DEUTSCHES ZENTRUM
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BfR
Bundesinstitut für Risikobewertung

GERMAN FEDERAL INSTITUTE
FOR RISK ASSESSMENT

Models used to detect skeletal anomalies: applications, limitations and future perspectives

Frank Schulze

Testing for teratogenicity: historic background

- late 1950/early 1960s: **Thalidomide** was sold in Germany
- broad **public discussion** about regulation and use of **teratogenic substances**
- exemplified **limitations of animal testing** strategies at that time
- direct consequence: **preclinical testing for teratogenicity** of drugs became part of German Law (Arzneimittelgesetz der BRD von 1978)

source: http://www.contergan.grunenthal.info

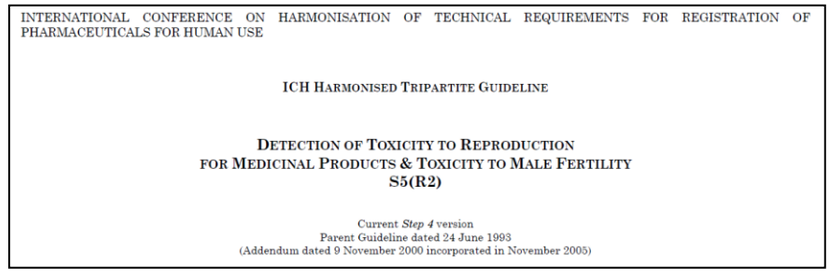
Testing for teratogenicity: present

International Council for Harmonisation (ICH): **safety guideline S5 (R2)**

- ➢ medicinal products

Organisation for Economic Co-operation and Development (OECD):
guideline 414

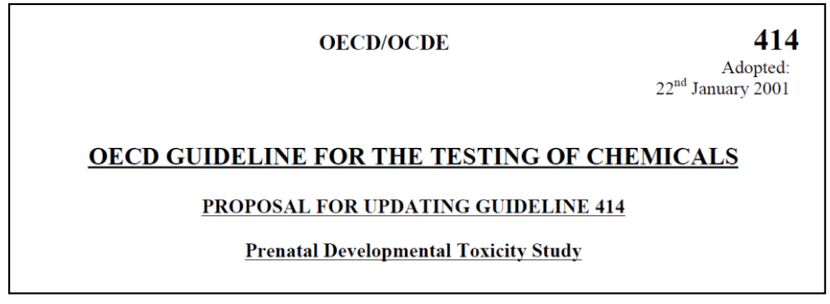
- ➢ general testing of chemicals


INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

DETECTION OF TOXICITY TO REPRODUCTION
FOR MEDICINAL PRODUCTS & TOXICITY TO MALE FERTILITY
S5(R2)

Current *Step 4* version
Parent Guideline dated 24 June 1993
(Addendum dated 9 November 2000 incorporated in November 2005)


