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OPTIMIZATION OF A DEFINED SERUM-FREE MEDIUM FOR THE PRODUCTION OF THERAPEUTIC HUMAN MYOBLASTS

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Duchenne Muscular Dystrophy (DMD) is a genetic disease affecting one boy out of 3500, which is due to a mutation in the dystrophin gene, inducing progressive and irreversible muscle degeneration. Cell therapy is the only means by which a DMD patient could recover part of his muscular mass and strength. We are presently collaborating with a team at the Quebec City University Hospital who is developing a cell therapy based on the graft to DMD patients of myoblasts obtained from biopsies on healthy and compatible donors. To do so, cells collected from donors need to be extensively multiplied. The standard culture medium allowing this multiplication contains foetal bovine serum (FBS). We have developed a serum-free medium (SFM) that supports cell growth to an extent and at a rate similar to the ones in FBS-containing medium. Cells grown in the developed SFM also show some typical myoblast phenotypes and functionalities such as: the presence of desmin and myosin heavy chain (MHC) and the capacity to fuse and form myofibers *in vitro*. However, cell morphology as well as the capacity to graft to muscle fibers *in vivo* are different for cells grown in the two culture media. We have therefore hypothesized that morphology was a marker of cell functionality *in vivo*, and have chosen to optimize our SFM with an objective function composed of both medium cost and cell morphology, while constraining to maintain cell growth rate and extent.

To do so, we have used 1) statistical design of experiment response surface methodology combined with 2) undecimated wavelet transform multivariate non-invasive image analysis to screen the SFM components for optimum growth, medium cost and cell morphology. The optimized SFM (MSFM) we have found through this process is 55% less expensive than the original SFM, and the human myoblasts grown in this medium exhibit comparable growth, morphology and muscle fiber graft capacity as cells grown in FBS-containing medium.