# BD Three Dimensional Calcium Phosphate Scaffold

**Catalog No. 354617**

***Guidelines for Use***

**FOR RESEARCH USE ONLY**

**NOT FOR CLINICAL, DIAGNOSTIC OR THERAPEUTIC USE**

BD Biosciences
Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen

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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.

Bone metabolism is a complex process that involves the resorption of existing bone by osteoclasts and the subsequent formation of a new bone matrix by osteoblasts. These activities are essential for bone remodeling, regeneration, and repair. For tissue engineering applications, 3D bone biomaterials must be capable of supporting the functional properties of osteogenic cells. For example, calcium phosphate ceramics have been shown to interact strongly and specifically with bone [7-9]. However, while materials such as hydroxyapatite are capable of stimulating new bone formation, bone cells are unable to remodel this bioceramic. The *BD 3D Calcium Phosphate Scaffold* is a proprietary mineralized calcium phosphate bioceramic that is ideal for *in vitro* and *in vivo* analyses of bone metabolism. It has been shown that this material supports bone remodeling *in vivo.* In addition, studies support the conclusion that this scaffold is suitable for *in vivo* analyses of cartilage regeneration.

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).
8. DeRuijter, J.E., et al., *Tissue Engineering*, 7: 279-289 (2001).
9. Langstaff, S., et al., *Biomaterials*, 22: 135-150 (2001).

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## MATERIALS PROVIDED

- Catalog # 354617 packaged as one 48-well plate with 24 3D scaffolds

| 1 Physical Specifications for BD 3D Calcium Phosphate Scaffold | | | |
|---|---|---|---|
| Scaffold Material | Calcium Phosphate | Average Pore Size: | 200-400 μm |
| Hydration Capacity: | 30 μl | Weight/wet weight | 45 mg/ 99 mg |
| Dimensions (diameter/height/volume): | 5mm x 3 mm x .058 $cm^3$ | | |
| Porosity: | 60 +/- 10 ppi (pores per linear inch) | | |

## 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 # 14-L5)
- **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.***

## CALIFORNIA PROPOSITION 65 NOTICE

**WARNING**: This product contains a chemical known to the state of California to cause cancer, birth defects and/or other reproductive harm..

**Component**: EtO Sterilization

## PROCEDURES FOR USE

### a) In Vitro Analyses

#### i) Cell seeding and culturing conditions

Certain cell types (e.g., MC3T3-E1 osteoblasts) 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 50 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 $5.0 \times 10^4$ – $2.5 \times 10^5$ 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 μg/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 Calcium Phosphate 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 μl 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 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's instructions (Sigma , Cat # 14-L5).

**v) Typical results (FOR REFERENCE ONLY)**

The following results were obtained by culturing MC3T3-E1 osteoblasts with the *BD 3D Calcium Phosphate Scaffold*. When MC3T3-E1 cells were cultured on the BD 3D Calcium Phosphate Scaffold, cell growth increased dramatically within 1-2 days and was maintained for up to 10 days in culture (**Fig. 1**). The increase in cell growth was found to correlate with an increase in cellular DNA content (**Fig. 2**). In addition, increased cell growth correlated with elevated levels of alkaline phosphatase activity (**Fig. 3**), which is a marker for osteoblast differentiation. Taken together, these findings indicate that the BD 3D Calcium Phosphate Scaffold provides a suitable environment for *in vitro* analyses of osteoblast growth and differentiation. **Results will vary using different cell types and culturing conditions.**

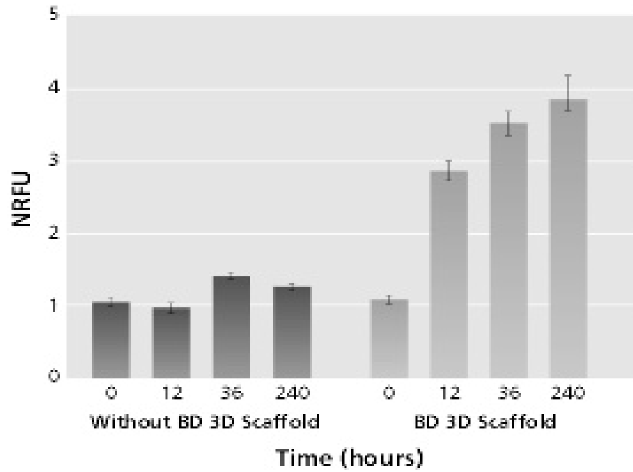


**Figure 1:**
*The BD Oxygen Biosensor System was used to analyze cellular growth. The consumption of oxygen by proliferating cells was measured by fluorescence detection, which is expressed in normalized relative fluorescence units (NRFU).*

