COMPUTATIONAL METHODOLOGY TO DETERMINE FLUID RELATED PARAMETERS OF NON REGULAR THREE-DIMENSIONAL SCAFFOLDS

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Abstract

The application of three-dimensional (*3D*) biomaterials to facilitate the adhesion, proliferation and differentiation of cells has been widely studied for Tissue Engineering purposes. The fabrication methods used to improve the mechanical response of the scaffold produce complex and non regular structures. Apart from the mechanical aspect, the fluid behavior in the inner part of the scaffold should also be considered. Parameters such as permeability (k) or wall shear stress (WSS) are important aspects in the provision of nutrients, the removal of waste metabolic products or the mechanically-induced differentiation of cells attached in the trabecular network of the scaffolds. Experimental measurements of these parameters are not available in all labs. However, fluid parameters should be known prior to other types of experiments. The present work compares an experimental study with a computational fluid dynamics (*CFD*) methodology to determine the related fluid parameters (k and WSS) of complex non regular poly(L-lactic acid) scaffolds based only on the treatment of microphotographies images obtained with a microCT (μCT). The *CFD* analysis shows similar tendencies and results with low relative difference to those of the experimental study. The correlation between the computational and experimental results validates the robustness of the proposed methodology.

Keywords: Tissue Engineering, Scaffolds, Permeability, Darcy's Law, Computational fluid dynamics.

1. Introduction.

Tissue Engineering is a technique that combines cells, three-dimensional biomaterials (scaffolds) and chemical/mechanical stimuli to replace damaged or diseased tissues. It involves several intrinsic properties for the ideal scaffold such as a three-dimensional (*3D*) interconnected pore network, adequate surface properties to enhance cell adhesion, proliferation, migration and differentiation, a biocompatible and bioresorbable substrate with controllable degradation rate,

appropriate mechanical properties to match those of the surrounding tissue when implanted, an architecture which promotes formation of the native anisotropic tissue structure and a reproducible architecture of clinically relevant size and shape.[1-8]

One of the most important functions of a scaffold is related with its inner structure. Scaffolds are expected to be highly porous, with at least 70 % porosity, to allow cells to migrate through the internal structure of the scaffold. High percentages of porosity should be coupled with good pore interconnectivity to achieve not only good cell homing but also satisfactory nutrient distribution and waste removal.[9-11]

Interconnected porosity and pore size are important factors in the mechanical characterization for a scaffold applied in Tissue Engineering.[12] The permeability (k) of scaffolds, a property directly related to the degree of pore interconnectivity, is a key factor influencing the scaffold's ability to enhance tissue regeneration. Permeability quantifies the ability of a porous medium to transmit fluid through its interconnected pores or channels when subjected to pressure. Permeability, therefore, controls the flow of nutrients to cells located inside the scaffolds.[7,13]

Recent developments in design and fabrication technologies have allowed the creation of three-dimensional scaffolds with controlled microstructure but not always with a regular shape. The desirable way to evaluate the scaffold *3D* architecture after the fabrication process would be a nondestructive, noninvasive, and quantitative technique.[14] Nondestructive high-resolution images can be obtained with microCT (μCT) techniques in the order of a few microns per pixel. This technique allows the reconstruction of small samples.[3] With recent advances in computer-aided design (*CAD*) software's, μCT techniques and imaging analysis, scaffold properties can be analyzed from a numerical point of view, using computational fluid dynamics (*CFD*). Usually the permeability values computed from *CAD*-based models are substantially higher than those from

μCT-based models. The relative difference between measured and computed values has been strongly reduced by means of *μCT*-based models.[15]

In many Tissue Engineering applications, porous poly(L-lactic acid) (*PLLA*) scaffolds have been used to guide tissue regeneration.[8] For this work, the scaffolds were prepared by the freeze extraction and particle leaching process using dioxane as *PLLA* solvent and spherical polymeric particles as a macroporogen.[16] A variation of the *PLLA* scaffold morphology was performed varying the PLLA/dioxane proportion during the fabrication process. These techniques provide complex non regular scaffolds with controlled pore size and porosity.

In this work we propose a robust methodology for designing an optimal structural scaffold for tissue engineering. The methodology is applied to *3D* structures with extremely complex and heterogeneous architectures. First, a morphological study of the internal structure is obtained from the *3D* reconstruction of the scaffolds. The image analysis determines the real structural parameters such as porosity and pore size (d). An experimental permeability study and *CFD* analysis are then carried out for different fluid flow rates to characterize the intrinsic permeability of the scaffold. In Tissue Engineering applications, low flow rates are used to evaluate the behavior of the interconnected structure. With the scaffold geometry and the *CFD* analysis it is possible to calculate variables which cannot be evaluated by other means such as the wall shear stress (WSS).

2. Materials and Methods

2.1. Fabrication of the poly(L-lactic acid) (PLLA) scaffolds

Medical grade poly(L-lactic acid) (PURASORB PL 18) with a viscosity of 1.8 dl/g and an average molecular weight of 165446 Da supplied by Purac Biomaterials (The Netherlands) was used to fabricate the scaffolds. Different solutions of *PLLA* in 1-4 dioxane (98% pure, obtained from Sigma Aldrich) (at 10, 15 and 18 wt.% of *PLLA*) were homogeneously mixed with PEMA spheres (from

Elvacite, 2043 acrylic resin) in mass proportion 1:1 at room temperature and then immediately frozen with liquid nitrogen. The frozen structures were immersed in pre-cooled ethanol and kept at -20°C during two days, with at least three changes of the ethanol to remove almost all the dioxane crystals. Afterwards, the extraction of PEMA macroporogen took place in ethanol at 40°C under continuous stirring. Various changes of solvent were needed to eliminate all the PEMA spheres; until no polymer deposit was left on a glass when a drop of the extraction liquid was evaporated. After extraction, the scaffolds were dried in air atmosphere for 24 h and then in vacuum to constant weight, first for 24 h at room temperature and then at 40°C. In this way, scaffolds with increasing size of micro and macro pores were prepared, since the amount of solvent affects not only the micropore size, but the macropore because it swells the PEMA spheres.. Hereafter, the samples will be referred to as PL-1:1-x%, x being the weight percentage of *PLLA* in the dioxane solution.

2.2. Permeability test

Permeability (k) is a structural variable that describes the interconnectivity and the capacity of a porous material to absorb liquid without altering its internal structure. To determine a relation between interconnected porosity and pore size, a permeability test has been developed under the Darcy Law and is available for Reynolds number lower than 8.6 (1).[9,10,13,15,17]

$$k = \frac{\mu t}{A} \frac{Q}{\Delta P} \qquad (1)$$

where k is the intrinsic permeability (m^2), μ the dynamic fluid viscosity (deionized water $\mu = 10^{-3}$ Pa s), t the specimen thickness, A the cross-sectional area, Q the volumetric flow rate and ΔP the total pressure drop across the scaffold sample (Pa). The total pressure drop measured with the scaffold specimen inside the chamber is $\Delta P_{scaffold}$ whereas $\Delta P_{chamber}$ is the measurement for the empty chamber (2).[15] Due to the test configuration, the measured pressure drop is attributed to the scaffold microstructure and the section change.

$$\Delta P = \Delta P_{scaffold} - \Delta P_{chamber} \quad (2)$$

The permeameter used is shown schematically in figure 1. The fluid was taken from an open reservoir and induced into the circuit with a peristaltic pump. A fluid damper (KH-07596-20 Pulse Dampener – Coleparmer Masterflex) was used to achieve a continuous flow through the circuit and avoid the peristaltic pulse produced by the pump. The permeameter chamber has a cross sectional geometry that facilitates placing the scaffold samples. In this way, undesirable movements of the considered structures are prevented. The $\Delta P_{scaffold}$ and $\Delta P_{chamber}$ were measured between two points of the permeameter chamber using a pressure meter (Testo 510 with a precision of ± 0.1% and operative range from 0 to 2000 hPa).

For each polymer concentration (10, 15 and 18 wt.% of *PLLA*), five samples were tested. Cylinders of 6 mm diameter and 3.11 ± 0.17 mm thickness were used. All the samples were immersed in a saline phosphate-buffered (*PBS*) solution during 48 hours before testing. In accordance with the experimental protocol, the fluid flow through the scaffold was varied by controlling the flow rate (20, 40, 60 ml min^{-1}). The $\Delta P_{scaffold}$ generated in each case was measured. The obtained ΔP was averaged out to determine the permeability of the structure using *Darcy's Law*. The mean, standard deviation and standard error were calculated. The results are presented as mean ± standard error.

2.3. MicroCT (µCT) imaging analysis

Microtomography was carried out to define the trabecular and pore distribution, as well as their uniformity in the *3D* structure.[6] The image files (DICOM- Digital Imaging and Communication in Medicine) provided by the *µCT* were the main input for building the geometric model of the scaffold. Images of the whole sample were obtained by a rotational scanning of 360 degrees. A GE Healthcare eXplore locus SP *µCT* was used, with an x-ray filter number 2, 45 kV voltage and 120

mA power. The resolution of the equipment was 8 μm. 348 DICOM files were obtained for each sample (one image for each rotation of 1.03 degrees). The quality of the final model and its similarity with the original sample are directly related to the degree of resolution and the number of segmented images. The selected volume for reconstruction must be representative of the entire sample. The volume segmentation was made by sweeping all the scanned slides (Mimics - The Materialise Group, Leuven, Belgium). The reconstructed volume was approximately 7.06 mm^3 (3 mm diameter and 1 mm thickness). Finally, a set of *STL* files were generated, this being the standard format used in computer-aided design. These files describe the surface of a three-dimensional geometry through a mesh of triangles. Details of the pre-processing are illustrated in figure 1.

A histogram of the diameter of the pores (d) was measured for each structure on the final *STL* geometrical models. The pores of the scaffold were selected and segmented using a threshold of the grey scale to transform the porous geometry into groups of voxels. The voxels were classified as hole or material, and each voxel identified as a hole defined a sphere. In the post-processing, any sphere included within another was eliminated. Finally, with the same grey scale it was possible to define the structural porosity.

2.4. Numerical discretization and fluid flow modelling

Three different *3D* CT-based scaffold geometrical models were built and meshed with the commercial software FEMAP (PLM Siemens, Plano, TX, USA), see figure 2. For each scaffold, several flow rates were tested prior to reconstructing the test room with the numerical software (see figure 2). Specifically, 10 flows for each scaffold were tested, representing a total of 30 *CFD* analyses.

To predict the pressure and velocity fields inside the scaffolds, the commercially available finite

volume code Ansys CFX (Ansys Software, Canonsburgh Pennsylvania, USA) was used to set up and solve the fluid dynamic problem. As already mentioned, the scaffold was inserted in a CAD-built test channel which, due to its geometry, perfectly reproduced the experimental channel (see figure 2).

Due to its intrinsic geometrical complexity, the grid was carried out through tetrahedral elements. About 5 millions cells were defined for each scaffold. To establish the appropriate element size, a mesh independent study with a fixed flow rate was previously conducted. Velocity profiles were compared at different channel sections before and after the scaffold. It was clearly demonstrated that for a number of elements greater than 5 million, increasing refinements produced higher computational costs but differences in velocities of less than 1%.

The culture medium was regarded as an incompressible and homogeneous Newtonian fluid with the properties of water (a viscosity of 10^{-3} Pa s and a density of 10^{3} kg m^{-3} at a temperature of 21°C). Due to the slow flow regime (Reynolds number Re < 50), a spatial and temporal constant flow rate was assigned to the inlet corresponding to 0.1, 0.5, 1, 1.5, 2, 3, 4.5 ml min^{-1}, thus being within the range of applicability of *Darcy's Law*. Unlike the study of Truscello et al., 2012[15] which approximated the outlet condition imposing zero pressure at the model outlet, in this work the same flow rate as the inlet was also imposed at the model outlet, assuming stationary conditions during both experimental and numerical analysis as well as rigid walls. In this way we also guaranteed the same conditions as the experimental analysis. No-slip conditions were finally imposed at the walls of the scaffold as well as at the channel walls. Steady-state Navier–Stokes equations were used to describe the flow problem. The pressure drop across the scaffold was obtained for all models and used to calculate the permeability coefficient.

2.5. Computational fluid dynamics (CFD) numerical approach

The governing equations were solved using finite volume discretization by means of the advanced coupled multi grid solver technology ANSYS CFX (v5, Ansys Software, Canonsburgh Pennsylvania, USA). The convergence criteria used in all simulations was 1×10^{-8}. This factor was used to reduce the initial mass flow residual during the simulation progress. The simulations were carried out on the 16 noded, Dual Nehalem (64 bits), 16 processor cluster with a clock speed of 2.33GHz and 32 GB memory for each node.

[Figure. 1]

[Figure. 2]

3. Results

A morphologic study can be made from the *3D* reconstruction of the scaffold microtomographies. A change in the polymer concentration (wt.% of *PLLA*) determines the trabecular structure and establishes its correlation with the uniformity and pore distribution. Additionally, with the image analysis the average pore size and the porosity are calculated for each three-dimensional structure. In our working range, an increase in the wt.% *PLLA* increases the uniformity of the scaffolds, because for large concentration of solvent irregular swelling of porogen spheres occur and zones with large pores, defects and broken trabeculae are found (see figure 3). Increasing the wt.% *PLLA* leads to a reduction in the structure porosity and the mean pore size (see figure 4 and table 1). These structural characteristics support the results obtained from the present study for the working range of the PLLA %.

[Figure. 3]

[Figure. 4]

[Table. 1]

From the experimental study, it can be deduced that the total difference pressure (ΔP) increases when the wt.% *PLLA* rises (see table 2 and figure 5a) whereas the intrinsic permeability (k) shows the opposite tendency. The structural permeability decreases when the wt.% *PLLA* increases (see table 3 and figure 5b). The results of the computational simulations confirm these trends (see tables

2 - 3 and figures 5).

In figure 6, the flow through the scaffold is shown by means of *3D* streamlines. These results refer specifically to the PL-1:1-15% scaffold and 20 ml min^{-1} flow. For this reason, ΔP is generated between both chambers and as a result between the scaffold faces located orthogonally to the fluid flow. This ΔP is represented in figures 7 and 9 for high and low flow rates, respectively, for the PL-1:1-15% scaffold. In particular it can be seen, as expected, that increasing the flow results in an increase in ΔP (see figure 7).

[Figure. 5]

[Figure. 6]

[Figure. 7]

[Table. 2]

[Table. 3]

For the scaffold difference pressure ($\Delta P_{scaffold}$), the results of the experimental and the *CFD* data showed the greatest variation for the PL-1:1-18% with a relative difference of 20.21%. This difference was generated by a flow of 20 ml min^{-1}. For the PL-1:1-10% and PL-1:1-15% the difference was 1.45% and 1.54%, respectively (see figure 5a). In the experimental study, the PL-1:1-10% showed the greatest dispersion in the permeability (k) data reported with ± $5.9x10^{-11}$ m^2 (see table 3). However, the highest relative difference again appeared for the PL-1:1-18% with 13.61%. Finally, for the PL-1:1-10% and PL-1:1-15% the difference was 9.58% and 2.24%, respectively (see figure 5b).

The previous results showed only small discrepancies between the experimental and *CFD* data for high flow rates through the *3D* structure (20, 40 and 60 ml min^{-1}). Additionally, a second experimental and *CFD* study was performed to evaluate the behavior of the *3D* structure at low flow rates (1.5, 3.0 and 4.5 ml min^{-1}). This study was focused in particular on the PL-1:1-15%

scaffold. The $\Delta P_{scaffold}$ and k results for all the flow rates are shown in table 4 and figures 8 - 9.

[Figure. 8]

[Figure. 9]

[Table. 4]

The greatest relative differences between the experimental and CFD data $\Delta P_{scaffold}$ results occurred for 1.5 ml min^{-1} with 62.58% and for 4.5 ml min^{-1} with 36.37%. For 3.0, 20, 40 and 60 ml min^{-1}, the differences were 13.35%, 11.56%, 2.08% and 1.05%, respectively. The relation found for the $\Delta P_{scaffold}$ showed a linear tendency with R^2 = 0.99. In the reported k data, the greater relative differences appeared for 1.5 and 3.0 ml min^{-1} with 26.28% and 24.81%, respectively. For 4.5, 20, 40, 60 ml min^{-1} the differences were 4.3%, 6.63%, 7.94% and 6.85%, respectively.

The study of the interconnected structure under low and high flow rates was used to determine the wall shear stress (WSS) of the PL-1:1-15 scaffold (see table 5 and figures 10 - 11 - 12). An increase in the flow rate increased the WSS on the trabecular structure. This tendency showed a linear regression with a quadratic correlation coefficient R^2=0.99. For flow rates equal to 1.5 and 60 ml min^{-1}, the WSS increased by 27.54%.

[Figure. 10]

[Figure. 11]

[Figure. 12]

[Table. 5]

As the considered flow is laminar, due to the relatively low flow rates hypothetical seeded cells would follow the flow profile with minimal advective transport in directions perpendicular to the flow. Only cells close to the pore wall of the scaffold would come into contact with the surface. To study how the flow may impact seeded cells, the WSS spatial distribution was analyzed as a function of the flow rate for different scaffold designs. Figure 11 depicts the PL-1:1-15 scaffold. The WSS spatial distribution is shown on the scaffold surface as a function of the steady flow.

Figure 12 represents the same situation for high flow rates.

Increasing the flow rate from 0.1 ml min^{-1} to 4.5 obtained WSS values in the range 1.3 - 6 Pa (see figure 11). The WSS spatial distribution along the scaffold was strongly dependent on the complexity of the porous structure, as expected. Overall, the level of WSS found throughout the surface was low and heterogeneously distributed, as shown in figure 11. As is usual, low WSS values were due to the effect of the flow separation regions along the porous sections. For this reason, these regions are characterized by relatively low velocities. In contrast, higher WSS values are due to the impact of the flow to the scaffold. This can be seen especially on the outer surface (see figure 11). Inside the scaffold, the high WSS values are due to the local acceleration of the flow along a single pore just before the flow separation. The same trend can be observed for high flow rates (see table 5 and figure 12). In this case an increase in the WSS values was observed.

4. Discussion

The intrinsic permeability is a function of the pore size and porosity in a *3D* structural scaffold. Thus, the smaller the pore size, the higher the trabecular area in contact with the fluid. This effect increases the frictional resistance to the flow and thus permeability decreases. Furthermore, as shown in our previous publication [Acosta Santamaría, 2012], when the amount of solvent increases the morphology of the scaffold becomes more heterogeneous in the sense that some zones with broken trabeculae are formed that give greater permeability to the structure.

Comparing the results obtained in this work with those of other authors, one of the main differences is associated with the evaluation of the intrinsic permeability of *3D* geometries with very complex and heterogeneous scaffold architectures. Truscello et al., 2012 proved that *CFD* models based on high resolution μCT images are accurate for the prediction of the permeability of regular scaffolds. Dias et al., 2012 computationally estimated the permeability of the scaffold using the

homogenization approach applied to the problem of a fluid flow through a homogeneous porous media.[15,18]

As in other works, the scaffold structure was studied with different percentages of porosity and pore size.[15,18] However, the present work also shows the *3D* architecture behavior under high and low fluid flow rates for different design parameters. Additionally, the *CFD* analysis determined similar tendencies and results with low relative differences compared with those of the experimental study. For the scaffold pressure difference ($\Delta P_{scaffold}$), the results between the experimental and *CFD* results revealed the greatest difference for slow fluid flow rates (relative difference between 13.35% and 62.58%). For fast fluid flows, these relative differences were between 1.05% and 11.56%. Similarly, the greater differences for the permeability data reported were for slow fluid flow rates (relative difference between 4.3% and 26.28%). For fast fluid flows, these relative differences were 6.63% and 7.94%. The relative differences found in this study could be associated with heterogeneous scaffold architecture. However, the correlation between the computational and experimental results validates the robustness of the design methodology here described. The correlation for $\Delta P_{scaffold}$ showed a linear tendency with R^2 = 0.91. The permeability correlation showed the same tendency with R^2 = 0.96, which is similar to the results found by Truscello et al., 2012 being R^2 = 0.91.[15]

CFD modeling, as previously discussed, can determine the flow, pressure field and other flow variables such as wall shear stress (WSS) and/or wall shear rates within a scaffold, down to the pore-sized level. The eventual deposition of seeded particles on the scaffold surface under steady flow conditions basically depends on many parameters such as advective transport[19,20] and diffusivity, colloidal interactions[21], concentrations of ligands and receptors, binding strengths and bondforming kinetics[22] and especially on the scaffold microstructure, as demonstrated in other studies.[23] Because of the highly irregular scaffold architectures, many of these parameters may vary

throughout the scaffold in the flow modeling when performing perfusion experiments.

