Complications in Orthopaedics and Traumatology
25-26 April 2019
Kursaal Oostende

Cellular Therapies for Bone Regeneration: an Update

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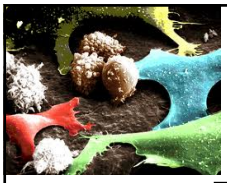
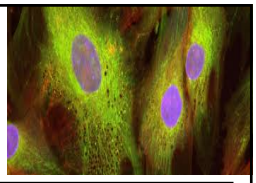
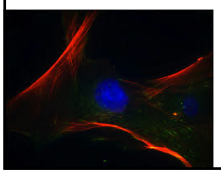
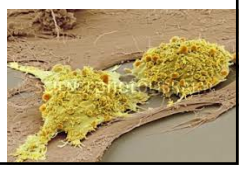
outline

- Why the interest?
- Background information
- Open questions – in my opinion as a surgeon …
- Case examples
- What the future holds…

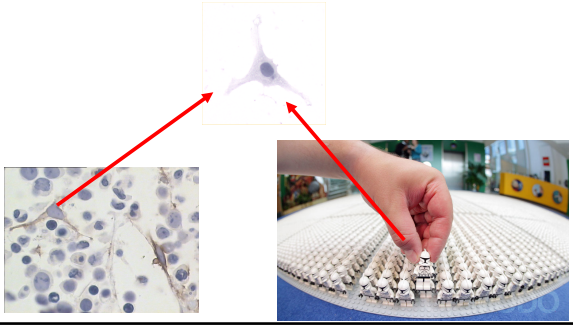



NCBI
PubMed

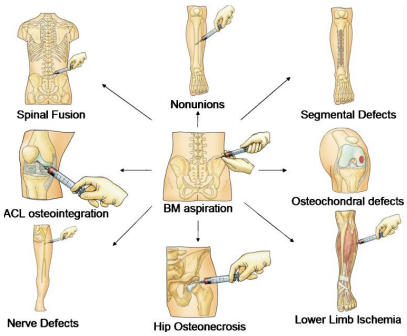
Stem cell	380,800
MSC	29,150
Osteoprogenitor cell	1,386

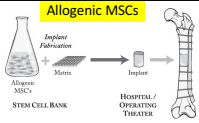
- BONE is in a CONTINUOUS REGENERATION process
- LIVING TISSUE to augment LIVING TISSUE
- In NONUNIONS a Decreased number of MSCs is found



MSCs: Applications

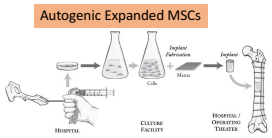


Allogenic MSCs

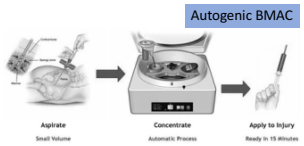


CELLULAR BASED THERAPIES

Autogenic Expanded MSCs



Autogenic BMAC

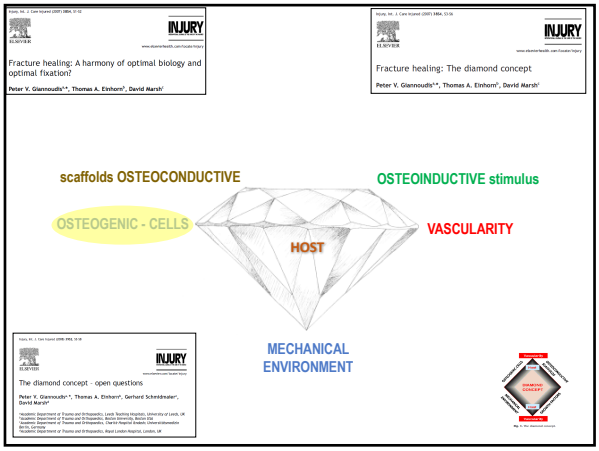


Leeds Institute of Molecular Medicine

UNIVERSITY OF LEEDS

The Leeds Teaching Hospitals NHS
NHS Trust

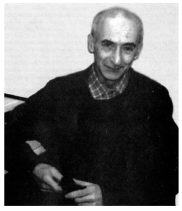
Leeds Major Trauma Centre

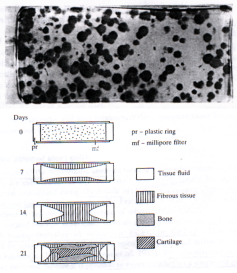


MSC:

Colony-forming, multipotential progenitor cells able to form bone and cartilage *in vivo*

Alexander Jakovlevich Friedenstein (1924-1997)





from A. Friedenstein, 1968

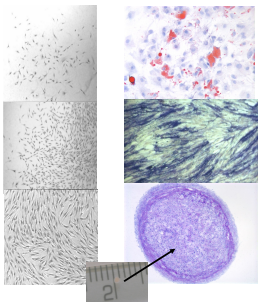
Culture-expanded MSC:

Multipotential stromal cells

Clonogenicity | Multipotentiality | Phenotype

Mark Pittenger, 1999

ISCT definition 2006



Negative:

CD45-
CD14- or CD11b-
CD19- or CD79a-
CD34
HLA-DR

Positive:

CD73+
CD105+
CD90+

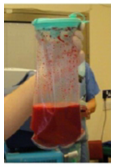
Two approaches for **Bone Repair** with **MSCs**

1) CULTURED EXPANDED MSCs

Unlimited Numbers
Phenotypically pure

2 procedures & lengthy expansion 3-4w subject to in vitro aging ATMPs regulation

2) MINIMALLY MANIPULATED ex vivo & in situ MSCs



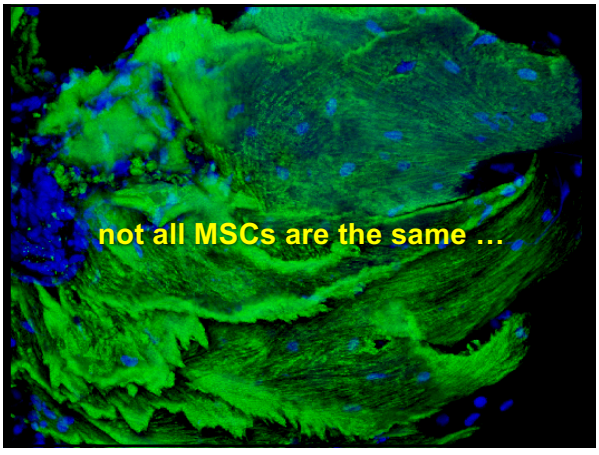
Limited Numbers
depending on technique
depending on tissue source

Minimal loss of Native phenotype & functionality

Lower regulatory burden
Lower cost

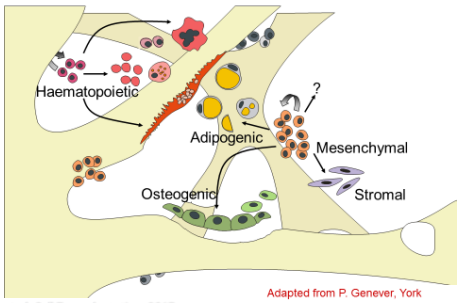
Cells ready for use within few minutes (single stage)

Cuthbert et al, PlosOne, 2016



Native MSC is not the same as cultured MSC

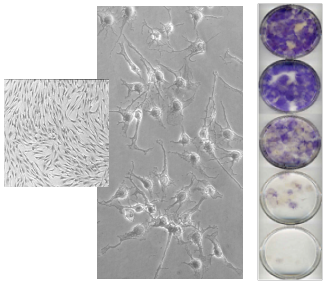
Native MSC in the bone marrow



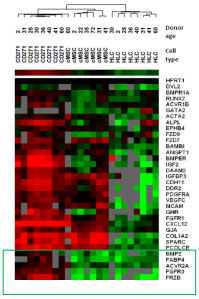
Ganguly et al, Cell Transplantation, 2017

Differences between In Vivo and In Vitro:

Morphology, Proliferation, Gene &Protein expression



Jones et al, Arthritis Rheum, 2002 and 2010

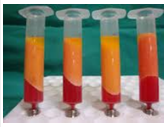


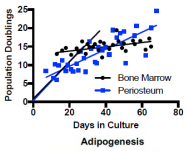
Churchman et al, Arthritis Rheum, 2012

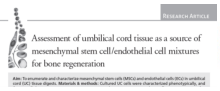
Factors affecting:

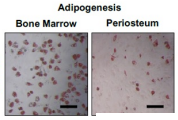
Tissue specificity

Adipose Umbilical cord Bone Marrow Periosteum







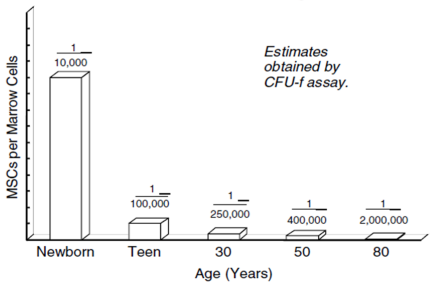


Owston et al, in preparation

Factors affecting:

Age and Co-Morbidities

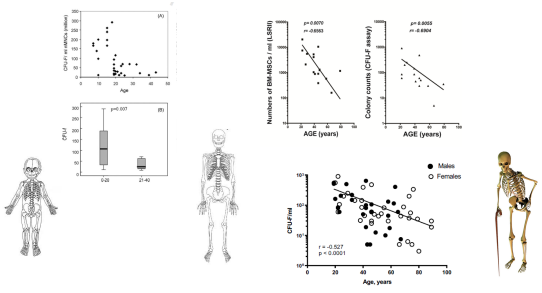
Human MSCs Decline With Age:



Caplan, J Pathol 2009; 217: 318-324

Factors affecting:

Age and Co-Morbidities



Stolzing, Mech Age Dev, 2008

El-Jawhari et al, JBJS, 2017

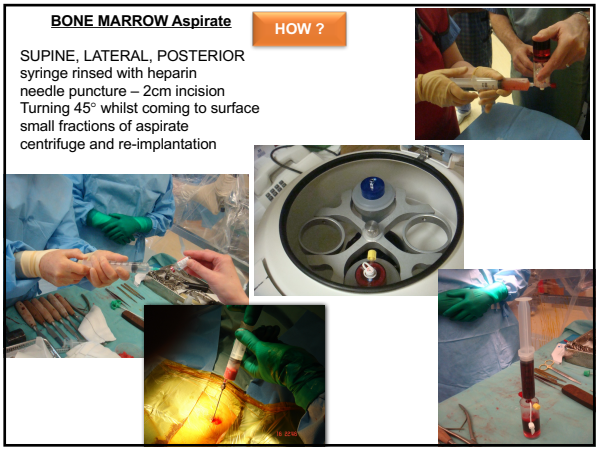
In my practice …

in vivo minimally manipulated MSCs

BONE MARROW Aspirate

HOW ?

SUPINE, LATERAL, POSTERIOR
syringe rinsed with heparin
needle puncture – 2cm incision
Turning 45° whilst coming to surface
small fractions of aspirate
centrifuge and re-implantation



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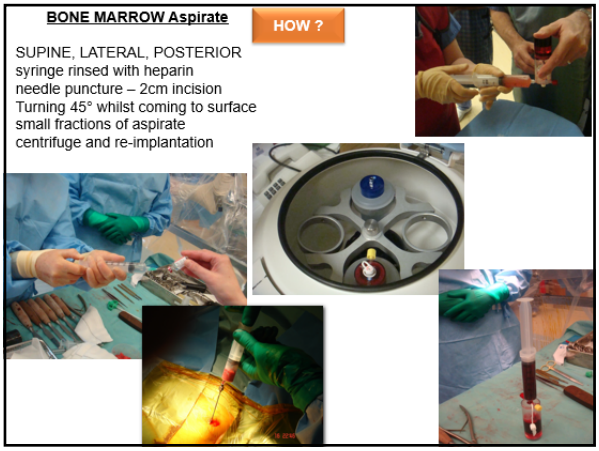
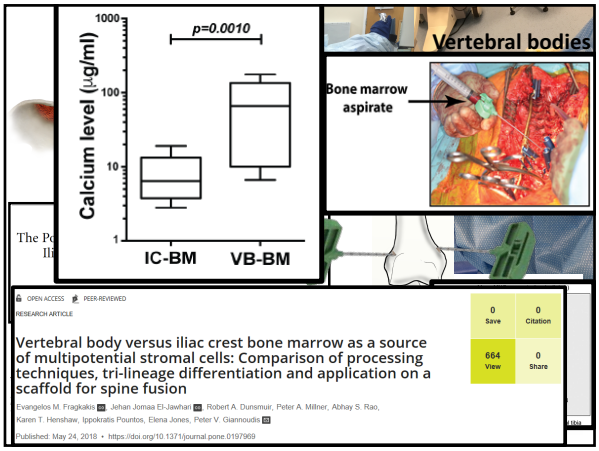


In my practice …

in vivo minimally manipulated MSCs

OPEN ISSUES

1) **WHERE FROM?**



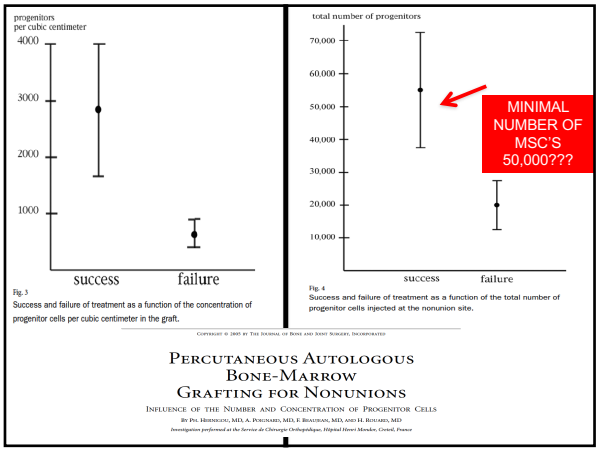
In my practice …

in vivo minimally manipulated MSCs

OPEN ISSUES

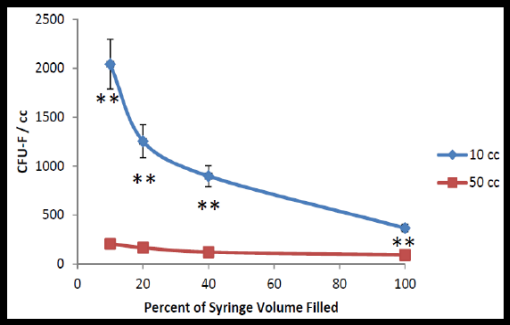
1) WHERE FROM?

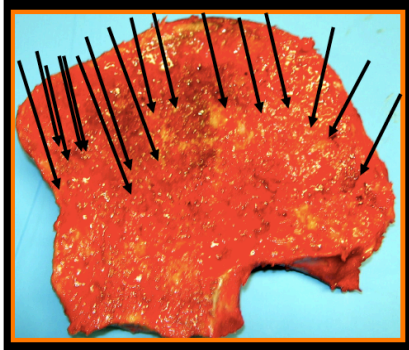
2) **WHAT IS THE MINIMUM NUMBER I NEED TO IMPLANT?**



MSCs obtained by aspiration.

Hernigou; Interntional Orthop 2014



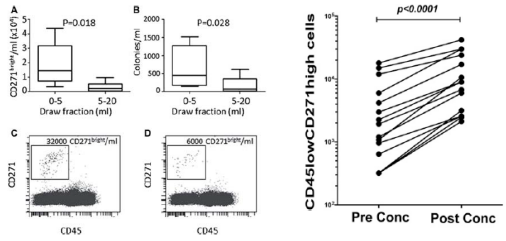


How many aspirations

- 2ml x 20
- 4ml x 10
- 6ml x 10
- 4ml x 15
- 4ml x 30
- 5ml x 30

At the end you need concentration to inject in a small volume

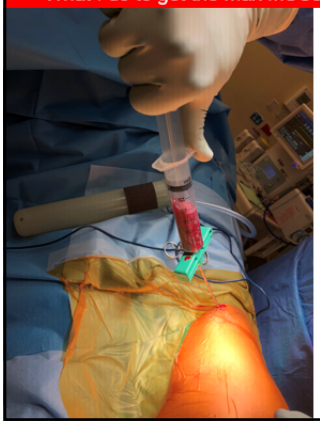
Harvesting and concentration



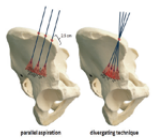
Cuthbert et al, Cytotherapy, 2012

El-Jawhari et al, JBJS, 2017

What I do to get the max MSCs possible …



1. Use of BMAC / Centrifuge
2. Volume of aspiration (60cc)
3. **Change Needle orientation**
4. **Slow Speed**
5. **High Vacuum pressure**
6. Geometry of aspiration syringe
7. Site of aspiration
8. **Concentrating device**



In conclusion, we compared 3 systems for autologous bone marrow concentration to analyze the variability that exists between the systems in producing a concentrated population of progenitor cells. We found that, compared with the Biomet and Arteriocyte systems, the Harvest system produced a greater number and concentration of progenitor cells as measured by CTPs in the concentrated marrow. Thus, it is possible that the Harvest system can provide greater healing capacity, and thus better union, fusion, and cartilage formation rates when compared with the Biomet and Arteriocyte systems.

Hegde et al *J Orthop Trauma* • Volume 28, Number 10, October 2014

In my practice …

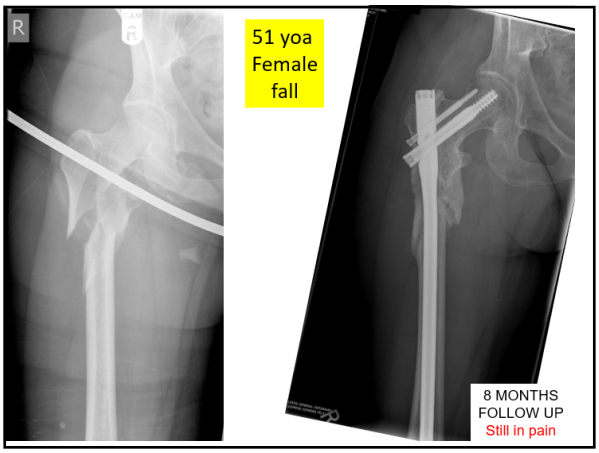
in vivo minimally manipulated MSCs

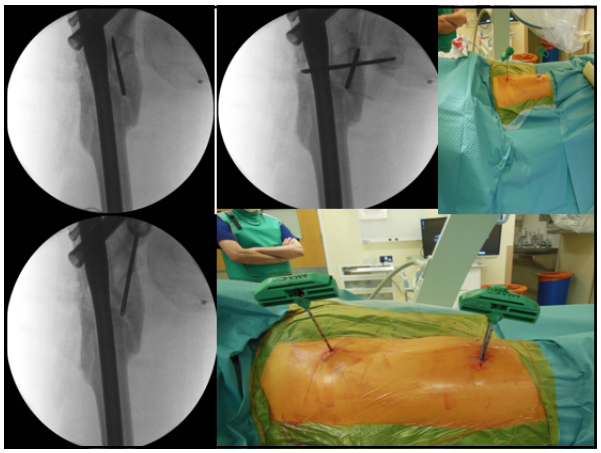
OPEN ISSUES

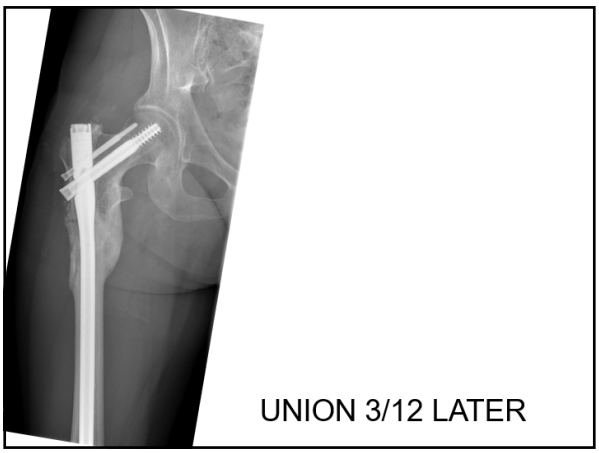
1) WHERE FROM?

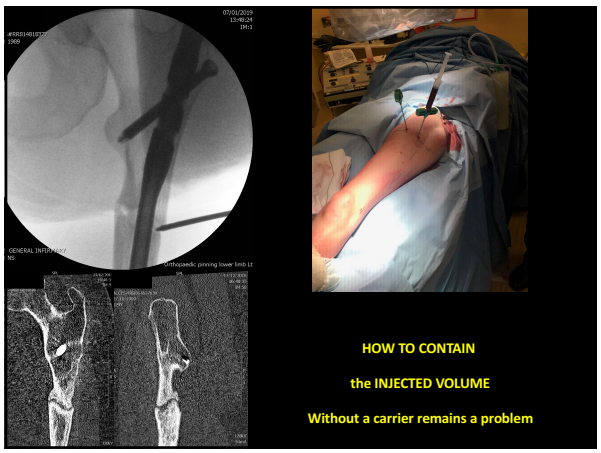
2) WHAT IS THE MINIMUM NUMBER I NEED TO IMPLANT?

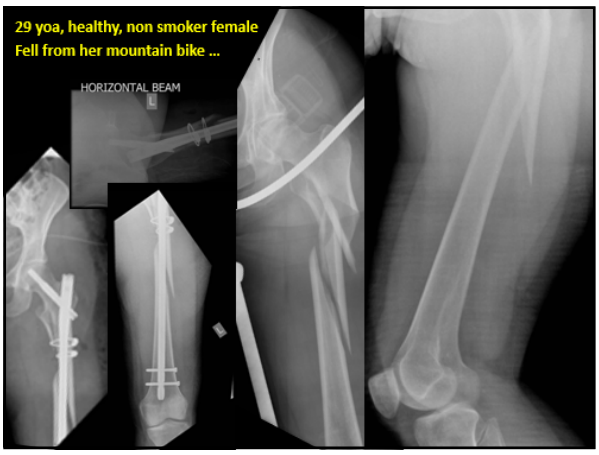
3) **HOW TO CONTAIN THEM?**

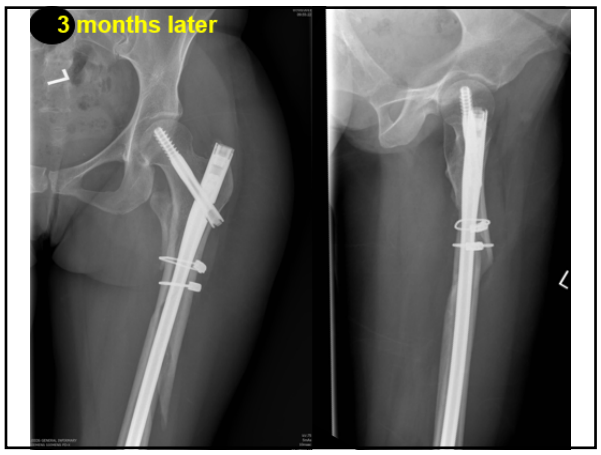

51 yoa
Female
fall
8 MONTHS
FOLLOW UP
Still in pain

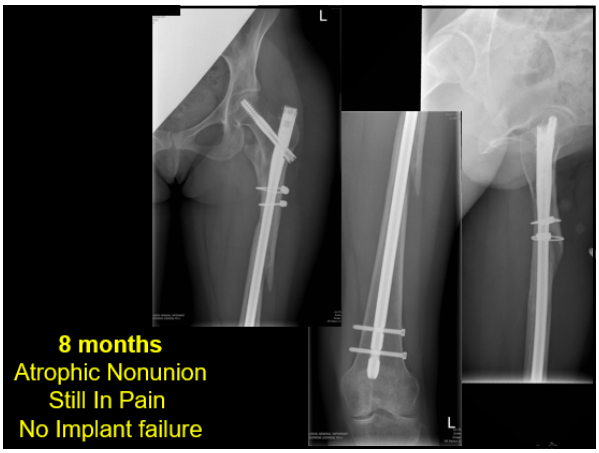


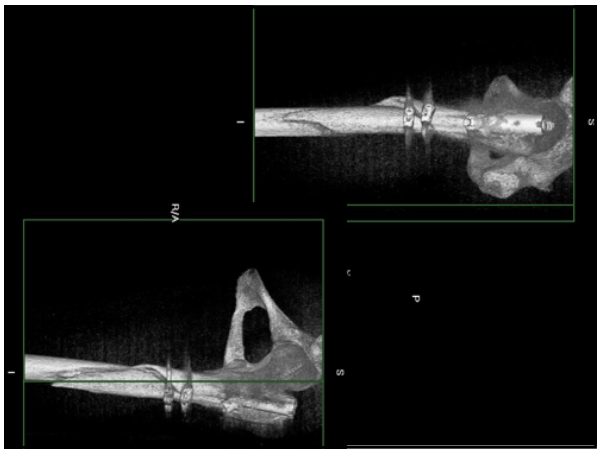

UNION 3/12 LATER

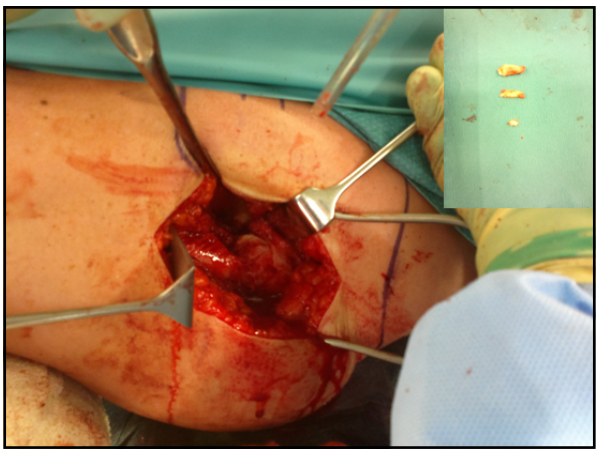

HOW TO CONTAIN
the INJECTED VOLUME
Without a carrier remains a problem

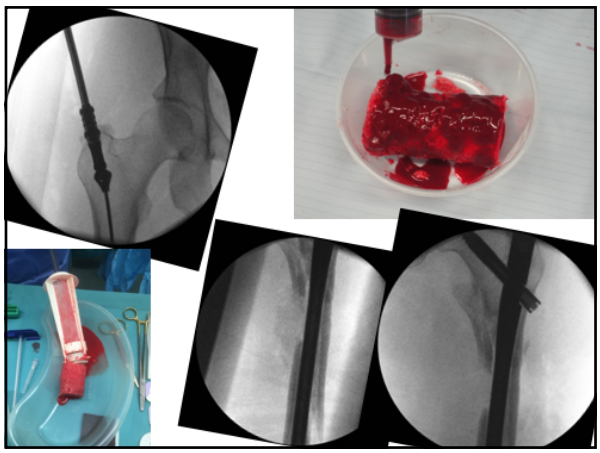

29 yoa, healthy, non smoker female
Fell from her mountain bike ...
HORIZONTAL BEAM

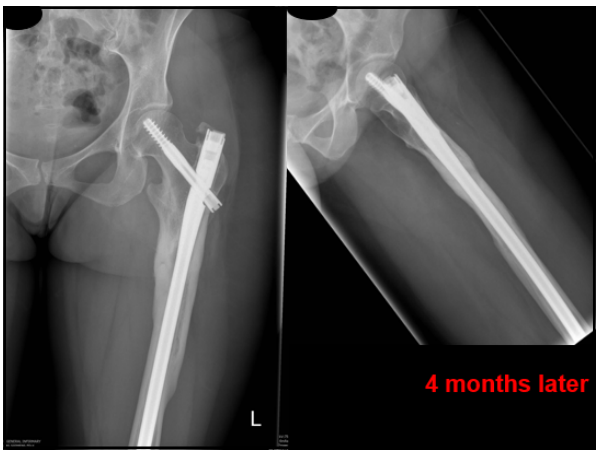

3 months later

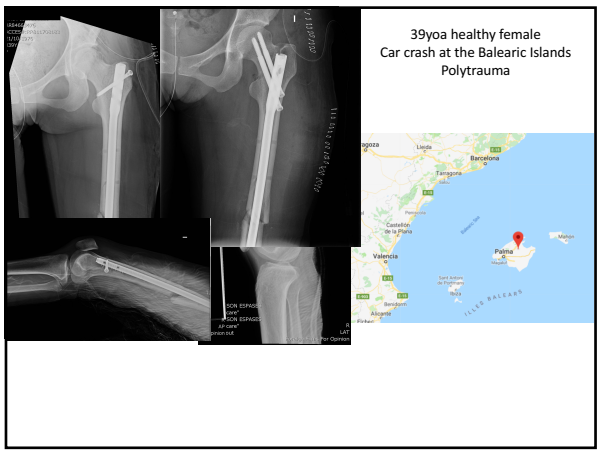

8 months
Atrophic Nonunion
Still In Pain
No Implant failure





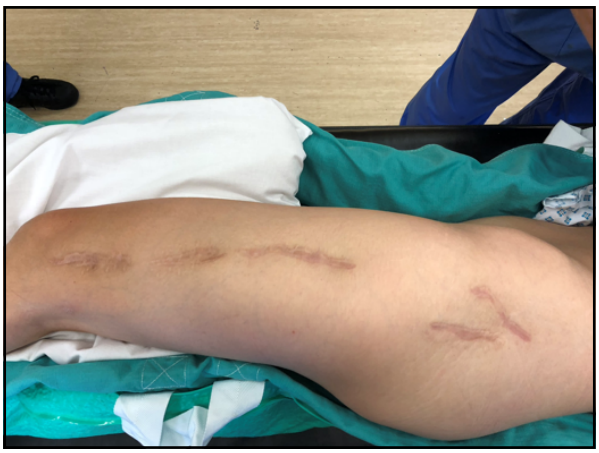


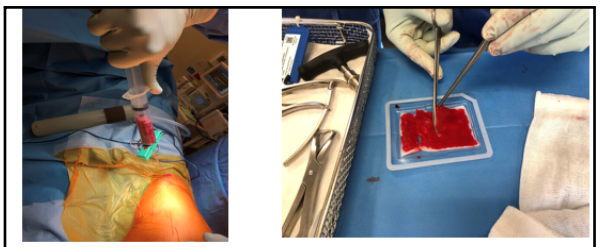

4 months later

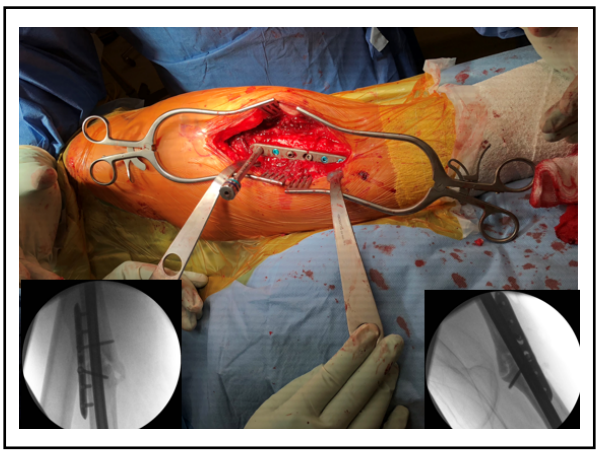

39yoa healthy female
Car crash at the Balearic Islands
Polytrauma

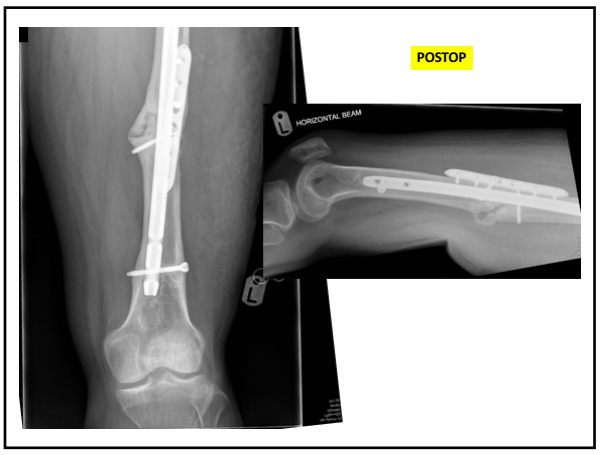

39yoa healthy female
Car crash at the Balearic Islands
Polytrauma – EXFX – IMN
7 months later in Leeds clinics
In PAIN – No Infection
External Rotation of 29 degrees

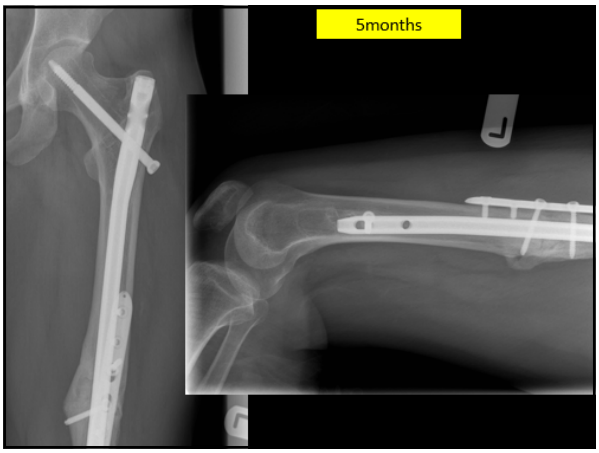

12 months later
Alignment improved
No evidence of infection
still in PAIN
4 surgeries
Open approaches x2




BMAC
Collagen sponge - BMP2




POSTOP
HORIZONTAL BEAM



What the future holds…

- Biological stimulation: autologous PRP, BMPs
- Bioactive scaffolds / gene modified matrices / implantable devices
- MSCs quantity and quality
- MSCs response to biomechanical stimulation

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THANK YOU

Biological stimulation of MSCs *in vivo:* PRP quality

UNIVERSITY OF LEEDS

Optimising Proliferation and Migration of Mesenchymal Stem Cells Using Platelet Products: A Rational Approach to Bone Regeneration

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ABSTRACT: This study investigates how mesenchymal stem cell's (MSCs) proliferation and migration abilities are influenced by various platelet products (PP). Donor-matched, clinical-, and control laboratory-standard PPs were generated and assessed based on their platelet and leukocyte concentrations. Bone marrow derived MSCs were exposed to these PP to quantify their effect on in vitro MSC proliferation and migration. An adapted colony forming unit fibroblast (CFU-F) assay was carried out on bone marrow aspirate using clinical-standard PP-loaded electrospun poly(ε-caprolactone) (PCL) membrane to mimic future clinical applications to contain bone defects. Clinical-standard PP had lower platelet (2.5 fold, $p < 0.0001$) and higher leukocyte (14.1 fold, $p < 0.0001$) concentrations compared to laboratory-standard PP. It induced suboptimal MSC proliferation compared to laboratory-standard PP and fetal calf serum (FCS). All PP induced significantly more MSC migration than FCS up to 24 h. The removal of leukocytes from PP had no effect on MSC proliferation or migration. The PP-loaded membranes successfully supported MSC colony formation. This study indicates that platelet concentrations in PP impact MSC proliferation more than the presence of leukocytes, whilst MSC migration in response to PP is not influenced by platelet or leukocyte numbers. Clinical-standard PP could be applied alongside manufactured membranes in the future treatment of bone reconstruction. © 2019 The Authors. *Journal of Orthopaedic Research*® Published by Wiley Periodicals, Inc. on behalf of Orthopaedic Research Society. J Orthop Res

Table 1 The effectiveness of bone marrow (BM) injections for nonunions

First author/year	Number of nonunions	Type	Mean age (y), *n* (range)	United, *n* (%)	Time to union (months), *n* (range)	Comments
Healey/1990 [45]	8	Femur 8	27 (6–60)	5 (62.5)	4.95 (1–9)	Nonunion occurred after reconstruction for lower limb cancer resection 50 ml percutaneous injection
Connolly/1991 [18]	10	Tibia 10	30 (18–82)	8 (80)	6.7 (5–10)	100–150 ml of BM aspirated and injected Fractures were immobilised by cast
Garg/1993 [32]	20	Tibia 15 Humerus 3 Ulna 2	35 (18–65)	17 (85)	5 (3–7)	Percutaneous injection of 15–20 ml of BM
Sim/1993 [102]	11	Tibia 8 Femur 1 Humerus 1 Ulna 1	38 (19–62)	9 (81)	2.5 (1–5.75)	Percutaneous injection of 50–200 ml of BM
Garg/1995 [33]	Case report	Tibia	12	1 (100)	18	~20 ml of BM were aspirated and injected in pseudoarthrosis while limb was placed in cast After 3 weeks the procedure was repeated
Matsuda/1998 [69]	7	Femur	53 (24–70)	4 (57)	na (5–9)	Percutaneous technique with injection of 150 ml of BM Two infections encountered resulting in failed union
		Humerus 12 Forearm 10				4–6 weeks after initial injection
Wang/2001 [113]	56	Tibia	na (19–72)	53 (94)	na	BM aspirated and injected percutaneously at nonunion site The procedure was repeated every month 2–3 times
Wilkins/2003 [115]	69	Tibia 36 Femur 16 Humerus 4 Others 13	42 (15–81)	61 (88)	8.1 (2–36)	Percutaneous technique with BM combined with allograft demineralised bone matrix
Goel/2005 [38]	20	Tibia 20	37.5 (24–60)	15 (75)	3.5 (1.5–5.5)	A maximum of 15 ml of BM was aspirated and percutaneously injected
Hernigou/2005 [47]	60	Tibia 60	40 (18–78)	53 (88)	3 (1–4)	An average of 306±24 ml was aspirated, concentrated to 20 ml and percutaneously injected
Tetreault/2007 [107]	Case report	Clavicle	39	1 (100)	na (within 12)	Percutaneous injection of 10 ml of BM

OVERALL SUCCESS RATE 80%

Bone Marrow Aspirate Concentrate In Vivo Minimally Manipulated MSCs