Human fetal mesenchymal stem cell secretome enhances bone consolidation in distraction osteogenesis

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Background

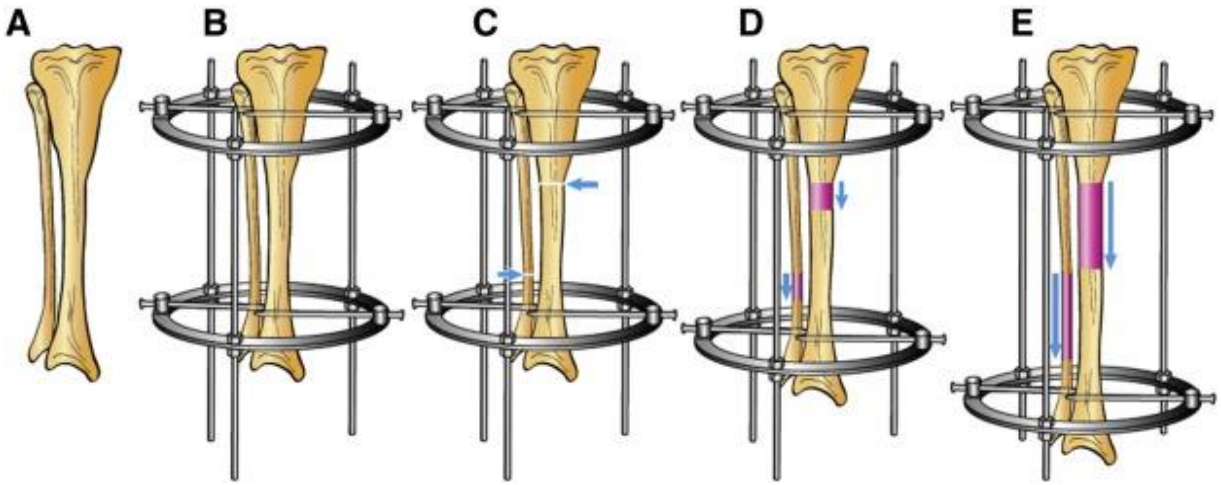
Distraction osteogenesis

Mesenchymal stem cells

Stem cell secretome

Background:
Distraction osteogenesis (DO)

- Controlled gradual traction between osteotomy cuts



Quelle: https://ars.els-cdn.com/content/image/1-s2.0-S1549963414002226-gr1.jpg

- "Tension-stress principle"
- Pro: correct a variety of orthopedic deformations and malformations
- Cons: undesirably long treatment period, external fixation

Background:
Mesenchymal stem cells (MSC)

- Various adult mesenchymal stem cells have been transplanted into damaged area to promote tissue repair
- Pure cell transplantation -> poor differentiation and survival rates ……PARACRINE EFFECTS [5,6]
- Serum-free conditioned medium derived from human adult MSCs (hAMSCs) was applied to accelerate bone formation in animals
- Human Fetal MSCs (hFMSCs) demonstrated recently to have growth promoting potential.
- They have superior cell proliferation capacity, more robust osteogenic potential and lower immunogenicity, compared to hAMSCs.

Background:
Stem cell secretome

- Regulatory and trophic factors including growth factors, cytokines, exosomes, and microRNAs
- Effect: enhances angiogenesis, reduces inflammation, promotes tissue repair, inhibits fibrosis and cell apoptosis

- Benefit of cell-free secretome application is to avoid:
  - immune incompatibility
  - Longer waiting time
  - Higher costs for cell preparation

Methods

Methods:

- **Secretome preparation**
  - **In vivo: 100µl secretome added into 3ml osteogenic induction medium (OIM)**
  - **In vivo: 100 µl secretome locally injected**
  - **Concentration: 100µg/µl and 3mg/µl**
- **In vitro:**
  - **Cell viability assay**
    - **Density 5000 rat bone marrow-derived MSCs (rBMSCs)/well**
    - from hFMSC secretome (0,10µg/µl,25µg/µl, 50µg/µl, 100µg/µl, 200µg/µl)
    - 24h and 72h incubation
    - via MTT reduction assay.

Methods:

- In vitro:
  - **Osteogenic differentiation of rat bone marrow-derived MSCs (rBMSCs)**
    - 5000 cells/cm^2
    - **Alkaline phosphatase (ALP) staining**
      - Secretome from rBMSCs, hFMSCs and hAMSCs (100 µg/µl)
      - hFMSCs secretome (0,10µg/µl, 25µg/µl, 50µg/µl, 100µg/µl, 200µg/µl)
      - 3 days
    - **Alizarin Red S staining**
      - Secretome from rBMSCs, hFMSCs and hAMSCs (100 µg/µl) – 7 and 14 days
      - hFMSCs secretome (0,10µg/µl, 25µg/µl, 50µg/µl, 100µg/µl, 200µg/µl) – 7 days
    - **RNA extraction and quantitative real-time PCR**
      - hFMSC secretome (100µg/µl) – 3 and 10 days
  - **Mixed rat peripheral blood lymphocyte (rPBL) reaction**
    - 1x10^5 rPBLs
    - hFMSCs and hAMSCs (0,10µg/µl, 25µg/µl, 50µg/µl, 100µg/µl, 200µg/µl)
    - Serum-free α-MEM as baseline control
    - 1,3, and 5 days of culture
    - Determination by bromodeoxyuridine (BrdU) incorporation assay

Methods:

- In vivo:
  - 24 12-week-old SD male rats
  - Subjected to a right tibia transverse osteotomy procedure
    with a closed fracture at the midshaft near the fibula-tibia junction
  - Monolateral external distraction fixator was placed to fix the proximal and distal segments
  - 3 groups:
    - PBS group (n=8);
    - medium group (n=8)
    - secretome group (n=8)

- **Distraction protocol:**
  - Latency phase :5 days
  - Active lengthening phase: 10 days
    - 1mm/day in two steps every 12h
  - Consolidation phase: 6 weeks
    - Injection of 100µl PBS, serum-free α-MEM or secretome **every 3 days** until termination

    - At the beginning: subcutaneous injection of calcein (10mg/kg)
    - 3 days before termination: subcutaneous injection of xylenol orange (30mg/kg)
  - Bilateral tibias were harvested, strapped free of muscle, and processed for further examinations

Methods:

- In vivo:
  - **Digital radiographs and Micro-computed tomography (µCT)**
    - weekly anterior-posterior x-ray including the distraction zone was taken until termination
    - The structural change within the distraction zone was quantitatively assessed with µCT
      - Three dimensional reconstructions of the mineralized callus were performed
      - Sagittal images were used to performe 3D histomorphometric analysis
      - Region of interest was defined as the distraction zone between the two closest proximal and distal half-pins
  - **Four-point bending mechanical testing**
    - 24h after termination
    - Control: Contralateral tibia
    - Four-point bending device with a 250N load cell
    - The modulus of elasticity, ultimate load, and energy to failure were obtained and analyzed

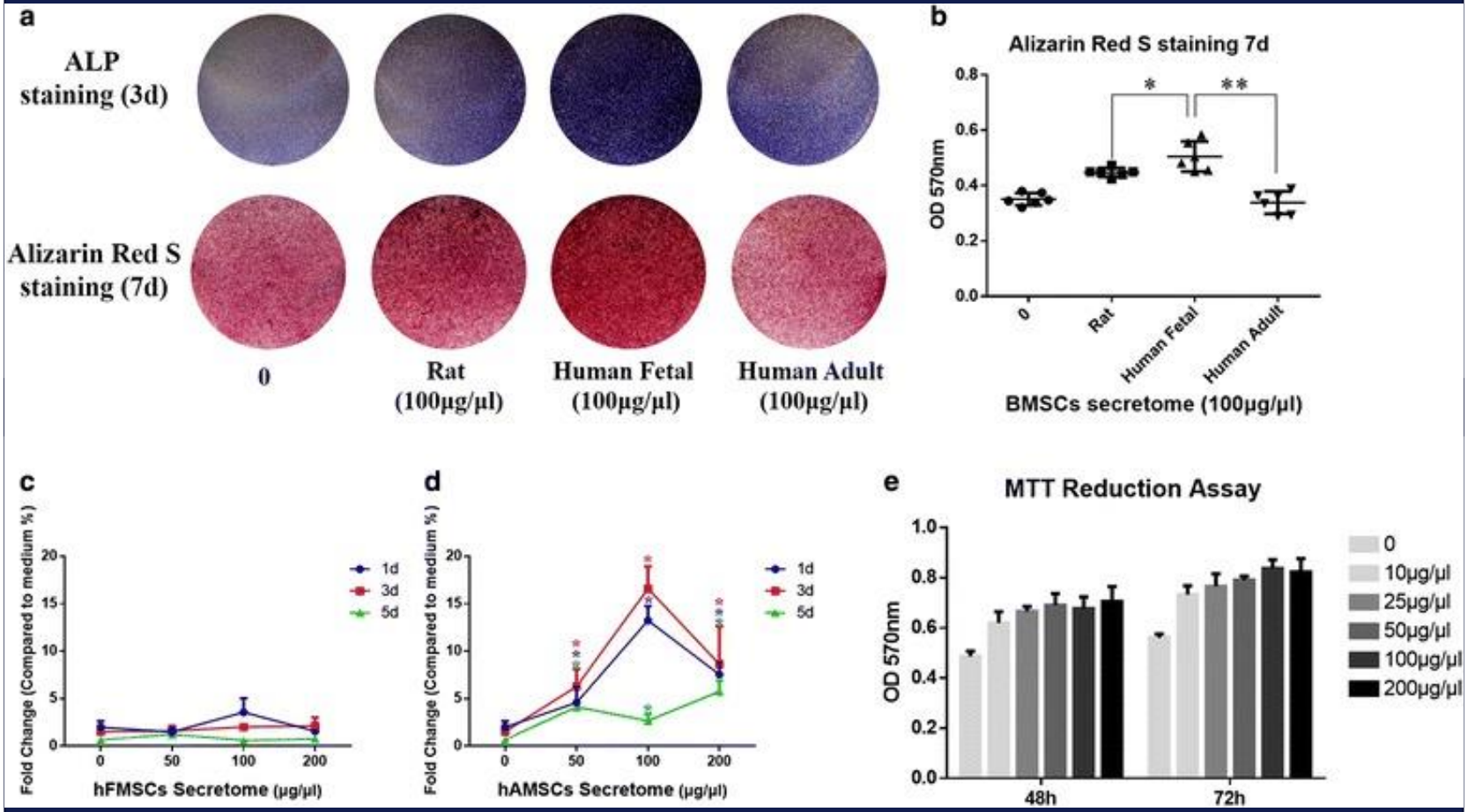
Methods:

