Development of a miniaturized chip for personalized therapy of Cystic Fibrosis

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Abstract — Cystic fibrosis (CF) is the most common genetic protein disorder. Hundreds of different mutations may be responsible for causing the disease, but the most common is F508del. This mutation affects a gene responsible for the production of cystic fibrosis transmembrane conductance regulator (CFTR) protein, resulting in misfolding and premature degradation of F508del-CFTR. Since this ion channel is affected the secretions become thicker and more viscous which causes dysfunction of external secretion glands of different organs.

In this work, a microfluidic device for personalized therapy of CF has been developed. It was chosen to use this attractive technology instead of conventional laboratory techniques due to considerably advantages to cell culture and as it allows achieving complete protocols in a single chip of smaller dimensions.

Thus different chips were developed to allow immobilization and culture of CFBE cells. With the control of a variety of conditions like physicochemical conditions, contamination, without bubbles and with a continuous perfusion of medium it was possible to culture the cells during 72h without any microbiological contamination and with an acceptable percentage of viability.

With these developments, now it’s possible to manipulate and apply different strategies to see the response of CFBE cells and in this manner see what is the best treatment take into account the features of each patient. Have the potential to open a new era of precision medicine and personalized health in patients with CF, transforming the way each of us understand our own health and the way in which medicine is practiced.

Keywords: Cystic Fibrosis, CFBE, CFTR, Microfluidics, Cell Culture.

1. INTRODUCTION

Cystic Fibrosis is an autosomal recessive disease that affects the chromosome 7. This disease affects both sexes, with a higher prevalence in Caucasians of Northern European descents. People with CF have inherited two copies of the defective CF gene, one from each parent. On the other hand, people who have only one copy of the defective CF gene are called carriers and they don’t have the disease.

It was firstly described as "cystic fibrosis of the pancreas" to be associated with a cystic dilation of the pancreatic ducts. Only few years later it was discovered and isolated the gene responsible for CF, responsible for encoding the protein Cystic Fibrosis Transmembrane Conductance Regulator (CFTR).

This protein functions as an ion channel in the apical membrane of epithelial cells regulating the passage of chlorine across cell membranes. In CF, the most common mutation that leads to the absence of functional CFTR protein is the F508del, which affects about 85% of all CF patients. In this mutation, the aberrant CFTR protein is produced, but doesn’t suffer the process of maturation and migration to the appropriate membrane, staying retained in the endoplasmic reticulum (ER) and degraded later. Leading to a defect in the ionic transport, which result in an accumulation of a layer of viscous and thick mucus in the epithelial surface of several organs, most notable the airways.[1],[2]

There is no cure for CF, the existing therapies serve only to prevent and alleviate the symptoms and consequent complications. The treatment is based on a regular treatment routine that includes airway clearance, medication, physical activity and good nutrition. The treatments can also include lung transplantation or CFTR modulator therapies. The intention with this work is to use and enhance the potential of the CFTR modulators to treat CF. The Lumacaftor (VX-809), one of the FDA-approved CFTR-modulator facilitate the trafficking of defective CFTR proteins, allowing those proteins to reach the cell surface and transport chloride.[3]

The cells used in this work, was the CF Bronchial Epithelium cells (CFBE), a cell line generated by the transformation of bronchial epithelial cells from F508del-homozygous CF patients, which make them an excellent preclinical model of CF with high biomedical predictive value. The microscopy images below show the morphology of CFBE cells in suspension in different stages of the normal evolution. They are spherical in shape with a regular size around 10 μm, which makes them exceptional for the formation of monolayers.[4]

Figure 1 - Normal aspect of CFBE cells. The first image correspond to CFBE cells before the adhesion, in the second image the cells in a confluence state and finally in a sub-confluence state.
1.2 Microfluidics

Microfluidics is a technology characterized by the study and engineered manipulation of fluids using channels with dimensions in the scale of sub-millimeters. In the last years, the microfluidic devices have received more attention and developments, because of their potential applications in various fields as well as due to its variety of advantages and so few disadvantages associated. Can be highlighted a quickly and low cost analysis, capabilities in the control of the spatio-temporal dynamics, real-time data acquisition, reduced reagent consumption, automation and miniaturize sample preparation.

The flow of a fluid through a microfluidic channel is commonly characterized by the Reynolds number:

\[ \text{Re} = \frac{\rho v L}{\mu} \]  

(1)

Where \( \rho \) is the density of the fluid, \( v \) is the velocity inside the channel, \( L \) the diameter of the channel and \( \mu \) is the fluid viscosity. Desribes the ratio inertial to viscous forces in a fluid and depending on the quantity it is possible to distinguish two types of fluid flow: laminar \((<2000)\) and turbulent. In microfluidics systems, due to low flow velocities and small dimensions implies a laminar flow. In laminar flow regime there are no turbulences and is characterized by a smooth and constant fluid motion, where two or more streams flowing in contact not mix except by diffusion.\[5,6\]

The microfluidic devices can be fabricated using different materials including silicon, glass, polymers or a combination of these materials. However, the most used is Poly(Dimethylsiloxane) (PDMS), due to their attractive features, namely its elastomer properties, gas permeability, optical transparency, biocompatibility, low cost, the simplicity of fabrication and finally a important feature for this work is the non-toxicity to proteins and cells. The major issues with PDMS microfluidic devices are their hydrophobicity, water permeability and evaporation that can lead to reduction of cell medium and in addition insufficient cell adhesion. Nevertheless these issues can be easily overcome with the use of ECM proteins, surface treatments or with the use of humidifiers.\[7\]

In terms of fabrication, the most common technique to build systems in PDMS uses soft lithography, which encompasses a collection of fabrication approaches to rapid prototyping. The main idea behind this approach is described in figure 2.\[8\]

1.3 Cell-chips

Microfluidic technology allows the construction of devices containing components with different functionalities, which create the opportunity for biologic research, leading to the idea an entire “lab on a chip”. The features and advantages of this technology have opened the doors for an increasing number of applications as drug screening, immunoassays, chromatography, DNA sequencing, cell culture studies, single-cell manipulation and electrophoretic separations.\[9\] The advantages for cell assays over traditional cell culture are: There is a design flexibility, which can be adapted taking into account the needs of individual cell types; allows greater control of fluid flow in defined geometries; miniaturization and high throughput experimentation; increased sensitivity; automation, reduced reagent consumption and single cell handling. The more important advantage is the ability to more closely mimic a cell natural microenvironment for example by a active delivery of nutrients to cells and removal of metabolic waste products over a continuous perfusion.\[10\]

Cell culture using microfluidic devices is a relatively recent area where the establishment of techniques to maintain and grow cells in vitro is still in development. Is a field that requires an understanding of different areas, including biochemistry, engineering, physics and biology. Changes in device material, surface coating, cell number per unit surface area, physicochemical properties (pH, oxygen levels, temperature, and osmolality) and cell type may all affect the outcome of standard protocols. The combination of these factors constitutes the cell microenvironment.

A cell culture medium serves as the biochemical microenvironment of the culture composed by different compounds that help in the control of some physicochemical parameters and also to provide nutrients and growth factors, essential to replicate the natural environment of the cells. It is expected that the physicochemical properties of a culture maintain unchanged throughout an experiment. As such the pH, CO₂, temperature, osmolality and O₂ levels must be measured and maintained within the optimal levels. For most of mammalian cells, incluing CFBHE cells, the temperature should be kept around 37°C to ensure the maximum chance of survival and growth. The temperature and CO₂ levels in a microfluidic device can be monitorized from the incubator controls or alternatively with a hot-plate in the case temperature. To maintain the pH in a relatively constant range between 7.2-7.4 (the optimal physiological pH for most of mammalian cells) the media normally is buffered with sodium bicarbonate \((\text{NaHCO}_3)\), which is dependent on CO₂ concentration or with not dependent buffers like \(4-(2\text{-hydroxyethyl})\text{-}1\text{-piperazineethanesulfonic acid}\) (HEPES) or red phenol.\[11\]-\[13\]

In terms of culture, an effective cell adhesion is essential for a good development of an experiment. In this way is common to coat the cell culture material with pre-immobilized cell-attachment proteins like gelatin, fibronectin (FN), laminin, poly-L-lysine or collagen to promote the adhesion of cells. After the seeding of cells in a microfluidic culture chamber the cells are allowed to adhere to the substrate in static conditions without nutrients. The duration of this time to attachment may
vary between 2h and overnight depending on cell type, cell density or culture substrate properties.

There are many practical issues to designing and operating a microfluidic system for routine culture of adherent cells. Involves many decisions, including the choice of materials, microfluidic layout, fabrication process and sterilization technique which must be compatible with the cell type that will be used. A practical issue to perform live cells assays in microfluidics devices is the need to retain cells at defined locations in order to observe their behavior. Different cell trapping strategies can be used for this purpose such as optical, chemical, magnetic, electric, hydrodynamics or acoustic methods. The most common strategy of achieving cell trapping is through hydrodynamic cell trapping, which is no more than variations of surface topography to separate particles from the flow and immobilize them on certain regions.

2. MATERIALS AND METHODS

2.1 Harvesting of cells

The cells were cultured in T75 cell culture flask using Eagle’s minimal essential medium (EMEM) supplemented with 10% (v/v) of fetal calf serum (FBS), 1% of glutamine and 1% of antibiotic penicillin-streptomycin (pen-strep). The T75 flask was maintained at 37°C in 5% of CO2 until reaching a confluence between 80%-100%.

2.2 Microfluidic Fabrication

The first step in the manufacture of the microfluidic device was to make a design using AutoCAD software.

This design was used to generate an aluminum hard mask through soft lithography. Once deposited the Al in a clean substrate of glass, a 1.4 μm thick layer of a positive PR was spin-coated onto the substrate and then the 2D design was transferred to the PR using the Direct Write Laser machine (DWL). After the PR developing, the sample was subjected to Al etchant to remove the exposed regions.

The hard mask was then used to create the SU-8 mold. To achieve the desired height a negative SU-8 50 was spin-coated over a clean silicon wafer. The sample was pre baked and soft baked to eliminate the solvent and to create a uniform PR layer. The hard mask was placed on top of the substrate with the aluminum facing the PR and exposed to the UV-light during different times, depending on the complexity of the design. After exposure the sample was again baked soft baked to promote the reaction of photo-acid. In the developing stage the substrate was immersed in a solution of propylene glycol mono-methyl ether acetate (PGMEA) washing away the unexposed areas of SU-8. To finish, the mold was hard baked at 150°C for 40 min to harden the mold.

The preparation of PDMS (SYLGARD 184 silicon elastomer kit) started by mixing a 1:10 ratio of a curing agent and PDMS monomers. This preparation was degassed, poured over the mold and baked during 1-2h. The holes of inlets and outlets were drilled. The structures were sealed in a glass with a PDMS layer, applying a plasma treatment.

2.3 Cell Counting

To monitor the growth of cells and to count the number of viable cells an image analysis software image j was used. Since it is not always practical and faster to count by hand an it was chosen to use an automated method.[14]

2.4 Experimental Setup

The experimental setup consists of the microfluidic device mounted on the stage of an inverted microscope (Olympus CKX41) equipped with a camera for image acquisition. The images were obtained using the Olympus CKX41 companion software cellSens, recorded with a exposure time of 170 ms, gain of 4 dB in a gray scale.

A cut off of 200 nm (Whatman FP 30/0,2 CA-S) was used to filter cell medium and FN and the obtained liquid was respectively placed in a 10 mL and 1 mL syringe. In the case of medium was used a syringe of 10 ml in order to fit the amount necessary for the longevity assays. For the cases of the cell suspension, ethanol and dyes for viability assays were also used a 1 mL syringe (CODAN). The syringes were topped with a 20-ga and connected to the inlets through a BTPE 90 nm tubing (INSTECH), after that were purged to remove any air. A syringe pump (New Era Pump Systems, Inc.) was used to deliver fluid into the device. For the adhesion of cells and longevity assays an incubator and hot plate were used to maintain the right conditions for the growth of cells. All the steps until cell insertion were performed on a sterile workbench to avoid contamination.

