Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biomedical Engineering

Shape up before you ship out: morphology as a potential critical quality attribute for cellular therapies

Kanupriya R. Daga[1,2,a], Priyanka Priyadarshani[1,2,a], Andrew M. Larey[1,2,a], Kejie Rui[1,2], Luke J. Mortensen[1,2] and Ross A. Marklein[1,2]

Abstract

Cell therapies have been explored for regenerative medicine; however, this immense promise has been met with limited clinical success. While many clinical trials aim to demonstrate product safety and efficacy, a number of issues remain related to product heterogeneity that must be addressed in order to fully realize the potential of cell therapies. A critical unmet need in cell manufacturing is a lack of critical quality attributes (CQAs) that predict the quality of different cell products. To address this need, researchers have begun exploring the potential of morphological profiling, using various imaging approaches and analytical tools, to identify morphological features that could serve as CQAs and enable better quality control of cell manufacturing, and thus improved clinical outcomes. Here, we present recent efforts that successfully use morphology as a CQA, as well as identify other potential uses of morphological profiling to improve cell manufacturing and ultimately accelerate clinical translation.

Addresses

[1] School of Chemical, Materials and Biomedical Engineering, University of Georgia, Athens, GA, USA

[2] Regenerative Bioscience Center, Rhodes Center for ADS, University of Georgia, Athens, GA, USA

Corresponding author: Marklein, Ross A (ross.marklein@uga.edu)

[a] These authors contribute equally.

Current Opinion in Biomedical Engineering 2021, **20**:100352

This review comes from a themed issue on **Molecular & Cellular Eng: Cell and Gene Therapy Manufacturing**

Edited by **Krishnendu Roy**

For complete overview of the section, please refer the article collection - Molecular & Cellular Eng: Cell and Gene Therapy Manufacturing

Received 11 June 2021, revised 10 September 2021, accepted 15 September 2021

https://doi.org/10.1016/j.cobme.2021.100352

Keywords

Cell Therapies, High Content Imaging, Critical Quality Attributes, Regenerative Medicine, Morphological Profiling.

Introduction

Although the promise of advanced cell therapies has been apparent for decades, there are very few Food and Drug Administration-approved cell therapy products (21) compared with more traditional pharmaceutical drug products. Most biologics are cord blood-derived products for hematopoietic cell transplantation and engineered T cells for treating a limited number of cancers (e.g. lymphoma) [1]. To fully realize the potential of cell therapies, a number of challenges must be addressed to ensure high quality (i.e. safe and effective) cell therapies become licensed and available for widespread patient use.

- Functional heterogeneity: defined here as differences in therapeutic function of cells derived from different donors, tissue sources, and manufactured under myriad conditions.
- Lack of reliable critical quality attributes (CQAs): the CQA is defined by the International Conference on Harmonisation (ICH) guideline ICH Q8 (R2) as 'a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.' [2] Combined with inherent functional heterogeneity, a lack of reliable CQAs makes it difficult to predict how a given product will perform before patient administration.
- Scale-up: As cell therapy doses can range from 10^7—10^9 cells per patient [3], significant manufacturing and scaling are necessary (in many cases *ex vivo*) to achieve adequate doses for a single patient (autologous) or many patients (allogeneic).
- Unknown effects of manufacturing changes: related to scale-up, changes in manufacturing such as different culture media, culture vessels, isolation techniques, cryoprotectants, and so on can have significant effects on product quality. Demonstrating product comparability after major manufacturing changes during clinical trials has been difficult owing to a lack of CQAs and understanding of how these changes impact quality.

Identification of meaningful CQAs could significantly accelerate translation of cell therapies as these CQAs would help address many of the other challenges, for example, CQAs to identify 'good donors' versus 'bad donors' or CQAs to demonstrate comparability of cell-derived products when switching from one manufacturing condition (e.g. reagent) to another. Efforts to identify novel CQAs using omics approaches

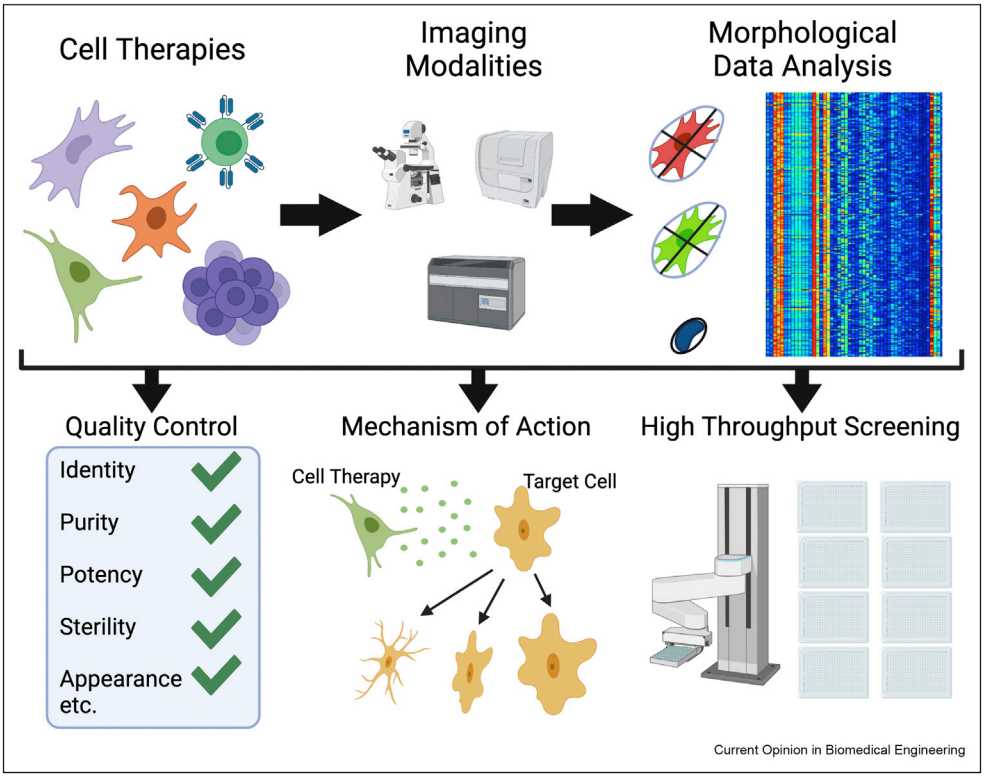
(population-based or single cell-based) have yielded promising candidate CQAs, but these approaches can be costly, require significant data processing, cannot be performed in a standard cell manufacturing laboratory, and in many cases are not high throughput. Cell morphology has emerged as a promising candidate CQA as it can effectively represent a visual readout of intracellular signaling. This review serves to illustrate how morphological profiling can be used to not only improve cell therapy quality control but also aid in elucidating mechanism of action (MoA) and guiding future manufacturing strategies (Figure 1).

Morphological profiling — current state of the art

High content imaging (HCI) is a powerful technique that can acquire images in a high-throughput manner using automated microscopy. Morphological profiling most commonly refers to the shape and intensity distributions of cells or subcellular components (e.g. organelles). Owing to the broad availability and diversity of fluorophores, ease of cell segmentation and imaging speed, widefield fluorescent imaging has been the most used approach to acquire morphology of cells and their organelles. However, advances in image quality and image processing have led to the broader use of label-free contrast modes such as brightfield and phase contrast imaging techniques that can potentially enable nondestructive imaging of live cultures [4]. Without disruption of cellular processes, nondestructive imaging is a cost-effective technique to monitor and control the quality of manufactured cells.

Morphological profiling enables quantification of features including staining intensities, textural patterns, size, and shape of the labeled cellular structures, as well as spatial relationships between cells and among subcellular structures [5]. Morphological profiling performed on fixed samples imaged using fluorescent microscopy provides high resolution, well-defined approaches for extracting morphological data; however, this approach is destructive and typically only provides a snapshot of the current cell state. Therefore, label-free, nondestructive imaging strategies are being developed to enable in-process monitoring of cells, which affords greater feedback control. Besides widely studied brightfield and phase contrast imaging techniques [6,7], quantitative oblique back-illumination microscopy was developed in previous studies to separate white blood cells from umbilical cord blood collection bags in a noninvasive manner [8,9].

Figure 1



Morphological profiling as a tool to accelerate translation of cellular therapies. Cellular therapies can be imaged using different types of imaging modalities to extract morphological data. The morphological data can then be used to assess cell quality, mechanism of action, or in a high-throughput manner to systematically screen manufacturing conditions that can impact final cell quality. Figure created using BioRender.

Furthermore, autofluorescence imaging and lifetime measurement are promising label-free nondestructive strategies to accurately classify T-cell activation as well as mesenchymal stromal cell (MSC) senescence under controlled expansion conditions by correlating MSC morphology with differentiation potential [10–12]. Emerging strategies have explored the potential of moving into 2-photon phenomena using multiphoton autofluorescence intensity and lifetime imaging to measure T-cell activation [11] and second-harmonic generation of collagen production for osteogenic differentiation. Finally, high-throughput imaging systems based on flow cytometry are being used to enable morphological profiling (and sorting) of large numbers ($>10^6$) of cells, which would enable imaging of suspension cells, as well as adherent cells harvested during the manufacturing process, for example, during passaging or before cryopreservation [13].

After acquiring a large image set using HCI, image preprocessing should be performed to obtain analyzable and quantitative information. General strategies to recover image features include illumination correction, noise reduction, and background subtraction. After image preprocessing, the use of open-source image analysis software (e.g. CellProfiler, CellCognition Explorer, BioImageXD) enables custom, automated segmentation, and assessment of quantitative morphological data from thousands of images at subcellular, single-cell, or population levels [14,15]. These image analysis approaches produce large, multivariate morphological data sets that are then filtered and/or merged and analyzed using dimensionality reduction techniques (e.g. t-distributed stochastic neighbor embedding [16], principal component analysis [17], uniform manifold approximation, and projection [11]), which are necessary to effectively characterize morphological responses to microenvironmental cues in the context of cell manufacturing.

In short, with destructive and nondestructive imaging techniques as well as advanced computational analysis, cell morphological data sets can be used to reliably and reproducibly identify cell quality and subpopulations with desired properties, such as proliferation or immune-suppressive function, in an effort to improve cell manufacturing and clinical outcomes [18].

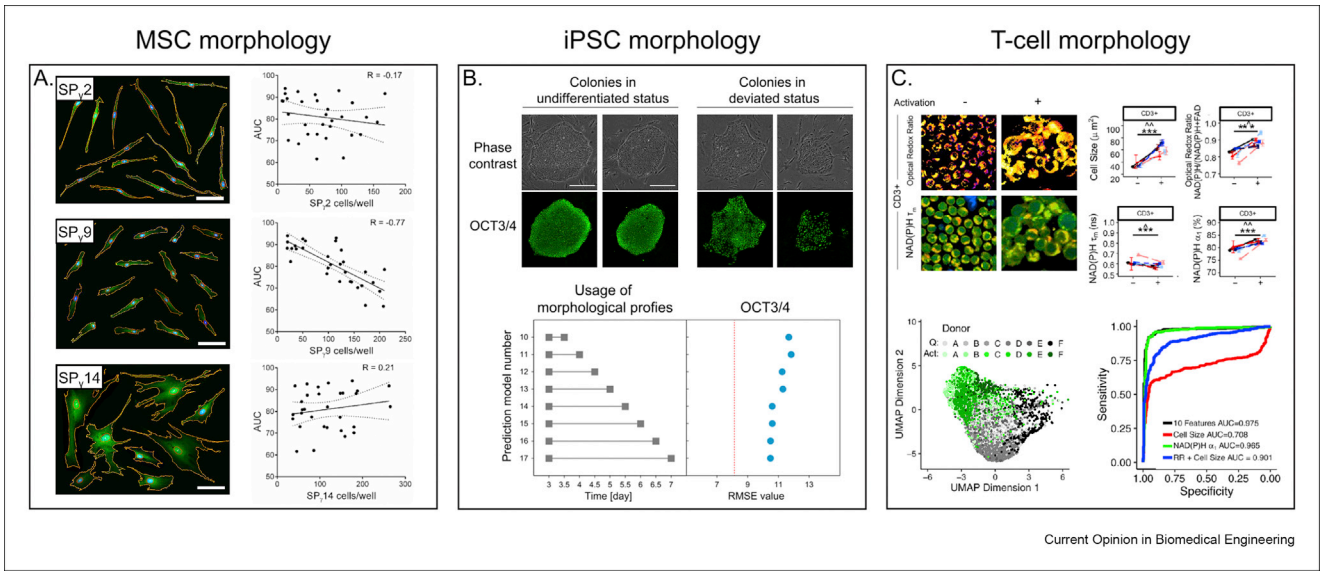
Morphology as a predictor of cell quality

The heterogeneity and highly responsive nature of cells necessitate rigorous quality control measures to be implemented throughout manufacturing. By not addressing cellular heterogeneity, there could be major safety concerns (such as tumorigenesis) or efficacy concerns (i.e. minimal therapeutic effect) [19]. To mitigate these concerns, efforts are being made to identify robust CQAs that are predictive of cell quality. Cell evaluation techniques that assess traditional biological outputs such as protein or gene expression are very commonly used to determine cellular behavior and predict CQAs [20]. However, conventional biological analytical techniques are invasive, which requires disruption of cellular processes. Moreover, such analytical techniques are also costly and time-consuming [20,21]. There is an emerging need for flexible, cost-effective techniques to efficiently control the cell processing variables and manufacturing of reproducible high-quality cell-based products. Recent advances in imaging techniques have presented image-based quality assessment as an attractive alternative to investigate cell phenotypes and make inferences about cell quality. Image-based analysis is being successfully implemented in different research laboratories and clinical settings combined with (or independent of) more common biological analytical techniques to assess cell quality [22,23].

Image processing technologies are being developed to obtain single-cell and multicellular morphological data that could be predictive of cell quality [24,25]. Using the widely investigated cell therapy candidate MSCs as an example, Marklein et al. [16] developed a machine learning-based approach for assessing the functional heterogeneity in MSCs based on identification of morphological subpopulations (Figure 2a). It was discovered that the amount of a specific morphological subpopulation, when stimulated with a functionally relevant inflammatory cytokine (interferon-gamma), could predict the immunosuppressive capacity of MSCs from multiple donors/passages. Imboden et al. [17] related differences in MSC morphology with eight common surface markers using label-free imaging combined with artificial intelligence-based methods (deep convolutional neural network). In another study, nondestructive, label-free assessment of induced pluripotent stem cell (iPSC) colony morphology was performed to examine the quality (i.e. expression of pluripotent markers) of iPSCs during culture. The use of time-course imaging process for tracking iPSC colonies helped to identify the morphological parameters that can be used for monitoring of iPSC loss of pluripotency (as indicated by Oct3/4 staining) (Figure 2b) [26]. Using another nondestructive imaging modality, label-free autofluorescence lifetime imaging, Walsh et al. [11] were able to determine the heterogeneity in T-cell population activation, which could help in the prediction of T cell immunotherapy function (Figure 2c). These studies demonstrate that functionally relevant morphological parameters provide insight into identify novel CQAs for therapeutic cell manufacturing.

The relationship between cell morphology and function has been significantly strengthened owing to identification of mechanotransduction regulators, for example, yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ). YAP/TAZ can

Figure 2



Morphology predicts cell quality of MSCs, iPSCs, and T cells (**a**) Representative images of IFN-γ-stimulated MSC distinct morphological subpopulations. MSC subpopulations correlate strongly with overall immunosuppressive capacity. (Scale bar: 100 μm) ($P < 0.05$). Reproduced with permission from (Marklein et al., 2019) (**b**) Representative images of iPSC colony unstained (phase-contrast imaging) and green stained with OCT3/4 (fluorescence image). Blue plots indicate the root mean square error (RMSE) values of Oct3/4 staining area predictions. iPSC colony morphology is highly correlated with the undifferentiated marker in the final stage, with a lower correlation in the initial stages. Scale bar: 400 μm. Reproduced with permission from (Yoshida et al., 2019) (**c**) The representative optical redox ratio and NAD(P)H τm autofluorescence images of quiescent (columns 1, 3) and activated (columns 2, 4) CD3+ T cells from two different donors. Autofluorescence lifetime imaging classified quiescent and activated T-cells based on function (Scale bar: 20 μm), (***p < 0.001). Reproduced with permission from (Walsh et al., 2021). IFN-γ, interferon-gamma; iPSC, induced pluripotent stem cell;

regulate stem cell self-renewal, maintenance of pluripotency, and differentiation [27,28]. In a study by Wang et al. substrate stiffness and micropatterning-altered YAP/TAZ activation and MSC differentiation. Specifically, MSCs seeded on stiff substrates had larger surface areas and higher nuclear localization of YAP/TAZ, whereas MSCs seeded on soft substrates adopted more rounded morphology, lower spread areas, and had cytoplasmic localization of YAP/TAZ [29]. In another study, surface topography (in the form of structured grooves) impacted overall iPSC colony shape, which was guided by single-cell localization of both pluripotent markers (Oct4/nanog) and YAP/TAZ intracellular localization [30]. Considering the critical role of YAP/TAZ in maintenance of the stemness and phenotype of stem cells, Bonnevie et al. developed a neural network model that integrates morphology, YAP/TAZ signaling, and biochemical cues of multiple cell types to measure the cell behavior at a single-cell level [31].

Advances in cell image analysis provide a unique opportunity to associate morphological responses and biological processes to predict cell growth and differentiation, as well as cell responses to changes in their microenvironment that are manufacturing conditions. These tools could be used to better understand functional heterogeneity of cell therapies to identify CQAs for cell manufacturing [19,25]. Moving forward, a transition toward nondestructive, automated imaging may be necessary as in-process sampling could enable real-time monitoring of manufacturing parameters to better meet desired specifications and identification of 'out of spec' or 'poor' batches of cells.

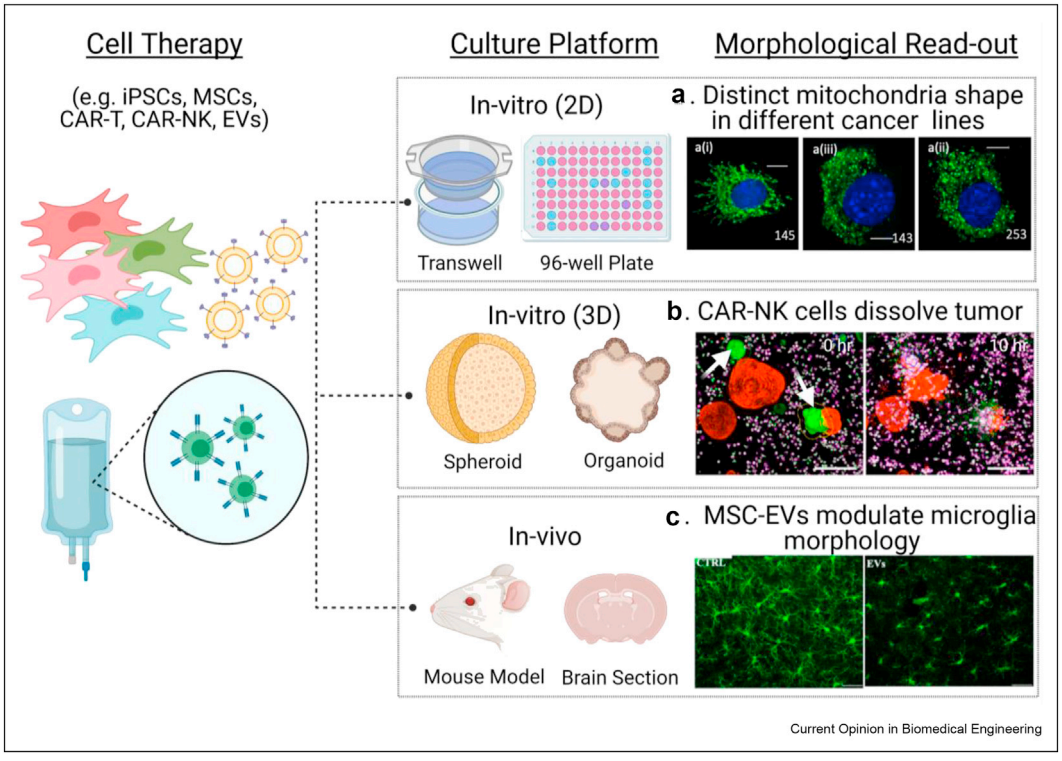
Elucidating mechanism of action based on cell morphology

Besides characterizing properties of the cell therapies themselves, it is crucial to develop potency assays that can be indicative of a therapeutically relevant function that is MoA. This MoA can take the form of modulating a specific target cell type (or multiple cell types) associated with a particular disease. As heterogeneity is a hallmark of all cellular systems [32], there is a need to develop improved approaches to assess cell heterogeneity with respect to target cell types to better understand how cell therapies exert their therapeutic effect. Advanced single-cell profiling based on genomic and proteomic technologies has revealed great insight; however, these approaches require significant resources in terms of computational infrastructure, equipment, and materials [33,34]. Morphological profiling using HCI provides a cost-effective, high-throughput approach to profile target cells under healthy and diseased conditions as a potency assay to determine cell

therapy MoA. This is predicated on the fact that many cell types possess distinct morphologies that enable them to function properly or improperly in healthy and diseased conditions, respectively. For instance, brain-resident microglia transition from a ramified morphology to an ameboid morphology in response to neuro-inflammation [35]. Similarly, tumor metastasis also leads to morphological changes in cancer cells and can be used as prognostic markers to indicate the cell state [36–38]. In addition, distinct morphological responses were observed for multiple neuronal cell types (microglia, astrocytes, neurons) in a porcine model of ischemic stroke [39].

Morphological profiling as a readout of cell therapy potency can be performed in multiple formats: *in vitro* (two-dimensional [2D] and three-dimensional [3D]); and *in vivo* (Figure 3). *In vitro* 2D cultures present the most simplified platform for morphological assessment of individual cells and their subcellular structures, allowing preliminary screens of target therapeutics in a high-throughput manner. For instance, cancer cells defined in terms of their mitochondrial morphology (Figure 3a) [40] can be treated with potential cell therapies to reveal the activated pathways and thus the MoA. Transwell systems, such as an air-liquid interface model using human small airway epithelial cells, allow for modeling the tissue microenvironment in 2D toward a clinically relevant outcome involving multiple cell types [41]. Although 2D morphology may not fully reflect morphology observed *in vivo*, it can provide valuable insight into the various phenotypic attributes that can be further validated and explored in more complex *in vitro* models and *in vivo*. Morphological profiling of more complex *in vitro* cultures, such as 3D spheroids or organoids, may have greater clinical relevance owing to recapitulation of the *in vivo* microenvironment. As shown by Schnalzger et al. chimeric antigen receptor (CAR) natural killer 92 cell specificity and efficiency can be tested in a tumor organoid coculture system [42] through serially combining z-stack images of the complete 3D structure (Figure 3b). Nonetheless, the complexity

Figure 3



Morphological screening of therapeutic cells viable across all culture platforms. (**a**). Representative images of KPDC145 (Drp1–/–), KPDC253 (Drp1+/+), and KPDC143 (Drp1+/–). Qualitative analysis of mitochondrial morphology indicates that KDPC145 is morphologically distinct from KPDC253 and KPDC143. Scale bars: 10 μm. Reproduced with permission from (Rohani et al., 2020). (**b**). Exemplary data with DsRED-expressing normal organoids (red), GFP-expressing EGFRvIII-positive tumor organoids (green), and anti-CD45-APC-labeled EGFRvIII-CAR NK 92 cells (magenta) at 0 h and 10 h of coculture. Maximum intensity projections are shown. Outlines of tumor organoids are automatically detected. Scale bars: 200 μm. Reproduced with permission from (Schnalzger et al., 2019). (**c**). Representative image of microglia cells stained for Iba-1 in medial hippocampus CA1 of control (CTRL) and EV-treated (EVs) 3xTg Alzheimer's Disease (AD) mice. Scale bars = 30 μm. Reproduced with permission from (Losurdo et al., 2020). Figure created using BioRender.

involved with establishing the right microenvironment as well as the extracellular matrix is a significant challenge for organoid-based cultures and may not be transferable between cell types. Morphology as a readout of *in vivo* therapeutic performance has been successfully implemented, for example, using a mouse model of Alzheimer's disease by quantifying microglia morphology after treatment with MSC extracellular vesicles (Figure 3c) [43]. However, *in vivo* studies cannot be performed in a rapid, high-throughput manner, and it is likely that a combination of screening methods using different culture platforms will be needed to determine MoA. This information can then be used to inform the manufacturing of cell therapies (in combination with CQAs) to more effectively and reproducibly target cell types associated with specific diseases.

High-throughput screening of morphological responses

Advances in HCI and computing have enabled image-based high-throughput screening (HTS) of cell phenotypes. Inspired by drug screening approaches, this high-throughput morphological profiling combines simultaneous testing of millions of perturbations (genetic, chemical) with broad morphological outputs, allowing functionally similar grouping of treatments and cell responses [44,45]. The ability of high-throughput morphological profiling to rapidly assess the response of cells to different treatments makes the technique desirable in the context of cell manufacturing. A typical HTS workflow consists of cell culture and plating in multiple well plates; high-throughput, multichannel image acquisition; high content morphological feature extraction; and hit identification, the last two steps often performed using machine learning [46]. One such well-recognized assay is the cell painting assay, which uses six fluorescent dyes to stain eight broadly applicable cellular components and organelles for profiling with 1500 morphological features per cell [5].

High-throughput morphological screens have been applied in a cell manufacturing context to assess the identity and potency of multiple cell types. For iPSCs, unstained and unlabeled live murine iPSCs were screened for the pluripotency level using a novel 'phase distribution' imaging system. Known mitochondrial structural changes related to functional changes in metabolic shifts from oxidative phosphorylation to glycolysis when reprogramming somatic cells to iPSCs allowed intracellular, organelle-based morphological profiling analogous to whole-cell morphological profiling. Phase distribution imaging distinguished and quantified the degree of somatic cell reprogramming and, conversely, iPSC differentiation, serving as a screening tool to identify higher quality (i.e. greater pluripotency) iPSCs before more rigorous biological assays [47]. High-throughput morphological profiling has been used to efficiently group functional responses to treatments such as growth factors and material cues as well [48,49]. Notably, the phenotypic response of MSCs to 2176 surface

Table 1

Candidate morphological features and biomarkers for screening applications.

Morphological Feature	Definition	Biomarker	Definition
Mean radius [7]	The median distance of any pixel in the object to the closest pixel outside of the object	Type IIα1 collagen [7]	Chondrogenic marker gene
Circularity (form factor) [7,16,36]	Calculated as 4*π*Area/Perimeter2	Oct-3/4 [20]	Master regulator of pluripotency transcription factor
Aspect (length-width) ratio [16,21,36] [37]	Ratio of the number of pixels in the major axis to the minor axis of the best fitting ellipse to the mask	Caspase 3/7 [48]	Apoptosis marker
Major axis length [21]	The number of pixels in the major axis of the best fitting ellipse to the mask	GFAP [21,39]	Astrocyte surface marker
Minor axis length [21]	The number of pixels in the minor axis of the best fitting ellipse to the mask	VCAM-1 [49]	Cellular adhesion marker gene
Area [16,36] [37]	Number of pixels in the mask	TNF-α [22]	Activated macrophage-secreted cytokine
Compactness [16]	The mean squared distance of the object's pixels from the centroid divided by the area	IL7R [38]	Interleukin receptor that plays a role in cancer progression
Perimeter [16]	The total number of pixels (2D) or voxels (3D) around the boundary of each region in the image	YAP/TAZ [30,31]	Proliferation transcription factor

TAZ, transcriptional coactivator with PDZ-binding motif; YAP, yes-associated protein.

topographies was investigated in terms of migration, proliferation, protein synthesis, apoptosis, and differentiation using quantitative image analysis after clustering the surfaces into 28 archetypical cell shapes. Transcriptomics analysis revealed a strong link between cell shape, molecular signatures, and phenotype, meaning manipulation of cell shape may be a useful route for achieving different molecular signatures and functions [49]. Considering the relationship between cell shape and molecular readout, Table 1 shows examples of morphological features and biomarkers associated with function that could be used in screening applications. These morphological features and biomarkers can be analyzed at a subcellular, cellular, and population level to better understand cellular heterogeneity and provide mechanistic insight into how manufacturing can impact cell function.

High-throughput morphological profiling is widely used for prediction of treatment efficacy — screening target cell morphological response to therapy to inform efficacious therapies and MoA. Wu et al. probed cancer cell heterogeneity and MoA using 216 morphological features. The results show single cell-derived clone morphological traits are heritable and correlate with specific genomic and transcriptomic phenotypes. Furthermore, unsupervised clustering of morphological subtypes predicts tumorigenic and metastatic potential in an *in vivo* mouse model of breast cancer [38]. CRISPR technologies have enabled genome-scale, multivariate gene perturbation morphological screens. Thousands of perturbations were screened and related to phenotypic changes in cellular morphology or the staining pattern of a marker of the nuclear pore complex in both HeLa and U2OS cells [50]. Such HTS approaches have been commonly adapted to iPSC-derived and cancer organoids to retain features of complex physiology and disease while still allowing screening at scale. For instance, iPSC-derived kidney organoid culture conditions were screened with fluorescent staining to enhance differentiation. Subsequently, genetically induced polycystic kidney disease organoids were screened with multiple compounds, revealing a role of myosin in polycystic kidney disease. Thus, in this study, HTS was used first for manufacturing of organoids and then for target response to treatment [51].

Conclusion

HCI shows increasing promise to fulfill an unmet need for robust and simple measurement of CQAs of cell therapeutics. As we have discussed in this article, imaging-based readouts of cell shape, organelle distribution, and metabolism — broadly referred to as cell morphology — are associated with a range of relevant biomanufacturing outcomes. Offline cell potency assays are one critical area of need and morphological imaging (in 2D and 3D) can provide valuable insight into *in vitro* or even *in vivo* outcomes, for instance, by imaging the anti-cancer effect of CAR-T cells in tumor organoid cultures. Other emerging functional applications of HCI include elucidating the method of action of intrinsically multifunctional cell therapeutics. Much of the current cutting-edge HCI promises to move toward integration with manufacturing pipelines by identifying quality attributes such as differentiation, cell activation, and immunomodulatory subpopulations across iPSCs, MSCs, and T-cells. However, challenges and opportunities remain in idevntifying key targets and desired outcomes, as well as in organizing and integrating data sets across manufacturing platforms. Furthermore, this work exists at the nexus of many fields; requiring collaboration between data scientists, imaging hardware development experts, biomanufacturing specialists, and clinical end-users to maximize the potential impact on cell therapies. As technologies advance from labeled off-line analysis strategies to inline, nondestructive label-free imaging outcomes, HCI is poised to transform the cell biomanufacturing field and human health.

Author contributions

KRD: Investigation, Visualization, Writing – Original Draft, Review & Editing. AML: Investigation, Writing – Original Draft, Review & Editing. PP: Investigation, Visualization, Writing – Original Draft, Review & Editing. KR: Investigation, Writing – Original Draft, Review & Editing. LJM: Supervision, Writing – Original Draft, Review & Editing. RAM: Conceptualization, Project Administration, Investigation, Visualization, Supervision, Writing – Original Draft, Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

KRD, AML, and PP are supported through the National Science Foundation Engineering Research Center for Cell Manufacturing Technologies No. EEC-1648035. KR is supported through NSF No. CBET-2040118.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. US Food and Drug Administration: ***Approved cellular and gene therapy products***. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products.

2. US Food and Drug Administration: ***Guidance for industry: Q8(R2) pharmaceutical development***. https://www.fda.gov/media/71535/download.

3. Olsen TR, Ng KS, Lock LT, Ahsan T, Rowley JA: **Peak MSC-are we there yet?** *Front Med* 2018, **5**.

4. Toda K, Tamamitsu M, Nagashima Y, Horisaki R, Ideguchi T: **Molecular contrast on phase-contrast microscope**. *Sci Rep* 2019, **9**:1–7.

5. Bray MA, Singh S, Han H, Davis CT, Borgeson B, Hartland C, Kost-Alimova M, Gustafsdottir SM, Gibson CC, Carpenter AE: **Cell Painting, a high-content image-based assay for morphological profiling using multiplexed fluorescent dyes**. *Nat Protoc* 2016, **11**:1757–1774.

6. Schaub NJ, Hotaling NA, Manescu P, Padi S, Wan Q, Sharma R, George A, Chalfoun J, Simon M, Ouladi M, *et al.*: **Deep learning predicts function of live retinal pigment epithelium from quantitative microscopy**. *J Clin Invest* 2020, **130**:1010–1023.

7. Lam J, Bellayr IH, Marklein RA, Bauer SR, Puri RK, Sung KE: **Functional profiling of chondrogenically induced multipotent stromal cell aggregates reveals transcriptomic and emergent morphological phenotypes predictive of differentiation capacity**. *Stem Cells Trans Med* 2018, **7**:664–675.

8. Costa PC, Ledwig P, Bergquist A, Kurtzberg J, Robles FE: **Noninvasive white blood cell quantification in umbilical cord blood collection bags with quantitative oblique back-illumination microscopy**. *Transfusion* 2020, **60**:588–597.

9. Ledwig P, Sghayyer M, Kurtzberg J, Robles FE: **Dual-wavelength oblique back-illumination microscopy for the non-invasive imaging and quantification of blood in collection and storage bags**. *Biomed Opt Express* 2018, **9**:2743–2754.

10. Wang ZJ, Walsh AJ, Skala MC, Gitter A: **Classifying T cell activity in autofluorescence intensity images with convolutional neural networks**. *J Biophot* 2020, **13**.

11. ** Walsh AJ, Mueller KP, Tweed K, Jones I, Walsh CM, Piscopo NJ, Niemi NM, Pagliarini DJ, Saha K, Skala MC: **Classification of T-cell activation via autofluorescence lifetime imaging**. *Nat Biomed Eng* 2021, **5**:77–78.

This paper demonstrates the suitability of a non-destructive imaging approach based on autofluorescence lifetime imaging to effectively classify T cell activation. This approach could be readily incorporated into cell manufacturing of T cell immunotherapies (such as CAR T cells) to monitor in-process cell quality and provide feedback or select for lots with desired (or undesired) properties.

12. Samimi K, Guzman EC, Trier SM, Pham DL, Qian TC, Skala MC: **Time-domain single photon-excited autofluorescence lifetime for label-free detection of T cell activation**. *Opt Lett* 2021, **46**:2168–2171.

13. Isozaki A, Mikami H, Tezuka H, Matsumura H, Huang K, Akamine M, Hiramatsu K, Iino T, Ito T, Karakawa H, *et al.*: **Intelligent image-activated cell sorting 2.0**. *Lab Chip* 2020, **20**:2263–2273.

14. Kang MS, Cha E, Kang E, Ye JC, Her NG, Oh JW, Nam DH, Kim MH, Yang S: **Accuracy improvement of quantification information using super-resolution with convolutional neural network for microscopy images**. *Biomed Signal Process Contr* 2020, **58**.

15. Sommer C, Hoefler R, Samwer M, Gerlich DW: **A deep learning and novelty detection framework for rapid phenotyping in high-content screening**. *Mol Biol Cell* 2017, **28**:3428–3436.

16. ** Marklein RA, Klinker MW, Drake KA, Polikowsky HG, Lessey-Morillon EC, Bauer SR: **Morphological profiling using machine learning reveals emergent subpopulations of interferon-gamma-stimulated mesenchymal stromal cells that predict immunosuppression**. *Cytotherapy* 2019, **21**:17–31.

This paper demonstrates the ability of morphological profiling to identify subpopulations of MSCs that correlate with a clinically-relevant function i.e. immunomodulation of T cells. The combination of high content imaging and machine learning enabled comprehensive profiling of MSCs from multiple donors and passages

17. * Imboden S, Liu XQ, Lee BS, Payne MC, Hsieh CJ, Lin NYC: **Investigating heterogeneities of live mesenchymal stromal cells using AI-based label-free imaging**. *Sci Rep* 2021:11.

This paper directly relates morphology of mesenchymal stromal cells with their multivariate surface marker expression to better understand single cell heterogeneity. This non-destructive approach lends great insight into cell heterogeneity that could be applied to other cell-types and therapeutic applications.

18. Mantripragada VP, Tan K-l, Vasavada S, Bova W, Barnard J, Muschler GF: **Characterization of heterogeneous primary human cartilage-derived cell population using non-invasive live-cell phase-contrast time-lapse imaging**. *Cytotherapy* 2020.

19. Sasaki H, Matsuoka F, Yamamoto W, Kojima K, Honda H, Kato R: **Image-based cell quality assessment: modeling of cell morphology and quality for clinical cell therapy**. In *Computational modeling in tissue engineering*. Edited by Geris L, Springer Berlin Heidelberg; 2013:207–226.

20. Smith D, Glen K, Thomas R: **Automated image analysis with the potential for process quality control applications in stem cell maintenance and differentiation** 2016, **32**:215–223.

21. Fujitani M, Huddin NS, Kawai S, Kanie K, Kiyota Y, Shimizu K, Honda H, Kato R: **Morphology-based non-invasive quantitative prediction of the differentiation status of neural stem cells**. *J Biosci Bioeng* 2017, **124**:351–358.

22. Pavillon N, Hobro AJ, Akira S, Smith NI: **Noninvasive detection of macrophage activation with single-cell resolution through machine learning**. *Proc Natl Acad Sci U S A* 2018, **115**:E2676–E2685.

23. Cheng S, Fu S, Kim YM, Song W, Li Y, Xue Y, Yi J, Tian L: **Single-cell cytometry via multiplexed fluorescence prediction by label-free reflectance microscopy**. *Sci Adv* 2021, **7**.

24. Imai Y, Yoshida K, Matsumoto M, Okada M, Kanie K, Shimizu K, Honda H, Kato R: **In-process evaluation of culture errors using morphology-based image analysis**. *Regener Therap* 2018, **9**:15–23.

25. Kerz M, Folarin A, Meleckyte R, Watt FM, Dobson RJ, Danovi D: **A novel automated high-content analysis workflow capturing cell population dynamics from induced pluripotent stem cell live imaging data**. *J Biomol Screen* 2016, **21**:887–896.

26. Yoshida K, Okada M, Nagasaka R, Sasaki H, Kanie K, Kato R: **Time-course colony tracking analysis for evaluating induced pluripotent stem cell culture processes**. *J Biosci Bioeng* 2019, **128**:209–217.

27. Pocaterra A, Romani P, Dupont S: **YAP/TAZ functions and their regulation at a glance**. *J Cell Sci* 2020:133.

28. Oliver-De La Cruz J, Nardone G, Vrbsky J, Pompeiano A, Perestrelo AR, Capradossi F, Melajová K, Filipensky P, Forte G: **Substrate mechanics controls adipogenesis through YAP phosphorylation by dictating cell spreading**. *Biomaterials* 2019, **205**:64–80.

29. Wang X, Hu X, Dulińska-Molak I, Kawazoe N, Yang Y, Chen GJSr: **Discriminating the independent influence of cell adhesion and spreading area on stem cell fate determination using micropatterned surfaces** 2016, **6**:1–13.

30. Abagnale G, Sechi A, Steger M, Zhou Q, Kuo C-C, Aydin G, Schalla C, Müller-Newen G, Zenke M, Costa IG, *et al.*: **Surface topography guides morphology and spatial patterning of induced pluripotent stem cell colonies**. *Stem Cell Rep* 2017, **9**:654–666.

31. Bonnevie ED, Ashinsky BG, Dekky B, Volk SW, Smith HE, Mauck RL: **Cell morphology and mechanosensing can be decoupled in fibrous microenvironments and identified using artificial neural networks**. *Sci Rep* 2021, **11**:5950.

32. Goldman SL, MacKay M, Afshinnekoo E, Melnick AM, Wu S, Mason CE: **The impact of heterogeneity on single-cell sequencing**. *Front Genet* 2019, **10**:8.

33. Manzoni C, Kia DA, Vandrovcova J, Hardy J, Wood NW, Lewis PA, Ferrari R: **Genome, transcriptome and proteome: the rise of omics data and their integration in biomedical sciences**. *Briefings Bioinf* 2018, **19**:286–302.

34. Suhre K, McCarthy MI, Schwenk JM: **Genetics meets proteomics: perspectives for large population-based studies**. *Nat Rev Genet* 2021, **22**:19–37.

35. Shahidehpour RK, Higdon RE, Crawford NG, Neltner JH, Ighodaro ET, Patel E, Price D, Nelson PT, Bachstetter AD:

Dystrophic microglia are associated with neurodegenerative disease and not healthy aging in the human brain. *Neurobiol Aging* 2021, **99**:19–27.

36. Alizadeh E, Castle J, Quirk A, Taylor CDL, Xu WL, Prasad A: **Cellular morphological features are predictive markers of cancer cell state**. *Comput Biol Med* 2020, **126**.

37. Hasan MR, Hassan N, Khan R, Kim YT, Iqbal SM: **Classification of cancer cells using computational analysis of dynamic morphology**. *Comput Methods Progr Biomed* 2018, **156**: 105–112.

38. Wu PH, Gilkes DM, Phillip JM, Narkar A, Cheng TWT, Marchand J, Lee MH, Li R, Wirtz D: **Single-cell morphology encodes metastatic potential**. *Sci Adv* 2020, **6**.

39. Spellicy SE, Scheulin KM, Baker EW, Jurgielewicz BJ, Kinder HA, Waters ES, Grimes JA, Stice SL, West FD: **Semi-automated cell and tissue analyses reveal regionally specific morphological alterations of immune and neural cells in a porcine middle cerebral artery occlusion model of stroke**. *Front Cell Neurosci* 2021:14.

40. Rohani A, Kashatus JA, Sessions DT, Sharmin S, Kashatus DF, Hacker Mito: **A set of tools to enable high-throughput analysis of mitochondrial network morphology**. *Sci Rep* 2020:10.

41. Bluhmki T, Bitzer S, Gindele JA, Schruf E, Kiechle T, Webster M, Schymeinsky J, Ries R, Gantner F, Bischoff D, *et al.*: **Development of a miniaturized 96-Transwell air-liquid interface human small airway epithelial model**. *Sci Rep* 2020, **10**.

42. Schnalzger TE, Groot MHD, Zhang CC, Mosa MH, Michels BE, Roder J, Darvishi T, Wels WS, Farin HF: **3D model for CAR-mediated cytotoxicity using patient-derived colorectal cancer organoids**. *EMBO J* 2019, **38**.

43. * Losurdo M, Pedrazzoli M, D'Agostino C, Elia CA, Massenzio F, Lonati E, Mauri M, Rizzi L, Molteni L, Bresciani E, *et al.*: **Intranasal delivery of mesenchymal stem cell-derived extracellular vesicles exerts immunomodulatory and neuroprotective effects in a 3xTg model of Alzheimer's disease**. *Stem Cells Trans Med* 2020, **9**:1068–1084.

In this study, morphological profiling of microglia was used to demonstrate the effect of mesenchymal stromal cell-derived extracellular vesicle potency in a mouse model of Alzheimer's. The change in microglia morphology was indicative of a greater therapeutic outcome and could be quantified on a single cell level.

44. Chandrasekaran SN, Ceulemans H, Boyd JD, Carpenter AE: **Image-based profiling for drug discovery: due for a machine-learning upgrade?** *Nat Rev Drug Discov* 2021, **20**:145–159.

45. ** Boyd J, Fennell M, Carpenter A: **Harnessing the power of microscopy images to accelerate drug discovery: what are the possibilities?** *Expet Opin Drug Discov* 2020, **15**:639–642.

This expert opinion review highlights the key aspects of high throughput screening using image-based approaches. While focused on drug discovery, this generalizable approach can be further applied to cell therapies to both inform manufacturing and better understand mechanism of action

46. Chessel A, Salas REC: **From observing to predicting single-cell structure and function with high-throughput/high-content microscopy**. *Single Cell Biol* 2019, **63**:197–208.

47. Nishimura K, Ishiwata H, Sakuragi Y, Hayashi Y, Fukuda A, Hisatake K: **Live-cell imaging of subcellular structures for quantitative evaluation of pluripotent stem cells**. *Sci Rep* 2019, **9**.

48. Way GP, Kost-Alimova M, Shibue T, Harrington WF, Gill S, Piccioni F, Becker T, Shafqat-Abbasi H, Hahn WC, Carpenter AE, *et al.*: **Predicting cell health phenotypes using image-based morphology profiling**. *Mol Biol Cell* 2021, **32**:995–1005.

49. Vasilevich AS, Vermeulen S, Kamphuis M, Roumans N, Eroumé S, Hebels DGAJ, Jvd Peppel, Reihs R, Beijer NRM, Carlier A, *et al.*: **On the correlation between material-induced cell shape and phenotypical response of human mesenchymal stem cells**. *Sci Rep* 2020, **10**:1–15.

50. de Groot R, Luthi J, Lindsay H, Holtackers R, Pelkmans L: **Large-scale image-based profiling of single-cell phenotypes in arrayed CRISPR-Cas9 gene perturbation screens**. *Mol Syst Biol* 2018, **14**.

51. Czerniecki SM, Cruz NM, Harder JL, Menon R, Annis J, Otto EA, Gulieva RE, Islas LV, Kim YK, Tran LM, *et al.*: **High-throughput screening enhances kidney organoid differentiation from human pluripotent stem cells and enables automated multidimensional phenotyping**. *Cell Stem Cell* 2018, **22**:929 [+].