A Serum-Free Workflow for the Isolation, Expansion and Differentiation of Human Myogenic Progenitors

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Introduction

In vitro culture of human skeletal muscle myogenic progenitors (hSKM MPs) is a useful tool for modelling skeletal muscle biology and disease, and for drug screening. hSKM MPs also hold potential for cell based therapies. Traditional culturing methods have relied on undefined serum-containing media to support the expansion and differentiation of MPs. A common challenge in the field, however, is to identify serum-free (SF) *in vitro* conditions that support reproducible, optimal expansion of these cells whilst maintaining their undifferentiated state. To address this issue, we have developed a defined (MyoCult™-SF Expansion Medium), serum-free culture medium that supports the derivation, expansion and differentiation of MPs from human skeletal muscle tissue.

Methods

Myogenic progenitor cultures were derived from human skeletal muscle tissue following enzymatic digestions with collagenase and dispase. Derivation of hSKM MPs was assessed by Pax7 immunostaining and EdU incorporation (12 h pulse) after 7 days in culture. Myoblasts were then purified using a column-free anti-CD56 immunomagnetic isolation kit (EasySep™). Long term expansion of CD56$^+$ MPs was assessed over 5 - 6 passages. Comparisons of MyoCult™-SF medium were made against 'Serum Medium' containing 20% FBS supplemented with 20 ng/mL bFGF. Myogenic differentiation was assessed by immuno-fluorescent staining using an anit-myosin (MF20) antibody, allowing quantification of fusion index.

Results

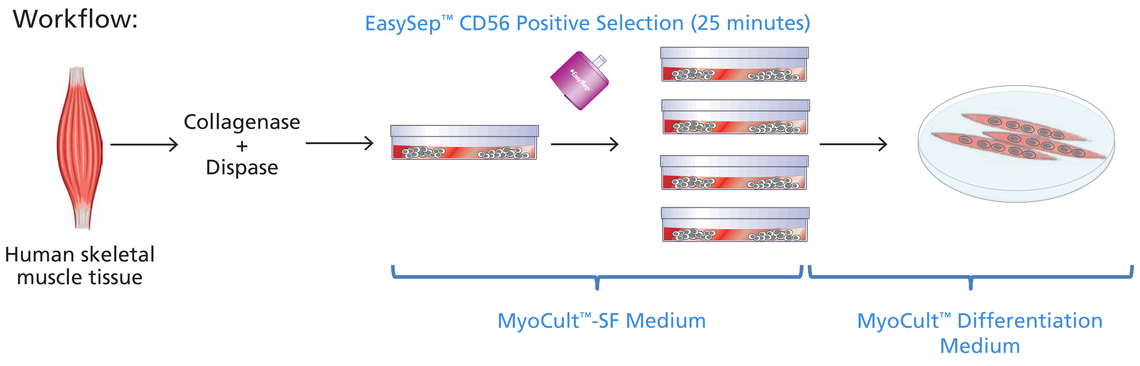
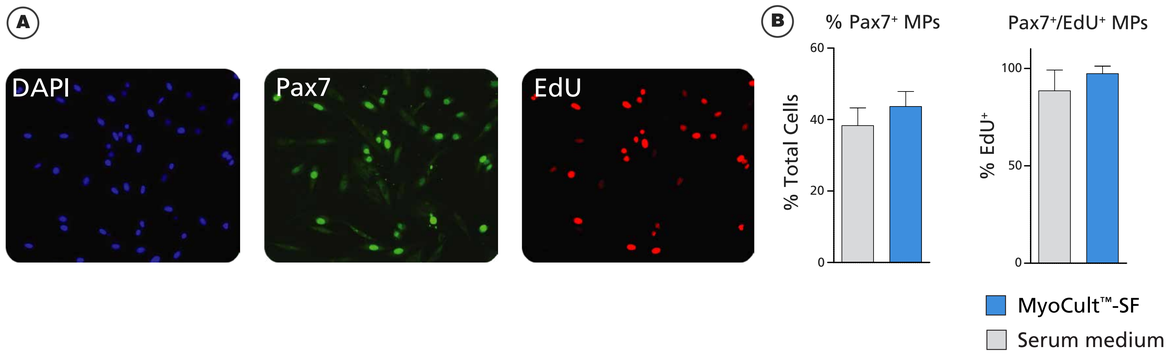
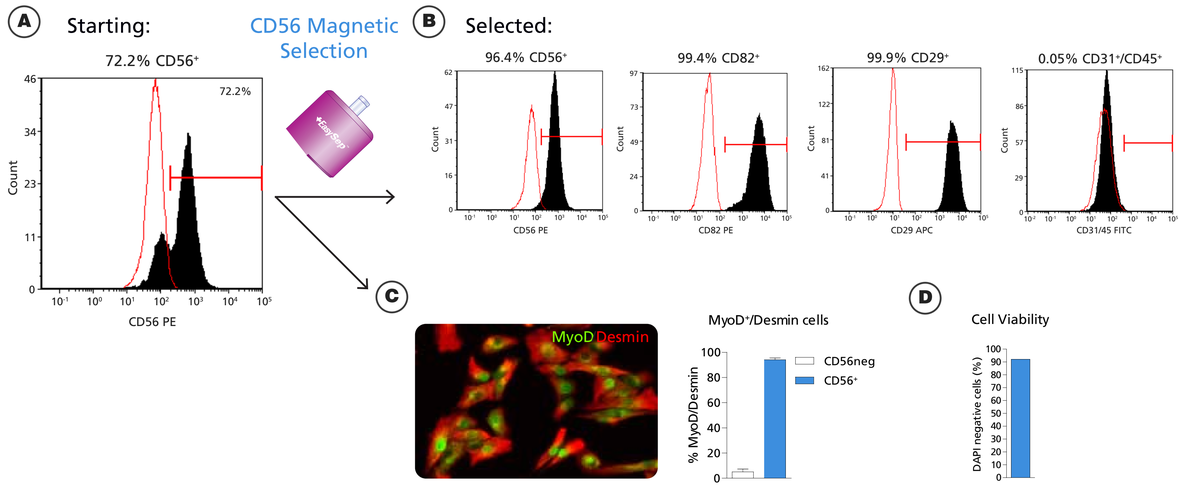


Figure 1. Identity and Proliferation of hSKM Cells Cultured for 7 days in MyoCult™-SF Medium



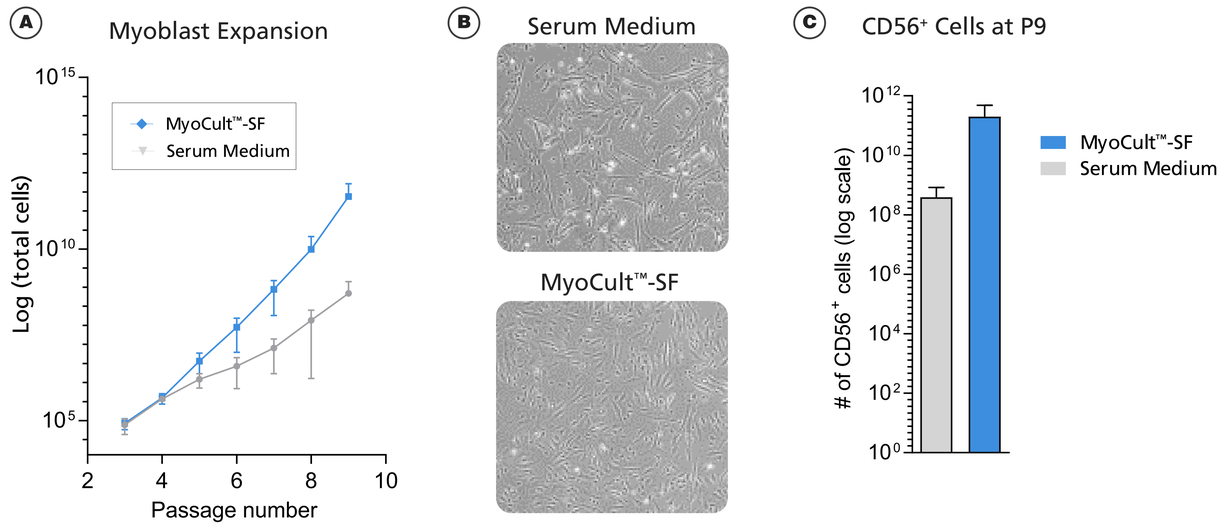
(A) Immuno-fluorescent images of Pax7 and EdU co-staining. **(B)** Quantification of immuno stainning. Quantification based on two independent experiments with at least two donors.

Figure 2. Human Myoblasts Can be Purified in Less Than 25 Minutes



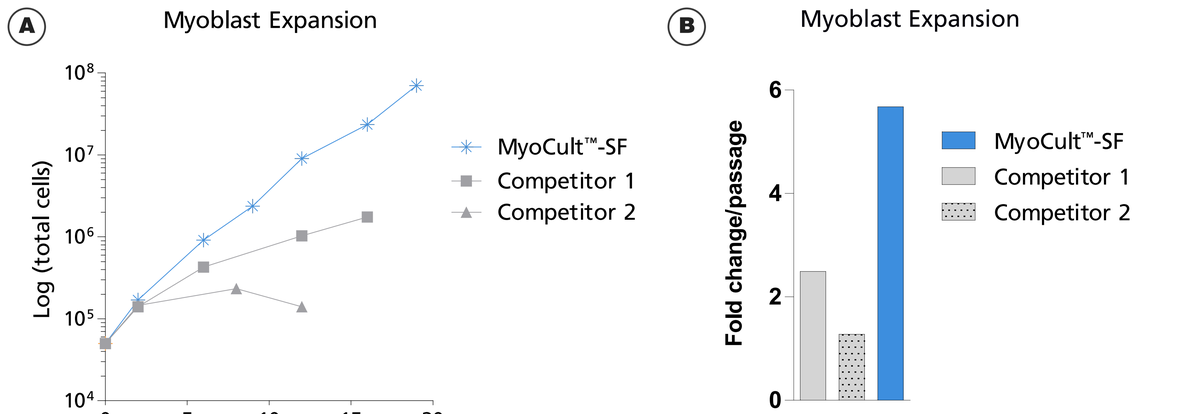
(A) % CD56$^+$ cells in starting population. **(B)** Cell surface antigen profile of cells after CD56 purification (CD56$^+$/CD29$^+$/CD82$^+$ and CD31/45neg). **(C)** % MyoD$^+$ cells after CD56 purification. **(D)** Cell viability and recovery after immunomagnetic separation.

Figure 3. Robust Expansion of Human Myoblasts Over Multiple Passages



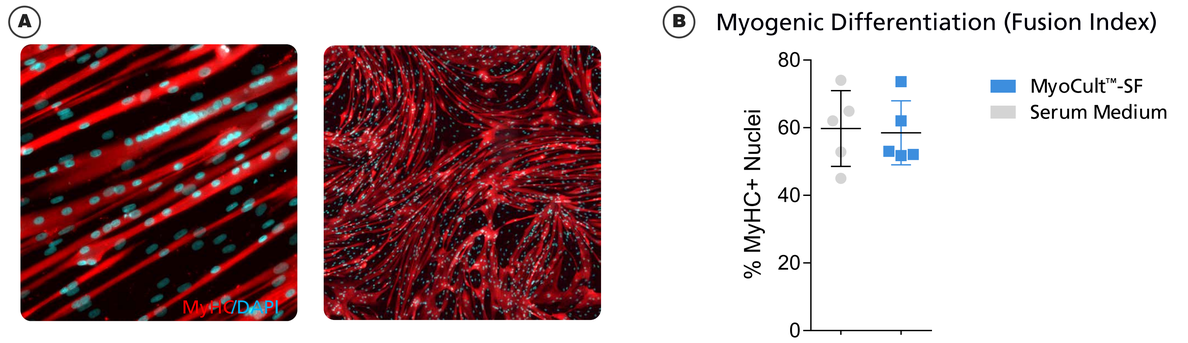
(A) Expansion rates of cells cultured in MyoCult™-SF compared to serum medium over 9 passages **(B)** Phase contrast images highlighting morphology of cells grown in medium with and without serum. **(C)** Summary of average fold expansion per passage.

Figure 4. Superior Expansion in MyoCult™-SF Compared to Serum-Free Competitors



(A) Expansion rates of MyoCult™-SF in different SF media over 6 passages. **(B)** Summary of average fold expansionper passage.

Figure 5. hSkM MPs maintain robust differentiation potential into multinucleated MyHC$^+$ myotubes following derivation and expansion in MyoCult™-SF Expansion Medium



hSkM MPs were expanded for 3 - 5 passages in serum-free conditions, seeded into 24-well plates, grown to 85 - 95% confluence and switched to MyoCult™ Differentiation Medium. Half medium changes were performed every other day for 10 days. **(A)** Immuno-fluorescent images of MyHC staining. **(B)** Quantification of myogenic fusion index.

Conclusions

- MyoCult™-SF Medium is a serum-free culture medium that supports the derivation and expansion of Pax7$^+$ human myogenic progenitors
- CD56 immunomagnetic isolation is a simple and rapid method of purifying viable human myoblasts
- Human myoblasts can be expanded for >9 passages in MyoCult™-SF Medium at rates superior to traditional serum-containing media
- MyoCult™ Differentiation Medium supports the differentiation and maintenance of highly organized elongated myotube cultures for at least 10 days without detachment, providing a culture system amenable to downstream biochemical and physiological assays