BD™ PuraMatrix™ Peptide Hydrogel
BD™ PuraMatrix™ Peptide Hydrogel is a synthetic matrix that is used to create defined three-dimensional (3D) micro-environments for a variety of cell culture experiments. To achieve optimal cell growth and differentiation, it is necessary to determine the appropriate mixture of this material and bioactive molecules (e.g., growth factors, extracellular matrix [ECM] proteins, and/or other molecules). BD PuraMatrix Peptide Hydrogel consists of standard amino acids (1% w/v) and 99% water. Under physiological conditions, the peptide component self-assembles into a 3D hydrogel that exhibits a nanometer scale fibrous structure (Figure 1). The hydrogel is readily formed in a culture dish, plate, or cell culture insert.

The resulting hydrogel has been shown to promote the differentiation of hepatocyte progenitor cells¹, rat pheochromocytoma cells (PC12)², and hippocampal neurons.³ Studies have also demonstrated that BD PuraMatrix Peptide Hydrogel supports the attachment of a variety of primary (e.g., neuronal, fibroblast, keratinocyte) and transformed (e.g., MG-63, SH-SY5Y, HEK293, NIH3T3) cell types.⁴ Other potential applications include stem cell proliferation, tumor cell migration and invasion, angiogenesis assays, and in vivo analyses of tissue regeneration.

BD PuraMatrix Peptide Hydrogel is biocompatible, resorbable, and devoid of animal-derived material and pathogens. For in vivo studies in animals, the soluble material can be injected and will subsequently form a 3D hydrogel upon contact with the physiological environment.

**Application Focus**

**Differentiation of Hepatocyte Progenitor Cells¹**

Rat hepatocyte progenitor cells (Lig-8) were encapsulated in BD PuraMatrix Peptide Hydrogel and cultured overnight in defined medium at 37°C. Samples were then used for bromodioxyuridine (BrdU) uptake and in situ immunofluorescence analyses. As shown in Figure 2, Lig-8 cells form spheroid colonies when cultured within the 3D hydrogel and express the hepatocyte markers CCAAT/enhancer binding protein α (C/EBPα) and cytochrome P450 1A1/1A2 (CYP1A1/1A2) in a manner that is independent of cellular mitotic activity. Therefore, while some cells are proliferating, the entire colony exhibits differentiation potential.

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**Figure 1.** Electron micrograph of BD PuraMatrix Peptide Hydrogel (bar, 100 nm).

**Figure 2.** Lig-8 cells cultured in BD PuraMatrix Peptide Hydrogel. All cells in spheroid colonies, arrested or not, undergo differentiation. Spheroids were isolated, transferred to adherent cultures, and incubated with BrdU for 24 hours.

(A) spheroid colony (phase contrast)
(B) same optical layer as A immunostained for C/EBPα (red)
(C) same optical layer as A immunostained for BrdU (green)
(D) spheroid colony (phase contrast)
(E) same optical layer as D immunostained for CYP1A1/1A2 (red)
(F) same optical layer as D immunostained for BrdU


**References**

Analysis of Neurite Outgrowth using PC12 cells

PC12 cells were cultured on BD PuraMatrix Peptide Hydrogel according to a surface plating protocol. To examine the differentiated morphology of these cells, samples were incubated in the presence of nerve growth factor (NGF) and subsequently analyzed by scanning laser confocal microscopy. PC12 cells cultured under these conditions were found to differentiate and exhibit pronounced neurite outgrowth (Figure 3). Moreover, human SY5Y neuroblastoma cells and a variety of primary neurons were shown to exhibit comparable activity on this material (Table 1).

| Table 1. Neurite outgrowth from neuronal cells on BD™ PuraMatrix™ Peptide Hydrogel. |
|-----------------|-----------------|------------------|
| Cell Type       | Neurite Length, µm | Cell Source†     |
| NGF-treated Rat PC12 | 400-500          | Cultured cell line |
| NGF-preprimed PC12    | 400-500          | Cultured cell line |
| Human SY5Y neuroblastoma | 400-500        | Cultured cell line |
| Mouse cerebellar granule neurons | 200-300       | Primary cell‡ |
| Mouse hippocampal neurons | 100-200        | Primary cell‡ |
| Rat hippocampal neurons | 200-300         | Primary cell§ |

† Cells were seeded onto BD PuraMatrix Peptide Hydrogel. The cell-bearing hydrogels were transferred to dishes with fresh medium. Maximum neurite length was estimated visually with scale bars 3 to 7 days after cell attachment for primary cells and 10 to 14 days for the cultured cell lines.
‡ Seven-day-old mouse
§ One-day-old rat

Differentiation of Neurons Derived from Hippocampal Slices

Hippocampal tissue slices (~200 µm thickness) were isolated from 7.5-day-old postnatal rats and placed on top of a microporous membrane insert for up to two weeks. The inserts were either uncoated (control samples) or coated with BD PuraMatrix Peptide Hydrogel (~500 µm thick). When cultured on the hydrogel, the hippocampal slice exhibited tissue growth and cell migration into the material (Figure 4). While neuronal migration was not observed in the control samples (Figure 4g), neuronal and glial progenitor cells were found to migrate from the tissue slice into the hydrogel layer (Figure 4f and 4h). These results suggest that BD PuraMatrix Peptide Hydrogel may be useful for promoting neural cell differentiation and tissue growth in vivo.