The wall shear rate has been described as crucial for cell suspension and deposition seeding techniques[23], shear stress has also frequently been described as an important mechanical stimulus able to induce cell proliferation and differentiation in different cell types.[24-27] A better knowledge of the WSS in the trabecular could help us to understand the heterogeneous cell behaviour in the internal part of the scaffolds. In this context, numerical simulations could be helpful to determine the cell differentiation process using a mechanobiological model such as that proposed by Lacroix and Prendergast, 2002.[25]

Due to the high variability of scaffold heterogeneity, it is quite complicated to make a precise comparison between WSS values that are strongly non-homogeneous and those reported in similar studies in the literature. Other studies such as that of Gutierrez et al., 2008 have attempted to assess bioreactor hydrodynamics under steady-state conditions, modeling *3D* scaffolds as non-porous solids with fluid flowing around and over the non-moving surfaces.[28] Singh et al., 2005 studied the flow around a geometrically defined scaffold finding WSS values of the order of 2, 4 and 8 Pa, respectively, depending on the rotation of the bioreactor.[29] These values are near to those found in this work for low flow rates. In contrast, Porter et al., 2005 found very small values (in the order of [mPa]) even using similar flow rates compared to those obtained here (0.1-2 Pa).[30] The differences in the WSS can partially be explained by the different porous distribution inside the scaffold. Moreover, Porter et al., 2005 used smaller samples for their numerical analysis which could have a significant effect.[30]

It should be noted that when considering other scaffold architectures and other flow rates, other hydrodynamic solutions may be obtained and, as a consequence, different WSS values, cell depositions and mechanical stimuli. Additional research into the probabilistic behavior of seeded

cells, a parameter whose influence has been neglected in this study, as well as into diffusive and advective transport should be undertaken in order to support the results found here. In this way, cell deposition and proliferation could be predicted in more detail.[23,31] As a long term result, the ability to track individual cells and compute their local hydrodynamic environment would allow the creation of a computational/experimental framework that may lead to a better understanding of biological cell processes in porous scaffolds. This study can be considered as a first step in this direction.

5. Conclusions

The present study evaluates heterogeneous structures with different pore size, interconnectivity distributions and diverse estimated flow rates. The pressure difference and the intrinsic permeability tendencies obtained for PLLA scaffolds from a CFD modeling study were similar to the reported experimental data. For high fluid flow rates, the results revealed small relative differences between the numerical and experimental methodologies whereas for low fluid flow rates, these differences were more significant.

An optimal reconstruction model for 3D complex geometry, appropriate pre-processing and image analyses, and the computational fluid dynamics methodology implemented in this study could be used as alternative tools to assess various mechanical variables for scaffold structures (porosity, pore size and trabecular distribution, structural difference pressure, intrinsic permeability and wall shear stress).

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Tables

Table 1. Porosity (%) and macropore size diameter (d) of the PL-1:1 series scaffolds obtained from the μCT reconstruction model and image analysis.

PLLA wt.%	Porosity (%)	Macropore Size Average (μm)
10	79.2	144.88
15	72.94	126.94
18	70.91	100.87

Table 2. Scaffold pressure difference ($\Delta P_{scaffold}$) for the PL-1:1 series scaffolds obtained from the experimental study and the CFD simulations.

PLLA wt.%	ΔP Scaffold (Pa) - Experimental Data				ΔP Scaffold (Pa) - CFD Data			
	20 (ml/min)	40 (ml/min)	60 (ml/min)	Average (ml/min)	20 (ml/min)	40 (ml/min)	60 (ml/min)	Average (ml/min)
10	79.24 ± 14.9	180.78 ± 19.4	293.26 ± 28.2	184.43 ± 20.4	72.2	167.87	321.23	187.10
15	138.46 ± 12.5	311.61 ± 26.0	518.51 ± 37.7	322.86 ± 25.1	122.45	318.11	513.07	317.88
18	203.34 ± 16.8	464.65 ± 82.7	851.29 ± 156.4	506.43 ± 84.1	134.99	374.0	703.20	404.06

Table 3. Intrinsic permeability (k) for the PL-1:1 series scaffolds obtained from the experimental study and the CFD simulations.

