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Development of a microfluidic paper-based analytical device for the determination of salivary aldehydes

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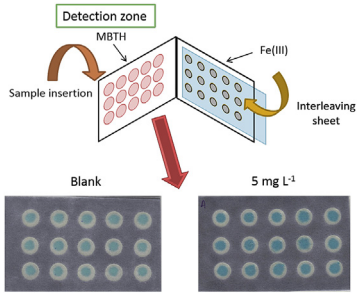
HIGHLIGHTS

- A microfluidic paper-based analytical device for salivary aldehydes is reported.
- Aldehydes react with 3-methyl-2-benzothiazolinone hydrazone and iron(III).
- The colour intensity of the blue coloured formazan dye is measured.
- The working range, LOD and RSD (n = 5) are 20–114 μM, 6.1 μM and less than 12.7%.
- The device is stable for more than 41 days when stored in a freezer (≤-20 °C).

GRAPHICAL ABSTRACT



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ABSTRACT

A low cost, disposable and easy to use microfluidic paper-based analytical device (μPAD) was developed for simple and non-invasive determination of total aldehydes in saliva with a potential to be used in epidemiological studies to assess oral cancer risk. The μPAD is based on the colour reaction between aldehydes (e.g. acetaldehyde, formaldehyde), 3-methyl-2-benzothiazolinone hydrazone (MBTH) and iron(III) to form an intense blue coloured formazan dye. The newly developed μPAD has a 3D design with two overlapping paper layers. The first layer comprises 15 circular detection zones (8 mm in diameter), each impregnated with 8 μL of MBTH, while the second layer contains 15 reagent zones (4 mm in diameter). Two μL of iron(III) chloride are added to each one of the second layer zones after the addition of sample to the detection zones in the first layer. All hydrophilic zones of the μPAD are defined by wax printing using a commercial wax printer.

Due to the 2-step nature of the analytical reaction, the two paper layers are separated by a cellulose acetate interleaving sheet to allow for the reaction between the aldehydes in the saliva sample with MBTH to proceed first with the formation of an azine, followed by a blue coloured reaction between the azine and the oxidized by iron(III) form of MBTH, produced after the removal of the interleaving sheet. After obtaining a high resolution image of the detection side zone of the device using a flatbed scanner, the intensity of the blue colour within each detection zone is measured with Image J software.

Under optimal conditions, the μPAD is characterised by a working range of 20.4–114.0 μM, limit of detection of 6.1 μM, and repeatability, expressed as RSD, of less than 12.7% (n = 5). There is no

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statistically significant difference at the 95% confidence level between the results obtained by the μPAD and the reference method (Student's *t*-test: 0.090 < 0.38). The optimized μPAD is stable for more than 41 days when stored in a freezer (≤-20 °C).

1. Introduction

The ingestion of aldehydes or their production in the human body is associated with numerous day to day activities (e.g. smoking, alcohol consumption). These compounds are known to be toxic and the body of evidence regarding their carcinogenic properties is growing rapidly. The carcinogenic potential and health risks associated with formaldehyde and acetaldehyde has been well-established [1,2]. Tobacco smoke is one of the major sources of aldehyde contamination in indoor air with reported formaldehyde and acetaldehyde concentrations of up to 69 and 1234 μg per cigarette, respectively [3,4]. In addition, there has been growing evidence for the tumour-producing effects of the intake of alcoholic drinks due to the conversion of ethanol to acetaldehyde [5].

Oral cancer embodies approximately 5% of malignant lesions worldwide, but this proportion is increasing rapidly. Individuals who are high producers of acetaldehyde upon exposure to ethanol, due to enhanced activity of alcohol dehydrogenase, may be at an increased risk of developing oral cancer [6,7]. It has also been reported that while alcohol presents a five-fold increase in oral cancer risk, tobacco smoke, which contains carcinogenic formaldehyde, increases the risk by roughly a factor of six [8]. Both risk factors account for 67% of all incidences of oral cancer [9]. Moreover, the emergence of the concept of ethanol-induced oral cancer caused by salivary acetaldehyde has invariably led to the hypothesis that widespread use of alcohol-containing mouthrinses in modern society may also contribute to the uprise of oral cancer. Ethanol is used as a solvent for the active agents in many commercially available mouthrinses, with concentrations ranging from 6% to 26.9% [10,11]. As these mouthrinses are kept in direct contact with the oral mucosa for longer periods of time (e.g. 30 s and longer) than alcohol-containing beverages, it would appear important to investigate the relationship between the use of alcohol-containing mouthrinses and oral cancer [11,12]. Hence, there is a widespread need for the availability of sensitive, accurate and precise diagnostic methods to assess patients' risk of oral cancer development.

Saliva often contains compounds of clinical interest (biomarkers) which are also found in blood. Therefore, it has the potential to provide a cleaner and non-invasive alternative to blood in terms of sampling and clinical diagnosis [13–15]. However, the limited sample volume collected, and the minute concentrations of biomarkers present there, are among the main key challenges to salivary analysis [15]. Due to the known toxicity and carcinogenicity of formaldehyde and acetaldehyde, it is therefore of interest to monitor these aldehydes in saliva as biomarkers for tobacco smoking and/or alcohol consumption [3,4,16]. Analytical techniques such as gas chromatography (GC) [4,17,18] or high performance liquid chromatography (HPLC) [19,20] are frequently used for the determination of aldehydes in biological fluids such as saliva due to their relatively high sensitivity, although the associated equipment is costly and bulky and must be run by trained laboratory personnel. Therefore, alternative analytical techniques with sufficient sensitivity are needed to improve the efficiency, cost effectiveness and scalability of the analysis of saliva and other biological fluids.

Previously, a simple gas-diffusion flow injection analysis (GD-FIA) method has been successfully developed for the determination of salivary acetaldehyde in our laboratory using a spectrophotometric method involving the reaction between 3-methyl-2-benzothiazolinone hydrazone (MBTH), iron(III) and aldehydes [21]. In this reaction an aldehyde reacts with MBTH to produce an azine which further reacts with the oxidized product of the reaction between Fe(III) and MBTH to generate a blue-coloured formazan dye (Scheme 1).

To further improve the scalability and cost efficiency of this aldehyde analysis, we have explored the possibility of using paper-based microfluidics as a tool for the chair-side determination of salivary aldehydes. The use of microfluidic paper-based analytical devices (μPADs) was first reported by the Whitesides group in 2007 [22] for the simultaneous detection of urinary glucose and protein. Since then, there has been a rapidly growing interest in the development of μPADs in the area of clinical diagnosis due to their pronounced simplicity, low cost and portability [23,24]. μPADs have also been successfully applied to various salivary analyses to diagnose diseases such as diabetes [25], dengue fever [26], dental caries [27], and periodontitis [28]. These analyses can also be conducted by personnel without specialized laboratory training which therefore opens up the possibility for their application in point-of-care clinical diagnostics [24,28].

This paper describes the development of a novel low cost μPAD for the accurate and reproducible determination of salivary aldehydes, consisting mainly of formaldehyde and acetaldehyde, using the MBTH spectrophotometric method. The proposed paper-based microfluidic methodology has the potential to be used as a diagnostic tool to assess patients' risk for developing oral cancer.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical reagent grade and all solutions were prepared in deionized water (18 MΩ cm, Millipore Synergy 185). Working acetaldehyde solutions in the concentration range from 15.9 to 114.0 μM were prepared daily from a standardised stock solution of 22.7 mM acetaldehyde (Sigma–Aldrich). To ascertain the sensitivity of the μPAD to formaldehyde (Chem-Supply), solutions of this aldehyde in the same concentration range as that of the acetaldehyde solutions were tested and prepared from a standardised 47.5 mM formaldehyde stock solution.

Solutions of both aldehydes used in this study were standardised by potentiometric titration as recommended by the International Organisation for Standardisation [29]. The acetaldehyde stock solution was stored at 4 °C to minimise evaporative losses. A 14 mM MBTH solution was prepared daily by dissolving 15.1 mg of MBTH (Sigma–Aldrich) in 5 mL of deionized water. The oxidizing agent, which consisted of 160 mM $FeCl_3$ was prepared by dissolving 216 mg $FeCl_3$ (Sigma–Aldrich) in 5 mL of 0.005 M HCl (Ajax FineChem).

A 1.85 mM MBTH solution in 0.1 M hydrochloric acid and a 3.70 mM $FeCl_3$ solution were used in conducting the spectrophotometric standard reference method [30].