Osteogenesis and angiogenesis of a bulk metallic glass for biomedical implants

Sun et al. Bioactive Materials 2022

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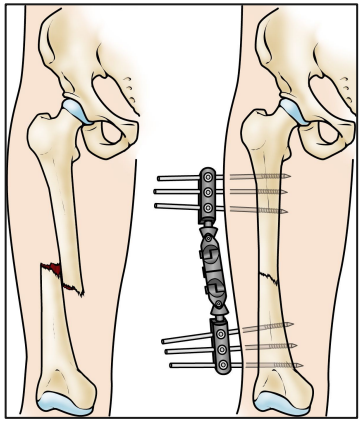
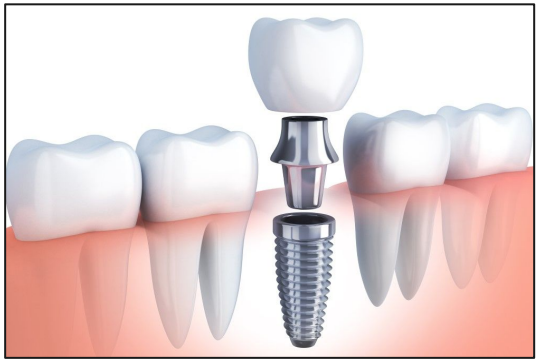
3A BIOMED - PHELMA
15/12/2022

Implants in orthopedic surgery



← Bone fracture fixation

Dental implant system →



What is important ?

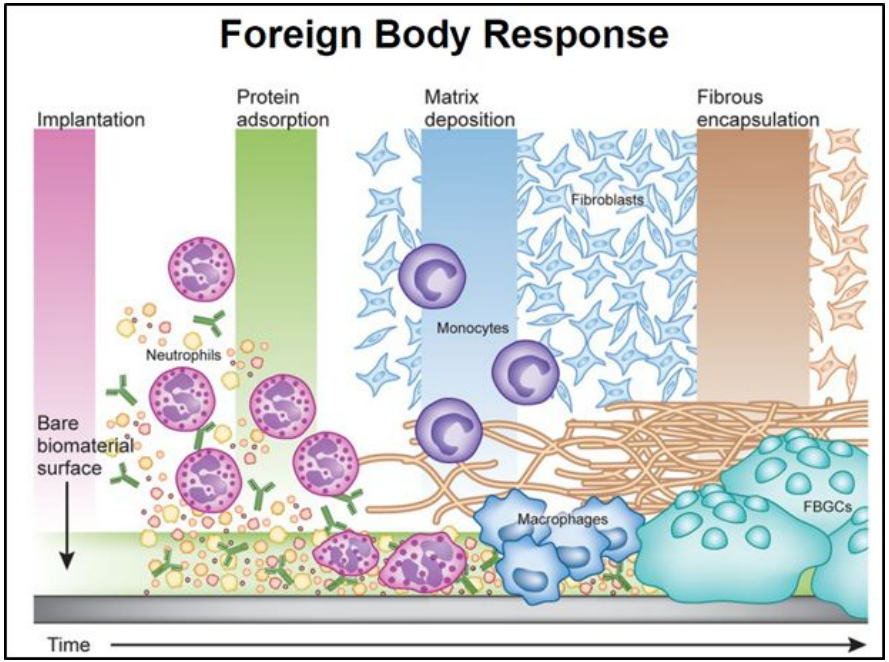
1) Biocompatibility

2) Mechanical behavior

3) Long-term safety / stability

Biocompatibility

What is the reaction when implanting a foreign-body ?

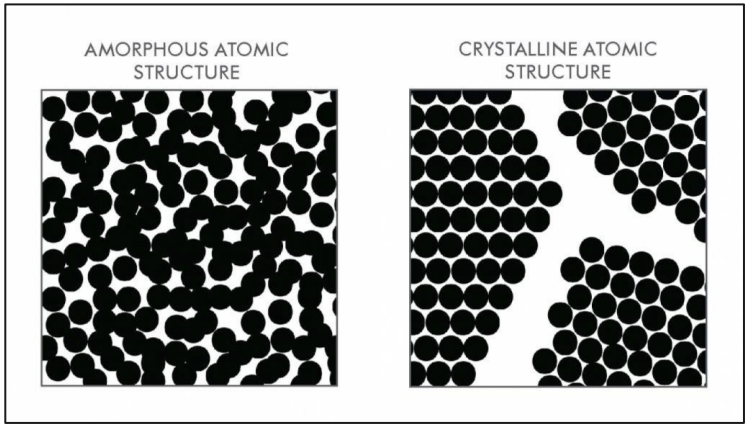


Vroman effect
Protein with the highest mobility will adsorb first.

Bulk Metallic Glasses (BMGs)

- Co-Cr alloys / 316L stainless steel
- Toxic effect

A new type of material : BMGs



https://www.bulkmetallicglass.it/en/index.php

- Amorphous solid material
- Glass-like atomic structure
- Irregular, very strong and elastic

The three protagonists

Ti-based BMGs

+ Improved wear resistance

- Young's modulus 80-120 GPa > human bone 10-40 GPa

$Zr_{16}Ti_2Cu_{25}Al_{12}$ (ZT1)

(poly-ether-ether-ketone) PEEK

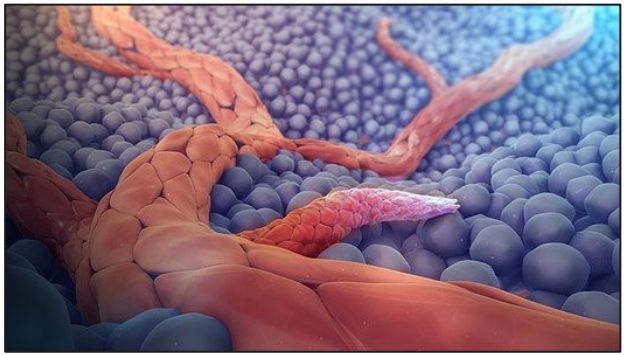
Cp-Ti (pure titanium)

What is angiogenesis and osteogenesis ?

Osteogenesis and angiogenesis

Osteogenesis : formation of bone

Angiogenesis : formation of new blood vessels from pre-existing vessels



Therefore, what is the problematic of this study ?

Problematic

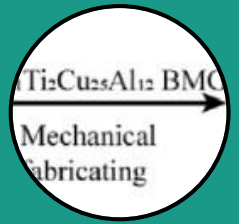
*“ Is **ZT1** a good candidate for **orthopedic applications** regarding its **biocompatibility and biomechanical properties** in in-vitro and in-vivo measurements ? ”*

Challenges of the study

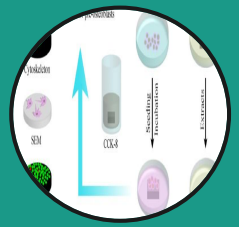
1 **Characterization of the materials**

2 **Osteogenesis properties**

3 **Angiogenesis properties**

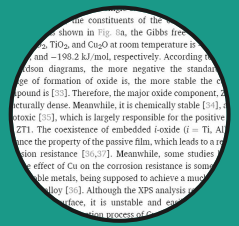


Characterization of the materials

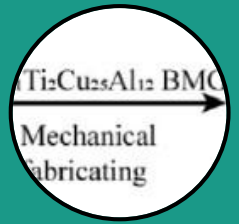


In-vitro measurements

In-vivo measurements



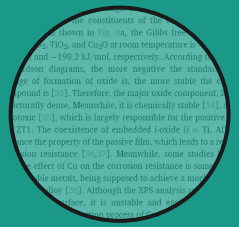
Discussion



Characterization of the materials

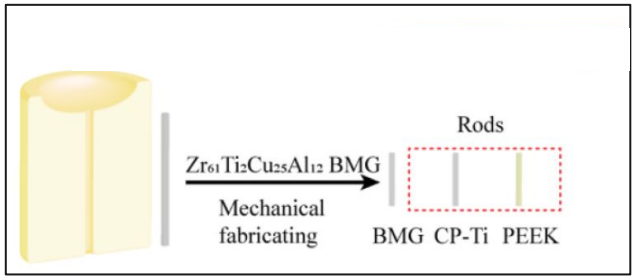
In-vitro measurements

In-vivo measurements



Discussion

Materials preparation

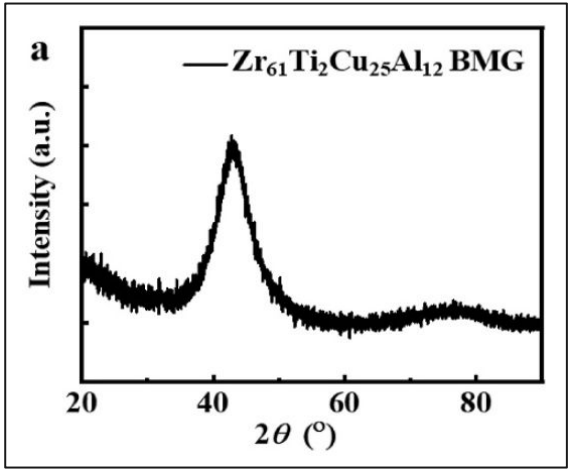


Rods preparation
→ *in-vivo* evaluations

Plates preparation
→ hydrophilic characterization
→ bio-corrosion tests
→ *in-vitro* evaluations

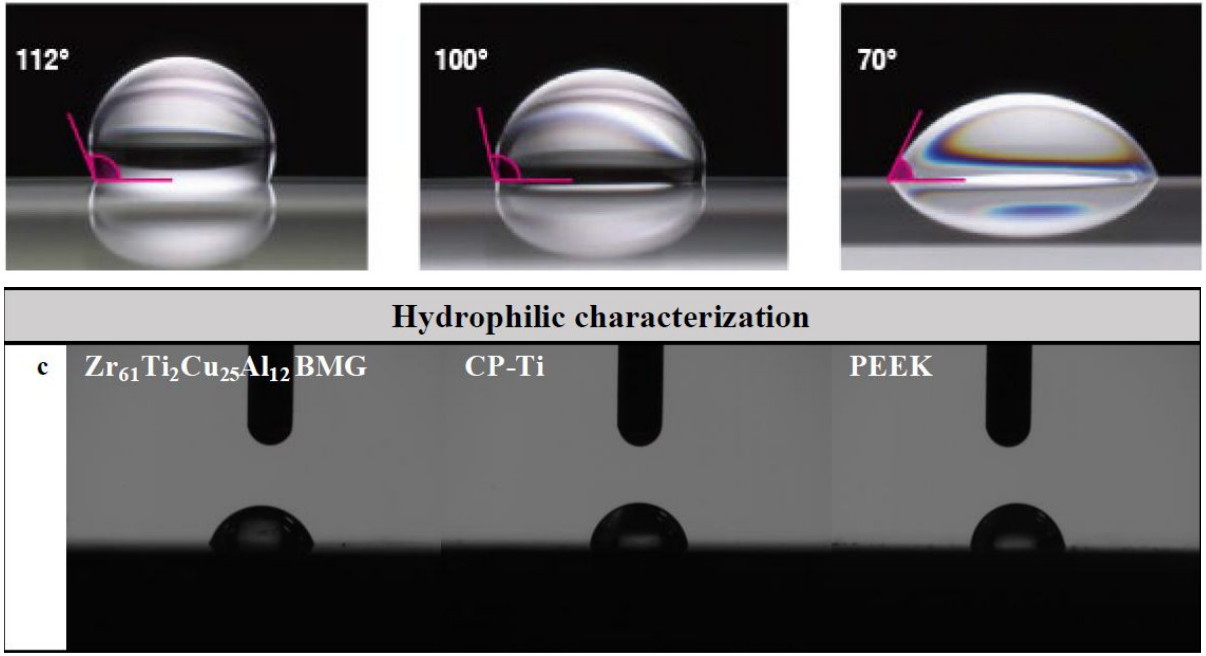
Structure study

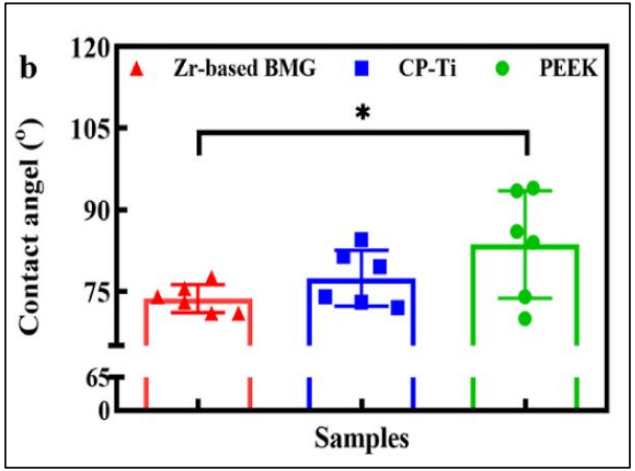
• Glassy structure characterized by X-ray diffraction (XRD)
→ typical broad scattering signal



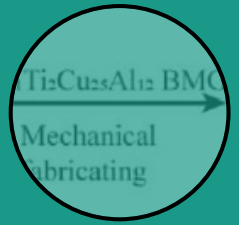
Amorphous structure

Hydrophilic characterization





ZT1 displays the most hydrophilic surface characteristic



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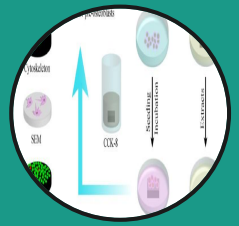
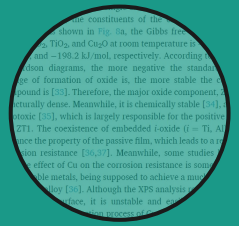


***In-vitro* measurements**

In-vivo measurements



Discussion

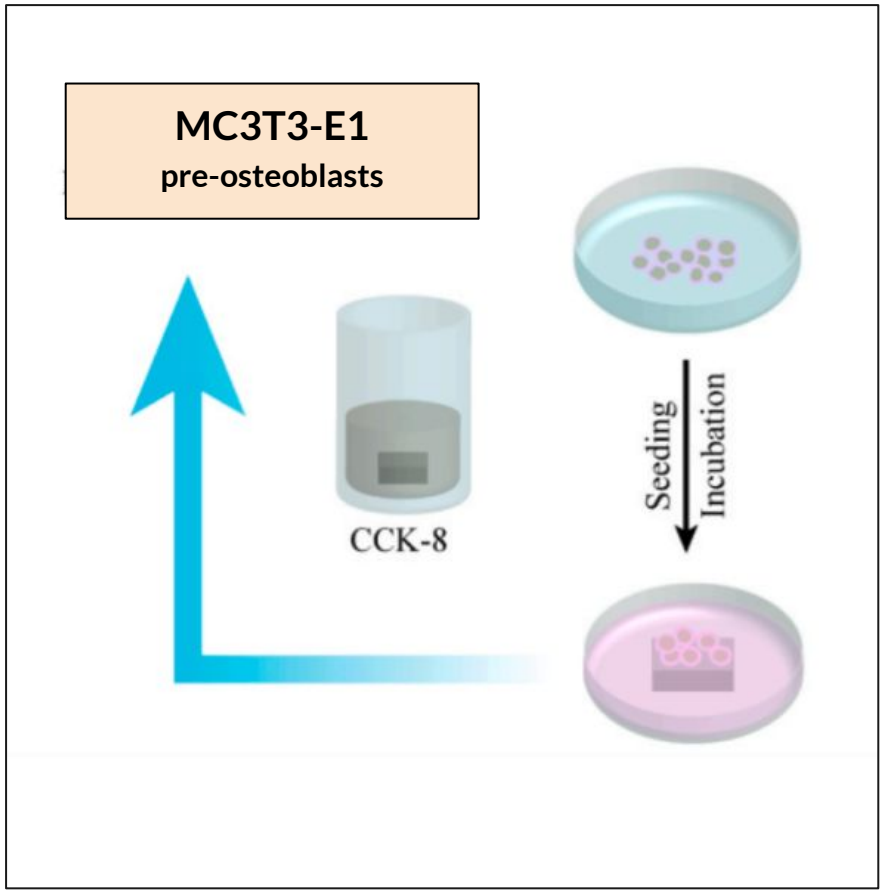
What is Cytocompatibility ?

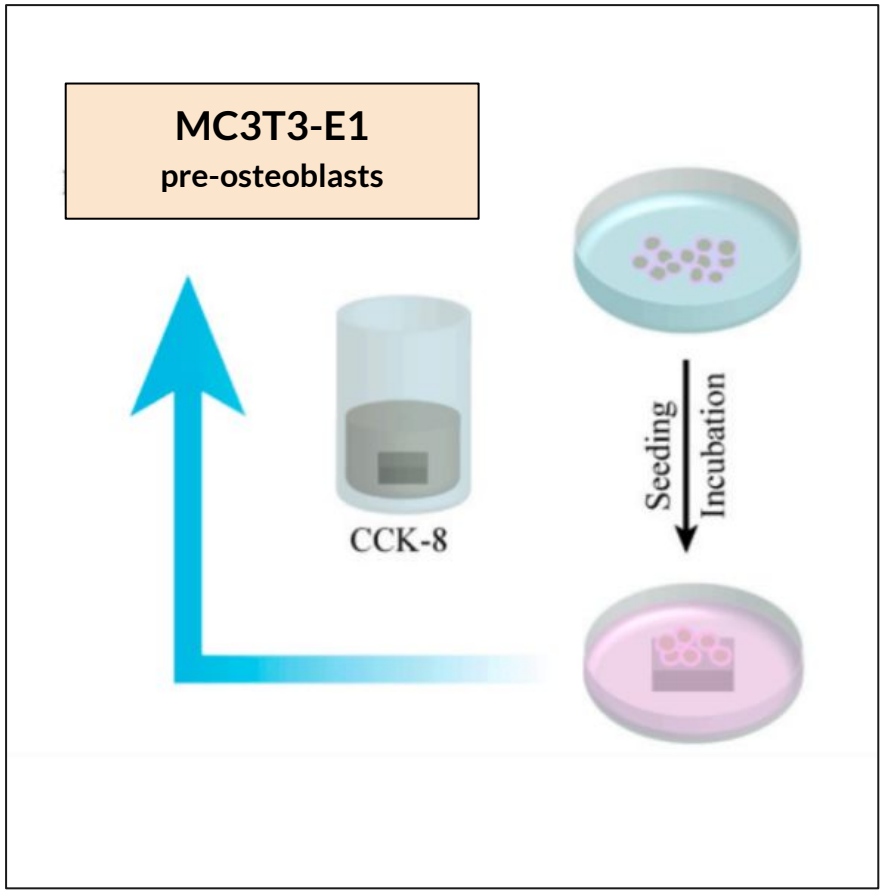
- **cytocompatibility :** property of not being harmful to the cell

How to assess Cytocompatibility ?

- Cell viability
- Cell adhesion
- Cell morphology
- Cell proliferation

→ **Study of cytocompatibility with MC3T3-E1 pre-osteoblasts**


MC3T3-E1
pre-osteoblasts
Seeding
Incubation
CCK-8



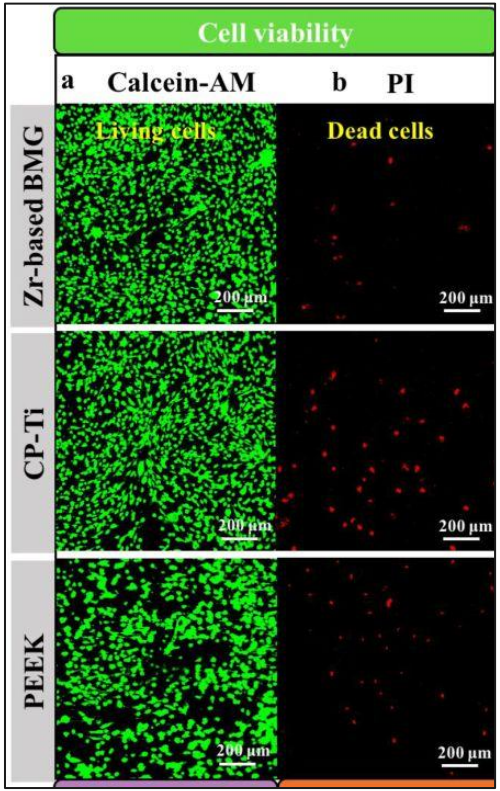
What is MC3T3-E1 pre-osteoblasts ?

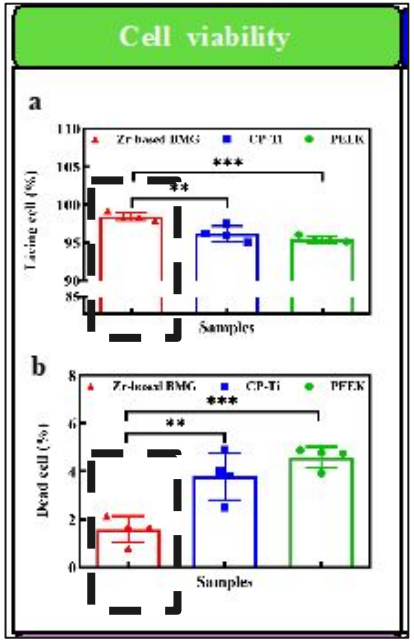
- **MC3T3** = Osteoblast precursor cell line (from mouse skull)

- **MC3T3-E1 sub-line** = spontaneously transformed (immortalised) cell line.

