DEVELOPMENT OF A PRIMARY SKELETAL MUSCLE CELL CULTURE MODEL FROM HUMAN MUSCLE BIOPSIES

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PURPOSE. We aimed to develop and validate a culture model of primary human skeletal muscle cells from human muscle biopsies.

MATERIAL & METHODS. Muscle biopsies were obtained post-mortem from the vastus lateralis (8 men, 73 ± 12 years and 10 women, 77 ± 15 years). The biopsies were enzymatically digested before separation of satellite cells (CD56+) from fibroblasts (CD56-) by two methods: pre-plating or immunomagnetic cell sorting using an anti-CD56 antibody. We also compared the effects of two culture media on myoblasts proliferation (10%FBS vs. 10%FBS + 2%Ultroser G (UG)) using cell doubling time as parameter. Two differentiation media (2%FBS vs. 0.5%UG) were tested on the myogenic index. Finally, we studied the expression of several muscle cell markers during myogenesis by qRT-PCR and immunofluorescence.

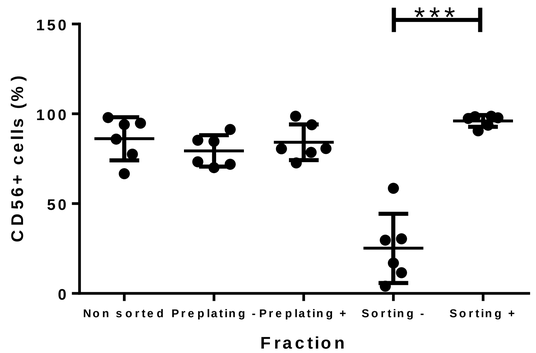

Figure 1.
Comparison of CD56+ cells between cell fractions after pre-plating or immunomagnetic beads cell sorting. The results are expressed as mean with SD (n=6). A p value of 0,05 or less is considered significant (***=p<0,001).

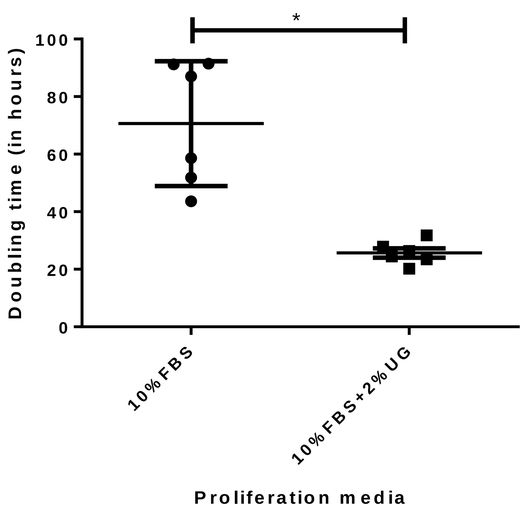

Figure 2.
Comparison of the proliferation media 10%FBS and 10% FBS supplemented with 2%UG on the cell doubling time criteria. The results are expressed as mean with SD (n=6). A p value of 0,05 or less is considered significant (*=p<0,05).

RESULTS. The cells obtained from the enzymatic digestion of the biopsy contained a varying proportion of CD56+ satellite cells (86,15 ± 12,09%) (Figure 1). The cell population obtained **after immunomagnetic cell sorting** (sorting +) contained **96,11 ± 3,27%** of CD56+ cells. Furthermore, this method **significantly separated fibroblasts from satellite cells (p=0,0004)**. In comparison, the ratio of CD56+ obtained by the preplating method was lower (84,19 ± 9,94%). The **cell doubling time was significantly shorter** with culture media containing **10%FBS and 2%UG** than with the media containing only 10%FBS (**25.70 ± 3,96h** vs. 70.64 ± 21,68h, **p = 0.0313**) (Figure 2). The myogenic index was not significantly different between the two differentiation media (2% FBS vs. 0,5% UG). Finally, the expression profile of MyoD1, and the increase in desmine and MYHs expression, indicated the phenotypic transition of myoblasts to myotubes (Figure 3 and 4).

