Micro-CT evaluation of subchondral bone response in the treatment of osteoarthritis with adipose derived stem cells (ADSC) in rabbit

Andrea Ferrari¹, Valentina Masciale¹, Giovanna Desando², Carola Cavallo³, Federica Sartoni¹, Lucia Martini¹,², Francesca Veronesi¹, Andrea Facchinì²,³,⁴, Brunella Grigolo²,³, Milena Fini¹,², Annapaola Parrilli⁵

¹ Preclinical and Surgical Studies Laboratory, Rizzoli Orthopaedic Institute, Bologna, Italy
² Laboratory of Immunorheumatology and Tissue Regeneration, Rizzoli Orthopaedic Institute, Bologna, Italy
³ Laboratory RAMSES, RIT Department, Rizzoli Orthopaedic Institute, Bologna, Italy
⁴ Department of Clinical Medicine, University of Bologna, Italy
⁵ Biocompatibility, Technological Innovations and Advanced Therapies Laboratory–BITTA, Rizzoli Orthopaedic Institute, Bologna, Italy

Aims
Osteoarthritis (OA) is one of the most common rheumatic disease. According to the World Health Organization, the prevalence of rheumatoid arthritis in the world is between 0.3% and 1%, with enormous consequences both socially and economically. OA is characterized by the progressive destruction of articular cartilage, possibility of subchondral bone remodeling, and synovia deterioration. This chronic condition, depresses the quality of life due to pain and constant need of analgesic therapy. The currently available treatments for OA are effective only on a short term and there is a largely unmet medical need for durable disease-modifying treatments. Different musculoskeletal applications with ADSCs have already been reported with encouraging results for the regeneration of cartilage and bone even if the mechanism of action is not clearly defined yet. The main aim of the current study was to explore the efficacy of an intra-articular injection of ADSCs in preventing cartilaginous damages following the onset of OA in a rabbit model. The harmonious relationship between bone and cartilage in OA has been widely discussed. Several groups have applied micro-CT techniques to non-destructive evaluation of trabecular bone and it has been shown to be an accurate tool to precisely measure changes in bone stereology as well as bone volume and microarchitecture.

Method
OA was induced surgically by bilateral Anterior Cruciate Ligament Transection (ACLT). 2x10⁶ and 6x10⁶ autologous ADSCs were isolated from rabbit inguinal fat and administered by an intra-articular injection into the affected hind limbs, 8 weeks after OA development. Animals were sacrificed at short (16 weeks) and long (24 weeks) follow-up. Local bio-distribution was verified by injecting CM-Dil labeled ADSCs. Cartilage and synovial tissues were scored using histological examinations supplemented by Laverty’s scoring systems. Protein expression of some extracellular matrix molecules (collagen I and II), catabolic (Metalloproteinase-1 and -3) and inflammatory markers (Tumor Necrosis Factor- α) were determined by immunohistochemistry. The specimens were also scanned with the high resolution microtomography system Skyscan 1172 at a source voltage of 100kV, at a source current of 100μA and using an aluminum filter of 0.5mm. Each sample was rotated until 180 degrees with a
rotation step of 0.4 degrees and a frame averaging of 4. The pixel size was 6.5μm. The reconstructions were performed using software NRecon (version 1.6.2.0) and the resulted jpg images had 4000X4000 pixels with a pixel size of 6.5 μm. No corrections were used but the specific misalignment for each acquisition and a moderate ring artifact reduction.

The images datasets in 8-bit jpg format (approximately 1200 images) were analyzed and binarized by CTAn software (version 1.10.1.3 -Skyscan, Belgium) to calculate epiphyseal bone volume BVF in %, epiphyseal Trabecular Thickness TbTh in μm, epiphyseal Trabecular Separation TbSp in μm, subchondral cortical bone thickness SubChondr. B in μm and osteophytes volume V in mm$^3$.

For BVF, TbTh and TbSp the femur part of the knee was divided into two compartments (medial condyle and lateral condyle) and three cylindrical sample volumes with a diameter of 1.5 mm were placed in separate anatomical regions (anterior, central and posterior) within each compartment. This method generates six distal femur cylindrical volumes from which compartmental BVF, TbTh and TbSp were obtained after individual global thresholds determined for each 3D dataset. Subchondral bone thickness was calculated by an operator on 5 central sagittal slices for every specimen spaced out 250 μm. In every slice the thickness was calculated in 10 different points along the subchondral cortical bone.

To quantify the volume of osteophytic bone, an operator identified and outlined osteophytes within each contiguous coronal image section. This 3D analysis was carried out over all the image volume, which included approximately 12 mm of the distal femur.

Precision errors are expressed as the percent coefficient of variation (CV in %).

**Results**

The micro-CT analysis led to different results depending on the considered morphometric parameter. All the mean results are shown in Figure 1 and in Figure 2 the microtomographic section and the 3D models of all groups at different times are shown.

**BVF**

There are no statistically significant differences in the bone volume fraction of the trabecular femoral bone.

**Tb.Th. and Tb.Sp**

There are no statistically significant differences even if in the group at 24 weeks the values are slightly lower in all zones (anterior, central, posterior) and more differences between medial and lateral condyles are in the posterior anatomical site of the condyles (in the medial condyle these parameters are higher).

**Subchondral cortical bone thickness**

The higher thickness value was found in the medial condyle of the control group at 24 weeks where the load is greater.

**Osteophytes Volume**

The parameter shows more differences in values over time. In the experimental groups that are no significant differences in medial condyles over time, beside there are an increase in volume in lateral condyles. In the control group the osteophyte volume decrease between 16 and 24 weeks in both condyles. This decreasing is more evident in the medial condyle.
Figure 1: Histograms of the mean results of the morphometric bone parameters evaluated.

Figure 2: Micro-CT sections and 3D virtual models by CTVox of all different groups at the experimental times of 16 weeks (blue) and 24 weeks (red).
Conclusion

Our data reveal a positive contribution of ADSCs in promoting cartilage repair and contrasting degenerative events occurring during OA as suggested especially by the histological and immunohistochemical analysis. Micro-CT suggested no meaningful remodeling of subchondral bone except for the new formation and growth of osteophytes.

References: