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# Design and development of a dual-flow bioreactor mimicking intestinal peristalsis and permeability in epithelial tissue barriers for drug transport assessment

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**Key words:** Bioreactor, Flow Simulation, Permeability, 3D Print, Small Intestine

**Abstract:** We present a bioreactor system which combines a semi permeable membrane that simulates the osmotic nutrients interchange in the small intestine circulation and rhythmic peristaltic movement. This custom-designed presents a semipermeable membrane bioreactor, with peristaltic flow and compression variation that allows adjustment of luminal flow rate. In addition, this system is also capable of achieving the drug distribution in the small intestine model from the apical compartment to the basal compartment by the semipermeable channel. This dynamic bioreactor can mimic the human small intestine with increased accuracy to overcome many of the limitations and accuracy with the previously described in vitro small intestinal models, providing a more representative model of the small intestine.

## Introduction

The major site for digestion, drug and nutrient absorption is the small intestine, the interaction with commensal microbiome, and development of mucosal immunity because is the primary site for many diseases, such as bacterial, viral and parasitic infections and inflammatory bowel disease (Turner, 2009).

Bioreactor models are replicates of the structure and biomechanical function of biological tissues that allow mimicking and predicting physiological responses to a variety of stimuli (Peterson and Artis, 2014). Although traditional static models and conventional substrates for cell culture, like plastic flasks, dishes, plates, wells or cover slips, offer ease of use and have low cost, they are not fully representative of the biological responses, as they do not mimic key aspects of the biomechanical environment. There is currently great interest in developing biomimetic *in vitro* models that better simulate the complexity of the biological peristalsis and permeability as alternatives to *in vivo* experiments. In particular, significant efforts are focused to mimic the dynamics of the cellular environment (Burnham and Carroll, 2013; Mak *et al.,* 2014).

The drug-absorption models are currently in monolayer cell culture with intestinal epithelial cells or in animal models. The monolayer cell culture models provide limited utility due to the nature of the architecture and substrate utilized. Animal models allow the development of novel therapies for intestinal diseases, but they are costly and often do not reflect the corresponding human disease, and many new drugs that have successfully passed animal trials fail during the human clinical trial phase (Marx, 2015; Barbara *et al.,* 2016; Gayer and Basson, 2009). The bioreactors offer an alternative to animal models for quantitative studies and tests of new drugs and their permeation in a verifiable, cost-efficient manner, through a biologically driven approach *in vitro* (Rigottier-Gois, 2013).

An *in vitro* model should model the *in vivo* situations, emulating the biomechanical dynamic microenvironment. In *in vitro* tests, physiological barrier models represent a key access point for transport and absorption of substances such as food, nanoparticles, or drugs (Larregieu and Benet, 2013). The chemical substances introduced into the blood circulation are transported across biological membranes such as skin, gastrointestinal, or bronchial epithelia, which can modify their kinetic and metabolic profiles. Moreover, these compounds can modify cell function, increasing, for example, the permeability of the barrier. In all these models, the method for culturing cells is almost always the same (Hilgers *et al.*, 1990).

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The capacity of biological membranes to restrict the passage of exogenous molecules is the basis of the defense mechanism of the human body, which tries to keep out foreign materials (Kim *et al.,* 2007). This feature, indeed, limits the movement of solutes and pathogens in order to prevent them from reaching the systemic circulation. On the other hand, overcoming these barriers is one of the major challenges in the field of drug delivery, where the aim is actually to enhance the transport of drugs in order to have therapeutic effects while minimizing the applied dose (Kim and Ingber, 2013). The study of nanoparticle (NP) translocation across biological barriers is of interest to both toxicology and drug delivery research, because these new materials are promising carriers of pharmaceuticals (Kim and Ingber, 2013). Unfortunately, the very same properties that enable their passage across biological barriers such as lung, intestine and skin, and into downstream cells and organs, can also lead to toxic effects (Cei *et al.,* 2017).

Under physiological conditions, the intestines experience rhythmic peristaltic motions characterized by wave-like muscle contractions traveling along the bowel wall as well as intestinal fluid flow regulated by constrictions (Maurer *et al.,* 2017). To mimic the dynamics of the original intestinal microenvironment there have been used bioreactors that integrate perfusion-based systems into static cultures. The recent bioreactors were designed and fabricated for small intestinal tissue-engineering with tubular polymer scaffolds. Nowadays, human-gut-on-a-chip models use fluid flow as well as cyclic mechanical strain to intestinal epithelial cells grown on a membrane at levels similar to those experienced *in vivo* in the living intestine (Lee *et al.,* 2016).

The design and construction of a bioreactor must highlight the hydrodynamic features that should meet the flow conditions of the tissue of interest. Computational flow dynamics (CFD) is an important tool used in biomechanical models that helps to define the system hydrodynamics that mimic the *in vivo* biological conditions. Bioreactor design is based on the scaffold hydrodynamic insight analysis and some of the controllable variables, such as morphology (size of the pore, materials, etc.) and initial conditions (flow rate, flow volume, etc.). These variables are used to solve Navier-Stokes equations, which help us to define the bioreactor velocity fields and to approach both the flow and the bioreactor performance (Ramirez-Fernandez *et al.,* 2016).

More recently, a biomechanical bioreactor mimicking the cyclic contraction and relaxation of the intestine tissue using an electro-responsive elastomeric membrane was designed for the *in vitro* remodeling of human intestine, but the native intestinal epithelium was a monolayer lining the 3D tubular tissue architecture and the apical surface of the epithelium (Chen *et al.,* 2015).

In this work, we present a bioreactor system which combines a semipermeable membrane that simulates the osmotic nutrient interchange in the intestine circulation and rhythmic peristaltic movement. This custom design presents a semipermeable membrane bioreactor with peristaltic flow and compression variation that allow adjustment of luminal flow rate. In addition, this system is also capable of achieving the drug distribution in the small intestine model from the apical compartment to the basal compartment by the semipermeable channel. This dynamic bioreactor can mimic the human small intestine with increased accuracy to overcome many of the limitations and accuracy of the previously described *in vitro* small intestinal models, providing a more representative model of the small intestine.

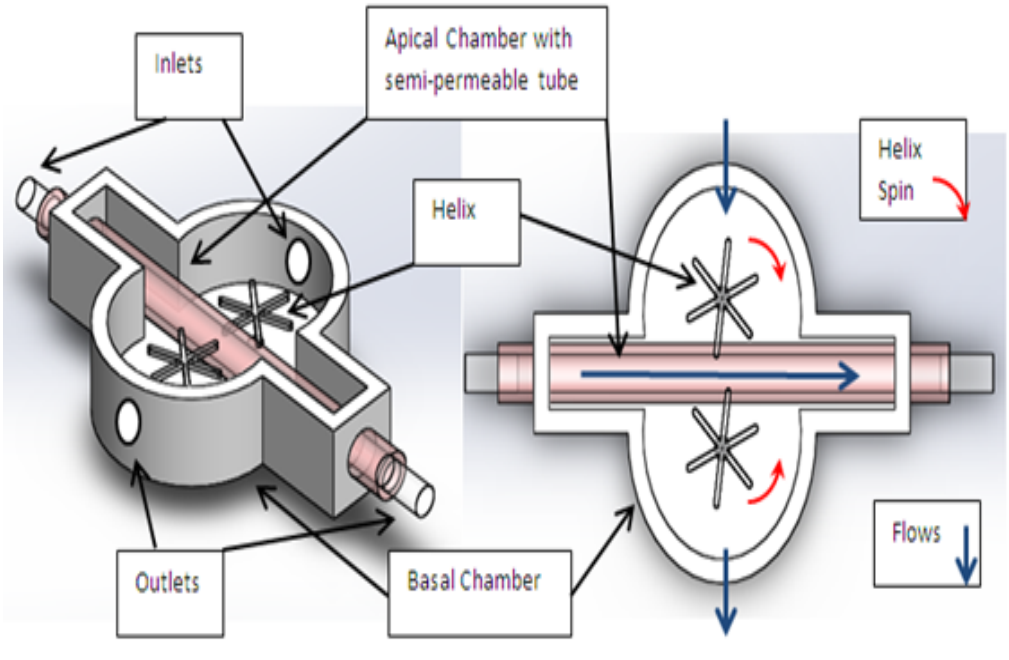


**FIGURE 1.** Bioreactor design. The flow goes through the apical chamber (blue arrows), where the cell culture is placed, the dialysis tube, and the helix rotation (red arrows) squish it; then the media pass to the basal chamber by the dialysis membrane where it circulates and combines with the basal chamber flow (blue arrows) to analyze it.