OECD/OCDE

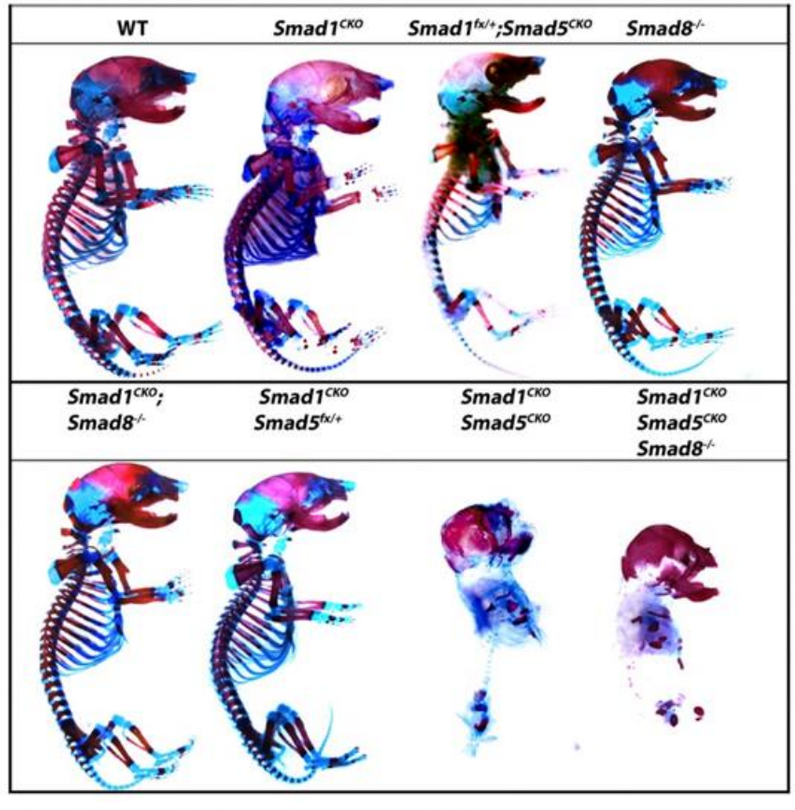
414
Adopted:
22nd January 2001

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

PROPOSAL FOR UPDATING GUIDELINE 414

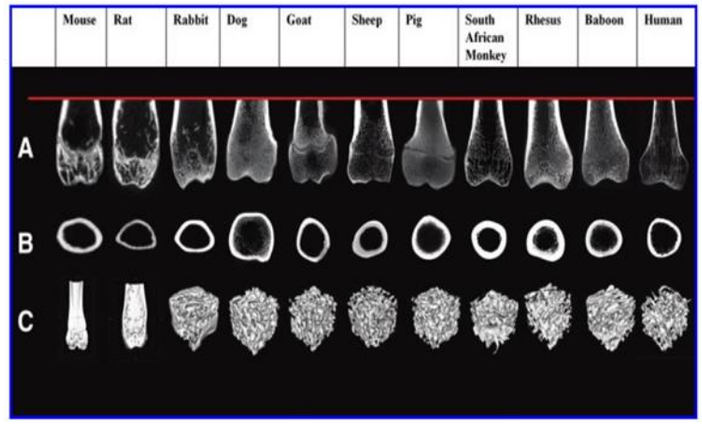
Prenatal Developmental Toxicity Study

- testing in **two distinct species**
- animal recieves **test substance during pregnancy** (beginning to estimated end)
- pregnant animal is sacrificed and **fetuses** are removed for **further testing**
- **removal** of soft and connective **tissues** followed by **staining** for chondrogenic and mineralised parts of the **skeleton**



Retting et al., *Development*, 2009

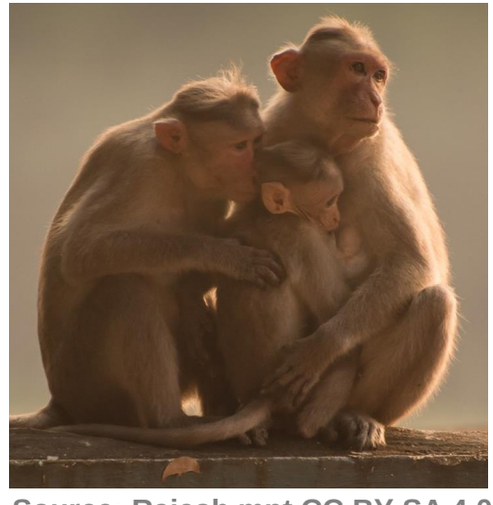
Testing for teratogenicity: current limitations



Muschler et al., *Tissue Engineering Part B*, 2009

Research into Thalidomide exposes **limitations of animal testing**:

- **mice or rats** fail to predict thalidomide teratogenicity in humans
- species-specific **differences** in **physiology and metabolism** [1]
- distinct **effective doses** in between different species [2]
- using animals that are **phylogenetic closer** to humans (e.g. nonhuman primates) **does not** facilitate the **identification** of all human teratogens [3]



Source: Rajesh mpt,CC BY-SA 4.0 commons.wikimedia.org

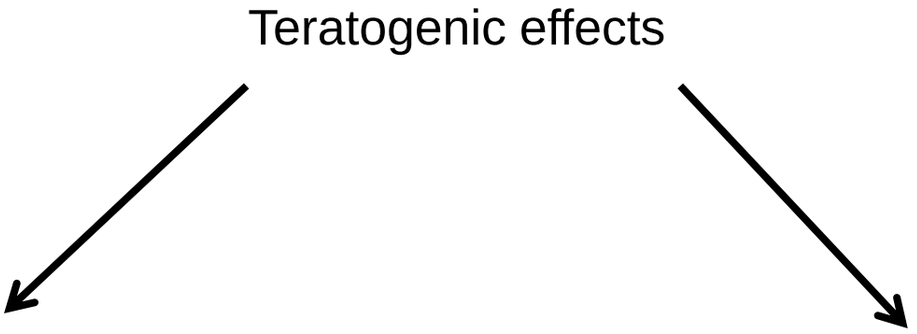
EU regulation: Registration, Evaluation, Authorization and Restriction of Chemicals (**REACH**)

- testing of **≥ 68.000** substances within the next decade
- approximately **9** [4] – **54** [5] million animals would be needed
- 70% - 90% animals for reproductive/develpomental toxicity testing [5]
- **cost and time** demanding

[1] Lu, J. et al. *J Pharmacol Exp Ther,* 2004
[2] Newman, L. M. et al. *Reprod Toxicol,* 1993
[3] Schardein, JL. *Chemically Induced Birth Defects,* 1985
[4] ECHA press release, ECHA/PR/09/11, 2009
[5] Rovida, C. et al. *Altex*, 2009

Testing for skeletal teratogenicity: alternatives

Spoiler: The complexity of embryogenesis and maternal-fetal interaction will not be recreated as an *in vitro* model in the foreseable future!



The **specific** inhibition of tissue or organ growth due to exposure to a given substance.

e.g. : **Tetracycline**-based anitbiotics are incorporated into bone matrix instead of calcium

-> deformations, **disruption of endochondral ossification** and therefore **longitudial bone** growth

Alternatives: ***In vitro* assays** that display **key events** in human **bone formation**

Teratogenicity is based on **secondary effects** of the substance in question.

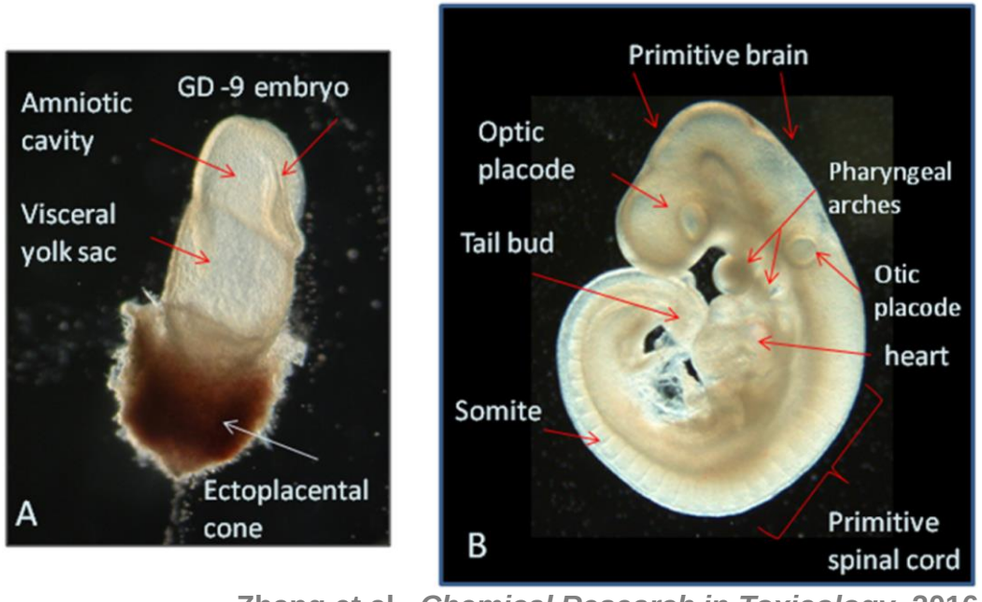
e.g. Non-physiologic exposure to **retinoic acid** causes spatial disruption of symmetry axes by **altering Hox-gene expression**.

-> deformations of **skull and limbs** but also eyes and central nervous system

Alternatives: **Change of organisms** towards smaller animals (conserved development process across vertebrae species, higher throughput, less ethical dilemma)

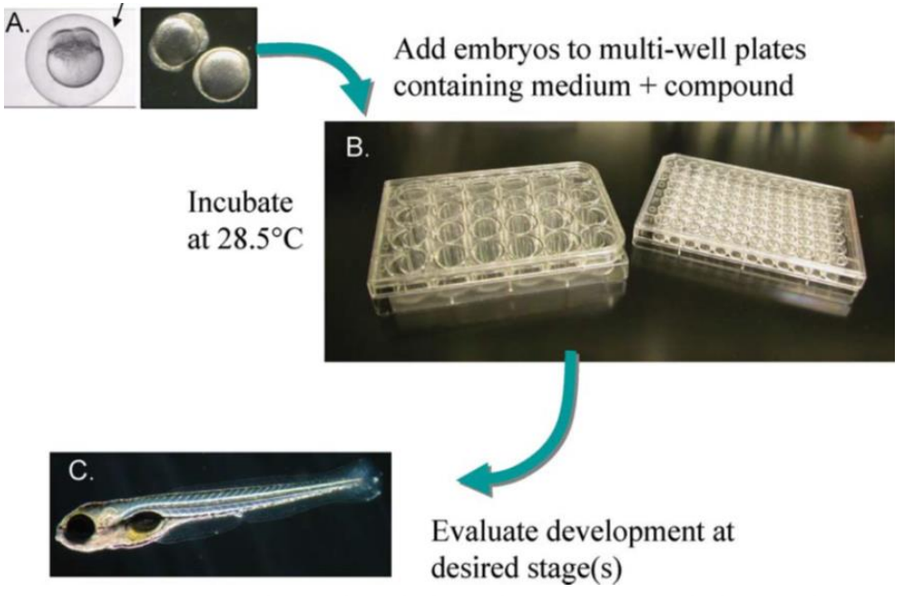
Alternatives for testing skeletal teratogenicity

Whole embryo culture (WEC)



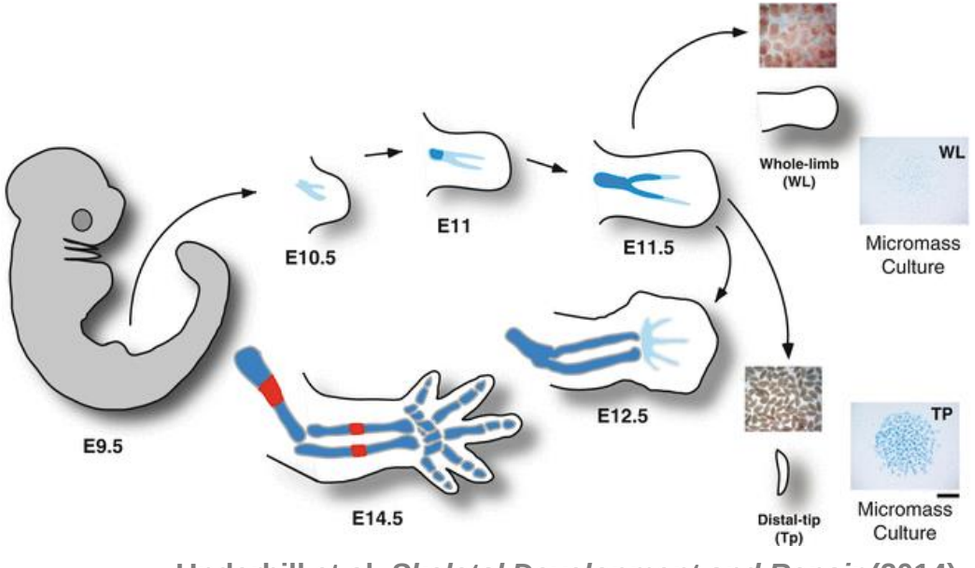
Zhang et al., *Chemical Research in Toxicology*, 2016

Zebrafish embryo culture



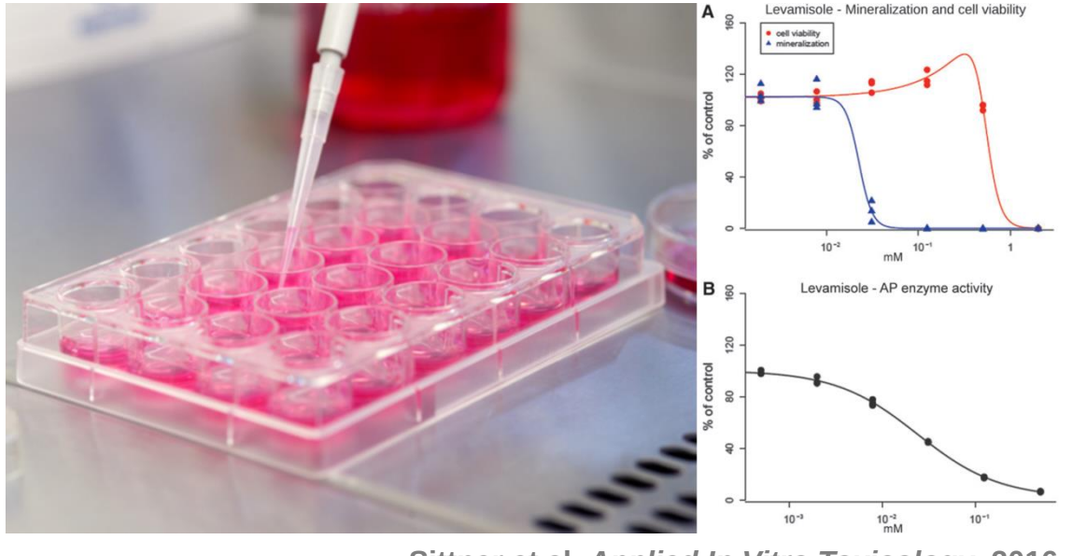
Rauch et al., *Birth Defects Research (Part C)*, 2010

Limb bud micromass culture



Underhill et al. *Skeletal Development and Repair* (2014)

Osteo EST

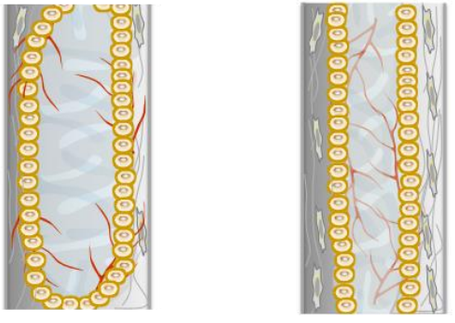


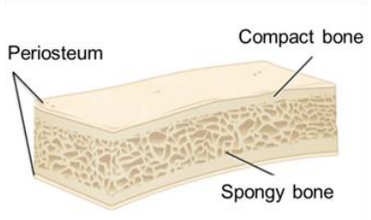
Sittner et al. *Applied In Vitro Toxicology*, 2016