- Caution when extrapolating these results to normal cells

Cell viability

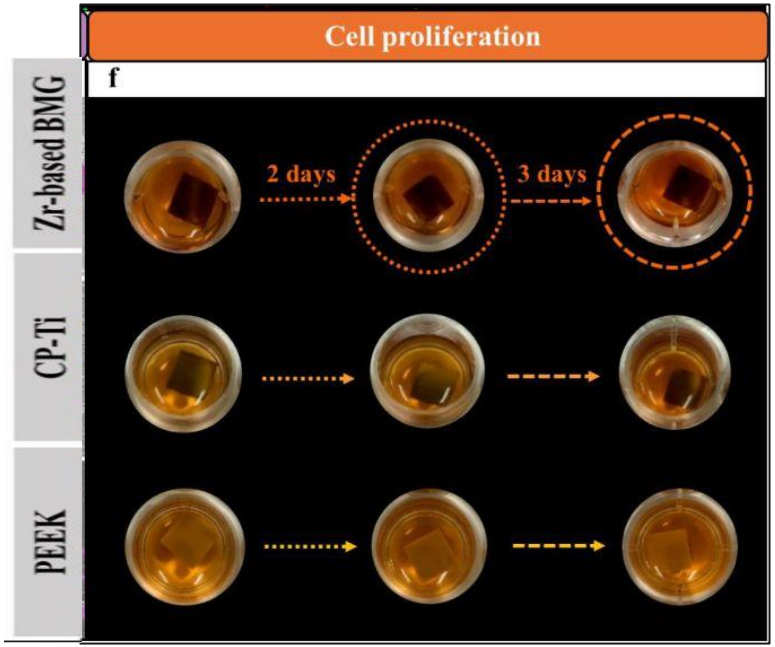


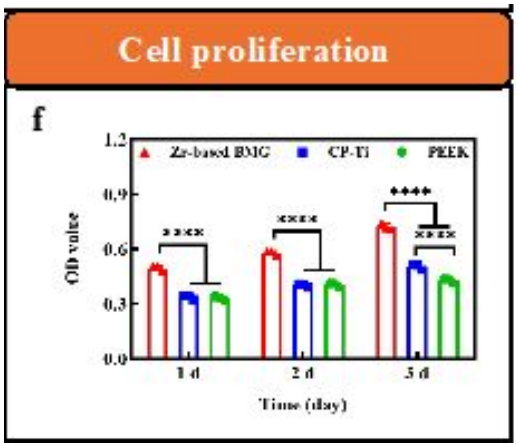


- live/dead staining assay
- living cells in green
- dead cells in red

ZT1 has the better cytocompatibility

Cell proliferation

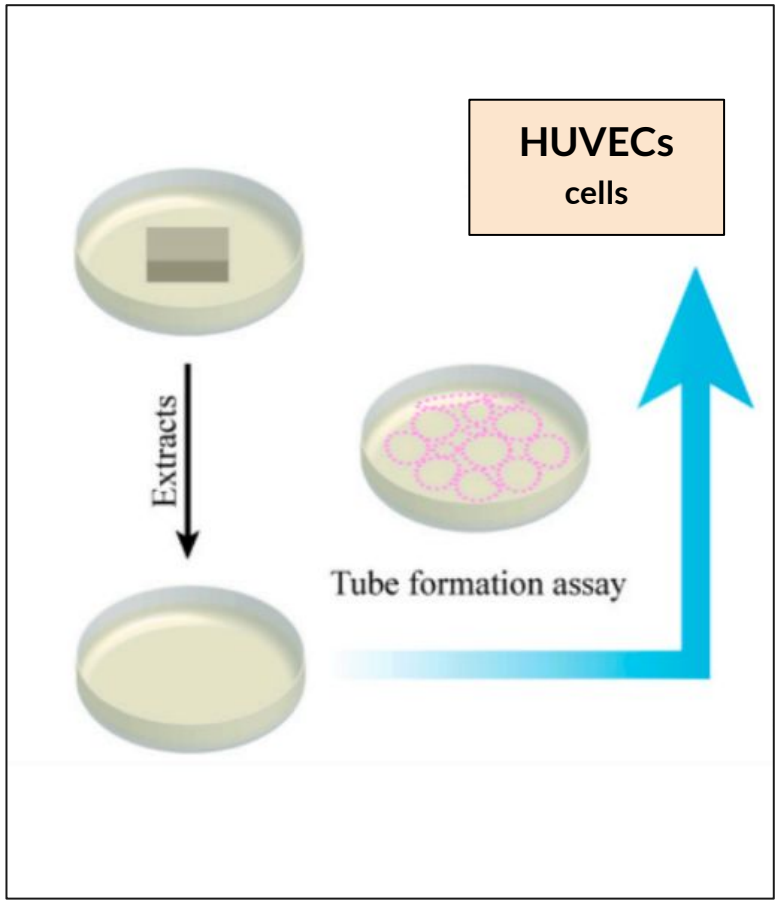


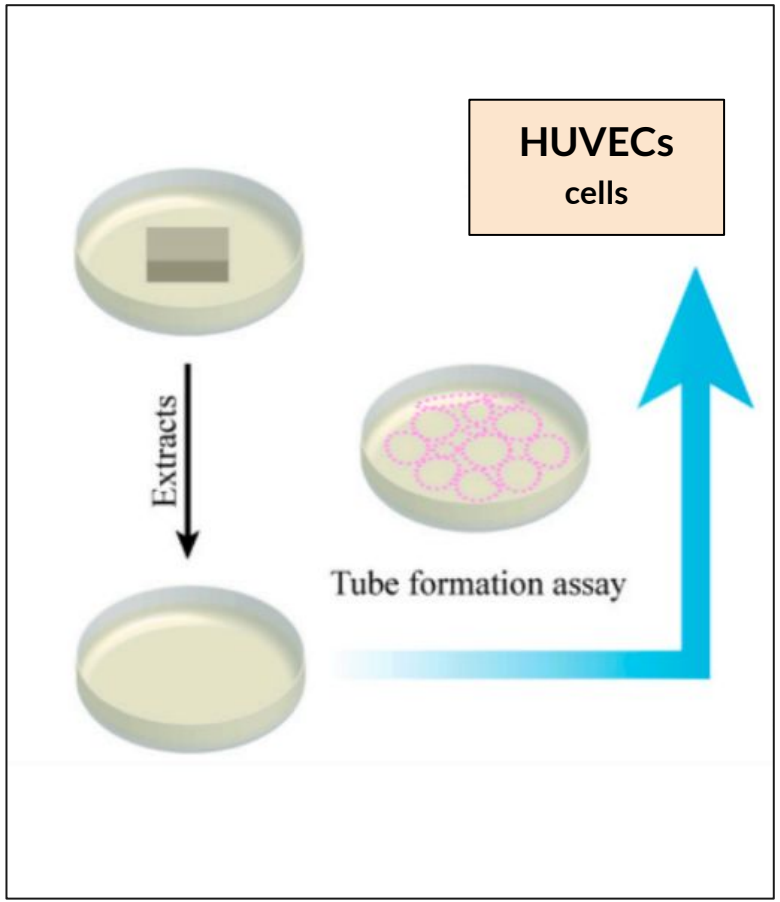


- cell proliferations after 2 days / 3 days in CCK-8 assay.
- the darker / the more it proliferates

→ **ZT1 stimulates proliferation**

Angiogenesis measurements with HUVECs


HUVECs
cells
Extracts
Tube formation assay



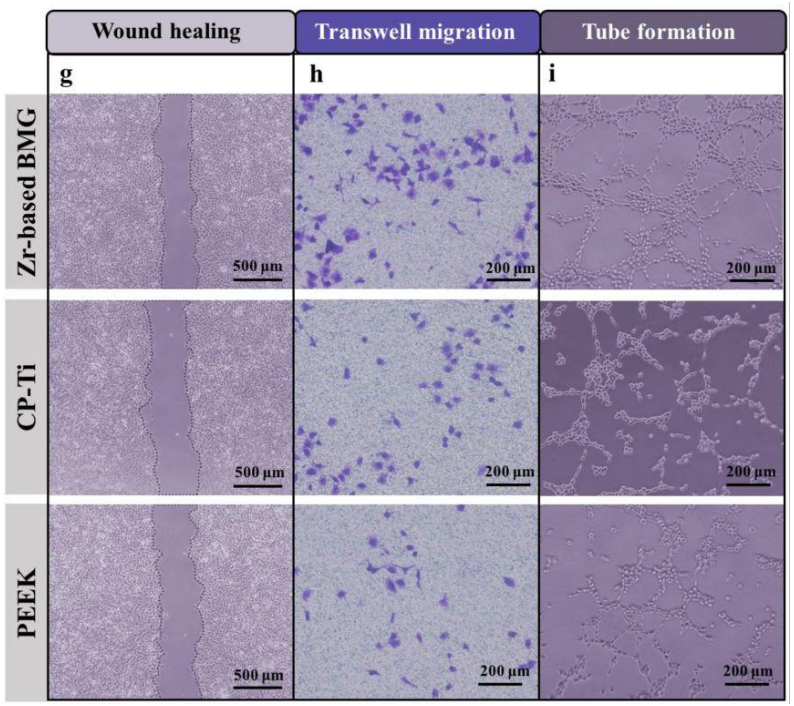
What is HUVECs ?

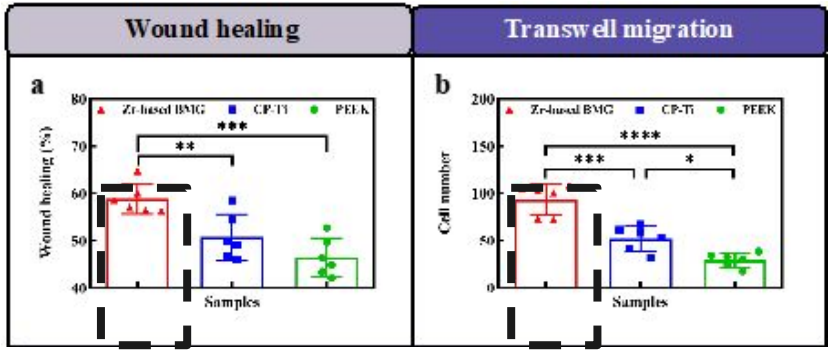
- Human Umbilical Vein Endothelial cells

- Model system for the study of the function and pathology of endothelial cells (e.g. angiogenesis)

- Easily to proliferate
- Low cost

Angiogenesis assessment



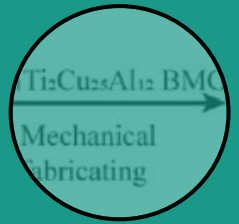


- wound narrowing after 24h for ZT1
- higher transwell migration for ZT1
- tube formation for ZT1

