NON-DESTRUCTIVE CHARACTERISATION OF THE INFLUENCE OF SURFACE MODIFICATION ON THE MORPHOLOGY AND MECHANICAL BEHAVIOUR OF RAPID PROTOTYPED Ti6Al4V BONE TISSUE ENGINEERING SCAFFOLDS

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Abstract
Bone tissue engineering (TE) is a multidisciplinary field of science that puts efforts in designing and developing smart constructs for the healing of large bone defects. Achieving controlled surface properties is also essential in the design and production of biocompatible implants [1,2]. The constructs mostly consist of a combination of a porous structure, based on biocompatible materials, with cells. They should support cell seeding, proliferation and differentiation, and be suitable for *in vivo* implantation [3]. Interaction between the metallic implants (scaffolds) and the surrounding biological environment depends mostly on the surface properties, i.e. surface chemistry and surface topography. In TE, the tendency is to evolve from the use of open porous foams with a random structure to scaffolds with a complex, but highly controllable designed morphology that can be useful for the production of a new generation of bone implants [3,4]. Selective laser melting (SLM), a relatively young rapid prototyping technique, offers the opportunity to produce micro-porous structures with morphological properties that are not random, but highly controlled through robust computer design. Micro-CT based characterisation of the scaffolds can be used for the optimisation of the design, the modelling and the production and, in the final stage, for the improvement of the properties of the scaffolds that are to be applied for bone regeneration.

1. Introduction

Bone tissue engineering (TE) is a multidisciplinary field of science that puts efforts in designing and developing smart constructs for the healing of large bone defects [1,2]. These constructs mostly consist of an open porous structure based on biocompatible materials combined with cells to support local cell delivery and *in vivo* implantation [3]. In TE, the tendency is to evolve from the use of open porous foams with a random structure to scaffolds with a complex, but highly controllable designed morphology useful for the production of a new generation of bone implants [3,4]. Selective laser melting (SLM), a relatively young rapid prototyping (RP) technique, offers the opportunity to produce micro-porous structures with global morphological properties that are not random, but highly controlled through robust computer design [4,5]. Many different parameters influence the cell behaviour within a scaffold and the strut surface roughness (SSR) is one of them [6]. Despite the advantage of SLM to allow a high control of the morphology at the mesoscale, at this moment functional constraints caused by working close to the technical limits of the production device prevent production of 3D porous scaffolds with a desired and controlled surface morphology at a cell-relevant level (microscale). Therefore, a modification of the as-produced SSR is needed to support the desired cell response, based on cell behaviour modelling and direct *in vitro* biological experiments [6]. It is obvious that any surface modification performed after production will change the topography of the struts surface as well as the local and global mechanical properties of the structure. Thus, the influence of the applied struts surface roughness modification (SSRM) procedures on the morphology (both meso- and microscale) and the mechanical behaviour of the scaffolds needs to be determined. In this study, the struts surface of open porous Ti6Al4V scaffolds produced by SLM [5] has been modified by chemical and electrochemical polishing. The characterisation of the mechanical behaviour of the tested micro-porous structures prior to and after surface modification is performed by the combined use of microfocus X-ray computed tomography (micro-CT), in-situ mechanical loading and advanced 3D images analysis [4,7]. For determining the influence of the SSRM procedure on the struts roughness, a non-destructive SEM image-based measurement protocol is applied. The quantification of the scaffold morphology as produced and after surface modification is done by micro-CT. The main goal of these experiments is the characterisation of the 3D SLM porous structures prior to and after surface modification in order to optimise the design, the modelling and the production of the porous structures and hence to improve the properties of the porous structures i.e. scaffolds to be applied for bone regeneration.

2. Materials and methods

2.1. <u>Materials</u>

Open porous bone scaffolds produced by selective laser melting (SLM) of bio-inert Ti6Al4V powder were used in this study. The metal powder (fig.1) was provided by Concept Laser GmbH, Germany [5]. The powder particles had a spherical shape with sizes distributed between 25 and 45 µm. The scaffolds were designed by using Magics software [Materialise NV, Haasrode, Belgium] and produced on a non-commercial SLM machine [5,8] equipped with Yb:YAG fibre laser with beam spot size 80 µm and a maximum power 300 W on the powder bed [5]. The designed unit cell had a diamond shape like structure (fig.2a and d) with strut and pore size of 0.1 mm and 1 mm respectively. The tested samples were built, in a closed and argon flushed chamber, layer-by-layer using a metal powder layer thickness of 30 µm. The scan speed was 260mm/s at 40W of laser power. After melting the powder should have a density of 4.42 g/cm^2, a stiffness of 110 GPa and a yield strength of 920 MPa. The diameter and height of the samples presented in fig.2 was 6 ± 0.5 mm and 12 ± 0.5 mm respectively.

