USF Nanofilm 3D Cell Culture Scaffolds

A Platform for Collaborative Discovery in Stem Cell Biology

Ultra Small Fibers, Inc. (“USF”) — Scientific Overview

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Or visit ultrasmallfibers.com

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Need for Better 3D Cell Culture Scaffolds:

Addressing the missing biophysical dimension in current cell culture systems

- **The Problem**

  Stem cell fate is determined by both **biochemical and biophysical** cues from their microenvironment

  While biochemical tools are highly advanced, physiologically relevant biophysical cues are largely absent from current cell culture systems.

  Current cell culture systems fail to accurately reproduce the biophysical microenvironment:

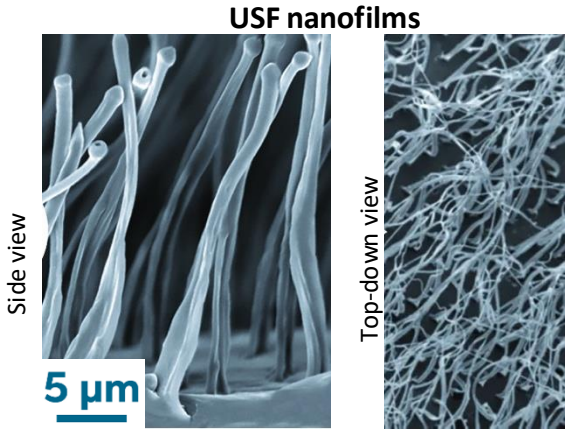
  - **2D cultures** fails to recapitulate complex cellular microenvironments
  - **Existing scaffolds** lack biomimicry, reproducibility and tunability
  - **3D organoids** often introduce experimental artifacts and can be difficult to scale and reproduce

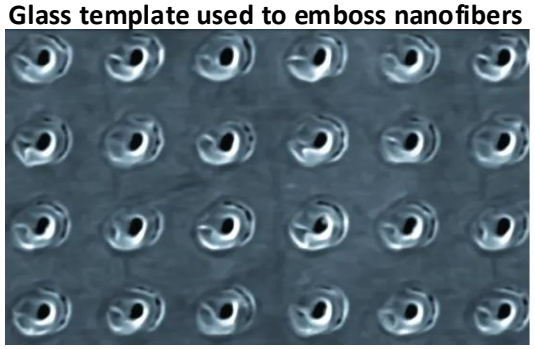
- **The Result:**

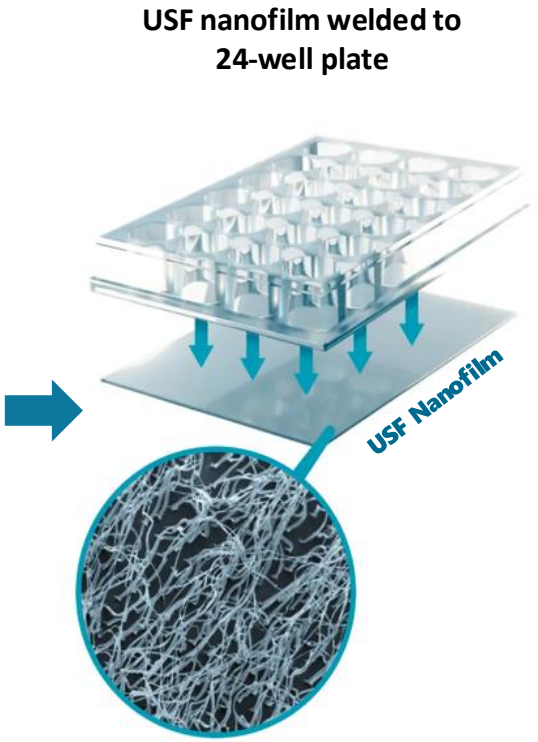
  High variability, increased development time, and higher research costs

The Solution: USF Nanofilm Cell Culture Scaffolds

Reproducible biomimetic microenvironments that can be tuned to optimize stem cell fate







Nanofilms (embossed nanofibers on polymer films)

- Fibers are integrally attached and will not detach
- Highly precise and reproducible in spacing, diameter
- Customizable (fiber density and patterning)
- Sealed to standard well plates for easy integration into existing workflows
- Other form factors will be developed, including large format for bioreactors

Nanofilms provide natural 3D scaffold and microenvironments

- Fiber diameters match native biological scaffolds
- Fibers mimic collagen
- Each fiber density provides a unique cellular microenvironment
    - Customizable fiber spacing replicates a range of biomimetic microenvironments
- No need for hydrogels
- Nanofibers can deliver essential biophysical signals that promote physiologically relevant cell behavior

See ultrasmallfibers.com for well plate animation, product, and other details

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Fundamental Advantages of USF Nanofilms Over Current 3D Scaffolds

Feature	USF Nanofilm Scaffolds		Animal-Derived Mimics (Ex: Matrigel, rat tail collagen)	Synthetic/Semi-Synthetic Hydrogels	Electrospun Nanofiber Scaffolds
	USF Assessed Q2 2026	USF Projected			
Biomimicry	**High**	**High**	Moderate to High	Low to Moderate	Moderate
Tunability	**Moderate to High**	**High**	**Low**	Moderate	Low to Moderate
Reproducibility	**Moderate to High**	**High**	**Low**	Moderate	**Low**
Established Protocols	**Low**	**High**	High	Moderate	Moderate
Transparency	**Moderate**	**Moderate to High**	Moderate to High	**Low to** High	Moderate
Shelf Life	**High**	**High**	**Low**	Low to Moderate	High
Ease of Handling	**Moderate**	**High**	Moderate	Moderate	Moderate to High
Coatability	**Moderate to High**	**High**	Moderate	Moderate	**Low**
Cell Health	**High**	**High**	Moderate	Moderate	Moderate
Scalability	**High**	**High**	**Low**	Moderate	Low to Moderate
Production Costs	**Moderate**	**Low to Very Low**	**Expensive**	Moderate to Expensive	**Expensive**

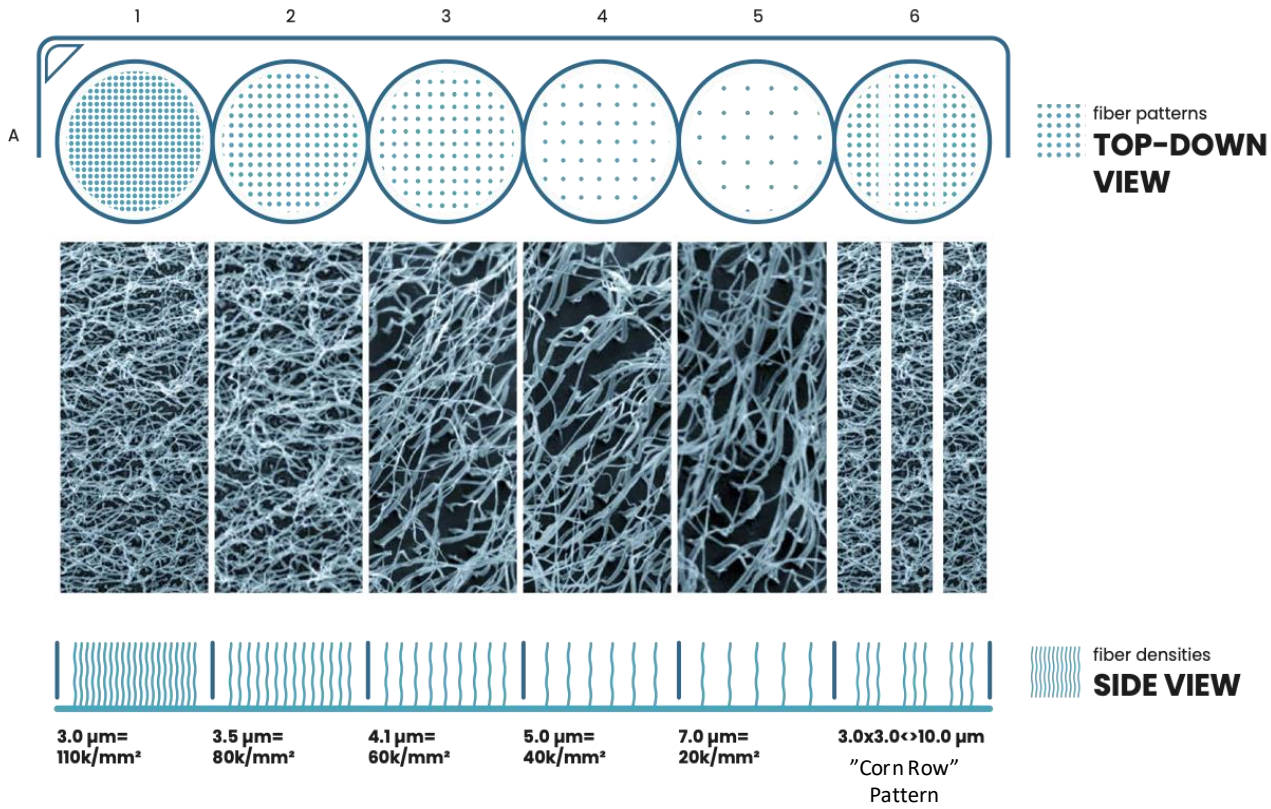
Free Tuning Plates Accelerate Researcher Adoption and Protocol Development

- **Objective:** Acquire data/validation while developing application-specific protocols in collaboration with partner labs

- **USF provides free 24-well "Tuning Plates"**
  - Each column = distinct fiber density/pattern (6 total)
    - Researchers can test cellular behaviors in unique microenvironments to identify the optimal fiber density for their cells and applications
  - Each row = different coating or experimental condition (4 total)

- **Customizable Options**
  - Order off-the-shelf plates with selected fiber density across all wells
  - Inquire about custom fiber patterns

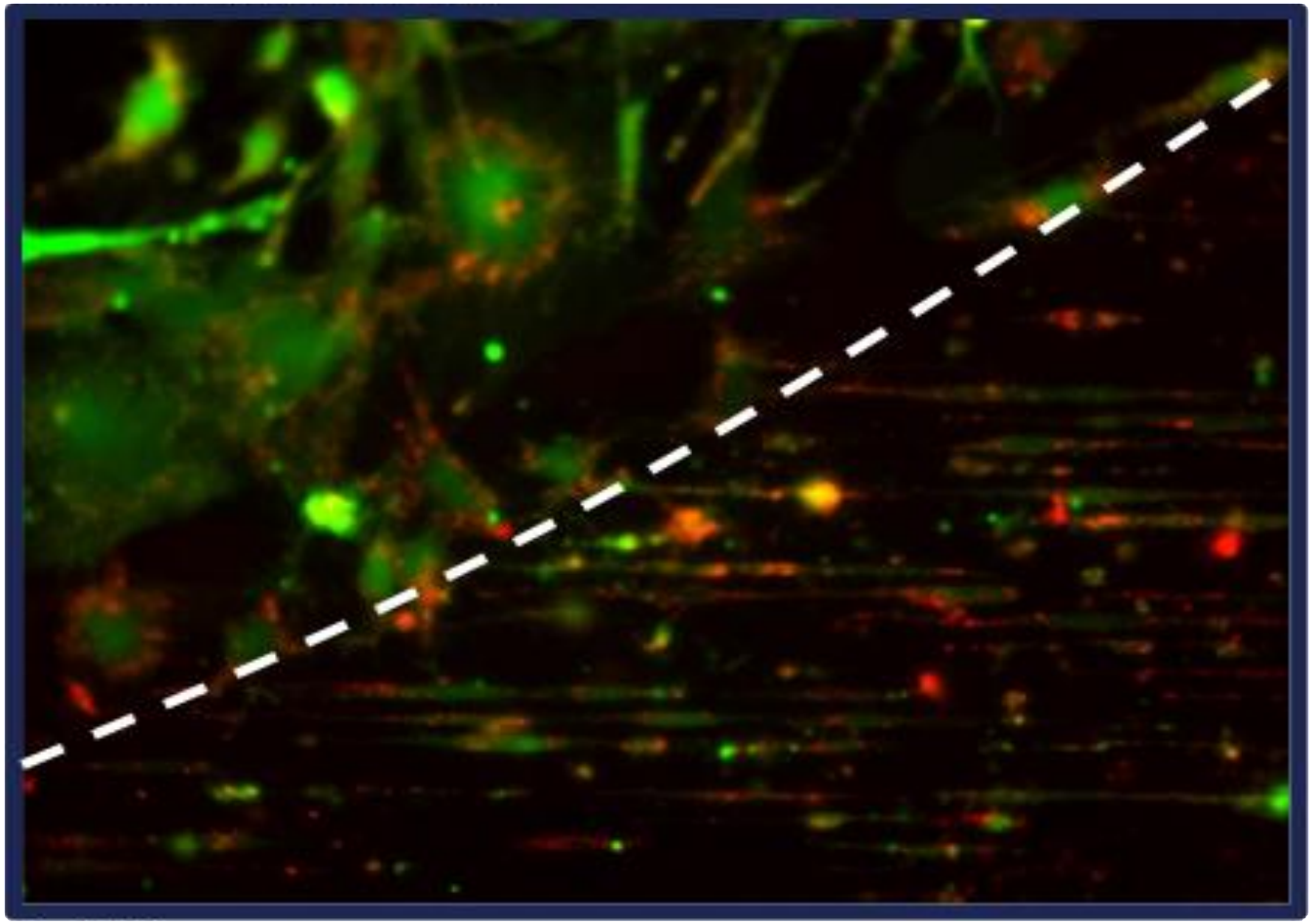
- **Collaboration Model**
  - Collaborating labs share data and protocols; USF builds a growing library of validated application-specific microenvironments
  - Partners gain access to **crowd-sourced application and protocol hub** for USF plates



USF nanofilm 24 well "Tuning Plate" *has 6 columns - each with different fiber densities or patterns creating unique microenvironments. Simultaneously test and compare cell culture behavior.*

Nanofibers Alone Drive Profound Changes Cell Morphology

2D Surface (no nanofibers)
Cells are larger and flattened



Calcein AM
LysoVieW

USF nanofibers
Cells are smaller, elongated and aligned

MEF cells show markedly different morphology in the presence, or absence, of nanofibers.

KEY FINDINGS AND INFORMATION

Cells: MEF (mouse embryonic fibroblasts)

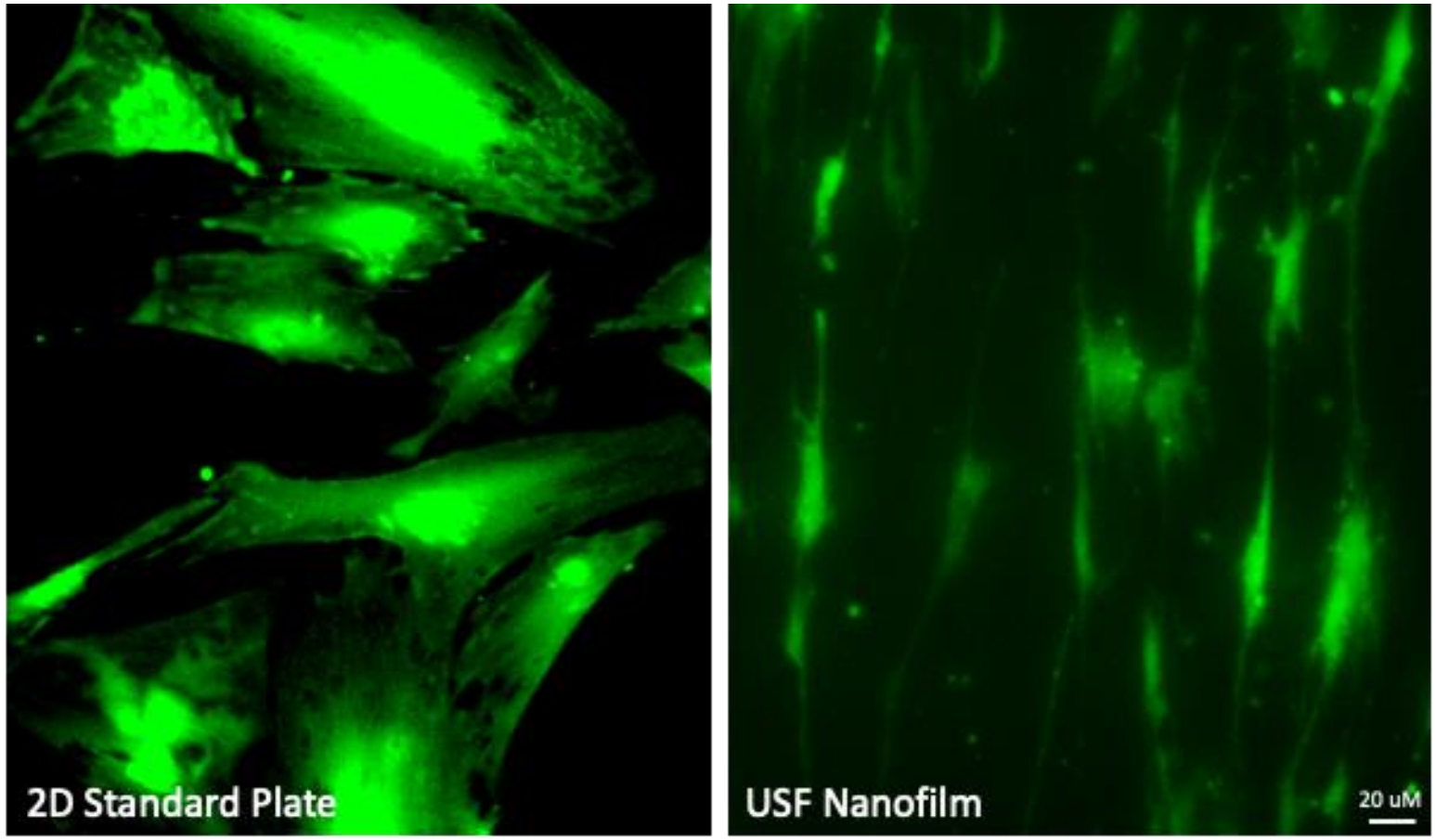
Identical cells and conditions. Nanofibers within a portion of a well were melted to created a 2D surface immediately adjacent to nanofibers

Biophysical cues drive the difference. Cells within USF nanofiber region are smaller, elongated, and aligned; cells on standard 2D region are large and flattened

* Dashed white line denists border between 2D Surface and USF nanofiber regions

Custom “Corn Row” Pattern

USF Nanofilms promote natural alignment of Fibroblasts



Calcein AM

USF Nanofilm guides fibroblasts to self-organize along nanofiber scaffolds, producing in vivo-like alignment and morphology that flat 2D surfaces cannot replicate.

