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Published in:
European Cells & Materials

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

P397 The use of pooled human platelet lysate for isolation and *ex vivo* expansion of skeletal myoblasts for clinical use

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Introduction: *Ex vivo*-expanded autologous myoblasts are being used in clinical trials for the treatment of various skeletal muscle conditions, including stress urinary incontinence caused by urethral sphincter damage. The use of fetal calf serum (FCS) as a growth media supplement in these procedures still raises various technical and ethical concerns. As an alternative to xenogeneic supplements, human platelet lysate (hPL) has recently gained increasing attention for *ex vivo* expansion of mesenchymal stem cells in clinical studies. However, little is known regarding the ability of hPL to support *ex vivo* expansion of skeletal myogenic precursors.

Aim: The aim of this study was to assess the performance of hPL for the isolation and expansion of human skeletal myoblasts in comparison to FCS.

Materials and methods: Skeletal myoblasts were isolated and expanded in a commercial medium formulation (MyoTonic, Cook Myosite) supplemented with either 10% FCS or 5% pooled hPL (Stemulate, Cook Regentec). Cells were assessed during multiple passage expansion by analysis of cell proliferation, cellular phenotype, and gene expression profiles. The ability of media to preserve the differentiation capacity of the cells was evaluated in cells from early and late passages by immunocytochemistry and qRT-PCR after induction with MyoTonic medium supplemented with 2% horse serum.

Results: Analysis of cell proliferation over five passages revealed that myoblasts cultured in hPL-supplemented medium displayed a significantly higher cumulative cell number over time, due to consistently larger growth rate. Cells grown in hPL media evidenced a lower activation during propagation, as revealed by a significantly lower transcription of the skeletal myogenic factors MYF5 and MYOD. The percentage of CD56 positive cells appeared to decrease over time with passaging irrespective of the media type, suggesting a progressive depletion of myogenic precursors over time. Nevertheless, the cells expanded in hPL evidenced a robust capacity for differentiation at early (P1) and late passages (P5), as demonstrated by the formation of myosin-rich mature myotubes displaying high levels of myogenin activation.

Conclusions: Our results suggest that the hPL supplemented medium supports isolation and efficient expansion of skeletal myoblasts while preserving their differentiation capacity. In perspective, hPL may represent an efficient alternative to FCS for the ex vivo expansion of myoblasts aimed for cell based therapies.