Modelling of bone scaffolds and *in silico* screening of graft performance

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Introduction

In the tissue engineering field, the use of 3D synthetic grafts is a promising strategy to be used as provisional biodegradable scaffolds as well as to regenerate damaged tissues by promoting the self-healing capacity of the patients. The porous architecture of the scaffolds, in terms of porosity, pore size distribution and connectivity, strongly affects its cell infiltration capacity and water permeability. Both parameters are critical to ensure a suitable scaffold performance once grafted, in terms of stimulating the growth of the damaged tissue and to guarantee the diffusion of nutrients and removal of waste products by cells, respectively. The *in silico* simulation by PoreXpert was used to evaluate the scaffolds' production batches regarding the porous structures and liquid flow properties through the material, as well as to carry out a first screening of scaffolds candidates prior to *in vitro* cell tests and preclinical studies in animals.

Modelling of the scaffold's properties

PoreXpert v.1.6.567 software was used to develop a 3D-network model having identical percolation properties as those of the analysed scaffolds (**Figure 1**). Raw data were obtained from scaffolds processed by USC

research group using supercritical foaming technology [1–6] and measured by mercury intrusion porosimetry (MIP)-cumulative curves using an Autopore IV 9500 model (Micromeritics, Norcross, GA, USA).

The *in silico* model consisted in a cubic structure formed by 1,000 pores (of cubic shape) connected by up to 3,000 throats of arbitrary cylindrical shape). By applying a Boltzmann-annealed simplex algorithm, the pore and throats specific characteristic were estimated and optimized, as well as the correlation level from the raw MIP data.

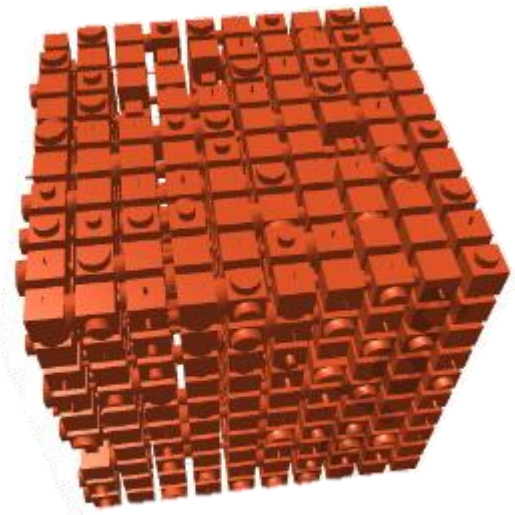

Figure 1. Representative image of the 3D-network model mimicking the analysed structures.

The water permeability estimation (25ºC, 1.03 bar) was performed by applying the equation (**Eq. 1**) and assuming a single-axis flow.

$$k_w = \frac{\pi}{8}\omega_{cell}(Farcs)\frac{l_{cell}}{A_{cell}} \qquad \textbf{(Eq. 1)}$$

where l_{cell} and A_{cell} represent the length and the cross-sectional area of the unit cell, respectively, and $\omega_{cell}(Farcs)$ is an averaging operator over the whole unit cell operating on the flow capacities of the pore throat-pore arcs

parallel to the single-axis. PoreXpert calculates the term $\omega_{cell}(Farcs)$ by means of the Dinic's network analysis algorithm.

A scheme of the workflow developed to perform the *in silico* screening of the scaffolds is depicted in (**Figure 2**).

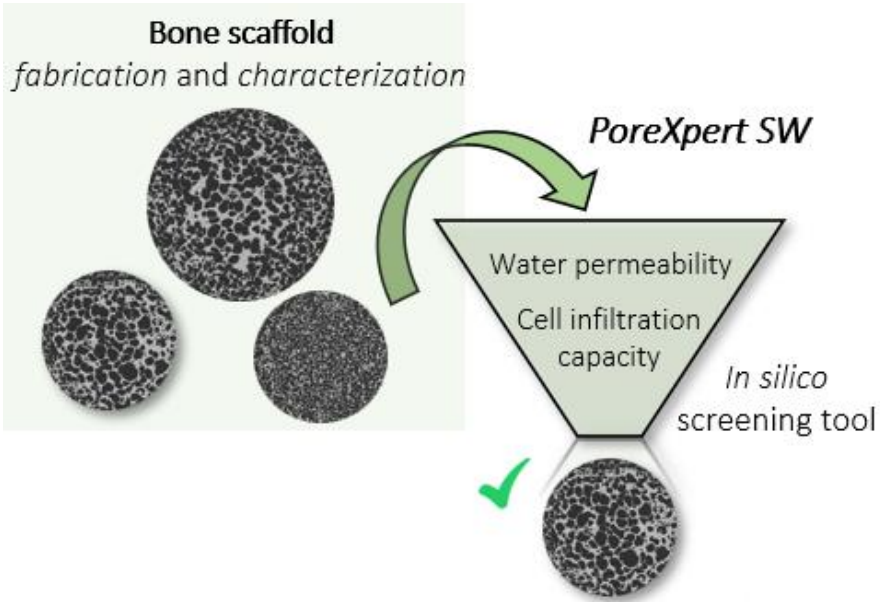

Figure 2. Scheme of the workflow developed by USC research group to perform the *in silico* screening of scaffold performance once grafted.

Under the former parameters, even slight morphological modifications on the analysed scaffolds can be detected and discrepancies on the permeability values of 1-log magnitude identified, as reported by Santos-Rosales et al. [5]. In this regard, the incorporation of additives to enhance the water permeability of the scaffolds was tested with strong correlations with *in vivo* results in murine models of calvarial defects [4].

For the suitable scaffold performance once grafted, the porous architecture must be not only interconnected, but also display throats of a suited size to avoid the retention of cells (**Figure 3**). As an example, a scaffold with 100% interconnectivity but very narrow pore throat sizes and/or intricate pore−throat−pore paths will show poor transport properties for biological fluids and cell recruitment. Therefore, the pore and throat size distributions obtained from the PoreXpert model assisted in establishing cut-off porous

properties for scaffolds with promising biological performance. Namely, the filtration module from PoreXpert software allows the simulation of the cell spread capacity in the scaffolds. Particularly, this *in silico* prediction of cellular behavior is of utmost relevance in the case of human mesenchymal stems cells (hMSCs), which exhibit multilineage differentiation and therefore, of interest in multitude of anatomical sites. In this sense, the particle size set for the simulation was defined at 26.5 ± 5 µm, corresponding to the average hMSCs value.

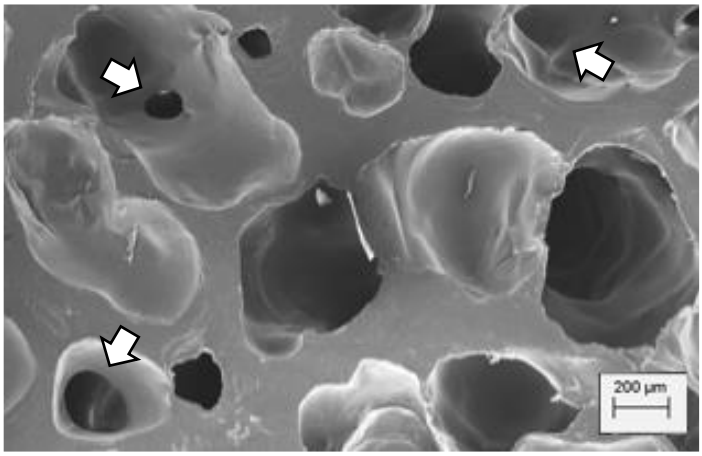

Figure 3. Scanning electron image (SEM) of bone scaffolds with interconnected pores (white arrows) required for the cellular colonization of the structures.

The filtration algorithm of PoreXpert software is based on the permeability algorithm determining the preferential flow routes. If the throat is blocked by a particle, the flow routes are recalculated. The algorithm keeps on running until all particles were filtered through the scaffold or the cell unit became clogged [2]. High cell infiltration values suggest that the developed scaffolds may have full accessibility for cells once grafted. Thus allowing a homogeneous tissue ingrowth instead of restricting it to the outer surface of the scaffold [5].

Conclusions

This application note places great value on PoreXpert software as an *in silico* screening tool for the development of scaffolds widely used in the tissue engineering field. Meaningful parameters regarding the scaffold biological performance once grafted can be predicted by a suitable choice of the software parameters. Thus, PoreXpert modelling offers a cost-effective preliminary screening of conditions before undergoing further *in vitro/in vivo* testing of the designed scaffolds. The research group from USC has a lifelong expertise in producing polymeric scaffolds for bone repair and correlating and validating results from simulated PoreXpert models into biologically relevant graft performances.

Acknowledgements

This research was funded by MICINN [PID2020-120010RB-I00], Xunta de Galicia [ED431C2020/17], Consellería de Sanidade, Servizo Galego de Saúde, Axencia de Coñecemento e Saúde (ACIS, CT850A-G), Agencia Estatal de Investigacón [AEI] and FEDER funds. Work supported by Ignicia Programme from Axencia Galega de Innovación (Xunta de Galicia, IN855A 2021/13).

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