Regulatory science insights into cellular products and practical microscale technologies for their assessment

Kyung Sung

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Cellular and Tissue Therapies Branch
Division of Cellular and Gene Therapies
Office of Tissues and Advanced Therapies
Center for Biologics Evaluation and Research

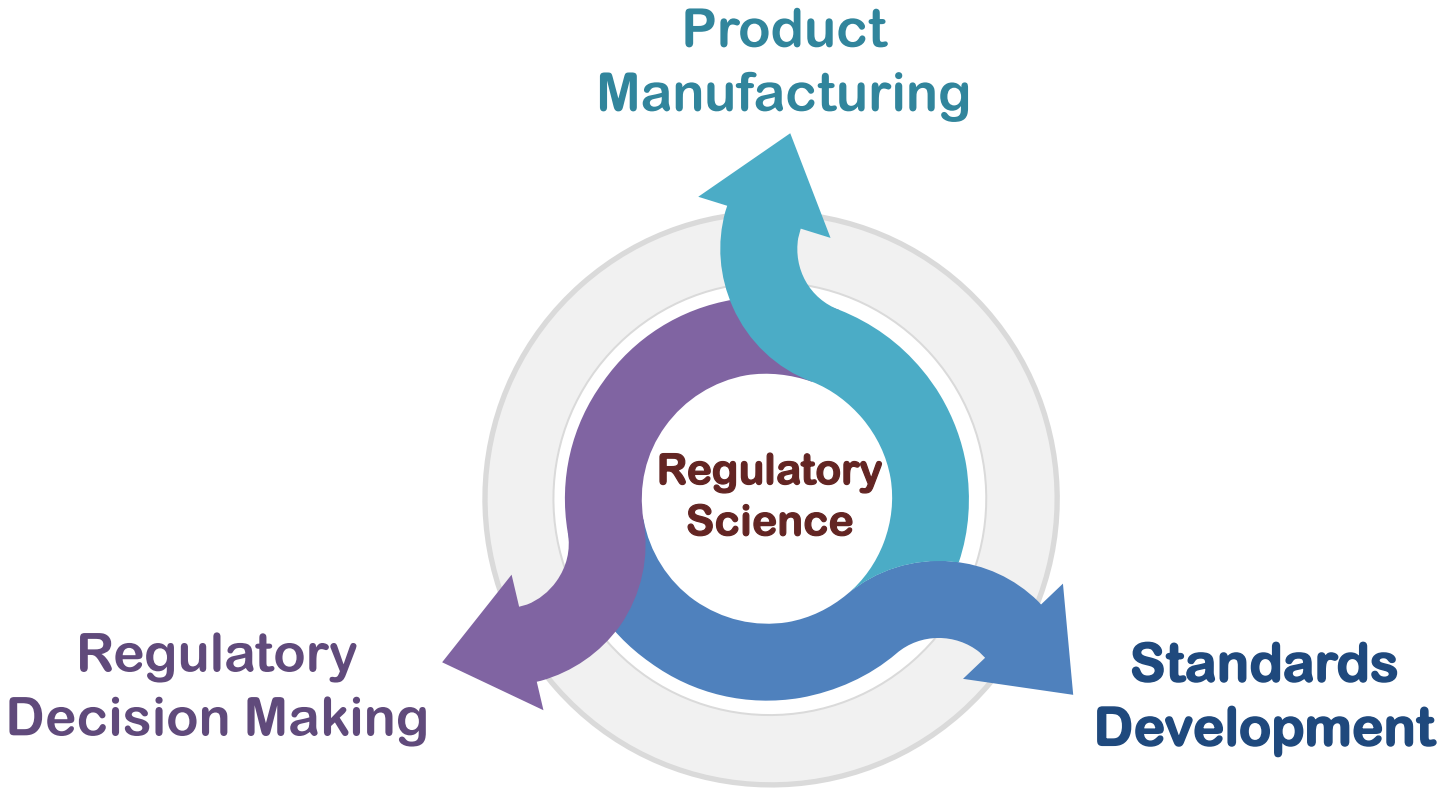
Overview of cellular product regulation

Kyung Sung

Practical microscale technology for product assessment

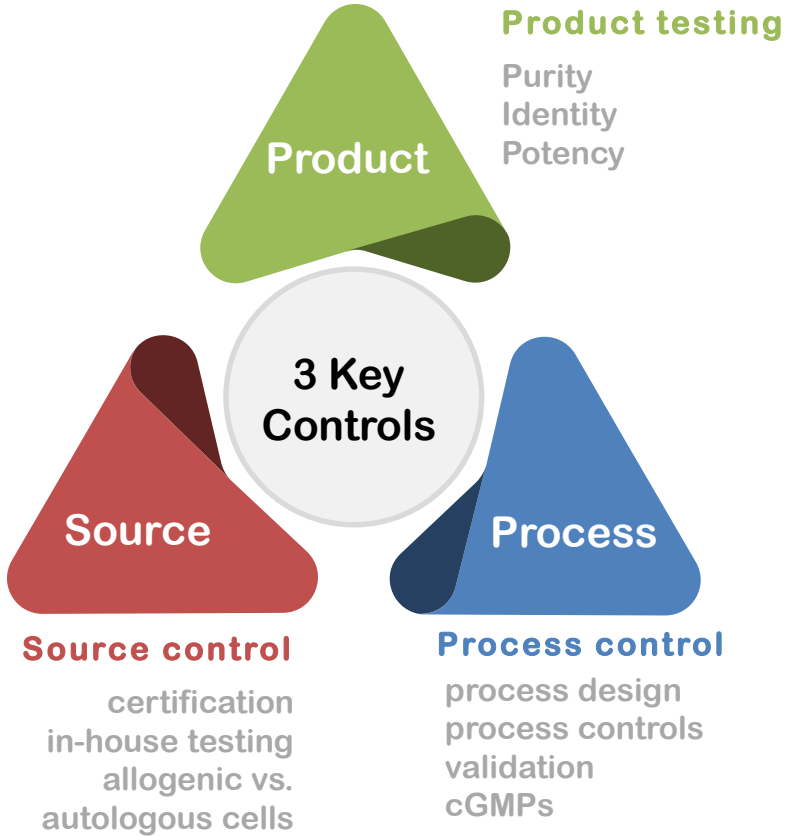
Johnny Lam

The Role of Regulatory Science



Chemistry, Manufacturing, and Controls (CMC)

- CMC = Product manufacturing and testing
- How do you make the product?
- What do you use to make the product?
- Product safety and Quality testing
- Product Stability
- Other controls – product container, labels, tracking



Biologic Product Specification:
Codified in Regulation (21 CFR 600 - BIOLOGICS)

Product should be characterized with reference to its:

- Safety
  - Sterility (bacterial and fungal sterility)
  - Endotoxin
  - Mycoplasma
  - Test for opportunistic viruses
- Purity
  - Free of extraneous materials
- Identity
  - Specific test to distinguish it from others
- Potency
  - Assay for biological function
- Constituent Materials
  - Ingredients, Preservatives, Excipients, etc.
- Stability

Why do you need specifications

- Demonstrate Product Consistency
- Control purity and impurity profiles of the final product.
  - Identify characteristics that predict safety and clinical effectiveness
  - Detect cells with undesired characteristics
- Demonstrate control of the Manufacturing Process.
  - Quality Assurance/Quality Control Program
- Ensure product integrity and stability.
- Identify product parameters that anticipate adverse events.

Biologic Product Specification:
Codified in Regulation (21 CFR 600 - BIOLOGICS)

Product should be characterized with reference to its:

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  - Er
  - M
  - Te
- Purity
  - Fr
- Identit
  - Sp
- Potency
  - Assay for biological function
- Constituent Materials
  - Ingredients, Preservatives, Excipients, etc.
- Stability

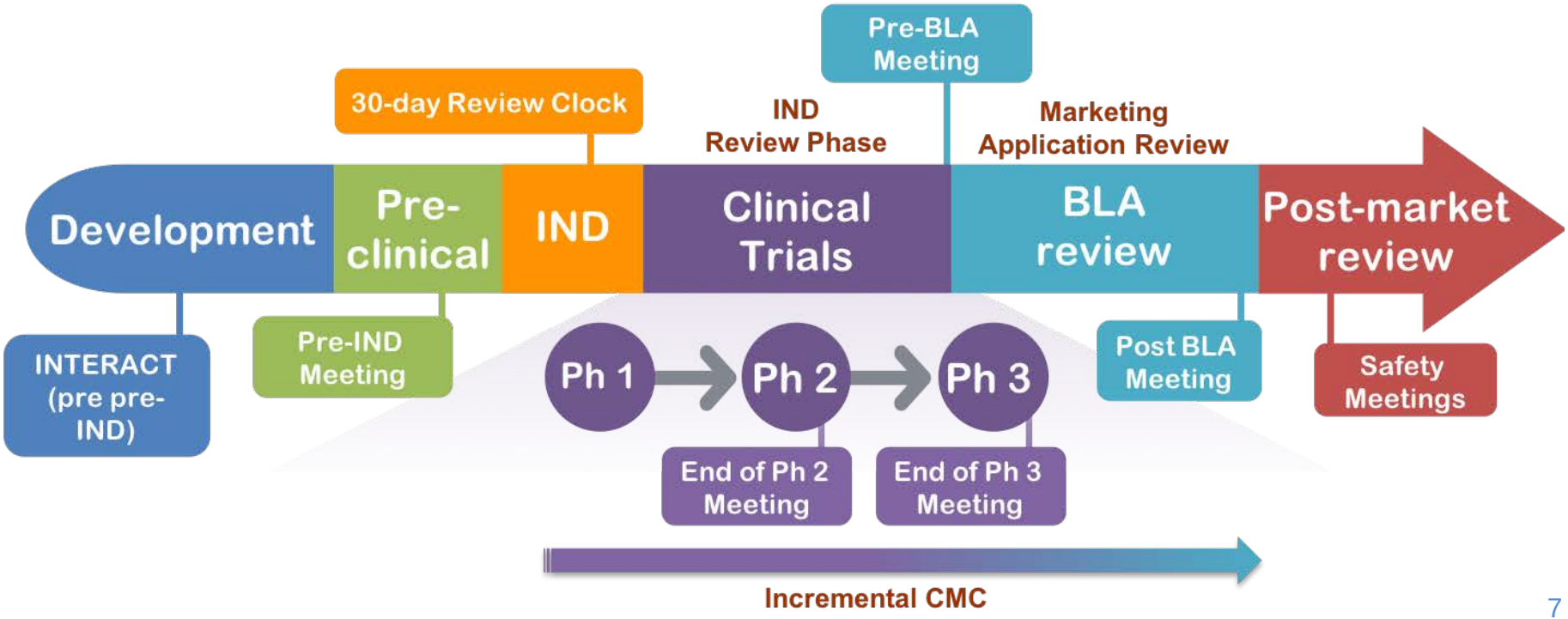
Ideally, testing should predict performance in vivo

Why do you need specifications

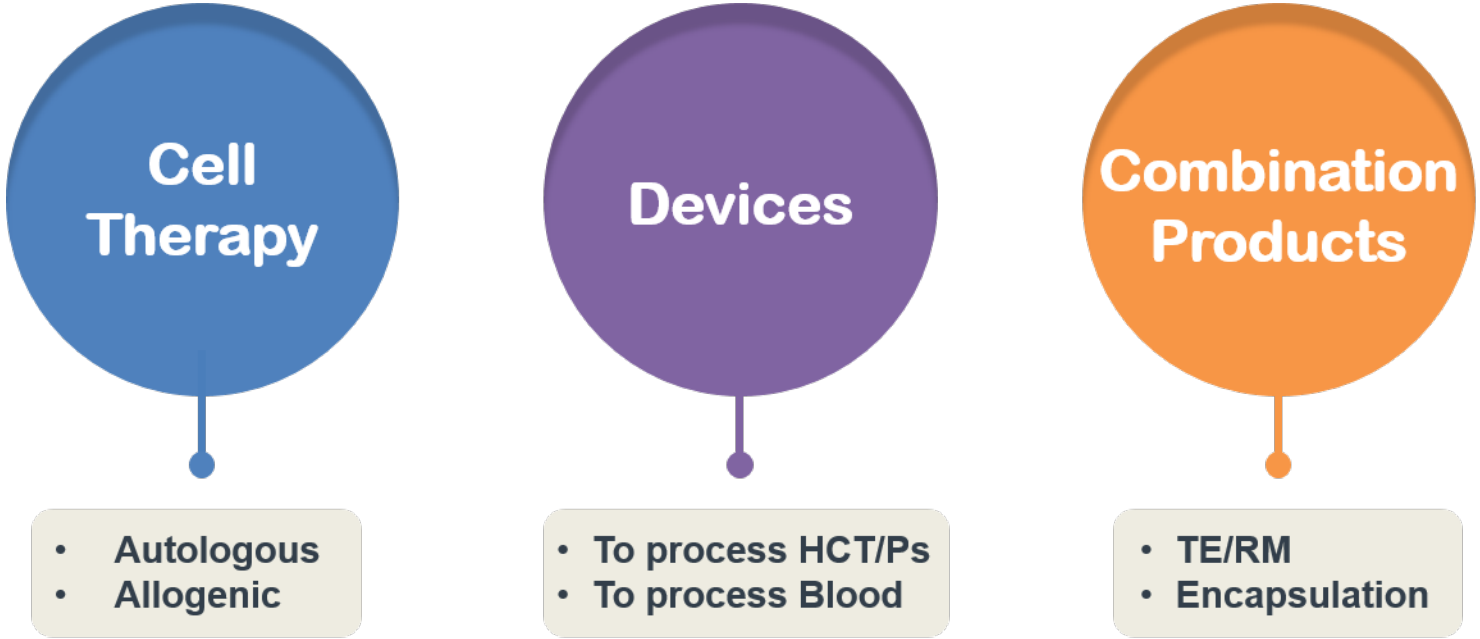
- Demonstrate Product Consistency
  - y profiles
  - redict safety
  - haracteristics
  - e
  - Quality Assurance/Quality Control Program
- Ensure product integrity and stability.
- Identify product parameters that anticipate adverse events.

Interactions with FDA Throughout the Product Lifecycle

Product development is an iterative process, with frequent FDA and sponsor interaction.

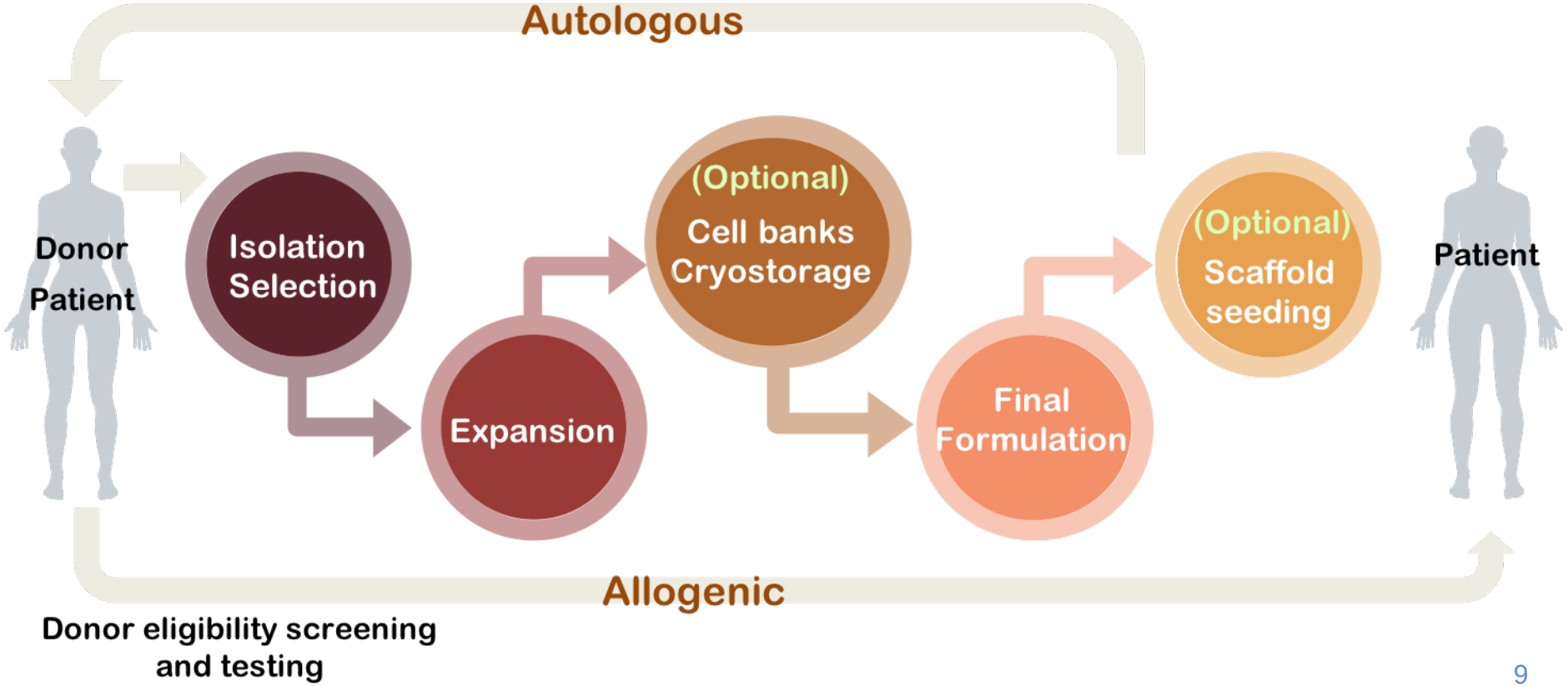


Product Classes Reviewed by Cellular Therapy Branch



… and many others…

Cellular Product Manufacturing Process



Devices in Cellular Therapy Branch

Devices -

- Intended to process HCT/Ps (Human cells, tissues and cellular and tissue-based products) or other biologics ex vivo to generate a device output at point-of-care
- Intended to collect, process, and/or store HCT/Ps
- Intended to process blood, a blood-BM mixture or BM

Devices Concentrating Blood/BM

510(k) cleared for an orthopedic indication(improving bone graft handling)

Input

Whole blood

Blood + BM

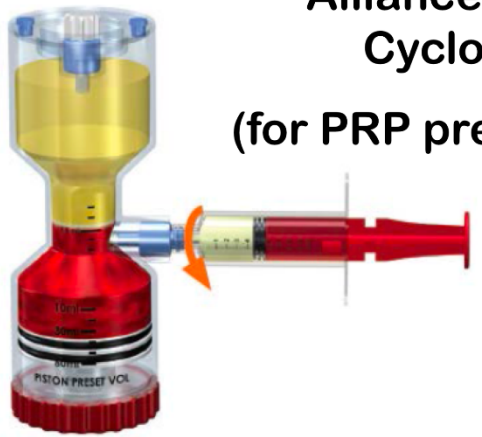
- Centrifugation
- Filtration
- Density gradient

Output

PRP
PPP
PRF

Alliance Spine Cyclone®

(for PRP preparation)



Devices in Cellular Therapy Branch

ReCell® Autologous Cell Harvesting

- An autograft-sparing technology indicated for use at the patient's point-of care for preparation of an autologous epithelial cell suspension to be applied to a prepared wound bed.
- The suspension is used to achieve epithelial regeneration for definitive closure of burn injuries, particularly in patients having limited availability of donor skin for autografting.
- PMA approved, September 2018

Cell-Device Combination product

- A product composed of different categories of regulated articles:
  - Device-biologic, biologic-drug, drug-device, biologic-drug-device (not biologic-biologic, etc)
- Both components are:
  - intended for use together
  - required to mediate the intended therapeutic effect
- Can be:
  - Physically or chemically combined
  - Co-packaged; or packaged separately but cross-labeled
- Guidance:
  - Early Development Considerations for Innovative Combination Products (2006): http://www.fda.gov/RegulatoryInformation/Guidances/ucm126050.htm

Tissue-engineered and regenerative medicine products (TEMPs):

Cell-scaffold constructs

Cells (and other biologics)

+

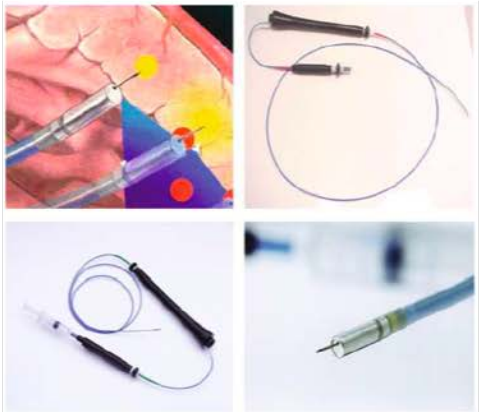
Delivery device (catheters, injection/spray devices, etc):

Cell-Device Combination product

Delivery Device

- **Cell or gene therapy + delivery device (catheters, injection/spray devices, electroporation devices)**
- **Compatibility between device and biologic is reviewed.**

MyoStar® Injection Catheter



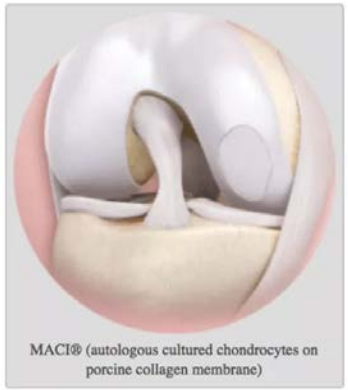
Cellectra 5PSP
Electroporation Device

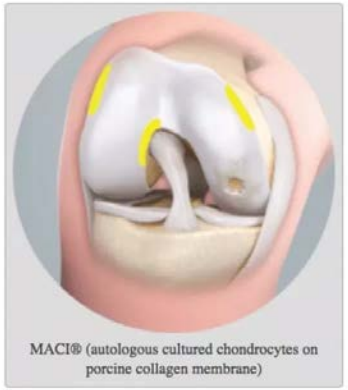
PharmaJet Needleless
Injection Device

Cell-Device Combination product

Tissue Engineered Product

- Expanded autologous chondrocytes (biologic constituent) + porcine-derived Type I/III collagen scaffold (device constituent)
- BLA approved in December 2016
- Indicated for the repair of single or multiple symptomatic, full-thickness cartilage defects of the adult knee, with or without bone involvement.





Regenerative Medicine Therapies

- Defined in Title 3, Section 3033 of the 21st Century Cures Act (signed into law December 13, 2016)
  - Cell Therapies
  - Therapeutic tissue engineering products
  - Human cell and tissue products
  - Combination products associated with the above
  - Genetically-modified cells*
  - Gene therapies that lead to a durable modification of cells or tissues*

- 21st Century Cures Act created the Regenerative Advanced Therapy designation program, which FDA generally refers to as Regenerative Medicine Advanced Therapy or RMAT

- Reviewed and regulated by OTAT in CBER

* Expedited Programs for Regenerative Medicine Therapies for Serious Conditions: Draft Guidance for Industry

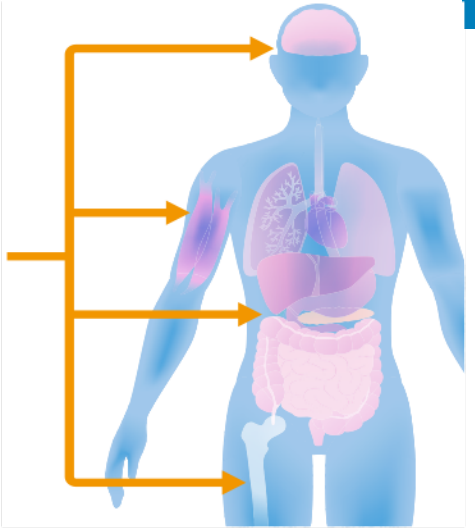
Regenerative Medicine Advanced Therapies

Sources

- Human tissue cells
- Stem cells
- Biomaterials
- Bioactive molecules

Therapeutic tissue engineered products

Human cell & tissue products



Manufacturing & Regulatory challenges

- Cellular heterogeneity
- Patient to patient variability
- Limited shelf life/limited sample volume
- Limited availability of starting material for test method development
- A wide range of manufacturing protocols

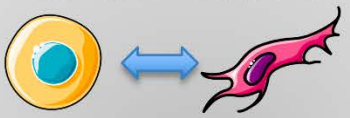
New methods and **quality attributes** may be needed to reliably predict biological functions of manufactured products.

Microscale Biomimetic System

Tools to help understand cell and tissue complexity

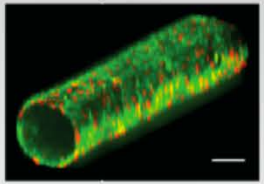
Local activities at a human tissue site

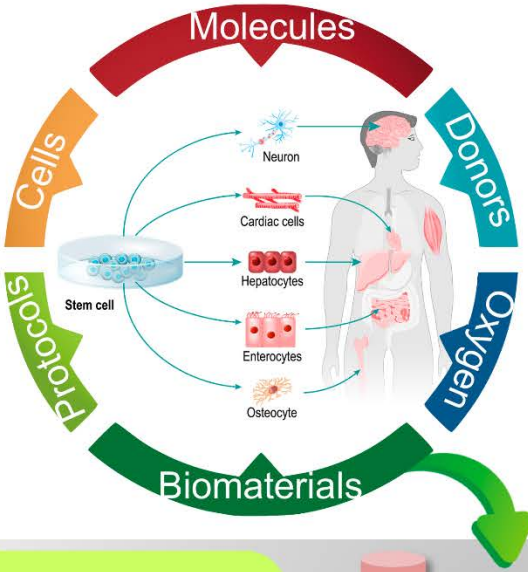
Cell-cell interactions



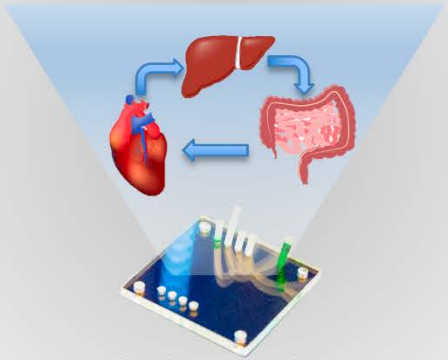
Cell-biomaterial interactions

Cells in structure (Blood vessels)



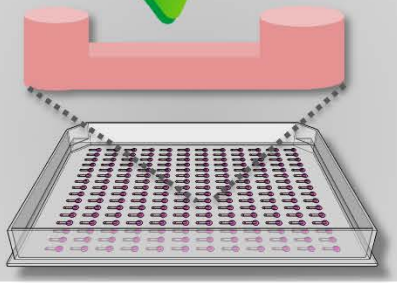


Organ-level activities



Body-on-a-chip

HTS via microchannel arrays

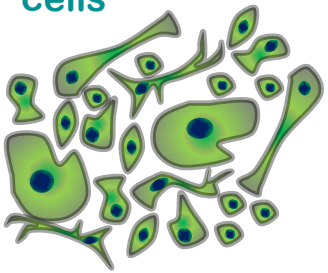


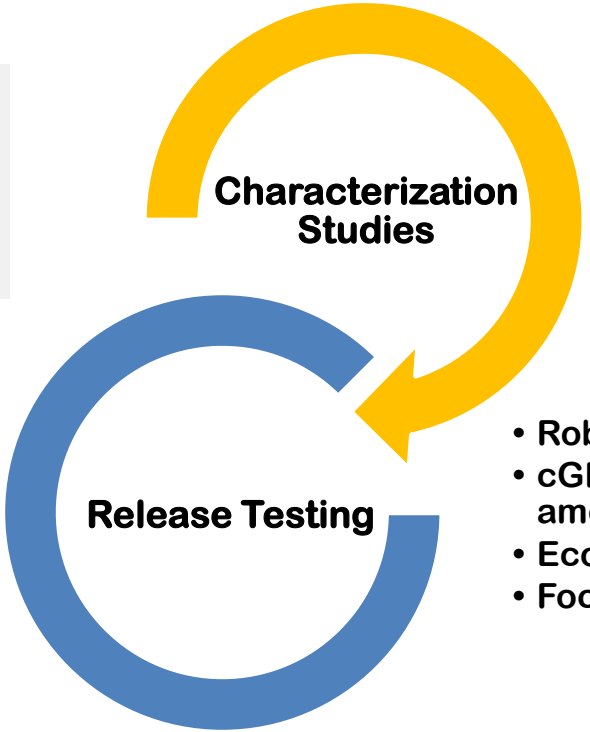
Automation

Goal of Manufacturing: Cellular Products

- Robust, reproducible process
- Safe and effective final product
- Starting cells suitable for further manufacture

Manufactured cells



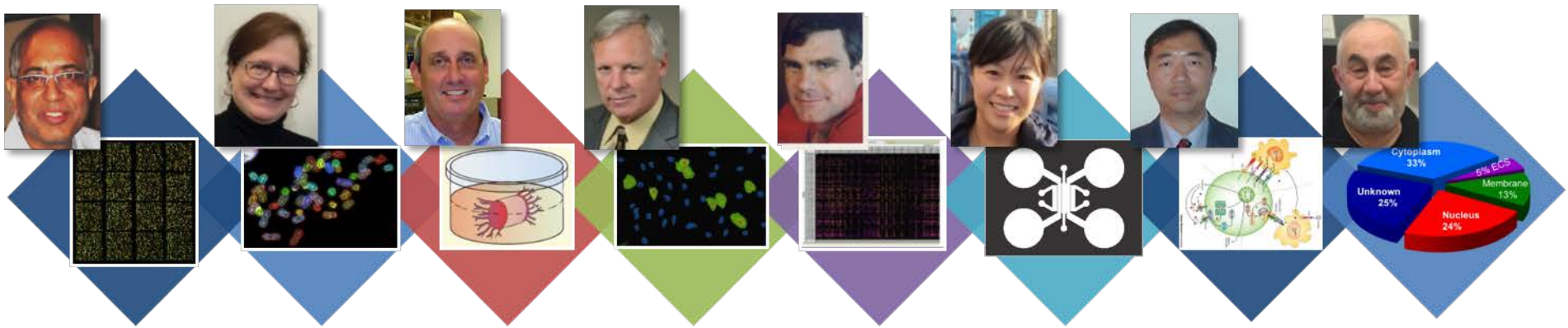


- Sophisticated, powerful, comprehensive
- Labor intensive, expensive, finicky

- Robust, Rapid
- cGMP amenable
- Economical
- Focused

FDA's MSC Consortium

- **Bone-marrow derived MSCs as proof-of-concept project**
  - **Develop strategies to determine identity/potency assays that predict safety and effectiveness**



Puri Lab
Genomics

Hursh Lab
Epigenetics
Karyotypes

McCright Lab
in vivo, in vitro models of wound repair

Bauer Lab
in vitro quantitative differentiation

Moos Lab
single cell PCR, NGS

Sung Lab
3D in vitro tissue microfluidic models.

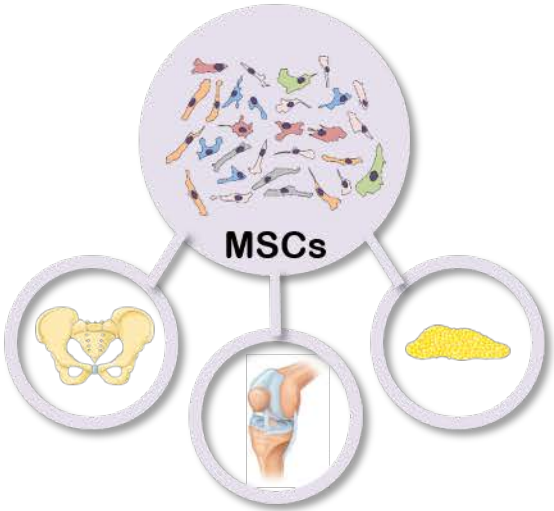
Wei Lab
In vitro, in vivo immunosuppression

Alterman Lab
Proteomics

Multipotent Stromal Cells (MSCs)

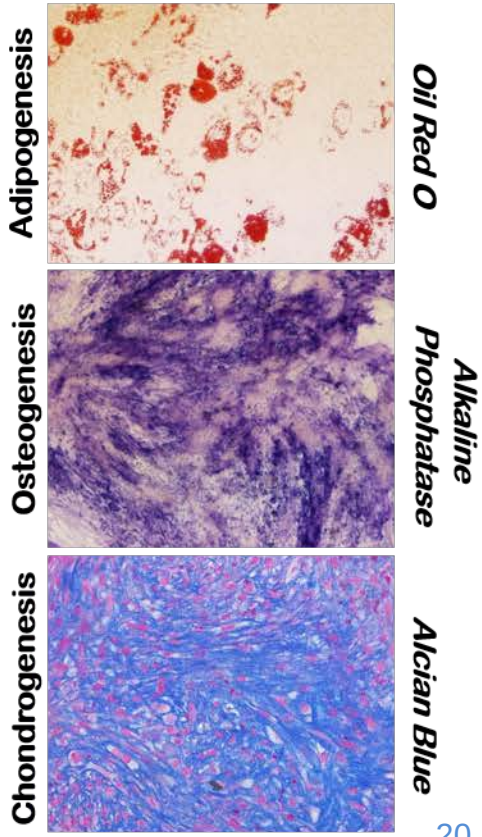
Adult, multipotent stromal cells derived from various tissue sources

- **Differentiates into bone, cartilage, and fat**
- **Easily harvested with little donor morbidity**



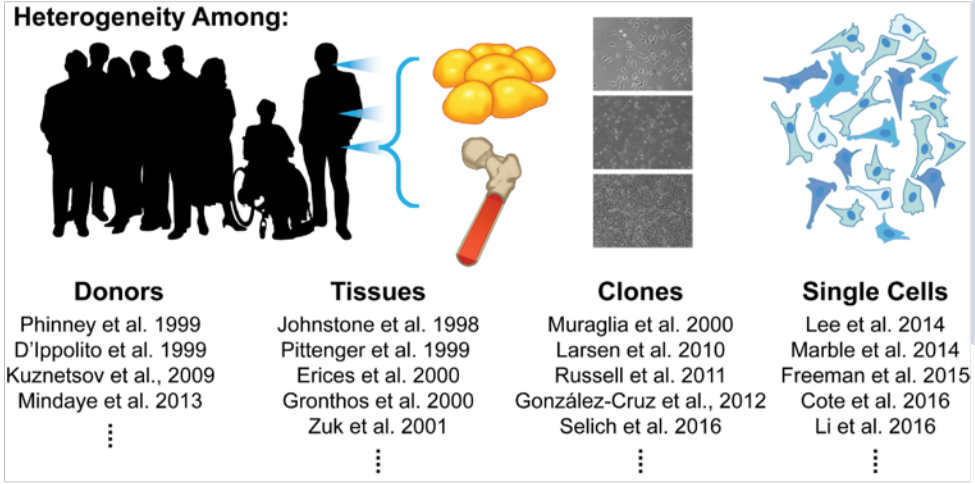
Most frequently used methods to assess differentiation are qualitative

Can we develop ways to identify Quality Attributes that predict safety and effectiveness?

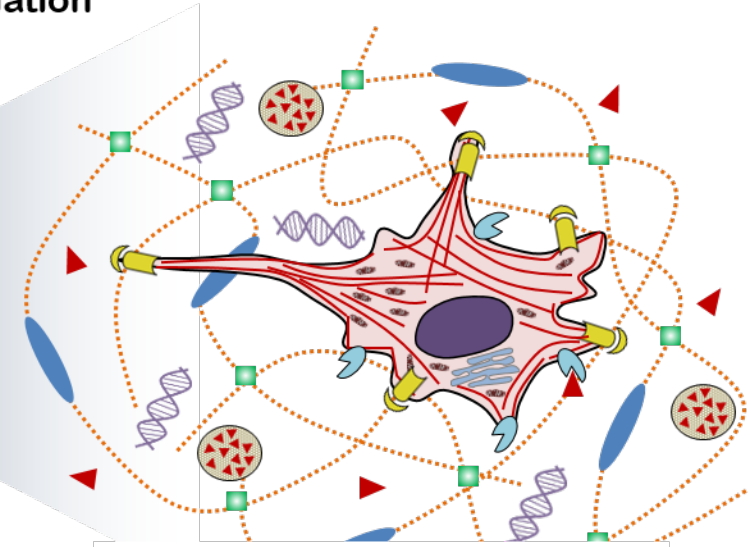


MSC Functional Heterogeneity

- MSCs characteristically exhibit variability in their functional capacity depending on a number of factors
- Heterogeneity may limit their therapeutic potential
- Need for quantitative bioassays to measure differentiation

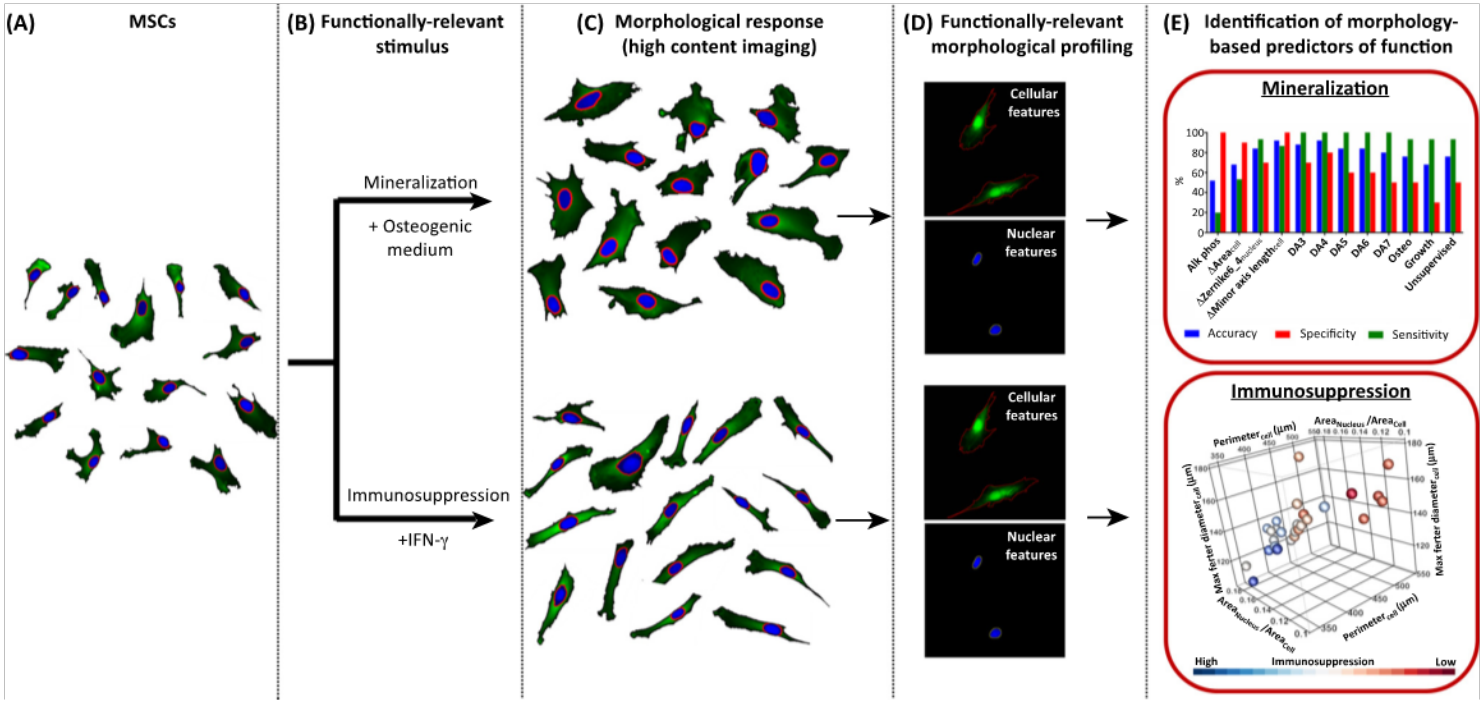


McLeod et al., *eCM* (2017), Marklein and Lam et al., *TIBTEC* (2017)



Cell morphology is a phenotype readout that may represent intracellular signaling events and is related to cell function

Predicting MSC Quality



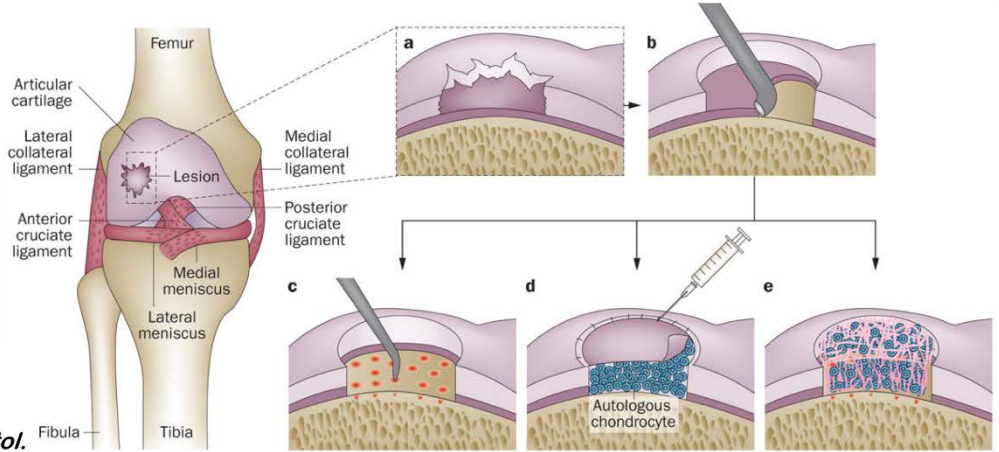
Osteogenic Potential of MSCs

Immunosuppressive Potential of MSCs

Marklein and Lam et al., *TIBTEC* (2017); Marklein et al., *Stem Cells* (2016); Klinker et al., *PNAS* (2017); Lo Surdo et al., *Cytotherapy* (2013)

Cartilage regeneration

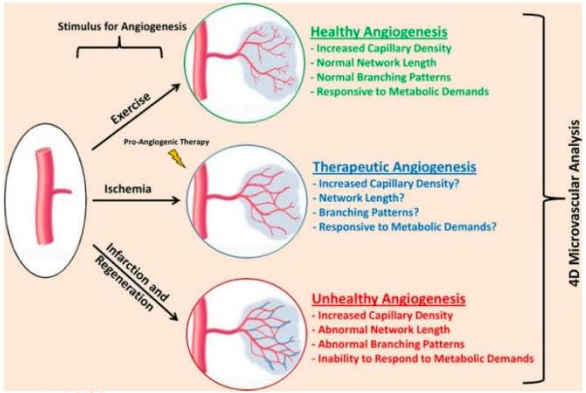
(*Chondrogenesis*)



Makris, 2014, Nat. Rev. Rheumatol.

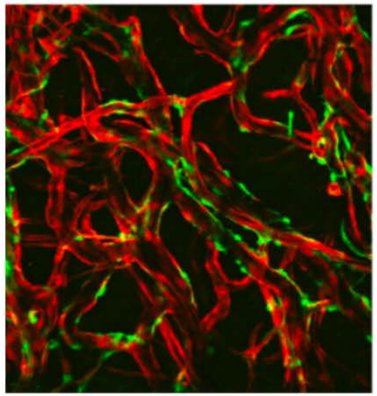
New vessel generation

(*Vasculogenesis/ Angiogenesis*)



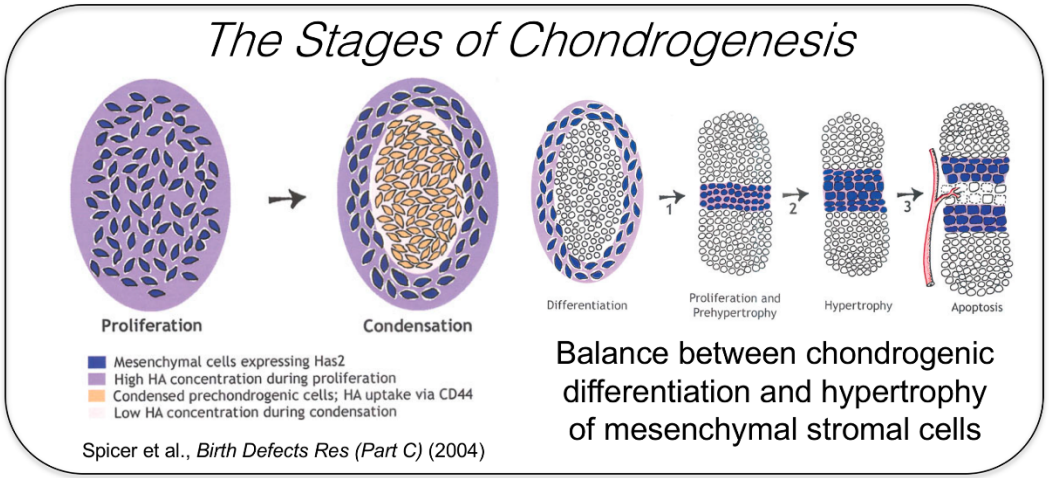
Quality over Quantity

Durand, Circ Res., 2017

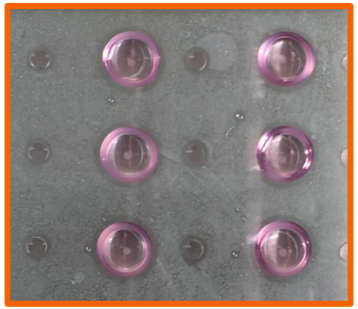


Tao, 2016, Stem Cell International

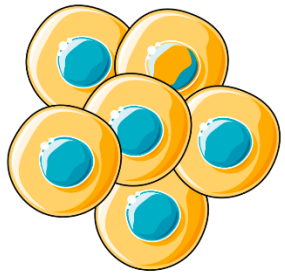
Chondrogenic Potential of MSCs



Hanging Drop

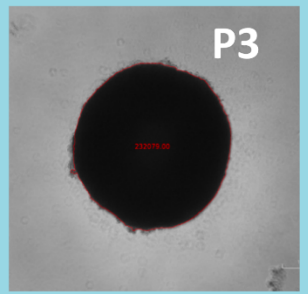


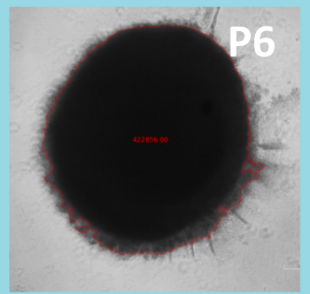
MSC aggregates



Spheroid Culture – Improved Functionality

- Recapitulates cell-cell contacts during condensation
- MSCs in 3D (vs. 2D) exhibit enhanced survival, increased functional trophic potential, and are more stem-like
- Spheroid morphology represents a phenotypic readout of MSCs in 3D





Chondrogenic Potential of MSCs:

Correlation with MSC Aggregate Morphological characteristics

Objective

To develop a simple, adaptive, and functionally-relevant assay to predict the chondrogenic differentiation potential of MSCs

Hypothesis

Differences in MSC aggregate morphology, which represents a functionally-relevant 3D phenotypic readout, are early indicators of chondrogenic differentiation capacity.

High-Throughput Generation of MSC Pellets from Multiple Donors

Objective

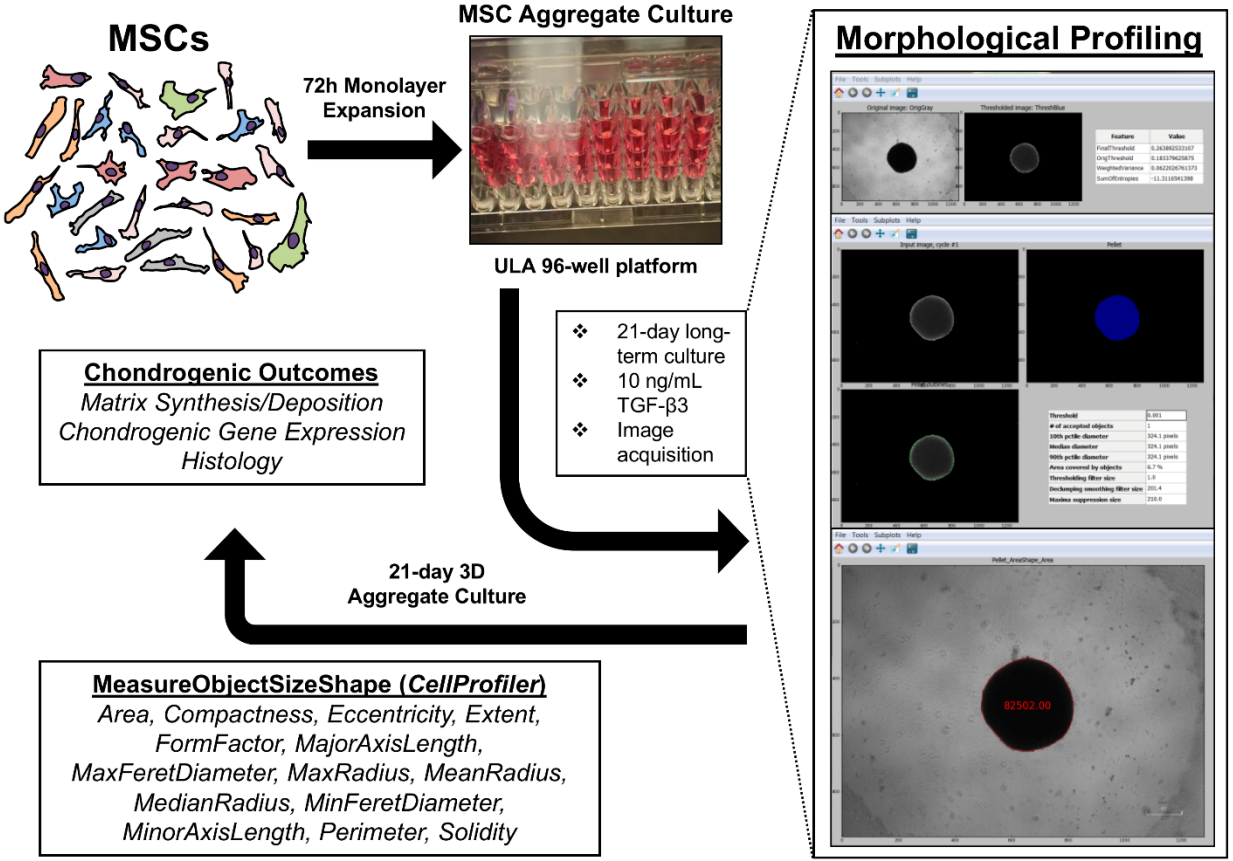
To assess aggregate morphology over time and evaluate the correlation of emergent morphological phenotypes with MSC chondrogenic potential

Group (n=12-15)	Cell Line	Passage
RB9ep/lp	RB9	Early, Late
RB12ep/lp	RB12	Early, Late
RB14ep/lp	RB14	Early, Late
RB16ep/lp	RB16	Early, Late
8F3560ep/lp	8F3560	Early, Late
167696ep/lp	167696	Early, Late
PCBM1632ep/lp	PCBM1632	Early, Late
PCBM1641ep/lp	PCBM1641	Early, Late

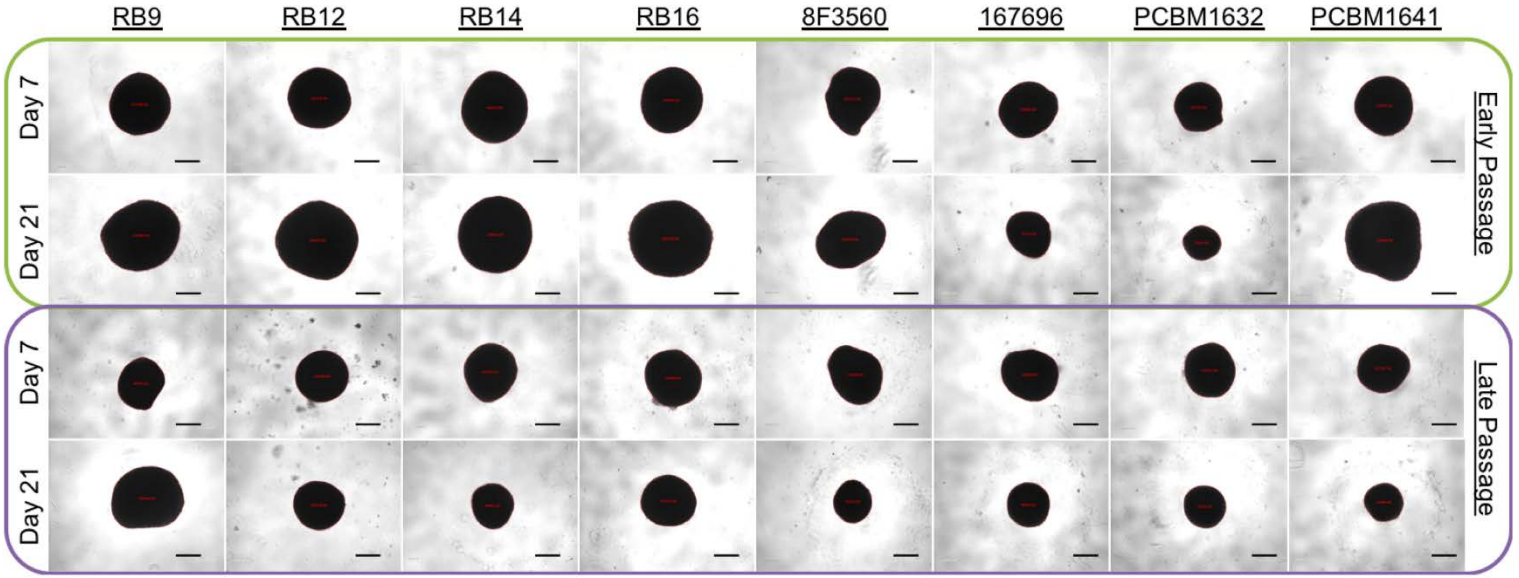
Experimental Notes:

- 100,000 cells per pellet
- Early passage: p2/p3; Late passage: p5
- Aggregate Morphological Characterization
- Biochemical Analysis: DNA, GAG, Histology
- Gene Expression

Experimental Scheme



Morphology of MSC Aggregates

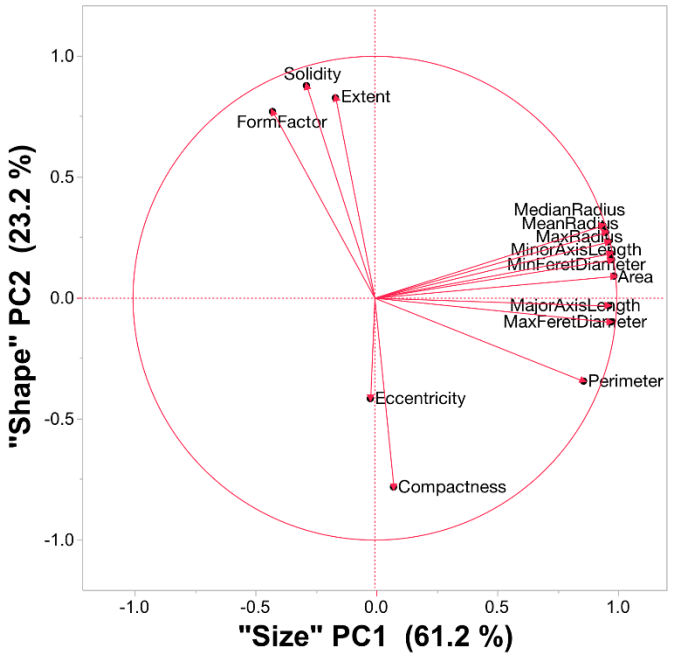


MeasureObjectSizeShape (*CellProfiler*)

Area, Compactness, Eccentricity, Extent, FormFactor, MajorAxisLength, MaxFeretDiameter, MaxRadius, MeanRadius, MedianRadius, MinFeretDiameter, MinorAxisLength, Perimeter, Solidity

MSC aggregates exhibit donor- and passage-dependent differences in aggregate morphology

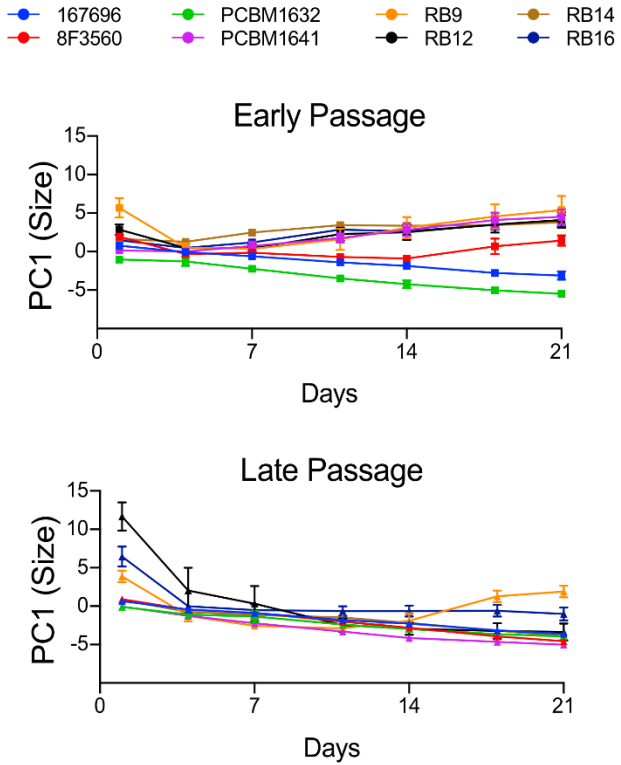
Compartmentalizing Measures of MSC Aggregate Morphology

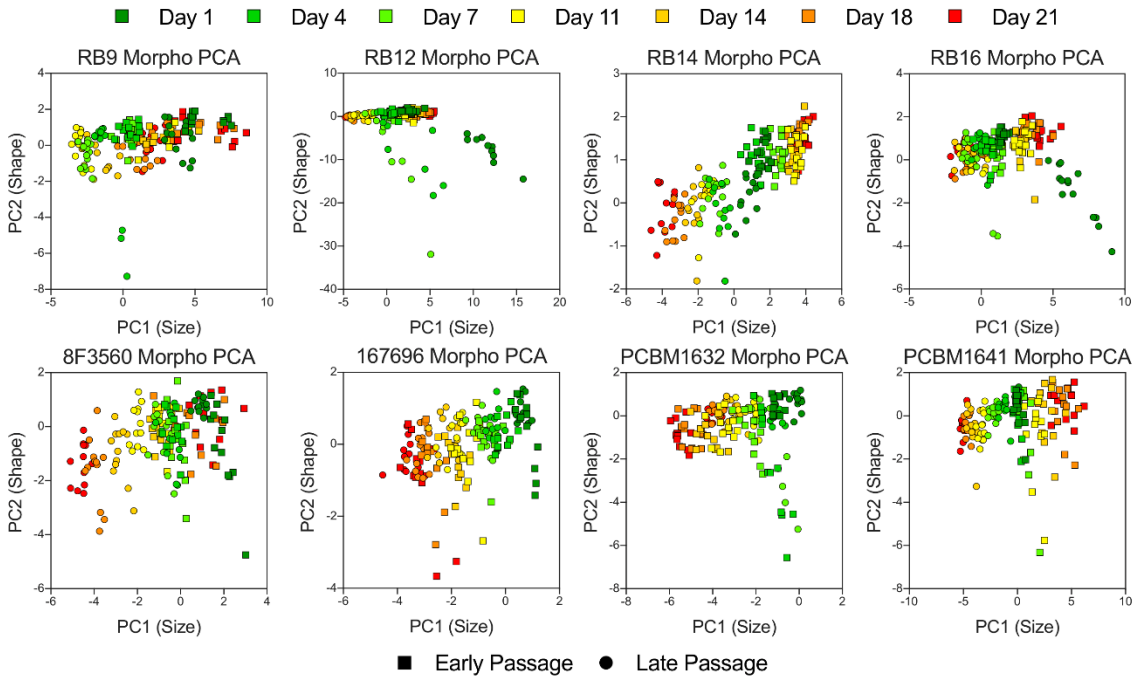


Feature	PC1 (61.2%)	PC2 (23.3%)
Area	***0.99059***	0.08963
MajorAxisLength	***0.96916***	-0.03278
MaxFeretDiameter	***0.97767***	-0.10053
MaxRadius	***0.96701***	0.23114
MeanRadius	***0.95658***	0.27053
MedianRadius	***0.94435***	0.29927
MinFeretDiameter	***0.9809***	0.15742
MinorAxisLength	***0.97592***	0.18306
Perimeter	***0.86504***	0.34466
Compactness	0.07914	***-0.78223***
Eccentricity	-0.01688	***-0.41659***
Extent	-0.16094	***0.8261***
FormFactor	-0.42133	***0.76959***
Solidity	-0.28121	***0.87664***

*High-dimensional morphological data of aggregates can be reduced to individual composite measures for **Size** and **Shape***

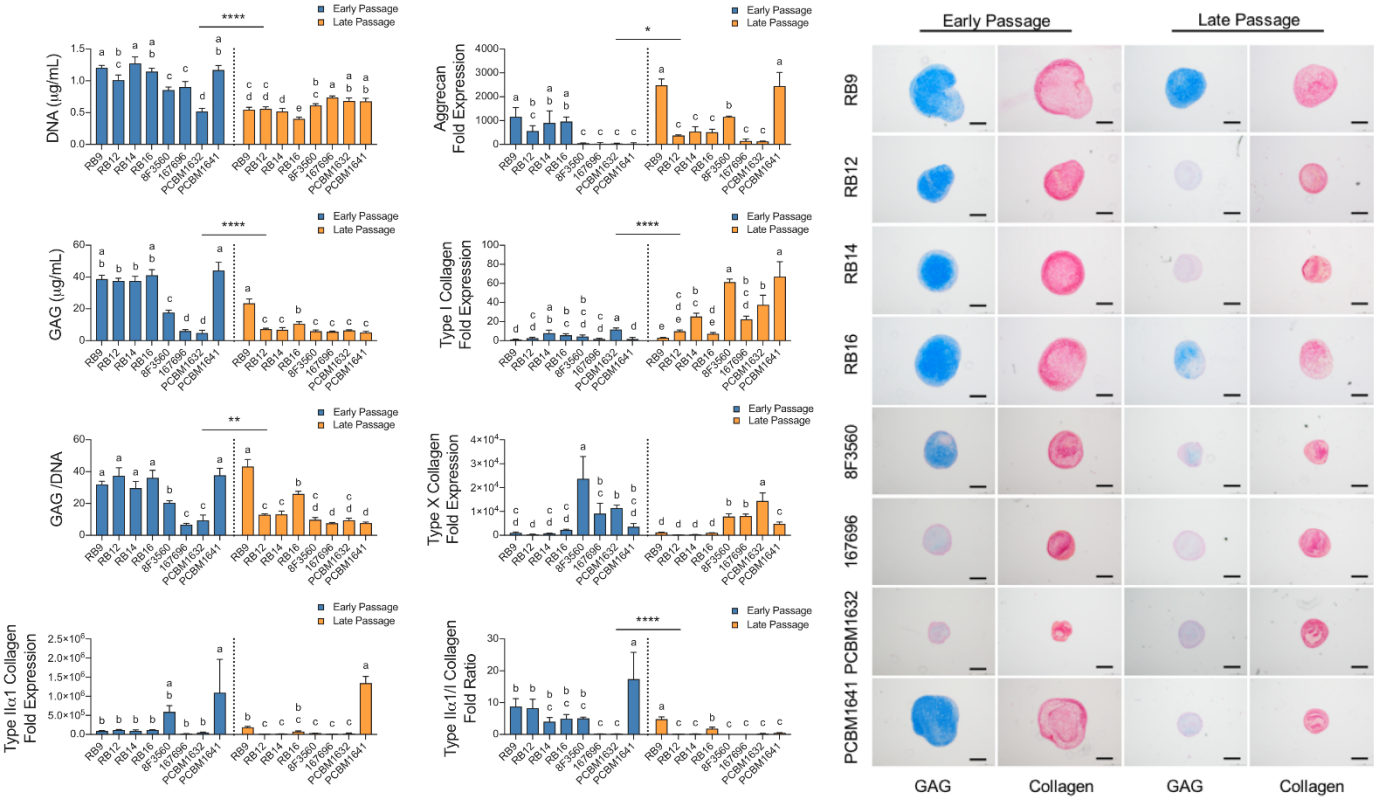
Morphological Dynamics of MSC Aggregates





MSC lines display donor and passage-dependent morphological landscapes in 3D culture

Chondrogenic Outcomes of MSCs



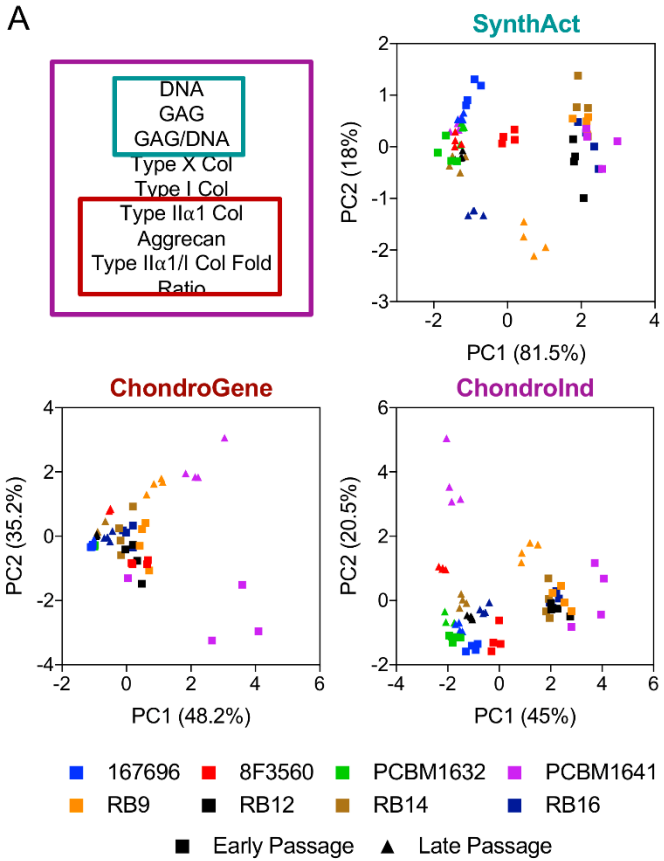
Chondrogenic outcomes of MSC lines in aggregate culture vary depending on both the donor source and passage

Compartmentalizing Measures of Chondrogenic Differentiation

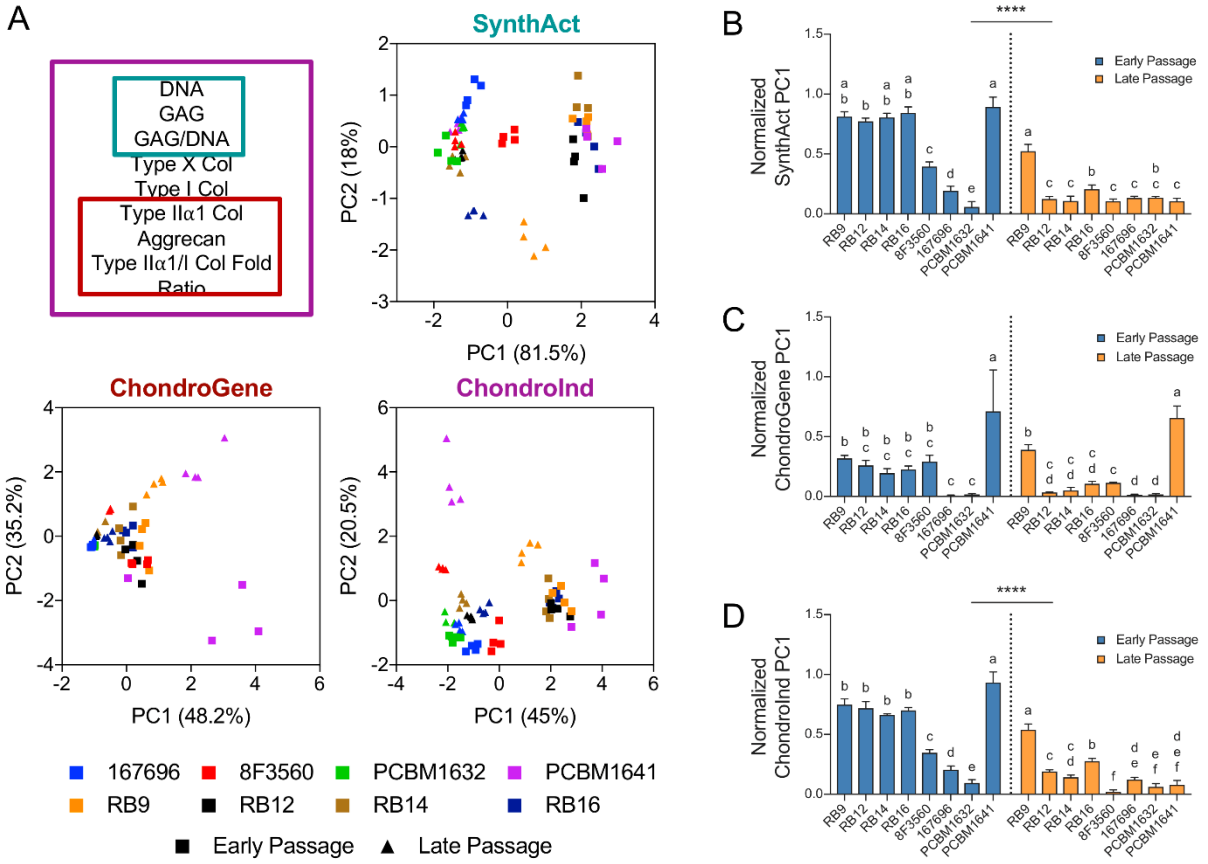
- Singular measures from individual assays may not fully elucidate extent of chondrogenic differentiation

- Composite metrics derived from Principal Component Analysis (PCA)
  - Supervised variables allow for composite scores representative of various aspects of differentiation (synthetic activity, overall gene expression etc.)

Compartmentalizing Measures of Chondrogenic Differentiation

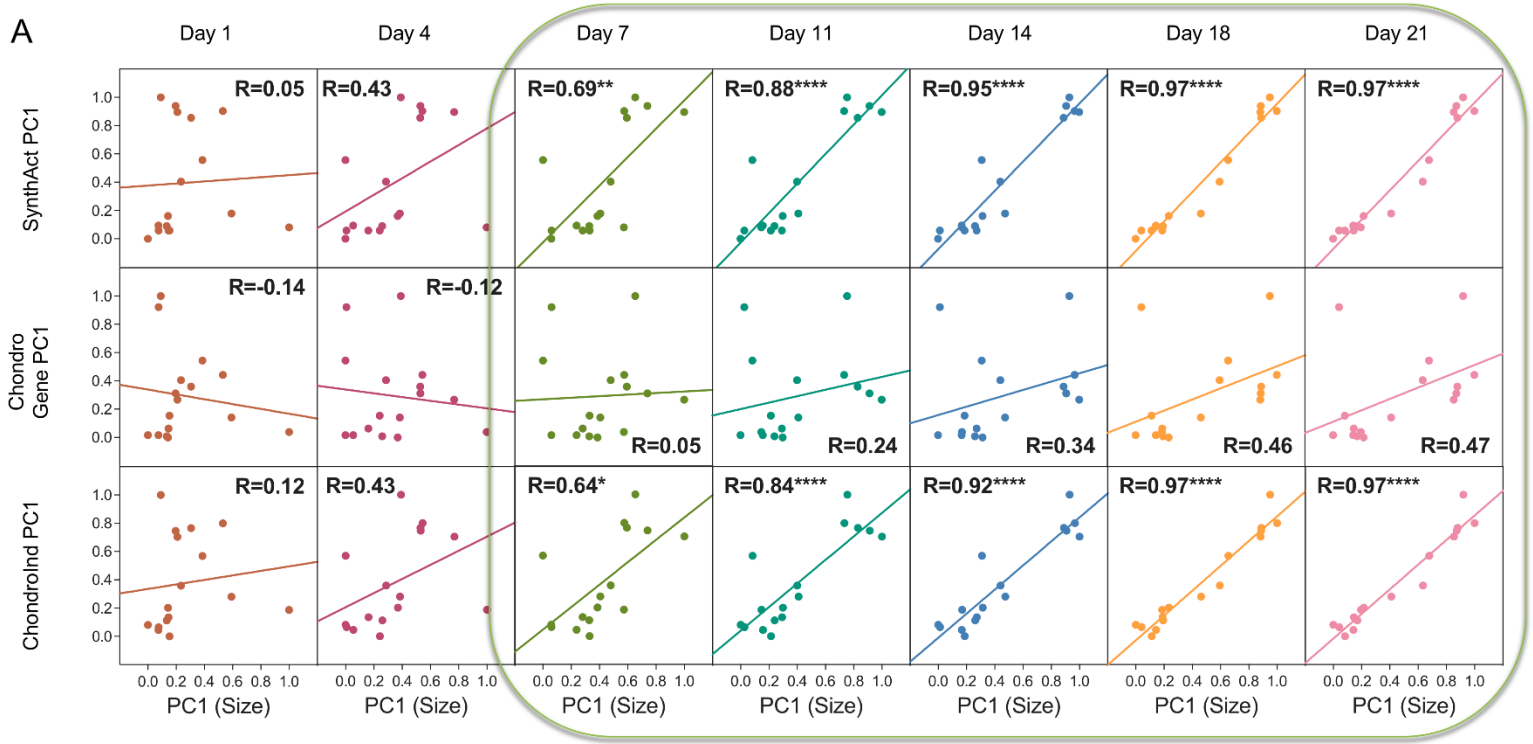


Compartmentalizing Measures of Chondrogenic Differentiation



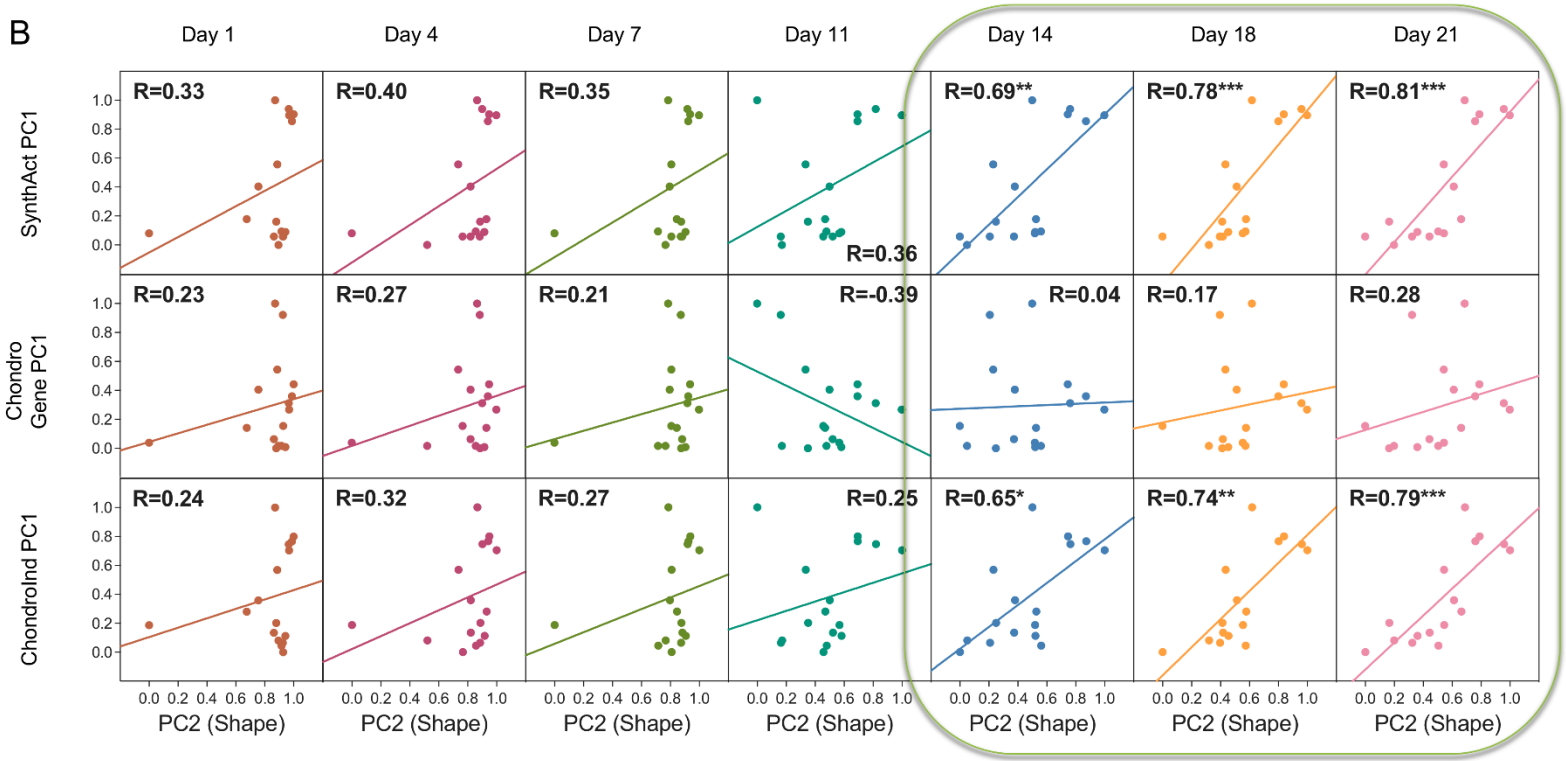
Quantitative composite scores measuring ***functional matrix accumulation***, ***chondrogenic gene expression***, *and* ***both***

Correlating Chondrogenic Outcomes with MSC Aggregate Morphology



*Synthetic activity, but not chondrogenic gene expression, correlates with emergent **Size** features by Day 7*

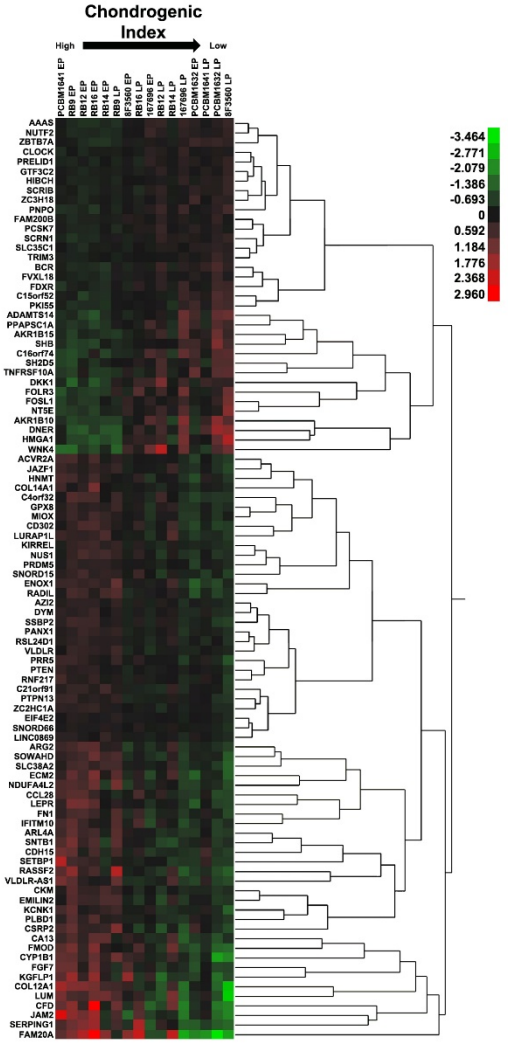
Correlating Chondrogenic Outcomes with MSC Aggregate Morphology



*Synthetic activity, but not chondrogenic gene expression, correlates with emergent **Shape** features by Day 14*

*Gene Expression Microarray was performed on **undifferentiated** MSC lines to identify inherent differences in gene expression*

***107 probes** were identified to be significantly correlated with MSC chondrogenic capacity*

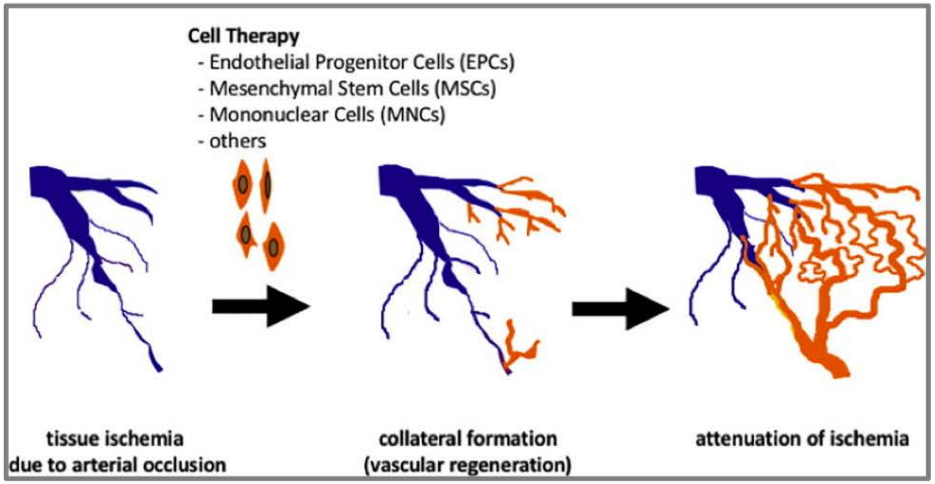


Discussion

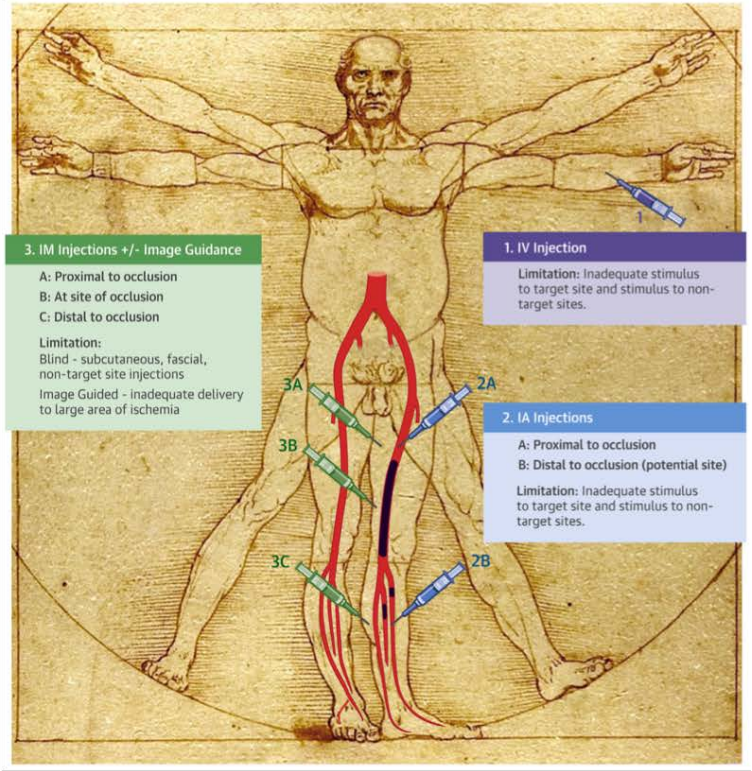
- ***Functional matrix accumulation*** **but not** ***chondrogenic gene expression*** **correlated strongly with aggregate morphology, highlighting a disconnect between chondrogenic phenotype and gene expression.**

- **By developing a simple non-destructive approach that can capture such differences in aggregate morphology between multiple MSC donors/passages, we provide a method for the early estimation of chondrogenic differentiation capacity that may have important implications for the manufacturing of quality MSC products.**

Cellular Therapies for Vessel Regeneration (vasculogenesis/angiogenesis)

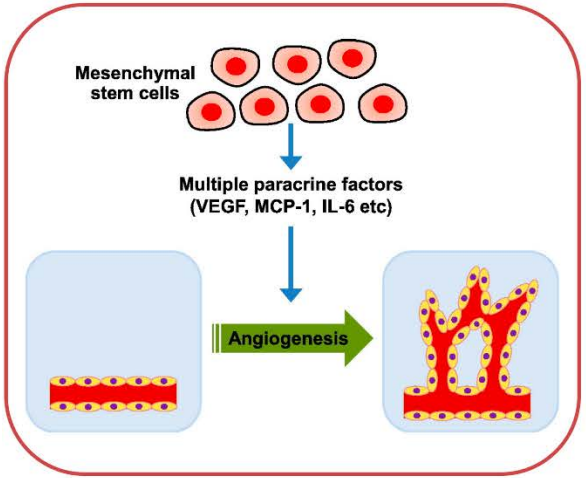


Fujita et al., Advanced Drug Delivery Reviews, 120 (2017), 25-40

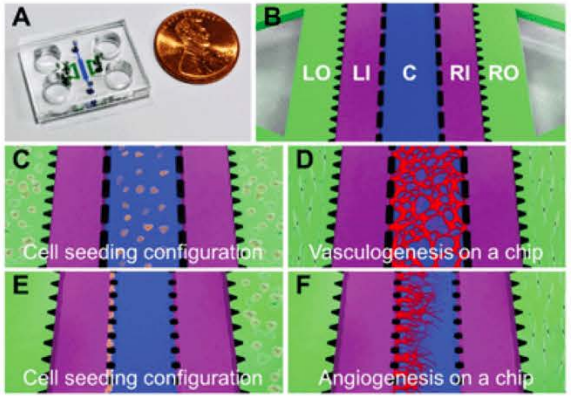


Iyer et al., J Am Coll Cardiol Basic Trans Science, 2017. 2(5), 503-12

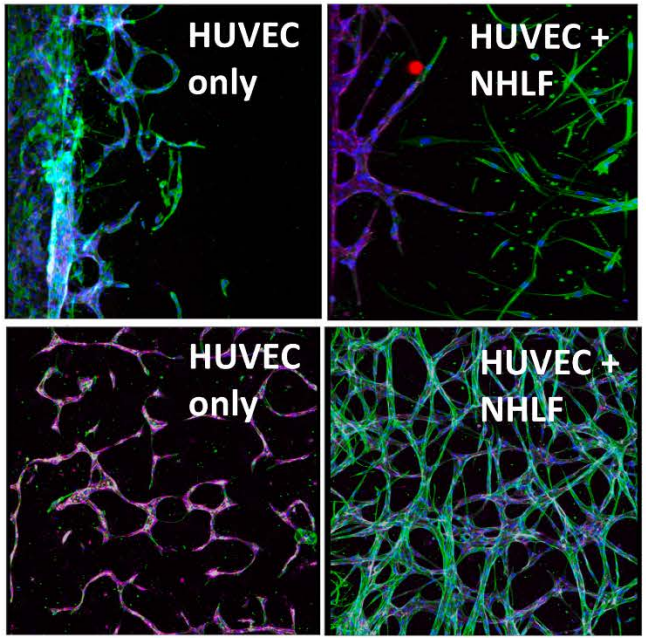
Angiogenic Potential of MSCs



Kwon, 2014, Vascular Pharmacology

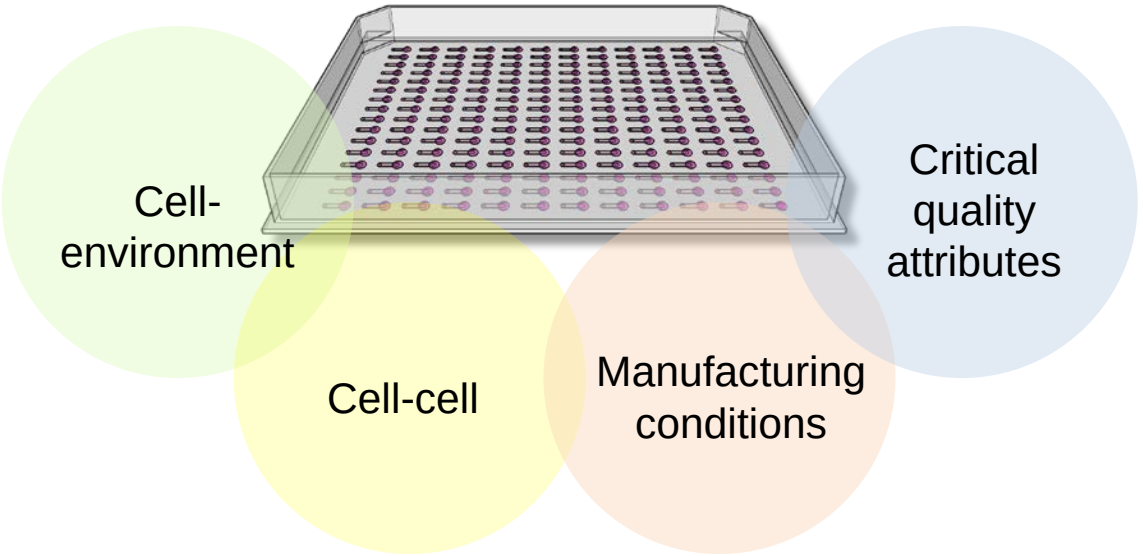


Kim, 2013, Lab Chip



Conclusions

Practical microscale biomimetic system



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