# Morphological and kinetic signatures of skeletal muscle cells predict myotube formation for regenerative medicine

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## 1. Introduction

Autologous human skeletal muscle derived cells (SMDC) are being investigated for skeletal muscle regeneration for conditions such as incontinence [1–3]. However, the outcomes of these therapies are unpredictable, which is unsatisfactory given the high cost of manufacturing. The impact of differences among different donors on cell quality cannot be ignored; however, methods to evaluate these differences are limited.

Aim: This project investigated the use of the HoloMonitor® label-free live cell imaging cytometer, which is based on the principle of quantitative phase imaging, enabling non-invasive visualization and quantification of living cells, to evaluate whether SMDC morphological or kinetic characteristics could serve as predictors of myotube formation through correlation with the fusion index.

## 2. Materials & Methodology

- Human SMDC from six donors (Lonza) were expanded in vitro for 72 hours while being continuously monitored using the HoloMonitor®.
- Eight morphological and kinetic characteristics including cell area, eccentricity, optical thickness, and motility were extracted for analysis.
- After monitoring, myogenic differentiation was induced over 7 days, and the outcomes were quantified through immunostaining for myosin heavy chain (MF-20) to calculate the fusion index of the myotubes (Fig. 1).
- One-way ANOVA was used to identify statistically significant differences between the donors. Spearman's rank correlation coefficient was calculated between the different cell features and the fusion index.

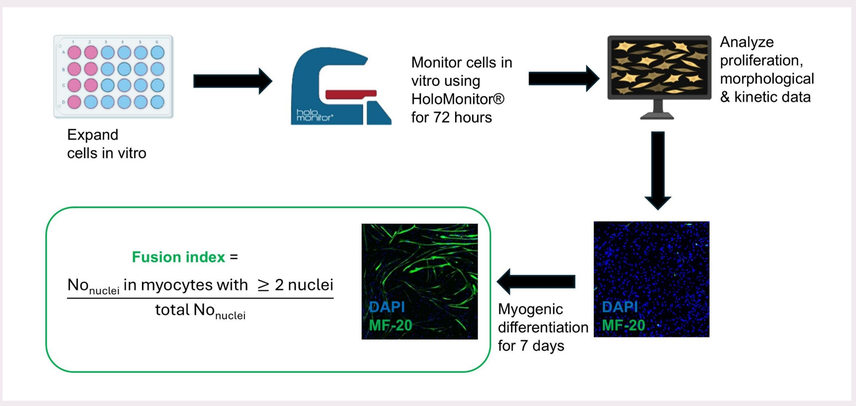



Fig. 1: Illustration of the experimental workflow chart (some icons have been created using BioRender.com)

## 3. Results: Proliferation

- Variability between donors was obvious (Fig. 2), as 17F and 21TL reached 50% confluence within the monitoring period, while 25M barely increased in cell count or confluence. 20F, 16M, and 35M all showed similar proliferation rates, showing slight but consistent growth both in cell count and confluence, nevertheless, nowhere near the proliferation rates of 21TL and 17F.

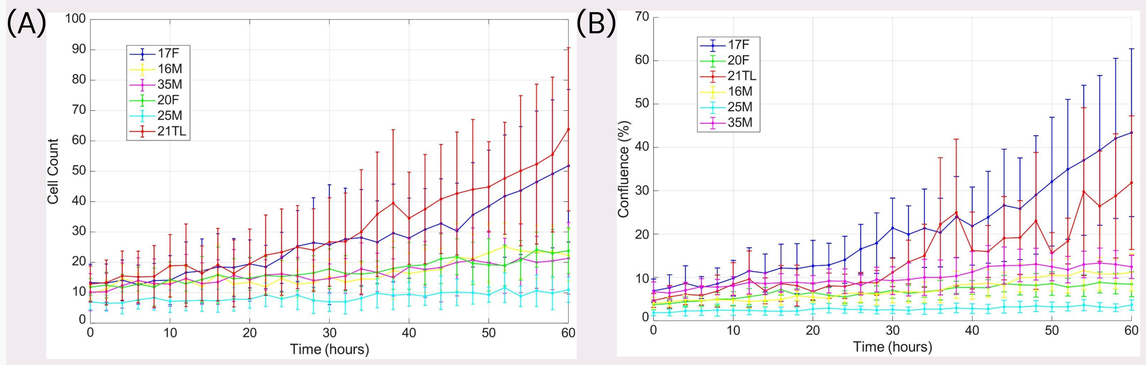



Fig. 2: Growth curves displaying the combined mean ± standard deviation, using error bars, of 6 FOVs for each donor. (A) Growth curve based on the cell count. (B) Growth curve based on the confluence.

## 4. Results: Morphological & kinetic features

- All donors, apart from 25M, seem to increase in mean cell area when comparing timepoint 12h with timepoint 36h.
- All means, except irregularity at 12h and eccentricity at 12h and 36h, were significantly different from each other ($p < 0.0001$), highlighting inter-donor differences.
- Both eccentricity and irregularity are very constant compared to the other characteristics and also have the smallest variance with eccentricity having a slightly smaller relative standard deviation across donors than irregularity.

Table 1: (A) Mean morphological & kinetic features for all donors at 12 h. (B) Mean morphological & kinetic features for all donors at 36 h.

A

| Characteristic | 17F | 16M | 25M | 35M | 20F | 21TL |
|---|---|---|---|---|---|---|
| Mean Area | 1078.63 ± 416 | 937.20 ± 444 | 800.21 ± 249 | 1314.09 ± 228 | 994.18 ± 358 | 875.41 ± 247 |
| Mean Eccentricity | 0.78 ± 0.0431 | 0.78 ± 0.105 | 0.79 ± 0.107 | 0.79 ± 0.0641 | 0.82 ± 0.0208 | 0.79 ± 0.0917 |
| Mean Irregularity | 0.54 ± 0.0864 | 0.55 ± 0.0310 | 0.49 ± 0.0786 | 0.54 ± 0.0694 | 0.54 ± 0.0759 | 0.55 ± 0.0821 |
| Mean Optical Thickness | 2.27 ± 0.600 | 1.85 ± 0.278 | 2.60 ± 0.721 | 1.89 ± 0.356 | 2.02 ± 0.441 | 1.78 ± 0.493 |
| Mean Optical Volume | 2463.70 ± 859 | 1836.71 ± 879 | 1837.82 ± 314 | 2307.02 ± 382 | 2322.16 ± 1568 | 1484.27 ± 344 |
| Mean Migration | 87.35 ± 33.8 | 85.29 ± 15.7 | 57.21 ± 30.0 | 50.91 ± 11.1 | 67.78 ± 16.8 | 65.19 ± 11.7 |
| Mean Motility | 151.91 ± 60.2 | 180.34 ± 37.9 | 156.11 ± 71.1 | 139.10 ± 36.0 | 151.87 ± 49.2 | 129.04 ± 17.5 |
| Motility Speed | 26.95 ± 9.59 | 31.78 ± 8.58 | 17.01 ± 9.71 | 18.15 ± 3.27 | 21.84 ± 4.08 | 20.07 ± 3.25 |

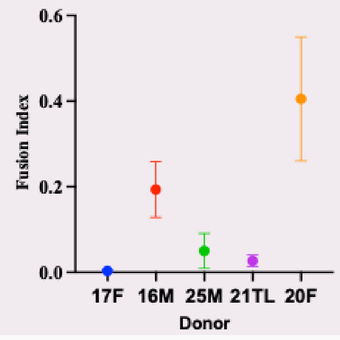
B

| Characteristic | 17F | 16M | 25M | 35M | 20F | 21TL |
|---|---|---|---|---|---|---|
| Mean Area | 1538.67 ± 163 | 1008.82 ± 97.0 | 738.91 ± 280 | 1377.91 ± 460 | 1049.58 ± 227 | 1080.66 ± 286 |
| Mean Eccentricity | 0.78 ± 0.0331 | 0.79 ± 0.0311 | 0.81 ± 0.0977 | 0.80 ± 0.0420 | 0.80 ± 0.0709 | 0.74 ± 0.0719 |
| Mean Irregularity | 0.61 ± 0.0287 | 0.54 ± 0.0319 | 0.54 ± 0.0935 | 0.54 ± 0.0430 | 0.58 ± 0.0300 | 0.62 ± 0.0501 |
| Mean Optical Thickness | 1.80 ± 0.315 | 1.84 ± 0.373 | 2.38 ± 0.597 | 1.84 ± 0.102 | 2.13 ± 0.453 | 1.32 ± 0.303 |
| Mean Optical Volume | 2703.35 ± 438 | 1739.79 ± 278 | 1682.47 ± 780 | 2567.47 ± 805 | 2327.59 ± 644 | 1398.19 ± 276 |
| Mean Migration | 120.71 ± 35.8 | 93.81 ± 26.3 | 57.56 ± 22.2 | 65.68 ± 22.9 | 73.82 ± 17.7 | 67.51 ± 13.4 |
| Mean Motility | 241.92 ± 60.9 | 225.07 ± 75.2 | 215.84 ± 104 | 204.96 ± 52.7 | 187.37 ± 36.6 | 141.31 ± 40.1 |
| Motility Speed | 32.27 ± 2.74 | 29.59 ± 3.53 | 23.55 ± 9.31 | 21.03 ± 5.76 | 19.77 ± 3.54 | 19.58 ± 3.18 |

## 5. Results: Differentiation

- The fusion index was quantified for each donor resulting in indices ranging from 0.34% ± 0.23 for donor 17F to 40.52% ± 14.4 for donor 20F (Fig. 3). This highlights the high heterogeneity in differentiation potential between SMDC acquired from different donors.

Fig. 3: Mean fusion index ± standard deviation for each donor (except 35M) after 7 days of differentiation. One-way ANOVA confirmed statistically significant differences between means ($p < 0.0001$).




## 6. Results: Correlations Between Pre-Differentiation Features and Fusion Index

The characteristics eccentricity (r = 0.8), irregularity (r = -0.67) and optical thickness (r = 0.6) exhibited moderate correlation with the fusion index, while all other characteristics only showed low or no correlation (Table 2).

Table 2: Spearman correlation coefficients (r) with associated p-values for the relationship between cell characteristics and fusion index across various timepoints and total mean.

| Characteristic | 12h (r, p) | 24h (r, p) | 36h (r, p) | Total mean (r, p) |
|---|---|---|---|---|
| Mean Cell Area | −0.10, 0.95 | −0.20, 0.78 | −0.60, 0.35 | −0.20, 0.78 |
| Mean Eccentricity | 0.53, 0.47 | 0.62, 0.30 | 0.60, 0.35 | 0.80, 0.13 |
| Mean Irregularity | 0.00, > 0.99 | −0.21, 0.77 | −0.56, 0.40 | −0.67, 0.27 |
| Mean Optical Cell Thickness | −0.10, 0.95 | 0.10, 0.95 | 0.60, 0.35 | 0.30, 0.68 |
| Mean Optical Cell Volume | −0.10, 0.95 | −0.10, 0.95 | 0.00, > 0.99 | 0.00, > 0.99 |
| Mean Cell Migration | −0.20, 0.78 | −0.20, 0.78 | −0.20, 0.78 | −0.20, 0.78 |
| Mean Cell Motility | 0.20, 0.78 | −0.30, 0.68 | −0.30, 0.68 | −0.40, 0.52 |
| Mean Cell Motility Speed | 0.10, 0.95 | 0.10, 0.95 | −0.30, 0.68 | −0.10, 0.95 |

## 7. Conclusion

Evaluating SMDC during early proliferation using the HoloMonitor® might enable prediction of myotube formation, despite statistical significance not being achieved due to small sample size. This study has created a solid basis for creating a workflow to evaluate the therapeutic potential of donor-derived SMDC.

**References:** 1. O. Boyer et al., Annals of Surgery, 2018, 267(3), 10.1097/sla.0000000000002268; 2. R. J. Jankowski et al., International Urology and Nephrology, 2018, 50(12), 10.1007/s11255-018-2005-8; 3. K. M. Peters et al., Journal of urology : official journal of the American Urological Association, Inc., 2014, 192(2), 10.1016/j.juro.2014.02.047.

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