Evaluation of Polymeric Scaffold Morphology before and after Hydration

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**Aims**
In recent years micro-CT has gained increasing interest also for the evaluation of polymers. One of the most important parameter in tissue engineering scaffolds is biomaterial porosity. Porosity as a measure of void spaces in the materials is expressed normally in percentage. There are several methods that can be used to evaluate scaffold architectural and structural characteristics. Researchers usually use theoretical methods and Scanning Electron Microscopy (SEM), flow and mercury porosimetry, gas pycnometry and adsorption. Analysis of polymer porosity using images obtained from micro-CT acquisition allows the qualitative and quantitative evaluation of scaffolds even if they are biomechanically fragile, produced in gel and after immersion in a liquid.

Using a Micro-CT system is extremely helpful to widen the knowledge on polymeric scaffolds and bone response or carry out quality tests on products. The quality of resolution reached (in this study the pixel size was 2.5 μm) is hard to achieve by other 3D techniques.

The aim of our study was to analyze the morphology of some different polymeric scaffolds and their changes after their hydration in a physiological solution using a micro-tomographic technique. These polymers were analyzed as an example of micro-CT use on dry and wet material morphology characterization in the ambit of a larger project of the healing of bone defects treated with polymeric biomaterials.

**Method**
The following materials were scanned and analyzed as an example of micro-CT use in material morphology characterization:

- A gel (GEL) composed of a 25 % PDLLA/PGA 50/50 copolymer dispersed in 10% poly-ethyl-glycol 1500 (PEG), 21% PEG 600 and 27% PEG 400, 15% Dextran and 2% H₂O matrix. It was purchased sterile in a 1-ml syringes.
- A scaffold (BLOCK) composed of 20 % PDLLA/PGA 50/50 copolymer dispersed in 20% poly-ethyl-glycol 1500 (PEG), 20% PEG 600 and 40% PEG 400 matrix. It was produced in a block-formulation provided as rods, 6 mm in diameter and 4 mm in height.

The specimens were scanned with the high resolution micro-CT system Skyscan 1172 at different source voltages and corresponding source current without using any filter between source and detector.

The gel-formulation material (GEL) was placed in eppendorf tubes. Block-formulation material (BLOCK) was scanned by directly positioning it in the acquisition chamber. After the acquisition of the dry materials, each biomaterial was put in an eppendorf test-tube and a few ml of physiological solution was added to complete immersion. They were left in immersion for 72 hours to allow the solution to soak the material completely and reach certain homogeneity along all the volume.

All the materials where scanned at 60 kV of source voltage and 167 μA, with a rotation of the specimen of 180 degrees and a rotation step of 0.30 degrees. The image pixel size was 2.5
μm and the scan duration was nearly one hour every specimen (s/w Skyscan 1172 version 1.5 build 8). The reconstructions were performed using s/w NRecon (version 1.6.0.3) and the jpg images had 4000X4000 pixels with a pixel size of 2.5 μm. No corrections were used except for the specific misalignment for each acquisition and an accurate ring artifact reduction because of the small rotation step and the small pixel size. The analyses were performed using s/w CTAnalyser (version 1.9.2.5) and were elaborated on the same cylindrical Volume Of Interest (VOI) traced from a circular Region Of interest (ROI) of 2 mm in diameter copied for 546 cross-sectional slices corresponding to nearly 1.4 mm that can be considered as the height of the cylinder. The VOI was positioned inside each specimen so that it was totally enclosed. A binarization of the VOI was subsequently carried out with a global threshold considering as object (white colored binarization) the pores. After the binarization both 2D and the 3D parameters were calculated. Three specimens of each type of material were scanned and analyzed. The results are the average of the analyses on each sample with the relevant standard deviation.

**Results**
The 2D and 3D analysis are reported in table 1.

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<tr>
<th></th>
<th>2D</th>
<th>3D</th>
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<tbody>
<tr>
<td></td>
<td>total porosity</td>
<td>total porosity</td>
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<tr>
<td>Gel</td>
<td>13.84 ± 2.79%</td>
<td>13.12 ± 2.52%</td>
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<tr>
<td>Gel + saline solution</td>
<td>7.25 ± 4.30%</td>
<td>6.70 ± 3.99%</td>
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<tr>
<td>Block</td>
<td>49.17 ± 2.75%</td>
<td>48.16 ± 2.80%</td>
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<tr>
<td>Block + saline solution</td>
<td>41.63 ± 2.60%</td>
<td>40.67 ± 2.59%</td>
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Table1 - 2D and 3D total porosity data of the materials before and after 72 hours of saline solution.

The parameter of the ratio between the volume of the maximum pore 3D detected and the total volume of all the pores detected in 3D can suggest an evaluation of the global connectivity of pores. The porosity percentage shows decreases (GEL) or preservation (BLOCK) of the empty space values. The micro-CT images of all materials before and after hydration are depicted in figure 1 and 2. In figure 3 are depicted the 3D models of the cylindrical VOI's of the materials before and after the hydration.
Figure 1- Three spatial planes of GEL before (left) and after hydration (right)

Figure 2- Three spatial planes of BLOCK before (left) and after hydration (right)

Figure 3- Three-dimensional models of the materials: (a) GEL; (b) GEL + physiological solution; (c) BLOCK; (d) BLOCK + physiological solution
Conclusion
One advantage of the non-destructive micro-CT technique is the possibility to evaluate the deviations in porosity of a material depending on the environment it is in. Many techniques are used by researchers and each of them have some limitations \(^1, 4, 5\). Therefore, micro-CT is not expected to be the only methodology for the thorough qualitative and quantitative analysis of the microstructure of the polymeric scaffold but the perfect pre-clinical investigation on scaffolds may require the use of different techniques in a complementary way \(^6\).

The biomaterials studied as scaffolds for tissue engineering or prosthesis surgery, such as spacers or bone fillers have to interact with a human or animal physiological environment. In the preclinical phase of research, besides the normal *in vitro* steps to test cytotoxicity etc, it would be useful to know the change in porosity of the material in contact with liquid solutions.

An image analysis by micro-CT offers wide ranging possibilities and opportunities and thus can be considered as a key method for qualitative and quantitative evaluation of innovative biomaterials.

References: