The engineering of scaffold-free cartilage using microtissues generated under altered growth factor stimulation regimes

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Disclosures: Not applicable

INTRODUCTION

Scaffold-free tissue engineering strategies using cellular aggregates, microtissues or organoids as 'biological building blocks' could potentially be used for the engineering of scaled-up articular cartilage grafts. However, little is known about how different chondrogenic growth factor stimulation regimes during cellular expansion and differentiation influence the capacity of cellular aggregates or microtissues to fuse and generate functional cartilage. In this study human bone marrow mesenchymal stem/stromal cells (MSCs) were additionally stimulated with TGF-β1 +/- BMP-2 during monolayer expansion and differentiation, and their capacity to undergo chondrogenesis in pellet culture and microtissue format was assessed over 3 weeks of culture.

METHODS

Human bone marrow MSCs were expanded in culture under 1) regular expansion media composed of DMEM, P/S, FBS and FGF-2; 2) regular media + TGF-β1; 3) regular media + TGF-β1 + BMP-2. For the pellet culture and microtissues system, a traditional TGF-β3 supplemented chondrogenic media (CDM) was prepared as described previously (Burdis et al., 2022) and additionally supplemented with (or without) BMP-2. The MSC derived microtissues were fabricated as described previously (Nulty, Burdis and Kelly, 2021 and Burdis et al., 2022) and cultured for 21 days at 5 % O_2. After 21 days, the MSC derived microtissues were harvested and histological and biochemical analysis were carried out to evaluate chondrogenesis.

RESULTS

MSCs displayed a higher proliferative potential during expansion with TGF-β1 or TGFβ1 + BMP-2. Next, the chondrogenic potential of the human MSCs was explored in pellet and in high throughput microtissues systems. After 3 weeks of culture, MSCs stimulated with BMP-2 during expansion and differentiation deposited higher levels of GAGs and collagen in both the pellet and microtissues systems; they also stained negative for calcium deposits. In addition, there was negative staining for collagen X in all the groups. When the fusion capacity of the microtissues was investigated, it was observed that all the groups were completely fused after 24h and were negative for calcium deposition (Figure 1). After 3 weeks of culture, it was observed that MSCs stimulated with TGF-β1 during expansion and additionally with BMP-2 during chondrogenic differentiation deposited the highest levels of sGAG. Furthermore, there was negative staining for collagen X in all the groups.

DISCUSSION & CONCLUSIONS

The main findings of this study are: (1) stimulation with TGF-β1 and TGFβ1 + BMP-2 during monolayer expansion positively impacts the proliferation of human MSCs; (2) in the pellet and microtissues system, superior chondrogenesis was observed following stimulation with BMP-2 during both the expansion and differentiation phases; (3) microtissues maintain their fusion capacity in all cases. This study demonstrates the importance of carefully optimizing MSC expansion and differentiation conditions when developing modular tissue engineering strategies using cellular aggregates or microtissues.

SIGNIFICANCE/CLINICAL RELEVANCE

In this work, we explored the chondrogenic differentiation potential of human bone marrow MSC derived microtissues under different growth factor expansion and differentiation regimes. As human bone marrow MSCs are used in numerous different tissue engineering and regenerative medicine applications, it is vital to identify high-throughput methods capable of generating large number of cells with robust differentiation potential. Furthermore, the chondrogenic growth factor stimulation regimes identified in this study supports the development of superior cartilage grafts, which may lead to better articular cartilage regeneration outcomes in the future.

REFERENCES

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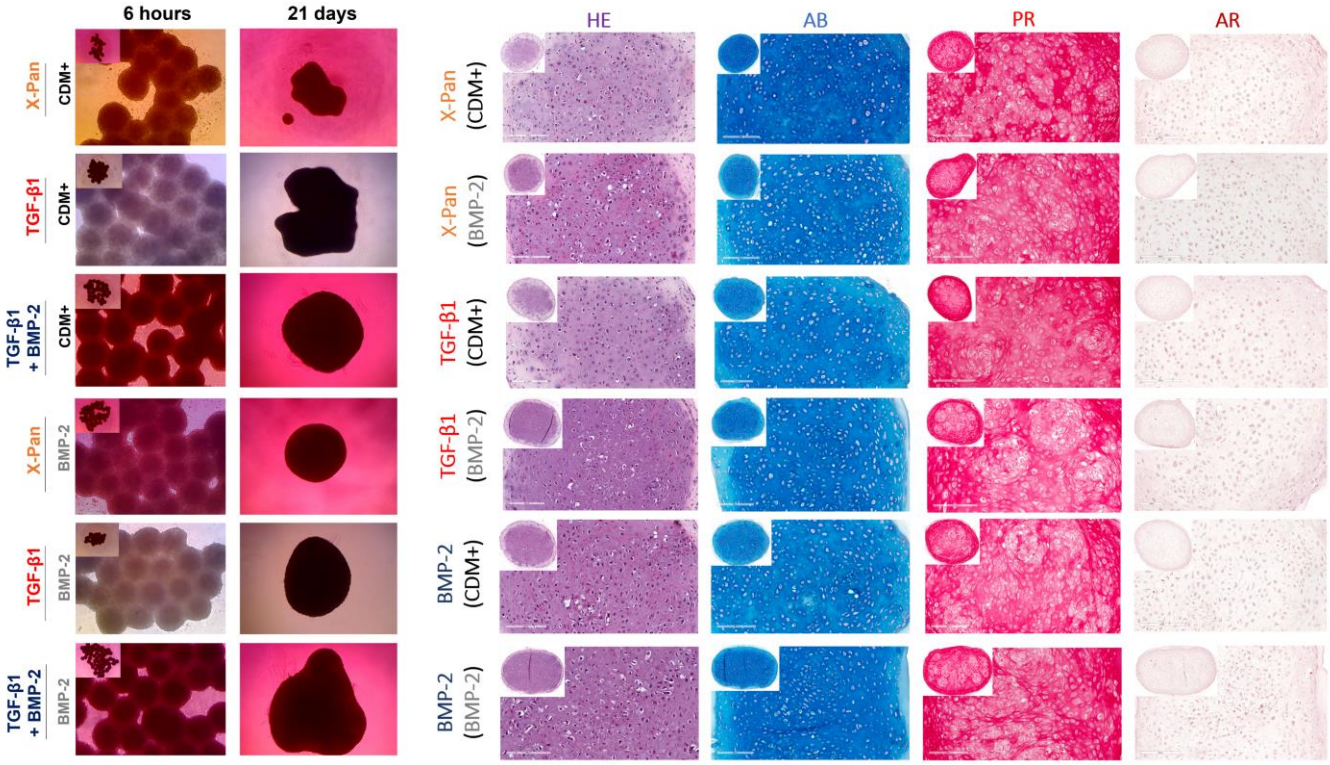

Figure 1: Fused microtissues are negative for calcium deposition under altered growth factor stimulation regimes. Phase contrast of fused microtissues at 6h and 21 days cultured under altered growth factor stimulation regimes. Hematoxylin and Eosin (HE), Alcian Blue (AB), Picrosirius red (PR) and Alizarin Red (AR) stains of fused microtissues induced in regular chondrogenic media (CDM+) and CDM+ with BMP-2 at day 21 of culture. The human bmMSCs were previously expanded in regular expansion media (X-Pan), expansion media + TGF-β1 (TGF-β1) and expansion media with TGF-β1 + BMP-2. Scale bars in histological sections: 200µm.