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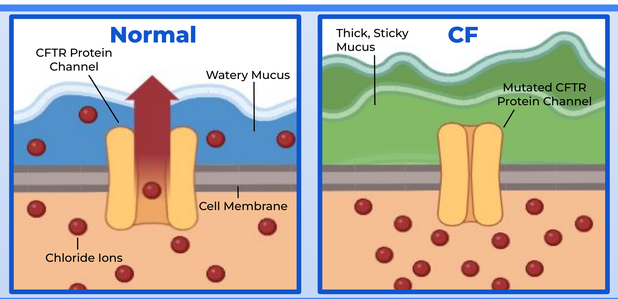
MEET THE MUCLEAR SCIENTISTS!

What is Cystic Fibrosis (CF)?

Cystic Fibrosis (CF) is an inherited genetic disorder, primarily affecting the respiratory system.

It is caused by mutations in the CFTR gene, which codes for a CFTR protein channel responsible for the movement of chloride ions and water in and out of cells.

In CF, the faulty CFTR protein leads to the production of thick, sticky mucus that clogs airways and traps bacteria, resulting in frequent lung infections and long-term respiratory decline. Notably, the most common mucins (a major macromolecular component) found in CF mucus includes MUC5AC and MUC5B.



Impacts of Cystic Fibrosis

Whilst an estimated 160,000 people around the world are affected by CF; In the UK, over 11,000 people are expected to have CF (that's 1 in every 2500 babies born), making CF both a domestic and global issue.

On an individual level, CF patients suffer a substantial impact on their quality of life owing to a "high symptom burden":

- **Respiratory Strain:** Persistent coughing, wheezing and shortness of breath impairs physical function and the ability to carry out daily tasks.
- **Time Consuming Treatments:** Demanding treatment regimens including daily airway clearance and physiotherapy can disrupt daily life requiring up to 1-3 hours.
- **Stressful Treatments:** Managing multiple complex regimens can lead to emotional and mental fatigue.
- **Absenteeism:** Missed school or work due to illness and appointments can cause social isolation.

Additionally, treating CF costs the NHS over £100,000 per patient annually, amounting to a total of £30,000,000 per year in medication.

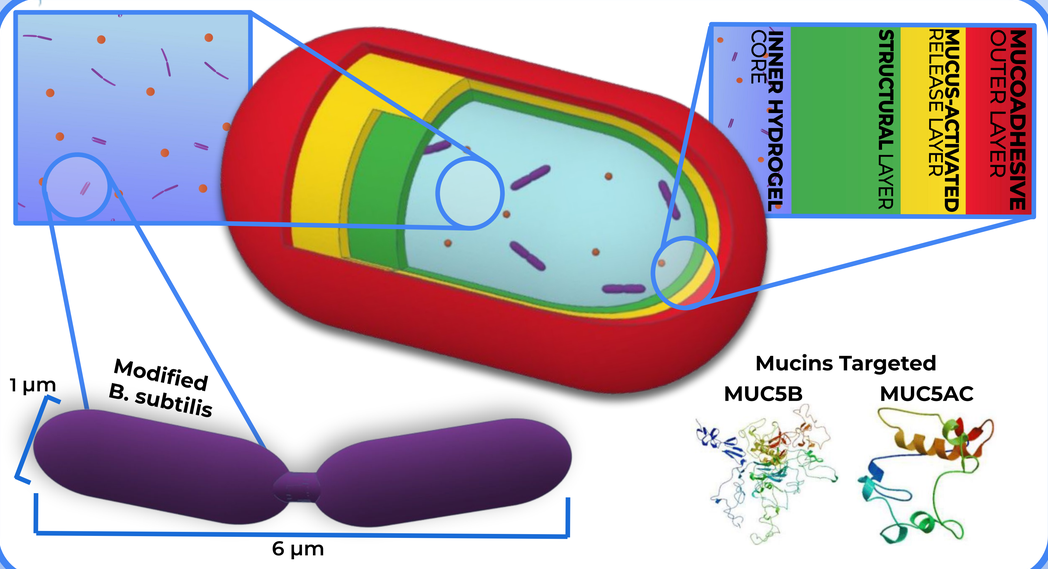
Current Treatments

The most common pharmaceutical treatments for CF include:

- **Mucus Thinners:** Tablets containing mucolytics which break down thick and sticky mucus
- **Kaftrio:** A triple combination tablet of CFTR modulators (elexacaftor, tezacaftor and ivacaftor) which improve the function of the CFTR protein channels

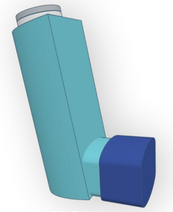
Pros & Cons

Mucus Thinners	
- Help you cough up mucus more effectively - Relieves chest congestion - Improves lung clearance and reduces flare ups	- Side effects include nausea, vomiting and sore throats - Not recommended for young children, pregnant women or those with asthma - Not actively targeted
Kaftrio Drug	
- Large lung-function boost - CFTR-targeted treatment - Widely approved and inclusive for over 90% of CF patients.	- Strict drug interactions and dietary rules - Needs to go through the circulatory system to then reach lungs - Doesn't directly clear mucus immediately



Delivery System - Capsule & Inhaler

Capsule Layers	Outer Mucoadhesive Layer	Mucus-Activated Release Layer	Structural Matrix	Hydrogel Core
Material Used	Chitosan and Alginate mixture	HNE-cleavable peptide linker	PEG	Alginate-based hydrogel
Description	• Enables adhesion to CF mucus. • Stable in saliva and upper airways preventing premature adhesion • Positively charged chitosan ionic bonds with negatively charged mucus	• Responds to high human neutrophil elastase (HNE) concentration which is typical in CF mucus • HNE passes through outer layer and cleaves peptide bond activating release • CF-specific release	• Maintains shape and structural integrity • Prevents leakage before contact • Provides strength during inhalation and transit • Porous when mucus-activated layer is cleaved	• Suspension for engineered bacteria and CFTR modulators • Suspension contains glucose to sustain bacteria till release • Maintains pH stability • Ensures even delivery of drugs and bacteria


3D Render

Metered Dose Inhaler (MDI)

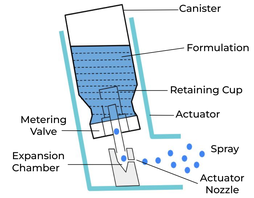
A compact and pressurized device, designed to deliver a specific amount of medication directly through the respiratory system.

Key Features:

Canister: Holds medication (CFTR modulation and capsule solution).

Metering valve: Ensures a consistent dose.

Actuator: Directs the spray into the mouth and airway.



2D Cross Section

Cost & Feasibility

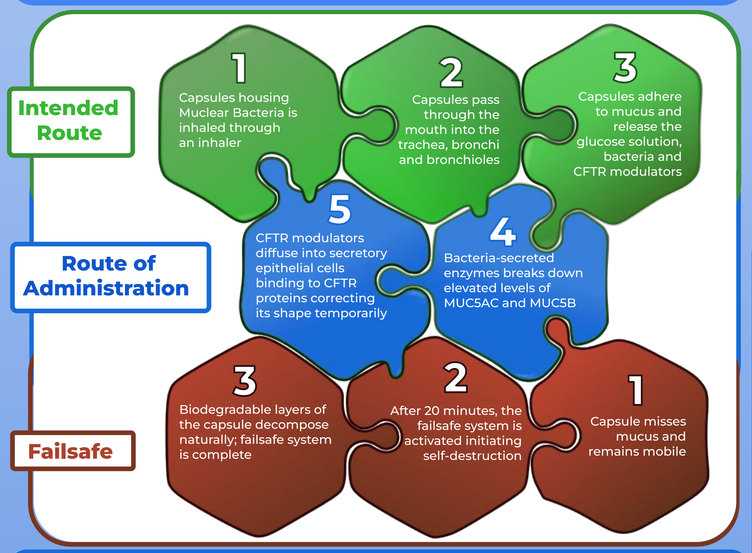
Component	Subcomponent	Estimated Cost/Dose (£)
Nanosized Capsule	Outer Mucoadhesive Coat	0.05
	Mucus Activated Release Layer	0.10
	Structural Matrix	0.01
	Internal Hydrogel Core	0.05
Solution	Kaftrio	~40
	Glucose solution	0.75
	Engineered Bacillus subtilis	0.01
	Processing of bacteria	0.50
Inhaler Device and packaging	Inhaler Device	0.80
	Packaging	0.10
	Solution Processing	0.15
Estimated Total		**42.52**

Treatment Benefits

Affordability: Generally, Kaftrio treatment costs roughly £280 per day, however as mentioned in the Kaftrio designated box, due to the efficient and direct delivery of Kaftrio within our capsules a much lower dosage can be used in our medication to yield similar results to current treatments. This will amount to around £40 per dose widening availability and equity in access.

Combined Functionality: Our capsule merges both the functionality of mucus thinners and the Kaftrio drug.

Feasibility & Social Acceptance: Whilst ambitious, our solution is grounded in contemporary science; key components such as B. subtilis for drug delivery and CRISPR-Cas9 gene editing are extensively proven methods. Moreover, our clinical trials and failsafe intervention secures scientific acceptance and its affordability ensures public appeal.



Kaftrio - CFTR Modulators

We have also incorporated the delivery of a CFTR modulator, specifically the Kaftrio drug, alongside our mucus-reducing bacteria. Notably, this will increase the overall efficiency of the modulator as the capsule will allow for precise delivery to airways affected by CF. Moreover, a lower dosage can be used as our delivery system circumvents the alternative oral route, allowing for a greater proportion of the drug to be conserved until it reaches the damaged CFTR protein.

The Kaftrio drug will be delivered within a micelle in the capsule; micelles are spherical structures formed by amphipathic molecules creating a hydrophilic (soluble in water) surface. In the context of the insoluble Kaftrio drug, the micelle will rectify Kaftrio's insolubility, which otherwise would lead to unpredictable delivery and it will be delivered directly to the surface of the epithelial cell as a result of its microscopic size, allowing for better diffusion into the cell.

Once absorbed, the triple combination drug will be initiated. Firstly, both elexacaftor and tezacaftor will aid the CFTR proteins in folding appropriately, preventing misfolds as a result of mutations. As well as this, they both work together to bring the CFTR protein to the plasma membrane of the epithelial cell. Finally, ivacaftor will bind to the CFTR protein, improving gating (increasing the frequency with which the protein channel opens) allowing for a healthy flow of chloride ions and water, which will ameliorate the hydration of the mucus, making it less thick and sticky. As with all modulators, the resolve is temporary.

Our Proposal

Objectives

1. Clear buildup of mucus by actively targeting high concentrations of mucins; MUC5B and MUC5AC.
2. Deliver targeted CFTR modulator doses to the defective CFTR protein channels.
3. Improve on the overall precision, accuracy and efficiency of mucus thinners and the Kaftrio drug.

Concept

We propose a targeted, nanosized, inhalable capsule designed to treat CF; delivered in solution within an inhaler.

Once inhaled, the capsule will specifically adhere to mucus located in the biochemical conditions of CF in the bronchi and bronchioles. Once bound to mucus, the capsule coating will dissolve, releasing the bacterium (B. subtilis) engineered to break down mucus.

While this happens, the CFTR modulator will diffuse into the CFTR protein channels, correcting them temporarily.

Mechanism

- **Solution**: Bifunctionality enables widespread dispersion of treatment over the lower respiratory tract whilst also providing a source of sustenance for the bacteria.
- **Capsule Coating**: 4 structural and mechanistic layers composed of a mucoadhesive outer coat, a mucus-activated release layer, a PEG structural matrix and an inner hydrogel core.
- **Bacteria**: Modified to produce the enzymes (B. subtilis) targeting elevated levels of MUC5AC and MUC5B
- **CFTR Modulator (Kaftrio):** Delivered in a micelle, it will work together to improve the functionality of the defective CFTR channels by correcting the misfold in the CFTR protein.

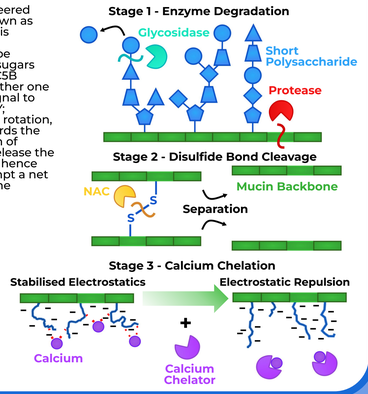
Bioengineered Bacteria

To break down the excess MUC5AC and MUC5B, we have engineered a non-pathogenic strain of Bacillus subtilis; a probiotic bacterium chosen for its historical use in drug delivery as a result of its naturally low pathogenicity.

Additionally, with the aid of CRISPR-Cas9, B. subtilis will be genetically modified in order to remove and alter genes coding for highly immunogenic surface antigens, allowing for complete immune system evasion and preventing what would otherwise cause the bacteria to be rapidly compromised, neutralised or even contribute to inflammation; to further ensure biosafety, attenuation modifications are made to remove and alter any residual virulence-related genes in B. subtilis associated with the secretion of enzymes capable of damaging tissue.

Additionally, our B. subtilis is engineered with mucin-sensitive receptors known as MCPs (Methyl-accepting Chemotaxis Proteins) integrated into its native chemotaxis system. The MCPs will be receptive to mucin fragments and sugars (characteristic of MUC5AC and MUC5B concentrations). On binding with either one of these, the MCP will transmit a signal to proteins CheA, thus activating CheY; controlling the direction of flagellar rotation, allowing the bacteria to move towards the area with the highest concentration of MUC5AC and MUC5B (as it would release the most mucin fragments and sugars; hence the chemotaxis system would prompt a net movement towards the area with the highest concentration).

Finally the already modified B. subtilis will undergo further CRISPR-Cas9 gene editing so as to safely produce proteases, glycosidases, NAC (N-Acetyl-cysteine) and calcium chelators specifically targeting the elevated levels of MUC5AC and MUC5B. This set of enzymes will allow for a 3-step method (enzyme degradation, disulfide bond cleavage and calcium chelation) executed to degrade the structure of both mucins by dismantling the mucin backbone.



Health & Safety - Failsafe

Regulators such as the MHRA and FDA require built-in biosafety for genetically modified organisms hence we've come up with a fully-functioning, automatic failsafe system to ensure that B. subtilis only survives and functions in CF airways and nowhere else.

In essence, the failsafe system consists of the timed activation of mucin dependency within the bacterium; without mucin, after a given amount of time, the bacterium will die. It is split into 2 principal phases:

Phase 1 - Repressilator (Timed Activation of Mucin Dependency)

A repressilator is a synthetic circuit typically made of 3 repressor genes that turn each other off in a loop, creating a delay before triggering an output. These genes are arranged in a loop such that they inhibit each other in a cycle. Attached to the loop is a reporter gene that activates after a set number of cycles.
The repressilator circuit will be integrated into a plasmid within B. subtilis and in order to fine-tune the repressilator timing, both cycle length and the number of cycles need to be controlled; gene strengths and feedback loops will be modified in order to alter the length of a cycle to an adequate duration such that after a set number of cycles a precise time is achieved, activating the reporter gene. Particularly, we have settled for 20 minutes; this is ample time for the capsule to have reached stage 3 of the route of administration and if not, it is an apt time to initiate the failsafe system before potential implications.
Once the reporter is activated, it expresses a TF (transcription factor which regulates gene expression by binding to specific DNA sequences) which binds upstream of the mucin-sensitive promoter allowing it to respond to mucin signals.

