INVESTIGATION OF THE INFLUENCE OF SURFACE ROUGHNESS MODIFICATION OF BONE TISSUE ENGINEERING SCAFFOLDS ON THE MORHOLOGY AND MECHANICAL PROPERTIES

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Aims

Bone tissue engineering (TE) is a multidisciplinary field of science focusing on healing large bone defects by designing and manufacturing constructs that combine open porous biomaterials (= scaffolds) with osteogenic cells to support cell seeding and *in vivo* bone formation [1]. 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 [2]. 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 [3,4]. Achieving controlled surface properties is also essential in the design and production of biocompatible scaffolds [2,5], since the strut surface roughness (SSR) influences cell behaviour within a scaffold [5]. 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. It is obvious that any surface roughness modification performed after production will change the topography of the struts surface as well as the local and global mechanical properties of the structure. Therefore, in the present research 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 has been determined.

Materials and methods

In this study, the struts surface of open porous Ti6Al4V scaffolds produced by SLM has been modified by chemical and electrochemical polishing. Scaffolds were designed by creating a CAD design (fig. 1a) using Magics software [Materialise NV, Haasrode, Belgium] and were produced on a non-commercial SLM machine equipped with a Yb:YAG fibre laser with a beam spot size of 80 µm and a maximum power of 300 W on the powder bed. The unit cell applied for the design (fid. 1b) had a diamond shape-like structure (fig.1a, 1c and 1d). The porous samples were built, in a closed and argon flushed chamber, layer-by-layer using a metal powder layer thickness of 30 µm. The diameter and height of the samples presented in fig.1a, 1c and 1d was 6.0 ± 0.5 mm and 12.0 ± 0.5 mm respectively. The designed strut size was 100 µm and the pore size 1 mm. SEM images (fig. 1e) show a large and highly inhomogeneous roughness caused by non–melted powder grains attached to the strut surface on the as-produced Ti6Al4V scaffolds.

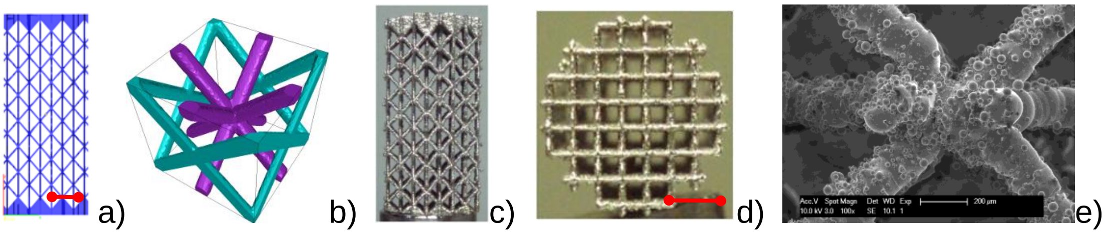

Figure 1. Examples of the Ti6Al4V porous scaffolds: a) CAD design of the scaffolds, b) the unit cell, c) cylindrical sample (side view) and d) cylindrical sample (top view) and e) SEM pictures of the unit cell showing highly inhomogeneous roughness caused by non–melted powder grains attached to the strut surface . Scale bars = 2 mm.

An appropriate roughness reduction procedure was applied by combining chemical and/or electrochemical polishing of the scaffolds, which apart from removing the inhomogeneities of the struts, allows to obtain a cell-friendly strut topology. In a first step, the produced samples (fig. 2a) were polished chemically (fig. 2b) in order to remove the attached non-melted powder grains. During chemical polishing samples were immersed for 10 minutes in a chemical solution with the following composition: HF + H_2O (hydrofluoric acid, HF, Riedel-de Haën, Germany, p.a. 48%). In a second step, electrochemical polishing was applied to obtain the desired surface morphology (fig. 2c). For electrolytic polishing, the electrolyte had the following composition: CH_3COOH + H_2SO_4 + HF (hydrofluoric acid HF, Riedel-de Haën, Germany, p.a. 48%; acetic acid CH_3COOH, Acros Organics, Belgium, p.a. glacial; sulfuric acid H_2SO_4, Fisher Scientific, United Kingdom, p.a. >95%). The combination of chemical and electrochemical polishing gives the opportunity to modify the strut surface in a controlled way.

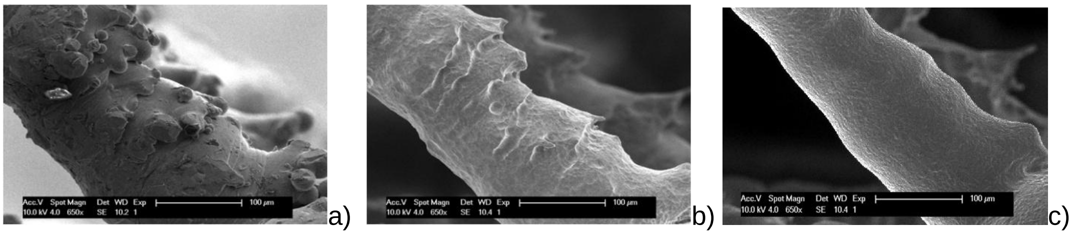

Figure 2. SEM images of the: a) raw strut with attached powder grains, b) strut after chemical polishing, c) strut after chemical and electrochemical polishing,

For determining the influence of the SSRM procedure on the struts roughness, a non-destructive SEM image-based measurement protocol was applied. Based on the profile lines on the surfaces of the struts, the following roughness parameters have been determined:

- 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.

The quantification of the scaffold morphology as-produced and after surface roughness modification was done by micro-CT, determining the influence of the surface roughness modification on the porosity, the available surface, the specific surface and the strut thickness. For this purpose, the Philips HOMX 161 x-ray system with AEA tomahawk CT software was used. The applied acquisition parameters are presented in table 1. The morphological parameters were determined using commercially available image analysis software, namely CTAn (SkyScan NV, Kontich, Belgium) [3].

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 Al	12.6 µm	0.5° over 187°	32 frames

The characterisation of the mechanical behaviour of the tested micro-porous structures prior to and after surface roughness modification is performed by continuous mechanical compressive loading, determining the E-modules, the strength and the strain at maximum stress. The samples were placed on an in house developed in-situ loading stage with maximum available load 3kN [3]. 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.

The main goal of all these experiments is the characterisation of the 3D SLM porous structures prior to and after surface roughness modification in order to optimise the design, the modeling, the production of the porous structures and the surface roughness modification and hence to improve the properties of the scaffolds.

Results

- *Surface roughness measurement*

Quantitative analysis of the surface roughness in function of the applied surface roughness modification was done on the basis of SEM images. Obtained profile lines of the strut surfaces determined on the basis of the pixels distribution in the SEM images are presented in figure 3. 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.

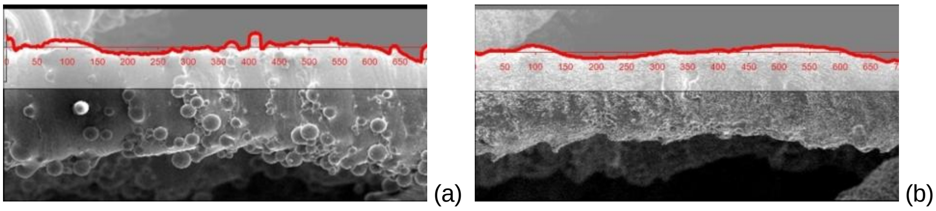

Figure 3. SEM images of a typical strut of the porous Ti6Al4V scaffolds with fitted profile lines generated during analysis: (a) prior to and (b) after surface roughness modification

As can be seen in fig. 3a, 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. 4) showed a difference between the roughness of the top and bottom surface of the strut for the samples prior to surface roughness 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).

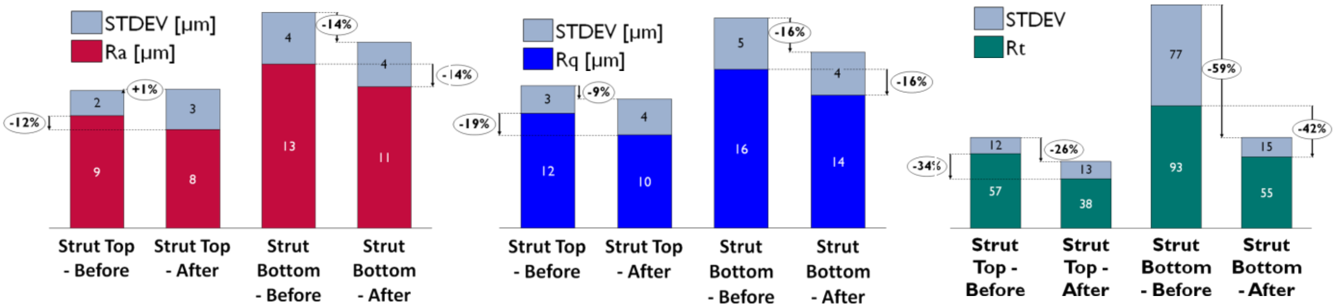

Figure 4. Strut surface roughness data of the Ti6Al4V scaffolds prior to and after surface roughness modification.

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 roughness 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 roughness modification was done on the basis of measurements of the changes in sample weight, as shown in Figure 5.

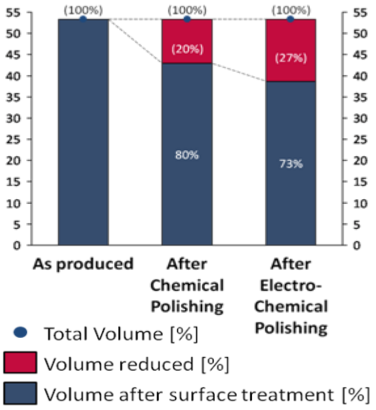

Figure 5. Average sample volume reduction in function of the applied surface modification

- *Morphological characterization*

Micro-CT based characterisation of the Ti6Al4V scaffolds prior to and after surface roughness modification was done in order to determine the influence of the surface roughness 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 6).

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

Tested Sample	Sample volume	Porosity	Avg. strut thickness
	micro-CT based characterisation		
	[mm^3]	[%]	[µm]
As-produced	57.47 ± 1.31	86.31 ± 0.16	213.81 ± 0.57
Surface modified	33.40 ± 2.19	92.03 ± 0.74	169.05 ± 7.58

Figure 6 shows changes in strut thickness distribution of the surface modified samples. Micro-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.

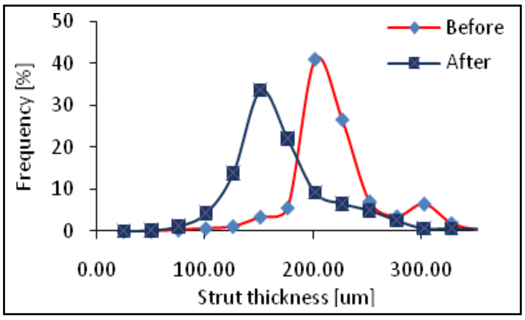

Figure 6. Strut thickness distribution prior to and after surface modification

- *Mechanical characterization*

In order to determine the influence of the surface roughness 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 roughness modification introduced changes in the mechanical behaviour of the porous structures. In figure 6, it can be seen that the applied surface roughness 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 roughness modification.

Table 3. Mechanical properties of the scaffolds prior to and after surface roughness 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 modified	7.02 ± 0.24	7.41 ± 0.88	226.15 ± 22.45

Conclusion

This study showed that thorough characterisation of the changes in scaffold morphological and mechanical properties due to surface roughness modification can be easily obtained by the SEM and micro-CT image-based analysis combined with mechanical testing. The 2D SEM image-based protocol can be applied to determine the strut surface morphology and can become a valuable tool for determining the roughness of complex porous structures. This will result in an optimisation of the design, the production and surface roughness 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.

Compression tests, performed on the as-produced and surface modified scaffolds, showed changes in mechanical properties as function of the applied surface roughness modification. Analysis of the mechanical properties combined with micro-CT based characterisation of the scaffolds, when related to the applied surface roughness 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.

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