# Manipulating time and space to achieve endochondral bone formation

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## INTRODUCTION

- **Autologous graft material for reconstruction of large bone defects is limited and harvesting is associated with additional patient risk highlighting a need for suitable graft alternatives [1].**
- **Chondrogenically priming mesenchymal stem cell (MSC) pellets *in vitro* will reproducibly result in endochondral bone formation *in vivo*[2].**
- **The chondrogenic priming time is lengthy for clinical applications and resulting bone quantity is insufficient to fill large defects [3].**
- **A scale up approach is necessary to achieve greater bone formation without the use of unmanageable cell numbers.**

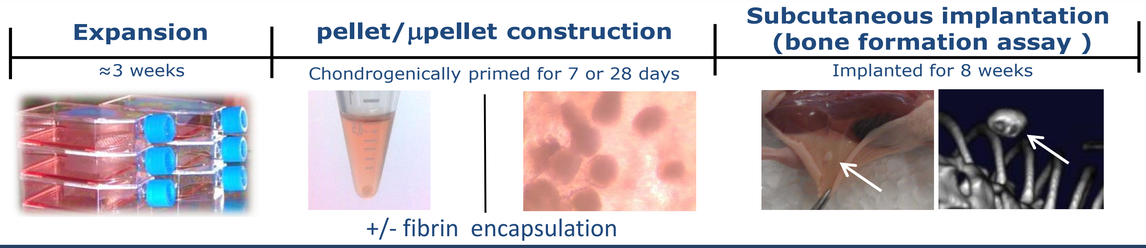
## AIMS

- **To determine if a shorter *in vitro* priming time could be utilised to achieve *in vivo* bone formation**
- **To determine if novel μpellet construct are capable of bone formation *in vivo***

## MATERIALS & METHODS

- **MSC culture-MSCs isolated from bone marrow aspirate were expanded in αMEM containing 10% serum and FGF2**
- **Pellet generation-2x10$^5$ MSCs were suspended in 500μL chondrogenic medium (serum free high glucose DMEM containing TGFβ-3), spun at 200g for 8 minutes**
- **μpellet generation-3x10$^6$ MSCs were seeded in chondrogenic medium to Aggrewell™ 800 plate, spun at 500g for 10 minutes (≈300 μpellets)**
- **Fibrin encapsulation-≈60 μpellets or 3 standard pellets were suspended in 40mg/mL fibrinogen, cross-linked with 1 IU thrombin per 2mg fibrinogen**
- **Analysis-*In vitro* samples analysed histologically and harvested for gene expression, *in vivo* samples analysed via μCT and histology. Linear mixed model with Bonferroni correction was used for statistic analysis**

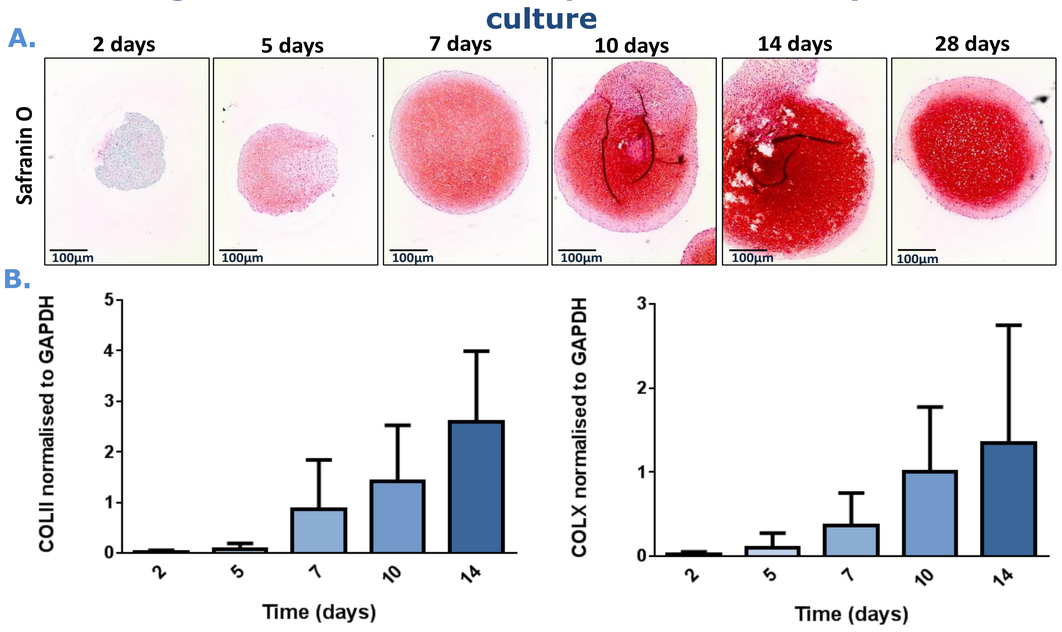
## *IN VIVO* EXPERIMENTAL TIME LINE




## RESULTS

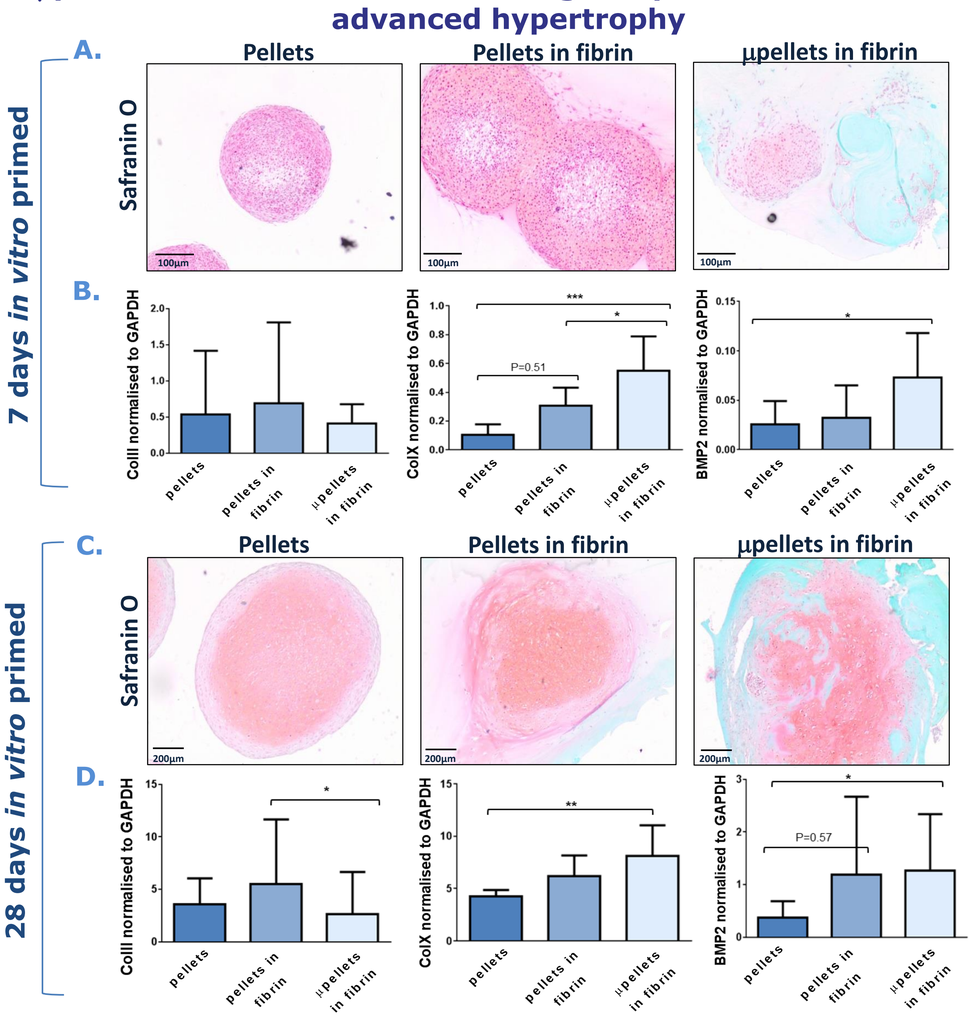
### *In vitro* results

**Chondrogenic characteristics are present after 7 days of *in vitro* culture**




**1A.** Safranin O (SafO) showing glycosaminoglycans (GAGs) are present after 5 days of chondrogenic priming and increases over time. **1B.** COLII and COLX expression increases after 7 days of priming (n=4).

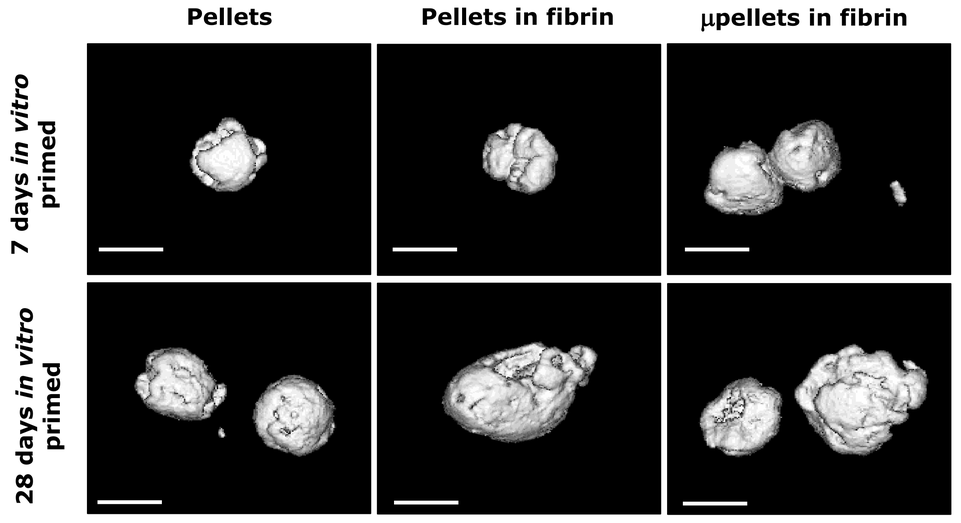
**μpellet constructs retain chondrogenic potential and exhibit advanced hypertrophy**




**2.** SafO showing similar GAG presence between constructs at 7 (**A**) or 28 (**C**) days of *in vitro* priming. COLII expression is maintained between constructs, however, ColX and BMP2 expression after 7 (**B**) or 28 (**D**) days of priming is upregulated in μpellet constructs. (Linear mixed model; Bonferroni correction, *P<0.05, **P<0.005, ***P<0.001 n=3)

