Synchrotron Radiation X-Ray micro-CT evaluation of Bone Augmentation

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The osteoconductive and regenerative materials are very important in bone regeneration process. We propose in this study to evaluate the interface between the old and the new regenerated bone. 60 rats femurs were used for this investigation. Under strict supervision, the holes were drilled in the rat femurs and then the entire defect is filled with different bone augmentation materials in order to promote the proliferation of neoformating bone. The samples were obtained after animals are x-rayed using dental equipment for evaluation of the healing process at 2, 4, 6, 8, 10, and 12 weeks, aiming to reveal the influence on the site of bone defect, reaction in the area, vascular reaction and radiopaque changes. A synchrotron radiation X-Ray micro-CT experiment was performed at the SYRMEP Beamline of the ELETTRA Synchrotron Radiation Facility (Trieste, Italy). The 1200 radiographic projections were acquired with a beam energy of 29 keV over 180° with a pixel size of 9 μm. A sample – detector distance of 15 cm was considered in order to have both absorption and phase-contrast signal, for a better viewing of the interfaces. For 3D visualization, data volumes were rendered directly without decomposing them into geometric primitives. A commercial software - VGStudio MAX - was used to generate 3D images and to visualize the distribution in 3D of different constituents.

Keywords: healing proces, bone defect, X-Ray micro-CT

Micro-CT is a non-destructive imaging technique, which uses X-rays to create high resolution (few microns) cross-sections images through a specimen which can later be reconstructed as a 3D model. In the last decade, micro-CT was intensively used for investigating bone [1,2] and bone tissue engineering [3,4], tooth investigations [5,6] and other medical and technological fields [7,8].

Micro-CT is similar to conventional computed tomography (CT) usually employed in medical diagnosis and in industrial applied research. Unlike these systems, which typically have a spatial resolution of about 0.5 mm, micro-CT is capable of achieving a spatial resolution of up to 0.2 microns, i.e. about three orders of magnitude higher. The use of X-rays delivered by Synchrotron Facilities has several advantages compared to X-rays produced by Laboratory or Industrial sources. It includes the possibility to take advantage of the high photon flux, which guarantees the achievement of high spatial resolution with high signal-to-noise ratio. Furthermore, the Synchrotron-produced X-ray beam is tuneable, thus allowing performing measurements at different energies and with the possibility to obtain monochromatic X-ray radiation, which eliminates beam hardening effects.

The interesting feature of X-ray micro-CT is that the 3D computed reconstruction could be sliced along any direction to gain accurate information on the internal geometric properties and structural parameters of the sample. The different phases present in the specimen can be easily visualized by performing a segmentation of the histogram.

In the pure absorption experimental set-up, the sample is as close as possible to the detector, as in this way the image blurring is reduced to minimum. By choosing a suitable sample-to-detector distance, the contrast is not given anymore only by pure absorption, but also by the phase differences among the scattered X-ray waves. The cross section for elastic scattering of X-rays in matter, which causes a phase shift of the wave passing through the object of interest, is usually much greater than the one for absorption. Because of that, in the last years the X-ray phase-contrast imaging technique was increasingly employed to investigate samples of interest in medicine. In particular, the phase-contrast effect, which puts into evidence edges between different materials, can be used for interface analysis and also for a better visualizing of the morphology of the different materials present in the investigated volume.

Experimental part

Biological material for the in-vivo experiments consist in 60 Wistar rats, 6 months old from the Animal Facility of the "Pius Branzeu" Center for Laparoscopic Surgery and Microsurgery with an average weight of animals of 300g (256 -331g), which were quarantined for two weeks before starting of the surgical experiments. The bone augmentation materials were implanted unilaterally in the medullary cavity of the femur of each animal. The distribution between sides will be equal. In the contralateral femur a defect was made in the same manner but without augmentation filing and used as histological control. For the entire healing period the animals will be kept two or three in a cage with ad-libitum supply of fresh water and rodent pellets food. The experimental protocol was submitted and approved by the Ethical Committee of the University of Medicine and Pharmacy, Timisoara.

After clinical examination, only the animal considerate healthy were used. Before surgery X-ray exams evaluation was performed for the femoral and tibia region for

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excluding eventually pathology at places of bone material augmentation insertion.

