Design of protective vessel and irrigation system for an organ-on-chip device

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Abstract: New devices have been in development in the biomedical engineering field which allow to mimic several physiological processes at once or individually. The present work introduces a design and computational simulation of the nutrient irrigation system, as well as the rapid prototyping of the protective vessel of an organ-on-chip (OOC) device as a way to manipulate and transport the system easily as a whole while maintaining the proper irrigation conditions in the media. The device was generated with the computer-aided design (CAD) software, SolidWorks® and the irrigation of the system was performed with the aid of SolidWorks Flow Simulation® module. The components of the presented OOC system were manufactured by 3D printing and by using the stereolithography technique. The results showed the flow velocity fields with values in the rage of 0.1830 m/s in the zone were the OOC is located, which indicates would allow a proper irrigation of nutrients to the cells in the chip. The proposed design of the OOC device as a whole, demonstrated to be an adequate storage and handling system for the OOC, in addition of providing a continuous irrigation of the medium.

Keywords: organ-on-chip; OOC; protective vessel; computer-aided design; CAD; irrigation system; 3D printing; flow simulation; biomedical device.

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1 Introduction

For many years now, *in vitro* technologies have been highly helpful by allowing researchers to understand the human physiology and its pathologies, as well as the development of biochemical and genetic analyses as well as the interaction between great varieties of tissues, which are of great importance to understand the intercellular signal transfers and tissue-tissue interactions (Ingber, 2016).

The existing models for the investigation of human physiology and medical industry show a large number of variables number that limit the medical advances and their studies. Two-dimensional *in vitro* models have many limitations, such as: the inability to generate a three-dimensional structure, lack of extracellular cell-matrix and cell-cell interactions, in addition to exhibiting low drugs resistance compared to three-dimensional models (Caballero et al., 2017; Kimura et al., 2018; Erge et al., 2015). On the other hand, *in vivo* models have been considered one of the most 'reliable' models in preclinical trials, they present difficulties such as high cost, slow results and a predictability deficit with human case studies, as well as the use of continuous animal species.

Nowadays, organ-on-chip (OOC) technology is a novel alternative for *in vitro* experimentation, which imitates the physiological conditions and organs and tissue functions. It offers advantages compared to the traditional *in vitro* and *in vivo* models. The OOC allows to determine the liquids flow control, as well as the channels geometry, which gives the chance to generate a continuous laminar like the *in vivo* capillary tissue that generates physical and chemical gradients (Halldorsson et al., 2015; Probst et al., 2018; Inamdar and Borenstein, 2011). In addition, the OOCs experimentation decreases the use of reagents and samples, reaction times are more efficient, are transportable, low production cost and allow to understand about drug dynamics mechanisms (Duval et al., 2017). The OOC Manufacture and their automatisation allow the high-performance cell culture implementation with low labour and reagent costs (Huang et al., 2010). To ensure

the adequate culture media irrigation in the OOC channels, irrigation systems have been developed that maintain a laminar flow.

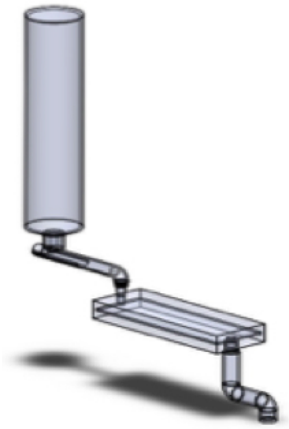
This work presents the design and manufacture of a protective vessel, as well as the flow simulation within the irrigation canals for an OOC all-together. The system as a whole, has been developed for a further experimental study of morphogenesis of healthy or pathological tissues and their nutrient distribution. The proposed OOC system presents an innovating *in vitro maintenance* system, that would enhance the storage and manipulation of an individual OOC, compared to conventional systems used for experimentation in laboratories for medical industry, cell differentiation, tissue assembly and cell maturation, therefore, it would be possible to achieve the viability of tissue samples for pharmacological tests, clinical trials and scientific research.

