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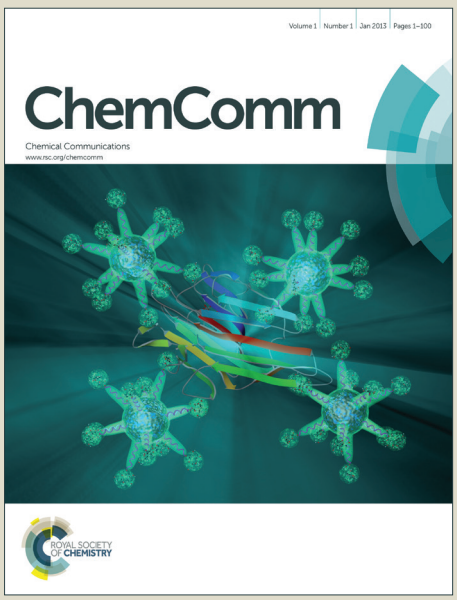

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COMMUNICATION

Paper-based skin patch for the diagnostic screening of Cystic Fibrosis

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A thin and flexible paper-based skin patch was developed for the diagnostic screening of Cystic Fibrosis. It utilized a unique combination of anion exchange and pH testing papers to enable a quantitative, colorimetric and on-skin detection for sweat anions.

Cystic Fibrosis (CF), associated with mutations of a single gene CF transmembrane conductance regulator (*CFTR*), is one of the most common inherited disorders, especially in Caucasian populations with a carrier prevalence of 1/3000.[1] One of its clinical manifestations is the abnormally elevated sweat anions (> 80 mM equivalent chloride).[2] This feature underlies the development of a sweat test that has been the gold standard to diagnose CF for as long as 65 years.[3] Currently, two sweat tests have been recommended by Cystic Fibrosis Foundation (CFF) and broadly used,[4] including Gibson-Cooke QPIT and Wescor Macroduct Sweat-Chek system. However, these tests, largely relying on cumbersome manual operations or expensive bench instruments, are still limited in the efficiency and availability. Particularly, they often need the transferring of sweat between steps of collection and detection. It would add complexity to the assay and increase the risk of sweat evaporation, a primary error in the sweat detection. In addition, the required volume of sweat (15 μL) is unfavorably large for infants, causing the issue of quantity not sufficient (QNS) that is the major restriction to newborn screening of CF.[5]

Compared with conventional sweat tests, on-skin (or wearable) analytical device with specific strengths of miniaturization, integration and convenience may represent a promising innovative direction.[6] Most of these techniques, however, is subject to biohazard concerns. For example, dark-brown silver chromate is useful to generate white precipitation for detecting sweat chloride, yet it is irritant and carcinogenic. Electrochemical method is common in bench and portable devices for sweat analysis, while the exposure of electrode to skin tends to cause burning and blistering. Consequently, these methods were excluded by the CFF guidelines from on-skin detection.[4]

Recently, microfluidic paper-based analytical devices (μPAD) have been vigorously developed.[7] By taking advantage of vertical stacking and the distinctive merits of paper,[8] such as capillary force, lightweight and ease of colorimetric detection, μPAD has the huge potential to inspire the development of on-skin detection.[9]

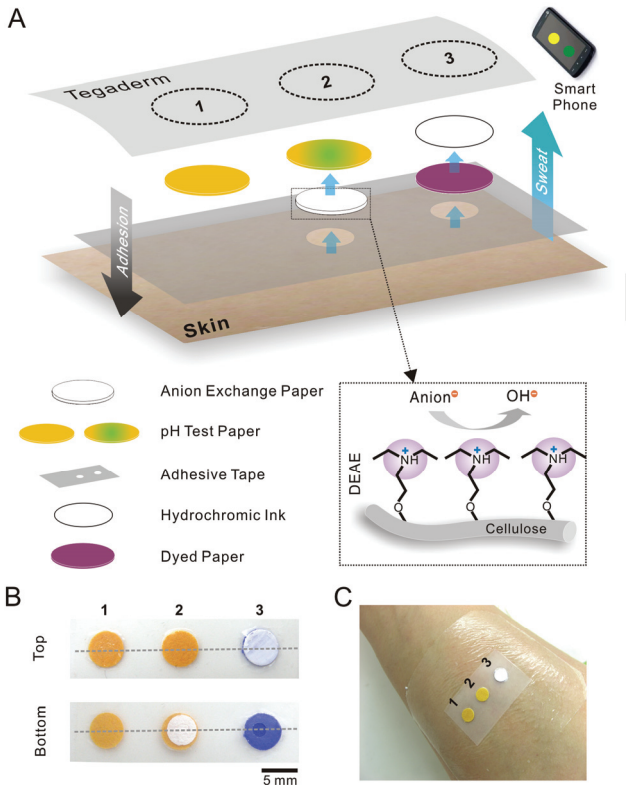

Fig.1 A. Schematics of the paper-based skin patch with three sections. Section 1) A sealed pH test paper (5 mm diameter) as a control of light source. Section 2) An anion exchanging paper (4 mm diameter,1.7 μeq/cm^2) and a pH test paper (5 mm diameter) are vertically aligned and stacked for detecting sweat anions. Inset image shows the anion exchanging via diethylaminoethyl (DEAE) groups modified on a cellulose fiber. Section 3) A purple paper (5 mm diameter) is covered by an opaque white hydrochromic ink that would become transparent in response to water. It is to indicate the volume of absorbed sweat. The blue arrows indicate the flow direction of sweat. The holes on adhesive tape are 2 mm diameter. B. The top and bottom photographic images of the skin patch. The cross-sectional view indicated by dash lines are provided in Fig. S1. C. The photographic image of the skin patch attached on the skin of inner forearm in the resemblance of a Band-Aid.

In this communication, we proposed a colorimetric detection of sweat anions integrated in a paper-based device as a skin patch for the diagnostic screening of CF (Fig. 1 and S1). The skin patch is consisted of vertically stacked functional papers grouped into three sections, and is attached on skin by a medical dressing (Tegaderm, 3M). The circular papers and the holes in the adhesive tapes were conveniently fabricated by Hole Punchers

with different sizes. The skin patch is not only transparent to allow colorimetric detection, but also lightweight, flexible, stretchable and adhesive to form a conformal contact to skin, showing the exact resemblance of a Band-Aid in both appearance and feeling (Fig. 1C). Additionally, the material cost of the skin patch is rather low (about 0.62 US $) because it is made of inexpensive materials like paper and tape (Table S1 and S2).

The key novelty of the paper-based skin patch is, in its section 2, the colorimetric detection of sweat anions (Fig. 1A). It is composed of an anion exchange paper (DE81, GE) and a pH test paper with a range of 1 to 14. The difference in diameter by 1 mm is for the convenience of stacking. After sweat stimulation by Pilocarpine Iontophoresis according to a previous report,[10] the patch would be tightly attached to the skin. Sweat is secreted from skin and flow upward into the above skin patch. In the process of anion exchange, anions in sweat would be absorbed on the paper to release hydroxide ions (Inset image in Fig. 1A). It would result in local alkalization, consequently changing the color of the pH test paper. It is noteworthy that the change of color is proportional to the quantity of sweat anions. The anion exchanging group is diethylaminoethyl (DEAE), a weak base that works under pH from 7.0 to 9.0 (corresponds the color of the pH test paper from yellow to green, Fig. S2). Notably, such mild pH range is biocompatible to the skin. The on-skin detection via the skin patch provides an integrated operation for eliminating manual operations as well as circumventing the need of transferring sweat to minimize the evaporation-induced errors (Fig. S4).

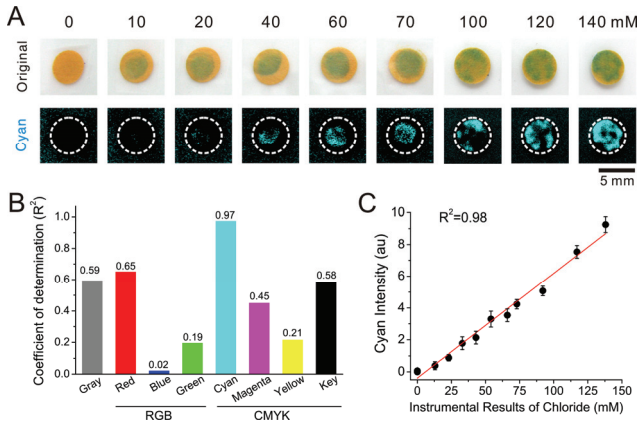

Fig.2 A. The original and converted cyan images of the section 2 in the skin patch for detecting 1.5 μL of chloride solutions in the clinically-relevant physiological range. Dot circles indicate the boundary of the pH test paper and the area of interest (AOI). B. The coefficient of determination of linear regression of each color. The color intensity was calculated as the mean intensity in dot circles. Cyan, rather than green and other colors, yields better quantitative correlations (R^2=0.97). C. The linear least-squares regression analysis shows a high correlation between the paper-based skin patch and a bench electrochemical instrument (R^2=0.98).

To examine the proposed colorimetric detection of sweat anions, we detected a series of standard solutions of chloride (the most abundant anion in sweat) on the paper-based skin patch (Fig. 2A). The appeared green color sometimes may be unevenly distributed on the yellow pH test paper. We attributed it to the fibrous nature of paper and incomplete contact between papers. It may be partially addressed by making the paper saturated (wet-out) and the contact close. The small size of paper (4-5 mm diameter) requires only minute volume of sweat (1.5 μL), which is, of note, ten times lower than the sampling volume (15 μL) in conventional methods. The low sampling volume would reduce the burden and time of sweat collection in favor of newborn screening of CF.[5]

We employed portable devices (such as a smart phone or a CCD camera) to capture images of the skin patch.[11] A controlled light source was employed to prevent from the variations of surrounding light. The pH test paper in the section 1 of the skin patch is sealed between the adhesive tape and the medical dressing to provide a standard color to confirm the consistency of the light (Fig. 1 and S3). We utilized ImageJ (NIH, US) to process images and convert them into eight different colors. Then we carried out linear regression analysis for each color (Fig. 2B). Because cyan shows the highest coefficient of determination (R^2=0.97), it would be the most suitable color for a linear quantitative detection of chloride concentration in the determined volume. Although the result may be counter-intuitive as the skin patch looks more green than cyan by eyes, it is coincident with one previous report that employed cyan color to quantify the color change from yellow to green.[12]

We compared the results of the paper-based skin patch with that of a clinically-approved bench instrument with an electrochemical detector (ABL 800 Analyzer, Radiometer). The percentage recovery at every concentration of the instrumental analysis is also provided (Table S4). The two methods show a high correlation (R^2=0.98, Fig. 2C). Furthermore, a triplicate experiment for detecting chloride solution at 100 mM demonstrated the production and functional reproducibility of the skin patch (Fig. S5). These results demonstrate that the synergy of an anion-exchange paper and a pH test paper is capable of the quantitative colorimetric detection of anions.

There are three primary anions in sweat. Besides chloride, we also detected the other two, lactate and bicarbonate, at approximately their physiological concentrations of 10 mM (Fig. 3A).[13] At the same concentration, the cyan intensity however is different and smaller than that of chloride. We attribute the disparity to the different molecular polarities (lactate < bicarbonate < chloride) and affinities of ion exchange. The results demonstrate that the paper-based skin patch is applicable to the detection of primary anions in sweat. Given that the cyan intensity is contributed by the three anions, it should be converted into the equivalent of chloride, for example in the detection of sweat conductivity,[2] by using the calibration curve in Fig. 2C.

