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Case report

Micro computed tomography as a new method of investigation: Biointegration performance of a bone substitute.

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Abstract

Introduction

X-ray computed microtomography (microCT) is a miniaturized form of conventional computerized axial tomography (CAT). The main purpose of this paper is to show one possible application of this new method of investigation: 3D inner display and morphometric characterization of a human bone sample previously grafted with a scaffold.

Case report

MicroCT enables 3D reconstruction of the internal structure of small X-ray opaque objects without sample destruction or preparation. Using this approach, the possibility of investigating the 3D morphometric characteristics of bone substitutes makes it possible to evaluate the biointegration performance.

Discussion

Since there is a close relationship between the properties of the materials and their microstructure, it is necessary to examine them using the highest levels of resolution before taking any action to enhance present materials or create new products.

Conclusion

This method of investigation is intended to be a valid aid in displaying, designing and manufacturing biomaterials with ideal features.

Introduction

Studies on biological and synthetic biomaterials have been carried out to restore form and function of lost bone structures. Bone healing is a quite complex and dynamic process that dates back to embryonic bone development and regeneration, and ends with the restoration of anatomical and functional standard conditions of the bone. As a scaffold, most present graft biomaterials are able to osteoconduct matrix or three-dimensional substrates to support new bone tissue formation[1,2,3,4,5,6,7,8].

The manufacturing production of scaffolds with ideal characteristics is one of the most challenging issues in bone tissue regeneration strategy.

To enhance the features of these devices, their morphometric architectural characteristics may be further studied and characterized using a 3D non-destructive and non-invasive method, a powerful investigative technique like X-ray micro Computed Tomography[9,10,11,12].

The main purpose of this paper is to show the possible applications of microtomography for 3D inner displaying and morphometric characterization of a human bone tissue grafted with an osteoconductive biomaterial. Quali-quantitative analysis has been carried out on human bone tissue of the same patient, harvested from the healing of a bone defect collected after extraction of the lower first molars; one extraction site has been cured only with a blood clot and the other with a bovine hydroxyapatite (HA) biomaterial graft.

After six months, bone samples from both sites have been extracted to be analysed by means of microtomography and histology. The microCT system Skyscan 1072 (Bruker-microCT, Kartuizersweg 3B, 2550 Kontich, Belgium) used in this study allows to obtain both radiographic images and their tomographic reconstruction data with a dedicated software package.

Case report

One healthy patient, aged 36, needing a surgical dental extraction of the lower first molars, has been selected. The patient was informed and signed an informed consent form according to the declaration of Helsinki that requires the patient adhesion to the clinical experimental study. One post-extractive site was implanted with the biomaterial used in this study, a bovine HA scaffold (Endobone, Biomet, USA; cancellous chips 0,5-1 mm) (test sample), while the opposite site was treated only with a blood clot (control sample).

Six months after surgery, at implant insertion time bone samples were extracted from test and control sites, using a trephine bur made of a surgical-steel bur (internal diameter 2 mm, external diameter 3 mm). Each bone sample was fixed in 10% formalin and before microtomographic analysis was carefully washed with physiological solution, and fixed on a suitable stub by plasticine.

The regenerated bone tissue of the test sample was analysed by means of 3D microtomographic and histological tests, and the results were compared with those obtained from similar evaluations carried out on the control sample. For both the test and control sample the morphometric parameters have been calculated, 2D and 3D dimensional images were processed with the same acquisition parameters to allow comparing qualitative and quantitative results (magnification, cross-section pixel size, rotation angle, rotation step, power source, aluminum filter).

The microCT system consists of a microfocus tube which generates a

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cone-beam of X-rays, a rotating specimen stub on which the object to observe is put, and an electronic detector system which acquires the images. The X-ray source is a 10W microfocus tube (tungsten), which can operate at currents and voltages up to 98 mA and 100 kV, respectively. The stub can rotate, normally at a 0.45° rotation step, for complete acquisition of 180° for a total time of about 2 h.

Comparing different resolutions of CT systems, it is possible to observe that medical-CT resolution is about 100 micron and laboratory microCT resolution range is from 5 to 10–20 micron, according to sample dimensions. This system is supplied with a 1 mm-thick aluminium plate, placed in front of the scintillator, to be used as a hardware beam hardening the minimizer filter[8,11,13].

Computed tomography is based essentially on two different processes:

- Acquisition of images and projection data;
- Reconstruction of images using specific algorithms.

An X-ray beam reduces its density while crossing a material, then it is collected by means of a system of detectors able to transform its intensity into an electric signal of corresponding value. Considering that the more attenuated an X-ray beam, the more dense the structures it passes through, a density map of the object can be acquired. After passing through a sample, a very thin and collimated X-ray beam reaches a detector that measures the attenuation occurred during the whole process.

The sample is then rotated to get as many projections as the angles of rotation. From these initial projection data (TIFF format) it is possible to reconstruct the cross section images (slices, in BMP format) of the object, using the software NRecon (version 2.23, Skyscan, Belgium), which is based on the cone beam algorithm[11,12,13,14,15].

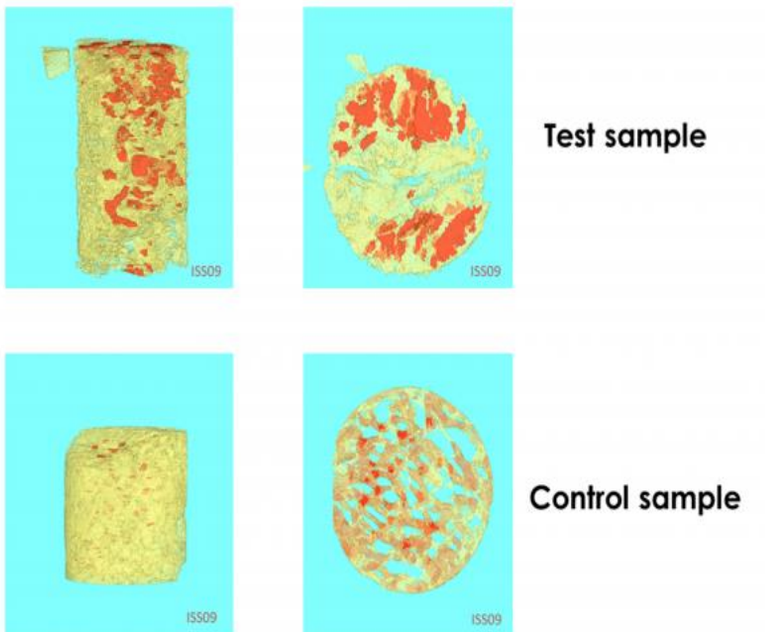

Figure 1: Top image shows 3D reconstruction color images of biomaterial test sample (all sample on left and a slice of the same sample on the right) while bottom shows 3D reconstruction color images of control sample (all sample on left and a slice of the same sample on the right).

These slices were also evaluated with the CTAnalyser software (vesion 1.11, Skyscan, Belgium) in order to obtain the 3D structure and morphometric parameters. To be processed this way, images have to be binarized. The process of "binarization" needs to choose a threshold value, and the resulting image is composed only of black pixels (bone) and white pixels (non-bone). After binarization, the CTAnalyser software allows to calculate morphometric parameters and creates the three-dimensional image of the internal structure of the analysed sample from images' slices in BMP format[13,15,16,17,18,19]. For each sample, an internal Region Of Interest (ROI) was selected and morphometric parameters (Table 1) for the corresponding Volume Of Interest (VOI) were calculated (Table 2).

Comparing the morphometric parameters of test and control samples, test sample showed Bone Volume over Total Volume (BV/TV) values lower than the control sample, while Bone Surface over Bone Volume (BS/BV) values were higher than in the control.

Table 1: Definition of morphometric parameters chosen for microCT analysis in this study.

Parameters	Units	Definition
Tissue Volume, TV	mm^3	total volume of the volume-of-interest (VOI). The 3D volume measurement is based on the marching cubes volume model of the VOI
Bone Volume, BV	mm^3	total volume of binarized objects within the VOI. The 3D volume measurement is based on the marching cubes volume model of the binarized objects within the VOI.
Bone Surface, BS	mm^2	the surface area of all the solid objects within the VOI, measured in 3D (Marching cubes method)
Trabecular Thickness, Tb.Th	mm	that represents the thickness of the trabecular bone. Tb.Th = 2BV/BS
Trabecular Separation, Tb.Sp	mm	that represents the distance between edges of the bone trabeculae. Tb.Sp. = 1/Tb(N-Tb.Th)

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Porosity value seems to have increased in the test sample of bovine hydroxyapatite, while light differences between test and control samples have been obtained for Trabecular Thickness (Tb.Th) and Trabecular Spacing (Tb.Sp) parameters.

After this quantitative analysis, figure 1 shows 3D coloured reconstruction of the samples. In radiology it is known that absorption coefficient depends on the density of crossed material. The observation of sample images shows that in all samples three main shades of grey can be identified and related to three different bone densities. Therefore, three threshold ranges were chosen (0-195, 0-155, 0-115).

In 3D sample images, for each range, a different colour was selected to identify structures with different density: yellow represents the least radiopaque component which may correspond to immature bone at first stage of calcification, orange represents areas that reached a higher stage of calcification, and red represents the areas with the highest radiopacity that may demonstrate the presence of residual scaffold.

The non-shaded areas presumably correspond to empty spaces or non-mineralized tissues (vascular structures, fibrous connective tissue, etc.)[19].

Comparing the images of test and control samples, it is possible to observe that more red areas are displayed on test sample slice images, probably corresponding to residual scaffold particles.

Control sample slices show few red areas but very large yellow and orange areas. By means of histological investigation (Figure 2), an interesting quantity of neo-formed bone is observed, while in the test sample there is less neo-formed bone together with some fibrous connective tissue and scaffold residues.

