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Review Article (Invited)

Chemical tongues as multipurpose bioanalytical tools for the characterization of complex biological samples

Shunsuke Tomita[1], Hiroka Sugai[1,2]

[1] *Health and Medical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8566, Japan*
[2] *Research Center for Autonomous Systems Materialogy (ASMat), Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Kanagawa 226-8501, Japan*

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Chemical tongues are emerging powerful bioanalytical tools that mimic the mechanism of the human taste system to recognize the comprehensive characteristics of complex biological samples. By using an array of chromogenic or fluorogenic probes that interact non-specifically with various components in the samples, this tool generates unique colorimetric or fluorescence patterns that reflect the biological composition of a sample. These patterns are then analyzed using multivariate analysis or machine learning to distinguish and classify the samples. This review focuses on our efforts to provide an overview of the fundamental principles of chemical tongues, probe design, and their applications as versatile tools for analyzing proteins, cells, and bacteria in biological samples. Compared to conventional methods that rely on specific targeting (e.g., antibodies or enzymes) or comprehensive omics analyses, chemical tongues offer advantages in terms of cost and the ability to analyze samples without the need for specific biomarkers. The complementary use of chemical tongues and conventional methods is expected to enable a more detailed understanding of biological samples and lead to the elucidation of new biological phenomena.

Key words: arrays, biosensing, machine learning, multivariate analysis, polymers

Significance

Chemical tongues offer a novel approach to characterizing complex biological samples. This versatile tool overcomes the limitations of conventional methods, which rely on specific biomarkers, thus enabling the analysis of samples with unknown compositions. By providing a comprehensive and cost-effective means of analyzing proteins, cells, and bacteria in biological samples, multipurpose chemical tongues have the potential to advance our understanding of biological systems and contribute to various fields, from fundamental research to medical applications.

Corresponding authors: Shunsuke Tomita, Health and Medical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8566, Japan. ORCID iD: https://orcid.org/0000-0002-0586-6976, e-mail: s.tomita@aist.go.jp; Hiroka Sugai, Research Center for Autonomous Systems Materialogy (ASMat), Institute of Innovative Research, Tokyo Institute of Technology, Yokohama, Kanagawa 226-8501, Japan. ORCID iD: https://orcid.org/0000-0002-5957-0011, e-mail: sugai.h.aa@m.titech.ac.jp

Introduction

With the aim of understanding biological phenomena, researchers undertake the analysis of biological samples consisting of proteins, cells, bacteria, and other components. A common method for the characterization of these samples is to precisely detect particular substances contained within the samples using either antibodies or enzymes [1–3]. However, many biological samples are composed of tens of thousands of components or more, and relying on information gained from the analysis of only a limited number of components may lead to overlooking important data and drawing erroneous conclusions. Although the developments in the area of omics technology aimed at a comprehensive detection has been remarkable [4–6], the technology is still associated with drawbacks such as high equipment costs, complicated measurement processes, and biases in the results, which depend on the experimental protocol used.

In our groups, we have been working on developing a bioanalytical tool known as a 'chemical tongue', which can overcome the challenges of conventional technologies by enabling the simple and comprehensive recognition of the characteristics of complex biological molecules/samples. In this review, we introduce the principles, material design, and examples of the applications of this tool to biological molecules/samples with a specific focus on our previous research. This review article is an extended version of the Japanese article [7].

Brief History of Mimicking Taste and Smell

A chemical tongue is an analytical technique that investigates target samples from multiple angles through 'non-specific' interactions with multiple probe materials and identifies or classifies the samples by recognizing the 'patterns' of the obtained probe responses [8–10].

The concept of the chemical tongue originates from the 1980s work of Persaud and Dodd [11], which led to the development of what are now known as 'electronic noses' or 'electronic tongues'. These analytical technologies share a common feature: they are both multisensor systems composed of low-specificity sensor units [12]. The terms 'nose' and 'tongue' are used to differentiate between technologies analyzing gases and liquids, respectively, based on the sensory functions they emulate. Electronic noses/tongues detect patterns of electrical changes using sensor arrays made from materials such as metal-oxide semiconductor materials, conducting polymers [13], or lipid/polymer membranes [14]. These analytical technologies have found extensive applications, particularly in the agricultural and food industries, and led to a variety of commercialized products [15]. Recently, their use has expanded into medical diagnostics [16–18].

In the 2000s, Suslick and colleagues applied this concept to chemical sensors that detect volatile molecules through chromogenic responses induced by interactions with dyes [19], coining the term 'chemical nose'. This approach was subsequently extended to liquids, establishing the chemical-tongue approach [8]. Notably, pioneering work by Rotello and colleagues since the late 2000s, primarily using gold nanoparticles, has demonstrated the potential applicability of chemical tongues to various biological samples [9,20,21].

Principles and Design Guidelines for a Chemical Tongue

The chemical tongues we have developed so far typically consist of a set of fluorescent polymer probes that mimic the function of human taste cells combined with multivariate analysis or machine learning to mimic the function of the brain (Figure 1) [22].