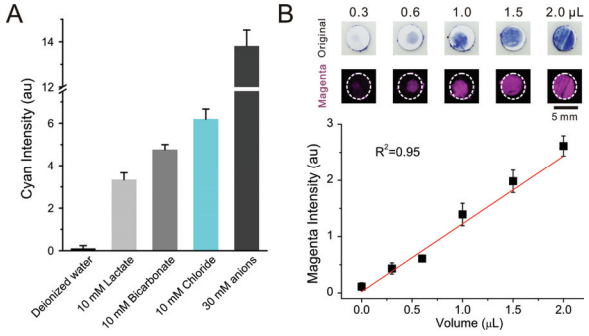

Fig.3 A. The detection of three primary sweat anions (lactate, bicarbonate and chloride) at 10 mM and their equal mixture at 30 mM on the paper-

based skin patch. Deionized water is a negative control. B. The original and converted magenta images of section 3 of the skin parch when pipetting liquid solutions with different volumes. The mean intensity of magenta color is in linear proportion to the liquid volume (R^2=0.95). The dot circles indicate the area of interesting (AOI)

Because the calibration curve in Fig. 2C is determined in a fixed sampling volume (1.5 μL), we developed another colorimetric method on the section 3 of the skin patch to indicate the volume of the absorbed sweat (Fig. 1 and 3B). A piece of purple chromatography paper was coated with a commercial hydrochromic ink (GC-YS-107, Gocolor). In response of water, the ink would become transparent to reveal the color of the purple paper (magenta) underneath. We found that the mean intensity of magenta color is linearly related with the liquid volume (Fig. 3B). Specifically, the magenta intensity around 2 indicates a volume of 1.5 μL. We also found that the existence of different anions shows minimum influence on the measurement (Fig. S6). This method is advantageous as being compatible with the colorimetric detection of anions.

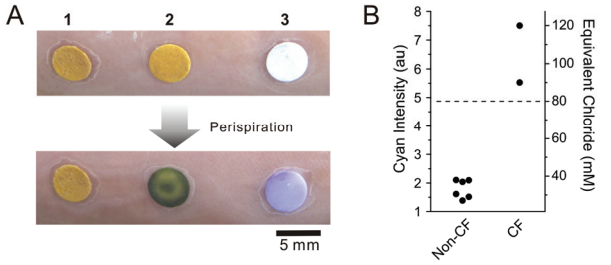

Fig.4 A. The photographic images of the paper-based skin patch attached on the skin of inner forearms of patients before and after perspiration. The numbers indicate corresponding sections of the skin patch. B. The cyan intensity is converted into the equivalent concentration of chloride. The detection results of the skin patch could discern CF patients from suspicious non-CF using a clinical reference value of 80 mM (equivalent chloride).

We used the paper-based skin patch to screen real patients from Peking Union Medical College Hospital, following ethical review and approval (Fig.4). The typical images of the skin patch before and after perspiration are shown in Fig. 4A. Sections 2 and 3 on the skin patch turn into corresponding colors in around 5 minutes, depending on individual's perspiration rate. Fig. 4B shows the screening results of eight suspected CF patients. The cyan intensity was converted into the equivalent concentration of chloride by using the calibration curve in Fig. 2C. Upon the clinical cut-off value (80 mM equivalent chloride), the skin patch could distinguish the CF patients from the non-CF individuals. The results are concordant with the diagnostic results based on QPIT, clinical manifestations and gene sequencing.[10] We also demonstrated a detailed comparison between the skin patch and two clinical standard methods (Fig. S4 and Table S1-S3).

In conclusion, we demonstrated a paper-based skin patch as an alternative sweat test and its clinical applications in the diagnostic screening of CF. The crux of the skin patch is the colorimetric detection of anions on the basis of ion exchange. Significantly, the simplicity of the detection makes it possible to integrate sweat test in the similar form of a lightweight and on-skin Band-Aid. Not only is the skin patch a new miniaturized wearable device, it is also capable of addressing some critical issues (like high expense and troublesome operation) in conventional diagnosis. It would open up new opportunities to the diagnostic screening of CF and many other Point-of-Care applications such as the monitoring of athlete fatigue and water quality.

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Notes and references

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