Klinikum rechts der Isar
Technische Universität München

In vivo experimental procedures

Summerschool Newgen, Patras, May 11th 2017

TraumaLab Munich

Experimental Trauma Surgery
Head: Prof. Martijn van Griensven, MD PhD

Something completely different?!?

You have heard talks on:

- Ceramics
- Glasses
- Metals
- Alloys
- Polymers
- Cements

- Bone
- biointerphase

Need to be tested....

Musculoskeletal Anatomy and Physiology

Anatomy

- Flat, Short, Long, Irregular bones
- Muscles – visceral, cardiac, skeletal
- Joints – freely & slight moveable, synovial fluid
- Cartilage, Ligaments, Tendons, Fascia, Bursae

Physiology

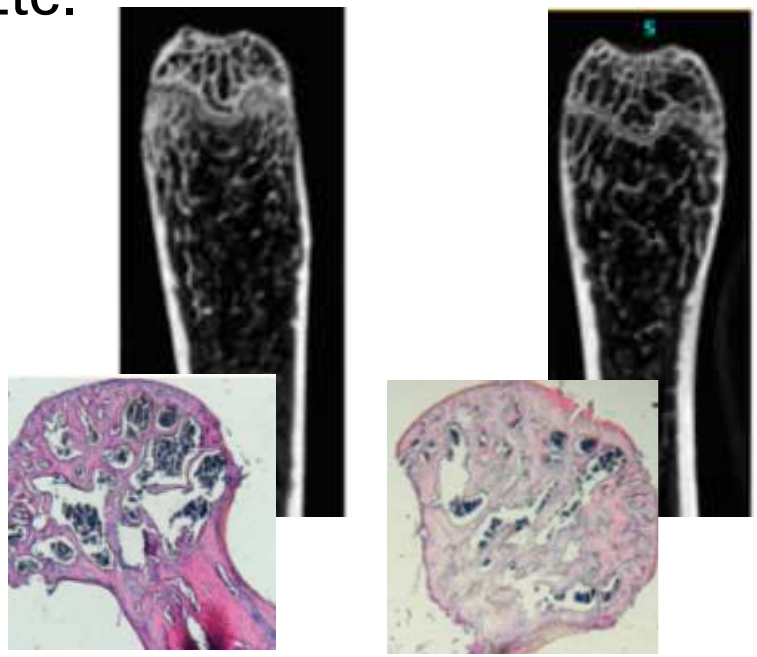
- Structure, shape, movement, protection, support, hematopoiesis

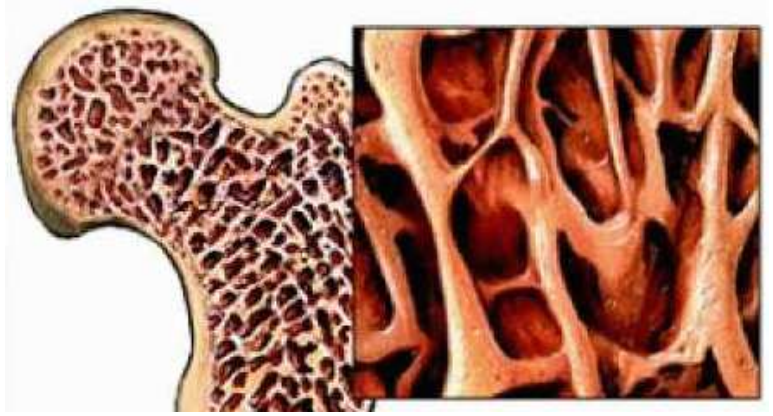
Aging & Co-morbidities In Bone Quality

Co-morbidities and bone healing

Increasing incidence of co-morbidities

- Age
- Osteoporosis
- Diabetes mellitus
- Etc.





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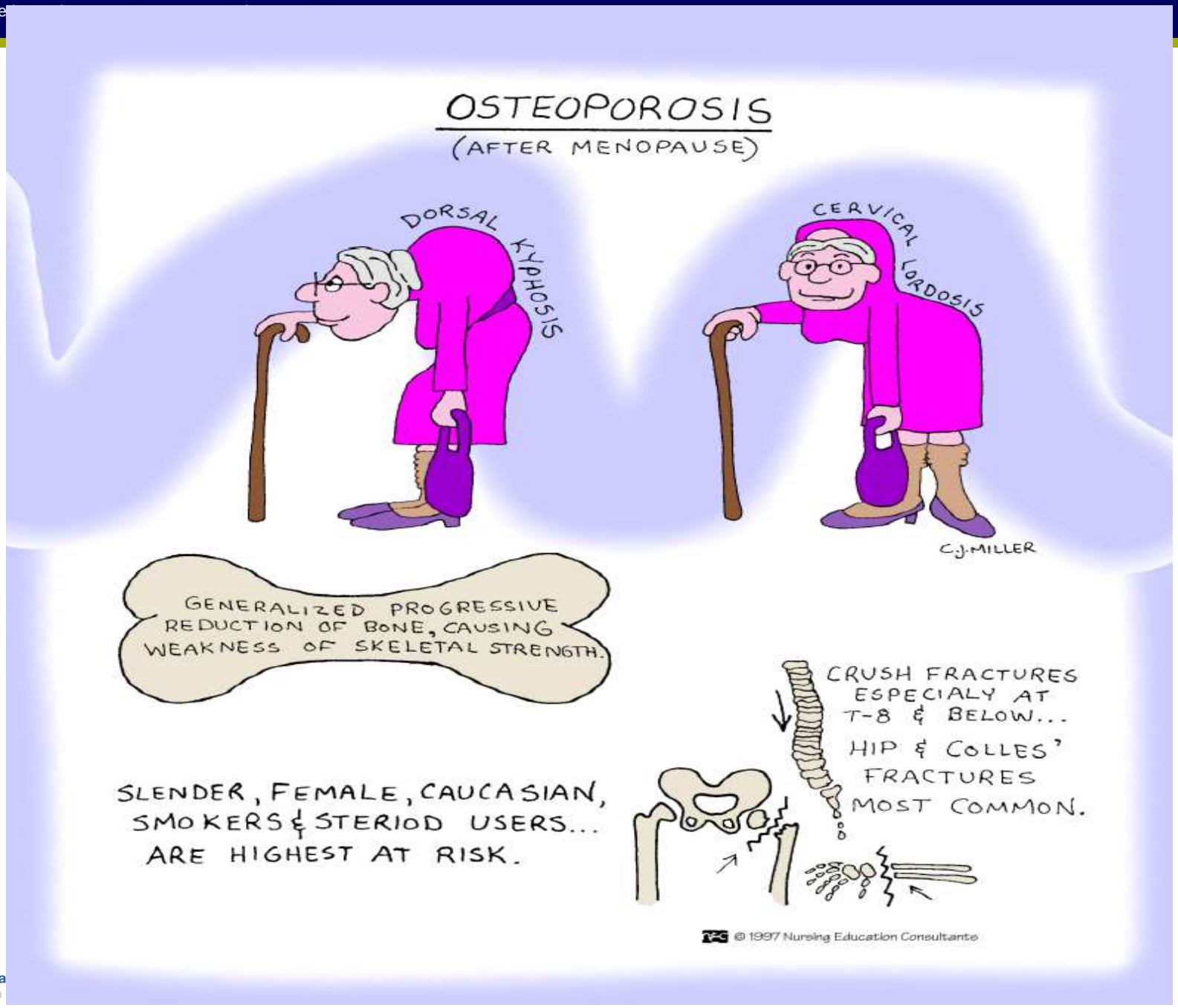

OSTEOPOROSIS
(AFTER MENOPAUSE)
DORSAL KYPHOSIS
CERVICAL LORDOSIS
C.J. MILLER
GENERALIZED PROGRESSIVE REDUCTION OF BONE, CAUSING WEAKNESS OF SKELETAL STRENGTH.
SLENDER, FEMALE, CAUCASIAN, SMOKERS & STERIOD USERS... ARE HIGHEST AT RISK.
CRUSH FRACTURES ESPECIALY AT T-8 & BELOW...
HIP & COLLES' FRACTURES MOST COMMON.
© 1997 Nursing Education Consultants

Bone Injury

- many different insults
  - Trauma
  - Infection
  - Tumor
  - metabolism
- Pathways involved in bone development also play an integral part in bone repair.

Nail Stabilization

- Ideal case – Femur or tibia
- Exchange nailing provides a good option for the tibia and femur
- Special equipment is often necessary to traverse sclerotic canals

Plate Stabilization

- Plates provide a powerful reduction tool
- Surgical technique should strive for absolute stability
- Locking plates have improved stability and fixation strength
- Other relative indications:
  - Absent medullary canal
  - Metaphyseal nonunions
  - When open reduction or removal of prior implants is required

Non-union definition

- FDA: 9 months elapsed time with no healing progress for 3 months.
- Problems
  - Subjective
  - Arbitrary
- Pragmatic: A fracture that has no potential to heal without further intervention

Non-union incidence

- 5-10% of long bone fractures
- Relative Risk depends upon:
  - Injury
  - Bone
  - Patient
  - Treatment

Tibial intramedullary nailing for nonunion

- Indications:
  - Correctable alignment
  - Demonstrated biology
  - Reconstructable canal
- Relative Contraindications
  - Previous infected pin sites
  - History of infection

Exchange nailing for tibial nonunion

- Indicated for isthmic fractures that are not infected
- Increase nail diameter by 2mm
- 95% success rate*
- Bone loss >50 circumference is a relative contraindication

*Zelle et al J Trauma 2004

Plating tibial nonunions

- Indications
  - No canal
  - Stiff deformity
  - Prior external fixation
  - Need for graft
- Relative Contraindications
  - Poor soft-tissues

Thus: not everything is perfect

There is a need to optimize the

treatment of large bone defects

including delayed- and non-union

"Virtually every major medical advance for both humans and animals has been achieved through biomedical research using animal models to study and find a cure for a disease and through animal testing to prove the safety and efficacy of a new treatment."

C. Everett Koop, M.D

Former U.S. Surgeon General

Why are animals used in research?

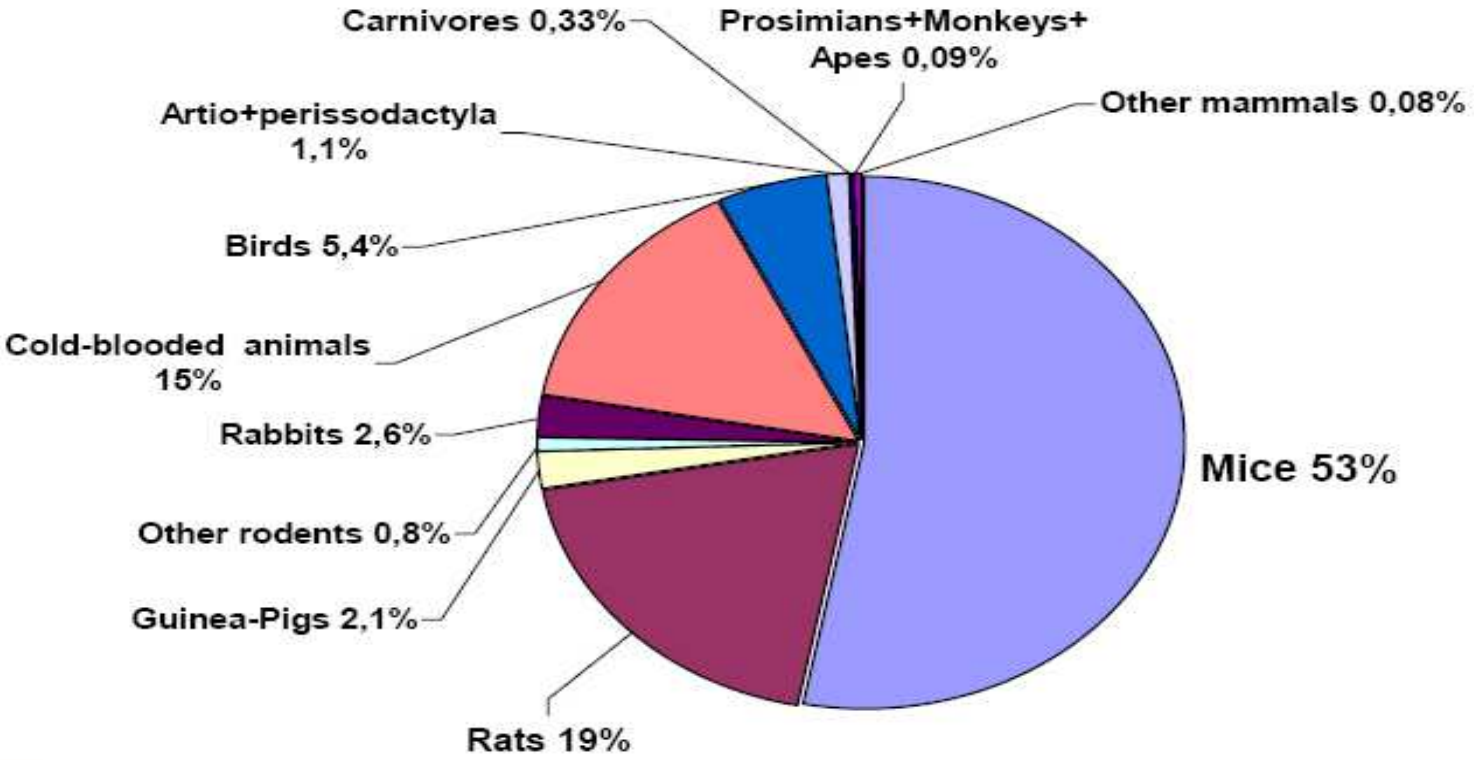
- organs and body systems similar to humans and other animals
- susceptible to the same diseases that affect humans
- short life span allows animals to be studied throughout their entire life
- environment easily controllable to keep experimental variables to a minimum

What animals are used in research?

- mice are used in research more often than any other animal species
- These mice, *plus* other rodents such as rats and hamsters, make up more than 90% of the total number of animals used
- Other animal species, *including* dogs, cats, rabbits, farm animals, fish, frogs, birds, nonhuman primates, and many others, make up the remaining 10% of animals used in research.

Europe 2005

Figure 1.1
Percentages of animals used by classes by the reporting Member States



Europe 2005

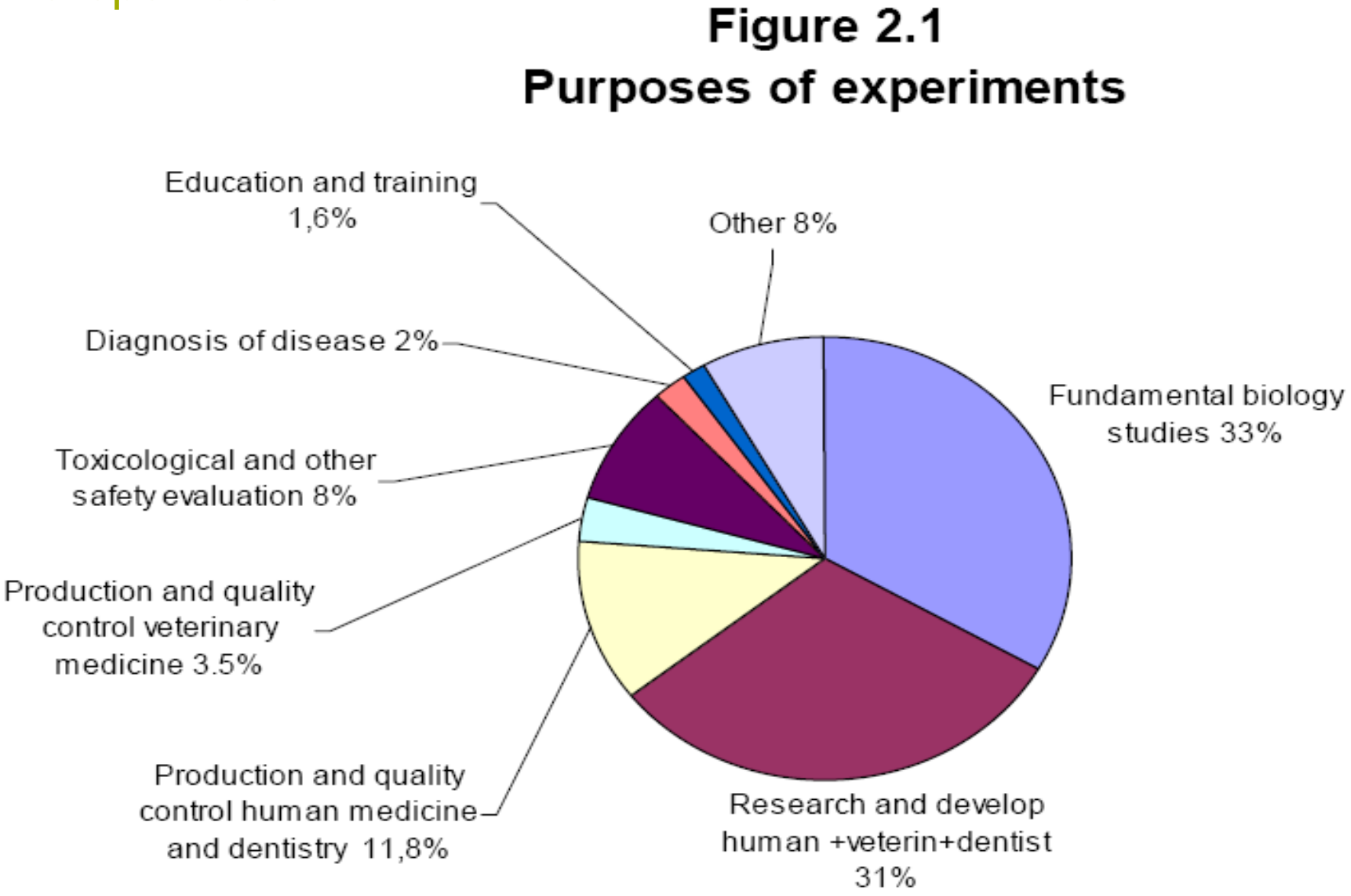
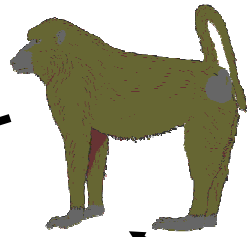
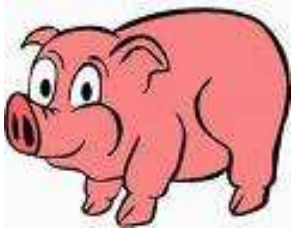
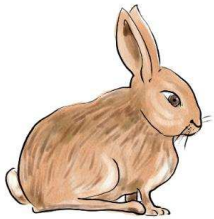


Figure 2.1
Purposes of experiments
Education and training 1,6%
Other 8%
Diagnosis of disease 2%
Fundamental biology studies 33%
Toxicological and other safety evaluation 8%
Production and quality control veterinary medicine 3.5%
Production and quality control human medicine and dentistry 11,8%
Research and develop human +veterin+dentist 31%

Animal models







3 R's

Replacement

Reduction

Refinement

Animal models are artificial

For an effective model,

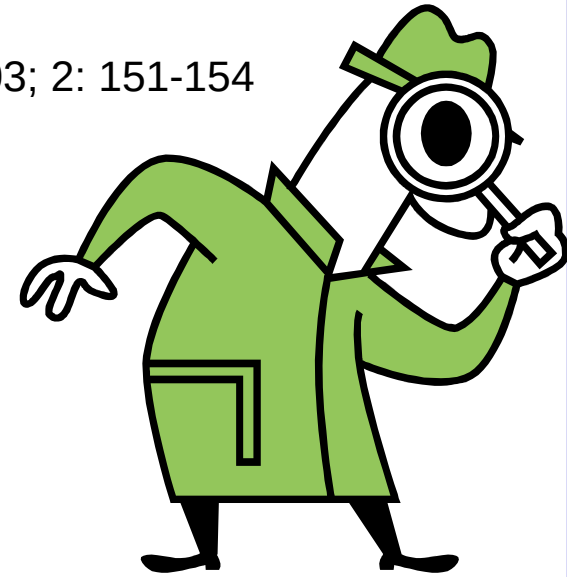
3 conditions must be met:

Full understanding of the animal model

Full understanding of the human disease

The above two cases must be substantially congruent in all important respects

David F. Horrobin, MD, PhD *Nature Reviews Drug Discovery*. 2003; 2: 151-154



Why Animal Experiments Fail

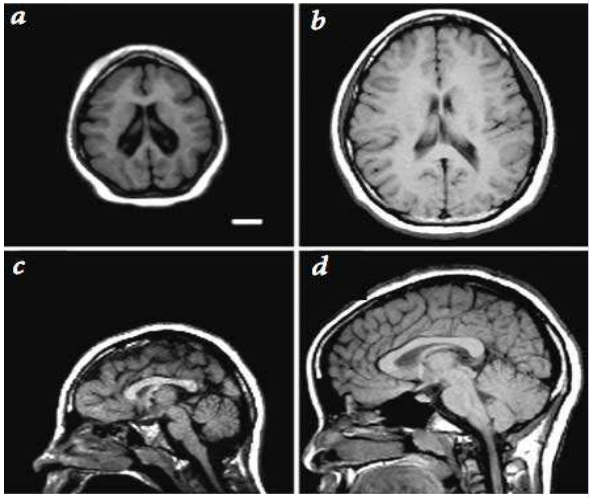
Even *minor* differences in physiology and anatomy can lead to *profound* differences in disease pathology and treatment effectiveness

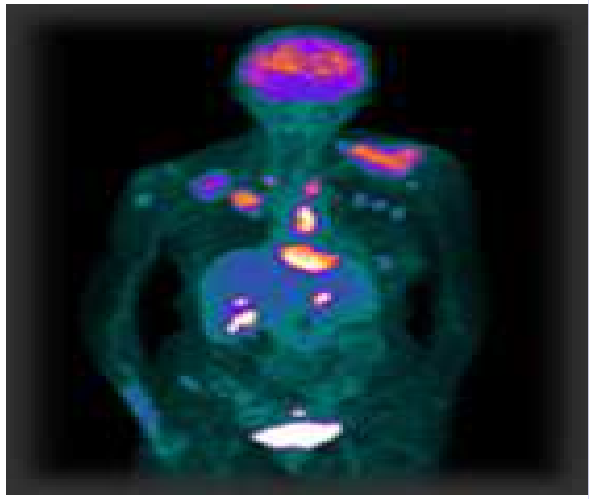
For a research model/method to work, it must be

predictive of human physiology and disease

What if we don’t test on animals?

- Epidemiological studies
- Computer modeling
- Human cell and tissue culture
- Post-mortem studies
- Genetic microarrays
- Human non-invasive studies
- Clinical Trials





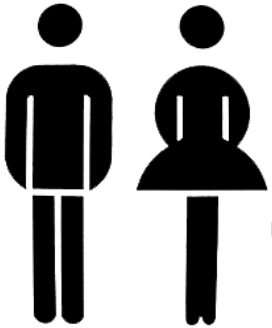
Which model for the bone area?

- Dependent on the research question
  - mechanism of bone healing
  - functionality
- Dependent on the locus of bone-healing
  - Dental („non weight bearing“)
  - Orthopaedic („weight bearing“)

Load bearing vs. Non-load bearing

Non-load bearing	Load-bearing
Material does not need to resist compression forces	Material needs to resist compression forces or is protected from them
Mainly flat bones; this results in nutrition of the bone defect by diffusion	Mainly long bones; large defects have long distances to the vasculature (i.e. more than the maximal 200 µm diffusion distance)
Mainly in the maxillofacial area; this means good soft tissue coverage	Soft tissue coverage can be problematic

van Griensven, Adv. Drug Deliv. Rev. 94:151-164, 2015



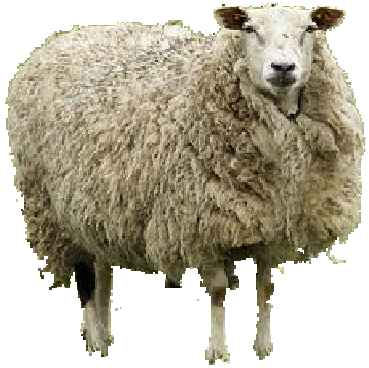
Load-bearing?

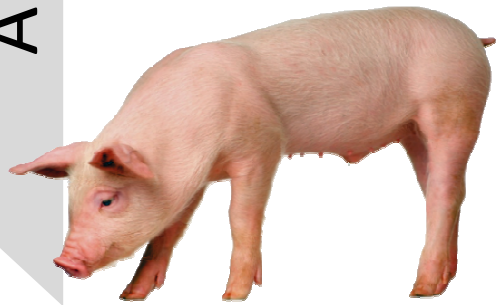
Non-load-bearing?

Mechanistic?

Functional?

