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The Flat Panel Volumetric Computed Tomography in In Vivo Tissue Engineering of Bone: Possibilities and Limitations

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Abstract

The scaffold-based tissue engineering of bones is an extremely promising concept with regard to the regeneration of major bone defects due to trauma, tumour or developmental abnormalities as well as for the treatment of pseudo-arthroses. The in vivo testing of implants is a significant phase in the development of specimens for the clinical application of suitable scaffolds. The collection of an optimal amount of information from these initial â??" clinical - tests demands, ideally, the most diagnostically conclusive studies possible. We tested the procedure of flat panel volumetric computer tomography (fpvCT) thus far virtually untried in the area of bone tissue engineering for the in vivo evaluation of small animal experiments and compared it with other methods (projection radiography, micro-CT, histology).

Index terms—

1 Introduction

n the scaffold-based tissue engineering of bones, experiments on small animals are the first practical test of the scaffold and a significant intermediate step on the road to the clinical testing of the material. As an experimental model the critical size defect (CSD, defect of critical dimensions) has proven its value [1,2]. Frequently utilised on the Ossa longa of animals, stabilisation of a defect requires sufficient osteosynthesis. Babis et al were able to demonstrate that stable osteosynthesis is a decisive condition for the mending of the scaffold [3]. This makes osteosynthesis a critical factor in the breadboard. Additionally, the correct location of the scaffold, the course of degradation and that of bone mending within the defect must be presented as accurately as possible and, ideally, in terms of their course.

Therefore, central issues with regard to the model of the critical size defect in scaffold-based tissue engineering of bone are the following:

? Is the scaffold situated correctly postoperatively (in the osteotomic cleft)? ? What is the degradation behaviour of the scaffold over time? ? Is there bone ingrowth into the scaffold?

? Is osteogenesis occurring in the scaffold?

? What characteristics demonstrate the osteogenic activity? ? How do various scaffolds perform in comparative terms? ? Is the defect closing? Therefore, suitable assessment methods are required for monitoring the course and outcome of the series of experiments, evaluating them and answering all relevant of the above questions. Significant here is above all the monitoring of the mending process in vivo, including in order to be able to recognise and evaluate the influe nce of the breadboard, above all that of osteosynthesis, upon the results.

2 II.

3 Status Quo

Presently, it is above all projection radiography, the micro-CT and histology that are used for the evaluation of in vivo experiments regarding scaffoldbased tissue engineering. Unfortunately, with these methods either resolution

and/or three-dimensional presentability are insufficient and/or the method is not compatible with the survival of the animal and an intact specimen.

Based on the high radiation dosage and the long exposure time, the micro-CT is not indicated for repeated tests on an individual in vivo, while additionally usually the volume to be studied must be significantly reduced [4]. Added to this is the fact that osteosynthetic material frequently causes very significant artefacts, so that this must usually be removed first. This at least partially destroys the specimen.

The same applies to histology: the bone scaffold structure must be cut. This results in a loss of part of the specimen. Additionally, the preparatory process is protracted and complex, and threedimensional presentation is not possible.

Projection radiography as a two-dimensional system can be repeated frequently over the course of time. Nonetheless, the bone mending process can only be assessed to a limited extent due to the lack of threedimensionality and this indeed can lead to erroneous assessments with regard to dual-plane exposures. In order to at least partially compensate for these disadvantages, some research groups such as Fialkov et al have chosen to use scores that they themselves have developed to assess roentgen images [5].

Conventional computer tomography permits three-dimensional representation, however with a maximal resolution of 0.5 x 0.5mm in the plane and 0.25-1mm in the z-axis. This is too low for the detailed representation of such bony structures as trabeculae and the scaffold [6].

Thus it is clear that a sparing procedure for the high-resolution, three-dimensional representation of the mending process in vivo over the course of time is still to be striven for.

Flat panel volumetric computed tomography provides a high-resolution, three-dimensional representation of tissue in vivo. Obert et al were able to visualise bones down to their trabecular structure in mice [4]. It is also possible to demonstrate vascular neoformation using contrast media [7,8]. This is a critical point in the tissue engineering of bones, because vascular neoformation or the ingrowth of vessels in the scaffold is a basic requirement for the formation of new bone in a defect.

Weinand et al utilised the fpvCT to measure a distal thumb phalanx in humans in order to use CAD technology with these data to produce a scaffold. After cell colonisation and implantation of the scaffold subcutaneously in a mouse, the fpvCT was used to monitor the course of the procedure [9].

Thus far the fpvCT has not yet been used to evaluate an in vivo model on a small animal based on critical size defect. Our objective was to determine whether this promising method represents an alternative to the already known methods for evaluating scaffoldbased tissue engineering.

4 III.

5 Material and Methods

The rabbit was obtained from the company Behring Aventis Marburg and allowed to become accustomed to its stables for a week before the operation. Premedication was effected with atropine, and anaesthesia induced with xylazine and ketamine IM. The left femur was shaved and disinfected, the operative field sterilely draped and disinfected again. In summary, a 12 mm piece was removed from the femoral diaphysis and a scaffold of calcium phosphate/PLGA was placed. Osteosynthetic supply was effected using a mandibular plate (Stryker) and 2.7 mm blocking screws. The screw length was chosen individually (10-16 mm). Caprofen was used for postoperative pain therapy. The first fpvCT evaluation took place two weeks, and the second four weeks, postoperatively. Thereafter an fpvCT was carried out every four weeks. After 20 weeks the rabbit was killed, the osteosynthetic material was removed and a micro-CT and a histological examination of the osteotomic cleft took place. Parallel projection radiographic studies were carried out.

The same anaesthetic method was chosen for the fpvCT as described above. panel roentgen sensors with a resolution of 1024 x 1024 pixels in each instance. The maximal Z-axis is 21cm per scan. A more precise description of the volume computer tomograph is contained in the literature [4,7,8]. Our images were obtained with 120 kV and 40 mA. The rotation time of a step was 8 seconds at a length on the Z-axis of 42 mm. Two steps were recorded, resulting in a Z-axis of 84 mm.

For the application of the contrast medium, after induction of anaesthesia a Braun cannula was introduced into the aural vein of the rabbit. 10 ml of contrast medium (Imeron 300, Altana, Constance) was injected 50 seconds before the scan. At an average number of exposures of 420, a voxel magnitude of 0.2 mm3 and a field of view of 102x102x84 mm3 were yielded in the reconstruction.

After four and 20 weeks, in each instance half the rabbits were killed. The left femur was removed, embedded in rigid plastic (Technovit, Fa. Kulzer) and the osteosynthetic material was removed. Then the micro-CT was carried out. The histological specimen was prepared after the micro-CT using the thin slice technique, and then dyed with toluidine blue.

The examination and evaluation of the fpvCT data was undertaken without knowledge of the results of the micro-CT and histology. The fpvCT data were reconstructed using a Linux-based network of seven 7 dual core 2.2GHz processor PCs and a cone beamfiltered back projection algorithm. The reconstruction time was approximately 13 minutes. The images were displayed on an Advantage Workstation, Version 4.1 from the company GE Medical Systems, based on a Linux PC with dual core 2.2GHz processor and 4GB RAM. The

evaluation was effected in maximum intensity projection (MIP) and volume rendering representation, viewing both the three-dimensional reconstruction and the sagittal, axial and coronary interfaces.

After evaluation of the fpvCT, the results were compared to those of the micro-CT and the histology.

6 IV.

7 Results

A total of 19 animals were observed over the defined experimental period. Of these, 8 animals had implanted scaffolds and one animal had an empty defect for 4 weeks and 8 animals with scaffolds and two animals with empty defects over 20 weeks.

Four animals were excluded for reasons of osteosynthetic insufficiency, and four animals experienced complications during the application of the contrast medium (see below).

8 a) Projection radiography

During postoperative roentgen controls, no scaffold could be demonstrated in the osteotomic cleft. An irregular shadowing was noted in some animals; however this could not be identified unequivocally, nor was it possible to determine precise contours.

Consequently, the correct positioning of the scaffold and the degradation could not be demonstrated or confirmed.

Bone formation in the osteotomic cleft was demonstrated in all animals. Nonetheless, it was impossible to differentiate with certainty between ingrowing bone and bone neoformation in the scaffold. Based on the growth sample one could only make conjectures. During the further course, in the presence of a virtually closed osteotomic cleft, no further differentiation was possible.