ZT1 enhances angiogenic ability

ZT1 has better **cytocompatibility** and **angiogenesis** than CP-Ti and PEEK

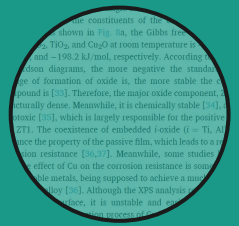
→ ***In-vivo*** **measurements**



Characterization of the materials

In-vitro measurements

***In-vivo* measurements**



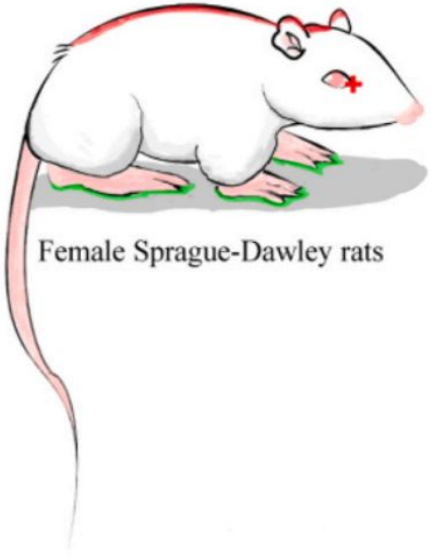
Discussion

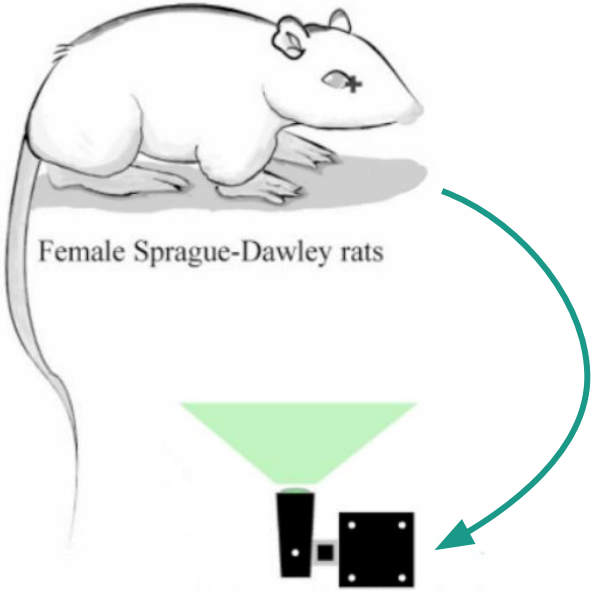
1 **Methods & Experiments**

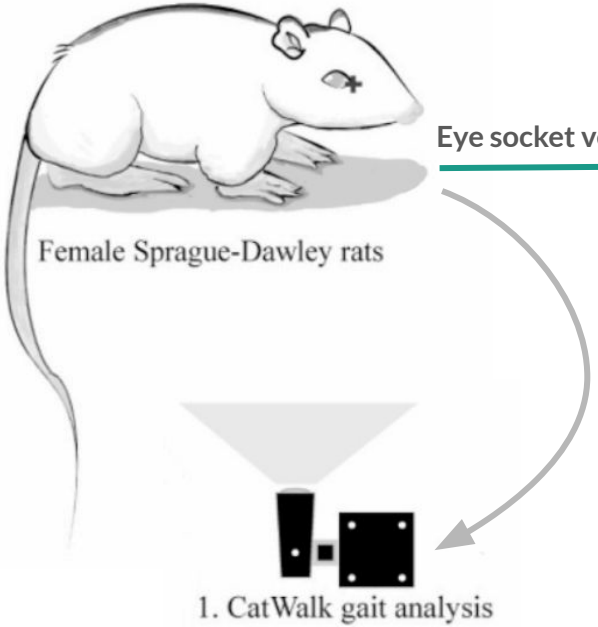
2 **Pain measurements**

3 **Osteogenesis measurements**

4 **Angiogenesis measurements**


Female Sprague-Dawley rats

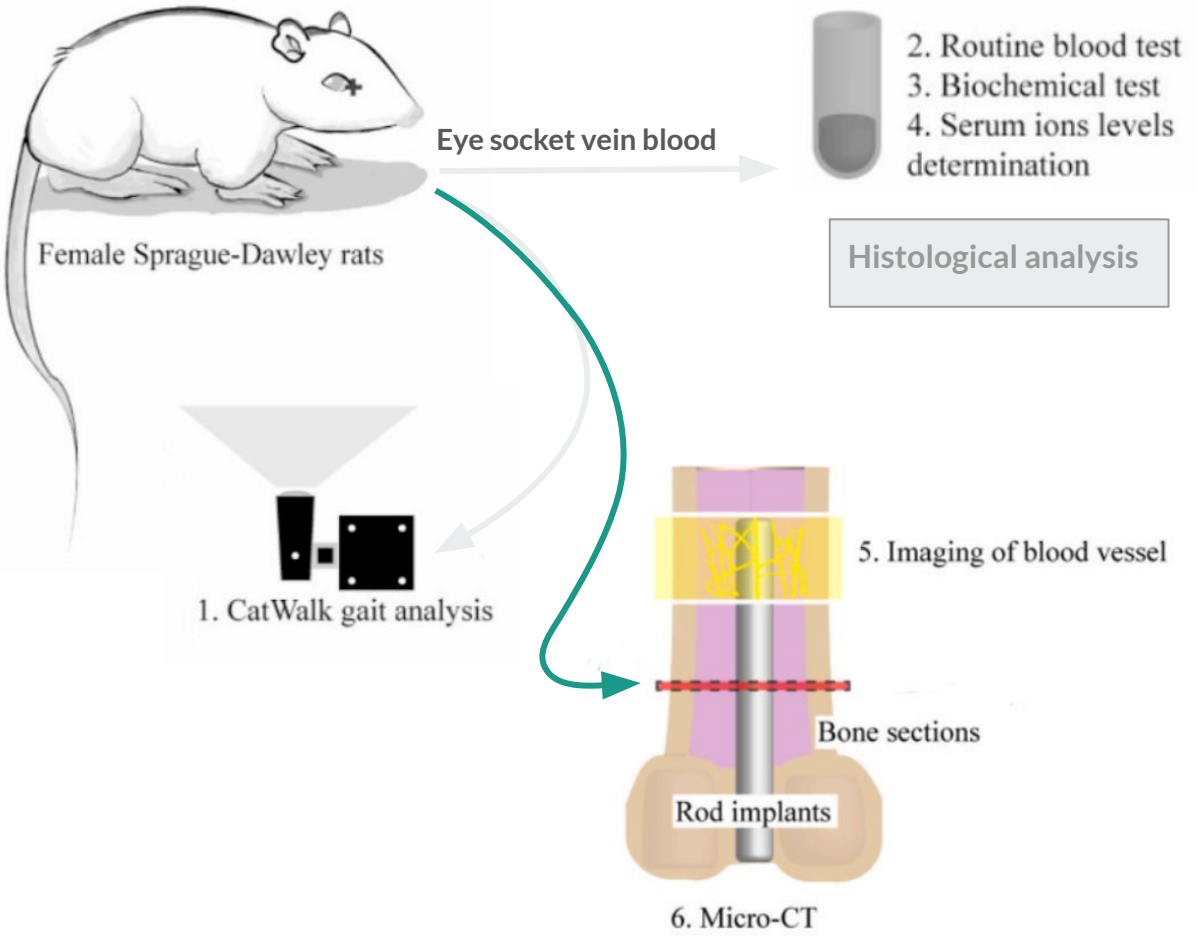

Female Sprague-Dawley rats
1. CatWalk gait analysis



Eye socket vein blood

2. Routine blood test
3. Biochemical test
4. Serum ions levels determination

Histological analysis


Female Sprague-Dawley rats
Eye socket vein blood
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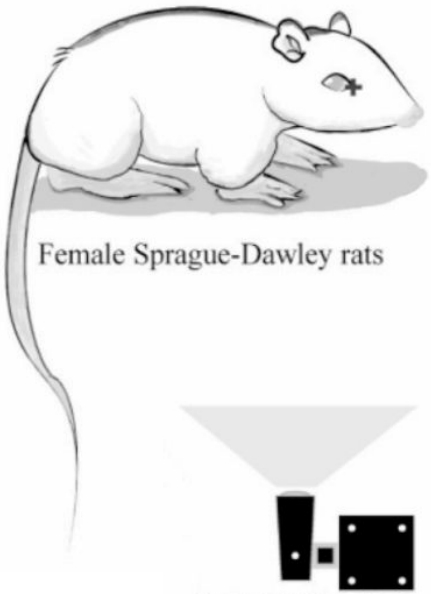
Histological analysis
1. CatWalk gait analysis
5. Imaging of blood vessel
Bone sections
Rod implants
6. Micro-CT



Female Sprague-Dawley rats

Eye socket vein blood

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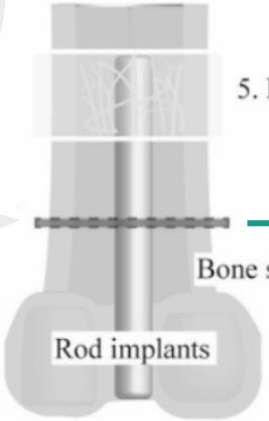
Histological analysis