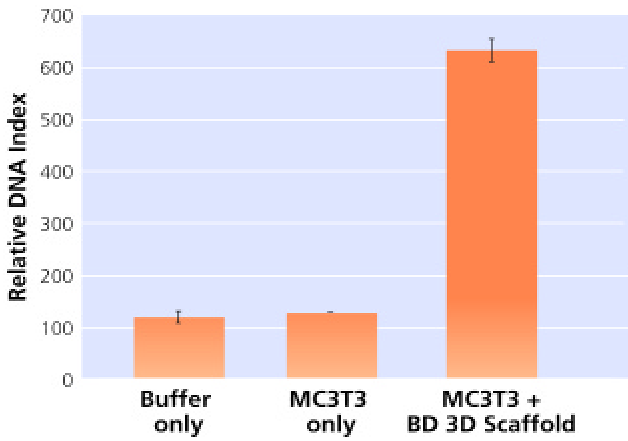


**Figure 2:**
*Cellular DNA content was measured using the Picogreen Assay Kit (Molecular Probes).*

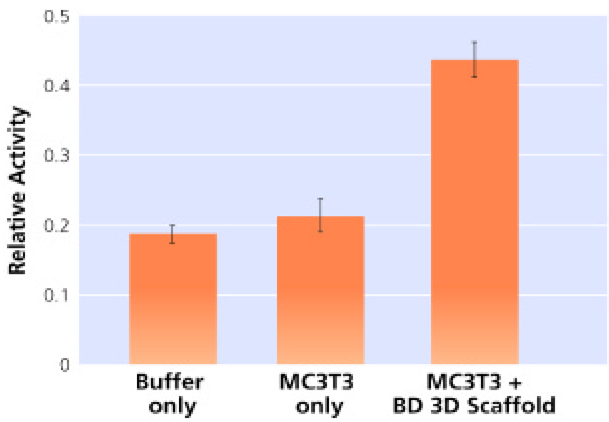


**Figure 3:**
*To assess osteoblast differentiation, alkaline phosphatase activity was measured using the AP Assay Kit (Sigma Diagnostics).*

## In Vivo Analyses

***The following protocols are intended to serve as general guidelines for the implantation of the BD scaffold into relevant animals. For certain* in vivo *applications, it may be necessary to employ alternative procedures.***

### i) Ectopic Bone Formation

Human bone marrow stromal cells (BMSC) (or other relevant cell types) can be loaded onto the *BD 3D Calcium Phosphate Scaffold* and implanted into immunodeficient mice to evaluate *in vivo* bone formation that is attributable to human cells.

BMSC are collected from culture dishes and then seeded on scaffolds as described above (see section a, part ii). The BMSC/scaffold composites are then subcutaneously implanted into 1 month-old recipient nude mice (CD-1 nu/nu; Charles River - 1-800-522-7287). Up to six scaffolds can be implanted on the back of each mouse. The scaffolds are retrieved for subsequent analyses at relevant time points.

### ii) Transfemoral Implantation

Male Wistar rats (100-125 grams) are anaesthetized and the lateral aspect of the thigh is then prepared for surgery according to established procedures. A lateral skin incision is made in the mid-femoral region, exposing the vastus lateralis and adductor muscles. The lateral cortex of the femur is then exposed and the periosteum scraped. A trans-femoral defect is created under continuous saline irrigation. The BD scaffold (with or without cells) is then inserted as a press-fit into the defect. The implant should be flush with the cortical surface of the femur. Following implantation, the integrity of the implant is confirmed and the musculature returned to its natural arrangement. The musculature and skin are then closed independently using standard degradable sutures. The procedure can be repeated on the contra-lateral side with a second implant of identical geometry and composition.

Animals are then terminated according to a preset schedule by cardiac perfusion of fresh Karnovsky's fixative. The femora are dissected to reveal the implant site and then retained in Karnovsky's fixative prior to further analysis.

### iii) Typical Results

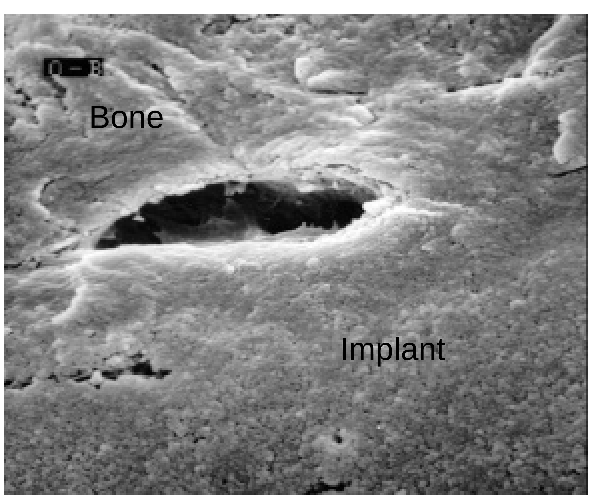


**Figure 4:** *Scanning Electron Micrograph (SEM) illustrating the bone-biomaterial interface at 23 days in vivo post-implantation of the mineralized calcium phosphate scaffold in a young adult male Wistar rat.9*

## GENERAL HINTS FOR USING 3D SCAFFOLD PRODUCTS

Handle all scaffolds under aseptic conditions.

## STABILITY

The *BD 3D Calcium Phosphate Scaffold* is stable for at least 12 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.