After 20 weeks, in the context of an empty defect the closure of the osteotomic cleft was suspected, because a continuous cortical line could be demonstrated on both planes (see **??**ig 2). Osteosynthesis could be assessed well on xrays. For example, the four osteosynthetic insufficiencies in the visualisation on two planes were observed immediately. For the most part there was avulsion of the screws distal to the osteotomic cleft.

9 b) Flat panel volumetric computer tomography

The data sets were evaluated at the workstation in maximum intensity projection. First the threedimensionally reconstructed femur was viewed, and then the interfaces parallel, perpendicular and axial to the lamina. Based on the isotropic voxels it was possible to set any other desired interface without any compromise in image quality.

In addition to the bone corticalis, trabecular structures were also shown quite well. In the sectional images one could even identify extremely fine fissures in the bone and changes in the bone structure (see **??**ig 3). All in all, there was only very minimal artefact formation due to the osteosynthetic material. Shadowing was seen parallel to the osteosynthetic material and raylike artefacts radiated from the lamina (see Fig 3). These, however, did not significantly hinder the evaluation.

Postoperatively one could identify the scaffold very well, and delineate it from the surrounding bone cleft. The degradation behaviour, as well, could also be observed very well up to 12-16 months postoperatively. At these times the scaffold was degraded to such an extent that it could no longer be shown sufficiently via fpvCT, nor could it any longer be differentiated from bone. The bone growing in from the outside could be clearly delineated from the bone formed in the osteotomic cleft on the fpvCT. Various different growth forms of the ingrowing bone could also be identified, thus yielding significant information concerning the breadboard. For example, cap formation beyond the medullary space radiating from the corticalis was demonstrated in nearly all the test animals, which enclosed the medullary space and thus made mending of the scaffold impossible (see **??**ig 5). The pfvCT was extraordinarily useful for the assessment of the osteosynthetic process. By way of the high-resolution representation of the entire femur, for the first time fine fissures in the bone between the screws could be identified. For example, one could derive significant information concerning the formation of screw fissures and thus osteosynthetic failure. Stressrelated remodelling around the screws in the bone could also be clearly identified (see **??**ig 7). To represent the vessels in the region of the femoral bone and the osteotomic cleft, a contrast medium CT was carried out on the test animals. It was expected that newly proliferating vessels would be identified. However, no blood vessels could be identified in the area of the bone and the osteotomic cleft. Vessels were only visualised in the large leg veins. In 4 test animals a fatal circulatory reaction occurred shortly after application of the contrast medium. However, this never occurred at the first administration, but only at the third or fourth test. We suspect stress-and volume-related acute circulatory insufficiency. In the absence of usefulness and considering the high risk for the animals, the contrast CT was then terminated.

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V.

11 Discussion

The fpvCt is a relatively new procedure for the high resolution, three-dimensional representation of tissue in vivo. It has been demonstrated in various publications that it is excellent for the representation of bone details and vessels and is superior to traditional computer tomography [4,6,[8][9][10][11]. At comparable radiation dosage and test duration, the fpvCT achieves significantly better local resolution (in our case 0.2mm3) than traditional computer tomography. By means of the technique of isotropes, that is to say cubic voxels, any chosen interface can be represented without compromise in quality. This is extremely useful above all in the precise assessment of bone growth. In comparison with the micro-CT, the advantage of the fpvCT is that it requires a much lower dosage of radiation, so that it can be used several times in one animal in vivo. The scan time is also significantly shorter (here 16 seconds).

Another decisive point is that studies of osteosynthesis were possible without significant artefact formation by the osteosynthetic material. This had not yet been demonstrated in the past. Additionally, the entire femur could be represented, something which had otherwise only been possible by way of projection radiography. For example, the entire osteosynthetic process could be observed in detail throughout the test period. This image material allowed significant conclusions to be reached with respect to the methodology of the critical size defect and, above all, osteosynthesis. For the first time, as well, the scaffold could be represented in vivo, allowing it to be demonstrated that the implant was in the correct location postoperatively and that the implant did not contract rapidly. Additionally, the degradation of the scaffold could be observed and the implant could be represented for a considerably longer time than is the case with projection roentgen. For a differentiation between bone neoformation on the one hand and the calcium phosphatase phase of the scaffold on the other, the resolution did not suffice, that is to say that no bone neoformation could be demonstrated in the scaffold. Based on the sclerotic zones in the scaffold, however, the suspicion is great.

