Research on the animal for the animal for diseases that are original to certain species, e.g., arthritis

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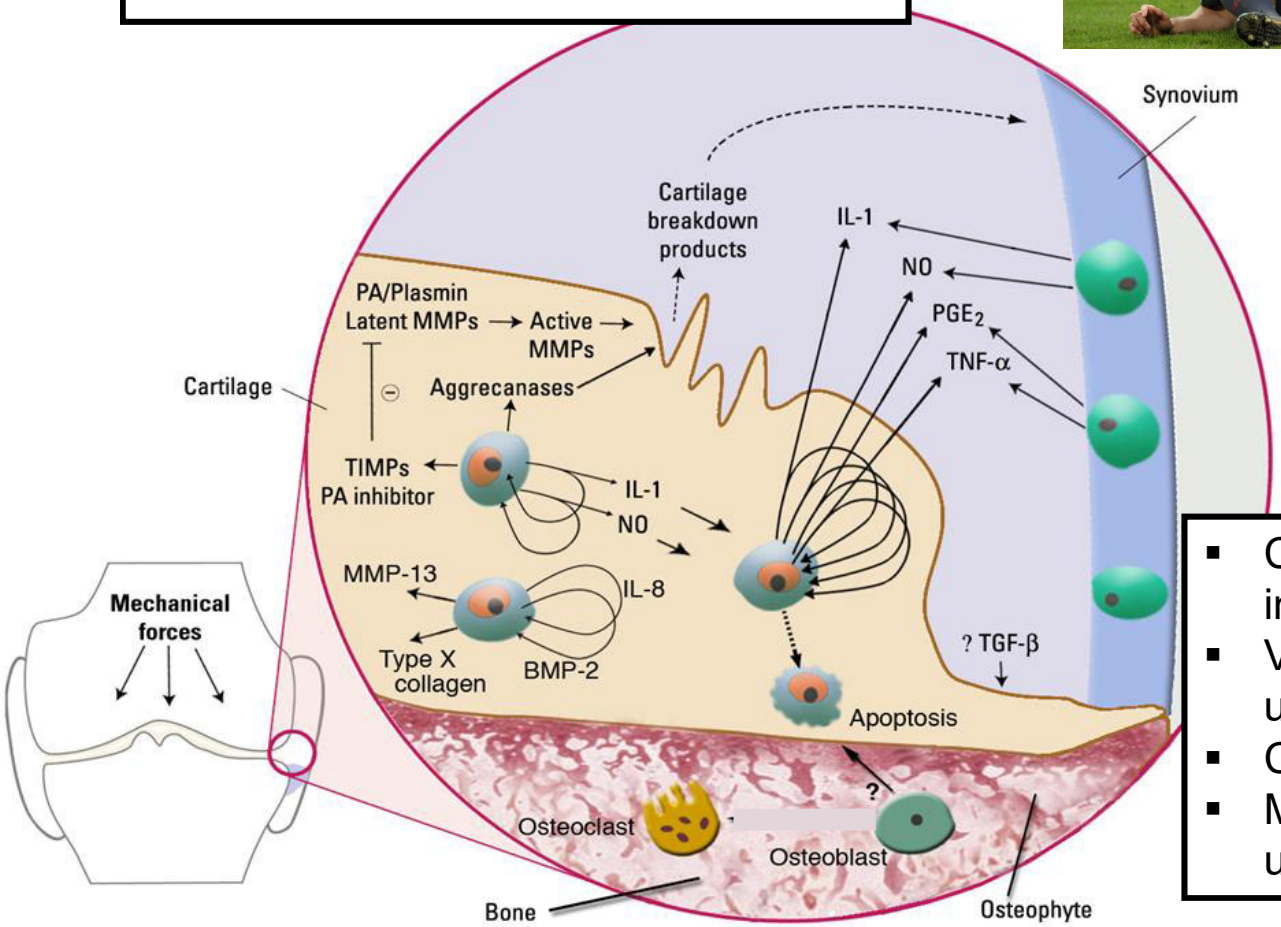
Osteoarthritis

- WHO: 9.6% of men and 18% of women over 60 suffer from OA
  Germany 2006: 7.1 billion euros in health care costs



- Chronic, degenerative and inflammatory
- Various causes, mostly unknown
- Cartilage loss
- Mechanisms not yet fully understood

Abramson et al. 2006 *Bull NYU Hosp Jt Dis*; 64:1-2

Therapeutic strategies and approaches

- **Cartilage loss**
  - Cartilage protection (hyaluronic acid, green-lipped mussel extract)
  - Autologous cartilage cell transplantation
  - Matrix-associated cartilage transplantation
  - Transplantation or injection of stem cells
- **Inflammation**
  - Paracetamol, NSAIDs and Cox-2 inhibitors, opioids
  - Disease-modifying osteoarthritis drugs (DMOADs) → monoclonal antibodies and soluble cytokine receptors
- **Cartilage loss and inflammation**
  - Gene therapy
  - Nanomedicine

→ A complete cure is currently not possible. Further developments are necessary!
Lack of understanding of the underlying mechanisms!

Animal models in orthopedic research

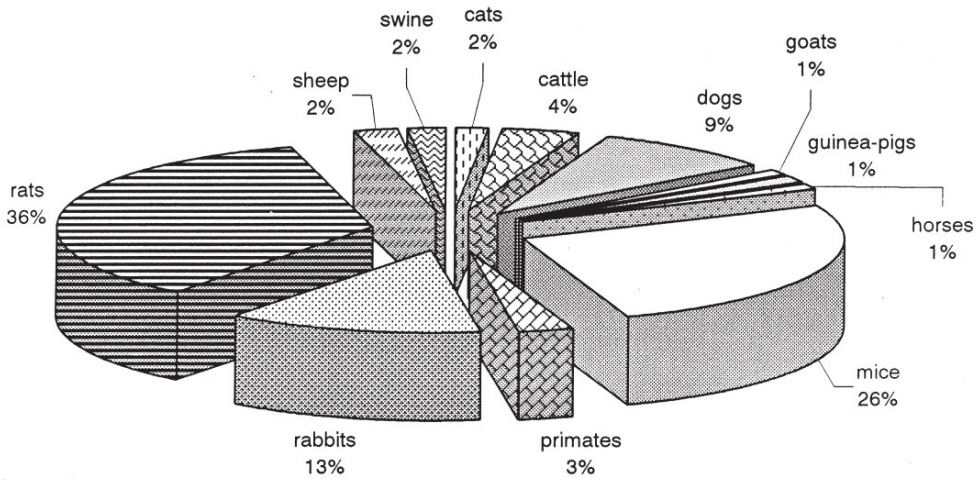


Figure 1. Mammals used for orthopedic research during 1970–2001. Average percentage expressed on the basis of total amount of 21,500 studies of animal species reported in the field of orthopedics.

Martini et al., *Comparative medicine* 2001; 51: 292-299

Animal models used in orthopedic research (1970 - 2001)

→ Rats (36%), Mice (26%), Dogs (4%), Sheep (12%)

Animal models in OA-Research

Why? → Models are systems for the study and understanding of complex interrelationships of human diseases

→ For the study of processes in a physiologically working organism

Osteoarthritis (OA)

Spontaneous

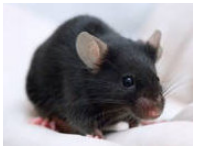
Cartilage loss
Inflammation
Pathogenesis

Age/ obesity (Hartley guinea pig)
Collagen mutation (Col II or IX)
Genetic predisposition
Trauma/ naturally occuring


Chemically- induced

Cartilage loss
Inflammation

Papain induced
Collagenase induced




Physically- induced

Cartilage loss

ACLT
Meniscectomy
Cartilage defects



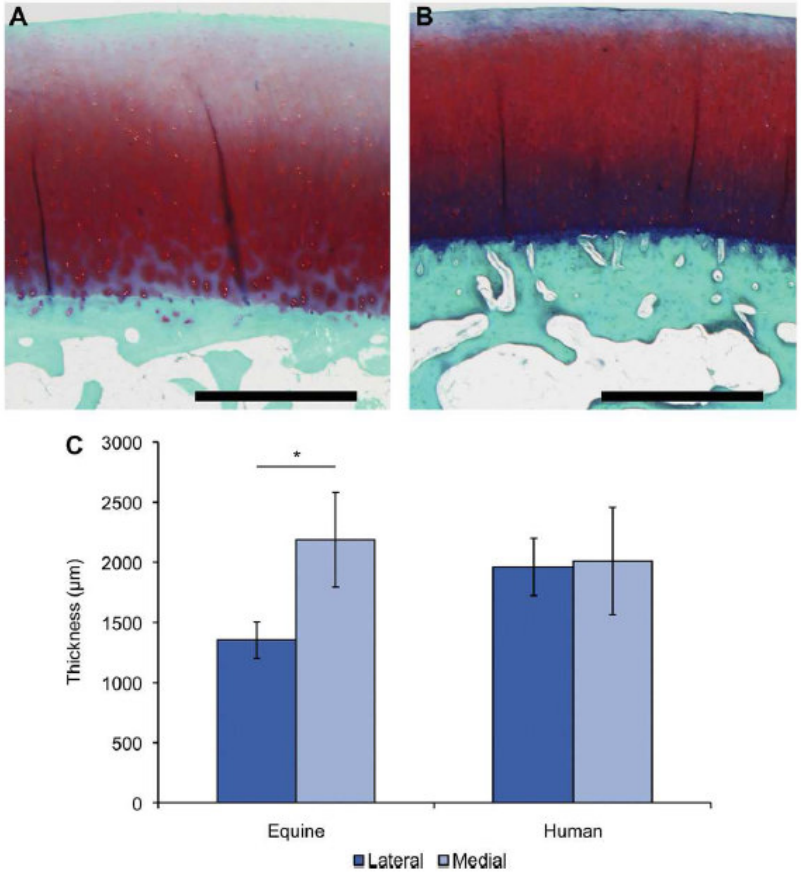

Cartilage thickness in different species

TABLE 1. MEAN CARTILAGE THICKNESS

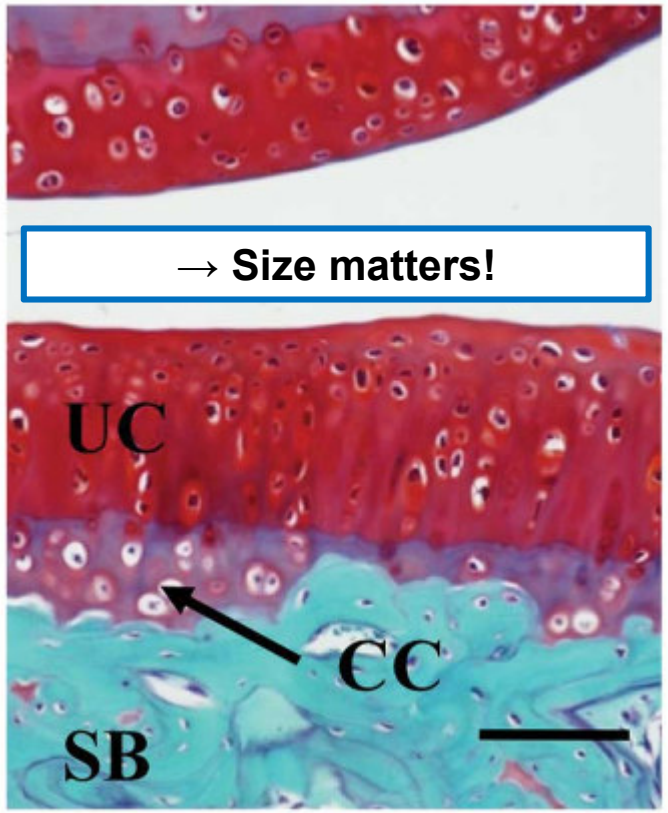
Species	*Murine*	*Lapine*	*Canine*	*Porcine*	*Caprine*	*Equine*	*Human*
MFC cartilage thickness (mm)	0.1	0.3	0.95	1.5	1.1	1.75	2.35

This table shows the mean cartilage thickness on the medial femoral condyle (MFC) from Frisble *et al.*[66] Values are reported in millimeters.

Frisbie et al. 2006 *Vet Comp Orthop Traumatol.*; 19 (3)



Malda et al. 2012 *Osteoarthritis & Cartilage;* 20



Watari et al. 2011 *Experimental and Therapeutic Medicine*

Animal numbers per study

Systematic Review: 1990 – 2007 → 113 studies

Table I

Overview of literature with reference to study cohort size, defect volume (mm^3) and cartilage thickness (mm). Cartilage thickness of the medial femoral condyle from Frisbie et al.[12]. *Defect volumes were calculated for cylindrical shaped defects using* $V = \pi r^2 h$, *for cube shaped defects using* $V = lwh$ *(r = radius, h = depth, l = length and w = width of the cartilage defect)*

Species	Medial femoral condyle cartilage thickness (mm)	Studies performed		Animal number utilized	Total volume (mm^3)	Cartilage volume (mm^3)	Subchondral volume (mm^3)
Murine	0.1	5	Mean	30	2.17	0.12	2.05
			SD	15.88	2.85	0.06	2.81
			Mode	30	5.3	0.18	0
Laprine	0.3	39	Mean	18.86	53	7.15	45.86
			SD	11.57	54.64	13.35	52.78
			Mode	16	21.21	2.12	19.09
Ovine	0.45	13	Mean	23.69	359.54	18.03	341.51
			SD	15.74	683.35	19.97	663.79
			Mode	20	n/a	12.5	0
Canine	0.95	16	Mean	34.82	82.39	18.43	63.86
			SD	46.85	197.94	17.4	181.9
			Mode	8	11.94	11.94	0
Porcine	1.5	10	Mean	9.56	107.43	43.76	63.67
			SD	2.35	87.87	24.05	78.9
			Mode	10	183.22	34.35	0
Caprine	1.1	13	Mean	30.55	251.65	45.71	63.67
			SD	20.39	448.46	35.1	78.9
			Mode	50	31.1	17.49	0
Equine	1.75	17	Mean	9	334.73	192.67	142.06
			SD	2.03	237.87	94.21	213.08
			Mode	8	137.44	137.44	0
Human	2.35	n/a	Mean	n/a	552.25	552.25	0

Ahern et al. 2009 *Osteoarthritis & Cartilage;* 17

→ The larger the animal, the fewer animals are needed!

The horse as a patient

- 33.7% of all horses treated in Germany suffer from a musculoskeletal disease
- The most common cause of retirement in equestrian sports is lameness
- Racehorses are particularly susceptible to knee problems and lameness (25%), which leads to high economic losses in the horse industry.
- 60% of lameness is caused by arthritic joint changes

"Comparative medicine" vs. "ONE HEALTH" → **no paradigm differences between human and veterinary medicine and both disciplines can help the other to develop further** (Zinsstag et al. 2011 *Prev Vet Med.*)

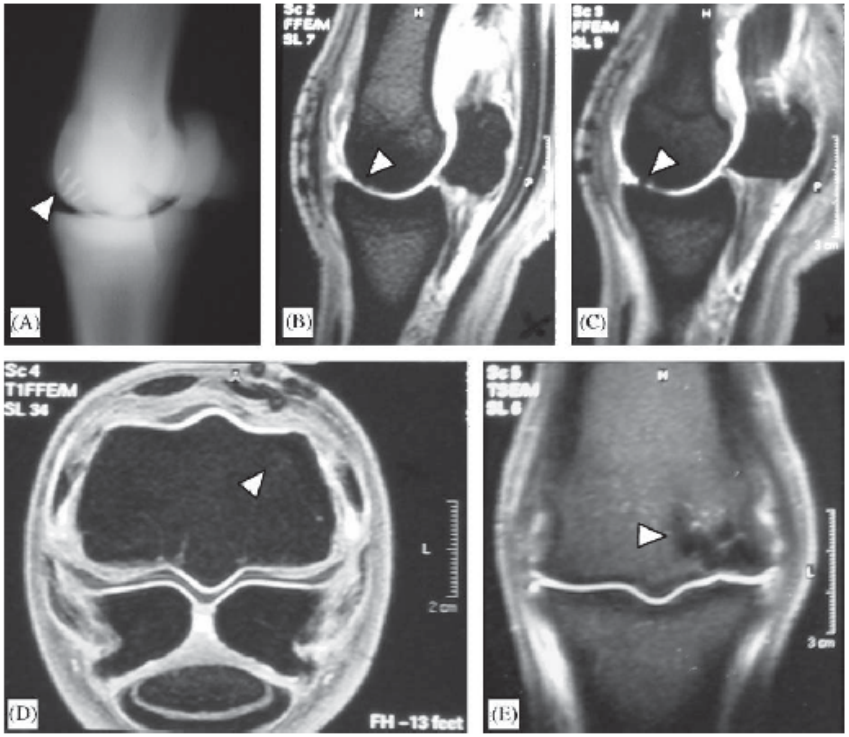
„…we should always remember that animals are also stakeholders and not just research tools.“

(Poole et al. 2010 *Osteoarthritis & Cartilage;* 18*)*

Lameness diagnostics

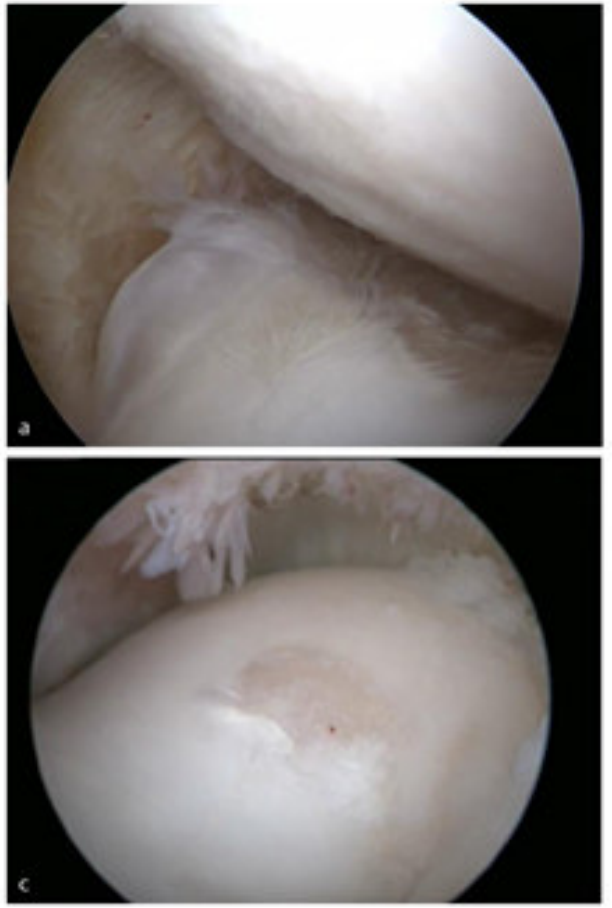
Imaging

MRI



Barnewitz et al. 2006 *Biomaterials;* 27

Arthroscopy



McIlwraith et al. 2010 *Osteoarthritis & Cartilage;* 18

Approach in OA-Research

Our Approach

Aim,
therapeutical approach

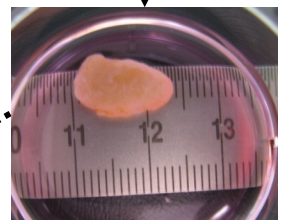
Simplified cell culture

Small animal model

Large animal model

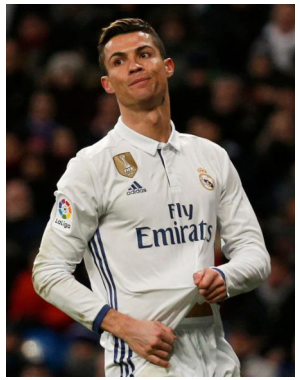
Aim,
therapeutical approach

Simplified cell culture



3D-Model

Large animal model = Patient

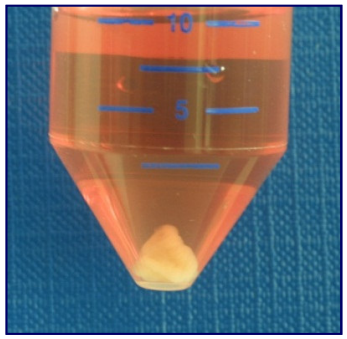


Development of an equine OA model

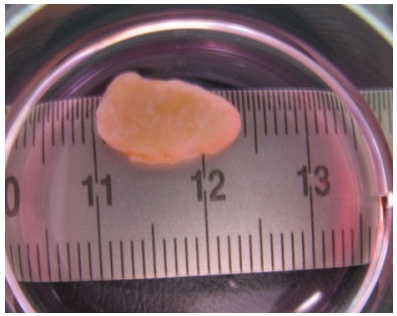
Cartilage biopsy

Isolation chondrocytes

Cell expansion



3 D tissue model

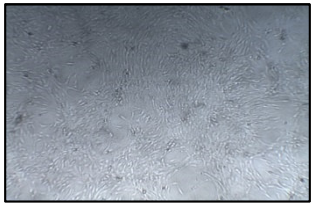


Loading and maturation

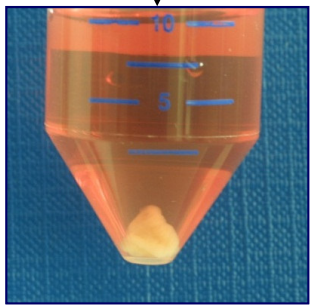
fzmb GmbH

in vitro OA model

Characterization of the cartilage model

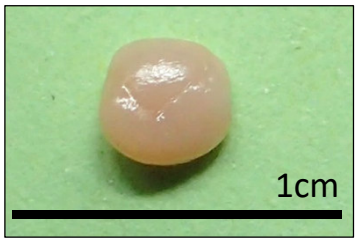


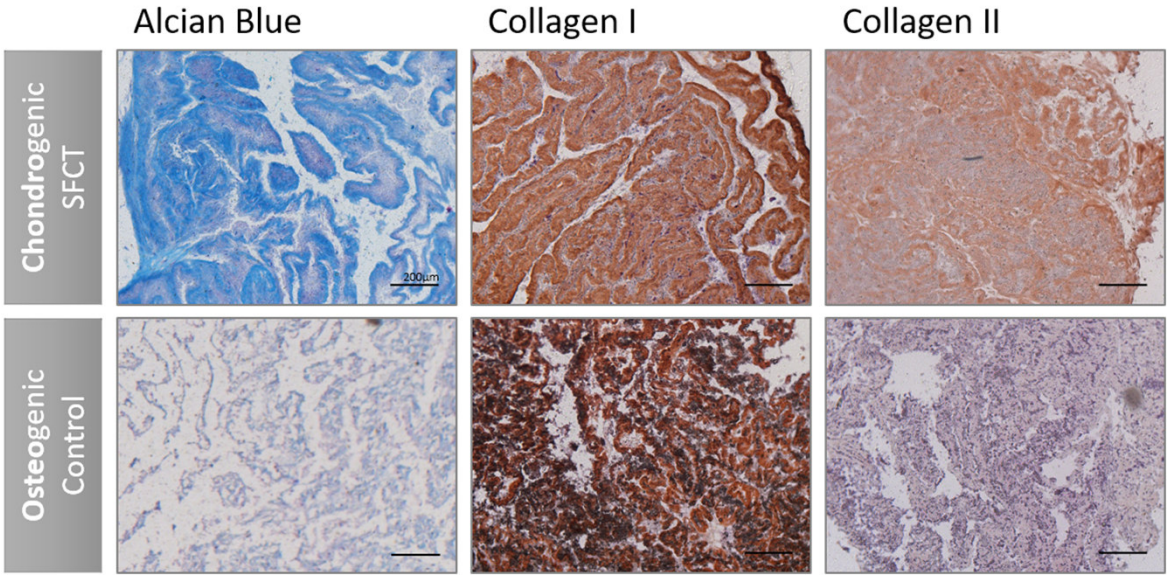
hMSC

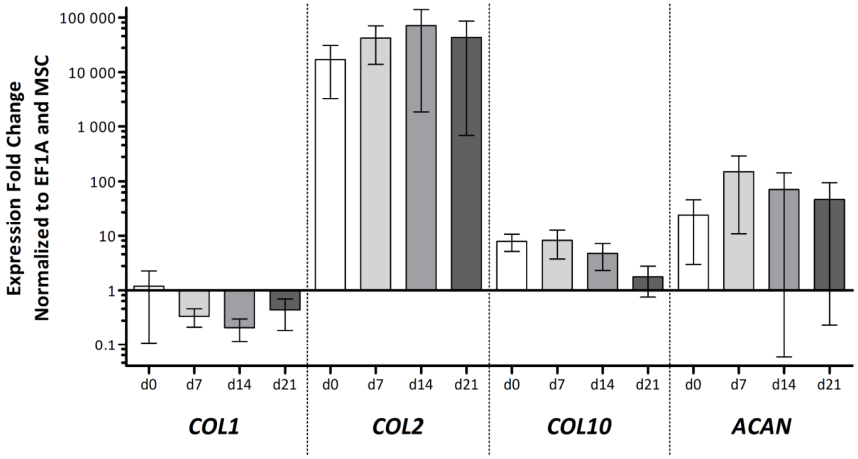


Maturation

fzmb GmbH





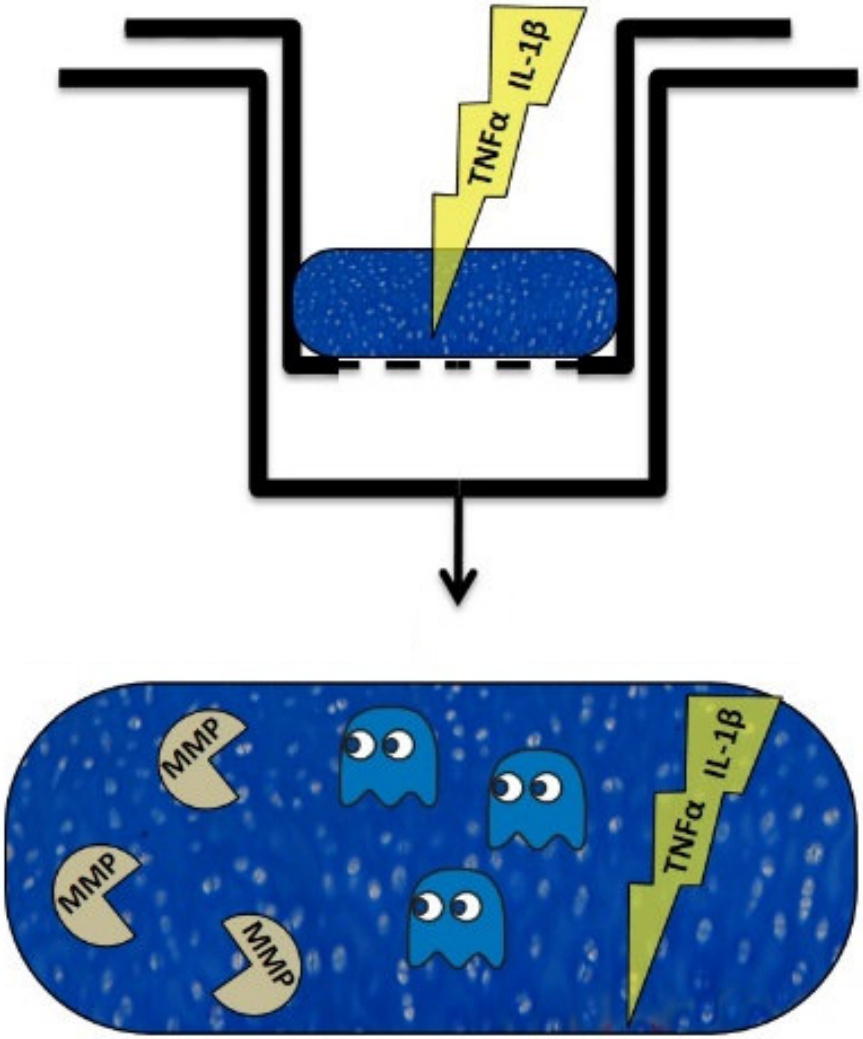


SFCT

Expression of characteristic genes

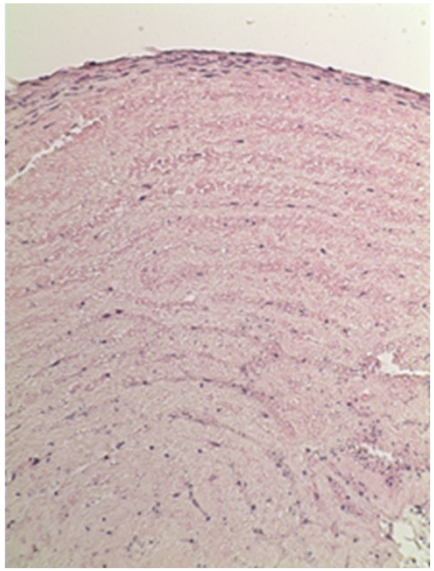
Weber et al. 2020 *Biofabrication*

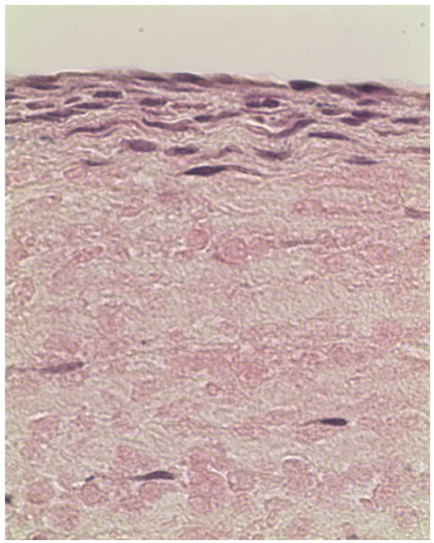
Development of an equine OA model



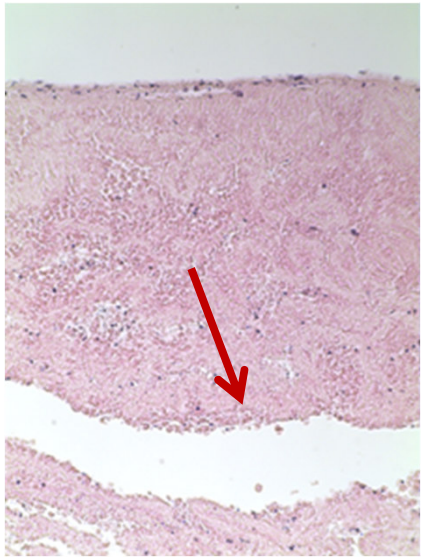
In vitro OA Model – What is it like?

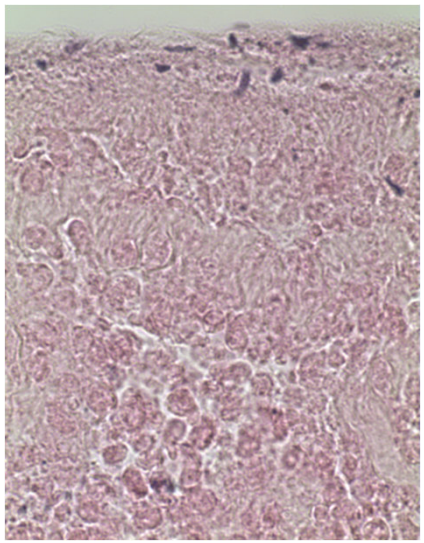
Initial stage



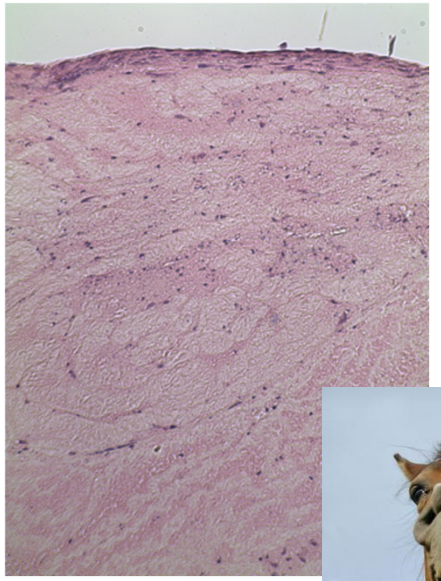


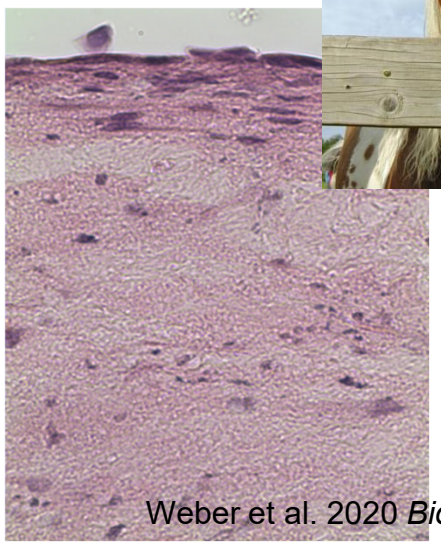
Arthrotic





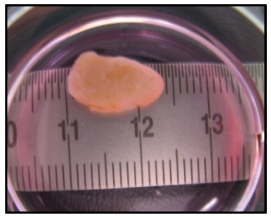
Recovered

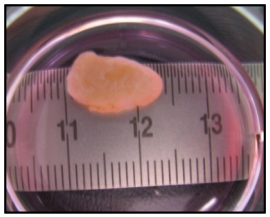




Weber et al. 2020 *Biofabrication*

Outlook

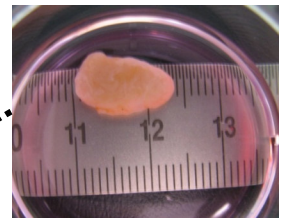




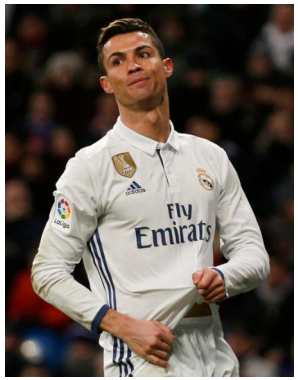
Equine OA-Model

human OA-Model

Goal



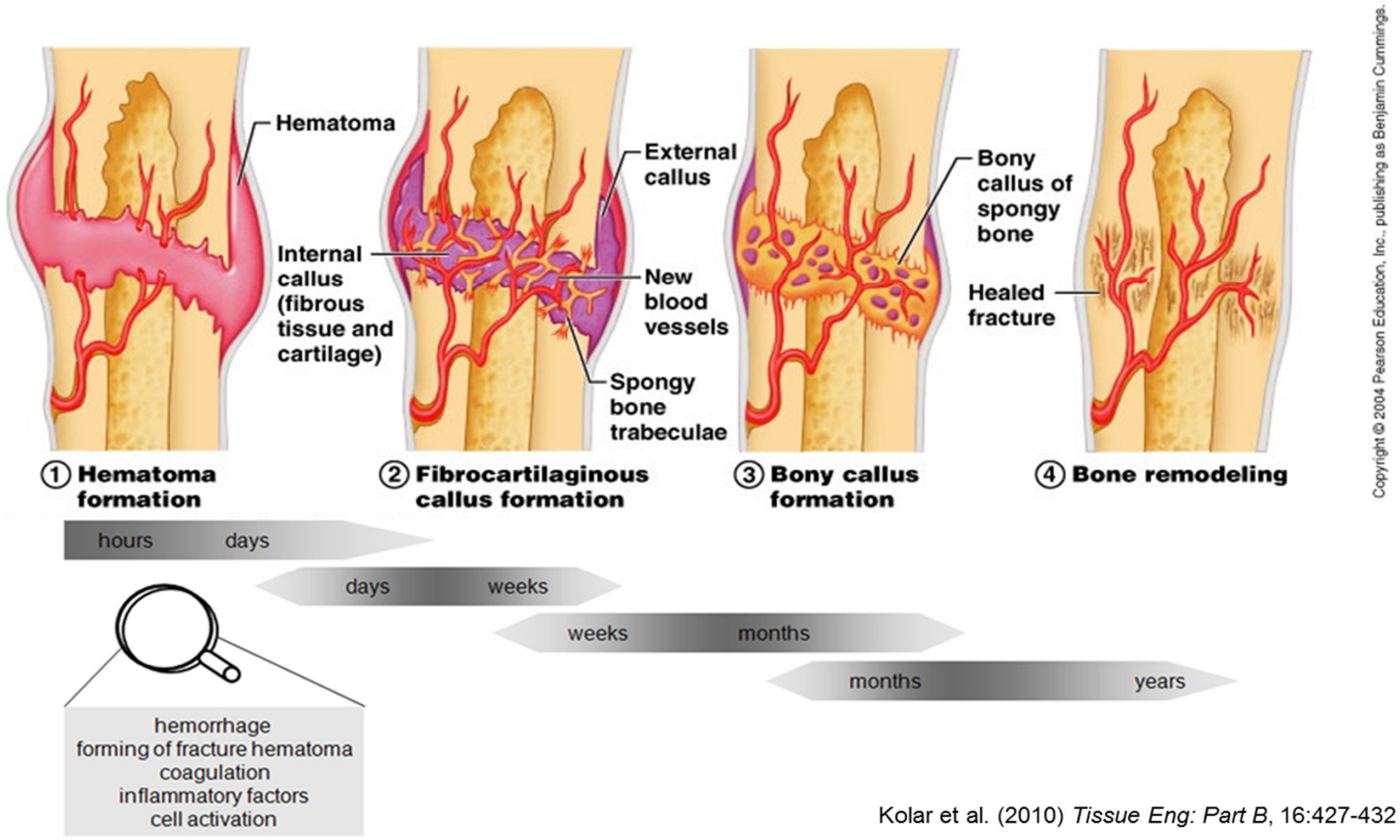
3D-Model



Large animal model = patient

→ Model should serve to save laboratory animals! A replacement is possibly conceivable. Further developments are necessary!

Fracture healing



Kolar et al. (2010) *Tissue Eng: Part B*, 16:427-432

→ Complex interplay, like in an orchestra!

Approach in fracture research

Aim

Therapeutical approach

Simplified cell culture

Small animal model



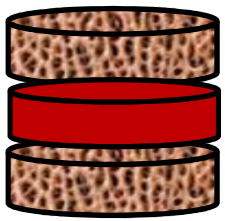
Large animal model

Our approach

Aim

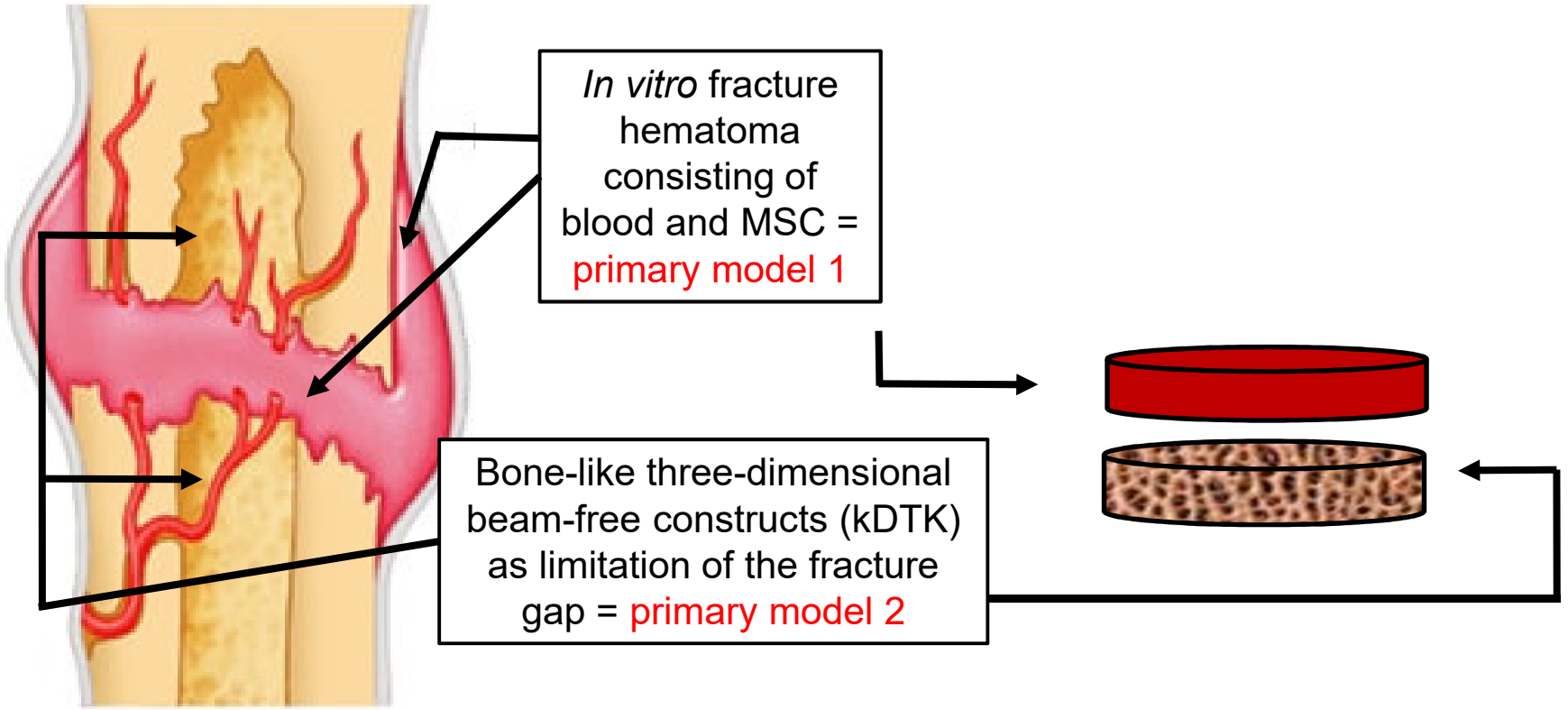
Therapeutical approach

Simplified cell culture



3D-Model

In vitro model fracture healing



Goal: To close the alternative method gap in the field of fracture research by providing a preclinical, adequate in vitro model → REDUCE, (Replace)

Fracture hematoma models *in vitro*

Cellular Composition

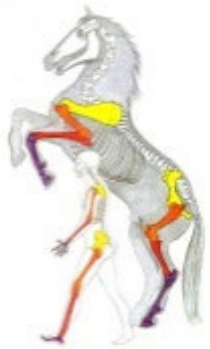
Cell Type	*Ex vivo* FH (< 72 h)	*In vitro* FH human (48 h HOX)
T helper cells	3.1% (1.4-15.4%)	1.9% (1.1-6%)
Cytotoxic T cella	1.9% (0.2-12.6%)	1.3% (0.9-2.5%)
Monocytes	4.9% (0.1-38.5%)	0.6% (0.3-1.7%)
MSCs	n.a.	23.4% (16.6-24.5%)
Granulocytes	64.2% (0.8-94%)	16.1% (5.6-24.7%)

RNA-Expression after 48 h

Gene symbol	*Ex vivo* FH (< 72 h)	*In vitro* FH human (48 h HOX)
RUNX2	↑**	↑
SPP1	↑*	↑****
VEGFA	↑*	↑***
IL8	↑***	↑**
IL6	↑***	↑
CXCR4	↑**	↑*
LDHA	↑***	↑**
PGK1	↑	↑

Matching characteristics of fracture hematoma model and *ex vivo* experiments with human patients fracture hematomas

Abstract: Learning from animal experiments to develop alternative methods



- Small animal models in particular are used in orthopedic research
- Transferability to humans is not always given
- New approaches and ways of thinking must find their way into biomedicine
- The horse plays an important role as a patient and is a very good model for humans
- The provision of adequate *in vitro* models opens up new possibilities and helps to spare animals

„…we should always remember that animals are also stakeholders and not just research tools.“

(Poole et al. 2010 Osteoarthritis & Cartilage; 18)

Thank you for your attention

Acknowledgment

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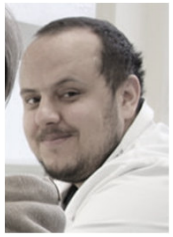

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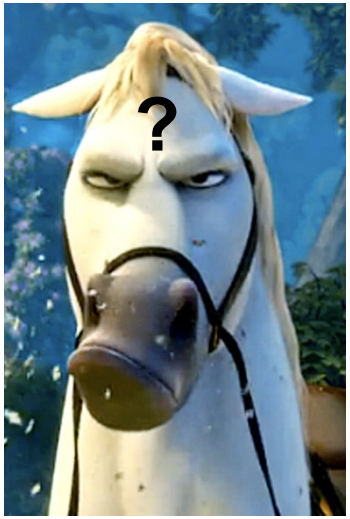

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