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Tumor-on-a-chip: new strategies of *in-vitro* cancer cell culture.

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1. INTRODUCTION

I had the opportunity to intern at the TargetsLab research center during the summer of 2021. This University of Girona-affiliated group has been researching Triple Negative Breast Cancer (TNBC) and lung cancer for years. I was entrusted with supporting former doctoral-level researchers in the construction of specialized scaffolds that could be used for the growth of the MDA-MB-231 TNBC cell line and would presumably facilitate and enhance the generation of tumoroids by imitating the human extracellular matrix. These forms are seen as a promising tool for cost-effective investigations on new cancer treatments that will be used in oncology's precision medicine.

Despite what I knew about fundamental cell culture methods, I was shocked by how culture equipment hadn't been upgraded to meet the biomedical field's expanding complexity. During my internship, I learned that imitating the human body's circumstances and dynamics as closely as possible was the key to growing organ-like cell cultures. Many other factors must be applied to the culture to simulate the human internal environment, like the flow and pressure of the liquids in contact with the cells or the oxygen and carbon dioxide concentration in the culture vessel.

I began studying dynamic culture methods and learned about "Organ-on-a-Chip," a micro-scale system meant to mimic the human body environment to generate human tissue models for disease modelling and pharmaceutical testing. Despite its many advantages, a brief market analysis found that relatively few companies developed dynamic culture equipment, which began at around $20,000

Since I started my degree, I've wanted to design a more accessible, sustainable, and decentralised science. After the investigation, I wondered how universities or research groups with fewer resources than pharmaceutical giants or huge educational institutions could use these new approaches and technology. Thus, the idea arose to create a system with the same objectives and features as existing dynamic culture equipment, but at a substantially lower cost and whose production could be handled by the same groups in a simple and cost-effective manner. The following is a proposal for an affordable device to produce dynamic 3D cell cultures. AutoCAD will be utilised for design, and fused filament fabrication (FFF) will be used for production.

2. RESEARCH QUESTION

Is it possible to fabricate a microfluidic system for the in vitro cell culture of tumor-like structures inspired by the technological concept of organ-on-a-chip and specifically tumor-on-a-chip using fused filament fabrication as the primary manufacturing method and inexpensive electronic components?

3. HYPOTHESIS

Manufacture of an in-vitro cell culture device incorporating a microfluidics system using cost-effective components and fused filament fabrication as the primary technology in the manufacturing process is possible by adapting the conceptualization of the models to the limitations of machinery and material and applying the appropriate post-processing.

4. OBJECTIVES

Open interest in 3D design, the use of biocompatible polymers, and fused filament fabrication in the conceptualization and manufacture of devices for cell culture using microfluidics, providing a

system that overcomes the major restrictions previously connected with this technique of production in terms of the manufacture of organs-on-a-chip and tumors-on-a-chip.

5. DESIGN

The device's design has to suit biomedical standards, such as resembling extracellular structures, as well as manufacturing and material constraints. Due to the fact that FFF printers employ polymers, a trade off had to be struck between miniaturisation, material strength, and intricacy in tiny components. Other variables included the size of the incubator, the modularity of the devices, and the thickness of surfaces printed using transparent filament. Multiple components make up the microfluidics system. For the culture chips, state-of-the-art models were reinterpreted to be applied to the FFF with the maximum miniaturisation factor possible. On one hand, we have a chip designed to withstand photometry, microscope, and other simple tests. On the other hand, a metastatic tumor-on-a-chip chip tries to mimic its microstructure and cell distribution. The tank for the culture medium was designed to fit the pump while preventing medium or blood loss. The rest of the parts fit these two.

5.1. CULTURE CHIPS

The design of the chips had three significant challenges: connecting the pump, printing the interior channels with enough detail, and making the bottom layer of the culture area transparent so the crop could be analysed. Adapting chip size to current restrictions resulted in a culture device smaller than a typical culture plate but larger than organs-on-a-chip. Needles wouldn't provide a tight connection with the polymer device (we'd have leaks), and perfusing medium through them wouldn't be efficient due to the chip's size. Therefore, the connectors were printed on the same piece, allowing a medium transport tube to be linked without further accessories. These connectors' external and internal dimensions were adjusted to fit inside a compact tube while remaining strong.

Using AutoCAD's "Difference" approach, chip covers were developed to seal the crop, maintain device pressure, and prevent leaks.

5.2. TANK

Originally, the culture medium tank had a single pump chamber. During the design phase, challenges developed, such as modifying the tank size to allow different configurations or experiments, sealing the tank to control oxygen and carbon dioxide concentrations, and implementing a filtration system before shutting the microfluidic circuit cycle. The final design was divided into two variants with the same conceptual design, two chambers, but distinct volumes, allowing this peripheral to be easily changed to the expanding number of chips.

5.3. CHANNELS MODULE

This device was meant to retain injection tubing through incorporated holes and stabilise the chip and its connections. Later, it was realised that the two concepts might be merged, allowing for uniform distribution and collection of the pumped material. Two 8 mm internal channels branch into 5 mm channels. It comprises methods for growing a single chip and up to three chips to replicate a body-on-a-chip, complex combinations, or a bioreactor. With a later-described adaptor, the modular device's connections can be united without several tanks. Each setup has two chip-based models.

5.3.1. ADAPTERS

Adapters fit infusion tubes and increase our design's capabilities. For this project, we have used two: the adapter that links the 5 mm channels of the channels module to the tubes that goes to the chips (its use is necessary for optimal device operation) and the adapter that may be inserted between the tank and the channel module so more than one of these can be utilised (its use is optional).

5.4. FILTERS

The two-piece filter manages the connection between the tank's two portions, retaining any tumour spheroids for further study. It houses filtering fibre scaffolds. It fits into the slots in the secondary chamber's partition wall for optimal installation.

5.5. BATTERY

Because the device will be in an incubator, a battery enclosure was made. This shell allows airflow between the culture basin and battery and encloses the battery to prevent humidity. A hole has been made at the back so that moisture can be easily detected.

6. MATERIALS

Biocompatible materials must be utilised because they will interact organic substances. PDMS, glass, and thermoplastic polymers are the most viable possibilities for manufacturing organ-on-a-chip devices according to the state of the art. Only the third material satisfies criteria due to production. PLA, ABS, PET, PETG, Nylon, TPU, polycarbonate, PCTG, PETT, carbon fibre, and PP are commonly used in FFF printers (polypropylene). The polymer's ease of printing was a critical factor in its selection because the models needed to be printable by non-experts. PLA is biocompatible, biodegradable, and the easiest material to print with an FFF printer. ABS is difficult to print, sensitive to temperature changes, and quickly warps. PET releases hazardous vapours when printed. PETG and PCTG are biocompatible, non-toxic, and easy to print. Nylon absorbs a lot of moisture, is sensitive to temperature changes, and printing is difficult, so it was abandoned. TPU is a flexible, biocompatible material that is easier to print than PP, hence it was used in some parts. Carbon fibre filaments are not biocompatible, difficult to print (requiring an aluminium nozzle and high printing temperatures), and expensive. PETT is less translucent than PETG, hence it wasn't used. PMMA is biocompatible, simple to manufacture, and transparent. Silicone is biocompatible, flexible, and transparent. Chloroform is a biological lab solvent.

6.1. PLA / PETG / PCTG

Most models are printed with PLA, PETG, and PCTG. Several variables to consider:

- Transparent filament must be used to print transparent chips. PETG has proven to be more durable and perfect for producing this component, as we need to apply pressure to the connecting pipes when installing the medium distribution tubes; however, a working assembly has been obtained using PLA.
- Consider the 5 mm channel adaptor while printing the channel module. If we need to remove the adapters because they are broken or to seal the entry for a specific application, we will need to apply heat to remove the portion without damaging the channel module. Therefore, PETG, which has a greater melting point than PLA, is preferred for the channels module. If the adapters are printed in this other polymer, the channel module won't melt.

6.2. TPU

As PLA/PETG printing was too difficult for straightforward mounting and installation, TPU was used for filter printing. This substance has a higher liquid absorption than other polymers, hence printed items will degrade faster than PLA/PETG parts.

6.3. PMMA

Transparent chip covers are made of PMMA. Despite positive results, a cutter was used to cut the polymer plates for these testing due to a shortage of laser cutter. The resulting parts didn't fit completely in the crop area hole, and the setup didn't resist the same stresses as the printed lid design. PMMA will be employed to build up cell cultures for optimal results and functioning.

6.4. SEALING

General treatment of the parts with chloroform has proven to be the most effective way to improve layer adhesion, making the models more durable and less brittle. It is also believed that the texture created by the manufacturing method on the infusion channel walls can be improved to prevent cell adhesion in undesirable areas .

6.5. SILICONE

Silicone tubes are used for medium transportation between the tank and the channels module and the channels module and the chips. Food/medical grade silicone was obtained from aquaponics circuit and respirator replacement parts dealers.

7. CONCLUSIONS

This project proposes a framework for creating microfluidic devices for cell culture, inspired by organ-on-a-chip and tumor-on-a-chip and manufactured largely with fused filament fabrication. This unique technology promises to simplify and minimise manufacturing costs, make it easier to adapt culture chips to the current application, be watertight, have an adjustable flow, be wire-free, and serve as a platform for future bio-inspired designs. To make the models work despite the required miniaturisation factor, the sizes of the various components and sections were adjusted using stress testing. To seal the parts and ensure that the system was impermeable, chloroform was used; it was also used to promote layer adhesion (and hence strength) and to smooth the pieces' texture to reduce cell adhesion. PLA, PETG, and PCTG are utilised to manufacture the majority of the models, TPU is used for the filtering system. PMMA caps for the chips must be made before culture tests to precisely monitor cells. These will be cut with a laser cutter and joined with chloroform. Cell culture and viability testing will determine the device's efficacy and future potential.

Three main improvements are recommended after assessing the current equipment. First, build a control circuit using an Arduino board as the main module and pH, oxygen, carbon dioxide, temperature, pressure, humidity sensors and thermistor, among others. Without a microscope or being present at the lab, we could monitor the culture. The thermistor lets us control the medium's temperature without an incubator. We could add another pump for more intricate O2 gradients. The second option is to incorporate sensor data into a machine learning-based prediction model to automate cell culture management and predict when it will no longer be viable. The third improvement would be to use a piece of PMMA as the chip bottom to maximise transparency and simplify microscopic examination. As a last extension, a chamber for administering chloroform to printed components could be built.

8. RESULTS

Figure 1. Final results. Images depict: (1,2) Assembly of the circuit in bioreactor mode; (3) individual culture channels module; (4) individual tank, lid, pump, and battery; and (5) assembly of the circuit in bioreactor mode utilising two channels modules and the extended tank.