

# **BD™ Three Dimensional Collagen Composite Scaffold**

**Catalog No. 354613**

***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 Collagen Composite Scaffold is a natural scaffold manufactured from a proprietary mixture of collagens that are derived from bovine hide. Overall, this material exhibits collagen fibrillar architecture, which is representative of the structure of collagen within the interstitial matrix.

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 USE IN HUMANS.**

## MATERIALS PROVIDED

Catalog # 354613 packaged as one 48-well plate with 24 3D scaffolds

### Physical and Mechanical Properties

**Scaffold Material:** Proprietary mixture of type I and III collagens derived from bovine hide

**Hydration Capacity:** 25  $\mu$ l

**Dimensions:** Diameter: 4.2-5.2mm  
Height: 3.9-4.5mm  
Volume: 0.039cm<sup>3</sup>

**Weight/Wet Weight:** 3.5mg/45 mg

**Average Pore Size:** 100-200  $\mu$ m

**Average Wet Tear Strength:** 0.48  $\pm$  0131 lb/mm

**Average Dry Compressive Stiffness:** 5.33  $\pm$  2.45psi (@ 75%  $\pm$  2% of uncompressed thickness)

**In Vivo Biodegradation:** Complete resorption at less than 30 days intramuscular; Complete resorption at about 60 days extravascular.

## 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)
- **Alkaline phosphatase (AP) Assay Kit** (Sigma Diagnostics, Cat # 140-LS)
- **Cytochrome P450 - Cyp3A4 substrate** 7-benzyloxyquinoline (BD Gentest, Cat. #451725),
- **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.*
- Collagen raw material source is from bovine hide. The animals are from the United States of America, and have never been fed mammalian protein. The sourcing and manufacturing of the collagen is in compliance with prEN 12442-1 (Animal tissues and their derivatives utilized in the manufacture of medical devices - Part 1: Analysis and management of risk) and prEN 12442-2 (Animal tissues and their derivatives utilized in the manufacture of medical devices - Part2: Sourcing, control, collection and handling).

## 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 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  $1.0 \times 10^4$  -  $5.0 \times 10^4$  cells in 100  $\mu\text{l}$  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  $\mu\text{l}$  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  $5.0 \times 10^4$  –  $2.5 \times 10^5$  cells in 250  $\mu\text{l}$  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  $\mu\text{l}$  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  $\mu\text{l}$  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 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 Collagen Composite 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 $\mu$ 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 family of 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 hepatocytes. Prepare lysates in the same manner as for the DNA analysis in **iii)** and analyze according to the method described on the BD Gentest website ([http://www.gentest.com/products/HTS\\_KITS/HTS/hts\\_summary.shtml](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 MC3T3-E1 osteoblasts. Prepare lysates in 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 fibroblasts and primary rat hepatocytes with the *BD 3D Collagen Composite 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 Collagen 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)** When primary rat hepatocytes were cultured on the *BD 3D Collagen Composite Scaffold*, cell growth increased within 1-2 days and was maintained for up to 14 days in culture. Cytochrome P450 activity was also maintained for up to 14 days in culture **(Figure 2)**.

Taken together, these findings indicate that the *BD 3D Collagen Composite Scaffold* provides a suitable environment for promoting the *in vitro* growth and differentiation state 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 Collagen Composite**  
**Raw Pico Green and Computed Oxygen Consumption Data**

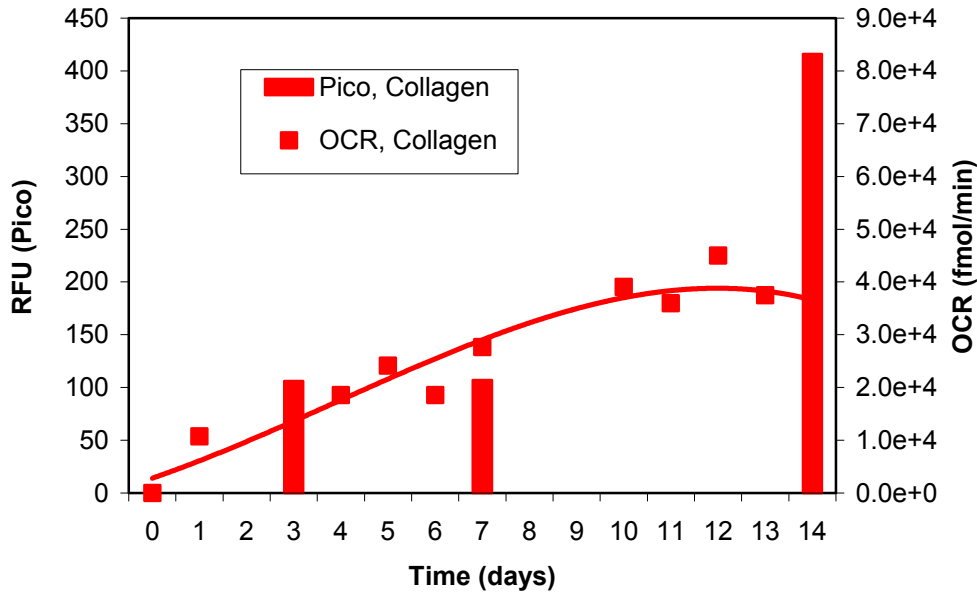


Figure 1:  
 The BD Oxygen Biosensor System (OBS) was used to analyze the Oxygen Consumption Rate (OCR) of WI38 Human Lung Fibroblasts cultured on the BD 3D Collagen Composite Scaffold. Cellular DNA was measured using the PicoGreen Assay Kit (Molecular Probes). The diamonds indicate the OCR and the solid line is a polynomial fit to the OCR points.

**Primary Rat Hepatocytes on Collagen Scaffold**

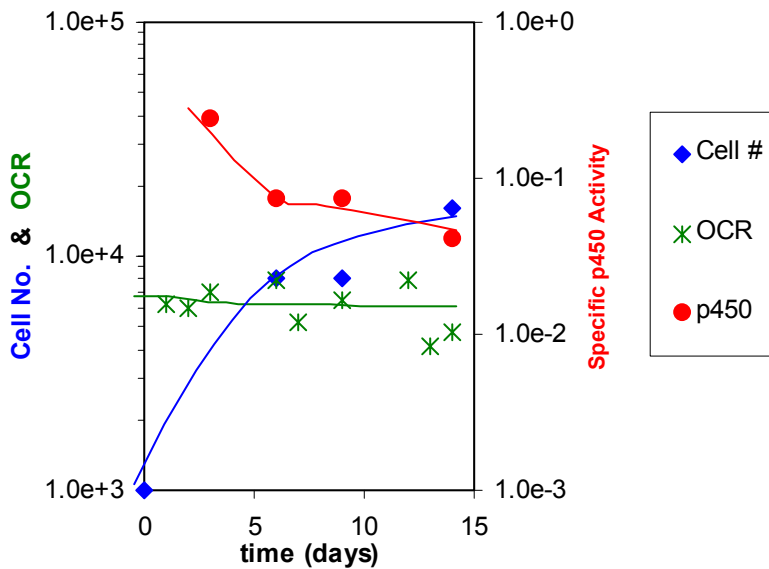


Figure 2:  
 The BD™ Oxygen Biosensor System was used to analyze cellular number and the OCR of primary rat hepatocytes cultured on the BD 3D Collagen Composite Scaffold. The BD Gentest Cytochrome P450 – Cyp3A4 Assay Kit was used to analyze the differentiation state of the hepatocytes. Oxygen Consumption 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.

## **STABILITY**

The *BD 3D Collagen Composite 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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