2.5 Flow rate calibration

As mentioned before to avoid the exchange of syringes of cell medium throughout the experiments it was necessary to use a bigger syringe of 10 mL. However, the syringes pumps are only calibrated for syringes of 1 mL, and as such it was done a manual calibration for syringes of 2.5 and 10 mL to know the real flow rate of each one. The manual calibration started by the drawing of some marks every 1 cm along the entire tube BTPE 90, then for different flow rates between 0 and 2.5 μl/min were seen the time needed to reach the next mark.

And as expected the real flow rate for a syringe of 10 mL is 0,13 μl/min, which is about 10 times higher than the flow rate displayed by the syringe pump.

3. RESULTS AND DISCUSSION

3.1 Device Design

Throughout this work different designs have been used in order to increase the cell capture efficiency and address other problems such as bubbles or cross-contamination. The two
first designs used have a chamber with the same cell trapping, varying only the number of inlets. The number of inlets was increased not only to prevent cross-contamination as also to avoid the exchange of syringes during the experiments that can lead to the appearance of air bubbles in the chamber or channels. The five inlets are dedicated to different solutions as: medium, cell culture, ethanol, FN and to the therapeutic agent VX-809. The cell inlet is the one farther away from the growth chamber and the medium the closest, in order to minimize the effect of any residual cells from the inlet area. Due to the increase of speed that occurs when fluid reaches the chamber, it was concluded that the trapping mechanism is not the best for the cells used in this work, once prevents the retention of the cells appropriately.

To overcome this problem a new chamber was designed with a hydrodynamic trapping mechanism for the particular cells used in this study. The cell barrier founds in figure 4 includes a set of squares of 100 µm X 100 µm with a gap of 5 µm between each other, in order to impede the passage of the cells, since they have a ~ 10 µm diameter. So, when the cells come into contact with this barrier, they tend to stop and settle near that. The only problem of this design is that the flow tends to go only in one direction and the cells tend to settle only in the middle of chamber.

So, when all the procedures were optimized a new design with a network of channels distributed along the chamber was developed in order to increase the number of cells in all the regions of the chamber and thus facilitate the visualization of protein during the traffic assays. As expected the quantity of cells in all the region of the chamber increase but the % of cell retention was lower compared with the retention in the previous case.

After all, the third design was the design that allowed better results.

3.2 Cell Density

During the cell culture assays were used a cell line of CFBF without the mutation F508 del, responsible for the traffic protein disorder. Different issues had to be overcome in order to achieve the cell culture in optimized conditions inside the microfluidic device.

Before each experiment was considered the density of cell suspension as it played a major role in the cell-loading step, and whereas a high cell density leads to faster waste accumulation and depletion of nutrient. When required the cell density was concentrated.

3.3 Chip Sterilization

A requirement for long-term assays with cells is an effective sterilization of microfluidic device as a way to prevent cell contamination. This was performed flowing a solution of 70% ethanol into the device and then exposing the device to UV-light during some minutes before the insertion of cells.

3.4 Cell Seeding

Cells in suspension with the desired density was loaded in a syringe into the device, however a controlled flow was necessary to ensure a homogeneous distribution of the cells in the chamber. Different flow rates were tested until getting the optimal flow rate. After some experiments the initial flow rate was defined at 2 µl/min and when the cells began to approximate the chamber this flow rate was gradually reduced to 0.4 µl/min in order to control de flow of cells.

Another issue was to ensure good cell attachment during cell seeding so that cells are not washed away upon media perfusion.

3.5 Surface Coating and Cell Adhesion

The second issue to be tackled was the adhesion/attachment of cells. Such as the first factor to be tested in a 24 well plate was the adhesion of cells doing a coating with different extracellular matrix (ECM) proteins, namely FN and collagen and also without an ECM protein. The adhesion of cells in the chip was firstly tested without the use of an ECM protein where the cells never adhered. So, the aim of this assay in a well plate was to mimic the procedure that is done in chip in order to understand what influence the adhesion of cells, which includes the material used.

With the results obtained in figure 5 (A) it can be concluded that when the channel is incubated with an ECM protein, the cells adhere better than in the same case without an ECM protein. Thus, the same procedure was performed on the chip only with FN and collagen IV to see which is the best ECM protein to promote the adhesion.