The analysis is performed as follows. First, an array of aqueous solutions of various fluorescent polymers with different chemical structures is prepared (Figure 1a and b). Microplates with 96 or 384 wells are commonly employed as containers for the arrays. When biological samples are added to the array, each polymer interacts with the components in the sample in distinct ways, and the strength of these interactions is output as a change in the fluorescence intensity. When the detected fluorescence responses of each polymer are combined, they form a 'fluorescence pattern' that is unique to the sample (Figure 1c). Finally, the obtained patterns are compared and analyzed using multivariate analysis or machine learning to evaluate the contents of the samples (Figure 1d).

The design of the probe materials is particularly important for the construction of a chemical tongue. As a prerequisite, the probes used must be able to interact with the target samples to generate fluorogenic (or chromogenic) responses. When targeting large molecules like proteins, or an assembly of biomolecules like cells, synthetic polymers [23–26], nanomaterials [27,28], DNAs [29], or their complexes [30–38] that can interact strongly with the targets at multiple points are often selected as the scaffold materials [22].

We propose a design method that introduces the following two functional units into a charged synthetic polymer scaffold (Figure 1b). One such functional unit is the 'recognition unit'. By introducing various chemical structures with, e.g., different hydrophobic properties and charges into the polymer, the affinity of the polymer for the constituent components of the sample can be modulated, enabling the sample to be examined from multiple angles. The other is the 'output unit'

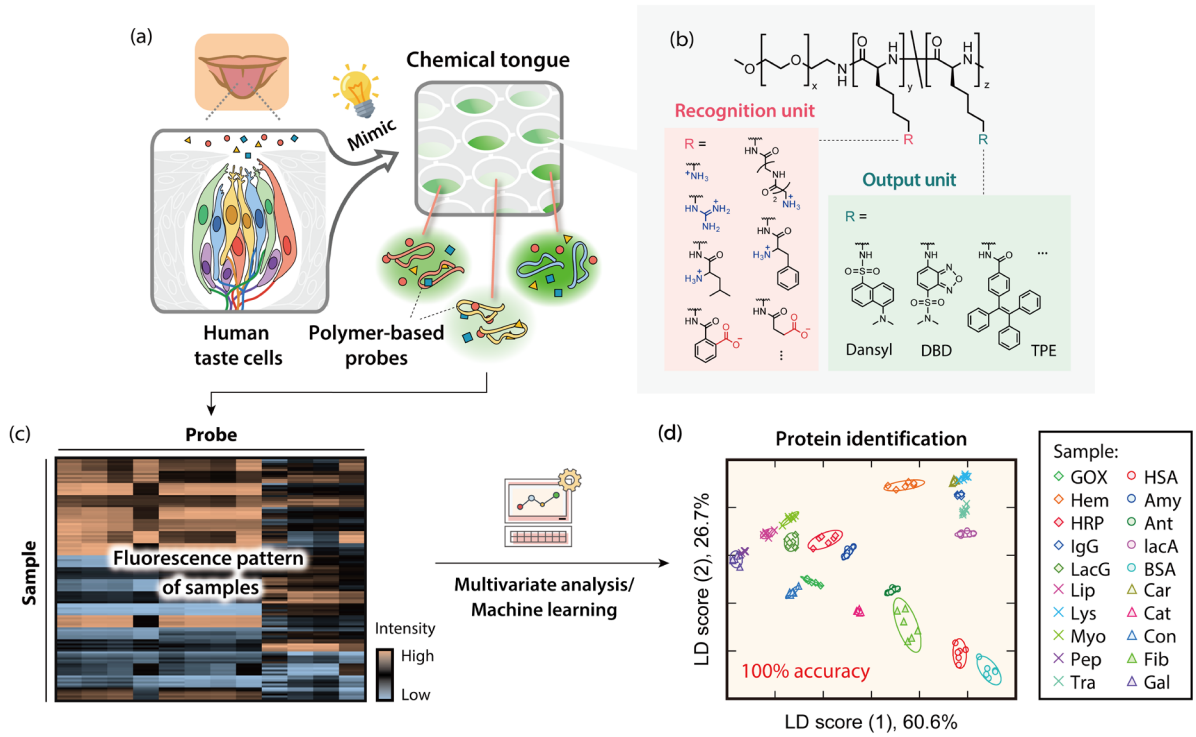

Figure 1 Schematic illustration of a chemical tongue. (a) The mimicking of taste using an array of fluorescent polymer probes sets. (b) Example of probe design for a chemical tongue. (c) Fluorescence patterns and (d) discrimination using multivariate analysis of 20 proteins. Reprinted with permission from [45]. Copyright 2019 American Chemical Society.

for converting the interaction between the polymer and the sample into a fluorescence signal. In the main, fluorophores that increase or decrease fluorescence in response to changes in the surrounding environment are used. 5-(Dimethylamino)-naphthalene-1-sulfonyl (Dansyl) or 4-(*N,N*-dimethylaminosulfonyl)-2,1,3-benzoxadiazole (DBD) groups that respond to polarity changes [39,40] and tetraphenylethylene (TPE) groups that respond to the inhibition of intramolecular rotational motion [41–44] are turn-on type fluorophores that generate fluorescence upon binding and have excellent sensitivity (Figure 1b). This high sensitivity is largely due to their lower background response in comparison to turn-off-type fluorophores [29,45].