ANIMAL MODEL





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van Griensven, Adv. Drug Deliv. Rev. 94:151-164, 2015

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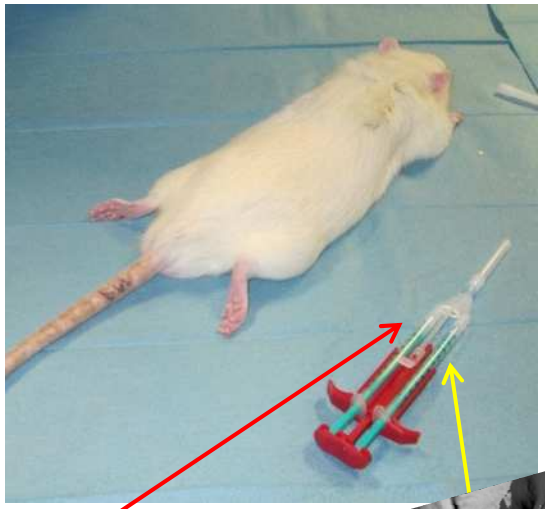
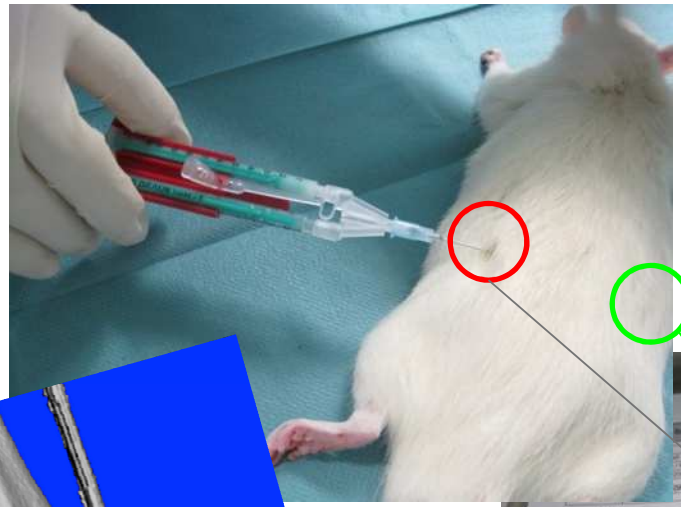
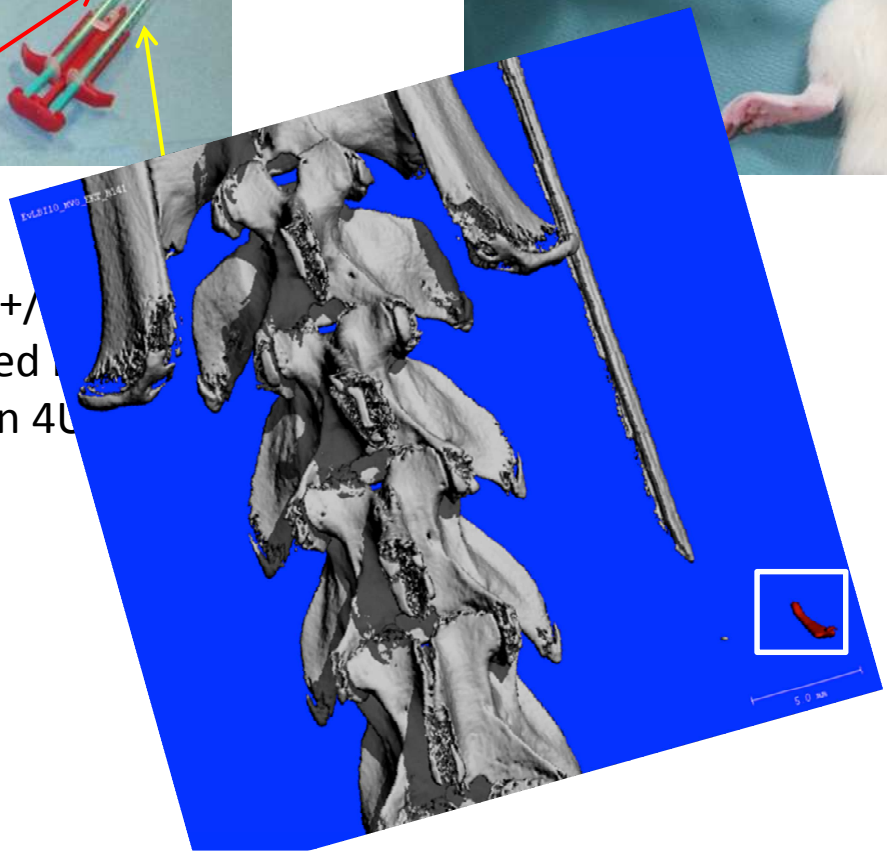
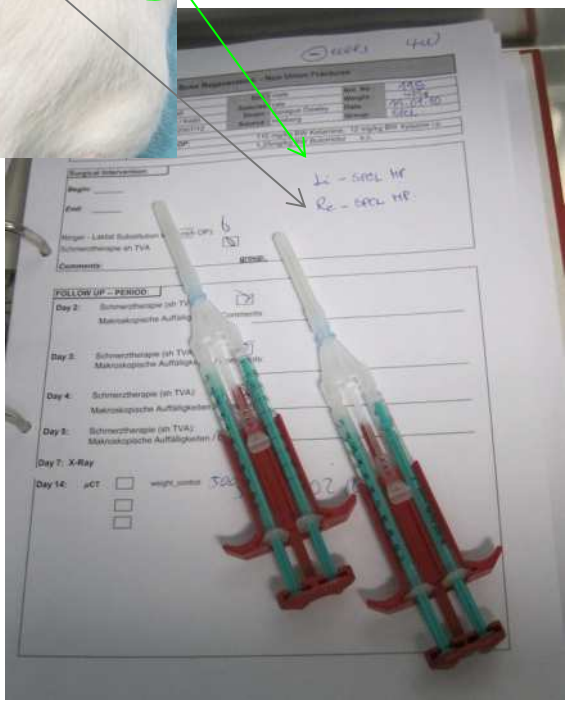
Ectopic implantation









Microparticles +/
hASC resuspended
150 µL Thrombin 4U

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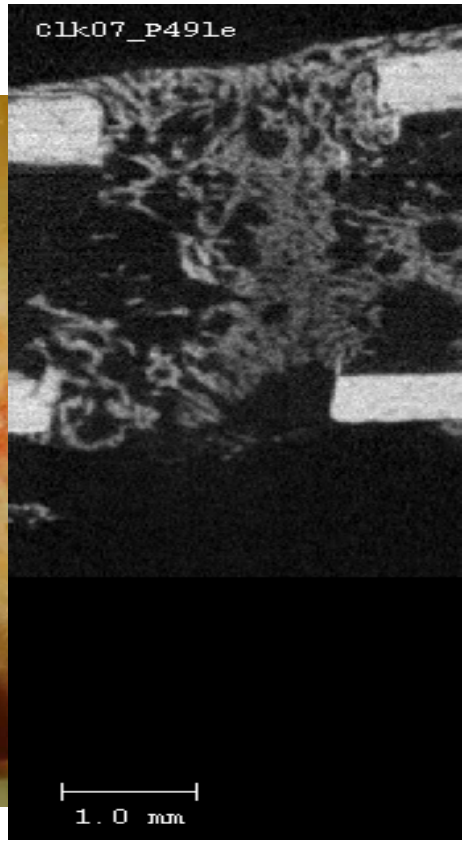
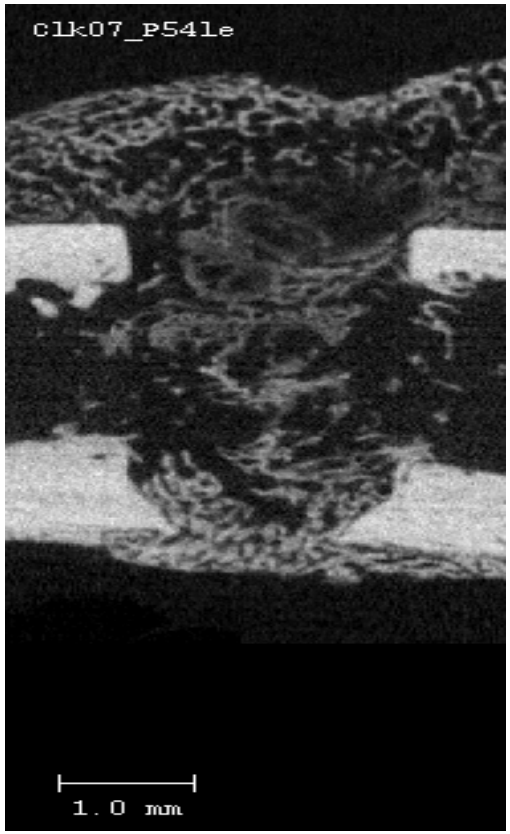
Drillhole rat femur



2 weeks



Fibrin + ASC + BMP-2

Fibrin + BMP-2

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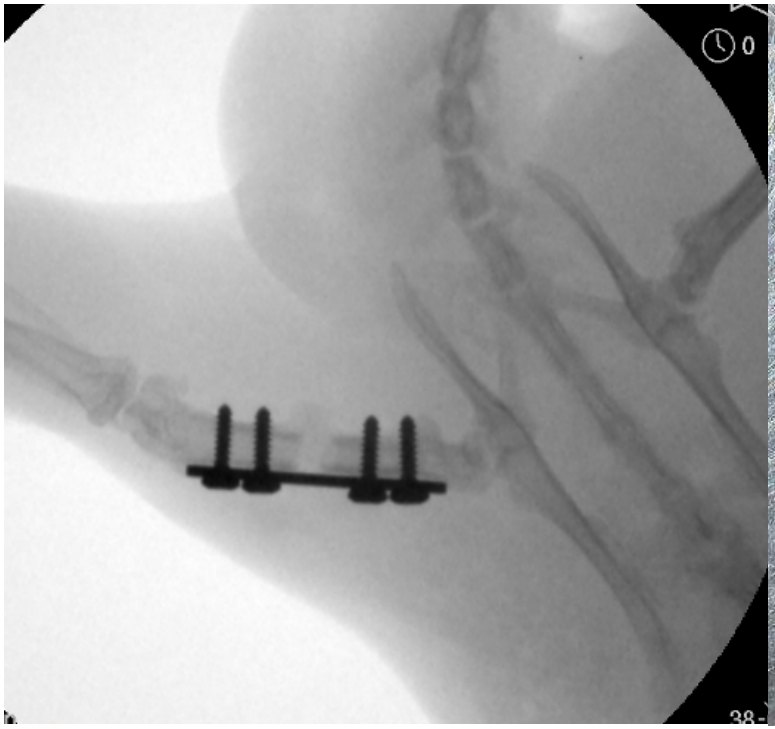
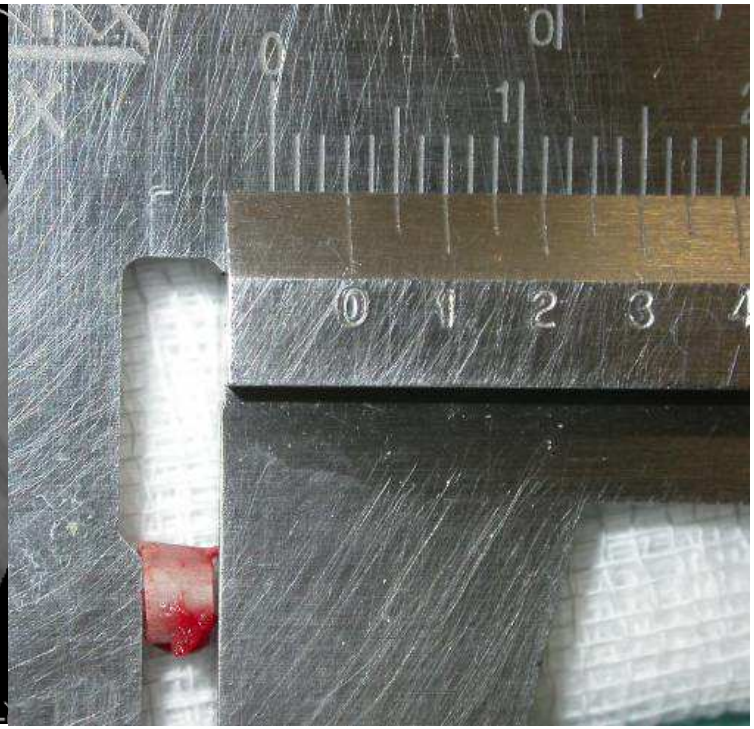
Keibl, …, van Griensven, Injury, 2011

Rat femur non-union

3mm defect, titanium plate,
4 cortical screws





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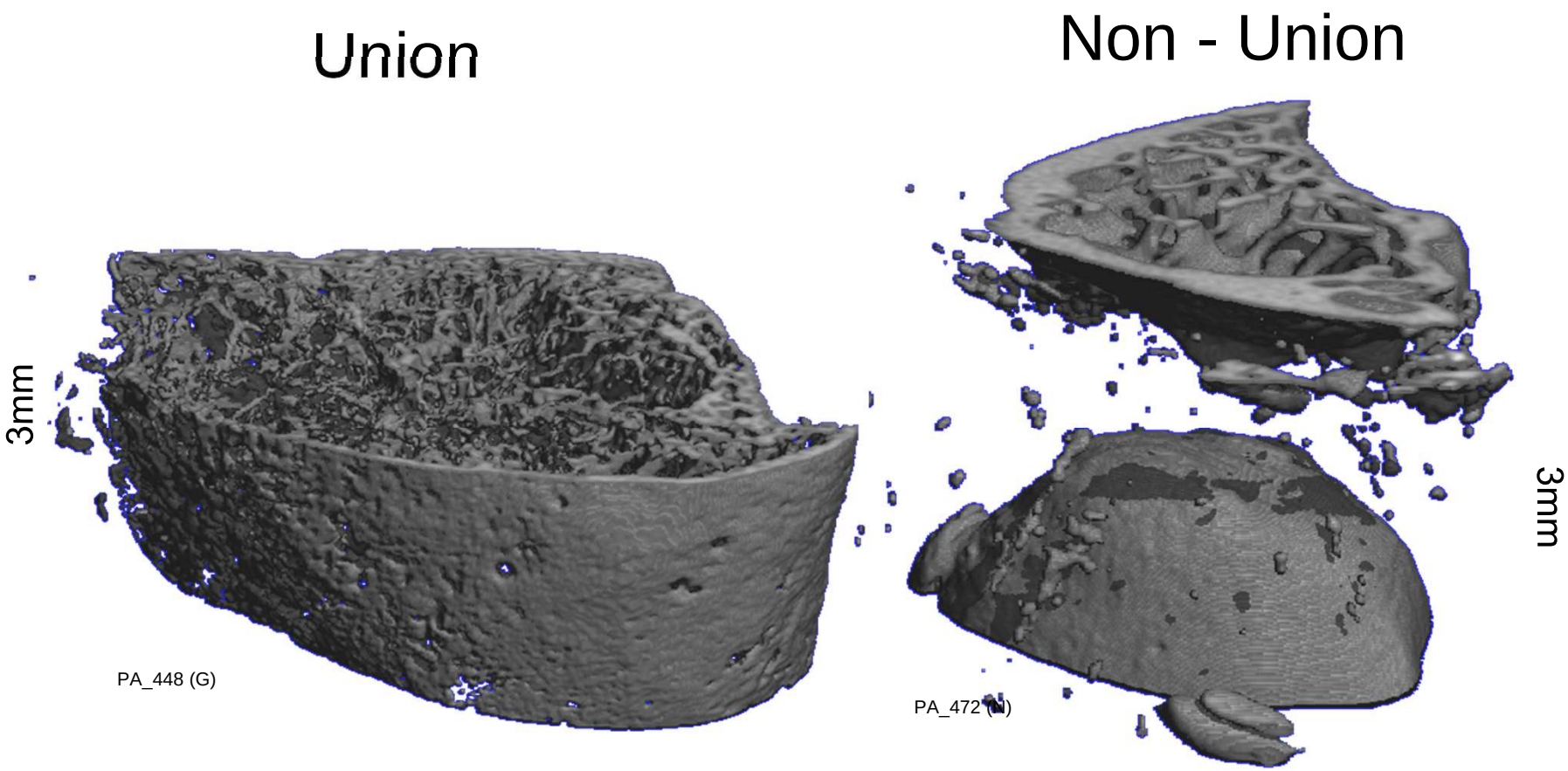
Schützenberger, …, van Griensven, et al. Arch Orthop Trauma Surg. 32(9):1363-1370, 2012

Bone regeneration with low dose BMP-2



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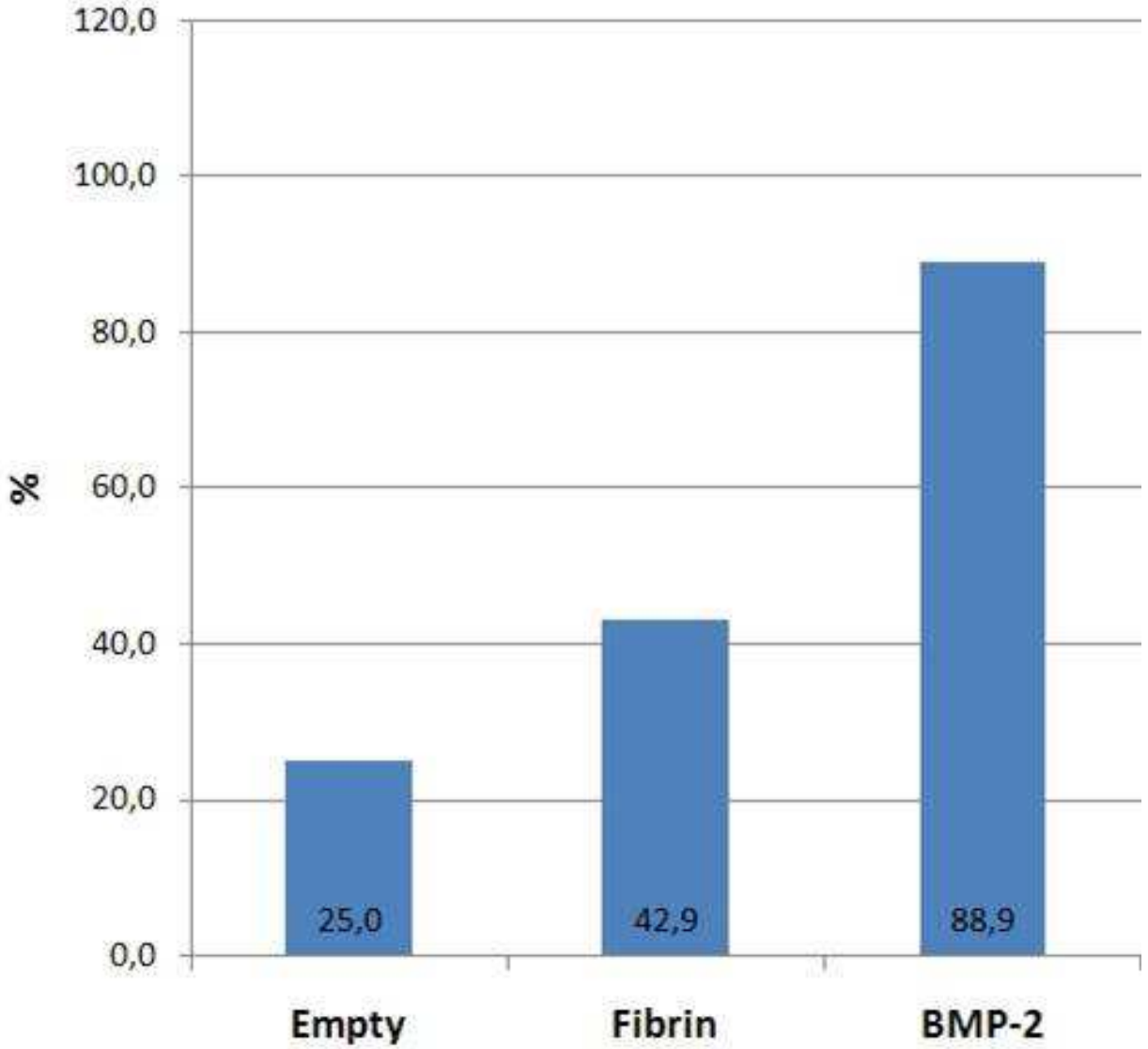
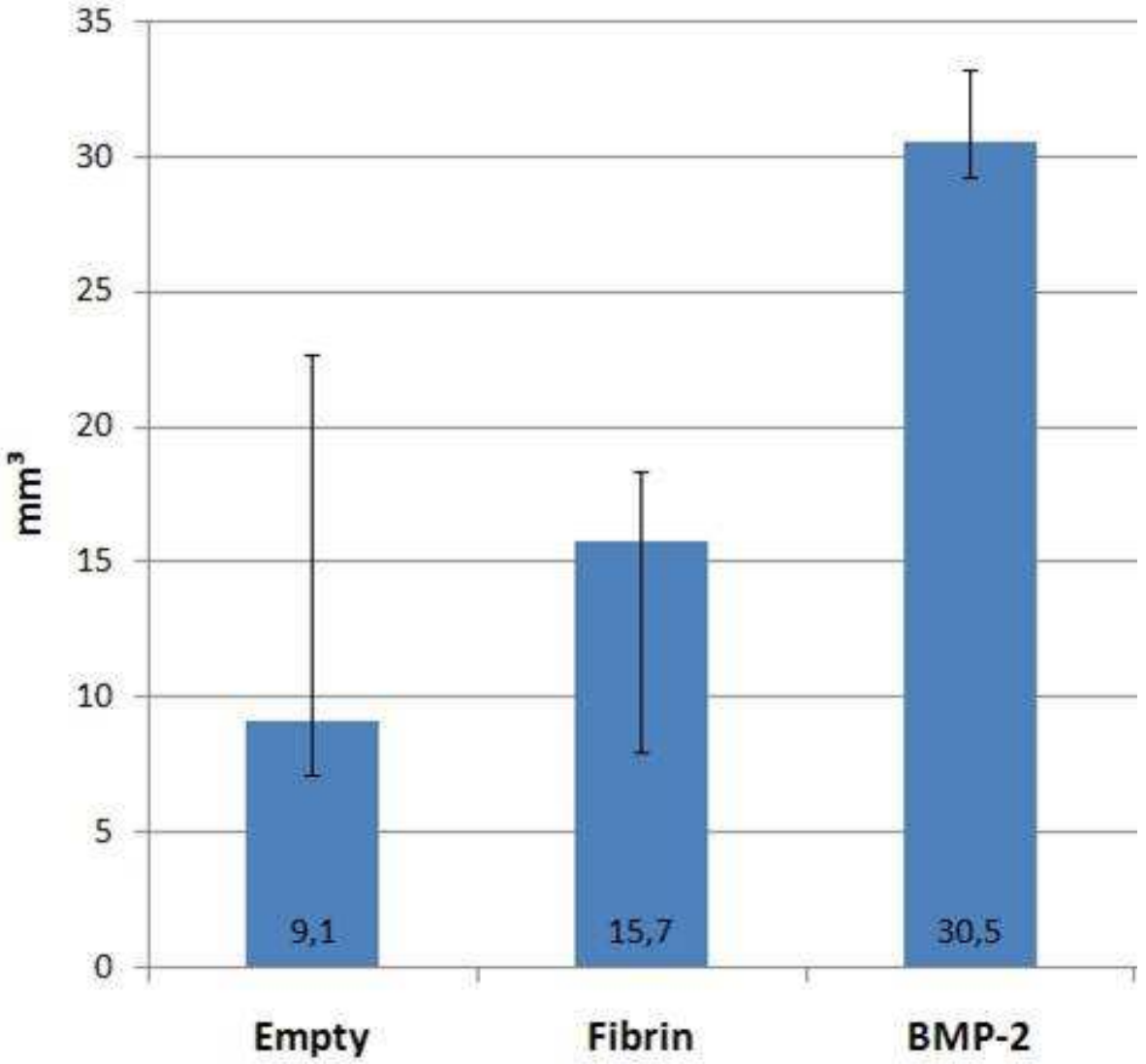
Schützenberger, …, van Griensven, et al. Arch Orthop Trauma Surg. 32(9):1363-1370, 2012

Percentage union



Schützenberger, …, van Griensven, et al. Arch Orthop Trauma Surg. 32(9):1363-1370, 2012

Bone volume (mm³)



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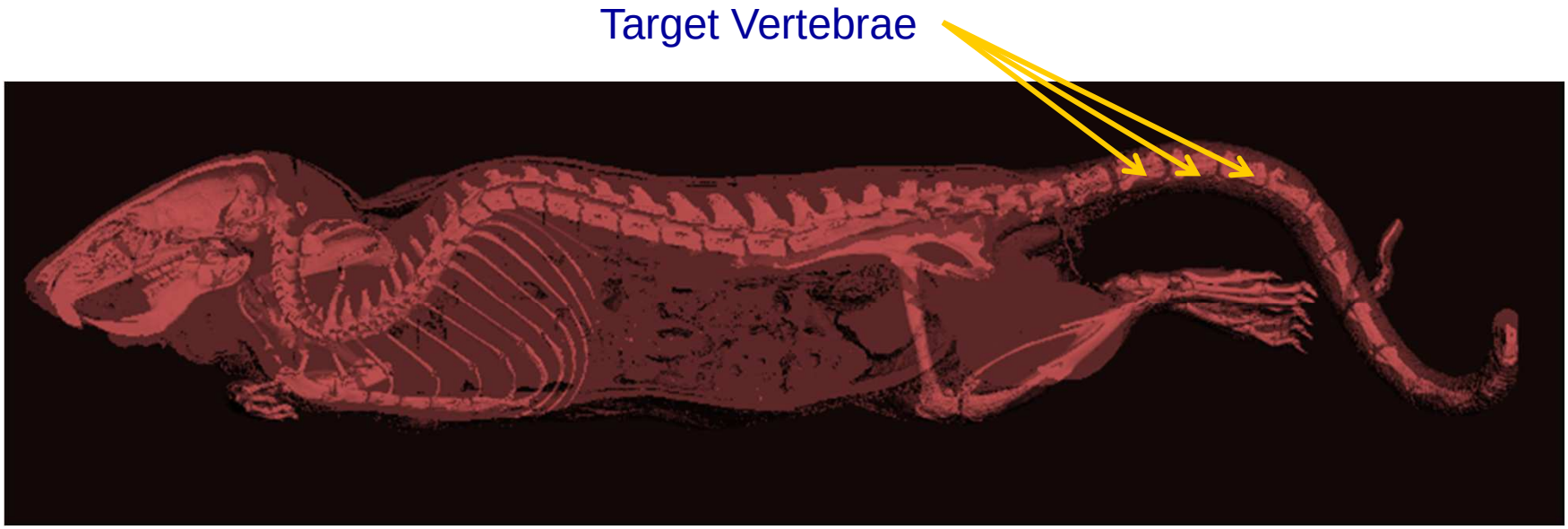
Rat Tail - Vertebrae

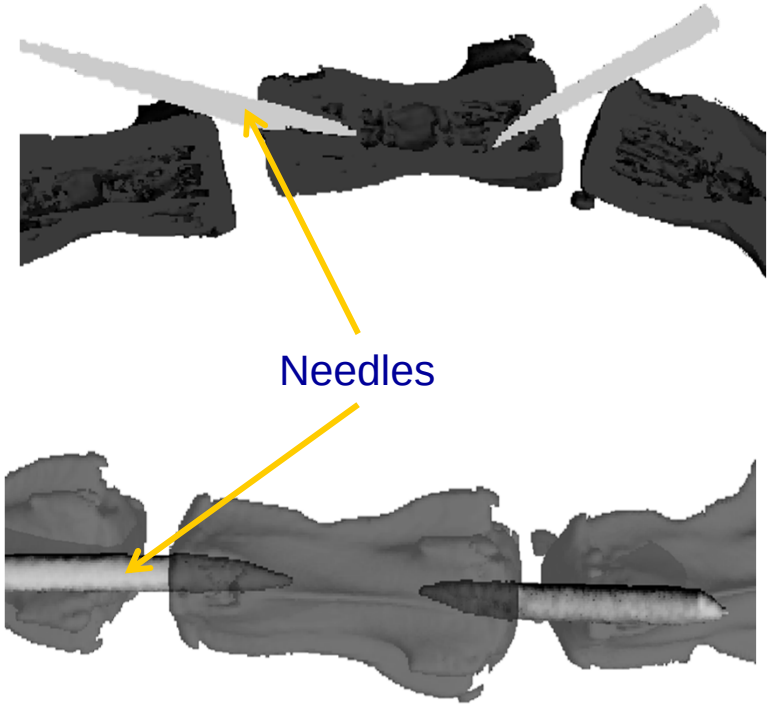


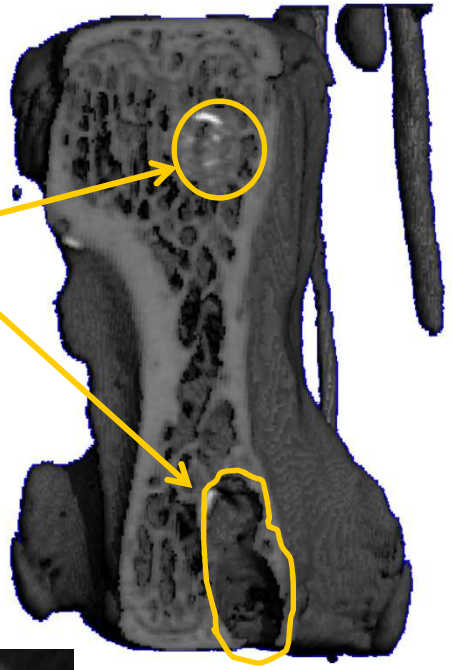
Development of an animal model for injectable bone augmentation materials for vertebroplasty

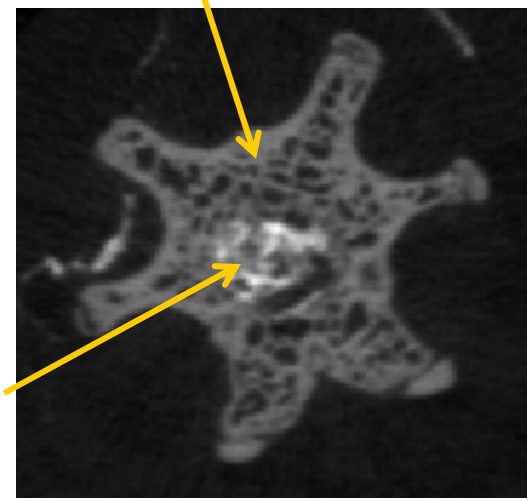
Easy to access / scan

Rat Tail - Vertebrae

Procedure – 1) Drill – 2) Rinse – 3) Fill







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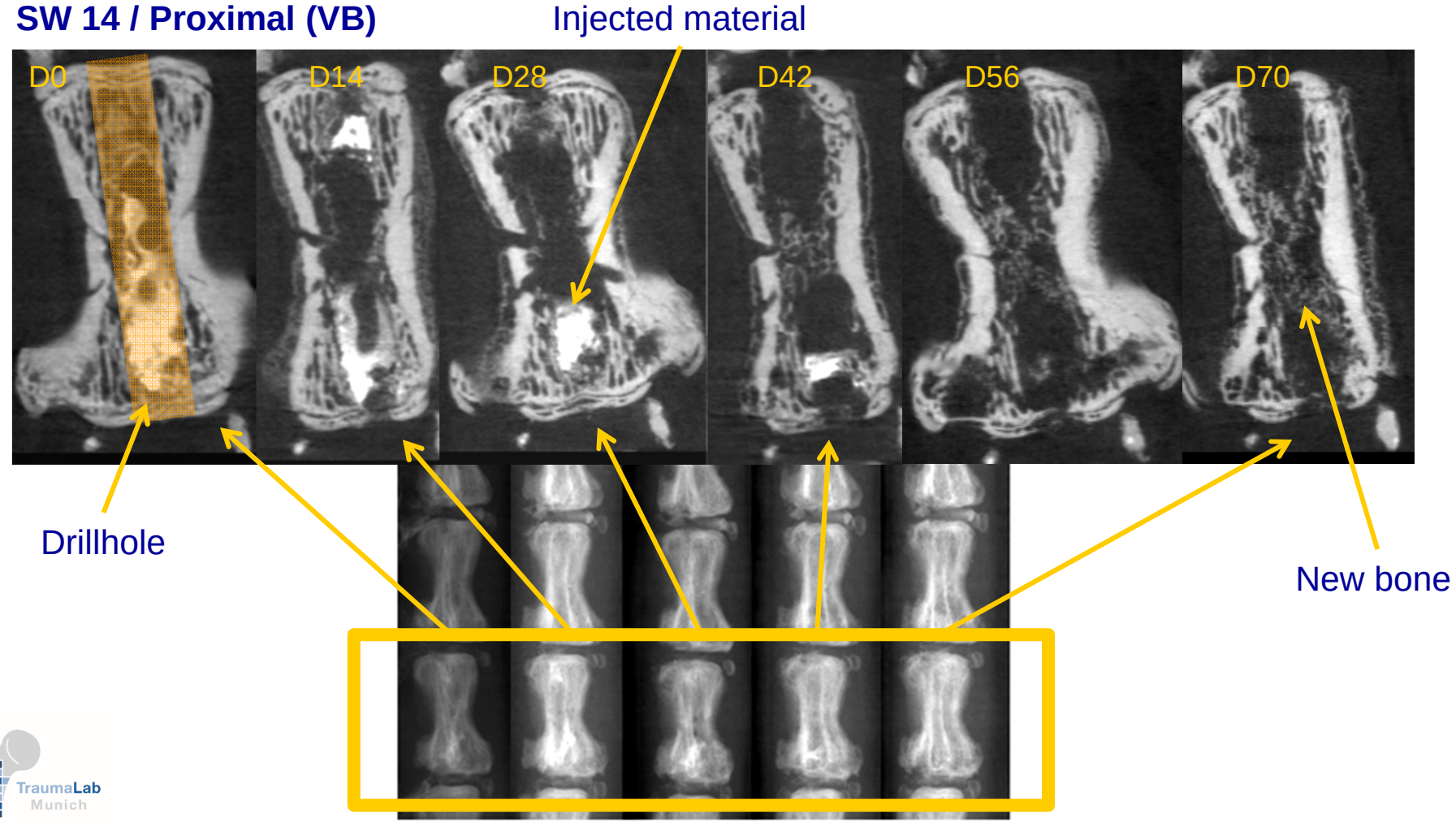
Applications: Rat Tail - Vertebrae

SW 14 / Proximal (VB)



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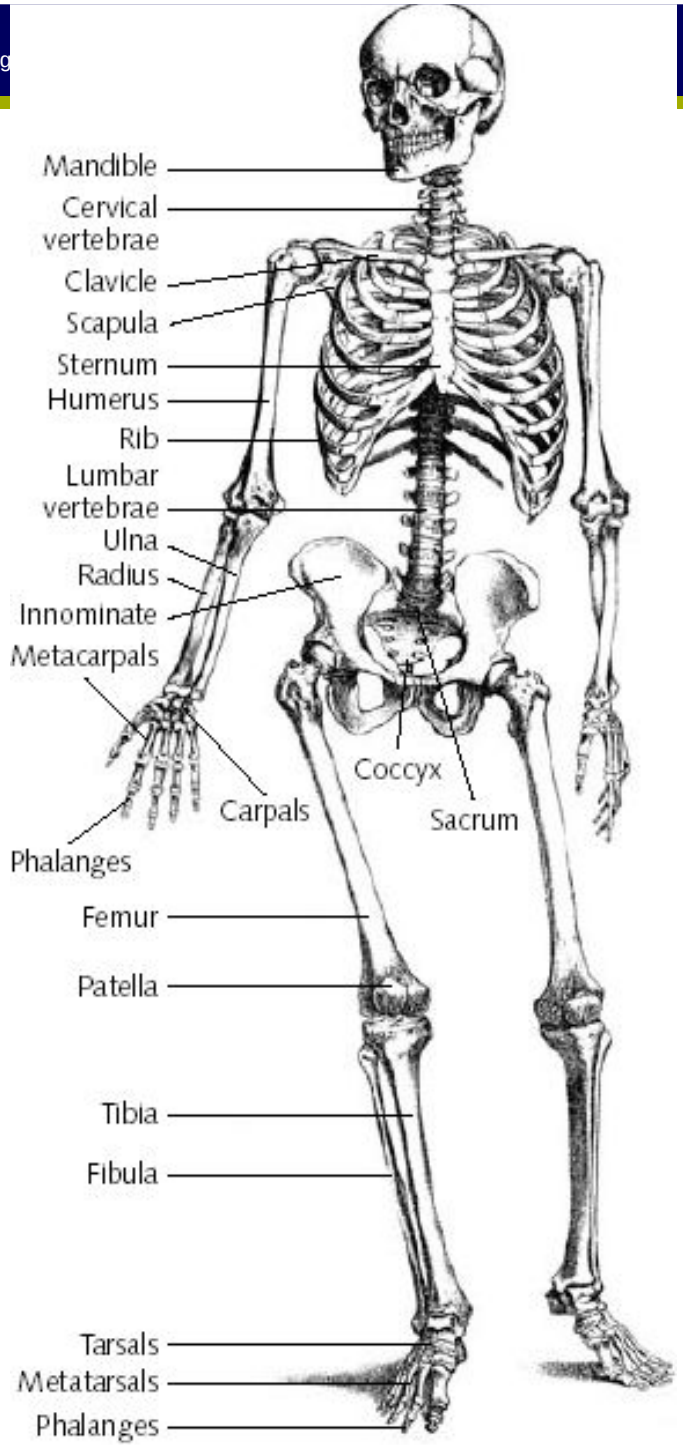
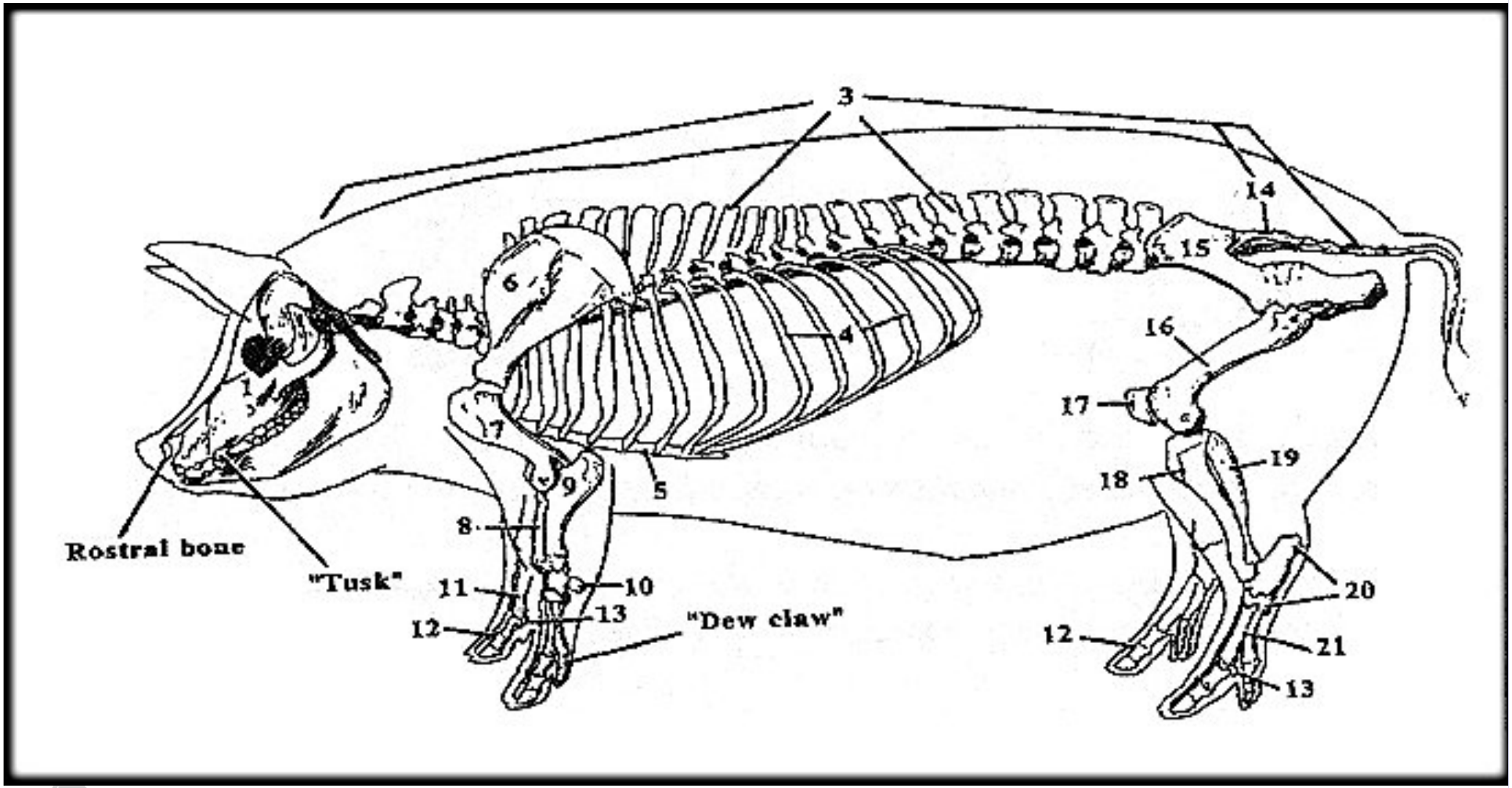
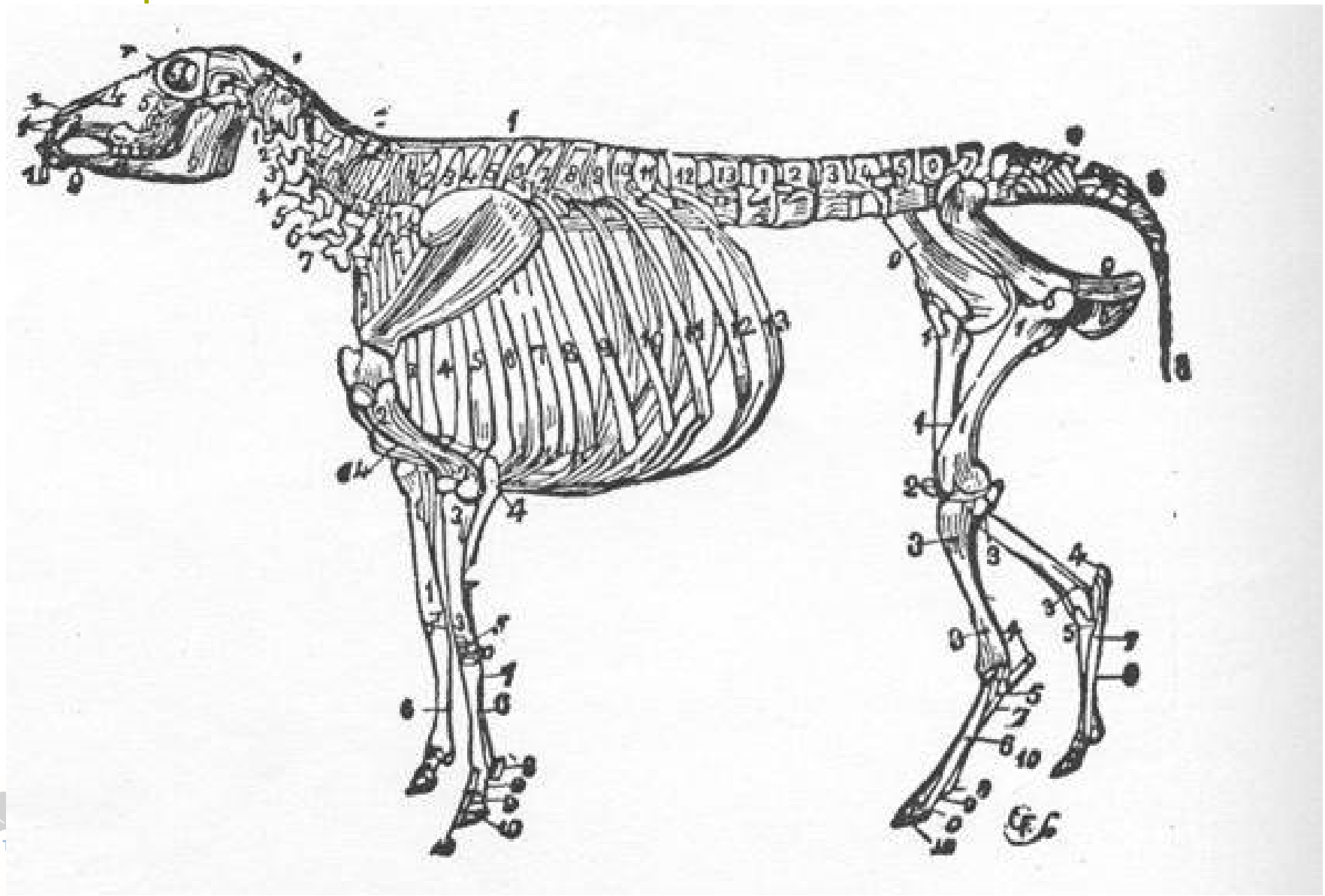

Mandible
Cervical vertebrae
Clavicle
Scapula
Sternum
Humerus
Rib
Lumbar vertebrae
Ulna
Radius
Innominate
Metacarpals
Carpals
Phalanges
Coccyx
Sacrum
Femur
Patella
Tibia
Fibula
Tarsals
Metatarsals
Phalanges

Pig skeleton

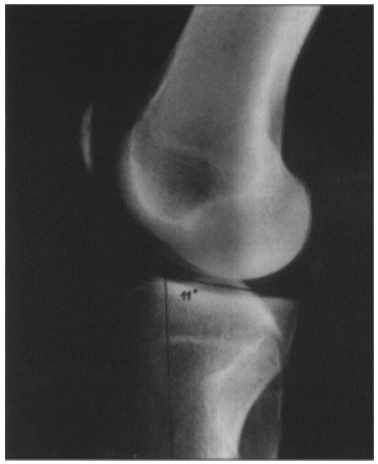


Sheep skeleton

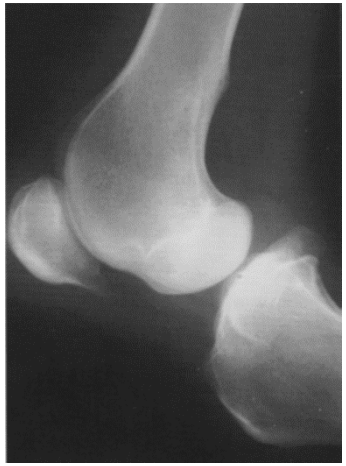


Skeletal Anatomy

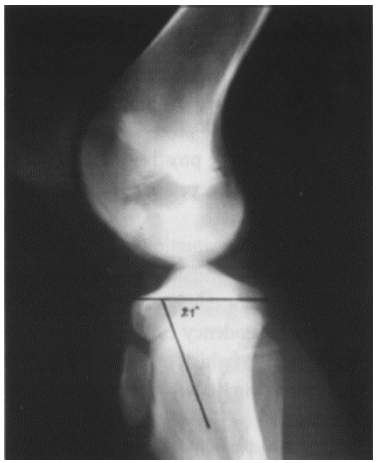
Human



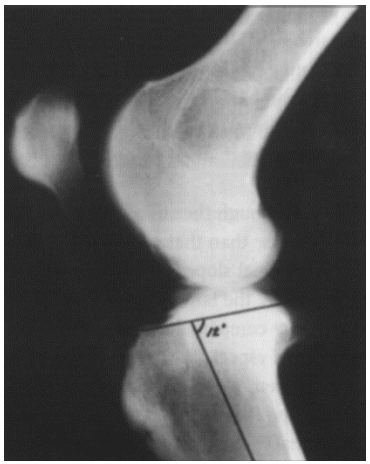
Goat



Sheep



Pig



Dog

Rabbit

Rat

Mouse

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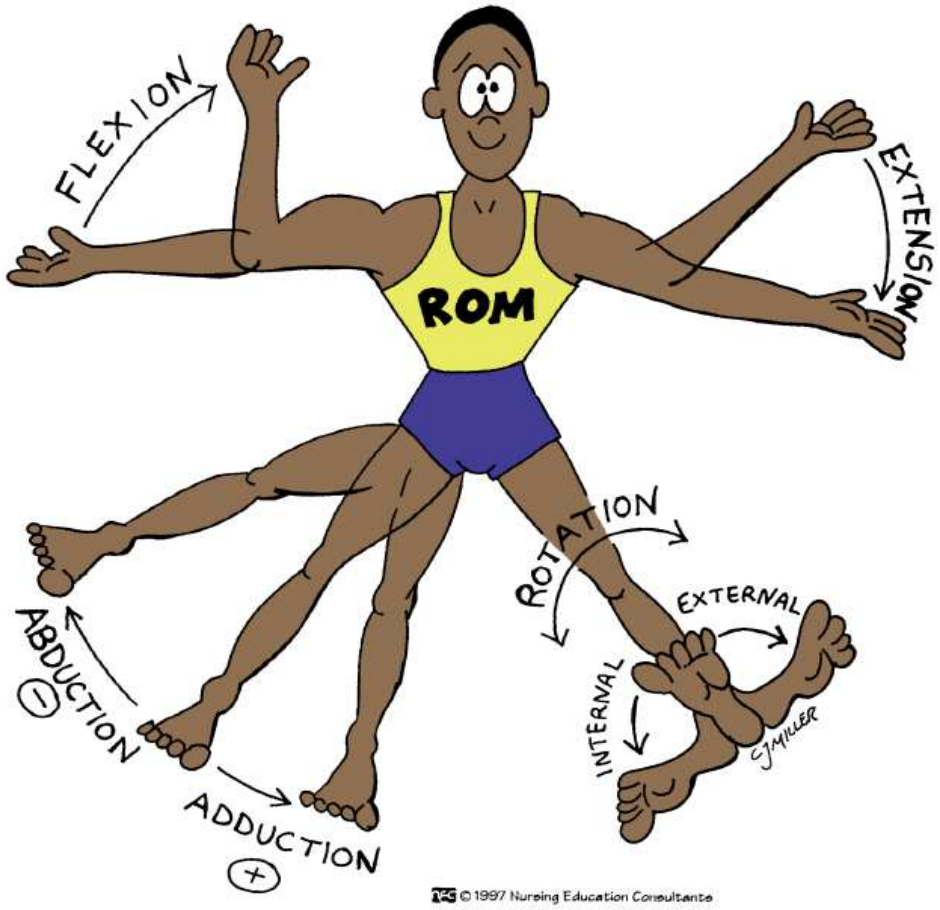
RANGE OF MOTION



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STANCE PHASE

Heel strike → Foot flat → Mid-stance → Push off

SWING PHASE

Acceleration → Mid-swing → Deceleration


2003 (c) D Wall

The Sheep

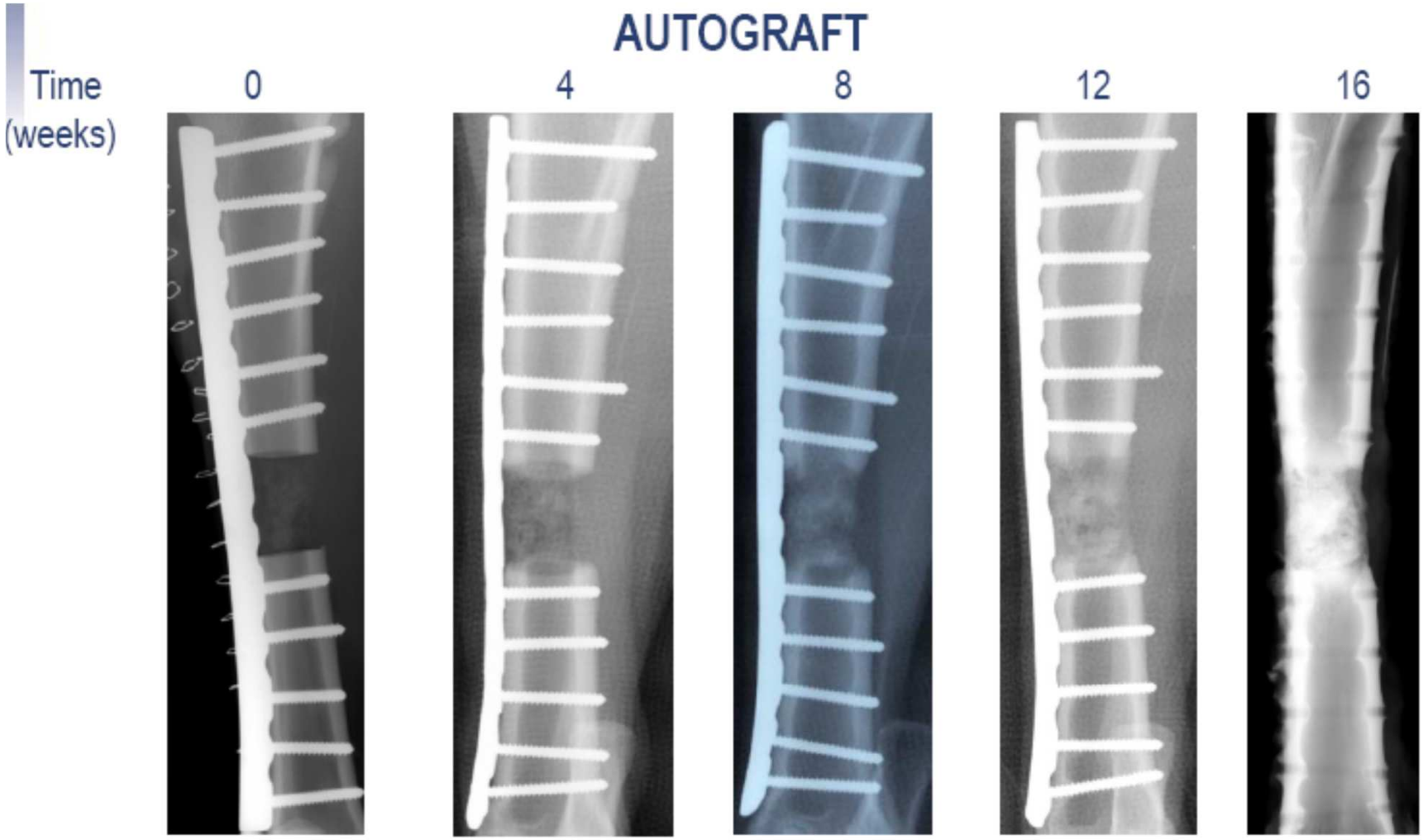
Advantages	Disadvantages
Relatively inexpensive to buy ($250) and house (<$10/week)	Variable quality, perhaps from commercial flocks
Range of sizes possible	Late skeletal maturity
Knee anatomy similar to humans	Disease status (Q fever, Toxo)
Arthroscopy feasible but not easy	Variable cartilage
Outdoor housing allows free exercise on long-term studies	Early post-op rehab hard to control
	Fat pad can obscure joint

The Pig

Advantages	Disadvantages
Readily available (including minipigs)	Rapid skeletal growth can be problematic (100 kg in 4 months!) so miniatures are preferred (<45 kg by 4 months)
Better student and facility acceptance than domesticated animals	Larger pigs can be noisy and extremely hard to manage in all bit a farm-type environment
Anatomy close to that of the human – cardiac, GI tract, bone	
Range of sizes possible	
Can be arthroscoped	

The „Zürich model“

- Segmental defect: 2.5 cm full-thickness defect in the mid-shaft of the sheep tibia (Meinel *et al.*, 2003) stabilized using an internal fixation plate.
- Positive and negative controls: autograft and empty defect.
- Results analysis:
  - radiographs were taken directly after surgery and every 4 weeks thereafter.
  - After 16 weeks, the animals were sacrificed and the tibia extracted for final analysis by µCT, histology and micro-radiography.



Bridging is observed in 6/6 sheep.

Looking to the kinetic of healing 16 weeks seems to be an appropriate follow up time.

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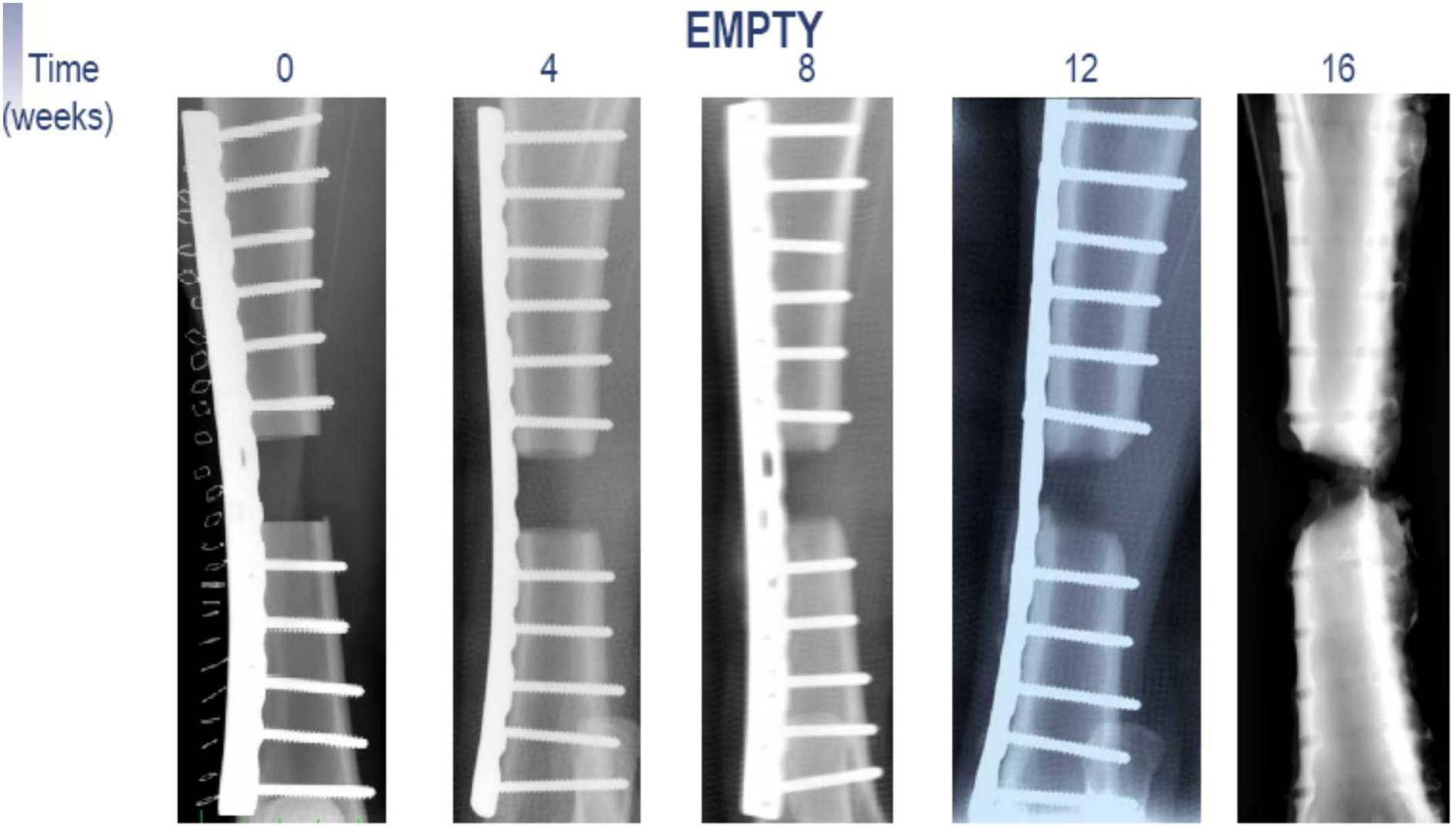

EMPTY
Time (weeks)
0
4
8
12
16

plate

- Lots of screws
- Instable
- difficult

Ovine tibia non-union

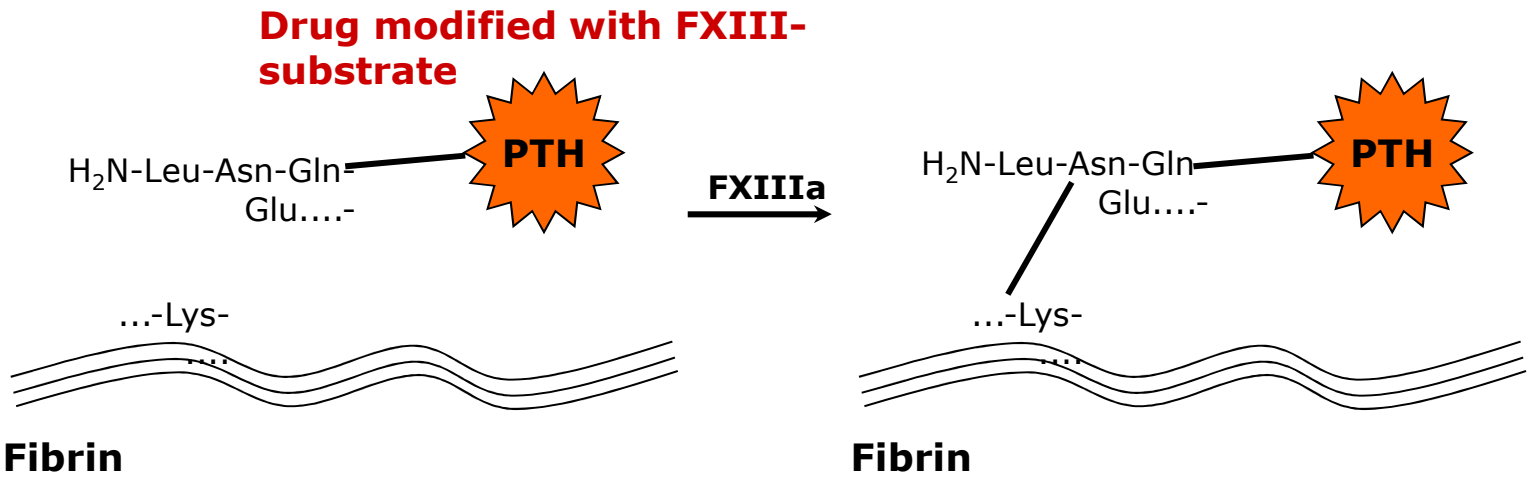
- 2.5 cm segmental bone defect
- intramedullary nail (UHN, Ø 9.5mm, 205 mm)
- n=10 per group
- Observation periods: 6 and 12 months
- Parameter: X-ray, µCT and histology

groups

- Controls
  - Empty
  - Autograft
  - BMP-7/osigraft
- Experimental groups
  - Fibrin + 0.4 mg/ml PTH
  - Fibrin + 1.0 mg/ml PTH
  - Tricos + 0.4 mg/ml PTH
  - Tricos + 1.0 mg/ml PTH

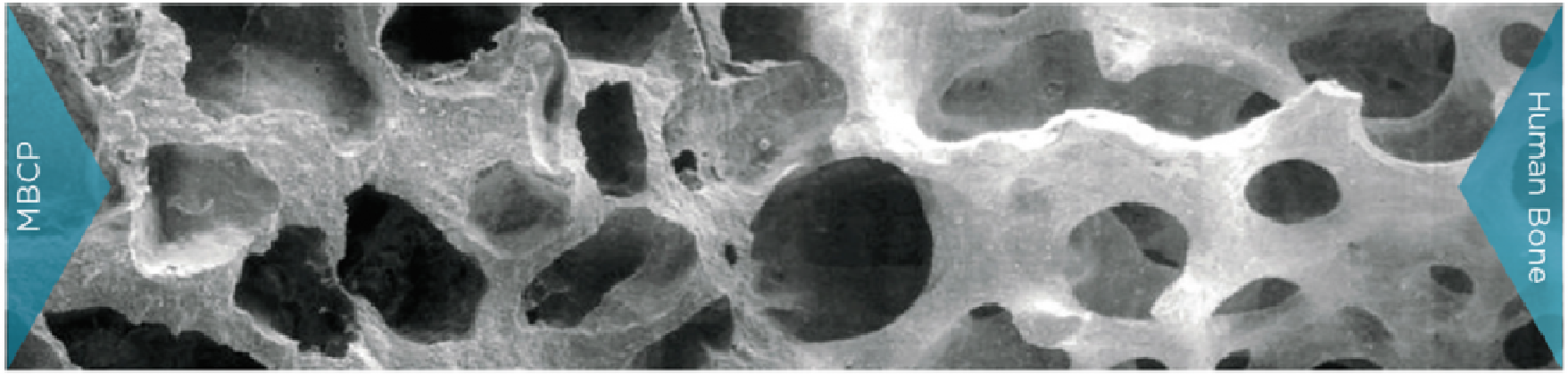
PTH release system with fibrin

- PTH1-34 used for osteoporosis therapy



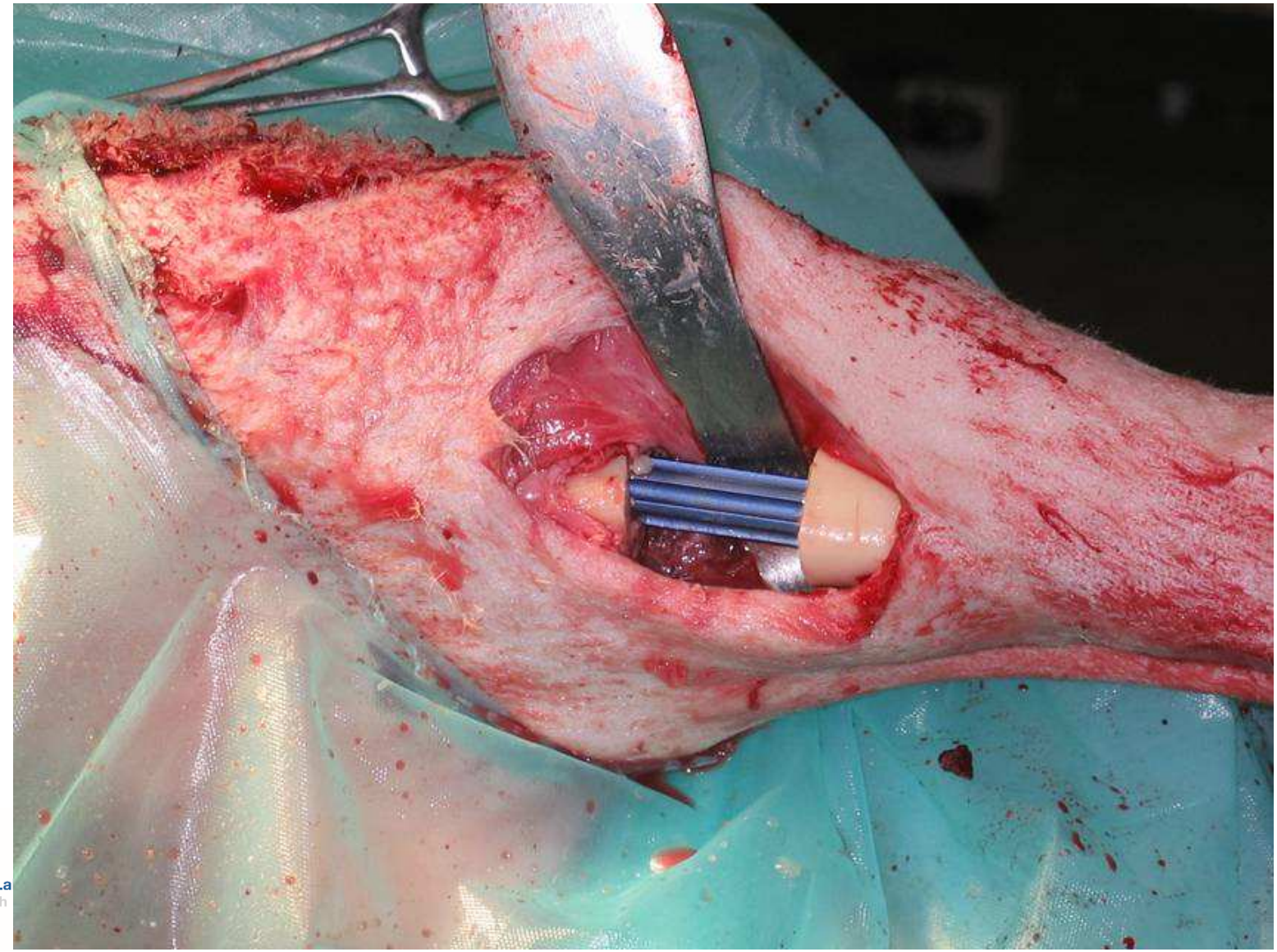
Drug crosslinked to Fibrin

Adding it to MBCP for osteoconduction

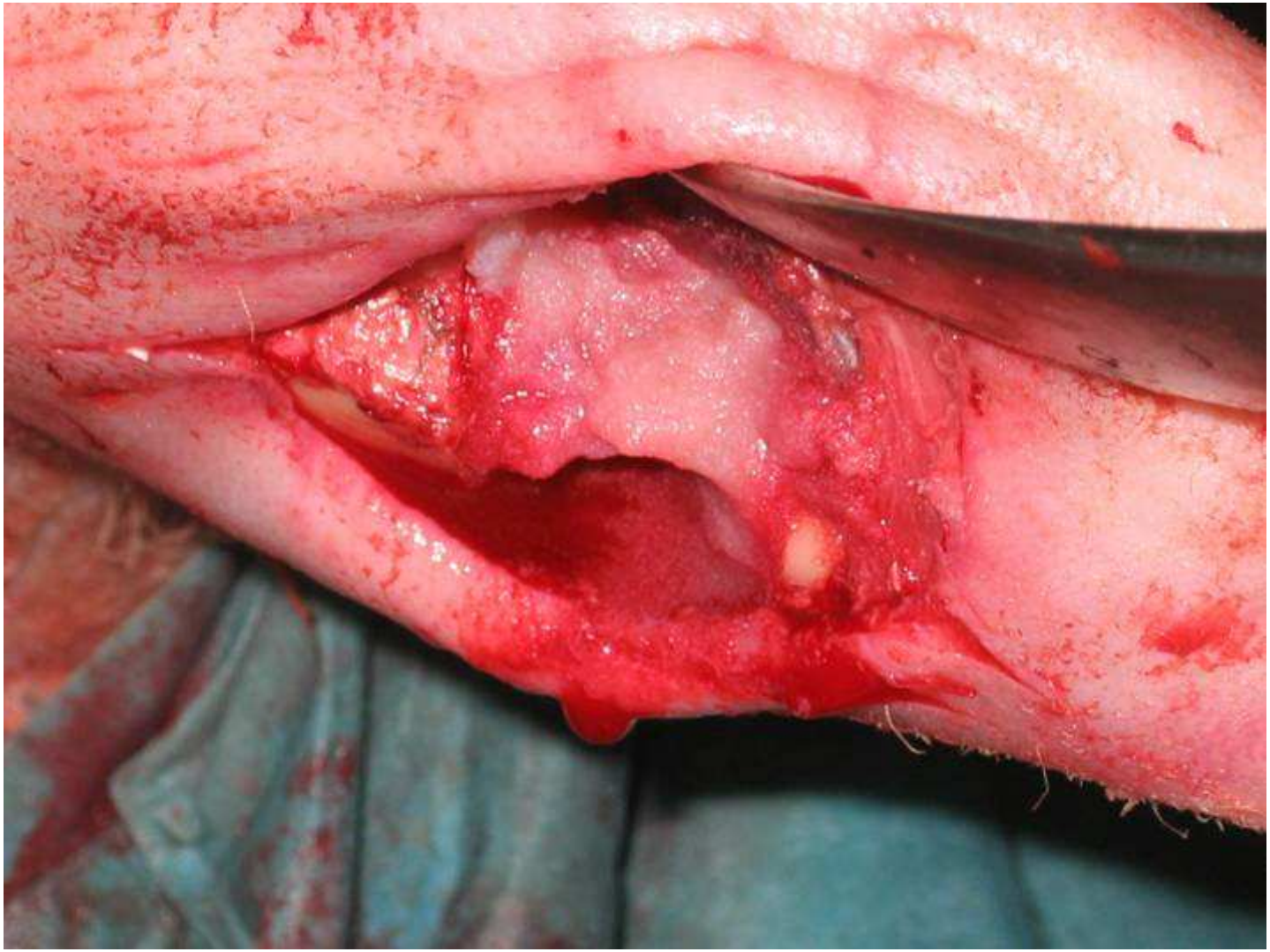


MBCP=micro-macro bi

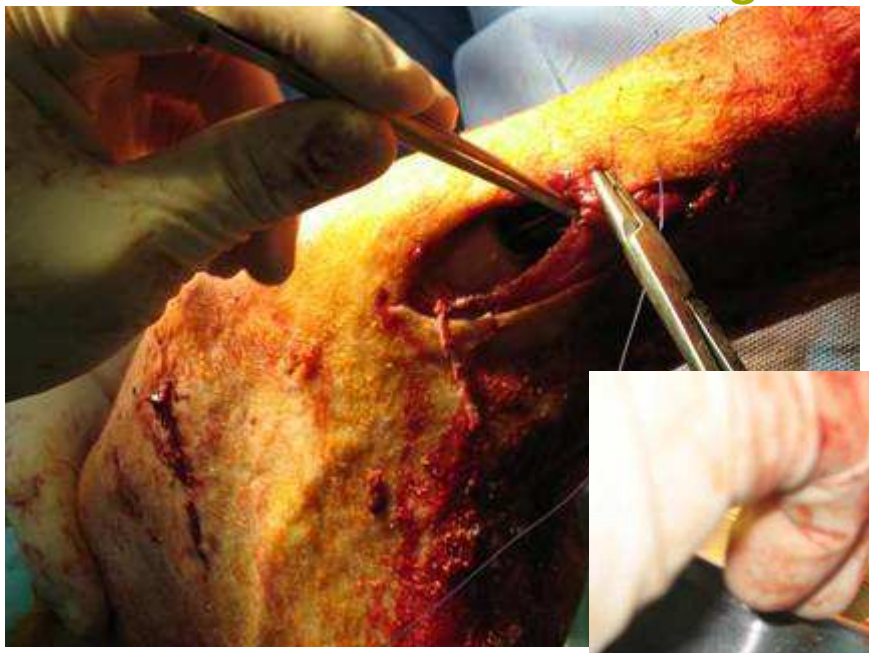
The defect…

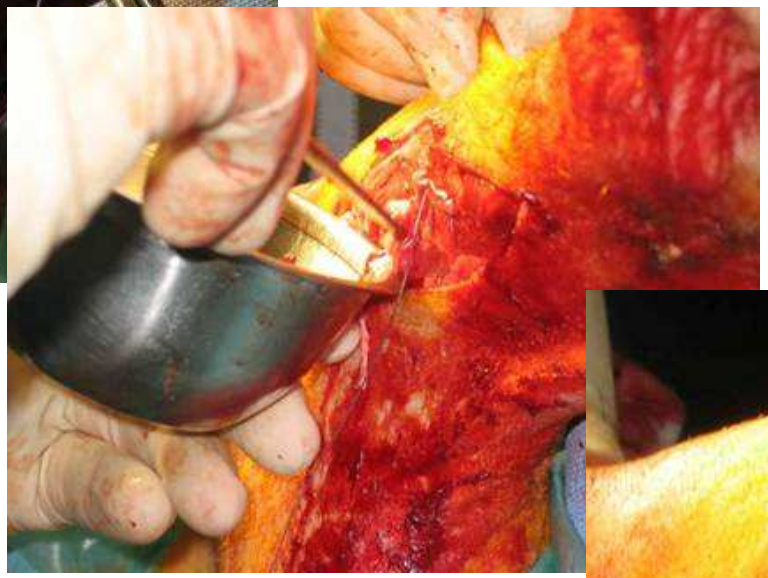


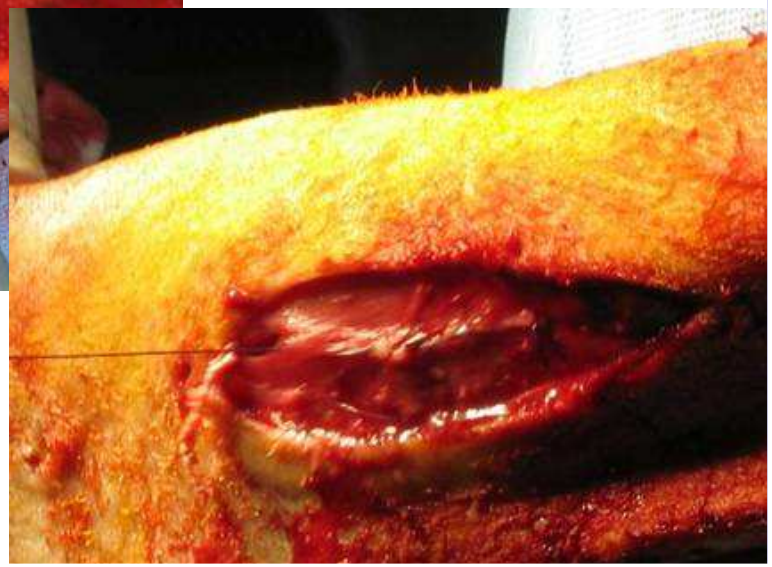
BMP-7 in collagen



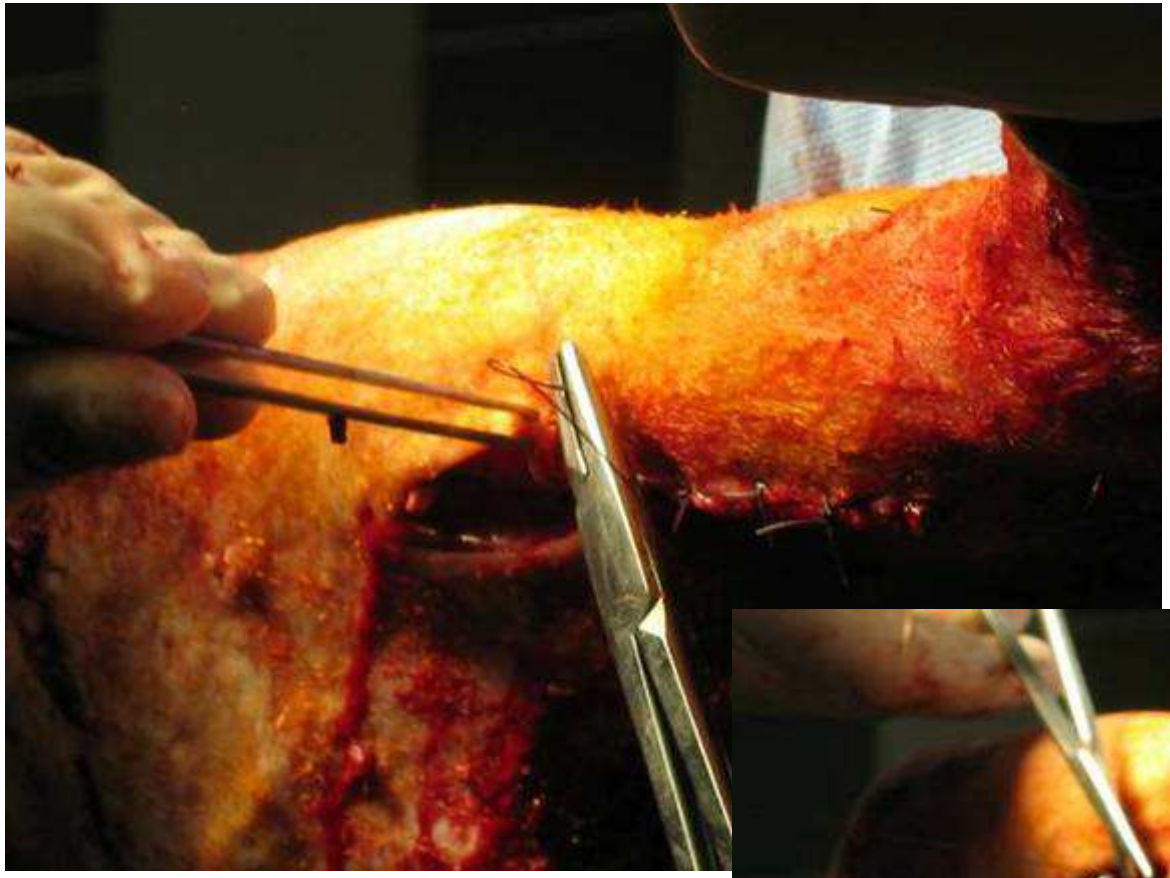
Administration of filling material

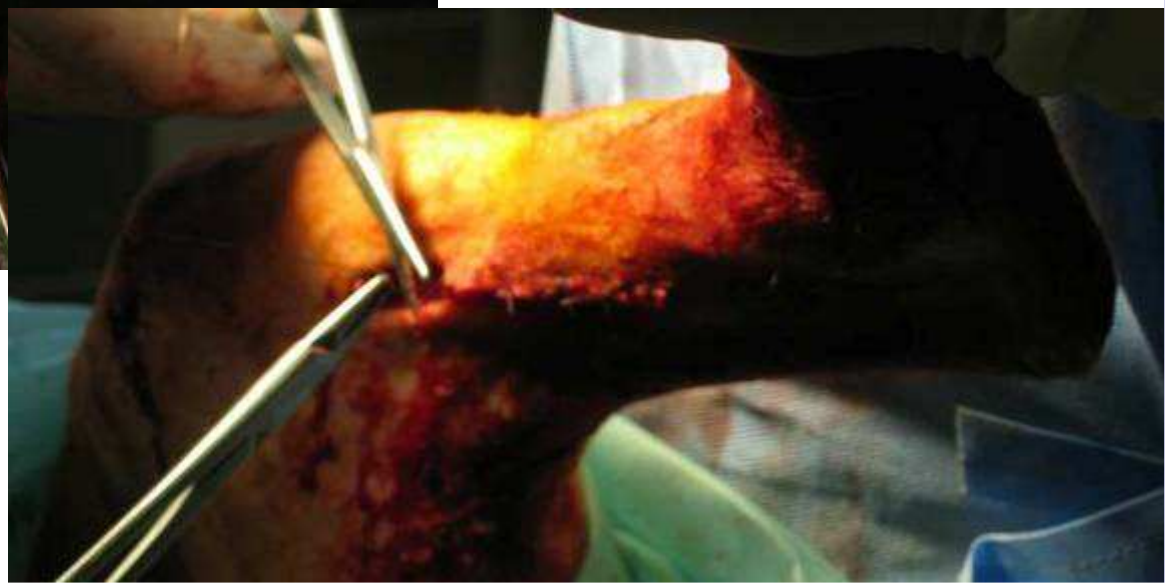






Skin closure





The caring team

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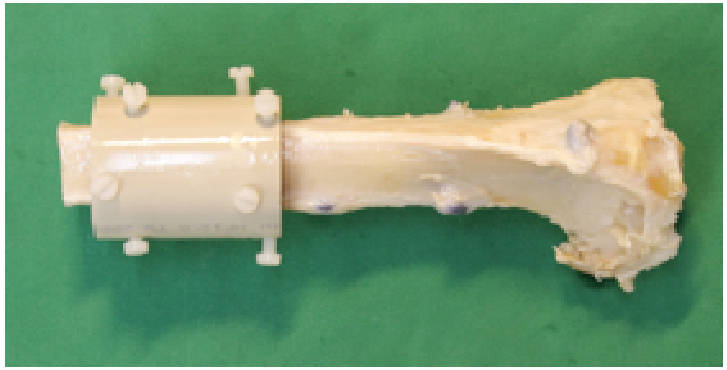
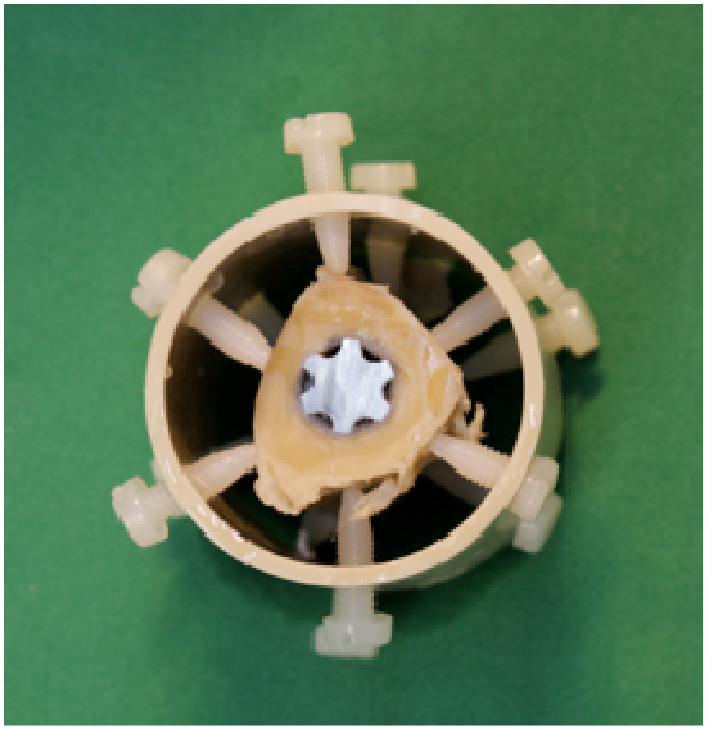
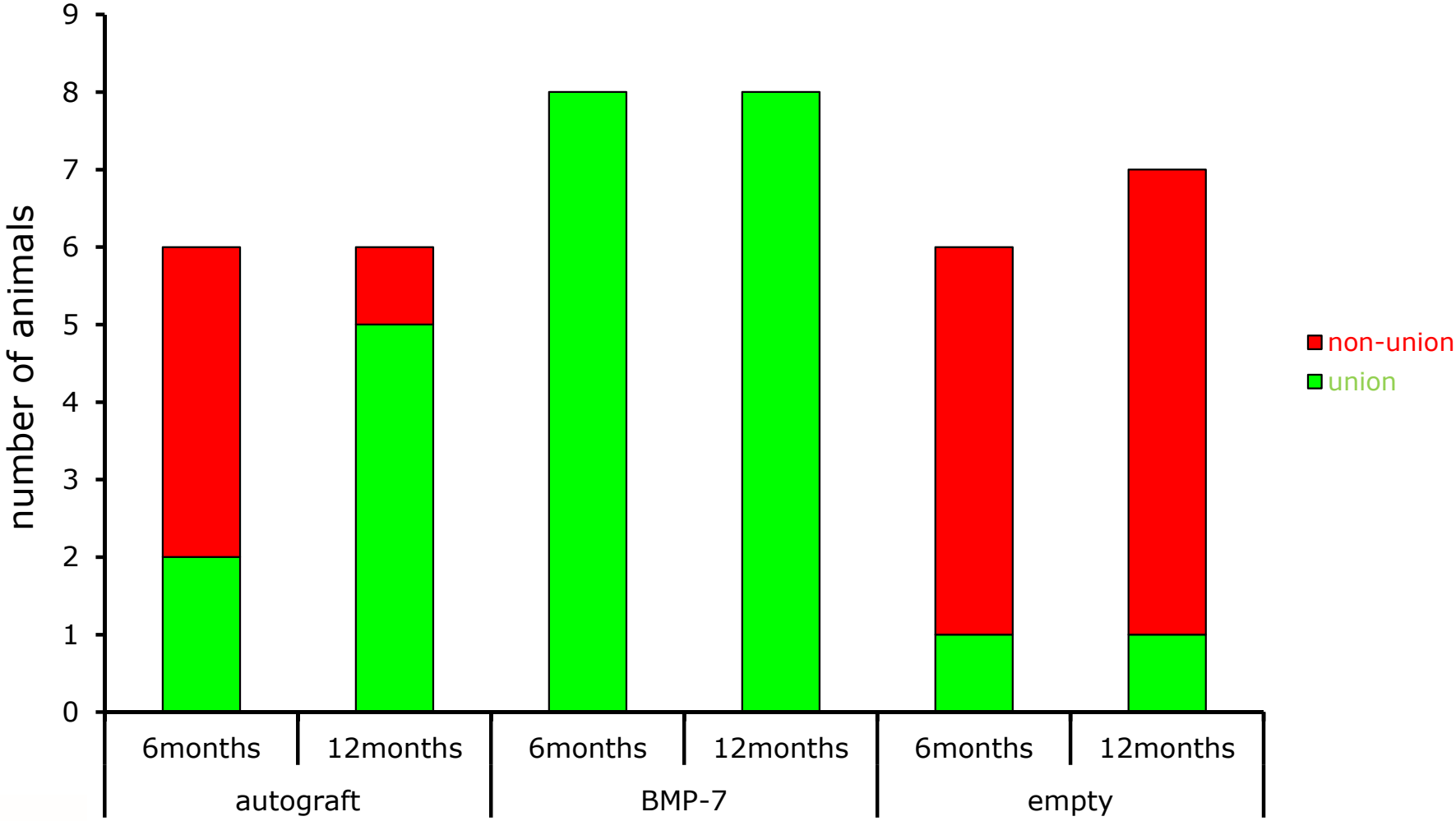
Processing for µCT and histology





United defects



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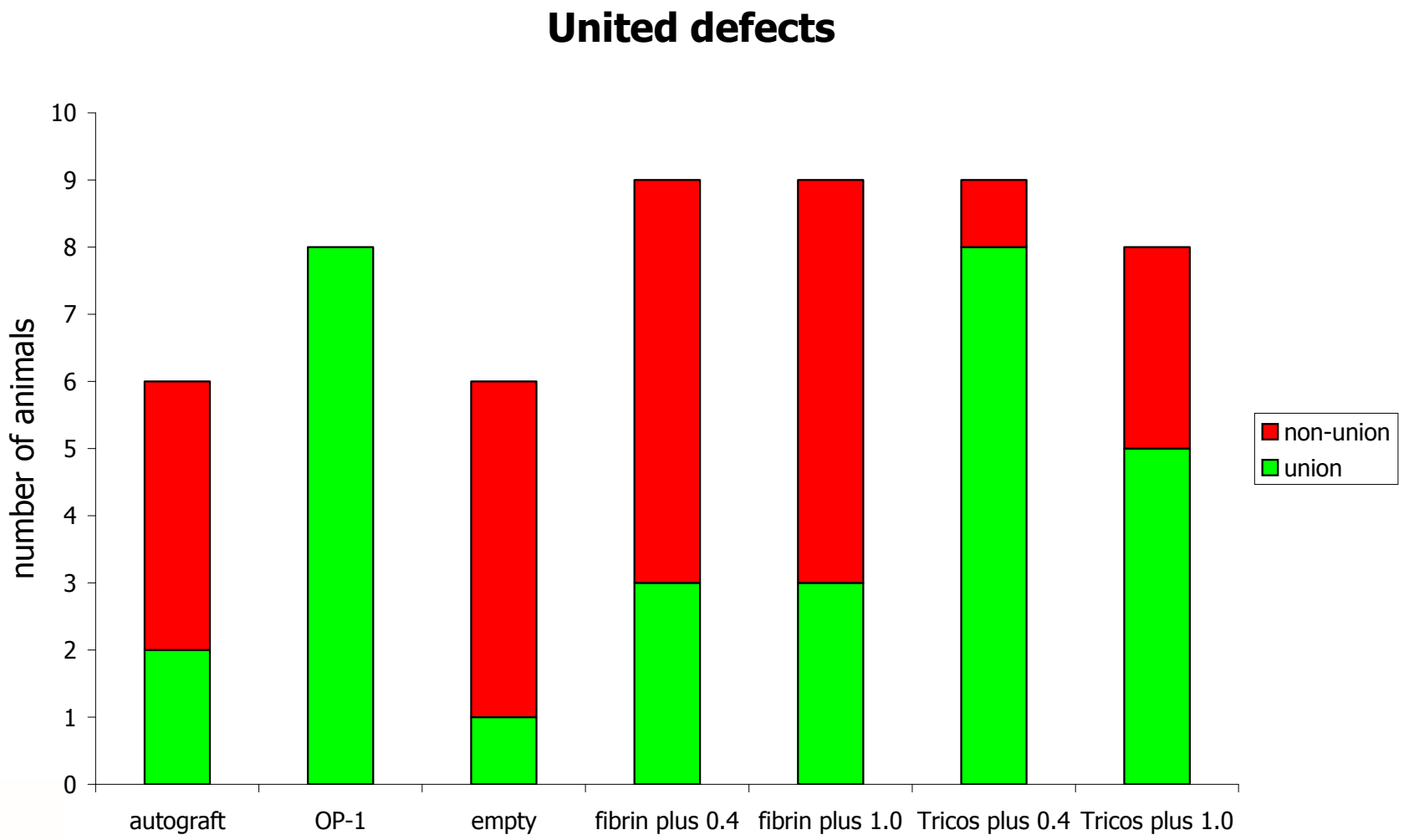
Union using PTH after 6 months



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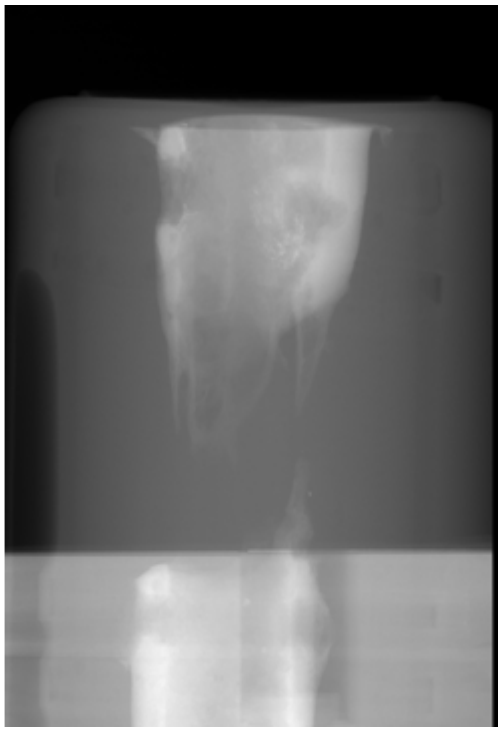
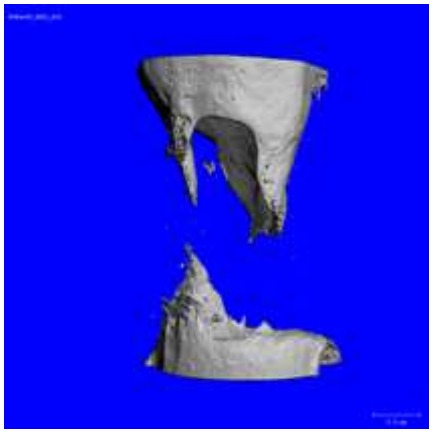
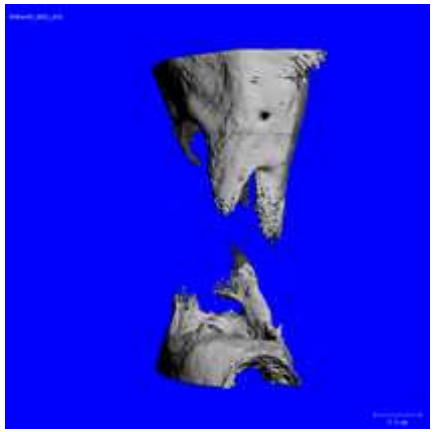
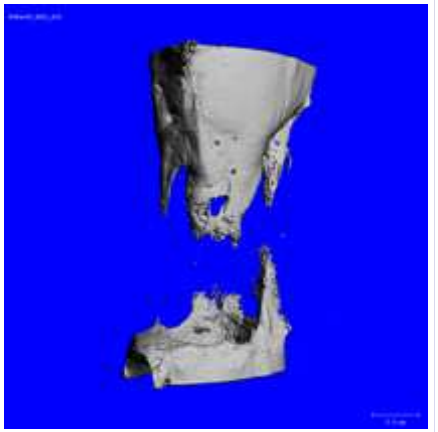
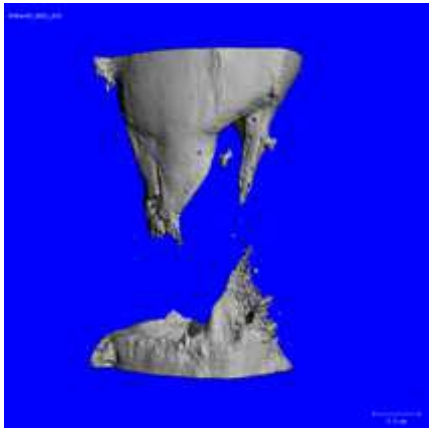
Empty

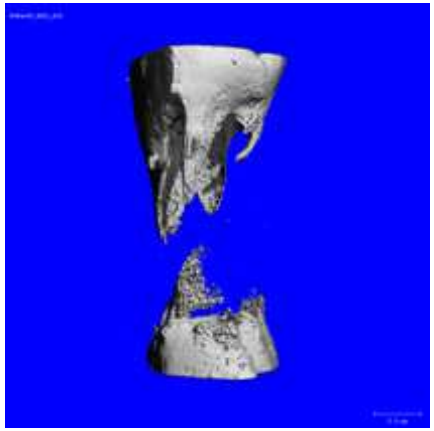


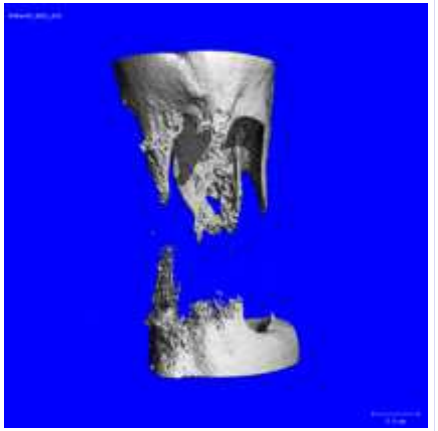












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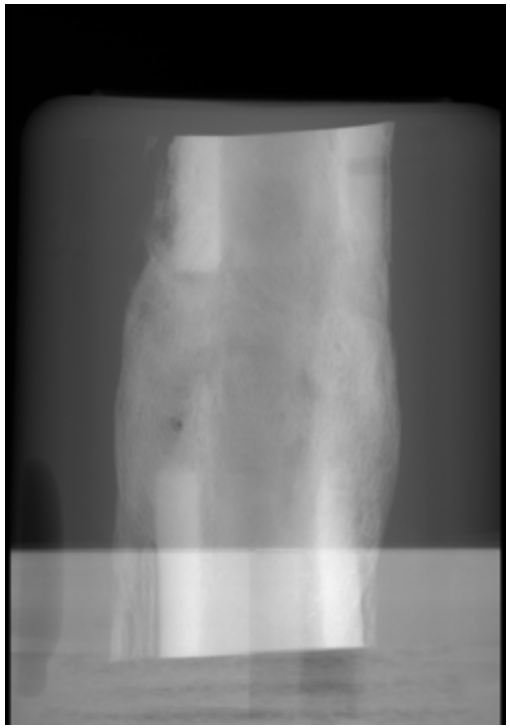
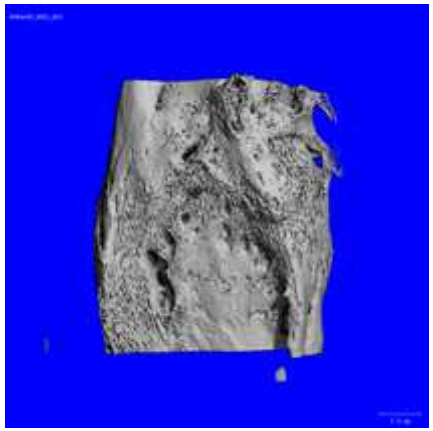
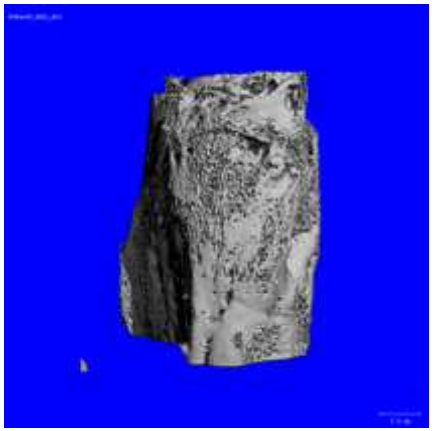
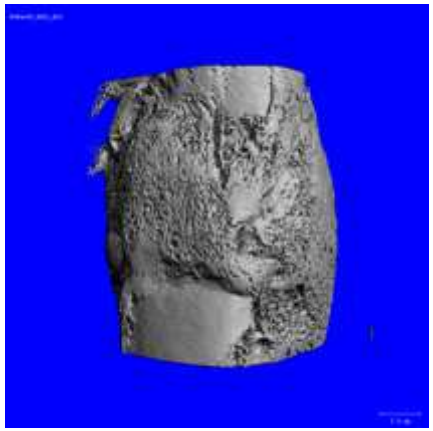
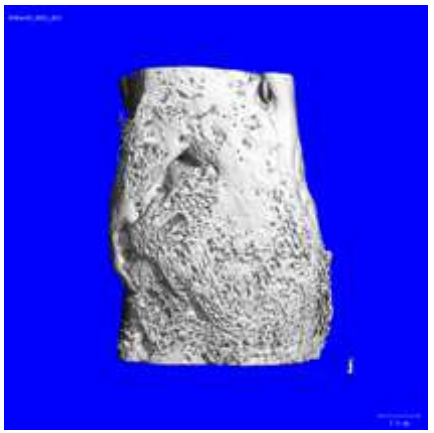
Autograft

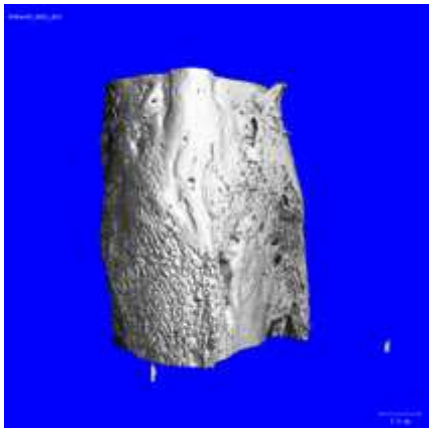












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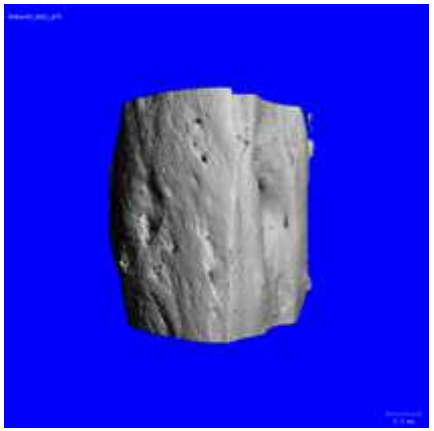
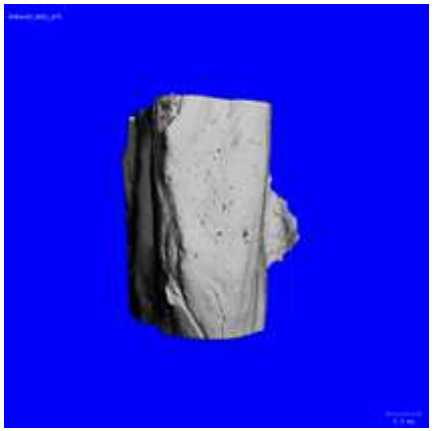
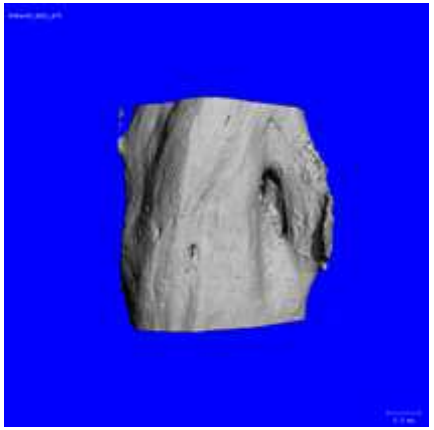
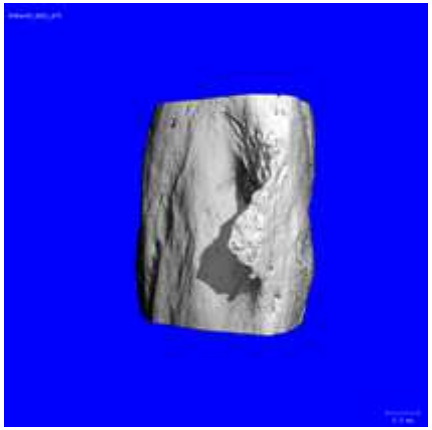
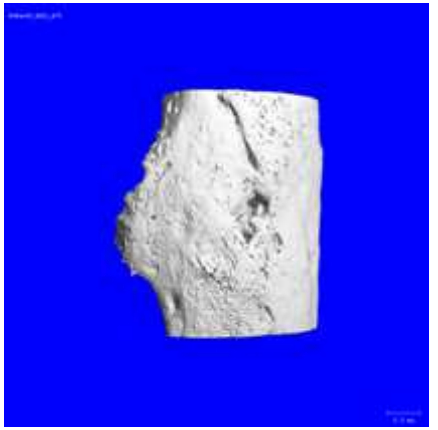
BMP-7 in collagen



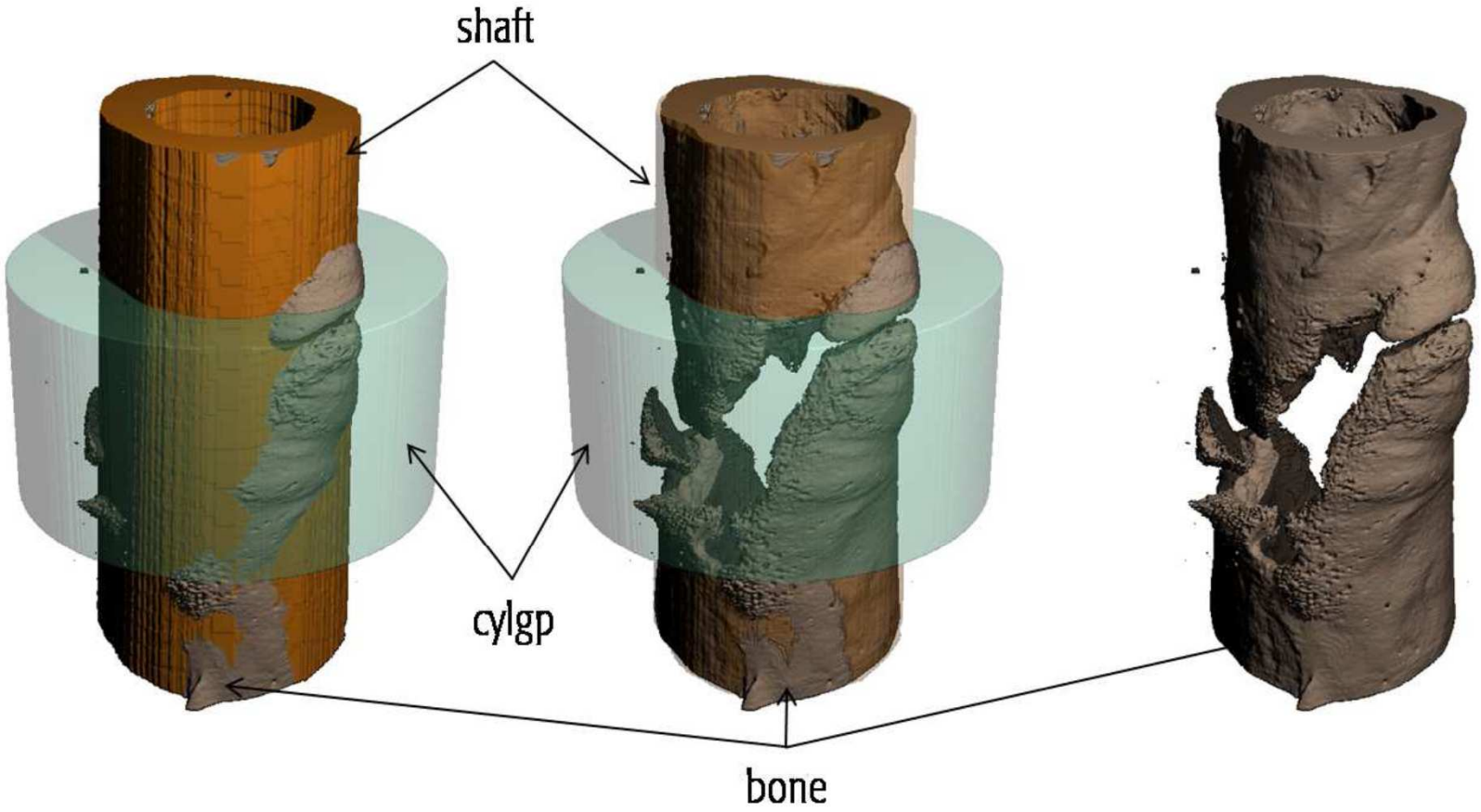




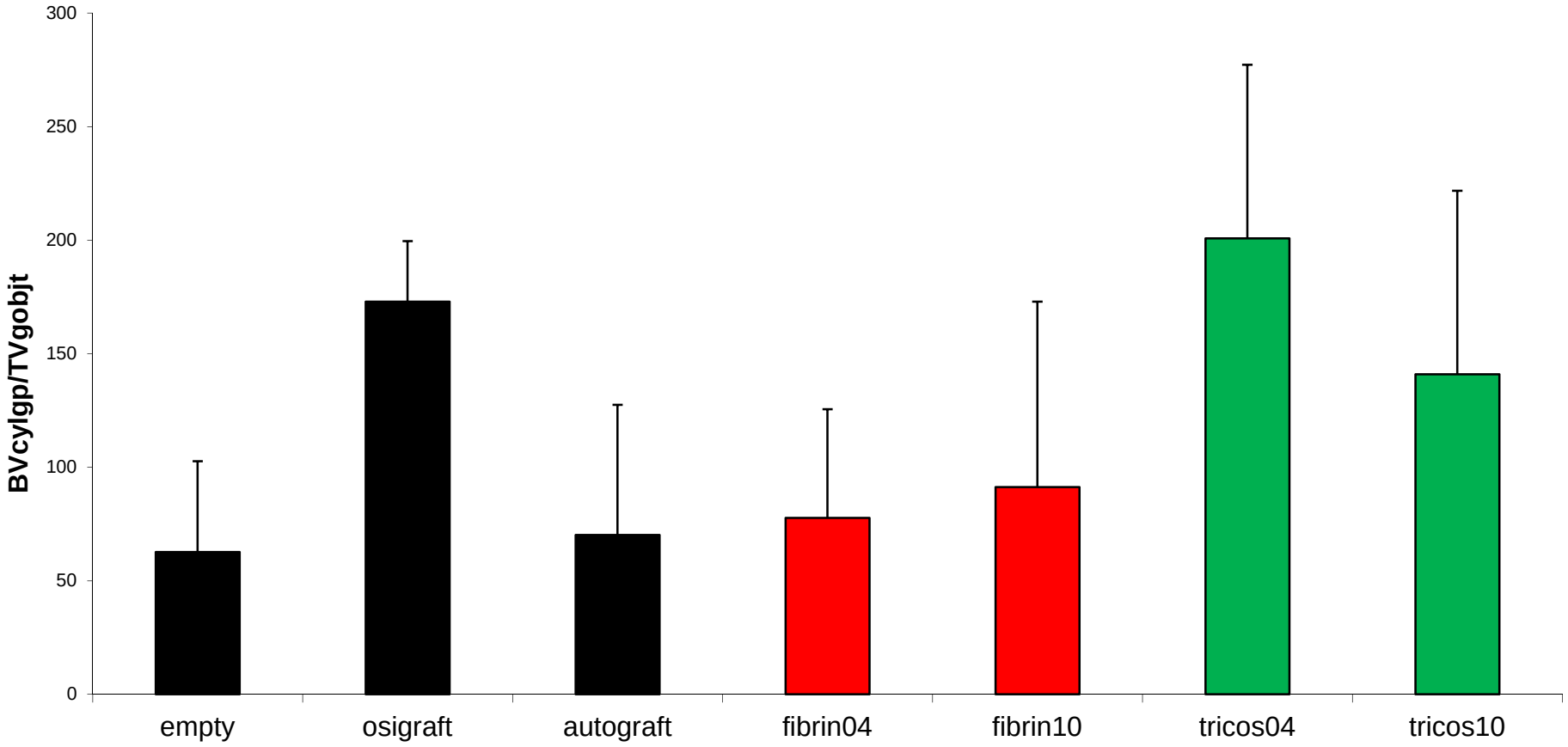




µCT evaluation



Bone volume of the gap (6 months)



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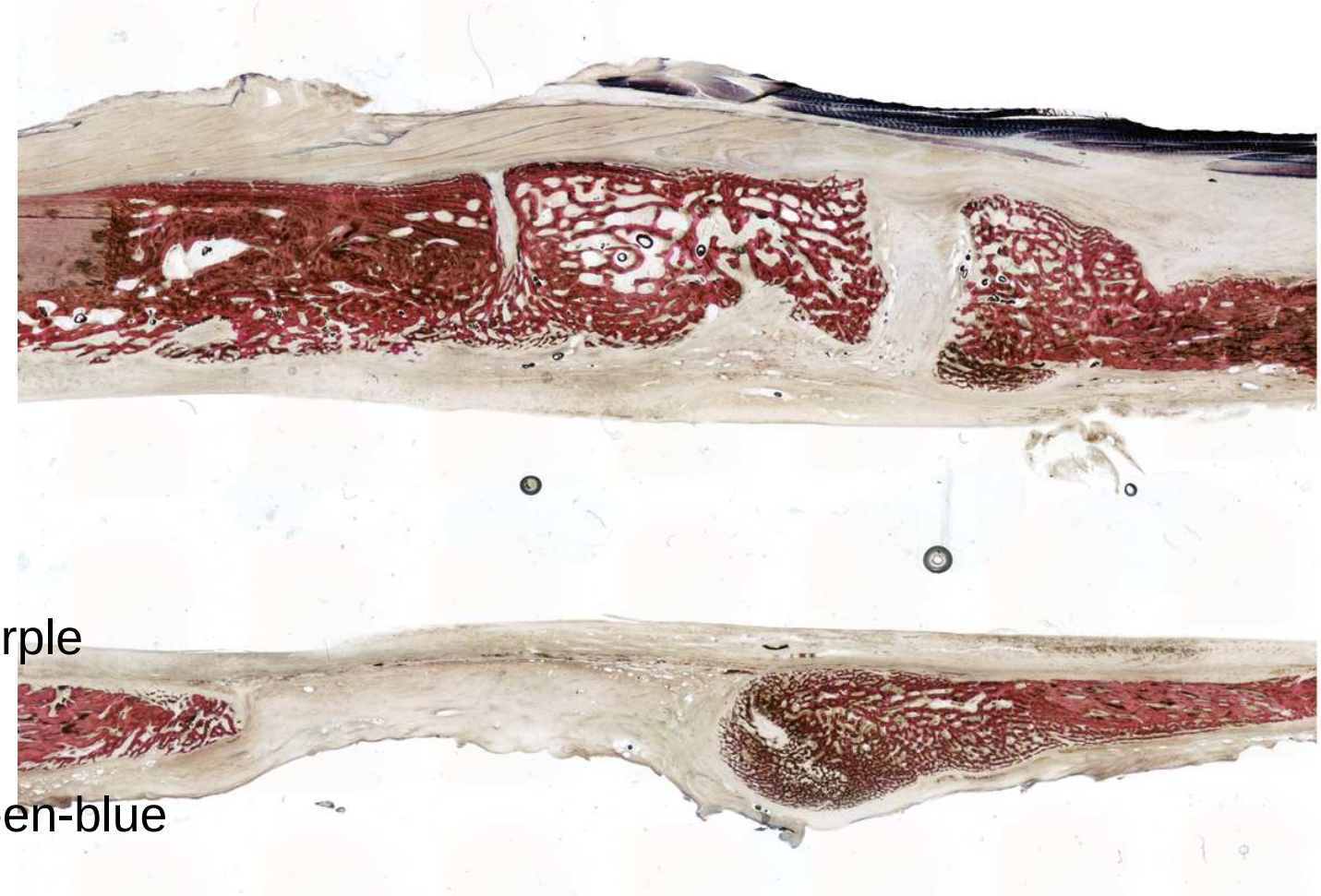
Levai-Laczko staining



- Bone: pink-red
- Cartilage: purple
- Collagen: blue-purple
- Elastic fibers: red
- Muscle fibers: green-blue

Histology

empty

autograft

BMP-7

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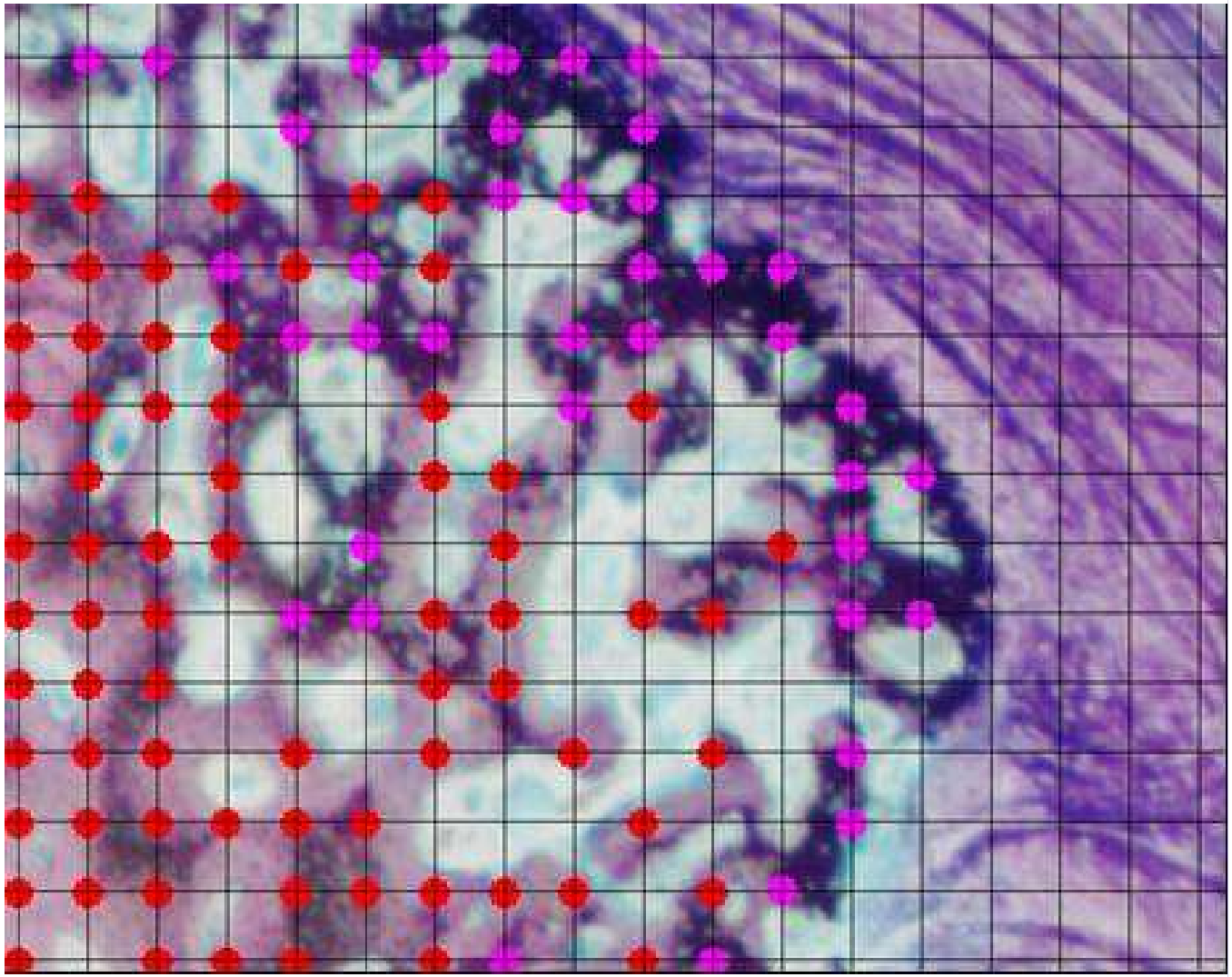
Morphometry: bone/cartilage

Red dot = bone
Purple dot = cartilage



Grid = 0.01 mm^2

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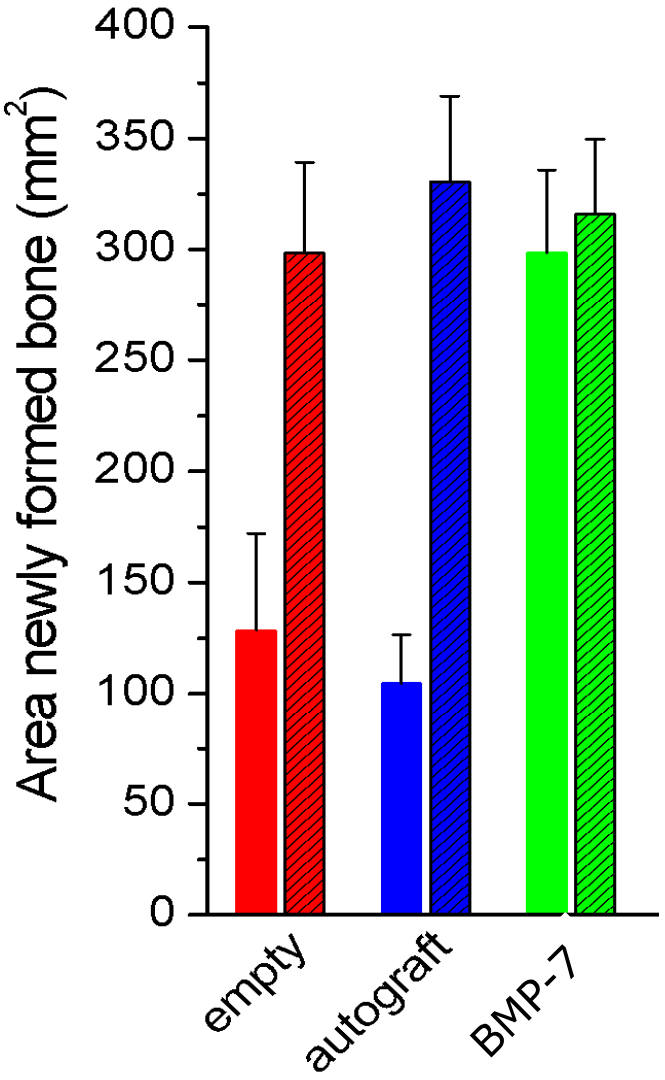
Newly formed bone

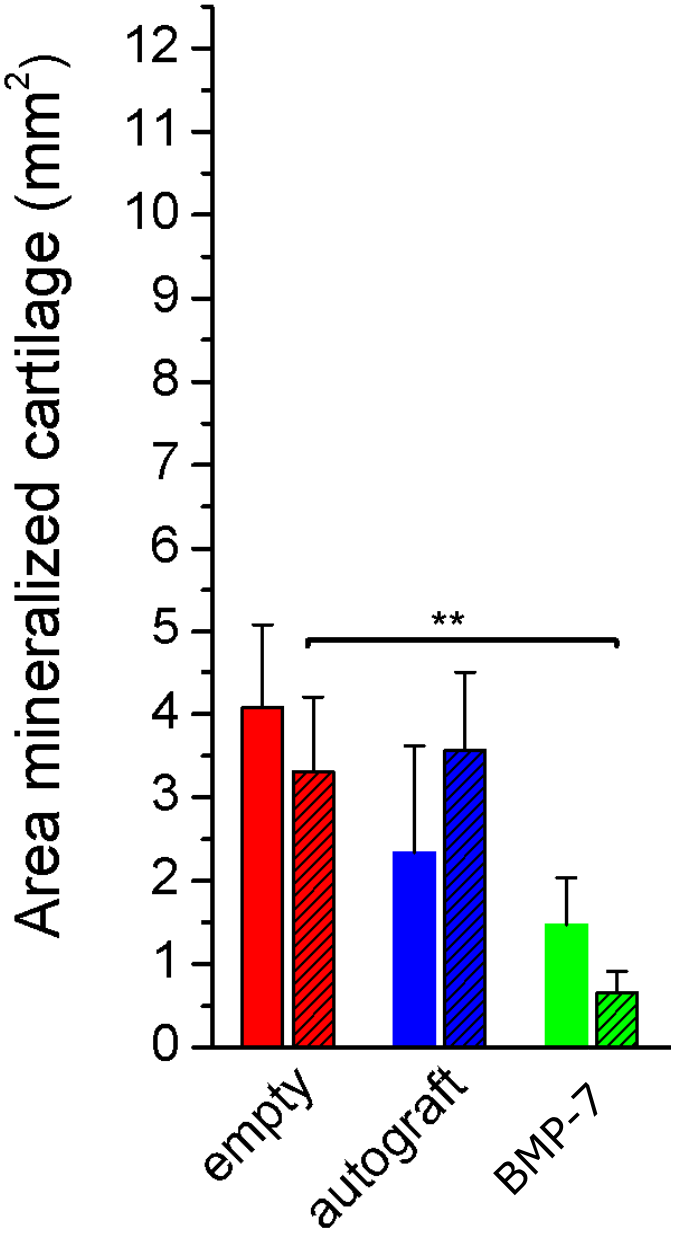


Mean±STD

Mineralized cartilage

BMP-7 functions via
direct ossification

Empty and autograft via
enchondral ossification



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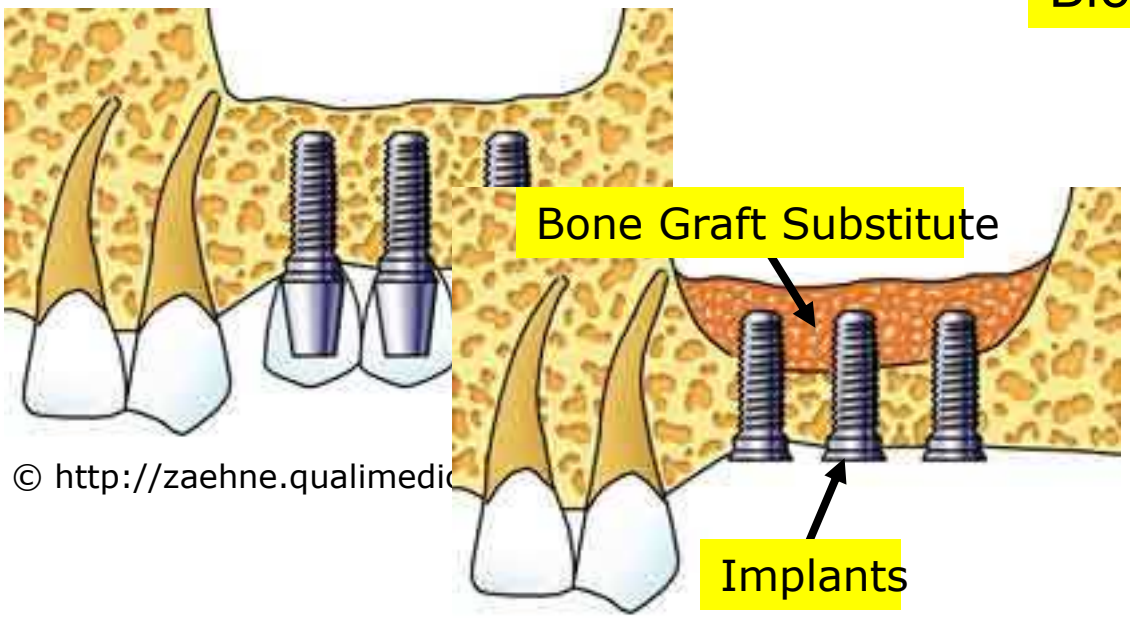
Sinuslift clinical study

$TCP + PTH_{1-34}$



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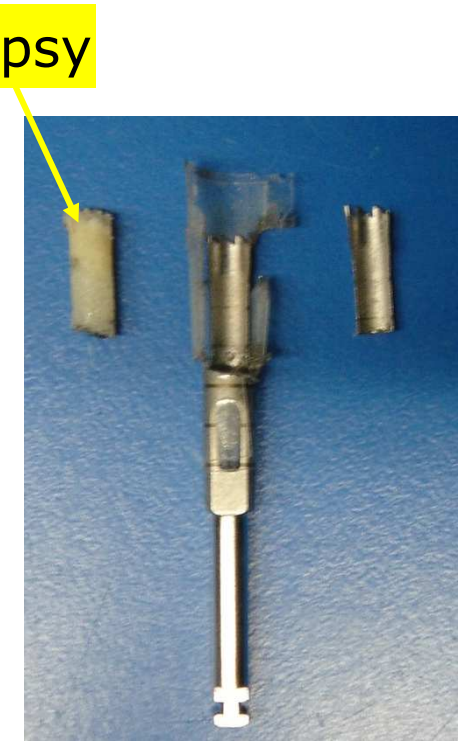

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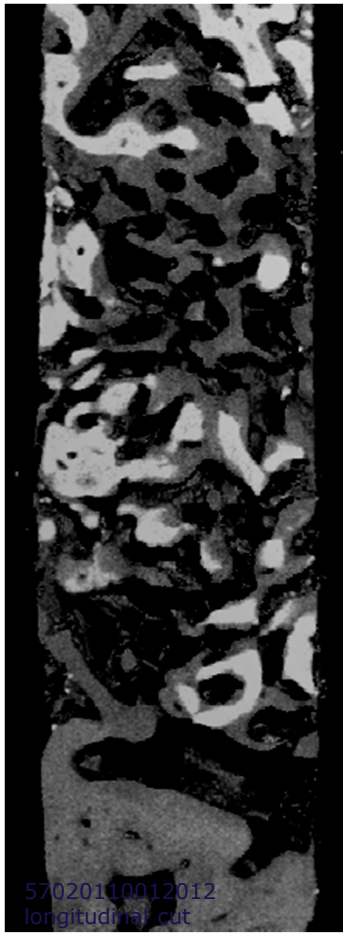
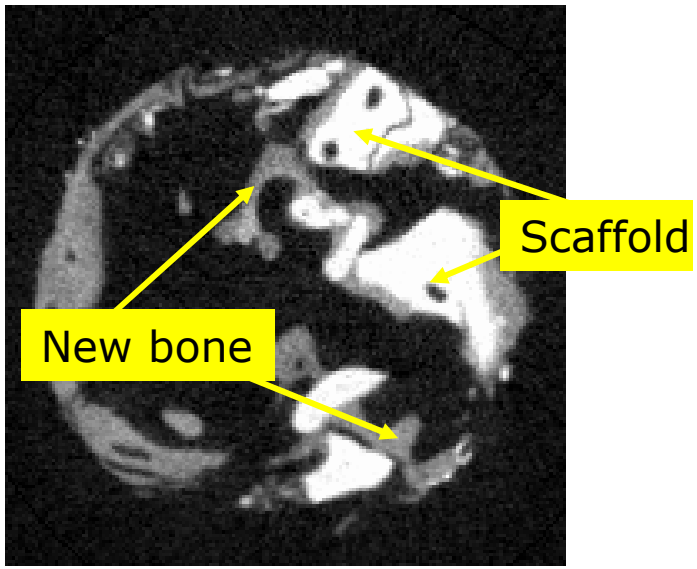
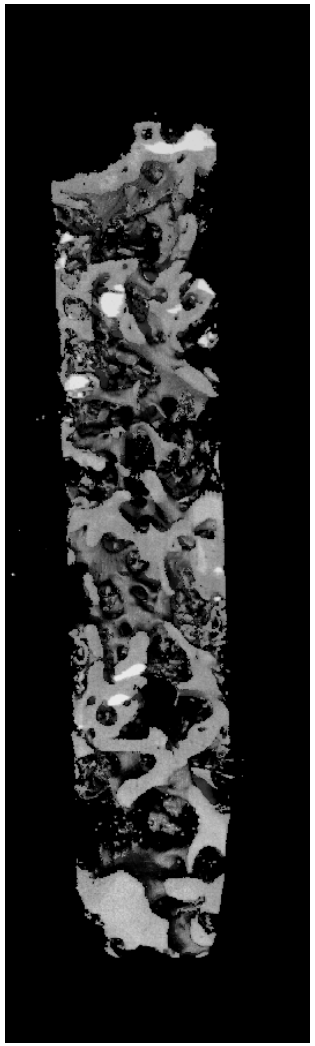
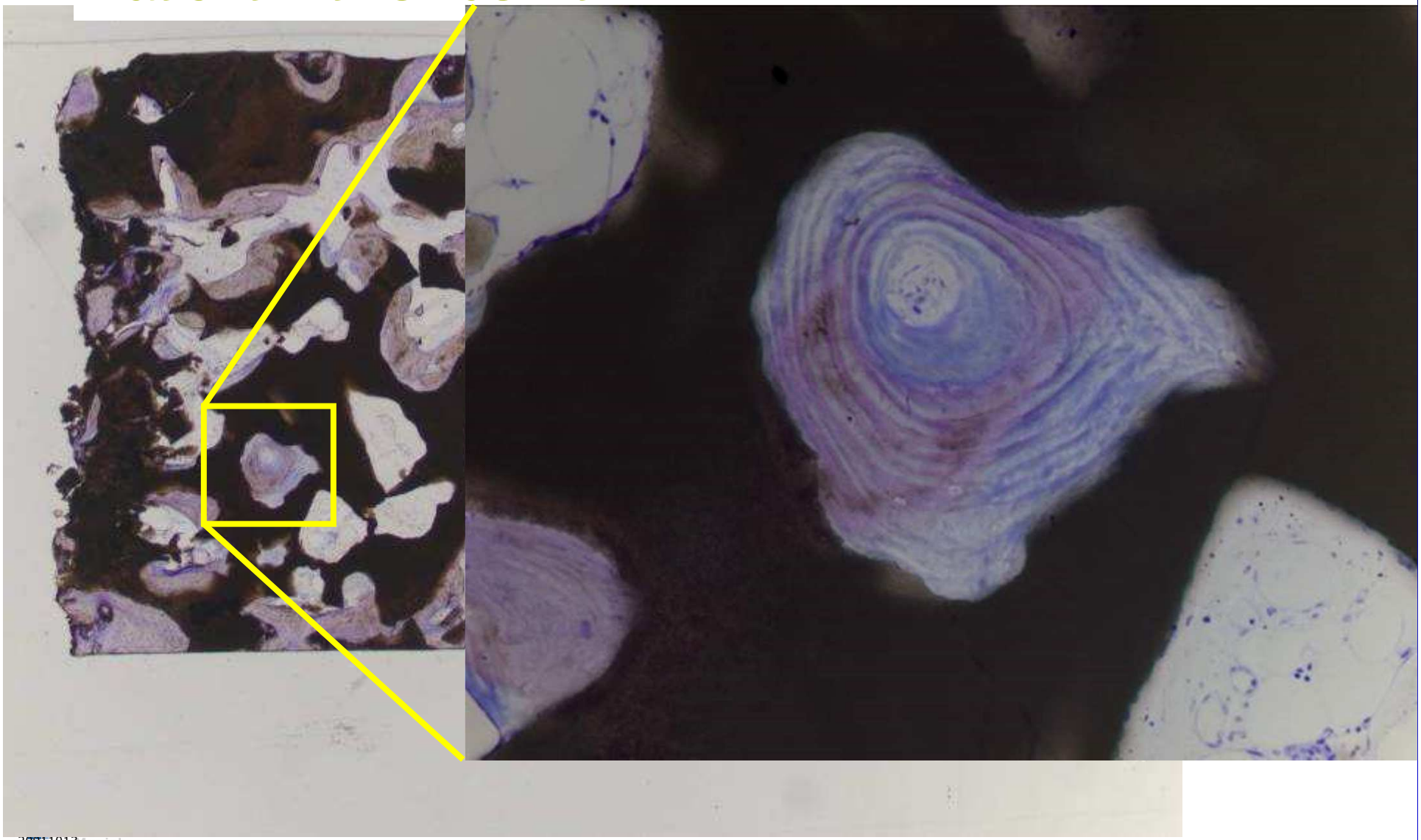
Bone regeneration







Patient with sinus lift

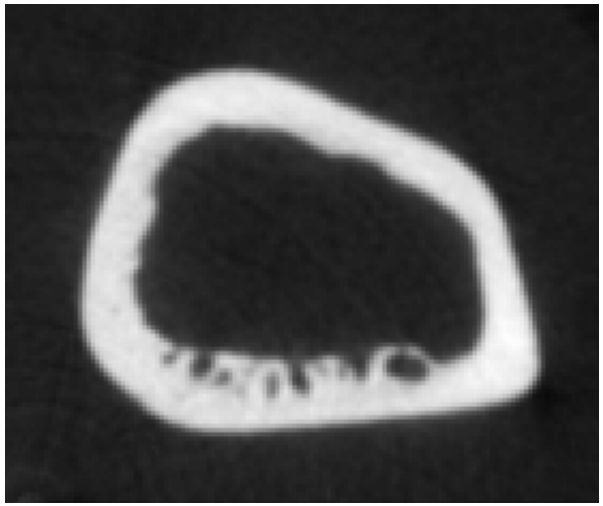


Co-morbidities in the models

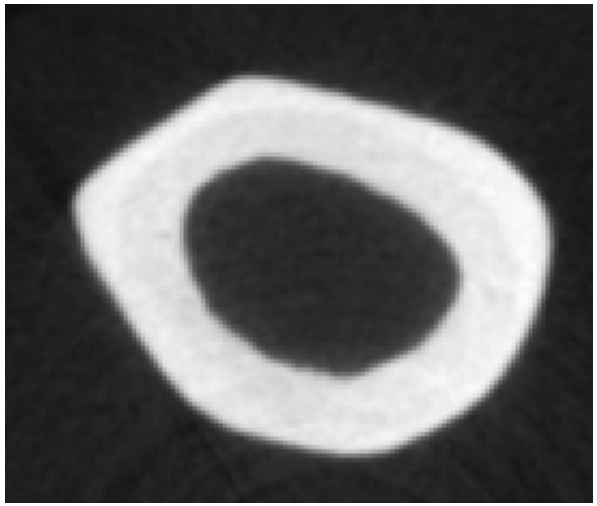
Mostly HEALTHY, YOUNG animals of ONE gender are used

- Osteoporosis
- Diabetes mellitus
- Age
- Etc.

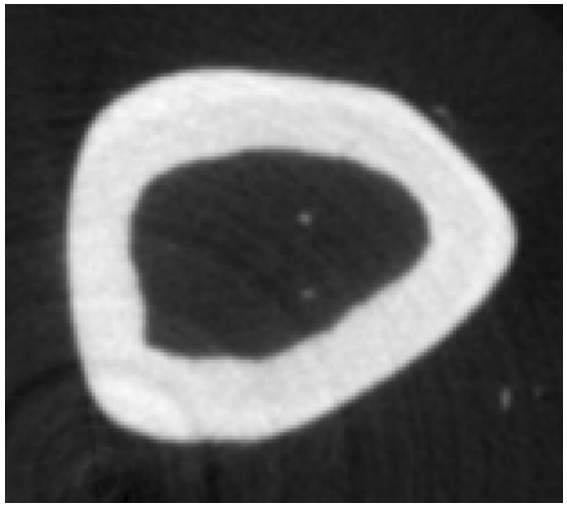
Ovariectomy in rats



Female ovx



Female



male

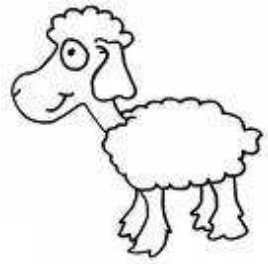
Human vs animal osteoporosis

Menopause

Severely **diminished circulating estradiol concentrations**

→ major factor contributing to the accelerated rate of bone loss.

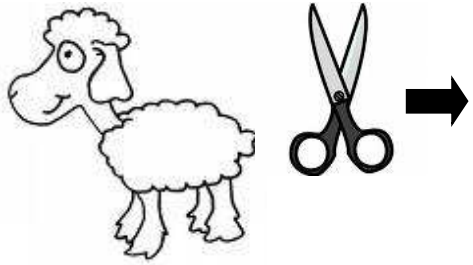
No menopause



Most other female animals, except >20y old Non-Human Primates, generally experience **lifelong estrous cycles**

→ no menopause, no natural major bone loss

Induced postmenopausal-like state



Cessation of estrous cycling can be brought by OVX.

→This approximately mimics what occurs endocrinologically and in some circumstances skeletally, in postmenopausal women

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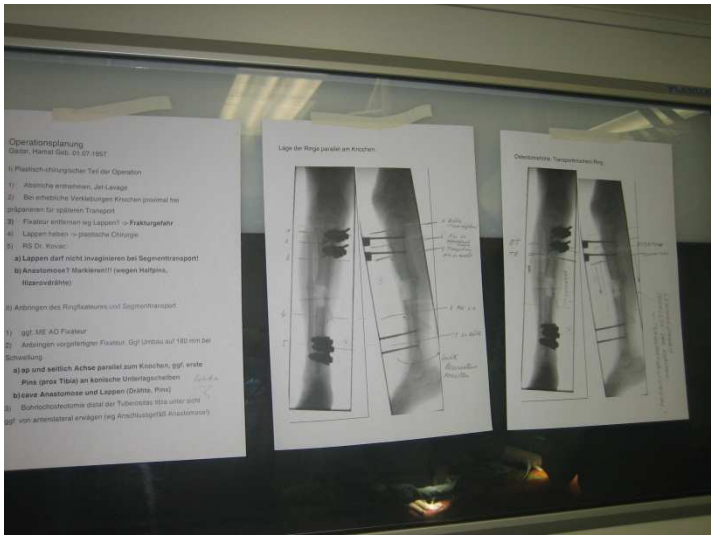
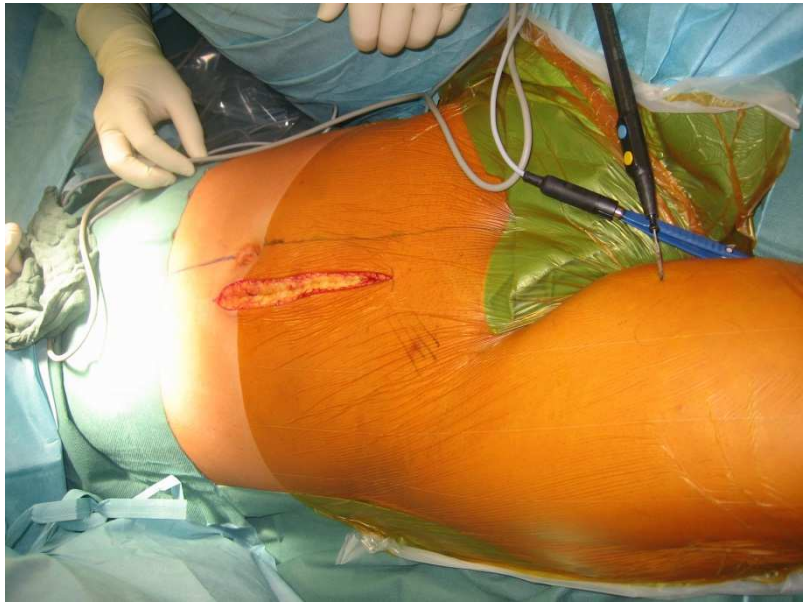
Clinical application

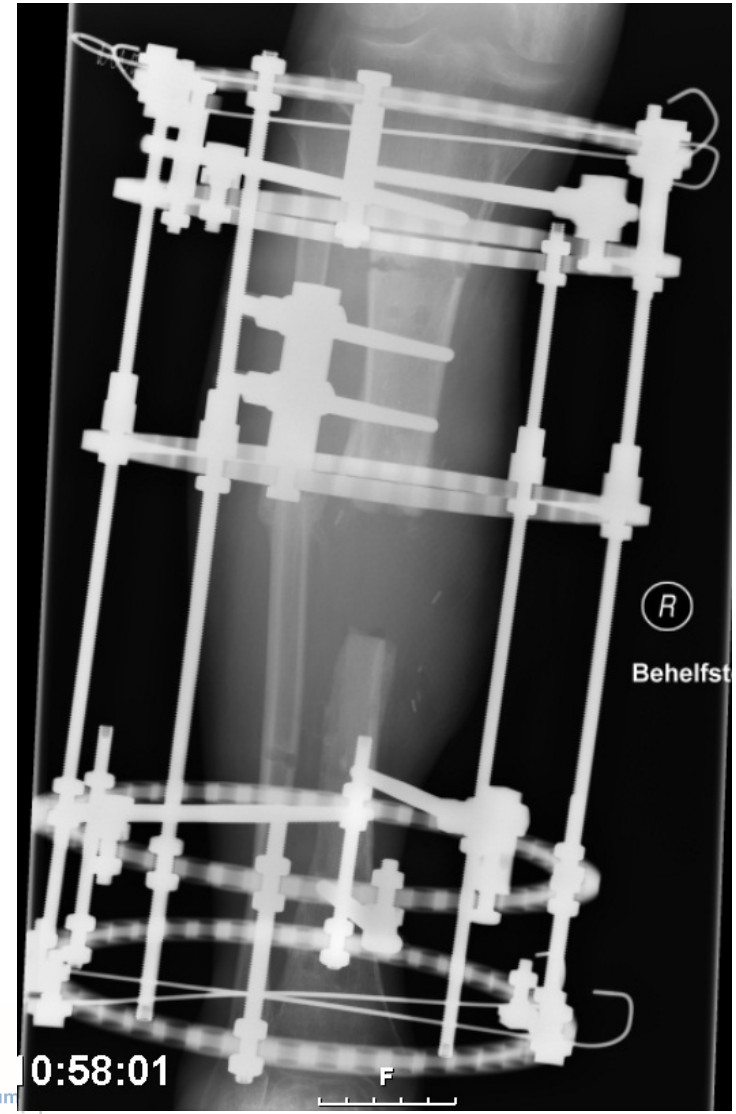
- Non-union
- Docking site at distraction osteogenesis

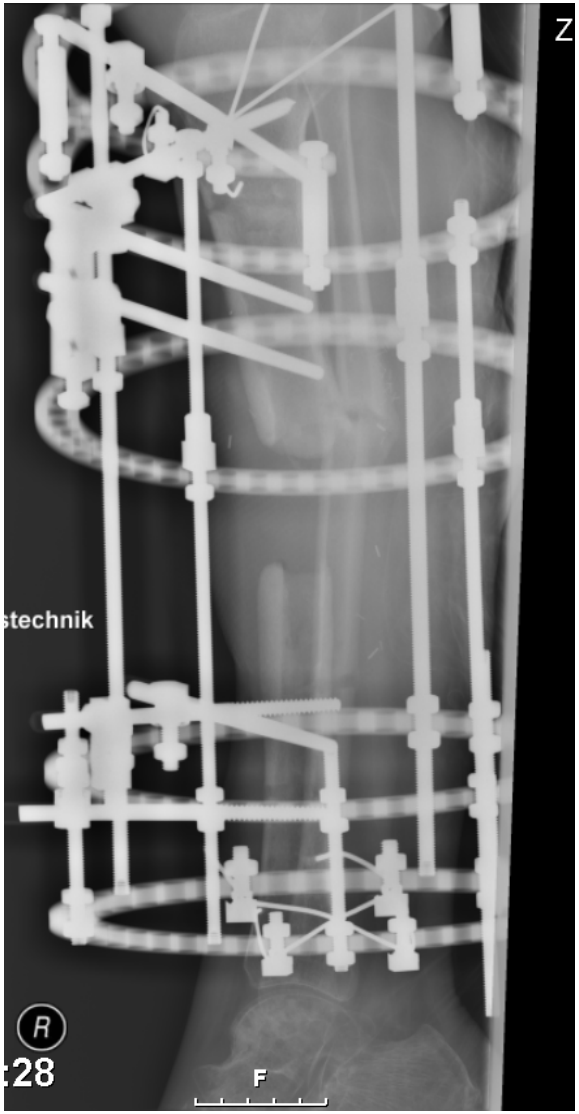
Non-union


Operationsplanung
Lage der Ringe parallel am Knochen
Osteotomielinie, Transportbruchteil Ring

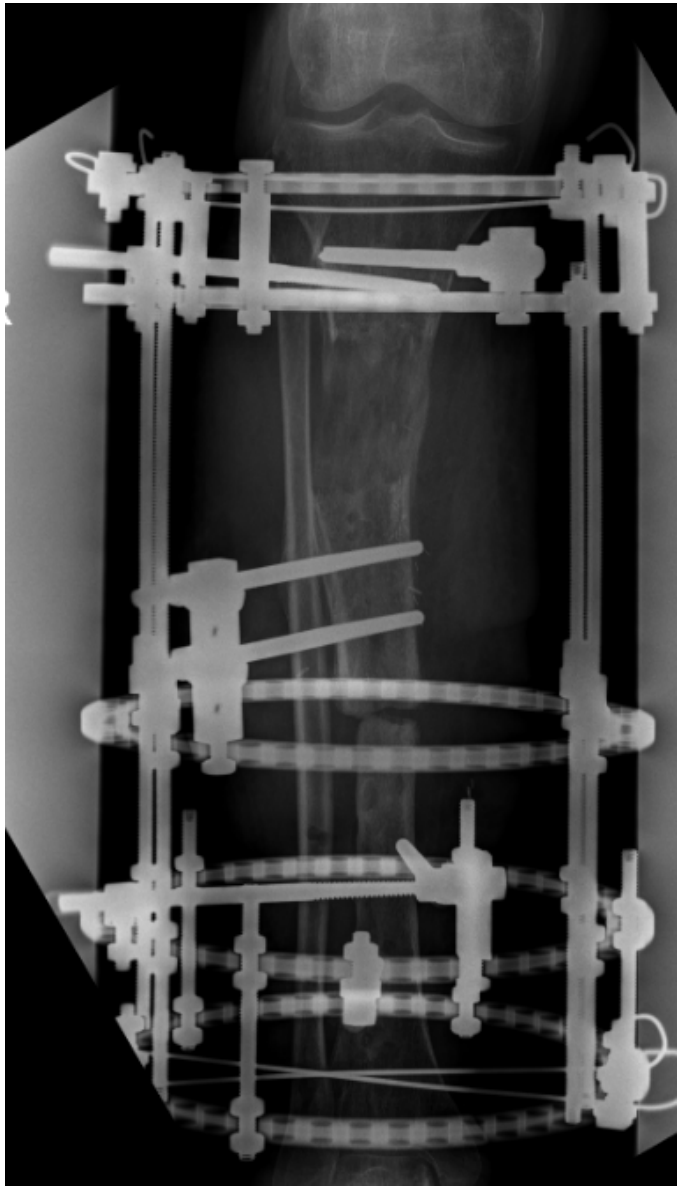


Distaction osteogenesis





Non-healing docking site

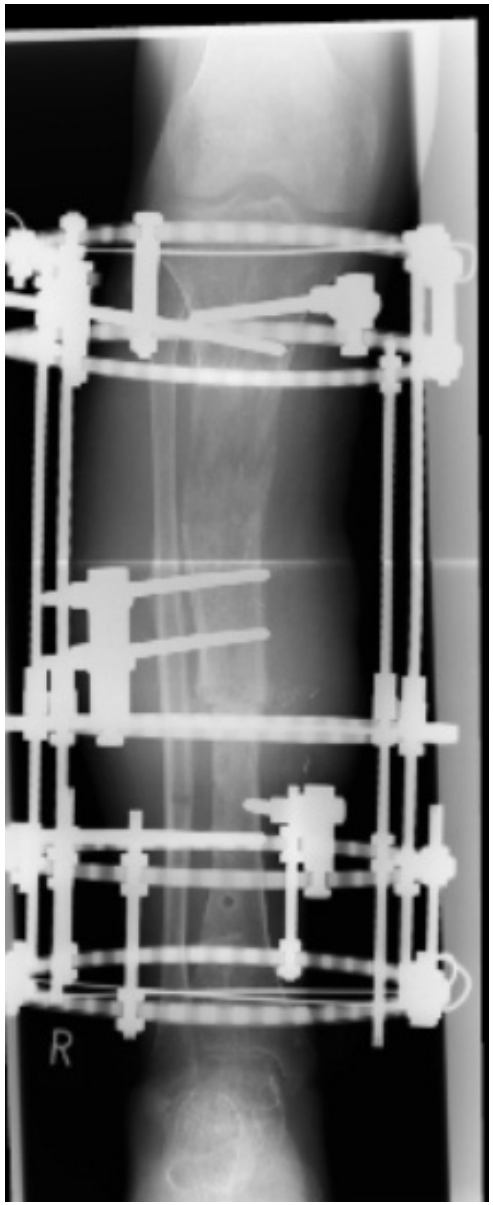


OP -procedure

Docking Operation

- Spongiosa
- TCP-collagen foam
- Bone marrow aspirate

1 month post-OP



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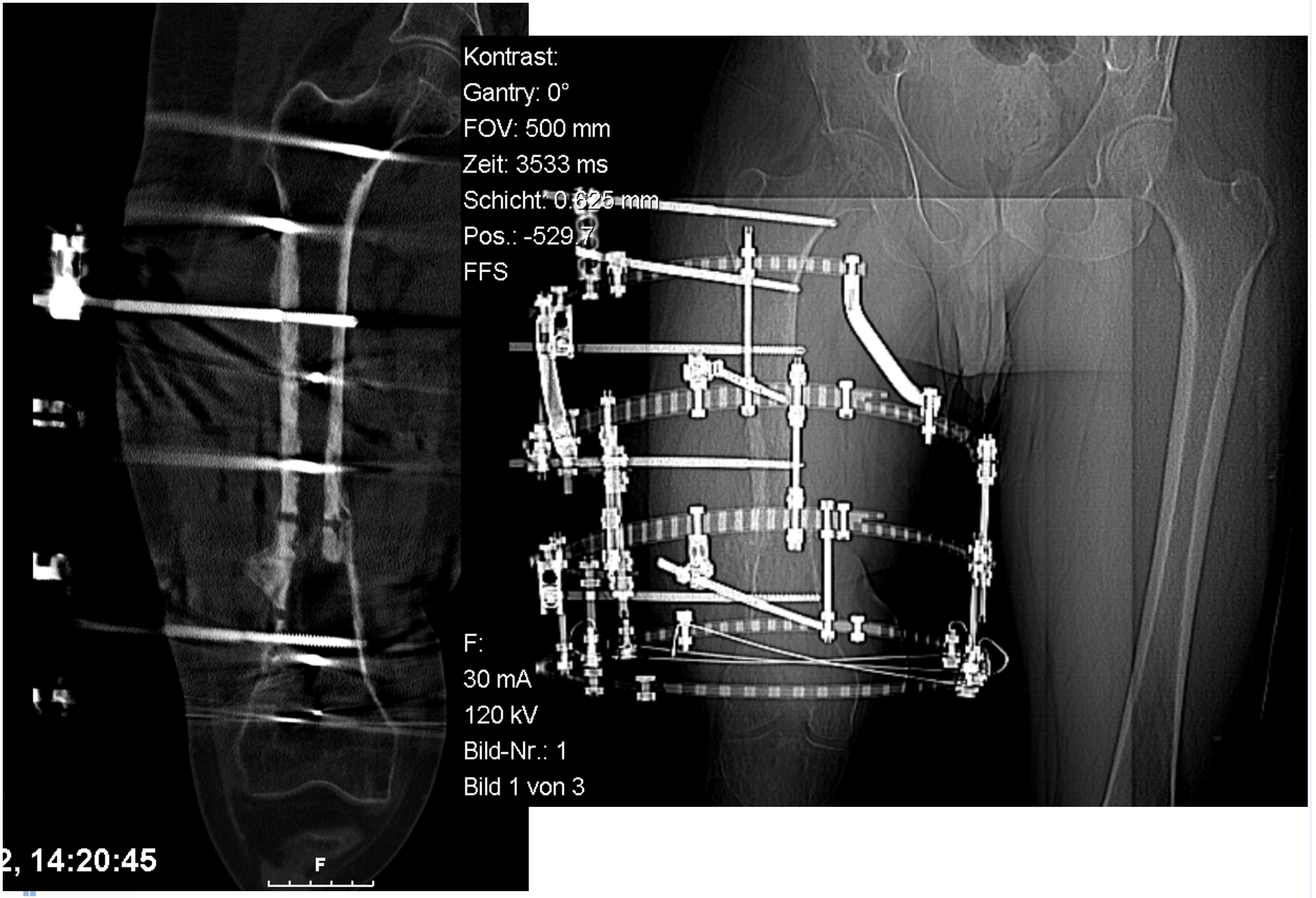
Tissue Engineering

- Stem cells
- TCP foam


Kontrast:
Gantry: 0°
FOV: 500 mm
Zeit: 3533 ms
Schicht: 0.625 mm
Pos.: -529.7
FFS
F:
30 mA
120 kV
Bild-Nr.: 1
Bild 1 von 3
2, 14:20:45
F

6 months

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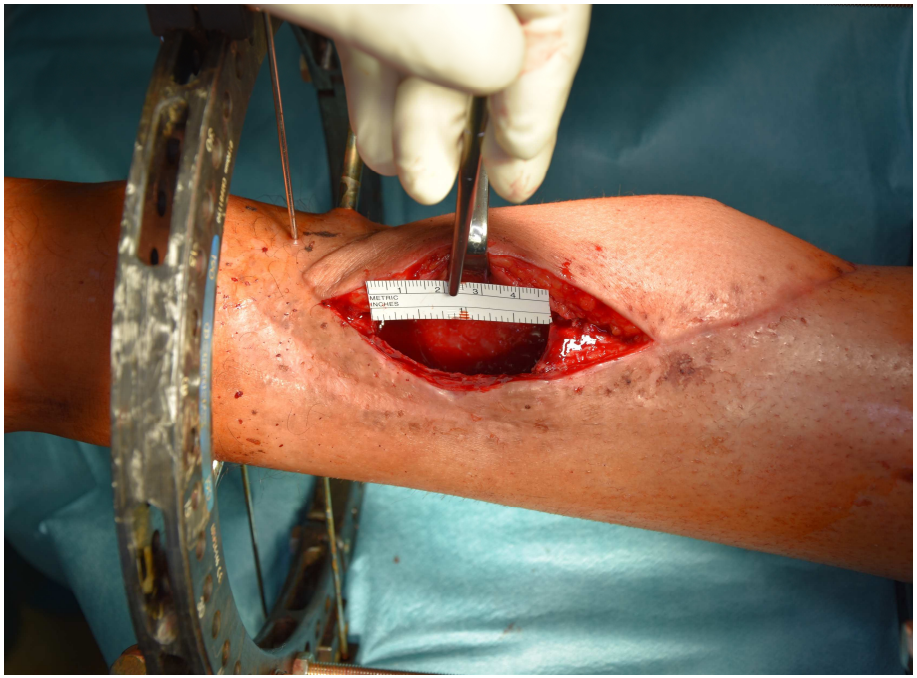
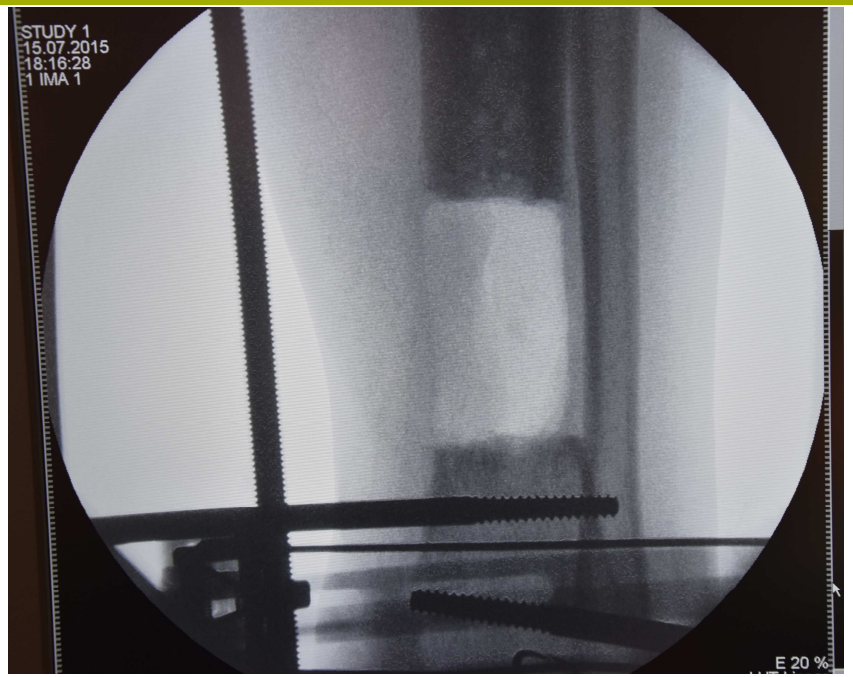
Non-union

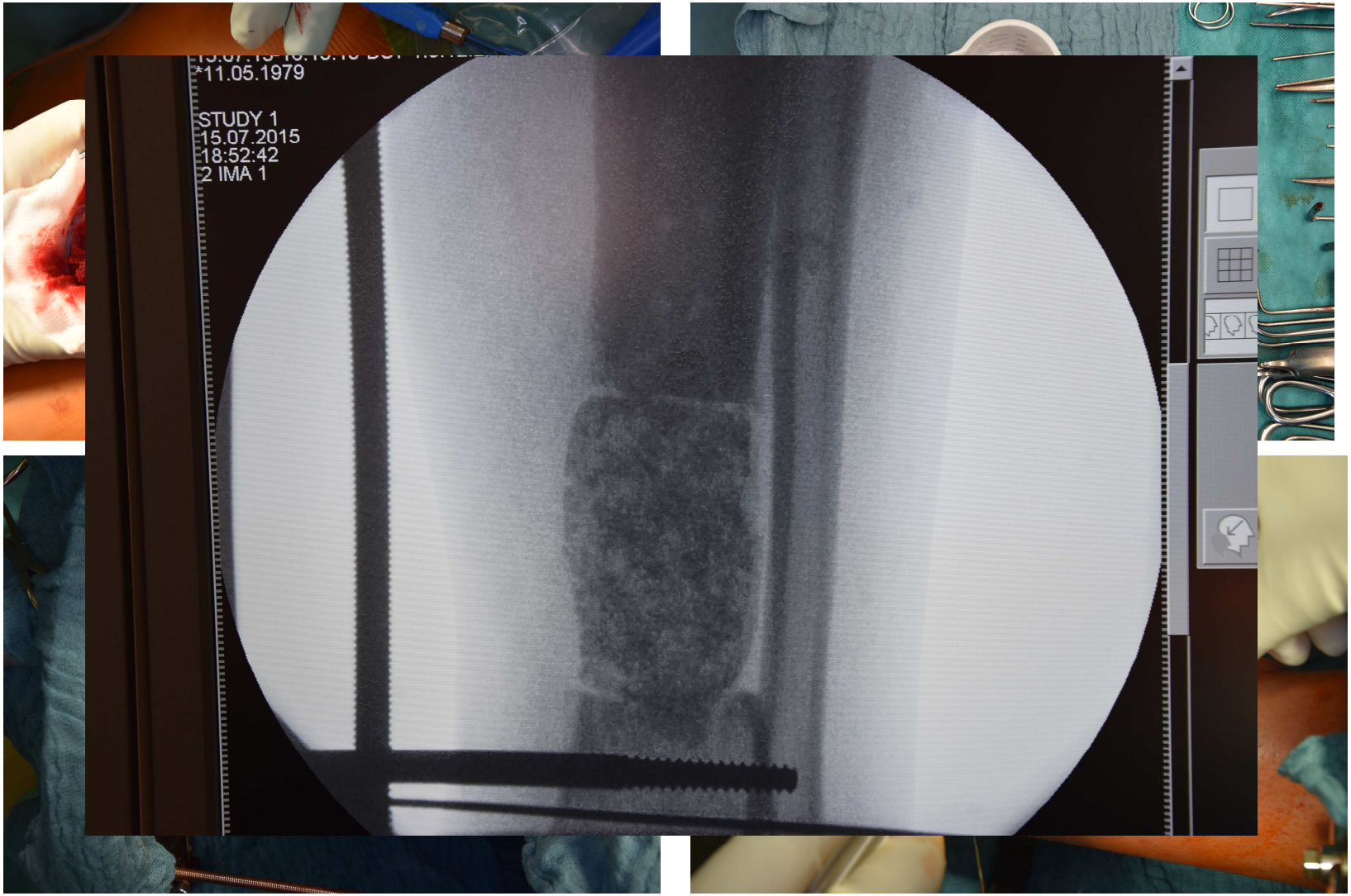
Bioglass

Masquelet





Bioglass administration within Masquelet membrane



8 months after implantation

Too less mobility of the patient!

11 months post operation

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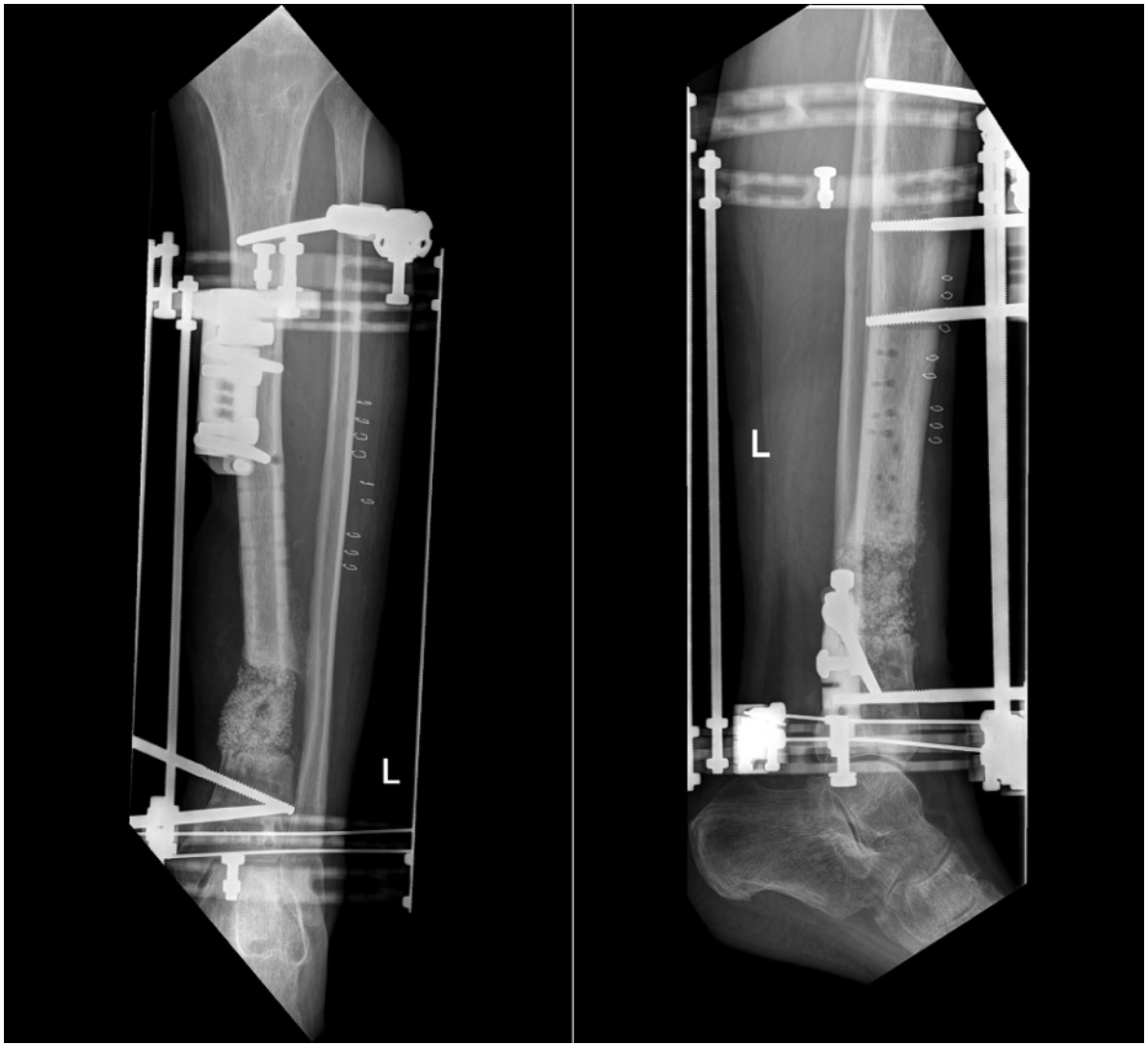
1 year post operation: plate broke due to heavy running



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NON-UNION

presentation

2.5 years non-union

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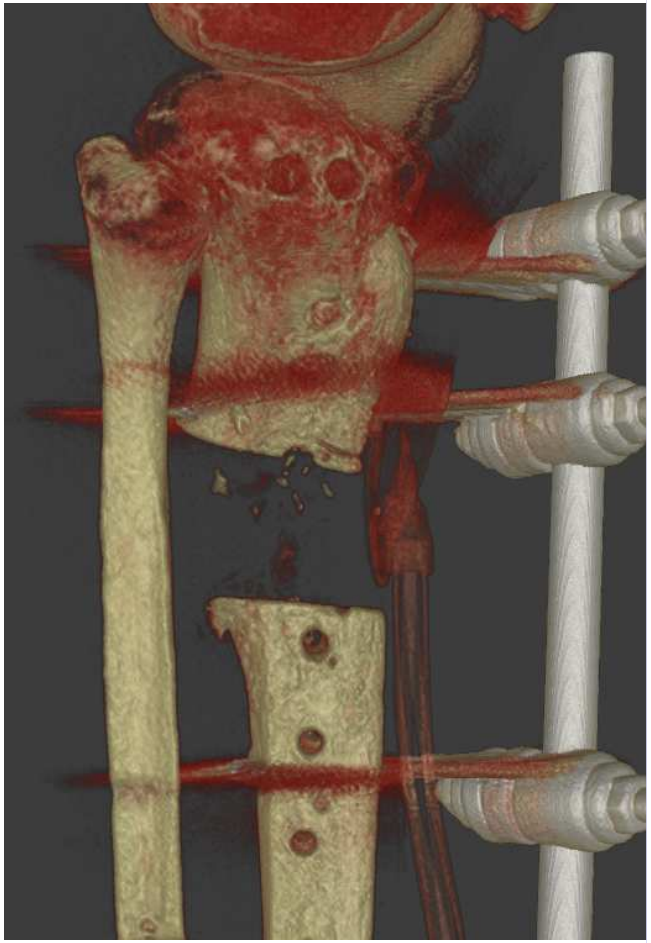
First operation

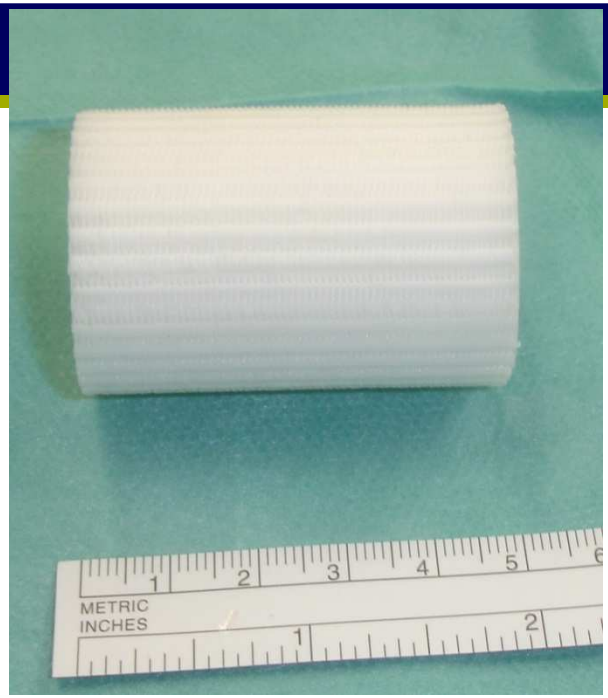
Resection

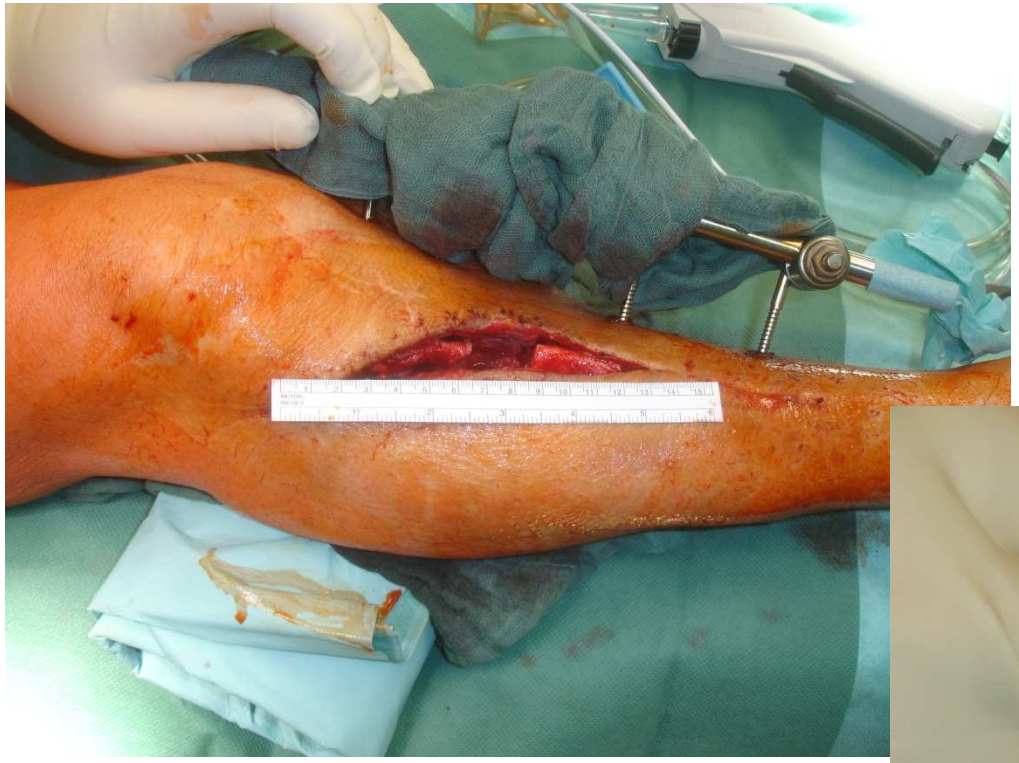
Drainage

Antibiotics



PCL-TCP scaffold





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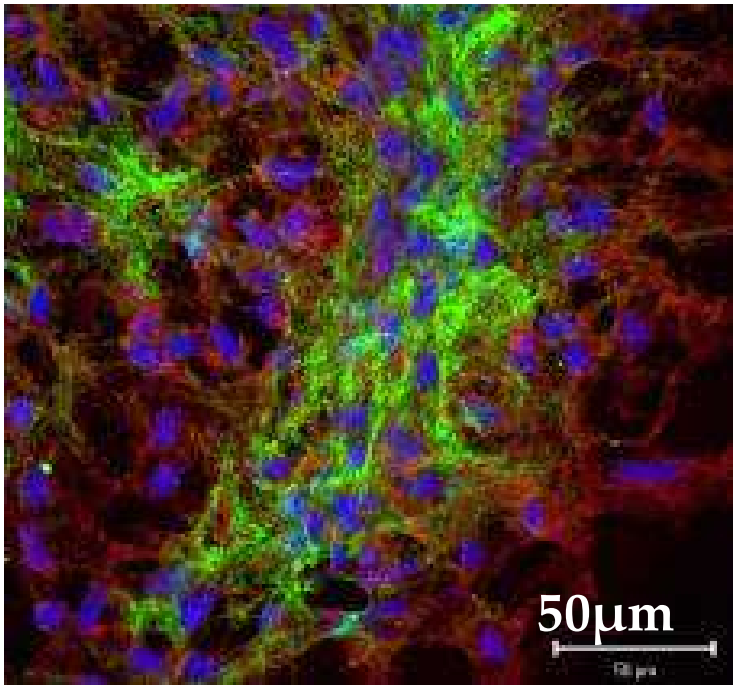
Bone specific immunohistochemistry

Bone matrix

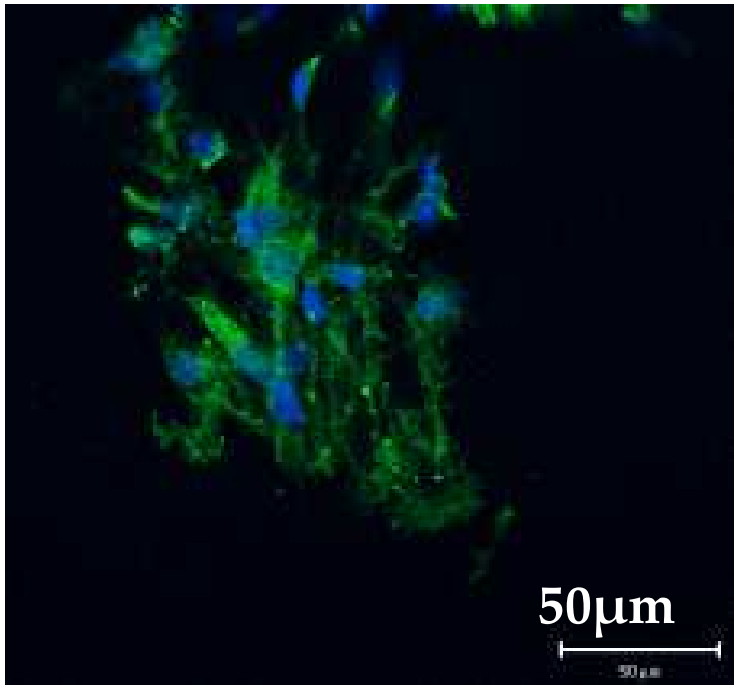


Anti-Kollagen I : green fibrils (FITC)

Nuclei : blue (DAPI)

Red : cytoskeleton (Phalloidin 546)

Bone Gap Junctions



Anti-Connexin 43 : green Punkte (FITC)

Nuklei : blue (DAPI)

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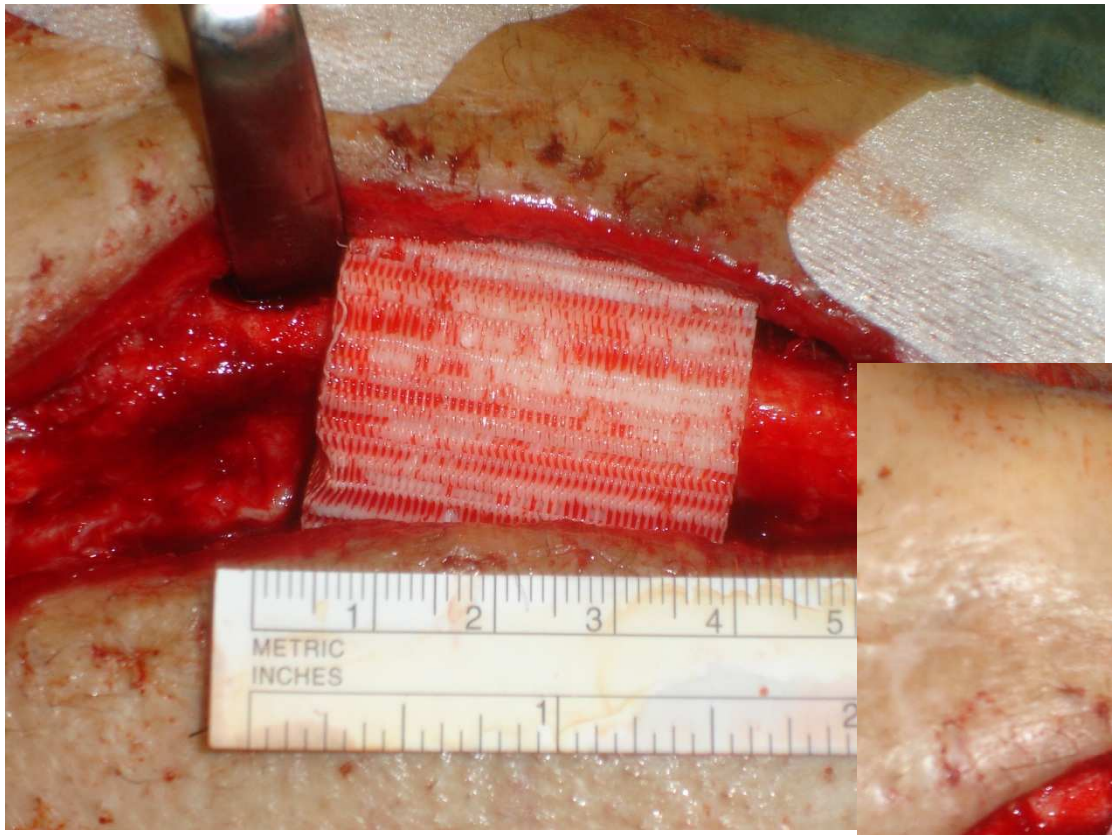
Scaffold on intramedullary nail



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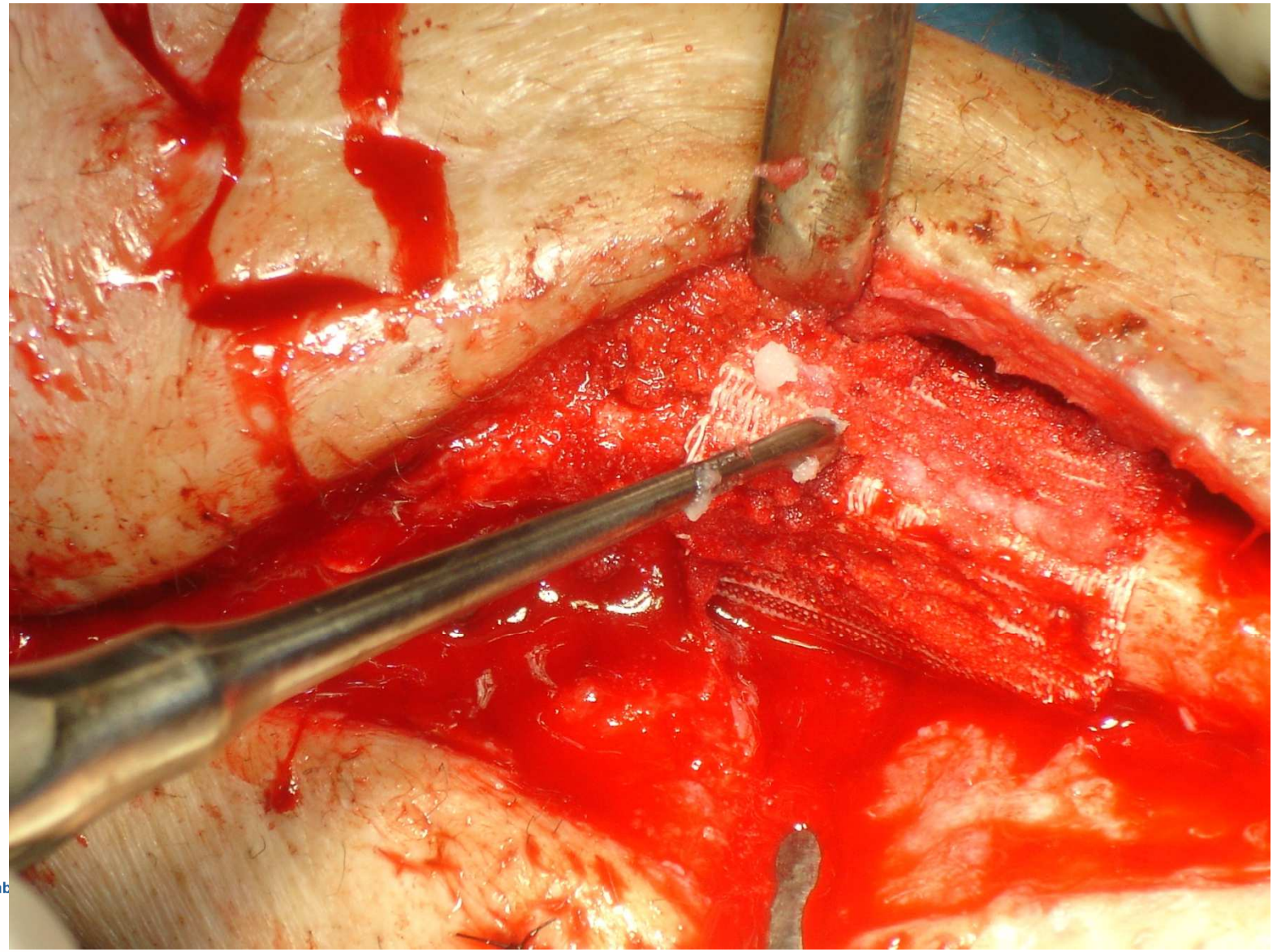
Applying BMP-7 + MSC



PCL cover

Scaffold + MSC + BMP-7 + „cover“

End of operation

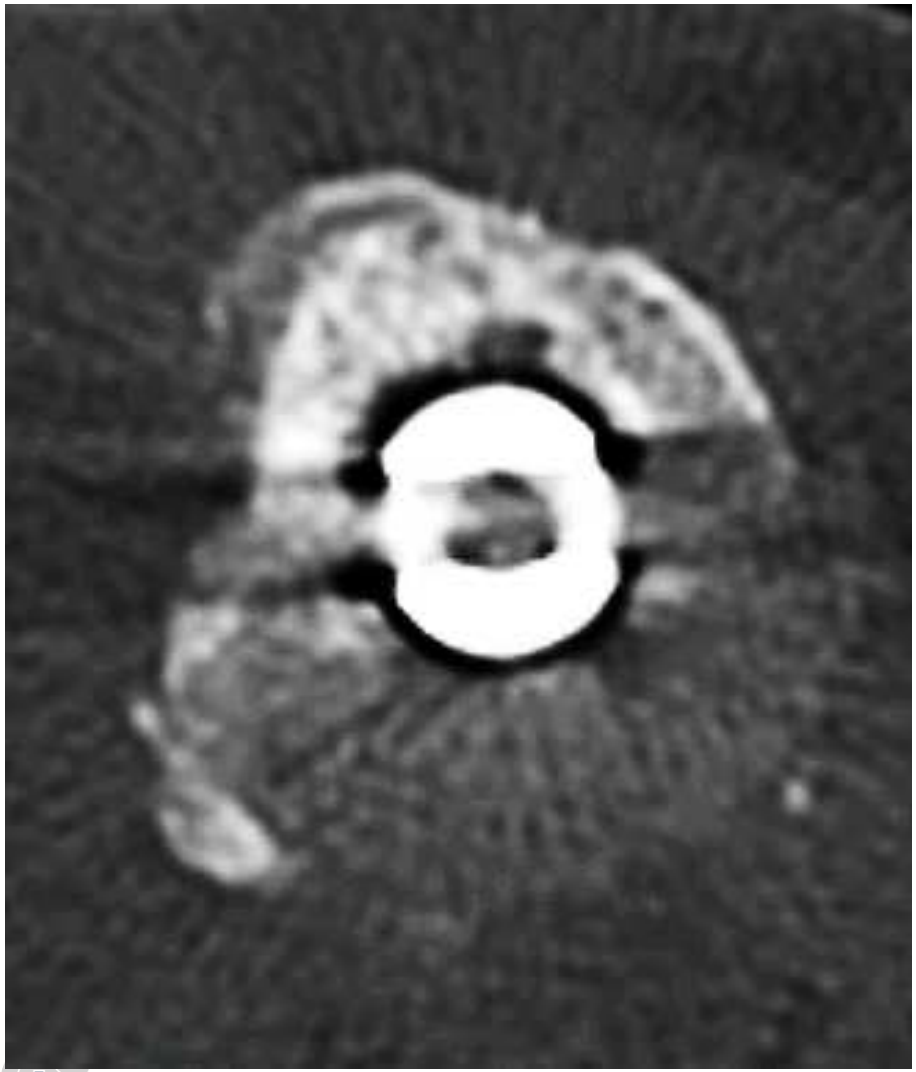
1 week post-OP

6 weeks post-OP

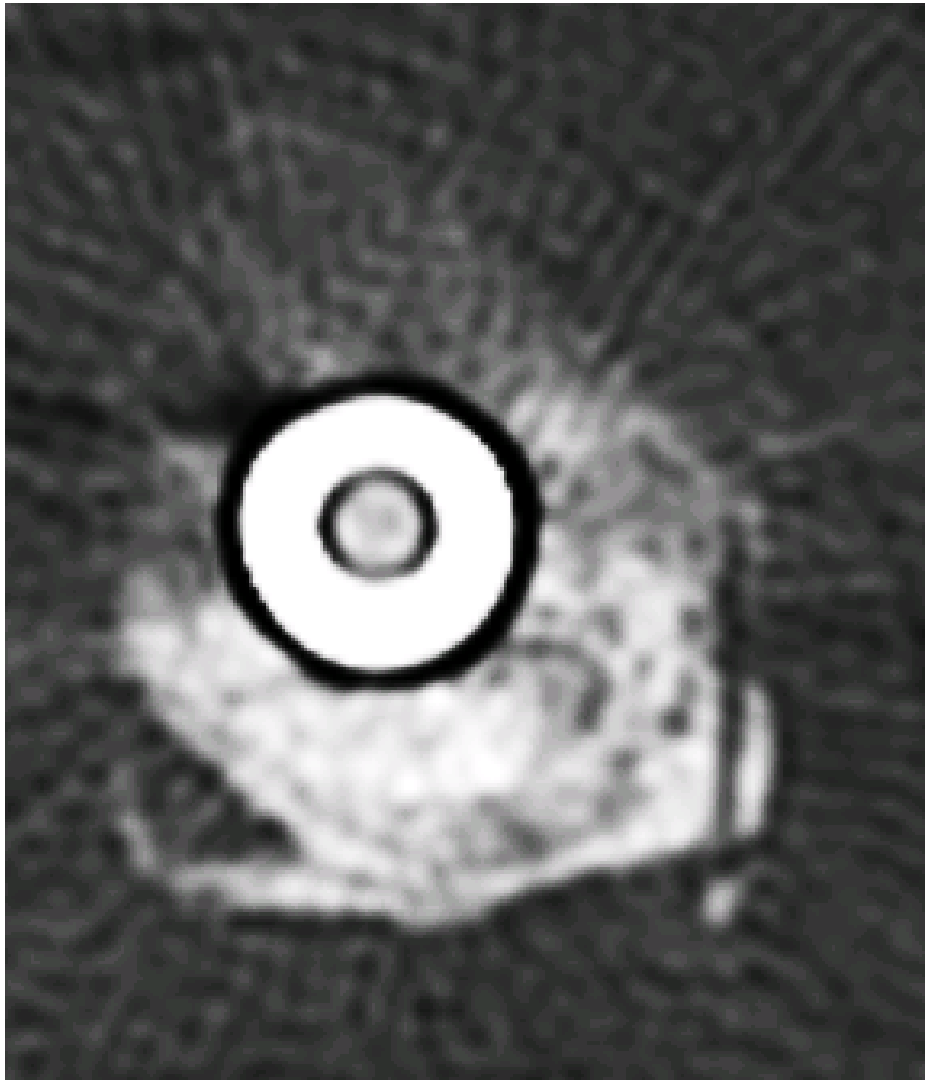
- First bone formation visible

3 months post-OP

6 months post-OP



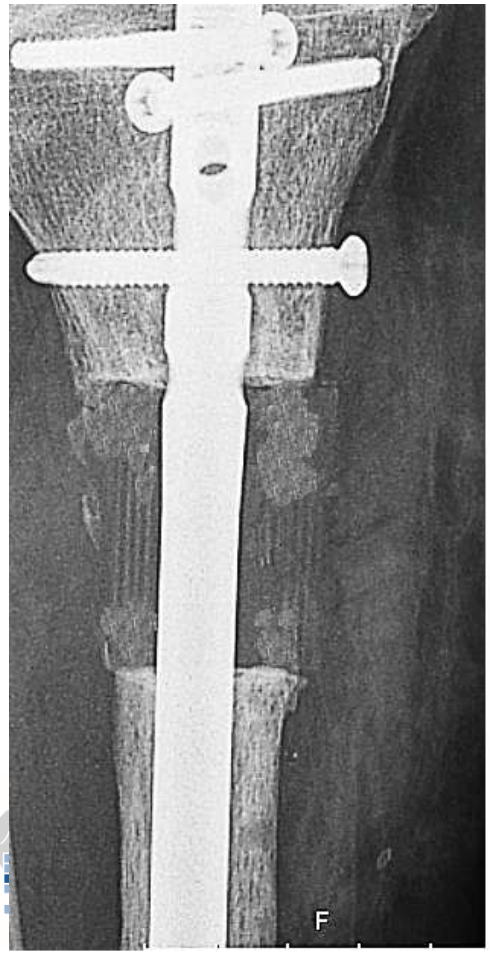
proximal



distal

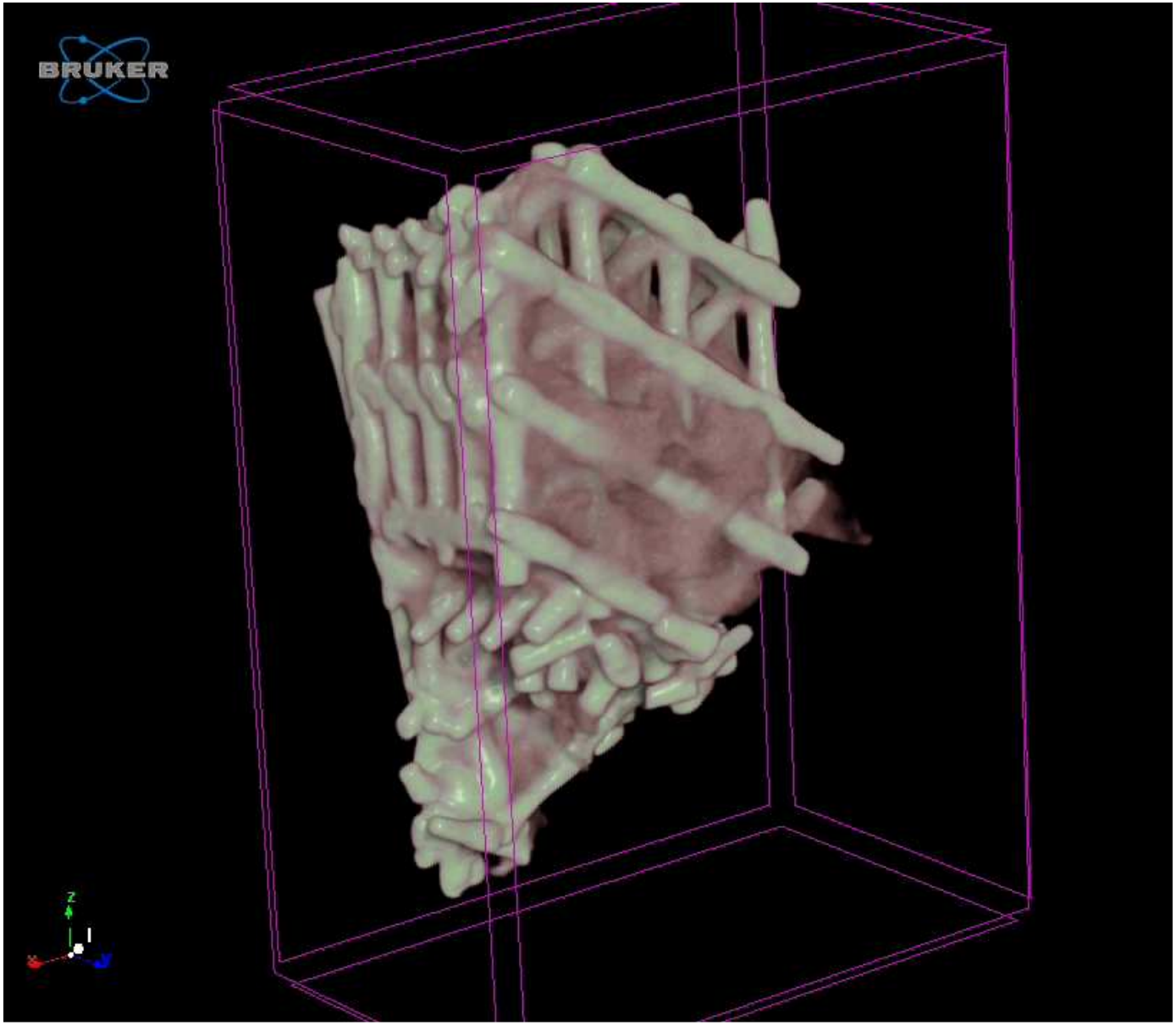
8 months post-OP

- Walking without aid
- Mountain tour 4 hours



1.5 years

biopsy



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Conclusion

- Think before you set up an animal model
- 3Rs
- If you do it right, you get a lot of information


Munich, Germany

Thank you!

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