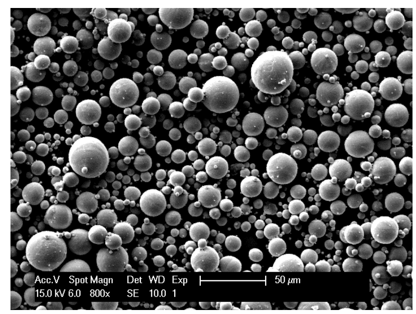


Figure 0. Scanning electronic microscope (SEM) image of the Ti6Al4V powder used for the SLM production of the porous scaffolds.

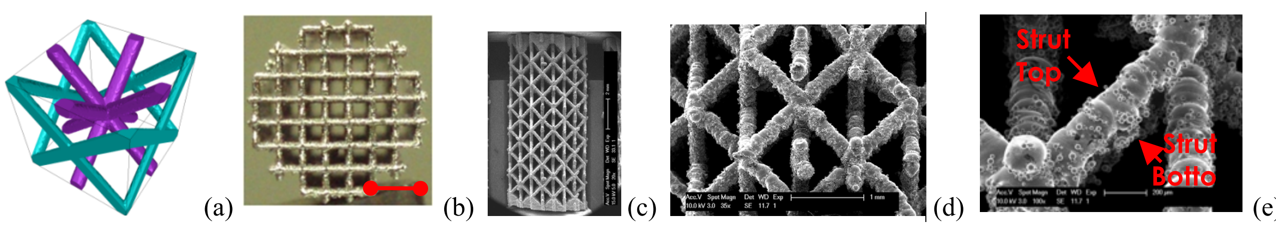


Figure 2. (a) The designed unit cell of the Ti6Al4V porous structures, (b) the top view of a typical SLM produced Ti6Al4V porous and by light microscopy and (b) a complete scaffold, (d) Typical unit cells, and (e) a single strut where non-melted powder grains are clearly present on the top and bottom surface. (fig 2.a scale bar = 2 mm)

2.2. <u>Surface roughness modification</u>

Scanning Electronic Microscope (SEM) based investigation of the struts surface of the SLM-produced Ti6Al4V scaffolds (fig. 2b,c) revealed a large and highly inhomogeneous roughness caused by non–melted powder grains attached to the surface (fig. 2e). Greater roughness was observed in the bottom part of the struts (fig. 2e) according to the building direction applied during production of the scaffold by SLM. In order to optimise the strut surface topology, an appropriate procedure for roughness modification, in this case reduction should be applied to the as produced scaffolds. For this purpose a combined chemical and electrochemical polishing procedure has been performed. During chemical polishing the samples were immersed for 10 minutes in a chemical solution based on hydrofluoric acid HF (Riedel-de Haën, Germany, p.a. 48%). This procedure allows removing all the non-melted attached powder grains from the scaffolds surface. As a second step, smoothening of the strut surface has been performed by electrochemical polishing. The experimental setup is shown in figure 3. The electrolyte, based on the "in-house" optimised chemical solution, was stirred during polishing with a magnetic stirrer. Using the cylindrical platinum basket as a cathode (fig.3) allowed to obtain more homogenous conditions for polishing so that 3D open porous structures with complex architectures can be polished. The combination of chemical and electrochemical polishing allowed to modify the strut surface in a controlled way and hence smooth surfaces can be obtained (fig. 4b) as well as nanopits-like morphologies (fig. 4d) [9,10,11].

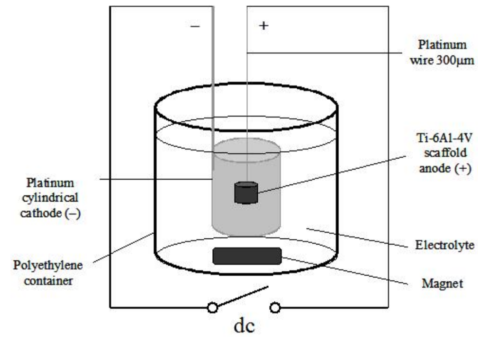


Figure 3. The experimental setup built for the electrolytic polishing of the Ti6Al4V porous structures.

