

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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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
- **3D organoids** often introduce experimental artifacts and can be difficult to scale and reproduce
- **Existing scaffolds** lack biomimicry, reproducibility and tunability

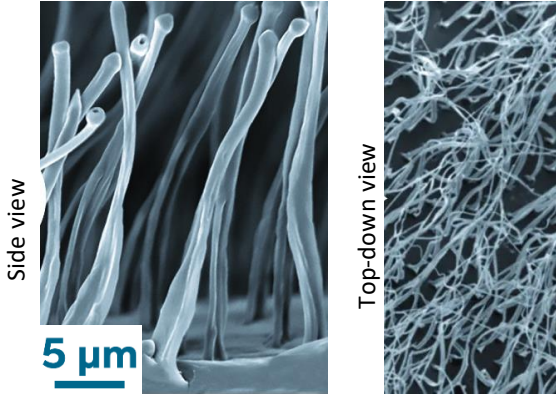
■ The Result:

High variability, increased development time, and higher research costs

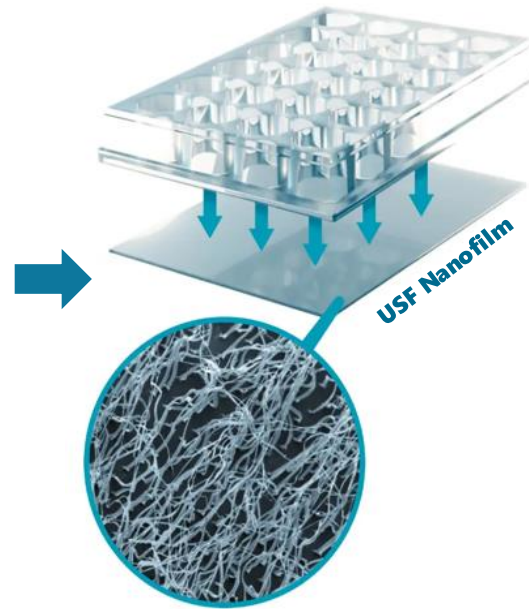
The Solution: USF Nanofilm Cell Culture Scaffolds

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

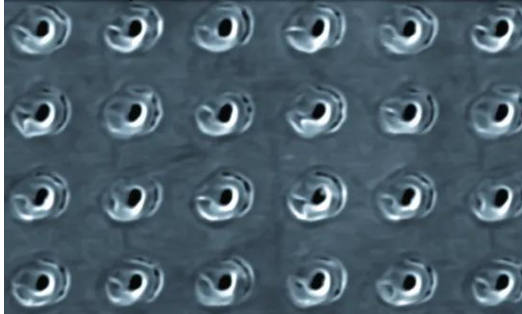
USF nanofilms



USF nanofilm welded to 24-well plate



Glass template used to emboss nanofibers



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

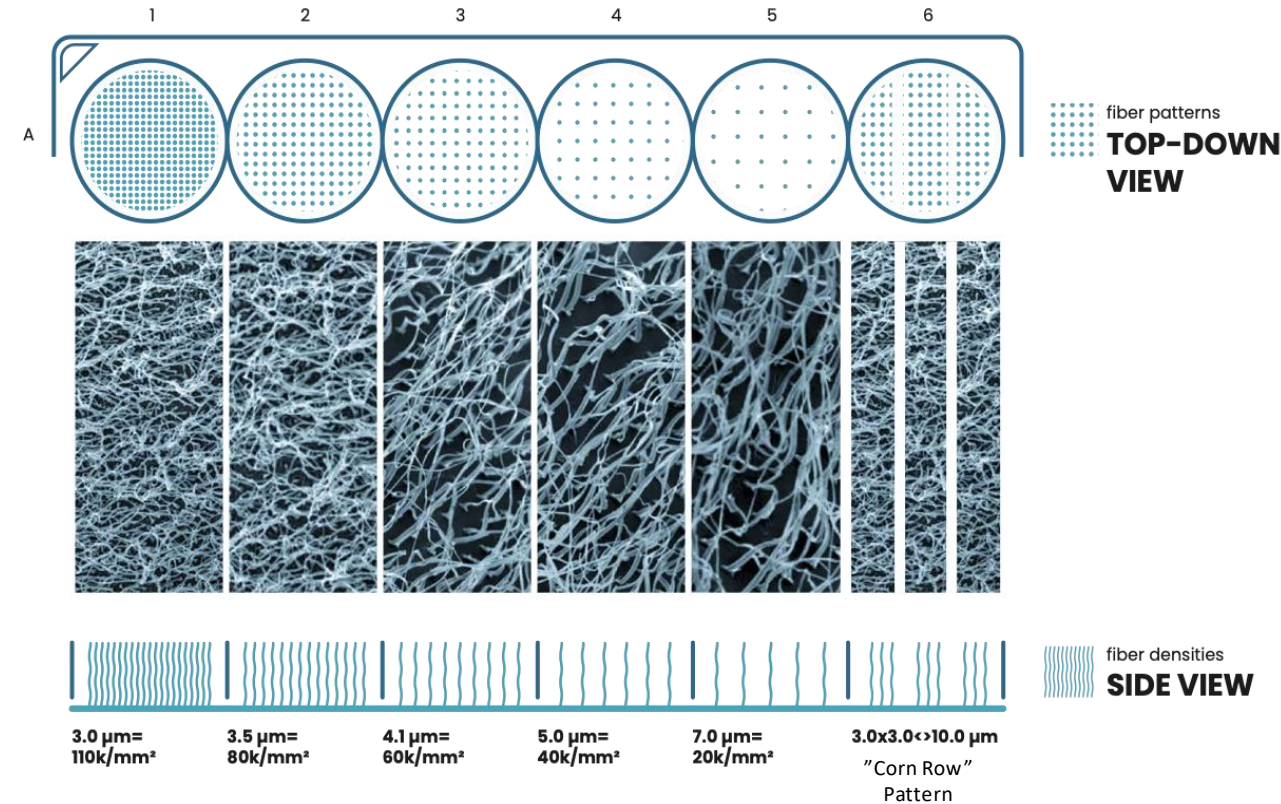
- Fiber diameters match native biological scaffolds
- Fibers mimic collagen
- Each fiber density provides a unique cellular microenvironment
 - Customizable fiber spacing replicates a range of biomimetic microenvironments
- No need for hydrogels
- Nanofibers can deliver essential biophysical signals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

