Instituts thématiques Inserm
Institut national de la santé et de la recherche médicale

UNIVERSITE DE NANTES

Régénération osseuse maxillofaciale à partir de cellules souches et de biomatériaux

Pierre Layrolle, PhD
Directeur de recherche DR1

Inserm U1238, Sarcomes osseux et remodelage des tissus calcifiés
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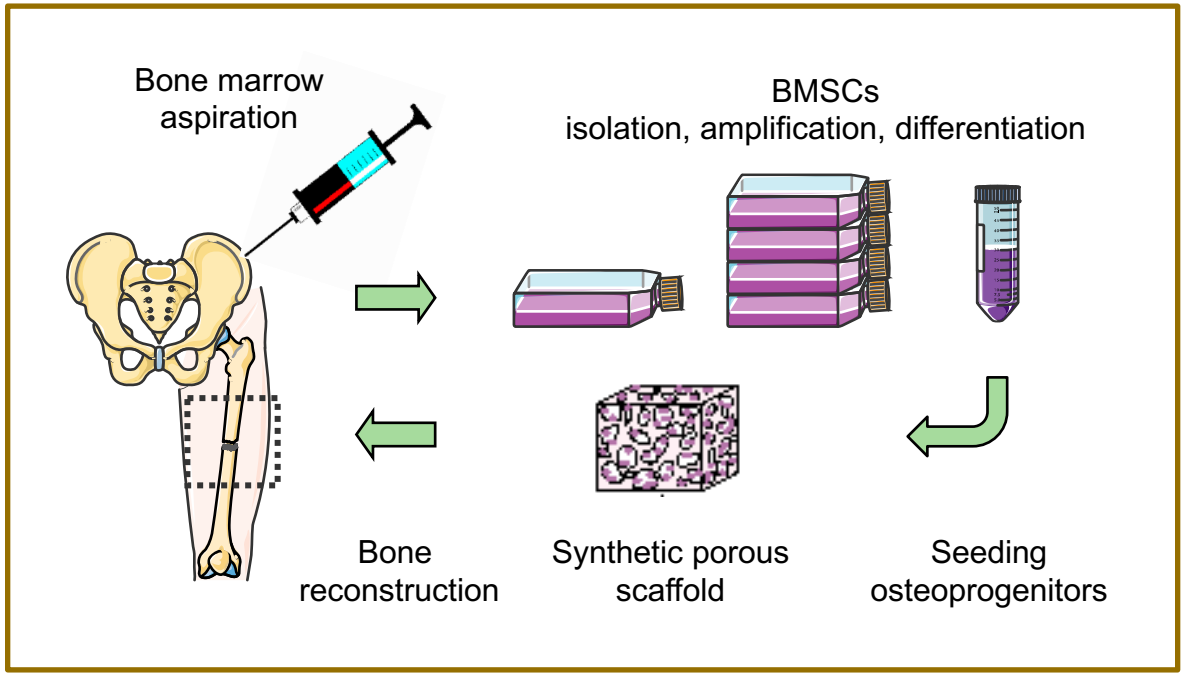
Introduction

- 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



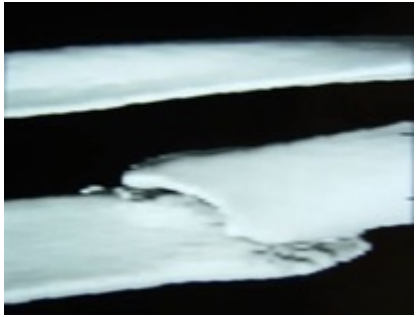
Cordonnier et al. Adv Funct Mater 2011
Rosset et al. Orthop Traumatol Surg Res. 2014
Stanovici et al. Curr Res Transl Medicine 2016

4 clinical trials

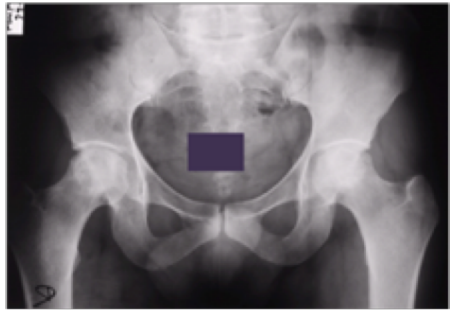
Reborne

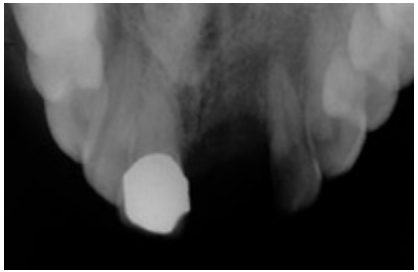
SEVENTH FRAMEWORK PROGRAMME

Long bone
non union fractures

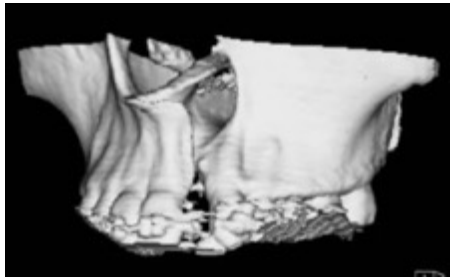


Osteonecrosis
of the femoral head





Maxillo-facial defects
before implants



Cleft palates

Reborne

SEVENTH FRAMEWORK PROGRAMME

XPAND

KitoZyme

Université de Liège

Inserm

EFS

CHRU

CENTRE HOSPITALIER UNIVERSITAIRE DE NANTES

ALCIMED

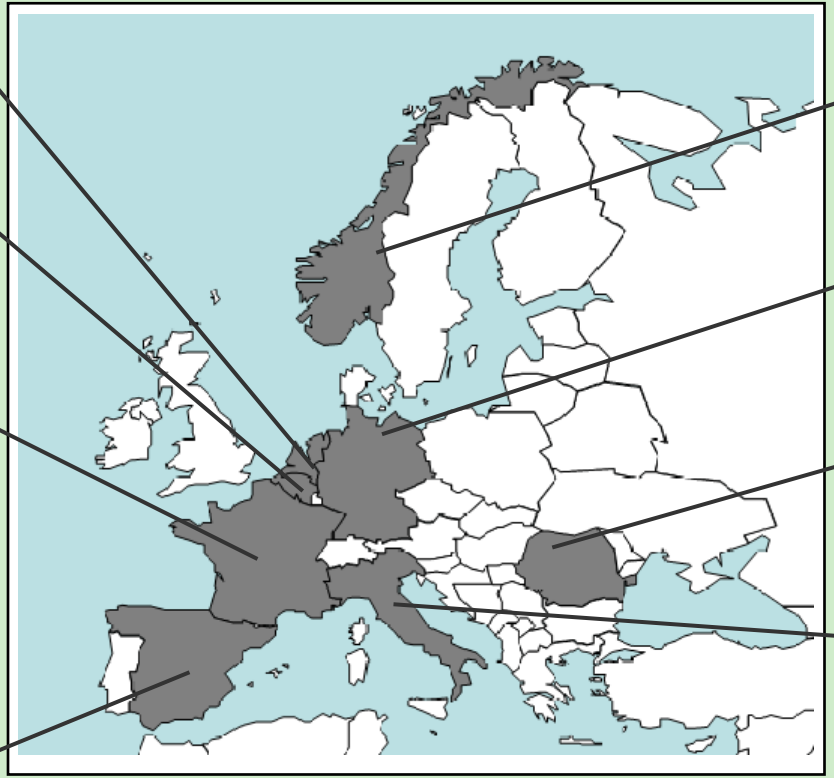
CHU HENRI MONDOR

cea

Biomatlante

UPC

UAM UNIVERSIDAD AUTONOMA DE MADRID



Deutsches Rotes Kreuz

uulm

Universitatea de Medicina și Farmacie "Victor Babeș" Timișoara

MEYER

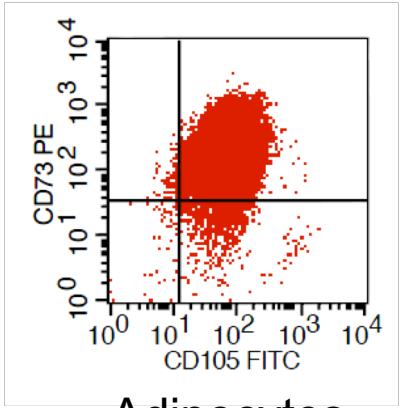
Azienda Ospedaliero-Universitaria

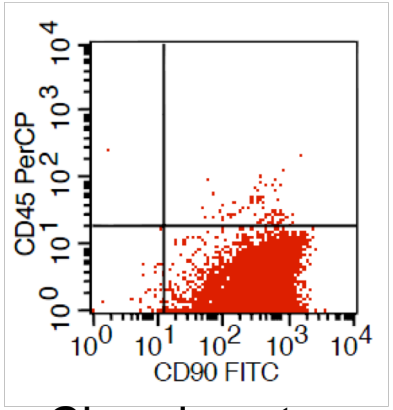
Istituto Ortopedico Rizzoli

UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

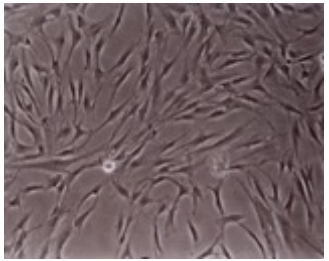
12 million € 2010-2015

Human Mesenchymal Stem Cells

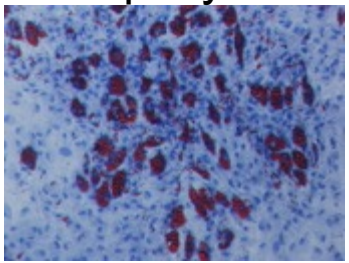




MSC

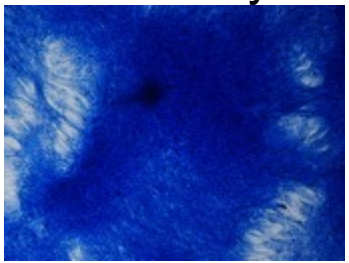


Adipocytes



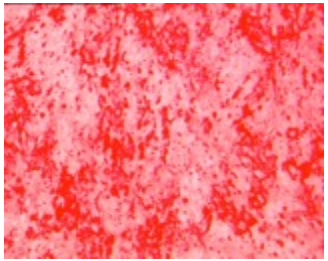
Red Oil

Chondrocytes



Alcian blue

Osteoblasts

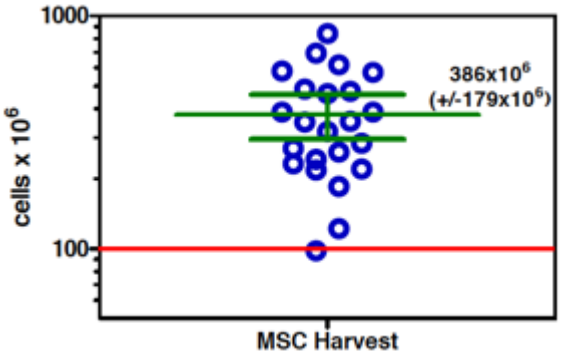


Alizarin red

Delorme et al. Blood 2008

Platelet Lysate for culturing hMSC

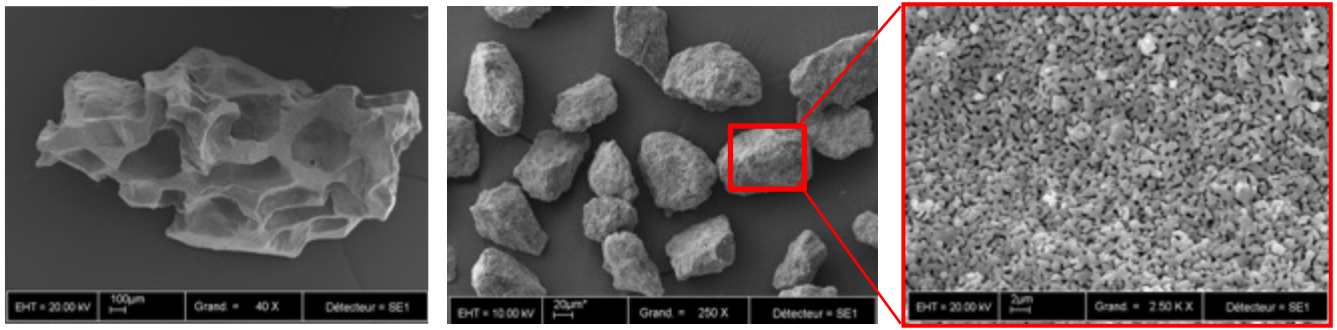
- Platelet Lysate from human blood
- Isolation and grow of hMSC in αMEM + 10% PL
- Xenobiotic free culture medium
- PL increased cell proliferation
- Approx. 400 x 10^6 hMSC produced in 21 days
- PL enhanced osteoblastic differentiation



Chevallier et al. Biomaterials 2010

Biomaterial

BCP = HA/β-TCP 20/80

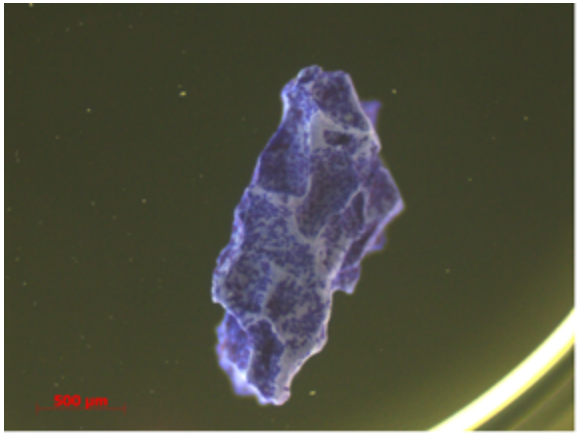


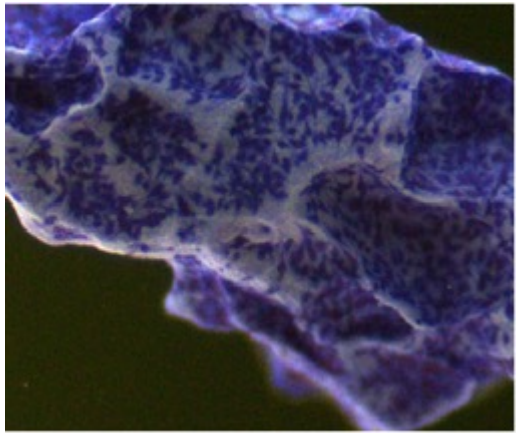
Manufactured by Biomatlante (CE and FDA approved)

Biomatlante
Biologics Solutions

Cell dose of hMSC on bioceramics

Meadhbh Brennan





Methylene blue

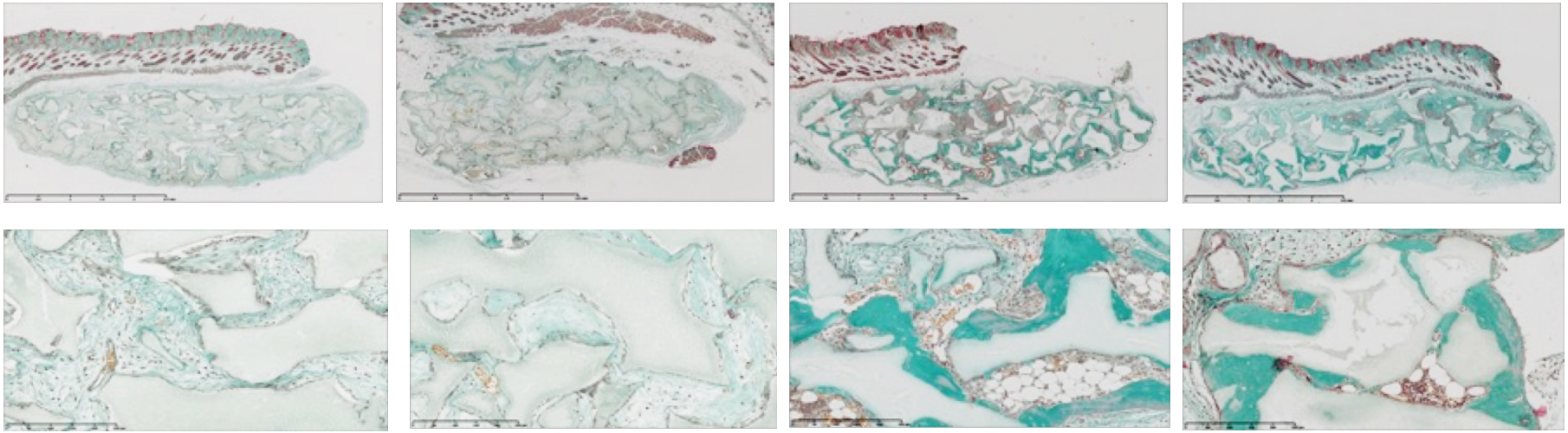
Subcutis implantation
in nude mice for 8 weeks

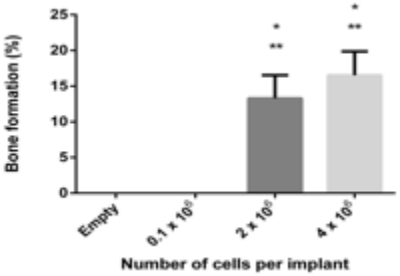
Histology

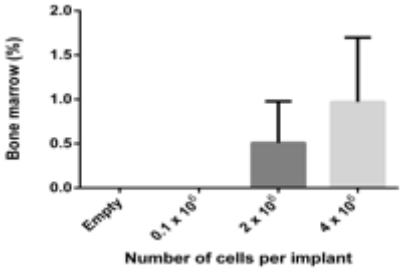
Brennan et al. Stem Cell Res Ther, 2014

Histology @ 8 weeks

BCP	BCP + 0.1x10^6 hMSC	BCP + 2x10^6 hMSC	BCP + 4x10^6 hMSC

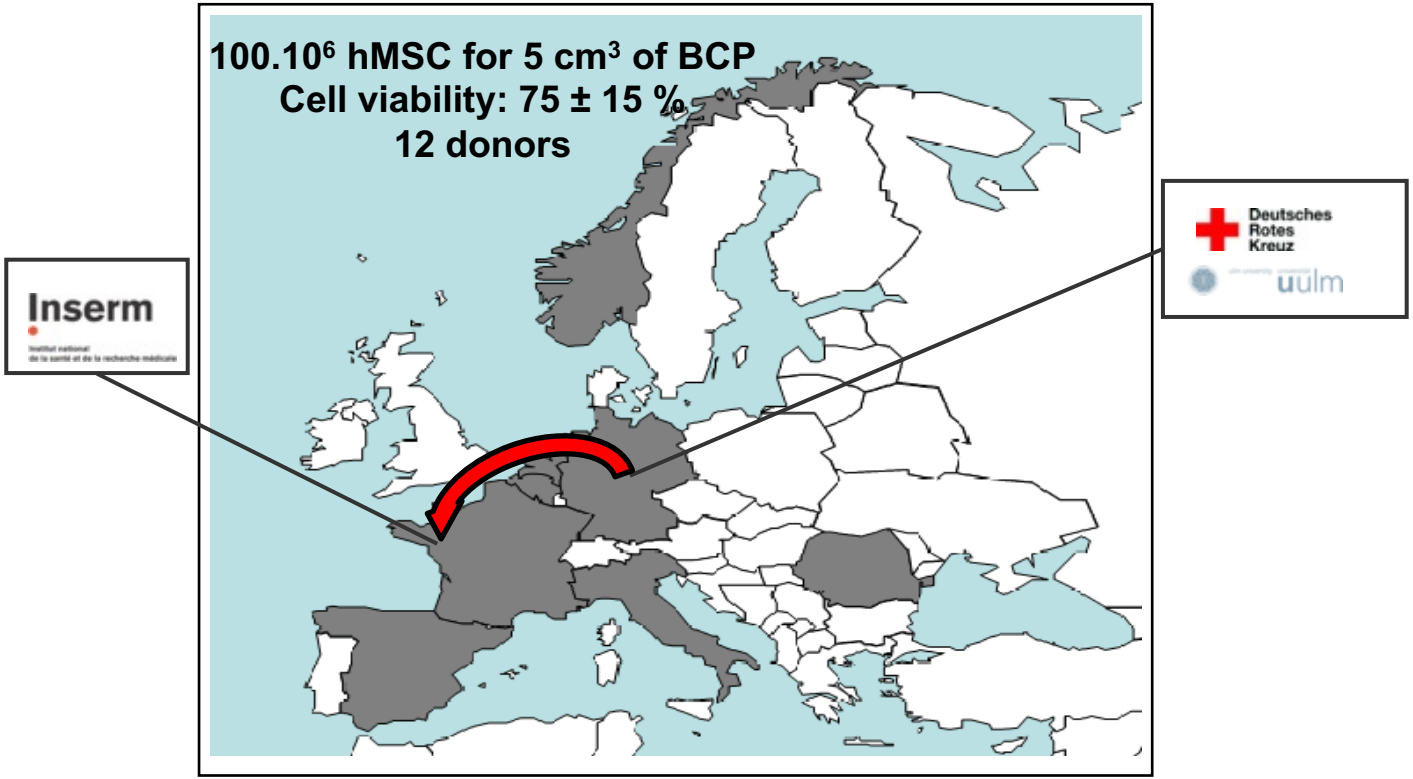






Masson trichrome staining

Production and transportation of hMSCs



Mixing cells and biomaterial

5 cm^3 BCP
2.5 g

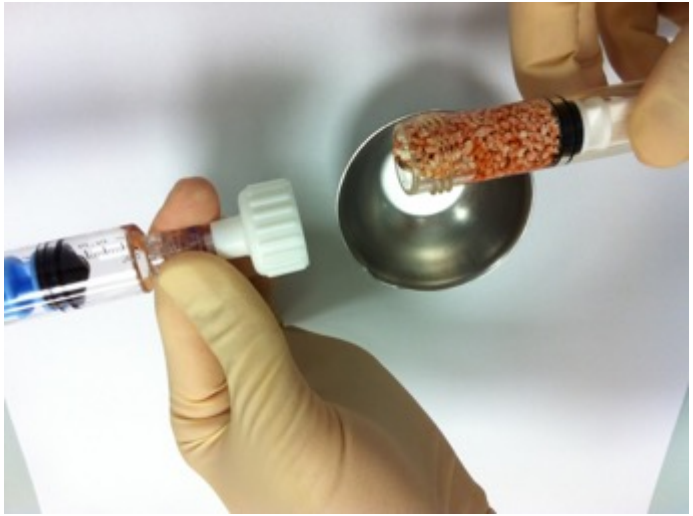
100 million hMSC

Brennan et al. Stem Cell Res Ther, 2014

Mixing cells and biomaterial

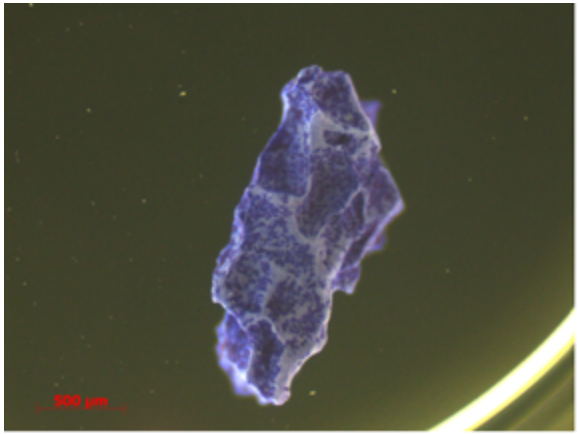
Brennan et al. Stem Cell Res Ther, 2014

Mixing cells and biomaterial

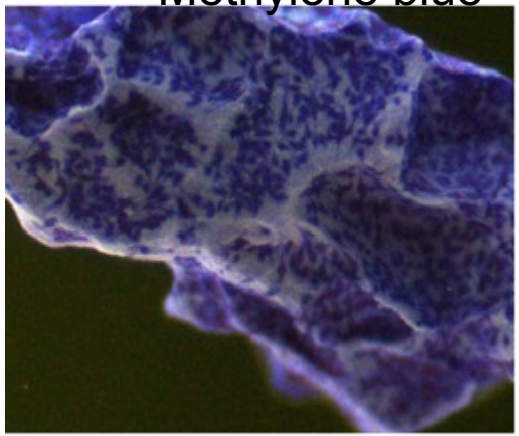


Brennan et al. Stem Cell Res Ther, 2014

hMSC on bioceramics

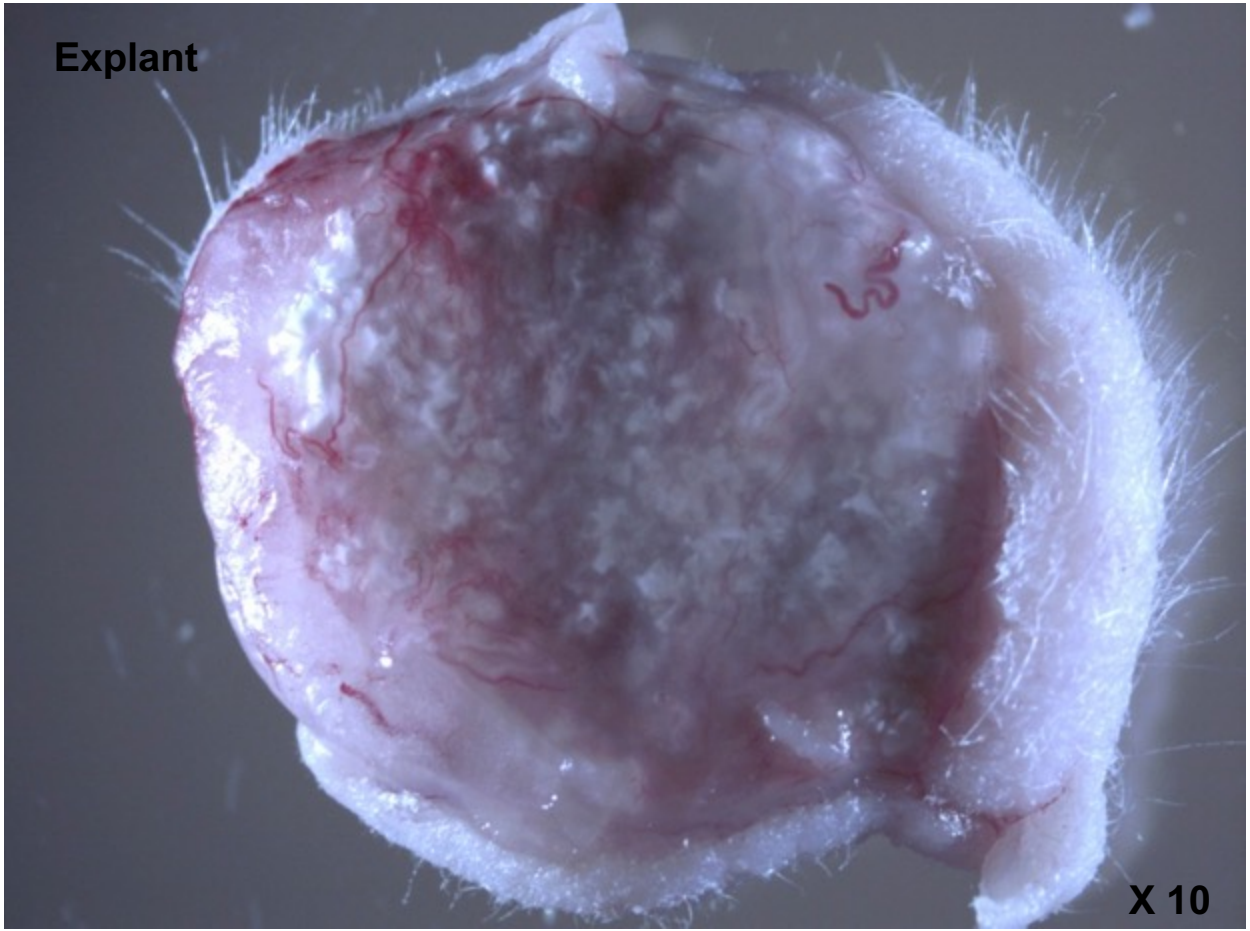


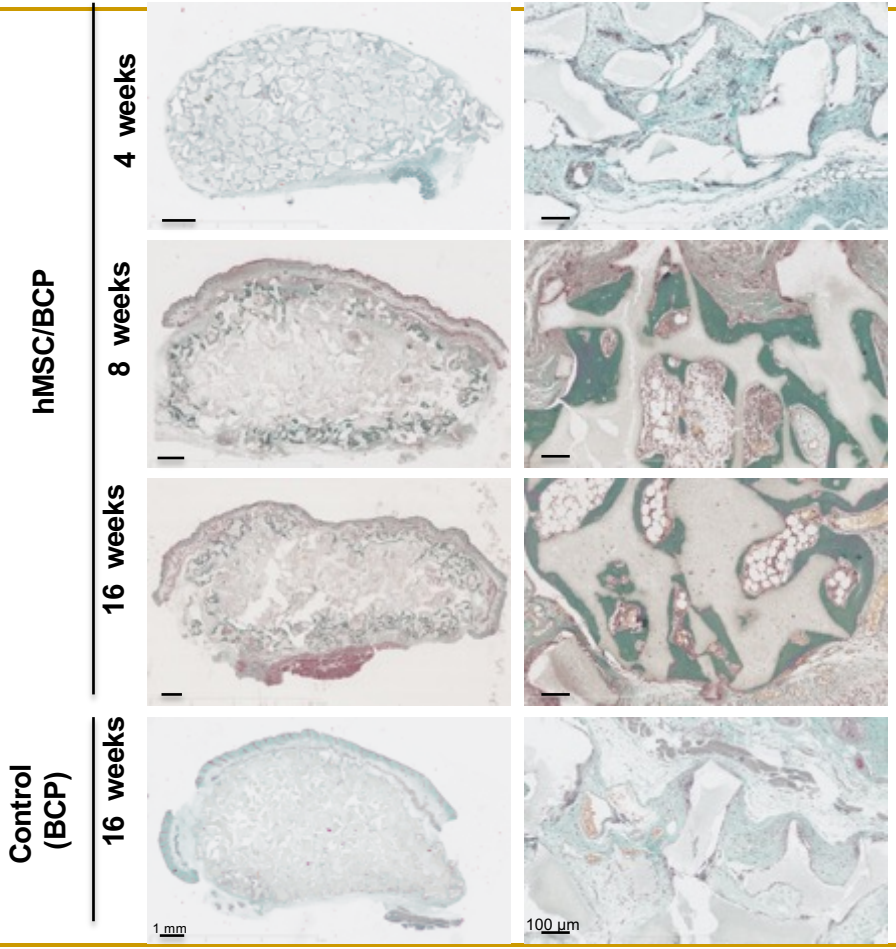
Methylene blue



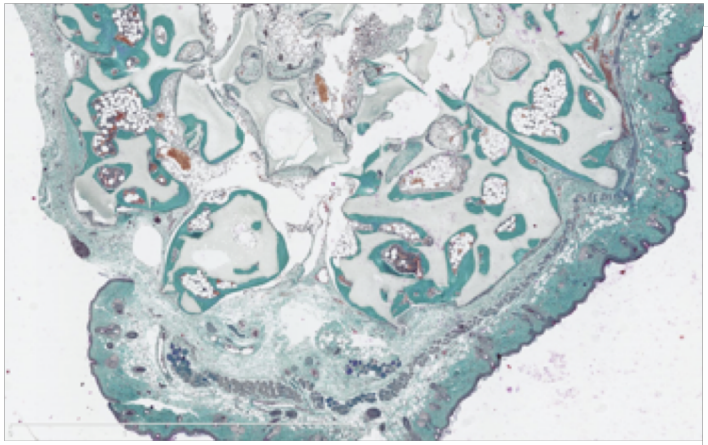
Subcutis implantation
in nude mice for 8 weeks

Histology

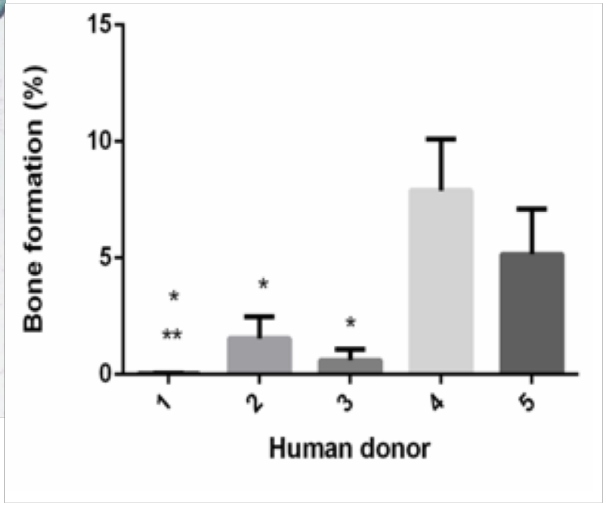

Explant
X 10


hMSC/BCP
4 weeks
8 weeks
16 weeks
Control (BCP)
16 weeks
1 mm
100 µm

Histology

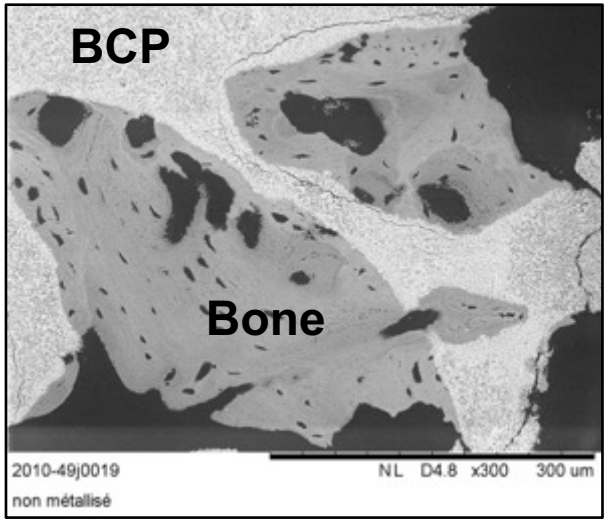


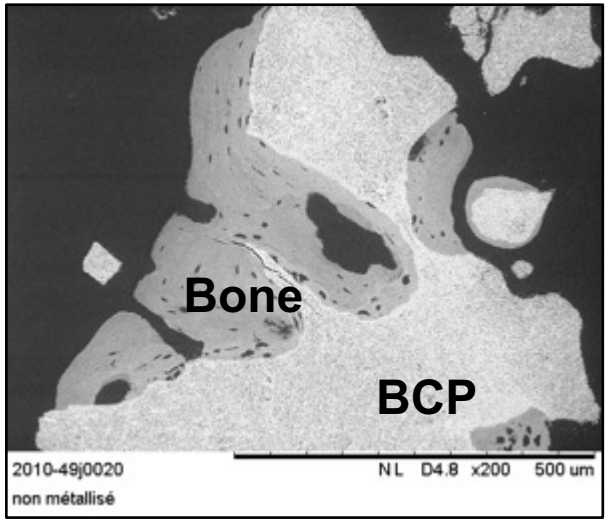
Goldner trichrome staining



Ectopic bone formation for 12 donors,
but high variability between donors

BSEM

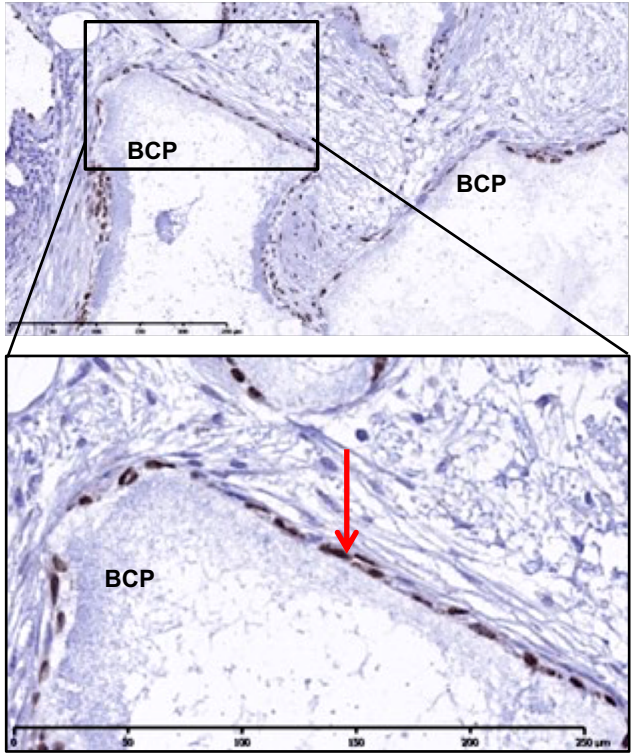




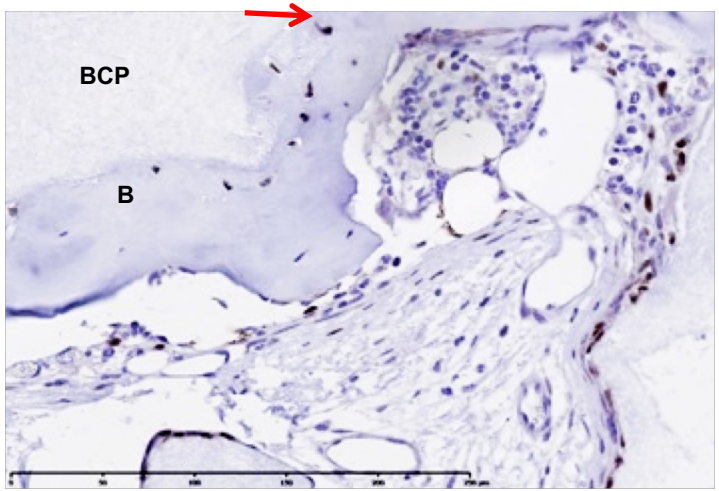
Brennan et al. Stem Cell Res Ther, 2014

hMSC engraftment

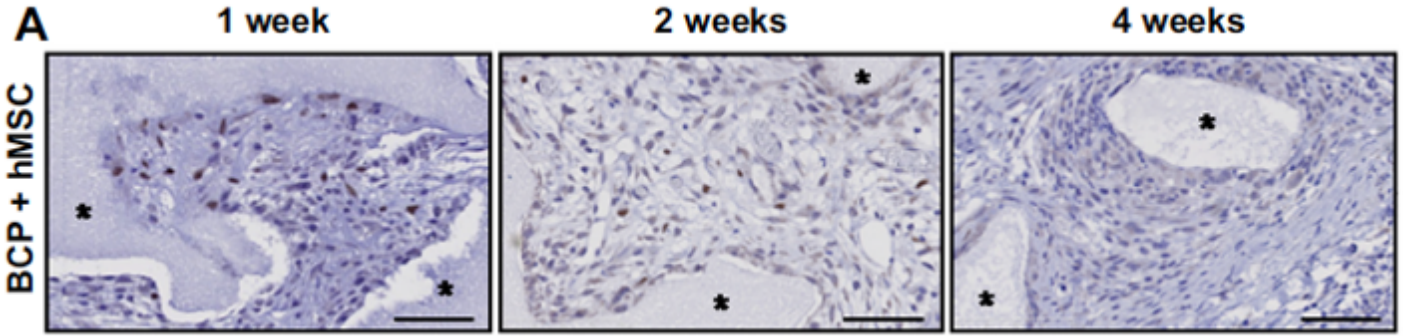
2x10^6 hMSC with 50mg BCP implanted subcutaneously for 8 weeks in nude mice

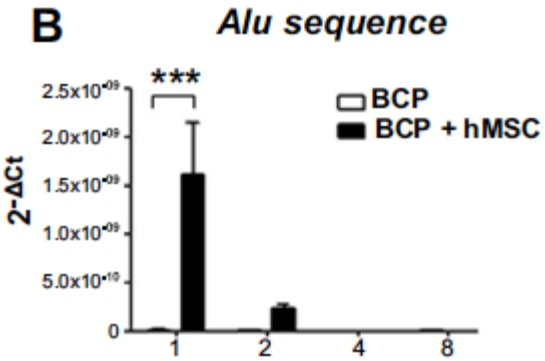


In situ hybridization using the human-specific repetitive *Alu* sequence for identification of human cells (brown –red arrow)



hMSC engraftment in muscle of nude mice

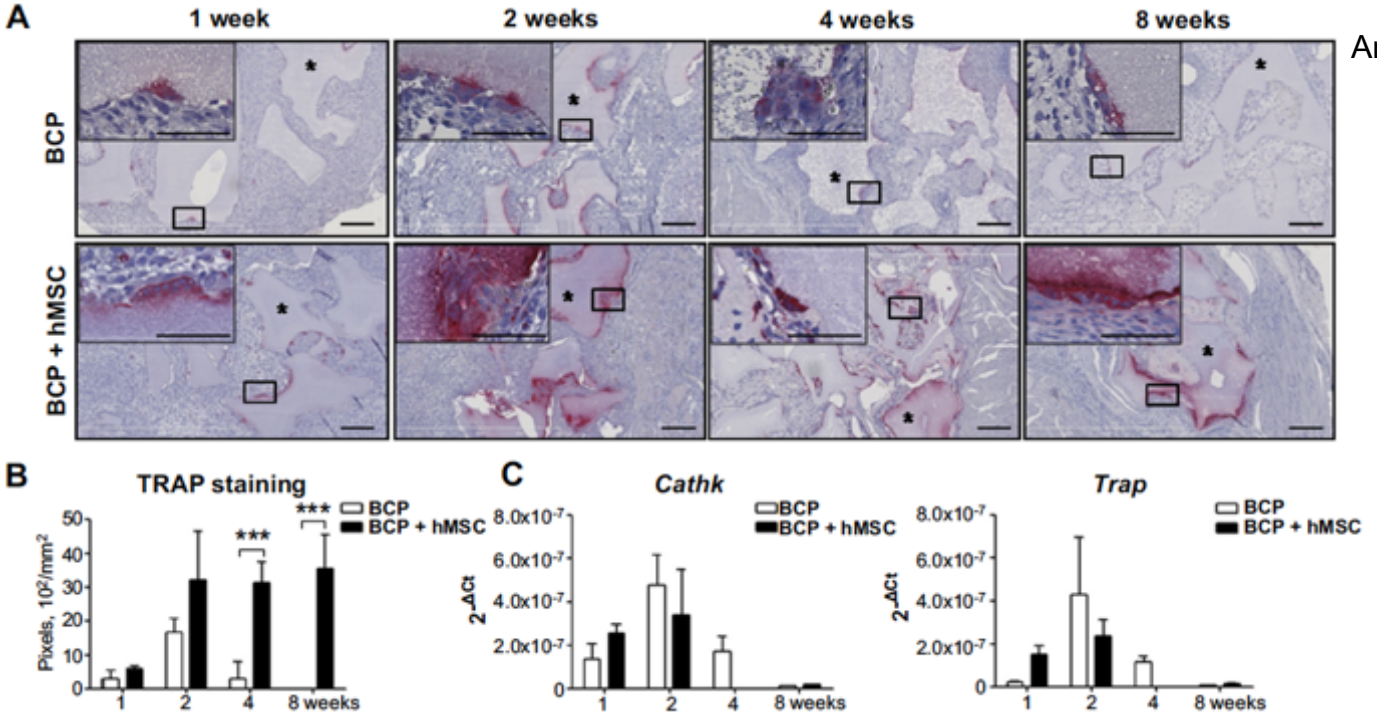




Gamblin et al. Biomaterials 2014

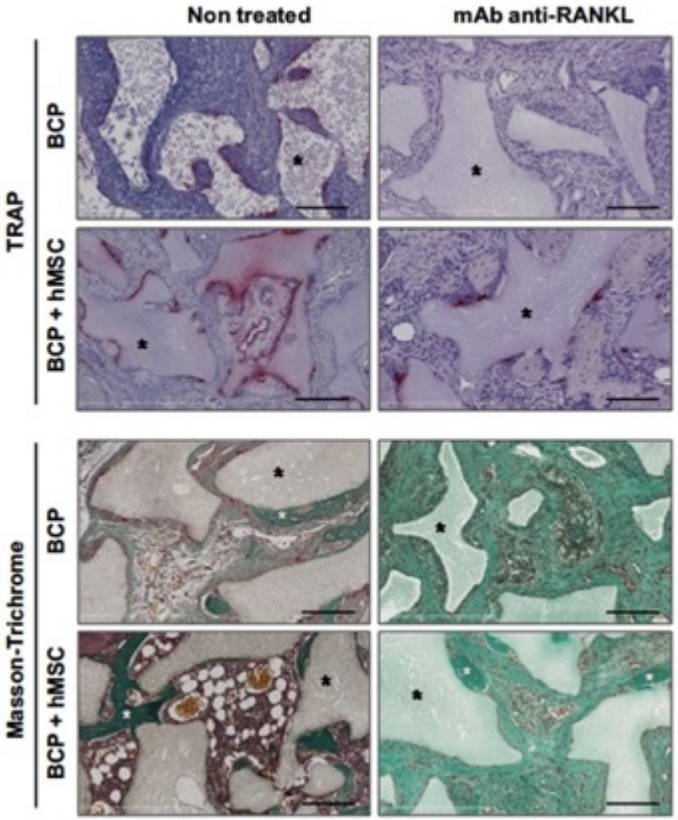
Osteoclastic recruitment with hMSC

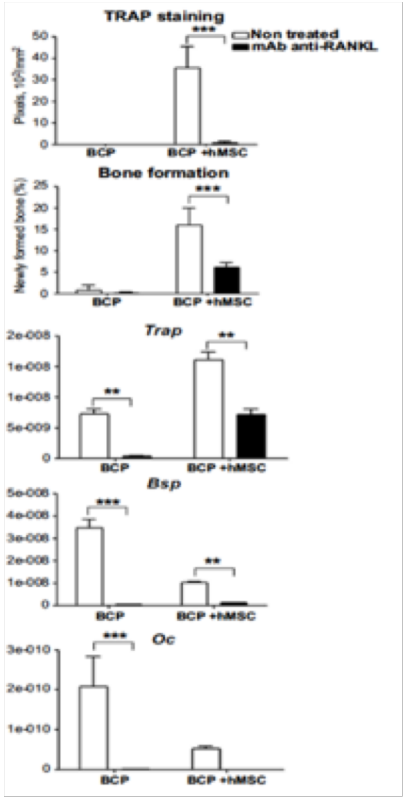
Anne-Laure Gamblin



Gamblin et al. Biomaterials 2014

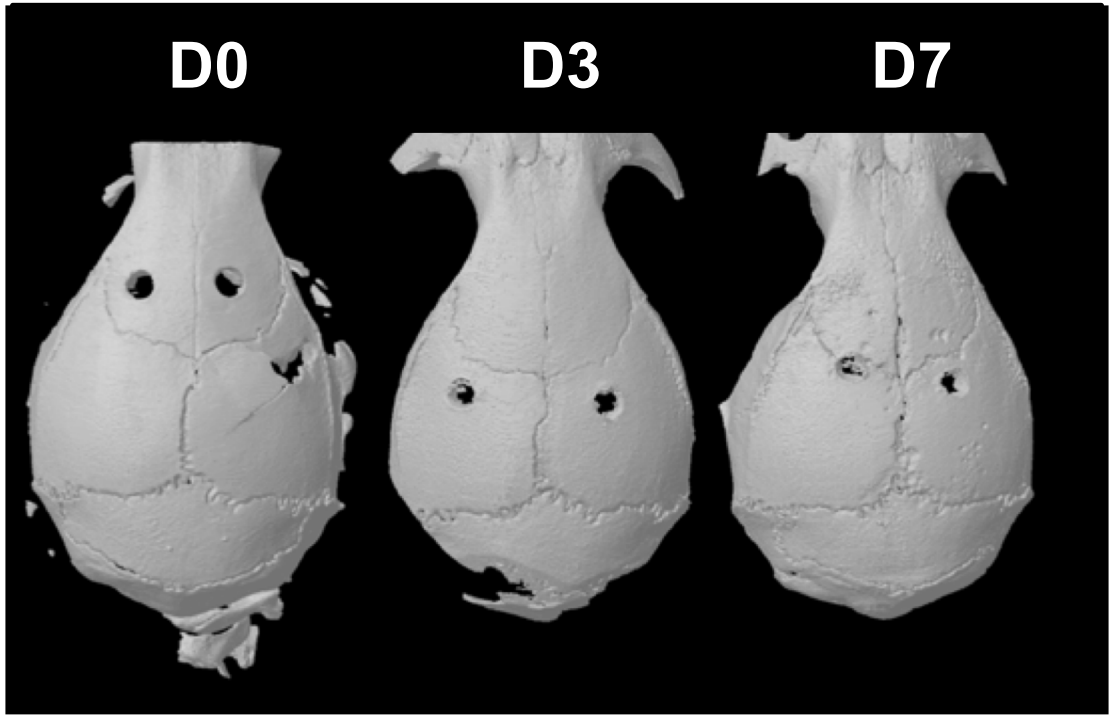
Anti-RANKL treatment decreases bone



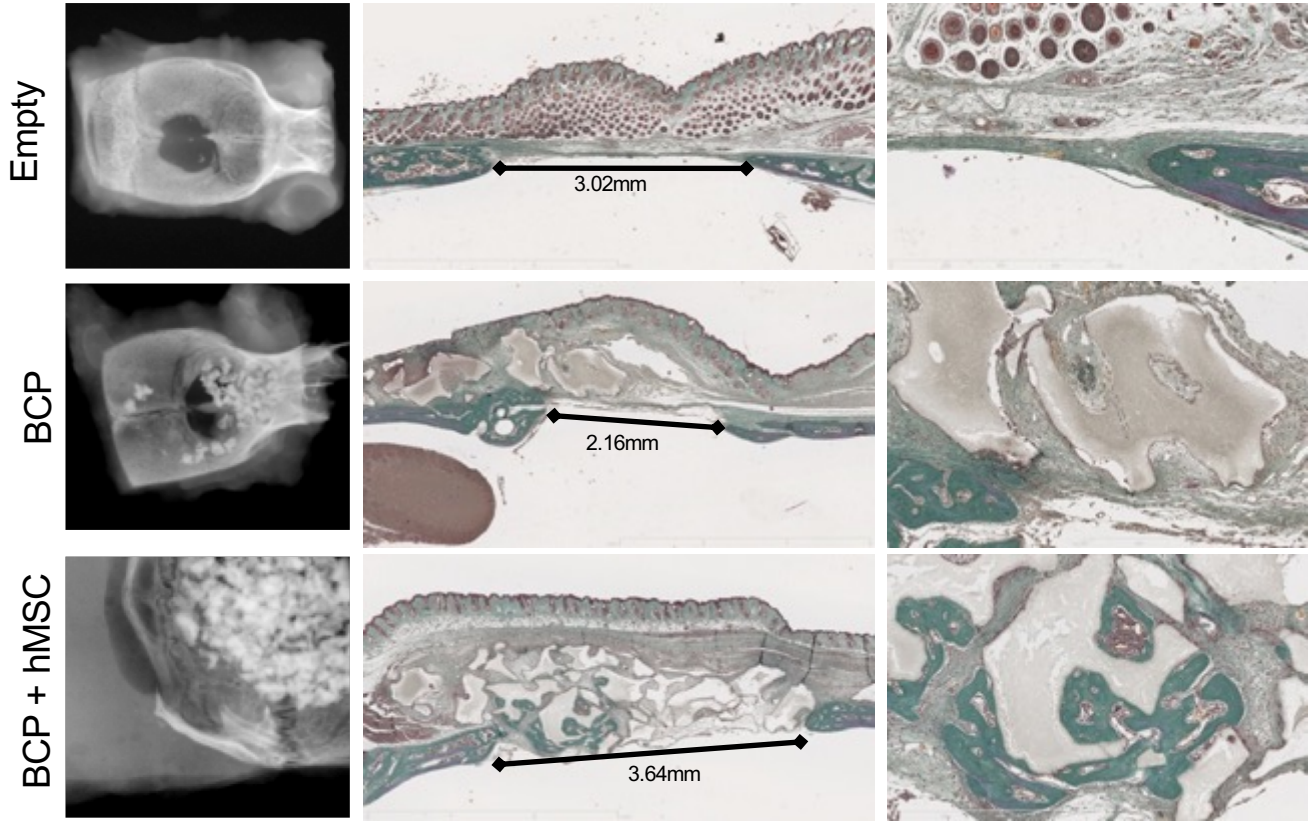


Bone regeneration with hMSC/BCP?

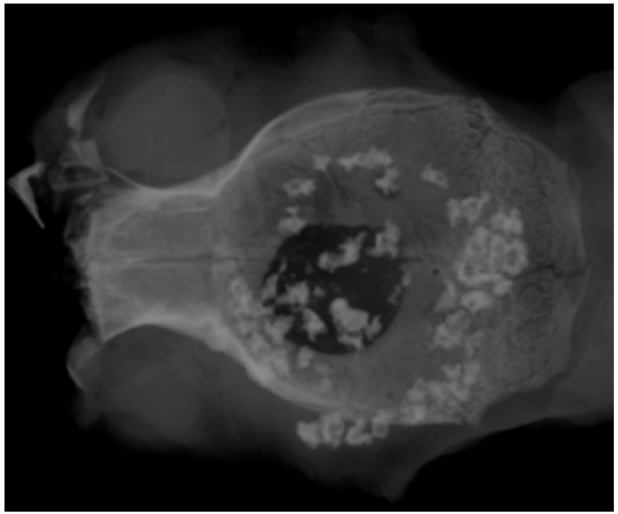
Calvaria defect (1 mm) in nude mice

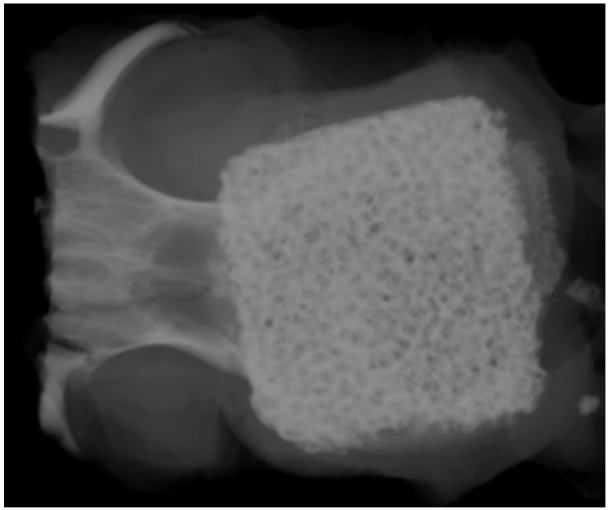


Calvaria defects (4 mm) at 8 weeks

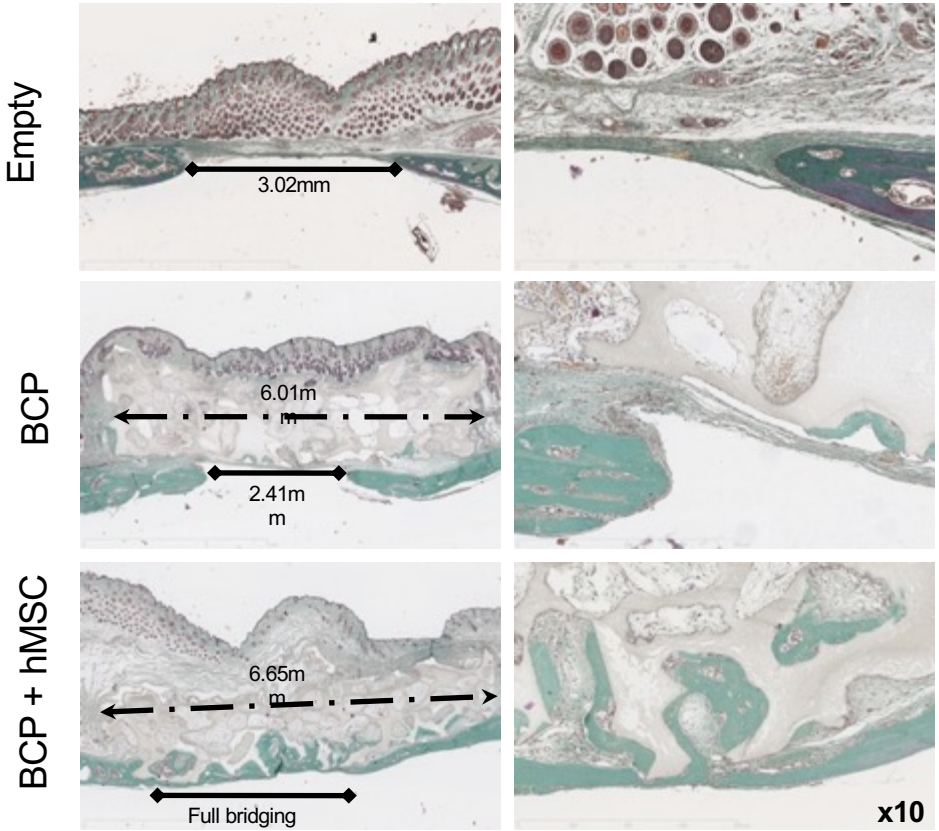


Calvaria defects (4 mm) in nude mice





Histology at 8 weeks



Submission to Medicinal agencies

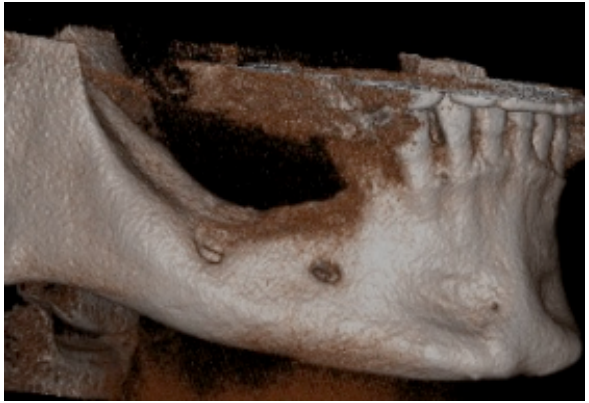
Folders for Paul Ehrlich Institute (Germany)

Maxillo 1

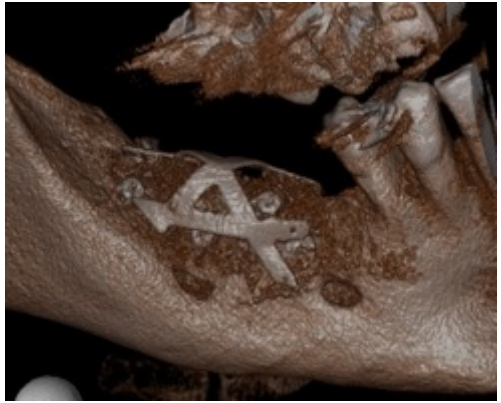
Reborne

- Bone augmentation prior to dental implants

Pre-op



Post-op



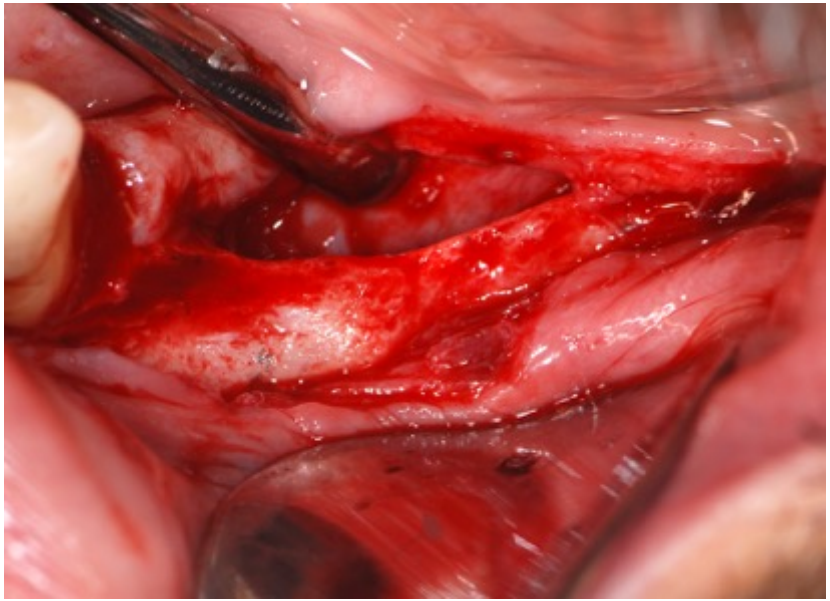
Prof. Sølve Hellem, Dr Cecilie Gjerde ; Univ Bergen
Completed study with 11 patients

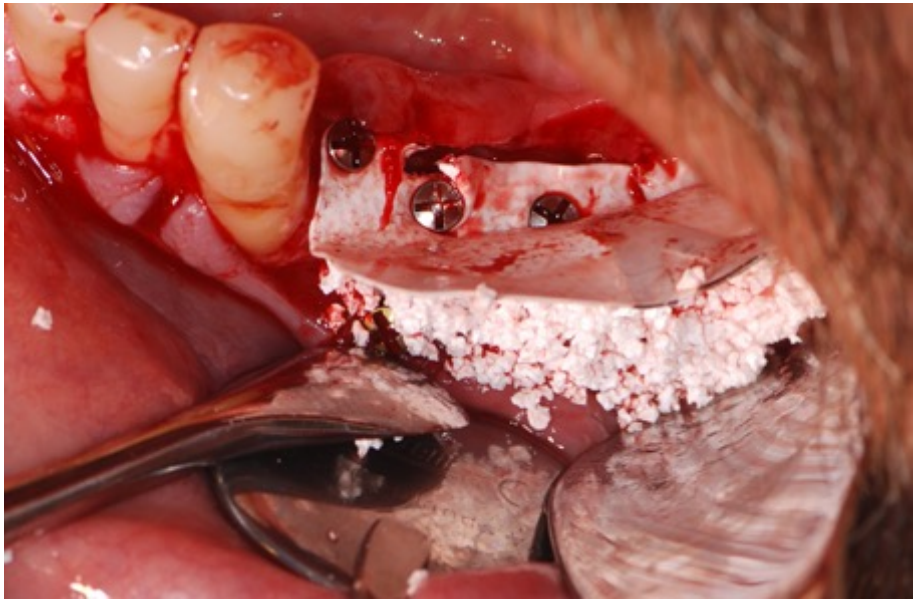
Gjerde et al. Stem Cell Res Ther, 2018

Maxillo 1

Reborne

- Bone augmentation prior to dental implants

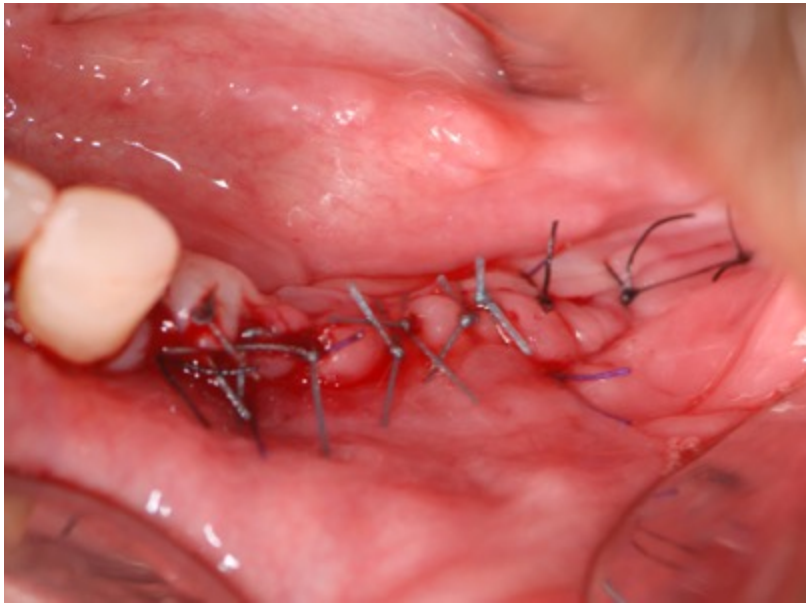




Maxillo 1

Reborne

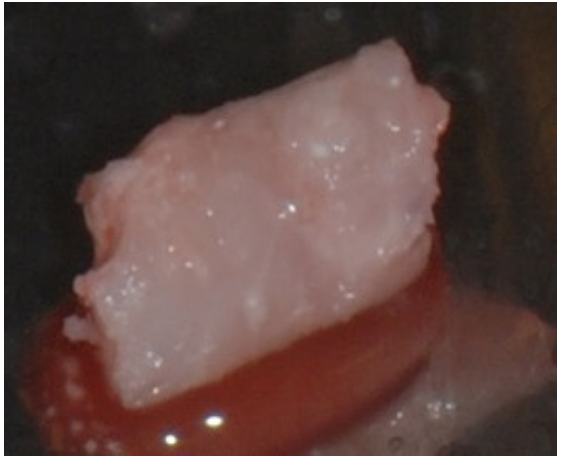
- Bone augmentation prior to dental implants

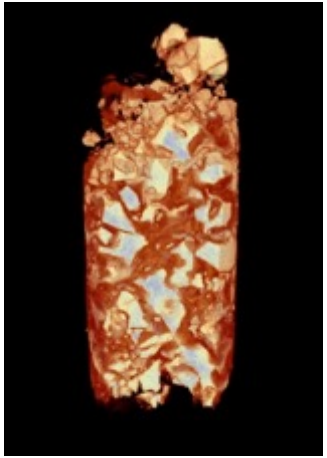


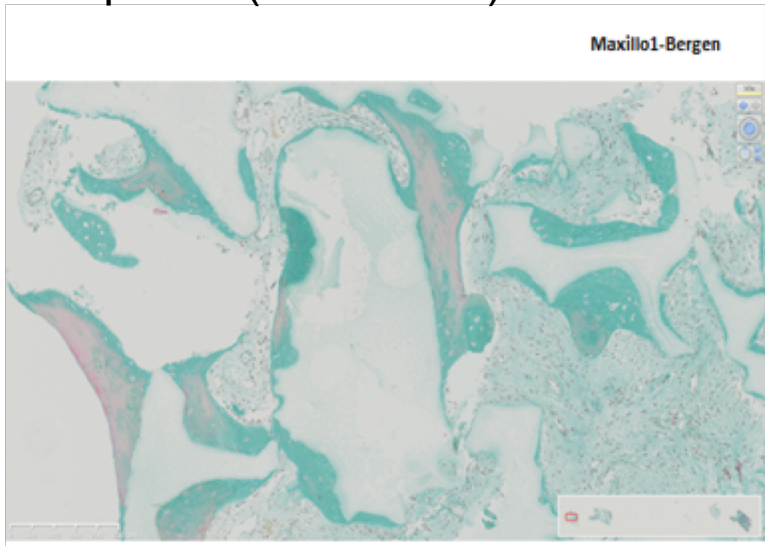
Maxillo 1

Reborne

- Biopsies taken at insertion of dental implants (+5 months)



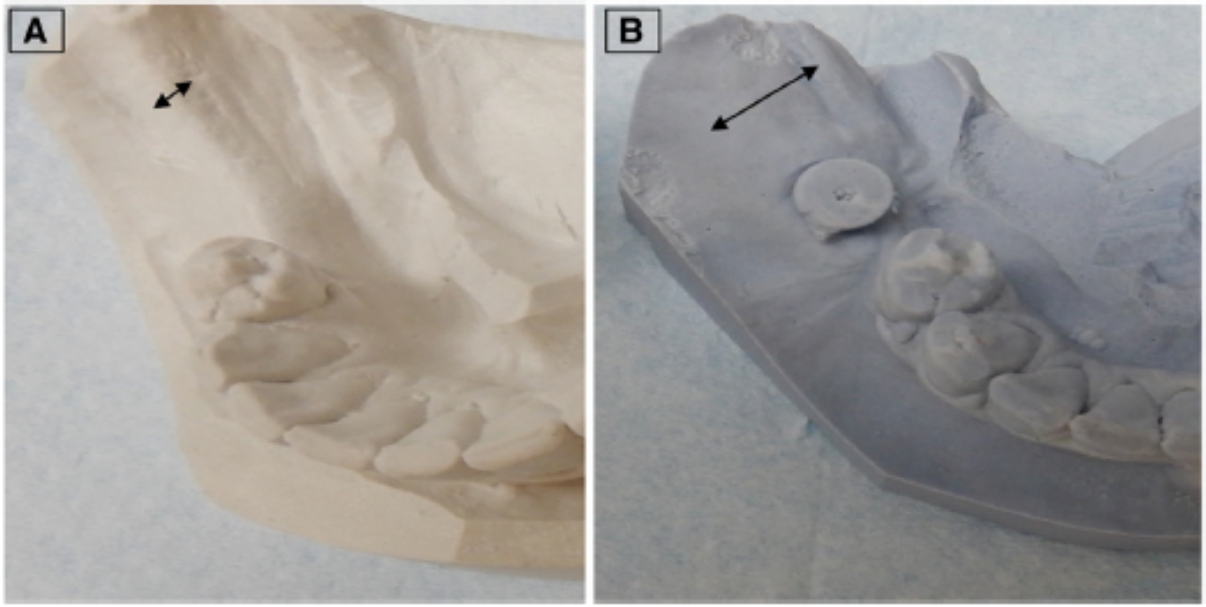




Bone formation in contact to BCP granules (=14)

Gjerde et al. Stem Cell Res Ther, 2018

Maxillo 1



CBCT analysis
mean increase in bone width : 4.05 mm
mean increase in volume : 887.2 mm^3

Maxillo 1

Reborne

functional dental implants after 3 years (n=14)

Gjerde et al. Stem Cell Res Ther, 2018

Maxillo 1

Reborne

Gjerde *et al. Stem Cell Research & Therapy* (2018) 9:213
https://doi.org/10.1186/s13287-018-0951-9

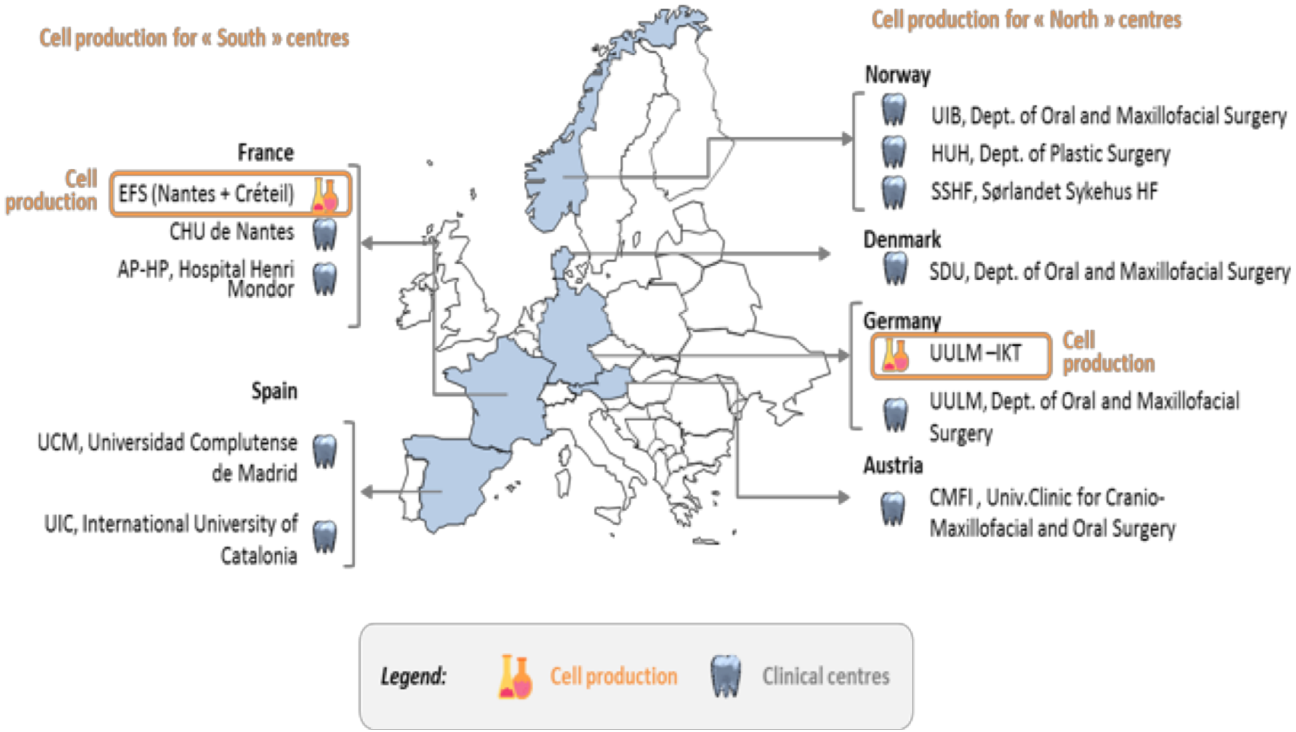
Stem Cell Research & Therapy

RESEARCH Open Access

Cell therapy induced regeneration of severely atrophied mandibular bone in a clinical trial