1. CatWalk gait analysis



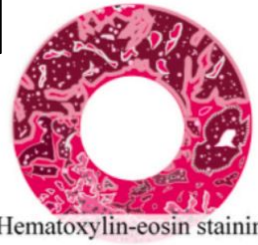
5. Imaging of blood vessel

Bone sections

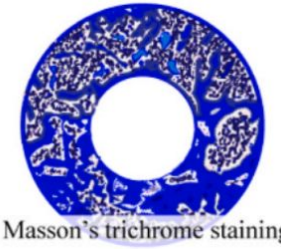
Rod implants

6. Micro-CT

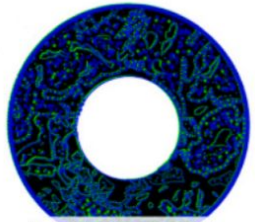
Immunofluorescence analysis



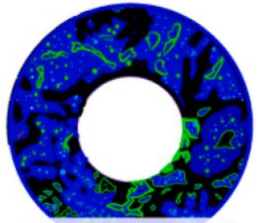
7. Hematoxylin-eosin staining



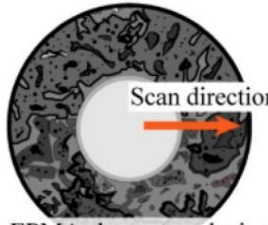
8. Masson's trichrome staining



9. Col-I immunofluorescence analysis

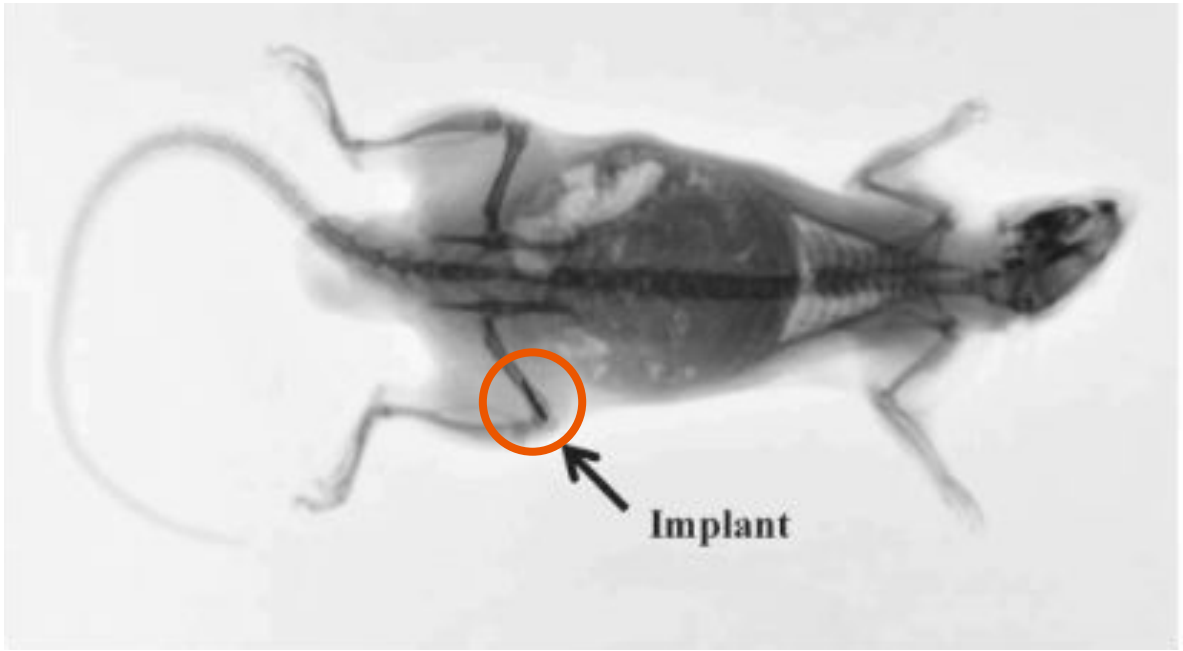


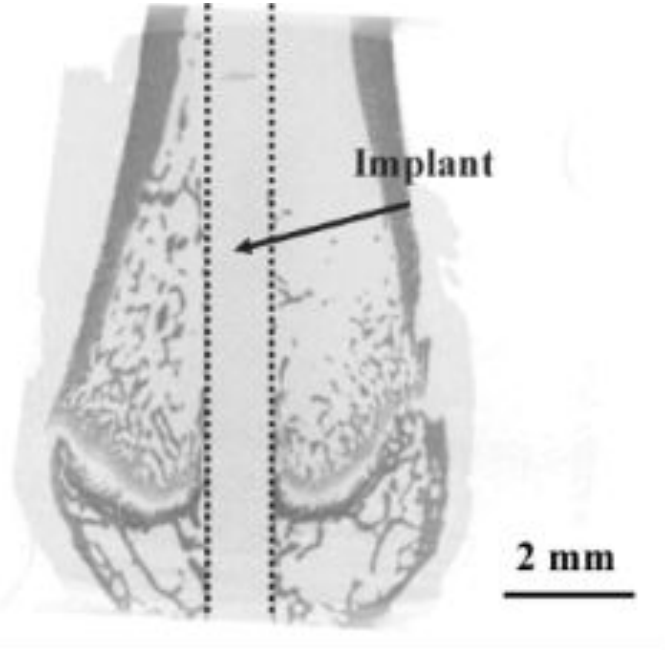
10. CD31 immunofluorescence analysis



11. EPMA element analysis at the implant-bone interface

Where is the implant ?





1 **Methods & Experiments**

2 **Pain measurements**

3 **Osteogenesis measurements**

4 **Angiogenesis measurements**

Gait analysis

- Estimate the acute effect of implants in the living body by measuring locomotion

⇒ **Pain levels can be assessed by measuring locomotion**

Gait analysis

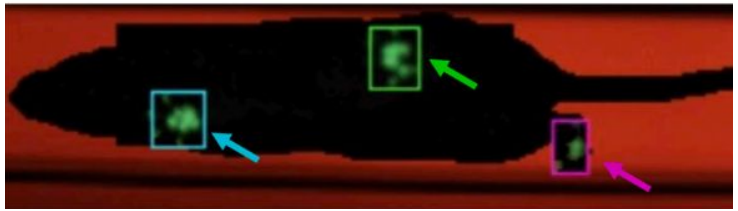
• Estimate the acute effect of implants in the living body by measuring locomotion

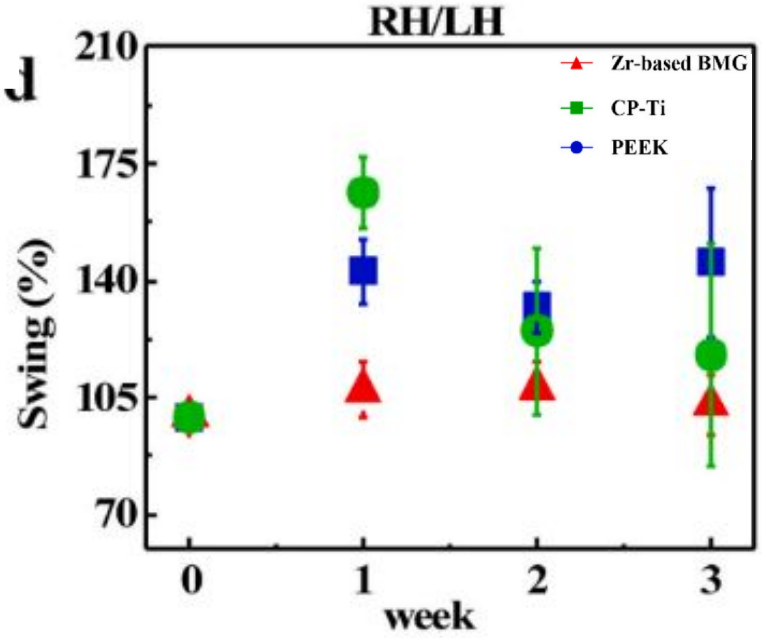
⇒ **Pain levels can be assessed by measuring locomotion**

• Materials implanted into the right hind (RH) leg

⇒ **Painful feeling expected to occur in the RH**

Gait analysis





- Longer swing time indicates a stronger pain feeling

- ZT1 tends to a lower pain level during recovery which can reduce the sensitivity of mechanical allodynia* induced by the implants

- *Allodynia is extreme sensitivity to touch

1 **Methods & Experiments**

2 **Pain measurements**

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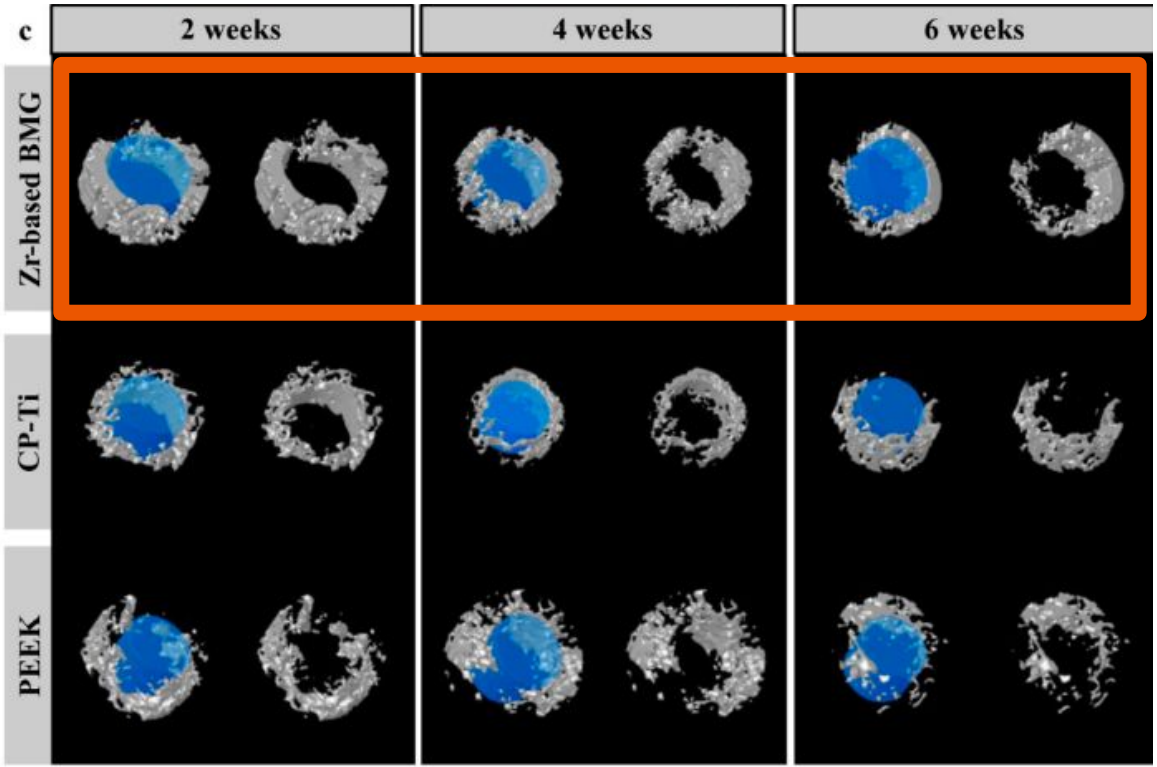
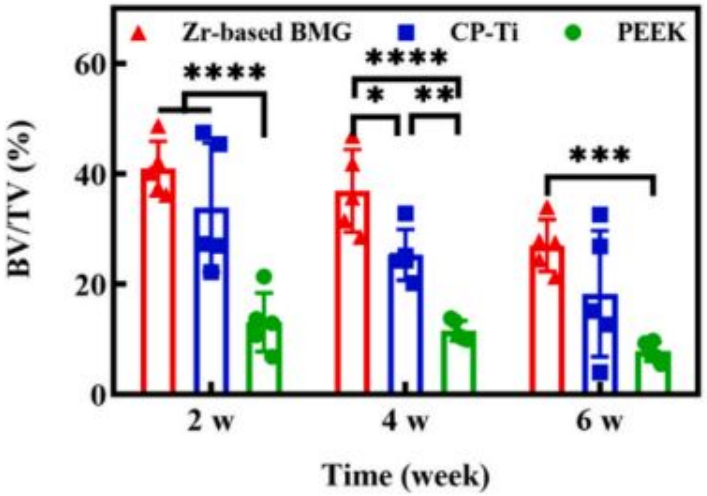
High-resolution microtomography evaluation



• Microarchitecture of the cancellous bone surrounding **the ZT1 is fairly better** than CP-Ti and PEEK.

High-resolution microtomography evaluation



• % of bone volume/total volume (BV/TV) significantly higher for ZT1 than PEEK

Higher *in-vivo* osteogenic potential for ZT1 compared with PEEK and CP-Ti

Histological analysis

- Hematoxylineosin (HE) and Masson's trichrome staining

- Hematoxylin stains cell nuclei a purplish blue

- Eosin stains the extracellular matrix and cytoplasm pink

- Masson's trichrome stains collagen, collagen fibers, fibrin, muscles, and erythrocytes.

⇒ **Assess the osteointegrations**

Histological analysis

- Hematoxylineosin (HE) and Masson's trichrome staining
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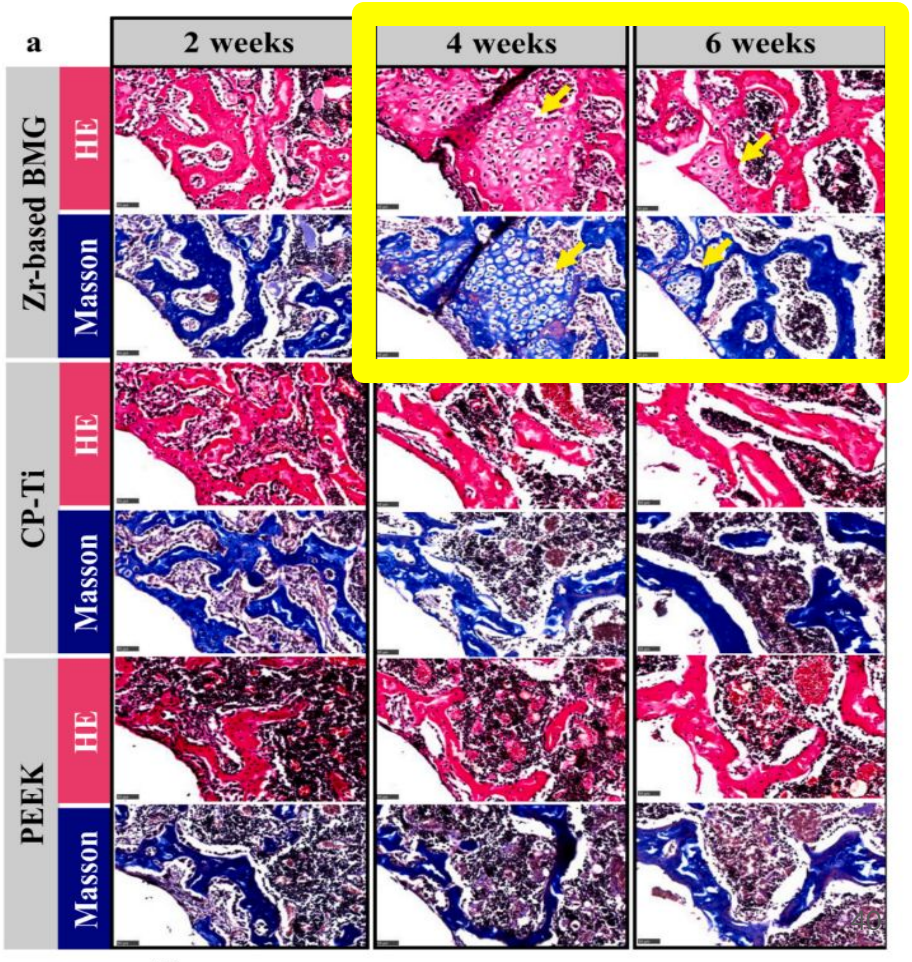
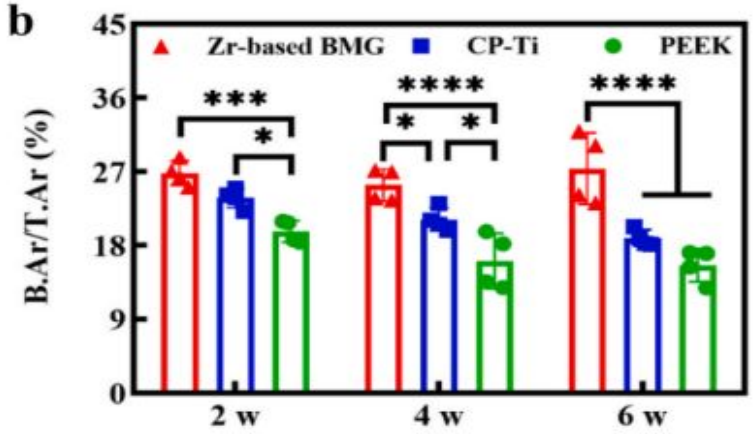
⇒ **Assess the osteointegrations**



Histological analysis



• Quantitative analysis of the bone area ratio (B.Ar/T.Ar)
→ significant differences between the materials

• ZT1 stimulate active bone regeneration and early bone remodeling

⇒ **Better osteointegration than CP-Ti and PEEK**

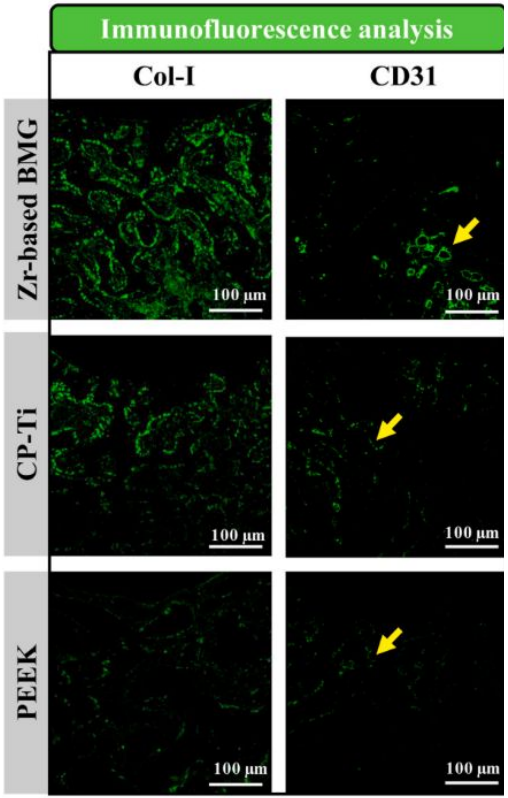
1 **Methods & Experiments**

2 **Pain measurements**

3 **Osteogenesis measurements**

4 **Angiogenesis measurements**

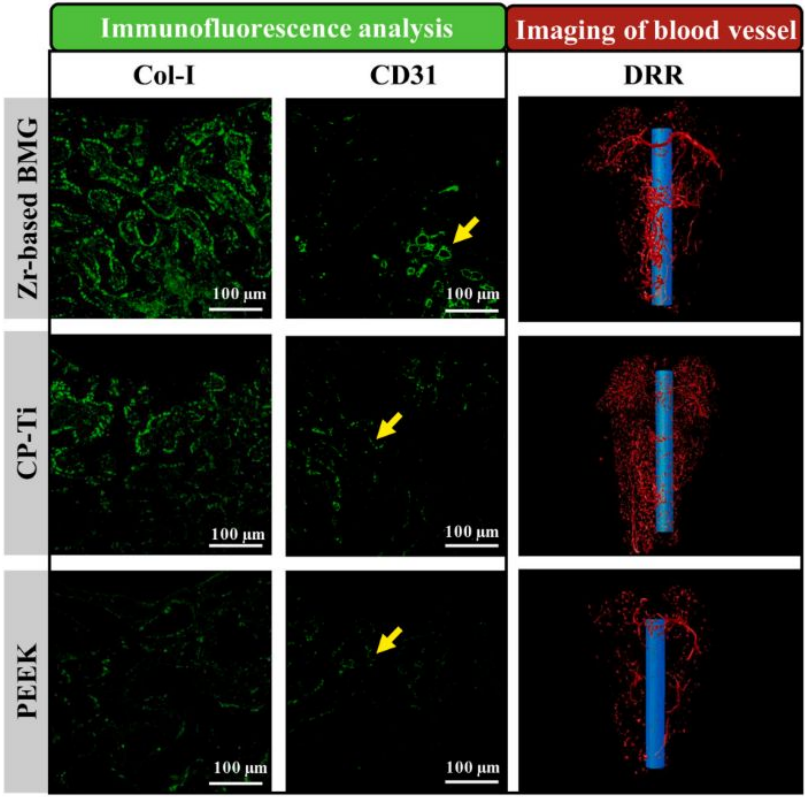
Immunofluorescence analysis and imaging of blood vessels



- **Col-I** = osteoblastic marker used to characterize early osteogenesis

- **CD31** = specific endothelial cell surface marker to characterize the angiogenesis