3D models of intramembranous ossification

bone development – intramembranous ossification

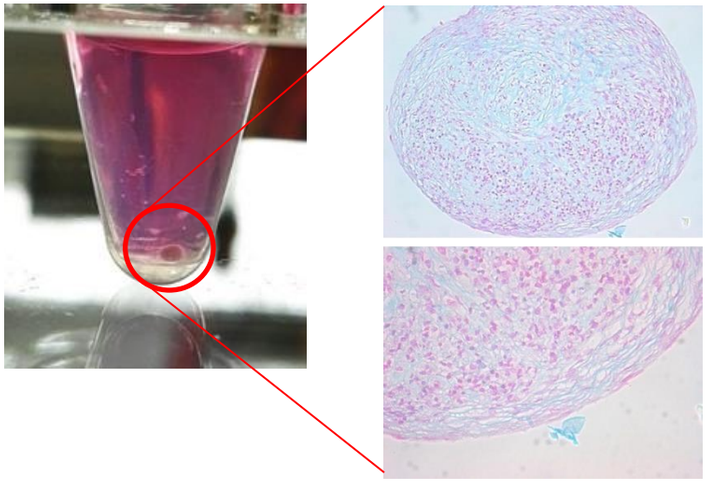
proliferation and condensation of osteoblasts formation of an ossification center → **mineralisation** → **vascularisation** → **remodelling**



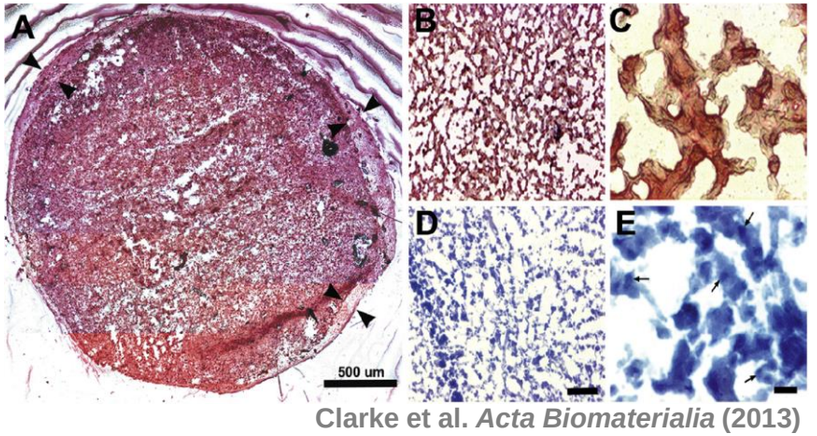


In vivo situation

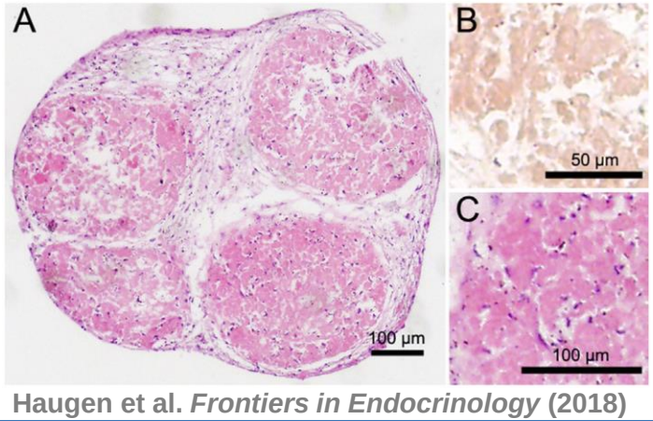
Source: BfR/OpenStax



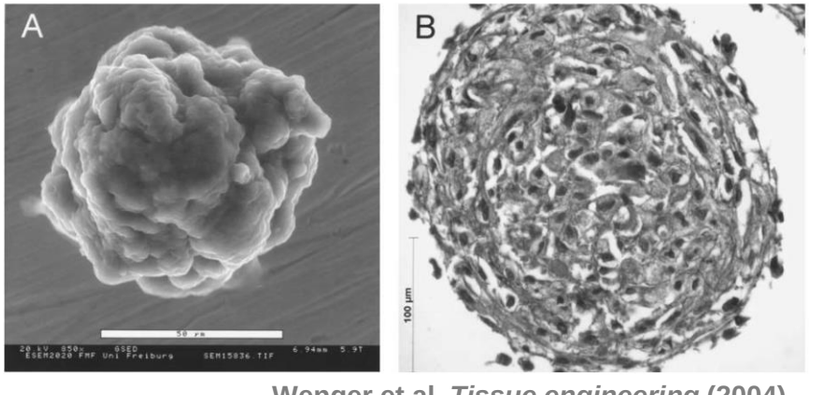
Schulze et al. unpublished data



Clarke et al. *Acta Biomaterialia* (2013)



Haugen et al. *Frontiers in Endocrinology* (2018)



Wenger et al. *Tissue engineering* (2004)

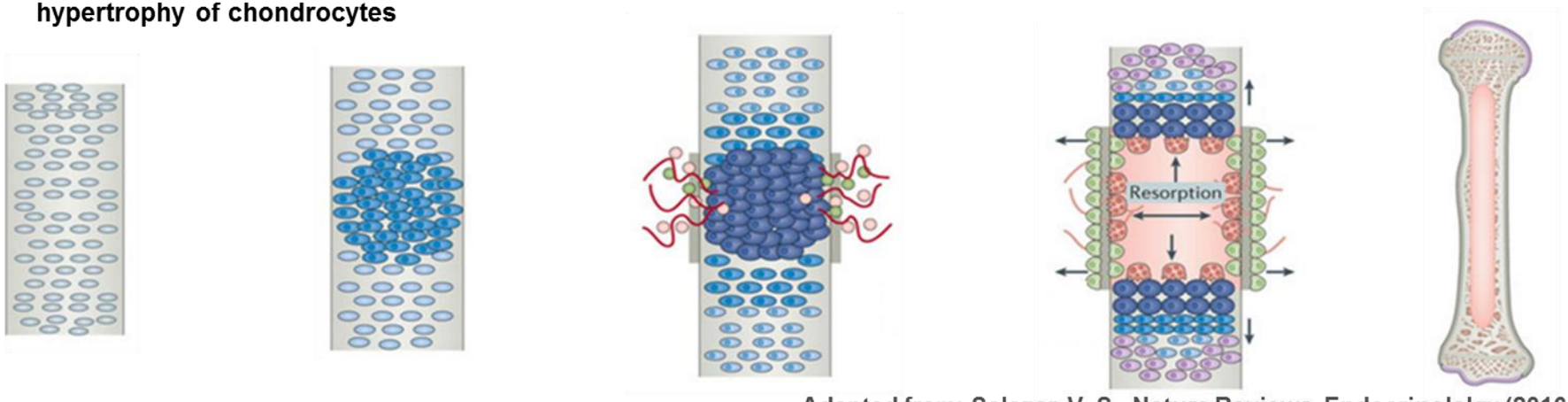
3D models of endochondral ossification

bone development – endochondral ossification

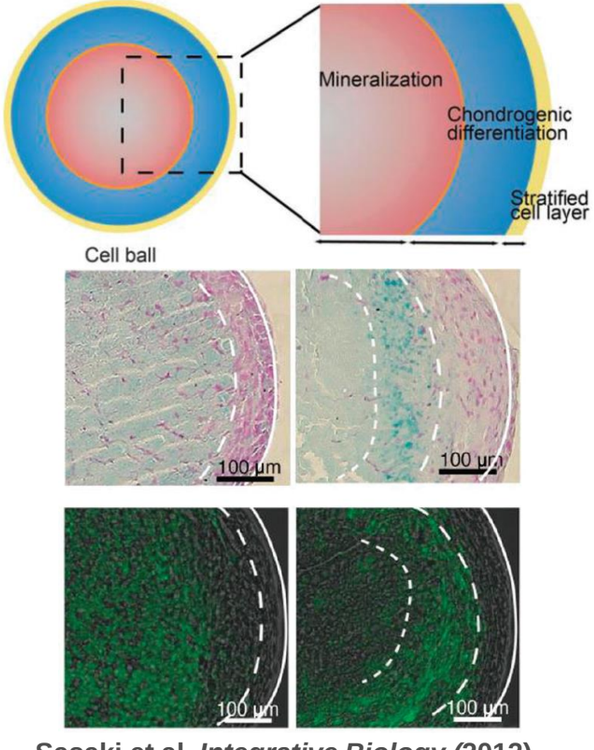
proliferation, condensation and subsequent hypertrophy of chondrocytes

mineralisation and vascularisation

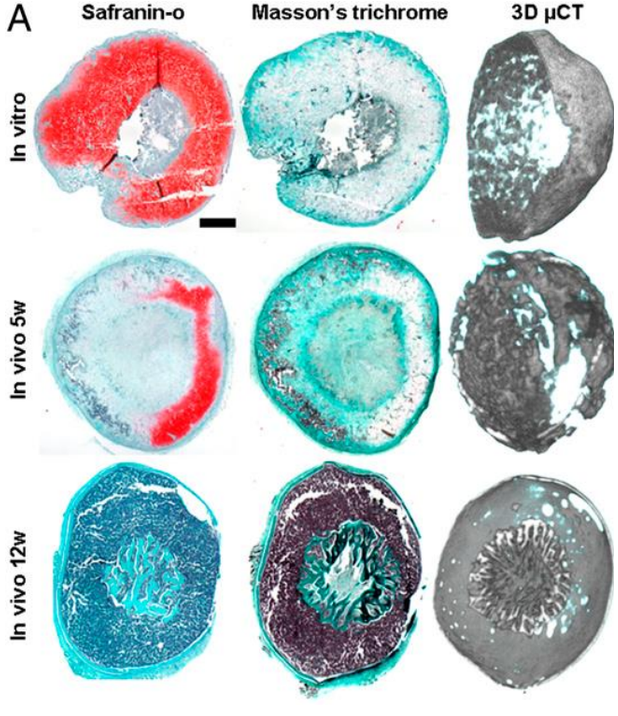
remodelling



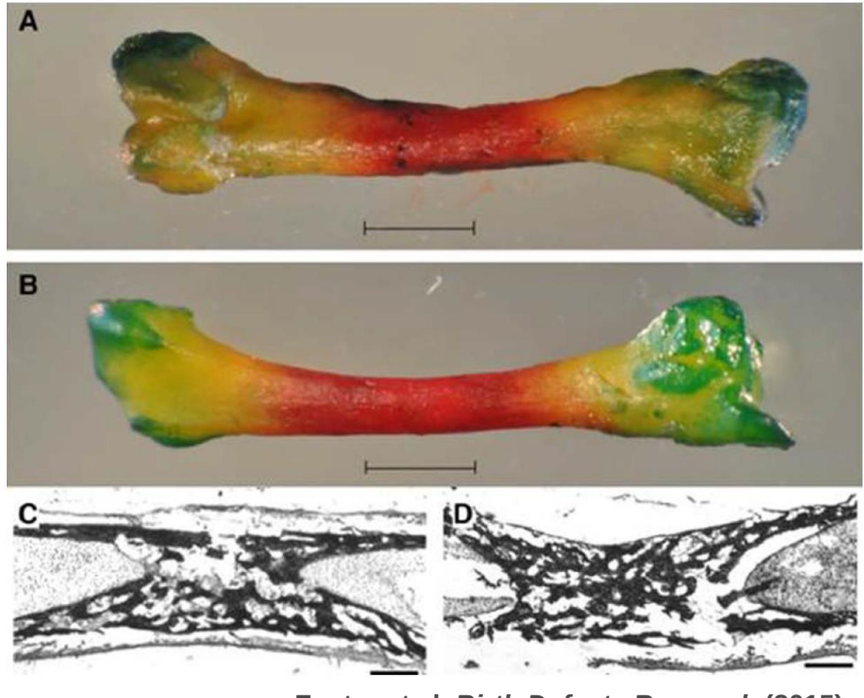
Adapted from: Salazar, V. S., Nature Reviews Endocrinololgy (2016)



Sasaki et al. *Integrative Biology* (2012)



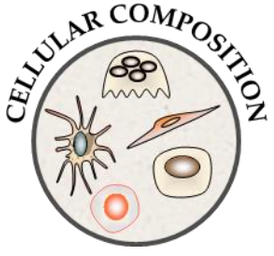
Scotti et al. *PNAS* (2013)



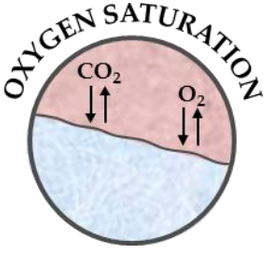
Foster et al. *Birth Defects Research* (2015)

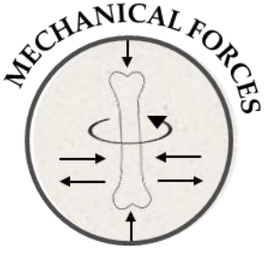
Recreating key parameters in bone biology: combining organoids and bioreactors

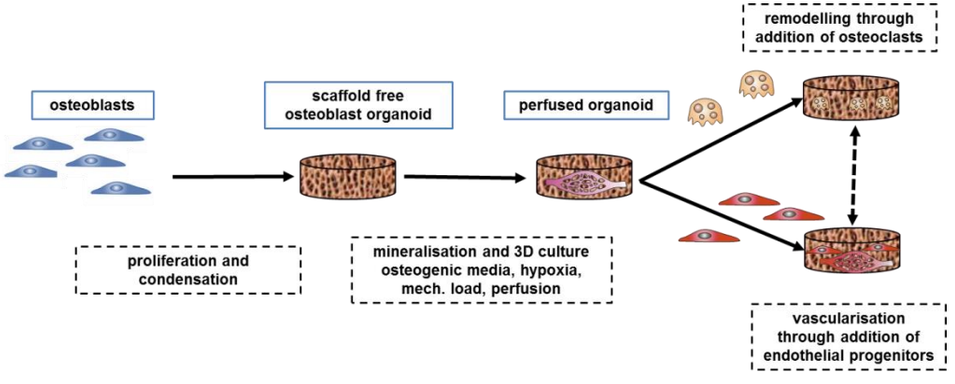
Biological Parameters

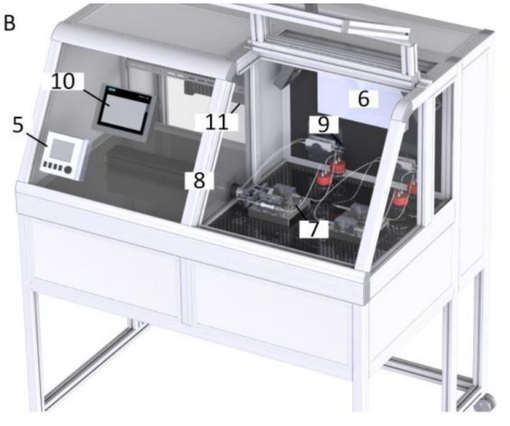


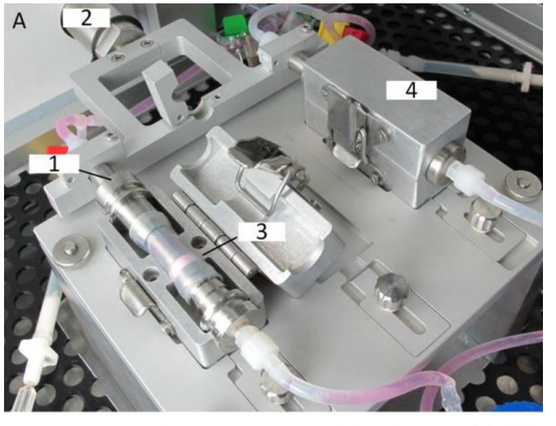
Physical Parameters



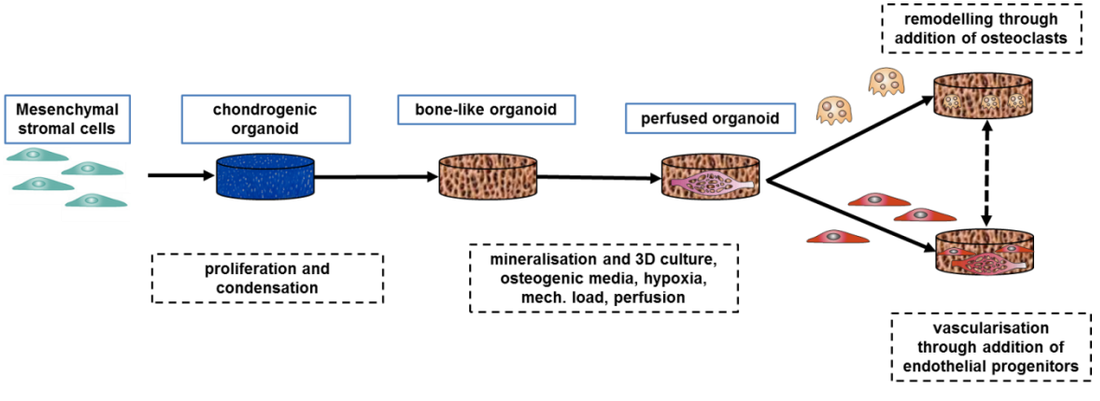


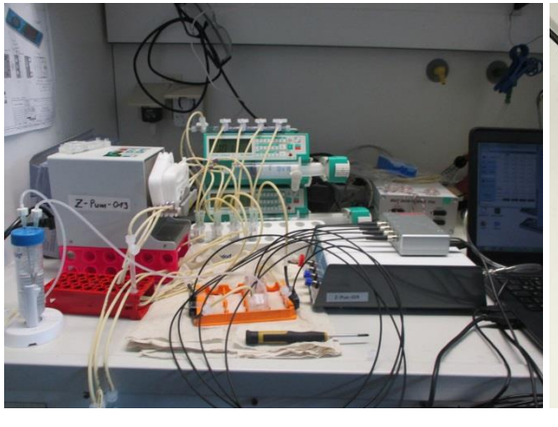






Ramani-Mohan et al. *J. Tissue Engineering and Regenerative Medicine* (2015)





Schulze et al. unpublished work

Conclusion

While **direct effects** on skeletal development **can be detected** *in vitro*, **indirect effects** cannot due to a **lack of complexity** and systemic interaction.

Investing in reliable *in vitro* test systems will be beneficial since:

- they allow **supplementation** of ***in vivo*** testing
- **low-throughput** sophisticated **3D models** can help elucidate biology and **key events** bone development
- **key events** -> simplified model (multi-titer) for **high throughput** applications
- **low-cost** and **high throughput** *in vitro* methods can help to **prioritize chemicals** for testing *in vivo*
- potential for the **reduction** of test animals
- **combination** with other organ/tissue models (placental barrier, liver) can **elevate physiologic relevance**

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Thank you for your attention

German Federal Institute for Risk Assessment
Max-Dohrn-Str. 8-10 ● 10589 Berlin, GERMANY
Phone +49 30 - 184 12 - 0 ● Fax +49 30 - 184 12 - 47 41
bfr@bfr.bund.de ● www.bfr.bund.de/en