

BD™ Three Dimensional OPLA® Scaffold

Catalog No. 354614

Guidelines for Use

FOR RESEARCH USE ONLY

NOT FOR CLINICAL, DIAGNOSTIC OR THERAPEUTIC USE

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INTENDED USE

Biological tissues and organs consist of specialized cells that are situated within a complex molecular framework known as the extracellular matrix (ECM). In addition to providing tissues with the appropriate three-dimensional (3D) architecture, ECM molecules have been shown to promote signaling pathways that influence key cell functions such as migration, proliferation, and differentiation. These diverse attributes of the ECM contribute to the structural and functional properties of each tissue type.

A variety of 3D biodegradable scaffolds are used as artificial substitutes for the ECM. These materials may consist of natural molecules and/or synthetic polymers. In contrast to conventional 2D cell culture systems, 3D scaffolds provide an adhesive substrate that also serves as a 3D physical support matrix for *in vitro* cell culture [1,2] as well as *in vivo* tissue regeneration [3,4]. Accordingly, the use of bioengineered 3D scaffolds is rapidly becoming the most promising experimental approach for mimicking the native structure of living tissues. Since tissue and organ function are dependent on the presence of an appropriate population of differentiated cells, a considerable amount of research is focused on the development of 3D culture systems for the expansion and differentiation of pluripotent stem cells [5] or lineage-restricted progenitor cells [6,7]. These cells are being used to study the signaling pathways that mediate cell differentiation and to identify optimal microenvironments that support cellular function.

The BD 3D OPLA[®] Scaffold is a synthetic polymer scaffold that is synthesized from D,D-L,L polylactic acid. This material has an open-cell faceted architecture, which is effective for culturing high density cell suspensions.

1. Martin, I., et al., *J. Orthopaedic Res*, 16:181-189 (1998).
2. Wei Tan, B.S., et al., *Tissue Engineering*, 7: 203-210 (2001).
3. Evans, G.R., et al., *Biomaterials*, 20: 1109-1115 (1999).
4. Saito, N., et al., *Nature Biotech*, 19: 332-335 (2001).
5. Solchaga, L.A., et al., *J. Orthopaedic Res*, 17: 205-213 (1999).
6. Martin, I., et al., *Endocrinology*, 138: 4456-4462 (1997).
7. Ripamonti, U., et al., *Matrix*, 12: 202-212 (1992).

THIS PRODUCT IS FOR RESEARCH USE ONLY; NOT FOR CLINICAL, DIAGNOSTIC OR THERAPEUTIC USE. NOT FOR HUMAN USE.

MATERIALS PROVIDED

Catalog # 354614 packaged as one 48-well plate with 24 3D scaffolds

Physical and Mechanical Properties

Scaffold Material: Synthesized from D,D-L, L polylactic acid

Hydration Capacity: 30 μ l

Weight/Wet Weight: 5.2mg/34 mg

Average Pore Size: 100-200 μ m

Dimensions: Diameter: 4.2-5.2mm
Height: 3.9-4.5mm
Volume: 0.039cm³

Stress @ 25% Strain: 0.23 \pm 0.01 MPa

Load @ 25% Strain: 40.3 \pm 1.5 N

Modulus of Elasticity @ 25% Strain: 0.92 \pm 0.03 MPa

Maximum Load: 63.1 \pm 2.4 N

In Vivo Biodegradation: Structurally functional at 13 weeks (dental barrier membrane); complete resorption at 12 months (dental barrier membrane).

SUPPLEMENTAL/OPTIONAL MATERIALS (NOT SUPPLIED)

- **Media:** MEM (Gibco, Cat #12561) supplemented with 5-10% FCS (heat inactivated) and antibiotics (100 units/ml penicillin, 100 mg/ml streptomycin)
- **BD Oxygen Biosensor System** (BD Biosciences Discovery Labware, Cat # 353830)
- **Picogreen Assay Kit** (Molecular Probes, Cat # P-7589)
- **Cytochrome P450 - Cyp3A4 substrate** 7-benzyloxyquinoline (BD Gentest, Cat. #451725),
- **Alkaline phosphatase (AP) Assay Kit** (Sigma Diagnostics, Cat # 104-LS)
- **Human Fibronectin** (BD Biosciences Discovery Labware, Cat # 354008)
- **BD Falcon Conical Centrifuge Tubes** (BD Biosciences Discovery Labware, Cat # 352070)

PRECAUTIONS

- **Removal of Scaffolds from Package:** *Carefully and slowly remove foam plug from the plate to avoid removing the scaffold along with it.*
- **Scaffold Transfer:** *Transfer scaffolds as gently as possible using sterile tweezers that have a ridged gripping surface.*

PROCEDURES FOR USE

In Vitro Analyses

i) Cell seeding and culturing conditions

Certain cell types (e.g., MC3T3-E1 osteoblasts, WI-38 human lung fibroblasts, and primary rat liver hepatocytes) can be seeded directly onto the dry scaffold in the following manner. Place each scaffold in a separate well of a 96-well plate. For static seeding, add approximately 1.0×10^4 - 5.0×10^4 cells in 100 μl of media to the scaffold. Incubate the seeded scaffold for one hour at 37°C in the absence of agitation and then supplement with an additional 150 μl of media. Media changes should be carried out as necessitated by the culture conditions and growth properties of the respective cell type.

Alternatively, dynamic seeding may be used to load cells onto the scaffold. Place the scaffold into a 50 ml conical tube (BD Biosciences Discovery Labware, *BD Falcon Conical Centrifuge Tubes*, Cat # 352070) and then add a cell suspension containing approximately 5.0×10^4 – 2.5×10^5 cells in 250 μl of media. Incubate the tube with gentle agitation (~50 - 100 rpm) on an orbital shaker at 37°C for at least 2-4 hours. A longer incubation time with agitation (e.g., 16 - 24 hours) may promote an increased level of seeding efficiency. To maintain appropriate pH during extended incubation times, add an equal volume of fresh media after 8-10 hours. After the incubation with agitation is completed, gently place the seeded scaffold into a well of a 96-well plate containing 250 μl of media for the remainder of the experiment. Gently transfer scaffolds using sterile tweezers that have a ridged gripping surface.

For some cell types (e.g., stem/progenitor cells), it may be beneficial to modify the scaffold surface by pre-coating with serum or an ECM molecule prior to cell seeding. For example, place the scaffold in a well of a 96-well plate and pre-incubate for 30-60 minutes at room temperature in 250 μl of culture media containing 5-10% fetal calf serum. Alternatively, pre-incubate the scaffold in the same manner using a solution of fibronectin [BD Biosciences Discovery Labware, Cat # 354008 (see directions that accompany product); dilute fibronectin stock solution to 50-100 $\mu\text{g/ml}$ in serum-free media or buffer at pH 7-9]. After the pre-incubation is completed, proceed with cell seeding as described above.

ii) Non-invasive evaluation of cell growth using the *BD Oxygen Biosensor System*

The *BD Oxygen Biosensor System* is a novel detection system for real-time non-invasive monitoring of cell growth. As oxygen becomes depleted, the biosensor fluoresces, providing a linear signal that can be directly correlated to cell growth. Since the fluorescence intensity is expressed in arbitrary units, normalized relative fluorescence values are obtained by dividing the values in each well at selected time points by the initial reading in the corresponding wells.

Perform cell seeding in a 96-well oxygen biosensor plate (BD Biosciences Discovery Labware, *BD Oxygen Biosensor System*, Cat # 353830) or place a pre-seeded scaffold into this plate. Samples are then analyzed according to the protocol that is provided with this system. The data shown in **Figure 1** is indicative of the utility of this system for analyzing cell growth on the *BD 3D OPLA® Scaffold*.

iii) Analysis of DNA content using the *Picogreen Assay Kit*

Cellular DNA content can be measured to confirm that an increase in cell growth correlates with an increased level of DNA. To process material for the analysis of DNA content, add 250 μ l of cell lysis solution (0.2% v/v Triton X-100, 10 mM Tris (pH 7.0), 1 mM EDTA) to each well containing a cell-scaffold sample. The sample can then be processed for DNA analysis or frozen at -70°C .