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

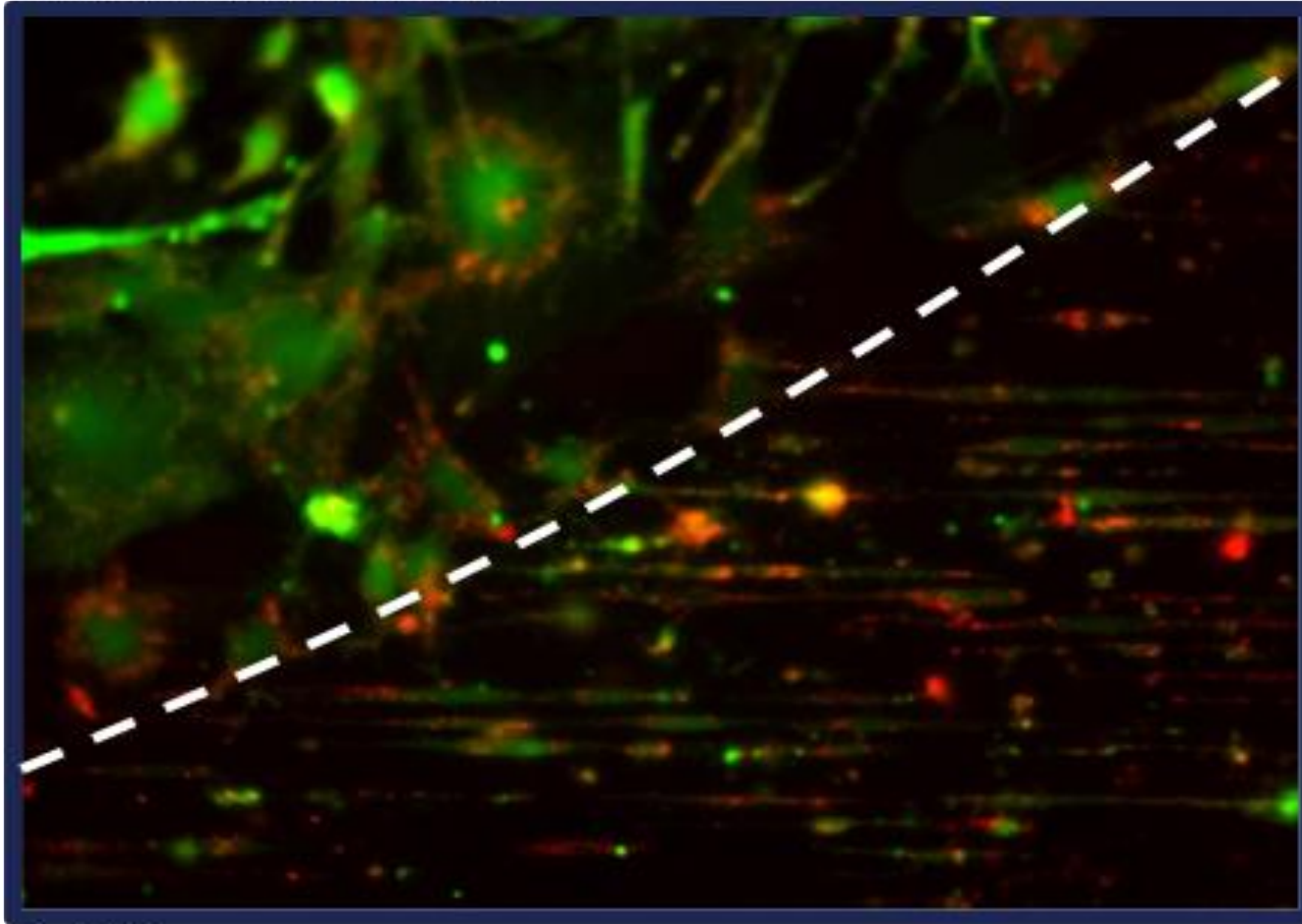


USF nanofilm 24 well “Tuning Plate” has 6 columns – each with different fiber densities or patterns creating unique microenvironments. Simultaneously test and compare cell culture behavior.

