

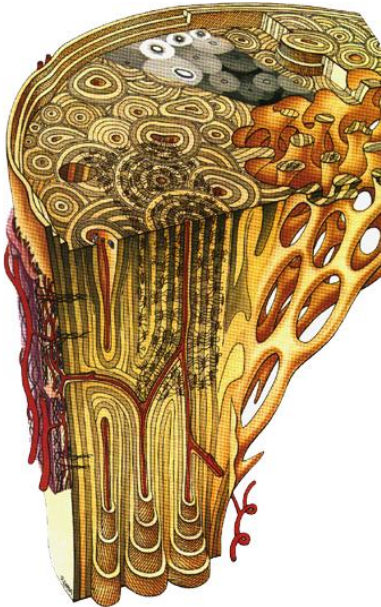
Bone regeneration using stem cells and biomaterials

Pierre Layrolle, PhD
Director of research

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Faculty of Medicine, University of Nantes, France

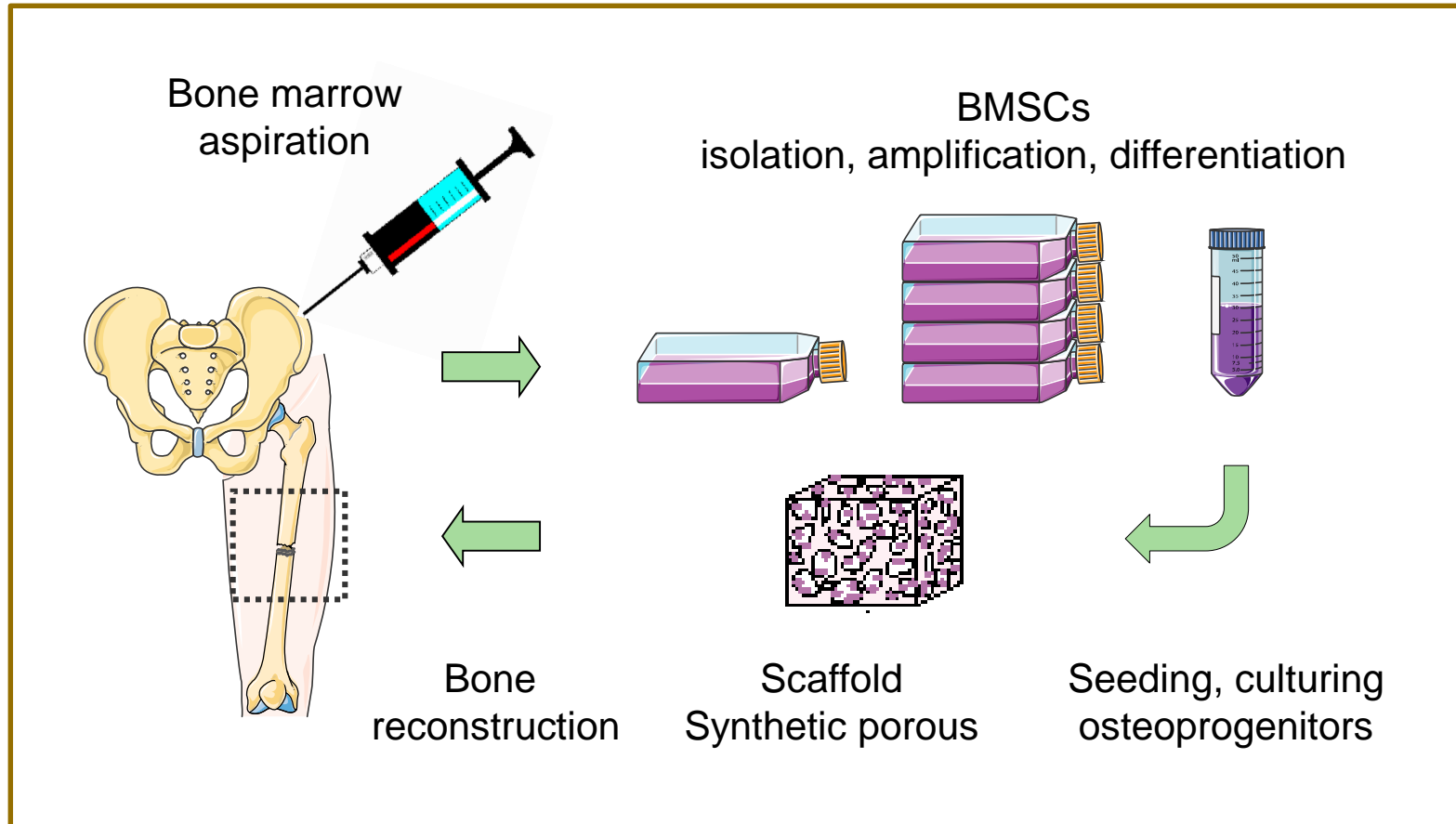
pierre.layrolle@inserm.fr

Introduction



- Bone is the most transplanted tissue (1 million procedures annually in Europe)
- Autografts is the gold standard but requires another surgery, limited bone stock and pain
- Synthetic biomaterials with mesenchymal stem cells may be an alternative

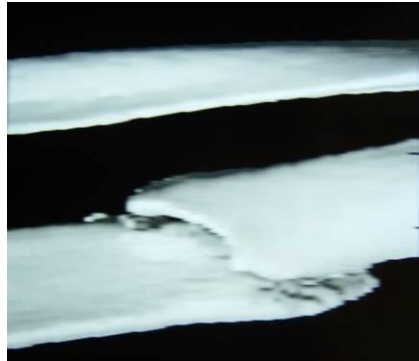
Bone tissue engineering



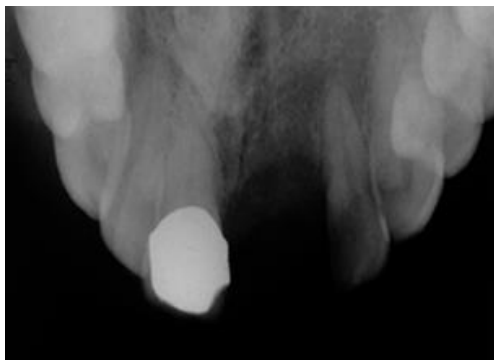
Cordonnier et al. Adv Funct Mater 2011

5 clinical trials

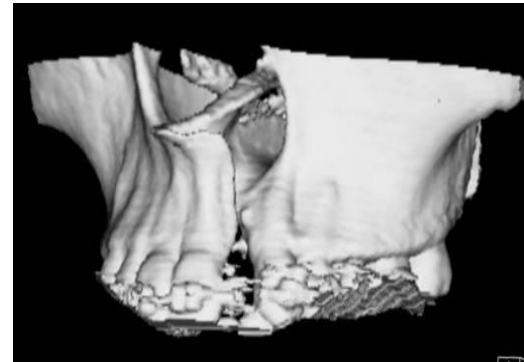
Long bone fractures



Osteonecrosis of the femoral head



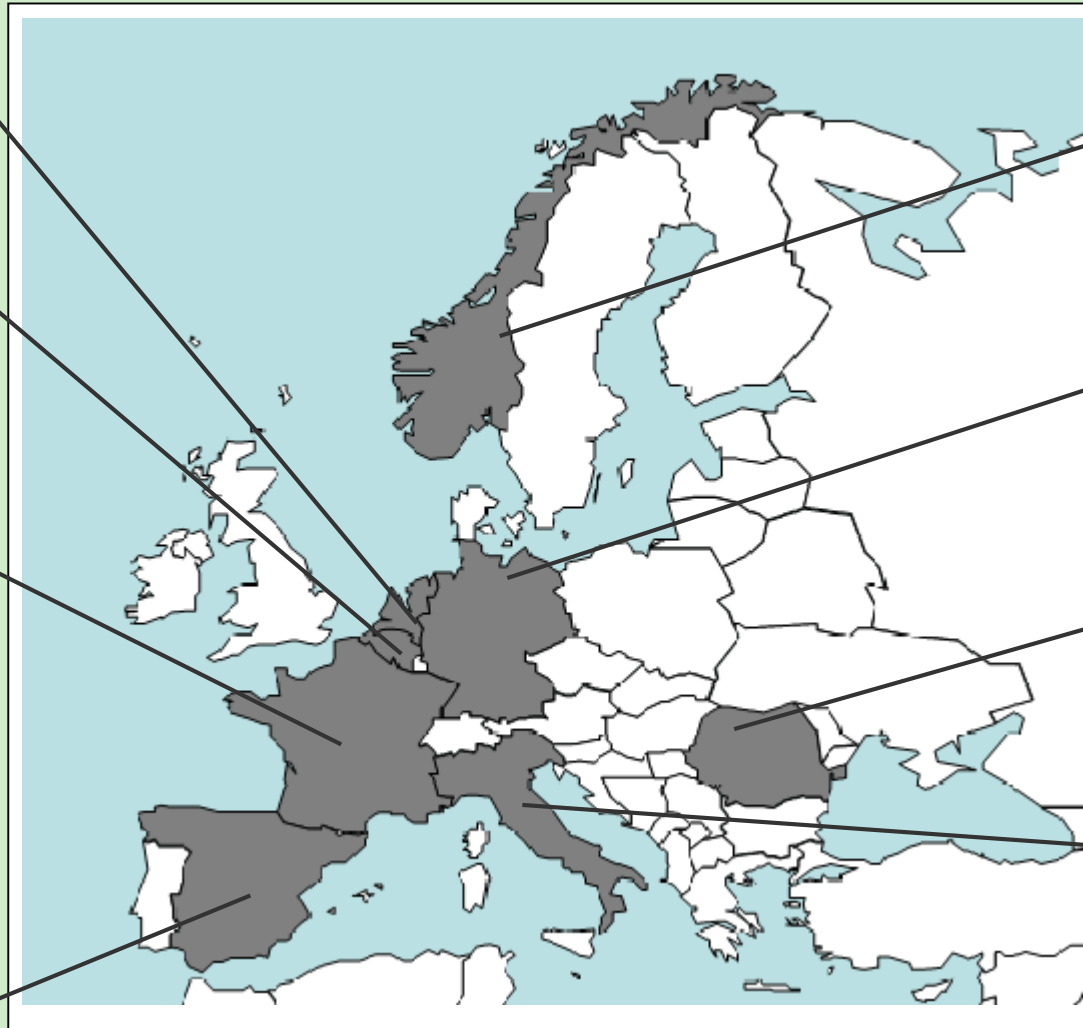
Maxillo-facial defects before implants



Cleft palates

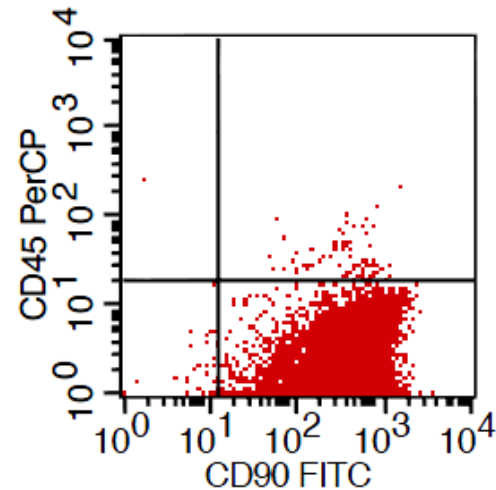
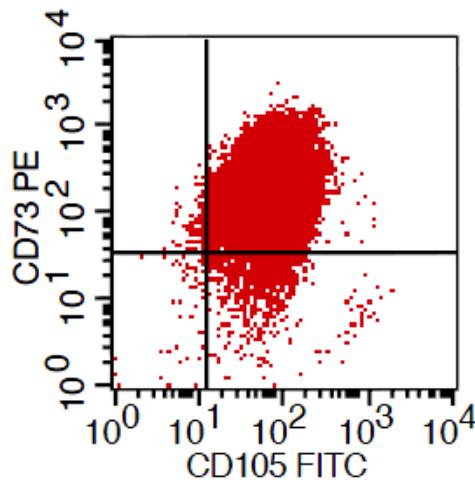
Consortium

Reborne



12 million €, 2010-2014

Human Mesenchymal Stem Cells

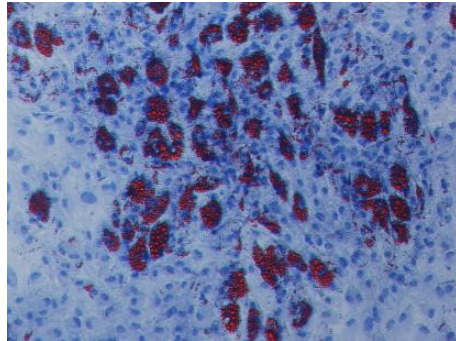
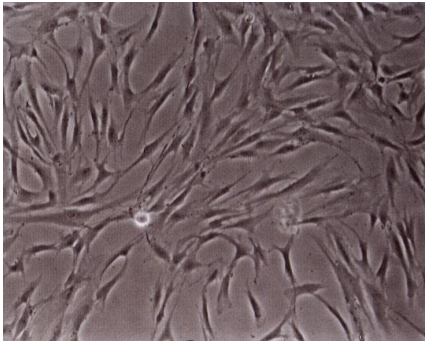


MSC

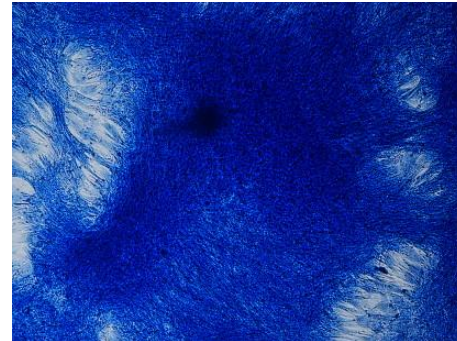
Adipocytes

Chondrocytes

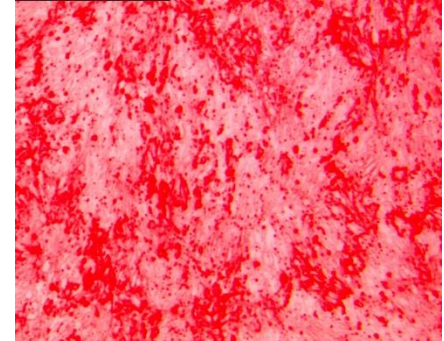
Osteoblastes



Red Oil



Blue Alcian

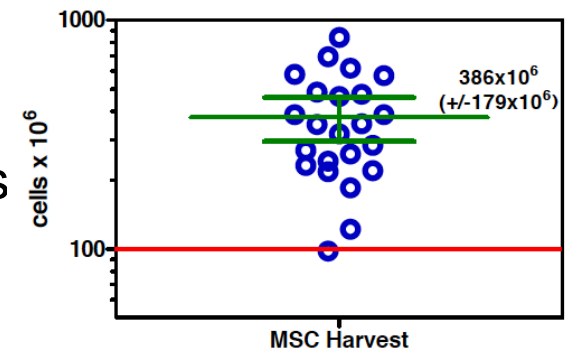


Red Alizarin

Delorme et al. Blood 2008

Platelet Lysate for culturing hMSC

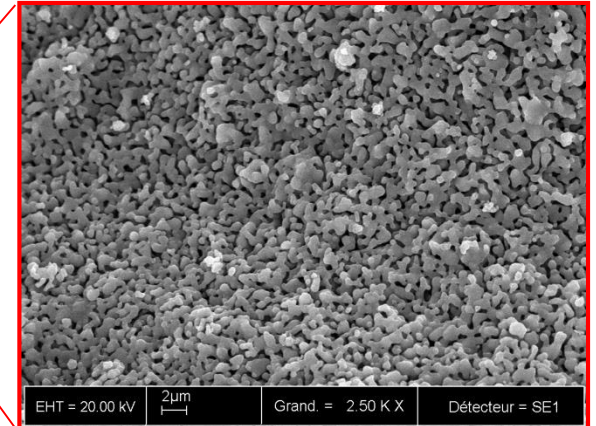
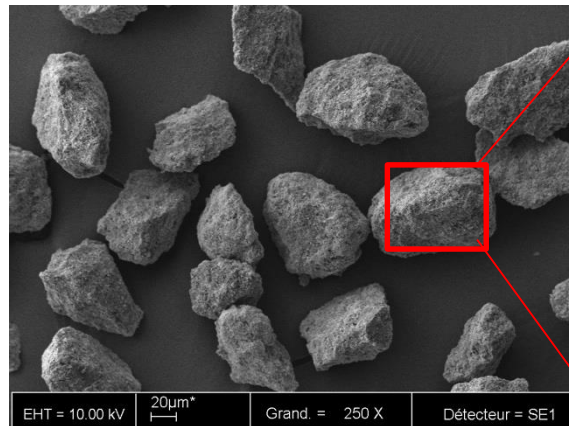
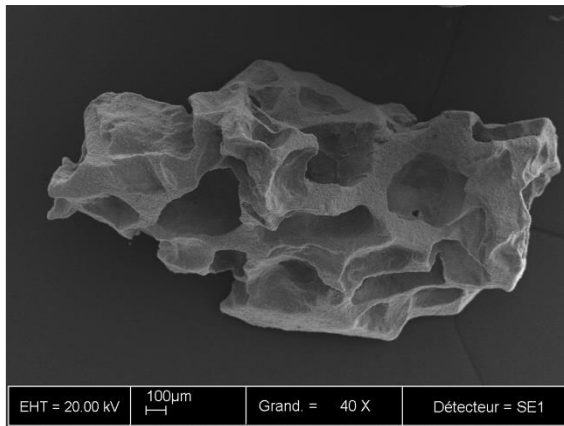
- Platelet Lysate from human blood
- Isolation and grow of hMSC in α MEM + 10% PL
- Xenobiotic free culture medium
- PL increased cell proliferation
- Approx. 400×10^6 hMSC produced in 21 days
- PL enhanced osteoblastic differentiation



Chevallier et al. Biomaterials 2010

Biomaterial

BCP = HA/ β -TCP 20/80

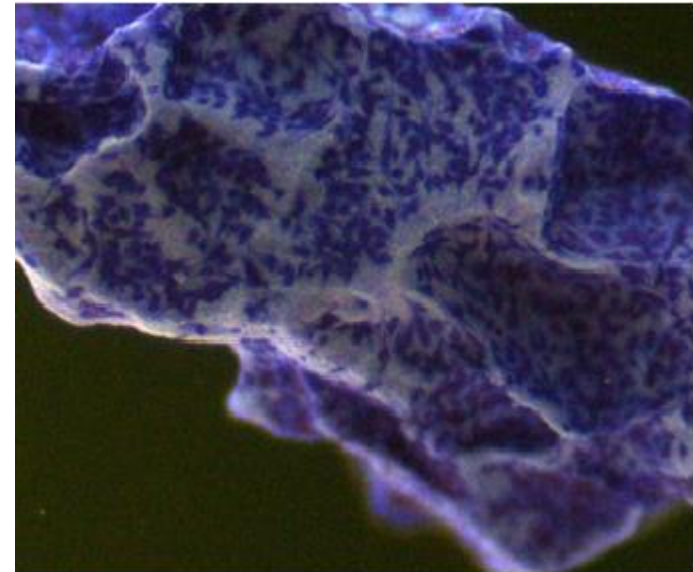
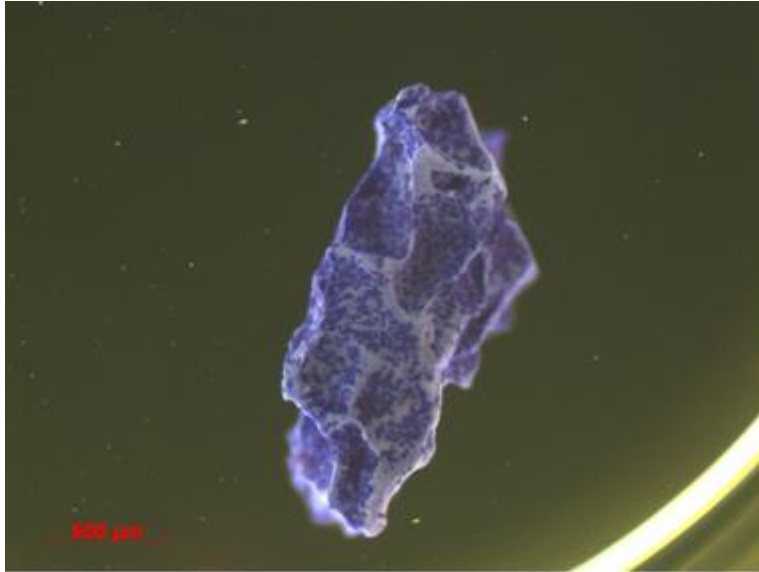


