Nanofiber Solutions

3D cell Culture

- Ellipsoidal cells around 10-30 um thick
- Have nearly 100% of their surface area exposed to other cells in the matrix
- Higher expansion rate and better control of differentiation

2D cell culture

- Flat cells around 3 um thick
- Only 50% exposed to fluid and a small percent to other cells
- Poor behavioral response in culture

Executive Summary

Cell culture is commonly carried out in cheap yet convenient flat plastic substrates. Mammalian tissue is composed of various proteins that make up a nanofibrous extracellular matrix (ECM). Thus, the study of cells on flat 2-D surfaces cannot capture the complex structural and chemical cues which exist *in vivo*. Researchers are realizing the advantages of moving towards using 3-D environments to more accurately study cellular biology but, have been hesitant due to complex and time consuming methodologies or expensive equipment.

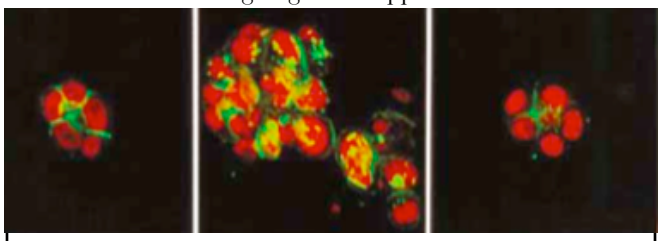
To address the problems of life science researchers, Nanofiber Solutions has developed patent pending nanofiber coated multiwell plates which mimic the complex topography of the ECM found *in vivo*, and allow researchers to more accurately study cellular functionality, morphology, protein/gene expression and drug interaction/toxicology *in vitro*. Nanofiber Solutions' products allow for live cell imaging, even while cultured on a 3-D nanofiber substrate, and cells can be easily removed after culture for post-processing assays. In addition, these nanofiber coated multiwell plates can be used for high-throughput cell culture and cancer research by being compatible with automated plate readers.

In the next 2–3 years, it's really going to be surfaces that are pulling us ahead. This is what we are waiting for. This is the last piece of the holy grail.

-Nature Methods 8, 293–297, 2011

Advantages of Moving Towards 3-D Scaffolds for Cell Culture

As a biologist, little thought is given to the materials which make up the products that we use in our research day-in and day-out. However, it is these materials which form the 'building block' or 'scaffold' of all cell culture research. The intricate detail going into understanding the cellular response is all dependent on the topographical patterns and surface chemistry of the scaffold used to grow these precious cells. Countless studies have shown the importance of choosing the appropriate tissue culture scaffolds: one that mimics the nanofibrous nature of the extracellular matrix for a wide variety of applications including basic cell biology, drug discovery, tissue engineering, cell migration and invasion, and stem cell expansion and differentiation. Cell culture is mainly performed on tissue culture polystyrene (TCPS) multiwell plates as it is cheap, optically clear, and many cells grow well on it. In reality, however, living organisms are made up of an extracellular matrix that presents both aligned physical structure and support to the cells.



Role reversal: unlike in 2-D cultures, breast tumor cells in 3-D culture (left) that become malignant (center) can be made to revert to their original state (right) when an antibody against β-integrin is added to the system.[1]

Not surprisingly, drugs developed using TCPS as an *in vitro* substrate experience a >99% failure rate in clinical studies.

The importance of the 3-D substrate is summarized by Abbott[1]:

> **There's a big difference between a flat layer of cells and a complex, three-dimensional tissue. But until recently, many biologists have glossed over this fact.**

Key Research Articles Which Show The Importance of Using Nanofibers for 3-D Cell Culture Research

1997: Antibodies against β1-integrin completely changed the behavior of cancerous breast cells grown in 3-D culture: they lost their abnormal growth pattern. This result had never been observed in 2-D cultures.[5]
2001: On comparing the growth and development of fibroblasts in 2-D and 3-D cultures, Yamada's group found that in 3-D culture, the cells had higher mobility, division rate and formed the characteristic asymmetric shape that fibroblasts have in living tissues.[4]
2002: Friedl's group showed that unlike in 2-D culture, 3-D studies provide subtle clues on how metastasizing cells escape the drug of interest, thus proving that substrate choice can influence the success or failure of the clinical performance of a promising drug.[6]
2003: Korn's group showed that 3-D cell culture studies increase the possibility of using gene therapy for the treatment of cancer. While both normal and malignant breast cells had high expression of adenoviral receptors in 2-D culture, only malignant cells carried large number of receptors in 3-D culture.[2]