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.

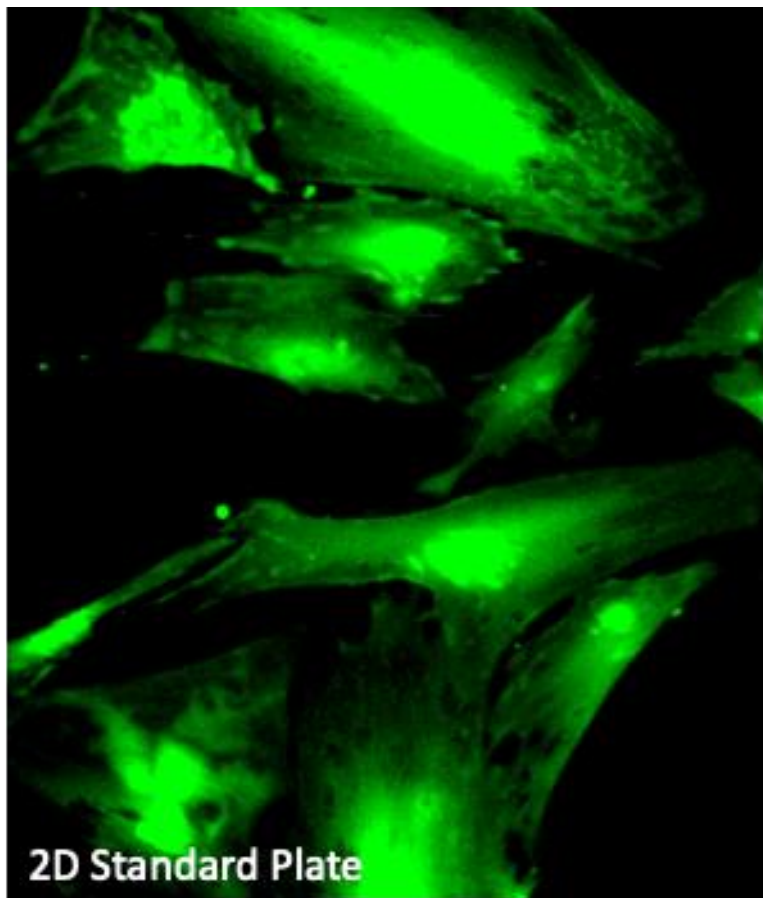
KEY FINDINGS AND INFORMATION

Cells: MEF (mouse embryonic fibroblasts)

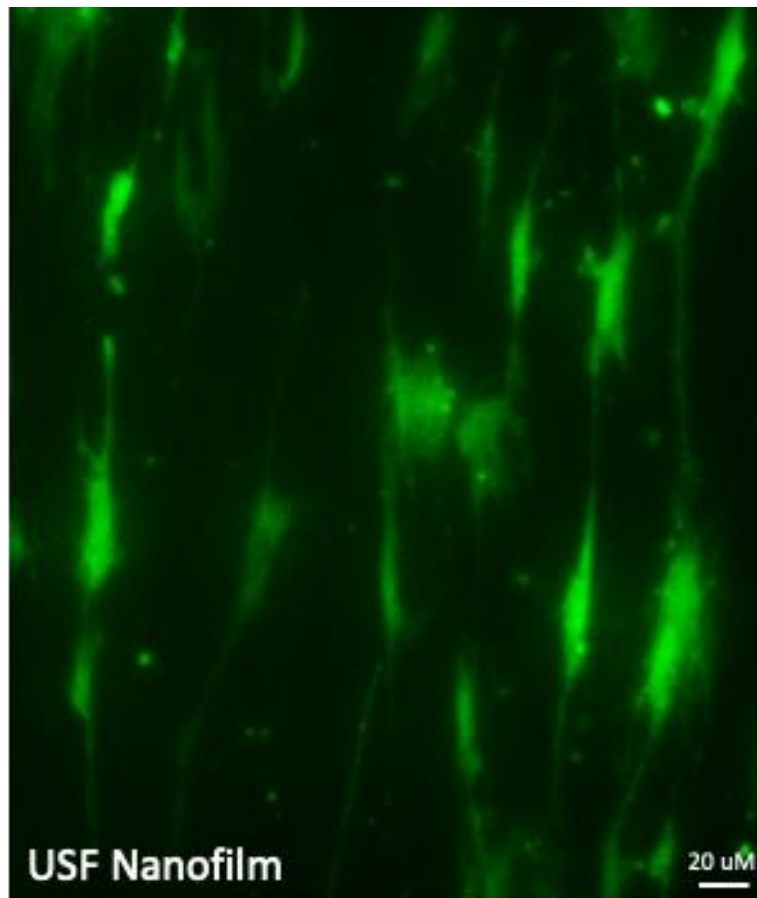
Identical cells and conditions. Nanofibers within a portion of a well were melted to create a 2D surface immediately adjacent to nanofibers

