CYTOO
PHYSIOLOGICAL ASSAYS FOR HCS

MYOSCREENΦ™ A fully mature human striated muscle model for HCS

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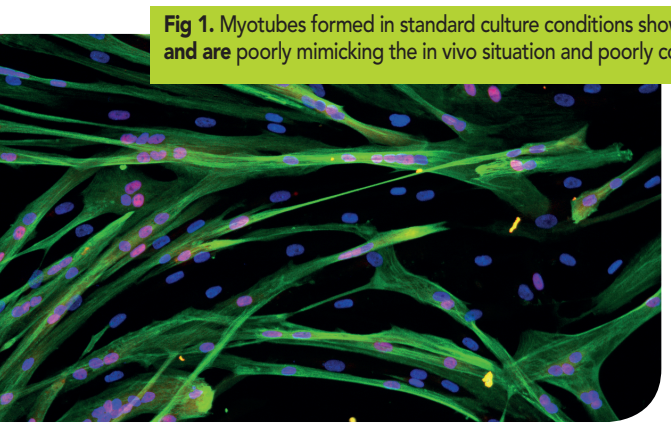
INTRODUCTION

The development of cellular models with higher physiological relevance coupled to more informative and standardized readouts are prerequisite to develop more predictive cell-based assays compatible with High Content Screening (HCS). Current models can show limited use in phenotypic screens due to the lack of predictivity: (i) cells grown in monolayers do not recapitulate the native tissue physical constraints, (ii) the heterogeneity in morphology and the availability of relevant readouts limit their use in HCS.
In this context, CYTOO has developed a physiologically relevant striated muscle model with standardized micro-tissue organization. When cultured on CYTOO micropatterns, human primary myoblasts differentiated faster into myotubes and displayed a higher level of sarcomere striation and nuclei alignment compared to standard culture conditions. Moreover, the use of micropatterns greatly standardized myotube formation and morphogenesis. Thanks to the development of new image analysis algorithms and the reduced variability of myotube morphology, the achieved cellular model enabled accessing new parameters for myotube characterization upon drug treatment. To demonstrate the compatibility of such model with HCS, the robustness of the assay was evaluated for atrophy and hypertrophy detection. Finally, various healthy donors were characterized and attributed a Myotube ID and evaluated for atrophy, hypertrophy and myotoxicity.

Standardized and homogeneous myotube morphology

Conventional culture conditions show high heterogeneity

Fig 1. Myotubes formed in standard culture conditions show **very heterogeneous sizes and shapes and are** poorly mimicking the in vivo situation and poorly compatible with High Content Analysis



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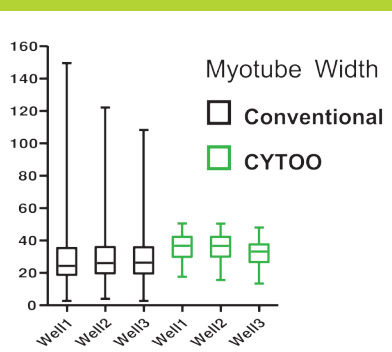
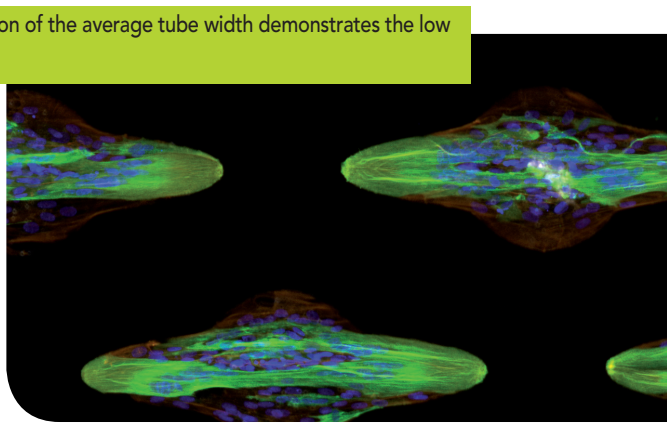
MyoScreenΦ™ demonstrates aligned and standardized myotubes

Fig 2. Myotubes standrardization. Quantification of the average tube width demonstrates the low variability of micropatterned myotubes

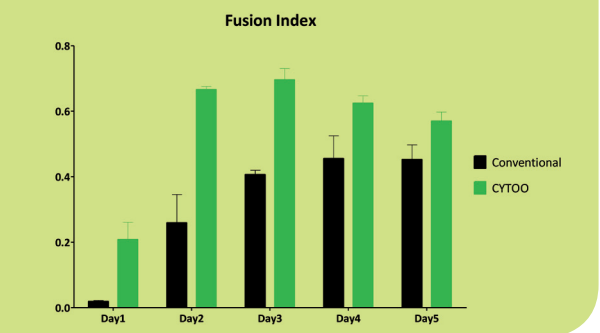




Advanced, quicker differentiation and maturation of myotubes

Higher myotube striation

Fig 3. Evolution of the fusion index over 5 days in CYTOO and conventionnal culture conditions.



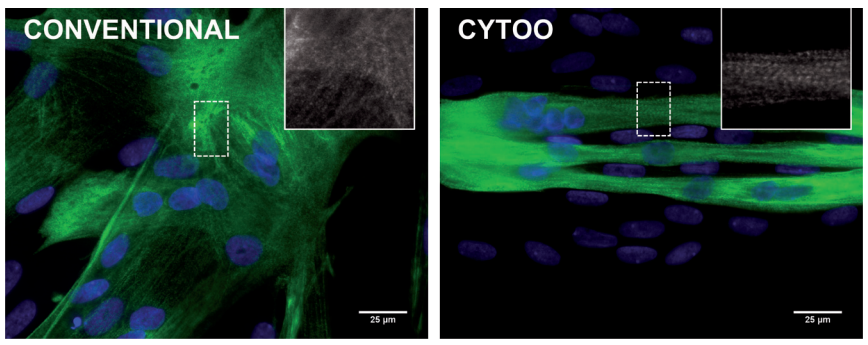
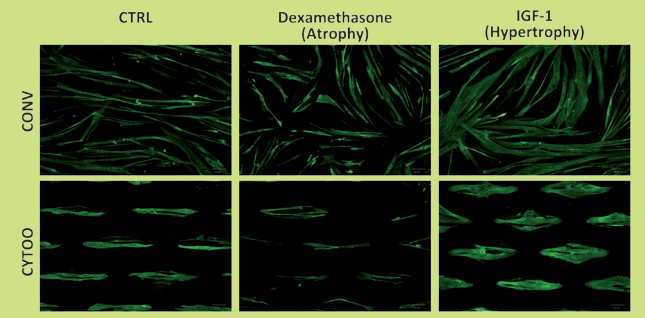
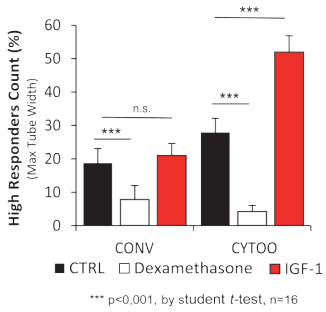


Fig 4. Level of sarcomere striation on both standard and micropatterned conditions after 5 days of differentiation: analysis reveals higher striation level of myotubes on micropatterns.

A robust cellular model to detect atrophy and hypertrophy





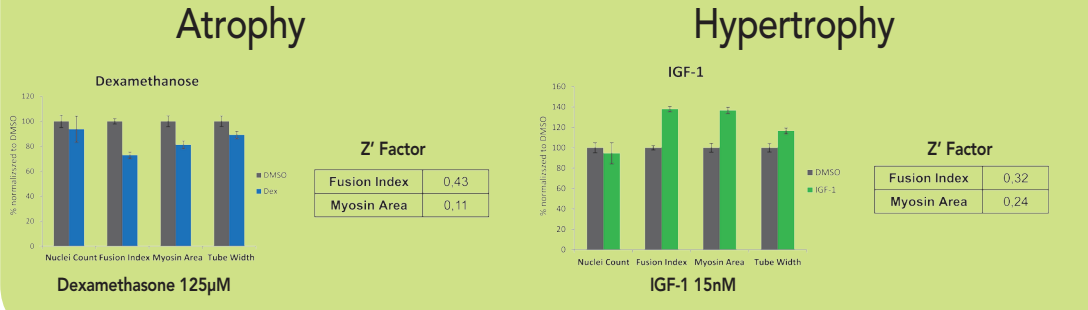


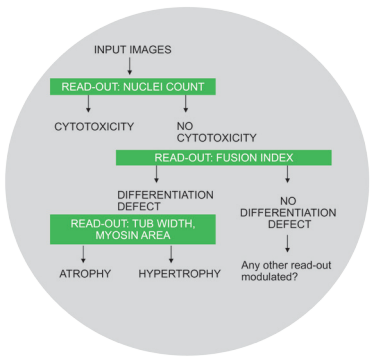
Fig 5. Comparison of the detection of compounds inducing atrophy (Dexamethasone) and hypertrophy (IGF-1) on conventional and micropatterned culture supports of differentiation: analysis reveals higher striation level of myotubes on micropatterns.

Fig 6. Computation of Z' factors of several read-outs in presence/absence of atrophic and hypertrophic compounds

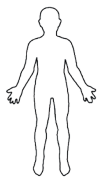
Multiplexed read-outs for drug MOA characterization

Multi-parameter Data Analysis

PARAMETERS	CLASS
#1: Nuclei number	↘ Toxicity / ↗ Proliferation
#2: Fusion index	↘↗ Differentiation
#3: Tube Width	↘ Atrophy / ↗ Hypertrophy



Donor choice for improved assay response



Donor ID
Sex
Age
Ethnicity
BMI
Diabetes
Heart disease
Smoke
Medication
Tissue Location

Myotube ID
Cell Passage
Nuclei / well
Tubes / well
Fusion Index
Myosin Area
Tube Width
Striation

Assay Validation
Response to Atrophy
Response to Hypertrophy
Response to Myotoxicity

MyoScreenΦ™ key features and benefits

- Standardized myotubes
- Highly mature model
- HCA/HCS compatibility
- Drug MOA characterization
- Robust atrophy/hypertrophy assays

CONCLUSION

MyoScreenΦ™ is a first-in-class striated muscle micro-tissue model for drug screening.
Our results showed that this model is highly robust and compatible with High Content Screening with increased Z' factors compared to standard assays for atrophy and hypertrophy. Enhanced image analysis capacities also allowed the simultaneous screening of compounds inducing atrophy, hypertrophy, cytotoxicity and globally affecting myogenesis.
Last but not least, MyoScreenΦ™ can be used in drug discovery, in particular to:

- Identify hypertrophic compounds to counteract sarcopenia and cachexia, and increase muscle mass,
- Early detect potential myotoxic compounds,
- Establish musculoskeletal disease models and discover novel active NCEs and NBEs,
- Establish equivalent rodent models to reduce the use of animals.