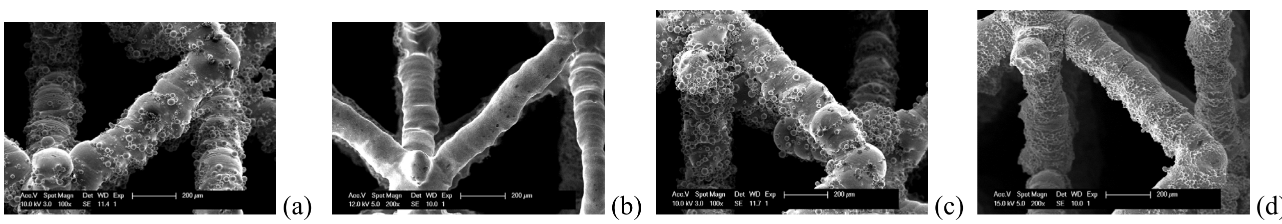
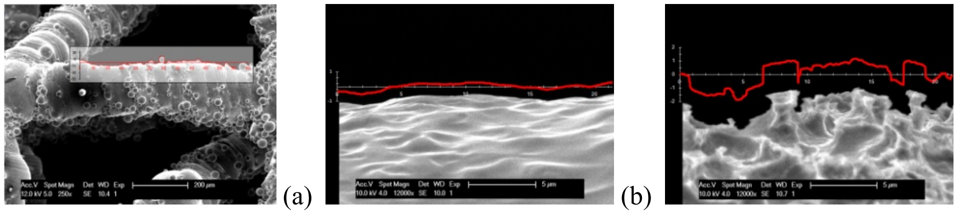


Figure 4. SEM images of a typical strut of the open porous Ti6Al4V scaffold produced by SLM prior to surface modification (a,c) where non-melted powder grains are present on the surface and after surface modification, showing a smooth surface (b) and nanopits like surface (d).

2.3. Strut surface roughness measurement protocols

As the surface roughness determines the microscale morphology of the scaffold surface in quantitative way [12], appropriate surface topology analysis of the 3D porous structures is needed. Since commercially available profile measuring systems fail when determining the SSR of the advanced porous structures, and because a high surface roughness of the struts compared to their dimensions causes difficulties for the quantitative determination of the surface morphology, new protocols for a quantitative analysis of the surface morphology are proposed on the basis of scanning electron microscope (SEM) images. For this purpose, a SEM image-based protocol for strut surface roughness measurements has been developed. The roughness was determined on the basis of 2D digital images where the profile line of the tested strut surface has been selected and used for the calculations. As the images of the struts were taken with a Philips XL40 SEM which requires no sample preparation, the measurements could be performed in a non-destructive way.



(a) (b) (c)

Figure 5. SEM images of a single strut showing the as-produced surface roughness on the global strut scale (a) and the local roughness of the struts after surface treatment showing a very smooth surface (b) or nanopits (c).

The 2D images of the scaffolds were binarised with Matlab software (MathWorks™) and the profile lines of the struts edges were selected. In the next step, images (bitmap format) were processed with Chropek.jar software (developed by Łukasz Sznajder, Poland) which extracts the pixels distribution of the profile lines. The data obtained in this way was transformed to Microsoft Excel in order to calculate the following roughness parameters:

- the arithmetic average deviation – $R_a = \frac{1}{n}\sum_{i=1}^{n}|y_i|$
- the root mean square deviation of the roughness profile from the mean line – $R_q = \sqrt{\frac{1}{n}\sum_{i=1}^{n} y_i^2}$
- difference between highest peak and deepest valley – $R_T = R_P - R_V$

where n = number of data points in X direction, y = the surface height relative to the mean plane, R_P = the highest point and R_V = the lowest points in the evaluation length.

2.4. μ-CT based characterisation

Micro-CT analysis of the scaffold morphology prior to and after surface modification was done by using the Philips HOMX 161 x-ray system with AEA tomahawk CT software. The applied acquisition parameters are presented in table 1. Morphological parameters were determined using commercially available image analysis software, namely CTAn (SkyScan NV, Kontich, Belgium) [5].

Table 1. Micro-CT acquisition parameters used for imaging the Ti6Al4V SLM scaffolds

Voltage	Current	Filter material	Voxel size	Rotation step, angle	Frame averaging
90 kV	0.39 mA	1 mm aluminium	12.6 μm	0.5° over 187°	32 frames

2.5. Mechanical properties

Mechanical scaffold properties were determined by compression testing. The samples were placed on an in house developed in-situ loading stage with maximum available load 3kN [4]. As a first step, a preload of 0.01kN was applied and afterwards compression at a constant rate of 0.2mm/min was maintained until final failure. Obtained load and displacement data were used to analyse the mechanical properties of the as-produced and surface modified scaffolds.

3. Results and discussion

3.1. Surface roughness modification

A thorough analysis of the strut and node surface morphology is also important from a modelling point of view, especially for porous structures produced by a RP technique. Working close to the technical limits of the technique can obstruct the production of 3D porous scaffolds with a desired and controlled surface morphology. Including detailed surface roughness information in finite element (FE) models of the porous structures can improve the analysis of the local mechanical properties at the micron level.

The surface modification on the Ti6Al4V scaffolds introduced significant changes of the strut and node surface morphology. The non-melted powder grains, attached to the surface, were removed and a nanopit-like surface was obtained. In figure 6, SEM pictures of nodes and struts prior to and after surface modification are presented.

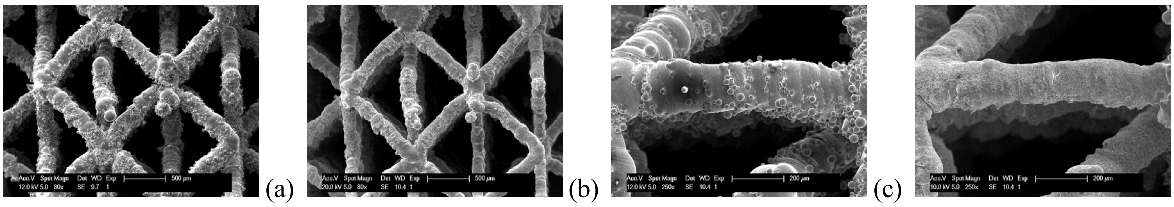
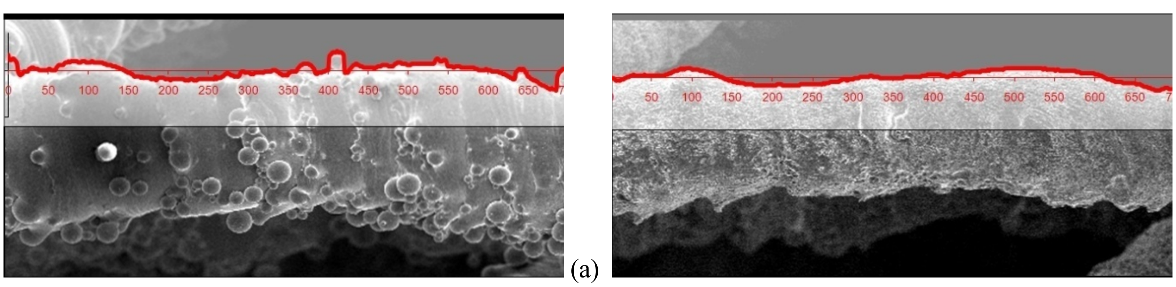

(a) (b) (c) (d)

Figure 6. Typical SEM images of the node and strut morphology of the open porous Ti6Al4V scaffold: (a,c) before and (b,d) after surface modification.

Correlation between nano-porous surfaces and cells (osteoblasts and fibroblasts) were examined in [13]. Reproducible nanopatterns on the surface of 2D Ti (and its alloy) plates, created by etching in a chemical solution based on strong acids like H_2SO_4, revealed accelerated cell growth compared to the untreated Ti and glass cover slips, suggesting that a nanopits like surface is better for cell proliferation and differentiation [13]. Although a positive cell response was found on the nanopits like surface of the Ti plates, experiments with scaffolds with similar surface morphology should be performed in order to determine the influence of 3D structures on cell behaviour.

3.2. Strut surface roughness measurement

Quantitative analysis of the surface roughness in function of the applied surface modification was done on the basis of the SEM image-based protocol described above. Obtained profile lines of the strut surfaces determined on the basis of the pixels distribution in the SEM images are presented in figure 7. It can be seen that the profiles clearly reflect the strut surface topology and can be used for determining the surface roughness of the examined samples.