2 Materials and methods

2.1 Design of irrigation system for the OOC

The components of the proposed OOC system (protective vessel and irrigation of an OOC) were designed with the aid of a computer-aided design (CAD) software (Solidworks®). Firstly, the nutrient reservoir was designed to simulate the intracellular blood circuit like a cylindrical geometry (standard syringe-like shape) with a total volumetric capacity of 35 ml, nutrients are delivered as a gravity driven irrigation system. The irrigation system was placed 12 mm above the OOC's base level in order to obtain a pressure gradient to allow a laminar and constant flow of nutrients throughout the OOC channels. The dimensions of the OOC to be located in the vessel are referenced to a standard microscope slide, whose dimensions are of 76 × 26 × 8 mm. Connectors were designed to smooth the flow between the reservoir and the inlet of the OOC, and from the outlet of the OOC to the final tubing discharge. The connectors were designed o be easily assembled between the components, and at the same time, to avoid any possibility of pollution of the cells in the OOC.

Figure 1 OOC's irrigation system, isometric view



To properly manufacture the components of the irrigation system, the capabilities of the 3D printer were considered while the design process was taking place.

2.2 CFD analysis initial conditions and boundary conditions

Once the geometrical design was finished, the Solidworks® Flow Simulation module was started, and the model of the proposed irrigation system was prepared with the simulation parameters as shown in Table 1. The OOC and its irrigation system were simulated taking as reference the hollow slide dimensions and being able to show the fluid behaviour in the section.

Table 1 Values and simulation conditions

Inlet conditions	Pressure: 101,325 Pa (1 atm)
Outlet conditions	Flow rate: $1.4960e-05\ \frac{m^3}{s}$
Fluid properties	Fluid temperature: 310.15 K (37°C)
	Density $\rho = 1000\ \frac{kg}{m^3}$

Table 2 Printing operation basic configuration

Basic configuration					
Grade		*Filling*		*Speed and temperature*	
Layer height (mm)	0.2	Lower/upper thickness	1.2	Print speed (mm/s)	15
Layer thickness (mm)	1.2	Filling density (%)	25	Printing temperature (°C)	210
Enabling retraction	☑			Plate temperature (°C)	65
Support		*Filament*		*Machine*	
Type of support	N/A	Diameter (mm)	1.75	Nozzle size (mm)	0.4
Platform adhesion type	N/A	Flow (%)	100		

Table 3 Advanced settings for printing operation

Advanced configuration							
Retraction		*Grade*		*Velocity*		*Refrigeration*	
Velocity (mm/s)	30	Thickness of the initial layer (mm)	0.2	Travel speed (mm/s)	30	Minimum layer time (sec)	3
Distance (mm)	8	Line width of the initial layer (%)	100	Lower layer speed (mm/s)	30	Enable fan	N/A
		Cut the bottom of the object (mm)	0.0	Filling speed (mm/s)	30		
		Double extrusion overlay (mm)	0.15	Lower/upper speed (mm/s)	30		
		Outer layer speed (mm/s)	30				
		Inner layer speed (mm/s)	30				

2.3 Manufacture of the proposed OOC system (protective vessel and irrigation system)

Once the design of the protective vessel and irrigation system were finished, manufacture by 3D printing and stereolithography was done. To achieve this, the Ultimaker Cura V15.04.6® software from Ultimaker® was used to print the PLA-based parts with the 4max Anycubic printer, while resin-based parts were manufactured with the Anycubic Photon® printer and its slicer software, Photon Slicer V1.3.6. Basic information and operational data for 3D printer setup is shown in Tables 2 and 3, whereas Table 4 shows the parameters used for resin printing.

Table 4 Printer configuration for resin printing operation

Configuration for resin printing	
Layer thickness	0.05 (mm)
Normal exposure time	10 (s)
Out of time	60 (s)
Lower layer	8 (s)

3 Results and discussion

3.1 CFD flow simulation

A lateral view of the fluid irrigation system is shown in Figure 2. Three important sections, S1 through S3, were taken in consideration in which the change of the fluid's velocity is much more visible. The narrow transition section of the reservoir identified as S1, has the highest velocity values calculated in the simulation, with a maximum speed of $7.304\ \frac{\text{m}}{\text{s}}$, generated by the reduction of the passage's area where the fluid travels through. Subsequently, the fluid travels from section S1 through the connector to section S2 to the OOC inlet. Due to its truncated conical geometry, this section shows an increase in velocity that goes from $0.304\ \frac{\text{m}}{\text{s}}$ to $4.258\ \frac{\text{m}}{\text{s}}$. The flow stabilises while flowing through the OOC while it section S3, where the values of the fluid's velocity slightly decreases compared to sections S1 and S2, with values from $0.913\ \frac{\text{m}}{\text{s}}$ to $1.217\ \frac{\text{m}}{\text{s}}$.