Cecilie Gjerde[1*], Kamal Mustafa[1*], Sølve Hellem[1], Markus Rojewski[2,3], Harald Gjengedal[1], Mohammed Ahmed Yassin[1,4], Xin Feng[1], Siren Skaale[1], Trond Berge[1], Annika Rosen[1], Xie-Qi Shi[1], Aymen B. Ahmed[5,6,7], Bjørn Tore Gjertsen[5,6,7], Hubert Schrezenmeier[2,3] and Pierre Layrolle[8]

MAXIBONE – Cell production sites and clinical centers



6 million € 2018-2021

Randomized Controlled Trial

Primary objective:
to perform a randomized clinical study on 150 patients for maxillofacial bone augmentation with autologous MSC (ATMP) and BCP biomaterial (100 patients) versus autologous bone graft (50 patients) prior to dental implants

Sponsor: University of Bergen

Regulatory pathway : VHP to the Norwegian Medicine agency, EMA, National Ethical committees, submission in October 2018

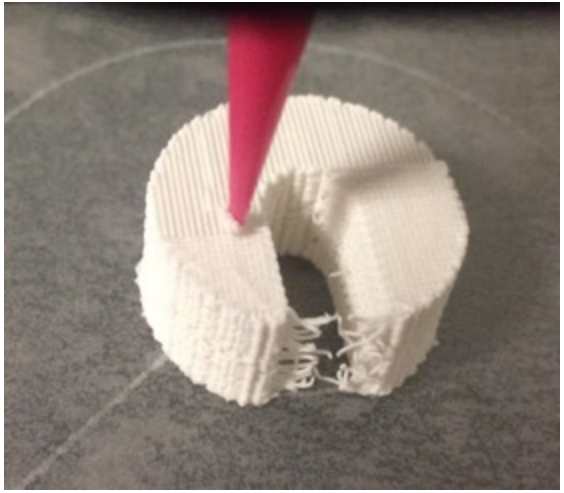
Begin of inclusions: March 2019 (20 patients/centre, 8 centres)

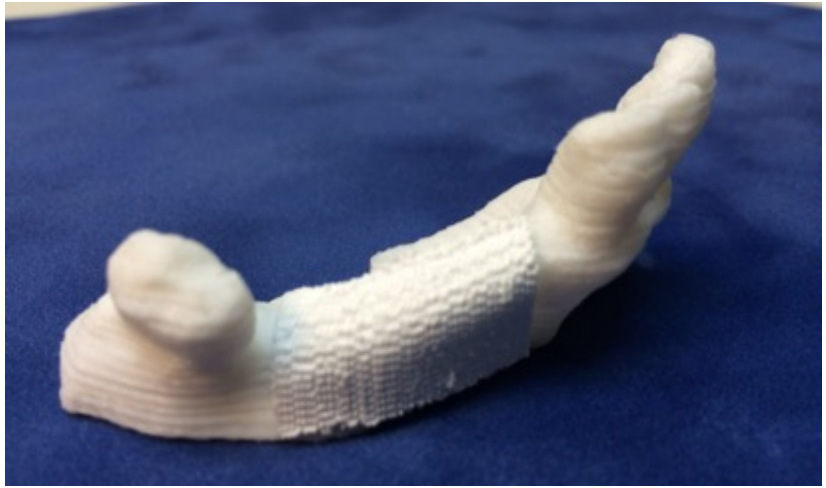
End of inclusion: March 2020

End of follow up and analysis of results (eCRF): June 2021

End of project: December 2021

3D printing of biomaterials from CT scans

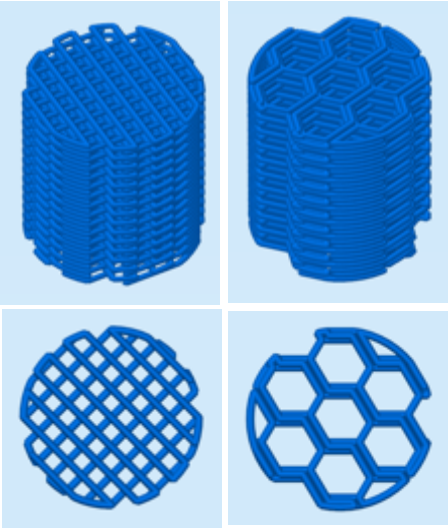


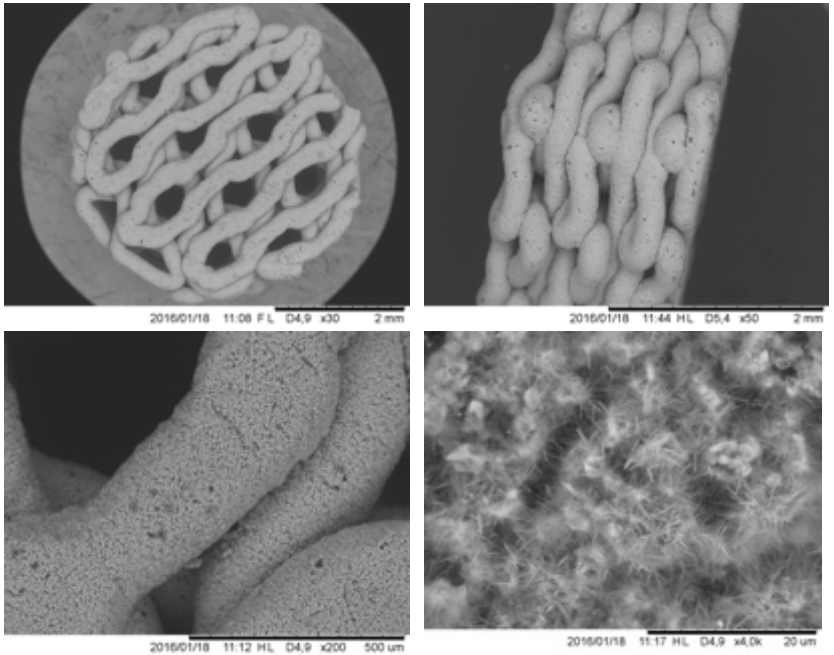


3D printing of biomaterials

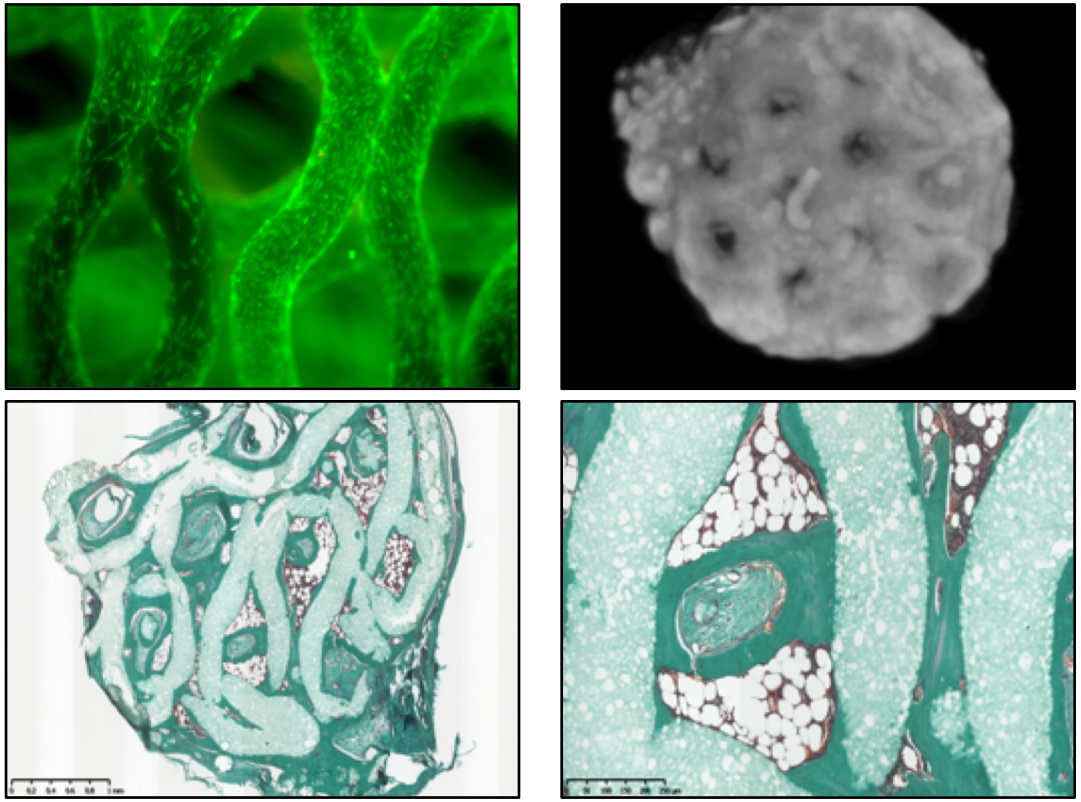
Control of shape, porosity, composition, cristallinity

Composition: CDA, CO3-AP
Porosiy: 60%, ortho et honeycomb





3D printed biomaterials + hMSC



Conclusions & perspectives

- 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 critical sized bone defects
- 4 multicentric clinical trials have been performed
  - 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
- Regeneration of large bone defects with personalized biomaterial and allogeneic cells

Acknowledgements

European Commission

Reborne

2010-2015
12 m€, 24 partners
4 clinical trials

ORTHOunion

2017-2020
6 m€, 11 partners
100 patients

Maxibone

2018-2021
6 m€, 12 partners
150 patients

MARIE CURIE ACTIONS

PARAGEN
2017-2019
246 k€