(a) (b)

Figure 7. SEM images of a typical strut of the porous Ti6Al4V scaffolds with fitted profile lines generated during analysis: (a) prior to and (b) after surface modification

As mentioned previously (point 2.2), SEM investigation revealed a high and inhomogeneous roughness of the strut surface especially at the bottom of the struts, which was caused by attached non-melted powder grains. In order to characterise inhomogeneities of the scaffold strut surfaces, the roughness of the top and bottom of the strut were analysed separately. Obtained results (fig. 8) showed a difference between the roughness of the top and bottom surface of the strut for the samples prior to surface modification. The average as-produced scaffold roughness (R_a) ranged between 9 and 13 µm for both the top and bottom side of the struts, but the difference between highest peak and deepest valley (R_t) for the top and bottom side of the struts was large (36 µm).

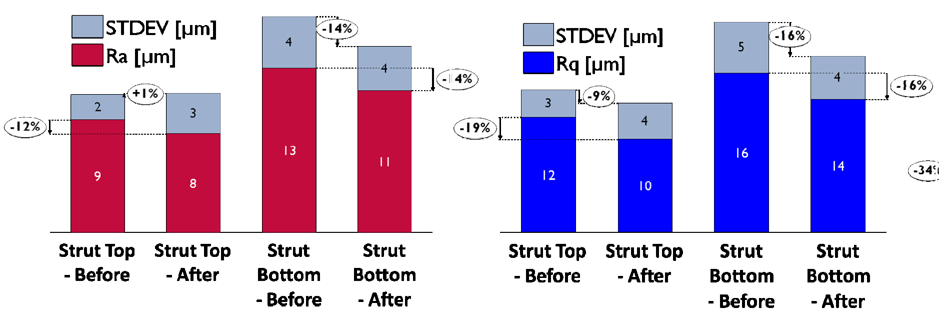


Figure 8. Strut surface roughness data of the Ti6Al4V scaffolds prior to and after surface modification.

The surface roughness of the scaffolds after polishing showed a smaller standard deviation, especially for R_t, which allowed to assume that the scaffold morphology after surface modification was more homogenous. A higher strut roughness reduction at the bottom side can be explained by the different current density distribution present during electrochemical polishing, which caused a higher dissolution rate of the rougher surface areas. Assessment of the sample volume reduction in function of the applied surface modification was done on the basis of measurements of the changes in sample weight, as shown in Figure 9.

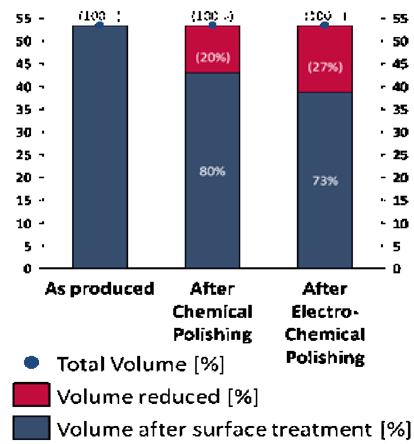


Figure 9. Average sample volume reduction in function of the applied surface modification

3.3. Micro-CT based characterisation

µ-CT based characterisation of the Ti6Al4V scaffolds prior to and after surface modification was done in order to determine the influence of the surface modification on the global scaffold morphology. Changes in porosity, average strut thickness and sample volume as well as strut thickness distribution were investigated (Table 2 and Figure 10).

Table 2. Morphological characteristics of the scaffolds prior to and after surface modification detrmined on the basis of weight measurement and micro-CT based image analysis.

Tested Sample	Sample volume	Porosity	Sample volume	Porosity	Avg. strut thickness
	Weight reduction		µ-CT based characterisation		
	[mm^3]	[%]	[mm^3]	[%]	[µm]
As-produced	53.14 ± 0.36	84.47 ± 0.11	57.47 ± 1.31	86.31 ± 0.16	213.81 ± 0.57
Surface treated	38.52 ± 2.35	88.76 ± 0.65	33.40 ± 2.19	92.03 ± 0.74	169.05 ± 7.58

Comparison of the sample volume reduction analysed via weight reduction and on the basis of µ-CT scanning showed a significant difference. This can be explained by the relatively low spatial resolution of the µ-CT images causing some details of the struts surface morphology to be overestimated. Figure 10 shows changes in strut thickness