Immunofluorescence analysis and imaging of blood vessels



- **Col-I** = osteoblastic marker used to characterize early osteogenesis

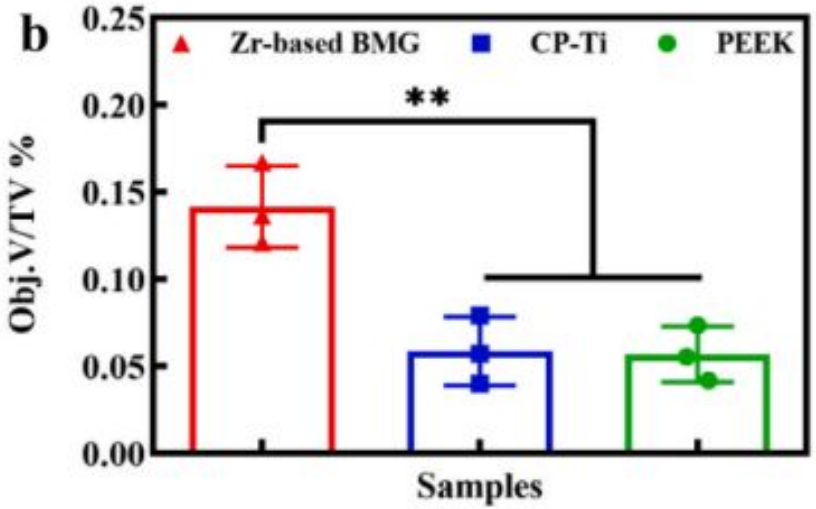
- **CD31** = specific endothelial cell surface marker to characterize the angiogenesis

- **Digital reconstructed radiographs** (DRR) depict the formation of blood vessels around the samples

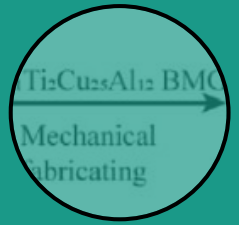
- **More immunofluorescence and more blood vessels are found in the ZT1**

Imaging of blood vessels

• **ZT1** shows higher vascular volume than the CP-Ti and the PEEK



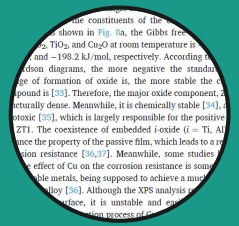
⇒ **It promotes the formation of blood vessels** (larger vascular volume postoperatively)



Characterization of the materials

In-vitro measurements

In-vivo measurements



Discussion

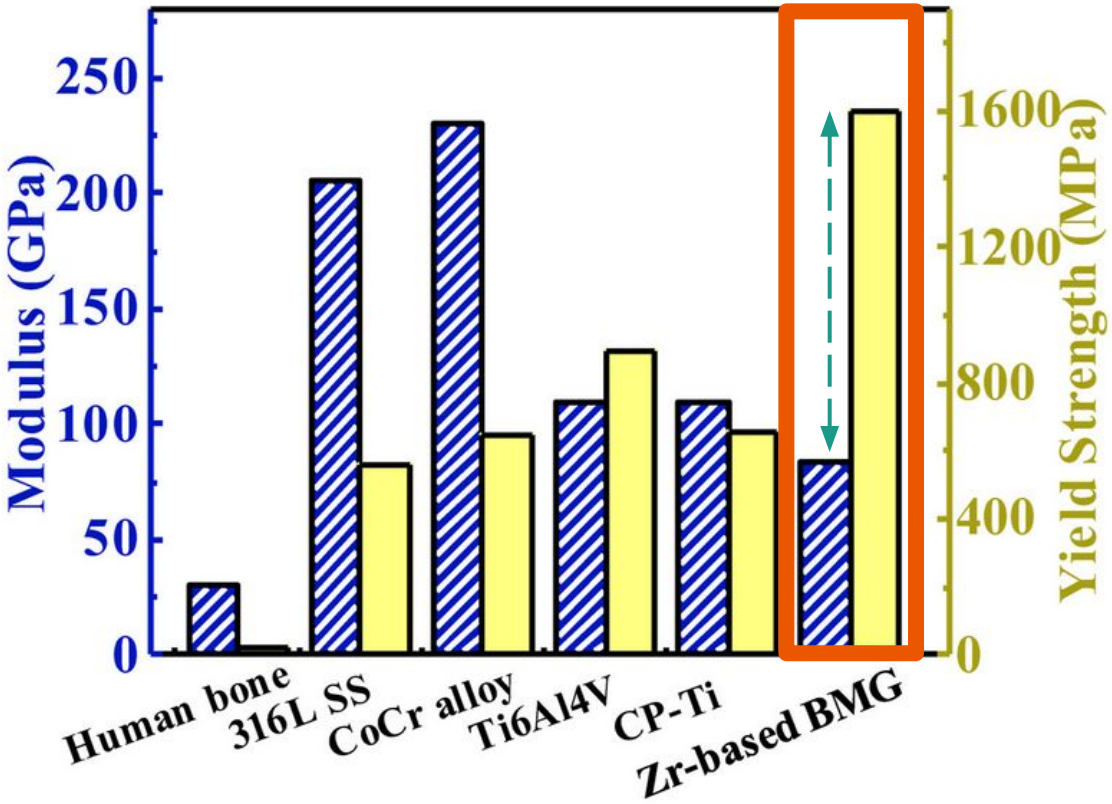
ZT1 properties

Biocompatibility
- Wettability
- Chemically stable
- Oxide films for increasing lifetime

- Warning concerning the Cu ions release that can affect blood vessel growth.

What about Biomechanical properties ?

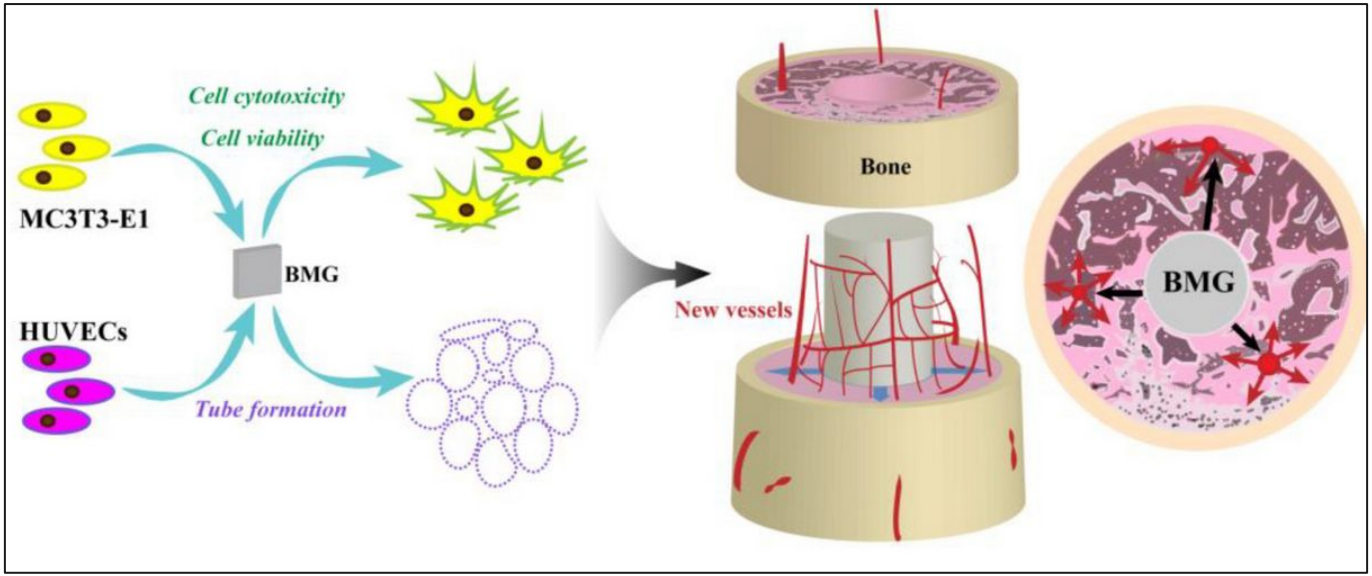
Biomechanical properties



• ZT1 has a more suitable Young's modulus (83 GPa) and a significantly high yield strength (1688 MPa)

Conclusion & Outlooks

Sum-up


Cell cytotoxicity
Cell viability
MC3T3-E1
BMG
HUVECs
Tube formation
New vessels
Bone
BMG

Outlooks

ELSEVIER

Composites Part B: Engineering

Volume 164, 1 May 2019, Pages 800-808

The role of alumina-zirconia loading on the mechanical and biological properties of UHMWPE for biomedical applications

D. Duraccio [a], V. Strongone [b, c], G. Malucelli [b], F. Auriemma [c], C. De Rosa [c], F.D. Mussano [d], T. Genova [d], M.G. Faga [a]

- Ultra High Molecular Weight Polyethylene (UHMWPE)
- Alumina is used for bone implants (osteoblast interaction)

- Nice frictional and wear behavior
- Nice reliability of tissue ingrowth

Material of choice for hip and knee joint.

What are the three protagonists studied ?

ZT1, PEEK and CP-Ti

Where was implanted the material ?

In the right hind leg

How can we assess the pain level ?

Using gait analysis

How many  were present in this presentation ?

None

Thank you for your attention !