- In vivo:
  - **Histology and immunohistochemistry**
    - Static histomorphometric analysis:
      - Trichrome Goldner staining
      - Von Kossa staining
    - Dynamic histomorphometric measurements:
      - Singled-labeled surface (sL.S)
      - Double-labeled surface (dL.S)
      - Ratio of mineralizing surface to bone surface (MS/BS)
      - Mineral apposition rate (MAR)
      - Bone formation rate per unit of bone surface (BFR/BS)
      - Bone formation rate of bone volume (BFR/BV)
      - Bone formation rate of tissue volume (BFR/TV)
    - Immunohistochemistry staining
      - Osx
      - OCN

Results

Results

ALP & Alizarin Red S staining, different MSC secretome

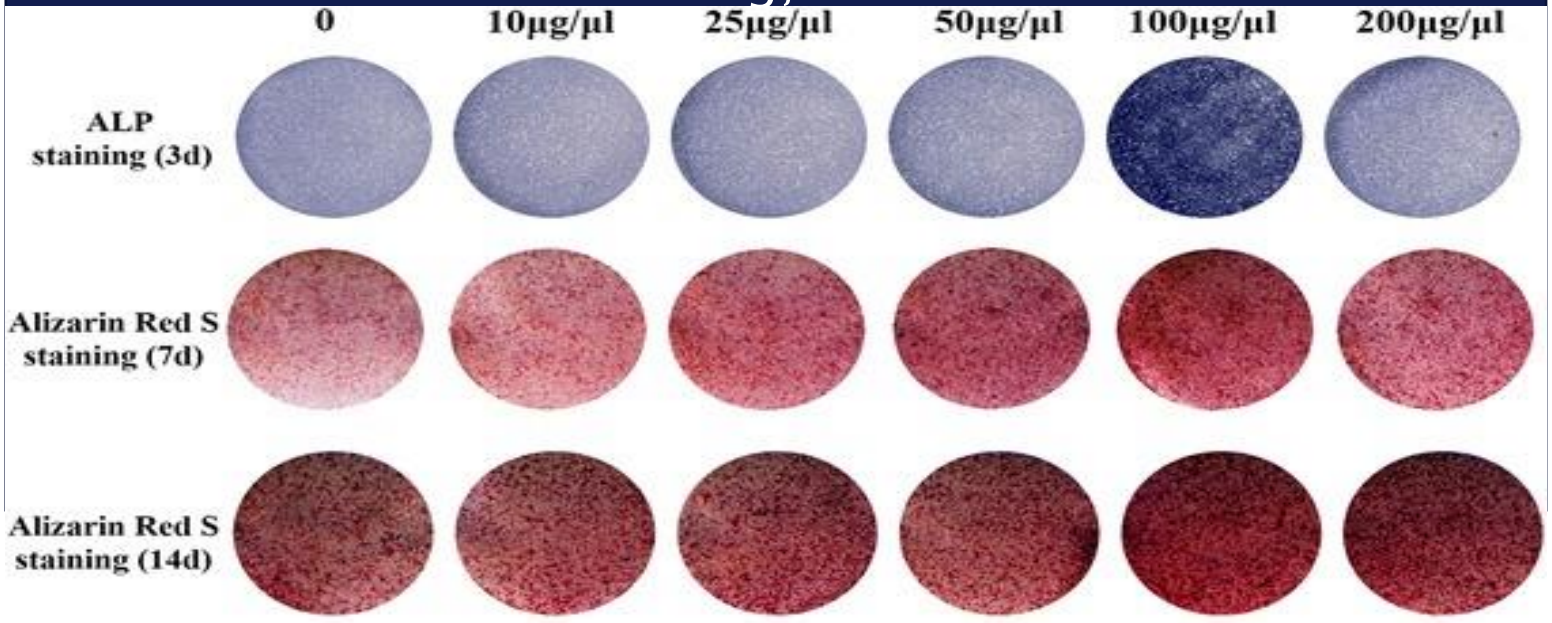


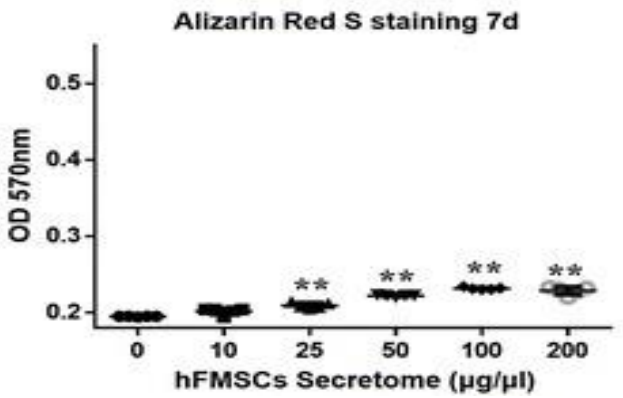
Immunogenicity

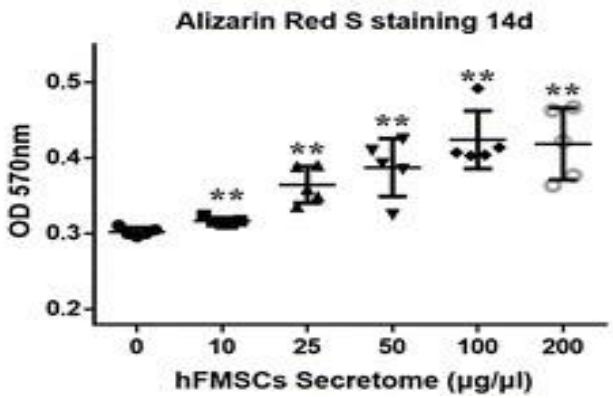
Cell viability

Results

ALP & Alizarin Red S staining, different concentration

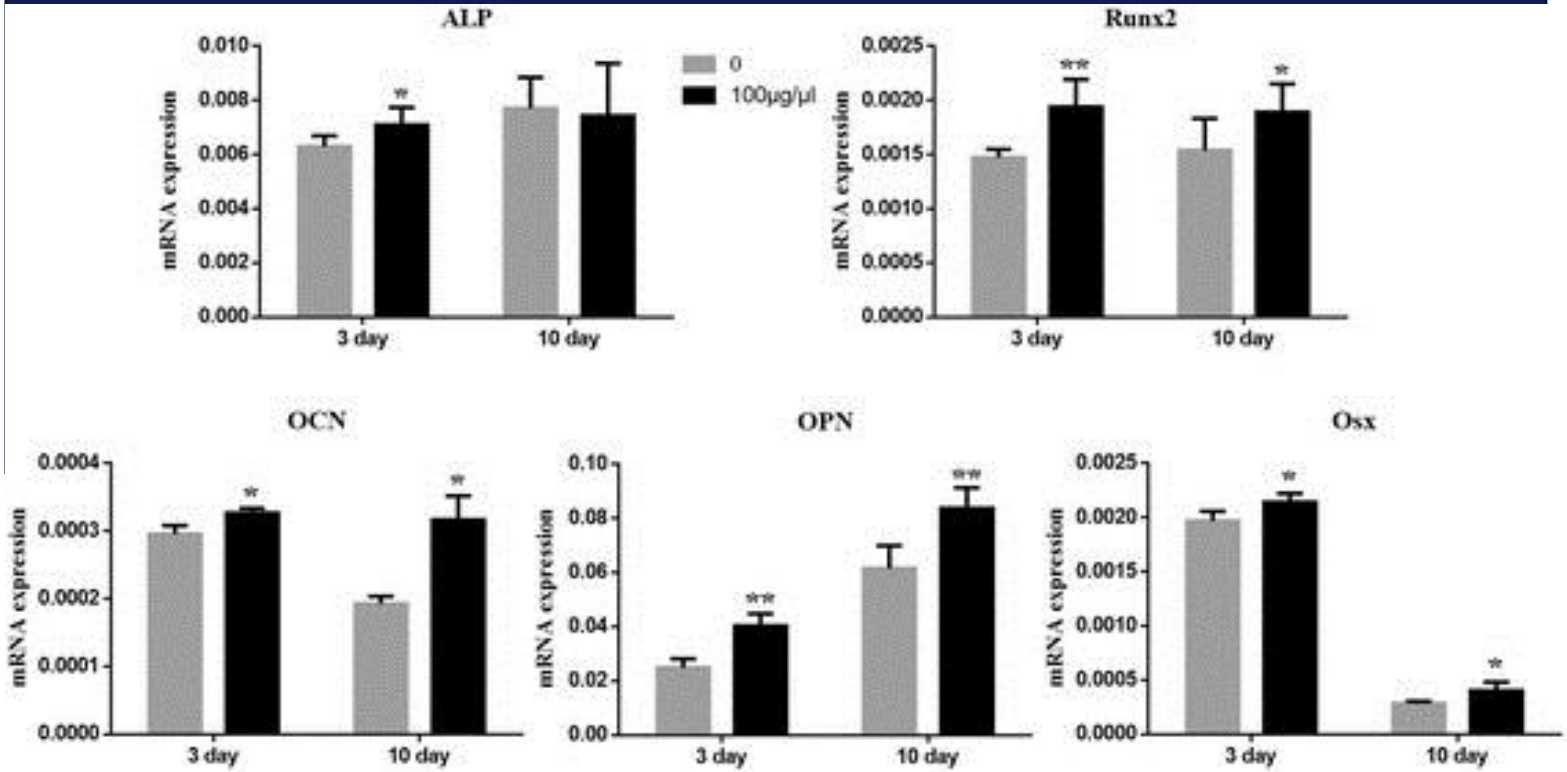






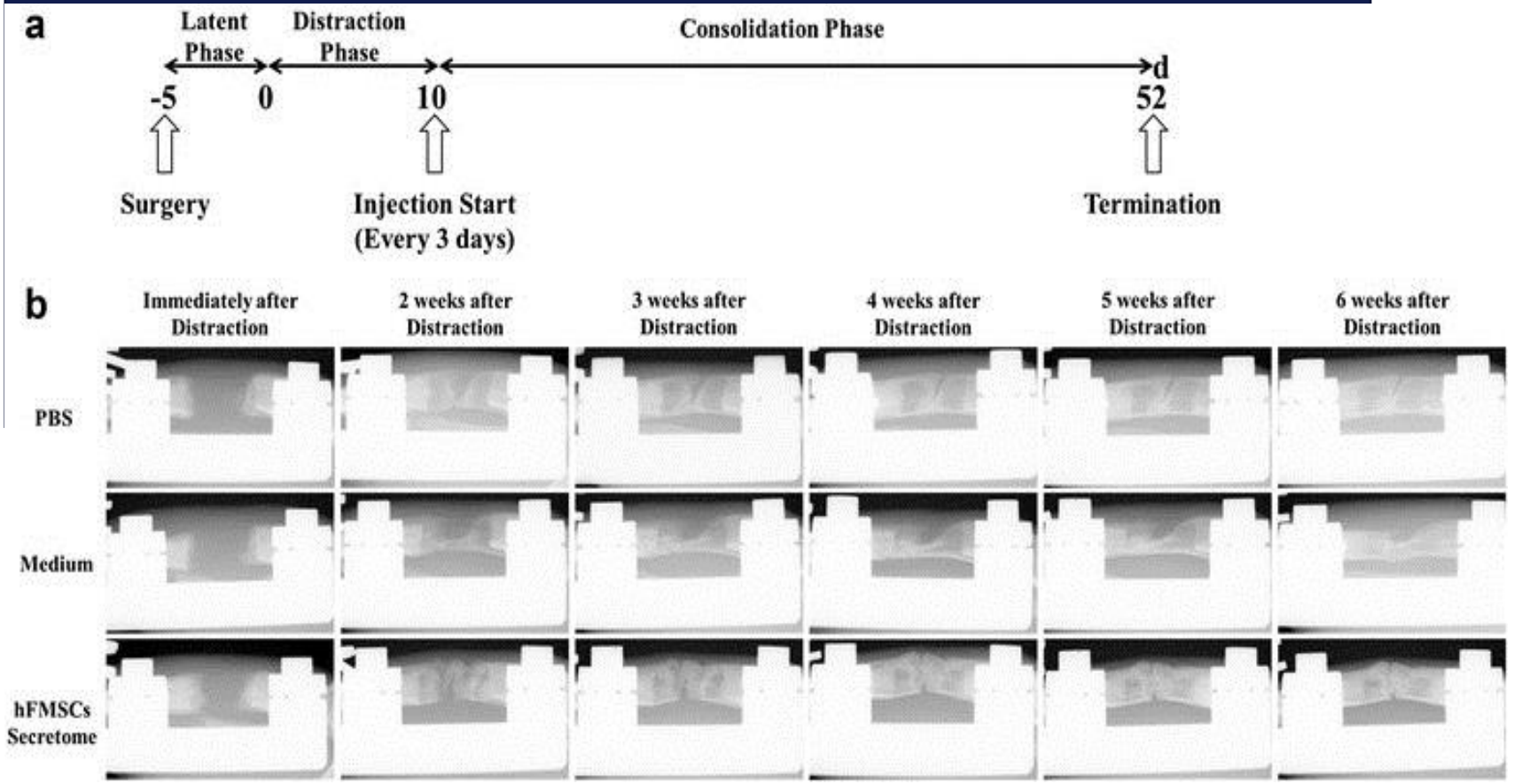
Results

PCR



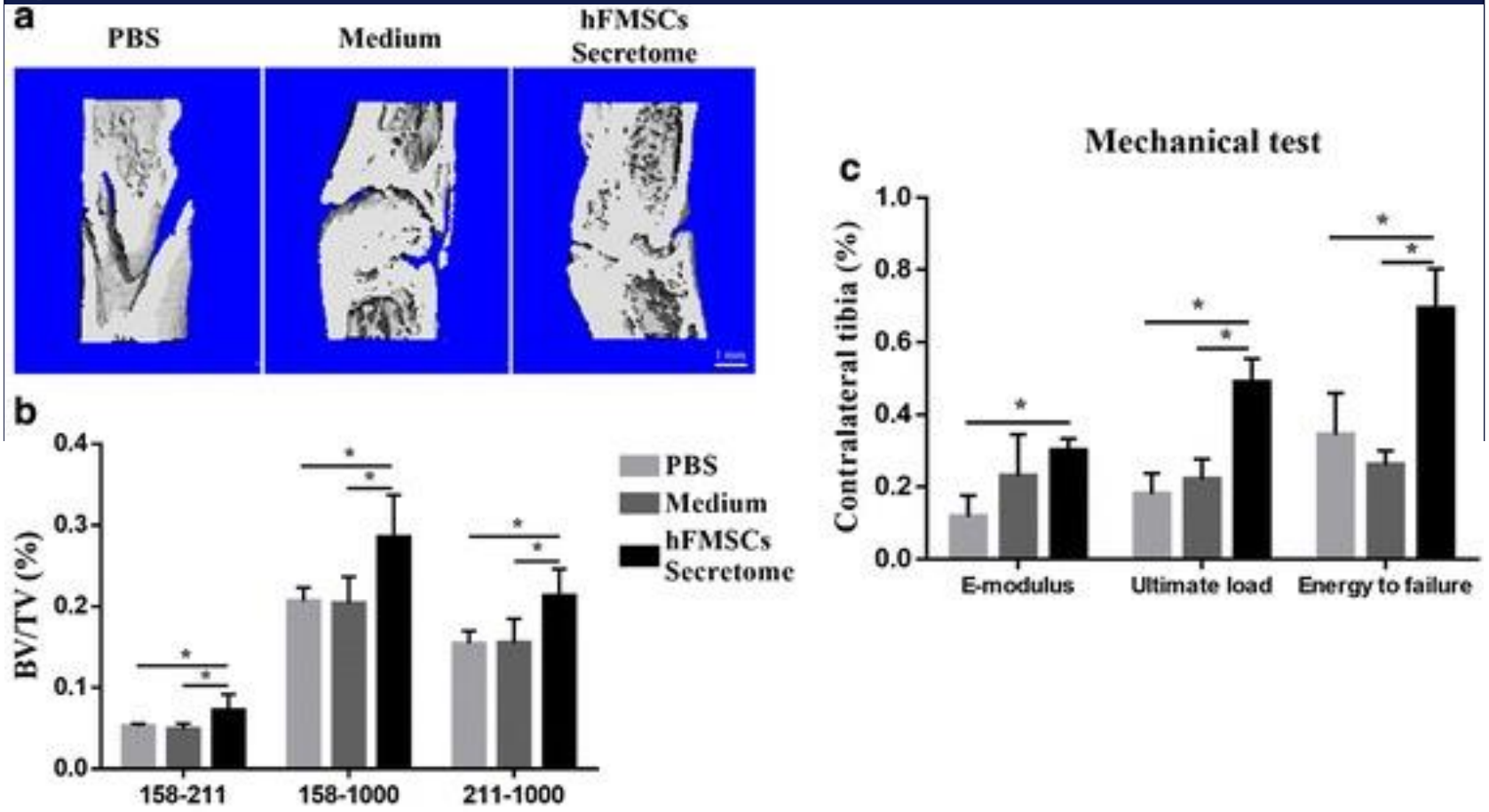
Results

In vivo: experimental design and x-rays



Results

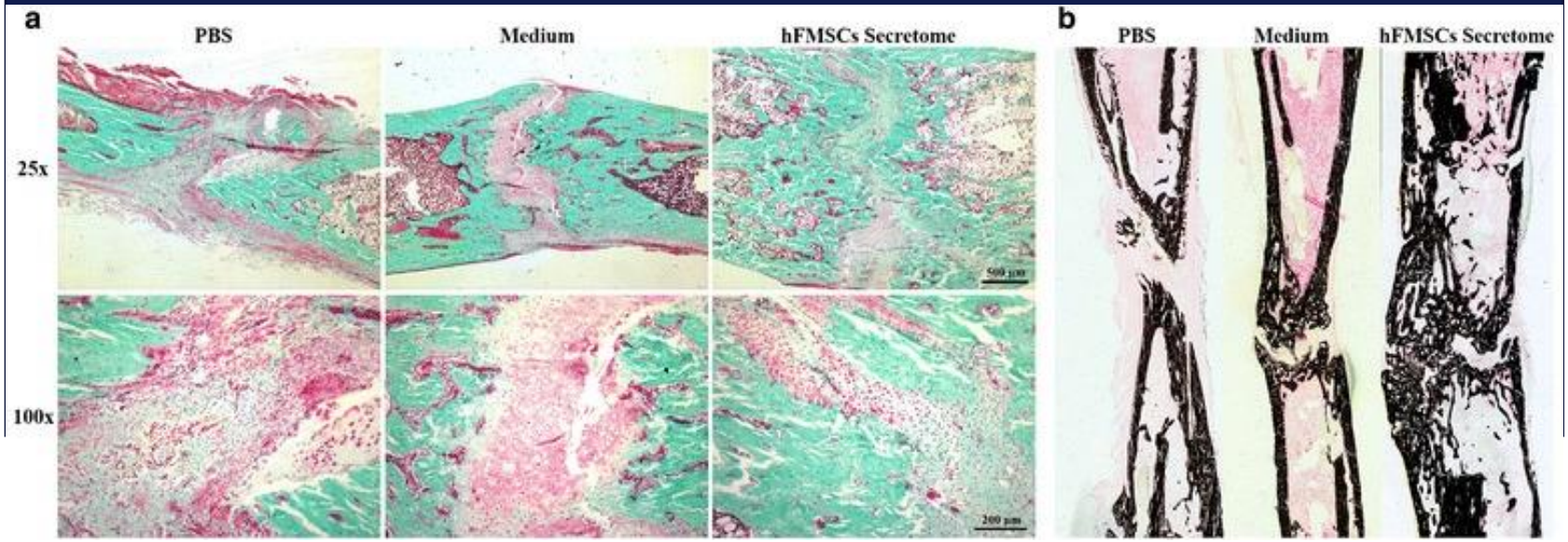
µCT and mechanical test



Results

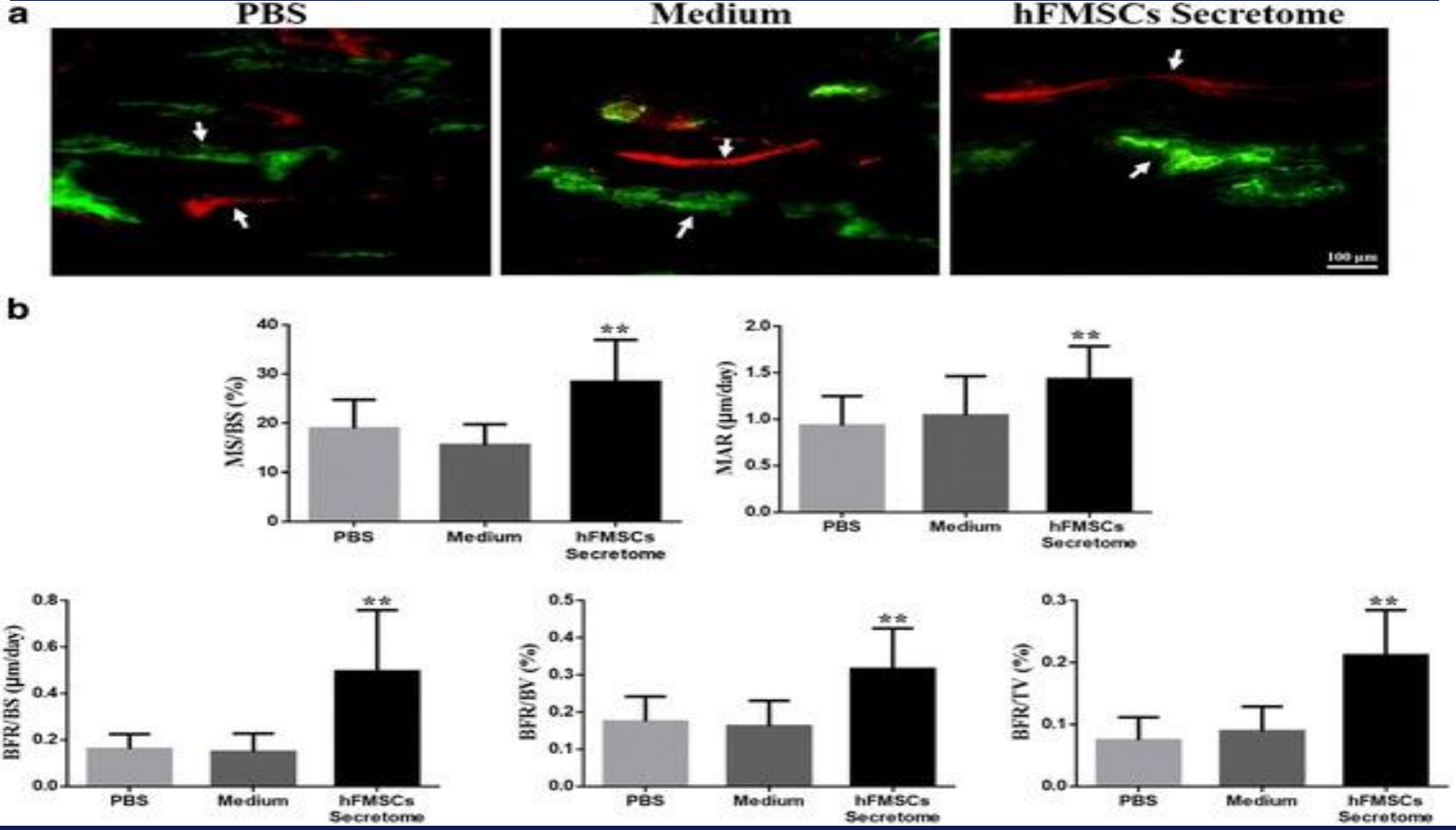
Trochrome Goldner

Von Kossa



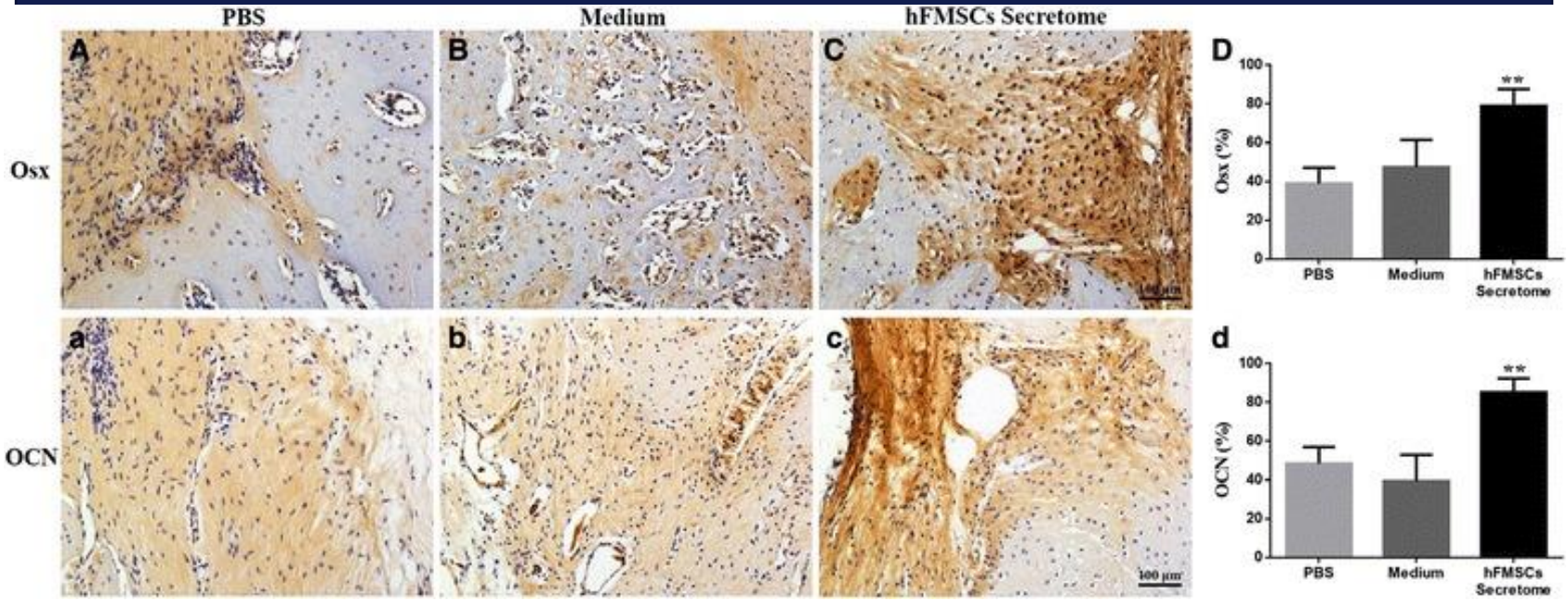
Results

Dynamic histomorphometric measurements



Results

Immunhistochemical analysis of Osx- and OCN positive cells



Discussion

Discussion

- hFMSCs are the most promising cell source for bone tissue engineering application because of their lower immunogenicity and higher proliferative and osteogenic capacity compared to hAMSCs
- Through gene modulation the proliferative and differentiation capacities of hFMSCs can be retained as well as their osteogenic potentials may be enhanced, but further studies are still needed.
