The association of BMP7 and Mesenchymal Stem Cells Promote Bone Allograft Integration

INTRODUCTION:
Massive bone allografts are widely used in orthopaedic reconstructive surgery to replace bone defects due to trauma or oncologic resections (1). The effectiveness of the clinical outcome depends on bone healing time and type of graft-host integration: the larger the amount of bone to be replaced, the more difficult the integration process becomes. This process may involve only 20% of the graft in 5 years, as shown by studies on retrieved allografts (2) (3). Limited incorporation and modest bone remodeling can cause allograft failure. We previously demonstrated that the association of PRP (Platelet Rich Plasma) with MSC improves allograft integration(4). The rationale to use BMPs and MSC is to induce a biological stimulus that should speed up graft incorporation, which will reduce the rate of failures, such as fracture and non-union, of massive bone allografts in clinical practice.

METHODS:
All experiments were performed with the approval first by the Rizzoli Orthopaedic Institute Ethics Committee and, at the same time, by the Italian Ministry of Health in accordance to 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. Twenty Alpine sheep were randomly assigned to four groups of five animals each. Under General anesthesia a critical full size defect 3 cm wide was made in the diaphysis of the metatarsal bone. The defect was replaced with a sterile allograft. In the Control Group the allograft was implanted alone. In the MSC Group, an implant consisting of 4 × 107 MSCs in 5 mL of sterile rat tail-derived collagen. In the OP-1 Group, an implant consisting of rh-OP1 (Ossigraft 3.5 mg, Stryker Biotech, Massachusetts, USA) in bovine collagen was applied around the grafted bone. In the MSC + OP-1 Group, an implant consisting of 4 × 107 MSCs in 2 mL of physiologic solution mixed with rh-OP1 (Ossigraft 3.5 mg) in bovine collagen was applied inside and around the allograft. In order to investigate allograft integration, all the animals were radiographed in AP view projection at 1, 2 and 4 months. Just before sacrifice, under general anesthesia, the femoral vessels were isolated at the groin and the femoral artery was cannulated, then, India Ink was pumped in the femoral artery of the animal to evidence the vessels of the metatarsal bone. The osteotomy lines were sectioned in a sagittal plane passing through the polar line including part (10 mm) of the grafted bone (osteotomy sides) leaving about 2 cm of the graft which was cut in a transverse plane (central side). All specimens were evaluated non-decalcified. The slides were evaluated for histomorphometric analysis. Statistical analysis was performed with the Mann-Whitney test calculated with the Monte Carlo method for small groups.

RESULTS SECTION:
The area of the periosteal callus increased with time in the Control Group and in the MSC Group, achieving the maximum score at the 4th month both in the proximal and distal osteotomy line. In the OP-1 Group the callus increased in the first month and remained stable after the 2nd month. In the MSC + OP-1 Group, instead, callus formation increased in the first month, but it decreased after the second month, becoming almost not visible at 4th month. Radiographic images taken at 4 months showed resorption of the graft in the OP-1 Group, in which the cortical bone was hardly detectable, while in all the other groups the allograft maintained the usual radio-density. Histology showed that in the Control Group the integration of the graft was caused mainly by external callus formation with no or extremely low presence of new bone within the graft. In the MSC Group as well the new bone formation occurred mainly starting from the periosteal callus, which was however less pronounced compared to the Control Group. On the other hand, in the OP-1 Group and in the MSC + OP-1 Group integration processes occurred mainly by direct new cutting cones penetration, the remodeling processes were predominant inside the graft and the external callus was almost not present. However in the OP-1 Group the graft after 4 months appeared to be almost completely reabsorbed, with a high presence of newly formed vessels and woven bone. In the OP-1 + MSC Group the graft was not reabsorbed, the remodeling processes were coupled with a good amount of new bone apposition and it was evident a fair amount of newly formed bone within its structure. The high presence of new bone was evident even in the most internal part of the graft, with new osteonic systems created around blood vessels. Histomorphometric measurements evidenced that the percentage of new bone formation within the graft was markedly higher in the MSC + OP-1 Group. When new vessels formation inside the graft was examined, in the MSC + OP-1 Group the vessel extension was significantly higher compared the control Group, achieving a mean length of 2.560 mm ± 0.85 in the proximal cut, and a length of 2.936 mm ± 1.022 in the distal cut, versus 0.69 mm ± 0.78 and 0.29 mm ± 0.5 of the Control Group (p=0.002, p<0.0005 in the proximal and in the distal cut respectively).

DISCUSSION:
In this study we were able to demonstrate that the addition of autologous expanded MSC to rh-OP1 improves significantly the new bone formation inside a massive bone allograft, both at the junction sites and in the central cortex of the graft. The need of Mesenchymal Stem Cells is also confirmed by the observation that MSC contribution from peripheral blood is extremely poor (39) (40), thus osteoblast precursors able to regenerate bone in this animal model can be provided only by host bone marrow and periosteum, and not from the surrounding tissues. The addition to the allograft bone of MSC without growth factor (OP-1) was not as effective, the amount of new bone inside the graft was not significantly higher compared to the control Group. In conclusion, this experimental study demonstrated that the addition of MSC and OP-1 is able to increase the integration of a massive bone allograft, with a high amount of new bone formation inside its structure. On the other hand, MSC alone, as well as OP-1 alone, were not able to increase the amount of newly formed bone inside the graft compared to the control Group. We believe that these results are extremely promising for bone reconstruction in difficult clinical applications that require massive graft implantation, however there is the need for more studies that could better explain the exact mechanism by which MSC and BMP work together.

REFERENCES: