Transforming Growth Factor-Beta (TGF-β) Responsive Cells in Human Periosteum Explants Regulate Cartilage Differentiation and Typing

Richard H Liu, Caleb Clemens Witsken, Weijia Zhou, KATHY B CAO, Cade Hines, Caroline L Keller, Lorenzo D Deveza

INTRODUCTION:

Degenerative cartilage conditions are among the most common health conditions, with diseases like osteoarthritis alone affecting almost a quarter of US adults. Yet, current regenerative therapies are limited by poor repair capacity and the formation of biomechanically inferior fibrocartilage. The periosteum serves as a potent reservoir of mesenchymal stem cells with remarkable chondrogenic potential, most notably demonstrated during bone repair where it critically supplies the mesenchymal progenitor cells essential for regeneration. Yet, the molecular signals that regulate their differentiation and the formation of differential cartilage types still remain poorly understood. Thus, advancing understanding of how this potential is promoted and optimized can hold profound implications for regenerative cartilage therapies and beyond. Our present work aims to clarify these unresolved areas by evaluating how TGF-β1 and TGF-β3, under serum and serum-free conditions, influence the chondrogenic potential of human periosteum-derived cells and guide cartilage typing toward hyaline or fibrocartilage phenotypes.

METHODS:

Human periosteum explants were cultured in six conditions to assess chondrogenic potential: basal chondrogenic media (BM), BM + TGF-β1, and BM + TGF-β3, each with (+) and without (-) fetal bovine serum (FBS). After 49 days, the explants were taken out of culture and were prepared for further analysis. Histological staining with H&E, Alcian Blue, and Safranin O staining was performed for both day 0 and day 49 time points, and subsequent analysis was performed via light microscopy. Stain intensity quantification of these slides using ImageJ then allowed for numerical comparison of cartilage formation across conditions. Further immunohistochemical analysis was also performed to reveal collagen typing results. Flow cytometry was conducted using fluorophore-conjugated antibodies and spectral analysis to assess the expression of TGF-β receptors (R1 and R2) and mesenchymal stem cell markers.

RESULTS:

TGF-β1 and TGF-β3 both significantly enhanced chondrogenesis in human periosteal explants, with the most robust effects observed under serum-free conditions. In contrast, explants cultured in basal media alone - regardless of serum presence - exhibited minimal cartilage formation. Tissues treated with either TGF-β isoform in the absence of FBS displayed stronger chondrogenesis, as evidenced by more intense Alcian Blue and Safranin O staining.

On morphological analysis, these serum-free samples formed more amorphous cartilage, with randomly positioned, sparser nuclei - reminiscent of hyaline cartilage. Conversely, explants exposed to TGF-β in the presence of FBS showed reduced matrix staining with a more linearly arranged, dense nuclei distribution - more reminiscent of fibrocartilage. These structural observations were bolstered by immunohistochemical analysis. Type II collagen - more associated with hyaline cartilage - was more abundantly expressed in serum-free conditions, whereas type I collagen - more associated with fibrocartilage - was more predominant in serum-present conditions.

Finally, analysis with flow cytometry revealed that TGF-β receptors I and II were strongly coexpressed in periosteum-derived cells, and also exhibited high association with populations positive for established mesenchymal stem cell (MSC) markers (CD271, CD105, CD200). These findings suggest the presence of a TGF-β-responsive chondroprogenitor subset within the periosteum. Taken together, these findings underscore the importance of TGF-β signaling in periosteal chondrogenesis and demonstrate how culture conditions may be leveraged to optimize both the extent of cartilage formation and its phenotypic identity.

DISCUSSION AND CONCLUSION:

Our findings demonstrate that TGF-β1 and TGF-β3 promote chondrogenesis in human periosteal explants, with heightened formation of hyaline-like cartilage in serum-free conditions. These findings illuminate the importance of the growth environment in shaping not only the quantity but also the quality of regenerated cartilage. Furthermore, understanding the interplay between growth factors and serum components may be critical for optimizing regenerative strategies and therapies, particularly in applications where hyaline cartilage is preferred, such as joint surface repairs. Ongoing work will leverage flow cytometry to characterize TGF-β responsive periosteal subpopulations, with the long-term goals of enabling targeted, cell-based therapies, advancing current cartilage regeneration therapies, and opening new avenues for tissue engineering and beyond.

Figure 1: Histological staining shows evidence of cartilage formation and chondrocyte differentiation in TGF-β environments

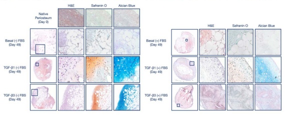

Figure 2: Color saturation analysis shows improved cartilage formation in serum-free TGF-β conditions

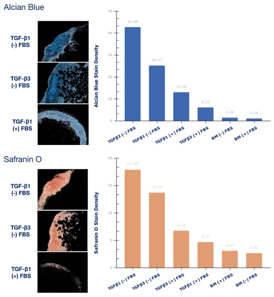

Figure 3: Collagen immunostaining demonstrates the formation of type II and type I collagen

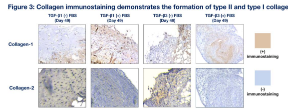

Figure 4: TGF-β responsive populations show strong overlap with MSC populations