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Integration of high-resolution micro-computed tomography in the quality control of 3D-printed scaffolds

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Abstract – Additive manufacturing, and among all stereolithography, has shown great potential in the field of biomedical science. This technology offers a relatively simple means to manufacture objects with complex external and internal features and high spatial resolution, compared to more traditional manufacturing methods. Successful clinical reports of patient-specific implants manufactured using 3D printing foster great promises, particularly in the field of craniofacial reconstruction. However, along with the ability to produce highly defined prostheses, the community still needs tools to ensure the quality of the final products. In this study, we demonstrate that high-resolution micro-computed tomography is an efficient system to control the quality of a 3D-printed structure. As a proof of concept, using stereolithography-printed microscaffold scaffolds with various mineral content, we showed that micro-computed tomography allows quantification of shape and morphometry of the specimens; it allows mapping differences between the 3D-printed scaffolds and their original Computer-Aided Design models, and can quantify the influence of the mineral content on the structures. As a perspective, the development of micro-computed tomography quality control routines would help ensuring the quality of implants preoperatively, and as such, has the potential to improve surgical outcomes.

Keywords – micro-computed tomography, 3D printed scaffolds, quality control, patient specific implant, stereolithography

1. INTRODUCTION

Additive manufacturing (AM) technologies for tissue engineering and regenerative medicine have recently emerged with great promises to traditional alternatives, such as graft treatments. [1] The last advanced AM techniques for tissue engineering include 3D (bio-)printing, rapid-prototyping (i.e. stereolithography, SLA), solid-free form fabrication, selective laser sintering and fused-deposition modelling [1]. AM allows the production of customized implants using patient’s own preoperative data, which is of tremendous interest in traumatology (e.g. craniofacial reconstruction [2]). AM also allows highly complex internal structures to be produced and tested in a very timely fashion.

Despite the worldwide enthusiasm for patient-specific 3D-printed implants, routine techniques for characterizing manufacturing precision are still lacking. First and foremost, one needs to compare the physical dimensions and geometries of the implants to the Computer-Aided Designs (CAD), since deviations from the CAD design may lead to misfits during reconstructive interventions. Additionally, the 3D-printed materials are often biofunctionalized; for example, in orthopedics by mixing nano-hydroxyapatite in the resin to make the implants osteo-promotive. But variations in resin/particles formulation may not only change the implant’s biological properties, but can also directly influence its homogeneity and/or geometrical properties, such as shape, porosity, surface area, etc.

We propose the use of high resolution micro-computed tomography (microCT) as a quality control tool for 3D printed implants. MicroCT has become a relatively mainstream technology in biomedical research laboratories, which would facilitate its standardization as a quality control tool. Standard desktop microCT scanners can scan objects with a wide range of dimensions, from 10⁻³ to 10⁻¹ m at a wide range of resolutions, from 10⁴ to 10⁻² m; and can be operated in batch modes for high-throughput screening.

Here, to illustrate this novel approach, the morphological characteristics, homogeneity and degree of similarity to the initial CAD model was derived from microCT scans of fifteen 3D-printed scaffolds.

2. MATERIAL AND METHODS

In brief, twenty microporous polymeric scaffolds were 3D-printed from the same source CAD design, but using three different resin compositions varying in mineral content. The scaffolds were microCT-scanned. The macrostructure’s morphometry was derived from the image data and compared
to that of the source file. Image data were then aligned in space and variability within groups were assessed and mapped.

Resins preparation

Three-armed poly(trimethylene carbonate) (PTMC) was synthesized by ring-opening polymerization of trimethylene carbonate (TMC, purchased from Huizhou Foryou Medical Devices Co. Ltd., China) initiated by trimethylolpropane (from Fluka analytical). Briefly, in a molar ratio of 96.8/1, TMC/trimethylolpropane was loaded in a flask under dry N2 atmosphere. Using 0.13 wt. % of Tin(II) ethylhexanoate (Sn(Oct)2, from Sigma-Aldrich) as a catalyst, the polymerization was conducted at 130 °C for 3 days. The formed PTMC (number average molecular weight of 10 kg/mol as determined by 1H-NMR [3]) was thereafter dissolved in dichloromethane under dry N2 atmosphere. In order to obtain a photo-crosslinkable compound, PTMC macromer with methacrylate end-groups (PTMC-MA) was subsequently prepared by reacting PTMC hydroxyl groups with methacrylic anhydride (from Sigma-Aldrich), as described, in order to obtain a degree of functionalization of 99 % [3, 4].

| TABLE I: RESIN FORMULATIONS USED FOR STEREOLITHOGRAPHY (IN WT. %) [4]. |
|-----------------|----------------|----------------|
| PTMC            | PTMC 20        | PTMC 40        |
| nHA             | 0.0            | 9.1            |
| propylene carbonate | 40.0          | 54.5           |
| Lacrim® TPO-L photo-initiator | 5             | 5              |
| Orasol Orange G dye | 0.15          | 0.10           |
|                 | 0.08           |                |

Three different resin formulations were prepared with a varying nano-hydroxyapatite content (nHA, supplied by Xpand Biotechnology BV, Bilthoven, The Netherlands) in order to fabricate structures with 0, 20 and 40 wt. % nHA (weight percentage in relation to PTMC-MA), named PTMC, PTMC 20 and PTMC 40 respectively (Table I).

Scaffolds design and preparation

The scaffolds were designed using K3DSurf and Rhinoceros3D (k3dsurf.sourceforge.net, www.rhino3d.com respectively) with dimensions 6 × 3.5 mm (diameter × height), and given a gyroid internal morphology with minimal pore size of 600 μm, porosity of 70 % and interconnectivity of 100 % (Figure 1).

The 3D macroporous scaffolds were manufactured from the photo-crosslinkable resins using an Envisiontec Perfactory® SXGA® Standard UV stereolithography system (SLA). A light intensity of 180 mW/dm2 was used with an irradiation time of 9 seconds per layer and the platform was elevated by 50 μm between each layer.

Scaffolds characterization

First, in order to characterize the precision of the SLA technique compared to the CAD model, the CAD model (Figure 1) was transformed into DICOM images with AMIRA and imported into the microCT database. Subsequently, SLA-fabricated scaffolds were scanned with a cabinet cone-beam microCT (µCT100, SCANCO Medical AG, Bruttisellen, Switzerland) with the x-ray tube operated at 70kVp/57μA, 500 ms acquisition time, 3000 projections/360°. Two-dimensional CT images were reconstructed into 3072 × 3072 pixel matrices with 3.3 μm nominal resolution.

It was first Gaussian-filtered (σ = 0.5, support = 1) to reduce noise. The scaffolds were then segmented using material specific global thresholds (PMTC: 0.24, PTMC20: 0.64 and PTMC40: 1.28 cm–1 respectively) to account for the difference in x-ray absorption. For each scaffold, morphometry and material density were assessed the volume and density of the whole object using direct voxel counting algorithms and the structural morphometry within a 5 × 2 mm region of interest (ϕ × h) to account for boundary effects using a standard volumetric spatial decomposition algorithm [8]. The impact of HA concentration on the printed geometry was assessed by performing an ANOVA analysis on the morphometric factors in function of the mineral concentration. P < 0.05 was considered significant. Finally, the segmented images were co-registered with the CAD designs, using rigid registrations. The aligned images were subtracted and the differences were mapped, the correlation factor between scan and CAD models were computed.

All image processing was performed using IPL (Scanco Medical AG) and statistics with R (www.r-project.org).

3. RESULTS

A 3D macroporous scaffolds with gyroid-like porosity was designed and subsequently twenty units were SLA-fabricated for this study. Five scaffolds were excluded because of damage occurring during handling, resulting in n=5/group. The scaffolds included in the study showed macroscopically similar shapes and porosity. As shown in Figure 2, besides colour, the differences between scaffolds of different groups were limited and certainly challenging to quantify visually.
Qualitative characterization of the scaffolds with microCT scanning showed that geometry and porosity of the different groups were relatively close to the CAD design but revealed subtle differences compared to the CAD design and between the groups (Figure 3). PTMC scaffolds had more structural distortions (1st & 2nd rows), probably resulting from the lower elastic modulus of the resin. PTMC 40 had pronounced “layering”, probably because of slower setting dynamics with higher nHA content. Variations in height were also observed (3rd row). Finally, with 40 wt. % nHA, we observed the formation of a rougher surface and pore inhomogeneity.

The co-registration analysis allowed to visually mapping the macroscopic analogies or discrepancies between scaffolds and the CAD design (4th row).

Interestingly, in these 3D-printed scaffolds, the mineral fraction had a significant influence on all volumetric indices. Volume and surface increased proportionally to nHA content, while porosity decreased. But with post-hoc analysis, only the PTMC group differed significantly from the CAD model, and only in terms of total surface. Although PTMC scaffolds were smaller than PTMC 20 and 40, their porosity matched the CAD model best (0.73 vs 0.7). Four out of six structural indices were significantly affected by the mineral content. The structure was thicker with mineral-containing scaffolds, and the spacing smaller. The degree of anisotropy was not affected, showing that the symmetry of the scaffolds is well preserved independently of the resin composition. Pore connectivity was preserved throughout all groups. In terms of structure, PTMC scaffolds also matched CAD best. Finally, the coefficient of correlation to the CAD model was only marginally affected by nHA content, suggesting that the various changes in volume and structure compensate each other overall.

TABLE II: QUANTIFICATION OF IMPORTANT ARCHITECTURAL AND PHYSICOCHEMICAL PARAMETERS OF SLA-SCAFFOLDS. DATA IS SHOWN AS MEAN (SD)

<table>
<thead>
<tr>
<th>Index</th>
<th>CAD</th>
<th>PTMC</th>
<th>PTMC 20</th>
<th>PTMC 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/V [mm²]</td>
<td>6.46</td>
<td>7.34(0.62)</td>
<td>5.54(0.29)²</td>
<td>5.56(0.38)²</td>
</tr>
<tr>
<td>DA</td>
<td>1.14</td>
<td>1.12(0.03)</td>
<td>1.15(0.044)</td>
<td>1.08(0.03)</td>
</tr>
<tr>
<td>S [mm²]</td>
<td>86.53</td>
<td>63.7(7.7)</td>
<td>80.96(2.04)²</td>
<td>91.87(0.06)²</td>
</tr>
<tr>
<td>v [%]</td>
<td>0.7</td>
<td>0.73(0.03)</td>
<td>0.61(0.02)²</td>
<td>0.57(0.06)²</td>
</tr>
<tr>
<td>V [mm³]</td>
<td>13.5</td>
<td>8.83(1.66)</td>
<td>14.71(1.34)²</td>
<td>16.7(2.22)²</td>
</tr>
<tr>
<td>Tb.th [µm]</td>
<td>0.31</td>
<td>0.27(0.02)</td>
<td>0.36(0.02)²</td>
<td>0.36(0.02)²</td>
</tr>
<tr>
<td>Tb.Sp [µm]</td>
<td>0.71</td>
<td>0.75(0.06)</td>
<td>0.56(0.03)²</td>
<td>0.49(0.08)²</td>
</tr>
<tr>
<td>d [mg/ccm]</td>
<td>na</td>
<td>-37.6(2.8)</td>
<td>238(11)²</td>
<td>523(10)²</td>
</tr>
<tr>
<td>ζ</td>
<td>1</td>
<td>0.77(0.04)¹</td>
<td>0.77(0.07)¹</td>
<td>0.77(0.04)¹</td>
</tr>
</tbody>
</table>

Lexicon:
- V: Volume, p: porosity, S: surface, DA: degree of anisotropy, SMI: structural model index, Con.D: connectivity density, Tb: trabecular, N: number, th: thickness, Sp: separation, D: mineral density, ζ: correlation coefficient to CAD, ANOVA: *: p<0.01, §: p<0.05; Tukey post-hoc tests: ¹: Different from CAD, ²: from PTMC, ³: from PTMC 20

The 3 µm-resolution microCT slices (Figure 4) revealed that the mineral fraction was not homogeneously distributed within the resin but that aggregates of maximum 50 and 100 µm in diameter were present in PTMC 20 and PTMC 40 respectively. Furthermore, a dense layer of <10 µm in thickness appeared at the surface of the PTMC 20 scaffolds, probably as a consequence of the manufacturing processes.

MicroCT analysis also offer the possibility to directly derive the morphometrical indices of the scanned structures using the algorithms originally developed for characterization of bony structures (hence the nomenclature). Table 2 shows the values and statistics for volumetric and structural indices, as well as the material mineral density and the coefficient of correlation to CAD.
4. DISCUSSION AND CONCLUSIONS

Additive manufacturing technologies hold great promises in the biomedical field, including bone tissue engineering. Indeed, medical applications represent a fertile market for 3D printed devices, and is expected to reach a colossal business of $2 billion in the next decade [5]. The driving force of these technologies relies on the possibility to manufacture customized implants with an unprecedented degree of complexity. For instance, BioArchitects (New York, USA) has just won a 510(k) FDA clearance for his 3D printed patient-specific titanium-based craniofacial implant; meanwhile Anatomics (Victoria, Australia) reported in 2014 a first skull replacement by an artificial 3D printed acrylic-made prosthesis in a patient [2]. But market growth based on complex medical products require thorough quality standards to be viable on the long-term, since quality issues can have dramatic consequences for both patients and businesses.

Here, we introduce microCT scanning as a quality control tool; and as a proof of concept, we characterized the manufacturing quality of bone scaffolds manufactured by stereolithography (SLA). SLA is one of the fastest high resolution prototyping systems [1]. This was shown recently by Schüller-Ravoo et al. [7], who reproduced an anatomically-relevant microvasculature network, which remained extremely challenging to approach with classic techniques such as gas forming, salt leaching or freeze-drying. In contrast to other AM technologies, SLA is only applicable to photopolymers, and consequently, we used a resin of PTMC macromers terminated with methacrylate groups and evaluated the impact of varying the resin’s mineral content on the quality of the fabricated scaffolds, both qualitatively and quantitatively.

Although visually similar, significant differences were found both compared to the CAD design and between the experimental groups. Remarkably, out of fourteen parameters, only two were not significantly affected by the HA content.

MicroCT derived parameters are notoriously sensitive to user-defined ROIs. But here, using an image registration approach, a single ROI was propagated throughout the entire sample of scaffolds, ensuring that parameters’ variations were either due to manufacturing processes or from material differences. Noting that the coefficients of error are relatively low (0.1 ± 0.05) for all groups independently of the parameter, we can confidently link the variations in structure to the variations in mineral content.

Finally, the image registration and overlap approach could be of great interest in the field of patient specific implants, as a means to certify a printed structure perfectly fits the anatomical defect of the patients before undergoing reconstructive surgery.

In conclusion, 3D printing is currently revolutionizing numerous industrial fields along with personalized medicine. However, in order to successfully reconstruct geometrically-challenging anatomical defects, patient-specific implants must be manufactured with great precision. We propose here to integrate high resolution microCT as a practical tool for quality control. MicroCT allows for comparing the fabricated implants with the original designs with great details; it allows controlling for variations within batches, and quantifying the impact of processes or material changes on complex structures. Such new control method is necessary to prevent implant misfit due to inappropriate geometry. As perspective, a routine quality control using microCT along with defined metric standards could benefit the patient-specific implant industry.

5. REFERENCES


6. ACKNOWLEDGMENTS

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