The polymer materials we primarily use, such as poly-L-lysine, have primary amino groups, which allow for easy functionalization due to their nucleophilicity, enabling access to a broad chemical space. This facilitates the realization of multifaceted interactions and sharp fluorescence responses, both of which are crucial for the construction of a chemical tongue. Furthermore, the bifunctionalization approach using recognition and output units eliminates the need for the frequently used complexation of different materials, reducing effort and human error, thereby providing a simpler and more accurate analytical system. This concept is based on issues identified in our earlier work using enzyme-polymer complexes [46,47].

By processing the fluorescence patterns obtained from the array of polymer solutions (Figure 1c) using multivariate analysis or machine-learning techniques (*vide infra*), biological molecules/samples can be distinguished based on differences in the patterns. For example, as shown in Figure 1d, we succeeded in discriminating 20 purified proteins with high accuracy using only three types of fluorescent polymer [45]. Considering the ratio of probe number to target protein number, this demonstrated excellent discriminative ability compared to other reports (see the Supporting Information of Ref. [45]). The required number of probes largely depends on the nature and number of the target samples to be distinguished. For instance, if the target proteins have similar structures, if they are present at lower concentrations, or if there are 50 different target proteins, using only these three polymers may not provide sufficient accuracy. In such cases, it would be necessary to add polymers with different properties that can output different characteristic information about the proteins.

Accuracy can be improved not only by using different probes but also through other methods [39]. Changing pH values of the solution can alter the charge properties of the target samples and probes, leading to different interaction driving forces and thus different readable information. Modifying the wavelength conditions used for measurement can be

effective for probes whose spectral shapes change upon interaction. These approaches allow for an efficient increase in the variables available for analysis.

For practical applications, minimizing the size of the chemical tongue is preferable. To this end, one straightforward but time-consuming approach is to systematically investigate the accuracy while reducing the number of variables one by one. However, a more efficient method has been proposed, which uses multivariate analysis to reduce variables based on their contributions to the overall performance [41,48].

Analysis of the Pattern Information

As shown in Figure 2a, the fluorescence patterns obtained from the chemical tongue are mostly multivariate, which makes it difficult to analyze the patterns in isolation. Therefore, multivariate analysis or machine-learning techniques are used to transform the complex patterns into a visually interpretable format or to create evaluation models and quantitatively assess their accuracy. This section provides an overview of two frequently used multivariate-analysis methods. These analysis methods can be easily performed using relatively inexpensive software such as SYSTAT (Systat Software Inc). Alternatively, the Python programming language, which provides user-friendly data analysis libraries and toolkits, has become much more accessible with the use of artificial-intelligence assistant tools equipped with natural language processing for efficient script creation.

Multivariate analysis can be categorized into 'unsupervised' and 'supervised' methods. The difference between the two is whether or not the 'sample-classification labels' are assigned to the pattern data used for the analysis and are taken into consideration during the analysis (Figure 2a). Labels can include information such as the type of protein (e.g., lysozyme or hemoglobin), concentration (e.g., 100 nM or 200 nM), or condition (e.g., healthy or unhealthy). Generally speaking, unsupervised analysis is used to understand the relationships between patterns, while supervised analysis is used to estimate the accuracy of evaluation models.

Hierarchical cluster analysis (HCA) is a representative unsupervised multivariate-analysis method, which sequentially links pairs of patterns that are separated by close distances. Figure 2b shows a dendrogram obtained from analyzing the fluorescence patterns of eight samples, [(i) to (viii)], each measured ten times using the six fluorescent probes (A to F)

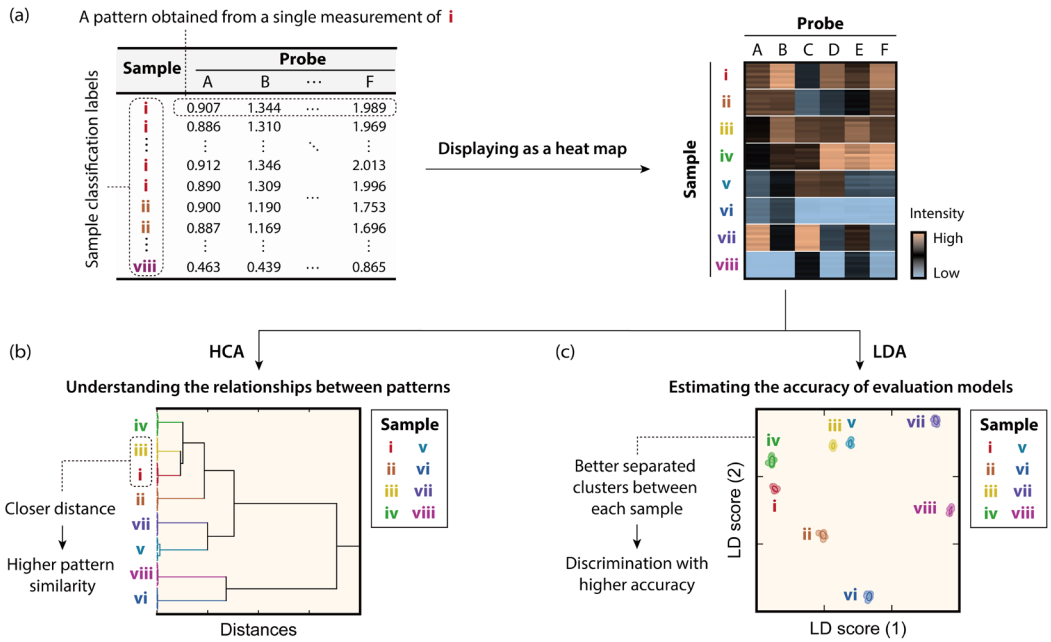

Figure 2 Typical fluorescence patterns and multivariate-analysis results. (a) Fluorescence patterns of 8 target samples [(i) to (viii)] obtained using six probes (A to F). Results were obtained from ten independent trials for each sample. (b) Dendrogram obtained by unsupervised HCA, exhibiting combinations of patterns connected in order of proximity. The ten measurement patterns for each sample form distinct clusters, suggesting statistical differences between the fluorescence patterns derived from each sample. Additionally, the distances between clusters provide insights into the similarities between the patterns. (c) Score plot obtained from supervised LDA. This plot was created by projecting sample fluorescence patterns onto a two-dimensional plane that maximizes the separation of patterns with different labels, i.e., visually representing the discriminatory ability of the chemical tongue.

shown in Figure 2a. Despite performing the analysis without using sample-classification-label information, the ten patterns obtained from repeatedly measuring the eight samples each form a cluster. This means that the variation between the patterns of the different samples is higher than the variation between trials of the patterns of the same sample. In other words, there are large differences between the fluorescence patterns derived from samples (i) to (viii). Furthermore, among the eight different samples, (i) and (iii) form a cluster first. This means that among the eight samples, the patterns of (i) and (iii) are the most similar. This similarity can also be observed in the heat map in Figure 2a, albeit that using HCA allows for clearer visualization and quantification of the differences. The ability to quantify such intuitive differences is one of the advantages of using multivariate analysis.

Linear discriminant analysis (LDA) is a commonly used supervised multivariate-analysis method. As mentioned above, this method analyzes the fluorescence patterns with label information assigned in advance. Figure 2c shows an example of the analysis results, often called an LDA score plot. This figure is created by projecting the fluorescence patterns onto a two-dimensional plane with the aim of separating the patterns with different labels as much as possible. The horizontal axis [the first discriminant score, LD score (1)] is the axis that separates the samples the most, and the vertical axis [the second discriminant score, LD score (2)] is the next axis. Each point in the score plot corresponds to a fluorescence pattern obtained from a single measurement. Ellipses in this score plot represent confidence intervals of ± 1 standard deviation for the individual samples. In Figure 2c, the ten points derived from (i) to (viii) form clusters without overlapping with the other samples, suggesting that the eight samples can be distinguished with high accuracy. This figure is often used to estimate the discrimination performance of the chemical tongue prior to model evaluation, as it is generally consistent with the cross-validation results explained next. Additionally, it is used as a visualization tool to help readers understand the characteristics of the developed chemical tongue, as simply presenting accuracy values such as 90% or 100% may not effectively convey the characteristics.

When high accuracy is expected from the LDA score plot, the performance of the chemical tongue is evaluated using a method called cross-validation. In cross-validation, the dataset of fluorescence patterns is divided into training data for creating the evaluation model and test data for evaluating it. The details are omitted here, but methods called leave-one-out cross-validation and k-fold cross-validation are commonly used.

In addition to the methods introduced above, various multivariate analysis and machine-learning methods can be used depending on the size of the data set, its quality, and its purpose [8]. Other commonly used methods include principal-component analysis (PCA) and support-vector machines (SVMs). PCA, which is an unsupervised multivariate analysis method similar to HCA, is used especially when a more detailed understanding of the relationship between the obtained patterns is required. For example, PCA is used to clarify the contribution of each probe to the clustering of patterns. SVMs are representative machine-learning methods, and a specific type of SVMs called support-vector regression (SVR) has increasingly been used for regression problems, i.e., quantitative analysis [40,49,50]. However, for those performing chemical-tongue analysis for the first time, using representative methods such as HCA and LDA should be sufficient to represent and understand the characteristics of the obtained patterns.

Evaluation of Cell Samples

The following section introduces several examples of biological sample evaluation using chemical tongues. In Figure 1d, we showed an example of discriminating purified proteins using a chemical tongue. However, an important feature of chemical tongues is that they can compare and analyze complex biological samples of unknown composition based on the obtained fluorescence patterns. This allows the determination of whether a sample is normal and the classification of its state. In this section, we introduce cell-sample evaluation using our polymer-based chemical tongues as an example.

Identifying cancer cells is important for determining the location of the primary tumor site. Generally, cancer cells are identified by detecting biomarkers for the desired cell type using antibodies [51]. However, given that biomarkers are produced as a result of a combination of multiple complex cellular processes, their detection does not provide definitive evidence of the cellular state. Chemical tongues can recognize the comprehensive molecular composition of the cell surface, potentially solving this issue. This approach was first proposed by Rotello and colleagues in 2009 [52]. Since then, researchers have demonstrated the effectiveness of this approach using various probe materials [53–59].

Our methods exploit the mechanism by which the fluorescent polymers, such as those shown in Figure 1b, can interact in distinct ways with various components of the cell surface, such as proteins, lipids, and polysaccharides (Figure 3a). Therefore, by adding a cell suspension to an array of fluorescent polymers it is possible to obtain a cell-specific fluorescence pattern. We analyzed ten types of mammalian cells using a chemical tongue composed of PEG-*b*-PLL-based polymers, in which the amino groups of lysine side chains were modified with different amino acids and fluorescein, that exhibit a turn-off fluorescence response (Figure 3b). The LDA plot of the fluorescence response patterns showed that the clusters of each cell type were separated without overlap (Figure 3b), whilst leave-one-out cross-validation also showed 100% discrimination accuracy [45]. In addition, we have demonstrated that breast-cancer cell lines (MDA-MB-453 and MCF-7) with different malignancy levels in a composite of mixed samples can be distinguished (Figure 3c) [60]. Other

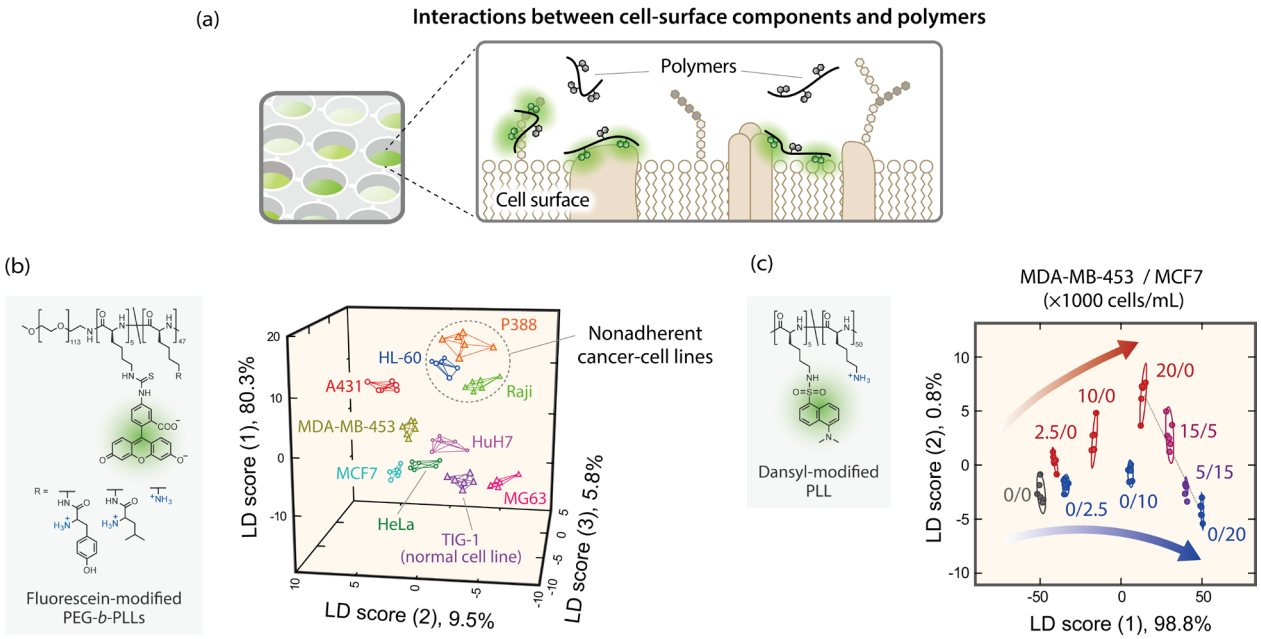

Figure 3 Recognition of cell surfaces using a chemical tongue. (a) Schematic illustration of the underlying molecular mechanism. LDA score plots of (b) mammalian cell lines using fluorescein-modified PEG-*b*-PLLs and (c) mixed samples of breast-cancer-cell subtypes with different malignancies using Dansyl-modified PLL. Reprinted with permission from Refs. [45] and [60]. Copyright 2019 American Chemical Society.

groups have also reported the effectiveness of the chemical-tongue approach for differentiating malignancy levels [52,53,61].

As described above, chemical tongues can identify cell types and detect metastasis without relying on the detection of biomarker molecules. Therefore, this technology is expected to have a wide range of applications, from fundamental work on elucidating cellular mechanisms to applied fields such as cytology.

Furthermore, chemical tongues can be used not only for recognizing cells themselves but also for recognizing components secreted by cultured cells into the medium (Figure 4a). To this end, we synthesized cationic polymers with different scaffolds, each appended with Dansyl fluorophores, to construct chemical tongues (Figure 4b). Figure 4c shows the results of applying this tool to monitor the stem-cell-differentiation-induction process [62]. First, bone marrow-derived mesenchymal stem cells (UE7T-13) were seeded and differentiation induction into osteoblasts was initiated. After a set period of induction, the medium was changed to one containing 1 vol% fetal bovine serum and samples of the medium were collected after 48 hours. When these samples were analyzed using a chemical tongue, the clusters were distributed without overlap in the LDA score plot and they could be distinguished with 96% accuracy using leave-one-out cross-validation (Figure 4d). Furthermore, it was found that the positions of the clusters shift over time depending on whether or not the stem cell induction as initiated. This suggests that the chemical tongue can extract information about changes in secreted components due to stem cell differentiation, even when a large amount of serum proteins is present in the samples.