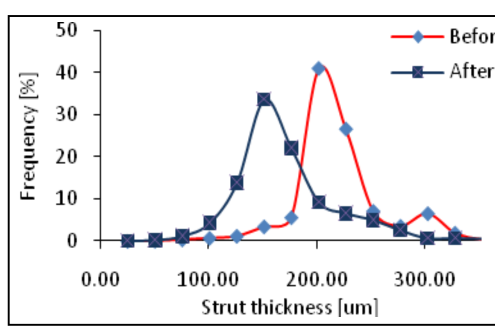


Figure 10. Strut thickness distribution prior to and after surface modification

distribution of the surface modified samples. μ-CT image based investigation provides the possibility to analyse, visualise and quantify (2D and 3D) the changes in strut surface morphology in a non destructive way. 2D scaffold images, where longitudinal sections of the struts could be visualised, were selected for the as-produced (fig. 11a,b) and surface modified (Fig. 11c,d) samples. Overlapping (fig 11e,f) of these images was done by using an automated image registration software, namely MIRIT [14]. This procedure allows visualising the 2D changes of the strut surface morphology. Figure 11f shows a detail of the pore and node overlap image of the μ-CT images prior to and after surface modification, showing that a higher surface reduction at the strut and node bottom side after electrochemical polishing. These results, combined with surface roughness measurements, can be used to unravel the polishing mechanisms of the Ti6Al4V porous scaffolds and to prove that the applied surface modification leads to a more homogenous scaffolds morphology. 3D visualisation of the porous structures (Fig. 12), using SkyScan [SkyScan NV, Kontich, Belgium] software, showed strut and node thickness changes, which suggest that despite the low spatial resolution (12.6 voxel size) of the μ-CT images, μ-CT still can be used for evaluating the influence of surface modification on the scaffolds morphology.

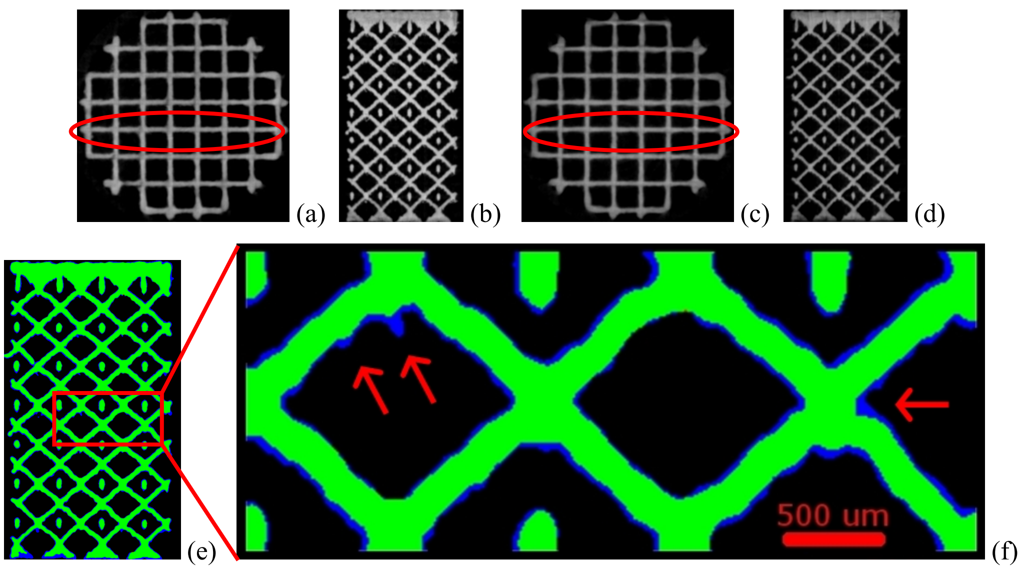


Figure 11. μ-CT based images of transversal and longitudinal scaffold sections (a,b) before and (c,d) after surface modification, and(e,f) combined images of the longitudinal scaffold sections prior to and after surface modification

