

Lab-on-a-chip technologies for diagnosis and monitoring of airway inflammation



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This brief review discusses the challenges involved in diagnosis and management of chronic inflammation of the airway and the potential role that lab-on-a-chip technologies can play in enabling personalized health monitoring. Challenges and opportunities are discussed in the context of technology development, and various solutions are discussed, along with future opportunities.

INFLAMMATION

Chronic inflammation plagues hundreds of millions of persons worldwide. In particular, respiratory conditions, such as asthma, affect roughly 300 million persons worldwide. Despite its high prevalence, a clinically sensitive yet minimally invasive molecular diagnosis for monitoring asthma, such as through blood testing or exhaled breath condensate (EBC) analysis, remains particularly difficult to achieve. Expensive procedures, which are either invasive and costly or lack clinical sensitivity, are still the gold standard. For example, spirometry is an inexpensive and noninvasive test; however, it has relatively low clinical sensitivity. Other tests, such as chest x-rays, show greater sensitivity compared with spirometry, yet are not as specific. Bronchoalveolar lavage is a diagnostic procedure that can be used to sample the inner lining of the respiratory airway and to perform molecular analysis with high accuracy, yet the procedure is rather invasive.

Biomolecular analysis of markers in the breath or EBC is a noninvasive method of sampling the airway; however, target concentrations are much lower compared with those in samples obtained from a bronchoalveolar lavage procedure. As an example, the exhaled nitric oxide (eNO) test has a lower sensitivity compared with the bronchial challenge test yet has higher specificity. This test requires breathing for 10 minutes and is chemiluminescent based.

Much effort has been focused on finding biomarkers in EBC and also blood-based inflammatory biomarkers. In the context of asthma, detection of protein biomarkers in EBC can result in low-cost diagnosis and monitoring of disease progression, allowing for ubiquitous screening in even resource-limited settings.

There is an intuitive advantage of measuring inflammatory biomarkers in EBC compared with detecting eNO in breath. The whole inflammatory cascade is what leads to excess levels of proteins and metabolites in EBC, and thus measuring biomarker levels in EBC allows direct monitoring of the inflammatory cascade, whereas nitric oxide is a gas, a fleeting molecule, and an indirect byproduct of the metabolic pathway. Also, it has been shown that in general, the efficacy of adapting asthma therapy based on eNO alone compared with clinical symptoms for asthma-related outcomes in both adults and children does not result in outcome improvement.¹ Nitric oxide molecules in breath oxidize and convert to nitrite in EBC, which is an intermediary state before conversion to nitrate. Nitrite is more stable compared with nitric oxide, and studies have shown that nitrite levels in asthmatic patients are often increased when nitric oxide levels appear to be normal.

In the context of point-of-care diagnostics and disease management, another potential advantage to monitoring biomarkers in EBC is that protein assays have been proved to be miniaturizable, whereas gas analysis platforms are often bulkier. Ultimately, however, measures of protein biomarkers and eNO are both complementary approaches, each of additional value to the other.

The difficulty for developing an EBC-based biomarker screening platform is 2-fold. The first difficulty is the lack of sensitivity, which can be attained with techniques such as ELISA or Luminex (Luminex, Austin, Tex). As a result, concentrations of inflammatory biomarkers, such as proteins, are at the lower compressed region of the dynamic range outside of the linear region, and thus quantification is difficult. More sensitive protein detection platforms are required to properly quantify inflammatory marker levels.

The other difficulty results from lack of a single biomarker with sufficient sensitivity and selectivity because of disease heterogeneity. Thus any effective screening tool would require multiplexing capability to analyze a panel of markers. Another limit is the lack of sensitive yet inexpensive tools capable of multiplexed rapid analysis of a panel of proteins.

BIOMARKER PANELS

Growing evidence shows that noninvasive measurement of airway inflammation by using analysis of inflammatory markers in EBC can provide more sensitive diagnostic value and be useful for asthma management. Given disease heterogeneity, a single marker is insufficient for characterizing specificity in relation to asthma. Thus the capability to rapidly analyze a panel of markers is significantly important. Various studies have shown that a combination of markers can provide greater sensitivity and specificity in diagnosis. For example, Robroeks et al² studied pulmonary marker levels and found that eNO and the EBC markers 8-isoprostane, IFN- γ , and IL-4 indicated asthma control with a

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sensitivity of 82% and specificity of 80%. Technologies that combine the benefits of sensitivity and quantification ability of ELISA, the rapidness and portability of lateral flow devices, and the multiplexing ability of techniques like mass spectrometry are necessary.