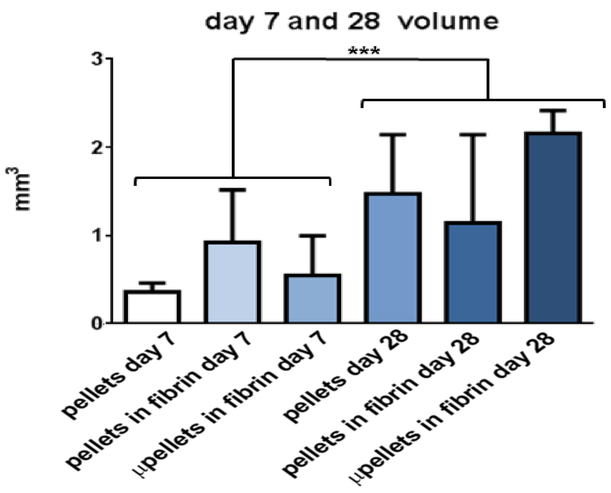
### *In vivo* results

**μpellet constructs produce similar quantities of calcified tissue to standard pellets**



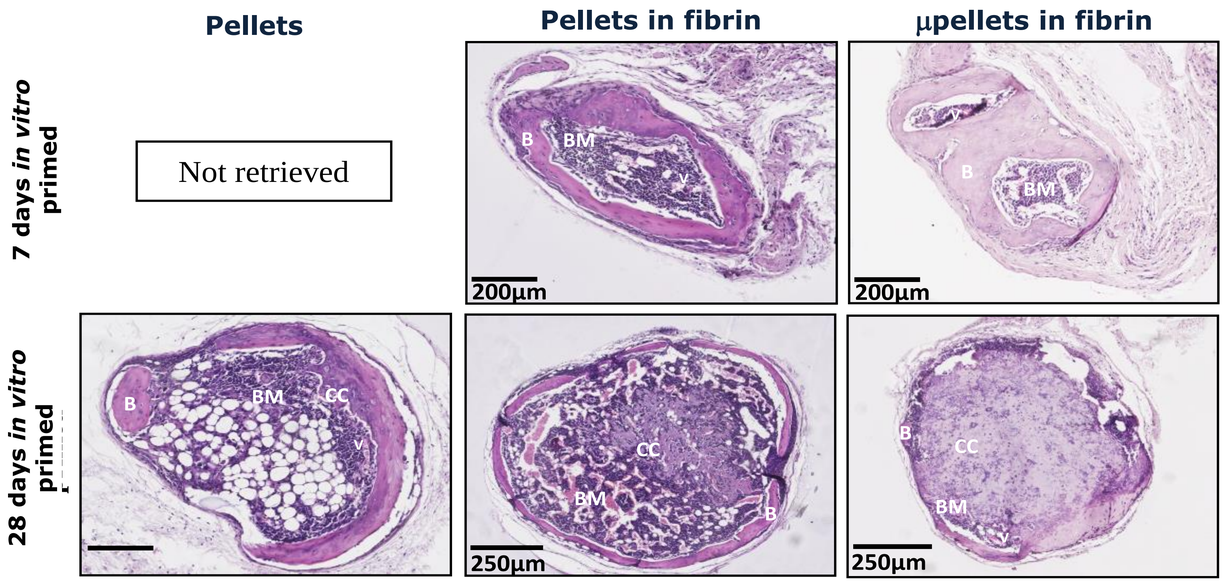

**3.** μCT of pellets, pellets in fibrin, or μpellets in fibrin after 7 (top row) or 28 (bottom row) of *in vitro* priming following 8 weeks implantation all showing areas of calcification (white bar for scale). Bridging is observed between individual μpellets.

**Priming time influences quantity of calcification formed *in vivo***




**4.** μCT of pellets, pellets in fibrin, or μpellets in fibrin quantified via conversion of original measurements, via linear transformation, to Hounsfield units applying global thresholding. Longer *in vitro* priming resulted in greater calcification (Linear mixed model; Bonferroni correction, *P<0.05, **P<0.005, ***P<0.001 n=9).

**Bone formation is achievable after only 7 days of *in vitro* culture**




**5.** H&E of pellets, pellets in fibrin, or μpellets in fibrin after 7 (top row) or 28 (bottom row) of *in vitro* priming following 8 weeks implantation (B=bone, BM= bone marrow, CC= calcified cartilage).

## DISCUSSION & CONCLUSION

- **μpellet constructs retain a similar chondrogenic potential to standard pellets, displaying signs of early hypertrophy**
- **7 days of *in vitro* chondrogenic priming is sufficient for bone formation *in vivo***
- **μpellets, due to their small size, could be optimised as an injectable therapy**
- **Given the shorter priming time coupled with injectability, this approach could offer promise for a minimally invasive therapy to replace some autologous bone transplantation procedures.**

## REFERENCES

1. Amini, AR. Critical reviews in biomedical engineering. 2012.
2. Farrell, E. Tissue Engineering Part C Methods. 2009.
3. Scotti, C. Proceedings of the National Academy of Sciences. 2010.

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**The authors declare no conflicts of interest.**