In addition, this technology can be applied to evaluate various cellular processes, such as the non-destructive monitoring of cell aging [63] and responses to anticancer drugs [64,65]. Our proposed application of chemical tongues to analyze the secreted components of cells sheds light on underexplored areas by leveraging the rich information contained in these secretions. Unlike previous approaches that have focused on cell-surface recognition, our method avoids cell damage, making it promising for novel applications such as monitoring and quality evaluation of cultured cells.

Detection of Bacteria

The rapid detection of bacteria is critical in various fields, including in medical diagnosis, environmental management, and food safety [66,67]. Common bacterial-detection methods include colony counting, genetic detection, and immunological techniques. While these methods offer high specificity, they often require extensive sample preprocessing

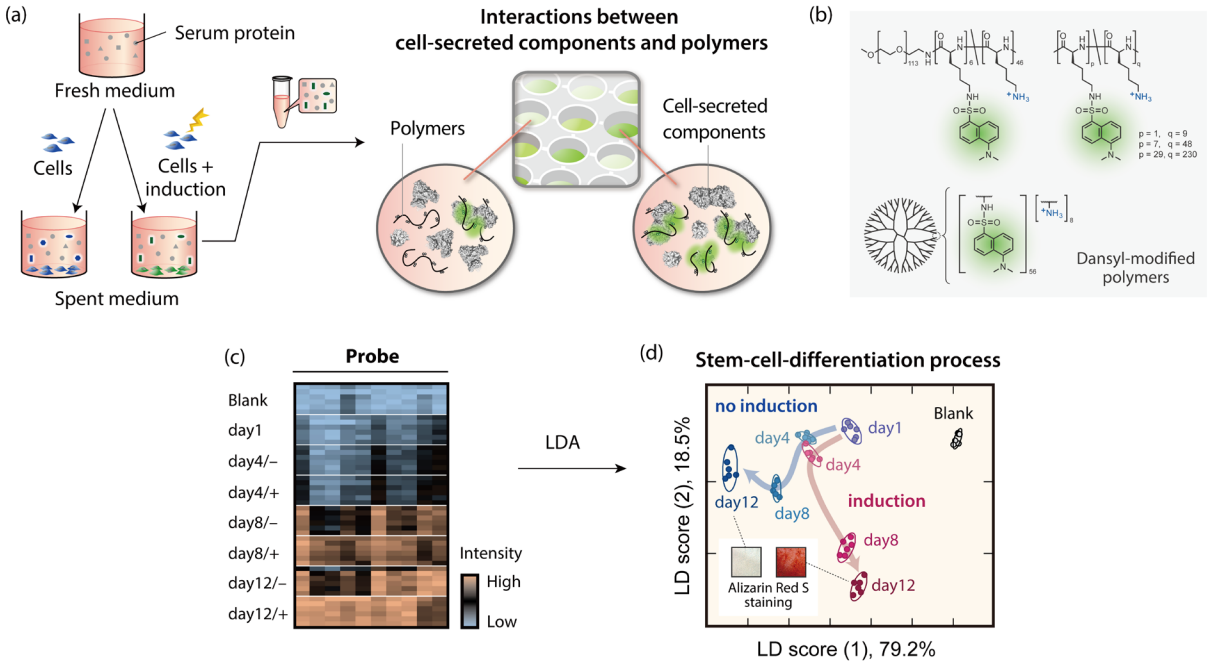

Figure 4 Recognition of cell secretory components using a chemical tongue. (a) Schematic illustration of the recognition process. (b) Molecular structures of cationic polymers modified with Dansyl fluorophores. (c) Fluorescence patterns and (d) LDA score plot of media collected during the differentiation-induction process of mesenchymal stem cells into osteoblasts. Reproduced from Ref. [62] with permission from the Royal Society of Chemistry.

and are time-consuming. The outer membrane surface of bacteria, which are prokaryotic organisms, is composed of various biomolecules such as proteins, lipids, and glycans [68]. By exploiting these characteristics of the bacterial surface, the use of a chemical tongue enables the rapid detection and identification of a wide array of bacteria [21,25,35,41,69–74] via an approach similar to its application in mammalian cells (Figure 3). Aiming to create chemical tongues for microbial analysis with both high sensitivity and accuracy, we used a set of fluorescent polymers that incorporate aggregation-induced-emission (AIE) luminogens, i.e., TPE moieties, as output units (Figure 5a). This system has been successfully used to identify 16 bacterial "species" and even "phyla", which are higher taxonomic groups (Figures 5b and 5c). Furthermore, the chemical tongue is capable of identifying variations at the "strain" level, the smallest classification unit (Figure 5d). Distinguishing between bacterial strains belonging to the same species is typically challenging when using standard metagenomic analyses, such as 16S ribosomal RNA gene sequencing. Therefore, this technology holds promise as an on-site detection tool in the medical, environmental, and food sectors.

The bacterial community residing in the human intestine (gut microbiota) plays a large role in many diseases, and it has become clear that improving the composition of the gut microbiota may lead to the improvement of lifestyle-related diseases and advances in cancer treatment [75–77]. We have also been working on developing chemical tongues that can recognize the characteristics of such bacterial communities (Figure 5e) [44]. TPE-based chemical tongues can be used to distinguish the gut microbiota derived from healthy mice and those with a sleep disorder (Figure 5f). Our application of chemical tongues to the microbiome offers a novel approach to characterize the microbiota. This method provides insights from a completely different angle than that from the standard method for analyzing the gut microbiota, which is an amplicon-sequencing analysis [78,79]. In addition, since the method based on a chemical tongue can be performed more rapidly, easier, and cheaper than conventional technologies, it can be expected to be applied more frequently in the future to monitor individual health conditions.

Comparison of Methods Based on Chemical Tongues with Conventional Methods and their Future Prospects

This review provides an overview of the bioanalytical tool known as a chemical tongue, which mimics the mechanism of the human taste system. Here, we compare this technology to conventional methods based on specificity. Table 1 summarizes the characteristics of the chemical tongues that we have reported.

Sensitivity. The sensitivity varies depending on the performance of the probes. For example, when using polymers that

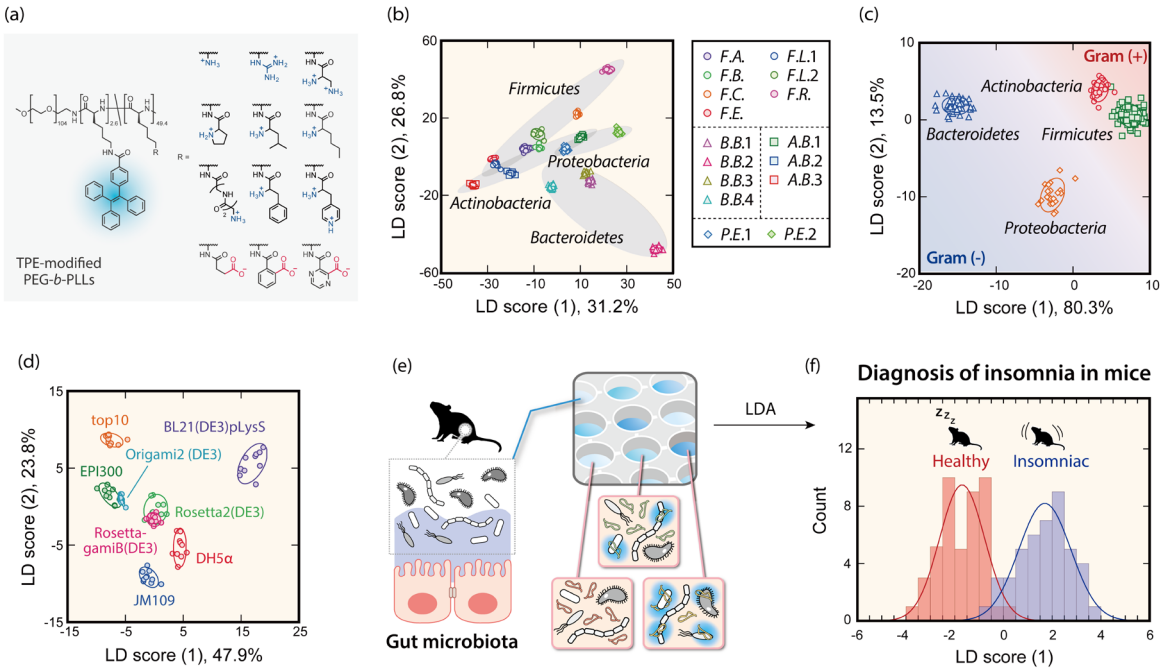

Figure 5 Recognition of bacterial samples using a chemical tongue. (a) Molecular structures of PEG-*b*-PLLs modified with TPE fluorophores and various functional groups. LDA score plots of bacteria wherein the analytes are labelled according to (b) species, (c) phylum, and (d) strains. (e) Schematic illustration of the recognition of gut microbiota in mice using a chemical tongue and (f) the LDA score plot of the gut microbiota derived from healthy and insomniac mice. Reproduced from Ref. [44] with permission from the Royal Society of Chemistry.

can interact strongly with samples at multiple points, proteins can be detected and distinguished at concentrations on the order of several nM in buffer solution. By using probes with smaller dissociation constants, higher sensitivities comparable to antibodies or enzymes can be achieved. However, in the case of chemical tongues based on non-specific interactions, the sensitivity decreases when interfering components such as serum are present. By using highly specific probes such as aptamers [38,80], it is possible to detect proteins at concentrations on the order of tens of nM while avoiding the influence of interfering substances [29].

Cost. The chemical-tongue technologies that we have reported to date permit the synthesis of the required probes for analysis at a relatively low cost, on scales from milligrams to grams. The cost of probes is minimal because the concentration used during analysis is low, typically 100-1000 nM, which corresponds to 20-80 μL per well for a 384-well microplate. Therefore, the main costs are consumables such as pipette tips, microplates, and equipment for detection (e.g., microplate readers). This is an advantage compared to conventional technologies that use expensive antibodies or enzymes.