3.2 Verification of filling times and fluid flow velocity

The Bernoulli theorem and Torricelli's theorem were used to calculate irrigation system filling by dropping. Bernoulli's principle was used to analyse pressure, flow rate and velocity, calculations were performed by sections from point 1 through point 8 as shown in Figure 3 (irrigation system lateral view).

Figure 2 Flow simulation, velocity field (see online version for colours)

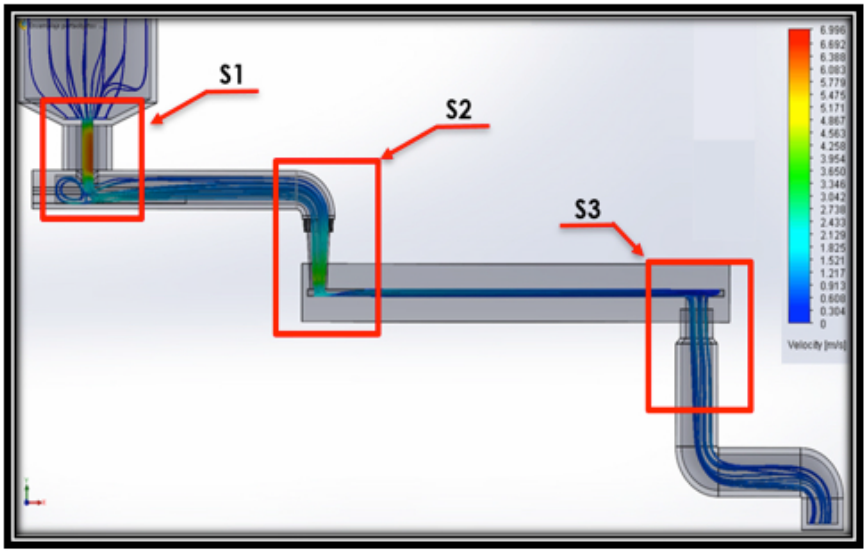


Figure 3 Irrigation system lateral view (mm units) (see online version for colours)

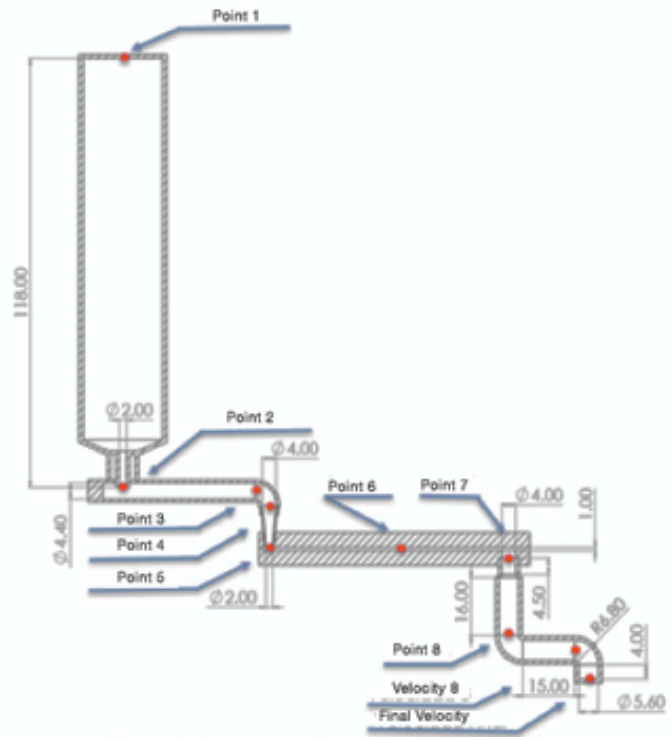
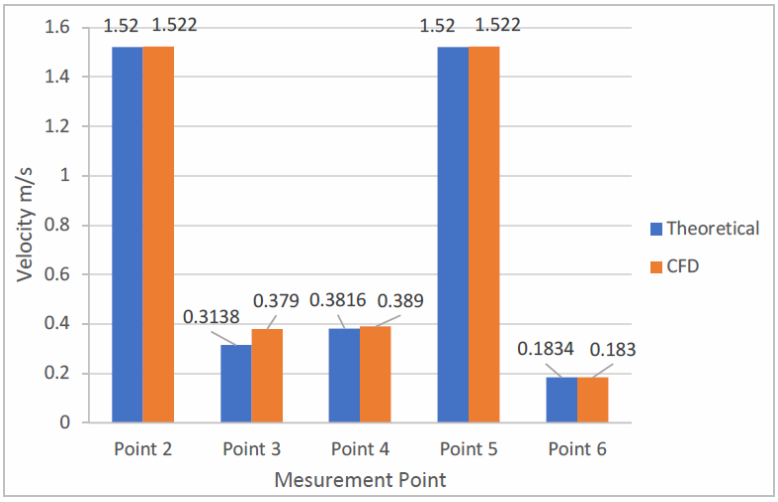


Figure 4 shows the irrigation system velocities by sections of the analysed flow rates theoretical and computational

Figure 4 Velocities comparison table, theoretical calculations vs. fluid simulation (see online version for colours)

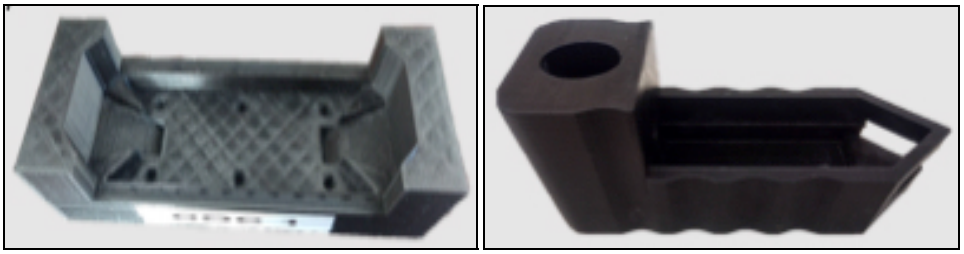


3.3 Manufacturing of the OOC system (protective vessel and irrigation system)

The proper manufacturing of the diverse components lied on the 3D printer's precision and specifications of the stereolithography equipment. As mentioned earlier, a dimensional tolerance of 0.4 mm was contemplated for a tight and smooth mechanical assembly.

The support of the OOC is the main structure where the rest of the components lie together; moreover, the structure's purpose is not only to provide a means of attachment, but to provide extra protection against incidents as well as to guarantee the welfare, protection of the OOC and integrity of the cells incorporated within. To achieve the purpose, the need to integrate multiple components forced to redesign the simple and compact OOC carrying vessel shown in Figure 5(a), to be a more robust and volumetric structure as shown in Figure 5(b). The final prototype turned out to have larger dimensions, but the addition of the grooved external walls provided a better grip from the user to handle the OOC's protective vessel [see Figure 5(b)].

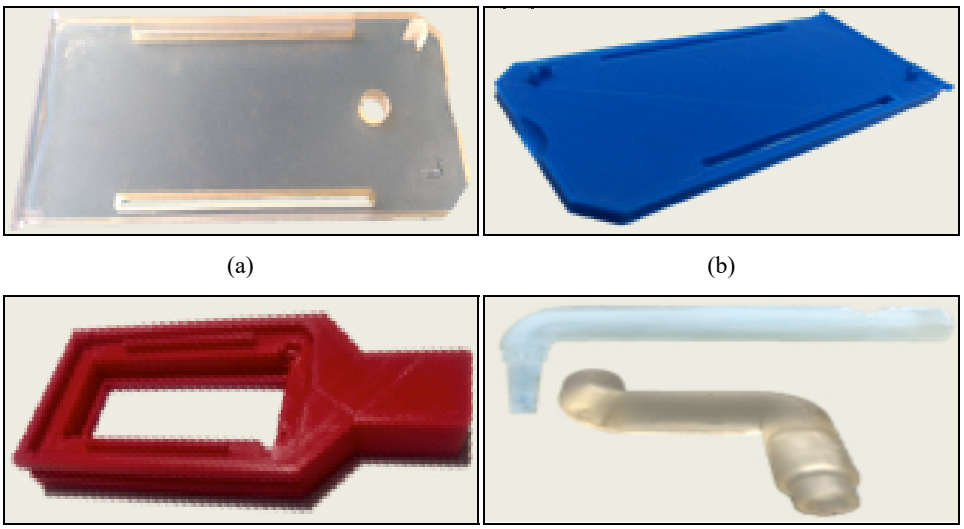
Figure 5 Prototypes for the OOC's protective carrying vessel, (a) first version (b) final version of OOC's protective and carrying vessel



(a) (b)

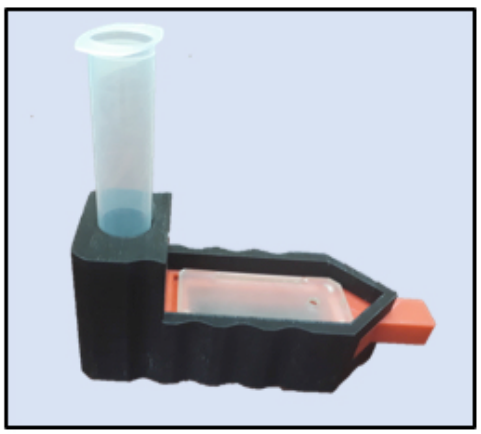
The second component of the OOC system that provides protection to the OOC is a cartridge-like case which reduces the user's direct contact, risk reduction of cross-linked contamination as well as protection against accidental internal or external damage of the OOC. The protecting cartridge-like case refers to the structure where the OOC is housed, the diverse components of the cartridge-like case are shown in Figures 6(a), 6(b) and 6(c), which were manufactured with PLA.

Figure 6 Protective case components, (a) upper lid made with photoresin (b) lower lid made with PLA (c) cartridge-like case made with PLA, other components (d) irrigation system connectors (see online version for colours)



(a) (b)

Figure 7 Final assembly of the OOC system (protective vessel and irrigation system) (see online version for colours)



Moreover, two connectors were manufactured [Figure 6(d)]: a connector for the irrigation system between the reservoir of nutrients and the OOC, and a connector functioning as outlet pipe to discharge the cell's waste from the OOC. Figure 7 shows the assembly of

the final OOC system (protective vessel and irrigation system) manufactured by rapid prototyping and stereolithography.

4 Conclusions

The present work had the objective of designing and manufacturing an OOC system, comprising of a nutrients irrigation system and protective carrying vessel for an OOC. The vessel physical was designed to bring protection against accidents and external pollutants to the OOC, as well as providing easy transportation of the OOC system as a whole structure. The resulting characteristics of the final design are highly valuable for the user while handling and performing experimental work, which consists in other activities, of constant manoeuvring of equipment and transportation in the laboratory installations. Moreover, by using the various techniques and materials for additive manufacturing and stereolithography, it was convenient to fulfil the need of having translucent pieces for easy visualisation of the OOC and at the same time to provide a proper protection. The use of PLA to manufacture other components was much more convenient when transparency is not indispensable.

Regarding the fluid flow simulation through the irrigation system showed flow velocities with close values to those calculated analytically, thus the OOC possesses low velocity values which allow a laminar fluid flow and evenly distributed along the OOC, which is very desirable for the proper growth and alignment of the crop that could be studied in the OOC. On the other hand, the increase in the velocity values at the connectors does not represent a risk for the crop in the OOC, since the flow manages to stabilise along its path.

It is concluded therefore, that the OOC system (protective vessel and irrigation system) will provide a constant laminar flow for OOC *in vivo* experiments, and the appropriate protection to avoid pollution and damage to the OOC.

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