# Chronic Obstructive Pulmonary Disease: an Overview and a related iGEM Project

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## Abstract

Chronic obstructive pulmonary disease accounts for over 3.3 million deaths worldwide annually, with especially high rates in Southeast Asia, Europe, and North America. Current detection methods are costly and sometimes inaccurate. COPDetective utilizes miRNA biomarkers to provide a novel diagnostic tool for COPD. Our project uses padlock probes with rolling circle amplification/Cas12a to quantify miRNAs via fluorescence. Furthermore, we developed our corresponding VBD3 (Visual Based Disease Detection Device), a portable fluorescent quantifier that analyses and quantifies the fluorescent signals generated, thus giving diagnosis results.

## 1. The Problem

Chronic Obstructive Pulmonary Disease (COPD) is the third leading cause of death globally (World Health Organization, 2023). Within China, the problem is most prevalent in the southwestern provinces (Fang et al., 2018).

### 1.1. Pathophysiology

COPD is triggered by prolonged exposure to harmful particles or gases. Inflammation occurs in the airways, lung parenchyma, and pulmonary vasculature. Multiple inflammatory mediators are

released, with oxidants and excess proteases leading to the destruction of air sacs. Airway obstruction/collapse could also trigger hyperinflation and gas exchange function impairment (Singh et al., 2019) (Stockley, 1999). Emphysema and chronic bronchitis significantly contribute to the development of COPD in patients. Furthermore, triggers such as bacterial/viral pneumonia and other environmental irritants could exacerbate the pre-existing COPD symptoms (Cm et al., 2005).

## 1.2. Symptoms

COPD can come in a variety of symptoms, the most common being breathing difficulty, mucus production, and wheezing. Others that may occur include skin cyanosis (low arterial oxygenation) (Changizi & Rio, 2010), lower extremity edema (Singh et al., 2019), and "barrel chest" (increased anterior-posterior chest wall diameter (Mattos et al., 2009).  People with COPD are also more susceptible to heart conditions, lung cancer, and a variety of other conditions (*COPD - Symptoms and Causes - Mayo Clinic*, 2020).

*Bronchitis and Emphysema, two main causes of airway obstruction (Mayo Clinic, 2020).*
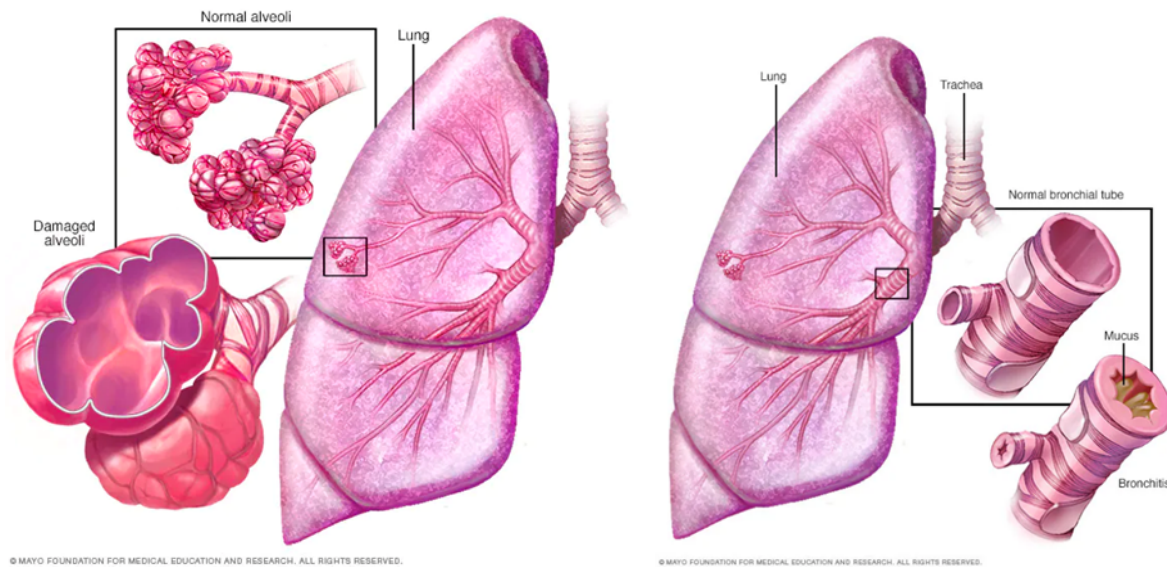
### 1.3. Current diagnostics

COPD is commonly misdiagnosed or undiagnosed. According to Koch et al. (2019), only 2.6% of the COPD population were aware of their condition, and 9.7% had a prior pulmonary function test. Conventional diagnosis of COPD is primarily based on tests such as pulmonary function tests, CT scans, arterial blood gas analysis, and chest X-ray (*COPD - Diagnosis and Treatment - Mayo Clinic*, 2020b). However, these tests are often costly and time-consuming. Several tests may be required, as well as cross-referencing of family and medical histories. According to Du et al. (2021), early screening costs approximately ¥6366. However, their statistics also showed that early screening patients had an increase in quality-adjusted life years by 0.28 units.

Thus, with a reagent cost per reaction of about $1, and an analytical hardware of just over ¥200 ($35), our project offers a more cost-effective and convenient method for COPD screening.

## 2. miRNA

MicroRNAs are a class of small noncoding RNAs that function in post-transcriptional regulation of gene expression and cellular activities such as growth, differentiation, and apoptosis (Saliminejad et al., 2018). The majority of miRNAs are transcribed from DNA sequences, which are then further transcribed into primary, precursor, and mature miRNAs (O'Brien et al., 2018). In order to regulate mRNA expression, miRNAs interact with the 3' untranslated region (3' UTR) of target mRNAs, thus inducing degradation and translational repression (O'Brien et al., 2018).

The expression of miRNAs is often altered in diseases, including Chronic respiratory diseases (CRDs) such as asthma and Chronic Obstructive Pulmonary Disease (COPD) (Cañas et al., 2021). The use of miRNAs as biomarkers for COPD is a novel approach that could improve the diagnosis and treatment of COPD. miRNA profiling could be employed to detect COPD at early, asymptomatic stages before severe progressions. Furthermore, the underuse of spirometry has often led to the misdiagnosis of COPD. In this case, the use of miRNA profiling could be beneficial, since it is an effective tool for detecting hidden pathologies.

## 2.1. 5p & 3p structure

In the pre-miRNA stem-loop structure, the 5p strand is in the 5'-3' position, while the mostly complementary 3p strand is in reverse (McAlinden, 2015).

## 2.2. miRNA selection

After the literature review, we decided to design biosensing systems to target miR-223 as a biomarker for screening and monitoring chronic obstructive pulmonary disease. miR-223 is

upregulated in COPD patients (Roffel et al., 2021). We avoided downregulated miRNAs to ensure that detection would be successful in the initial phase.

## 3. Our approach

HS-China's COPDetective employs a composite approach to the early screening and diagnosis of chronic obstructive pulmonary disease (COPD). We developed the MB-ERC2 system for the microRNA biomarker associated with COPD in conjunction with VBD3, our fluorescence quantification/analysis device.

Our miRNA biomarker-based exponential RCA Cas12a/CRISPR (MB-ERC2) system uses linear ssDNA padlock probes along with rolling circle amplification (RCA) and Cas12a to detect and amplify miRNAs and quantify their fluorescence. We also developed our visual-based disease detection device (VBD3), an analytical hardware/software that quantifies the fluorescent signals generated by our reaction system.

Throughout our project, HS-China consulted stakeholders such as patients, doctors in the respiratory diseases field, and pharmaceuticals/therapeutics companies. Since COPD disproportionately affects those from developing countries (i.e. marginalized populations), we took initiatives to address inequalities, including fieldwork promoting general awareness in our team's home country, online educational videos, and online articles introducing COPD and our work.

With COPDetective, HS-China holistically addresses the problems of diagnosis of chronic obstructive pulmonary disease through a multitude of approaches including our miRNA biosensing system, cost-effective analytical hardware, and educational initiatives.

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