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TENOGENIC DIFFERENTIATION PROTOCOL IN XENOGENIC-FREE MEDIA ENHANCES TENDON-RELATED MARKER EXPRESSION IN ADIPOSE-DERIVED STEM CELLS

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Tendon injuries are common and current therapies often are unsuccessful. Cell-based therapy using mesenchymal stem cells (MSCs) seems to be the most promising approach to heal tendon. Moreover, providing safe and regulated cell therapy products to patients requires adherence to good manufacturing practices (GMP). Adipose-derived stem cells (n=4) were cultured in 6-well plates coated with type-I collagen in a chemically defined serum-free medium (SF) or a xenogenic-free human pooled platelet lysate medium (hPL). At passage 4, ASCs were induced to tendon lineage for 14 days using 100ng/ml CTGF, 10ng/ml TGFβ3, 50ng/ml BMP12 and 50μg/ml ascorbic acid in the SF (SF-TENO) or in the hPL (hPL-TENO) medium. Cells cultured without any supplements are used as control. Morphological appearance, cell viability and FACS were performed in undifferentiated cells to evaluate the xenogenic-free culture conditions; the gene and protein expression were performed by RT-PCR and immunofluorescence to evaluate to expression of stem cell- and tendon-related markers upon cell differentiation. SF-CTRL and hPL-CTRL showed similar viability and MSC's surface proteins and expressed the stemness markers NANOG, OCT4 and Ki67. Moreover, both SF-TENO and hPL-TENO expressed significant higher levels of SCX, COL1A1, COL3A1, COMP, MMP3 and MMP13 genes already at 3d ($p<0.05$) respect to CTRLs. Scleraxis and collagen were also detected in both SF-TENO and hPL-TENO at protein level in higher amount than CTRLs. In conclusion, ASCs exposed to CTGF, BMP12, TGFb3 and AA in both serum and xenogenic-free media possess similar tenogenic differentiation ability moving forward the GMP-compliant approaches for the clinical use of ASCs.

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