Micro-CT SKYScan 1172 potential in orthopaedic applications

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INTRODUCTION:
Bone fracture repair is a complex process divided into inflammatory, soft and hard callus reparative, and remodeling phases. Our current research focuses on the reparative phase, during which bone stiffness is restored. Animal models are frequently used to study fracture repair.
Microcomputed tomography (micro-CT) measurements are extensively applied in preclinical research on bone and are well validated to study microarchitecture.
Non-invasive characterization of fracture callus structure and composition may facilitate development of surrogate measures regarding mechanical function. As such, analyses of fracture calluses, based on micro-CT could enable more reliable clinical assessments of bone healing¹. Radiographs are inherently a two-dimensional assessment of the 3D callus structure and are correlated with callus cross-sectional area and bone mineral content. In comparison, Micro CT provides superior resolution to radiographs that is for us benefit, when studying bone healing in small animals.
The aim of this paper is to report two examples in which we use the micro-CT system, SkyScan 1172, in our laboratory for preclinical studies and to show how micro-CT improve our understanding of the effects of recombinant osteoinductive morphogen protein OP-1 and Ultra Sound (US) in the fracture healing compared to histology, hystomorphometry and radiographs.
OP-1 is involved in bone repair. In bone defect models without spontaneous healing, local administration of recombinant human OP-1 (rh-OP-1) induces complete healing. Moreover, massive bone allograft with OP-1 can be used in orthopaedic reconstructive surgery to replace bone defects due to trauma or oncologic resections².
US has been used to manage refractory fracture healing and its effectiveness has been confirmed³. However, the mechanism underlying the effect of ultrasound is not well understood, but US has been shown to stimulate the differentiation of a variety of cells including bone-marrow stromal cells, mesenchymal stem cells, chondrocytes and osteoblasts in vitro⁴.

MATERIALS AND METHODS:
The studies were approved by Ethical comettee, Health Italian Ministry and were performed according the European and Italian laws on animal experimentation (Italian D.L. January 27, 1992; N.I.H. No. A5424-01) and the Guide for the Care and Use of Laboratory Animals.
In the first study a group of Alpine sheep were assigned randomly to two groups: the control group and the OP-1 group. A 3 cm osteoperiosteal defect was performed in the middle part of the metatarsal bone in each group. In the control group the reconstruction of the bone defect was performed with the bone graft alone; in the OP-1 group the allograft was added with rh-OP-1 and applied around the defect². In the second study (US) in a group of adult male rabbits a complete longitudinal incision was made on the third medial of radii bilaterally with a Gigli’s saw. The right radius is treated with US and the left radius is control. At established experimental times, animals were sacrificed by pharmacological euthanasia under general anesthesia. Bone samples were excised, removed from soft tissues and were
analysed to investigate the osteotomy healing and periosteal callus presence with microCT, histological and histomorphometric approaches. Before histology we used micro-CT to see changes that occur in bone but inside: callus volume and density, new bone formation inside bone marrow and cortical bone microstructure. In addition the spatial arrangement of microstructural features and size of pores have been implicated in mechanical properties such as fracture toughness.

**MICRO CT**
During acquisition of OP-1 study we used a Al+Cu filter, a rotation step of the specimen every 0.4 deg (rotation until 180 deg) and a frame averaging 5. We used an image pixel size corresponding at 11µm and the source voltage and the source current were respectively 100kV and 100µA. Because of the size of the specimen (nearly 5cm) we used an oversized scanning mode in which the number of connected scans was 5.

During acquisition of US radii study we used an Al filter with a thickness of 0.5mm, a rotation step of the specimen every 0.3 deg (rotation until 360 deg) and a frame averaging 4. We used an image pixel size corresponding at 12µm and the source voltage and the source current were respectively 100kV and 100µA. Also in this case we use the oversize scanning mode with 5 connected scans.

The tiff images obtained in both studies were used to reconstruct the samples by the SkyScan NRecon software (version 1.4.4). We used in both cases the same post alignment for all the sub-scans to have a continuous dataset useful in particular for a 3D model construction. We have use CTVol (version 1.11) to visualize and move the 3D image of the specimens. For every specific case we used different kind of filter, like ring artifacts reduction or smoothing. The undersampling factor was 1 for each samples in both studies.

**IMAGES**

![Fig.1 Metatarsus treated with OP-1](image1)

![Fig.2 Two radii: on the right there is the control, on the left there is treated](image2)

In the image (Fig.1) of metatarsus with OP-1 reconstructed with CTAn, we can see the graft bone inside the medullary canal and an initial callus outside. In the normal radiography we can’t see the bone from this prospective but with micro-CT the internal structure of a specimen (Fig.1) is reconstructed as a set of flat cross sections which are then used to analyze the two and three dimensional morphological parameters of the object.
In the US study we can compare two radii (right is control and left is treated with US), reconstructed with micro-CT. As we can see in figure 2, we can compare the different quality of callus of radii, treated and control. So we can contend probably that the radio stimulate with US has more extensive callus than control has.

Furthermore with MicroCT we have examined the performance of the fracture in 3D across the volume of the specimen. We have created a 3D model, with the dataset of reconstructed images, that we have moved and filmed using CTVol (version 1.11). We have filmed the specimens in all parts, to create a 3D model on the move. Also in the film we can enter the object and show some characteristics of material of the object: trabecolae of bone, microarchitectural changes, canal network, as we can see in the figure 3.

![Fig.3 An image of metatarsus treated with OP-1 film](image)

**MICRO-CT Vs HISTOLOGY**

The analysis of bone microstructure, including the canal network, has largely been restricted to the two-dimensional realm. Although 2D histomorphometric techniques have and continue to provide a wealth of information regarding bone tissue dynamics, they can’t yield a complete picture of three-dimensional microstructure of cortical bone. Cortical microstructure exists and remodels in 3D; Therefore, a full understanding of its architecture will require 3D analysis, in a digital data format, that facilitates quantitative 3D architectural analysis.

In our laboratory we can study the performance of OP-1 and US or another device, before preparation and cut of the specimens needed for histology and histomorfometry (Fig.4, 5, 6); in fact the process of MicroCT is non destructive and requires no special preparation of the specimens.

With Micro-CT we can quickly obtain information about the specimens than the histology do: histology needs some weeks because of specimen preparation (disidratation in alcohols, inclusion in paraffina or acrylic resin and cut), on the contrary analysis of a specimens with Micro-CT spends few days.

Micro-CT can analyze more sections of a sample than the histology can do; moreover Micro-CT offers the unique possibility to visualize in 3D the microarchitectural changes occurring in healing bone.

Therefore micro-CT provides information about a specimen in more exhaustive way and faster than histology and radiographs do.
CONCLUSION
In conclusion during histological and histomorphometric analysis, Micro-CT is more useful because, shortly, can give us a careful qualitative and quantitative description of bone tissues: Bone Volume (BV), ratio between bone volume and tissue volume (BV/TV), Bone surface (BS), Bone specific surface (BS/BV), bone surface density (BS/TV), Trabecular thickness (Tb.Th), Trabecular separation (Tb.Sp), Trabecular Number (Tb.N), Euler Number (Eu.N), Porosity (Po). These are the most interesting parameters for our research, and it is very important that we can obtain more information before the specimens are processed with histological technique.

REFERENCES