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A Poly(vinyl alcohol) 3D Platform for the Evaluation of Hepatocytes Response to External Stimuli

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SUPPLEMENTARY INFORMATION

Determination of Scaffold Morphological Parameters

*Porosity***.** To estimate the percentage porosity (P%) of the scaffolds, images stacks were converted in binary images and inverted (so as to show as black the empty volume) and P% was estimated as the ratio of thresholded Voxels to Total Voxels of a specified region of interest (ROI of about 1-2 mm in height for a number of approximately 150-300 slices). To better characterize the porosity of the materials, also the Specific Surface Area (SSA) was calculated as the ratio of Total Surface Area to Total Volume of the sample.

Void size distribution (VSD) analysis. The pore size distribution (PSD) was estimated from μ-CT images with a 'destroy and rebuild' method. This one uses the Particle Analyser (PA) basic function of ImageJ, that analyses horizontal projections of each pore inside the matrix, and an algorithm, written in Visual Basic (Microsoft), which takes the coordinates ($Xc;Yc$) of the mass centres and the areas of the pores sections obtained with PA to reconstruct a good approximation of the whole porous matrix of the scaffold.

In our analysis, we made the assumption that pores preserve a spherical shape within final scaffolds; it can therefore be assumed that horizontal pores sections are circles and their mass centres coincide with the centres of the circles that best fit them (Figure S1).

The first step of PSD analysis consists of inverting and thresholding (making binary) a scaffold's ROI of μ-CT slices (~1-2 mm in height) in order to bring into evidence the empty volume of the matrix. Different voids inside this volume are then separated using a watershed algorithm. PA function is then run and as results it gives back information about the coordinates of mass centres ($Xc;Yc$) and the numerical value of areas of horizontal pores sections for each slice. During this procedure the correspondence between sections and three-dimensional pores is lost.

In the second step, our algorithm takes the $(Xc;Yc)_n$ (n is the slice number) coordinates of these sections and tries to reconstruct the global pore (Figure S1) grouping horizontal sections which have their coordinates between $|(X'c;Y'c)_{n+1} - (Xc;Yc)_n| \leq \delta$ (δ is an empirical parameter and its value is 3,5% of the mean pore diameter $\langle D \rangle$) (Figure S1). Sections belonging to an already reconstructed pore are not considered any more. At the end of the second step, a first approximation of the pores structure is obtained. This approximation is post-processed with a filter function to obtain a better valuation of the PSD. This filter removes from final analysis those pores situated on ROI 's edges. This is done because these pores are generally broken pores affected by some irregularity arising from the images analysis method. Finally, an equivalent volume and radius are calculated for each pore. The major advantage of this kind of analysis is the possibility to process a large number of pores, which gives statistical significance to the data obtained.

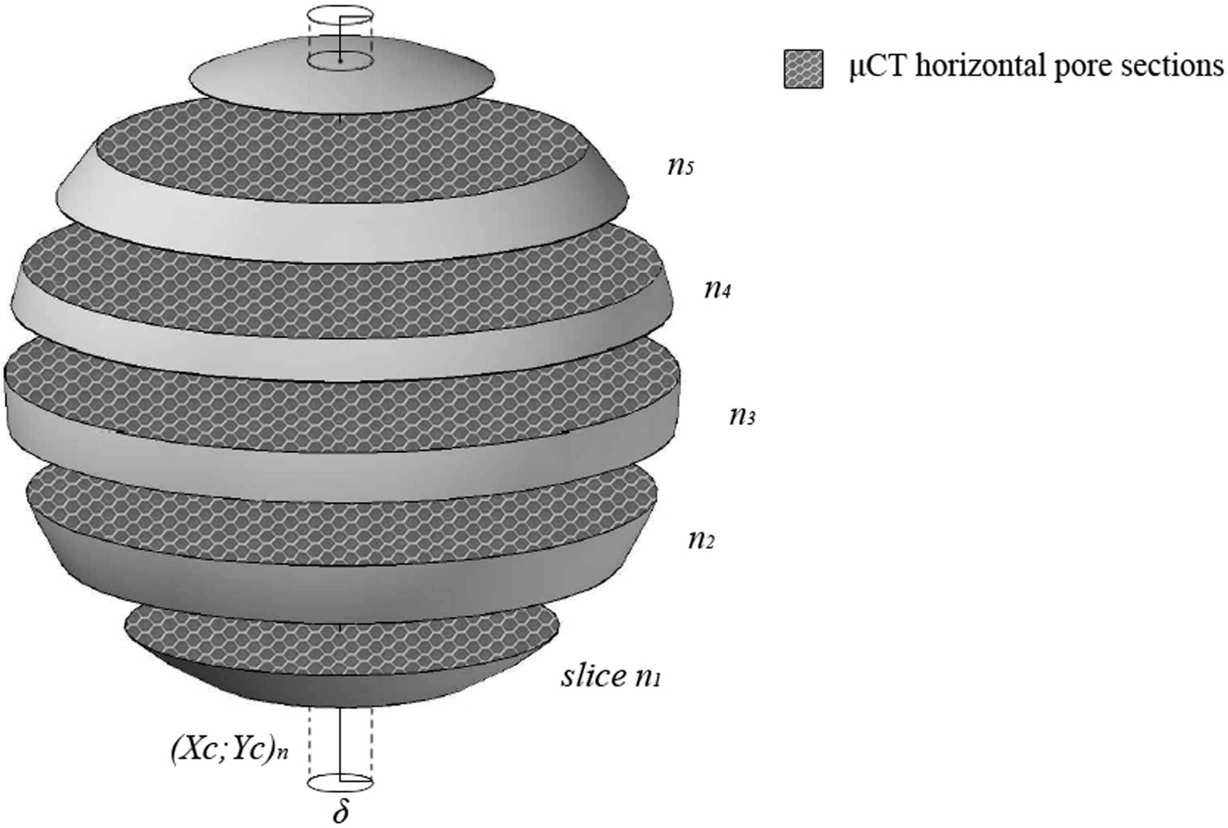

Figure S1. Graphical representation of PSD calculation method: the Analyse Particle function of ImageJ calculates the (Xc;Yc) coordinates of the mass centers of the µCT horizontal sections of the pore and the corresponding areas, while the Visual Basic algorithm reconstructs the whole pore and its volume.

Wall thickness distribution. Wall thickness was analysed with the use of the Local Thickness plugin and Colour Inspector 3D plugin of ImageJ applied on µCT images of the two samples. The Local Thickness plugin works on the 3D images stacks and computes a distance map by the Euclidian distance transformation using voids centres as reference points, and it gives back a coloured map of local wall thickness. A quantitative measure of thickness was extrapolated using the Colour Inspector 3D (CI3D) plugin on the coloured distance map (Figures S2). CI3D calculates the colour-histogram of the image with a percentage value of frequency for each colour. By creating a calibrated correspondence between a colour and a thickness value, a distribution of wall thicknesses was obtained.

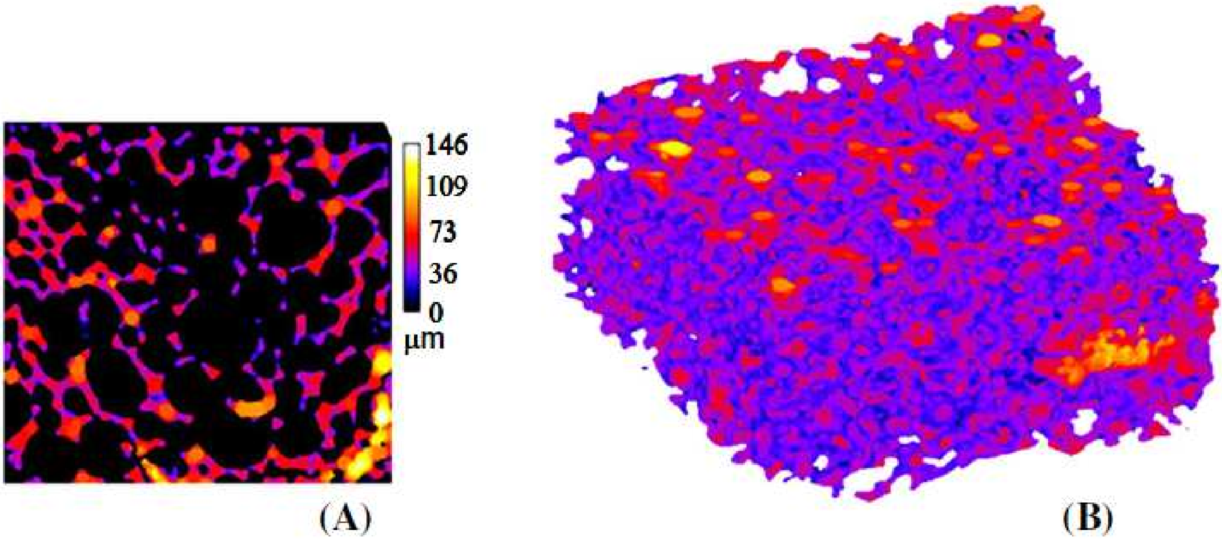

Figure S2. One slice (A) and 3D reconstruction (B) of the coloured map of wall thickness obtained for the a PVA scaffold.

Interconnect Size Distribution (ISD). Generally, this is the most difficult parameter to be extracted from µCT data, because interconnections can be placed in any spatial direction, and the horizontal projections of µCT images can lose some information. To allow interconnections measurement, a binary image of µCT slices was used (Figure 3a): the stack was inverted (to show as black pores structure, Figure 3b) and voids were separated using a watershed algorithm (Figure 3c). To obtain interconnections, the image containing the closed pores was subtracted to the inverted one. This operation gives rise for each slice, to a set of lines corresponding to the horizontal projections of interconnections (Figure 3d). In order to reconstruct the three-dimensional structure of interconnections, the Object Counter 3D (OC3D) plugin was run; this function joins interconnect projections in adjacent slices and reconstructs the corresponding surface. OC3D hence provides the areas of interconnections, from which the distribution of interconnections diameter is calculated. To take into account those interconnections that are not visible in the X-Y plane (horizontal plane) a re-slice of the stack in the ZY and ZX planes was performed, and the described passages of calculation were repeated.

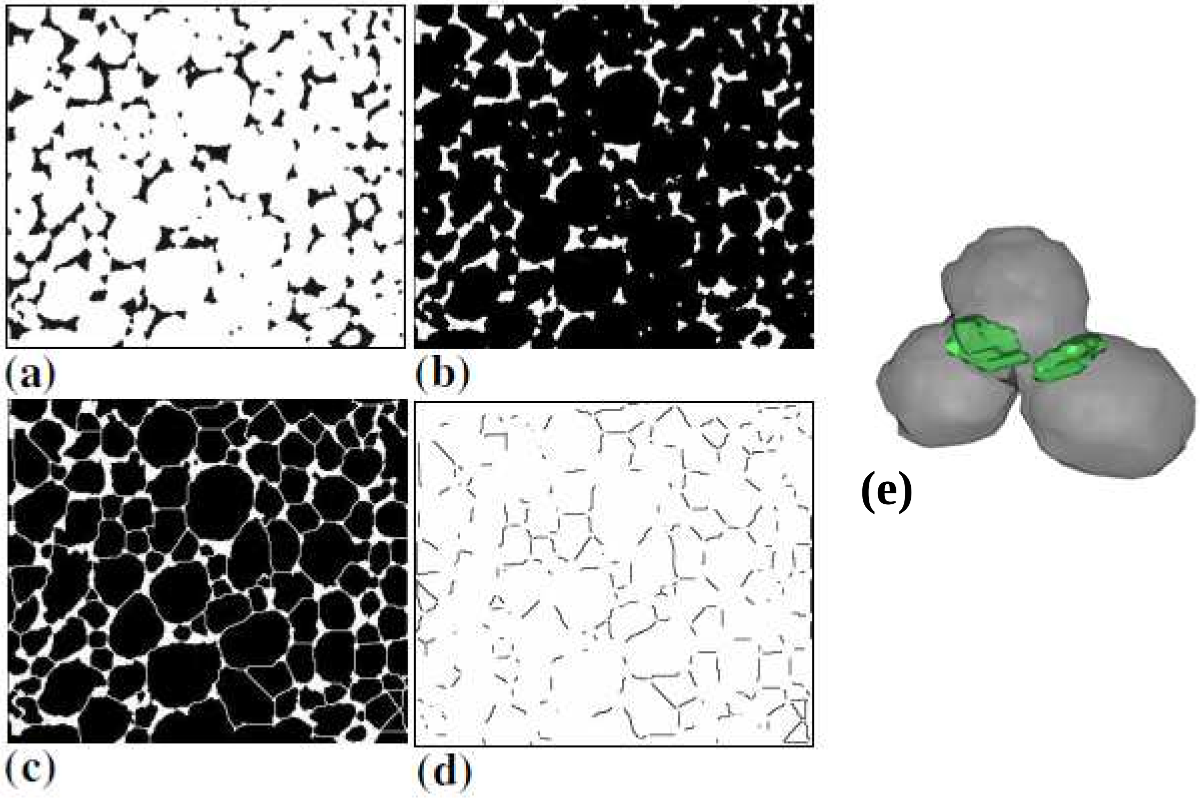

Figure S3. Example of interconnection analysis: (a) binary image of the slice; (b) inverted binary image; (c) separation of pores with watershed algorithm; (d) image difference between (c) and (b). (e) Representation of three-dimensional reconstruction of interconnections performed by Object Counter 3D.