Figure 3 The design that allowed better results. This design comprises 5 inlets of 200 nm width connected to a common channel that is consequently connected to a chamber with a barrier to trap the specific cells used during the work and ends in an outlet.

Figure 4 Cells trapped inside the culture chamber.
Unlike the results obtained in well plates, the adhesion of cells in the chip (figure 5 (B)) is more quickly achieved when the coating is done with FN instead of collagen. Therefore, in view of these results it was decided to do the coating only with FN in the remaining microfluidic assays.

The procedure with FN was also tested with and without incubation after the introduction of cells to see what impact had on cell adhesion. This experience led to two different results that are presented in figure 7: incubation at 37ºC, with 5% of CO$_2$ and humidity environment is a key factor in the adhesion of cells, without incubation the cells do not adhere.

To verify cell adhesion inside and outside the incubator the same temperature and buffer substance was used, the only difference was the CO$_2$ atmosphere and humidity environment. The buffer substance used is dependent on carbon dioxide (CO$_2$) and normally requires an external source of CO$_2$ in order to maintain the pH within the optimal range. So, the results outside the incubator can be explained by the imbalance between CO$_2$ and HCO$_3^-$, which consequently leads to changes in pH over time, thus not allowing cell adhesion.

**Figure 5** - Results obtained for the adhesion experiments. (A) Performed in the 24 well plate. (B) Performed in the cell chip.

**Figure 6** CFBF cell adhesion inside and outside of an incubator. With incubation, the cells adhere in less than 24h forming a monolayer of cells connected with each other. In the second case, only with temperature control the cells didn’t adhere, maintaining the spherical format.

### 3.6 Perfusion Assays

Once reached the intended adhesion, the microfluidic device was tested in terms of longevity, using a continuous perfusion of EMEM at 37ºC without conditioning to CO$_2$ and humidity ambient. The purpose of this step was the maintenance of cells with viability and without contamination in the system over a period of 3-4 days. So, it was decided to use a continuous perfusion instead of periodic perfusion to prevent accumulation of waste products that can be toxic to the cells and also to allow a constant flow of nutrients.
The results showed that the CFBE cells survived 4 days at 37°C with cell culture medium refreshed continuously.

3.7 Conditions and Parameters to Culture Maintain

Adequate culture conditions were essential to achieve good biological results. To be able to properly tailor the microenvironment it was necessary to integrate different aspects to apply the fundamentals of cell culture to microfluidics. Different properties like physicochemical properties of the cell culture medium, aseptic conditions, bubbles, shear stress and contamination were monitored and manipulated.

pH Control

As mentioned before, cells are very sensitive to changes, especially pH changes, which can lead to adverse effects on the culture, as for example loss in cell viability. Such as the medium was buffered with the most commonly buffer substance known as sodium bicarbonate to guarantee the pH control in the optimal range around 7.4. As mentioned before this buffer is dependent on CO₂, therefore during the adhesion assays the incubator provided an external CO₂ and in perfusion experiments the continuous perfusion of cell medium and the air diffusion was sufficient for equilibrium and cell survival.

Temperature

Temperature also has influence on dissolved CO₂ concentration. And as mentioned before the concentration of CO₂ has impact in the buffering system used to culture CFBE cells and consequently in pH. Thus, to help to keep the pH in the optimal range and to control the temperature, the temperature was maintained and monitored directly from the incubator controls during the cell attachment and with a hot plate during the perfusion assays.

Evaporation and O₂ levels

The choice of a PDMS for the device fabrication was an important decision because PDMS is gas permeable to O₂ so the diffusion from the air helped to keep the levels in a precise range. And apparently take into account the results of cell viability this was enough to keep the cells alive. However, in systems where the cell culture temperature is held at 37°C, the liquid within a chamber can quickly evaporate and pass through the PDMS, resulting in an increased osmolarity of the liquid. One possible way to overcome this problem is to place the microfluidic device in a closed humidity environment, but with the impossibility of this happening during the perfusion tests to circumvent the problem the cells were cultured in the device under continuous flow conditions.