Anesthesia, of the animals was achieved with Isofluran in concentration of 5% and O2 at 1L/min, for induction in the anesthesia chamber, and after that, animal is connected throw a facemask to an open breath circuit of anesthesia which inflows Isofluran at 1% and O2 at 1L/min. The hindlimbon the experimental side is shaved, and the entire dorsal aspect of the animal is disinfected.

Surgery is performed under sterile conditions. Access to the periosteum is made throw a 3 cm. longitudinal incision of the skin, on the lateral part of the thigh region, in the cranial third of the femur, followed by the blunt dissection of the quadriceps muscle with exposure of proximal diaphysis of femoral bone. All the maneuvers were performed under the microscope and the tissues are handled with microsurgical instruments to prevent as much as possible the tissue distruction. Group 1 (20 animals) will have a circular defect perpendicular to the bone, approximately 3mm in diameter and 2 mm deep using Surgic XT Plus, a surgical motors which provide speed and high torque accuracy for maximum safety during operation. Group 2 (20 animals) consist in making of a V-shaped defect with the top of "V" oriented transversal on the length of the bone. Group 3 (20 animals) consist in creating of a longitudinal bone defect in 3 mm long and 1 mm wide. Bone defects are performed with VarioSurg, which is a powerful ultrasonic saw. The ultrasonic approach to bone surgery reduces heat generation in order to minimize osteonecrosis and avoid damage to surrounding soft tissue.

The entire defect is filled with different bone augmentation materials in order to promote the proliferation of neoformating bone.

Closure of the surgical site was undertaken with Monosyn 5.0 continuous suture in 2 planes of the muscles and separated stiches of the skin with Prolene 5.0 thread. Antibiotic prophylaxis during the surgery was performed with Cefazolin + Gentamicin to prevent any infection with any Staph, strep, Gram(-) bacilli or anaerobes. Postoperatively all the animals received for 5 days analgesic medication with Buprenorphine.

Follow up period of the animal consist in daily clinical examination for 21 days with evaluation of the general clinical status (heart rate, respiratory rate, body temperature, mucosal appearance and healing of the incision, posture and locomotion).

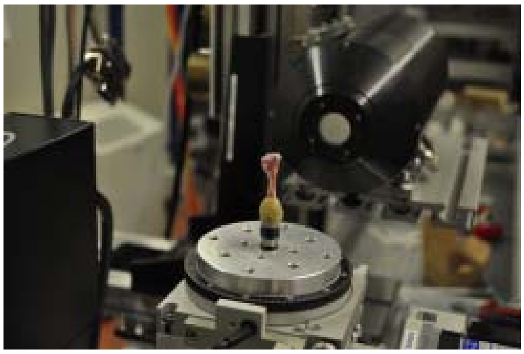


Fig.1. Micro-CT investigations using the Synchrotron Radiation at the SYRMEP Beamline of the ELETTRA Synchrotron Radiation Facility (Trieste, Italy)

Animals are x-rayed using dental equipment for evaluation of the healing process at 2, 4, 6, 8, 10, and 12 weeks, aiming to reveal the influence on the site of bone defect, reaction in the area, vascular reaction and radiopaque changes.

All rats are euthanatized (Thiopental overdose) at 84 days after surgery. The tight is exposed through an extended longitudinal lateral incision. Soft tissues were removed with preservation of the periosteum. After exarticulation of the knee and hip joints, the femurs are removed and the specimens with new bone formation will be gross examined, emphasizing the changing, after that is being prepared, together with bone structure for microCT and microscopy evaluation.

A synchrotron radiation X-Ray micro-CT experiment was performed at the SYRMEP Beamline of the ELETTRA Synchrotron Radiation Facility (Trieste, Italy – fig. 1 below). The 1200 radiographic projections were acquired with a beam energy of 29 kV over 180° with a pixel size of 9 μm. A sample – detector distance of 15 μm was considered in order to have both absorption and phase-contrast signal, for a better viewing of the interfaces (fig. 1).

The tomographic reconstruction was performed by means of the common filtered back-projection method [9].

For 3D visualization, data volumes were rendered directly without decomposing them into geometric primitives. A commercial software - VGStudio MAX - was used to generate 3D images and to visualize the distribution in 3D of different constituents.

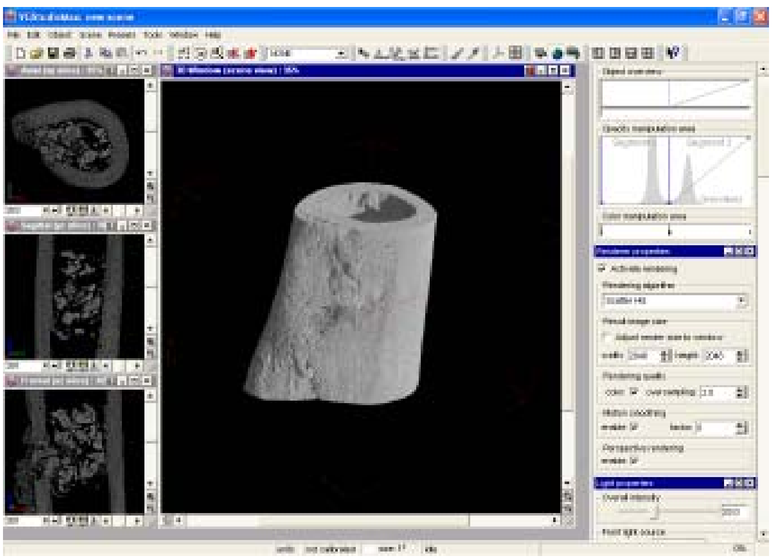
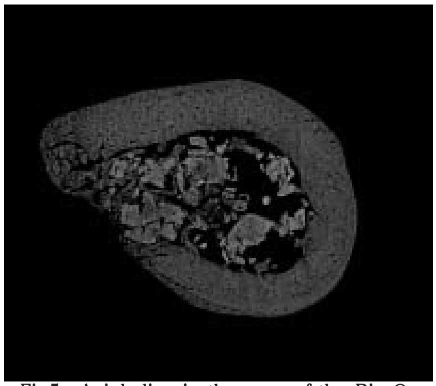


Fig. 2. VGStudio Max software for 2D and 3D visualization of the reconstructed femur

Fig. 3. 3D reconstruction of the area of the Bio-Oss insertion

Fig. 4. Frontal slice in the area of the Bio-Oss insertion



Fi. 5. Axial slice in the area of the Bio-Oss insertion

Results and discussions

Macroscopic evaluation of the bone augmentation samples reveals the formation of a fibrotic tissue over the surgical defect induced after 15 days. After 45 days the fibrotic capsule tissue contoured well the defect area. Over 60 days a new bone tissue was present in the defect area with discontinuity related to the old bone.

VGStudio Max software gives a comprehensive 3D visualization of the reconstructed specimen, allowing the segmentation of the grey histogram, in order to visualize only the phases of interest in the imaged volume. It allows a direct view of three orthogonal axis (Axial, Sagittal and Frontal), together with the 3D image that can be rotated or slices in any direction for a good visualization of the morphology of the reconstructed specimen. From the pictures below one can observe the area in which the drilling in the femur was performed, with the consequent insertion of Bio-Oss material (fig. 2).

On the 3D reconstruction the new bone together with the old bone and some of the unmodified augmentation materials could be observed (fig. 3). In order to have a better evaluation of different sections in the samples, frontal (fig. 4) and axial (fig. 5) could be generated. The amount of the remaining augmentation material could be spotted on the imagistic evaluation depending of the amount of healing time.

Conclusions

Evaluation of the bone grafting material/bone interface with noninvasive methods such as microCT using the synchrotron radiation could act as a valuable procedure that can be used in the future for usual research procedures.

The analysis of the interfaces permits to evaluate the level of the bone regeneration.

The interface between bone and osteoconductive materials was clearly observed. After the third month the results show good and open interfaces but also gaps inside the osteoconductive materials.Besides the possibility of navigating inside the structure, one additional advantage of this technique was pointed out:the remaining regenerative materials can be separated from the normal bone and the new bone can be visualized one month after the augmentation procedure

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