosensors with reduced susceptibility to the problems originating from non-enzymatic reactions (*e.g.* interfering species being non-enzymatically detected). B-ET using the DET capabilities of CDH from different origins as the recognition elements were reported by Cipri *et al.* (Cipri et al., 2016). The extensive diversity of CDHs facilitates the construction of recognition systems for other analytes of sugar origin, *e.g.* cellobiose and maltose (Bollella et al., 2017). The enzymes from the oxidoreductase group were also implemented in B-ETs (Al-Issa et al., 2015; Medina-Plaza et al., 2015; Weltin et al., 2014). However, the reported enzymatic activity was not high enough and peaks assigned to the enzymatic process were not satisfactory. There is a great emphasis on biosensor selectivity, the main drawback still being their cross-reactivity. False-negative results are rarely reported, but false-positive results are more frequent in the literature and depend on several factors, such as pH, sample complex, viscosity or ionic strength (Badea et al., 2016). Without sample clean-up

or extraction before the testing, matrix effects might be expected to lead to a significant overestimation of analyte concentration, mainly when colour samples are tested in colorimetric detection. Therefore, to avoid misinterpretations, positive results should be confirmed with the conventional analytical methods. A new generation of enzyme-based biosensors offer superior selectivity because they are able to operate in a potential range which is closer to the redox potential of the enzyme (Nguyen et al., 2019). The amplification of the enzymatic signal, increasing cross-selectivity and the sensitivity of enzyme-based biosensors can be enforced by a combination with a suitable electron mediator (*e.g.* conducting polymers, gold nanoparticles) (Mavrikou et al., 2017). A combination of enzymes (tyrosinase and glucose oxidase) with composites (poly-pyrrole (Ppy) or polypyrrole/AuNP (Ppy/AuNP) can also be used for the construction of the B-ET sensitive to phenols and sugars (Garcia-Hernandez et al., 2019). The use of Ppy/AuNPs as the electron mediator increases the sensitivity and cross-selectivity of the sensors, thus improving B-ET performance. A novel biocompatible matrix (MWCNT/Poly(L-Asp) with glassy carbon electrode for the immobilisation of xanthine oxidase was also presented (Yazdanparast et al., 2019). Modification of a specific enzyme using ZnO nanowires makes it possible to conduct a piezo-biosensing process of nanocomposites (Han et al., 2017). A new self-powered wearable gustation e-skin for mimicking TB has been completed by Zha *et al.* (Zhao et al., 2018) based on the enzyme-modified/ZnO nanowire arrays on a patterned flexible substrate. One of the development directions is the intensification of the research conducted on self-powered bionic systems which can convert human-motion energy and output triboelectric current without an external electricity source, involving new cell designs, new materials and new genetically engineered biological catalysts and providing innovative approaches for developing artificial gustation systems (Grattieri and Minteer, 2018; Zeng et al., 2018).

A bottleneck in the production of the enzyme-based biosensors for *in vivo* analysis is the reduction of signal and selectivity with respect to interferents and other interferences connected with a complicated composition of a matrix. The next generation of enzymatic biosensors will involve enzymatic engineering and implementation of hybrid materials to promote enzyme stability and minimization of endogenic interferences (Nguyen et al., 2019). It is expected that the development of enzyme modification, biocompatibility of platforms and immobilisation techniques will contribute to progress in the enzyme-based B-ETs regarding improvement of the metrological parameters of the biosensors used for the construction. From the historical standpoint, enzymes were the first elements of molecular recognition in biosensors. Enzyme-based devices constitute the largest category of biosensors and their development is connected with the optimisation of enzymatic microsystems, which opens many possibilities of biochemical analysis.

Fig. 7. 1) The diagram of enzyme-based electrochemical biosensor fabrication by covalent immobilisation of xanthine oxidase on glassy carbon electrode with Poly(L-Asp)/MWCNT nanocomposite: (A) MWCNTs casting, (B) electropolymerisation of poly (Asp), (C) xanthine oxidase immobilisation on a transducer. Adapted with permission from (Yazdanparast et al., 2019).
2) An example of enzyme-based biosensors array (B-ET) for wine sample analysis (A) by cyclic voltammetry and ANNs (C, D). Adapted with permission from (Cetó et al., 2014).

2.5. Molecularly Imprinted Polymers and Peptides

The materials, which are constantly gaining popularity in different applications including sensor construction, are molecularly imprinted polymers (MIPs) able to recognise biological and chemical analytes, which are synthesised using host-guest principles (Ahmad et al., 2019). They show selective molecular recognition sites created during the polymerisation and functional monomers of MIPs interact with the ligand to perform a well-stablished complex. This step is crucial as different interactions between these two species will rule both the selectivity and the further recognition process. One of the principal goals of molecular imprinting is to achieve binding affinities similar to

the high selectivity offered by natural receptors for their ligands. MIPs allow the detection of molecules in situations where a specific bioreceptor is missing, which makes them elements of biomolecular recognition to be employed in biosensors (Diouf et al., 2017; Menon et al., 2018) and B-ETs (Herrera-Chacon et al., 2018). Presented by Herrera-Chacon et al., 2018, MIPs-based B-ETs (Fig.8-1) with graphite epoxy composite electrodes and sol-gel were used for voltammetric detection of polyphenolic compounds present in wine. The reported principles are of generic use, suitable for a wide variety of examples where the conditions is that differentiated MIPs can be synthesised and template compounds are electroactive – a necessary condition to obtain the voltammetric transduction. The MIPs-based sensors can be improved by the implementation of nanomaterials (graphene, carbon nanotubes, nanoparticles, *etc.*) (Ahmad et al., 2019); they also can be used for direct and indirect detection (labelled/label free) (BelBruno, 2019). Compared to other biorecognition elements, MIPs can operate within a wide pH range, at higher temperatures and pressures and are characterised by high physical and chemical resistance offering the possibility of mimicking specific biological reactions in non-physiological media. Moreover, MIPs provide sensitivity and selectivity with chemical and physical stability, reusability and cost-effectiveness due to the unique characteristics of monomers (Idil et al., 2017). A serious limitation in the development of the MIPs-based B-ETs with electrochemical transduction is a complex process of MIP integration with the electrode surface, which is due to the formed isolation layer and inability of electron transfer between the MIP-trapped analyte and the electrode. There is a deficit in the availability of the facile immobilisation methods for MIPs onto transducers; such methods would allow for the rapid substitution of the molecule targeted by the sensor by a change in the imprinted template rather than the laborious redesign of the sensor, thus greatly increasing the versatility of the systems. The main difficulty in the search for an effective immobilisation method for particulate MIPs lies in the compromise between extraction or measurement time and reproducibility. One of the approaches was applied (Bates and del Valle, 2015) through a modified sol–gel to immobilise MIP microspherical particles onto epoxy-graphite electrodes (Bates and del Valle, 2015). The preferential occupation of the surface of the sol–gel membrane by the MIP particles allowed a short measurement time and the regeneration of the electrode.

Synthetic peptides are also used as an alternative to typical biological elements of analyte detection in aqueous samples (Karimzadeh et al., 2018). Peptide-modified electrodes (Fig. 8) can be used for analyte measurement as mono-electrode sensors (Wan et al., 2018; Yang et al., 2018) or as arrays of B-ETs (Serrano et al., 2014), in which each electrode is modified with a different peptide, providing a multivariate response. Peptides can selectively bind many compounds including metals (Wan et al., 2018), toxins (Liu et al., 2017) *etc.* (Karimzadeh et al., 2018; Xing et al., 2019). The specificity and affinity of the interaction between peptides and ligands can be adjusted using corresponding side-chains of amino acid residues (Wan et al., 2018). Owing to the high affinity of peptides with particular ligands, the problem of interference can be minimised and they can be used as the receptor elements of biosensors operating in real conditions (Liu et al., 2017). The application of MIPs and synthetic peptides (Fig.8) in the B-ETs design opens a wide spectrum of opportunities, where the aforementioned bioreceptors, combined with solutions known from ETs, can be complementary to typical biological systems of recognition, in situations of missing receptor elements. The developed endotoxin peptide-based biosensor, presented by Liu et al., 2017, may have potential applications in testing the cell culture medium, pharmaceutical products and bacterial contaminations in drinking and environmental water. Indeed, the design of artificial molecules, such as aptamers, biomimetics, molecular imprinting polymers, peptides, nucleic acids and ribozymes, provided a trustworthy support for biosensing technology in solving some of the problems related to specificity, sensitivity and stability.

Fig. 8. 1) B-ET made of MIPs and the modelling capabilities of ANN able to identify 4-ethylphenol and 4-ethylhuaiacol in wine samples. Reprinted with permission from (Herrera-Chacon et al., 2018). 2) Peptide-based biosensor preparation with a gold electrode and immobilised peptide with C-terminal cysteine residue for the quantitative determination of endotoxin by the recording of voltammetric responses. Copyright from (Liu et al., 2017).

Table 1 Advantages and disadvantages of biomaterials used for bioelectronic tongue development.

Biorecognition element	**Advantages**	**Disadvantages**
Tissues	- can respond to a single compound or a mixture of compounds, - can be employed directly, - signals from many cells can be detected simultaneously, - natural functions well maintained, - real-time detection of extracellular signals,	- challenges in maintaining functions for a longer time, - low stability, reproducibility and repeatability, - time-consuming preparation, - not fully unveiled signal of the transduction mechanism,
Cells	- signals similar to biological ones, - suitable for physical absorption, - fairly straightforward preparation process, - homology and consistency of cell line-derived taste cells allow for more stable cellular responses, - provide insights into physiological relevant responses at the cellular level,	- difficult to control taste cell types containing desired taste receptors, - primary taste cells diversity is limited by the number of living animals, - purification of taste cells is laborious and time-consuming, - expression efficiency of taste receptors in heterologous cell systems is usually low, - high costs due to accessory instrumentation, - problems with regeneration and storage life, heterogeneity in cell populations,
Taste receptors (nanovesicles)	- more stable and sensitive responsive capability for taste sensing, - generation of signals similar to cells, - production from cells ensures that most cell components are maintained, - nanovesicles highly useful with nanomaterials, - long-time storage at low temperature,	- extraction and purification are time-consuming and expensive, - low stability under ambient conditions, - difficult to regenerate after analysis,
Enzymes	- possibility of modifying the catalytic properties, - wide range of commercial enzymes, - highly useful with nanomaterials, - enzyme-substrate of high specificity,	- some enzymatic systems, *e.g.* (i) oxidases, are oxygen dependent, (ii) dehydrogenases are highly limited by the use of co-substrates, - enzymatic sugar biosensors can be heavily affected by the presence of other electroactive interferences that are always common in real industrial samples, - constrained by usage of mild enzymatic conditions as proteins may suffer denaturalisation, - limited number of substrates for which enzymes have been evolved, - limited interaction between environmental pollutants or food contaminants and specific enzymes, - lack of specificity in differentiating among compounds of similar classes such as organophosphate and carbamate pesticides, - reduced signal response and selectivity due to the presence of fouling agents and interference by chemicals present in the sample matrix,
MIPs/Peptides	- easily tuneable sensitivity and selectivity, - good biocompatibility, - good stability and reproducibility, - straight forward chemical synthesis, - prolonged storage, - high selectivity.	- limited to certain analytes only, - expensive and time-consuming synthesis.

3. Applications of Bioelectronic Tongues

The growing demand for fast, sensitive and selective analysis techniques capable of fulfilling market expectations stimulates the development of biosensors and B-ETs. The use of inexpensive, easy-to-use, mobile and fast devices for analysis of samples occurring in complicated matrices is an attractive alternative to classic analytical techniques (Cetó et al., 2016).

B-ETs offer tuning of the operation mode to the desired application (*i.e.* qualitative/quantitative analysis, prediction of total or individual indexes, classification/discrimination of samples, *etc.*) and they are capable of subjective correlation related to non-chemical indexing of the recorded data, for example the artificial reproduction of the descriptors identified by sensory panels. In addition, B-ETs are capable of working without a preliminary test, making them a viable alternative to conventional techniques. B-ETs ability to reproduce biological flavour systems sheds new light on the attempts at taste standardisation, coding information about tastes and reproduction of taste sensations (J. S. Lee et al., 2018). For the first time, the B-ET using *in vitro* system based on a cellular model overexpressing sweet and bitter receptors has been applied for molecular analysis of amino acids by Bassoli *et al.* (Bassoli et al., 2014). They suggested that the presented model could add some useful information to those obtained with *in vivo* studies and, therefore, they can help to clarify better taste responses at the molecular level. The ability to recognise and distinguish accurately flavour molecules is necessary for quality control, *e.g.* during developing drug formulations, food quality control and to ensure better understanding of taste functionality (J. S. Lee et al., 2018). Zhang *et al.* (W. Zhang et al., 2017) proposed a promising platform to recognise taste stimulations, helpful in studying chemosensory and coding mechanisms in a non-invasive manner (Fig. 5-2). Based on the results presented by Qin *et al.* (Qin et al., 2019), B-ET may be a promising tool for the detection and identification of bitter compounds targeting at certain bitter receptors, and they can be used in the fields of the pharmaceutical and food industry, being especially beneficial in finding more agonists of T2R38 helpful in lung and esophageal cancers (expanded in Section 2.2).

The bioelectronic tongue potential is especially evident in the applications related to the main assignments of biological counterparts, *i.e.* food, beverage, water analysis (Table 2). The use of biosensors and B-ETs in the food industry is mainly associated with food quality control, essential for consumer protection (J. S. Lee et al., 2018; Qin et al., 2019; Son et al., 2017; J. Wang et al., 2019; Zhao et al., 2018). The applications include biosensors for detecting pathogens, changes in food product composition and the presence of additives (Table 2). Noticeable interest is focused on the applications of B-ETs in the analysis of phenolic substances (Czolkos et al., 2016; Garcia-Hernandez et al., 2019; Herrera-Chacon et al., 2018; Medina-Plaza et al., 2015), widely used in industry as intermediate products (*e.g.* olive oil production), antioxidants, gasoline additives, cleaning agents, tannins, production of drugs, pesticides, *etc.* (Mark et al., 2019). Enzyme-based B-ETs have also been applied in the detection of analytes in food samples (Table 2). Typical examples of the enzymes employed in the biosensors for food-related applications are as follows: cholinesterase for organophosphorus and carbamate pesticide analysis; tyrosinase or laccase for analysis of phenols, quinones and related compounds; glucose oxidase for sugar content analysis; carboxyl esterase, alcohol oxidase, carboxypeptidase, L-aspartase, peptidase, aspartate aminotransferase for aspartame; xanthine oxidase to detect the level of freshness in fish; glucose oxidase, β-galactosidase, fructose dehydrogenase and other dehydrogenases to detect glucose in fruit juice; lactose or lactulose in milk and so on (de Sá et al., 2016; Monteiro and Almeida, 2019; Salvo-Comino et al., 2018). The enzyme attracting significant interest as far as the B-ET design is concerned is cellobiose dehydrogenase (CDH) because it has shown DET for a variety of substrates, including analytically relevant sugars, glucose and lactose (Bennett et al., 2019). The great variety of CDHs may allow the system to be tailored to also detect other analytically relevant sugars such as maltose or cellobiose (Scheiblbrandner and Ludwig, 2020). The findings are of potential interest for new biosensor applications in the food and dairy industry for detecting analytes and adjusting the presence of activators or interferents in one run using the same analytical method which is more cost and time

efficient (Cipri et al., 2016). Also a concept of the B-ETs for taste evaluation in pharmacy has been increasingly popular in the literature (Ahn et al., 2018; Qin et al., 2019; Wasilewski et al., 2019). Currently, there are no commercially available B-ETs based on the TRs. A progress in potential applications and successful commercialisation depends on solving some technical problems associated with the stability in aqueous media, repeatability and mass production of functional biorecognition elements. An alternative platform for the development of decoding methods, neural recordings, understanding of taste stimulus space and mechanisms of gustatory receptors are platforms using whole animals as sensing elements (Qin et al., 2017), where chemical information on tastants is decoded as neural response patterns. B-ETs can play an important role in many taste-related fields and present opportunities for potential commercial applications. The research on bioinspired artificial taste sensing systems is still a very active and promising field. Moreover, exciting opportunities, like chip-based sensor systems with an initial focus on an electronic taste chip system have been afforded by multiparameter sensing that mimics the sense of taste, overcoming the limitations of salty, sweet, sour, bitter, and glutamate sensing and moving into fingerprints of health and wellness (Christodoulides et al., 2019).

Examples of recent gustatory-inspired biosensors and B-ETs with a high application and implementation potential, including water, food, soil analysis, pharmaceutical industry, diagnostic analysis and other cases are shown in Table 2.

Table 2 Recent gustatory-inspired biosensors and B-ETs applications.

Biological recognition element	Transducer, data analysis	Target compound(s)	Sensitivity (range)/Selectivity	(Optional) application	Reference
Taste receptor cells (NCI-H716 and STC-1 cells)	Carbon screen-printed electrodes, stochastic resonance	Quinine	-	Sweet and bitter measurements	(Hui et al., 2014)
Functional unit of rat taste buds with type II taste receptor cells	MEA	Glucose, sucrose, saccharin, cyclamate	5 mM	Sweetness measurements	(Zhang et al., 2014)
Porcine taste bud tissues sandwich-type sensing membrane	Glassy carbon electrode	Sucrose octaacetate, denatonium benzoate, quercetin	Activation constants: 8.748×10^{-15}, 1.429×10^{-12}, 6.613×10^{-14} mol/L	Kinetics of receptors and ligands	(Wei et al., 2017)
Taste bud tissues of SD rats		Capsaicin, gingerol	1×10^{-12} mol/L	monitoring pungent substances, investigating ligand-receptor interactions	(Qiao et al., 2015)
Taste-bud tissue of SD rats fixed between two nuclear microporous membranes		Capsaicin, analgesic compounds	2.022×10^{-18} mol/L	Kinetics of capsaicin and competitive allosteric regulatory ligands	(Xiao et al., 2019)
DNA-mediated assembled taste and neuronal cells		Denatonium benzoate	5.0 mM	Bitterness measurements	(Yun et al., 2018)
Taste cells isolated from tongue tissue	Tongue extracellular matrix	Sucrose, NaCl, glycine, saccharin	0.01 mg/mL	Quality control of food products, healthcare	(J. S. Lee et al., 2018)
Neuron-gustatory cell with agarose gel coating	-	Denatonium benzoate	1 mM	-	(Le-Kim et al., 2019)
Caco-2 cells with expressed T2R38 bitter receptor	Impedance sensing	Phenylthiocarbamide, propylthiouracil	1 mM	Targeting bitter receptors, pharmaceutical and food industry	(Qin et al., 2019)
Human bitter (TAS2Rs) and sweet (TAS1R2/TAS1R3) receptors cloned into expression vectors	-	20 receptor agonists	3-100 mM	Molecular activity of D- and L-amino acids	(Bassoli et al., 2014)
Single taste bud cell isolated from rats	LAPS	Adenosine triphosphate	1 nM – 10 µM, low cross-selectivity	Recording of ATP release from a single taste bud cell	(Du et al., 2018; Wu et al., 2019)
Cardiomyocytes expressed from SD rats	MEA	Denatonium benzoate, diphenidol, monosodium glutamate	10^{-6} M	Umami and bitter taste detection, pharmaceutical industry	(Wei et al., 2019)
Taste receptor cells from SD rat	MEA	Sour compounds	-	Sour stimulation, elucidation of sour taste sensation and coding	(W. Zhang et al., 2017)

				mechanism	
Odorant Binding Proteins	Screen-printed electrodes with Graphene oxide	Docosahexaenoic acid, linoleic acid, lauric acid	10^{-9} – 10^{-4} mg/mL	Fat taste detection	(Y. Lu et al., 2017)
Human bitter taste receptor, hT2R4 expressed in *E.coli*	ITO-based electrolyte-semiconductor	Quinine, alpha-naphthylthiourea	50 – 500 nM	Pharmaceutical industry and food screening	(J. Wang et al., 2019)
Male mouse germ cells expressing bitter receptor T2Rs	8 chip impedance electrodes	Denatonium, N-phenylthiourea, 6-propyl-2 thiouracil, quinine	<1 mM	Bitterness measurements	(Hu et al., 2016)
Rat's gustatory cortex with a brain–machine interface	MEA	Denatonium benzoate	0.076 µM	Bitterness measurements	(Qin et al., 2016)
Rat's gustatory cortex with implantable brain-computer interface	MEA with neural decoding algorithms, PCA	Quinine, denatonium benzoate, salicin, NaCl, sucrose, HCl	1 – 100 mM	Bitterness measurements	(Qin et al., 2017)
Polypyrrole entrapping lactate oxidase, horseradish peroxidase	Thin-film gold microelectrodes	L-lactate	5.2×10^{-7} M, low cross-selectivity	Monitoring of malolactic fermentation in wineries	(Giménez-Gómez et al., 2017, 2016)
Tyrosinase with L-lysine membrane	Voltammetric, Au nanoparticles, ANNs	Hydroquinone, catechol	25 µM	*On-line* monitoring systems of wastes	(Yaoyu et al., 2014)
Peroxidase and glucose oxidase	Two miniaturized 8-channel array combining multiple enzymes combined with carbon and platinum electrodes, PCA, PLS	Phenolic compounds, pesticides	-	Evaluation of wastewater quality, prediction of organic pollutant indexes	(Czolkos et al., 2016)
A 90 mer long ssDNA aptamer (NG3)	FET with inter-digitated gold microelectrodes	*Plasmodium falciparum* glutamate dehydrogenase	0.1 pM – 10 pM, low cross-selectivity	Diagnosis of malaria (serum samples)	(Singh et al., 2019)
Human OR2J2, 2W1, TAAR5, and TAS2R38 cloned in the pET-DEST42 *E. coli* expression vector	Multi-channel carbon nanotube FET	Octanol, hexanal, hexanol trimethylamine, goitrin	~ 1 pM	Food freshness	(Son et al., 2017)
Nanovesicles with human taste receptors, heterodimeric GPCRs composed of hTAS1R2 and hTAS1R3	Single-walled carbon nanotube FET	Sucrose, glucose, as-partame, and saccharin	5 pM – 10 mM	Food and beverage industry	(Song et al., 2014)
Human umami ligand binding domain venus flytrap originating from T1R1 produced from *E. coli*	Graphene-based FET	Monosodium L-glutamate	1 nM, low cross-selectivity	Standardizing umami taste, beverage and food industry	(Ahn et al., 2018)
Nanovesicles with human T1R1/T1R3	Micropatterned graphene-based FET	Monosodium glutamate	100 nM, low cross-selectivity	Detection of tastes development of new artificial tastants	(Jang et al., 2016)
Tyrosinase, laccase	4 voltammetric electrodes, ANN	Catechol, m-cresol, guaiacol	-	Monitoring of pollutants in wastewater	(Cetó et al., 2015)
Flavin adenine dinucleotide glucose dehydrogenase	Osmium redox polymer, multi-walled carbon nanotubes on graphite electrodes	Glucose	5 mM, low cross-selectivity	*In vivo* biosensing and biofuel cells	(Bennett et al., 2019)

Cellobiose dehydrogenase	Gold/SAM electrodes, ANN	Lactose, glucose	~ 10 mM	Food and dairy industry	(Cipri et al., 2016)
Glucose oxidase, ascorbate oxidase, uricase	Metal electrodes (Pt, Au) with membrane coatings (nafion, chitosan)	Glucose	0.1 mM	Detection of glucose	(Al-Issa et al., 2015)
Glucose oxidase, lactate oxidase immobilized in a poly(hydroxyethyl methacrylate)-based hydrogel membrane	MEA	Glucose, lactate, oxygen, glutamate	1 – 5 mM	*In vivo* measurements of metabolism parameters	(Weltin et al., 2014)
Glucose oxidase, tyrosinase	Modified screen-printed electrode system, PLS	Glucose, polyphenol	-	Simultaneous sugar and phenols *in situ* analysis	(Medina-Plaza et al., 2015)
Membrane engineered Vero cells	Gold nanoparticle-modified carbon screen printed electrodes	Aflatoxin B1	0.05 ng/mL, low cross-selectivity	Screening of aflatoxin B1	(Mavrikou et al., 2017)
Tyrosinase and glucose oxidase	Polypyrrole or polypyrrole/ gold nanoparticles composites modified electrodes, PCA, PLS, SVM	Glucose and phenols	-	Food industry, Total Polyphenol Index prediction	(Garcia-Hernandez et al., 2019)
Xanthine oxidase	Multi-walled carbon nanotube and poly(L-aspartic acid) film on a glassy carbon electrode	Xanthine	3.5×10^{-4} µM, low cross-selectivity	Assessment of the meat products	(Yazdanparast et al., 2019)
Taste bud-inspired circuit based on glucose oxidase	Multi-walled carbon nanotube glassy carbon electrode	Glucose	0.644 µA mM^{-1}	Detection of tastes	(TermehYousefi et al., 2017)
Tyrosinase, laccase, Cu nanoparticles	4 voltammetric electrodes, ANNs	Catechol, *m*- cresol, guaiacol	0 – 80 mg/L, low cross-selectivity	Wine analysis	(Cetó et al., 2015)
Ascorbate acid oxidase, alcohol oxidase	Piezo-biosensing unit of ZnO nanowires Ti modified electrode	Ascorbic acid, ethanol	2×10^{-2} M, 10 mM	Food and beverages analysis	(Zhao et al., 2018)
Lactate oxidase, glucose oxidase, uricase, urease		Lactate, glucose, uric acid and urea	~ 0.5 mM	Disease diagnosis	(Han et al., 2017)
Primary motor cortex area of the mouse brain, alcohol oxidase	Polydimethylsiloxane/polypyrrole modified electrodes implants	Ethanol	0.0435 – 43.5 mM, low cross-selectivity	Beverage analysis	(Zeng et al., 2018)
Genetically modified acetylcholinesterase's B394 and B4	Two amperometric sensors incorporated in FIA system	Chlorpyriphos-oxon, malaoxon mixtures	-	Beverage analysis	(Mishra et al., 2015)
MIPs	MEA, ANN	4-ethylphenol, 4-ethylguaiacol	1.3 µg/mL, 2.4 µg/mL	Wine analysis	(Herrera-Chacon et al., 2018)
Glutathione, Cys–Gly, γ-Glu–Cys	Array of graphite–epoxy composite electrodes, FFT, ANNs	Cu^{2+}, Cd^{2+}, Zn^{2+}	0.1 – 1.5 µmol/L	Determination of heavy metals in environmental and biological samples	(Serrano et al., 2014)
Peptide (KKNYSSSISSIHC)	Gold electrodes	Endotoxin	0.04 EU/mL, low cross-selectivity	Testing of the cell culture medium, pharmaceutical products, bacterial contaminations	(Liu et al., 2017)

4. Prospects of Bioelectronic Tongues

Bioelectronic tongues constitute a combination of achievements in many fields, including biotechnology and electronics. Their development is mainly focused on the potential of the obtained biological receptor elements and the processing methods of the acquired data. It is expected that a combination of artificial senses of taste and smell, including with an electronic eye module or computer vision, can contribute to the improvement of a sample classification system using e-senses. The attempts of coupling two or three systems has so far led to the conclusion that the results subjected to fusion are more reliable than those treated separately (Buratti et al., 2018). It can be stated that a combination of different recognition systems brings many benefits to sensory analysis. However, the combined systems are still at a developmental stage and require more investigations to obtain a successful practical application (Wasilewski et al., 2019). Moreover, overcoming the developmental barriers is related to the implementation of micro-flow solutions and innovative nanomaterials, which can be used to widen the capabilities of B-ETs as far as analysis of a broad spectrum of analytes with the desired sensitivity and selectivity is concerned. Contribution from nanotechnology and microfabrication enables the construction of the high throughput biosensing platforms, which significantly extends the spectrum of potential applications of B-ET devices.

4.1. Microfluidics

Microflow technologies have created new perspectives in many branches of science (Shi et al., 2019). Microflow platforms in the form of microchips usually comprise a properly designed network enabling performance of many operations in a single device or in particular modules, such as sampling, filtration, extraction, concentration, separation, detection, *etc.* Operation with the volumes of nano-/microliter levels provides significant cost reduction and in some cases, where availability of particular material is limited, it is absolutely indispensable to conduct the measurements. Moreover, the integration of a preliminary sample preparation step in the microflow systems helps to eliminate sample losses and removes the problems associated with pollutants, which occur due to a system operating *offline*.

Accordingly, the microflow systems are convenient and profitable for industry where they can be used in highly effective screening tests involving a small number of samples (Loo et al., 2019). As far as the cell and tissue engineering are concerned, the microflow systems can provide universal solutions for the creation of 3D models and organ-on-a-chip systems by mimicking tissue microenvironments (Bhatia and Ingber, 2014). A significant decrease in scale is inevitably connected with the necessity to develop new technologies of the design, production, supply and control of the operation of the fundamental elements and systems for fluid flow in microsystems (Liu and Jiang, 2017). Microflow devices enable, among other concepts, controlled and reproducible synthesis of nanoparticles and nanovesicles (Molinaro et al., 2018, 2016), by physical transfer of membrane peptides to synthetic carriers, which results in new biological functions. The synthesis based on microflows maintains biological functions of transferred proteins, *in vitro* and *in vivo*, which turn out to be especially useful for obtaining functional receptor elements of biosensors. Special attention is drawn to the implementation of microflow technology for the extraction of gas substances to liquids (Warden et al., 2019), which significantly extends the potential application field over the biosensors operating in the liquid phase. In view of the unique properties of the microflow devices, they constitute an excellent platform for the integration of biomaterials in the ETs and B-ETs systems (Braunger et al., 2017; Cerdeira Ferreira et al., 2013; Daikuzono et al., 2015; Itoh et al., 2013; S. Wang et al., 2019). Coupling the microflow systems with primary TR cells and tongue ECM contributes to the improvement of fundamental metrological parameters of B-ETs (J. S. Lee et al., 2018). Phenotype properties and functionality of the TR cells for the recognition of flavour substances were improved

by the coupling of the tissues-derived matrix components with the microflow systems. This suggests potential utility of the microflow systems as an effective and standardised platform for the construction of the biosensors used in B-ETs. Currently, the microflow systems are also widely used as a platform for flow-injection analyses in microscale, they can also be combined with fluorescent and electrochemical detection (López et al., 2016). An impressive increase in the interest in the microflow techniques is reflected in a revolution of microfabrication techniques, notably integration into complex, portable integrated microflow systems to be employed by non-experts. The emergence of microfluidics has been a boon for cell-based biosensors as it enables the powerful concept of "cell culture on chip", which can be very helpful in the development of new-generation cell-based biosensors (Bae et al., 2018). They presented a microchemostat with independently parallelised culture chambers which collectively addresses the main limitations of the existing microbial biosensor platforms at the same time. The platform can provide long-term on-chip storage for future portable instrumentation, user-friendliness in cell preparation and device operation, enhanced sensing performance and multiple uses of microbial biosensors (Bae et al., 2018). However, some efforts are necessary to validate the methods and to prove the reliability and reproducibility of the microflow systems. The coupling of the microflow platforms with biosensors is a very promising direction of development, bringing practical implementation closer. New trends, such as 3D printing and multiplex detections in single chips, are emerging. The application of portable detection systems (wearable, flexible, portable and implantable biosensors) is of special interest and the microflow platforms seem to be the most suitable for such solutions. From the design viewpoint, the main challenges are connected with the development of a proper microflow architecture, including nanoarchitectonics and nanomaterials. In its perspective, it can enhance sensing as well as transduction in the biosensing devices. A growing number of works in the last decade recognises the utility of microfluidic platforms. Even though a revolution in technology and microfabrication techniques is taking place, the integration of components into complete and functional systems, small and thus potentially portable as well as simple to use by nonexperts are all required features. Moreover, additional efforts have to be made to validate the methods in order to demonstrate the reliability of microfluidic systems. Despite these already mentioned challenges, the incorporation of biosensors into microfluidic platforms constitutes an excellent and promising tool for the future of food analysis (López et al., 2016). Recent and future trends in biotechnology, biomimetic chemistry, nanotechnology and material science guarantee innovation in biosensors, and thus allow for their dissemination into new application fields (Fig. 9).

4.2. Nanoelectronics

Nanotechnology significantly improves, or even revolutionises, many fields of life and industry (Bhatia, 2017). It brings a prospect of developing a wide range of tools and manufacturing a more personalised, cheaper, portable, safer and easier implementation in potential applications. Physical, chemical and biological properties of nanomaterials substantially differ from their macroscopic counterparts. The unique properties of nanomaterials can be employed to design biosensors, an account of improving fundamental parameters, cutting costs and facilitate the construction of portable devices that can be used in the places where classic techniques are inaccessible (Sadana et al., 2018). The currently available biosensitive devices allow not only on-site measurements but also distribution/consumer level measurements. With the application of the aforementioned microflow systems, it is possible to implement materials at the nanoscale level. Due to the harmonisation of functional nanoparticles and biomaterials or artificial nanobiocomponents, analytes can be detected with characteristics corresponding to human counterparts (Ahn et al., 2018; Jin et al., 2012; Song et al., 2014, 2013). A combination of the biosensors into arrays enables multianalyte detection or even obtaining spatial distribution of a single analyte (Kulshreshtha et al., 2017). Among a variety of sensor

platforms, those based on nanomaterials provide the highest efficiency. The substantial progress in nanotechnology has expanded the spectrum of nanomaterials with different shapes and sizes, suitable for the application in biosensors, such as nanoparticles (Zn, Au, Fe, *etc.*), nanotubes, nanorods, nanowires, graphene, nanosheets, quantum dots, *etc.* (Kwon et al., 2019). A high surface-to-volume ratio provides proper environment for the immobilisation of biological elements, for instance enzymes (TermehYousefi et al., 2017) and nanovesicles (Jang et al., 2016). Nanomaterials practically contribute to a higher efficiency of a biosensor because the dimensions of nanomaterials are similar to those of enzymes, and thus they can be close to the reaction centre. As a result, the signal – due to enzymatic reaction – is efficiently transported by the transducer (Kwon et al., 2019). An enhanced electron transfer due to the high electrical conductivity of nanomaterials as a transduction platform, *e.g.* gold nanoparticles, reduces the limit of detection (LOD) and offers the ability to detect single molecules (Holzinger et al., 2014). Nanoscale transistors of more efficient, fast and energy-saving characteristics are being developed (Sivakumarasamy et al., 2018). For example, conductive polymer nanomaterials (CPs), as a result of their excellent efficiency and unique optoelectronic properties, are successfully employed in the new-generation biosensors (Kwon et al., 2019). A particular interest in carbon nanomaterials has been focused on carbon nanotubes and graphene (Wang et al., 2015) characterised by improved electron transport without their dissipation and increased electron conductivity, thus providing a convenient platform for the deposition of the biomaterials mimicking natural receptors (Cho et al., 2017; Song et al., 2014, 2013). The implementation of CN in the nanobioelectric sensors often calls for an appropriate chemical modification of the surface, including the change of ligands, charge and bioconjugation (Biju, 2014). Effective and selective bioreceptor immobilisation is possible thanks to, among other requirements, the application of the chemical modifications, which ensure high-quality biosensitive platforms, *e.g.* SWNT-FET-based nanobioelectronic tongue containing human sweet TRs, with heterodimeric GPCRs composed of hTAS1R2 and hTAS1R3 (Song et al., 2014). Excellent electrical properties make it possible to design new and improve the already existing biosensor arrays and can be used in multiplexed analyte detection (Jang et al., 2016; Kwon et al., 2015; Son et al., 2017). The manufacturing of natural receptors with high functionality is currently a main priority in the development of B-ETs based on nanomaterial receptor hybrid structures. Further progress in the biosensors can be expected with the amplification step to enable the detection of single molecules. To achieve this, ultrasensitive transducers and/or new detection techniques should be developed. The devices operating in nanoscale offer the possibility of the observation of chemical reactions and equilibria at a level of single molecules, which allows an investigation of the physical and chemical properties dependent on single molecules. The development of integrated biosensors accommodating bioreceptor material, transducer and indispensable electronic elements in a single device would lead to measurement process automation (Loo et al., 2019; Mishra et al., 2015). It is expected that the improvement of the techniques of immobilisation and functionalisation of nanomaterials with biomaterials will results in the broadening of potential fields of application of B-ETs. A progress in multiplexed nanobioelectronic tongues, pattern recognition, signal processing and parameter standardisation can facilitate recording and transfer of artificial taste codes. As soon as the components of flavourings are identified, it would be possible to restore particular tastes by combing the elements of the flavour compounds based on artificial codes. Enhancers of receptor responses will not only provide more sensitive biosensors, but also they may be used as taste modulating agents. Biomimetic electronic tongues capable of confirmation of the predicted taste sensations can provide a database of the so-called fingerprints of taste samples paving the way to investigate the dependence between the taste structure and chemical properties (Dagan-Wiener et al., 2017). In recent years, there has been enormous progress at the molecular biological levels of understanding taste mechanisms, but this has not been integrated well into developmental

neurophysiological, higher coding, integrative, digestive or perceptual functions. A concept of taste standardisation can facilitate the opening of new possibilities of developing devices for recording, transfer and reproduction of human taste, which can be applied in many branches of industry. Nanobiosensors, as highly sensitive, selective, rapidly responding, real-time, massively parallel, with no or minimum sample preparation, on a platform suited to portable and handheld devices can be implemented in the rapid detection of analytes for in field uses even by non-skilled personnel.

A summary of the recent B-ET development pathways is provided in Fig. 9.

Fig. 9. Future prospects of bioelectronic tongue development.

In reality, these above-mentioned technologies, including biomimetic chemistry, biomaterials, nanotechnology and microengineering, are still in the early stages, but they have the highest probability of making an impact on biosensors for the B-ET design in the near future. Their cross-cut disciplinary character will certainly lead biosensors and biosensor arrays to commercialisation and find extensive use in several application fields. Systems integration aspects to consider, include easy and fast (multi)sensor interrogation and interfacing with monitoring and control functions. Reliability is required within the foreseen operating environment, considering humidity, temperature and other parameters that affect stability. Also, a low rate of technology transfer and low level of development have deterred the development of new biosensors. The integration of micro- and nanostructured materials in biosensing devices provides excellent analytical performances for the analysis of different samples. Advances in microfluidics permitted the miniaturization of analytical systems, offering at the same time multiple advantages such as low volume samples, reduction in consumption of reagents and waste, decrease in analysis time with an increased sensitivity and reliability. One of the key contributions of these technologies in the biosensing field relies on the design of novel transducers, not only with enhanced transducing features, but also with improved immobilization of biomolecules while retaining their biological activity.

5. Conclusions

Mimicking biological senses enables an objective evaluation and standardisation of taste sensations. A variety of B-ETs is available for use in biosensing in many applications, offering specific, sensitive detection. However, these technologies have to prove their value in real sample analysis. Thus, versatile multifunctional sensing systems, which can operate in a variety of complex matrices, are needed. Meeting some of those requirements would create a significantly more complete range of analytical devices, operating in the field dominated by conventional analytical techniques. There is a continuous increase in the demand for simple and efficient instruments enabling simple, fast (but simultaneously selective and sensitive) analysis of compounds, without the high cost of equipment and its maintenance. Despite their high specificity and selectivity, biosensors still exhibit certain cross-sensitivity, for instance the affinity of enzymes with particular groups of compounds, instead of a single ligand. According to the authors, the sustainable development of B-ETs should be directed not only to the development of novel systems in new application fields but also to the improvement of the already existing solutions. The developed B-ETs based on a taste sensation mechanism may provide an innovative means to devise novel personal digitalised taste communication devices ensuring high accuracy and speed. Some directions will probably achieve significant progress in the near future. A key factor seems to be the further improvement of the reproducibility, repeatability and long-term stability of the designed biosensors as well as the development of strategies for their counterbalancing related to modelling. In addition, the calibration procedures, which are time and computing power consuming, are the key limiting factors. The signal transduction mechanisms of gustatory signals and their modification may provide a theoretical foundation for the improvement

of biosensor performance. The development of large-scale biosensor arrays composed of highly miniaturized signal transducer elements, should enables the real-time monitoring of multiple species. Multichannel biosensors are mandatory for direct detection in high-throughput screening systems. In our opinion, the interest in the bioelectronic devices, including B-ETs, will continuously grow and the development in the future should enable the implementation of commercial devices in analytical practice.

Acknowledgments: Financial support for this work was provided by the Polish National Science Centre grant PRELUDIUM 2019/33/N/ST5/01517.

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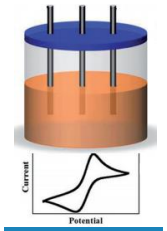
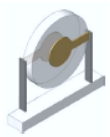
1906-1937

pH glass electrode (GE) and ion-exchange theories, widely used for the description of behavior not only of GEs but also of other ion-selective electrodes.



1961-1969

Conventional ion-selective electrodes and biosensors. Starting point of modern potentiometry and selective potentiometric response detection in biological membranes.



1970 →

Microelectronics in sensor development, first ISFET and piezoelectric biosensor. First miniaturized sensors/ biosensors capable to measure different parameters.

1985 →

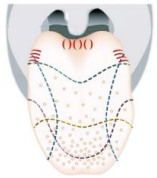
Multisensor arrays and sensor systems (Otto, Thomas). Rapid progress of semiconductor micromachining as the basis for integration into arrays. New trends in the biosensors technology, named micro total analytical systems (μTAS) or the lab-on-a-chip.

1996

"Taste" sensor (Toko). Concept different from conventional chemical sensors, troughs a new light on food and environmental samples analysis techniques.

1998

First electronic tongue (Vlasov, Legin, D'Amico, Di Natale). Promising device in quantitative and qualitative analysis of multispecies solutions.

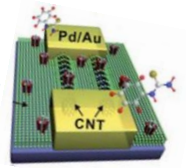


1999

Encoding taste receptors (Hoon *et al.*). Expansion knowledge of intracellular signaling pathways involved in taste transduction and critical steps in a comprehensive dissection of taste biology.

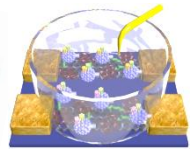
1999

First bioelectronic tongue (Bachmann, Schmid). Sensitive screen-printed amperometric multielectrode biosensor with Artificial Neural Network (ANN) employed for the rapid discrimination of the insecticides.



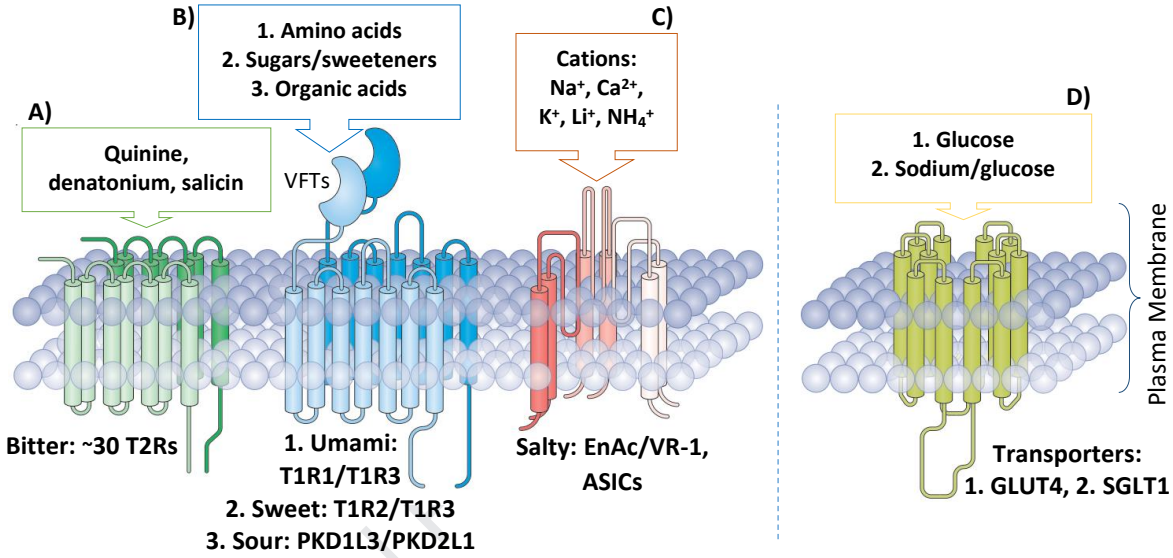
2010

Human-like nano-bioelectronic super-taster (Kim *et al.*) for detection of bitter tastants and distinguish between bitter and non-bitter tastants with similar chemical structures similarly to human tongue.



2016

Duplex bioelectronic tongue (Ahn *et al.*) expected to replace previous sensory evaluation strategies for the food and beverage industry and facilitate the functional study of dimeric GPCRs.



ELECTRONIC TONGUE	BIOELECTRONIC TONGUE	HUMAN TASTE

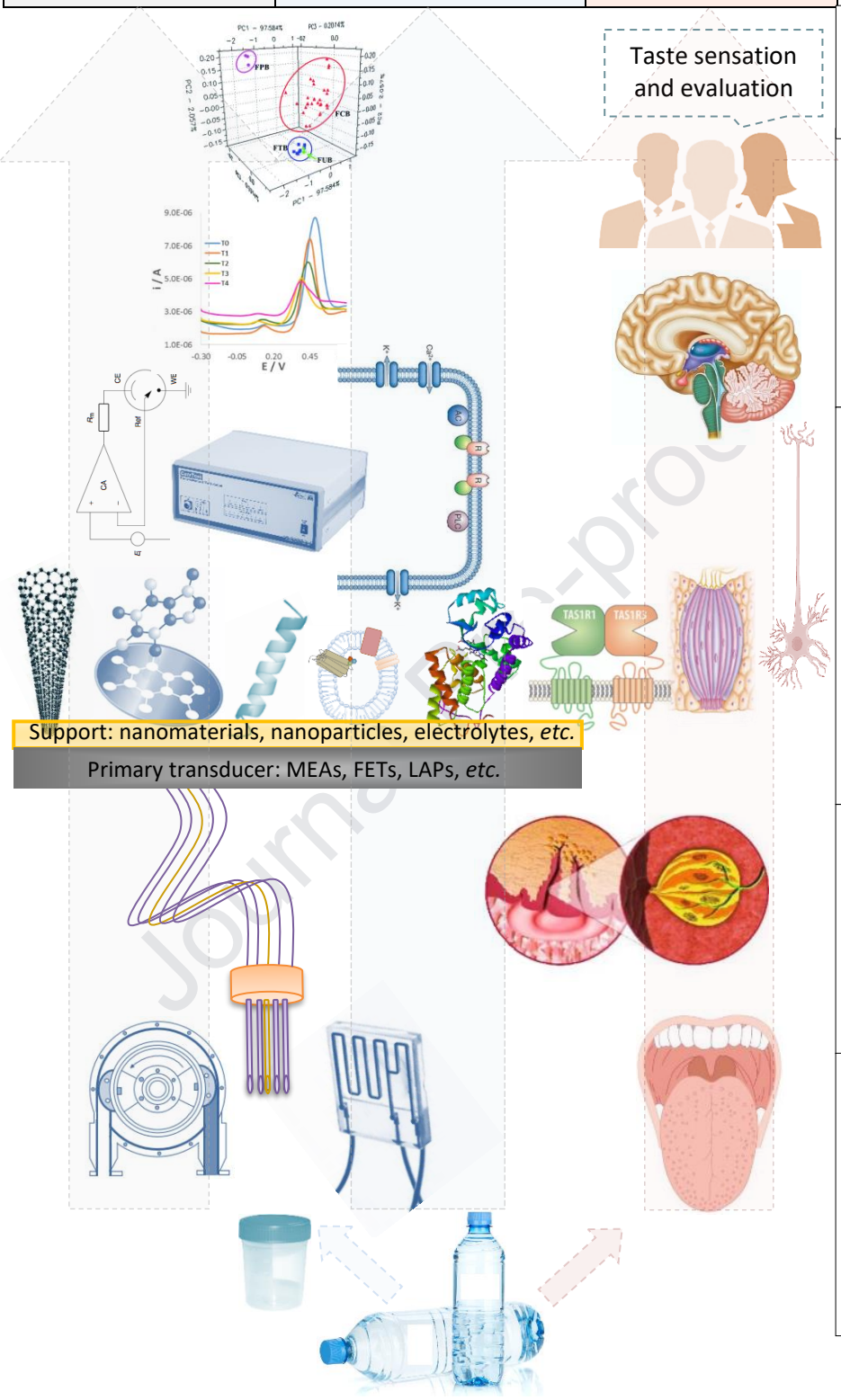
Results visualization and classification by chemometric tools to discriminate, identify or quantify the sample.

Chemical/ biological signals recording and data acquisition obtained from sensors/ biosensors array and initial data analysis. Conversion of biological signals.

Sample analysis by sensors/ biosensors array consist of primary transducers, supporting materials and secondary transducers (biosensing element in case of B-ET).

Sample transfer through pumps/valves, microfluidic systems.

Sampling system, optional step of sample collection.



Qualitative and hedonic sensations are perceived.

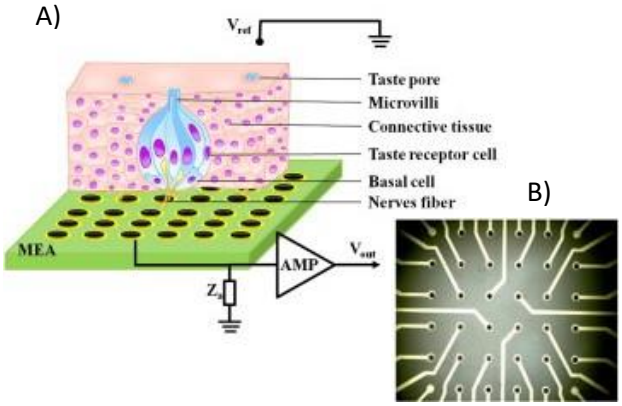
Through synapse and neurons an electrical impulse to the gustatory region of the cerebral cortex of the brain is transmitted.

Taste cells membrane depolarization trigger ions influx. GPCRs or channel receptors regulates the release of neurotransmitters onto nerve fibres and/or from adjacent taste bud cells.

Signal transduction cascades initiated taste receptors and stimulated by the chemical makeup of solutions.

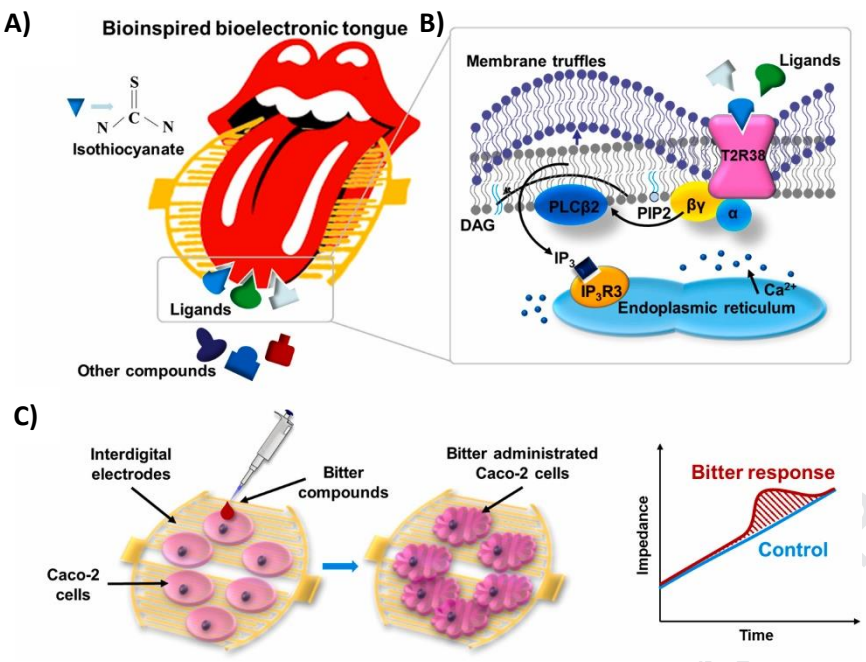
Liquid compounds, dissolved in saliva, interact with TRCs located on taste buds in the oral cavity, mostly on the tongue.

A)

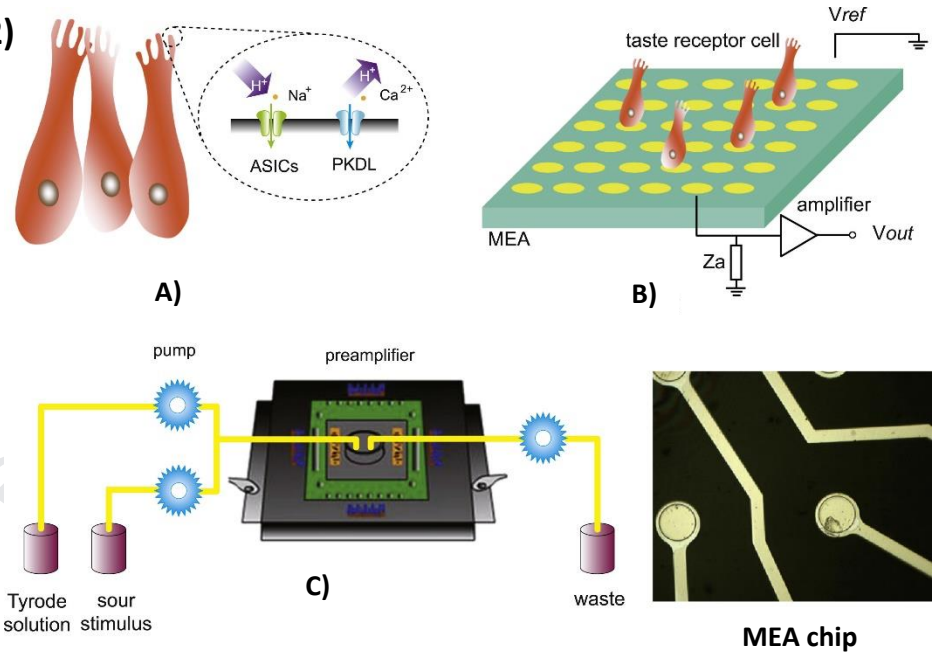


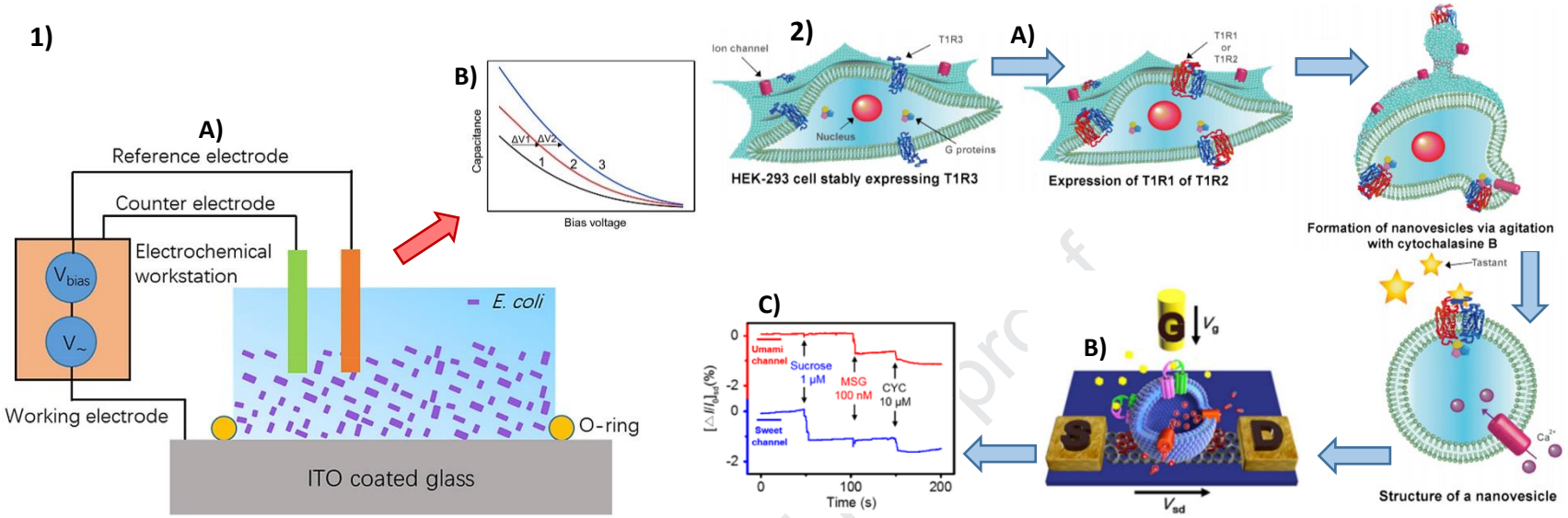
B)

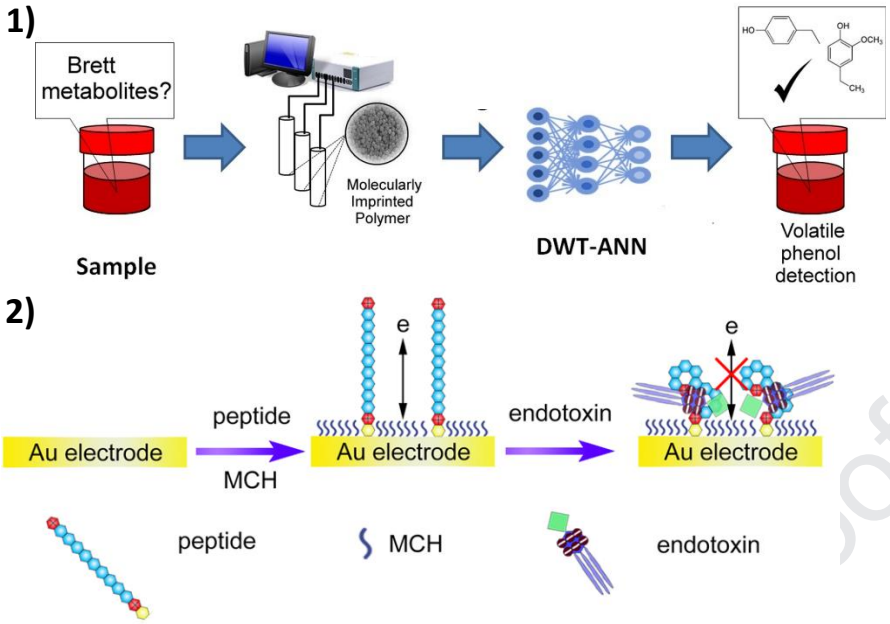
1)

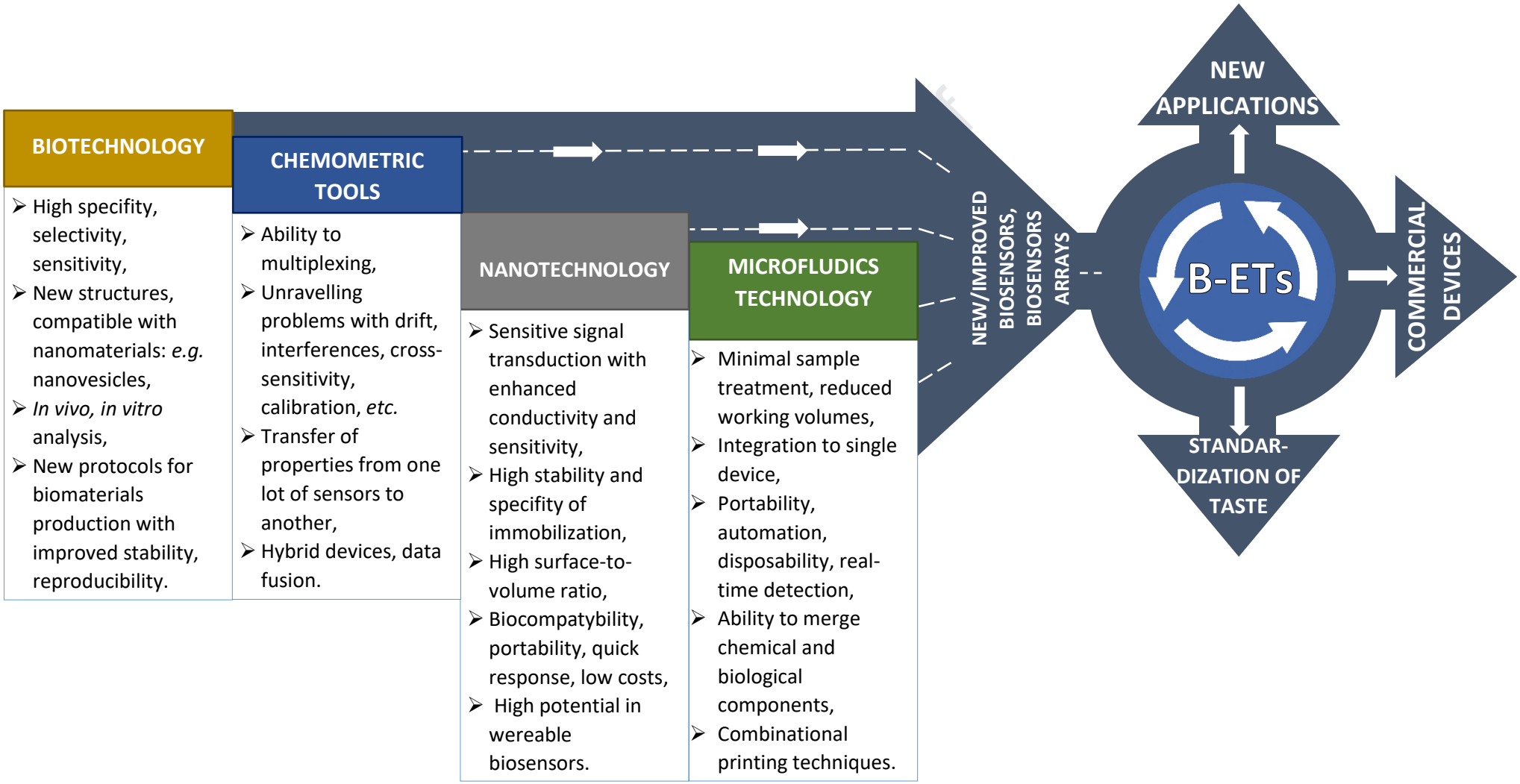

A)
Bioinspired bioelectronic tongue
Isothiocyanate
Ligands
Other compounds
B)
Membrane truffles
Ligands
T2R38
PLCβ2
PIP2
βγ
α
DAG
IP3
IP3R3
Ca2+
Endoplasmic reticulum
C)
Interdigital electrodes
Bitter compounds
Caco-2 cells
Bitter administrated Caco-2 cells
Impedance
Bitter response
Control
Time

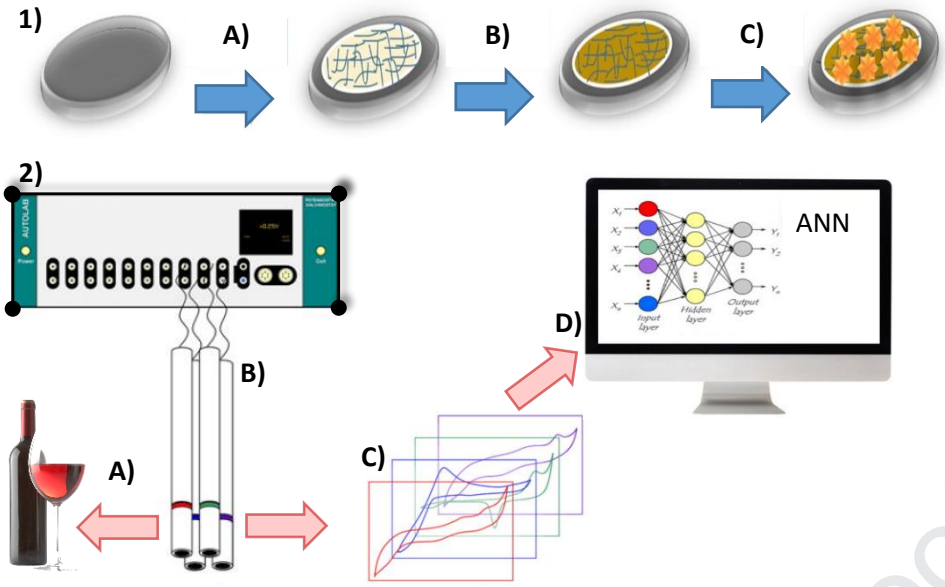
2)


A)
H+
Na+
Ca2+
ASICs
PKDL
B)
Vref
taste receptor cell
amplifier
MEA
Vout
Za
C)
pump
preamplifier
Tyrode solution
sour stimulus
waste
MEA chip


1)
A)
Reference electrode
Counter electrode
Electrochemical workstation
V_{bias}
$V_{\sim}$
Working electrode
E. coli
O-ring
ITO coated glass
B)
Capacitance
ΔV1
ΔV2
1
2
3
Bias voltage
2)
Ion channel
T1R3
Nucleus
G proteins
HEK-293 cell stably expressing T1R3
A)
T1R1 or T1R2
Expression of T1R1 of T1R2
Formation of nanovesicles via agitation with cytochalasine B
Tastant
Structure of a nanovesicle
B)
G
V_g
S
D
V_{sd}
C)
Umami channel
Sucrose 1 μM
MSG 100 nM
CYC 10 μM
Sweet channel
0
-2
0
-2
0
100
200
Time (s)




BIOTECHNOLOGY
High specifity, selectivity, sensitivity,
New structures, compatible with nanomaterials: e.g. nanovesicles,
In vivo, in vitro analysis,
New protocols for biomaterials production with improved stability, reproducibility.
CHEMOMETRIC TOOLS
Ability to multiplexing,
Unravelling problems with drift, interferences, cross-sensitivity, calibration, etc.
Transfer of properties from one lot of sensors to another,
Hybrid devices, data fusion.
NANOTECHNOLOGY
Sensitive signal transduction with enhanced conductivity and sensitivity,
High stability and specifity of immobilization,
High surface-to-volume ratio,
Biocompatybility, portability, quick response, low costs,
High potential in wereable biosensors.
MICROFLUDICS TECHNOLOGY
Minimal sample treatment, reduced working volumes,
Integration to single device,
Portability, automation, disposability, real-time detection,
Ability to merge chemical and biological components,
Combinational printing techniques.
NEW/IMPROVED BIOSENSORS, BIOSENSORS ARRAYS
B-ETs
NEW APPLICATIONS
COMMERCIAL DEVICES
STANDAR-DIZATION OF TASTE



Highlights:

- State-of-art in the field of bioelectronic tongues,
- Currents status and development prospects of different type bioelectronic tongues,
- Applications of bioelectronic tongues,
- Micro and nanotechnologies in bioelectronic tongues development,
- Critical evaluation in the field of bioelectronic tongues and future outlooks.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: