A novel human skeletal muscle in vitro model using opti-ox mediated cellular reprogramming of induced pluripotent stem cells

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Abstract

Skeletal myocytes play roles in a number of biological processes ranging from limb movement to the regulation of nutritional homeostasis, and are implicated in the pathophysiology of a variety of diseases involving muscle dysfunction. There is a pressing need for reliable models of mature human skeletal muscle to permit investigations into physiological and disease mechanisms, and to facilitate the generation of new therapeutics. While human induced pluripotent stem cells (hiPSCs) offer a promising starting material for skeletal muscle cells, their broad use has been hampered by difficult to reproduce, complex differentiation protocols. We have developed an optimised inducible system (opti-ox)[1] that enables tightly controlled expression of transcription factors improving cellular reprogramming approaches for the differentiation of hiPSCs. Through targeting of genomic safe harbour loci, we used opti-ox to achieve homogenous, inducible expression of the myogenic regulator MYOD1. MYOD1 induction leads to shutdown of the core pluripotency network, and activation of key myogenic factors including myosin heavy chain. opti-ox reprogrammed ioSkeletal Myocytes express Desmin, Dystrophin and Titin, and form contractile, striated and multinucleated myocytes by Day 10 post-revival. Critically for metabolic studies, robust expression of the insulin-regulated glucose transporter GLUT4 is also detected. Importantly, skeletal myocytes produce a highly pure MHC positive population of cells within 4 days of thawing, comparable to day 10 transdifferentiated fibroblasts, and are amenable to high-throughput screening (HTS). The scalability of opti-ox reprogramming provides a unique hiPSC based model for research, disease modelling and HTS focused on muscle, neuromuscular, and associated metabolic disorders.

1. Precise reprogramming of hiPSCs into defined muscle cells

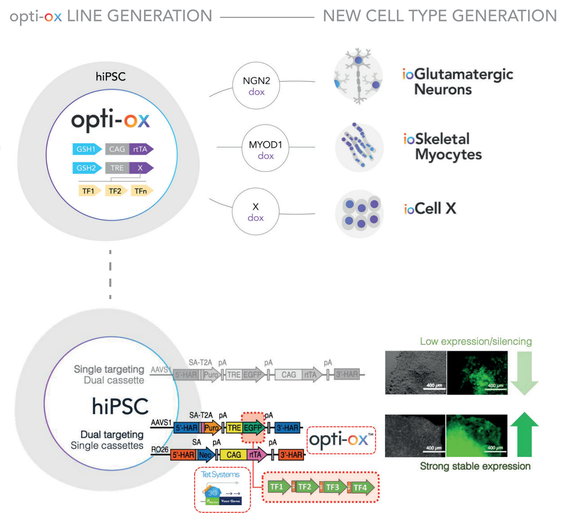
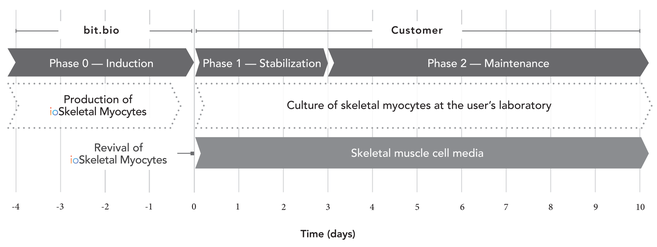


Figure 1: opti-ox technology for the optimal cellular reprogramming of human iPSCs into defined muscle cells. opti-ox dual cassette Tet-ON system ensures tightly controlled and homogeneous expression of reprogramming transcription factors by preventing silencing of the inducible expression cassette after genetic engineering of hiPSCs[1]. Human iPSC-derived ioSkeletal Myocytes, generated by MYOD1-driven opti-ox reprogramming, are functional within days and provide consistent and reliable human muscle cells for research, disease modelling and high throughput screening.

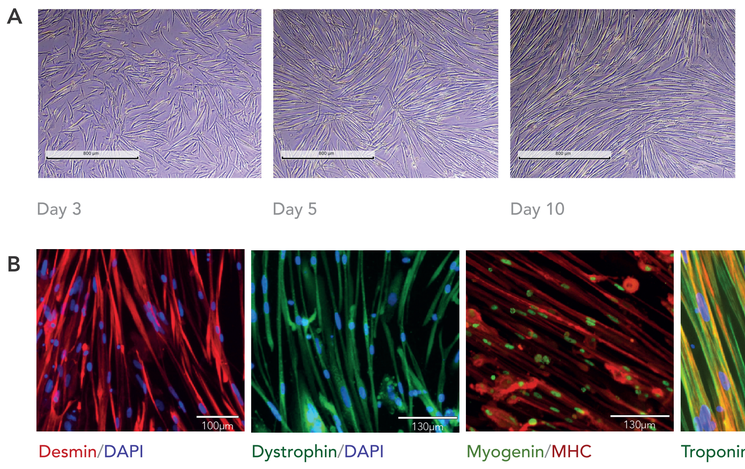
2. Human muscle cells ready for experimentation within days

Figure 2. ioSkeletal Myocytes are derived from hiPSCs by MYOD1[1,2] driven opti-ox reprogramming and arrive ready to plate. Cells are delivered in a cryopreserved format and are programmed to rapidly mature upon revival in the recommended media. The protocol for the generation of these cells is a three-phase process: 1. Induction (carried out at bit.bio); 2. Stabilization for 3 days with Doxycycline; 3. Maintenance during which the skeletal myocytes mature.



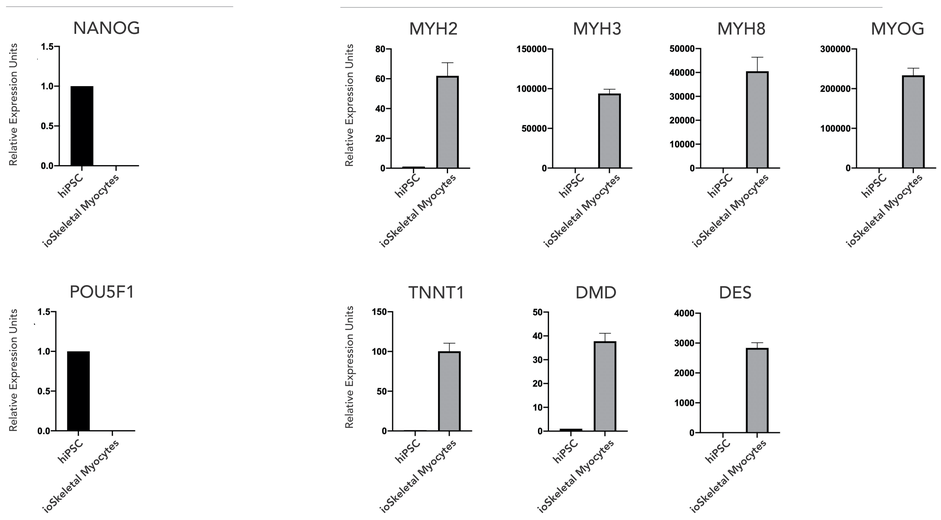
3. ioSkeletal Myocytes form contractile, elongated fibres over 10 days and express mature myogenic markers

Figure 3: Characterization of ioSkeletal Myocytes. (A) ioSkeletal Myocytes after revival over the course of the first 10 days of culture. Day 1 to 10 post-thawing; 4X magnification; scale bar: 800µm. **(B)** Immunofluorescence staining at day 10 post revival demonstrates robust expression of components of the contractile apparatus such as Desmin, Dystrophin, and Myosin Heavy Chain (MHC), along with the muscle transcription factor Myogenin. Cells also demonstrate expression of Troponin with visible striated fibres, and multinucleation.



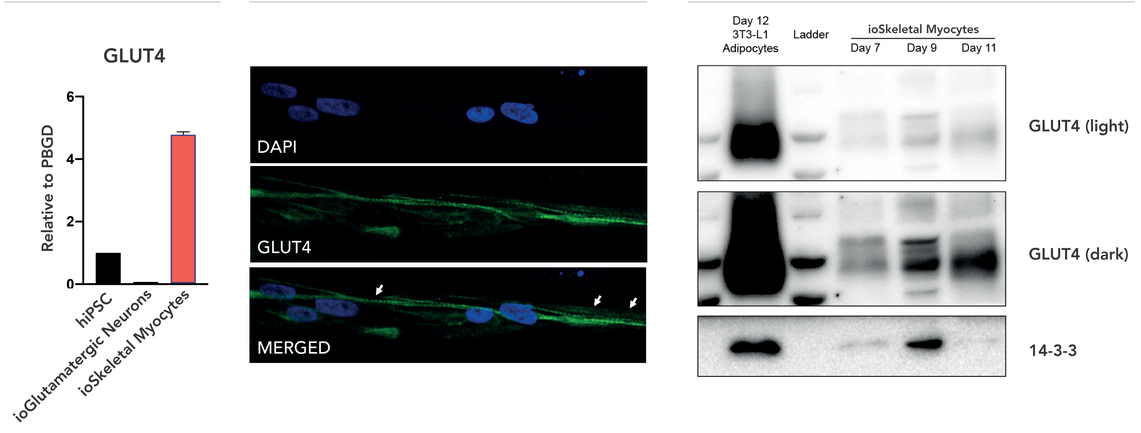
4. Cells demonstrate gene expression of key myogenic markers following reprogramming

Figure 4: ioSkeletal Myocytes gene expression. Following reprogramming, ioSkeletal Myocytes downregulate expression of the pluripotency genes **(A)**, whilst demonstrating robust expression of key myogenic markers **(B)**. Gene expression levels assessed by RT-qPCR (data expressed relative to the parental hiPSC, normalised to HMBS). Data represents Day 10 post-revival samples; n=7 biological replicates.



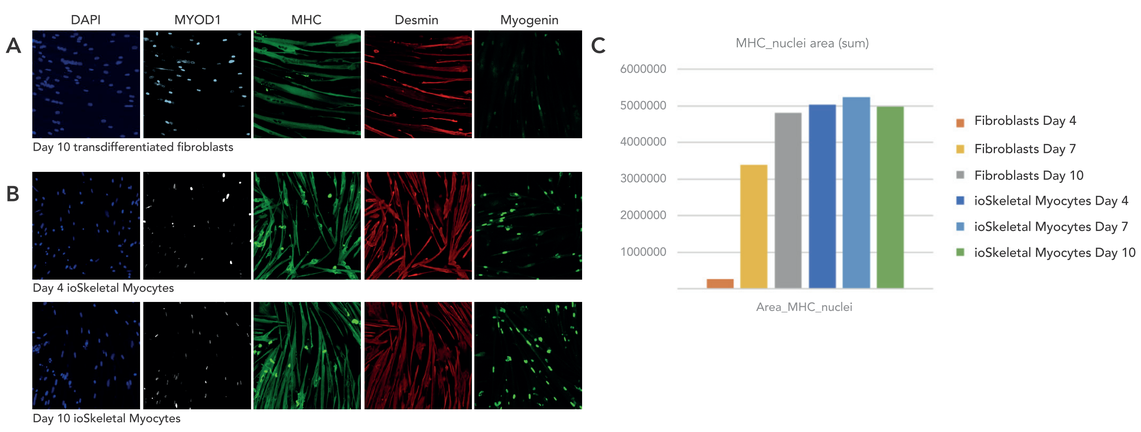
5. Cells express the insulin regulated glucose transporter GLUT4, critical for metabolic studies

Figure 5: Data demonstrates expression of the insulin regulated glucose transporter GLUT4, suggesting that ioSkeletal Myocytes provide a unique human cell model for metabolic research. (A) RT-qPCR at Day 10 post-revival demonstrating expression of GLUT4 in the Skeletal Myocytes, compared to undifferentiated hiPSCs and ioGlutamatergic Neurons. **(B)** Immunocytochemistry at Day 7 post-revival demonstrates expression of GLUT4 in peri-nuclear regions, and striations, in the ioSkeletal Myocytes[3]. **(C)** Western blotting of differentiated 3T3-L1 adipocytes and maturing ioSkeletal Myocytes demonstrates GLUT4 expression in a time-dependent manner[3].



6. Myocytes are suitabale for phenotypic based HTS

Figure 5: ioSkeletal Myocytes generate myocytes within as little as 4 days post-revival with a high degree of MHC+ cells. (A) Human fibroblasts were transduced with lentiviral vectors allowing inducible over-expression of MYOD1 to transdifferentiate them to myocytes in approximately 10 days. Transdifferentiated myotubes were stained for multiple myotube markers to assess the purity and degree of multi-nucleation[4]. **(B)** iPSCs stably and inducibly expressing MYOD1 using opti-ox technology (ioSkeletal Myocytes) can generate myocytes within as little as 4 days from revival with a high-degree of MHC+ cells (>95% purity), suitable for phenotypic based high throughput screens[4]. **(C)** Comparable total area of MHC positive cells are generated between ioSkeletal Myocytes and transdifferentiated fibroblasts[4]. These results suggest that the ioSkeletal Myocytes are amenable to HTS.



Summary

- Human iPSC-derived skeletal myocytes generated using opti-ox demonstrate robust expression of components of the contractile apparatus and form striated, multinucleated, myocytes by Day 10 post revival, that contract in response to acetylcholine (refer to bit.bio for data on contractility).
- ioSkeletal Myocytes are highly characterized and demonstrate expression of mature myocyte markers.
- GLUT4 expression in ioSkeletal Myocytes suggests they provide a unique human cell model for metabolic research.
- ioSkeletal Myocytes produce a highly pure MHC positive population of cells within 4 days post-reival, suitable for phenotypic based high throughput screens.
- ioSkeletal Myocytes provide a highly-defined, consistent and reliable human cell model for research, disease modelling and high-throughput screening focussed on muscle, neuromuscular, and associated metabolic disorders.

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1. Pawlowski M, et al., Stem Cell Reports, 2017. 2. Yang N, et al., Nature Methods, 2017.
3. Dougall Norris & Daniel Fazakerley, Wellcome-MRC Institute of Metabolic Science. 4. Shushant Jain et al, Charles River Laboratories