X-ray microtomographic characterization of highly rough titanium cold gas sprayed coatings for identification of effective surfaces for osseointegration

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ABSTRACT

Highly rough titanium coatings were successfully obtained by means of Cold Gas Spray (CGS) technique. The increase of surface roughness is beneficial for joint prosthesis to promote osseointegration. This is due to the increase in total effective surface area generated by the coating hence improving cell attachment. Based on this hypothesis, a CGS-titanium (CGS-Ti) coating sample was scanned by means of micro-computed tomography (micro-CT). A very high-resolution 3D study has been carried out of the inner structure and the complex surface generated from the CGS-Ti coating sample. This work shows the feasibility of using micro-CT scanning technique to study highly complex surfaces by means of a 3D modelling analysis. It allows a qualitative and quantitative description of the main features, morphology of the pores and surface roughness of the coating. Several numerical values were obtained to describe size, form and distribution of the closed/inner and open/outer pores. Additionally, surface roughness and open porosity were modelled in order to find the effective surface for osseointegration according to an open pore threshold of 150 µm.

The obtained results showed closed pores to be rather homogeneously distributed along the coating, presenting a size distribution between 45-153 µm in diameter and a median pore size of ~55 µm. 3D modelling of the surface and open porosity demonstrates that the roughness generated by CGS-technique allows a ~1.6-fold increase in the coating surface, which is of ~1.35-fold increase when considering only the effective surface for osseointegration. Although quantification of those micrometric structures can show slight deviations due to the inherent limitations of the method, mainly due to voxel size resolution, the obtained results were highly illustrative, representing a starting point for further investigations in the field of coatings for implant applications.

Keywords: Micro-CT; Porous titanium; Cold Gas Spray; Joint prosthesis; osseointegration

1. INTRODUCTION

X-ray microtomography (micro-CT) is one of the most useful and capable non-destructive techniques for carrying out detailed studies in material sciences for 3D visualization and quantification of highly complex internal structures of materials (Pyka, G. et al. 2014). In the biomedical field it has been mainly used for evaluating the porosity of scaffolds, i.e. titanium and hydroxyapatite (Moiduddin et al., 2017; Jones et al. 2007), but its use has not expanded yet in the coating materials research. In fact, studies of porous coatings and porous titanium structures were mainly devoted on the study of the improvement of mechanical properties and in promoting osseointegration (Vilardell et al., 2018; Singh et al., 2010).

Most of the research performed by means of micro-CT lies in the area of metal powder additive manufacturing (AM), resulting in a valuable method for evaluating surface and internal structures of AM built parts. Examples of its applicability can be found from the internal defects analyses and interconnectivity of customized mesh structures for cranial implants (Moiduddin et al., 2017), to analyses of the effect of jet blasting and sintering post-processes on pore and strut network of porous Ti structures (Kim et al., 2014). In the field of cementless joint prosthesis, it has also been applied to Vacuum Plasma Spray (VPS) coatings, where not only the surface topography is important for cell attachment but also pore size and pore connectivity for bone ingrowth (Jaeggi et al. 2009; Johansson et al. 2015). This technology is a well-established fast deposition process especially for oxygen sensitive materials. However, a high cost is imposed by the necessity of vacuum conditions. As an alternative, Cold Gas Spray (CGS) technology is a solid-state process which might be also promising to produce rough and porous coatings accomplishing the mechanical standards of joint prosthesis in a more cost-effective way and less heat input in comparison with VPS (Vilardell et al. 2018). A high surface roughness and internal porosity is achieved by spraying coarse particles at supersonic velocities through an accelerated gas. CGS is a solid-state process just implying the plastic deformation of the impinging particles resulting from their acceleration in an accelerated gas, whereas VPS implies particle melting due to the higher heat input involved in the process.

To the authors knowledge, few micro-CT studies of rough/porous CGS-Ti coatings have been reported so far. Zahiri et al. (2008) observed that the use of helium as a carrier gas in CGS decreases porosity in comparison with nitrogen by reconstructing CGT-Ti volumes in 3D rendered micrographs. However, no data regarding surface roughness, pore size and interconnectivity was performed for biomedical purposes. Currently, porous/rough CGS-Ti coatings for biomedical purposes have been produced by the use of porogen elements such as magnesium or aluminum. The obtained average porosity was >48%, with a pore size range between 70-150 µm and 71-91 µm, respectively (Sun et al. 2008; Qiu et al. 2013). However, both studies did not distinguish between open and closed porosity, nor the effective surface area. The porosity and pore size values were obtained through the cross-section areas of the coating by 2D qualitative image analyses (optical and electronic microscopies), which is less accurate than 3D analyses such as the ones provided by micro-CT. Only Zahiri et al. (2008) reported the suitability of the microtomography to evaluate the internal porosity of a CGS titanium coating as an effective methodology to provide a 3D observation of the internal coating structure and porosity network, but not with the specific purpose to achieve an internal porosity to be used in the field of biomedicine. Micro-CT analyses of VPS Ti coatings with a resolution of 5 µm voxels yielded an average pore size of ~80–140 µm, pore connection diameter of ~50 µm and Ti sinter neck diameter of ~30–40 µm (Jaeggi et al. 2009).

In the present study, the 3D internal porous network of a highly rough CGS titanium coating is characterized by micro-CT as a very valuable non-destructive characterization technique. The study evaluates qualitatively and quantitatively the inner/closed and open pores as well as the complex surface roughness resulting from the spraying of coarse particles.

2. EXPERIMENTAL PROCEDURE - METHODS

Commercial pure grade 2 Ti (CP-Ti) irregular powders from MBN Nanomaterialia SpA (Italy) were sprayed onto Ti6Al4V alloy substrates with a CGT KINETICS® 4000 (Cold Gas Technology, Ampfing, Germany) using nitrogen as the propellant gas.

Top surface area was examined by Scanning Electron Microscopy (SEM) using a JEOL 5510 operated at 20 kV. The cross-section area was prepared by grinding and polishing samples until 1 µm size diamond suspension. Thickness and roughness values were measured according to ASTM F1854 with Optical (Leica DMI5000-M) and Confocal Microscopy (Leica DCM3D). The roughness values of the samples were extracted from the global profile with a Gaussian filter (0.25 mm) according to ISO 4287.

The 3D study of the surface roughness and inner structure/porosity of the Ti coating, i.e. pore size and distribution, was carried out by means of micro-CT with the MultiTom Core X-ray CT system (CORELAB-UB).

A Ti coating piece of 7.5x7.46x2.95 mm was scanned at 170 kV and 12 W tube conditions, using a 0.5 mm filter of Cu, with a total of 3000 projections, an exposure time of 2500 ms per projection. Final voxel resolution obtained was of 12 µm. The acquired images were reconstructed using the ACQUILA software (www.XRE.be) and resulting 3D volumes were analysed using Avizo 9 (www.fei.com).

3. RESULTS AND DISCUSSION

3.1. CGS CP-Ti coatings

The top surface morphology and cross section of the obtained CGS CP-Ti coating show a highly rough surface topography, which provides an enhancement of the specific surface area (Fig. 1). CGS CP-Ti coatings have a thickness of 294±75 µm. The surface topography provided a global profile of Ra=40±2 µm and a microroughness of 13.2±1 µm. Additionally, good bonding interface could be observed between particles.

3.2. Data treatment and quantifications

With the aim to evaluate the sub-surface coating porosity, i.e. closed porosity, and the surface roughness where bone cells can grow, the region of interest (ROI) was centred on the coating itself (Fig. 2a). The plane surface (XY) was slightly reduced to an area of 4.51x6.23 mm^2 in order to avoid errors in the quantifications due to possible scattering radiation effects in the borders. The total volume studied was 16 mm^3.

Individual identification of the inner/closed pores was carried out by means of differential density segmentation based on grey value thresholding (Fig. 2b), resulting in a total of 165 closed pores, where only pores with a diameter 3 times the voxel size have been considered, i.e. ~36 µm equivalent diameter. Each of the pores was then characterized by measurements of its volume, anisotropy, elongation, flatness, equivalent spherical diameter and its barycentre. Finally, using the coordinates of the barycentre, it has been also possible to determine the surface (XY) and vertical distribution of the pores.

Closed pores of the CGS CP-Ti coatings are distributed homogeneously along the coating, both in the horizontal (Fig. 3a and 3b) and vertical dimensions (Fig. 3c). In addition, estimation of the equivalent diameter (Eq. diam.) of each pore showed distribution of pore sizes between ~45 µm and ~153 µm, and allowed calculation of their frequency of appearance (Fig. 3d). 70 % of the pores ranged between 45-60 µm diameters and a median pore size of ~55 µm.

In the study of open porosity or surface roughness, in-vitro and in-vivo studies of porous Ti alloy implants have reported pore size diameters from 100 µm up to 700 µm (Guoyuan et al. 2016, Prananingrum et al. 2016). However, the ideal pore size diameter to promote higher bone osseointegration is still under debate. It has also been suggested that an appropriate pore size should be of 100 µm, although subsequent studies have shown better osteogenesis for substitutes with pores >300 µm. Smaller pores (75– 100 µm) resulted in ingrowth of unmineralized osteoid tissue or were penetrated only by fibrous tissue (Hannink et al. 2011). Additionally, larger pores favor direct osteogenesis, since they allow vascularization and high oxygenation. For all these reasons, in this study, an aperture of pore size diameter of 150 µm was chosen to evaluate the surface roughness in terms of effective surface for cell deposition. Roughness in form of open pores with an aperture Ø<150 µm was not considered to be usable for cells grow (Xue et al. 2007). Thus, pores Ø<150 µm were initially isolated, and subsequently removed as they were not considered to contribute in osseointegration.–This process was carried out by homogeneous vertical growing and posterior reduction of 6 voxels of the surface (Fig. 2b), thus infilling the open pores and generating a new interpolated surface (Fig. 4). Again, those pores with a volume below a 36 µm diameter equivalent sphere were removed due to resolution limitations. Finally, the effective surface for cell grow ($S_{\text{effective}}$) has been estimated by removing the open pores (<150 µm) surface (S_{open}) from the total real coating surface (S_{coating}), and adding the interpolated open pore’s caps surface ($S_{\text{Int-Aperture}}$) (Fig. 4):

$$S_{\text{effective}} = S_{\text{coating}} - S_{\text{open}} + S_{\text{Int-Aperture}} \qquad \text{(eq. 1)}$$

The studied piece had a square surface of 28.10 mm^2 and the calculated surface of the coating itself was of 45.84 mm^2 (S_{coating}). The area for the estimated surfaces of the inner part of the open pores with apertures <150 µm (S_{open}) and that of the new surface generated ($S_{\text{Int-Aperture}}$) were of 16.91 mm^2 and 9.15 mm^2, respectively. Thus, the final effective surface of the CGS CP-Ti coating ($S_{effective}$) resulted of 38.08 mm^2. The feasibility of micro-CT technique enables the calculation of real surface of complex featured surfaces with high roughness and pores, despite the inherent limitations of the technique, mainly related to spatial resolution, i.e. voxel size. However, more comparative studies with different methodologies are needed in order to verify the results here obtained, which would likely supply additional information for the characterization of these very complex surfaces. Additionally, further research is required in the study of implant surfaces to achieve suitable osseointegration. Defining an optimal pore diameter, as well as pore morphology for bone ingrowth, is crucial to decide the best method to develop coatings for joint prosthesis.

4. CONCLUSIONS

The inner/closed porosity of a CGS CP-Ti coating was quantified, as well as analysed in terms of pore size and morphology, by micro-CT. High-resolution 3D analysis of the coating allowed the isolation and removal of open pores below a minimum surface of 150 µm, as considered less ideal for bone ingrowth. Finally, interpolation of the surfaces covering those open pores allowed calculation of the real surface for cell deposition, demonstrating this technique is highly convenient for a precise estimation of real surfaces in 3D complex environments.

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REFERENCES

Guoyuan, Li et al. (2016) In vitro and in vivo study of additive manufactured porous Ti6Al4V scaffolds for repairing bone defects, Scientific Reports volume 6, Article number: 34072.

Hannink, G et al. (2011) Bioresorbability, porosity and mechanical strength of bone substitutes: What is optimal for bone regeneration?, Injury. 42, S22–S25.

Jones, AC et al. (2007) Assessment of bone ingrowth into porous biomaterials using MICRO-CT, Biomater. 28, 2491-2504

Jaeggi, C. et al. (2009) 3D characterization of open porous vacuum plasma sprayed titanium coatings by means of high resolution micro computer tomography, Mater. Lett. 63, 2643-2645.

Johansson, P. et al. (2015) Nanosized Hydroxyapatite Coating on PEEK Implants Enhances Early Bone Formation: A Histological and Three-Dimensional Investigation in Rabbit Bone, Mater. 8, 3815-3830.

Kim, T.B. (2014) Additive manufactured porous titanium structures: Through-process quantification of pore and strut networks, J Mater Process Technol. 214, 2706–2715.

Moiduddin, K. et al. (2017) Structural and mechanical characterization of custom design cranial implant created using additive manufacturing, Electron J Biotechn. 29, 22–31.

Pyka, G. et al. (2014) The effect of spatial micro-CT image resolution and surface complexity on the morphological 3D analysis of open porous structures. Mater. Charact. 87,104-115.

Qiu, D. et al. (2013) A novel composite porous coating approach for bioactive titanium-based orthopedic implants, J.Biomed.Mater.Res. 101A, 862–872.

Singh R. et al. (2010) Titanium foams for biomedical applications: a review, Mater Tech. 25,127–136.

Sun, J. et al. (2008) Innovative fabrication of porous titanium coating on titanium by cold spraying and vacuum sintering, Mater. Lett. 62, 3623–3625.

Vilardell, AM et al. (2018) Hierarchical structures of anodised cold gas sprayed titanium coatings, TIMF. 96, 71-78

Prananingrum, W. et al.(2016) Bone ingrowth of various porous titanium scaffolds produced by a moldless and space holder technique: an in vivo study in rabbits. Biomedical Materials, Volume 11, Number 1

Xue, W. et al. (2007) Processing and biocompatibility evaluation of laser processed porous titanium. Acta Biomater. 3, 1007–1018.

Zahiri, S.H. et al. (2008) Characterization of Cold Spray Titanium Deposits by X-Ray Microscopy and Microtomography, Microsc. Microanal. 14, 260-266.

FIGURE CAPTIONS

Figure 1. Schematic illustration of CGS CP-Ti coatings. SEM and optical microscopy micrographs of top surface and cross-section of the coating

Figure 2. a) 3D surface of the CGS Ti-coating as-scanned with micro-CT, and the ROI showed in green; b) Micro-CT cross section with segmented: i) closed pores in light-red, and ii) and iii) process of segmentation of open surficial pores in red.

Figure 3. Distribution of closed pores of CGS CP-Ti coating, a) 3D view, b) surface view, c) cross section. d) Frequency of appearance of inner/closed pores by equivalent diameter.

Figure 4. 3D modelling of the different identified surfaces of the CGS-Ti coating. a) Calculated surfaces of the open pores with diameters < 150mm (S_{open}, in red), and newly interpolated surfaces for those open pores ($S_{Int\text{-}Aperture}$, in green). b) Final modelled effective surface ($S_{effective}$) of the coating resulting from the subtraction of the S_{open} and the sume of the $S_{Int\text{-}Apaerture}$ to the total coating surface ($S_{coating}$, in yellow).