Other features. One of the most important advantages of using a chemical tongue is that it can avoid the issue of 'specificity'. Creating antibodies or enzymes that can specifically recognize target molecules from scratch for new targets is challenging, and artificially designing and synthesizing highly specific probe materials is not a straightforward process either [81]. By using an array of fluorescent polymers and multivariate analysis, the problem of quality regarding specificity can be solved by harnessing the power of quantity. Additionally, a chemical tongue using fluorescent probes often employs a simple ‘mix-and-read’ type of analysis system in a microplate format. This simplicity facilitates integration with lab automation, potentially increasing throughput and reducing human error.

However, chemical tongues also have weaknesses. With conventional specific and comprehensive methods, the detection targets are clear, making it easy to link the obtained results to past biological findings. In contrast, chemical tongues are generally not suitable for obtaining information about individual components in a sample and are fundamentally unsuitable for applications such as detecting or searching for specific biomarker molecules.

What can be seen from the comparisons described above is that the characteristics of biological samples recognized by chemical tongues based on non-specific and specific approaches are completely different. Therefore, using these methods in a complementary manner may enable a more detailed understanding of the samples being analyzed. For example, chemical tongues may detect changes in sample conditions that cannot be captured by existing technologies or have so far been buried in excess information. In this case, the insights obtained by chemical tongues could inform subsequent specific or comprehensive methods, potentially establishing a novel scheme for a more in-depth analysis. Alternatively,

Table 1 Examples of biological molecule/sample recognition using various chemical tongues

Biological molecule/sample	Probes		Sample concentration	Ref.
	Scaffold materials and recognition units	Output units		
Proteins	PEG-*b*-PLL modified with different amino acids [a]	Fluorescein	20 μg/mL	45
Albumins from different sources and various chemically modified albumins	PLL dissolved in aqueous solutions with various pH and ionic strength [b]	Dansyl	3~20 μg/mL	39
Heat-treated antibodies	Complexes of graphene oxides and single-stranded DNA	Tetramethyl rhodamine	0.1 mg/mL	82
Proteases	single-stranded DNA	Tetramethyl rhodamine	10~50 μg/mL	29
Mixtures of proteases/inhibitor proteins at various concentration ratios	DNA aptamers	Tetramethyl rhodamine	0~120 nM	29
Cell suspensions	PEG-*b*-PLL modified with different amino acids [a]	Fluorescein	10000 cells/mL	45
Cell suspensions	PLL dissolved in aqueous solutions with various pH and ionic strength [b]	Dansyl	2500~20000 cell/mL	60
Serum-free media used for culturing various cell lines	Complexes of PEG-*b*-PAMA[c] derivatives and anionic enzymes	Enzymatic reaction	0.25 μg/mL (total protein concentration)	83
Serum-free media used for culturing mesenchymal stem cells differentiated into different lineages	Complexes of PEG-*b*-PAMA[c] derivatives and anionic enzymes	Enzymatic reaction	0.25 μg/mL (total protein concentration)	83
Serum-free media used for culturing fibroblasts with various numbers of cell divisions	Complexes of PEG-*b*-PAMA[c] derivatives and anionic enzymes	Enzymatic reaction	0.67 μg/mL (total protein concentration)	63
Serum-free media used for culturing liver cancer cell lines treated with drugs under various conditions	PLL dissolved in aqueous solutions with various pH and ionic strength [b]	Dansyl	10~50 vol% (concentration of collected media)	65
Media containing 1 vol% serum used for culturing various cell lines	PLL with different molecular weights, PEG-*b*-PLL, PAMAM dendrimers [d]	Dansyl	5 vol% (concentration of collected media)	62
Media containing 1 vol% serum used for culturing mesenchymal stem cell lines during differentiation induction	PLL with different molecular weights, PEG-*b*-PLL, PAMAM dendrimers [d]	Dansyl	5 vol% (concentration of collected media)	62
Isolated gut-derived bacteria	PEG-*b*-PLL modified with various functional groups	Tetraphenylethylene	OD_{600} = 0.04	44
Different isolated strains of *E. coli*	PEG-*b*-PLL modified with various functional groups	Tetraphenylethylene	OD_{600} = 0.04	44
Gut microbiota derived from normal and insomniac mice	PEG-*b*-PLL modified with various functional groups	Tetraphenylethylene	20 μg/mL	44

[a] Polyethylene glycol and poly-L-lysine block copolymer, [b] Poly-L-lysine, [c] Polyethylene glycol and poly(*N*,*N*-dimethylaminoethyl methacrylate) block copolymer, and [d] Polyamidoamine.

by leveraging the throughput and cost advantages of chemical tongues, they could be applied to screening compounds like pharmaceuticals or materials such as cell-culture substrates, facilitating their development and exploration. We hope that the future application of chemical tongues to various real samples will lead to the elucidation of new biological phenomena.

Conflict of Interest

There are no conflicts to declare.

Author Contributions

All authors contributed to the writing of the original draft as well as the review and editing of the final manuscript.

Data Availability

The evidence data generated and/or analyzed during the current study are available from the corresponding authors on reasonable request.

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