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

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Aim

Human platelet lysate (hPL) constitutes a clinically compliant supplement for the expansion of mesenchymal stem cells, representing an alternative to fetal calf serum (FCS)

The aim of this study was to assess the performance of hPL for the isolation and expansion of human skeletal myoblasts

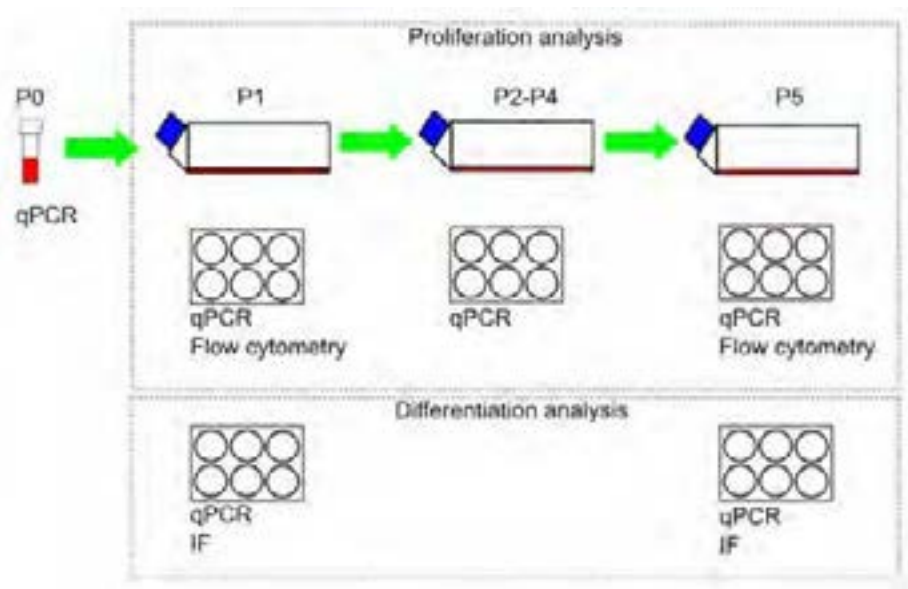

hPL

vs.


FCS

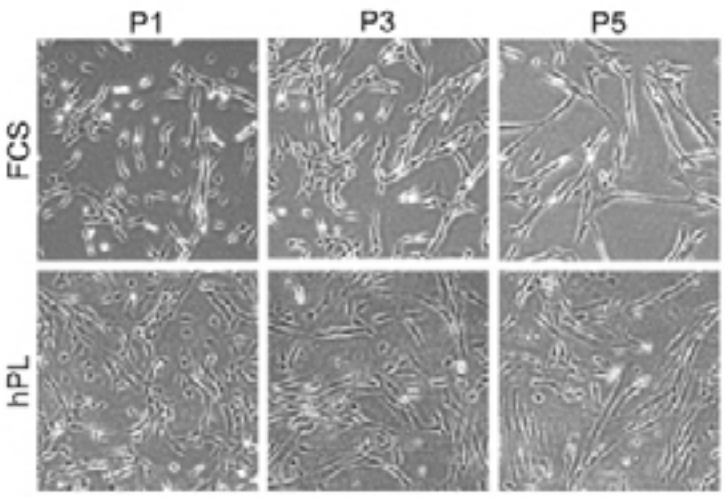
Methodology

- Human skeletal myoblasts were isolated and expanded in MyoTonic medium (Cook Myosite) supplemented with either 10% FCS or 5% pooled hPL (Stemulate™, Cook Regentec)
- Cell proliferation, cellular phenotype, and gene expression profiles were assessed over the course of 5 passages.
- Differentiation capacity was evaluated at passages 1 and 5 by immunofluorescence (IF) and qPCR

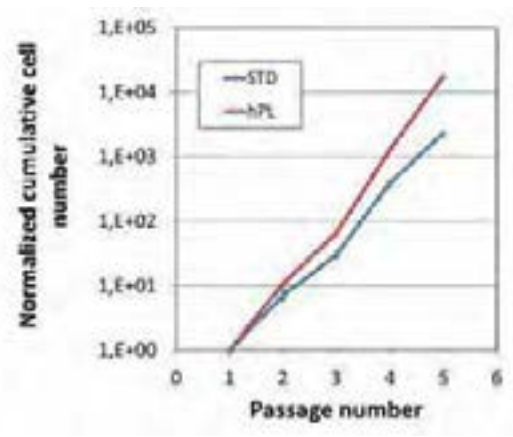


Results

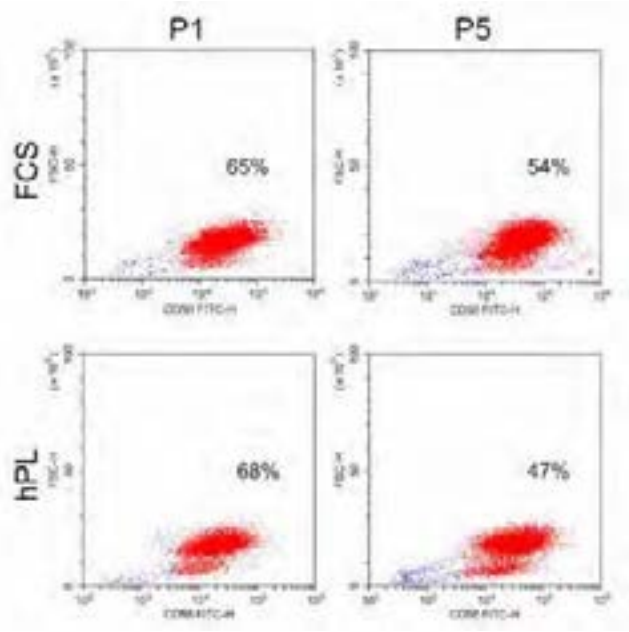
1. Passaging influences cell morphology



2. Significantly higher growth rate in hPL medium

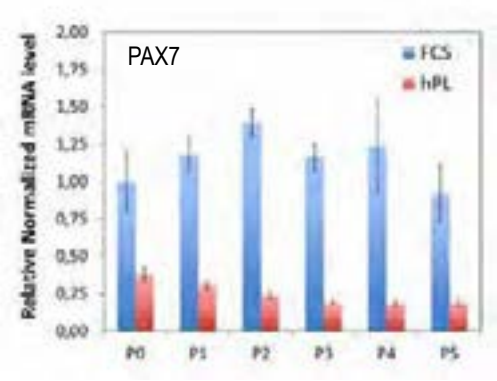


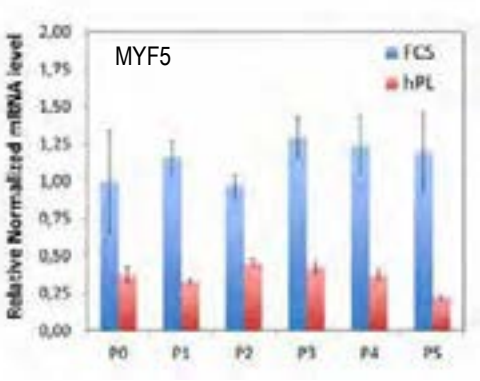
3. Similar decrease in CD56⁺ subpopulation over time

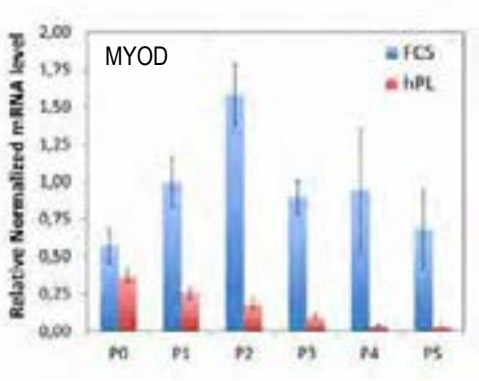


4. mRNA levels of myogenic transcription factors:

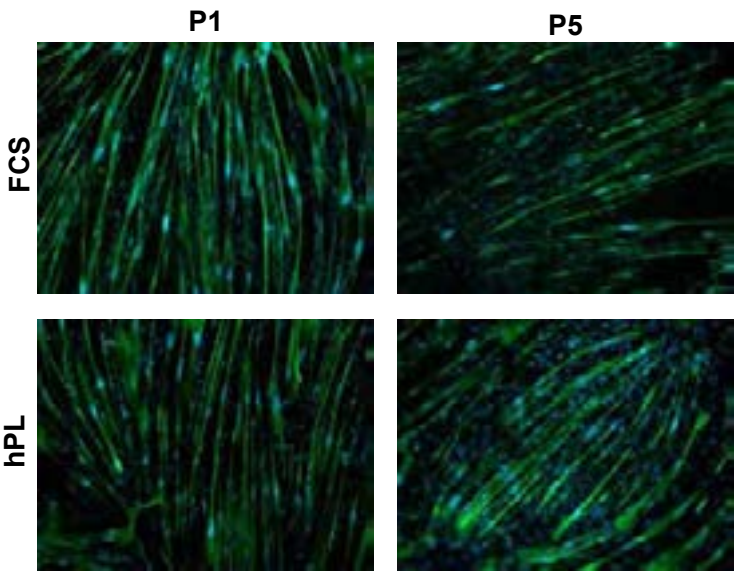
- Early genes PAX7 and Myf5 are stably expressed but displayed significant down regulation in hPL
- Intermediate gene MYOD displayed a down regulation over passaging







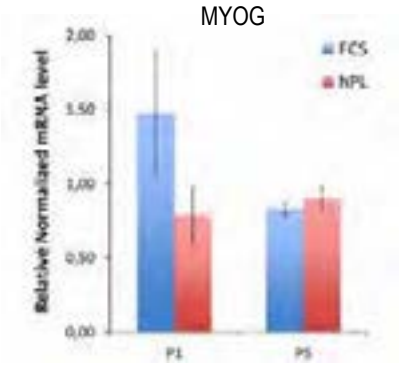
5. The differentiation capacity was preserved over passaging



MHC HOECHST

After 7 days in differentiation medium (MyoTonic+2% horse serum):

- Myosin (MHC) positive myotubes
- Preserved myogenin (MYOG) activation



Conclusions

- hPL supplemented medium efficiently supports isolation and expansion of human skeletal myoblasts
- hPL appears to maintain a lower expression level of the early myogenic differentiation genes
- Although it appears that CD56+ progenitors are being depleted by passaging, the differentiation capacity is preserved in hPL

Take home message

hPL may represent an efficient alternative to FCS for the *ex vivo* expansion of myoblasts aimed for cell based therapies.

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