Prior to the fpvCT studies, there had been considerable hope that vessels would be visualised. After the successful visualisation of neoangiogenesis in tumours in the mouse [7,8] we hoped to be able to show vascular neoformation in and around the osteotomic cleft in vivo by way of contrast media using the fpvCT. However, this did not occur. Indeed it was possible to show the larger femoral vessels, however no small vessels in and around the bones or indeed in the osteotomic cleft could be represented. This was probably attributable to the field of view that was too large in comparison with the very small vessels. On the other hand, however, no central necrosis could be demonstrated. This was a clear indication of newly occurring, intact vessel supply in the osteotomic cleft.

Another critical point was the death of four rabbits in the context of the application of the contrast medium. An allergic reaction was most improbable, because the deaths occurred at the earliest at the time of the fourth contrast medium application. We assume that the rabbits, already under considerable stress due to their transport and examination (induction of anaesthesia), suffered circulatory shock when the contrast medium was administered. Rabbits are animals that are quite sensitive to stress, making a change in location and an unfamiliar environment particularly dangerous for them. According to our experience, an accustomisation phase of one to two hours in a quiet and air conditioned room prior to the study significantly lowers the stress load and therefore the cardiorespiratory risk.

In addition to the great advantages with respect to the representation of bones, a disadvantage is certainly the rarity of the fpvCT. Because the method is still only rarely used, one must generally expect long travel times or, better yet, the entire test process could take place where the fpvCTs are located, in order to spare the animals long transport periods. Another disadvantage in comparison with projection radiography is the significantly greater cost per procedure, while on the other hand the process does afford considerably more accurate statements concerning the course of mending. However, the fpvCT is not sufficient as a sole evaluation method, because even though the scaffold can indeed be shown, no concrete statements can be made concerning bone and vascular neoformation in the scaffold and osteotomic cleft. Unfortunately, a program for the quantification of bone ingrowth in the osteotomic cleft does not exist yet, something which could facilitate objectivisation of the results. At the moment there is only qualitative analysis. This is, however, a very valuable instrument for observing processes in the bone and osteotomic cleft over the course of time, promising to yield significant information concerning the breadboard and methodology.

12 VI.

13 Conclusion

The fpvCT is more than simply an alternative to the projection roentgen and micro-CT. Under certain conditions, it can replace both of those evaluation methods. For example, qualitatively it is superior to the projection x-ray in every aspect, with its only disadvantage being higher costs and more test-related expenditures. The micro-CT can also be replaced if higher resolution can be done without. Beyond that, in our opinion the micro-CT offers no advantages over the fpvCT. The representation of very small vessels can be achieved by a smaller field of view, which would then require further examination and the administration of contrast medium. More extensive

1

Figure 1: Figure 1 :

knowledge could only be realised through histology, which in terms of certain issues cannot be replaced by the fpvCT. [1]

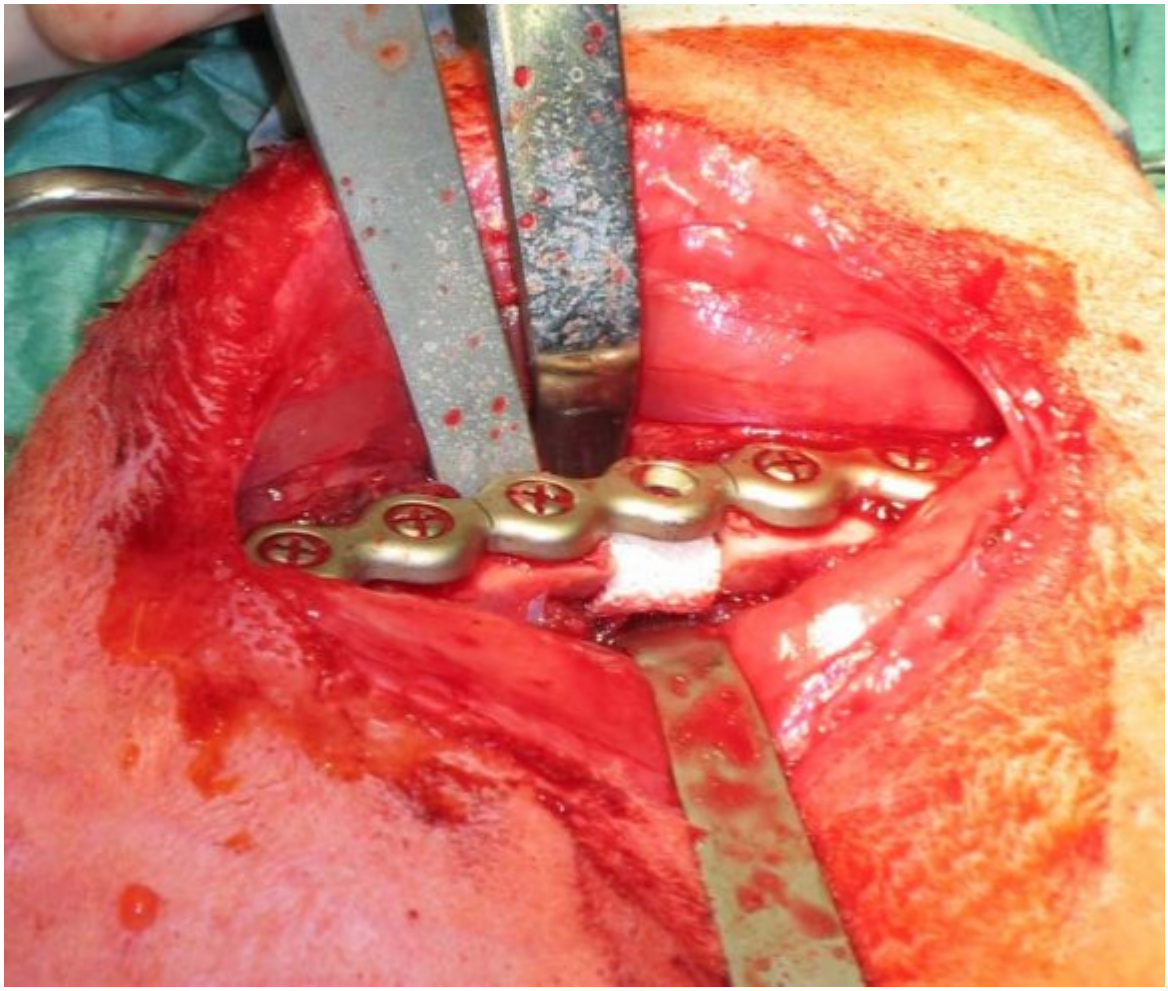

Figure 2:

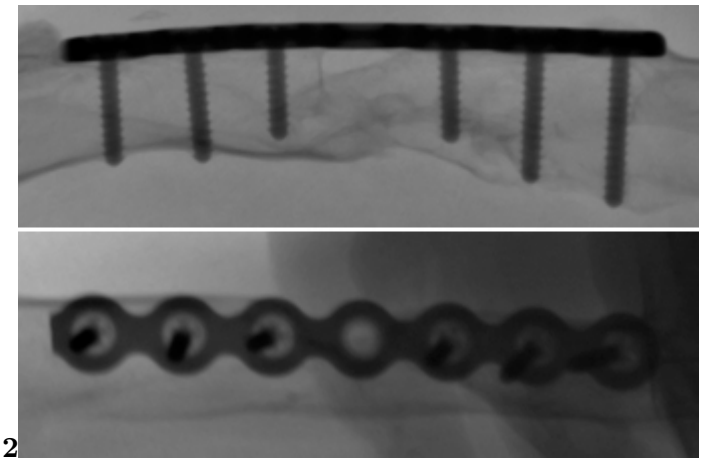

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Figure 3: Figure 2 :

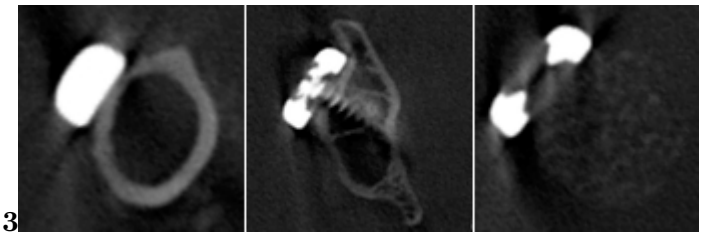

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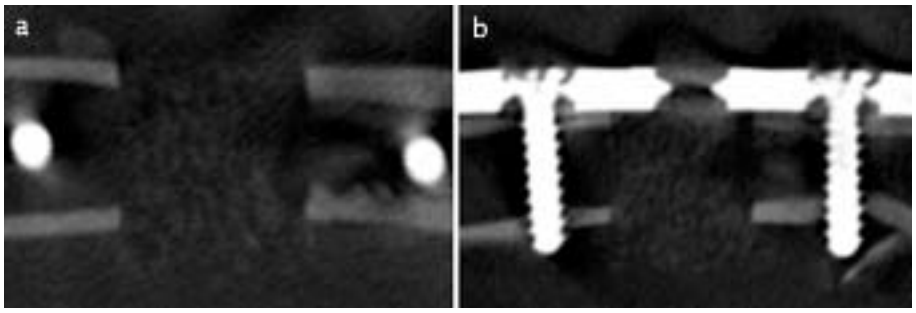
Figure 4: Figure 3 :



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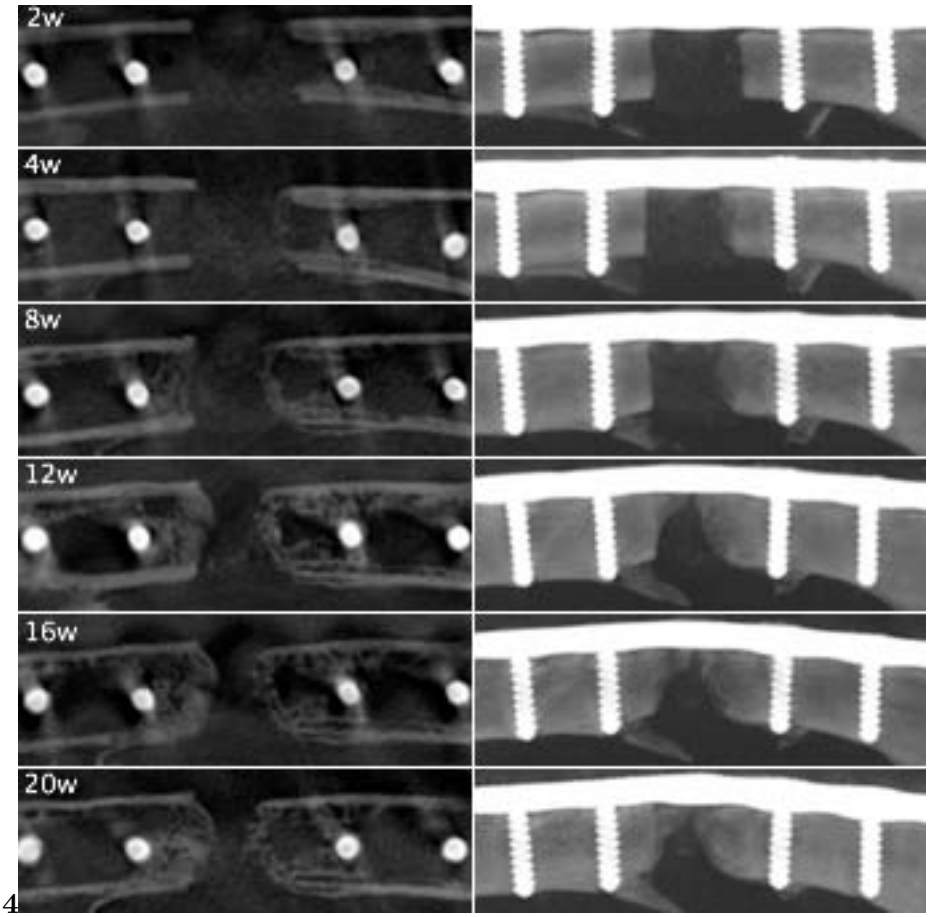

