Multi-scale biomechanical analysis of mineralized spheroids derived from human mesenchymal stem cells

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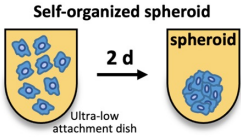
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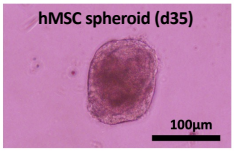
Introduction

1. Limitations of conventional 2D culture
• Conventional 2D culture systems fail to replicate cell–cell interactions and mechanical environments found in vivo.
• Bone formation is inherently a three-dimensional process.
2. Importance of mineralization in bone formation
• Bone tissue formation relies on calcium deposition and hydroxyapatite formation.
• However, the spatial and temporal dynamics of mineralization remain unclear.
3. Unresolved role of mechanical environment
• Mechanical factors/environments are believed to influence mineral nucleation and growth
• Yet, mechanical evaluation within 3D tissues is still limited.
4. Positioning and novelty of this study
• Use of scaffold-free spheroids as a 3D bone model.
• Multiscale analysis combining macroscopic compression and microscopic AFM.
• First integrated evaluation of evolving mechanical properties during mineralization.

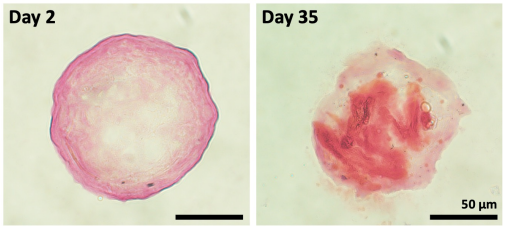
Spheroid culture

- Cell source:
  - human mesenchymal stem cells (hMSC)
- Fabrication of spheroids:
  - Self-organized spheroid
  - Ultra-low attachment U-bottom culture dish
  - 2,500 cells / spheroid
- Medium:
  - DMEM-high glucose
    + ascorbic acid, β-glycerophosphate, dexamethasone
- 35-day incubation





Alizarin-red staining



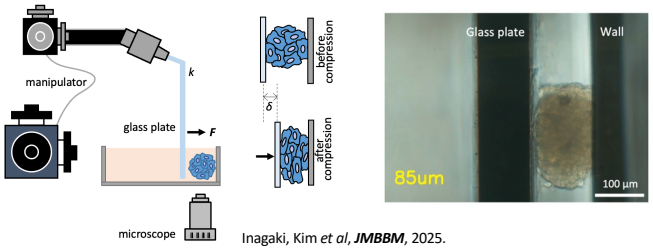
Alizarin-red staining: calcium deposition during osteogenesis

✓ **Long-term spheroid culture for hMSC induced mineralization.**

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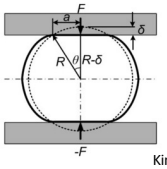
Uniaxial compression testing

- Uniaxial compression test system for spheroids
  - 1-mm thick glass plate
  - Three-axis micromanipulator (Narishige)
  - IX73 inverted microscope (Olympus)



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- Model fitting using force, spheroid radius, & displacement to calculate Young's modulus *E*

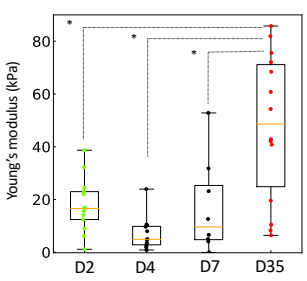


$$E = \frac{3(1-\nu^2)F}{(4\delta a)} + \frac{f(a)F}{\pi\delta}$$

δ: displacement
F: force
R: radius of spheroid
E: Young's modulus

Kim, K *et al*, ***J Biomed Mater Res A***, 2010

- Young's modulus *E*



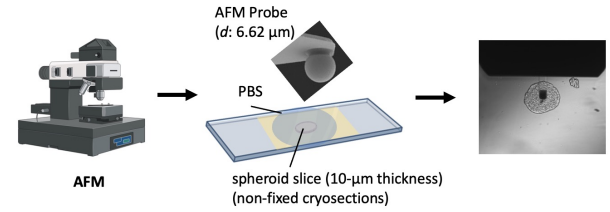
Mean ± SD
n = 8 – 13
from 3 independent experiments
One-way ANOVA
with Tukey post-hoc test
* $p < 0.01$

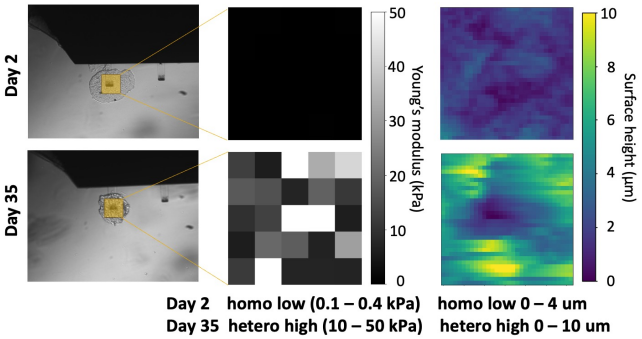
Measuring stiffness of spheroids
Day 2 : 17.9 ± 9.9 kPa
Day 4 : 7.0 ± 6.1 kPa
Day 7 : 17.0 ± 16.9 kPa
Day 35 : **44.4 ± 17.5 kPa**

✓**Long-term culture elevated the stiffness of hMSC spheroids.**

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Atomic force microscopy (AFM)





Day 2 homo low (0.1 – 0.4 kPa) homo low 0 – 4 um
Day 35 hetero high (10 – 50 kPa) hetero high 0 – 10 um

✓**35-day spheroid exhibited heterogeneity in Young's modulus and surface height.**

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Discussion

- Mineralization of spheroids induces stiffening.
- AFM revealed highly heterogeneous local stiffness and height (residual stress).
- Importance of multiscale mechanics
  - Discrepancy between macroscale and microscale stiffness
  - Surface (cytoskeleton) vs internal (mineralized ECM) contributions
    → Bone formation should be understood as a hierarchical process.
- Limitations and future directions
  - Achieving GPa-scale like bone tissue (optimization of cell types, differentiation protocols, and osteogenic supplements)

Acknowledgment

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