# Double Flow Bioreactor for In Vitro Test of Drug Delivery

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**Abstract.** In this work, double-structured polymeric scaffolds were produced, and a double flow bioreactor was designed and set up in order to create a novel system to carry out advanced in vitro drug delivery tests. The scaffolds consists of a cylindrical porous matrix, are able to host cells, thus mimicking a three-dimensional tumor mass: moreover, a "pseudo-vascular" structure was embedded into the matrix, with the aim of allowing a flow circulation. The structure that emulates a blood vessel is a porous tubular-shaped scaffold prepared by Diffusion Induced Phase Separation (DIPS), with an internal lumen of 2 mm and a wall thickness of 200 micrometers. The as-prepared vessel was incorporated into a three-dimensional matrix, prepared by Thermally Induced Phase Separation (TIPS), characterized by a high porosity (about 95%) and pore size adequate to accommodate tumor cells and/or mesenchymal cells. The morphology of the multifunctional scaffolds is easy-tunable in terms of pore size, porosity and thickness and therefore adaptable to various cell or tissue types. At the same time, a double flow bioreactor was designed and built up, in order to be able to carry out biological tests on the scaffold under dynamic conditions. The device allows a separate control of the two flows (one for the tubular scaffold, one for the porous matrix) through the scaffolds. Preliminary characterizations and tests carried out suggest the presented system as a candidate to suitably "in vitro" assess the effects of different drugs on various cell populations.

Keywords: Poly-L-Lactic Acid; Bioreactor; Phase Separation; Fluid dynamic, Vascular Tissue Engineering.

## MATERIALS AND METHODS

The double-structured scaffold was prepared by performing a Thermally Induced Phase Separation (TIPS) around the vessel-like scaffold. The vessel-like scaffold was placed perpendicularly into a cylindrical aluminum sample holder (height 40 mm, diameter 18 mm) and the TIPS protocol already published by the present authors [1,2] was followed. A representation of the produced bioreactor is depicted in figure 1. The double flow bioreactor is composed of a glass tube (36 mm diameter, 210 mm height) where the scaffold is located halfway in length. A recycled current of medium is able to perfuse the outer zone of the scaffold from top to bottom. The diameters of the columns and their heights were designed to fulfill the following requirements: A) the whole assembly could be easily allocated in an incubator of standard size; B) the volumes of medium that bioreactor may contain should be sufficient for long term cell cultures.

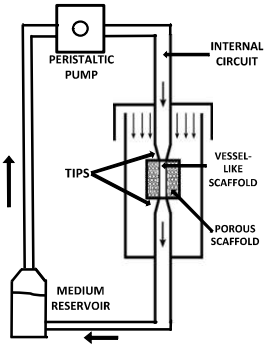



FIGURE 1: Schematic representation of the experimental set up

## RESULTS AND DISCUSSION

Fig 2A shows a cross section of the multifunctional scaffold. The presence of the support, during the TIPS process, assured a limited shrinkage of the tubular scaffold, whose lumen was estimated to be about 2 millimeters. It is easy to observe that the vessel-like scaffold is totally embedded into the porous structure produced via TIPS (Fig. 2C) and a continuous porous structure at the border of the vessel-like scaffold in communication with the macropores of the "main" scaffold can be detected (Fig. 2B). Moreover, SEM analysis allowed to confirm that the thickness of wall of the embedded tubular scaffold did not change during the thermally induced phase separation process (Fig. 2D).

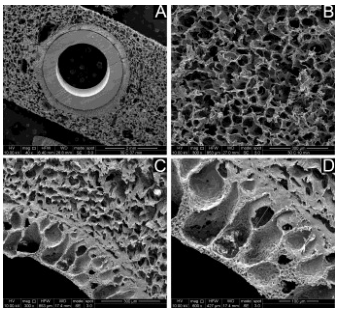
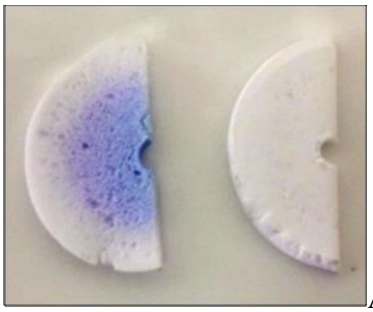



FIGURE 2: Micrographs showing the cross-section of the multifunctional scaffold (A); external porous matrix (B); detail of embedding zone (C); tubular scaffold wall (D).

To check whether the permeate flows homogeneously from the tubular scaffold to the three-dimensional matrix, a solution containing a dye (toluidine blue) was fed to the inside of the tubular scaffold for 8 hours. The cross-sections of the as-treated scaffolds were then observed under a stereomicroscope. As seen in Figure 3A, the porous matrix presents a radial homogeneous staining, proving that the fluid permeates and spreads uniformly from the tubular scaffold to the external porous matrix.In order to know the flow rate of the circulating liquid in the inner perfusion circuit and the pressure drop in the bioreactor, a specific experimental characterization of the system was carried out. Figure 3B indicates the flow rate of the internal perfusion circuit as a function of the rounds per minute (RPM) of the peristaltic pump. For each flow rate (Q) value examined, fluid velocity (v) and Reynolds number (Re) were calculated, in order to check the flow regime, which remains laminar for the whole range investigated.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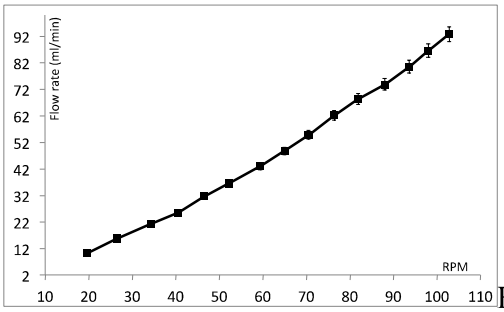

B

FIGURE 3: A) Multifunctional scaffold before (on the right) and after (on the left) the toluidine blue perfusion. B) Flow rates calculated as a function of the RPM of the peristaltic pump.

## CONCLUSIONS

In this work, a novel system for drug delivery assays was designed, developed, built up and tested. A double-flow perfusion bioreactor was coupled with a multifunctional scaffold mimicking a vascularized tissue. From the obtained data it is possible to postulate that the presented system meets all the necessary requirements for its use in drug delivery studies.

## REFERENCES

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