Manipulating time and space to achieve endochondral bone formation

C.A. Knuth[1], J. Witte-Bouma[1], R.Y. Ridwan[2], E.B. Wolvius[1], E. Farrell [1]

[1]Department of Oral and Maxillofacial Surgery, Special Dental Care and Orthodontics, Erasmus University Medical Center, Rotterdam , Netherlands

[2]Department of Genetics, Cancer Genomics Centre, Erasmus University Medical Center, Rotterdam, Netherlands

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.**

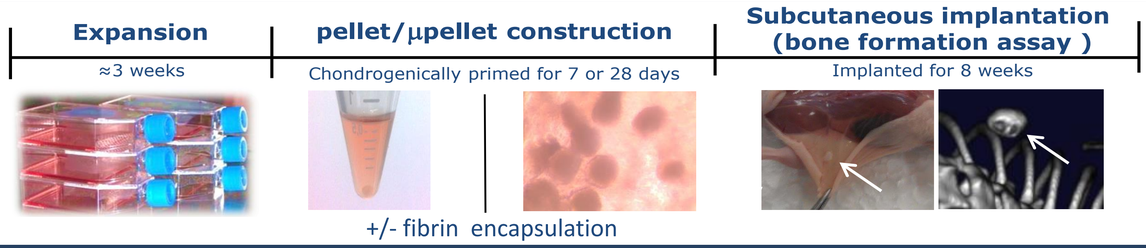
AIMS

- **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**

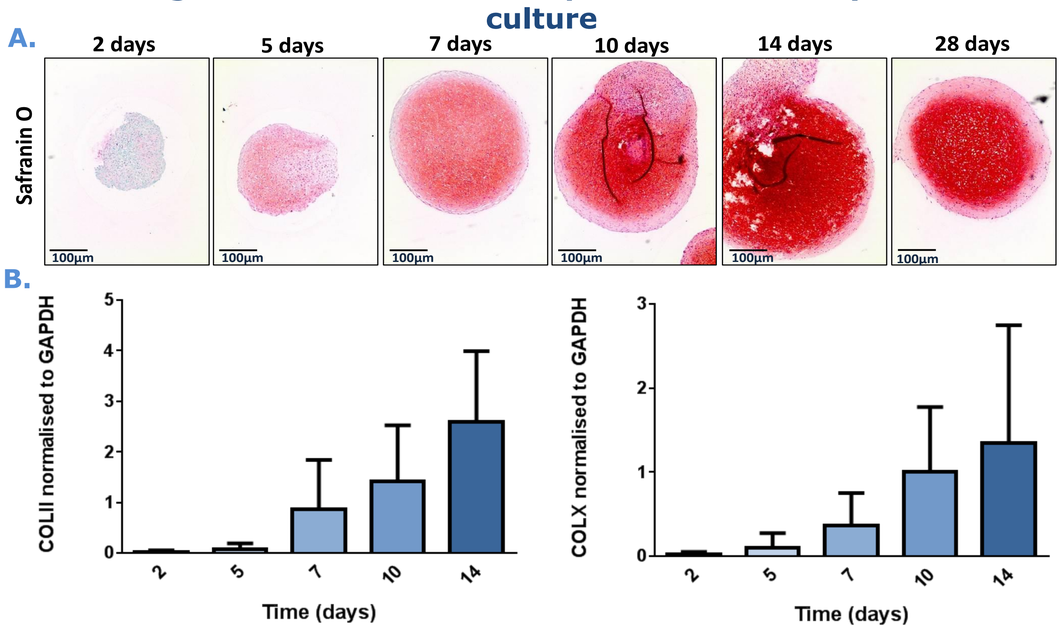
IN VIVO EXPERIMENTAL TIME LINE



RESULTS

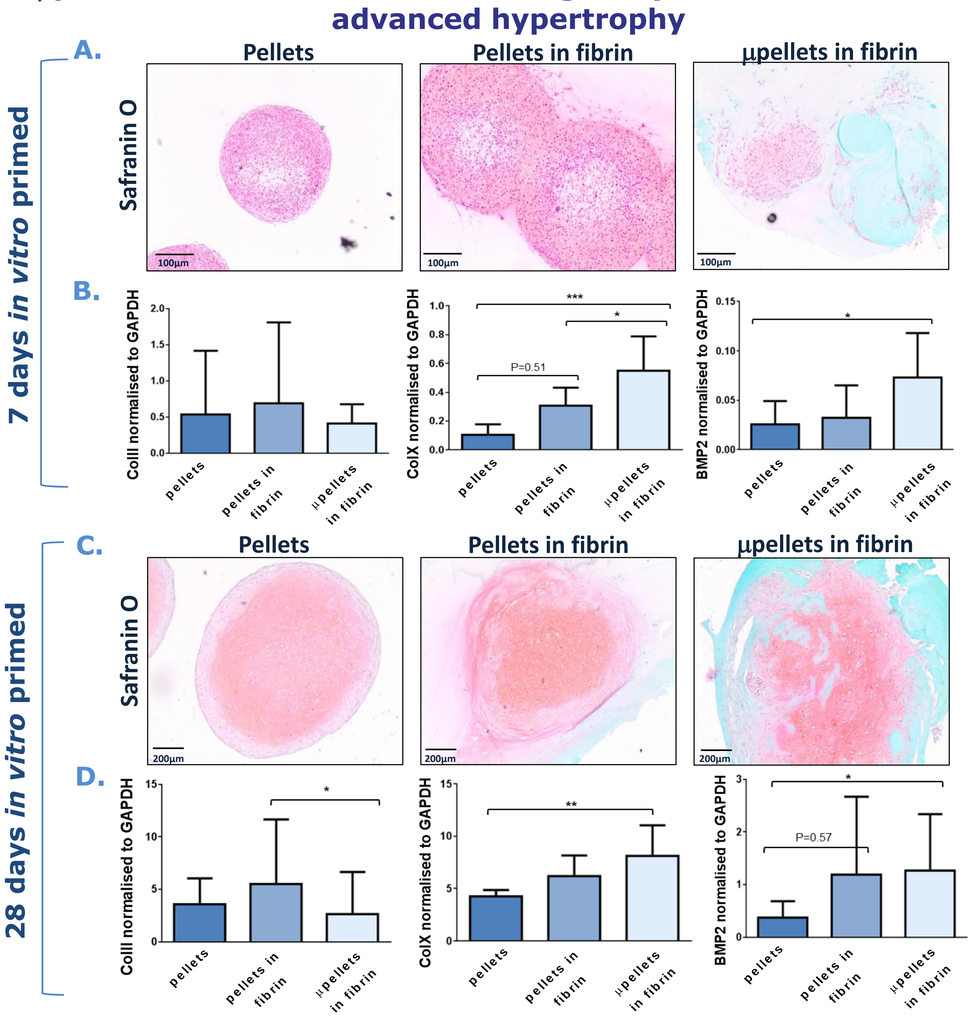
In vitro results

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).

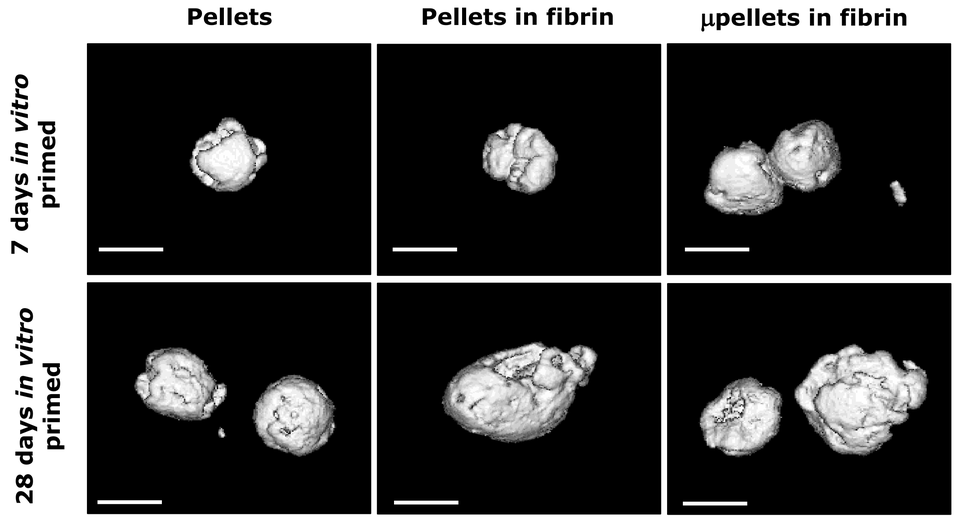
μpellet constructs retain chondrogenic potential and exhibit advanced hypertrophy



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)

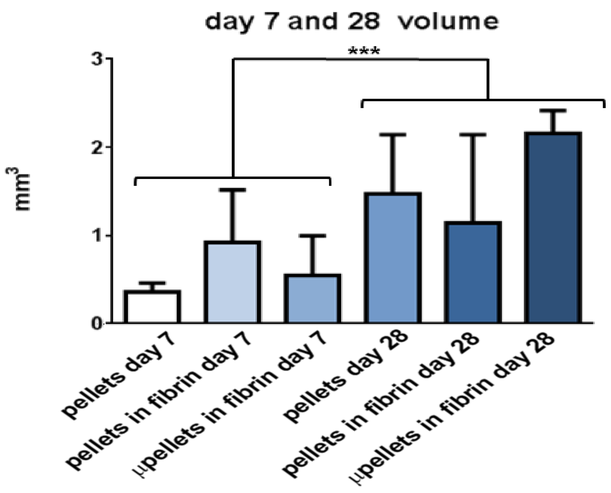
In vivo results

μpellet constructs produce similar quantities of calcified tissue to standard pellets



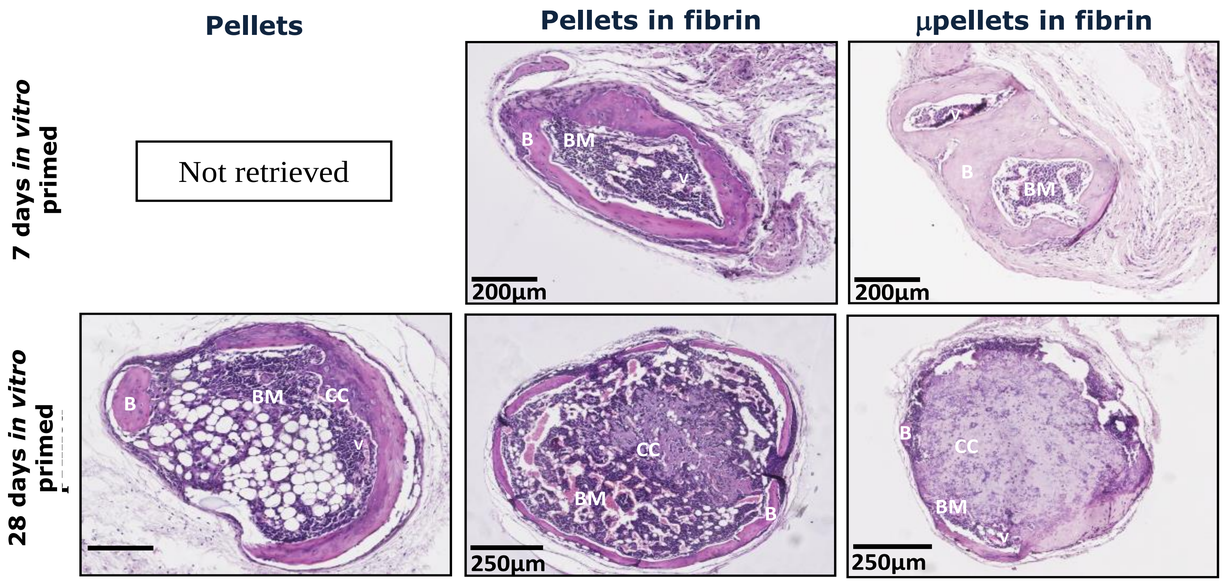
3. μCT of pellets, pellets in fibrin, or μpellets in fibrin after 7 (top row) or 28 (bottom row) of *in vitro* priming following 8 weeks implantation all showing areas of calcification (white bar for scale). Bridging is observed between individual μpellets.

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.

ACKNOWLEDGMENTS AND CONTACT

We would like to acknowledge Dr. Erwin Waarsing (Orthopaedics, Erasmus MC) for assistance with statistical analysis, and Nicole Kops (Orthopaedics) for assistance with animal model work.

Contact: Dr. Eric Farrell (e.farrell@erasmusmc.nl)

The authors declare no conflicts of interest.