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Figure 6: Figure 4 :

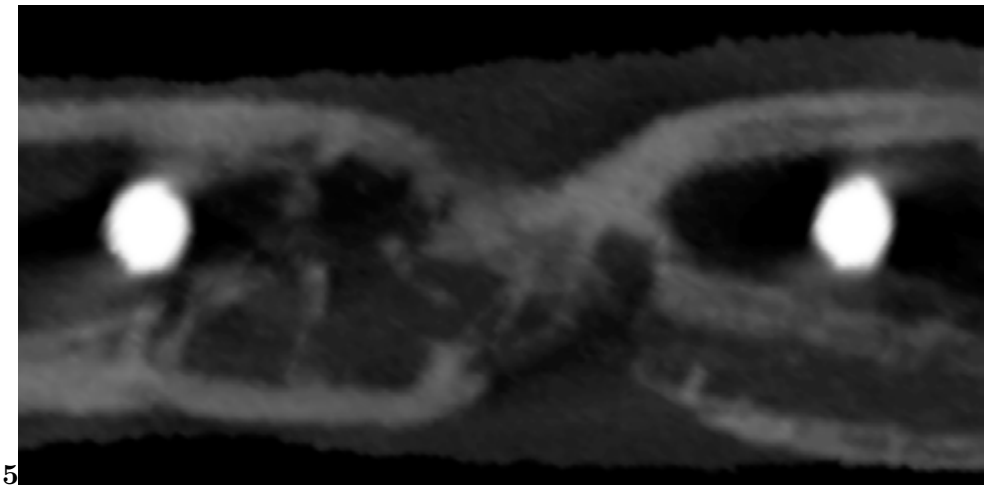

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Figure 7: Figure 5 :

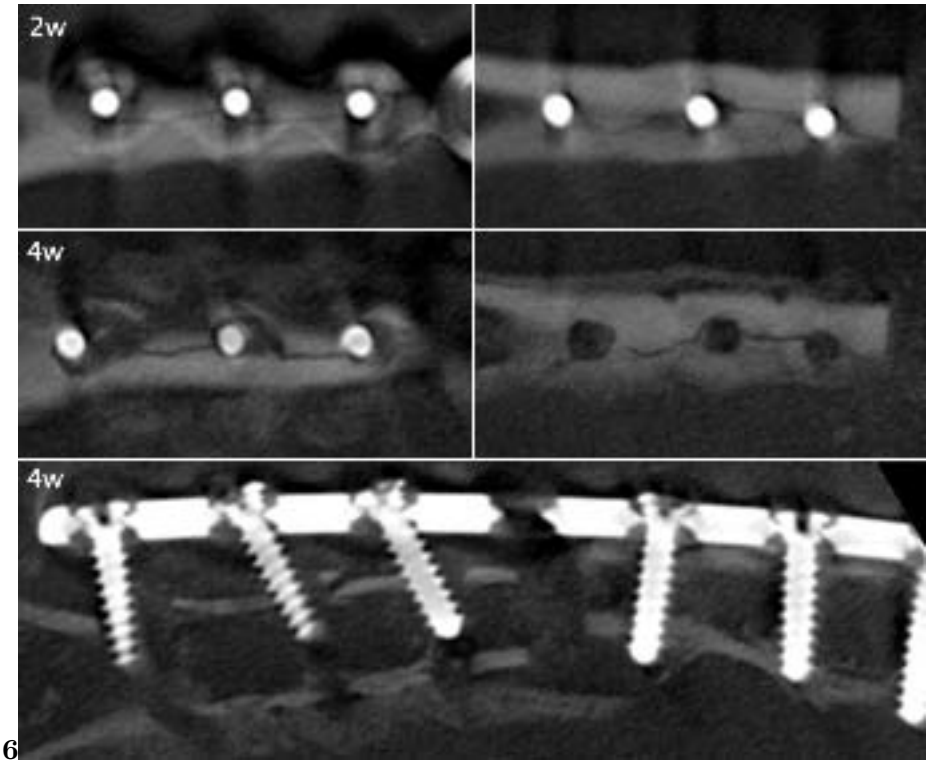

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Figure 8: Figure 6 :

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