

Stimulation with TGF- β 1 and BMP-12 Growth Factors induces tendon-specific markers in Adipose-Derived Stem cells (ADSCs) in serum-free culture conditions

Noelia Pilar D Falcon¹, Graham Riley² and Aram Saeed¹

¹School of Pharmacy – University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom,
²School of Biological Sciences - University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, United Kingdom



Background

- ✓ **Differentiation of stem cells** as a cell-based therapy is becoming increasingly attractive within the field of musculoskeletal tissue engineering.
- ✓ Recent studies focused on **the induction of tendon-specific markers** in cultured stem cells using different Growth Factors including Bone Morphogenetic Proteins and Transforming Growth Factor isoforms.

Relevance of serum-free media in tenogenic differentiation protocols

- ✓ The **inclusion of serum** in relatively high concentration is less favourable, since the components within serum may interfere with the induction of the markers
- ✓ Moreover, the use of serum represents a **limitation for the clinical applicability** of the cell-based therapies to human
- ✓ Therefore, *in vitro* studies with **low concentration or absence of serum** would be ideal

Viability of ADSCs

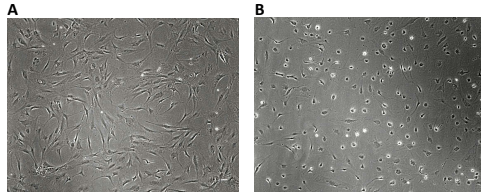


Figure 1. Image of ADSCs taken (A) 24 hours after culture in 10% FBS or (B) 24 hours after serum starvation. Scale bar is 100 μ m

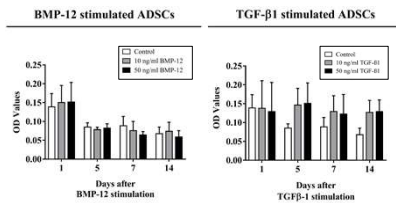


Figure 2. Metabolic activity of cultured ADSCs was analysed by MTS assay for over one, five, seven and fourteen days after stimulation with BMP-12 or TGF- β 1.

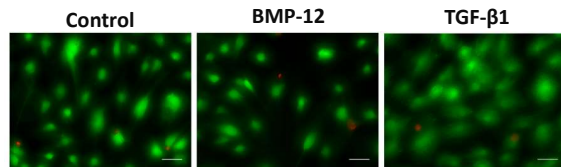


Figure 3. Live and dead assay in serum-free cultured ADSCs seven days after stimulation with BMP-12 or TGF- β 1. Scale bar is 50 μ m

Tenogenic and other tissue phenotypes gene expression

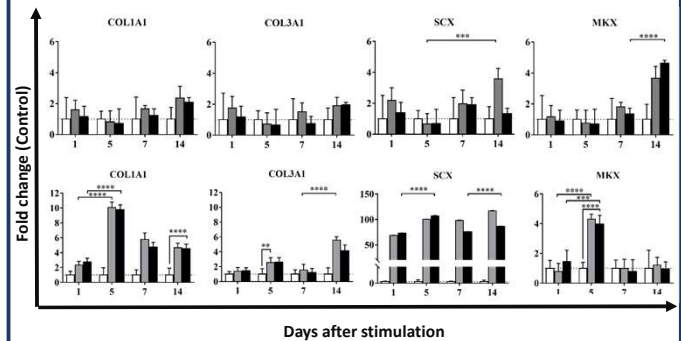


Figure 4. Characterisation of tenogenic induction in ADSCs stimulated with BMP-12 (top row) or TGF- β 1 (bottom row). White (control), grey (10 ng/ml), black (50 ng/ml)

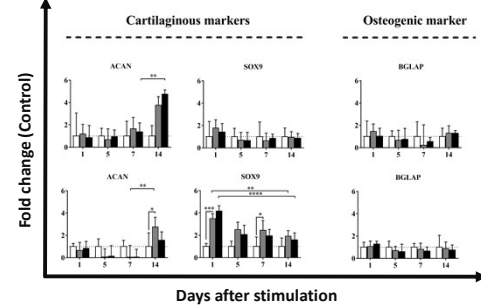


Figure 5. Cartilaginous and osteogenic induction in ADSCs stimulated with BMP-12 (top row) or TGF- β 1 (bottom row). White (control), grey (10 ng/ml), black (50 ng/ml)

Collagen type I and Scleraxis protein expression

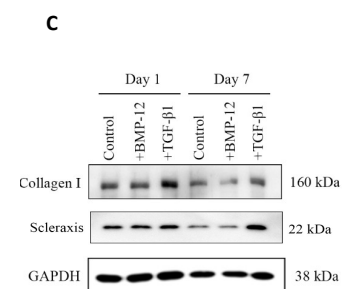
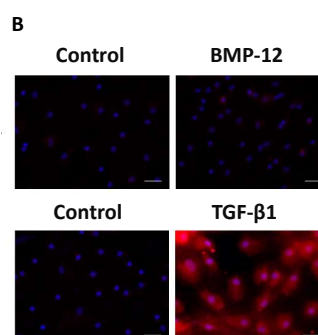
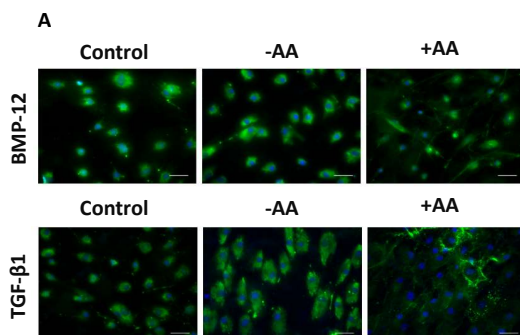


Figure 6. Immunocytochemical and western blot analysis of Collagen I and Scleraxis expression. (A) Collagen I with or without Ascorbic Acid (AA) or (B) Scleraxis Immunolocalisation in permeabilised ADSCs in serum-free media seven days after stimulation with BMP-12 or TGF- β 1. Scale bar is 50 μ m. In (C) Western blotting analysis of Collagen I or Scleraxis in ADSCs in serum-free media one and seven days after stimulation with BMP-12 or TGF- β 1

Conclusions

- ✓ BMP-12 induces a **generally late expression** of the selected tendon markers whereas TGF- β 1 induced their earlier expression.
- ✓ **Scleraxis protein expression** displays notable differences between BMP-12 and TGF- β 1. The addition of **Ascorbic Acid (AA)** resulted in increased deposition of Collagen I.
- ✓ Our results **enhance the existing protocols for the differentiation of ADSCs** towards the tenogenic lineage in serum-free conditions and contribute to the understanding and the development of tenogenic induction protocols.

References

Noelia D. Falcon, Graham P. Riley, and Aram Saeed. *Tissue Engineering Part C: Methods*. Jul 2019:389-400. <http://doi.org/10.1089/ten.tec.2019.0080>
Marta Bottagisio et al. Different combinations of growth factors for the tenogenic differentiation of bone marrow mesenchymal stem cells in monolayer culture and in fibrin-based three-dimensional constructs. *Differentiation* (2017).

Acknowledgments

