

Micro-ct analysis of porous alginate/poly (ϵ -caprolactone)scaffolds for bone tissue engineering

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AIM OF THE STUDY

The purpose of the study was to obtain a three dimensional analysis of PCL porous scaffold with structure, porosity and mechanical properties suitable for bone cells adhesion, growth and proliferation.

METHODS

Scaffold characterization and porosity measurements

To preserve scaffold morphology all the specimens were cut in a parallelepiped (height 1 cm, side 0.5 cm) with a lancet taking special attention to obtain parallel surfaces. The upper side of each parallelepiped was marked to keep the same orientation during MicroCT acquisition and compression test. (Fig 1)

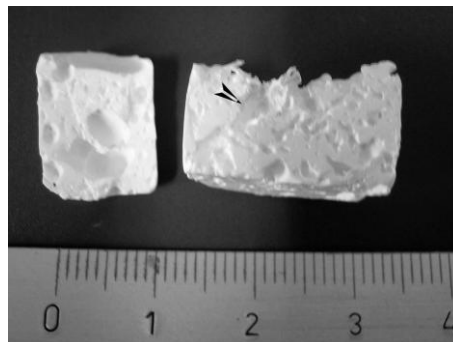


Fig 1. PCL scaffolds

Samples were scanned following standard processing procedure using Skyscan 1172 HR micro-CT (Skyscan, Aartselaar, Belgium). The MicroCT settings were used as follows: voltage 57 KV; current 167 μ A; exposition time 242 ms; Field of View (FOV) 1280 x 1024 pixel; isotropic voxel size 8 μ m. All the samples underwent a 360° rotation, rotation step 0.4 degree and a frame averaging of 1. The raw data acquired were reconstructed using N-Recon Software (Skyscan, Belgium): axial subsequent images (saved as bitmap format) were reconstructed using back projection algorithm. The bitmap images were analyzed by Ct-An Software: the selected volume of interest (VOI; 3 x 1.59 mm) was focused in the centre of the PCL scaffolds to avoid artifacts due to the cutting. All the samples were binarized using the same parameters. Porosity and pore quantitative analysis were obtained based on the structural indices measured for bone samples as reported by Hildebrand et al. (1999). Sample porosity was calculated as

$$\Phi = 1 - BV/TV$$

where Φ is the total porosity, BV is Bone Volume, TV Total Volume and BV/TV the percent of Bone Volume.

Trabecular Thickness (Tb.Th) (or pore wall) and Trabecular Separation (Tb.Sp) (or pore diameter) were computed by direct measurement.

3D images were reconstructed by CT Vol Software (Skyscan, Belgium)

Mechanical compression test

PCL/Ca²⁺ alginate threads scaffolds were then subjected to compression testing by Material Testing Stage (MTS, Skyscan, Aartselaar, Belgium) and were compressed in continuous mode. The maximum load applicable was 22 Kg with real time visualization of curve of displacement.

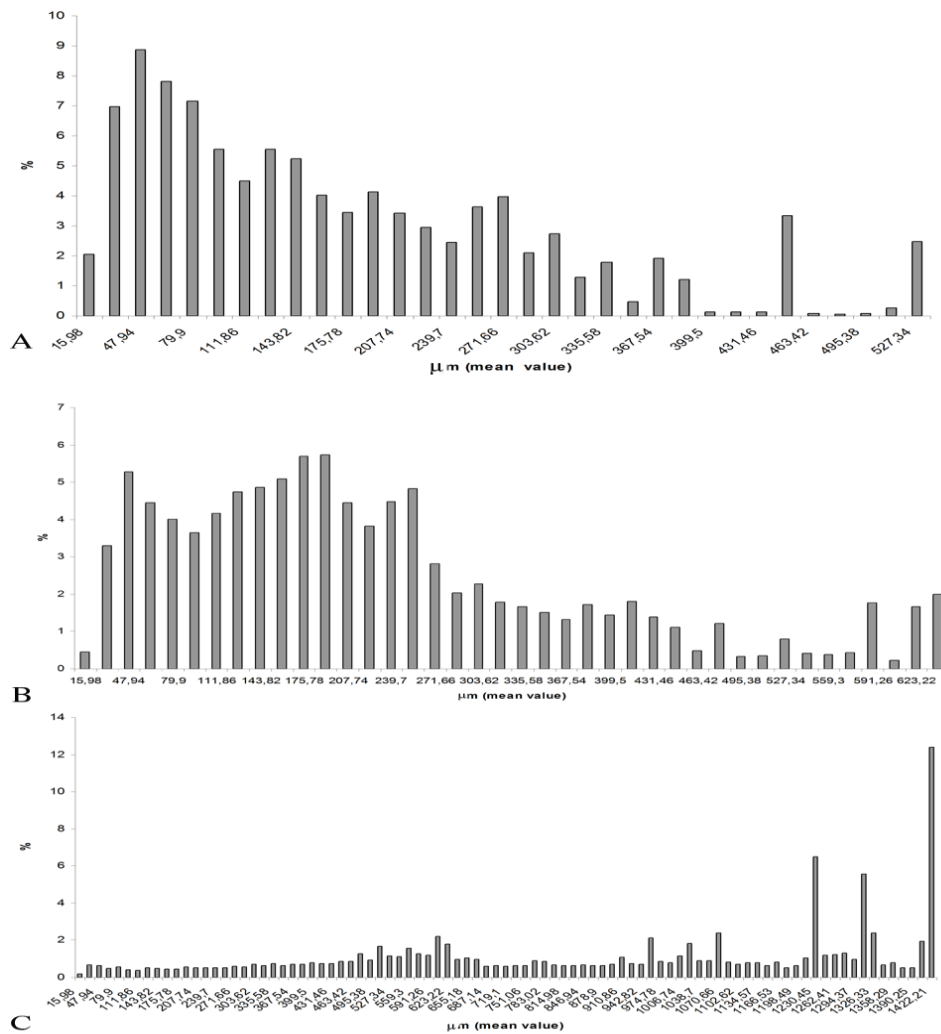
RESULTS

Micro-CT analysis revealed a porosity degree between 21% and 73% (Table 1). PCL/ 100% Ca²⁺ alginate threads scaffolds showed value of porosity and of Tb.Sp lower not only of PCL/ 200% Ca²⁺ alginate threads scaffolds, but also of natural trabecular bone. BS/BV and Tb.Th were, instead, in the range of bone values.

Table 1. MicroCT characterization of PCL/Ca²⁺ alginate threads scaffolds.

Sample	Φ	BS/BV (1/ μ m)	Tb.Sp mean value (μ m)	Tb.Th mean value (μ m)
PCL 100%	52%	0.024	231.45	91.24
PCL 200%	73%	0.018	913.22	103.01
PCL with no porogen	21%	0.017	172.61	101.54
Trabecular Bone (as reported by Hildebrand et al.,1999)	52-96%	0.007-0.034	450-1031	80-280

Φ = total porosity, BV = Bone Volume, TV = Total Volume, BV/TV = percent of Bone Volume, Tb.Th = Trabecular Thickness (pore wall), Tb.Sp = Trabecular Separation (pore diameter).



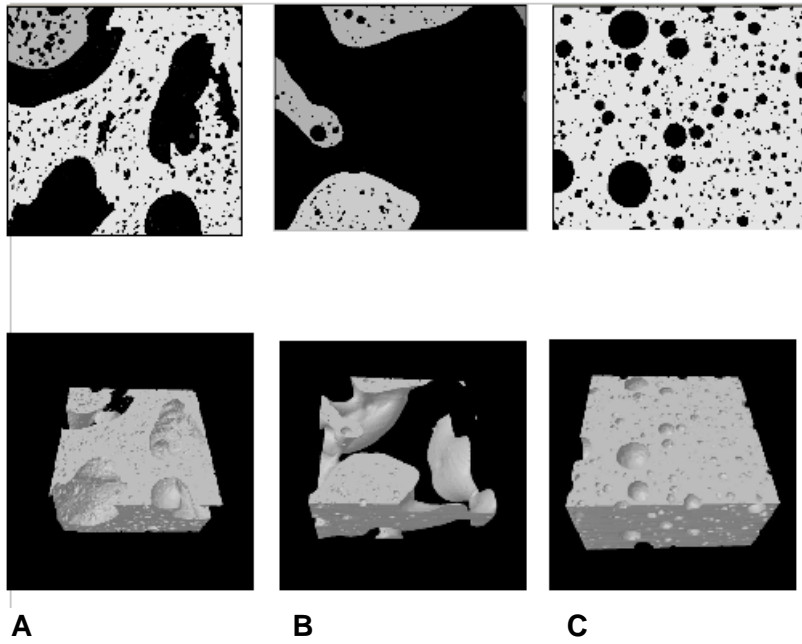


Fig 3. PCL scaffolds 2D and 3D images. (A PCL/100%Ca²⁺; B PCL/200% Ca²⁺; C PCL with no porogen)

Load displacement test showed that PCL/ 200% Ca²⁺ alginate threads scaffolds underwent the less displacement (3 mm vs 4.6 mm of PCL/ 100% and 4.5 mm of PCL with no porogen), its shift/deformation started at a less load (1.794 g; vs 3.447 g of PCL/ 100% and 3.200 g of PCL with no porogen) and its elastic recovery results to be the highest one (almost 2 mm vs 1.3 mm for PCL/ 100% and 0.6 mm of PCL with no porogen) (Fig 4).

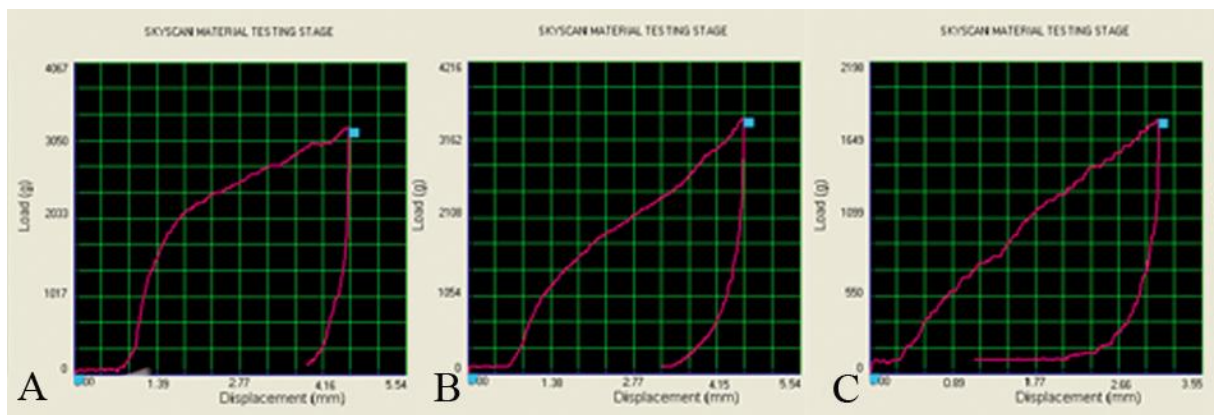


Fig 4 PCL scaffolds Load Displacement

CONCLUSIONS

Micro-CT demonstrated to be an accurate and unique method for three dimensional analysis of PCL scaffolds. Micro-CT analysis revealed that PCL/200% Ca²⁺ alginate threads scaffold was characterized by morphometric indices (values of porosity, interconnectivity and distribution of pores) very similar to natural trabecular bone (Hildebrand et al. 1999). Moreover it possessed a large (greater than the other two polymeric scaffolds) surface area, which has been demonstrated to favor cell

attachment and growth (Yang et al. 2001). Even if, it was shown that it possessed an high elastic recovery, which resulted to be unsuitable for orthopedic applications (Currey 1998, Karageorgiou et al., 2005.), it has to point out that PCL scaffolds did not possess any mineral component (which guarantee resistance at tensile strength) and the adding of hydroxyapatite or calcium phosphates could increase scaffold mechanical properties (Yu et al., 2007).

In conclusion, our results demonstrated that scaffolds with high porosity and an uniform distribution of different size pores could represent a suitable microenvironment for bone cells.

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