Figure 3. PC12 cell neurite outgrowth and differentiation on BD PuraMatrix Peptide Hydrogel. The image is a merged stack of multiple confocal optical sections. PC12 cells cultured in the presence of NGF attached to the hydrogel and projected extensive neurites. The black spots are holes in the hydrogel. The micrograph is representative of at least four independent experiments.

Data provided by 3D Matrix, Inc. and originally described in Holmes, T.C., et al., PNAS USA 97: 6728 (2000).

Figure 4. Hippocampal organotypic slice cultures on BD PuraMatrix Peptide Hydrogel develop extended tissue growth. Hippocampal slices were cultured on control membrane or BD PuraMatrix Peptide Hydrogel layers (~500 µm thick). Time lapse was carried out to follow tissue growth from the perimeter of the dentate gyrus region. (A) Time 0 (0 hour) of control slice culture; (B) Time 0 (0 hour) hydrogel (RAD16-I) slice culture; (C) 72 hours, control slice; (D) 72 hours, hydrogel slice; The red line indicates the original border of the tissue slice and the yellow line in d shows the extended tissue growth. The yellow arrow in d indicates the direction of tissue growth and extension. Black bars represent 100 µm. (E) 72 hours, control slice immunostained for GFAP (glial cell marker, green); (F) 72 hours, hydrogel slice immunostained for GFAP (green); (G) same optical layer as e immunostained for NeuN (neuron marker, red); (H) same optical layer as f immunostained for NeuN (red). Red lines in e and f and yellow in g and h indicate original perimeter of the tissue slice. The white line in e and g indicates the extended tissue scaffold in control cultures. The white line in f and h is used to compare the over extension obtained on hydrogel cultures. Yellow arrows in h indicate NeuN+ neurons (red) migrating into Area II in hydrogel slice cultures. White bar in f represents 100 µm.

**BD™ PuraMatrix™ Peptide Hydrogel**

### Features and Benefits

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<tr>
<th>Features</th>
<th>Details</th>
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<tr>
<td><strong>Purified Synthetic Peptide</strong></td>
<td>Highly defined material that promotes cell attachment</td>
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<td><strong>Composition (1% w/v)</strong></td>
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<td><strong>3D Hydrogel Structure</strong></td>
<td>Assembles into fibrous structure with average pore size of 50-200 nm</td>
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<td><strong>Easy Handling</strong></td>
<td>Easily mixed with cells and/or bioactive molecules (e.g., growth factors) prior to gelation; injectable for <em>in vivo</em> studies in animals</td>
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<td><strong>Transparent Hydrogel</strong></td>
<td>Samples are readily visualized using standard staining methodologies and microscopy</td>
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<td><strong>Established Protocols</strong></td>
<td>3D cell encapsulation cultures; Surface plating of adherent cells on microporous membrane inserts and microplates; cell recovery for sub-culturing or biochemical analyses; <em>in vivo</em> injection</td>
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### Characteristics

- Peptide sequence promotes cell attachment, but does not activate RGD-dependent integrin signaling
- In the presence of salt-containing solution, the peptide component of BD PuraMatrix Peptide Hydrogel self-assembles and forms a transparent 3D hydrogel
- Exhibits nanometer scale fibrous structure
- Biocompatible; devoid of animal-derived material and pathogens

### Technical Specifications

- 1% solution (w/v) of purified synthetic peptide
- Packaged material exhibits pH = 3.0
- Quality Control:
  - Tested and found negative for bacteria, fungi, and Mycoplasma
  - Cell viability > 80% based on cytotoxicity analysis of NIH3T3 fibroblasts
  - Identity confirmed using Mass Spectrometry
  - Demonstration of fiber formation using a self-assembly assay

### Ordering Information

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<th>Description</th>
<th>Qty</th>
<th>Cat. No.</th>
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<td>BD™ PuraMatrix™ Peptide Hydrogel</td>
<td>5 ml</td>
<td>354250</td>
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To place an order in the U.S., contact Customer Service at:

- tel: 800.343.2035 or 978.901.7300
- fax: 800.743.6200 or 978.901.7493

To place an order outside the U.S., contact your local distributor or nearest BD Biosciences office.

For information about GMP-grade material, contact 3D Matrix, Inc. at:

- e-mail: sales@puramatrix.com; internet: www.puramatrix.com