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Final report

Establishment of Automated System for Organ-ON Chip development platform

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# Introduction

The development of organs on chip can have a great impact in the current state of pharmaceutical and. In order to mimic the conditions that cells undergo in-vivo with their environment, biomaterial matrices are commonly used as the culture support for cells on OOC development. However, standardization of membrane coatings and repeatability of the protocol remains a problem, causing variability between test and cases. Current coating approaches on the Lung on Chip (LOC) PLATFORM rely on manually application of the coating, which even after the correct standardization of the coating protocol it can yield undesirable and unsatisfactory results. Furthermore, the integration of a high-throughput system for processing of organs on chip platform can accelerate and improve the current state.

Another, effort that can accelerate the field of Organ on chips is the development of a high-throughput imaging

# Automated Chip Coating Experiments

# Characterization of Coating Quality

A high quality of coating is a required to ensure that the system performs as expected.

## Sirius Red Assay

Coating Parameters

## Coating stability

# Sterilization of AX6 Platform

A key parameter to ensure the safety and reliability of the in-vitro system is ensuring a controlled and germ-free environment through the sterilization of the device. Biological, chemical or physical contamination can negatively impact the health of cells or their microenvironment. Ensuring that the platform is maintained with a high standard of cleanliness and preventing the growth of bacteria or other living microorganisms is critical for the reproducibility of the platform. Assembling and pre-processing the chip under a Biological Safety Cabinet or thoroughly disinfecting the components are commonly used practices to minimize the contamination risk. However, to ensure the complete elimination and inactivation of all biological agents a process of sterilization is often required. Sterilization is a harsh process that can potentially affect the surface properties and characteristics of the materials of the in-vitro device. The properties affected range from appearance properties such as colour or haze to mechanical properties relating to strength or flexibility. In some cases, sterilization chemicals can be absorbed by the plastic materials and may be slow to permeate out. At the same time, sterilization methods are varied and are catered for different material types. In a complex system such as the AX6 with composite materials and delicate coated surfaces, selecting the optimal sterilization method is challenging.

## Objective

The main purpose of this experiment was to select an optimal sterilization scheme for the complete disinfection of the AX6 platform, while maintaining the properties and characteristics of the in-vitro system (cell adhesion, stiffness, surface properties, …) unchanged. Furthermore, this experiment compares the direct effects of the studied sterilization methods on the coating solutions of Alveolix AG, the AX Treat and AX Sense.

## Materials and Methodology

### Coating of AX6 chip

The AX6 chips were assembled and sterilized using UV radiation as shown in the L-o-C production protocol (SOP 03.01.00). The coating solutions were applied both on the apical and basal side of the membrane, following the Chip Coating protocol (SOP 02.12.02). Three different conditions were tested to quantify the effects of the sterilization method on the Alveolix coating solutions. In condition 1, the AX6 was assembled and immediately sterilized. After sterilization the chip was apply the AX treat and AX Sense following the standard protocol. In Condition 2 the AX treat was applied to the PDMS membrane and subsequently sterilized. Finally, the AX Sense was apply following the standard protocol. In condition 3 the AX6 was fully coated both with AX Treat and AX Sense and the chip was then sterilized.

### Sterilization

#### Gamma Sterilization

Gamma sterilization uses a radioactive source, typically Cobalt-60 (60Co), which emits high energy gamma rays. Ionizing radiation can modify physical, chemical, and biological properties of materials. Currently, principal industrial applications of radiation are for sterilization of healthcare products (including pharmaceuticals), irradiation of food and materials modification (such as polymer cross-linking).

Gamma sterilization is a “cold” sterilization technique, where temperature is not a key parameter. Temperature may increase slightly in the product due to ionization, but gamma sterilization may be effective at ambient, refrigerated, or even frozen conditions. The key parameter is the dose received by the product. The dose is dependent on the presentation to the source and the time exposed to the gamma ray source.

#### Ethylene Oxide

Ethylene oxide (EO) is a medium temperature sterilization method (40–55 °C), and microbiocidal lethality is achieved by chemical reaction (alkylation) of proteins and DNA within bacteria. The alkylation process requires moisture to act as a catalyst to open the epoxy bond, so preconditioning and/or conditioning are an essential part of the ethylene oxide sterilization process

EO is a colourless, odourless, volatile, and toxic gas that is carcinogenic and highly explosive from 2.7 percent in air up to 100 percent. Extreme caution must be taken to use this highly reactive molecule.

EO sterilization is typically a three-step process, starting with preconditioning in a room or cell, then sterilization in a chamber, and finally, desorption of gas in heated aeration in a room or cell. Preconditioning is used to heat up and humidify the products in order to present them in homogeneous favourable conditions for efficient sterilization.

### Storage Conditions

All chips were stored at 4 degrees subsequently after the coating application. The sterilization process was performed by an external company, which meant that during the processing time of 2 weeks the chips storage temperature was not controlled. Furthermore, a control sample was also stored for the duration of the experiment at room temperature, to test the degradation of the coating.

### Cell Seeding

After the final processing steps, the AX6 chips were seeded with endothelial cells to examine the impact of the sterilization process on the integrity of the coatings. RFP positive VeraVec Endothelial cells were seeded apically with a cell density of 6000 cells per well. The cells were maintained on the AX6 membrane for a period of up to 7 days.

### Cell Imaging

The cells were image periodically using bright field and

## Results

## Conclusion

# LOC Autofluorescence Assay