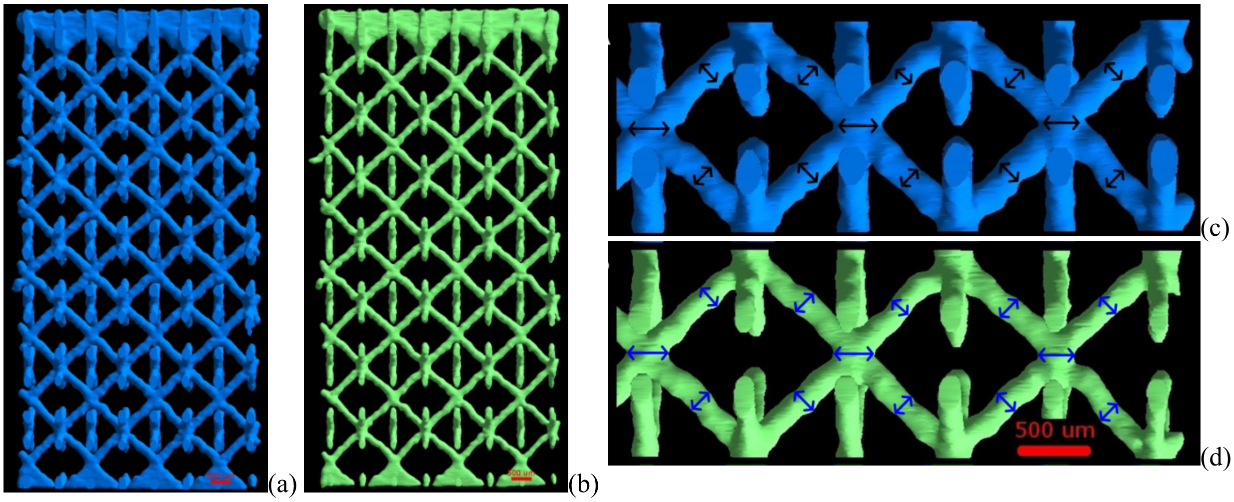


Figure 12. μ-CT based 3D visualisation of an (a) as produced, (b) surface modified porous scaffold and the comparison of the 3D visualisation of the struts and nodes prior to (c) and after surface treatment (d); arrows have the same length and indicate changes of the structure size after surface roughness reduction.

3.4. Mechanical properties

In order to determine the influence of the surface modification procedure of the Ti6Al4V scaffolds on the mechanical properties, compression tests of the as-produced and surface modified samples was performed (Table 3). It can be clearly seen that the applied surface modification introduced changes in the mechanical behaviour of the porous structures. In figure 2, it can be seen that the applied surface modification leads to a more smooth strut surface, but it also decreases the strut diameter, thus also significantly decreasing the mechanical properties. This is caused by the changed scaffold unit cell dimensions due to the reduction in strut size and the increase in the pore size during polishing. Since pores can be described as spaces between struts of the scaffold, changes of the pore size, revealed by micro-CT characterisation, are caused automatically by reduction of the strut thickness. To compensate for this strut thickness reduction related loss in mechanical strength, the effective strut thickness that determines the mechanical properties of the scaffolds should be accounted for both in the design and SLM-production to ensure desired mechanical properties after controlled surface modification.

Table 3. Mechanical properties of the scaffolds prior to and after surface modification

Tested Sample	Strain at max strength		Strength		Stiffness	
	[%]		[MPa]		[MPa]	
As-produced	6.04	± 0.32	13.00	± 0.62	397.07	± 29.95
Surface treated	7.02	± 0.24	7.41	± 0.88	226.15	± 22.45

4. Conclusions

This study showed that thorough characterisation of the changes in scaffold morphological and mechanical properties due to surface modification can be easily obtained by the SEM and µ-CT image-based analysis combined with mechanical testing. Since the 2D image-based protocol can be applied to determine the strut surface morphology, high resolution µ-CT imaging has the potential to become a valuable tool for determining the roughness of complex porous structures by applying this protocol to µ-CT images. This will result in an optimisation of the design, the production and surface modification protocols related to obtaining controlled morphological and mechanical properties of porous (metallic) materials. In a next step *in vitro* biological experiments are needed to evaluate the relation between these properties and cell behaviour.

In-situ compression tests, performed on the as-produced and surface modified scaffolds, show changes in mechanical properties as function of the applied surface modification. Analysis of the mechanical properties combined with µ-CT based characterisation of the scaffolds, when related to the applied surface modification, can be used for the optimisation of the design, the modelling and the production and hence to improve the properties of scaffolds that can be applied for bone regeneration.

The combined use of µ-CT imaging, 3D image analysis and non-rigid image registration with in-situ mechanical loading could potentially be applied in order to characterise strain distribution and to compare the local mechanical behaviour of the as-produced and surface modified porous materials.

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