



Premi de recerca per a estudiants
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Entrega de l'Abstract

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Títol de la recerca: Optimization of a protocol to purify CD56+ cells using magnetic beads
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Abstract (màxim 500 paraules):

INTRODUCTION AND OBJECTIVES:

Human myoblast primary cultures are a very valuable tool for research, diagnosis and evaluation of potential treatments of neuromuscular disorders. A major limitation of this approach is the contamination of primary cultures by muscle fibroblasts. To address this issue various methods have been devised. One of them is the use of magnetic beads coupled to the CD56 antigen which is expressed on the surface of muscle cells (MACS® Technique, Miltenyi Biotec). The aim of this project was to optimize this technique in order to obtain highly pure primary myoblast cultures derived from muscle biopsies of children affected by neuromuscular diseases. An additional goal was to study the viability of the purified cells, their potential to differentiate into mature myotubes and the expression of relevant muscle proteins.

METHOD:

We performed a series of experiments to evaluate the effect of



various modifications to the original protocol. Isolated myoblasts were characterized by flow cytometry and immunostaining using CD56 antibodies and desmin respectively.

RESULTS

The average percentage of myoblasts increased from 1-4% in the original cultures up to 87% in the purified fraction. We were able to increase cell viability from 50% to 90% by modifying the solution used in the washing steps. Results were reproducible when the technique was applied to different cell lines. Purified myoblasts expressed desmin, integrin α -10, porin and caveolin-3 in a pattern comparable to an immortalized human skeletal muscle cell line. They differentiated into myotubes albeit at low fusion indexes.

CONCLUSION:

To conclude, the use of CD56 MicroBeads (MACS® Technique, Miltenyi Biotec) is a simple, fast and reliable technique to obtain highly viable human myoblasts from muscle biopsies.

AUTHORSHIP:

The author has contributed to the realization of the experiments. The author initiated the process performing the muscle biopsies to diagnose neuromuscular disorders. Also the elaboration of the material and mediums used for the experiments, the growth and preparation of muscle cells was done before the separation with magnetic beads. She performed the separation using a protocol previously developed by the group.

The author also conducted all the analysis of the data and is involved in the writing of the paper.