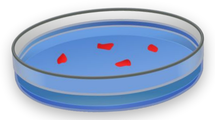
Phase 2 - Mucin-Dependency

The mucin-sensitive promoter is a DNA sequence that controls when a specific gene is turned on or off for transcription. The mucin promoter is linked to a TF that can detect mucin such that it only binds to the promoter when mucin is present, triggering the specific gene controlled by the mucin-sensitive promoter.
Specifically, the gene it controls codes for the synthesis of the enzyme tRNA synthetase, which attaches the correct amino acid to each tRNA during translation, thus making it key for protein synthesis; an essential survival factor since if amino acids aren't bound to the corresponding tRNA molecule, protein synthesis ceases, which will kill B. subtilis. Hence, after Phase 1, if B. subtilis is not in the presence of mucin: the transcription factor is not released, the mucin-sensitive promoter is not turned on, tRNA synthetase is no longer synthesised and no more protein synthesis occurs therefore B. subtilis is terminated.

Clinical Trials - Assessing Effectiveness

Stage 1 - In vitro testing

In order to test the capsule containing our CFTR modulators and genetically modified bacteria, we will have to begin with in vitro testing. We will use a 3D airway organoid model to accurately simulate the conditions in the airways of a CF patient; organoids will be extracted from multiple CF patients. Within the petri dish, the cells will be embedded within a matrigel matrix and will then be given growth factors, allowing it to grow into a 3D structure with mucus producing epithelial cells. We will maintain the temperature at 37°C and keep the CO_2 at 5% in order to accurately simulate the conditions inside the lungs. Capsules will then be introduced directly into the lumen of the organoid using microinjection.



Our initial focus will be testing the capsule's ability to adhere to the CF mucus and its ability to effectively release its contents. If this is successful, we will move on to testing the efficacy of the enzymes, the failsafe and the CFTR modulator. Furthermore, we will evaluate the effectiveness of the enzymes in degrading the CF mucus as well as the effectiveness of the CFTR modulator in correcting the CFTR protein channel in cell cultures derived from the CF patients. Once we have evidence that the enzymes can successfully lower mucus levels, the bacteria die after the failsafe is activated and the CFTR modulator improves CFTR function, we can move on to animal studies

Stage 2 - Animal Studies

CFTR knockout mice will be utilised to test the efficacy and toxicity of our treatment in a whole, living organism. The CFTR knockout mice are genetically engineered to lack the functional CFTR protein, allowing us to mimic the symptoms of CF in the animal. We will administer our treatment using inhalation, just like how we intend to administer it to human patients in the future. We will check for any adverse side effects, particularly the side effects of the bacteria on the mouse. If we see results of improved breathing and clearance of mucus without any serious side effects, we can move onto clinical trials.

Stage 3 - Human Studies

Phase 1 - Safety and dosage

This phase will involve 50 healthy volunteers and the primary aim will be to determine a safe dosage range. The drug will be administered and we will constantly be monitoring the volunteers for any adverse reactions such as difficulty in breathing and inflammation hence revalidating the drug's toxicity.

Phase 2 - Efficacy and dosage

This phase will involve 300 patients who have CF. We will monitor their levels of mucus and breathing ability, while continuing to monitor for any adverse side effects. This will allow us to analyse the optimum dosage of the treatment (minimum toxicity and maximum efficacy).

Phase 3 - Confirmatory Trials

Around 3000 CF patients will be selected to confirm the efficacy of our treatment and record any side effects in this wider group of patients. Double blind, placebo controlled trials will take place to test the efficacy of the treatment without any bias. If this is successful, we will then register for regulatory approval with the MHRA

Stage 4 - Post marketing surveillance

Once the drug is administered to a large mass of patients with CF, we will monitor the long term effects of the treatment. Due to the much larger patient population, we will be able to detect rarer side effects that were not identified during clinical trials.