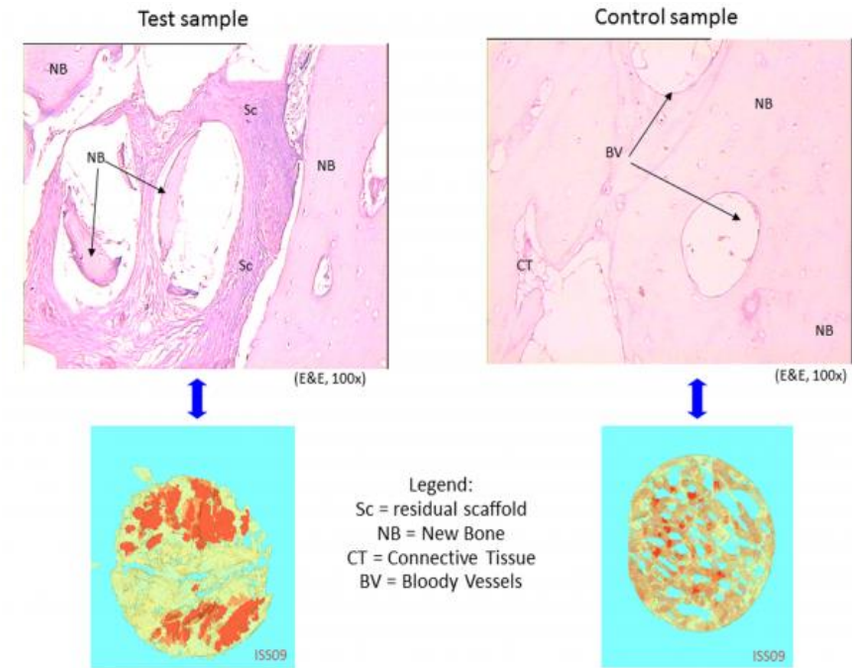

Figure 2: Histological images of test (left) and control (right) bone samples (hematoxylin eosin staining, 100x magnification).

Table 2: Morphometric parameters obtained for control and test bone samples.

Parameters	Test Sample	Control Sample
BV/TV (%)	31,06	36,42
BS/BV (mm^{-1})	104,20	78,52
Porosità (%)	68,94	62,69
Tb.Th (micron)	0,05	0,06
Tb.Sp (mm^{-1})	0,15	0,14

Discussion

The main features that the ideal scaffold should have are: high porosity, a large surface for cellular adhesion, and large pores to enable the penetration of neo-formed vascular structures[10,15,19,20,21,22].

Investigated parameters are all important for the graft to be successful. Hydroxyapatite is one of the most used biomaterials for regenerative purposes, thanks to its chemical and physical characteristics and biological relations that make it similar to and compatible with human bone tissue[1,2,3,10].

These results have been studied and evaluated by means of microCT that identified morphometric parameters and then quantitative properties of each bone sample (Table 1). X-ray computed tomography is an innovative investigation technique used to study histomorphometric characteristics of a bone substitute in a non-destructive, non-invasive way compared to other traditional but invasive methods like optical and electron microscopy[11,13,15,23].

MicroCT allows to analyse a sample by means of slices acquired at different levels and directions using a dedicated 3D reconstruction software[13,14].

The tissue sample grafted with Endobon shows more porosity than the control sample, due to the presence of more spaces occupied by non-mineralized tissues (fibrous, connective, vascular structures and empty spaces); this structure is confirmed by a lower volume

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percentage and a higher trabecular separation.

Observing the three dimensional reconstructions (Figure 1), which located different structures with different X-ray absorption, it is possible to study the qualitative aspects of the two bone specimens. On the one hand, after 6 months, Endobon-grafted bone tissue sample shows, at the regeneration site, biomaterial high persistence compared to the total volume, but also a relevant amount of new-formed bone tissue with a few areas of high mineralization. After the same time, the specimen extracted from coagulation healing site shows a more important bone tissue formation with many mineralized areas. These findings may suggest that biomaterial slows down the bone neoforming process.

The results are confirmed by the subsequent histological examination on these samples (Figure 2): the grafted bone sample showed a high presence of connective fibrous tissue, residual biomaterial particles and ossification thin tissue with histiocytic lacunae. At higher magnification, multinucleated giant cells were visible, due to granulomatous reaction and reabsorption of Endobon particles. In the control sample, mature structured bone tissue has been observed with histiocytic lacunae and atrophic marrow spaces containing adipose tissue.

Despite the undoubted advantages of the microtomographic technique compared to the traditional optical microscopy, only histological analysis has been able to confirm the presence of biomaterial scaffolding residues in the test bone sample. It is worth mentioning that only histological images can display the presence of different non calcified tissues, like fibrous connective tissue, vascular structures, adipose tissue and gaps that during 3D microtomographic reconstruction it is impossible to colour[19,24,25].

Conclusion

This study is intended to be a contribution to the application of microCT in biomedical engineering and surgical treatments.

The possibility of investigating proprieties and features of a small object without any alteration or destruction demonstrates that microCT is a non-invasive and conservative technique compared to other traditional but invasive methods, like optical and electron microscopy.

To this end, microCT analysis would be a powerful aid to tissue engineering for designing enhanced scaffold materials.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

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