

Features and Benefits of Nanofiber Solutions™ 3D Scaffolds

Q: What are the differences between Nanofiber Solutions™ 3D Scaffolds and other 3D products on the market?

A: Nanofiber Solutions™ products differ in that they can be customized to fit the researchers' needs and applications. Competitor products are available in limited formats. Nanofiber Solutions™ 3D Scaffolds are available in a variety of formats. Nanofiber Solutions™ 3D Scaffolds are made of biomimetic synthetic polycaprolactone (PCL) which is softer and closely resembles biological tissue and this in turn provides a better 3D environment for the cells to grow on. Some competitor products are made of stiff rigid polymers such as polystyrene that aren't biocompatible.

Q: Can the Nanofiber Solutions™ 3D Scaffolds be designed into a three-dimensional structure, e.g., pancreas, liver, heart?

A: Yes. This is our area of expertise and we can develop complex shapes/geometries to model different organs throughout the body. We recently made history in designing the first synthetic trachea implanted into a human patient. Please refer to the Company page of our website for more information (<http://www.nanofibersolutions.com/company.html>).

Q: Will your Nanofiber Solutions™ 3D Scaffolds support the growth of cells, and if so, what type of cells, i.e., adult differentiated cells, adult progenitor cells, adult germ layer lineage stem cells, adult pluripotent stem cells, adult totipotent stem cells?

A: Yes. The nanofibers support the growth of all types of cells. With stem cells, we can achieve faster expansion rates while maintaining higher percentages of pluripotent stem cells compared to traditional flat culture surfaces.

Q: Can you get reproducible data using Nanofiber Solutions™ 3D Scaffolds?

A: Reproducibility of data using Nanofiber Solutions™ 3D Scaffolds is very good. We set the industry standard for batch-to-batch consistency.

Q: Is there batch-to-batch variability?

A: Each batch of Nanofiber Solutions™ 3D Scaffolds are tested for its physical properties, porosity and suitability for cell attachment and viability. Only batches fulfilling strict criteria are released by our Quality System. Many of our customers find that Nanofiber Solutions products are far more reproducible than other sources of electrospun fibers to which they may have access.

Physical Properties

Q: Do cells proliferate at the same rate on Nanofiber Solutions™ 3D Scaffolds compared to tissue culture polystyrene?

A: The answer is: No! Cells typically show a more rapid expansion/growth rate on our 3D scaffolds compared to traditional 2D tissue culture polystyrene. For example, Stem Cells have shown a 3x greater expansion rate on Nanofiber Solutions™ 3D Scaffolds.

Q: How long can you culture cells for?

A: Depending on the cell type and the method of analysis to be performed, Nanofiber Solutions™ 3D Scaffold plates are usually recommended for cell culture experiments lasting up to several weeks. Ideally, the extra cellular matrix proteins left behind by previous passages should create an environment more suited for better long term expansion, but this has not been tested. The fibers will not degrade or detach from the plate. The fibers will maintain a tight bond to the bottom of the culture area. **Note:** Physical damage can occur if the fibers are touched with a pipette tip.

Q: How well does type-I collagen bind to the Nanofiber Solutions™ 3D Scaffolds?

A: Very well. Nanofiber Solutions can either make collagen nanofiber scaffolds or we can make synthetic nanofibers and coat them with collagen.

Q: Can the Nanofiber Solutions™ 3D Scaffolds form tubes of different sizes?

A: Yes. It is very easy for us to make tubes ranging from a few hundred micrometers in diameter to multiple centimeters in diameter.

Q: Can the Nanofiber Solutions™ 3D Scaffolds form tubes of different sizes within the same structure?

A: Yes. We can combine different shapes and sizes together into complex, 3-D structures.

Q: What are the physical dynamics, e.g., sheer force, tensile strength, stretchability, compressive force, etc.?

A: The physical properties Nanofiber Solutions™ 3D Scaffolds depend upon the type of polymer used in the manufacturing process. For example, something made from polyurethane (PU) will be very elastomeric like a rubber band, while something made from polyglycolic acid (PGA) will initially be more rigid and stiff.

Q: Will your Nanofiber Solutions™ 3D Scaffolds bind to carbohydrate moieties, e.g., glycosaminoglycans, glycoproteins, glycolipids?

A: Yes. We can purchase commercially available conjugated polymers or conjugate the polymers ourselves with relatively simple cross-linking protocols from the literature.

Q: How are the Nanofiber scaffolds attached to the tissue culture plate?



A: The Nanofiber inserts rest inside at the bottom of the plate and are not attached to the bottom. For the plates, the Nanofibers are electrospun onto a thin layer of tissue culture grade polystyrene then bonded to the plate bottom.

Q. What is average diameter of fibers in NanoAligned™ and Nanofiber Solutions™ ?

A: The average diameter of a PCL nanofiber is ~ 700nm. We can control the size of the fibers to make larger or smaller sizes if needed.

Q: What Nanofiber Solutions™ 3D Scaffolds made of?

A: Nanofiber Solutions™ 3D Scaffolds are made of polycaprolactone (PCL) and range in thickness from 200-700µm.

Q: Are other polymers available?

A: Other polymers (polystyrene, polyethylene terephthalate as well as many others) can be special ordered, please contact us for details.

Q: What is the porosity of Nanofiber Solutions™ 3D Scaffolds?

A: Nanofiber Solutions™ 3D Scaffolds have a porosity of greater than 90%.

Q: Are Nanofiber Solutions™ 3D Scaffolds biodegradable?

A: Yes and no. The nanofibers can be fully degradable or permanent depending on the polymer.

Protocols/Techniques

Q: Are there any protocols or technical notes available for the users?

A: A range of example protocols can be found in the Technical Resources section of our website (<http://www.nanofibersolutions.com/technicalresources.html>). These are being continually updated and expanded upon and currently include general information about choosing and using Nanofiber Solutions™ 3D Scaffolds; examples of how to grow specific cell types; compatible analytical techniques and specialized applications.

Q: What analysis techniques are compatible with Nanofiber Solutions™ 3D Scaffolds?

A: Nanofiber Solutions™ 3D Scaffolds are compatible with a broad range of general cell and molecular techniques. These include various biochemical assays, histology (tissue processing, fixation, embedding and sectioning), fluorescence microscopy, immunocytochemistry, in-situ hybridization, electron microscopy, bright field and phase microscopy; extraction of nucleic acid and total protein etc.

Q: Can you coat Nanofiber Solutions™ 3D Scaffolds?



A: Yes. Most standard coating methods for cell culture plastic are compatible with Nanofiber Solutions™ 3D Scaffolds including the use of extra-cellular matrix proteins, collagen, fibronectin, laminin, poly D/L lysine, etc.

Q: Can you remove cells from Nanofiber Solutions™ 3D Scaffolds?

A: Yes. Cells can be easily removed from the scaffolds using a combination of trypsin-EDTA or Accutase™ plus mild agitation if needed. For an example protocol, please visit the Technical Resources pages on our website (<http://www.nanofibersolutions.com/technicalresources.html>).

Q: Is gene analysis possible with cells extracted from Nanofiber Solutions™ 3D Scaffolds?

A: Yes. Cells can be removed from NanoECM™ 3D Scaffolds for gene analysis.

Q: Can you perform co-culture experiments?

A: There are several options available for performing co-culture experiments, including; 1) Two or more cell types can be seeded onto one Nanofiber Solutions™ 3D Scaffolds simultaneously; 2) Our Interactive Microenvironment System (IMEMS, part number: [IMEMO1 random](#), [IMEMO2 aligned](#)) allows co-culture of several different cell types for enhanced interaction and cellular response. Cells have chemical communication, but not physical contact which allows easy post-analysis of each cell type. Each layer within the IMEMS allows for high-resolution imaging of the cells after the culture period. Refer to our Technology page for more information on the IMEMS (<http://www.nanofibersolutions.com/technology.html>).

Q: Can you extract protein and nucleic acids from cells within Nanofiber Solutions™ 3D Scaffolds?

A: Yes, standard protocols for RNA and total protein extraction will work from cells grown on Nanofiber Solutions™ 3D Scaffolds.

Q: Can you explant tissues onto Nanofiber Solutions™ 3D Scaffolds?