KEY FINDINGS AND INFORMATION

Cells: Mouse Embryonic Fibroblasts (MEF)

MEFs align directionally along nanofibers

Fibroblasts adopt spindle-like morphology resembling *in-vivo* tissue organization on USF nanofilm plates

Natural Alignment of NRVMs in USF Nanofilms

Standard 2D Plate (Control)

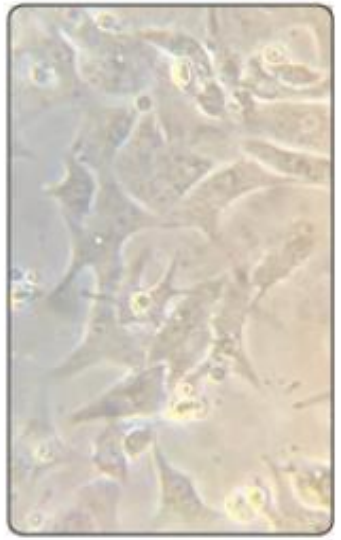
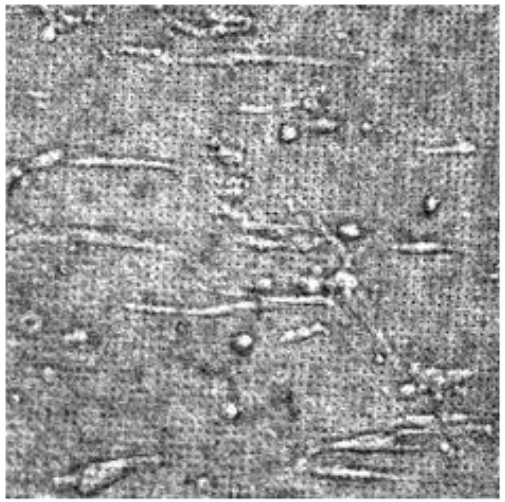
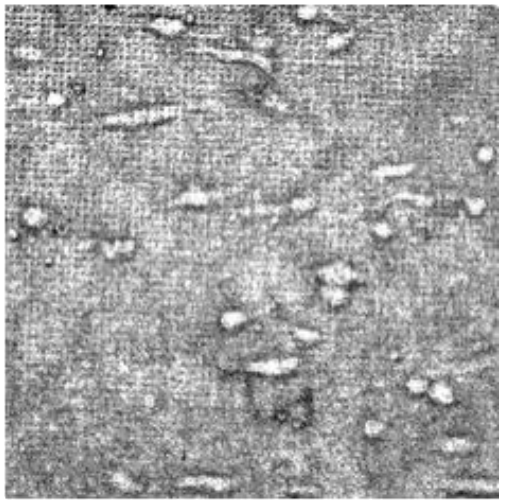


*image: Pereira et al., (2021) STAR Protocols

USF Nanofilm Plate



USF nanofilm – 5.0 um



USF nanofilm – 4.1 um

Data generated by Cara Barnett and Patrick Oakes, PhD, Loyola University Chicago using USF Plates

USF Nanofilm guides fibroblasts to self-organize along nanofiber scaffolds, producing in vivo-like alignment and morphology

KEY FINDINGS AND INFORMATION

Cells: Primary neonatal rodent ventricular cardiomyocytes

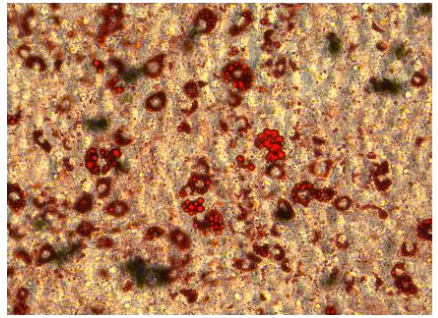
Cardiomyocytes align directionally along nanofibers

Cardiomyocytes adopt elongated morphology resembling *in-vivo* tissue organization on USF nanofilm plates

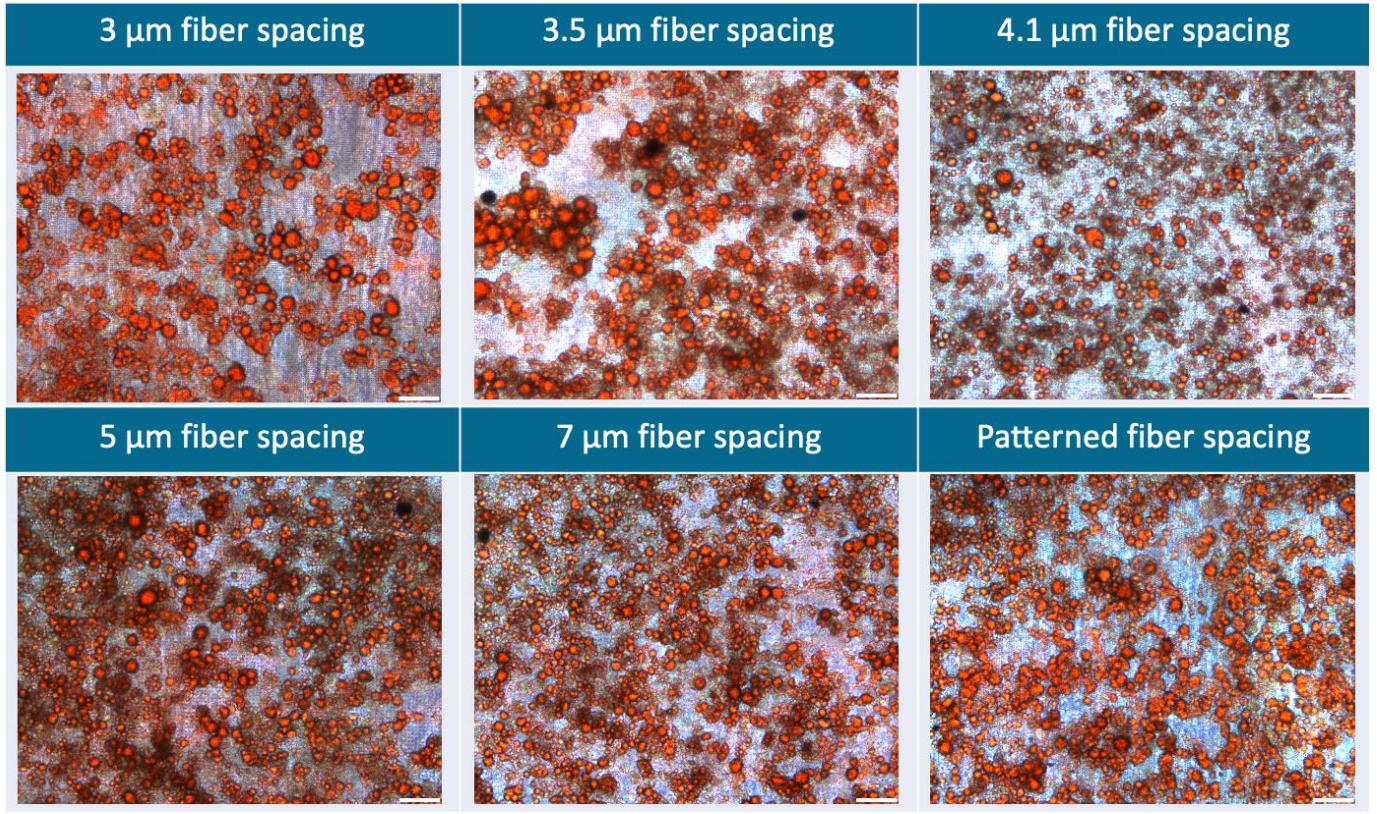
**Pilot experiment – protocol optimization ongoing*

USF Nanofilm Plates Enhance Adipose Cell Differentiation

Standard 2D Plate (Control)



USF Nanofilm Plate



Scale bar = 100um

Data generated in collaboration with Angelica Ross and Marcella Vaicik, PhD, Illinois Institute of Technology

USF nanofilm biophysical cues work synergistically with existing biochemistry to promote more effective differentiation

KEY FINDINGS AND INFORMATION

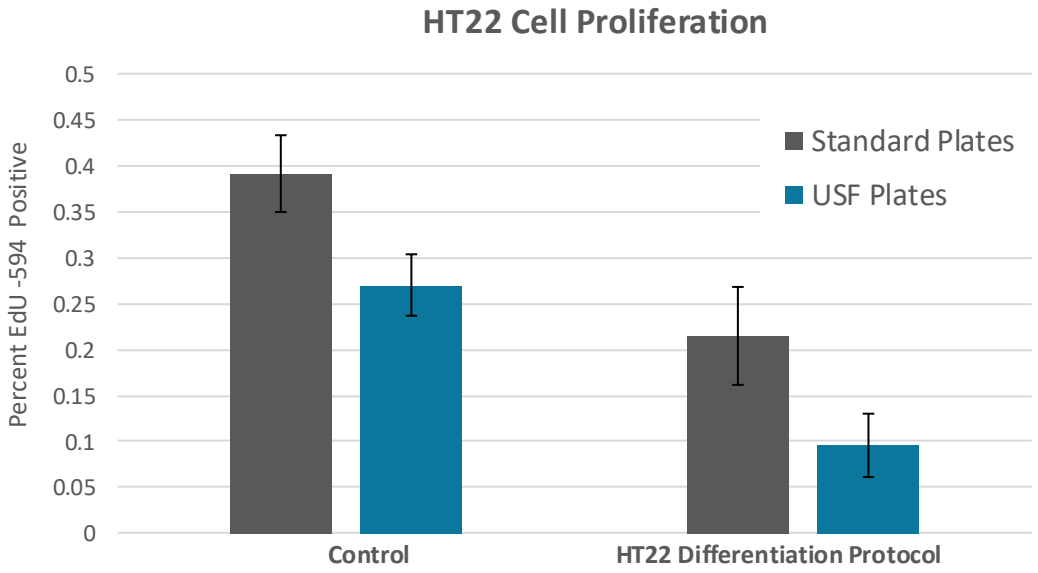
Cells: Adipose cells differentiated from 3T3 cells

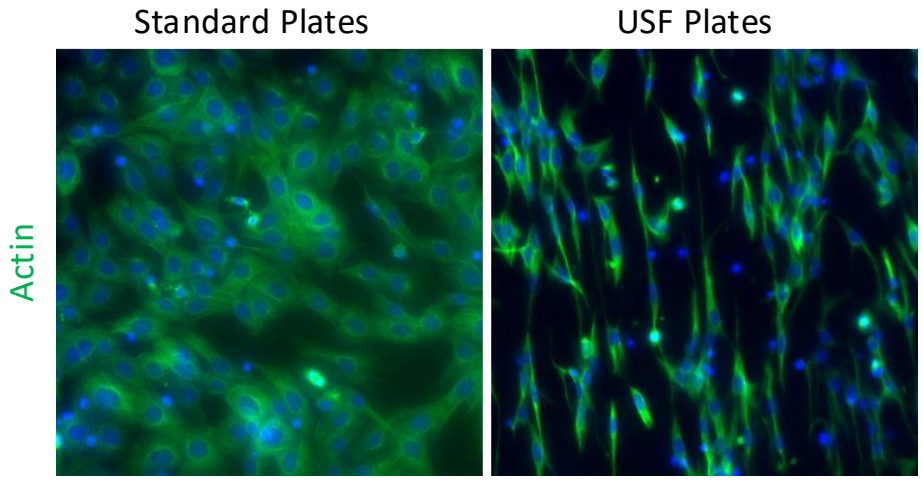
Biophysical cues from USF nanofibers more effectively direct cells toward adipogenic fate

All other protocol conditions between USF and standard plates remained identical

**Pilot experiment – protocol optimization ongoing*

USF Nanofilm Plates enhance HT22 Cell Differentiation





Biochemical differentiation signals combined with USF nanofilm biophysical cues work synergistically to promote more effective differentiation

KEY FINDINGS AND INFORMATION

HT22 cells can be differentiated toward a more neuronal phenotype

Decreased proliferation is a hallmark of neuronal differentiation

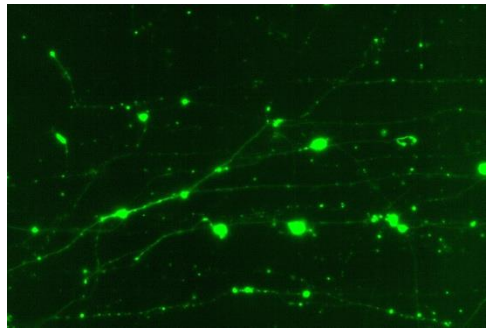
Differentiation on USF plates is more effective than on standard 2D plates

USF Nanofilm cells adopt neuronal-like morphology, suggesting a more complete differentiation response

USF microenvironments Direct Neuronal Patterning

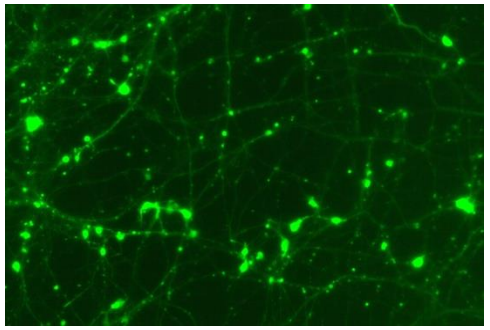
High Density Nanofiber Spacing

- Enhanced **isolation of individual neurons and axons**
  - Ideal for imaging and analysis of single cells or axons
  - Potential Applications: Neuronal morphology studies, regeneration studies, electrophysiology, etc



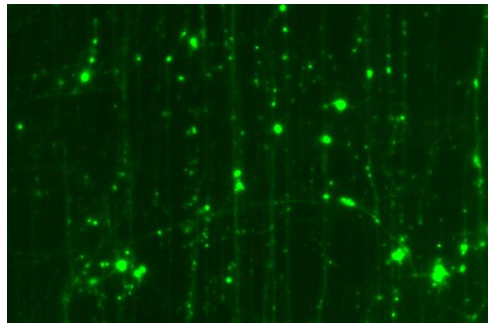
Low Density Nanofiber Spacing

- **Evenly distributed** neuronal cultures
  - Similar to traditional 2D culture but with reduced clumping
  - Improved reproducibility and analysis in imaging experiments
  - Potential Applications: Multi-cell disease modeling, high-throughput imaging, drug/compound testing, etc.



"Corn Row" Patterned Spacing

- Neurons grow in **aligned, straight lines**
  - Allow for directed neuronal growth
  - Greater spatial isolation of neurons and axons
  - Ideal for axon-focused research
  - Potential Applications: Axonal Transport and Trafficking, Live-Cell protein dynamics studies, axonal growth studies, etc.



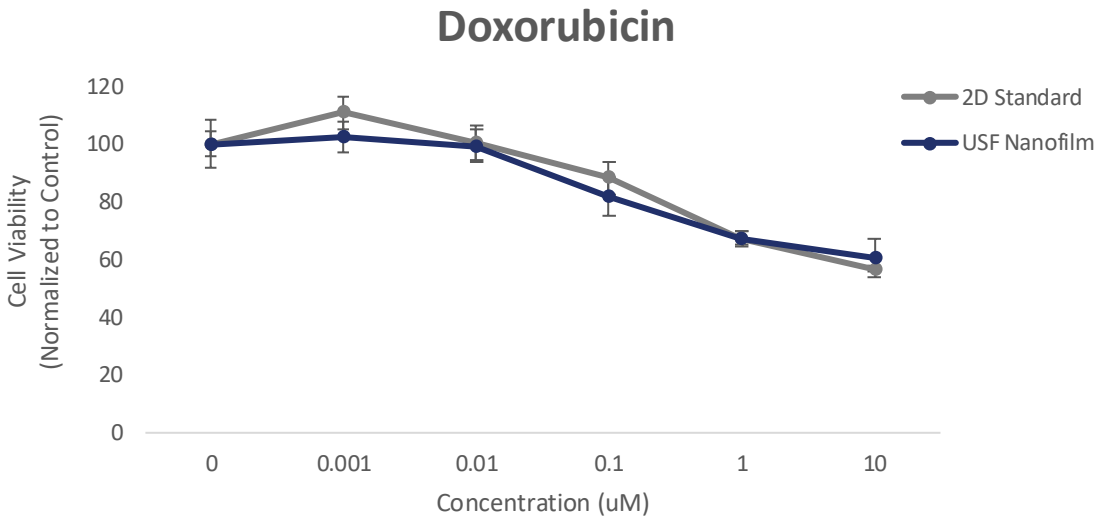
USF nanofilm technology creates targeted microenvironments that direct and influence neuronal patterning and growth

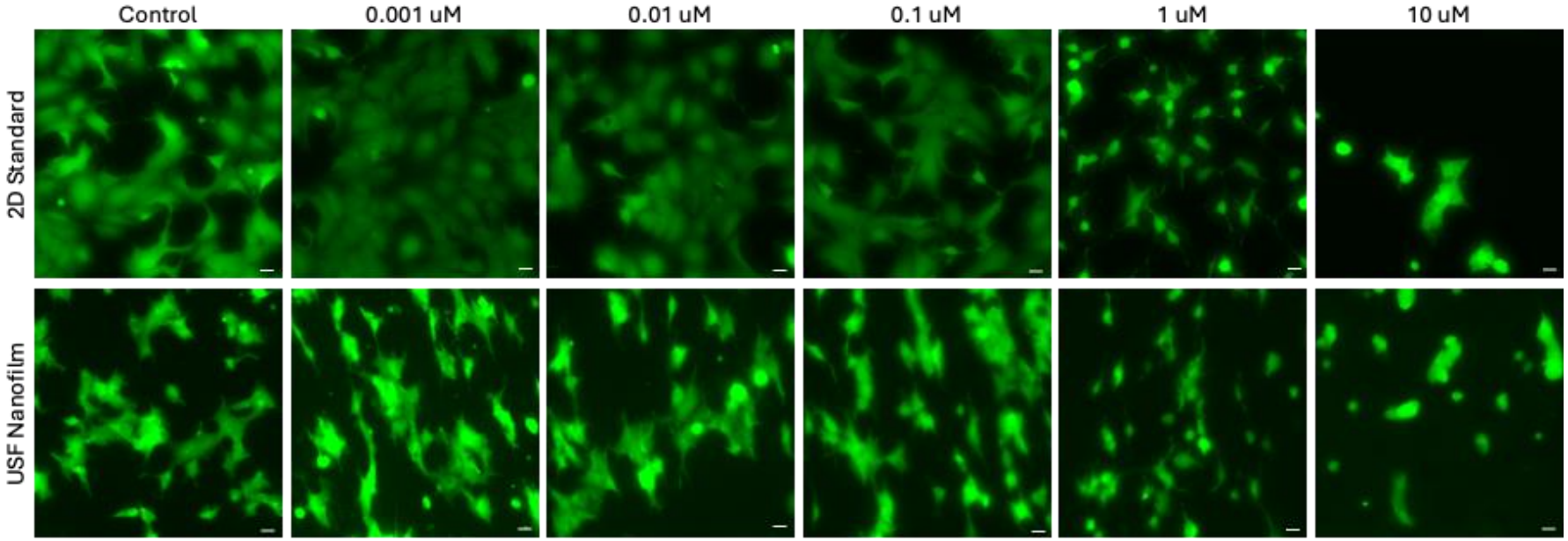
KEY FINDINGS AND INFORMATION

Cells: iPSC-derived human cortical neurons

USF technology enables the development of tailored neuronal models for specific research applications

Influence of Nanofiber Microenvironment on HeLa Cell Drug Response





KEY FINDINGS AND INFORMATION

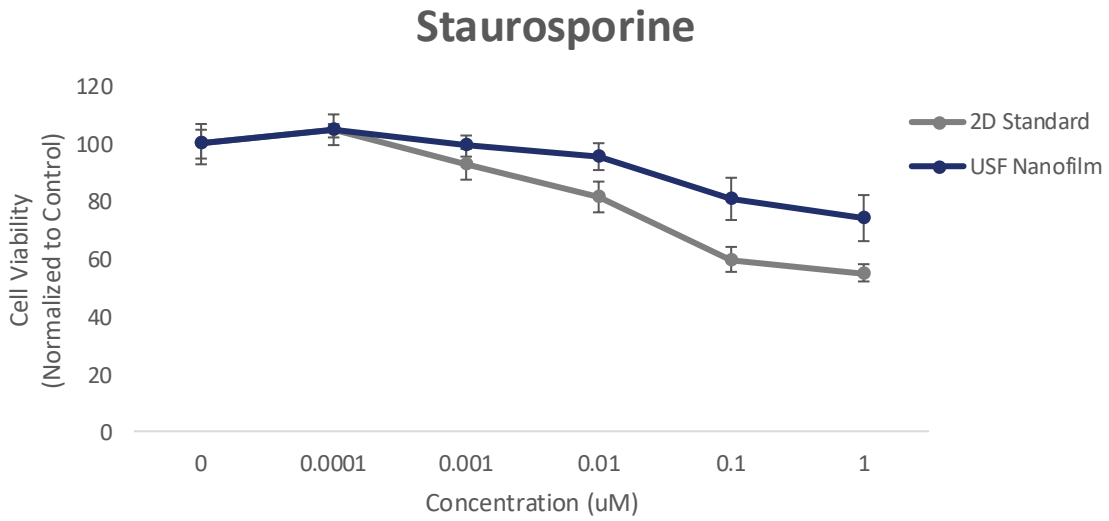
Drug Facts: Doxorubicin is a DNA-damaging chemotherapy drug.

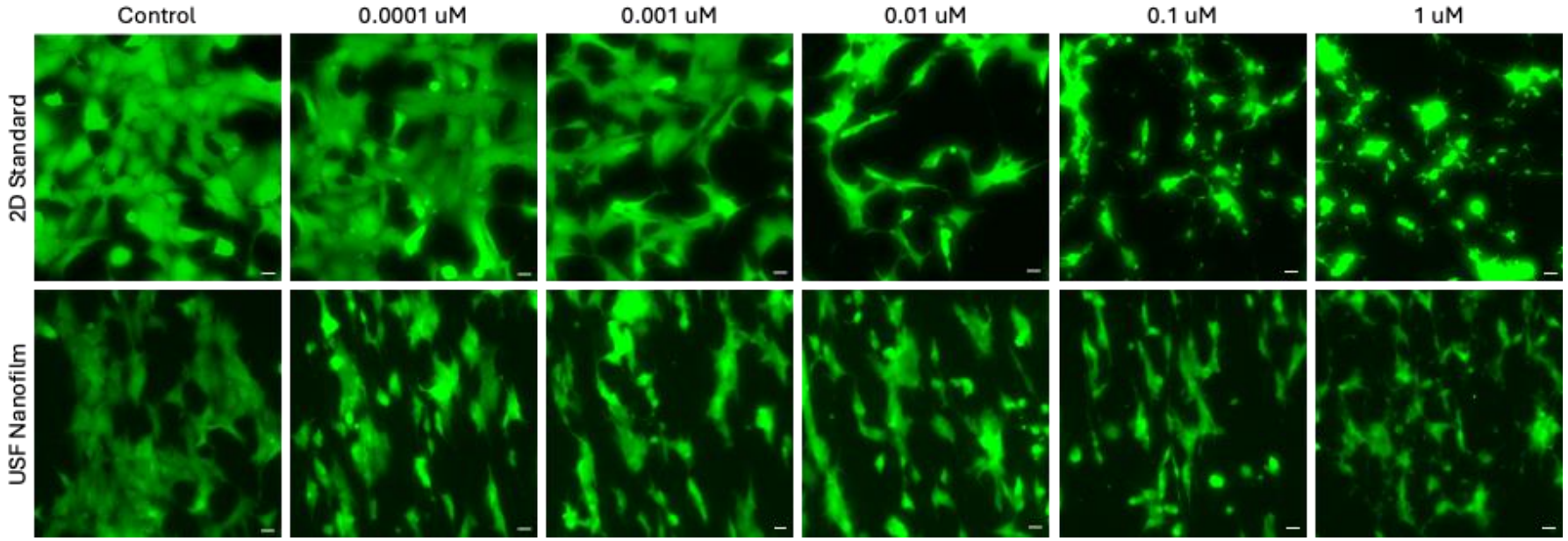
Minimal influence of microenvironment on drug response: Doxorubicin acts directly on DNA, making it less dependent on cell shape, adhesion, or microenvironment.

Similar viability curves: Consistent potency across both microenvironments suggests nanofibers do not impede drug activity or delivery.

5x5um Pattern, Scale Bar = 20um, HeLa Cells

Influence of Nanofiber Microenvironment on HeLa Cell Drug Response





KEY FINDINGS AND INFORMATION

Drug Facts: Staurosporine is broad protein kinase inhibitor and strong apoptosis inducer

Strong influence of microenvironment on drug response: Staurosporine inhibits survival signaling pathways (strongly influenced by cell–matrix interactions and cytoskeletal organization).

Clear, sustained separation of viability curves show a meaningful decrease in sensitivity to Staurosporine treatment

The importance: USF nanofilms appear to capture effects of ECM on drug response typically missed by standard plates.

5x5um Pattern, Scale Bar = 20um, HeLa Cells

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Why are drug responses different? Mechanism of Action Explains the Gap

DOXORUBICIN

MECHANISM

DNA intercalation + Topoisomerase II inhibition triggers apoptosis via nuclear damage

ECM / NANOFIBER INTERSECTION

Minimal. Once drug reaches the nucleus, cell death is largely independent of microenvironmental survival signaling.

Platform effect: SUBTLE
Curves converge

STAUROSPORINE

MECHANISM

Broad-spectrum kinase inhibitor blocks PKC, Akt, and pro-survival kinases, triggering apoptosis via survival pathway shutdown

ECM / NANOFIBER INTERSECTION

Direct. The nanofiber scaffold activates the survival signaling pathways that staurosporine block. This ECM-driven survival signaling partially offsets kinase inhibition, mirroring *in vivo* resistance.

Platform effect: STRONG
~20% viability difference at max dose

The drug's target pathway determines whether the microenvironment changes the outcome.
USF's platform captures this distinction — 2D models cannot.

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A Progressive Toolkit: From 2D to Organoids

USF nanofilm scaffolds add biophysical dimensionality at two stages of this progression — and may ultimately enhance a third. Each stage has an enduring role.

Current Best Practice: 2D

Flat plates +/- Matrigel, ECM gels, or emerging biochemical alternatives

Flat plastic or glass which can be augmented with animal-derived or synthetic hydrogel matrices to add biological context.

Fundamental limitations:

- Little biophysical tunability or architectural control
- Animal-derived matrices couple biochemical and biophysical signals — and vary batch to batch
- Poor physiological relevance for adherent-cell biology
- Fast, cheap, and scalable — will remain widely used

Cell Culture in USF 3D Scaffolds

USF's current commercial focus

Standard cell culture within a biomimetic nanofiber scaffold. Drop-in compatible with existing well-plate workflows.

What USF adds vs. current best practice:

- Optimized culture conditions for each specific cell type through independent control of biophysical and biochemical variables
- Biomimetic nanofiber architecture replicates key ECM structural cues
- Reproducible: defined, xeno-free chemistry with no batch variability
- Compatible with coatings, imaging, and standard assays; no animal-derived components

Data in hand: HT22, HeLa, fibroblasts, adipocytes, iPSC neurons

Co-Cultures in USF 3D Scaffolds

Promising but very early stage

The nanofiber canopy (40+ µm height) supports multi-cell cell growth within a structurally defined, reproducible architecture.

What this may enable:

- Disease modeling with greater physiological relevance
- Greater physiological complexity
- Retains reproducibility and biophysical tunability
- Scaffold-guided architecture — not random self-assembly
- Compatible with live-cell imaging
- Allows for the greater physiological complexity with the ability to track and analyze cultures in real time

Early exploration underway

Organoids (3D)

Speculative long-term opportunity

Self-organizing 3D structures with high physiological complexity. High discovery potential but current biomimicry, tunability, reproducibility and other issues are major limitations.

The USF hypothesis:

USF scaffolds establish a biomimetic, tunable, reproducible foundation for the first cell layers. Those cells deposit their own ECM — guided by the scaffold architecture — which in turn directs subsequent layers to self-organize into a biomimetic, reproducible organoid structure. ***The promise is real but is speculative and not yet tested.***

2.5D* Cultures

USF PLATFORM ADDRESSES STAGE 2 TODAY — EXPLORING STAGE 3 NOW, THEN BUILD TO STAGE 4

***2.5D refers to monolayer cultures that recapitulate the physiological complexity of 3D culture**

Collaboration Opportunity

What USF Offers

- Complimentary Tuning Plates for your application area
- Technical support and protocol guidance throughout evaluation
- Custom architectures designed for specific cell types or assay needs
- Flexible partnership structures: co-development, licensing, or OEM integration
- Roll-to-roll scalable manufacturing; compatible with large-format cell production needs

Where USF Adds the Most Value for Stem Cell Research

- **Stem cell differentiation:** Biophysical cues work synergistically with biochemical differentiation protocols to direct stem cell fate -- adipocyte and neural data in hand
- **Stem cell expansion and maintenance:** USF nanofiber substrates significantly increased expression of self-renewal factors Nanog and OCT4A in hMSCs, suggesting biophysical cues help maintain stemness during expansion. (Hofmeister et al., J. Biological Engineering, 2015)
- **Organoids:** A reproducible, scalable 2D alternative for researchers seeking physiological relevance without organoid complexity -- with potential as a biophysical foundation layer as organoid protocols mature.
- **Neuroscience:** Architectural control for neuronal organization and iPSC-derived neural models
- **Cell therapy manufacturing:** Large-format adherent cell culture scaffolds for scale-up of iPSC and cell therapy production

Intellectual Property

- **17 issued U.S. patents**, including broad foundational patents covering core platform architecture
- Patents covering nanostructure fabrication, biomimetic cell culture substrates, biomimetic lamellar tissue scaffolds; roll-to-roll production and surface wettability modification have international coverage in China, Europe, Korea, Mexico, and Hong Kong
- IP portfolio supports licensing, co-development, and OEM partnership structures

Suggested Next Step

Request free Tuning Plates and add the missing biophysical variable to your next experiment. USF will design the evaluation to match your specific cell types and research questions.

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