- Cell-free secretome harvested from hFMSC conditioned medium centrifugation is an easy and cost-effective procedure

Discussion

- Upregulated osteogenic marker genes
  - ALP - mineralization, osteoblastic differentiation
  - OCN - late stage of mineralization
  - Runx2 - production of bone matrix protein, osteoblast differentiation
  - OPN - bone formation, remodeling
  - Osx - osteoblast differentiation, bone formation

- Mechanisms as how hFMSC secretome augments bone formation and maintains vascularity are still unclear
- The effect of MSC secretome can be improved through enhancing survival of the progenitor cells that homed to the target site

Discussion

- VEGF is needed to promote osteogenic differentiation over chondrogenic differentiation of MSCs, what might be the reason for more cartilage stoll remaining the PBS group and medium group in the current study
- Further research is warranted to verify the current findings
- Limitations:
  - Effectiveness and potential immunogenicity of using human proteins in rats still need further careful investigation
  - The exact mechanism of how hFMSC secretome enhances osteogenic differentiation of rBmSCs without detectable immunological responses need further exploration
  - In vivo, Secretome contents likely change from what they would be in vitro

Conclusion

- hFMSC secretome improves the osteogenic differentiation potential of rBMSCs and accelerates bone consolidation during DO in a rat model
- A novel application of hFMSC secretome is proposed with a clinical potential to enhance bone consolidation of DO treatment for patients with limb discrepancy, severe deformities, and bone defects

Thank you for your attention