Osteocytogenesis *in-vitro* for advanced microphysiological modelling of mature human bone tissue

Madden, L.A. [1,2], Milazzo, R. [1,2], Kelly, D.J. [1,2,3], Hoey, D.A. [1,2,3]

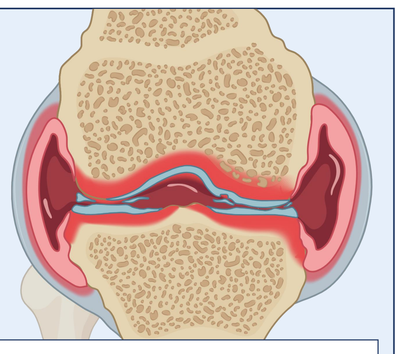
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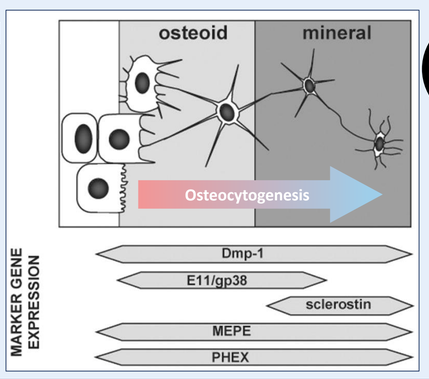
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Introduction

- Current microphysiological models of bone **do not incorporate human osteocytes**, the terminally differentiated and most abundant cell in bone tissue [1].
- Osteocytes are vital to effective modelling of bone homeostasis and drug response [2]
- ***In vitro* models of human osteocytic bone are needed**

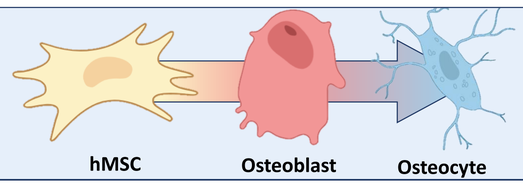


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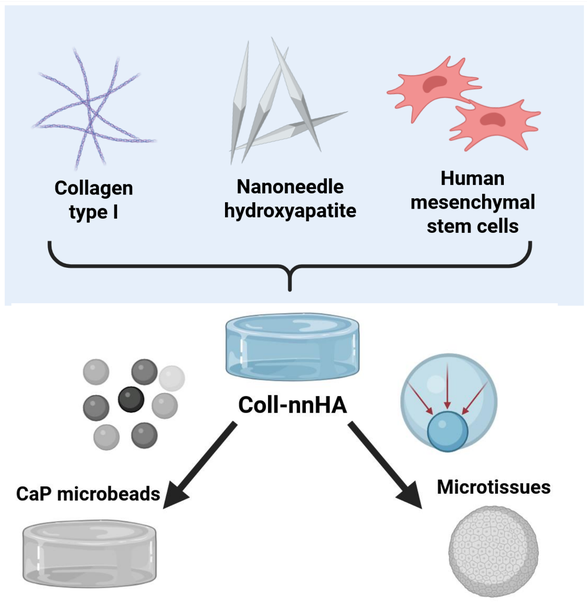


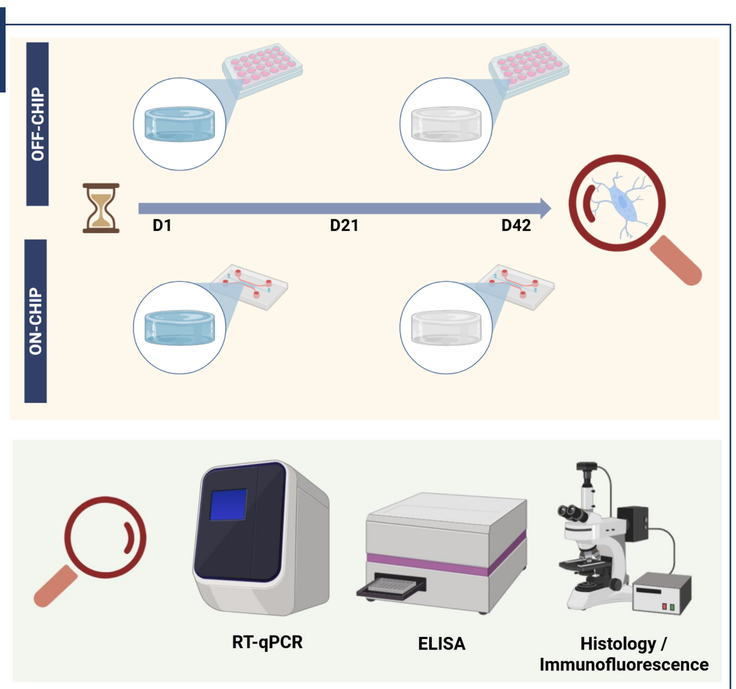
Objectives

- Induce **osteocytogenesis from human stem cells** using a **biomimetic hydrogel**
- Investigate applications of this gel in **microphysiological modelling** of mature human bone



Methods





Results

1 Coll-nnHA gel induces human osteocytogenesis *in vitro,* but not on chip

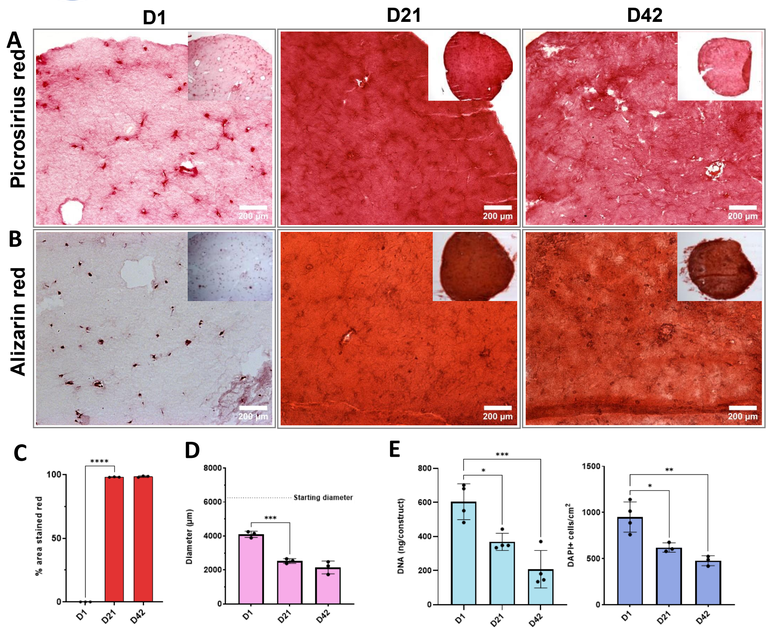
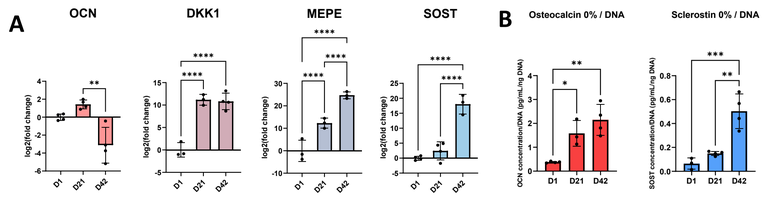


Fig 1: Coll-nnHA induces potent osteogenesis in hMSCs. Histological staining of (A) Picro Sirius red and (B) alizarin red of coll-nnHA gel culture shows (C) mineralisation until D21 of culture. (D) Gel diameter is reduced significantly over course of culture. (E) DNA content and DAPI+ staining quantification show loss of DNA over time. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.



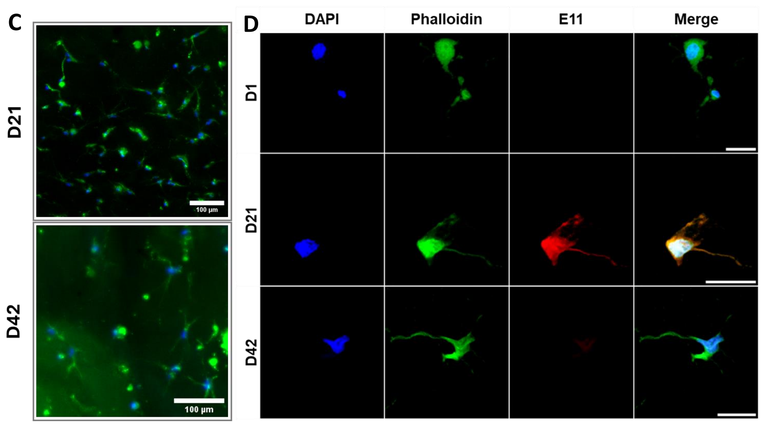


Fig 2: Progression through bone cell lineage to mature osteocyte is observed. (A) RT-qPCR results showing increase in osteocytic gene expression over time in culture. (B) ELISA protein assay results showing increase in osteocalcin and sclerostin secretion throughout culture. (C) DAPI/f-actin staining showing cell morphology and network formation through culture. (D) Immunofluorescence for E11. Dendrite formation observed at D21 of culture and not observed at D42. Scale bar = 20 µm. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.

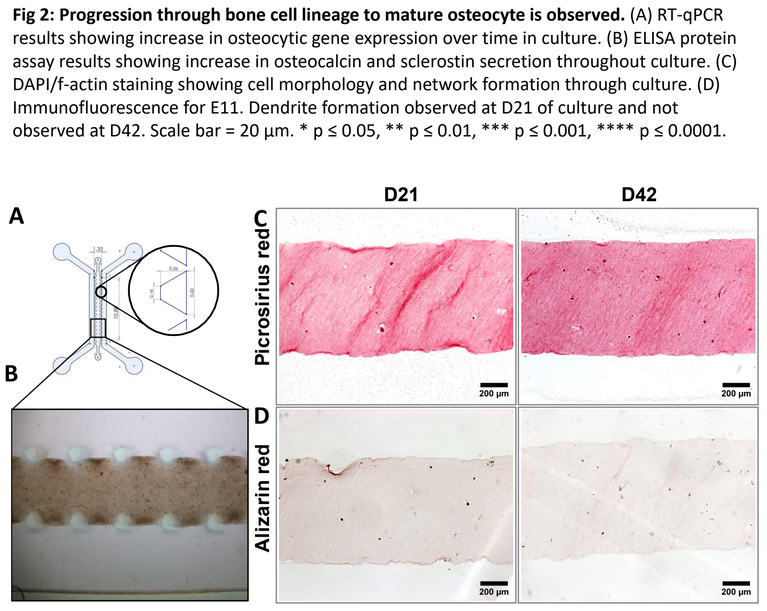


Fig 3: Coll-nnHA culture in OoC device does not undergo mineralisation. (A) Device schematic showing trapezoidal phase guides. (B) Coll-nnHA gel in chamber. (C) Picro Sirius red and (D) alizarin red staining showing lack of mineralisation throughout culture.

2 Addition of β-Tricalcium phosphate microbeads supports formation of osteocytic mineralised bone on-chip

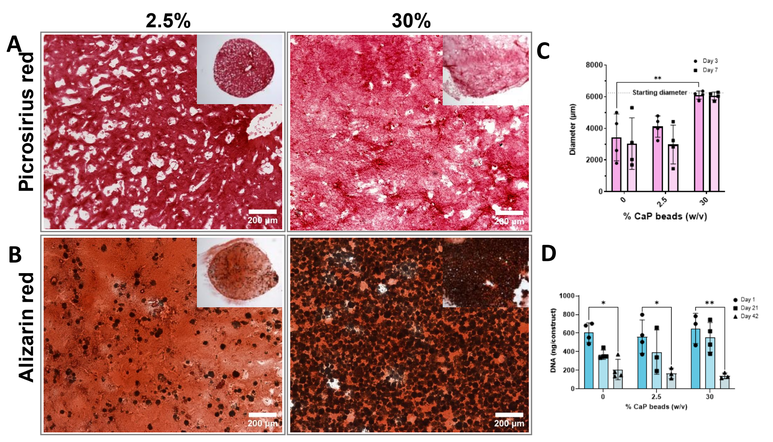


Fig 4: CaP bead supplementation prevents gel contraction and results in similar mineral content. Histological staining with (A) Picro Sirius red and (B) alizarin red of coll-nnHA gel culture with 2.5 and 30% CaP beads. (C) 30% w/v CaP beads is sufficient to prevent gel contraction. (D) DNA content quantification shows loss of DNA over time. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.

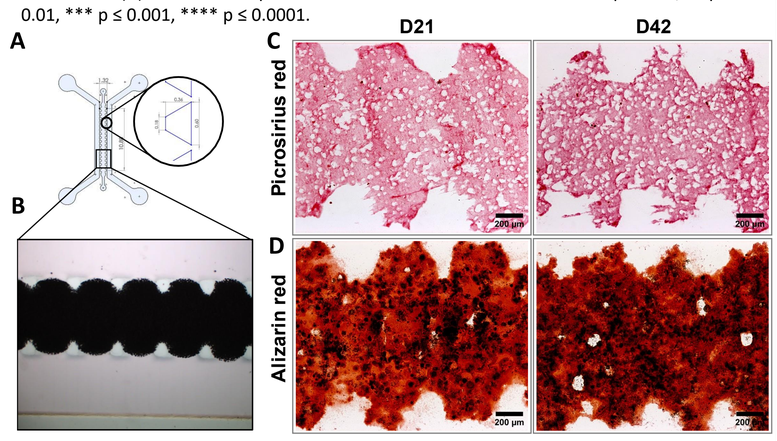


Fig 5: CaP bead supplementation enhances mineral content of coll-nnHA in OoC device. (A) Device schematic showing trapezoidal phase guides. (B) 30%% CaP bead-supplemented coll-nnHA gel inside chamber. (C) Picro Sirius red and (D) alizarin red staining of 30% CaP bead-supplemented hydrogel after 21 and 42 days of culture inside OoC device.

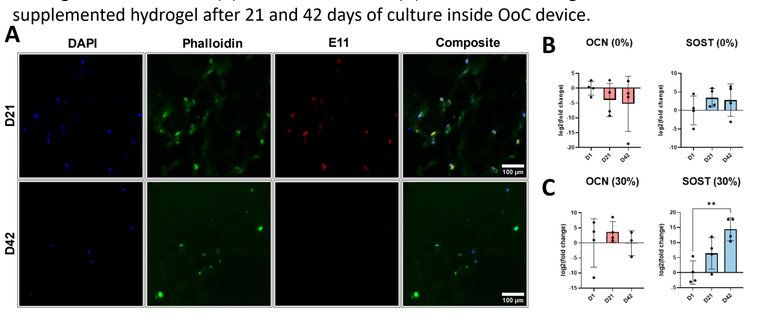


Fig 6: 30% CaP beads supports formation of osteocytic phenotype in OoC. (A) Immunofluorescence for E11. Expression of E11 at D21 and not D42. RT-qPCR shows lack of mature osteocyte marker SOST expression in unsupplemented OoC gel (B), while expression is observed with 30% CaP beads (C). * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.

3 Coll-nnHA contraction drives development of mineralised bone microtissues

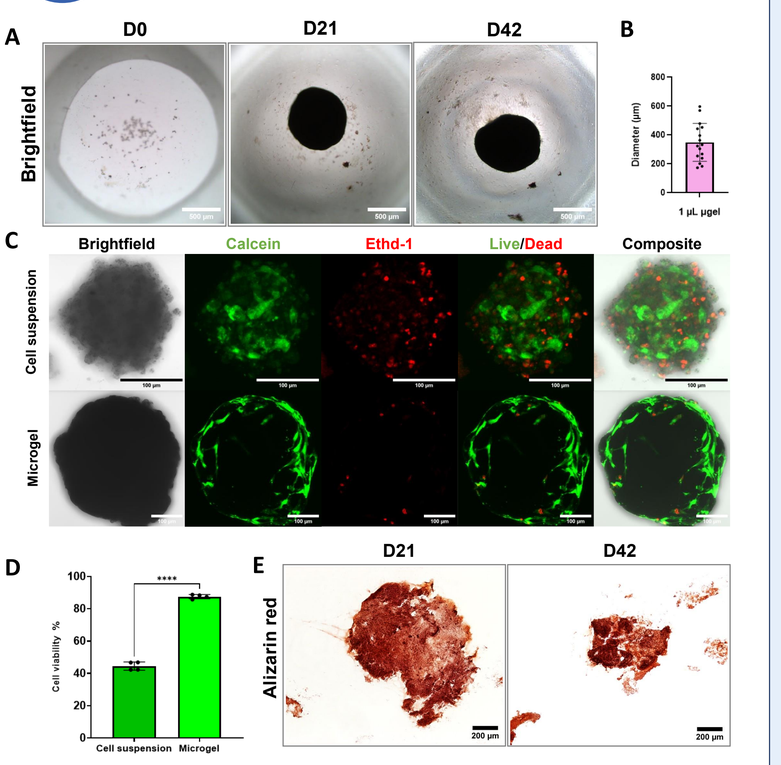


Fig 7: Gel contraction allows for formation of bone microtissues. (A) Coll-nnHA contraction allows formation of cell-laden microgels. (B) Microgel size after 21 days in culture. (C) Imaging and (D) quantification of Live/Dead cell viability. (E) Alizarin red staining of microgels after culture, showing potent mineralisation. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.

Future Work

Microtissues-on-chip

- ✓ **Pre-maturation** of microtissues may allow for seeding mature phenotype
- ✓ Can be seeded alongside **other cell types** to increase model complexity
- ✓ Represents significant step forward for **humanised bone models**

Disease modelling

- ✓ Modelling of disease using this approach will allow for **efficient drug testing**
- ✓ Currently developing **real-time optical biosensors** for OoC
- ✓ Incorporation of both offers **real-time feedback on drug activity**

Confirm presence of **osteocytic phenotype**
Test pre-maturation and seeding into **OoC device**

Test induction of **diseased phenotype**
Incorporate novel **optical biosensing technology**

Discussion & Conclusions

- ✓Coll-nnHA gel induces **mineralisation and rapid osteocytogenesis** in human MSCs
- ✓Coll-nnHA **fails to mineralise** when introduced to OoC
- ✓Supplementation with **30% CaP beads** recovers mineralisation loss in OoC environment
- ✓Coll-nnHA contraction can be used to form **bone microtissues**

Key Takeaway:
Coll-nnHA induces human osteocytogenesis *in vitro* and supports formation of bone-on-chip models

References

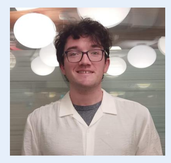
[1] Cope et al., Osteoarthritis Cartilage (2019)
[2] Bonewald L. F., JBMR (2011)
[3] Dallas et al., Ann N Y Acad Sci. (2010)

Acknowledgments

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