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P343 Adipose-derived mesenchymal stem cells cultured in tenogenic serum-free medium express tendon-specific markers

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Tendon injuries are common and present a clinical challenge, as they often respond poorly

to treatment and result in long-term functional impairment. Poor tendon healing responses are mainly attributed to insufficient or failed tenogenesis. For optimal treatment, enhanced understanding of tendon physiology is necessary. Among others, growth factors (GFs) and cytokines modulate the differentiation of tendons during embryogenesis and the healing process of injured tendons. Cell-based therapy using mesenchymal stem cells (MSCs) in combination with GFs and biomaterials seems to be the most promising approach to heal tendon injuries. Adipose-derived MSCs (ASCs) are multipotent and immunoprivileged, making them ideal candidates for therapeutic purposes. Moreover, providing safe and regulated cell therapy products to patients requires adherence to good manufacturing practices (GMP), and GMP guidelines should be adhered to throughout the process of isolating, expanding and differentiating MSCs. For these reasons, the aims of this study were: i) to investigate the effect of several GFs already known to be involved in tendon development/healing process on human ASCs proliferation and expression of tendon-related markers; ii) to develop a tenogenic GMP-compliant serum free medium. Subcutaneous fat was obtained from 5 healthy donors by lipoaspiration, after written consent. Primary cultures of the stromal vascular fraction were established and characterized by flow cytometry analysis to evaluate cell viability (7AAD(-) and SYTO 40(+) expression), and ASC surface marker expression (CD45(-), CD146(-) and CD34(+)) and then cryopreserved. After thawing, ASCs were expanded until P3 culturing in a commercial human platelet lysate-supplemented culture medium (hPL) or in a well-defined serum free medium (SF) developed in our laboratories. At P4, tenogenic induction was performed: ASCs were cultured in 6-well plates coated with the tendon matrix protein type-I collagen and in tenogenic medium (TENO) consisting in hPL or SF medium supplemented with 100ng/ml CTGF, 10ng/ml TGFβ3, 50ng/ml BMP12 and 50µg/ml Ascorbic acid (AA) for 1, 3, 7 and 14 days. Cells cultured without any supplementations at the same time points were used as control (CTRL). Morphological appearance (optical microscopy), cell proliferation (lactate assay), gene (RT-PCR) and protein (immunofluorescence, SIRIUS-RED staining) expression were performed in all groups at all time points. Both SF-TENO and hPL-TENO cells appeared more rounded and with more cytoplasmic content and proliferated faster than respective CTRL. Tendon-marker genes (*SCX*, *COL1A1*, *COL3A1, TNC, MMP3*, *MMP13)* were significantly upregulated already after 3 to 14 days of differentiation in respect to CTRL without any significant differences between hPL and SF groups. In the meantime, stem cell gene (*KLF4, NANOG, OCT4*) expression decreased in TENO cells vs CTRL. SCX protein expression and the increase of collagen-matrix deposition were also observed in all TENO cells vs CTRL. These results demonstrate that ASCs possess tenogenic differentiation ability when exposed to CTGF, BMP12, TGFb3 and AA in both hPL and SF medium providing insights of the earliest events of tendon development and move forward the GMP-compliant approaches needed for cell-therapy strategies.