Bubbles

The bubbles were an important factor to be controlled, since its appearance could lead to the rupture of cell membranes or can block channels impeding the passage of fluid in a proper manner. It was almost impossible to prevent the appearance of bubbles since they can arise from residual air or spontaneous formation at defect sites. However, the important was to remove bubbles when they appeared, preventing its expansion due to evaporation. For removed them, normally a pressure was applied to the system or the bubbles disappeared during the incubation step probably because the control of humidity by the incubator.

A problem that was encountered after several hours of perfusion was the formation of gas bubbles within chamber. In this case a pressure cannot be applied because it will lead to the removal and destruction of cells.

The only thing that varied from the assays before perfusion and the perfusion assays was the control of CO₂ and humidity. To understand why the bubbles appear different control tests with and without temperature control and another without cells were performed to see if the cells or temperature could have some impact in the appearance of bubbles. Was concluded that the temperature control combined with a carefully insertion of tubes have a significant impact on the surrounding environment, allowing the development of experiment without bubbles.

Contamination

In the first months of the work the contamination problems persisted, therefore was essential to understand what are the main sources of contamination and eliminate them to achieve the necessary conditions to proceed with the cell experiments.

The first action was the use of only one cell chip in each glass, since if various structures are sealed in the same glass and if it is badly sealed can lead to cross-contamination. During the microfluidic assays more actions were taken, in each experiment the chip was sterilized on two occasions as described in chip sterilization section. The cell medium was also supplied with antibiotics and as mentioned before during the incubation step the entrances of cell chip were kept closed. Antibiotics only was used as a last resort and only for short term applications, as the continued use leads to the
development of antibiotic resistant strains and allows contamination to persist.

So, all the steps were carried out with the utmost care and sterilization to avoid possible contamination that could compromise the experience and disabling cell growth. Thus it was possible to perform the whole experiment (6 days) without any contamination when this procedure was followed.

**Medium Residence Time and Shear Stress**

Medium residence time (MRT) and shear stress are two parameters that were considered in perfusion assays to achieve all the information necessary to guarantee cell viability.

The MRT is defined as the time needed for the complete change of medium in the culture chamber or channel. And was useful in the perfusion assays to know if the rate of medium renovation is sufficient for nutrient delivery, oxygen transport and to avoid the accumulation of waste products resulting from the metabolism of cells. The MRT of the chamber presented in figure 4 was calculated using the equation 2, obtaining the value of $MRT = 1.87s$ for a flow rate of 1µl/min, which is acceptable for the replacement of nutrients and to prevent the accumulation of waste.

$$MRT = \frac{V}{Q} = \frac{l}{v}$$

Where the $Q$ is the flow rate (in $m^3 s^{-1}$) and $(V)$ is the useful volume obtained from the width $(w)$, length $(l)$ and height $(h)$ (in $m$) of the chamber.

The shear stress obtained for the same design was $\tau = 0.325 \text{ dyn/cm}^2$ for a flow rate of 1 µl/min which is higher compared to the limit of shear stress to protect cells with a diameter of 10 µm which is around 0.3 dyn/cm², however with no visible ill effect on cells.

**3.8 Device Operation**

Prior to cell insertion, the microfluidic channels were sterilized with 70% ethanol (v/v). Then the channels were coating to promote adhesion, growth and proliferation by flowing a solution of FN and the chip was maintained in the hood with all the inlets open during 24 h to create a uniform surface chemistry for cell adhesion. After this 24h, the chip was irradiated with UV light in order to sterilize the chip one more time before the crucial step of cell insertion. EMEM supplied with antibiotics was added in order to remove bubbles and waste that could influence the flow of cells.

Cells were inserted initially with a higher flow rate and this flow rate was gradually reduced in order to control the flow of cells. All liquid injection was stopped when a sufficient amount of cells where inside the chamber. Before the incubation of chip, the tubes of all inlets were cut and plug with a closer plug metal adapter to prevent fluid losses, contamination and bubble formation.

The microfluidic device was then incubated in a humidified incubator during 24h at 37°C in a 5% CO₂ atmosphere in order to allow cell adhesion. The perfusion assays was then performed.