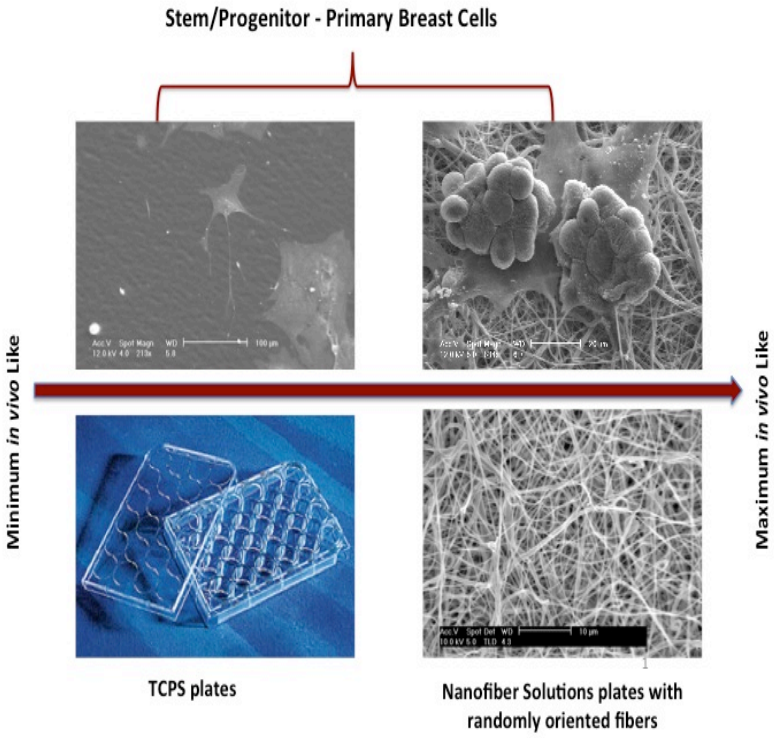
The Most Realistic *In Vitro* Substrate

3-D cell culture requires a substrate that mimics the ECM in real tissues. Most researchers currently use Matrigel, a cocktail of substances extracted from the ECM of Engelbroth-Holm-Swarm (EHS) mouse sarcoma. Although Matrigel has proved effective, its inventors admit that they are surprised that it hasn't yet been superseded. "I'm absolutely shocked that there isn't really anything better," says Hynda Kleinman, lead author of the papers that introduced Matrigel to the world.[1]

The batch to batch variability of Matrigel leads to poor reproducibility in experiments, while animal components lead to critical problems when investigating clinical treatments with stem cells. This means there is a critical gap when researchers look towards moving away from living tissues- which vary from batch to batch and are

THE NANOFIBER SOLUTIONS PLATFORM HAS THE ADVANTAGE OF:

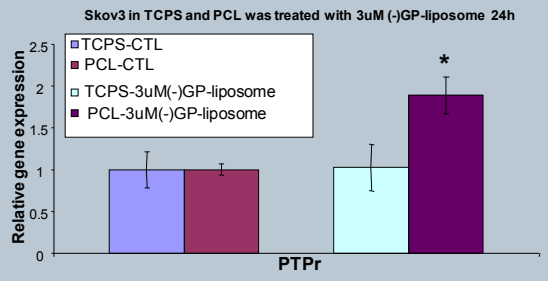
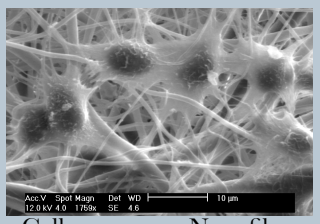
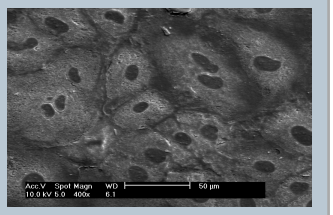
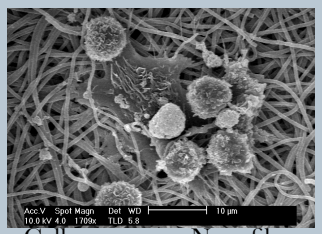
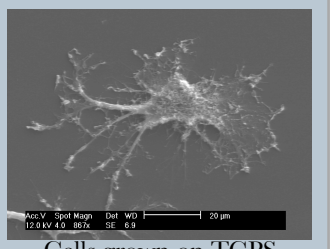
- ✦ A unique ability to up-regulate specific genes by as much as 340 fold in the presence of neighboring cells.
- ✦ Increased sensitivity in drug analysis assays.
- ✦ Live cell imaging to observe cell behavior.
- ✦ Fully compatible with existing protocols such as immunohistochemistry, viability assays, RNA analysis, protein expression, and automated plate readers.
- ✦ Nanofiber scaffold can be easily coated with proteins such as fibronectin, laminin, collagen, etc.



prohibitively expensive- towards synthetic materials that mimic the ECM, do not vary from batch to batch and are very cost effective. Nanofiber Solutions provides this answer through the electrospinning of a polymer into a nanofibrous sheet that mimics the ECM, allowing broad application from tissue engineering to drug discovery.

Nanofiber Solutions offer two types of multiwell plates: (1) Random nanofiber is best suited to mimic most environments *in vivo* that don't have a natural alignment or orientation.(2) Aligned nanofiber is best suited to mimic white matter, the central nervous system, cardiac tissue and skeletal muscle, or for performing cell migration assays. These nanofiber multiwell plates offer a better 3-D model for mimicking the *in vivo* environment and will allow the reduction in use of experimental animals and decrease the time to market for drug development.

Surfaces offer considerably more control over cells' microenvironments and so could better mimic the niches in which stem cells differentiate. "We have to stop thinking about the medium and the surface as two separate entities. We have to think about the whole environment of the cells," says Clive Glover, head of product management at STEMCELL Technologies.[3]

Type of Study	Effect of Using Nanofiber Solutions' Product	Corresponding Data	
Response to Chemothe-rapeutic Compounds	Ovarian cancer cells when cultured on Nanofiber Solutions' nanofiber (PCL-CTL) show an enhanced response to a chemotherapeautic compound when compared to standard tissue culture polystyrene (TCPS).		
Stem Cell Studies	Bone marrow derived stem cells cultured on Nanofiber Solutions' nanofiber specially treated to investigate idiopathic lung fibrosis.	 Cells grown on Nanofiber plates	 Cells grown on TCPS
	Umblical cord stem cells cultured on Nanofiber Solutions' nanofibers to maintain stem cell phenotype while allowing higher expansion rates.	 Cells grown on Nanofiber plates	 Cells grown on TCPS

	Nanofiber	Transwell	MATRIGEL	TCPS
Analysis	Quantitative and qualitative	Quantitative	Quantitative or qualitative	Quantitative or qualitative
Detection Time	Endpoint or real time	Endpoint	Endpoint or real time	Endpoint or real time
Chemoattractant Gradient	Yes	Yes	Yes	No
Adaptability to Automation	Good	Poor	Poor	Good
Physiological Relevance	Mimics white matter, central nervous system and other tissues of the body	Flat sheet with holes does not resemble anything i*n vivo*	Provides chemistry of a tumor which can be good or bad: however, lacks structural components	Does not mimic any *in vivo* environment
Microscopy	Confocal, epifluorescence, light microscopy, and electron microscopy	Epifluorescence is required	Confocal, epifluorescence, and light microscopy	Epifluorescence or light microscopy
Flexibility/Ease of Use	High	Fair	Low	High
Retail Price	Medium	Medium	High	Low

References

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4.Cukierman, E., Pankov, R., Stevens, D. R. & Yamada, K. M. Science 294, 1708–1712 (2001).
5.Wang, F. et al. Proc. Natl Acad. Sci. USA 95, 14821–14826 (1998)
6.Wolf, K. et al. J. Cell Biol. 160, 267–277 (2003).