To prepare the cell lysate, process the samples through a total of two freeze/thaw cycles. Freeze samples at -70°C and thaw at room temperature for 45-60 minutes. After the final thaw, break up the scaffolds with a pipette tip to improve cell lysis. Transfer the lysate to a fresh 1.5 ml tube. The lysate can be used immediately or stored at -70°C prior to analysis. The cell lysate is then analyzed using the *Picogreen Assay Kit* according to the manufacturer (Molecular Probes, Cat # P-7589).

iv) Analysis of Cytochrome P450 activity using 7-benzyloxyquinoline as a cyp3A substrate

The cytochrome P450 enzymes are mixed function oxidases that are important in fatty acid metabolism, steroid biosynthesis and drug metabolism. The cyp3A subgroup is common in the liver. It can be used to follow the differentiation state of the hepatocytes. Prepare lysates the same manner as for the DNA analysis in **iii)** and analyze according to the method described on the Gentest website (http://www.gentest.com/products/HTS_KITS/HTS/hts_summary.shtml).

v) Analysis of Alkaline Phosphatase activity using the *AP Assay Kit*

Total alkaline phosphatase activity can be monitored to follow the differentiation of the MC3T3-E1 cells. Prepare lysates the same manner as for the DNA analysis in **iii)** and analyze the lysates using the *AP Assay Kit* according to the manufacturer (Sigma Diagnostics, Cat # 104-LS).

vi) Typical results (FOR REFERENCE ONLY)

The following results were obtained by culturing WI-38 cells and primary rat liver hepatocytes with the *BD 3D OPLA[®] Scaffold Scaffold* in the *BD Oxygen Biosensor System*. Using the oxygen biosensor system, the oxygen consumption rate (OCR) of cells in culture can be measured and correlated with cell growth. When WI-38 human lung fibroblasts were cultured on the BD 3D OPLA Scaffold, cell growth increased within 1-2 days and was maintained for up to 14 days in culture. Furthermore, the increase in cell growth was found to correlate with an increase in cellular DNA content. **(Figure 1)** In parallel experiments with the BD 3D OPLA Scaffold, primary rat hepatocytes exhibited a marked increase in cell growth and a concomitant decrease in cytochrome P450 Cyp3A activity. These results indicate that primary hepatocytes dedifferentiate when cultured on the BD 3D OPLA Scaffold **(Figure 2)**.

Taken together, these findings indicate that the BD 3D *OPLA[®] Scaffold* provides a suitable environment for *in vitro* studies of a variety of cell types. Moreover, *in vivo* studies suggest that this scaffold is suitable for tissue engineering applications in animal model systems (data not shown). **Results will vary using different cell types and culturing conditions.**

WI-38 Human Lung Fibroblasts on BD 3D OPLA® Scaffolds
Raw Pico Green and Computed Oxygen Consumption Data

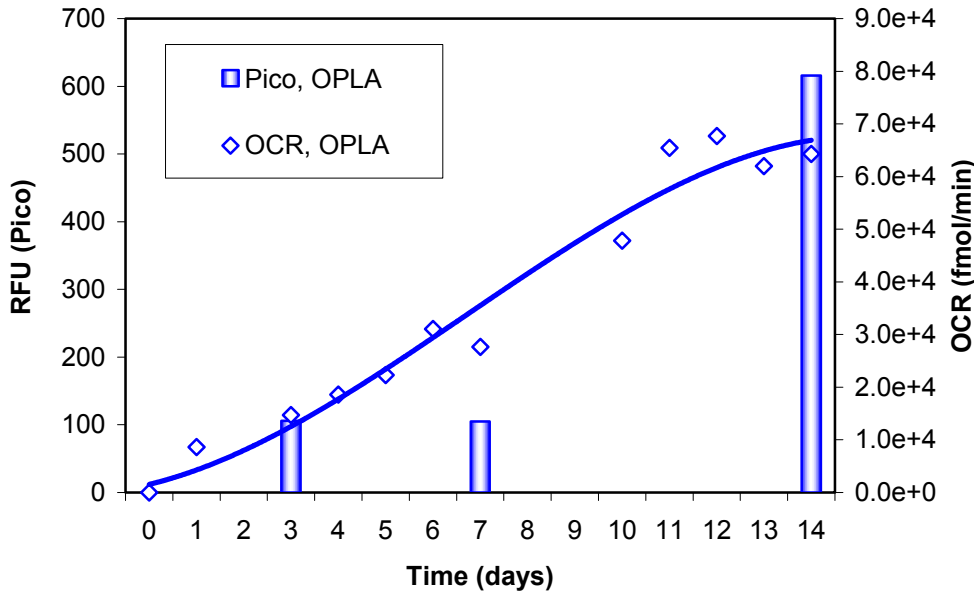


Figure 1:
 The BD Oxygen Biosensor System (OBS) was used to analyze the Oxygen Consumption Rate (OCR) of WI38 Human Lung Fibroblasts cultured on the BD OPLA® Scaffold. Cellular DNA content was measured using the Picogreen Assay Kit (Molecular Probes). The diamonds indicate the OCR and the solid line is a polynomial fit to the OCR points.

Primary Rat Hepatocytes on OPLA Scaffold

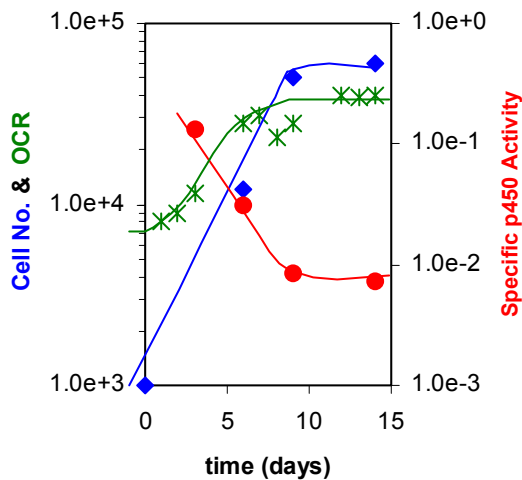


Figure 2:
 The BD™ Oxygen Biosensor System was used to analyze cellular number and the OCR of primary rat hepatocytes cultured on the BD 3D OPLA Scaffold. The BD Gentest Cytochrome P450 – Cyp3A4 Assay Kit was used to analyze the differentiation state of the hepatocytes. Oxygen Consumption Rate (OCR) (*), cell number computed from Pico Green fluorescence (◆), and specific P450 activity reported as fluorescence divided by cell number (●) as a function of time.

GENERAL HINTS FOR USING 3D SCAFFOLD PRODUCTS

Handle all scaffolds under aseptic conditions.

For sectioning and staining the scaffolds, the scaffolds should not be processed with protocols that use a rapid dehydration step. While rapid dehydration is typically used to fix cells on slides or plates, this method may lyse the cells in the scaffolds.

The BD 3D OPLA® scaffolds will dissolve in toluene. To prepare for sectioning and staining, treatment in butanol is recommended.

STABILITY

The *BD 3D OPLA Scaffold* is stable for at least 3 months from date of shipment when stored at **4-30°C**.

TECHNICAL SERVICE

For Technical Service/Questions call 1-800-343-2035.

For Customer Service call 1-800-343-2035.

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OPLA is a registered trademark of Kensey Nash Corporation.

Scaffolds are proprietary biomaterials of Kensey Nash Corporation, manufactured for BD Biosciences.

The OPLA is manufactured under one or more of the following U.S. Patents: 4,186,448; 5,736,160; 5,855,608; 5,935,594; 5,981,825; 6,005,161; and 6,264,701.