PROCEEDINGS

Bone Surgery

International Biobridge Conference

"GENERATION REGENERATION"

CINI FOUNDATION, VENICE-ITALY

2013 & 2014

BIOBRIDGE FOUNDATION

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"This book comprises a collection of clinical reports presented during the 7th and the 8th Biobridge Conferences held in Venice in September 2013 and September 2014 respectively."

BONE

BONE MARROW FOR BONE RECONSTRUCTION

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"Stem cell therapy in Orthopaedics: strategies for selection and delivery"

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"Pseudoarthrosis biological stimulus: infiltrative technique"

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Dr. Pietro Di Biase, Orthopaedics & Traumatology Department, San Donato di Arezzo Hospital, ITA
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Prof. Chian-Her Lee, Chief of Department of Orthopaedics, Taipei Medical University Hospital, Taipei, TAI
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Dr. Margherita Giorgetti, Director Hand Surgery & Reconstructive Microsurgery Department, Cisanello Hospital, Pisa, ITA
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BONE SURGERY

BONE SURGERY
BONE MARROW FOR BONE RECONSTRUCTION

Session: BMC: THE REAL STEM CELLS THERAPY – 22nd September 2014

Presentation: **Stem cell therapy in Orthopaedics: strategies for selection and delivery**

Lecturer: Prof. Frank Barry, Scientific Director of the Regenerative Medicine Institute, National University of Ireland Galway, IRL

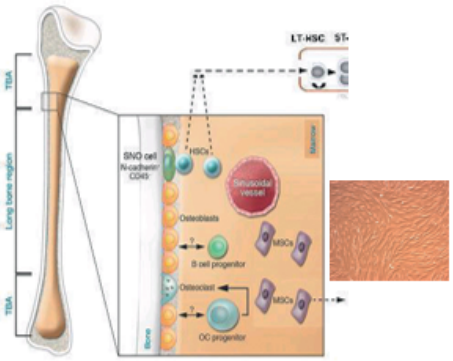
INTRODUCTION

Adult mesenchymal stem cells (MSCs) isolated from bone marrow and a variety of connective tissue have been extensively tested in the treatment of bone and cartilage repair and in osteoarthritis (OA). There are many aspects of the biology, selection and expansion of MSC poorly described and a more exhaustive characterization is necessary in tissue repair. The aim of our work was to address aspects such as the selection, concentration and characterization of bone marrow progenitors for therapeutic use in musculoskeletal repair with special attention to MSCs obtained with RegenLab RegenTHT tubes.

METHODS

The development of efficient and standardized methods for the isolation and characterization of MSCs has not advanced in recent years. The MSC's isolated by current methods are not homogenous and comprise a mixture of progenitors and other cells. Recently several antibodies have been developed which are routinely used by flow cytometry and other methods like CD 105, CD73 etc. (see below) to characterize MSCs. However, none of these represents a specific marker and consequently the homogeneity, reproducibility and consistency of isolated populations is not assured.

Phenotypic Properties of Mesenchymal Stromal Cells



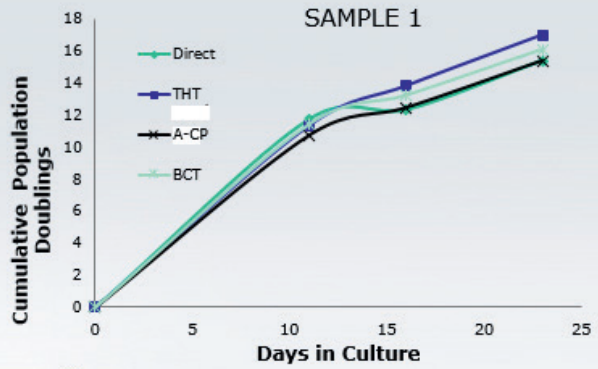
Surface markers	Differentiation potential	Secreted factors
CD44+	Osteogenic	VEGF, Ang-1, SDF-1,
CD73+	Adipogenic	PDGF, TSG-6, bFGF,
CD90+	Chondrogenic	FGF-7, IL-1, IL-6, IL-10,
CD105+	*Myogenic*	PIGF, MCP-1, TGFβ,
CD11b–	*Endothelial*	PGE-2, IDO, M-CSF, HGF,
CD14–	*Epithelial*	MMP-9, Sfrp, Thymosin β4,
CD34–	*Neuronal*	Plasminogen, Tenacin C,
CD45–		Thrombospondin
CD79a–		
HLA-DR–		

Different approaches are available for MSCs preparation: 1) removal of the culture-induced heterogeneity by using more precise selection strategies, 2) use clonally isolated populations, 3) use non-expanded progenitor cell preparations either extracted and cryopreserved or prepared intraoperatively using RegenTHT tubes.

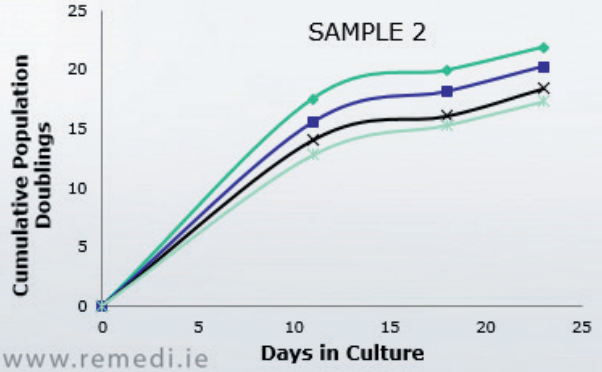
We compared different RegenLab devices for Bone Marrow aspirates (THT, BCT, A-CP) to the standard direct plating technique.

RESULTS

Growth of hMSC in Culture



Sample 1	% MNC recovered	CFU-F/ 10^5 MNC
Direct Plating	66	4.54
THT	73	4.03
BCT	22	9.90
A-CP	15	13.41



Sample 2	% MNC recovered	CFU-F/ 10^5 MNC
Direct Plating	66	1.56
THT	87	5.44
BCT	3.4	49.23
A-CP	7.3	32.86

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Flow Cytometry Results

	Direct	THT	A-CP	BCT
CD3	0.19	0.0	0.06	0.17
CD14	1.74	0.41	0.82	0.58
CD19	0.33	0.0	0.04	0.0
CD34	0.58	0.27	0.04	0.029
CD45	0.21	0.21	0.08	0.029
CD73	99.7	99.9	100	100
CD90	98.7	99.1	98.6	99.5
CD105	96.1	98.9	100	99.9
HLA-DR	1.89	0.32	1.76	2.23

Flow Cytometry

- hMSC QC panel
  - CD73
  - CD90
  - CD105

  Positive markers

  - CD3
  - CD14
  - CD19
  - CD34
  - CD45
  - HLA-DR

  Negative markers

CONCLUSION

RegenTHT, RegenBCT and A-CP tubes isolate mesenchymal stem cells from bone marrow aspirate having comparable proliferative activity and differentiation capacity to cells isolated using the standard direct plating technique.

RegenTHT tubes offer the best performances in terms of recovery and consistency of isolated cells from bone marrow aspirates.

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Session: BONE MARROW & SURGERY – 24th September 2013

Presentation: **Pseudoarthrosis biological stimulus: infiltrative technique**

Lecturer: Dr. Domenico Aloj, A.S.O. C.T.O – M. Adelaide of Turin, Turin, ITA

INTRODUCTION

Bone fracture is a partial or total break of the continuity of a bone. In relation to the site of trauma, fractures are classified in:

- **Epiphyseal fractures:** involving the portion of the bone wrapped in epiphyseal cartilage. In this type of fracture the cartilage breaks down and it is necessary to intervene surgically to achieve joint reconstruction. This class of fracture can be considered easy to heal because the epiphyses are highly vascularized;
- **Metaphyseal fractures:** the metaphysis is the portion of bone that serves as support for the epiphysis. It is formed by vascularized cancellous bone and it is the site of osteoclastic activity;
- **Diaphyseal fractures:** concern the central portion of the long bones.

The healing of a bone fracture can be direct (in the case in which it occurs spontaneously without the need for rigid fixation) or indirect (in case there is need to use external fixators and/or stimuli of biological or pharmacological nature). The process of bone healing passes through four stages, namely:

- **Haematoma and inflammation:** after the fracture there is formation of a hematoma due to the rupture of blood vessels. Subsequently, the hematoma is replaced by a clot, and it is infiltrated by cells such as macrophages, white blood cells, fibroblasts and mastoblasts. This process leads to the removal of the necrotic bone;
- **Granulation tissue formation:** the clot is populated by arterioles that bring oxygen and other cellular elements such as osteoblasts, chondroblasts and prosteocytes. At this stage, the formation of the fibrous callus occurs;
- **Callus formation:** the fibrous callus, 3-4 weeks after fracture, starts to turn into bone callus. Calcification of the tissue begins, that will lead to the transformation of the callus into structured bone;
- **Remodeling:** the remodeling phase begins six weeks after the fracture and can last for weeks or months. During this time, the bone begins to achieve its structure and to regain its previous mechanical strength. The remodeling phase is considered complete when the regenerative process at the level of the fracture comes to a halt.

The term pseudoarthrosis (PSA) identifies a fracture that doesn't reach resolution within 20 weeks; it is a fracture in which the fibrous callus does not develop into bone callus. This type of fracture will not heal without the contribution of mechanical stimuli and/or biological stimuli (Stem Cells, Platelet Concentrates).

The aim of the clinical investigation described below was to assess the contribution made by the use of Mesenchymal Stem Cells, Platelet Rich Plasma (PRP) and Plasma Rich Fibrin (PRF) in the promotion of the resolution of PSA.

METHODS

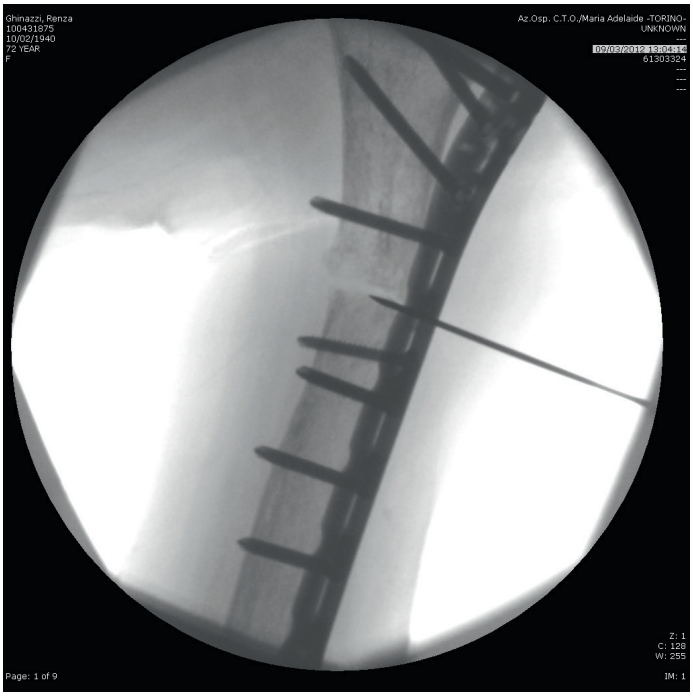
During the period 2011/2012 a total of 130 patients with nonunion fractures were treated at the Department of Traumatology, CTO Hospital of Turin. Of these fractures, 53% were treated with mechanical stimulus (reaching PSA resolution) while 47% were treated with the contribution of biological stimulus. Specifically, the patients for whom biological stimulus was used were suffering from nonunions of the upper and lower limbs: femur, tibia, humerus and radius. The average age of the subjects was 43 years. In all patients, medullary blood was withdrawn in order to obtain a concentrate of MSC (Mesenchymal Stem Cells) through the use of an appropriate medical device (Regen Extracell BMC, Regenlab®). The MSC preparation was administered percutaneously, either alone or in combination with PRP / PRF depending on requirement, directly at the site of PSA by availing of the same trocar used for the withdrawal of medullary blood. During the follow-up period, 25.7% of these patients underwent ultrasound scans with gas contrast (CEUS) to assess the presence of angiogenesis at the level of the fracture (comparison with a baseline control carried out before the application of biological stimulus).

CLINICAL RESULTS

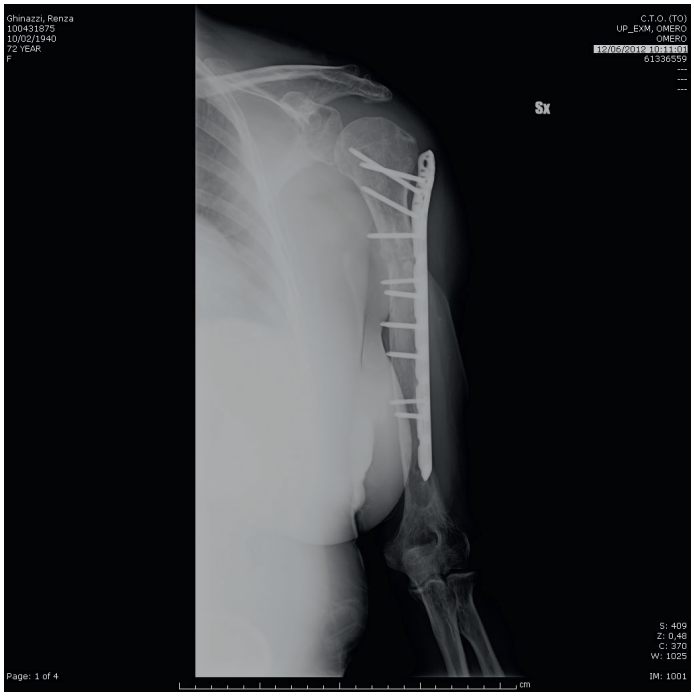
Of the 61 patients treated with MSC, 49 (80%) achieved complete healing of the fracture in an average timeframe of 4.9 months. The remaining 20% were re-treated with the use of a recombinant bone morphogenic factor (OP1).

There were no infections resulting from the treatment and the average time of hospitalization was one day.

PSA humerus, 9 months after the fracture



3 months after MSC infiltration



CONCLUSIONS

The injection of MSC at the site of PSA did not require surgical access to the lesion and it allowed resolution of the lesion in shorter timeframes than those associated with a variety of open surgical approaches. The technique developed is not invasive and it is easy to perform and results in a decrease in the length of hospital stay.

The use of ultrasound control with gas contrast medium (CEUS) is useful for the identification of suitable cases of viable PSA, i.e. those cases that are able to respond positively to the biological treatment described above.

Session: BMC: THE REAL STEM CELL THERAPY – 22nd September 2014

Presentation: **Long bone nonunion after open fractures: treatment with planting technique, autologous bone graft and mesenchymal cells**

Lecturer: Prof. Roberto Buzzi, Director of Orthopaedics & Traumatology Department, CTO Careggi, ITA

INTRODUCTION

Nonunion fractures with bone loss are characterized by non-viable segments with bone gaps, insufficient stability and sometimes loco-regional infection.
The Etiology is mainly due to open fractures (segmental, post debridement, blast injury).
The goal of treatment is the restoration of bone defect and adequate stabilization.

We describe here our experience in the management of nonunion following open fractures with plating technique in association with Regen Kit Extracell GLUE system and autologous bone grafting .

With the wave plate technique we can obtain correction of large bone defects, alignment and rotation, increased functional diameter of the nonunion site and a graft placement all around the nonunion site. With autologous bone grafting we can provide osteogenesis, osteoinduction, osteoconduction, structural support, and with mesenchymal cells in platelet-glue, we can promote the osteoinductive function and the osteogenetic reparative processes.

METHODS

At our Institution from July 2010 to December 2013 twenty-eight selected patients underwent definitive treatment of nonunion following open fractures with plating technique in association with the RegenKit Extracell GLUE system and autologous bone grafting. The patent population included 23 males and 5 females; patient average age was 41.3 years. There were no smokers and there were no associated co-morbidities.

There were 18 tibia shaft nonunions, 14 femur shaft nonunions and 1 humeral shaft nonunion. One patient was affected by bilateral tibia nonunion. Two patients were affected by ipsilateral femur and tibia nonunion. Another patient was affected by bilateral femur nonunion and tibia nonunion of one leg. Average bone Defect was 2.5 cm for the tibia, 3.5 cm for the femur and 1.5 cm for the humerus.

The MSC/platelet gel was intraoperatively obtained with the use of the Regen Kit Extracell GLUE system. The gel was directly mixed to the autologous bone graft and administrated onto the prepared bone surface at time of plate fixation. All procedures were performed by the same senior surgeon.

Radiographs and clinical outcome were evaluated at 1-3-6-12 months following surgery. Mean follow up time was 14 months (range 12 to 18 months).

CLINICAL FINDINGS

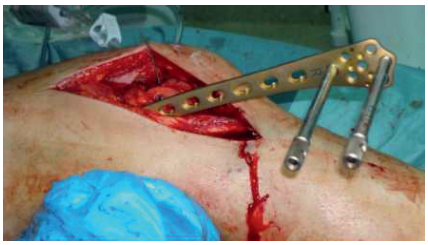
Bone union was obtained in all cases.

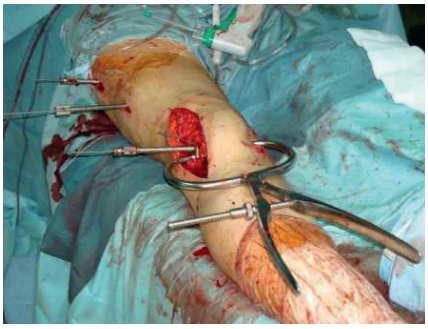
Mean time for radiographic union was 6 months for the tibia, 4 months for the femur and 3 months for the humerus.

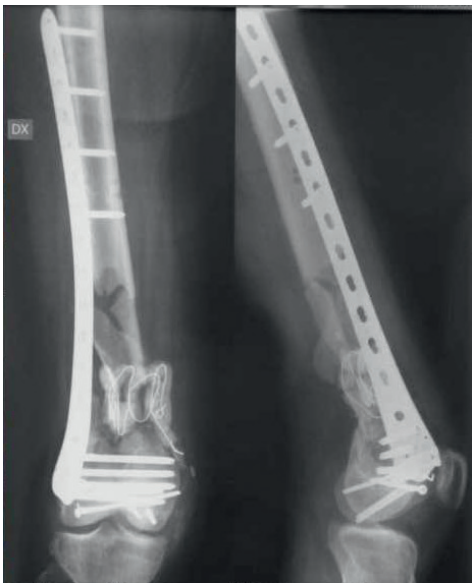
Clinical outcome was satisfactory in all cases with the restoration of proximal and distal joint mobility and absence of rotational, axial or length defects.

No other peri- or post-operative complications were observed, except for one case of nonunion with hardware failure that required re-intervention.

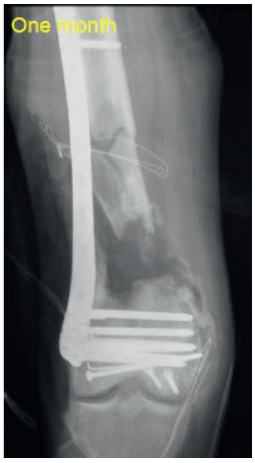
Individual cases with their operative procedures and clinical and radiological outcomes were presented and commented.

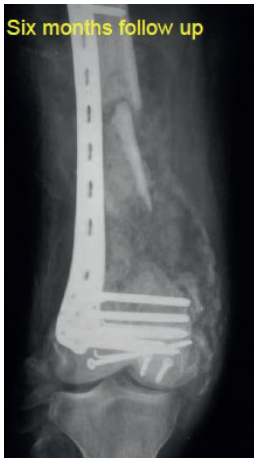


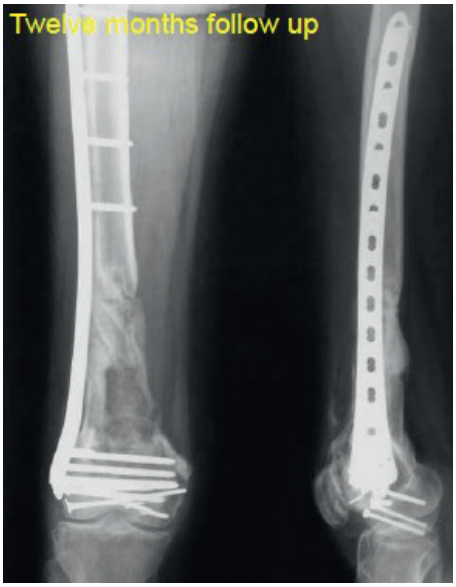




Female, 43 years
Automobile accident 14/07/2013
Multiple fractures:
- Right distal tibia
- Left femur neck







CONCLUSION

From our experience we can say that the RegenKit Extracell GLUE system associated to autologous bone grafting represents a valid adjuvant for the healing of nonunions with bone loss treated with plating technique.

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Session: BONE MARROW & SURGERY – 24th September 2013

Presentation: **Growth Factors, mesenchymal staminal cells and scaffold in bone regeneration: personal experience in 300 patients**

Lecturer: Prof. Rodolfo Capanna, Director of Orthopaedic Oncology Department, CTO Careggi, ITA

INTRODUCTION

Bone loss due to trauma or disease is an increasingly serious health problem. The requirement for new bone to replace or restore the function of traumatized, damaged, or lost bone is a major clinical and socioeconomic need. Current clinical treatments are problematic and often yield poor healing due to the complicated anatomy and physiology of bone tissue, as well as the limitations of medical technology. Bone tissue engineering offers a promising alternative strategy of healing severe bone injuries by utilizing the body's natural biological response to tissue damage in conjunction with engineering principles. Mesenchymal Stem Cells from the iliac crest, growth factors (autologous PRP), and biomaterial scaffolds form the foundation of the many bone tissue engineering strategies employed to achieve repair and restoration of damaged tissue.

This paper summarized the ten-year experience of the University of Florence at the Center of Orthopaedic Oncology & Reconstructive Surgery (Italy).

This new approach, identified as « IN VIVO CELL FACTORY », has been mainly investigated and used in the following clinical indications: distraction osteogenesis (1), pseudoarthrosis and open fractures (2), curettage & grafting in bone tumors (3), epiphyseal necrosis and allografts revascularization (4).

METHODS

In the case of the "IN VIVO CELL FACTORY APPROACH" used by University of Florence, the biological components as PRP (Platelets Rich Plasma) and MSC's (Mesenchymal Stem Cells) were obtained from the same patients through an autologous and extemporaneous procedure using the RegenKit Extracell GLUE system (not subjected to prior cell culture). The main scaffolds used were allograft products as mineralized bone matrix (chips, fibers and powder) and demineralized bone matrix.

The implant mode was chosen depending on clinical indication: Percutaneous Technique (1-2-3), apposition at Interface Host Bone & Cortical Graft (2), Open Surgery Peripheral Apposition (3), Conventional "enriched" grafting into a bone cavity and Cocktails plus Vascular Supply.

A total of 242 cases were treated, as follows:
- 131 major bone defects or wide cavity defects after benign bone tumor;
- 28 hip osteonecrosis Steinberg stage II a-b;
- 83 post-traumatic pseudoarthrosis (segmental defects);

CLINICAL FINDINGS

MAJOR BONE DEFECTS (131 cases):
- Follow up 58 months (6-96)
- Healing 94.5 %
- Healing time 6 months (3-14)

OSTEONECROSIS (28 cases):
- Follow Up 25 months (6-60)
- Healing 80 %
- Healing time 3 months (2-12)

PSEUDOARTHROSIS (83 cases):
- 5/2002 – 12/2012
- 51 male / 32 female
- R 36 / L 47
- Average age 44,6 yrs (16-83)

TRAUMATIC PSEUDOARTHROSIS:

• percutaneous injection	MSC/PRP only	60% healing (6 months)
• open surgery	G.F. +/- scaffold	89 % healing (6 months)
• open surgery	G.F. + scaffold + stem cell	100 % healing (6 months)

ONCOLOGICAL CASES:

- No donor site problems
- No surgical site complications
- No adverse effects in tumor local control
- 2 recurrences (1 ABC, 1 TGC) 5 %
- Faster bone healing than control (half time)
- More than one procedure needed for percutaneous cases (7 out of 14)

CONCLUSION

Through the study of the above cases emerges the strong evidence of synergistic effect between MSC's, BMP's or other GF's. The contribution of biological components improves the vascularity and the healing time. The carrier properties may enhance GF delivery thereby improving the final effect of treatment through a prolonged release.

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Session: BCM: THE REAL STEM CELL THERAPY – 22nd September 2014

Presentation: **Biologic chambers in biological reconstruction**

Lecturer: Prof. Rodolfo Capanna, Director of Orthopaedic Oncology Department, CTO Careggi, ITA

INTRODUCTION

In the 1950's Ilizarov introduced the technique of distraction osteogenesis where, preserving the periosteum, bone is cut with surgery and lengthened gradually; new bone formation (osteogenesis) is observed at the lengthening site and the periosteal chamber is able to induce ossification after distraction.

Masquelet has developed the technique of periosteal membrane induced by bone cement after diaphyseal resection of bone tumors, infection or fracture. This two stage surgery is able to offer an important option for mature and viable bone reconstruction, after filling the periosteal membrane with autologous cancellous bone.

In Orthopedic Oncology, is a well-known and confirmed experience that, after a curettage of a benign tumoral lesion, you obtain a bone chamber with bone healing after bone grafting in 90% of cases. Furthermore, if you put the bone graft in soft tissue instead of in the bone chamber, the transplant will be reabsorbed. For this reason, clinical experience suggests the addition of autologous bone graft at the osteotomy line, not immediately after primary surgery, but only after and below neo-periosteal appose.

The technique of combining a massive allograft with a vascularized fibular graft was introduced in 1993 by Dr. Capanna. This technique has shown its efficacy after intercalary resection in long bone or arthrodesis. The scientific rationale is to combine the unviable but biological chamber offered by allograft with a viable and vascularized fibula. Long term results showed how after 20 years more than 80% of cases had been integrated and successfully treated, with fibular hypertrophy and allograft incorporation.

The technique of vascularized fibular graft has evolved towards harvesting the fibula together with a vascularized periosteal stripping. This allows the association to the viable autologous fibula of a periosteal chamber that you can fill with bone chips. With this technique, it is possible to reconstruct diaphyseal massive bone loss in infected or irradiated fields with amazing results.

MATERIAL AND METHOD

'ILIAC CREST BONE GRAFT' ICBG
MSC's cells were obtained with RegenKit Extracell GLUE (RegenLab) after harvesting of Bone Marrow from the Iliac Crest.

Clinical Experiences and Results

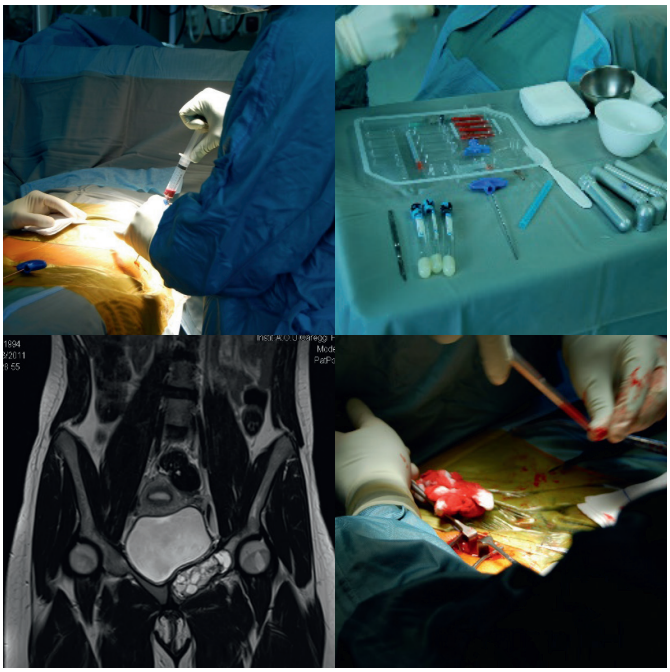
Biologic potential of VFG (vascularized fibular graft) + BMA (Bone Massive Allograft) mechanical resistance.

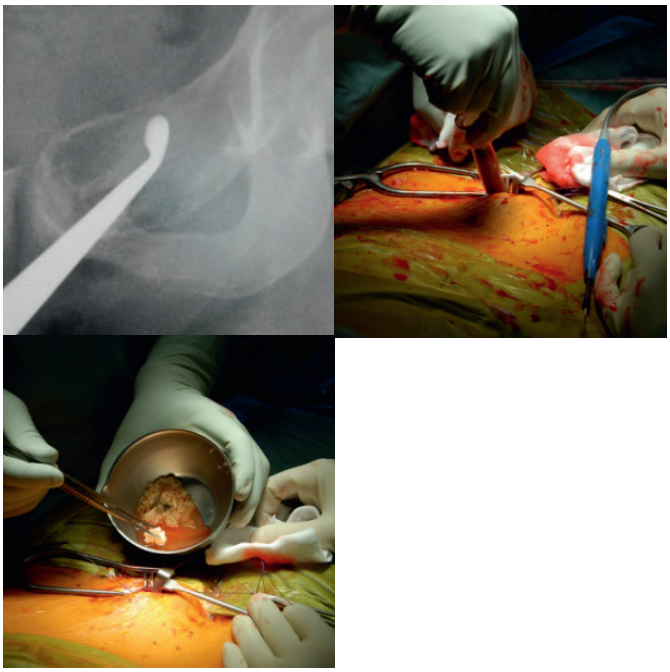
Very stable assembling for lower limb. Indicated in iuxta-articular resection in upper and lower limb.
Results on 52 pts. Average Follow up is 12 yrs.

The average time of union: VFG 6 month (range: 3-10) and Allograft 20 month (range: 10-34).
92% 1 year union. VFG improves allograft fusion.

ANEURYSMAL BONE CYSTS (ABS)

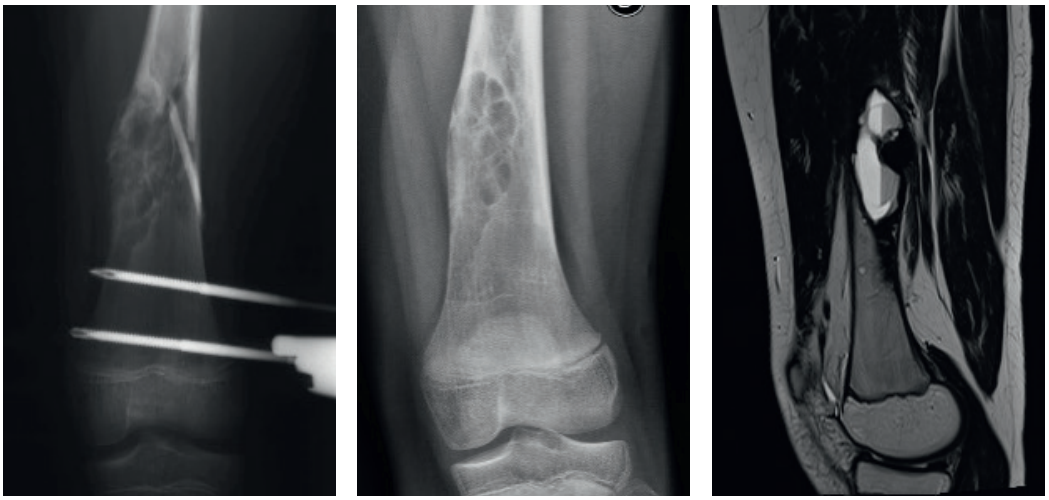
13 patients were treated with DBM (demineralized Bone Matrix) + ABM (Autologous Bone Marrow) → Biological chamber in ABS: Long bone (6), pelvis (5), scapula (1), and talus (1). Follow up at 45 months with 11 healed (85%).

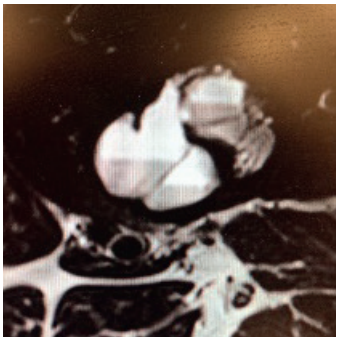


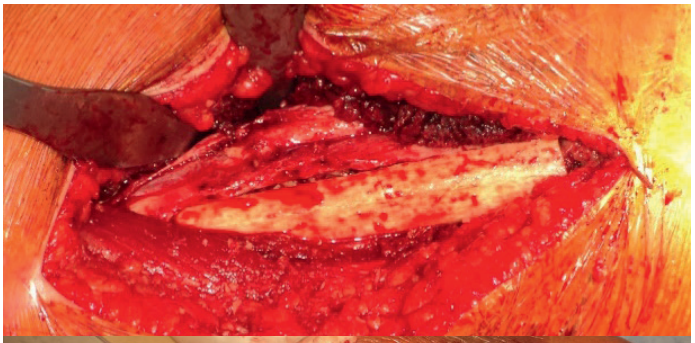


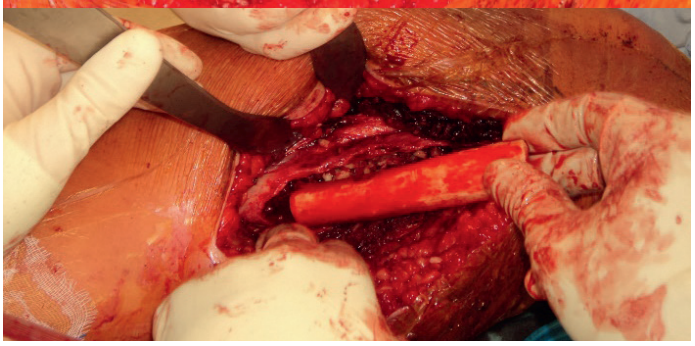
CLINICAL CASE:

BMA (Bone Massive Autogtaft) + BMC to treat benign bone tumors:







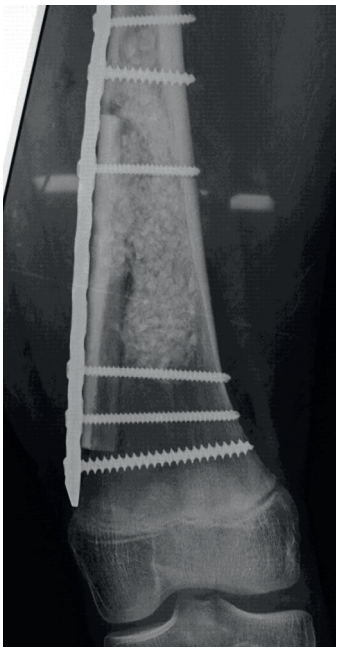


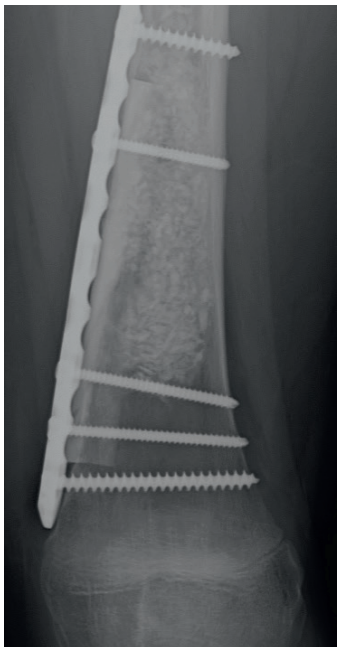
"BMA + BMC

Biological Chamber"

90% healing

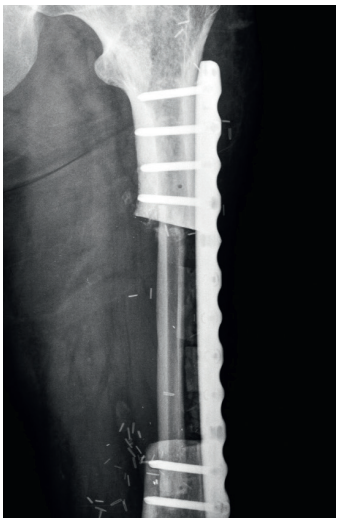
In Benign BT

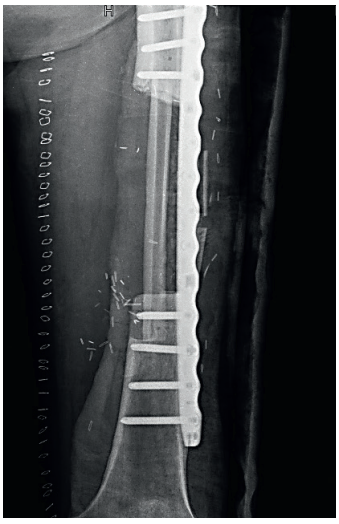


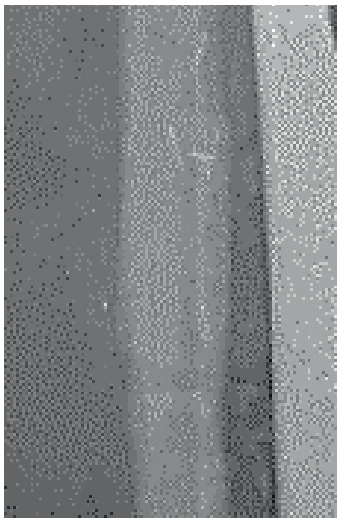


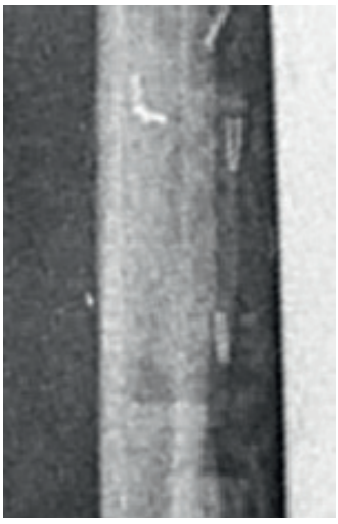
INFECTED/IRRADIATED PSEUDOARTHROSIS:

VFG (vascularized fibular graft) with interosseous membrane + ICBG (Iliac Crest Bone Graft)
Biological Chambers:
Complete healing, radiographically observed at 5th month.

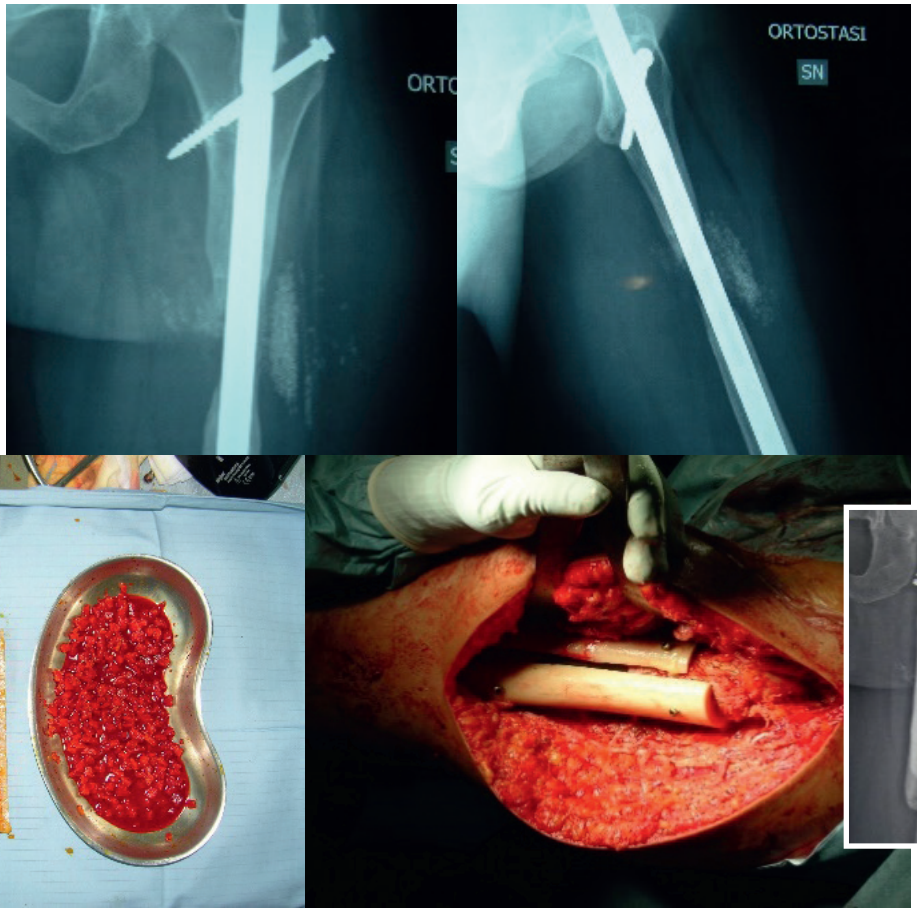








PSEUDOARTHROSIS
"BMA + DBM + ABM
Biological Chambers"
And again:
"Trabecular Metal + ICBG
Biological Chambers"



CONCLUSIONS

The concept of a "biological chamber" in different biological reconstruction application was shown to be real and efficacious.

Stem cells, GF & BMC offers encouraging results, reducing time of healing and improving overall results.

A longer FU is needed to validate schemes and treatment protocols, especially in high risks cases.

Session: BMC: THE REAL STEM CELL THERAPY – 23rd September 2014

Presentation: **The treatment of non union homer with bone graft and stem cells**

Lecturer: Dr. Salvatore Caruso, Orthopaedics & Traumatology Department, ASP of Siracusa, ITA

INTRODUCTION

Humerus diaphyseal fractures are estimated to account for 1% of all fractures. To reduce the risk of non-union, it is mandatory to perform an anatomic reduction, followed by the right choice of implant and a proper stabilization; indeed, the humerus is continuously influenced by torsional forces that, if not limited, lead to a pseudoarthrosis.

Post traumatic bone defects can negatively influence the outcome and they represent a treatment challenge.

BONE DEFECTS ETIOLOGY:
Management of Post-traumatic Segmental Bone Defects is defined by the lengths of defect:

Bone defects 0.1-1 cm → acute shortening
(femur, humerus)
Advantages: early fracture stability, reduction of soft tissue stress, improvement of vascularization, decrease of neurogenic pain, primary closing of soft tissue.

Bone defects 0.5-3 cm → bone grafting
(femur, humerus, tibia, forearm)
Autograft (high osteoinduction, high osteoconduction, high osteogenicity)

Homograft (poor osteoinduction, good osteoconduction, no osteogenicity)

Xenograft (no osteoinduction, modest osteoconduction, no osteogenicity)

Bone defects 2-10 cm → Bone carriage
(femur, tibia, forearm)
Intra surgery lengthening

Bone and Callus distraction

Bone defects ← 4 **cm** → Compression-Distraction Technique

Bone defects → **10 cm:** Vascularized fibular graft + homolateral fibula pro-tibia

MATERIALS AND METHODS

From 2009 to 2013 we treated 39 patients affected by post-traumatic bone defects: 24 male/15 female with an average age of 38 (range 20 to 66).

TREATMENT AND BONE MATRIX:

	Shortening	Bone Graft	Bone Carriage	Free Vascularized Bone Graft
Homerus (5 cases)	**2**	**3**		
Forearm (6 cases)		**4**	**2**	
Femur (8 cases)	**3**	**2**	**3**	
Tibia (20 cases)		2	**15**	**2**

	# cases
Acute Bone lost	**17**
Pseudoartrosis	**10**
Osteomyelitis	**12**

Plastic Surgery	14
Free lemb	4
Transposition lemb	8
Dermis graft	2

Biomaterial	12
OP-1	3; 3/bone graft
PRP	7 ; 6/bone graft
MSC's	2; 2/bone graft

Follow-up: 3.9 years av. (max. 5 y – min. 1 y) with radiograph and functional evaluation of outcomes. Average healing time was 16 Months (max. 22 M – min. 6 M).

BONE QUALITY:
Excellent: 8 cases
Good: 12 cases
Moderate: 18 cases
Bad: 1 case

FUNCTIONALITY:
Excellent: 9 cases
Good: 19 cases
Moderate: 9 cases
Bad: 2 cases

From 2009 to 2013, we treated 12 aseptic Humerus pseudoarthrosis:

6 ATROPHIC CASES → Homologous bone from Bone Bank + MSC's from bone marrow selection (RegenKit Extracell BMC, RegenLab).
4 HYPERTROPHIC → 2 cases with Homologous bone from Bone Bank + MSC's from bone marrow selection; 2 cases with MSC's + External Fixation in compression.
2 BONE DEFECTS → Homologous bone from Bone Bank + MSC's from Bone Marrow selection.
Average follow-up was 42 Months (min. 28M – max. 62M).
Average healing time was 4.8 Months (min. 2.5M - max. 11M).
11/12 were classified as healed

BONE QUALITY:
Excellent: 9 cases
Good: 1 case
Moderate: 1 case
Bad: 1 case

FUNCTIONALITY:
Excellent: 2 cases
Good: 6 cases
Moderate: 3 cases
Bad: 1 case

Patients	Score	Pain	Shoulder and Elbow functional movements	Angulation
8	Good	Absent	< 20%	0
3	Modest	After effort	20-40	< 10°
1	Bad	Permanent	> 40	Failure

CONCLUSION

Humerus non-union is a difficult fracture to treat. Our approach can be considered a confirmation of international literature as it allows a stable synthesis, an efficient biological support and leads to early functional recovery.

Mesenchymal Stem cells treatment can be considered appropriate every time a stimulus of new bone formation is required. It represents an additional solution in trauma and reconstructive surgery.

Biological stimulus alone cannot, however, achieve bone healing and mechanical factors must also be addressed.

BIOBRIDGE FOUNDATION

regenlab

Session: BMC: THE REAL STEM CELL THERAPY – 23rd September 2014

Presentation: **The treatment of post traumatic non union**

Lecturer: Dr. Salvatore Caruso, Orthopaedics & Traumatology Department, ASP of Siracusa, ITA

INTRODUCTION

Pseudoarthrosis, or non-union, is defined as a fracture which does not heal within 6-8 Months, in which the consolidation process is compromised. The etiology of non-union includes iatrogenic, biological, mechanical and metabolic causes.

Pseudoarthrosis can be Atrophic (Septic and Aseptic) and characterized by periosteal muscle damage, potential soft tissue damage, vascular deficit and lack of stability of the mechanical osteosynthesis.

The treatment of pseudoarthrosis includes both mechanical stabilization and biological stimulus through debridement and bone grafting.

MATERIALS AND METHODS

NON-UNION TREATMENT:

1. Check of the existing mechanical fixation (stability, quality) and the local condition.

2. Mechanical stimulus of bone-implant complex.

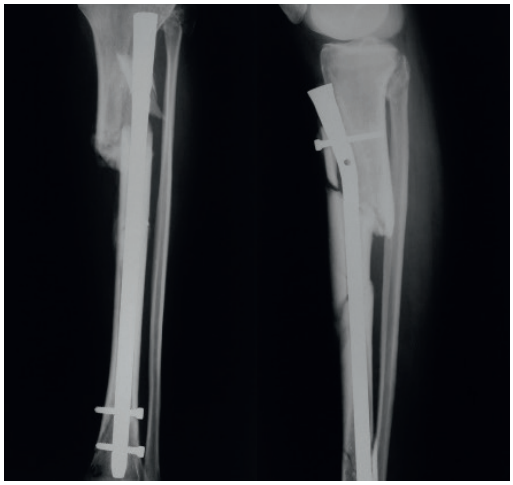
3. A surgical approach which is as biological as possible.

4. Cleaning of non-union source.

5. Debridement following Judet's techniques

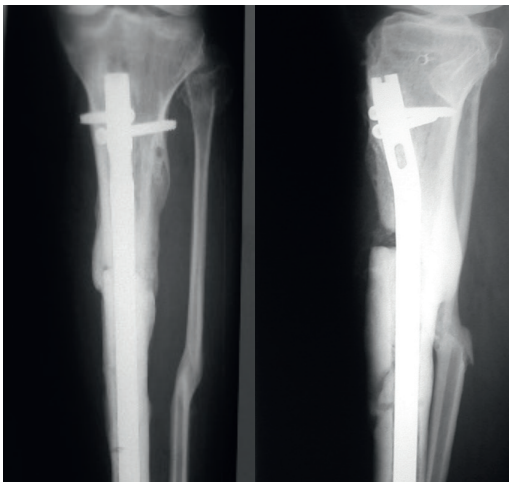
6. Addition of MSC's to the site. MSC's were selected using Regen Kit Extracell BMC (RegenLab).