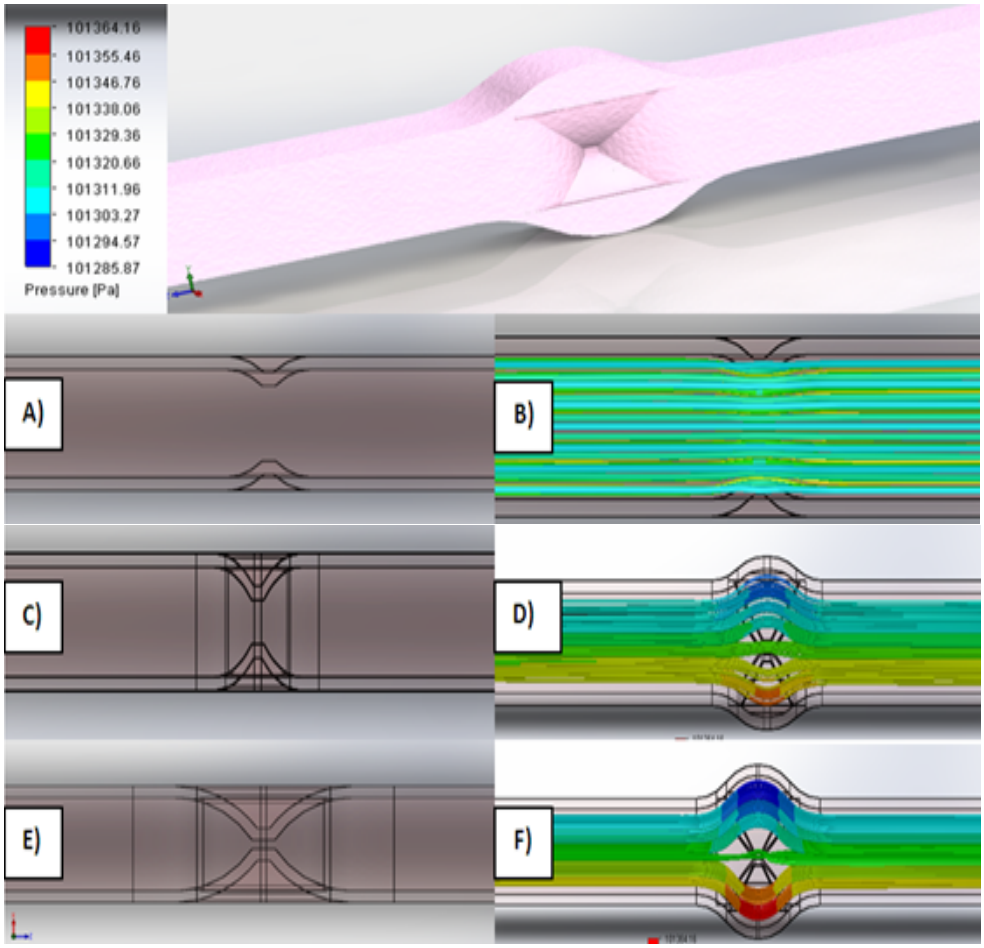


**FIGURE 2.** Colorimetric plot of velocity field and shear stress levels for different compress of the dialysis tube **(A, C, E)** show the lateral view for the lumen at 70%, 40% and 10% respectively. The lateral view simulation at 70% lumen with a small variation in the velocity field is shown in **(B)**. The isometric view at 40% and 10% lumen are shown in **(D)** and **(F)**, respectively, with their different velocity fields caused by the helix compress in the apical chamber.

## Materials and Methods

*Membrane bioreactor design*

The bioreactor is composed of two chambers:

(i) an apical chamber, with a 5 mm-diameter dialysis tube.

(ii) a basal chamber, with an inlet and an outlet with a wet volume of 5 mL, and two helices that rotate to squeeze the dialysis tube to simulate the peristalsis.

The dialysis tube is a commercial hemodialysis permeable membrane (D9527, Sigma-Aldrich), the chamber and the helix are made in Sylgard (SYLGARD 184, Sigma-Aldrich); as shown in Fig. 1, the bioreactor has two independent fluidic circuits in the apical and basal chambers, respectively. Each circuit is connected to a peristaltic pump (ISMATEC, PC N4, Wertheim, Germany) and a mixing chamber, which serves as a media reservoir and for oxygenation.

*Model design*

When the dialysis tube is inserted along with the bioreactor apical chamber, water flows through and around the dialysis tube; these aspects were considered for the hydrodynamic simulation. The flow simulation data assists in the calculation of mechanical shears applied to the cells and the tube to avoid static zones or zero flow within the bioreactor.

The geometric bioreactor model was designed at SolidWorks (systems Dessault, inc.). The model was then exported to COSMOSFloWorks (systems Dessault, inc.) software to solve Navier-Stokes equations to determine speed fields and work against the scaffold. The universal physical water features were: isothermal, Newtonian liquid, 1000 kg/m$^3$ density, 0.889 mPa viscosity and 5 cm/h, bioreactor continuous flow rate of 7 ml/h, and atmospheric pressure. For the dialysis tube, 50 µm pores and silicon chambers were used (Ramirez-Fernandez *et al.,* 2017).

The bioreactor flow system has two independent fluidic circuits, one for the apical chamber and another for the basal chamber, which collects the media into a sampler compartment for further analysis. As mentioned above, the bioreactor lies inside of an incubator that is temperature-regulated and provides 5% of $CO_2$; the gases penetrate in the chamber by diffusion. The internal bioreactor chamber isolation as the external bracket is made from food-grade polyurethane (Sigma-Aldrich). The hoses used to connect the peristaltic bomb to the reactor are made from medical-grade PVC, 6.35 mm diameter (Sigma-Aldrich). The helix (SYLGARD 184, Sigma-Aldrich) rotation were controlled by an Arduino Due system and two Stepper motors with their controllers at 6 rpm to mimic the peristaltic wave-like moves, as described by Lee *et al.* (2016).

*Permeation in dynamic environment*

The bioreactor mechanical conditions were based on the work by Giusti *et al.* (2014); the chambers were connected to the dynamic circuits and filled with 12 mL of deionized water and flow was applied at a rate of 7 ml/h, using a peristaltic pump (ISMATEC, PC N4, Wertheim, Germany), and 6 rpm

helix rotation. This dynamic regimen was kept for 3 weeks. As controls, bioreactors were filled with deionized water and kept only with the peristaltic pump connected in the first control, and to static conditions in the second control, for the same period. On days 7, 14 and 21 a permeability assay was performed to assess the passage of methylene blue (Sigma-Aldrich) from the apical chamber to the basal chamber. The compound was added to the mixing chambers that fed the apical circuits of the bioreactors in dynamic conditions, and to the apical compartments of the bioreactors in static conditions, at a concentration of 30 mg/l (Heppell *et al.,* 2015).

*Data analysis*

Three samples of 100 µl were taken from the mixing chambers that fed the basal circuits of the bioreactors (in dynamic conditions) and from the basal compartments of the bioreactors (in static conditions) after 1, 6 and 24 h and were placed on a 96-well plate (Corning) for analysis. Sample volumes were replaced by the same volume of deionized water in the corresponding mixing chambers/compartments. After 24 h of the testing period, the content of both circuits/compartments was replaced by fresh deionized water and this step was repeated until the water appeared clean. To determine the concentration of methylene blue in the collected samples, standards of known concentrations were prepared and the absorbance of all the samples (collected samples and standards) was measured at a wavelength of 665 nm using a spectrophotometer (Fluostar Omega, BMG Labtech, Germany).

Data was presented as mean ± SE of at least three independent experiments. Origin 8.1 software was used to test ANOVA. Multiple comparisons were made using Tukey test. A $P < 0.05$ was considered statistically significant.

## Results

*Flow simulation*

Bioreactor simulation is shown in Fig. 3 with three different helix compression percentiles at 7 ml/h in both frontal and lateral views showing the media flow. There is a speed gradient inside the central bracket, but once the media culture passes this point the speed stabilizes with a constant velocity magnitude. The water flow inside this particular bioreactor allows a better intake of flow conditions due its continuous replacement. In the frontal view, where a suction vortex can be appreciated at the bioreactor exit, there is no possibility for static zones inside the apical chamber and it assures the successful flow exchange. All velocity fields were counted for this image to observe the system behavior. The simulation represents the water flow throughout the entire system. The basal chamber flow through the dialysis tube is independent of the apical flow as we can see in the interchange chamber (Fig. 3).

*Basal chamber simulation*

Derived from the simulations, flow rates cover a wide range of magnitudes. Even though the fluid inlet conditions of the three models are the same, the results are significantly different. The velocity field decreases when the inlet volume decreases; but it conserves the perpendicular flow to the seeded cells and the nutrient interchange. According to the above research, we know that when the flow rate is the same, we can reduce the shear stress effectively by choosing reasonable materials, geometry and location.

*Construction*

Then we proceeded to the bioreactor building (Fig. 4) that was based on the obtained simulation design. The Chamber mold parts were processed by a 3D printer machine with polycarbonate feed, and then the structures were generated with Sylgard. The compresion helices were made in a photolitographic machine.

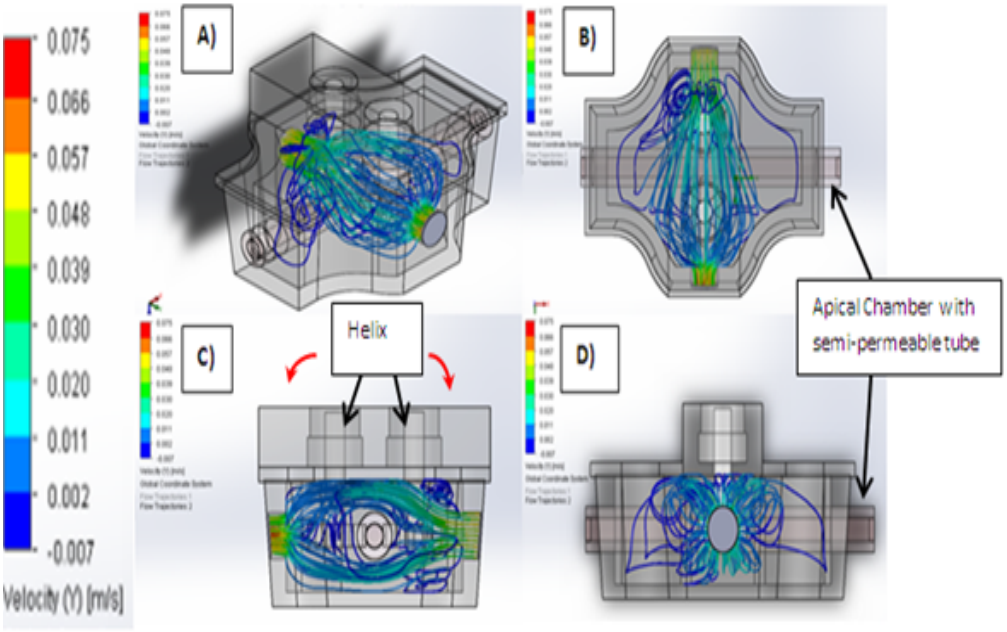


**FIGURE 3.** Colorimetric plot of velocity field and shear stress levels for the basal chamber show the **(A)** isometric, **(B)** top, **(C)** frontal, and **(D)** lateral views.

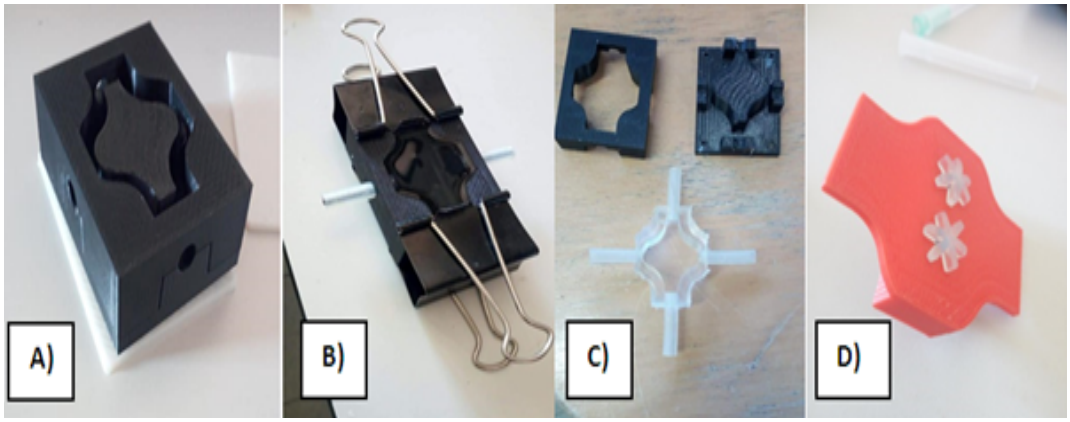


**FIGURE 4.** (A) Basal chamber molds. **(B)** Molds with Sylgard. **(C)** Bioreactor chambers (apical and basal). **(D)** Helix.

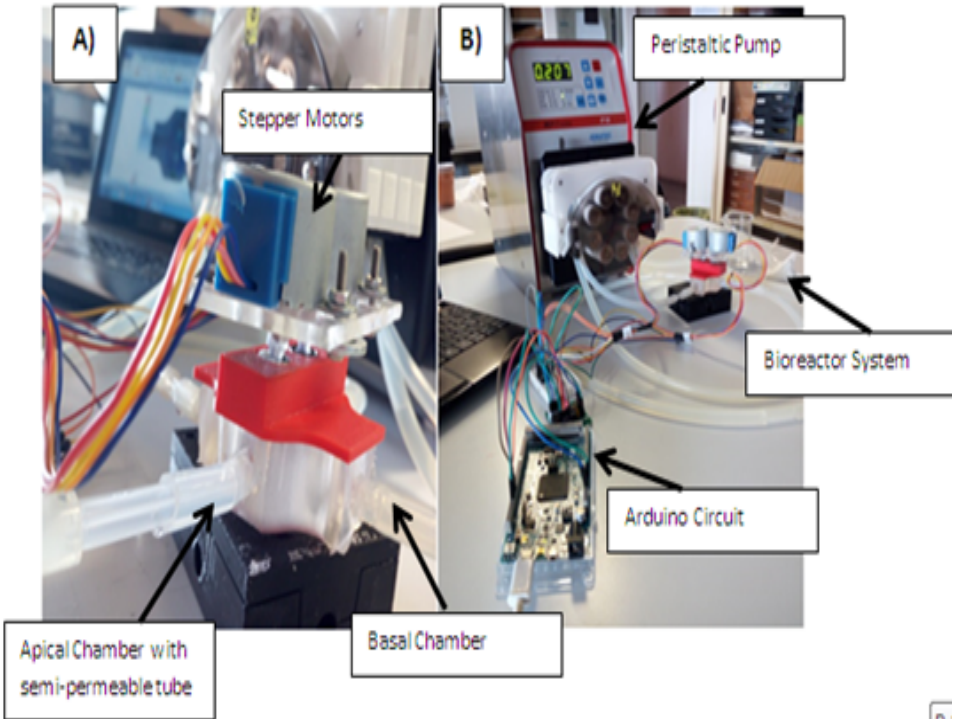


**FIGURE 5.** Final assembly.

Finally, we proceeded to the assembly of the final system with the hoses and the motors with their Arduino controls connected with the peristaltic pump (Fig. 5).

*Permeability assay*

The results, summarized in Fig. 6, indicated that in dynamic and squish conditions 12-13% of the compound permeated through the apical chamber and passed to the basal chamber after 3 weeks. This indicated that the dialysis tube was effectively permeable under these conditions. The permeation profile of the dynamic bioreactor was similar to the profile of the bioreactor maintained under static conditions, meaning that the flow did not affect the permeation of the compound. Actually, on the first 7 days, the values of some samples under static conditions were even higher than the dynamic ones, while in some cases it was the opposite. Nevertheless, the data presented low standard deviation values (0.6). In any case, it could be that the helix compress represents the main enhance for the permeation of the bioreactor. In that case, the "barrier effect" could have overcome the effect of the concentration gradient, which potentially was kept high for a longer period in the cases where it was being diluted by the moving fluid of the basal chamber.