**3.9 Cell Viability assays**

Viability testing was performed to access the live/dead condition of cells present inside the culture chamber and thereby to do the correlation between cell behavior and cell number. This test is judged based on morphological changes or changes in membrane permeability, which are inferred by an exclusion test of certain dyes. Different techniques can be used, however it was chosen to do a trypan blue (TB) dye exclusion test and also to use a mixture of two fluorescent dyes, blue Hoechst dye and red propidium iodide (PI). In the first case the principle of this end-point test is that live cells possess intact membranes not taking up this molecule, since the TB only enters in cells with compromised membranes like non-viable cells. Leaving the non-viable cells with a blue color. Generally, a good viability result is obtained when the % of viable cells is at least 95%.

Once recognized the cell density, 10 µL of a solution composed by 50% of EMEM and 50% of TB was introduced in the chip during approximately 5 min at 1 µL/min. The chip was incubated during more 10 min at 37°C, 5% of CO₂ and with control of humidity and the cells were examined under a microscope at low magnification.
Due to the strange fact that the cells before the assay seem alive and the viability test result contradicts this, it was tried to understand why this happened. After some assays with different concentrations of TB, it was achieved the conclusion that the concentration of TB has influence on the toxicity to the cells, leading to the cell death in some cases.

In both cases the number of dead cells was counted and the formula 3 was used to calculate the percentage of viable cells.

\[
\text{% viable cells} = \left(1 - \frac{\text{Number of dead cells}}{\text{Total number of cells}}\right) \times 100
\]  

In general, the cell viability after 3 days of perfusion was more than 50%.

4. Conclusions

The microfluidics is considered a revolutionary new technology, since has the potential to impact almost every area of society. One application area that holds the promise of providing great benefits for society in the future is in the realm of biomedicine, offering the biologist new ways in which to manipulate cells and observe their behavior. A major challenge is getting accurate protocols on a chip as the ability to create an environment almost as real.

The main goals of this work were the development of a chip capable to retain the CFBE cells as well as their culture with viability during 3 days and also protein traffic assays using the therapeutic compound VX-809.

As such, during this thesis four microfluidic devices with three different trapping mechanisms were developed, one with more capabilities to retain the cells than the others, which was a very difficult task along the work. The chip that leads to the best results, it was developed for the specific cells used throughout this work. It comprised 5 inlets for different solutions, preventing this way possible cross-contamination, a chamber composed by a barrier of squares with a space of 5 \(\mu m\) between each other and a final outlet.

Different challenges were overcome during 6 days of experiment like device sterilization, cell seeding, cell
attachment, cell culture, contamination and bubbles. The first challenge was the insertion of cells inside the devices and following retention in the cell culture chamber. For that it was necessary to optimize the cell density of cell suspension and the flow rate to flowing the cells, where for this was used a method of cut-and try. Once solved this initial problem the next challenge was to promote the adhesion of cells to the surface of the chamber. It was trying to coat the cell culture material with pre-immobilized cell-adhesion proteins like FN and collagen IV, where the best results were obtain with FN. After insertion of cells and adherence of them, the device was tested in terms of longevity during 3 days with a continuous perfusion of cell medium. In this step more challenges were overcome, it was necessary to identify and control different condition at the same time to provide an environment as natural as possible to the CFBE cells. It has been shown, as control variables such as pH, temperature, amount of O₂ and CO₂ influence the growth and development of CFBE cells within the microfluidic device. The pH was controlled with a buffer system, the temperature was maintained at 37°C with a hot-plate and a continuous perfusion and PDMS permeability helped to keep the O₂ levels, CO₂ and also help to avoid evaporation problems. The assays were done with CFBE cells that adhered to microfluidic chamber previously incubated with FN. Surviving then during 3 days under a continuous perfusion without contamination, bubbles and with more than 50% of viability.

The third objective of the work, which is the protein traffic assays, was not reached because the complexity, challenges and duration of the experiments (6 days) not allowed. However, with the developments done now it is possible to do these experiments and this innovative device opens the opportunity to test non-invasively whether a given therapy is suitable to a given patient thus allowing an improvement in health care and quality of life of CF patients.

REFERENCES