Clinical Case: G.R. Female, 40 year old smoker, AO 42.C2. 1st surgery four months before.

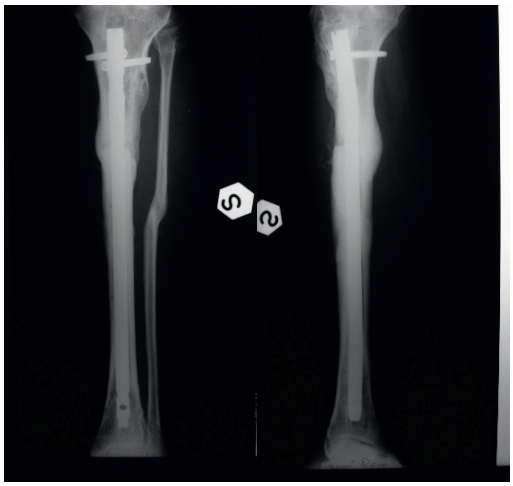


Treatment: intramedullary nail (dynamic) plus MSC's:

Follow up 1 Months



Follow up 6 Months



RESULTS

Between 2009 and 2013 the Ortho and Trauma department of "Umberto I" Hospital (Siracusa) treated 12 cases of tibial non-union. Six of those cases were treated using MSC's, together with bone grafting and hybrid external fixation techniques (compression-lengthening).

Healing time was shorter using bone grafting plus growth factors and MSC's.

CONCLUSION

Currently, the use of MSC's can be still considered an innovative approach in Orthopaedic surgery and the surgical techniques must be improved, through a better synergy between cellular biology and bone biomechanical properties.

MSC's and growth factors are a valid treatment option for tibial pseudoarthrosis, in association with bone grafting and hybrid external fixation techniques.
external fixation technique.

Session: BMC: THE REAL STEM CELL THERAPY – 22nd September 2014

Presentation: **The use of stem cells for the treatment of pseudoarthrosis of long bones: our experience**

Lecturer: Dr. Edoardo Crainz Fossati, Responsible of Orthopaedic Department, Le Scotte Polyclinic, ITA

INTRODUCTION

The use of stem cells from bone marrow is a technique known and used for years in orthopaedic surgery for the treatment of pseudoarthrosis and bone defects. Mesenchymal multipotent line MSCs are Immature, unspecialized and multipotent and they can differentiate into different adult cells. They also have crucial regenerative role on damaged cells (0.01 % of total marrow cells, 1/105 mononucleated cells).

MATERIAL AND METHODS

From 2010 to 2013 we treated 13 patients (14 PSA) with average age of 43y (range 29 – 74). In particular:

7 Femur, 4 humerus, 2 Tibia, 1 clavicle with a ratio female/male of 8:1.
Average follow-up 26 months (range 12 – 34), conducted by X ray at 1, 2, 3, 6 Months – 1 y

9 Patients were treated with graft from Iliac Crest + Homologous Bone grafting while 4 patients with autologous bone grafting.

Bone marrow concentration and MSC's separation were made using Extracell Glue kit (Regen Lab) and H-19 F Regen Centrifuge following protocol's indication (1° Centrifugation: 2 min @ 4000 rpm, then 2° Centrifugation: 8 min @ 3500 rpm).

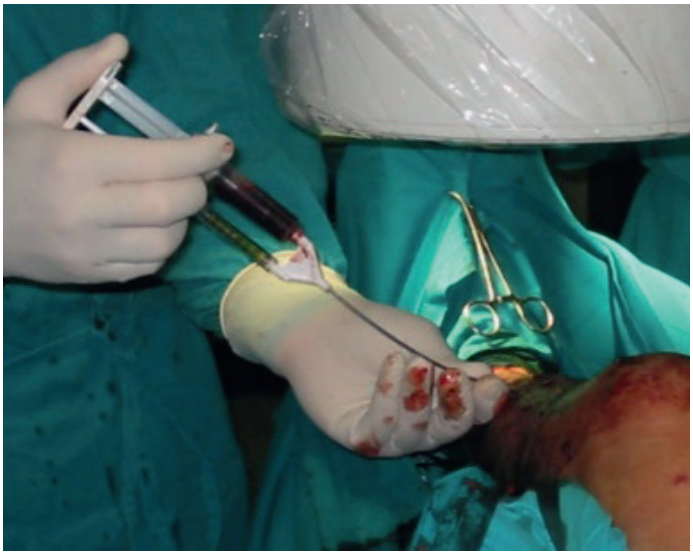
SURGICAL TECHNIQUES + STEM CELLS
→ 9 PATIENTS (OMOLOGOUS BONE GRAFTING)
→ 4 PATIENTS (AUTOLOGOUS BONE GRAFTING)

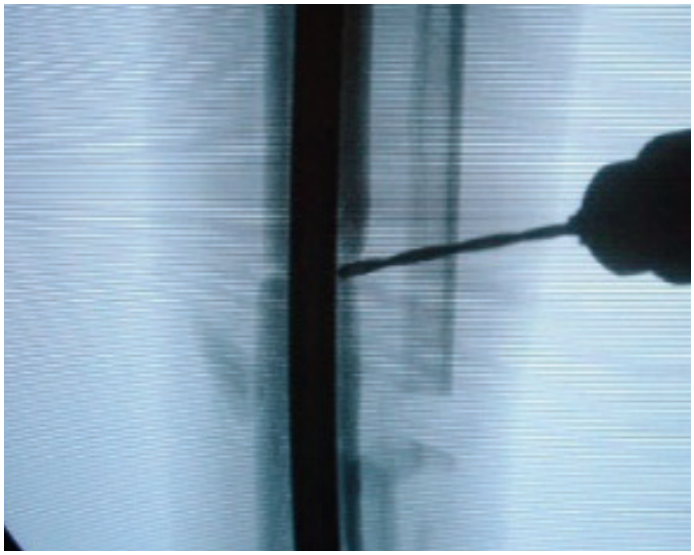
SURGICAL TECHNIQUE: ORIF + GRAFTING
10 PSA: 6 femur, 3 humerus, 1 clavicle

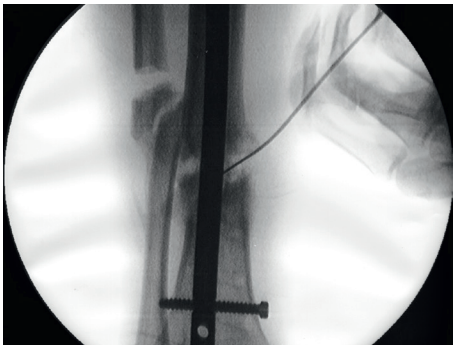
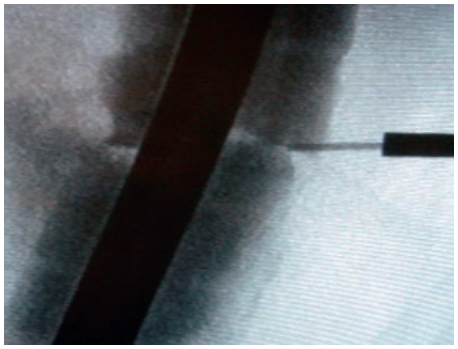
SURGICAL TECNHIQUE: NAILING + GRAFTING IN OPEN
2 PSA: 1 femur, 1 tibia

SURGICAL TECNHIQUE: OVERREAMED NAILING
2 PSA: 1 humerus, 1 tibia

SURGICAL TECHNIQUE: PERCUTANEOUS INJECTION
1 humerus, 1 tibia





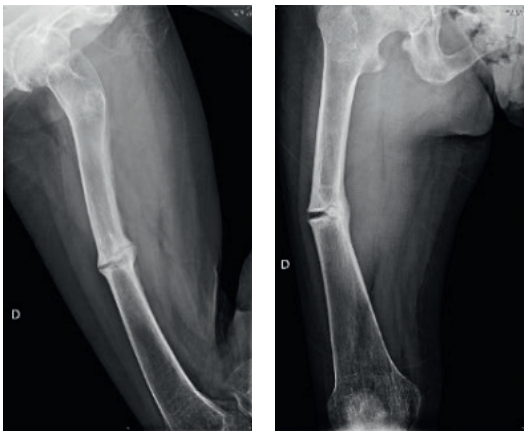




RESULTS

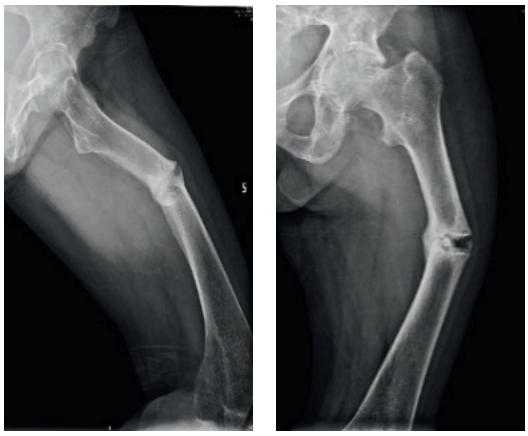
Average F.U. at 30 Months (range 12-42) by X Ray 1, 2, 3 and 6 months – 1 year.
We observed a clinical and radiographical healing in all patients with average time of 9.6 weeks.
Consolidation was always complete, with complete pain relief and no complications.

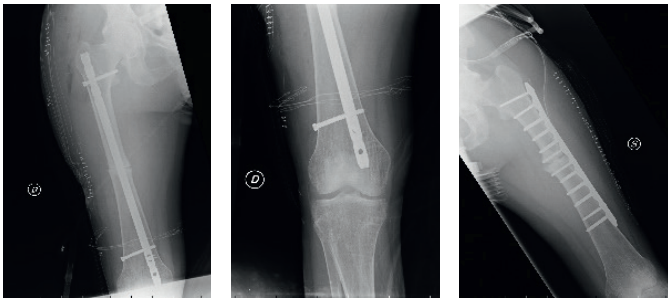
CLINICAL CASES
Phosphocalcic metabolic disease M.F.: 29 years old / F

Pre-op right

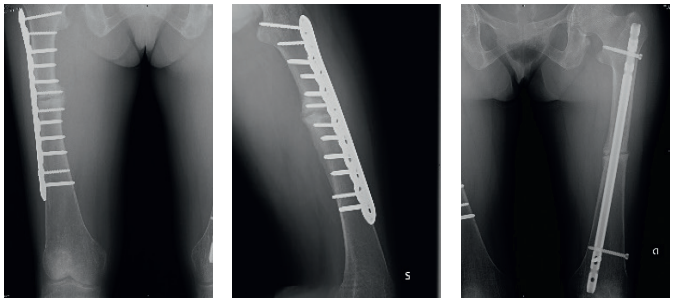


pre-op left





Post op. Right Left



Post op 7 Months Right Left

DISCUSSION AND CONCLUSION

A rationale exists for CLINICAL application of BMC/stem cells.

It is an easy and safe intraoperative procedure. It does not significantly prolong surgery and can be of limited cost. Use of BMC/stem cells is widely described in huge number of clinical trials (www.clinicaltrials.com - different methods for extraction, lots of problems unsolved, very different series, never homogeneous series and encouraging but however not conclusive results).

In our experience techniques can be easily applied on severe bone defects, AVN, Pseudoarthrosis, complex patterns, and comorbidities.

Clinical and radiographical results are really encouraging. There was a very satisfying "biological boost" in critical patterns and patients and good Outcome with every surgical technique.

Further studies need to consolidate the effectiveness of the whole procedure targeting a single techniques.

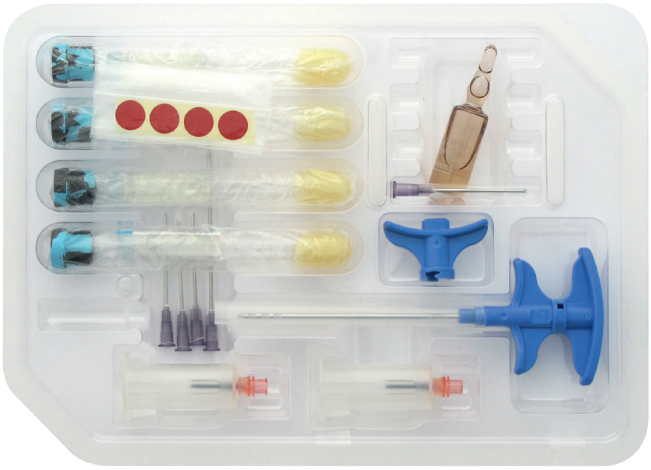
Session: BMC: THE REAL STEM CELL THERAPY – 22nd September 2014

Presentation: **Ten-year experience of non unions treated with autologous bone marrow concentrate and osteogenic protein-1**

Lecturer: Dr. Pietro Di Biase, Orthopaedics & Traumatology Department, San Donato di Arezzo Hospital, ITA

INTRODUCTION

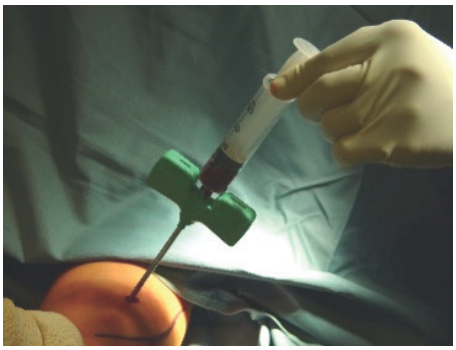
The treatment of non-union has seen in the last few years a lot of new proposals with a large number of studies on the use of growth factors and autologous bone marrow concentrate (BMAC). These methods can be used with open surgical techniques or with percutaneous techniques.



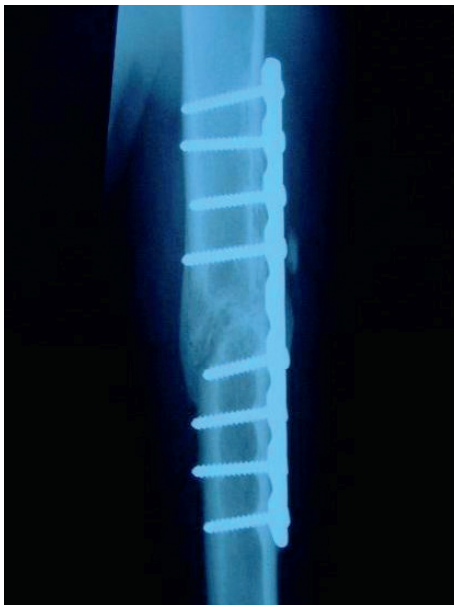
MATERIAL AND METHOD

MSC's were harvested and collected by using the RegenKit Extracell Glue system (RegenLab). The cells are separated and concentrated following harvesting of bone marrow from the iliac crest. Then the aspirate is transferred to sterile tubes and centrifuged (Sterile Centrifugation of the tubes x5). BMCs were obtained after 8 min x 3400 rpm (H-19F centrifuge by RegenLab).

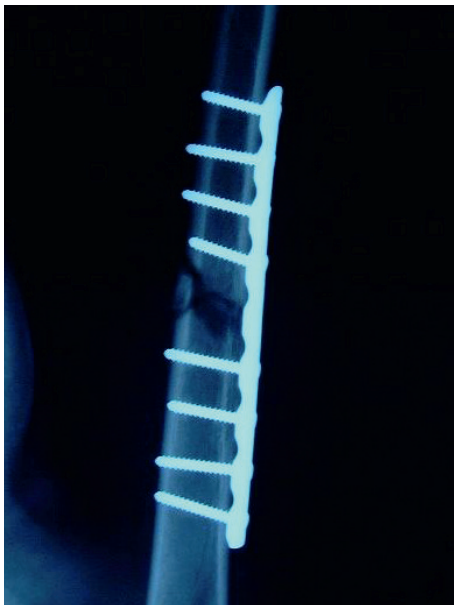
Percutaneous Technique (no bone gap): percutaneous injection of growth factors and MSC's to stimulate callus formation.



3 Months post op



5 Months post op.



CLINICAL EXPERIENCE

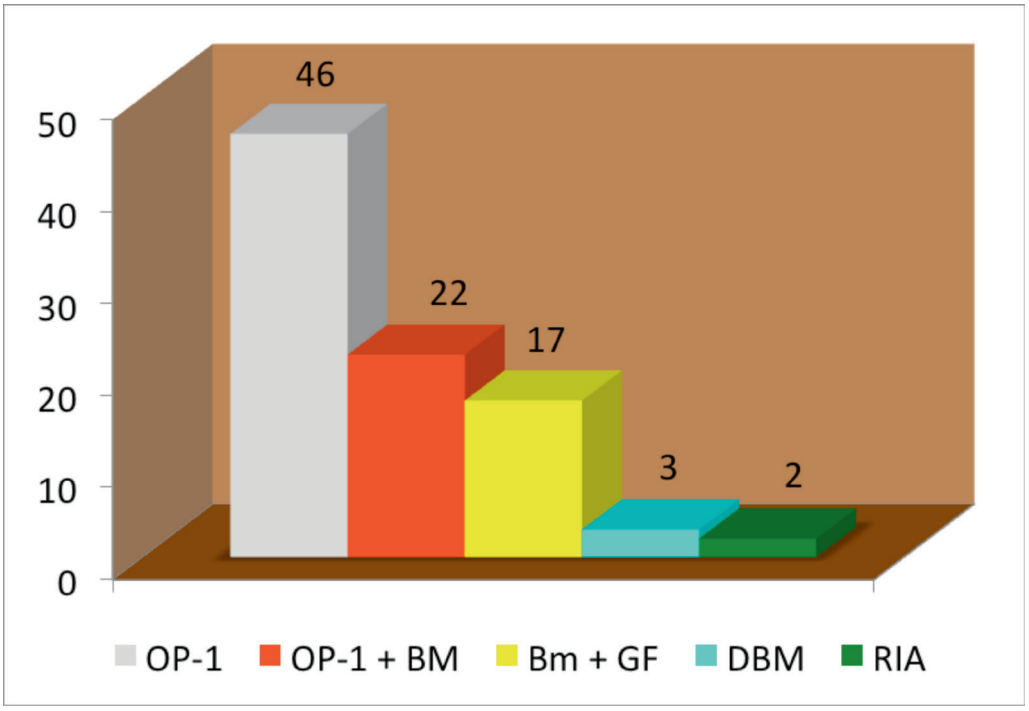
The purpose of this presentation is to summarize a decade of experience in the use of these methods of regenerative medicine. We reviewed 90 cases of non-union which were treated from 2000 to 2012 with an allograft enriched with BMAC, Osteogenic Protein 1 (OP-1) or a combination of the two. The non-unions were classified based on the site of the involved bone, which were in decreasing order of occurence: tibia, femur, humerus, ulna, radius, clavicle, ankle and astragalus. All patients presented with a failure of a previous treatment for non-union, with an average of 2.5 previous treatments (2-6). Due to failure of traditional methods of autologous grafting technique, all patients were treated with a combined allograft using BMAC or using OP-1. The BMAC we used was obtained with RegenLab Kit for autologous bone marrow concentration based on the amount required. To reproduce the osteoinductive and osteoconductive component required for bone regeneration, OP-1, allograft or synthetic scaffolds were added individually or in combination.

RESULTS

Overall, we achieved a successful union of the bone in 79% of cases. We did not find differences in results obtained using different volumes of bone marrow concentrate. The addition of a scaffold did not affect healing. Some patients were treated by percutaneous infiltration of BMAC without other factors and achieved 83% of successful healing. Patients treated with open surgery healed in 79% of the cases.

Non-union experience:
- 5/2002 – 12/2012
- 90 patients
- 49 males/ 41 females
- Right or dominant side 44
- Left or non dominant side 46
- Average age 47 yrs (16-83)

Biological stimulus used:



Results with OP-1 Treatment:
- Group A (OP-1) : 66 cases Recalcitrant non unions
- Lower limb
- Synthesis failure
- Comorbidities
- FU 9 mos on average (3-14)
- Healing 89%
- t 4 mos (1-11)

Results with Bone Marrow Aspirate Concentrate (BMAC) and Growth Factor Treatment :
- Group B: 24 cases
- Delayed healing
- Upper limb (≈ humerus)
- FU 15 mos on average (3-19)
- Healing 91%
- t 5 mos (2-10)

CONCLUSION

Summarizing the data collected, we can say that the techniques of tissue engineering applied by us were safe and effective for the patient and open up developments and applications in other diseases. In conclusion we can say that, after more than 10 years of using a graft enriched with BMAC and/or OP-1, the surgical technique has proven to be safe for the patient, non-invasive and effective.

Session: BMC: THE REAL STEM CELL THERAPY – 22nd September 2014

Presentation: **Bone graft merged with mesenchymal stem cells and platelet-rich plasma in the management of post traumatic extensive bone defects**

Lecturer: Prof. Paolo Ferrata, Director of the Orthopaedics & Traumatology Department, Siena University, ITA

INTRODUCTION

MSC (Mesenchymal Stem Cell) studies are widely reported in the literature, with proven efficacy and safety.

MSC's act by driving multiple effects: they directly differentiate into tissue-specific cells and thus substitute damaged or lost cells and modulate inflammatory processes (TGF-beta1, NO, prostaglandin-E2, HLA-G, hepatocyte growth factor, and IL-10). They indirectly influence tissue regeneration by secretion of soluble factors and also promote vascularization, cell proliferation, differentiation within the tissue (IGF-1, HGF, BMP, VEGF, IGF-2, bFGF, or pre-microRNAs).

Studies of the combination of MSC's with Bone allograft have demonstrated both biochemically and histologically that MSCs can adhere to and proliferate on highly washed morselized bone graft and, significantly, can withstand the forces equivalent to a standard femoral impaction bone grafting.

PRP (Platelet-Rich-Plasma) apparently has conflicting publications with poor comprehension of the exact involvement of its growth factors. However, validated knowledge on PRP concerns:

- Secretion of the growth factors begins within 10 minutes after clotting.
- More than 95% of the presynthesized growth factors are secreted within 1 hour.
- After the initial burst of PRP-related growth factors, the platelets synthesize and secrete additional growth factors for the remaining 7 days of their life span.

The great variety in the platelet concentration techniques that are available on the market may lead to extremely variable results: P-PRP (pure platelet-rich plasma), L-PRP (leukocyte platelet-rich plasma), P-PRF (pure platelet-rich fibrin), L-PRF (leukocyte platelet-rich fibrin).

Platelet growth factors support bone regeneration primarily via chemotactic and mitogenic effects on preosteoblastic and osteoblastic cells. But it seems that the potency of the growth factors liberated by PRP is too weak to induce bone formation in defects with low regenerative capacity and without a stable structure. PRP alone cannot induce bone formation but can support osteogenesis in the presence of an adequate number of precursor cells.

PRP with Leukocyte (L-PRP) has a documented anti-infectious activity which is comparable to gentamicin and oxacillin against methicillin susceptible Staphylococcus aureus (MSSA) and inhibits the growth of methicillin resistant Staphylococcus aureus (MRSA) and Escherichia coli.

MATERIAL AND METHODS

We present our reconstruction technique based on the combination of homologous bone graft with mesenchymal stem cells and PRP (platelet rich plasma). Since 2011 we treated with this method 12 patients. The mean age was 35 years (range 23-49); there were 8 men and 4 women.

Nine cases were PSA (3 femur, 1 humerus, 3 ulna, 1 radius, 1 tibia) and were treated as follows: 3 percutaneous (MSC + PRP), 6 open (MSC + PRP + bone allograft);

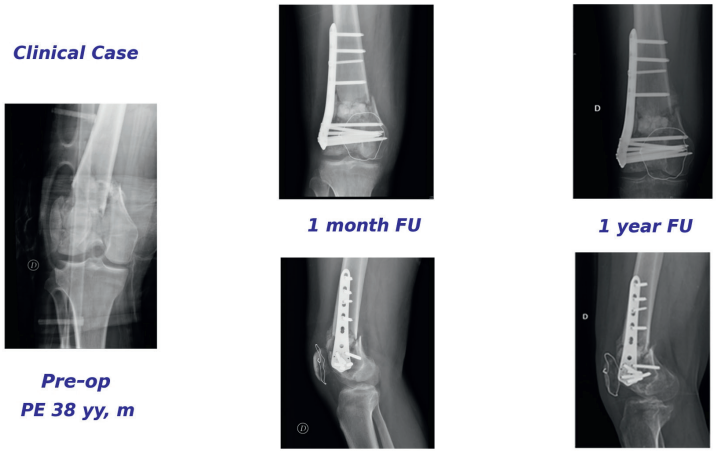
Three cases were post-traumatic bone loss and were treated with MSC + PRP + bone allograft (chips or cortical strut)

A Medical Devices Class IIb, Regen Extracell GLUE CE (MSCs + PRP + ATS) was used to harvest, concentrate and separate MSCs from Bone Marrow and to prepare PRP and Autologous Thrombin serum (Regen Lab).

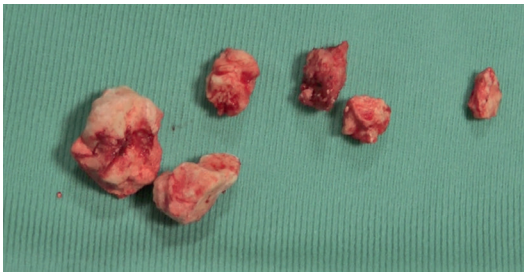
RESULT

Anteroposterior and lateral radiographs were taken preoperatively, postoperatively, at one month to assess the appearance of the callus and then every month thereafter, until bone healing occurred, to monitor the progression of the callus. Radiographic evidence of fracture union was observed at an average of twelve weeks (range, four to sixteen weeks). In our group we didn't have recurrence of pseudoarthrosis, infection or device loosening. This technique, associated to a meticulous fixation procedure, offers the possibility to fill in the bone gap produced by the trauma ensuring a fast and safer healing process.

Clinical cases: Ankylosed knee

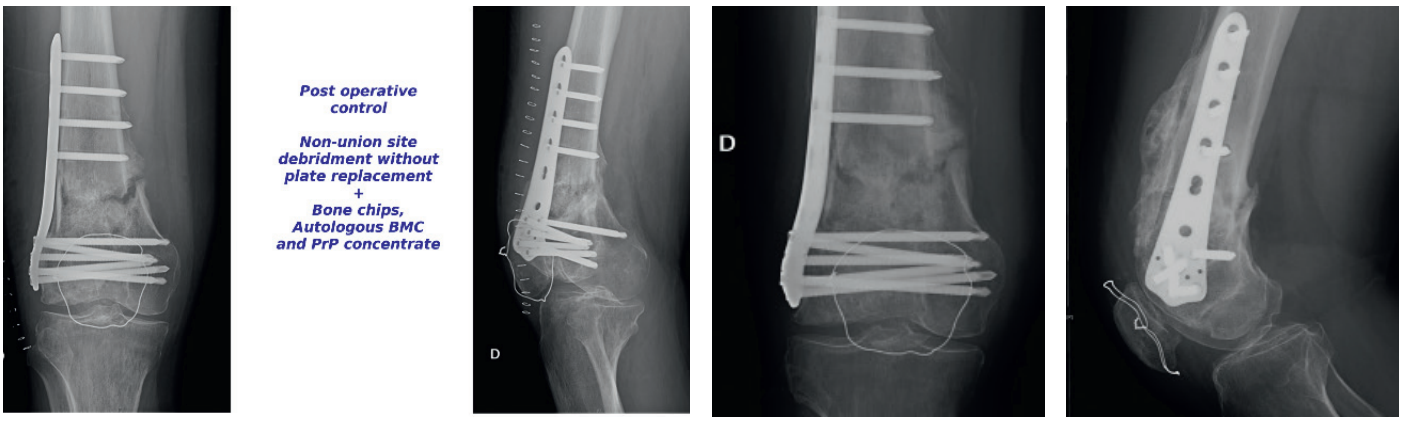


Technique: Soft tissue debridement and passive mobilization, then Bone debridement (Large bone defect). Removing of bone substitute: calcium-phosphate cement. It shows no integration and could interfere with osteogenesis and neoangiogenesis.



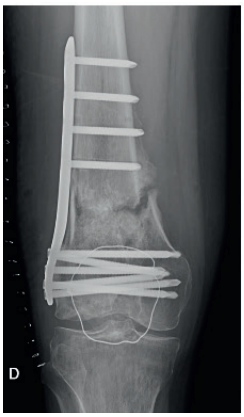
"Biological cocktail" described by Capanna: MSCs + PRP (Extracell Glue Kit Regen Lab) with Bone allograft. Filling in the defect

Results

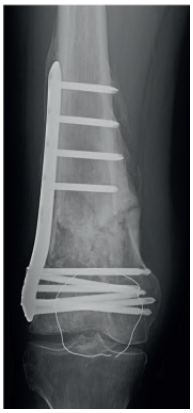


Post Op control

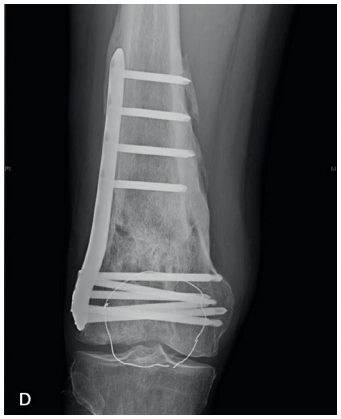
5 Months F.U. He maintains 0-100° of knee ROM. Full weight bearing, bicycling

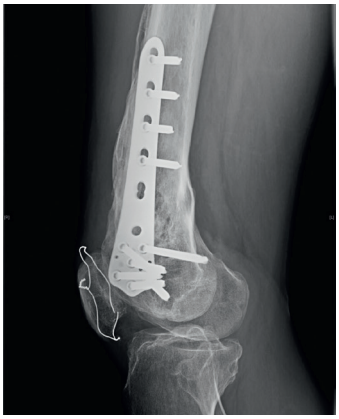


callus growth in 10 months



10 Months F.U





FU 28 months → soft running and bicycling

CONCLUSION

The "biological cocktail" increases the endogenous osteopotential, enhances neoangiogenesis in fracture site and prevents infection (L-PRP). It also provides adequate mechanical stability.

We introduce the "biological cocktail" in the two stages technique:

I stage → damage control (Irrigation – Early antibiotic prophylaxis – Debridement - External fixation)

II stage → early definitive reconstruction with bone allograft merged with MSCs and PRP as soon as the clinical and local condition allows to proceed.

These effects allow to extend the indication of use at the treatment of selected post-traumatic bone defects in acute setting.

Session: KEYNOTE LECTURES – 22nd September 2014

Presentation: **Hypes and hopes in cellular therapies in orthopaedic regenerative medicine**

Lecturer: Prof. Massimo Innocenti, Professor of Orthopaedics & Director of Orthopaedic Department at University of Florence, ITA

INTRODUCTION

Platelet rich plasma is widely used in orthopaedics and it's well known its benefit in treatment of tendon, cartilage and muscle pathology, as demonstrated in several clinical studies. Conflicting data from clinical studies appear in literature about use of PRP alone in treatment of bone pathologies. It's known that suitable colonization and vascularization of tissue-engineered constructs after transplantation represent critical steps for the success of bone repair. PRP is composed of numerous growth factors known for their proliferative, differentiate and chemo-attractant effects on various cells involved in wound healing and bone growth. Large bone defects are often present in reconstructive orthopaedic surgery, especially in knee and hip arthroplasties revision, but also in post-traumatic and oncological patients, challenging the surgeon to find a solution with use of less resources to reduce economic burden, reduce patient discomfort and morbidity (for example avoiding morbidity of the donor site when autologous bone is used). In our experience we prefer using homologous bone from cadaver, but we have to deal with problem of resorption and less osteointegration when compared to autologous bone. The problem of fewer osteoinduction and osteoconduction is not secondary.

Bone reconstruction in Traumatology includes management of non unions which are normally treated by direct percutaneous injection of concentrated stem cells or by Mini-Invasive procedures (elective indication in case of non union with stable fixation device).

High tibial osteotomy, avascular necrosis of femoral head, hip and knee revision surgery (aseptic / septic challenging cases) are typical pathologies in Orthopaedic surgery.

Cellular therapies in Orthopaedic surgery includes cells (Stem cells from Bone Marrow) + Growth Factors (PRP) + Scaffold (Homologous Morselized Bone; Ideal chips dimension are between 3 – 8 mm of cancellous bone from cadaver).

Hernigou et Al. demonstrated close relationship between clinical results and the number of stem cells. But the main question is: how many cells do we inject ?

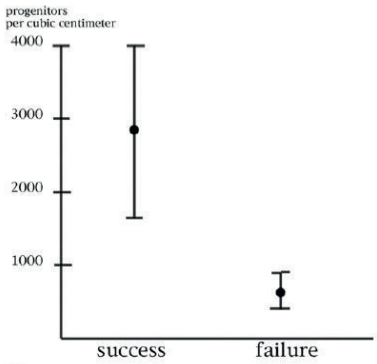


Fig. 3
Success and failure of treatment as a function of the concentration of progenitor cells per cubic centimeter in the graft.

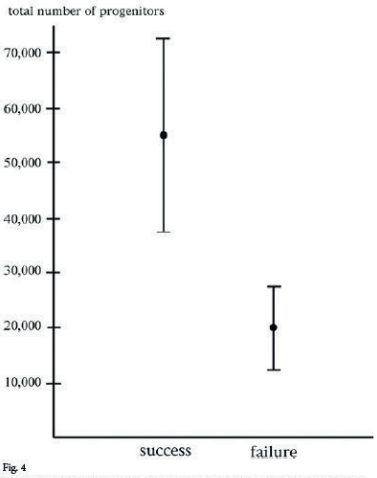


Fig. 4
Success and failure of treatment as a function of the total number of progenitor cells injected at the nonunion site.

We performed a prospective study on potential cell concentration from bone marrow with REGEN KIT®.

MATERIAL AND METHOD

Study population: 30 Patients, mean age is 51.3 years. Bone marrow aspirate was collected and divided into 4 Regen THT tubes, and then centrifuged in the operating room. The content of one of these tubes was used for a cellular efficiency and yield analysis. The sample was stratified on a lymphocyte separation medium gradient (density 1.077 g/ml, Lonza) and centrifuged at 1000xg for 30 min., washed and seeded on a 100 mm culture plate, in growth culture medium. A non-centrifuged bone marrow aspirate sample,

submitted to the same laboratory procedures was used as control. Forty-eight hours after seeding, adherent cells (MSCs) present in 30 ocular fields were counted directly on the growth medium using an appropriate standard grid; the number obtained was normalised using an appropriate conversion coefficient.

RESULTS

A number of MSCs in the vicinity of 1 x 104 cells/ml was obtained with the Regen Kit. Regen Kit which also resulted in almost total removal of erythrocytes with selection and enrichment of MSCs. The number of MSCs per mL was 8 times higher than in the control group.

CLINICAL EXPERIENCE

We perform polytherapy, using mesenchymal stem cells (MSC) concentrated from autologous bone marrow and platelet rich plasma, both of which were isolated using the Regenkit Extracell systems. MSC's ad PRP were combined with homologous bone as a scaffold, for the treatment of large bone defects in: revision total hip and knee arthroplasties, non unions, osteotomies and fusions. These effects are measured even by radiographic controls and clinical score after surgery.

Material and method:

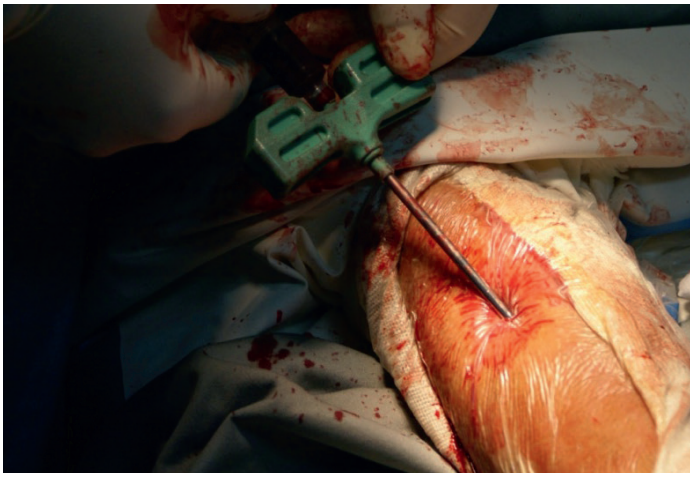
Autologous Stem Cells: Centrifugation (BMC Regenlab kit). Platelet-Rich Plasma: Centrifugation (PRP Regenlab kit). Scaffold: Homologous morcelised bone graft.

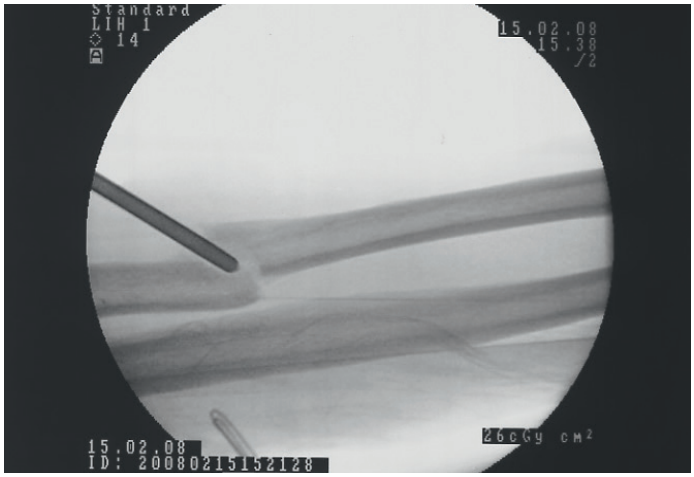
Non Union Data

• Male 24 years old radial non union: Direct percutaneous injection of concentrated stem cells. Healing at 4 Months follow-up. Mini-invasive procedure in non unions.

• Female, 42 years old: Atrophic non union → 2 cm gap: stable intramedullary nail. 3 Months post-operative follow-up Healed with non-invasive procedure (mini incision).

• Male, 25 years old, humeral atrophic non union. Stable plate, in situ treatment. Complete healing 4 months after mini open surgery and composite graft.





Male, 19 years old, 10 months post op: Non union and torsional defect. Mini open for Proximal parafocal osteotomy. New nail. Complete healing at 3 Months.

Data for High tibial osteotomy, VARUS KNEE INDICATION:

A Study with 47 PATIENTS was conducted. Middle age : 49,5 aa (29 – 58 aa): 35 males – 12 females. Patients were divided in 2 groups on treatment base:

1) Bone substitute	2) Autologous + MSC
N. Patients : 24	N. Patients : 23
Age: 48,4 aa (27-61)	Age: 51,2 aa (38 – 57aa)
8 M / 16 F	9 M / 14 F
BMI: 22,6	BMI: 23
pre op: 6° varus	pre op: 4° varus
Tomofix plate	Tomofix plate

Findings:

Bone substitute cases: We observed immediate weight bearing, with average consolidation time of 3.5 Months.

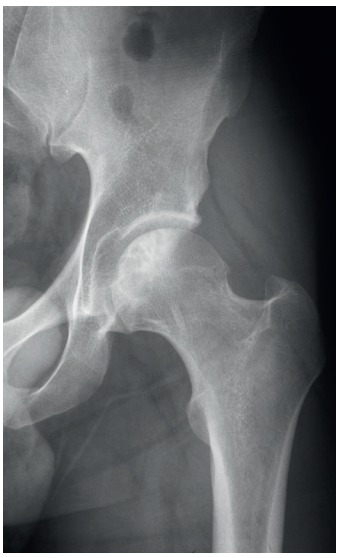
Polytherapy with homologous graft, PRP and MSC Immediate weight bearing Average consolidation time: 2 Months.

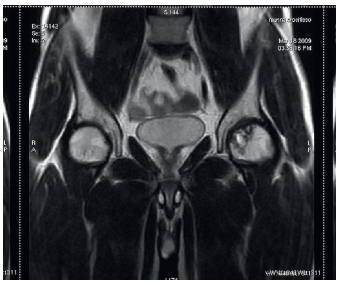
Femoral Head Necrosis Data: Core decompression and grafting

Core decompression has been the most employed operation for early stage osteonecrosis. Decompression of the necrotic site is a risk factor. So, as reported Brown et al., JBJS, 1993, a mechanical support of the necrotic area is required.

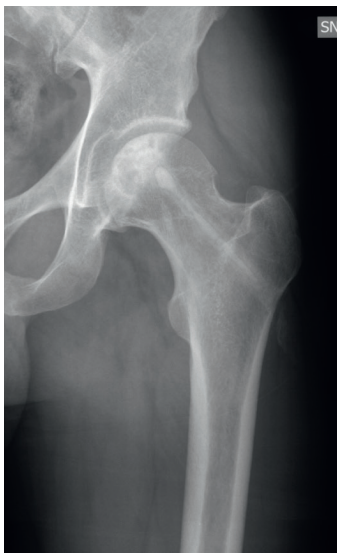
Surgical Technique: Patient positioning on traction, then amplioscopy. MSC preparation is used for fertilization of the homologous graft. Then proceed with core decompression. Necrotic bone must be removed with spoon and expansible reamer. The mixture of graft + MSC to fill necrotic area is applied. The injectable bone substitute was used to fill the space around the graft and in the femoral neck to assure mechanical support.

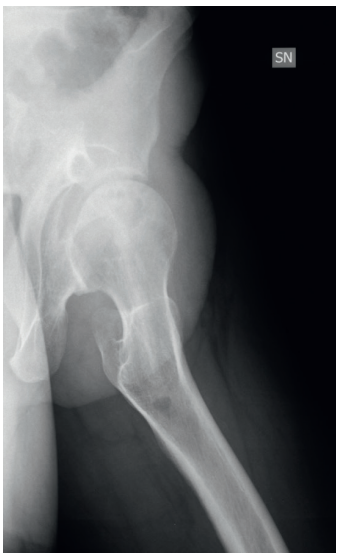
Steinberg Stage 2c, Male, 54 years old Left AVN





36 Months follow up





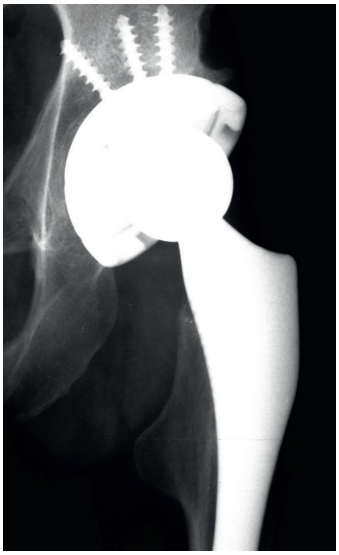
MSC + PRP and graft in Hip and knee revision: data

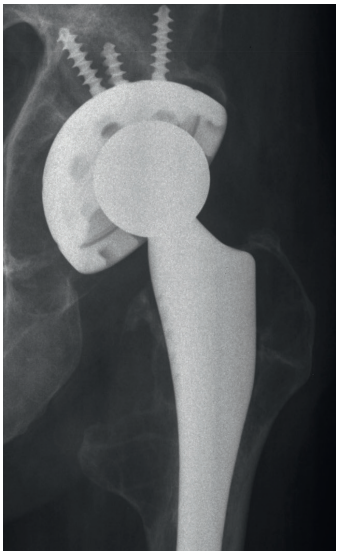
It is indicated in challenging cases where a further failure would be catastrophic

Materials: Trabecular metal cage, MSC, PRP and graft. Follow up with radiographic control

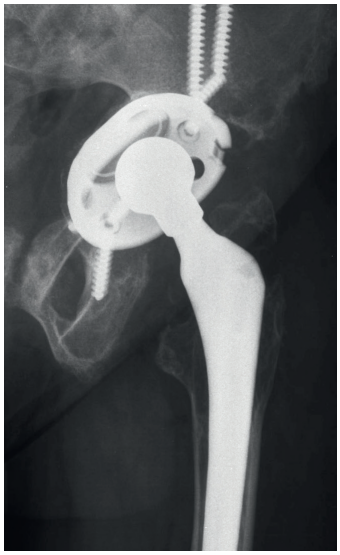
Clinical cases:

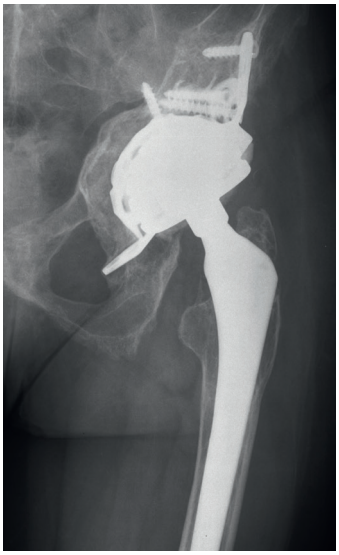
• 47 years old, acetabular ostheolysis





• 67 years old, 2° acetabular failure, iliac artery compressed. Iliac vessels are prepared, then the old implant is removed. MSC, Graft and new Cage were implanted.





CONCLUSIONS IN BONE RECONSTRUCTION

Simplified Tissue Engineering applied to bone reconstructive surgery is nowadays a safe and feasible procedure that often allows high success rate with less invasive techniques.

Biological composites made of Stem Cells-Growth Factors-Scaffolds are excellent solutions in challenging post-traumatic and degenerative cases.

Session: BMC: THE REAL STEM CELL THERAPY – 22nd September 2014

Presentation: **The treatment of long bone pseudoarthrosis with MSC derived from bone marrow: literature review and case series review**

Lecturer: Dr. Paolo Domenico Parchi, Orthopaedics Department, Cisanello Hospital, Pisa ITA

INTRODUCTION

The treatment of non-union has seen in the last few years a lot of new proposals with a large number of studies on the use of growth factors and autologous bone marrow concentrate (BMAC). These methods can be used with open surgical techniques or by percutaneous techniques. Pseudoarthrosis is one of the most frightening complications of a fracture with a percentage of incidence that goes from 5 to 10%. It is characterized by the non union of the bone fragments within 6 months from the fracture with absence of signs of healing in the previous 3 months. Treatments are various and comprise electrical stimulation, internal fixation, external fixation, bone grafting and cellular therapies. The latter treatment is nowadays viewed with great interest by the research community.

Bone marrow supplies mesenchymal cells and the growth factors necessary for bone consolidation.

Percutaneous bone marrow aspirate grafting is a minimally invasive treatment, with proven effectiveness for the treatment of atrophic pseudoarthrosis, with few reported complications.

The Healing rate increases in proportion to the injected MSC concentration.

It was demonstrated that the bone marrow graft infusion with less than 1000 progenitor cells/cm3 did not result in consolidation and that a total of more than 30.000 progenitor cells are required for the therapy to prove successful.

Automated and closed systems for the separation and the concentration of the mononuclear cells obtained from the bone marrow aspirate maximize the therapeutic potential, and increase the reproducibility of the cell isolation process.

MATERIAL AND METHODS

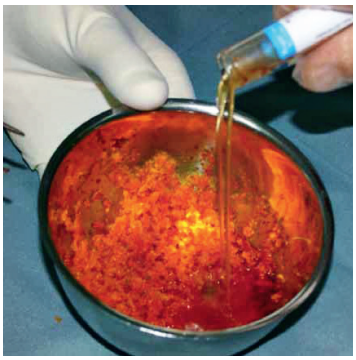
From Jan 2009 to Jan 2014, 30 cases of pseudoarthrosis were treated (27 Atrophic – 3 Hypertrophic)

- 27 cases were treated with the use of MSC derived from Bone Marrow. Infected pseudoarthrosis were excluded (1 Atrophic – 2 Hypertrophic). In particular:
- 14 male - 8 female. Age: 51.3 years old (22-84), from 15 traffic accidents, 5 job Injuries, 7 other.
- 6 Open Fractures, 21 Closed Fractures.

Regen Extracell Kit (Regen Lab) was used for MSC and PRP preparation.

Protocols:

1. Sampling of 20 ml of bone marrow from iliac crest.
2. Sterile transfer to the device.
3. Centrifugation (Centrigel Regen H19-F) for 12 minutes at 3200 RPM: Separation and concentration of mononuclear cells → 87%.
4. Union of the cell concentrate with the scaffold: DBM 10 cc.



We changed the fixation in 86% of the cases (23/27):

- Intramedullary Nails (8 cases), Plate Fixation (11 cases), External fixation (4 cases), No change (4 cases).

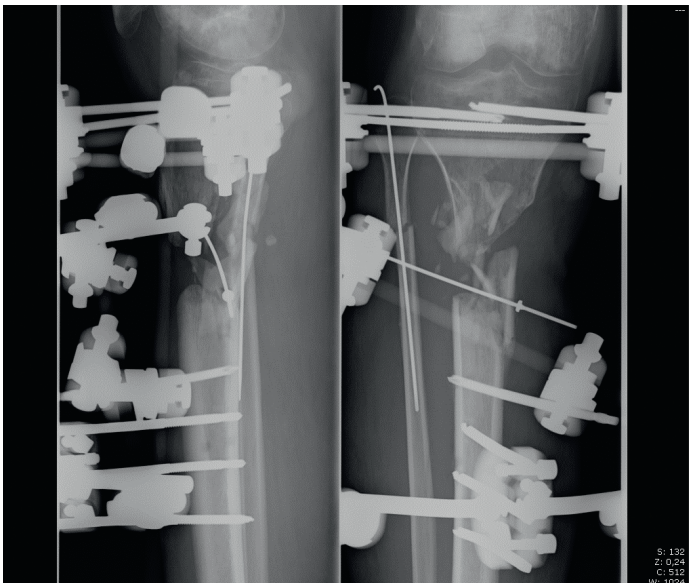
In 4 cases a second revision has been necessary.

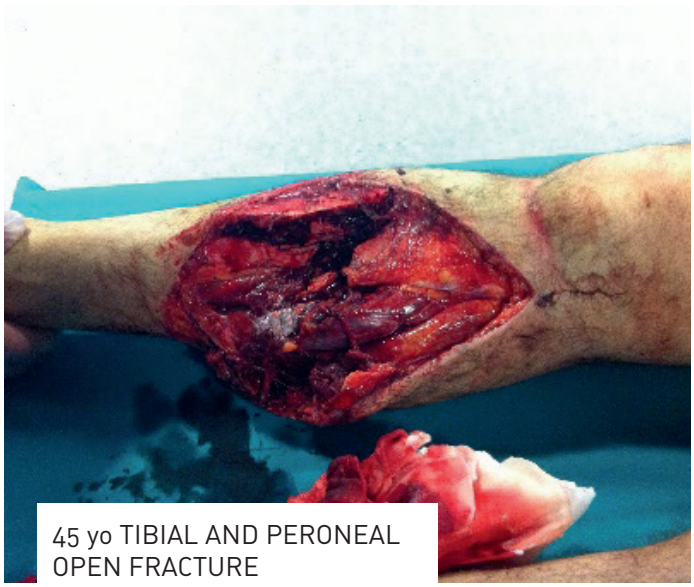
RESULTS

Follow-up at 10, 22 months (4-21).

- 75% (20 cases) complete non union healing. Average time to healing: 4.9 months
- 81% of healed cases had complete remission of clinical symptoms (VAS 0)
- 3 cases referred a residual non-constant pain (VAS ← 3)
- 7 Cases had no sign of healing on X-RAY (9 months)
- 4 of these cases referred an improvement of clinical symptoms (VAS 7 → 4)

Clinical cases

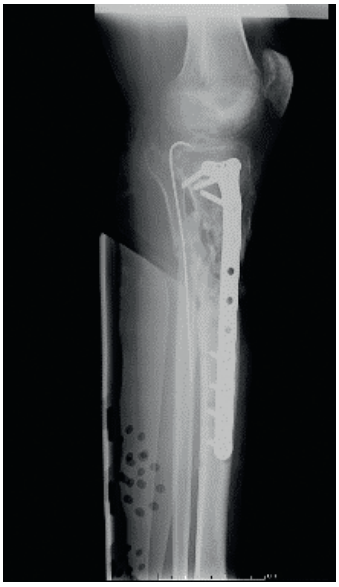


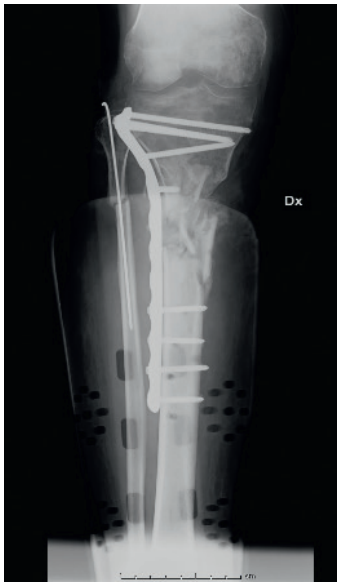


45 yo TIBIAL AND PERONEAL
OPEN FRACTURE
Gustilo 3A
Treated with EF
3 months no sign of healing

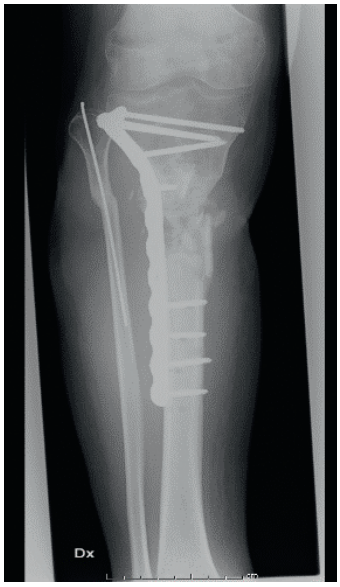
1. MECHANICAL NEEDINGS
2. BIOLOGICAL NEEDINGS

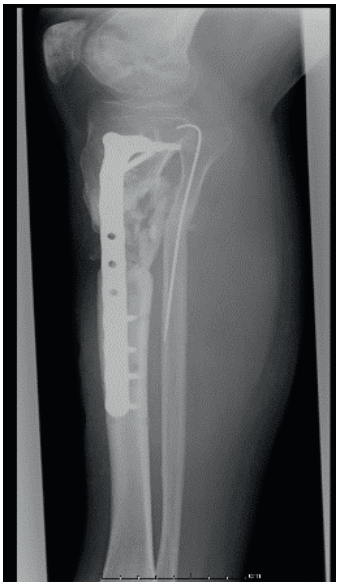
1st ATTEMPT





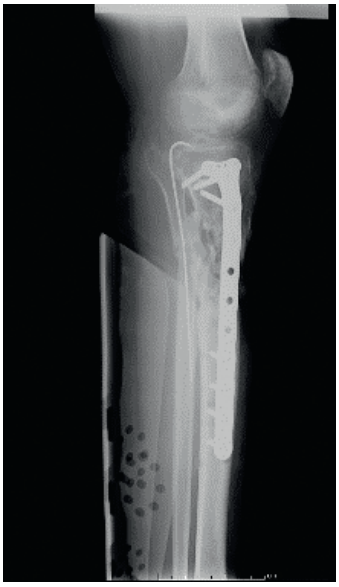
Open nonunion site curretage
New synthesis with Plate Fixation
Bone Marrow Aspirate (Regen Kit)

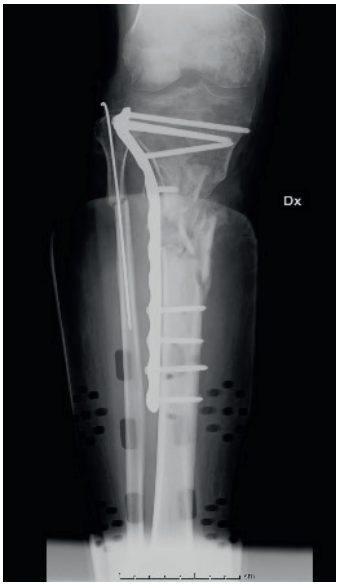




After 5 months from second surgery
Good Bone Formation,
BUT IT 'S NOT ENOUGH

2nd ATTEMPT





Open nonunion site curretage
New synthesis with Plate Fixation
Bone Marrow Aspirate (Regen Kit)

HEALING TIME: 4 MONTHS, VAS 2

LITERATURE REVIEW

19 patients with long bone nonunion.

All patients were treated with bone marrow-derived cells.

(Extracell BMC/Glue kit Regen lab)

Patients had a mean age of 42.3 years (min 20–max 73).

Mean follow-up time was 7.2 months (min 3–max 19).

Radiographic investigation showed complete healing in 78.9 % (15 cases) with an average healing time of 6.5 months.

Musculoskelet Surg (2014) 98:101–106
DOI 10.1007/s12306-013-0271-2

ORIGINAL ARTICLE

Long bone nonunions treated with autologous concentrated bone marrow-derived cells combined with dried bone allograft

M. Scaglione · L. Fabbri · D. Dell'Omo ·
F. Gambini · G. Guido

CONCLUSION

Bone marrow concentration allows the collection of a large number of MSC's.

In our experience, the use of MSC's derived from bone marrow in the treatment of long bone non-union is associated with good results in over 77% of the cases.

The exact number of MSC's remains an open question (we don't know the exact recipe). The number of MSC's is related to several factors (i.e. age).

This is only a preliminary study with limitation due to the small number of patients and to the multiple sites where the technique was applied.

BONE SURGERY

MENISCUS

BIOBRIDGE FOUNDATION

regenlab

Session: SPORTS MEDICINE & INFILTRATIONS & MUSCULOSKELETAL MEDICINE – 23rd September 2013

Presentation: **Knee meniscal tears: treatment by autologous platelet-enriched plasma**

Lecturer: Prof. Philippe Adam, Sports Medicine, Medipole Clinic, Toulouse, FRA

INTRODUCTION

Normal menisci are fibrocartilaginous C-shaped discs composed of collagen fibres, in which are embedded cells known as fibrochondrocytes that are able to synthesize the matrix. Menisci are integral to knee function, providing load transmission, shock absorption and joint stability. Meniscal ties are of functional importance giving rise to differing levels of mobility, with the lateral meniscus being more mobile and the medial meniscus poorly mobile and firmly attached to the joint capsule. The meniscus is typically avascular or poorly vascularised, with only the peripheral 10 – 30% of the meniscus vascularised. Only the outer third of the meniscus has pain fibres, which may not necessarily equate to an absence of pain if a tear occurs in the non-innervated region as causes of pain can be other injuries and/or osteoarthritis. Peripheral vascularity seems to play an important role in meniscal healing. However, deep meniscus is also exposed to the joint fluid and, as a result, growth factors can impact meniscal healing by stimulation of vascular proliferation.

There is a strong rationale for regenerative treatment of meniscal tears as most tears are degenerative and not traumatic in origin. Factors affecting success of meniscal tear repair include: the stability of the meniscal lesion and of the joint; the age, weight and morphotype of the patient (varus, valgus); associated injuries including osteoarthritis and instability of the knee (ACL and MCL tears). It is estimated that the rate of unsuccessful healing after meniscal suture is in the order of 25%.

Anterior cruciate ligament (ACL) reconstruction should accompany meniscal repair in the ACL deficient knee to improve mechanical stability. Tears amenable to repair include unstable tears greater than 1 cm in length and less than 3 cm, occurring in the outer 20% to 30% toward the periphery (vascular, red zone). Those tears occurring more toward the junction of the red-white zone may also heal.

There are a number of options for the treatment of meniscal injuries. What we term "regenerative conservative therapy" involving PRP and growth factors is suitable for the preservation of injured but stable meniscus. Unstable lesions can be addressed with surgical meniscal repair by suture. Partial or complete meniscectomy can be undertaken with the risk of increased development of degenerative changes in the knee after the removal of large amounts of meniscus.

The approach of the study described here was to regenerate meniscal lesions without being aggressive by injection of growth factors of platelet origin into the joint in proximity to the meniscus. The enrolled candidates for PRP treatment were patients with stable tears (II/III), cysts and sutured menisci.

METHODS

A total of 185 menisci were treated over a period of 10 months.

Group 1 → patients treated with PRP only: 126 cases (68%) with Grade II and III stable symptomatic lesions located in RR or RW area (91%, mainly medial meniscus) or meniscal cysts (9%, mainly lateral meniscus).

Group 2 → patients treated by PRP as a supplement to a surgical suture one month after surgery: 59 cases (32%), with bucket handle or other unstable meniscal lesions.

Before PRP injection, imaging of the knee was completed and the IKDC score was measured. PRP was freshly prepared (RegenKit) and 5 cc was injected into the joint in aseptic conditions under imaging in order to position the needle near the meniscus. In the case of cysts, needle aspiration of meniscal cyst (mucoid content) was conducted and PRP was injected into the cleft. After the PRP injection, ice was applied, antalgic drug prescribed in addition to two days of rest. Imaging was repeated at one month and clinical evaluation was conducted by the surgeon at two months.

CLINICAL FINDINGS

In Group 1, a positive outcome was observed for 92% of patients (116/126). From a clinical perspective, we observed a constant antalgic effect of PRP, which could be due to the anti-inflammatory effect of growth factors, and improved joint function. The imaging results indicated reduced inflammation of the joint and a decrease or stabilization of the meniscal lesion (cyst, cleft). The 10 failures appeared to have been due to an incorrect evaluation of the lesion stability, underlining that good diagnosis by imaging is essential

In Group 2, positive outcomes were achieved for 100% of patients. These results demonstrate that it is possible to improve the success rate for meniscal suture through treatment with PRP.

We also undertook a remote evaluation of the effectiveness of PRP treatment using a Satisfaction Survey through which we scored knee function before and after PRP treatment. Before PRP treatment, the average score was 4.25 (0 to 6/10), whereas after PRP treatment the average score had increased to 7.96 (5 to 10/10). Most of the improvement appeared to begin in the three weeks following PRP injection.

CONCLUSION

PRP injection alone is effective in the treatment of stable meniscal lesions. Precise diagnosis of the lesion by imaging (Postural CT Arthrography if necessary) is essential. Also in this study, PRP injection was found to be a very good complement to surgical suturing of the meniscus. Follow-up in these patients will be continued to evaluate results at one year (IKDC score).

Session: SPORTS MEDICINE & INFILTRATIONS & MUSCULOSKELETAL MEDICINE – 23rd September 2013

Presentation: **Arthroscopic repair of chronic isolated unreduced bucket-handle tear of menisci and chronic displaced discoid lateral meniscal tear with platelet-rich fibrin matrix augmentation**

Lecturer: Prof. Chian-Her Lee, Chief of Department of Orthopaedics, Taipei Medical University Hospital, Taipei, TAI

INTRODUCTION

Data in the literature indicates that the success rate of meniscus repair is anywhere from 70% to 90%. A number of factors influence successful outcome including rim width, tear length, anterior cruciate ligament (ACL) laxity and whether concomitant ACL reconstruction is undertaken. Further factors which influence success are whether we are dealing with an acute or chronic tear, whether it is the medial or lateral meniscus which is involved and whether the tear is in the red-red, red-white or white-white zone.

Chronic, isolated, displaced, bucket-handle meniscal tears and displaced discoid lateral meniscal tears with poor vascularity and healing ability are very challenging to repair and there are not a lot of options available to the surgeon. The purpose of the work described in this paper was to determine whether platelet-rich fibrin matrix (PRFM) augmentation can increase the meniscal healing rate in these cases.

PRFM is obtained by mixing autologous PRP with autologous thrombin serum to yield a membrane-like structure. PRFM contains all of the key growth factors of PRP.