Our bioreactor is an approach that combines different microfabrication techniques to produce chambers that resemble the architecture of the intestinal movements. The analysis of the structures and their flow indicated that the aimed topography was successfully accomplished. The cell tests will support the suitability of the bioreactor for the *in vitro* cell culture of intestinal cells. Additionally, the permeability data confirmed that the bioreactor was effectively permeable and suitable for exposure to flow. This work aims at the final integration of the cell culture in a bioreactor that can provide an adequate nutrient and oxygen flow to better recreate, in this way, the dynamic environment of the modelled epithelium (Ramirez-Fernandez *et al.*, 2017). Thus, the complete validation should be attained with further cell culture tests. This approach can potentially overcome some of the shortcomings of traditional *in vitro* models and could help settling the path towards the ideal intestinal *in vitro* model.

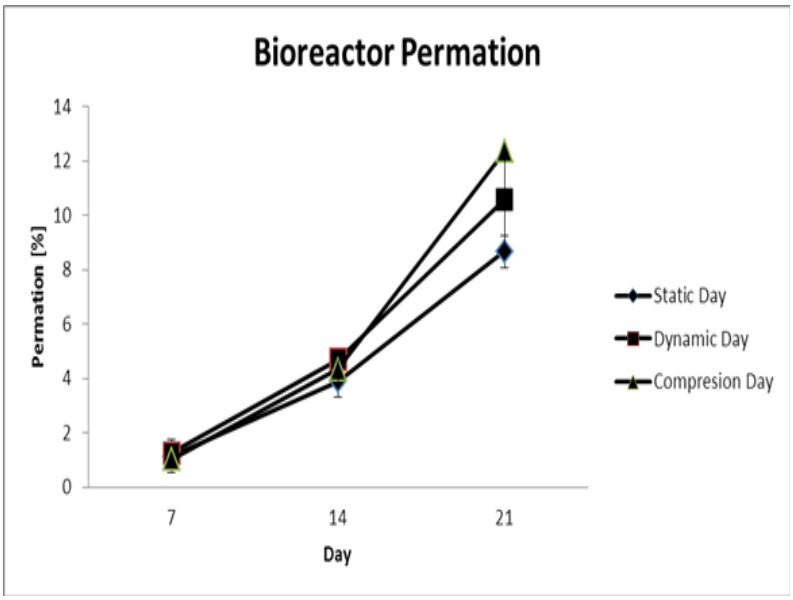


**FIGURE 6.** Permeability data from dialysis tube kept in the bioreactors for 3 weeks under 3 different conditions. For the first condition we considered the dynamic and compression conditions with a flow of 7 ml/h and helix squishing (triangle). For the second condition we used just the dynamic conditions with a flow of 7 ml/h without helix spin (square). Finally, we considered the static conditions without flow and helix spin (diamond). The assay was performed at days 7, 14, and 21 and the passage of the substance was measured. $P < 0.5$.

## Discussion

In the bioreactor design, the shear stress and medium transfer rate are the main technical indicators caused by fluid flow. The two factors are mutual restraints, but we can change some conditions, such as: scaffold materials, scaffold position, pore size, the number of capillary for medium supply to optimize the microenvironment in the bioreactor to make it more suitable for intestine cell growth (Ramirez-Fernandez *et al.,* 2016).

In order to improve the media interchange, we can improve the medium flow rate or double the number of supporting capillaries. Although, previous simulation results showed that doubling the medium flow rate can proportionally improve media change, but with a slightly increase (Ramirez-Fernandez *et al.,* 2015). Increasing the medium flow rate will reduce reaction time, and will improve support capillary to make the external circuit more complex.

The shear stress and the value of pressure are the most essential parameters which are widely concerned about (Ramirez-Fernandez *et al.,* 2014). The control of other parameters can enable the above mentioned parameters closer to the parameters for the human intestine.

To simulate the peristaltic movements we decided to use two helices with six radial blades that allowed us to model the compression movements of the intestine to pass nutrients through the lumen of the apical side. We modeled the system in such a way that the maximum point of compression was out of 50% of the total lumen of the pipe; the model can vary the occlusion percentage of the lumen since we simulated and modeled scenarios with different occlusions. To generate this difference in the bioreactor, we decided to leave the centers of the propellers fixed and to make the propellers interchangeable to adjust the different lengths of the pallets. This with the objective of not making the shafts of the mobile propellers since with each experiment it would be easier to change the propellers than to design a piece with the mobile helices, even an experiment is not common where the compression distance had to be varied during the experimental time course.

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