Manufactured by Biomatlante (CE and FDA approved)



Cell dose of hMSC on bioceramics

Methylene blue



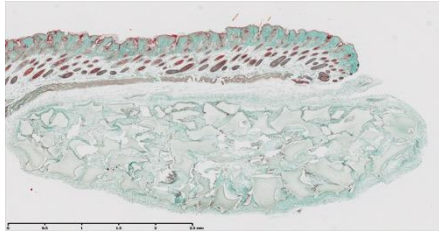
Subcutis implantation
in nude mice for 8 weeks



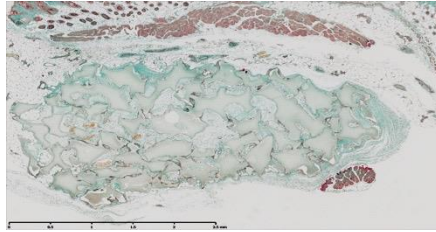
Histology

Histology @ 8 weeks

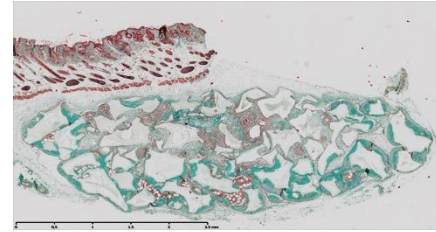
BCP



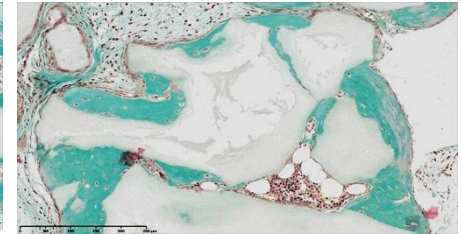
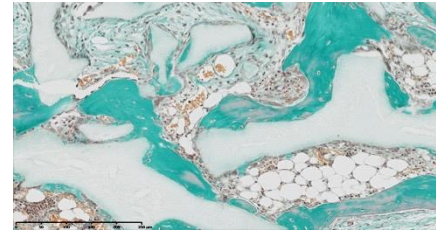
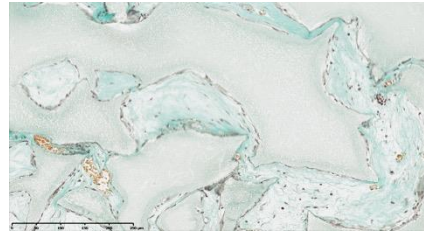
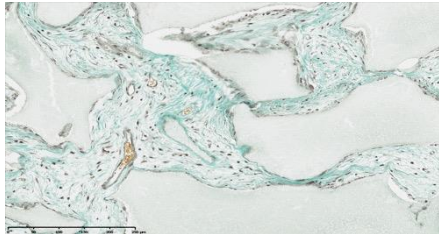
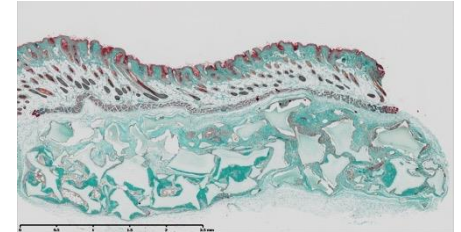
BCP + 0.1x10⁶ hMSC



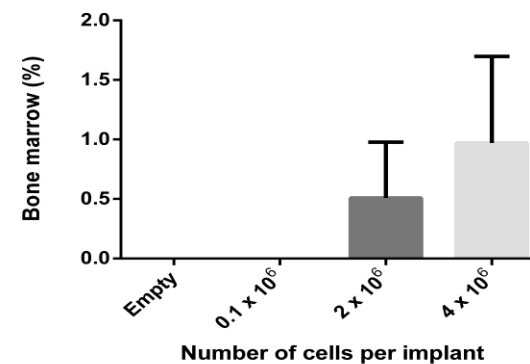
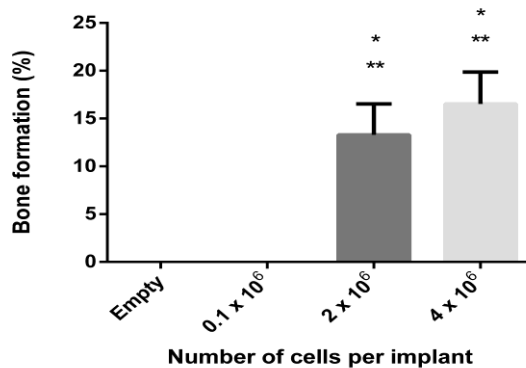
BCP + 2x10⁶ hMSC



BCP + 4x10⁶ hMSC



Masson trichrome staining



Production and transportation of hMSCs

$100 \cdot 10^6$ hMSC for 5 cm^3 of BCP
Cell viability: $75 \pm 15 \%$
12 donors

Inserm

Institut national
de la santé et de la recherche médicale

 **Deutsches
Rotes
Kreuz**

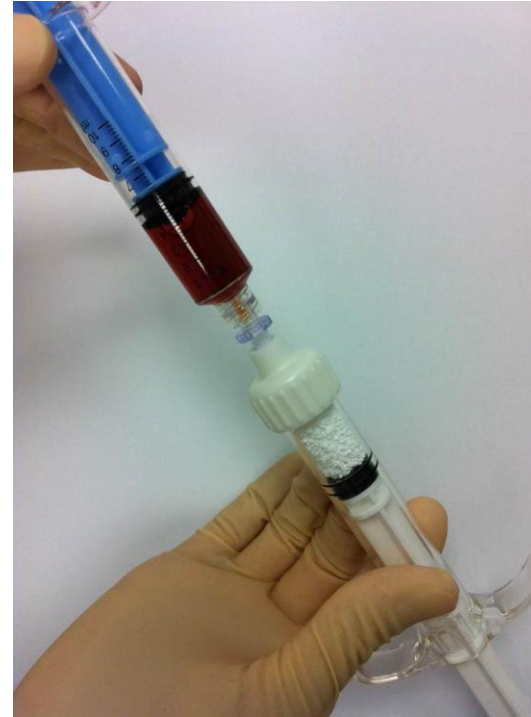
 ulm university universität
uulm



SOP for mixing cells and biomaterial



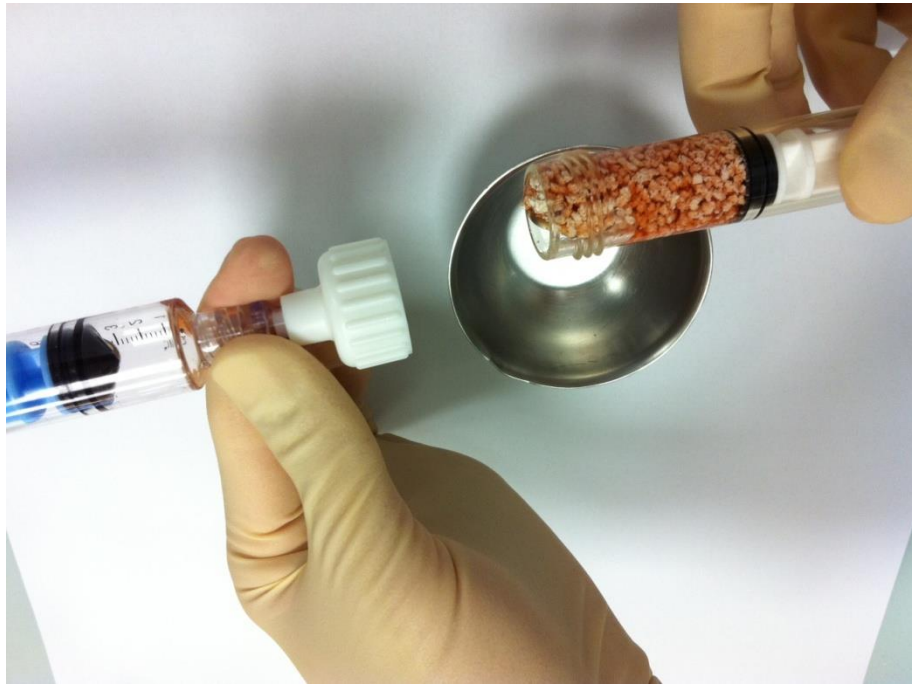
SOP for mixing cells and biomaterial



SOP for mixing cells and biomaterial

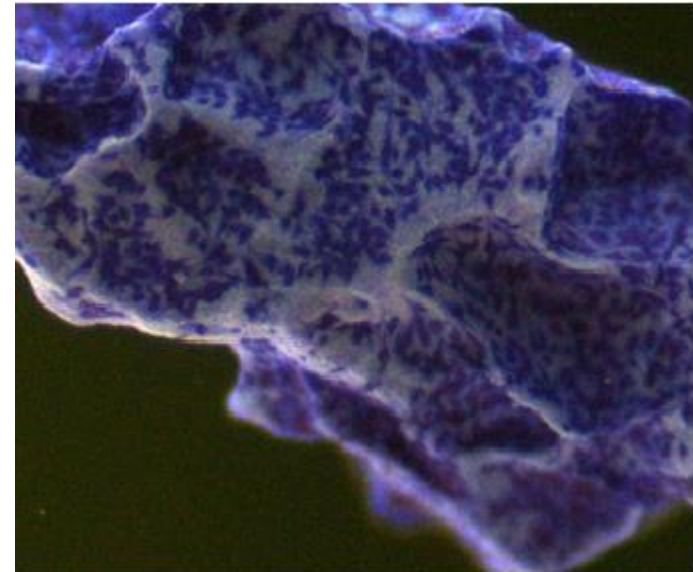
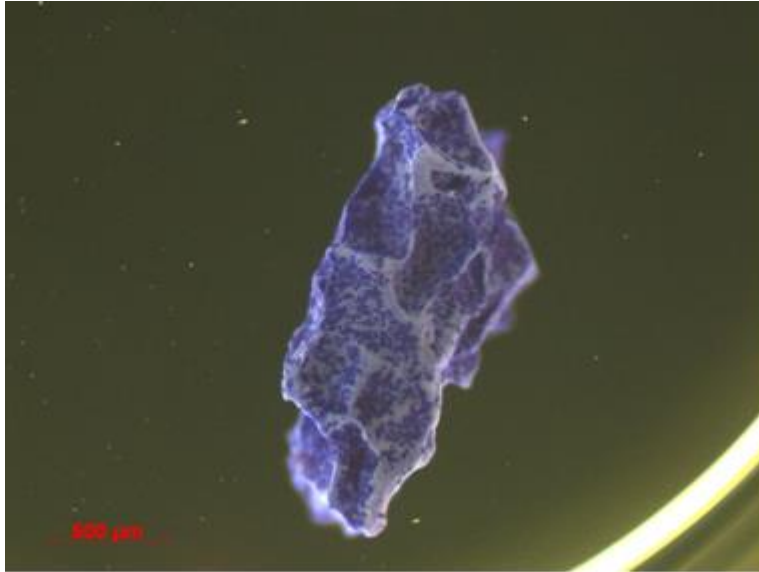


SOP for mixing cells and biomaterial



hMSC on bioceramics

Methylene blue

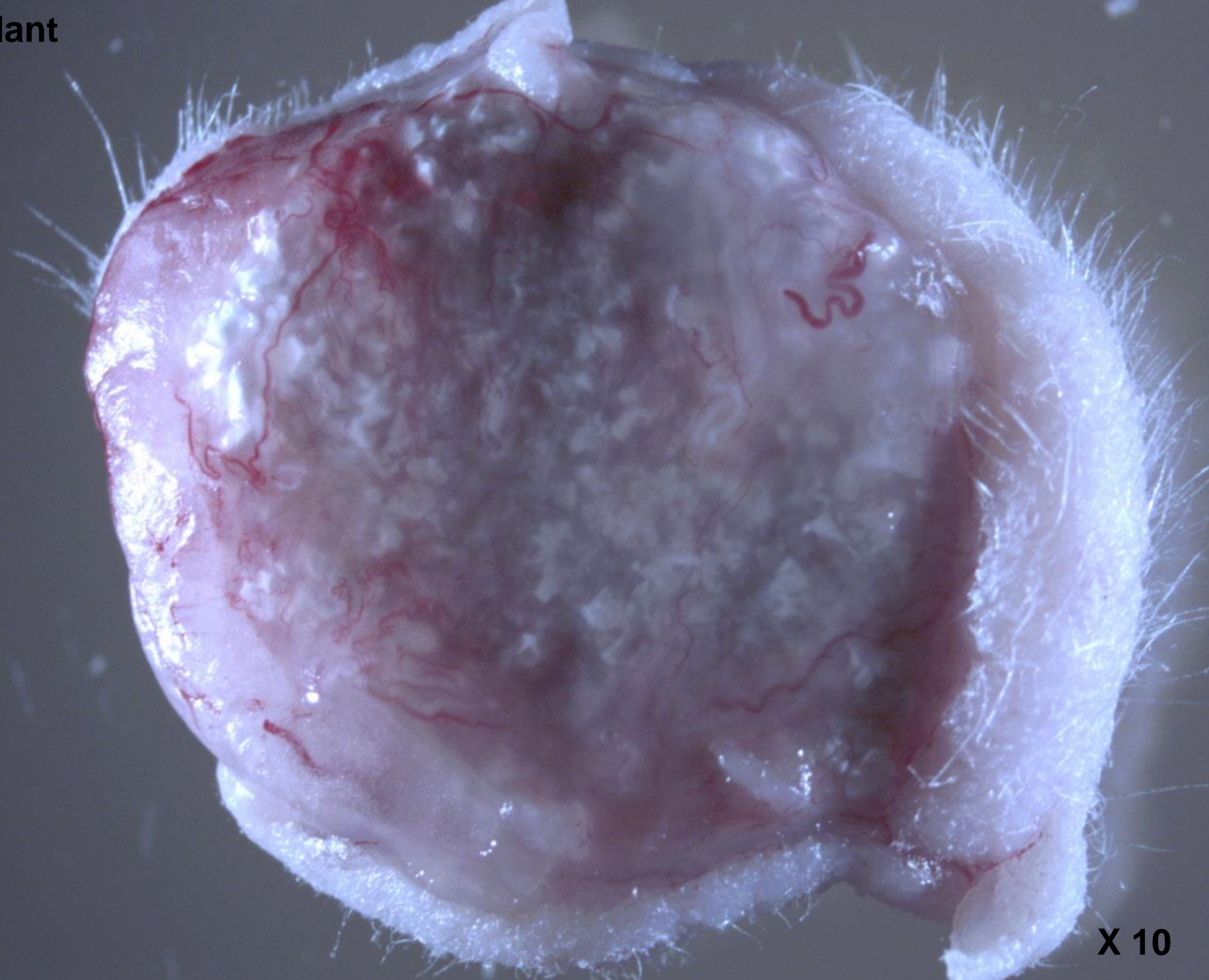


Subcutis implantation
in nude mice for 8 weeks



Histology

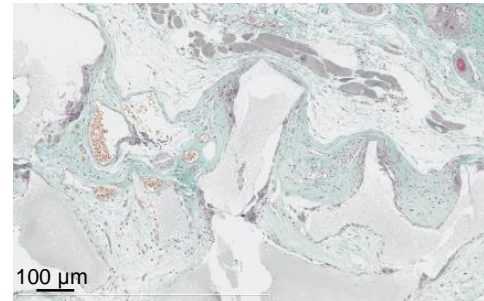
Explant



X 10

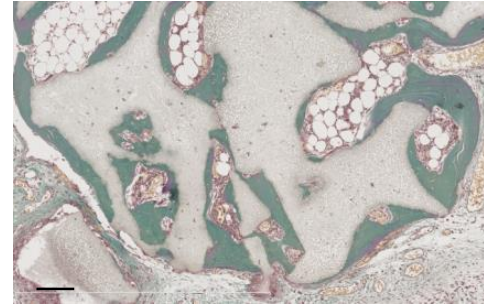
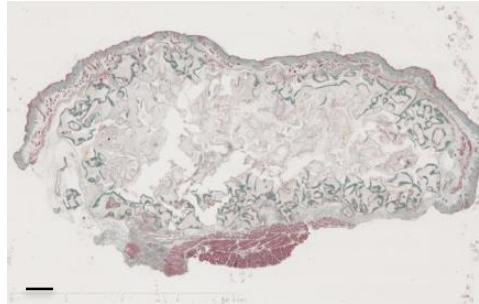
**Control
(BCP)**

16 weeks

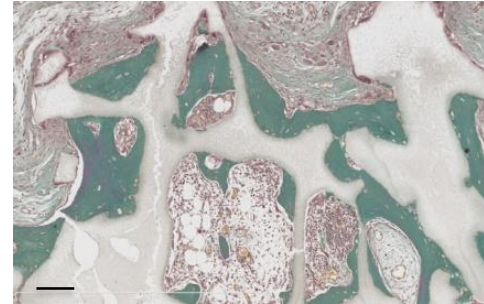


hMSC/BCP

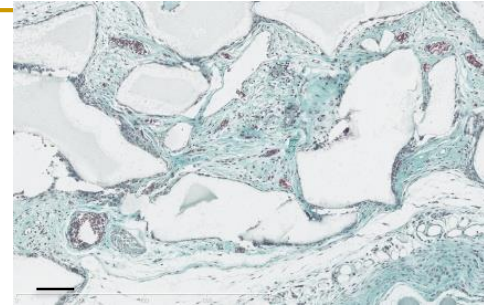
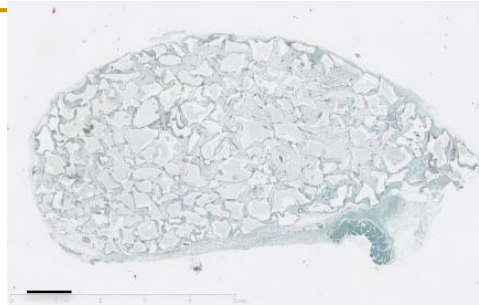
16 weeks



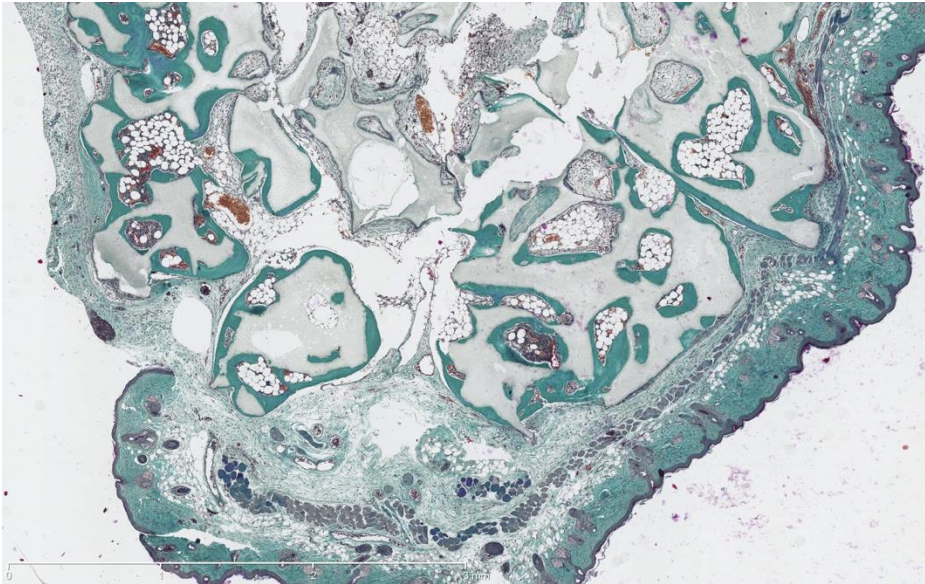
8 weeks



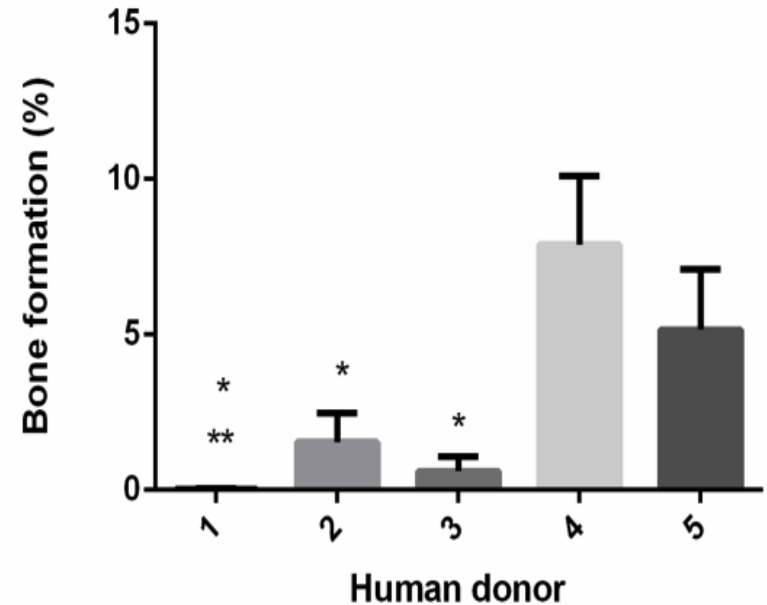
4 weeks



Histology

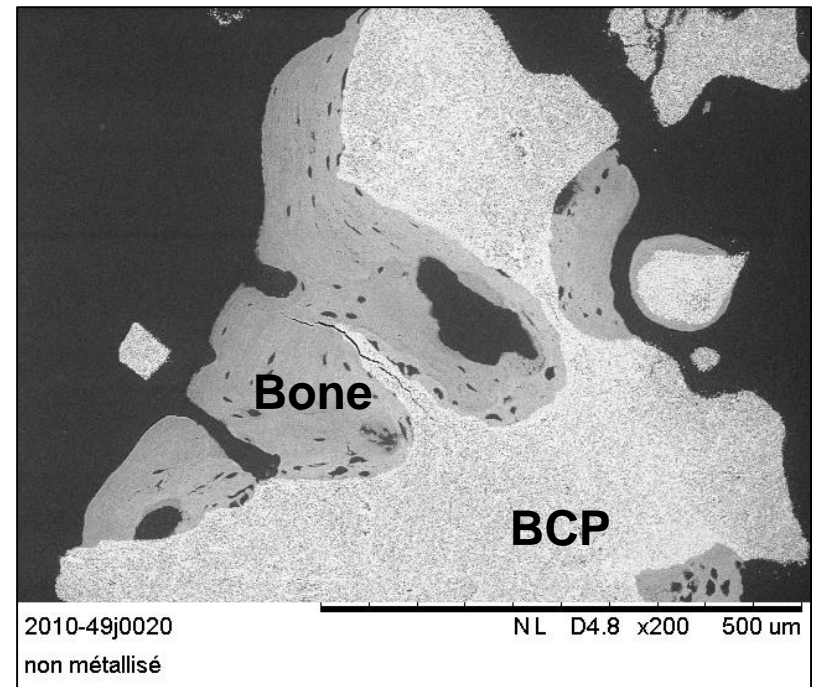
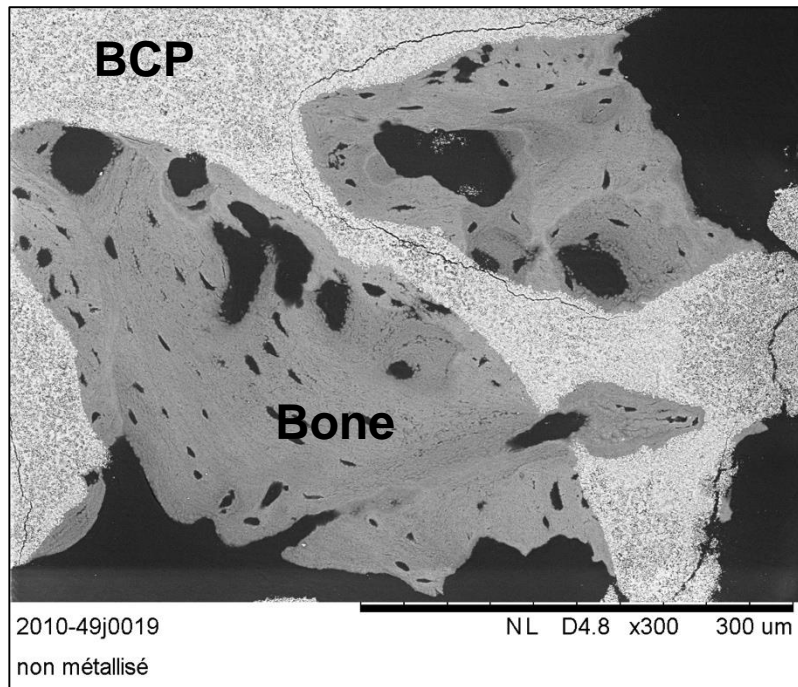


Goldner trichrome staining



Ectopic bone formation (6/6) x12 donors

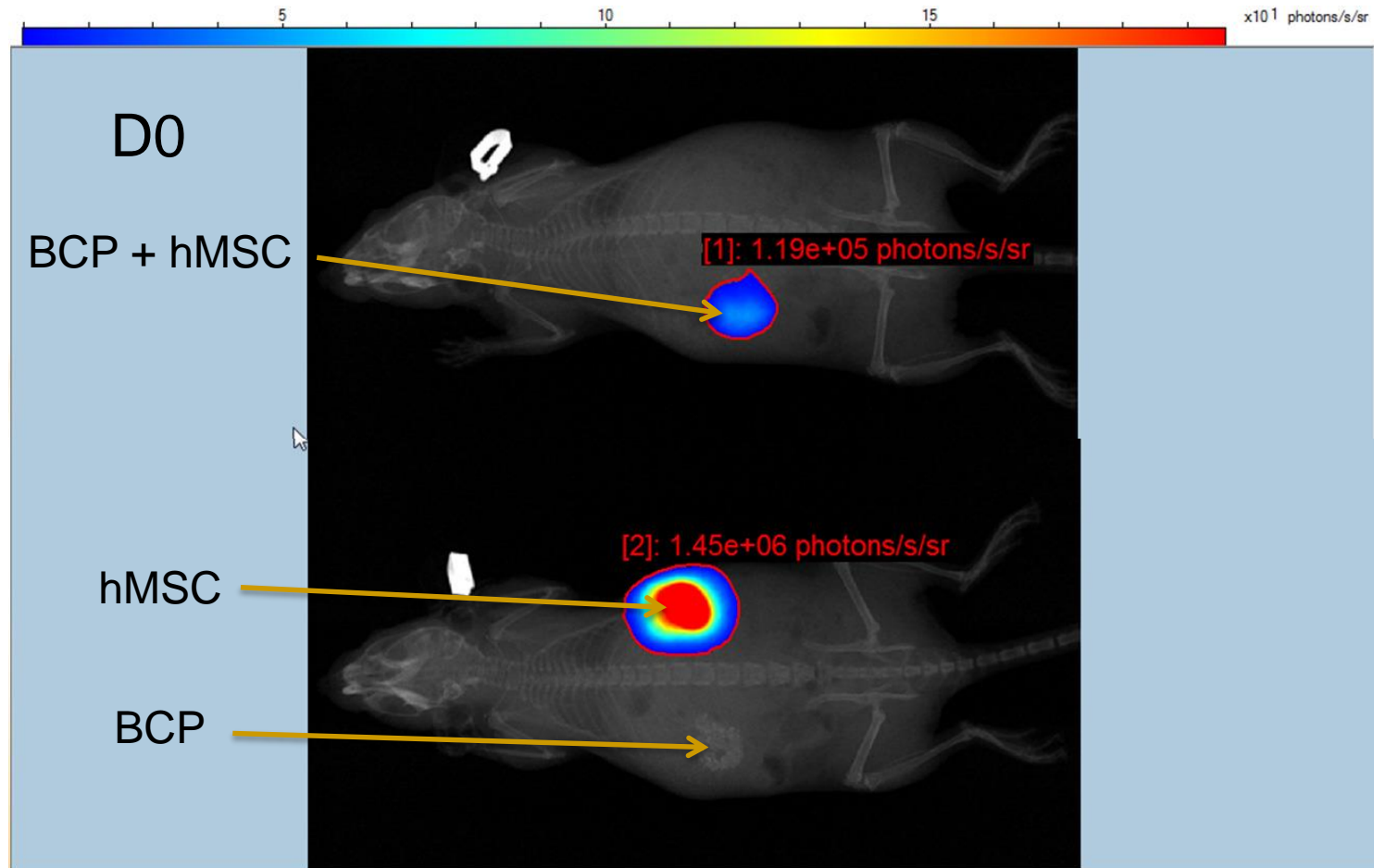
BSEM



Brennan et al. Stem Cell Res Ther, In revision

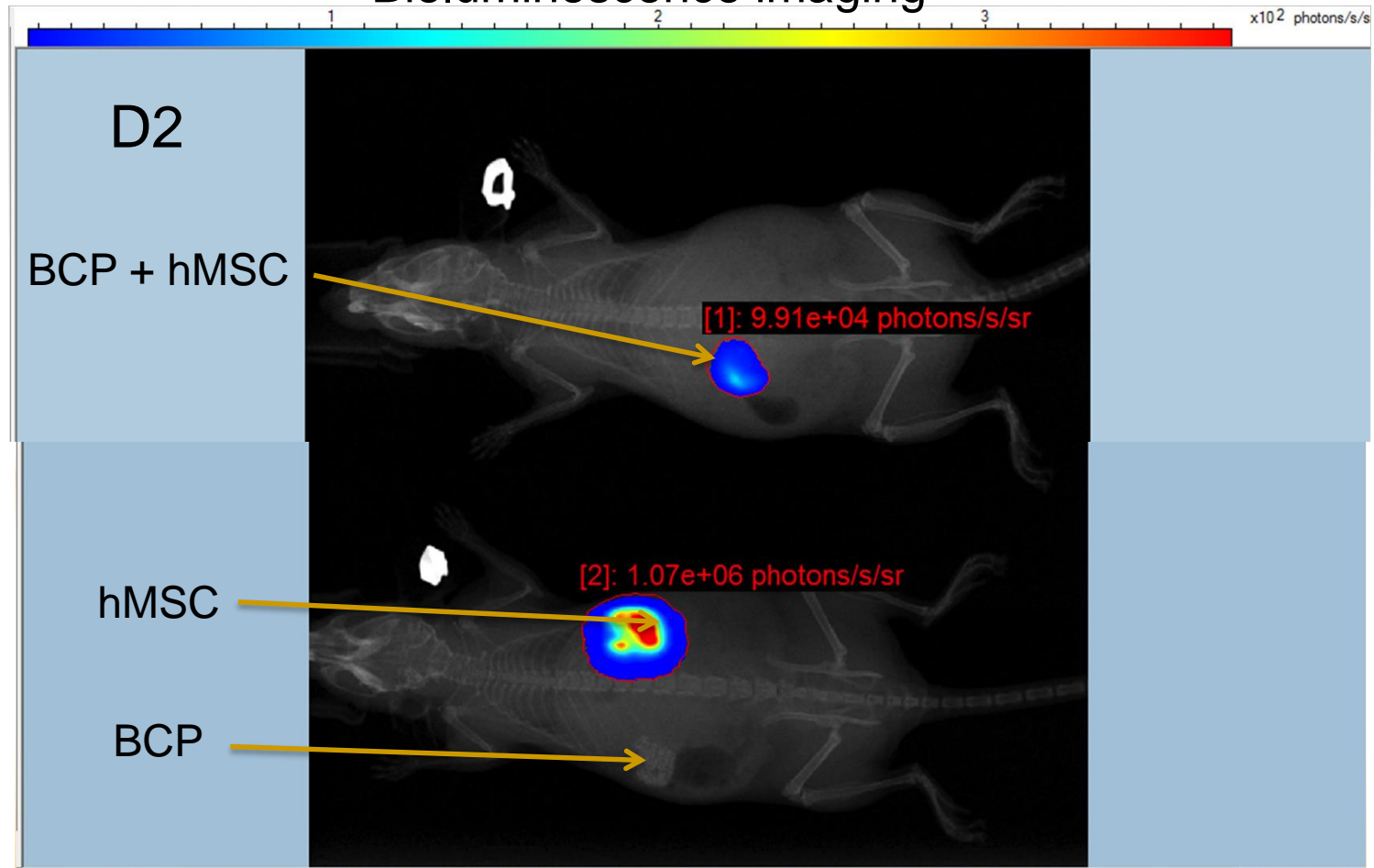
Viability of stem cells *in vivo*?

hMSC-EGFP-LucF
Bioluminescence imaging



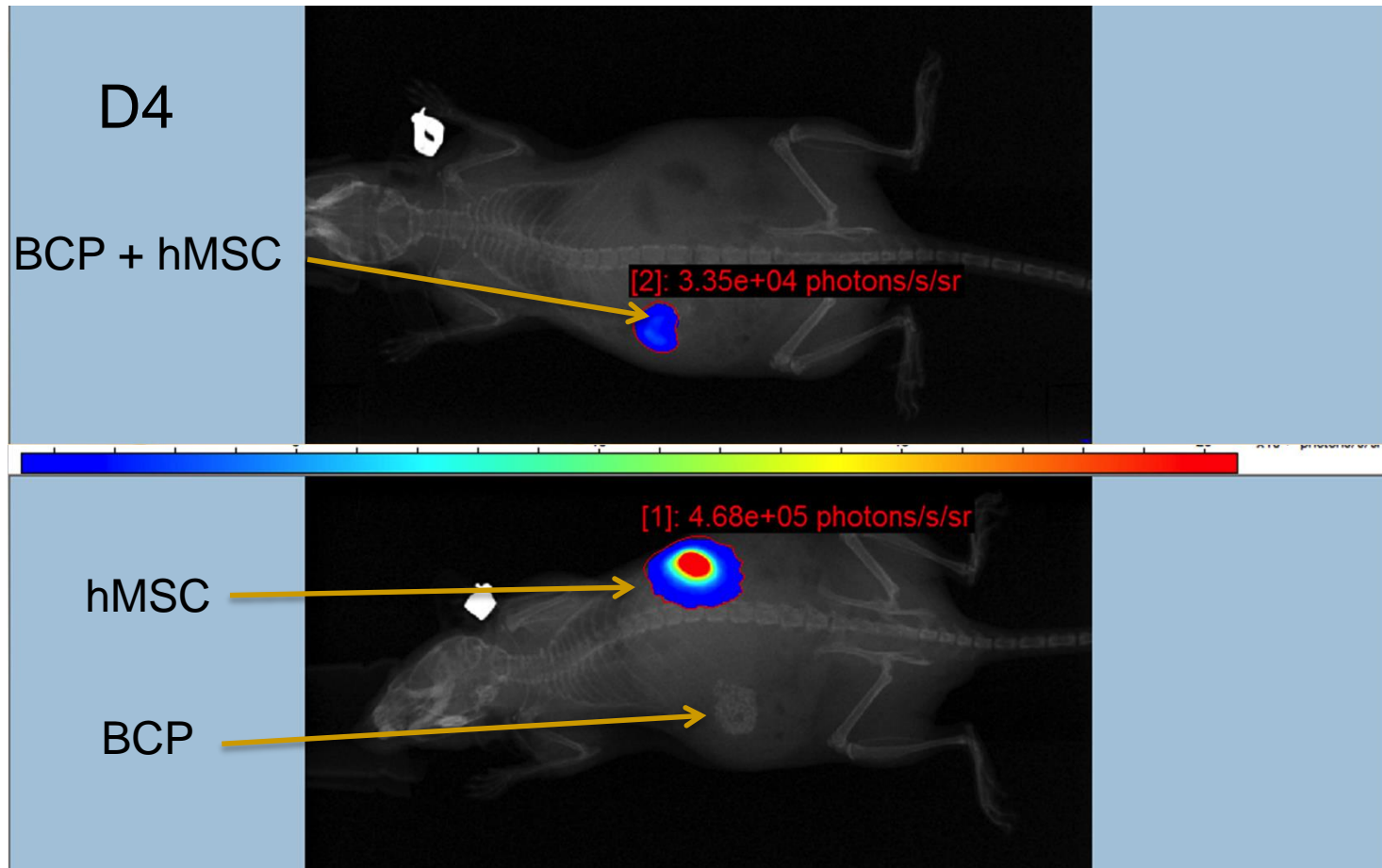
Viability of stem cells *in vivo*?

hMSC-EGFP-LucF
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Viability of stem cells *in vivo*?

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Bioluminescence imaging

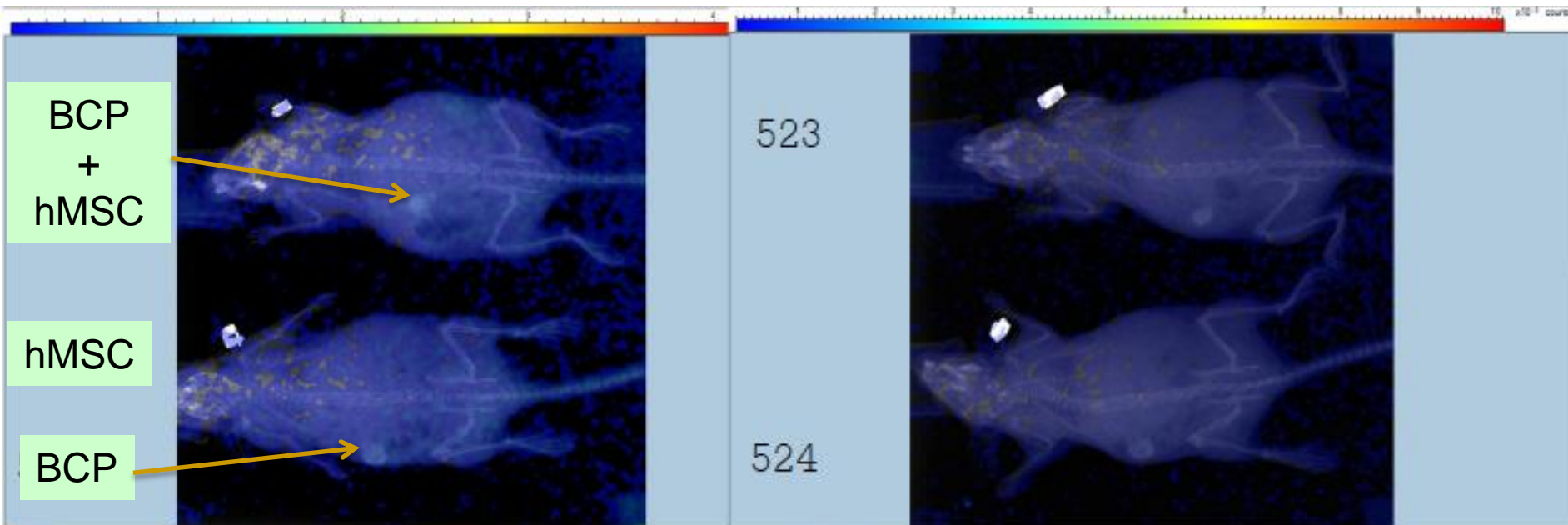


Viability of stem cells *in vivo*?

hMSC-EGFP-LucF
Bioluminescence

D7

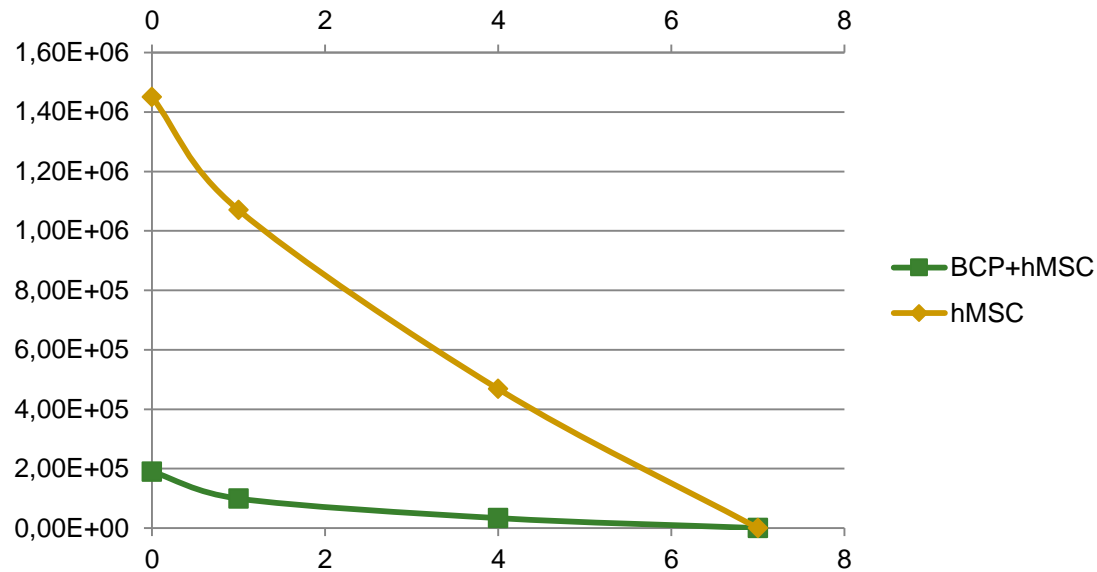
D11



Viable hMSC are not detected after 7 days

Viability of stem cells *in vivo*?

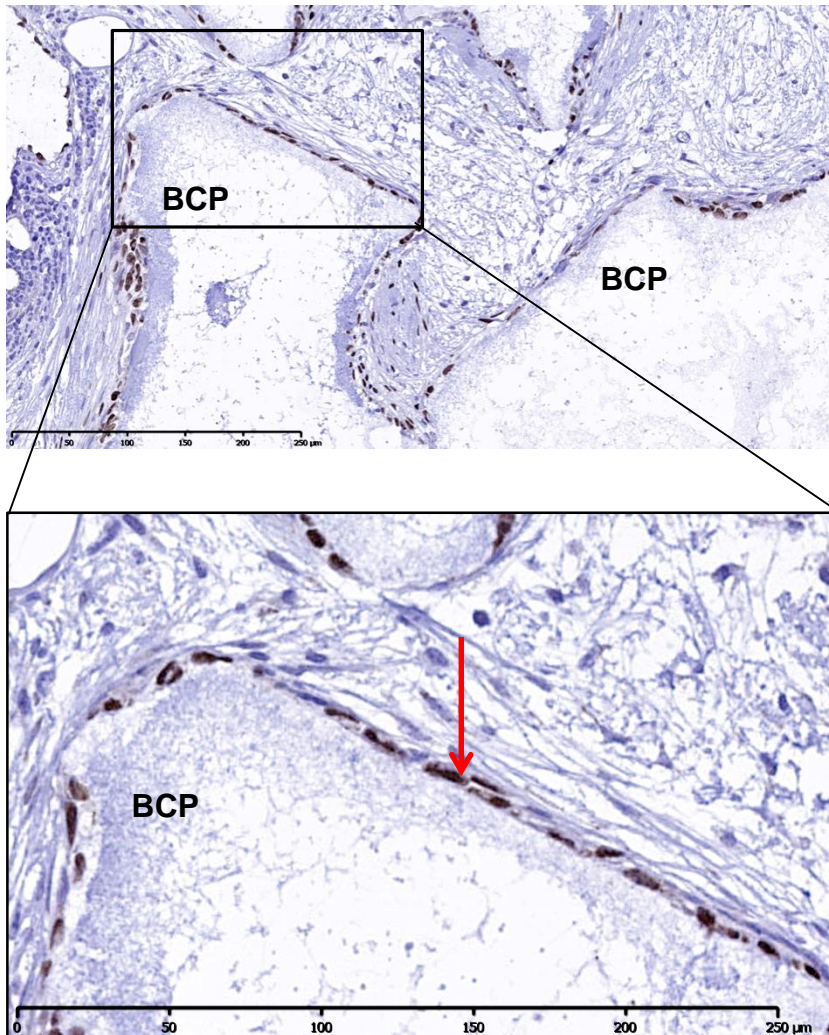
hMSC-EGFP-LucF
Bioluminescence



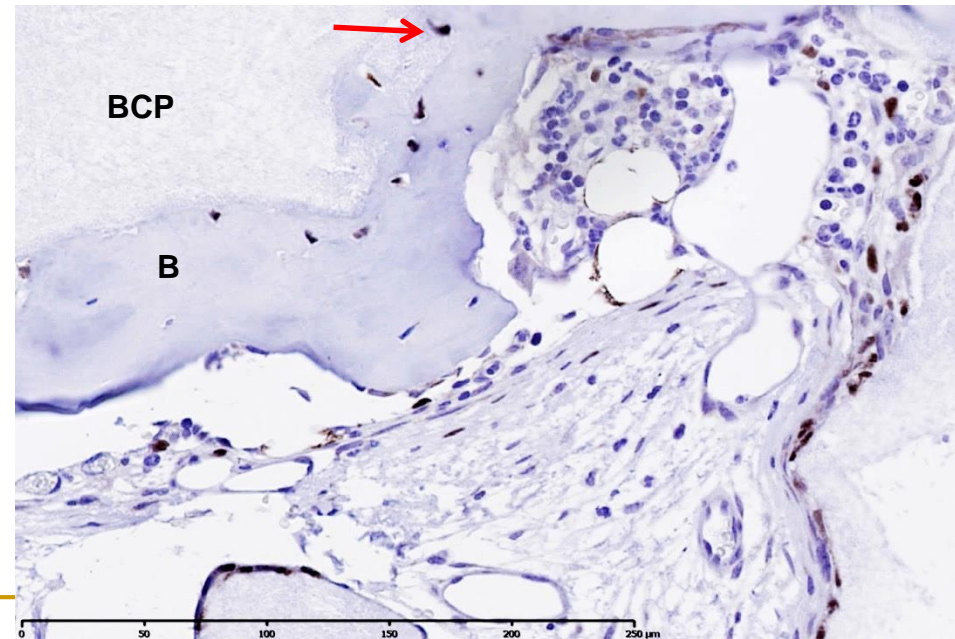
Viable hMSC are not detected after 7 days,
but bone tissue is formed at 8 weeks only in hMSC/BCP group
Is it a paracrine effect?

hMSC engraftment

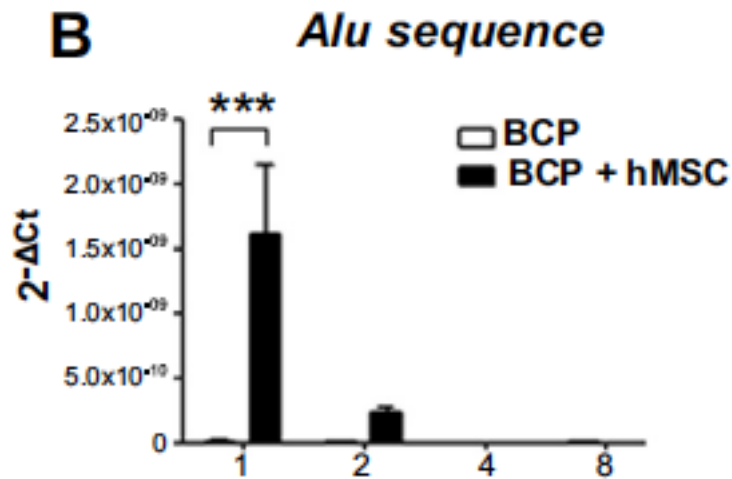
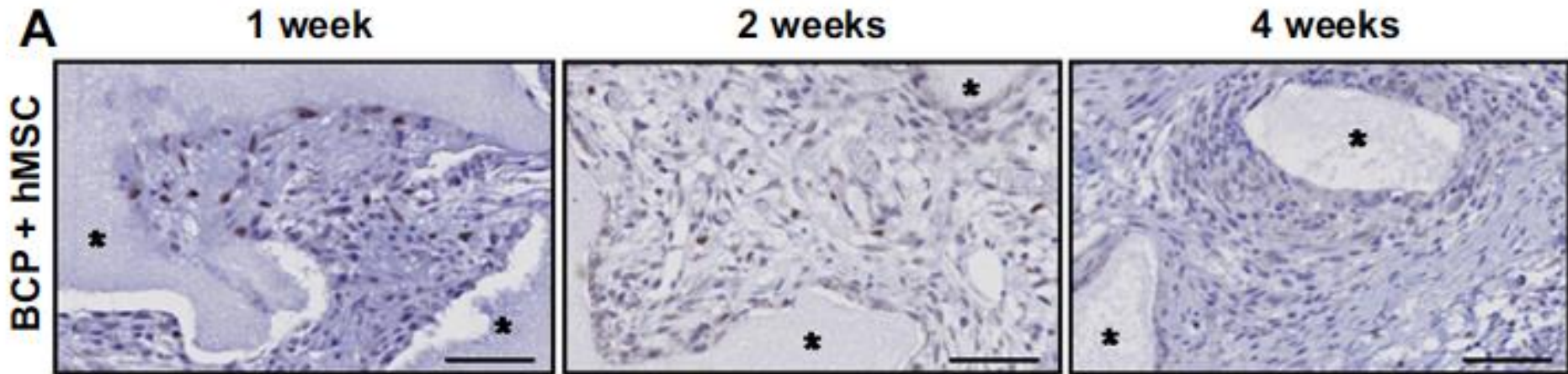
2×10^6 hMSC with 50mg BCP implanted subcutaneously for 8 weeks in nude mice



In situ hybridization using the human-specific repetitive *Alu* sequence for identification of human cells (brown – red arrow)

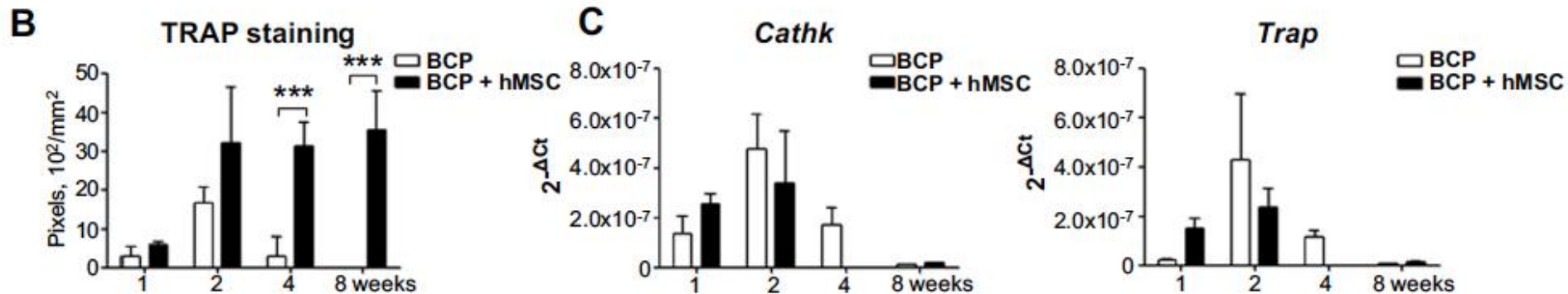
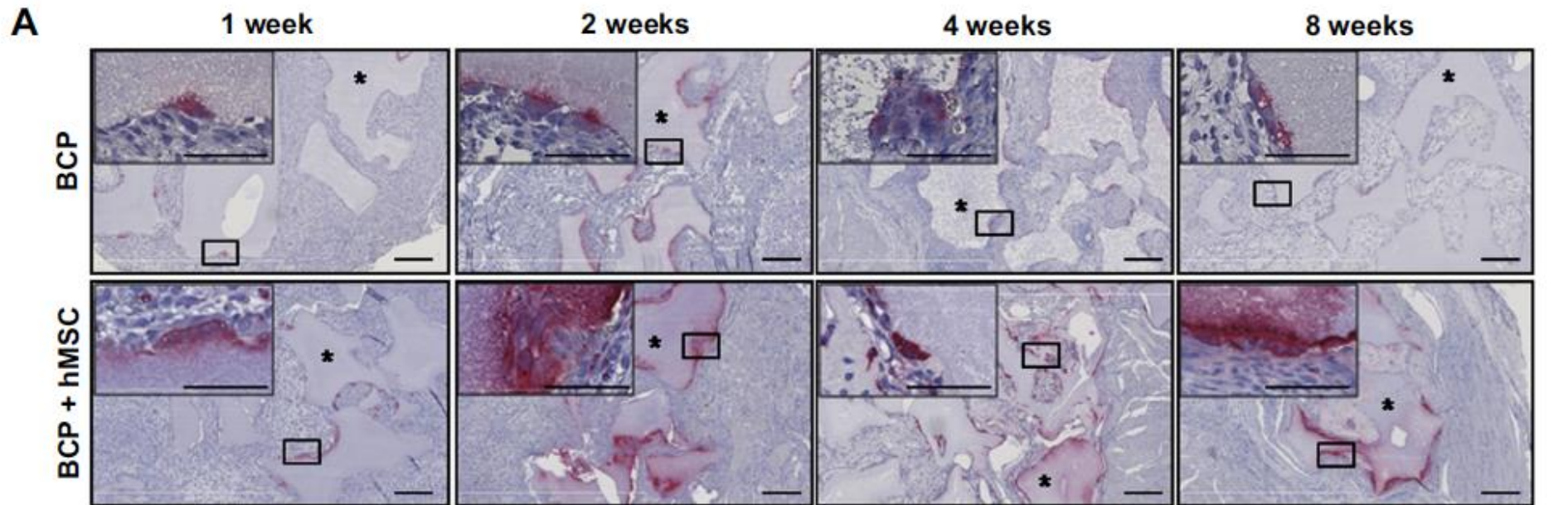


hMSC engraftment in muscle of nude mice

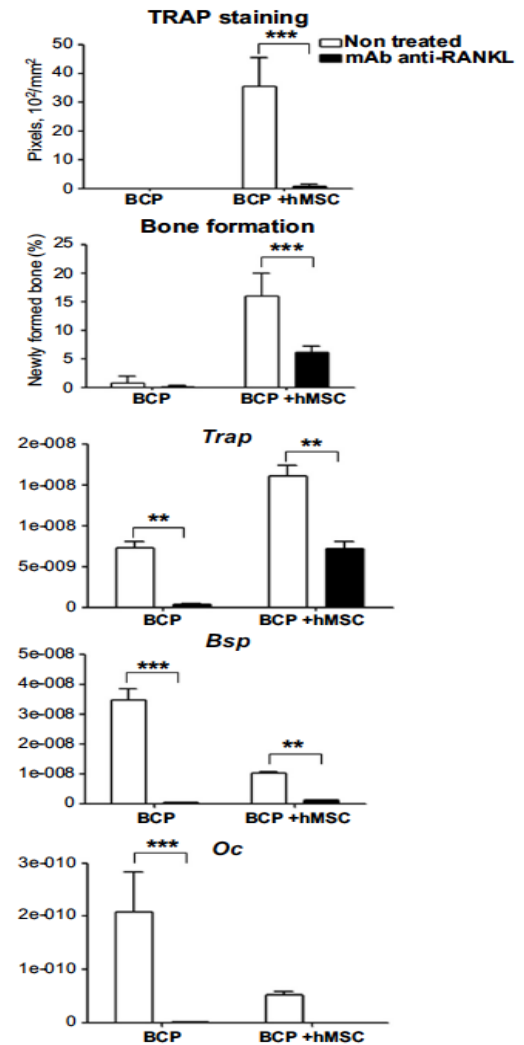
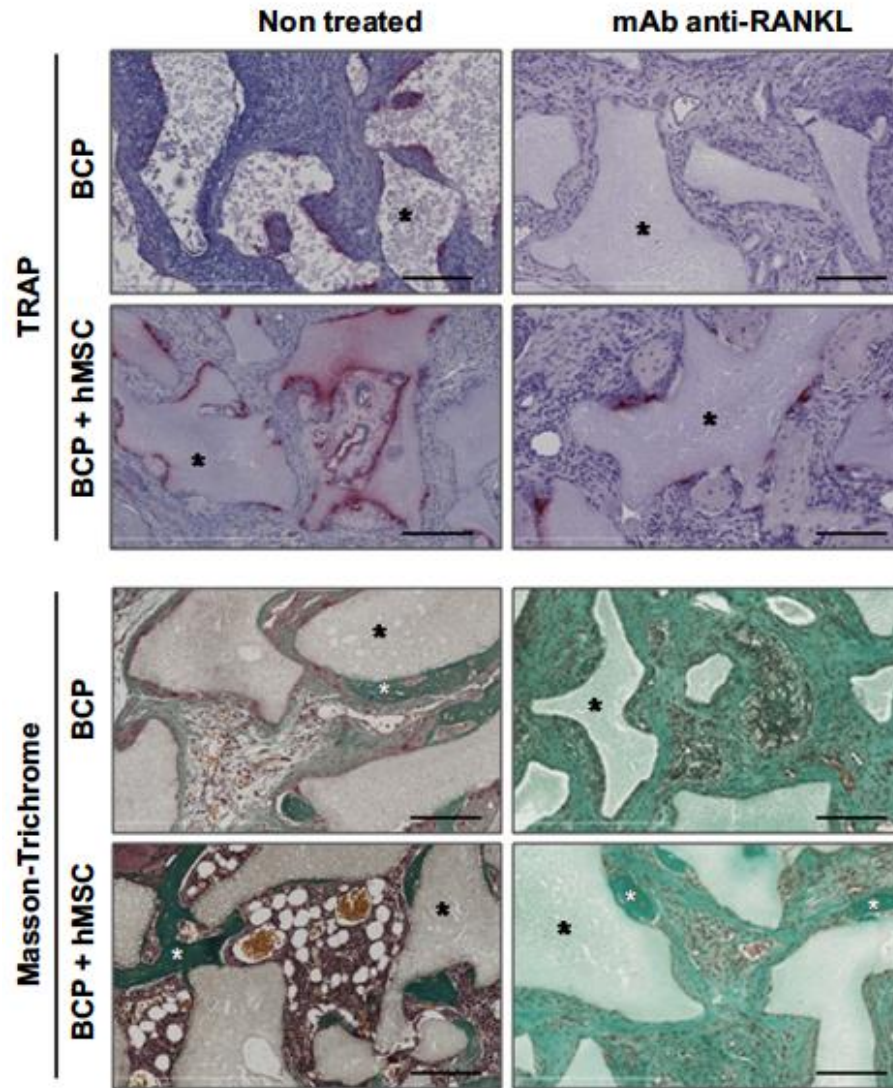


Gamblin et al. Biomaterials 2014

Osteoclastic differentiation with hMSC



Anti-RANKL treatment decreases bone



Source of stem cells: bone marrow or adipose tissue?

Background / hypothesis

- Adipose stem cells can mineralize *in vitro*, can they form bone *in vivo*?

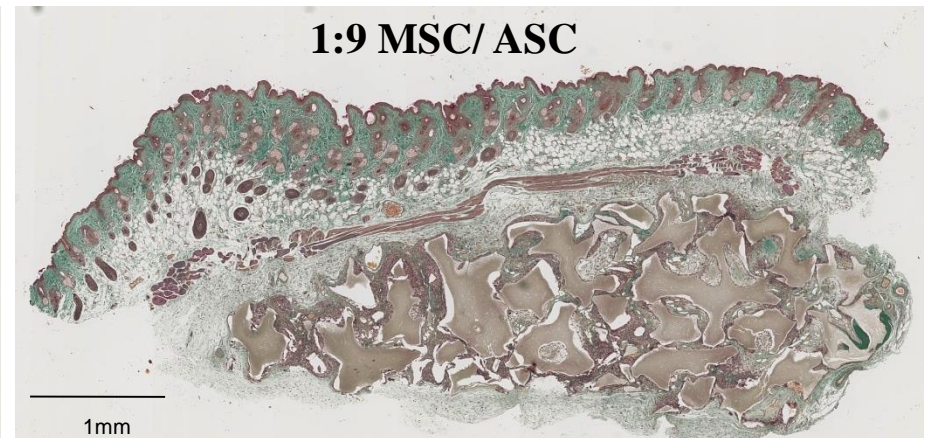
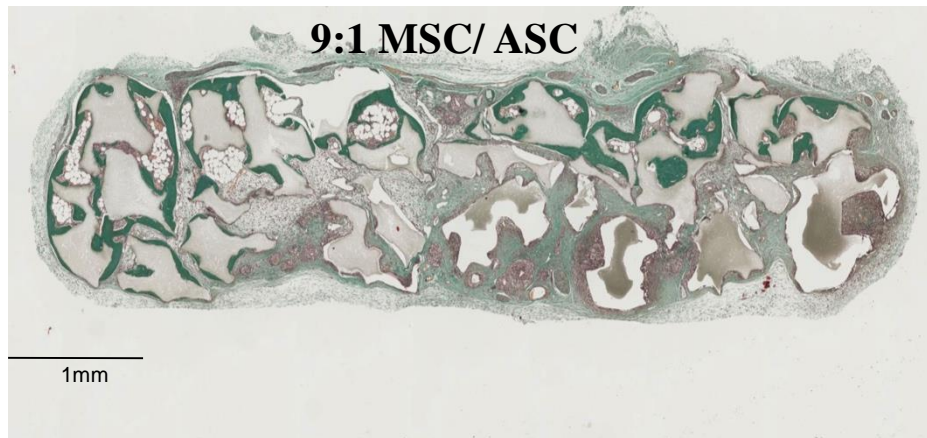
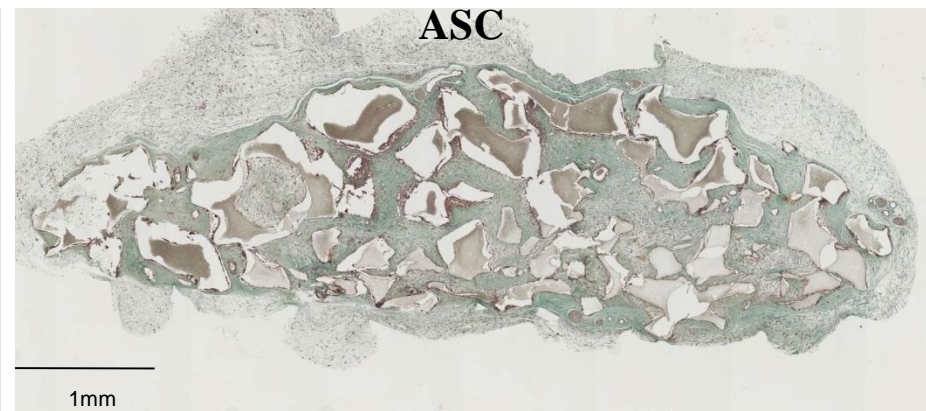
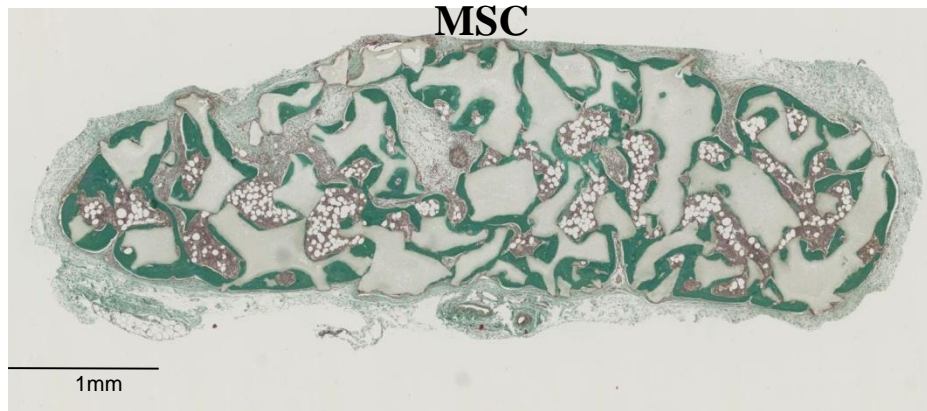
Brennan et al. in preparation

Source of stem cells: bone marrow or adipose tissue?

Methods

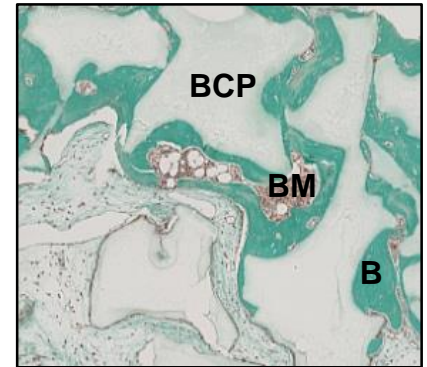
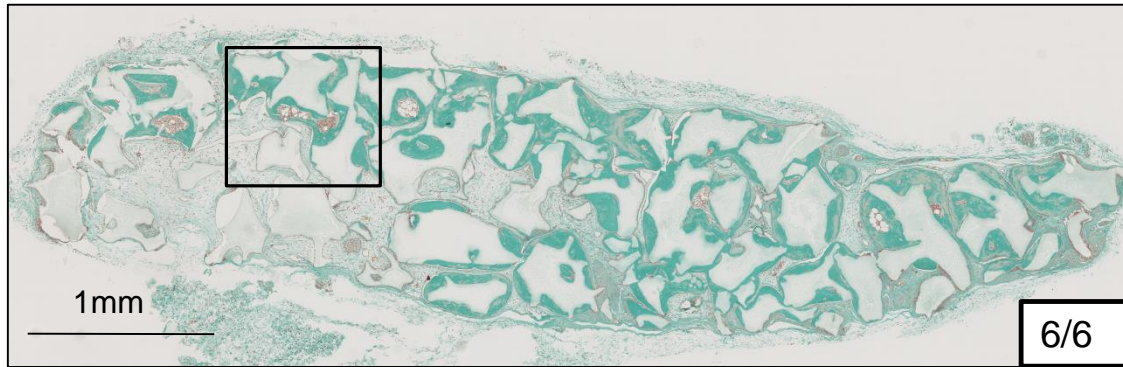
- BM-MSC or ASC cultured in 8% PL (6 donors)
- Osteogenic differentiation for 6 days (Dex or BMP4)
- Seeding 2×10^6 cells/50mg BCP granules for 1 h
- Implantation in subcutis of nude mice for 8 weeks

Source of stem cells: bone marrow or adipose tissue?

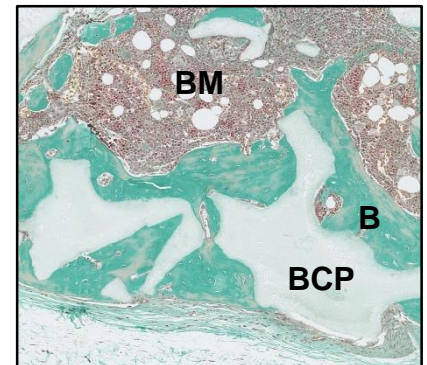
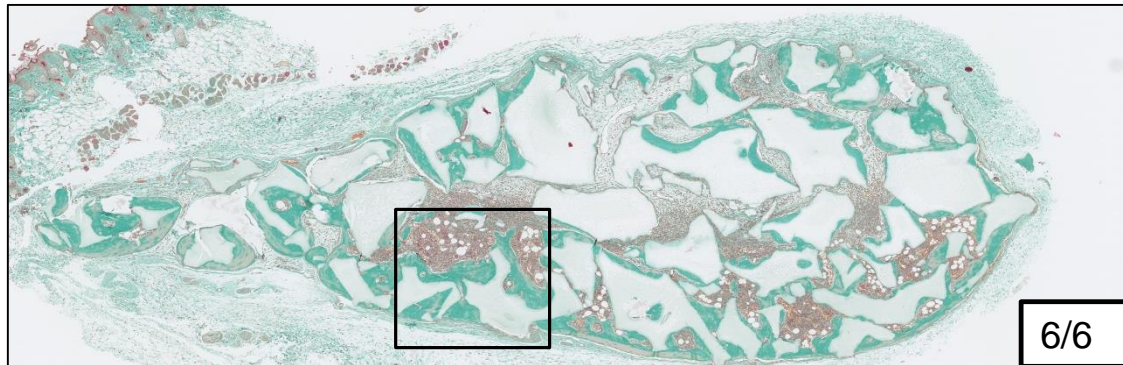


Bone formation

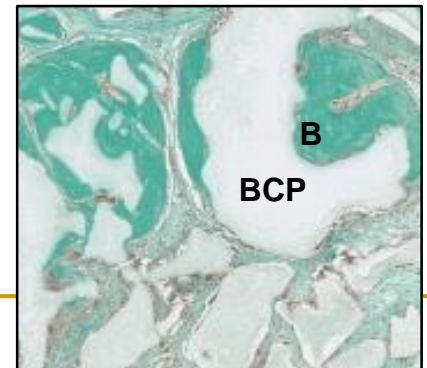
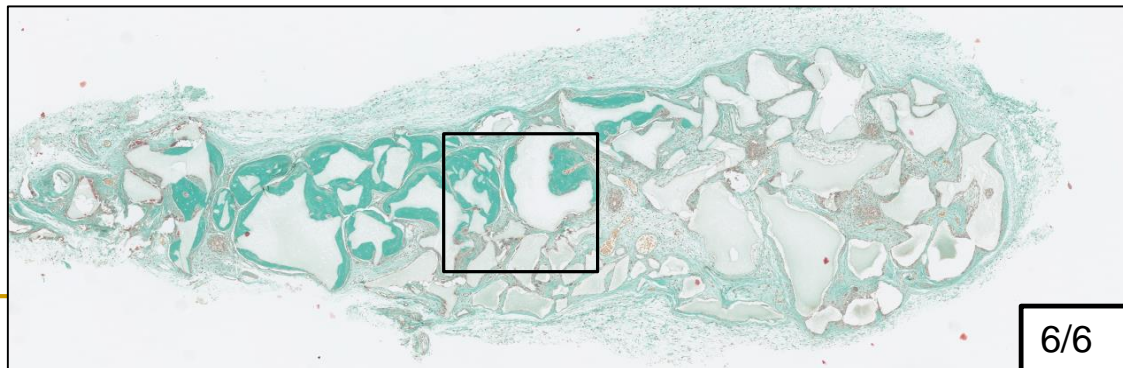
BM MSC



BM MSC Osteo

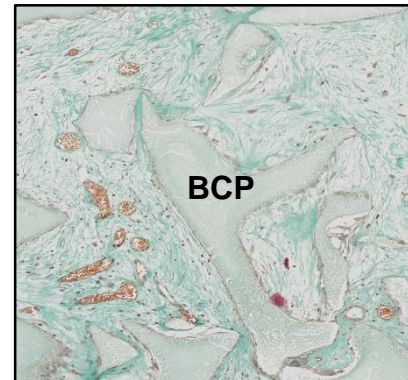
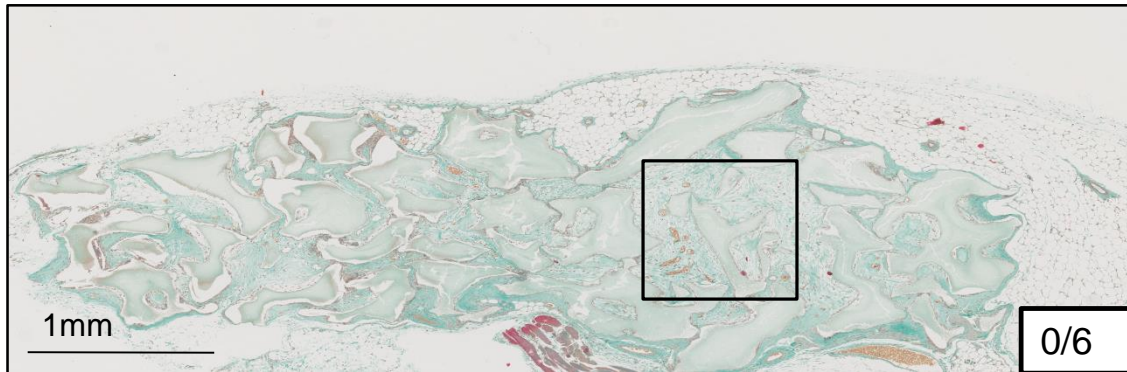


BM MSC BMP4

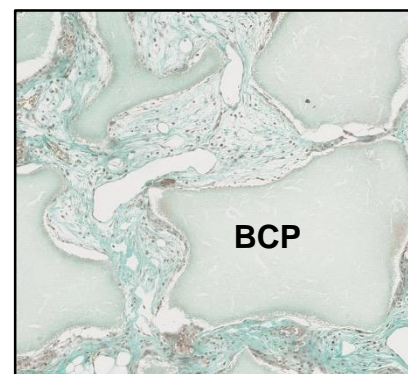
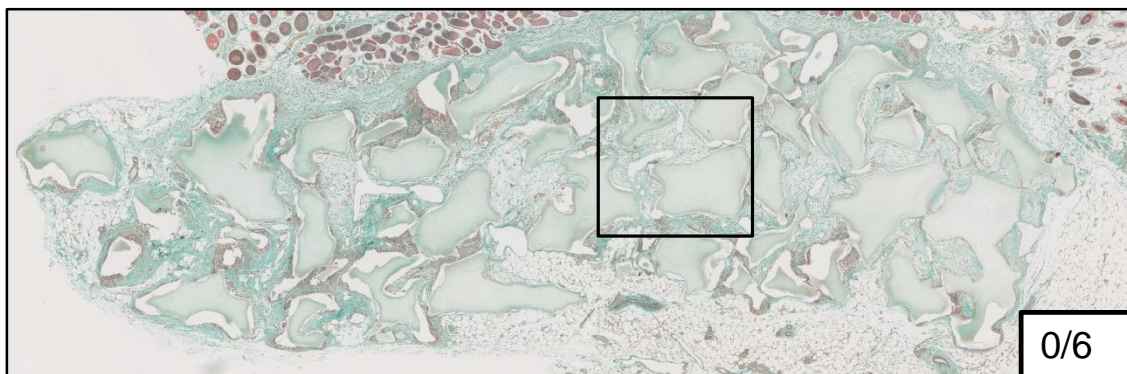


Bone formation

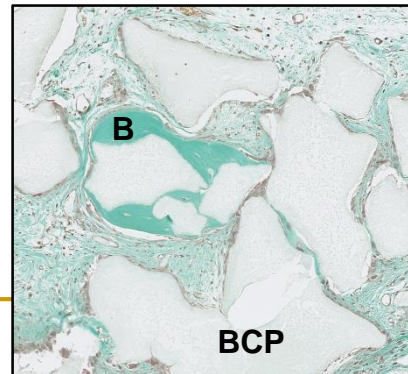
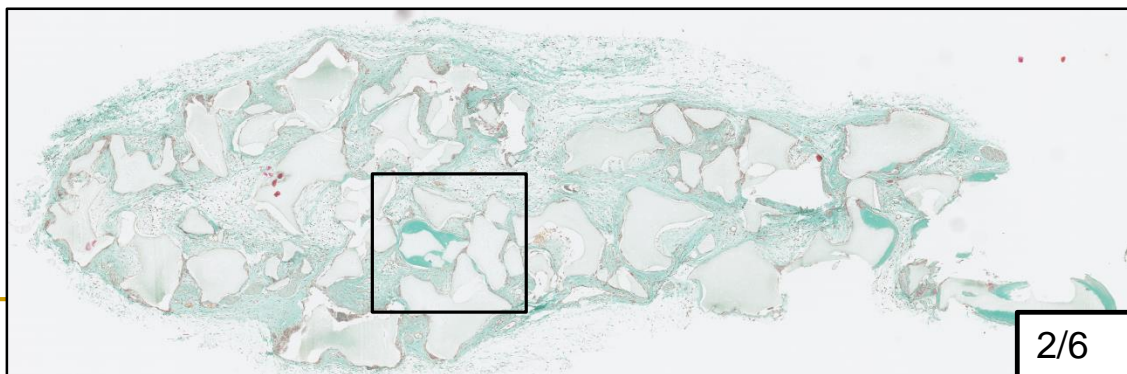
ASC



ASC Osteo

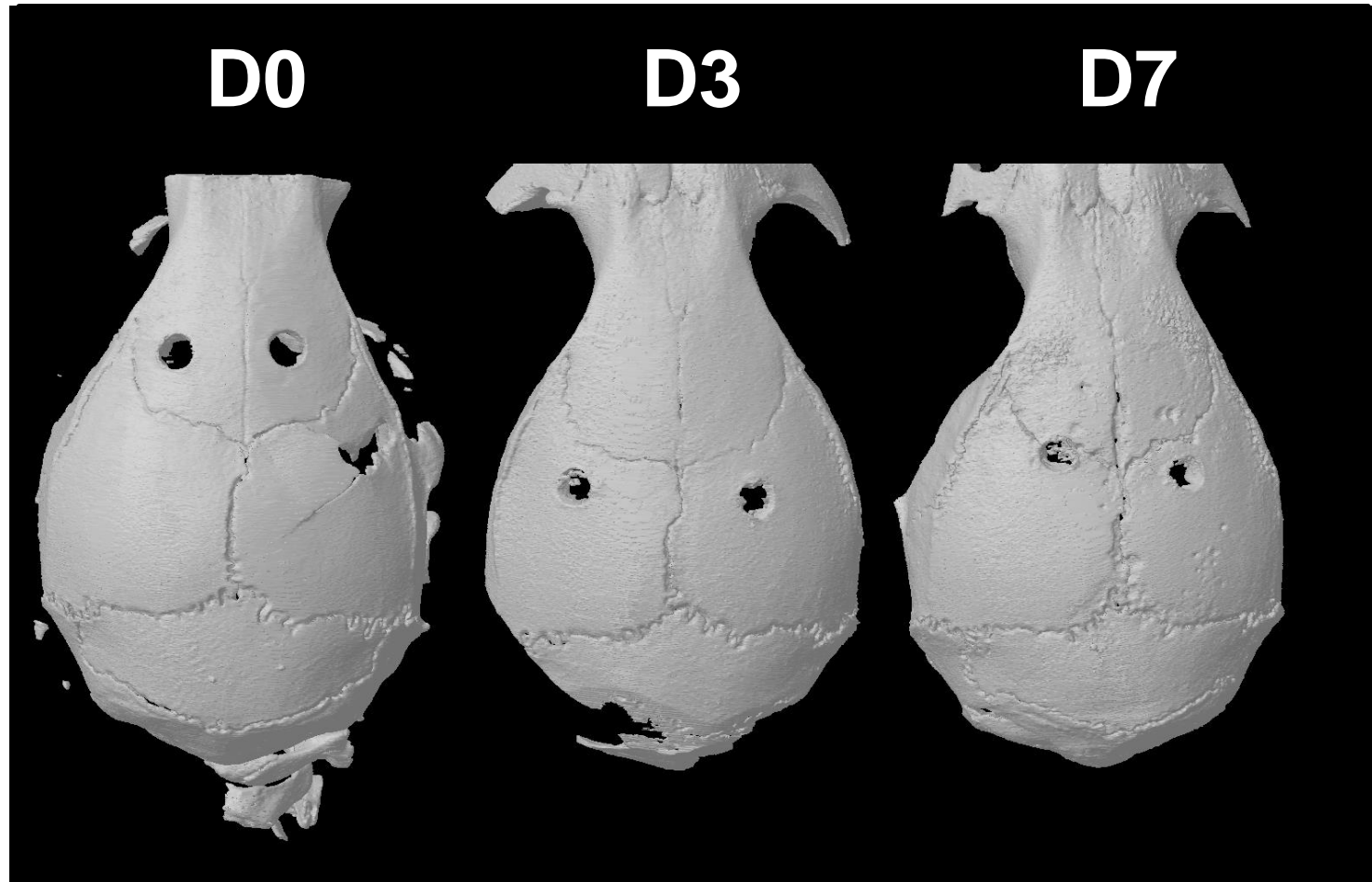


ASC BMP4



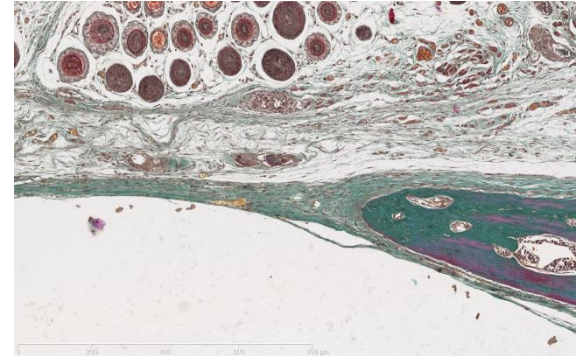
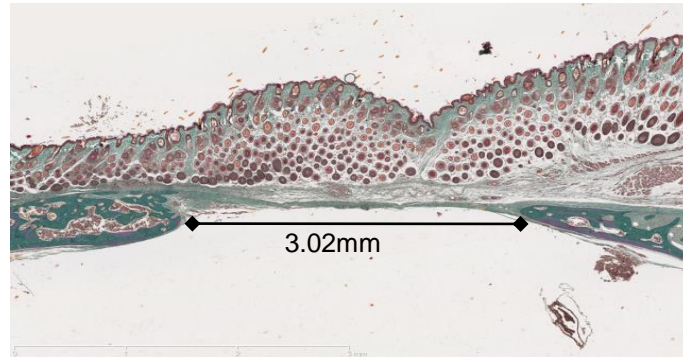
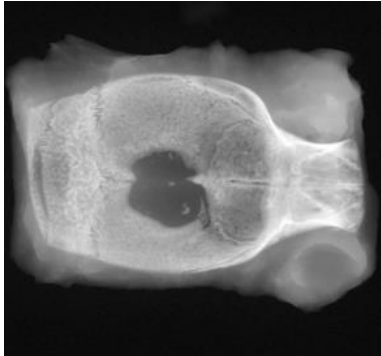
Bone regeneration with hMSC/BCP?

Calvaria defect (1 mm) in nude mice

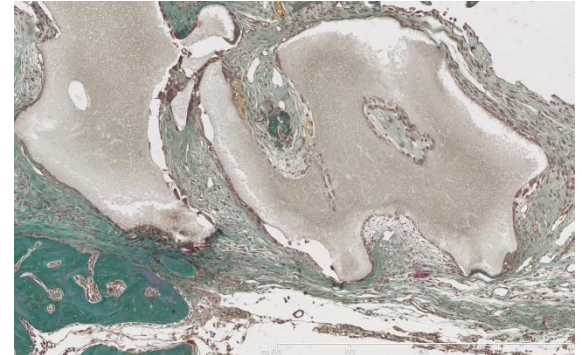
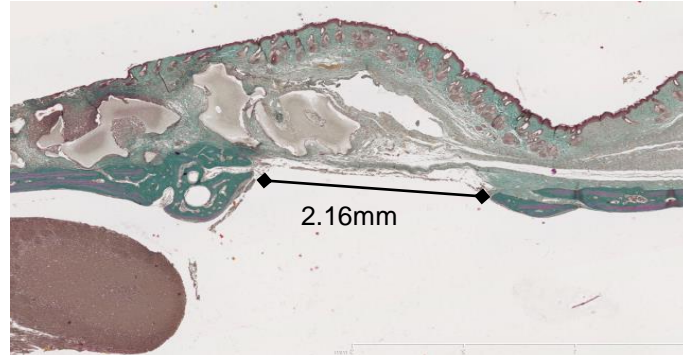


Calvaria defects (4 mm) at 8 weeks

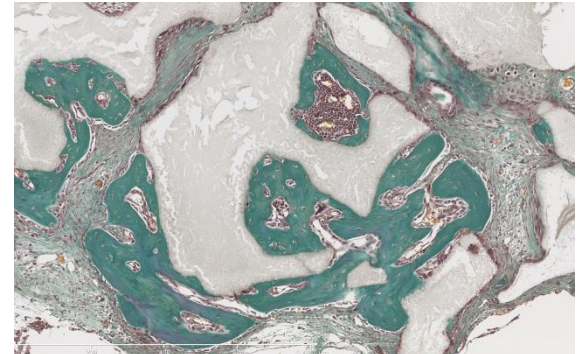
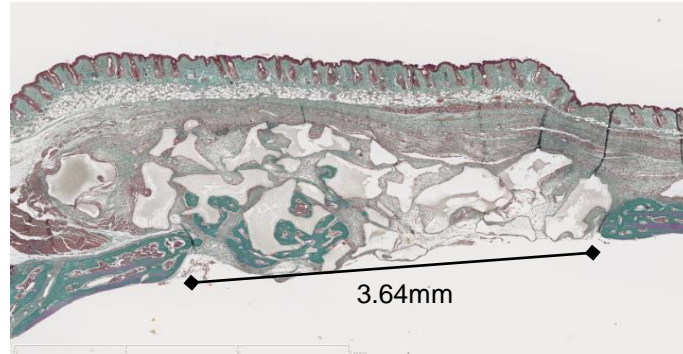
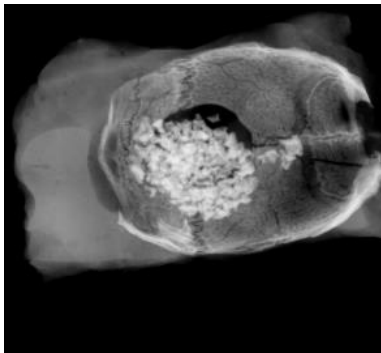
CT



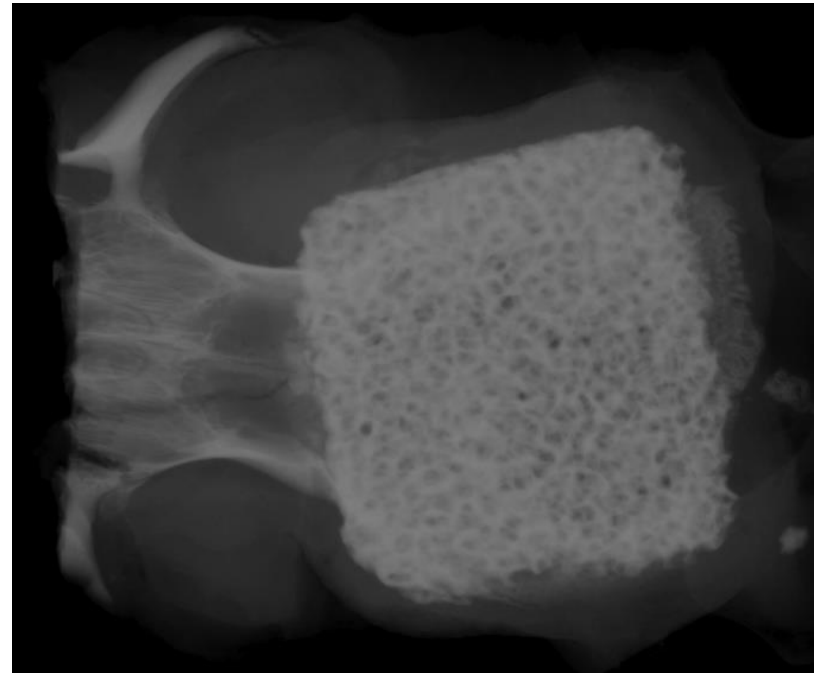
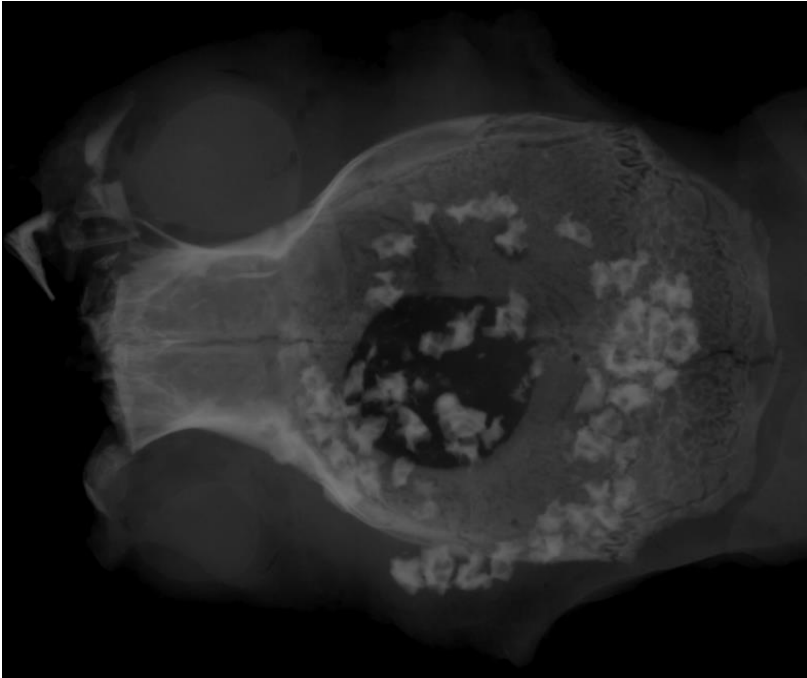
BCP



BCP + hMSC

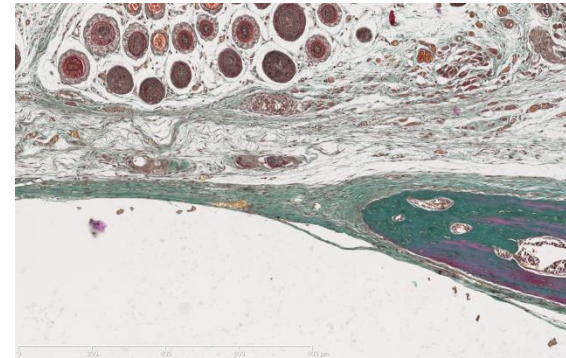
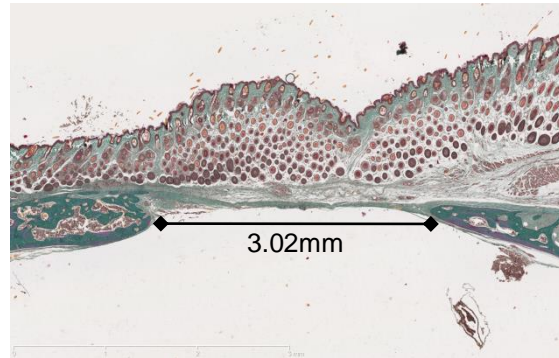


Calvaria defects (4 mm) in nude mice

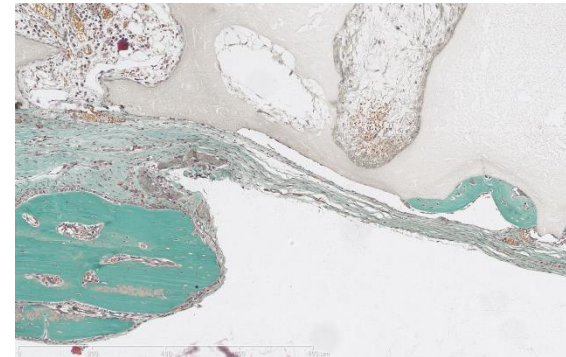
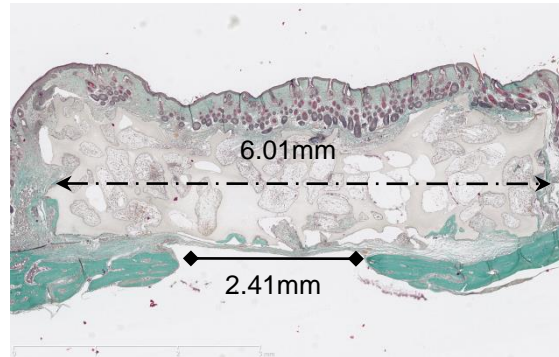


Histology at 8 weeks

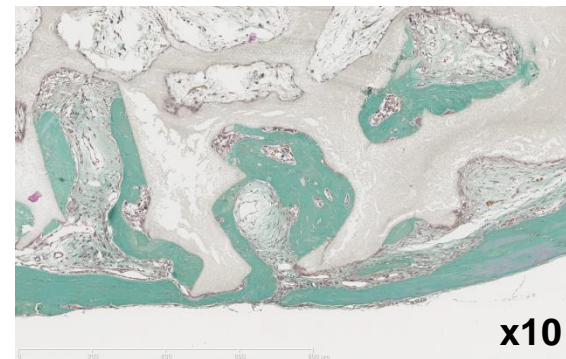
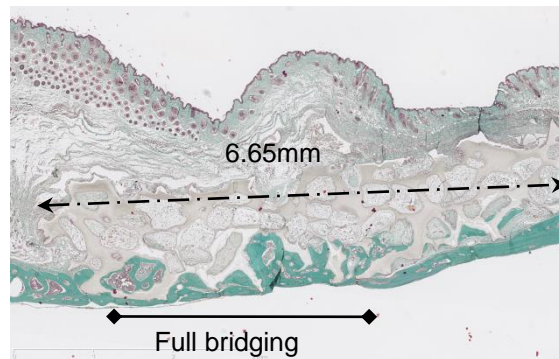
Empty
defect



Control
BCP



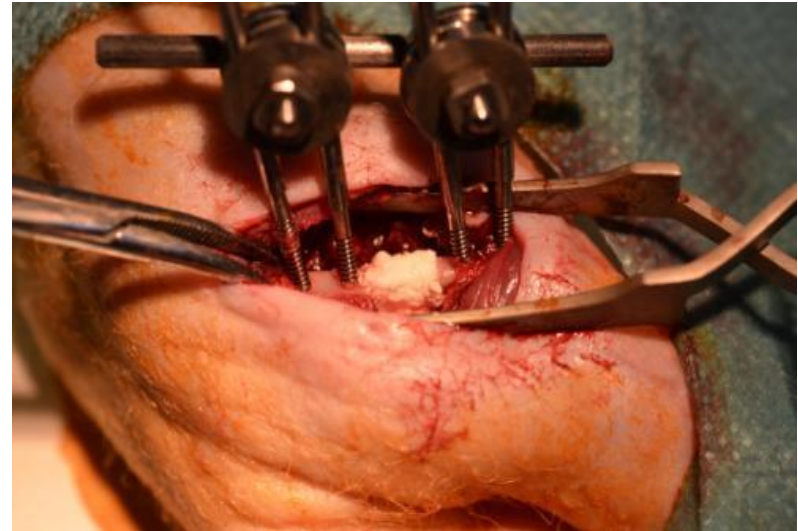
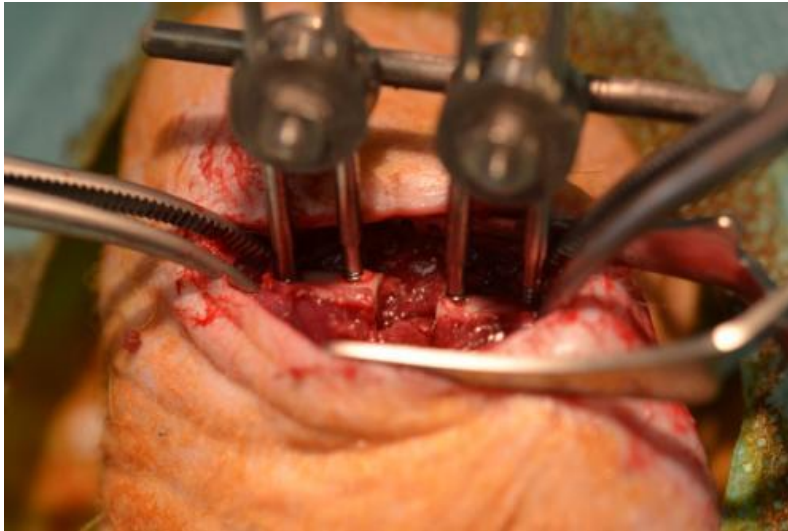
hMSC/BCP



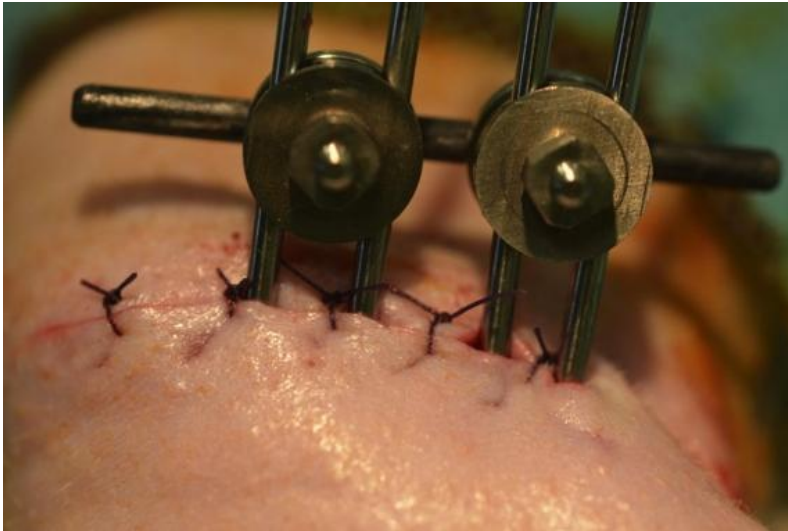
Femoral defects (5 mm) in nude rats



Femoral defects (5 mm) in nude rats



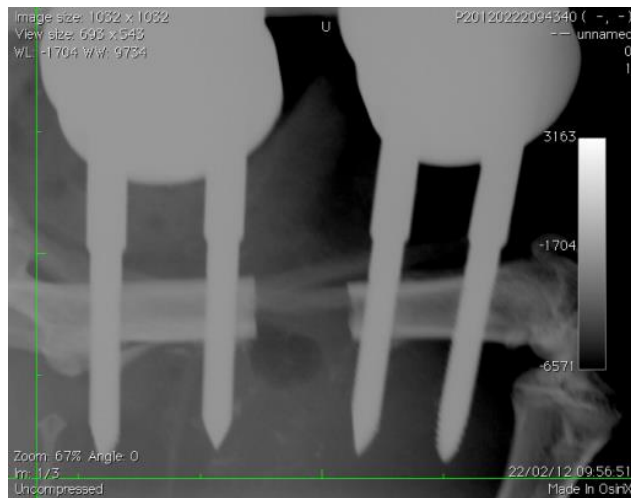
Femoral defects (5 mm) in nude rats



Stanovici et al. In preparation

Femoral defects (5 mm) in nude rats

Empty



BCP



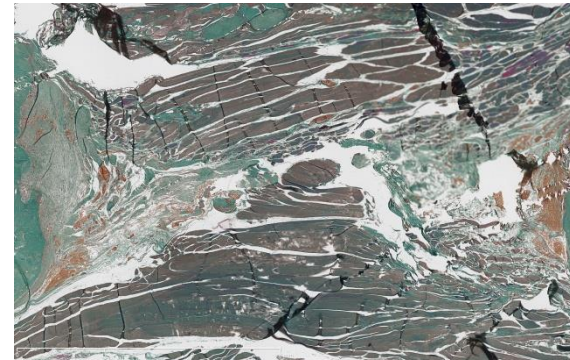
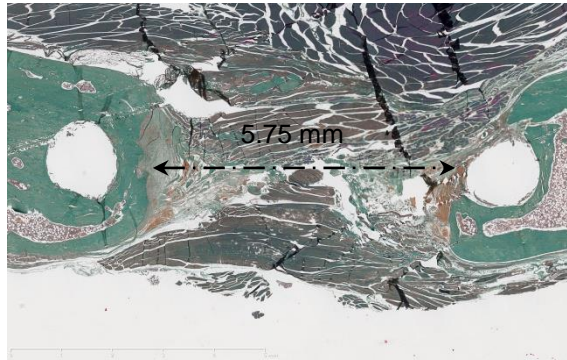
hMSC + BCP



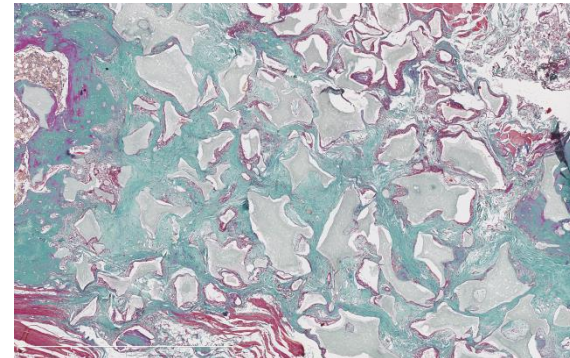
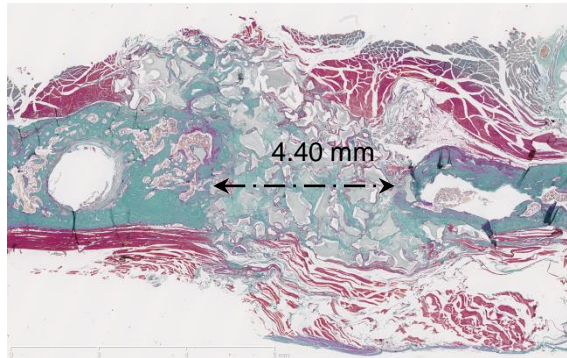
Stanovici et al. In preparation

Histology at 16 weeks

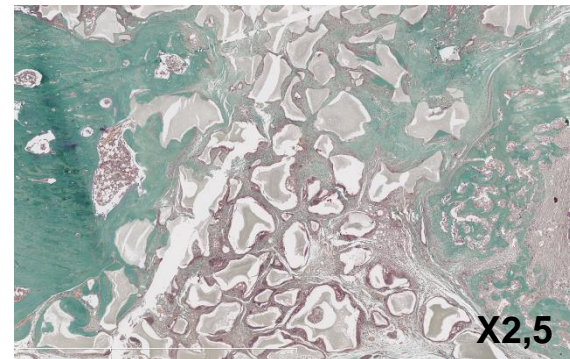
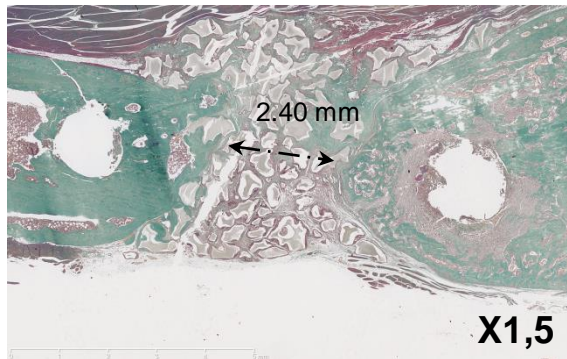
Empty defect



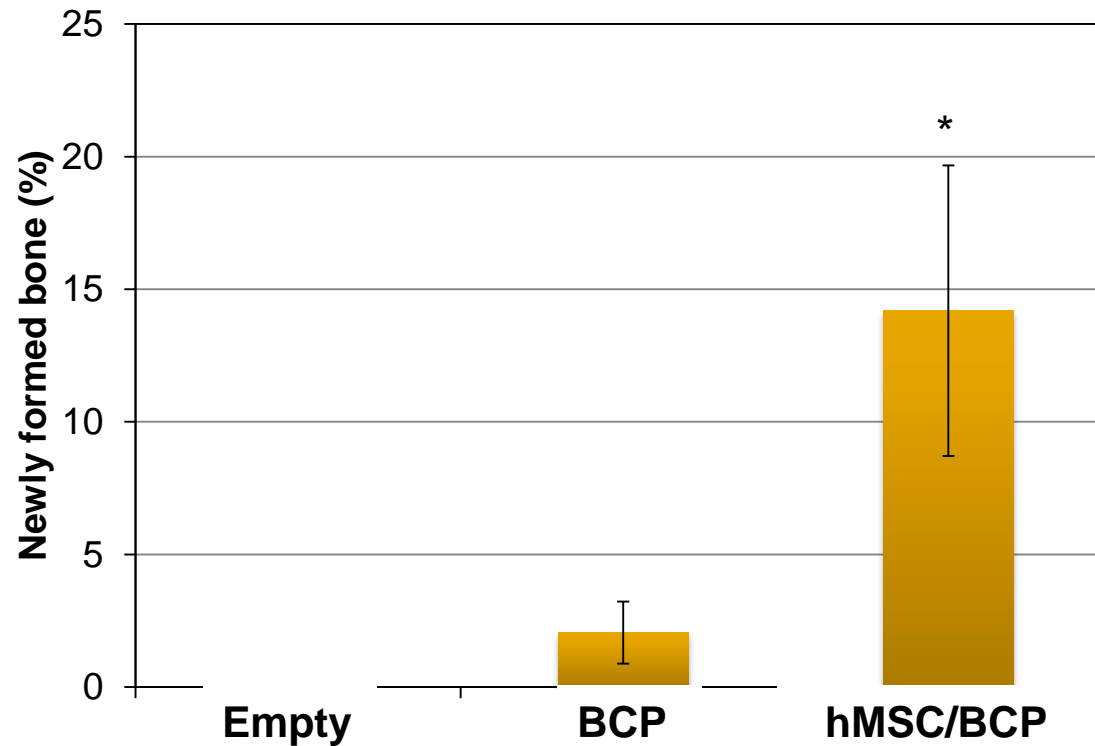
Control neg
(BCP)



hMSC/BCP



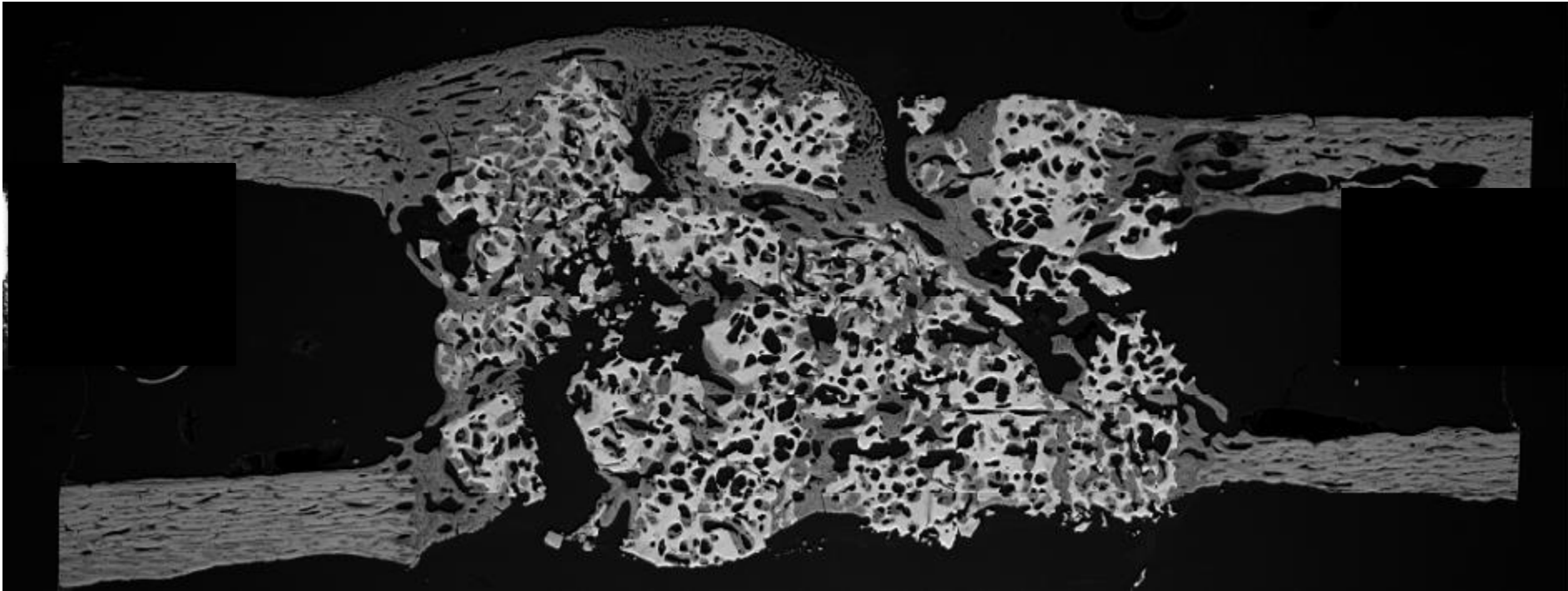
Histomorphometry at 16 weeks



Stanovici et al. In preparation

Regeneration of critical bone defects in sheep

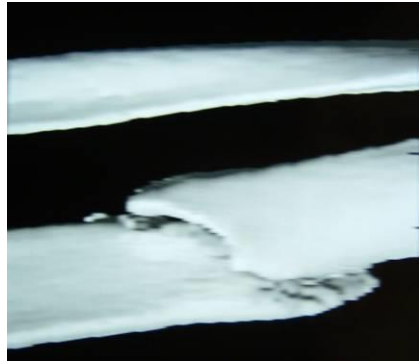
Segmental defect (2.5 cm) in metatarsus of sheep after 12 weeks regenerated with autologous MSC and granules



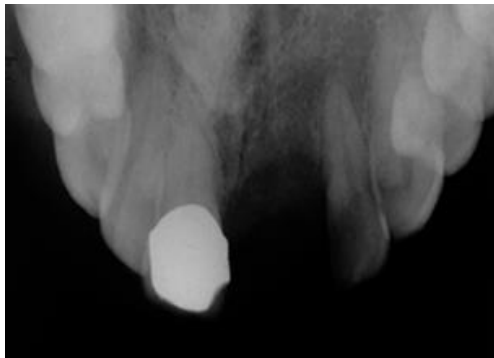
Cordonnier et al. COP 2012

5 clinical trials

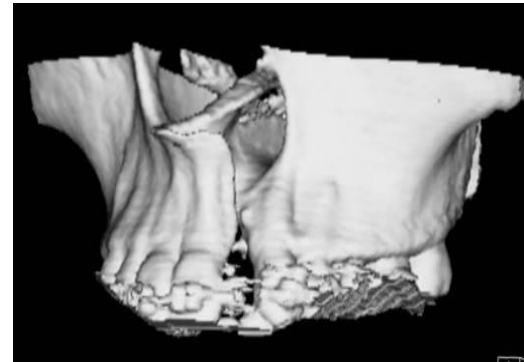
Long bone fractures



Osteonecrosis of the femoral head



Maxillo-facial defects before implants



Cleft palates

Ortho 1

- Delayed union or non-union fractures requiring bone graft
- Phase II multi-centre clinical trial approved in France, Germany, Spain & Italy
- 1st patient: 38 years old
- July 2009: motorbike accident, closed fracture right femur
→ IM nailing
- June 2013: complete weight bearing possible but with pain (VAS 5)
→ inclusion in Reborne, Prof. Philippe Rosset, CHU Tours

Pre-OP



Methods

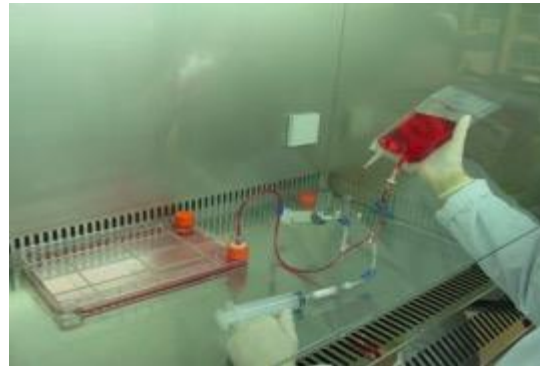
Bone Marrow aspiration
Posterior iliac crest (50 ml)



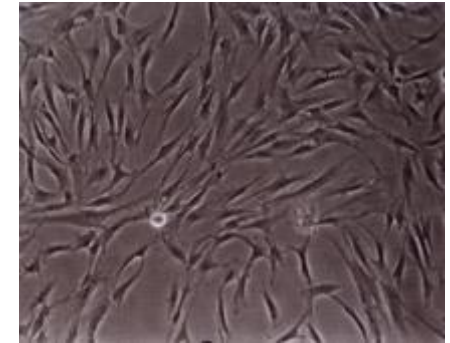
TRANSPORT

GMP Lab

Expanded MSC / 3 weeks



1272 cm² Cell Stack



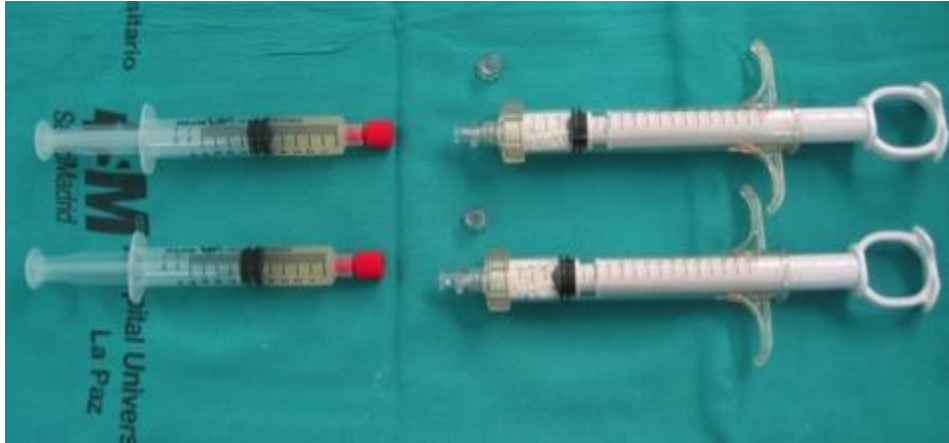
90% confluency

TRANSPORT



200 x 10⁶ MSCs in 10 ml

Technique



200 x 10⁶ MSCs

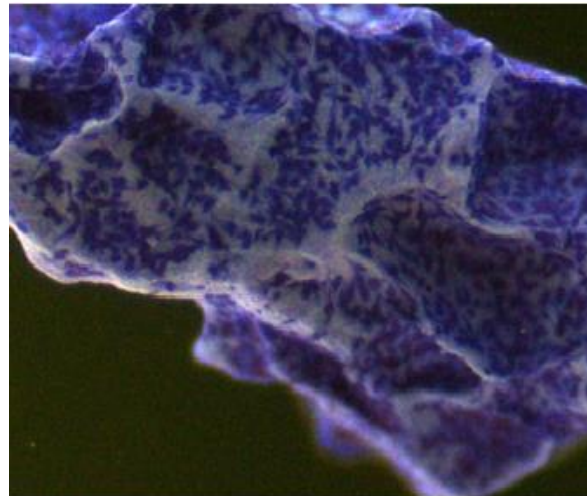
10 ml Biomaterial MBCP+[®]
(HA+ β TCP)



mixing



Incubation during
1 hour



1st patient 38 years old
motorbike accident in 2009, closed fracture femur



Pre-op



Post op



1 month



3 months



6 months

No pain
Full weight

Mrs GU C 57 y

domestic accident, closed fracture humerus, orthopaedic treatment -> pseudarthrosis



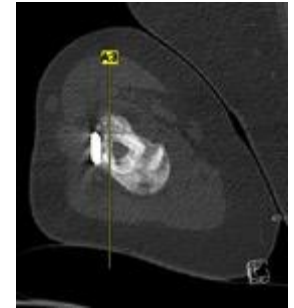
Pre-op



Post-op

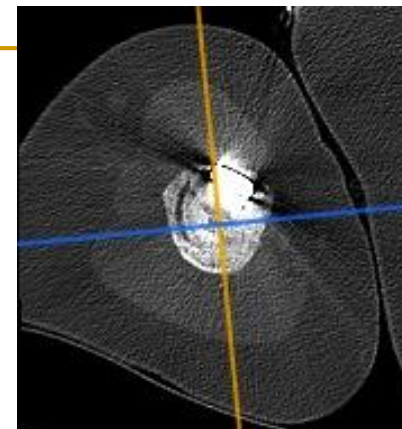


At 3 months
No pain
Lift weight OK



Mrs Bo 44 y

Traffic accident, closed fracture humerus -> pseudarthrosis

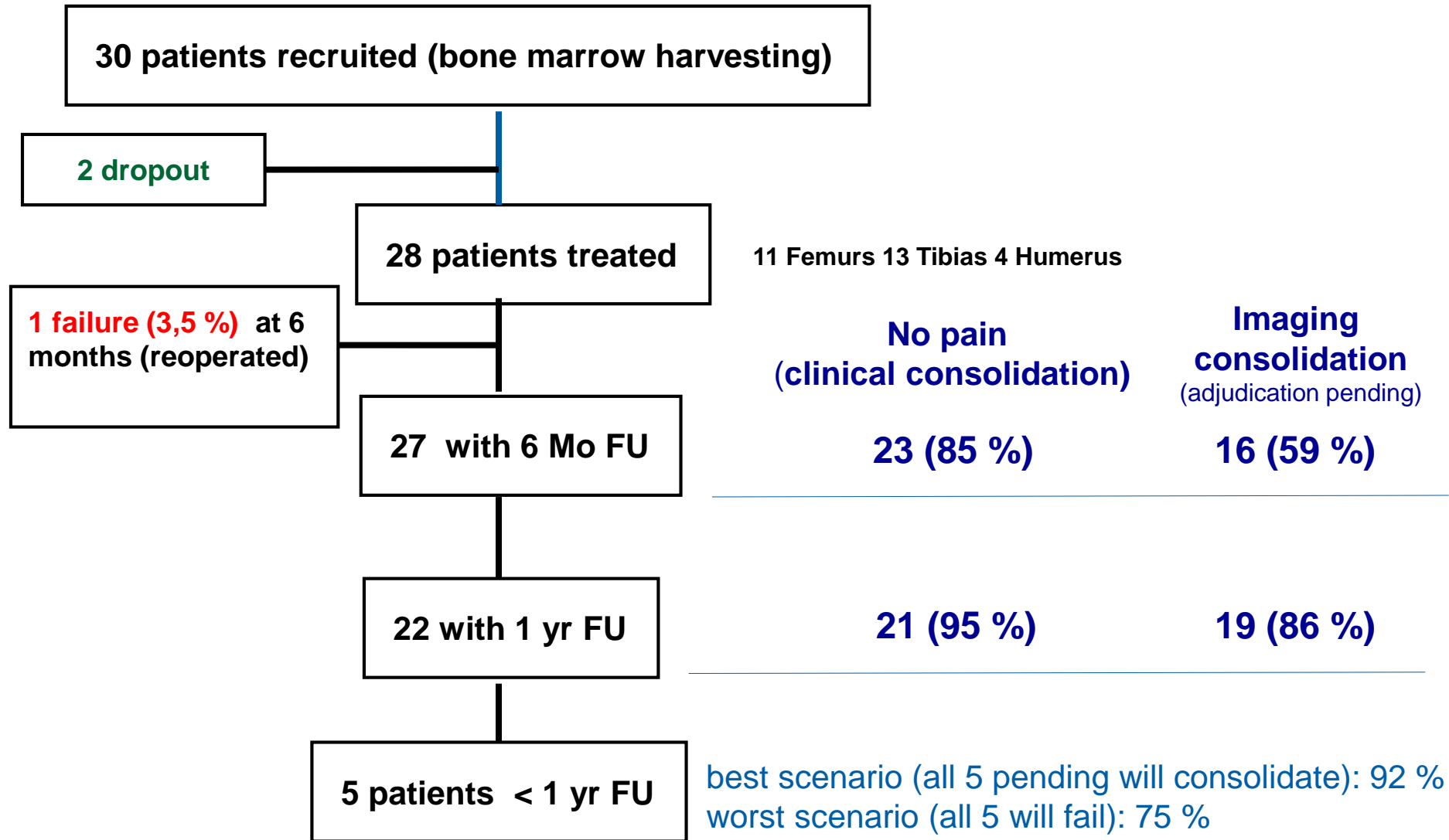


Pre-op

Post-op

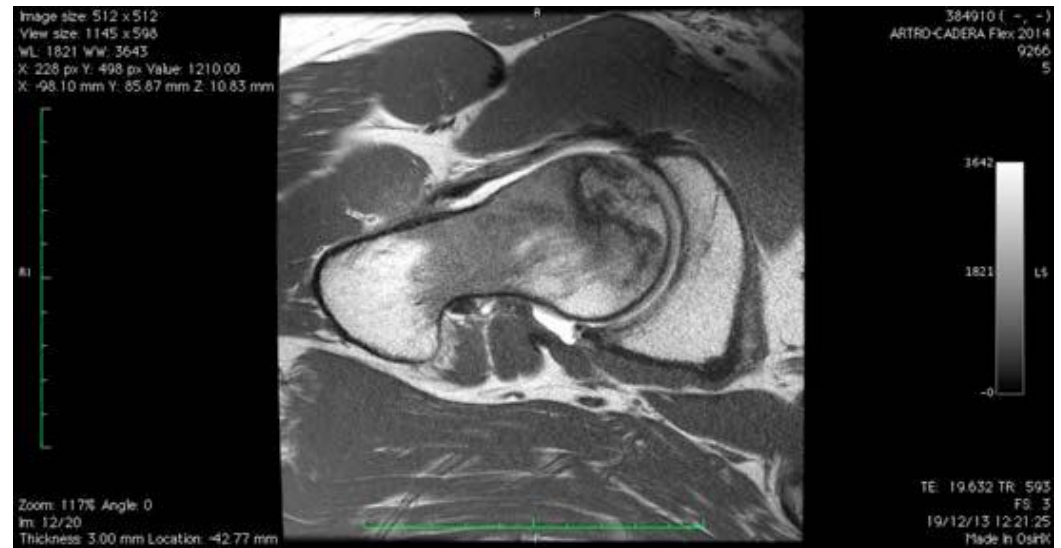
At 6 months No pain Lift weight OK

Preliminary results



Ortho 2

Osteonecrosis of the femoral head



Prof. Enrique Gomez Barrena, UA Madrid

Ortho 2

Injection of autologous cultured BM-MSC



25 patients included

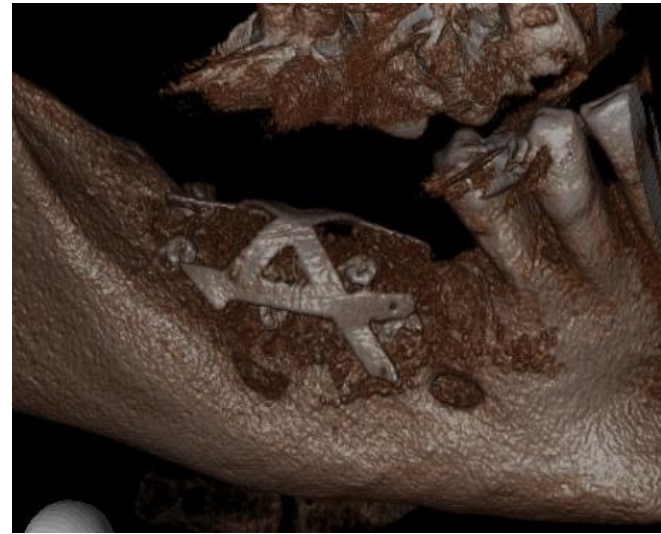
Maxillo 1

- Bone augmentation prior to dental implants

Pre-op



Post-op



Prof. Sølve Hellem, Univ Bergen

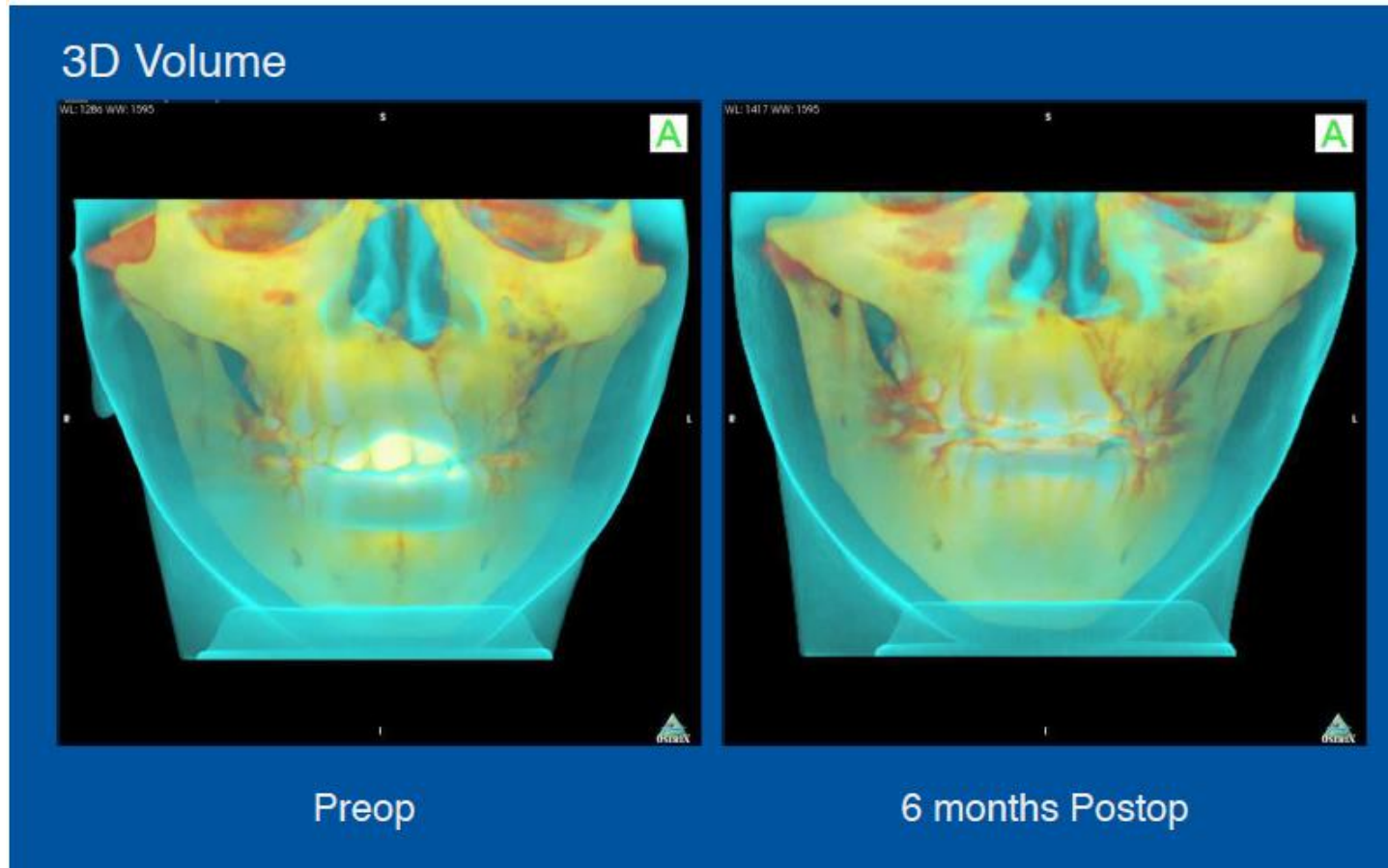
Completed study with 12 patients

Maxillo 2

- Reconstruction of cleft palates in children



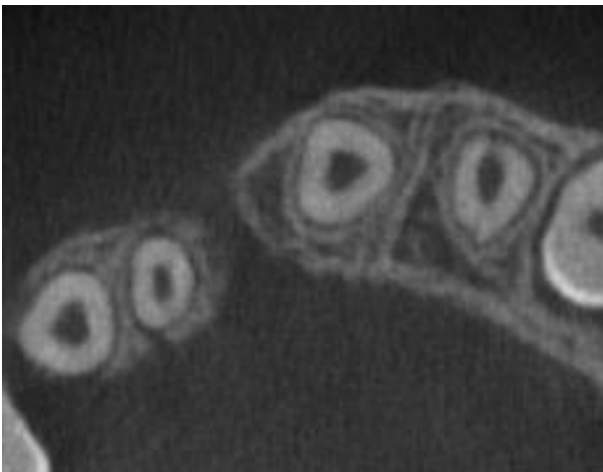
Universitair Medisch Centrum
Utrecht



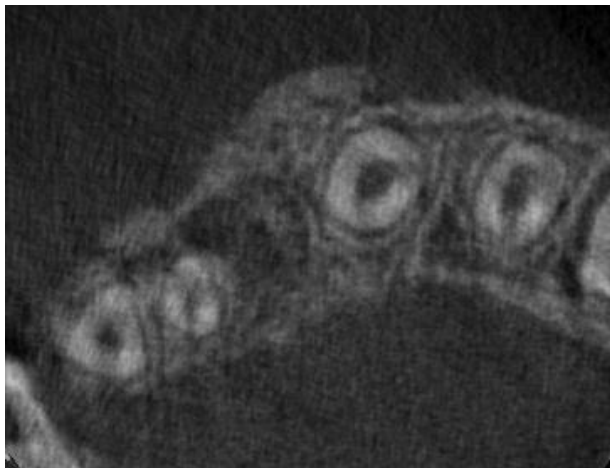
Maxillo 2

- Reconstruction of cleft palates in children

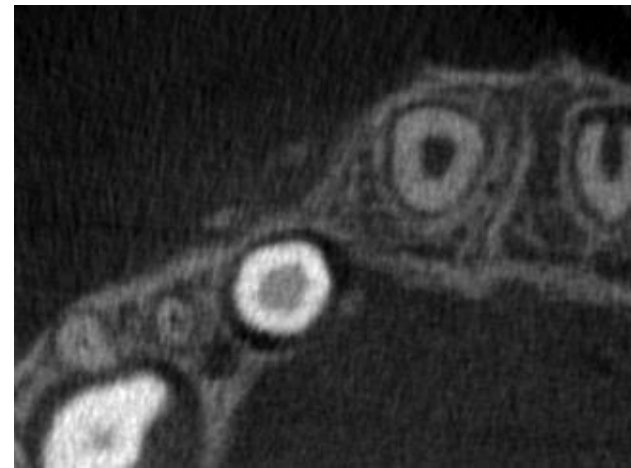
Pre-op 04.12.13



Postop 1 week



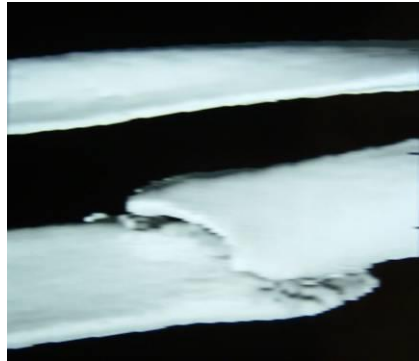
Postop 6 months



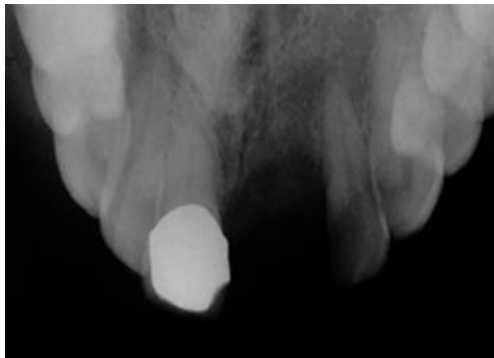
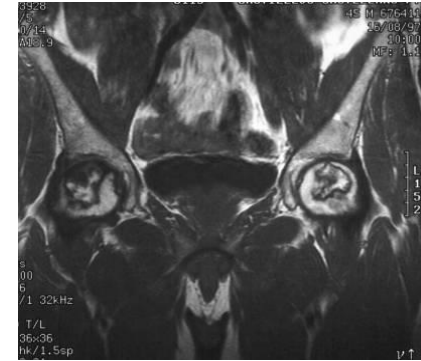
Completed study with 20 patients

5 clinical trials

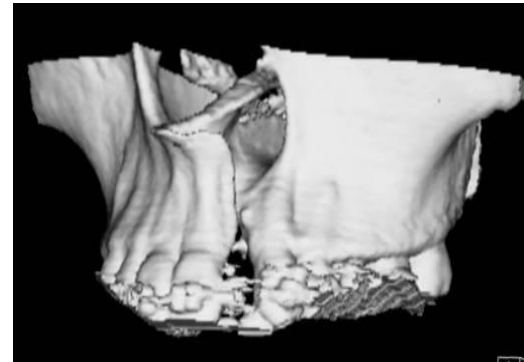
Long bone fractures



Osteonecrosis of the femoral head



Maxillo-facial defects before implants



Cleft palates

Submissions to Medicinal agencies

Inserm
Institut national de la santé et de la recherche médicale

Reborne

TECHNICAL FILE

« Evaluation of efficacy and safety of autologous MSCs combined to biomaterials to enhance bone healing in patients with delayed consolidation after long bone fracture requiring graft apposition or alternative orthobiologics »

REBORNE - OrthoCT 1

Sponsor N°	EudraCT N°	ID RCB N°
C11-12	2011-005441-13	2011-A00797-34

VERSION V2.1 OF 11/05/2012

CONFIDENTIAL

Sponsor:
Inserm - ESP Pôles Recherches cliniques
101, rue de Tolbiac, 75564 Paris Cedex 13
Contact:
Ms Domitille d'Alarçon - Project Manager

<p>European Project FP7 Coordinator: Dr. Pierre Laroche</p> <p>Address: Inserm U957 UPMC, Faculté de Médecine 1 rue Gaston Veil 44035 Nantes Cedex 1 France Tel: +33 (0)2 72 84 11 43 Fax: +33 (0)2 49 41 28 66 Email: pierre.laroche@inserm.fr</p>	<p>FP7 Co-coordinator: Dr. Luc Senécal</p> <p>Address: EFS Pyrénées Méditerranée Avenue de Grande Bretagne BP1210 31027 Toulouse Cedex 3 France Tel: +33 (0)5 62 17 08 91 Email: luc.senecal@efs.sante.fr</p>
<p>European Trial Coordinating investigator: Dr. Enrique Gomez-Barrera</p> <p>Address: Facultad de Medicina Universidad Autónoma de Madrid c/Arzobispo Morcillo 2 Madrid 28029 Spain Tel: +34 91 6375473 Fax: +34 91 4870303 Email: enrique.gomezbarra@uam.es</p>	<p>French Trial Coordinating investigator: Dr. Philippe Rosset</p> <p>Address: Département d'Orthopédie Service d'Orthopédie 2 Hôpital Trousseau CHU Tours 37044 Tours Cedex 1 Tel: +33 2 47 47 55 15 Fax: +33 2 47 47 55 12 Email: rosset@med.univ-tours.fr</p>

C11-12_IMR_V2.0_0010402 Page 1 / 107

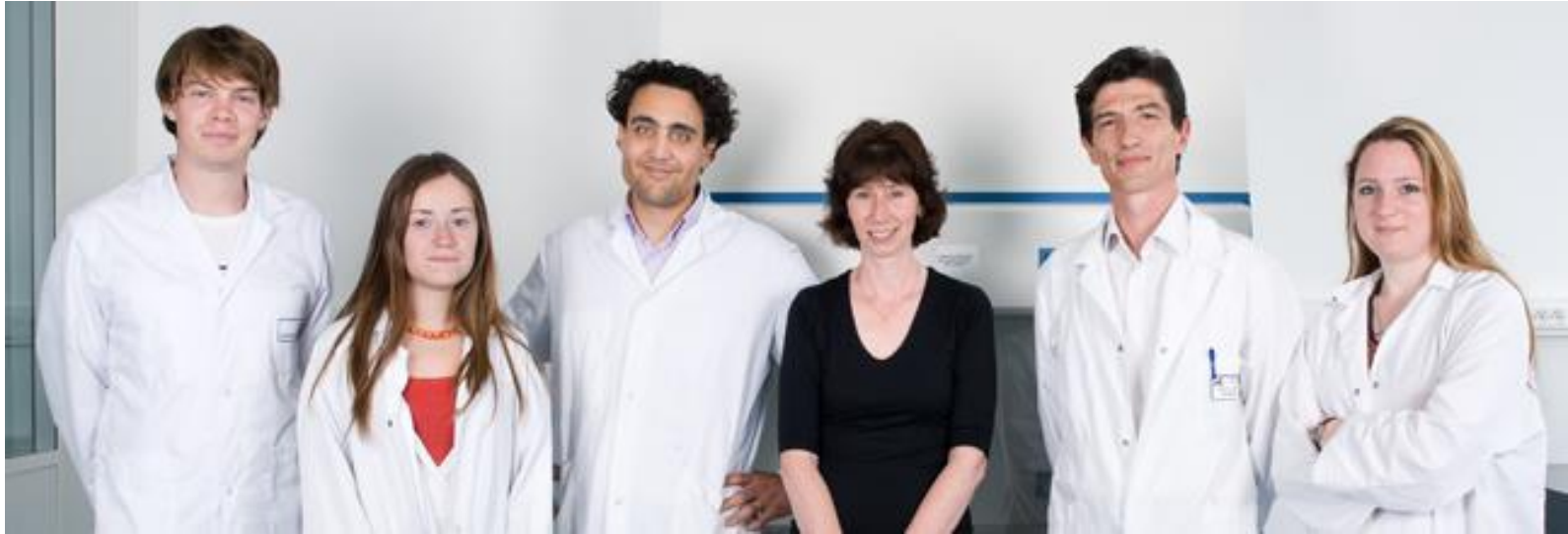


Folders for Paul Ehrlich Institute (Germany)

Conclusions

- Bioceramics are appropriate scaffolds for bone tissue engineering
- Human mesenchymal stem cells are easily isolated from bone marrow and amplified in culture
- hMSC mixed with BCP induced bone formation
- hMSC seeded on BCP regenerated large bone defects
- 4 multicentric clinical trials are underway:
 - regeneration of non-union fractures
 - Osteonecrosis of the femoral head
 - Bone augmentation in the mandible prior to dental implants
 - reconstruction of cleft palates in children

Acknowledgements



Jérôme Amiaud, AI histology
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