UNMET TECHNOLOGICAL NEED FOR ASSAYING PROTEINS

Currently, there is no clinical test performed for assaying protein biomarkers of asthma in EBC. Accumulating clinical data strongly point to the need to assay multiple biomarkers because individual biomarkers are often incapable of accurately defining disease pathology, progression, and thus diagnosis. At present, technology for such a multiplex proteomic assay that can be used at the point of care does not exist. The most routinely used protein assay for analyzing clinical samples is the ELISA. Although relatively inexpensive and amenable to multiplex analysis, ELISAs are not well suited for use at the point of care. ELISAs typically take on the order of hours to go from sample to answer, have at best a picomolar limit of detection, use at least 100 μL of volume for each target assayed (thus requiring large sample volumes when assaying EBC), and typically rely on bulky equipment for optical readout.

LAB-ON-A-CHIP-BASED SOLUTIONS FOR PROTEIN DETECTION

Various groups have developed miniaturized technologies with ultracompact footprints for performing multiplex detection of protein biomarkers for inflammation and autoimmune disease. These include optical, magnetic, and electrical sensing modalities. Hong et al³ demonstrated a fully integrated Complementary Metal Oxide Semiconductor biosensing platform using the on-chip interconnects as nanoplasmonic filters for enabling fluorescence detection of various analytes. The ability to miniaturize fluorescent readout to an instrument with such a tiny footprint can be useful for a plethora of applications in monitoring chronic inflammation. Mok et al⁴ demonstrated a miniaturized electronic digital assay for quantification of cytokines in serum. Xie et al⁵ developed an electronic method for barcoding microparticles, which can be used in conjunction with the digital protein assay for performing multiplexed detection of inflammatory markers in biological fluids. Choi et al⁶ fabricated a portable giant magnetoresistance-based biosensor with a smart-phone interface to perform a 1-step assay for quantifying markers of autoimmune disease in biological fluids. The abovementioned devices have focused on detecting biomarkers in blood primarily; however, these types of ultrasensitive yet compact technologies are examples of multiplexed protein assays that can be used to monitor biomarkers in EBC in the future.

NANOTECHNOLOGY AND ELECTROCHEMISTRY FOR NITRITE AND NITRIC OXIDE DETECTION

Recently, graphene-based thin films have emerged as promising electrodes for various sensing applications. Metals, such as gold and platinum, are resilient to corrosion yet exhibit slow electron transfer, whereas electrodes made from silver and aluminum exhibit low electron transfer resistances but corrode easily. Graphene-based electrodes are unique in that they are



FIG 1. A depiction envisioning the future of respiratory health monitoring using wearable devices and smartphones. An asthmatic patient wears a wristband into which he or she blows periodically to measure inflammatory markers in EBC. Data are processed and displayed on a smartphone, providing the user with therapeutic guidance. For example, increased levels of inflammatory markers can trigger the smartphone to alert the user to use an inhaler to prevent onset of an asthma attack.

resilient to corrosion while exhibiting a rapid electron transfer rate. Recently, Gholizadeh et al⁷ demonstrated the ability to quantify nitrite levels in EBC with high sensitivity using reduced graphene oxide electrodes. Submicromolar levels of nitrite were quantified by using thin-film electrodes, and studies were performed with human patient samples. Before this, Li et al⁸ used reduced graphene oxide electrodes in a field-effect transistor configuration for detection of nitric oxide levels in the gas phase at the part per billion level.

WEARABLE SOLUTIONS AND FUTURE RESEARCH DIRECTIONS

The technologies discussed above are all solutions with the capability of becoming portable handheld devices. Ultimately, patients with chronic inflammation will benefit from wearable devices that can continuously monitor health (Fig 1). Furniturewalla et al⁹ recently fabricated a smart wristband capable of monitoring biomarkers and cells in bodily fluids. One can envision asthmatic patients with a bracelet or necklace into which they

can blow periodically or wirelessly powered sensors mounted onto tooth enamel for monitoring biomarkers in EBC continuously.¹⁰ The same type of wearable smart wristbands monitoring biomarkers in sweat and blood can also be used to monitor inflammation in patients undergoing acute inflammation (eg, burns) and autoimmune disease (eg, sepsis), and potentially, smart bandages can be used for monitoring inflammation in wound healing. With the emergence of an ultracompact laboratory on a chip device and highly sensitive nanotechnologies, an enormous potential exists for personalized health monitoring.

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