METHODS

We studied a population of 80 patients treated for meniscal tears between 2010 and 2013, 62 of whom had a bucket-handle tear and 18 of whom had a discoid lateral meniscal tear. These two cohorts of patients underwent surgery as follows:

• Chronic displaced Bucket-handle tear of menisci; 62 (MM39/LM23) age:14-56 (average: 35.6 y/o), duration: 3-72 ms, average: 7.5 ms; FU 4-36 months, average 8.5 months
o Group I: concomitant ACLR= 24 (2 later retear à repair with PRFM)
o Group 2: chronic isolated tear without ACLR = 38

• repair alone without PRFM =22 (5 retear à 4 re-repair with PRFM, 1 resection)

• repair with PRFM = 16 (+2 +4 above = 22 cases) à 1 retear à re-repair

• Chronic discoid LM with tear = 18
o repair without PRFM = 12 (no retear)
o repair with PRFM = 6 (no retear)

The following surgical techniques were used:

1. Reduction meniscus à hybrid repair: Outside-in repair + all-inside repair with FasT-fix or Sequential device for posterior horn
2. Discoid LMà partial resection, then repair
3. Prepare PRFM
4. delivery PRFM through tube with gelform
5. Put PFRM in between capsule /meniscus or meniscus/ meniscus
6. Tied over the sutures finally
7. Long leg splint for 2 weeks

CLINICAL FINDINGS

Meniscal repair was considered to be clinically successful in cases where there were no meniscal mechanical symptoms (such as locking, catching, or giving way), no recurrent effusion, no or mild joint-line tenderness and a negative McMurray test.

Bucket-handle tear

In the cohort of 24 chronic bucket-handle tear of menisci repaired with concomitant ACLR, the success rate was 91.6%. In the cohort of 22 cases of chronic bucket-handle tear repaired without PRFM augmentation, the success rate was 77.2%.

Of the 22 patients with chronic bucket-handle tear of menisci who underwent repair with PRFM augmentation, there was only 1 case of re-tear. Therefore, in this cohort, the success rate, at 95.4%, was greater than that in patients not receiving PRFM. Furthermore, in this group of patients, the Lysholm knee score at final follow-up was 87.6 ± 3.8 (75 - 95) as opposed to the preoperative score of 56.9 ± 3.3 (35 - 76).

Discoid lateral meniscal tear.

In the cohort of 18 patients with chronic discoid LM tear, the success rate was 100% with no cases of re-tear.

CONCLUSION

There are a number of critical factors which contribute to successful bucket-handle meniscal repair (BHMT). Firstly, good tissue vascularity is essential. Secondly, a hybrid suture technique with stable repair should be used and, the ACL, once injured, must be reconstructed. Based on the results of this study, authors believe that for chronic displaced meniscal tears with poor vascularity, PRFM augmentation is a good choice for enhancing tissue healing. As confirmation, in this study they found that, for chronic isolated, displaced, bucket-handle tears of menisci, repair with PRFM increased the success rate from 77.2% to 95.4% which is similar to or better than the success rate for patients with concomitant ACLR (91.6%).

BONE SURGERY

BONE SURGERY
NEUROSURGERY & SPINAL SURGERY

Session: PRP, THE FOUNDATION OF CELL THERAPY – 22nd September 2014

Presentation: **An autologously generated platelet-rich plasma suturable membrane may enhance peripheral nerve regeneration after neurorraphy in an acute injury model of sciatic nerve neurotmesis**

Lecturer: Dr. Margherita Giorgetti, Director Hand Surgery & Reconstructive Microsurgery Department, Cisanello Hospital, Pisa, ITA

INTRODUCTION

The nerves are anatomical structures of the peripheral nervous system; they are formed by bundles of axons (originating from a group of neurons) that carry information to or from the central nervous system. The nerve also contains blood vessels useful to the supply of oxygen and nutrients.

In nerves sheaths of connective tissue are present that gradually become smaller, these cover the nerve bundles and bundles of axons. The color of the nerves is white/gray, depending on the presence of fibers myelinated or unmyelinated. A nerve lesion is an injury that affects one of the nerves in the body. Nerve lesions can be caused by a wide variety of situations and medical conditions, and they can cause an assortment of symptoms. The nerve lesions can have different causes, in specific degenerative diseases of the nervous system, tumors, burns, cuts and different traumas. In all cases, part of the nerve is damaged, and the myelin, the thick sheath that covers the nerve, may be partially removed.

The nervous tissue has scarce regenerative capacity; the regeneration of nervous tissue is one of the slowest processes in the body. In the case of nerve lesions, there is a need for surgery to repair the nerve, and scar formation is an inevitable result post-surgery. An extra-neural scar may lead to the adhesion of nerves to adjacent tissue, for this reason scarring can result in incomplete recovery.

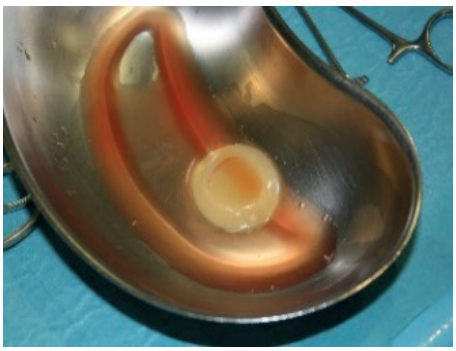
Therefore, it is essential to reduce epineural and extraneural scar formation to improve the outcome after nerve injury. Surgical repair, in combination with the use of platelet concentrate can regulate the connective tissue proliferation and can perform axonal migration into the distal stump.

The aim of the study reported below was to evaluate the usefulness of RegenLab PRP Membrane in nerve surgery.

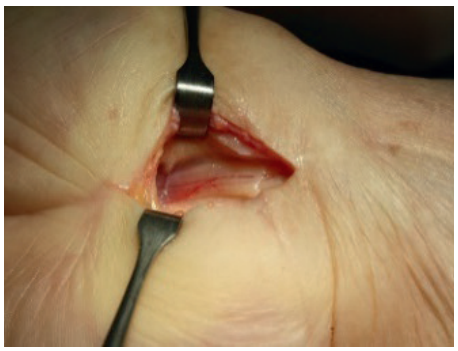
METHODS

In this study, 20 patients with nerve lesions or compression underwent surgery and were divided in two groups, each group was composed of 10 patients. A PRP membrane was applied directly in contact with the nerve in the first group of patients. The second group was treated by surgical decompression alone. Both groups were evaluated using clinical parameters and electroneurographic parameters.

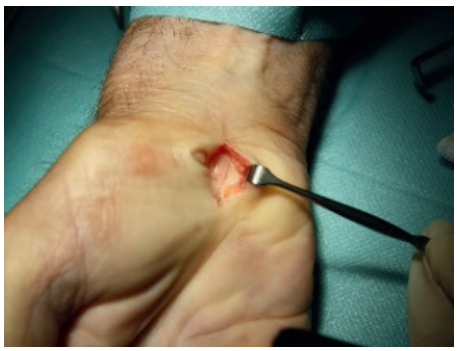
PRP Membrane



Median nerve appearance



Application of PRP Membrane



RESULTS

Both groups were evaluated using clinical parameters and electroneurographic parameters and there was a significant difference between pre-and postoperative values of the motor potential amplitude and sensory velocity in the group treated with the PRP membrane as compared o the control group.

Also clinically, the PRP group had a postoperative pain free anatomo-functional and recovery significantly better with respect the control group, 2 years after surgery.

Examination of Nerve Conduction

	MOTOR				SENSITIVE			
	Wrist Amplitude (mV)		Distal Latency (ms)		Wrist Amplitude (µV)		VdC (m/s)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
PRP	2,6	3,5	8,07	5,74	0,42	5,85	1,65	24,08
	Var. 32%		Var. 29%		Var. ….		Var. ….	
No PRP	3,93	5,12	6,06	5,45	1,04	6,4	3,38	21,06
	Var. 30%		Var. 10%		Var. …..		Var. …..	
Significance values Post-Surgery	P = 0,39		P = 0,8		P = 0,79		P = 0,3	
Significance values Pre/Post Surgery	Prp P=0,46		Prp P=0,15		Prp P=0,0006		Prp P<0,0001	
	NO Prp P=0,57		NO Prp P= 0,63		NO Prp P=0,01		NO Prp P=0,0032	

CONCLUSIONS

The mechanisms whereby PRP might improve tissue healing and regeneration are still unclear, however the data obtained in this study showed that the application of PRP fibrin membrane around the neurorrhaphy improves the nerve regeneration process.

The use of PRP as a suturable membrane acts only as a source of bioactive proteins but also as a nerve guide to control scarring and improve axonal regeneration.

Session: PRP, THE FOUNDATION OF CELL THERAPY – 22nd September 2014

Presentation: **Intradiscal injection of PRP indications, methods and first results**

Lecturer: Dr. Jean Denis Patet, NeuroSurgery & Spine Surgery, Genolier Clinic, Geneva, CHE

INTRODUCTION

Low back pain affects 3 to 4% of the active population every year with high social costs.

Different techniques have been developed for percutaneous treatment of the pathology of intervertebral discs. These are based on: laser, radiofrequency, corticoids or ozone or enzymes (chymopapaine). More recently, micromechanical devices such as the herniatome have been used.

All these techniques are well adapted to pathologies such as disc herniation, nerve root compression, or very important disc bulging.

At the present time percutaneous treatment is the preferred technique because it is very simple (one day surgery), non invasive, with good results on pain (60 to 70%) and without risk (no infection). Contraindications are presence of: migration of the hernia outside of the disc, narrow lumbar canal, substantial loss of height of the disc, vertebral instability, weakness or palsy.

The main problem for the percutaneous treatment is to find a treatment that decreases intradiscal inflammation, and if possible help the disc to regenerate. At this time corticoids are the only possibility.

Based on the good results obtained with PRP in decreasing inflammation and allowing mechanical restoration in all tendinous lesions, we started to use PRP in intra-discal injections after the degenerative part of the disc had been removed by percutaneous approach.

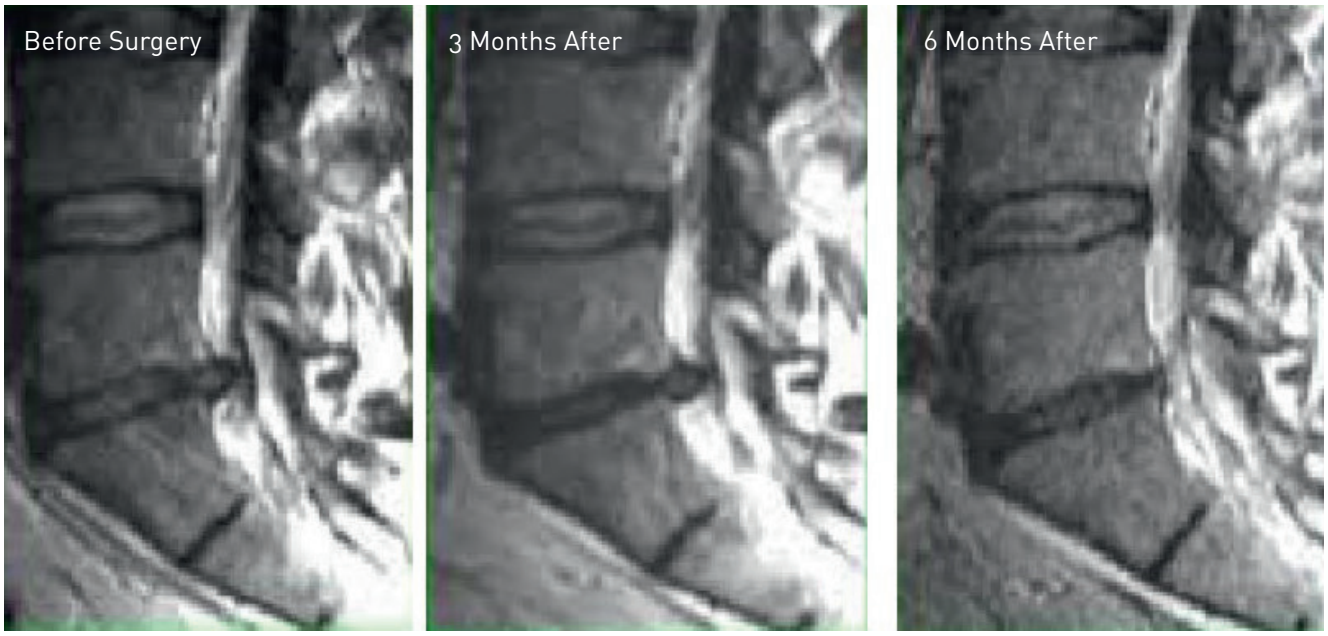
METHODS

Our initial cases with Regen PRP are presented here. The technique is also discussed.

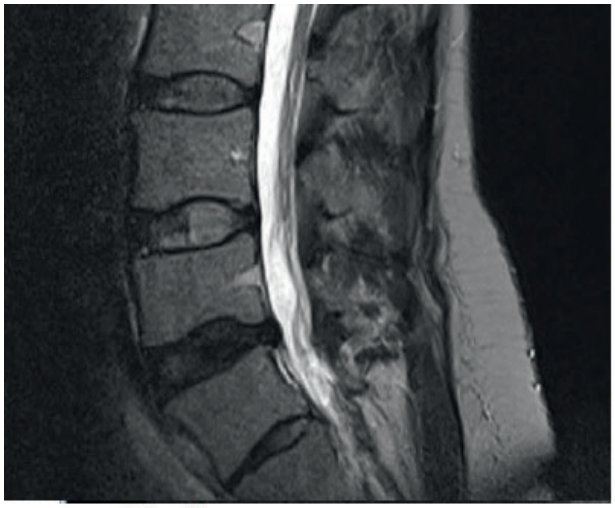
CLINICAL FINDINGS

Figures 1 and 2 illustrate MRI images of our first cases.

Case 1

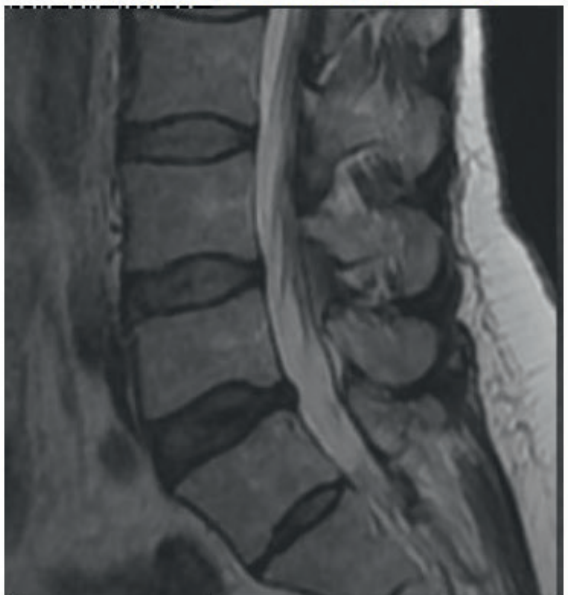


Case 2



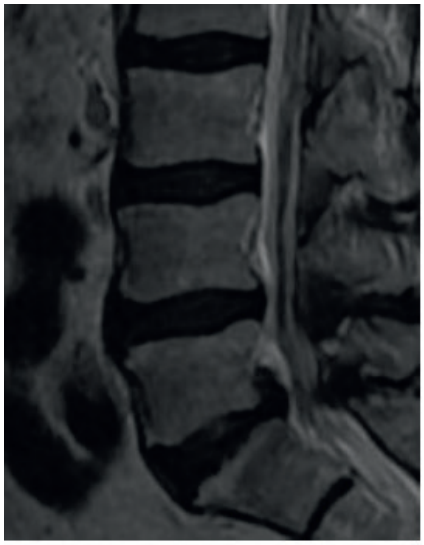
Before surgery

3 months after PRP



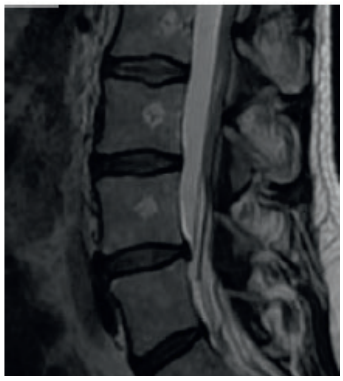
CONCLUSION

PRP is a valid therapeutic tool for reducing inflammation. At present, we are using PRP during percutaneous treatment of intervertebral disc pathology after excision of the herniation. PRP injection before herniation will also be tested in the future.



ACTUAL TREATMENT.
By percutaneous approach
Excision of the herniation
Disparition of inflammation: PRP

FUTURE
Prevention of degenerative degradation, before disc herniation with injection of PRP for black disc.



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