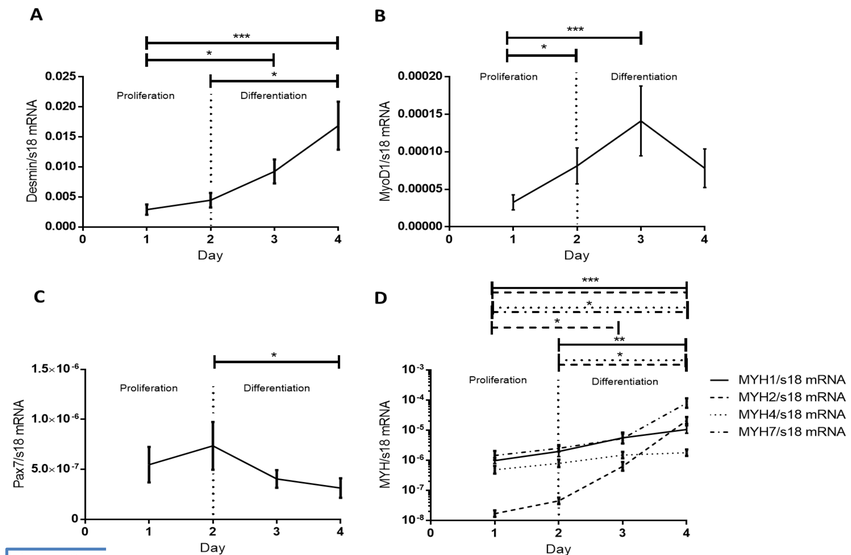

Figure 3.
Expression of myogenic markers through myogenesis highlighted by transcriptomic analysis. The dotted line represents the change of medium after 48 hours of proliferation. Results are represented as mean with SEM (n=7). (A) Desmin (B) MyoD1 (C) Pax7 (D) MYHs. A p value of 0,05 or less is considered significant (*=p<0,05; **=p<0,01; ***=p<0,001).

CONCLUSIONS. Immunomagnetic cell sorting allows better satellite cells and fibroblasts separation than the preplating technique. UltoserG serum substitute accelerates cell proliferation and allows the differentiation of myoblasts into myotubes. The markers MyoD1, desmine and MYHs are good markers of the myogenic differentiation of myoblasts. This *in vitro* model makes it possible to purify efficiently muscle satellite cells from human muscle biopsies. These cells can be used in *in vitro* studies to decipher the pathophysiological mechanisms of muscle disease and the mechanisms of action of treatments.

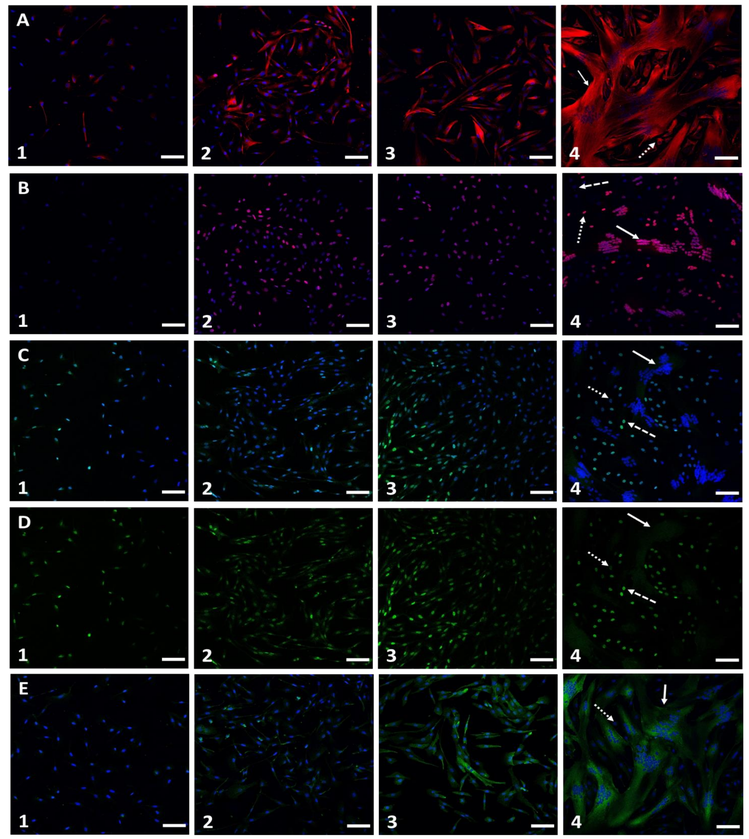

Figure 4.
Expression of myogenic markers through myogenesis highlighted by immunofluorescence. The numbers represent the days. The scale bars at the lower right represent 100µm (20x magnification). DAPI is represented in blue, desmine and MyoD1 in red and MYH and Pax7 in green. The interrupted arrows indicate myoblasts and the uninterrupted arrows indicate myotubes (A) Desmin (B) MyoD1 (C) Pax7 (D) Pax7 (not merged with DAPI) (E) MYHs (the antibody used target all isoforms of MYHs).

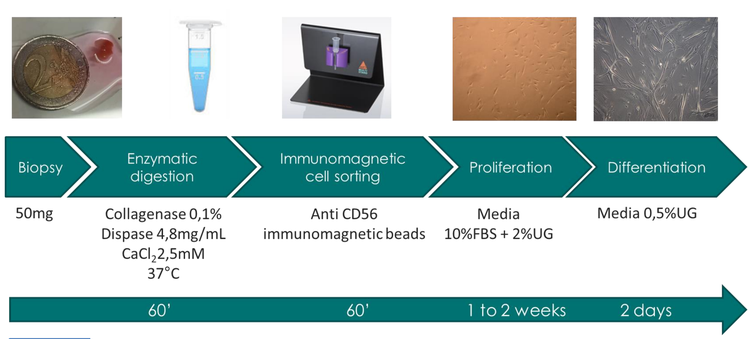

Figure 5.
Schematic representation of the skeletal muscle cell culture model.