Development of a muscle cell culture model from human muscle biopsies

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PURPOSE. The skeletal muscle account for about 40% of the total body mass. In addition to its role in locomotion, skeletal muscle also has endocrine functions. The skeletal muscle cell model can be used to study the effect of certain drugs or to study the differentially secreted proteins during certain pathologies. In this purpose, we have developed a cell culture model of skeletal muscle cells obtained from human muscle biopsies.

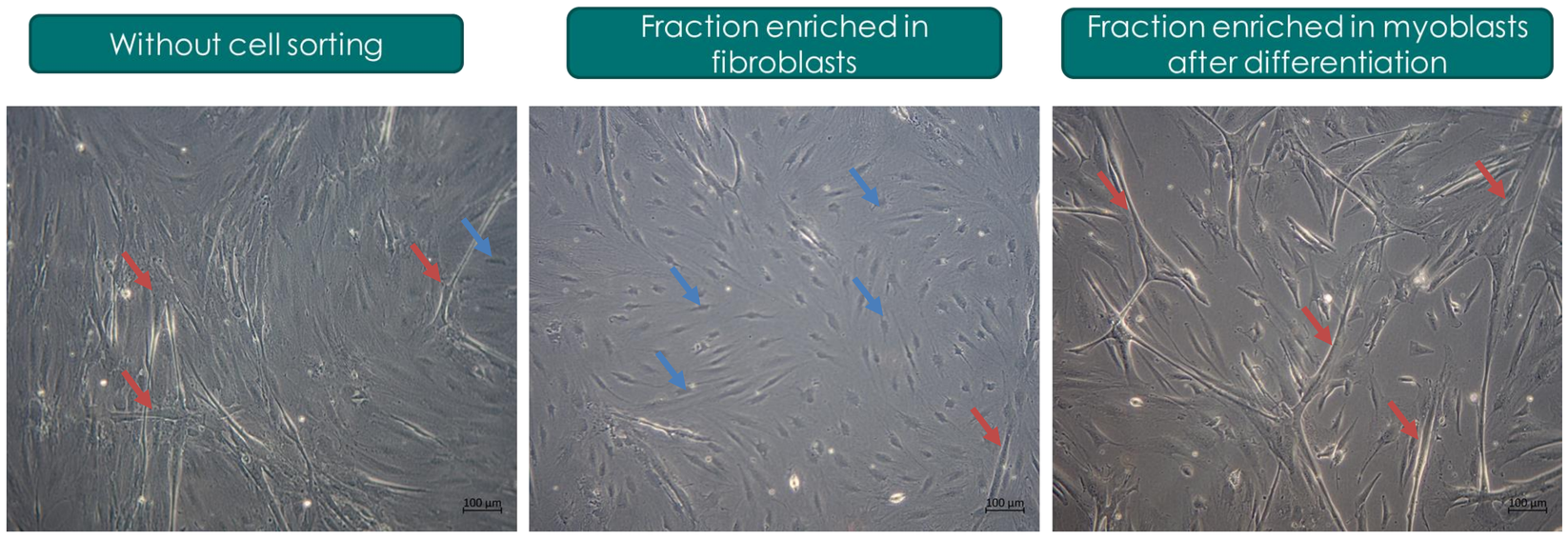


Figure 1,
The highlighting of the different populations is easy during observation with a phase contrast microscope (myotubes = red arrows; fibroblasts = blue arrows). The non sorted fraction contains myoblasts and fibroblasts (72,16% desmine positive cells), the fraction enriched in fibroblasts contains mostly fibroblasts (23,08% desmine positive cells) and the fraction enriched in myoblasts contains mostly myoblasts (95,59% of desmine positive cells) before differentiation in myotubes

METHODS. Human muscle biopsies from vastus lateralis were obtained from cadavers (n=16). Two enzymatic digestion methods of the biopsy were compared: collagenase/dispase/$CaCl_2$ vs. collagenase/trypsin. In order to purify the cell population into myoblasts, and to eliminate fibroblasts, we used magnetic beads sorting (MACS) of the cells directly after the enzymatic digestion. The beads, coated with an anti-CD56 antibody, were incubated for 15 minutes at room temperature, in order to prevent mortality. We have developed an immunofluorescence protocol to characterize skeletal muscle cells with an anti-desmine antibody. To select the best proliferation medium, we have compared 2 medium (all composed of DMEM containing 100 U/ml penicillin, 100 µg/ml streptomycin, 10 mM HEPES and 2 mM glutamine with addition of serums) on cell doubling time, by counting the cells with trypan blue. Similarly, in order to select the best differentiation medium, we have challenged 2 media on the following criteria: the fusion index (FI, ratio between the number of nuclei present in the myotubes and the total number of nuclei), the surface of the myotubes (SM), and the mortality (evaluated by a LDH assay) induced by the change of medium (M).

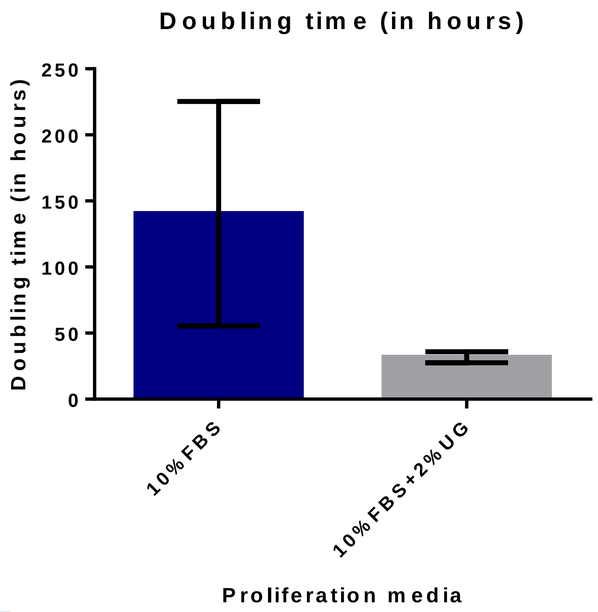


Figure 2,
Comparison of 2 proliferation media based on the cell doubling time (in hours). The medium DMEM supplemented with 10% Fetal Bovine Serum (FBS) and 2% UltroserG (UG) was selected out-off 2 proliferation media, because it had the best doubling time (31-hours).

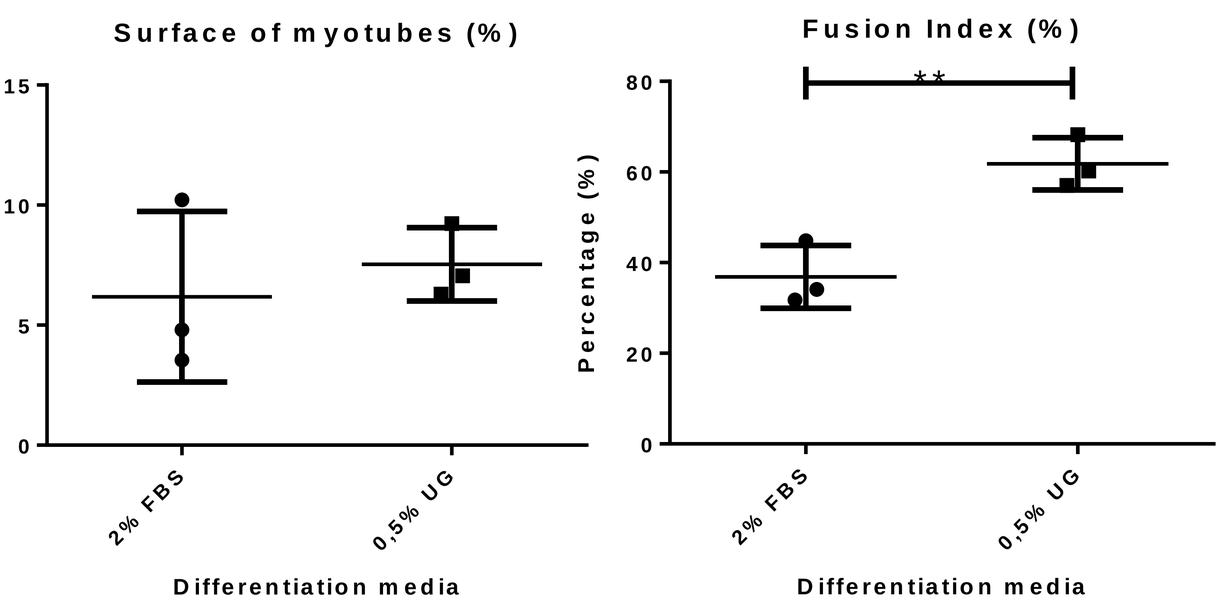


Figure 3,
Comparison of 2 differentiation media based on the surface of myotubes (in %) and the fusion index (in %). The medium DMEM supplemented with 0,5% UG was selected because it showed the best fusion index, the larger myotubes and the lower mortality (FI=61,82%; SM=7,53%; M=1,45%).

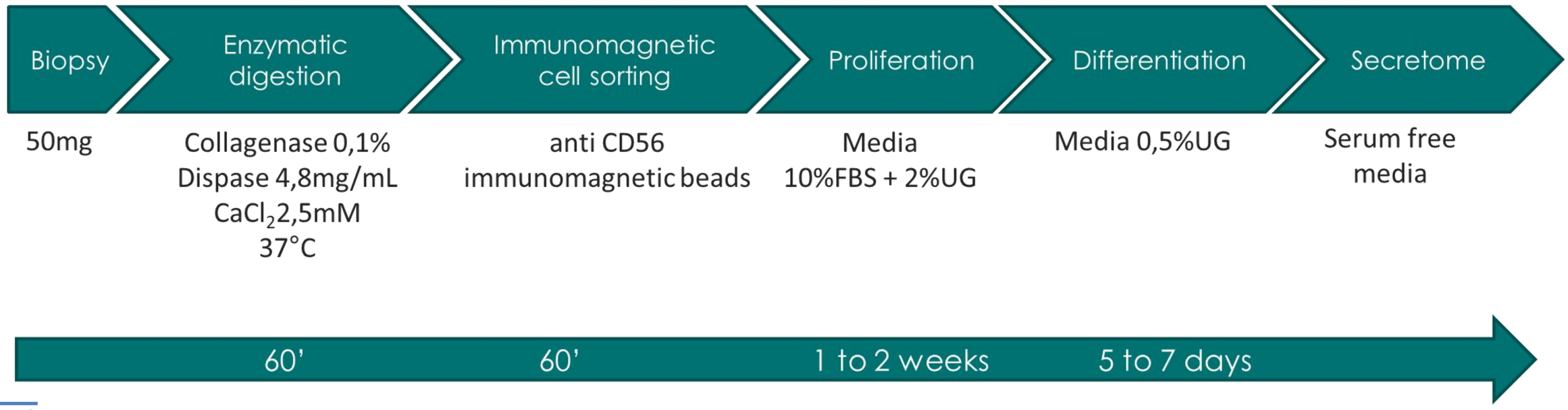
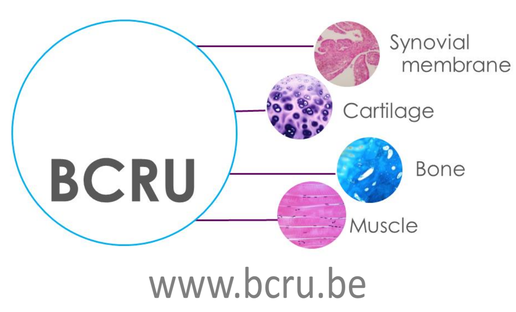


Figure 4,
Protocol of the skeletal muscle cell culture model

CONCLUSIONS. **Our human muscle cell culture model includes a digestion step with an association of collagenase/dispase/$CaCl_2$ and an anti-CD56 immunomagnetic beads step to eliminate contaminating fibroblasts. The proliferation is performed in DMEM supplemented with 10% FBS and 2% UG while the differentiation occurs in DMEM supplemented with 0,5% UG. This model may be used to study the effect of some drugs or to study the differentially secreted proteins during certain pathologies.**



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