PLLA wt.%	k (m^2) - Experimental Data				k (m^2) - CFD Data			
	20 (ml/min)	40 (ml/min)	60 (ml/min)	Average (ml/min)	20 (ml/min)	40 (ml/min)	60 (ml/min)	Average (ml/min)
10	$5.30\times10^{10}\pm 9.8\times10^{11}$	$4.24\times10^{10}\pm 4.6\times10^{11}$	$4.05\times10^{10}\pm 3.6\times10^{11}$	$4.53\times10^{10}\pm 5.9\times10^{11}$	4.85×10^{10}	4.17×10^{10}	3.27×10^{10}	4.10×10^{10}
15	$2.69\times10^{10}\pm 1.7\times10^{11}$	$2.40\times10^{10}\pm 1.7\times10^{11}$	$2.21\times10^{10}\pm 1.4\times10^{11}$	$2.43\times10^{10}\pm 1.5\times10^{11}$	2.87×10^{10}	2.21×10^{10}	2.05×10^{10}	2.38×10^{10}
18	$1.96\times10^{10}\pm 1.2\times10^{11}$	$1.86\times10^{10}\pm 2.6\times10^{11}$	$1.53\times10^{10}\pm 1.8\times10^{11}$	$1.78\times10^{10}\pm 1.8\times10^{11}$	2.65×10^{10}	1.91×10^{10}	1.52×10^{10}	2.03×10^{10}

Table 4. Scaffold pressure difference ($\Delta P_{scaffold}$) and intrinsic permeability (k) obtained from experimental and CFD data of the PL-1:1-15 scaffold.

	Experimental Data						CFD Data					
	ΔP (Pa)			k (m^2)			ΔP (Pa)			k (m^2)		
Q (ml/min)	1.5	3.0	4.5	1.5	3.0	4.5	1.5	3.0	4.5	1.5	3.0	4.5
PLLA 1:1-15%	13.36	21.96	38.40	$4.19\times10^{10} \pm 2.0\times10^{10}$	$3.70\times10^{10} \pm 1.2\times10^{10}$	$3.39\times10^{10} \pm 1.2\times10^{10}$	5.00	19.03	24.43	5.29×10^{10}	2.78×10^{10}	3.25×10^{10}

Table 5. Wall shear stress (CFD data), obtained for the PL-1:1-15 scaffold.

PL-1:1-15% - CFD Data						
Q (ml/min)	1.5	3.0	4.5	20	40	60
WSS (Pa)	4.1	5.4	6.0	12.1	18.3	23.1

Figure Legends

Figure 1. Schematic diagram of the materials and methods applied for the experimental study and the computational fluid dynamics (*CFD*) analysis.

Figure 2. Chamber and scaffold three-dimensional mesh models.

Figure 3. Scaffold microtomographies showing the distribution of macropores in the three-dimensional structure. (a) PL-1:1-10%. (b) PL-1:1-15%. (c) PL-1:1-18%.

Figure 4. Morphologic results obtained from the *μCT* reconstruction model and image analysis for the PL-1:1 series scaffolds. (a) Porosity and macropore size average diameter. (b) Macropore size distribution.

Figure 5. Experimental and *CFD* data obtained for the PL-1:1 series scaffolds. (a) Scaffold pressure difference - $\Delta P_{scaffold}$. (b) Intrinsic permeability - k. (c) Pressure difference correlation. (d) Permeability correlation.

Figure 6. Fluid flow through the scaffold structure (PL-1:1-15%) shown by means of three-dimensional streamlines.

Figure 7. Scaffold pressure difference ($\Delta P_{scaffold}$) for PL-1:1-15% scaffold determined for high flow rate and obtained from the *CFD* simulations.

Figure 8. Experimental and *CFD* data obtained for the PL-1:1-15 scaffold. (a) Scaffold pressure

difference - $\Delta P_{scaffold}$. (b) Intrinsic permeability - k.

Figure 9. Scaffold pressure difference ($\Delta P_{scaffold}$) for PL-1:1-15% scaffold determined for low flow rate and obtained from the *CFD* simulations.

Figure 10. Wall shear stress obtained for the PL-1:1-15 scaffold from *CFD* data.

Figure 11. Wall shear stress for PL-1:1-15% scaffold obtained from the *CFD* simulations for low flow rates.

Figure 12. Wall shear stress for PL-1:1-15% scaffold obtained from the *CFD* simulations for high flow rates.