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

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

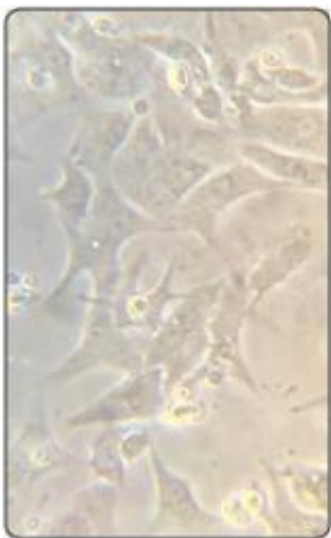
Cells: Mouse Embryonic Fibroblasts (MEF)

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)



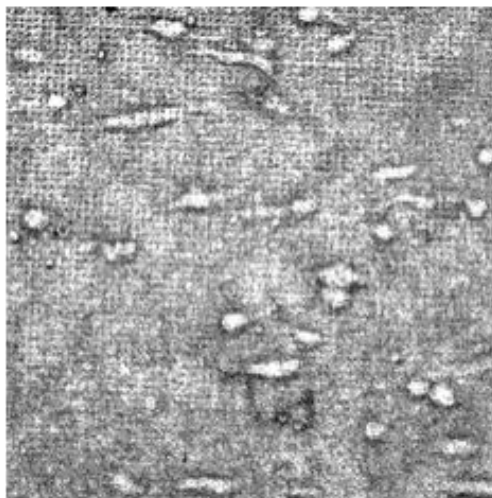
*image: Pereira et al.,
(2021) STAR Protocols



Nanofilm Plate



USF nanofilm – 5.0 um



USF nanofilm – 4.1 um

USF Nanofilm guides cardiomyocytes 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

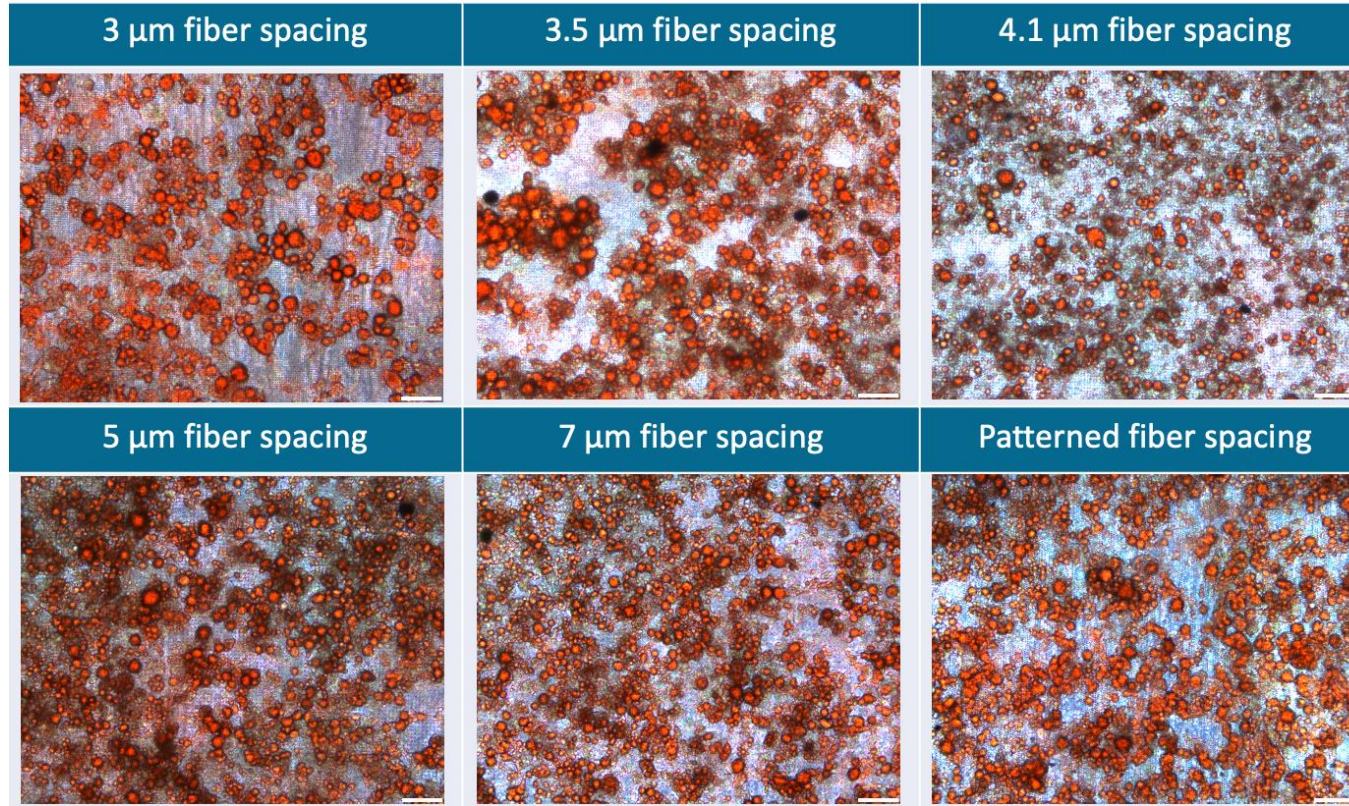
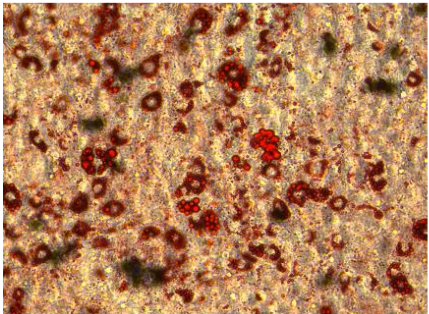
Cardiomyocytes adopt elongated morphology resembling *in-vivo* tissue organization on USF nanofilm plates

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

USF Nanofilm Plates Enhance Adipose Cell Differentiation

USF Nanofilm Plate

Standard 2D Plate (Control)



Scale bar = 100 μm

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

KEY FINDINGS AND INFORMATION

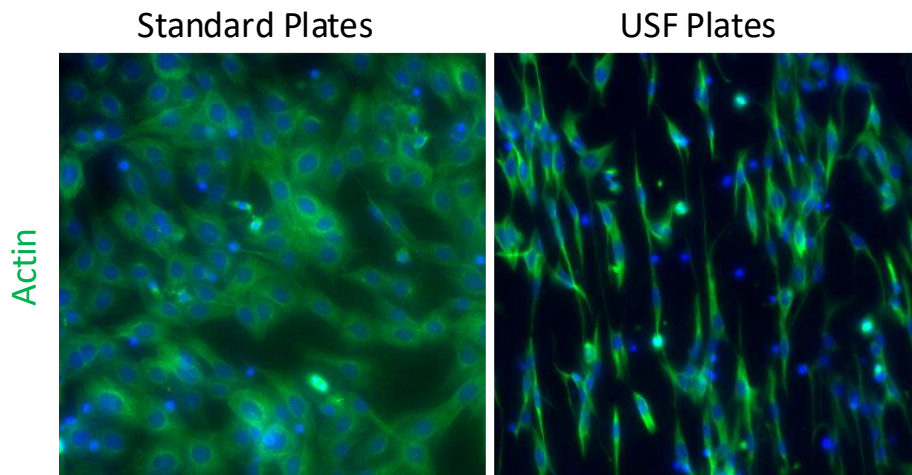
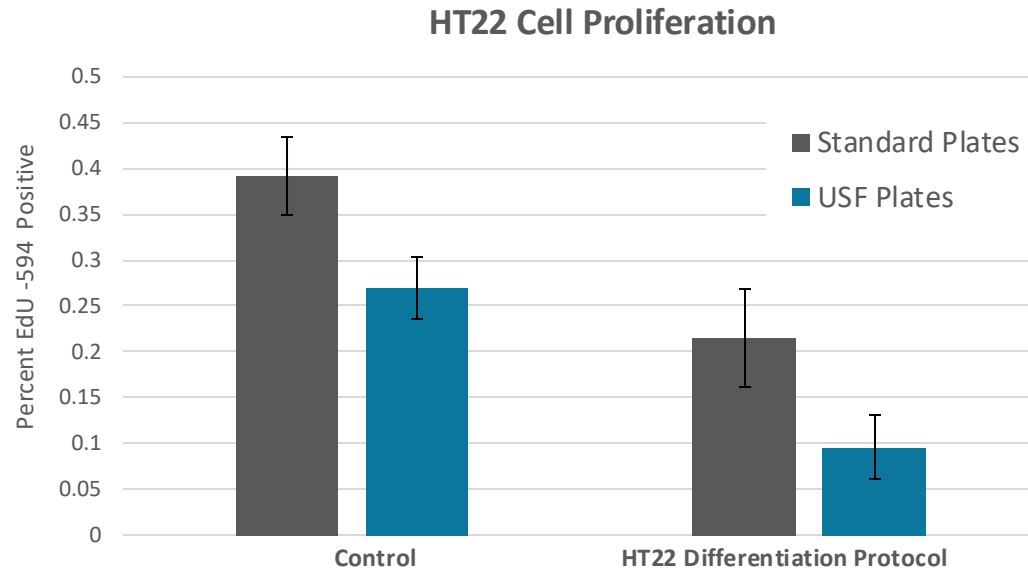
Cells: Adipose cells differentiated from 3T3 cells

Lipid accumulation is visibly greater on USF nanofilm across all six conditions. Quantification in progress.

All other protocol conditions between USF and standard plates remained identical

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

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

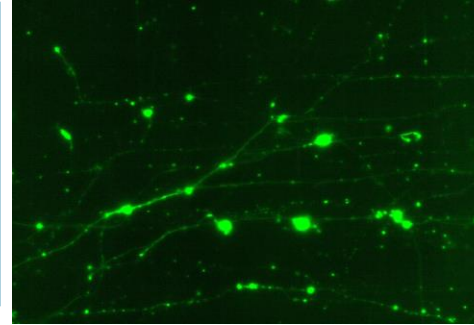
Differentiation on USF plates is more effective than on standard 2D plates

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

USF microenvironments Direct Neuronal Patterning

High Density Nanofiber Spacing

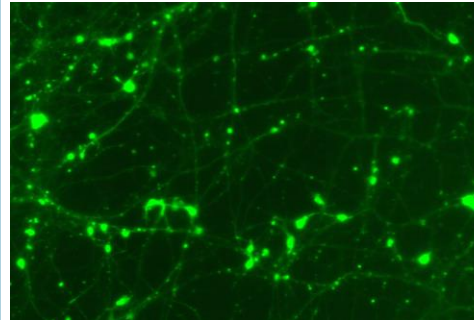
- Enhanced **isolation of individual neurons and axons**
 - Ideal for imaging and analysis of single cells or axons
 - Potential Applications: Neuronal morphology studies, regeneration studies, electrophysiology, etc



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

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.

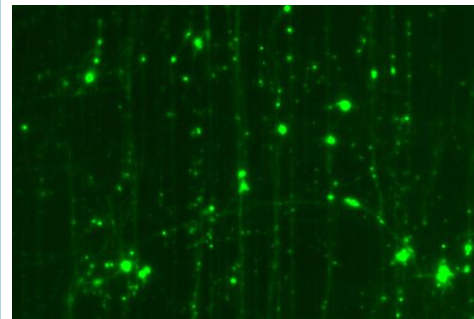


KEY FINDINGS AND INFORMATION

Cells: iPSC-derived human cortical neurons

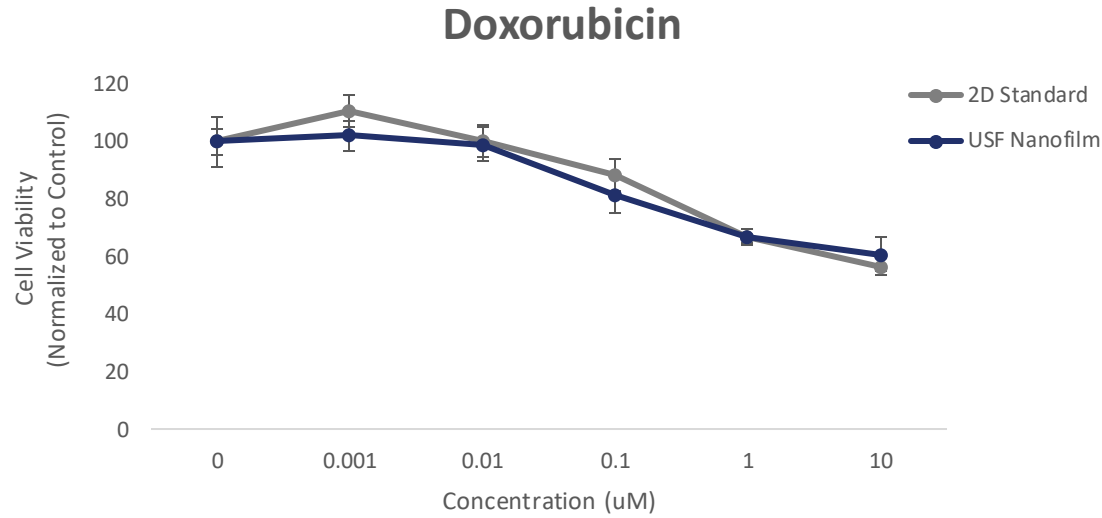
"Corn Row" Patterned Spacing

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

Influence of Nanofiber Microenvironment on HeLa Cell Drug Response

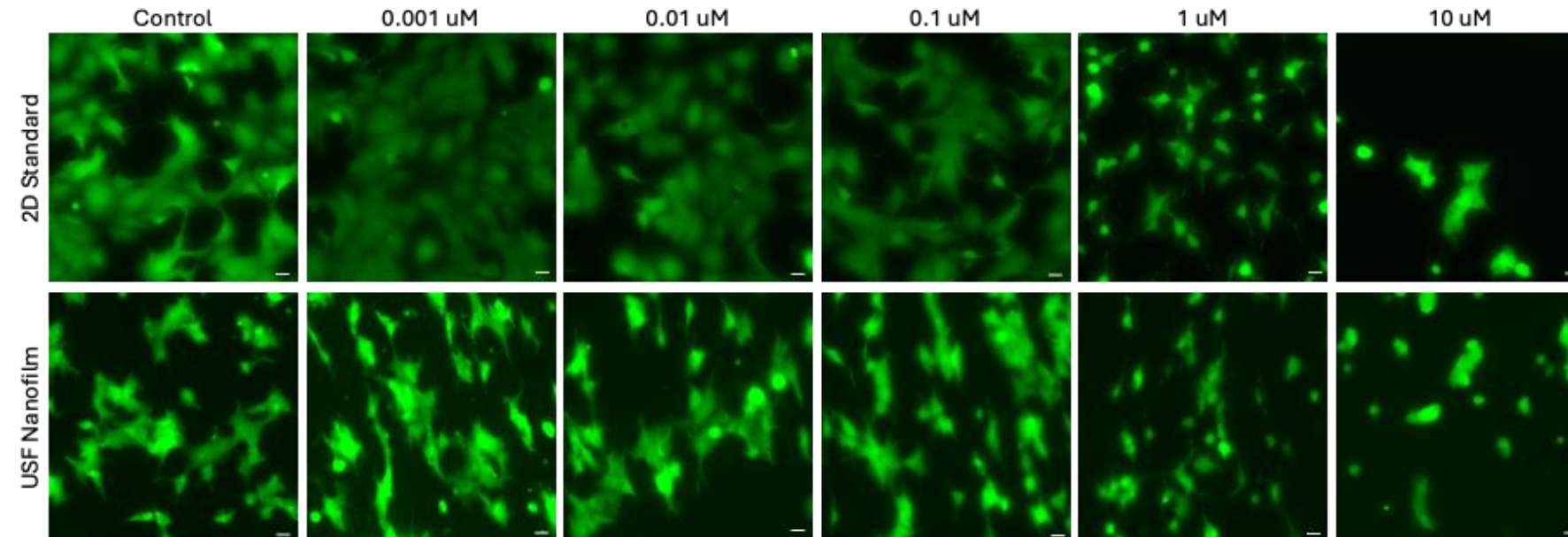


KEY FINDINGS AND INFORMATION

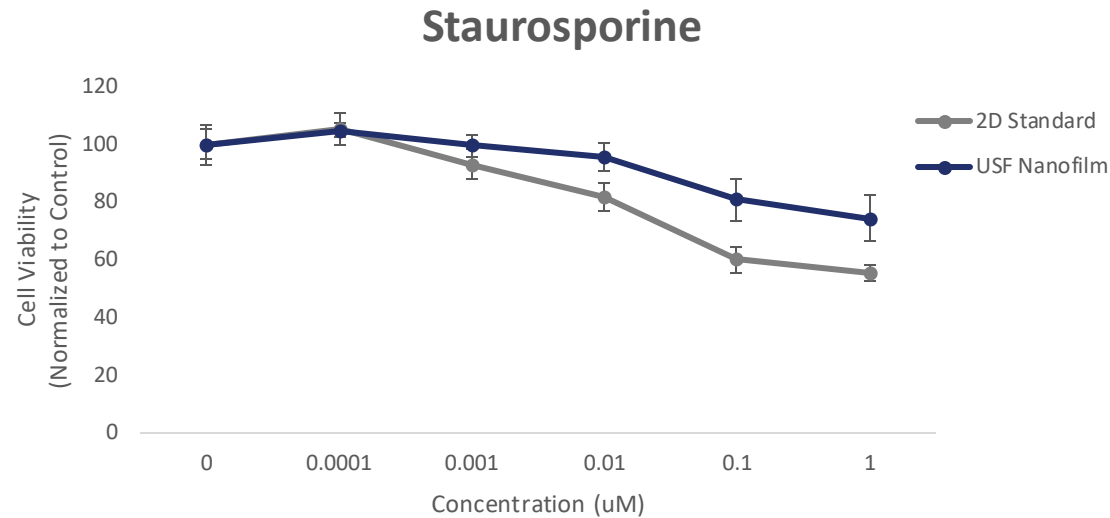
Drug Facts: Doxorubicin is a DNA-damaging chemotherapy drug.

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.



Influence of Nanofiber Microenvironment on HeLa Cell Drug Response



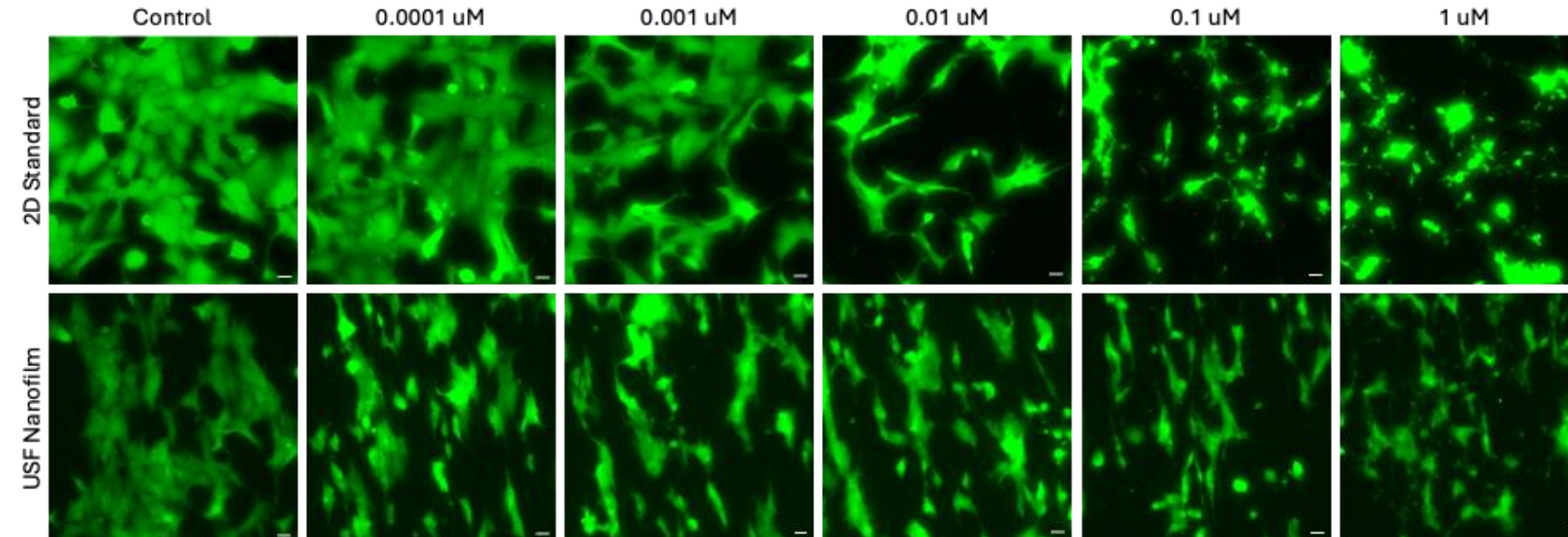
KEY FINDINGS AND INFORMATION

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.



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

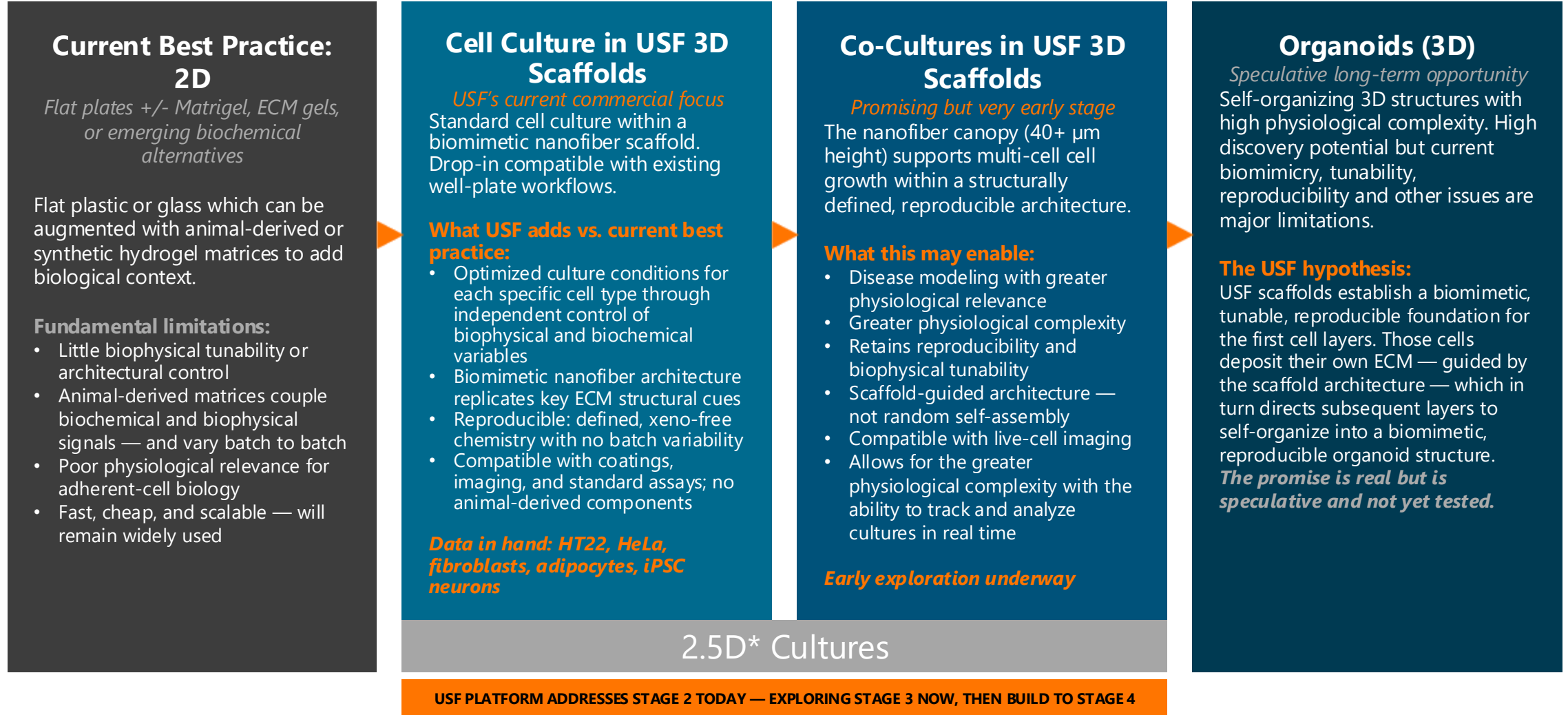
Direct. The nanofiber scaffold activates the survival signaling pathways that staurosporine block. This ECM-driven survival signaling partially offsets kinase inhibition, mirroring *in vivo* resistance.

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.

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.



**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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