A: It is possible to take explanted tissue and continue to let it grow in 3D in Nanofiber Solutions™ 3D Scaffolds. Please refer to our Technical Resources section on our website for publication references (<http://www.nanofibersolutions.com/technicalresources.html>).

Q: Can the Nanofiber Solutions™ 3D Scaffolds be placed in suspension and aerosolized?

A: No. Our Nanofiber Solutions™ 3D Scaffolds are continuous fibers that can be placed in suspension (for example, in a large commercial bioreactor) but it would be very difficult to aerosolize them.

Compatibility of Scaffolds

Q: Can you perform FACS analysis on cells cultured on Nanofiber Solutions™ 3D Scaffolds?

A: Yes. Cells can be removed from Nanofiber Solutions™ 3D Scaffolds using standard protocols with trypsin-EDTA, and Accutase™ for FACS analysis.



Q: Are Nanofiber Solutions™ 3D Scaffolds suitable for cell invasion and cell migration assays?

A: Yes. It is possible to measure the rate of migration using image analysis of histological sections, or by fluorescently labeling cells. Several cell migration videos are published on our website. Please go to our Technology section to view the live cell imaging videos (<http://www.nanofibersolutions.com/technology.html>).

Q: Is there a protocol for performing transfections on Nanofiber Solutions™ 3D Scaffolds?

A: Yes. Commercially available transfection kits are compatible with Nanofiber Solutions™ 3D Scaffolds.

Q: Are Nanofiber Solutions™ 3D Scaffold plates compatible with standard laboratory plate readers/instrumentation?

A: Yes. The physical dimensions are the same as standard tissue culture plates.

Q: Are NanoECM Scaffolds compatible with bioreactor systems?

A: Yes, our Nanofiber Solutions™ 3D Scaffolds can be customized to fit industrial bioreactors. Please contact us for more information (<http://www.nanofibersolutions.com/contact.php>).

Q: Are Nanofiber Solutions™ 3D products recommended for Stem Cell expansion?

A: Yes, we have seen 3-9x the cell expansion rates of Stem Cells compared to the same cells plated on traditional tissue culture polystyrene. Stem cells, when grown on Nanofiber Solutions™ 3D products do not differentiate down an undesirable phenotypic pathway.

Seeding/Growing

Q: Should I seed wells with the same number of cells I use on flat plates?

A: Not likely – One of the major benefits of Nanofiber Solutions™ 3D Scaffolds is that they mimic *in vivo* physical structures. This means there will be 2-3x more surface area on our scaffolds; which will require more cells for seeding purposes to obtain a true 3D cell environment.

Q: What happens if more than the recommended numbers of cells are seeded onto Nanofiber Solutions™ 3D Scaffolds?

A: The seeding densities recommended are guidelines and may need to be optimized depending on cell type. Nanofiber Solutions™ 3D Scaffolds will have 3x the surface area compared to standard polystyrene plates so cell seeding may need to be optimized for each cell phenotype. Please refer to the Technical Resources of our website for more information (<http://www.nanofibersolutions.com/technicalresources.html>).

Q: Do cells growing on Nanofiber Solutions™ 3D Scaffolds behave the same as cells growing in traditional 2D cultures?



A: No, there is now much evidence to show that cells growing in 3D retain their native morphology and functionality which represents a more accurate gene and protein expression profile as would be found in vivo. There are many papers highlighting these differences including some Nanofiber Solutions™ 3D Scaffolds publications referenced on the Technical Resources section of our website.

(<http://www.nanofibersolutions.com/technicalresources.html>).

Treatment

Q: Are Nanofiber Solutions™ 3D Scaffolds tissue culture treated?

A: Yes. Nanofiber Solutions™ 3D Scaffolds are surface plasma (tissue culture) treated to provide for excellent wettability and are ready to use out of the packaging.

Q: Do the Nanofiber Solutions™ 3D Scaffolds need to be treated or equilibrated before use?

A: Prior to use we recommend a 30 minute pre-incubation in the media plus any biological components of interest at 37°C, followed by aspiration.

Sterilization

Q: Are Nanofiber Solutions™ 3D Scaffolds supplied sterile?

A: Nanofiber Solutions™ 3D Scaffolds have been ultra violet light treated and are packaged within sterile Tyvek packs.

Q: Can you autoclave Nanofiber Solutions™ 3D Scaffolds?

A: No. The Nanofiber Solutions™ 3D Scaffolds cannot be autoclaved, high pressure and heat will destroy the fibers.

Q: Are other forms of sterilization possible for the scaffolds?

A: Yes. Gamma irradiated sterile products are available. In addition, many of our collaborators have used 70% ethanol solution successfully.

Q: Are Nanofiber Solutions™ 3D Scaffolds reusable?

A: Nanofiber Solutions™ 3D Scaffolds are a single use, disposable product.

Q: Do Nanofiber Solutions™ 3D Scaffolds have an expiration date?

A: No. For optimal performance Nanofiber Solutions™ 3D Scaffolds are recommended to be used within a year of purchase.

Q: Are Nanofiber Solutions™ 3D Scaffolds chemically resistant?



A: The surface properties can be affected by certain chemicals. Some solvents like acetone or xylene may swell or destroy the scaffold

Imaging

Q: Can Nanofiber Solutions™ 3D Scaffolds be imaged using standard light or visible microscopy?

A: Yes. Nanofiber Solutions™ 3D Scaffolds can be imaged using standard light and visible microscopy applications.

Q: Do Nanofiber Solutions™ 3D Scaffolds autofluoresce?

A: No significant levels of autofluorescence have been observed from Nanofiber Solutions™ 3D Scaffolds using standard visible wavelengths of light. Under confocal microscopy PCL exhibits a minor green autofluorescence.

Q: How can you see your cells growing in 3D on Nanofiber Solutions™ 3D Scaffolds?

A: Nanofiber Solutions™ 3D Scaffolds are optically transparent and cells growing on the fibers can be visualized using standard microscopy. Other options to visualize cells growing in 3D in Nanofiber Solutions™ Scaffolds include, but are not limited to, live cell imaging, fluorescent marker analysis, confocal analysis, histology using a range of cytological stains and electron microscopy. Please refer to the Technical Resource section of the website

(<http://www.nanofibersolutions.com/technicalresources.html>).

Q: How do you estimate cell confluence on Nanofiber Solutions™ 3D Scaffolds?

A: Visible inspection under a light microscope or simple dyes can be used to estimate cell culture confluence and viability. A full protocol for a simple method is available in the Technical Resources section of our website. An extensive number of end-point visualization techniques are also compatible, including live cell imaging, fluorescent marker analysis, confocal analysis, biochemical assays, histological analysis using a range of cytological stains and electron microscopy.

Other General Questions About Nanofiber Solutions™ 3D Scaffolds

Q: What formats of Nanofiber Solutions™ 3D Scaffolds are available?

A: Nanofiber Solutions™ 3D Scaffolds are currently available in the following formats: Random and Aligned orientations. Standard well plate formats available are: 6, 24, 96, and 384 wells/plate.

If desired, Nanofiber Solutions™ 3D inserts are also available in 6, 12, 24 well formats and can fit a range of culture plates from different manufacturers. Inserts are also available for 60 and 100 mm tissue culture dishes. Nanofiber Solutions™ 3D coated chamber slides and the 6-well Interactive Microenvironment System (IMEMS) complete our product line. For more information, please visit the Product page (<http://www.nanofibersolutions.com/products.html>).



Q: Are other formats available?

A: Custom-made formats of Nanofiber Solutions™ 3D Scaffolds are available for collaborations; please contact us for details (<http://www.nanofibersolutions.com/contact.php>).

Q: How many 24 well plate inserts are included in a single order (Part# 122402)?

A: The inserts come in 6, 12, or 24 formats. Each part order will come with the indicated number of inserts.

Q: Have any papers been published using Nanofiber Solutions™ 3D Scaffolds?

A: Numerous peer-reviewed scientific articles describe the development and application of Nanofiber Solutions™ 3D Scaffolds and can be accessed from the Technical Resources page <http://nanofibersolutions.com/technicalresources.html>. Scientists at Nanofiber Solutions are continuously researching novel applications for our technology, publishing and presenting data when appropriate.

