A Synthetic Hydrogel for 3D Cell Culture: BD™ PuraMatrix™ Peptide Hydrogel

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BD Biosciences

August 11, 2009

Topics for Discussion

- Extracellular Matrix (ECM) and 3D Cell Culture
- BD PuraMatrix Peptide Hydrogel
- Representative Applications
  - Primary hepatocytes and progenitors
  - PC12 neurite outgrowth
  - Primary neurons
  - Hippocampal slice culture
  - Endothelial cells

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The Extracellular Matrix

- Complex mixture containing glycoproteins, collagens, and proteoglycans
- Forms structural framework that stabilizes tissues and provides mechanical support for cell attachment
- Plays important role in cell functionality and differentiation
  - Receptor-mediated signaling
  - Regulation of gene expression

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Basal Lamina in Chick Embryo Cornea

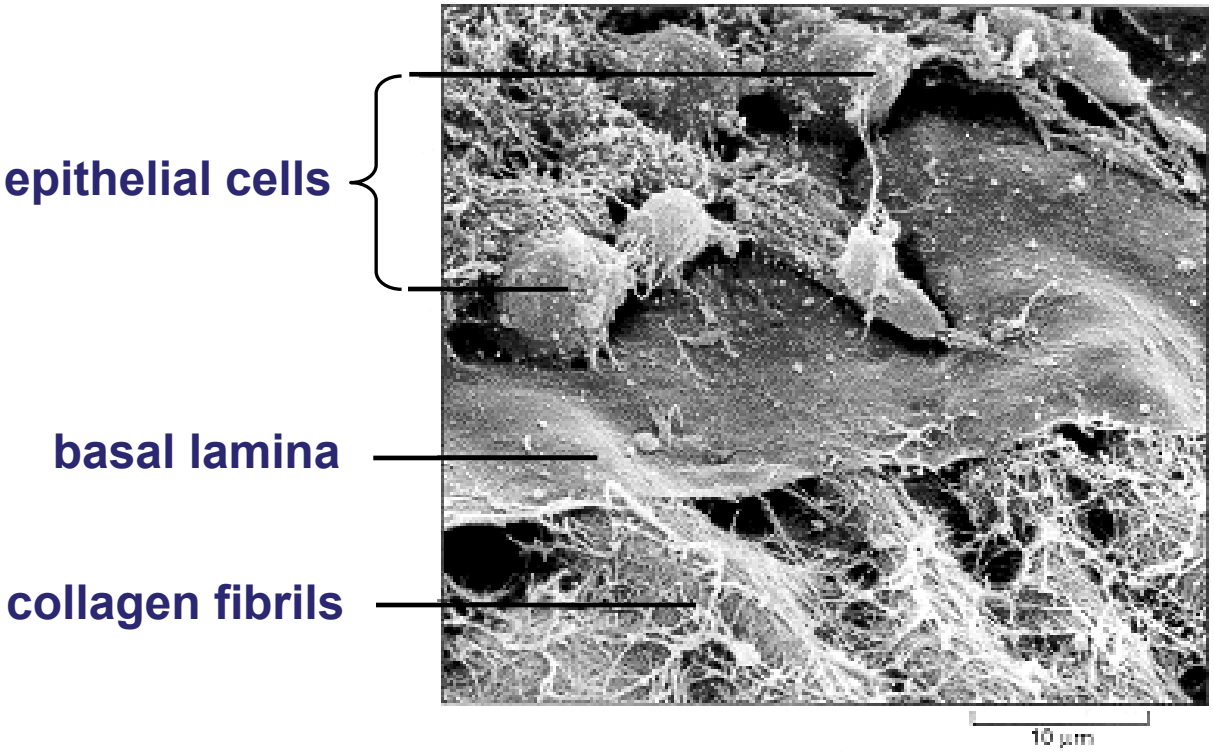

Figure: Molecular Biology of the Cell (3rd Edition)

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ECM Contributes to Intracellular Signaling Pathways

- ECM-based growth substrates provide a physiological environment that supports and promotes key cell functions
- ECM molecules interact with cell surface receptors (e.g., regulation of integrin signaling by fibronectin:integrin interactions)
- ECM appears to function in the storage and presentation of growth factors

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Factors that Influence Cell Differentiation and Functionality

- **Biological composition of the culture environment** (e.g., cell types, ECMs, growth factors)
- **Molecular interactions and cell adhesion** (cell:cell, cell:ECM, ECM:ECM, cell:growth factor, and ECM:growth factor)
- **Mechanical strength and structural properties** (degree of rigidity, 3D architecture)
- **Size scale** (i.e., pore or fiber size relative to cell size; microfibers vs. nanofibers)

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2D vs. 3D Cell Culture

	2D	3D
Growth Substrate	Rigid; inert	Mimics natural tissue environment
Architecture	Not physiological; cells partially interact	'Physiological'; promotes close interactions between cells, ECMs, growth factors
Cell Encapsulation	No	Yes
Growth Factor Diffusion	Rapid	Slow; biochemical gradients regulate cell-cell communication and signaling

Current Approaches for Cell Culture

- **2D Growth Substrates**
  - Tissue culture treated cellware (e.g., dishes, multiwell plates, flasks)
  - Pre-coated cellware (e.g., ECMs, attachment factors)
- **3D Growth Substrates**
  - Collagen gels
  - Reconstituted basement membrane (BD Matrigel™ Matrix)
  - BD Laminin/Entactin Complex
  - Hydrogels [e.g., self-assembling synthetic peptides, polyethyleneglycol (**PEG**) diacrylate, poly N-isopropylacrylamide (**PIPAAm**), alginate, agarose]
  - Macroscaffolds (BD 3D Scaffolds)

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BD Matrigel™ Matrix: *Reconstituted Basement Membrane*

Composition

- Laminin ~ 60%
- Collagen IV ~ 30%
- Entactin ~ 8%
- Heparan sulfate proteoglycan (perlecan)
- Growth factors (e.g., PDGF, EGF, TGF-β)
- Matrix metalloproteinases

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BD Matrigel™ Matrix: *Reconstituted Basement Membrane*

Representative '3D' Applications

- Hepatocyte differentiation
- Neuronal cell differentiation
- Mammary epithelial cell polarity
- Endothelial cell tubulogenesis
- *In vivo* tumor formation & angiogenesis

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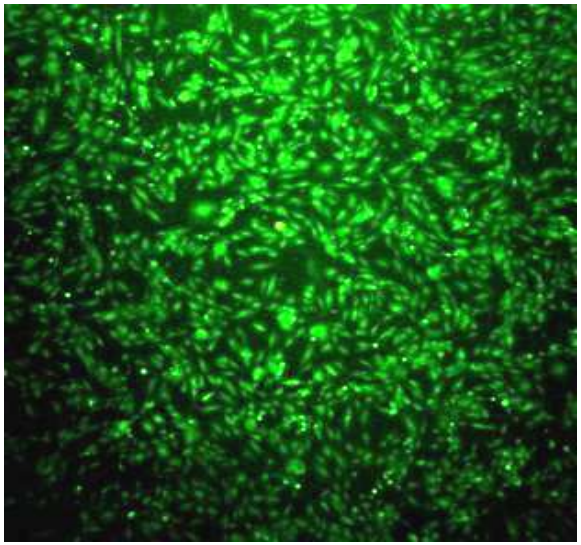
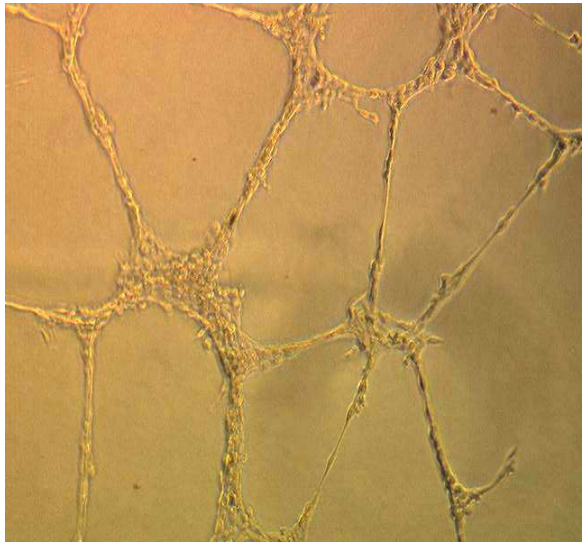
Human Microvascular Endothelial Cells Form Tubules on BD Matrigel Matrix



Collagen I

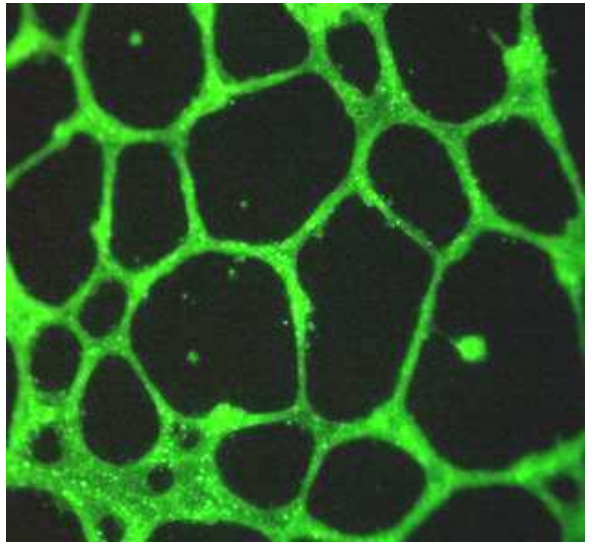
2D



BD Matrigel Matrix

3D

Bright Field



BD Matrigel Matrix

3D

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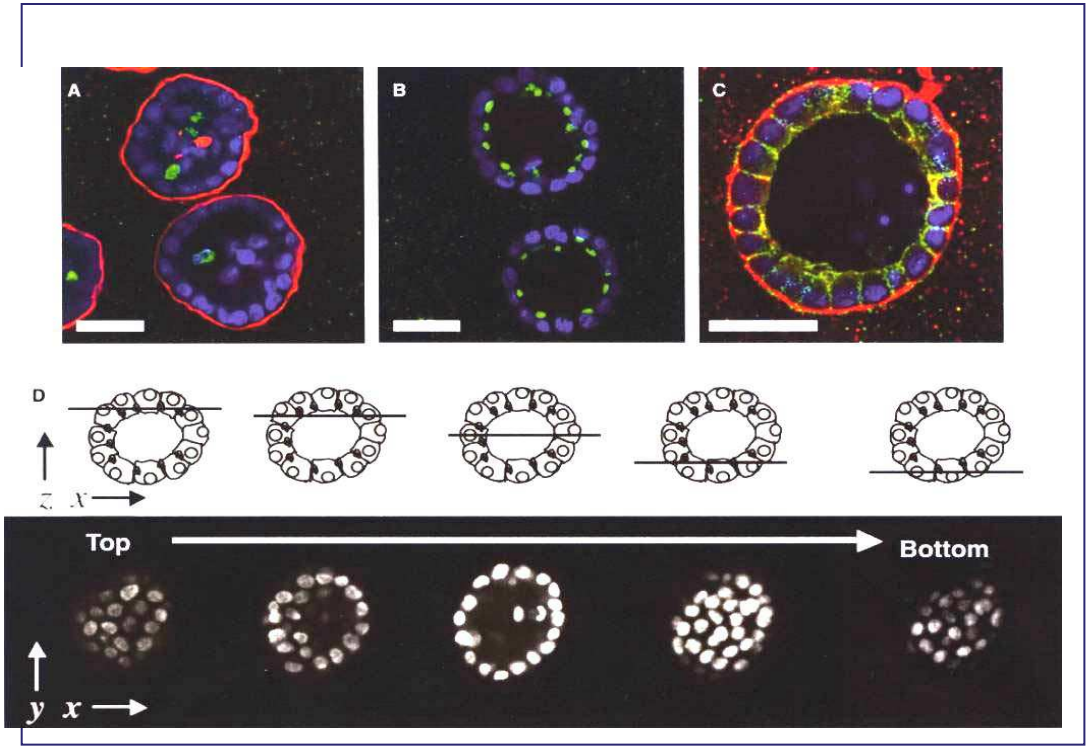
Mammary Epithelial Cells in BD Matrigel Matrix using 3D 'Overlay' Method



Data provided by Dr. Joan Brugge, Harvard Medical School

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High Concentration ECM Proteins

High Concentration Laminin/Entactin Complex

- Major component of basement membrane in Engelbreth-Holm-Swarm (EHS) mouse tumors
- Protein concentration: ≥ 10 mg/ml
- Purity >90% by SDS-PAGE
- Forms 3D gel that models tissue microenvironment *in vivo*
- Supports cell differentiation (e.g., mouse submandibular cells, endothelial cell tube formation)

High Concentration Collagen I

- Source: rat tail tendon
- Protein concentration: 8-11 mg/ml
- Full-length protein (not treated with pepsin)
- Purity >90% by SDS-PAGE
- Forms sturdy gel that provides maximal 3D support matrix

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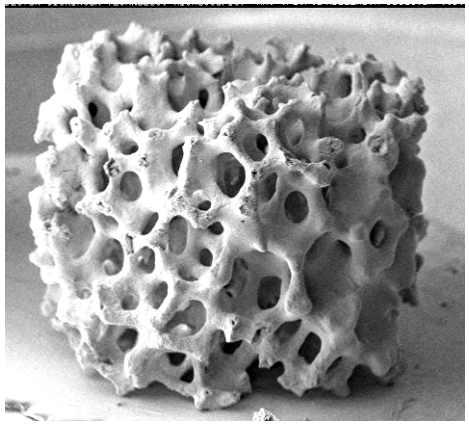
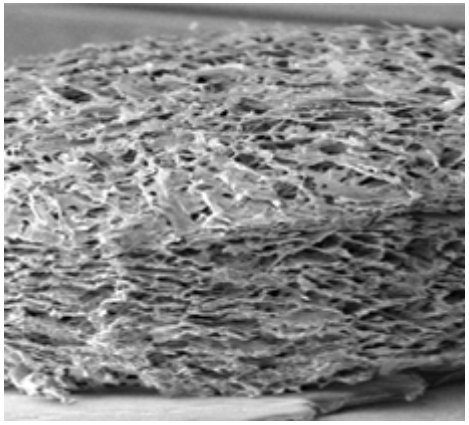
3D ‘Macroscaffolds’

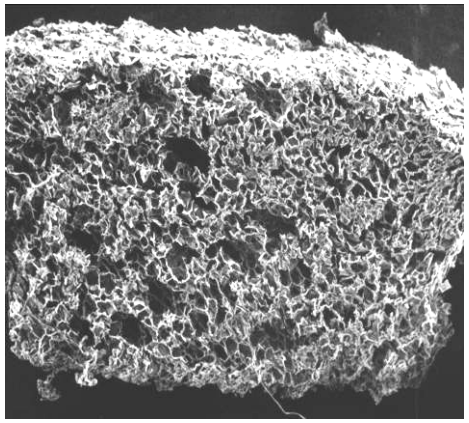


BD™ 3D Calcium Phosphate Scaffold

Cell types: *bone, cartilage*



BD™ 3D Collagen Composite Scaffold



BD™ 3D OPLA® Scaffold

Cell types: *epithelial, endothelial, smooth muscle, neuronal, bone, cartilage*

Skelite is a trademark of Octane Orthobiologics, Inc., Ontario, Canada. Collagen Composite and OPLA Scaffolds are proprietary biomaterials of Kensey Nash Corporation; OPLA is a registered trademark of Kensey Nash Corporation.

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Potential Limitations of Current Systems

- Composition of the environment not optimal for key cell type(s)
- Growth substrate or material not well defined
- Complex materials often exhibit lot-to-lot variability
- Presence of animal-derived components
- Synthetic materials likely to generate acidic breakdown products that are cytotoxic
- Size scale not optimal

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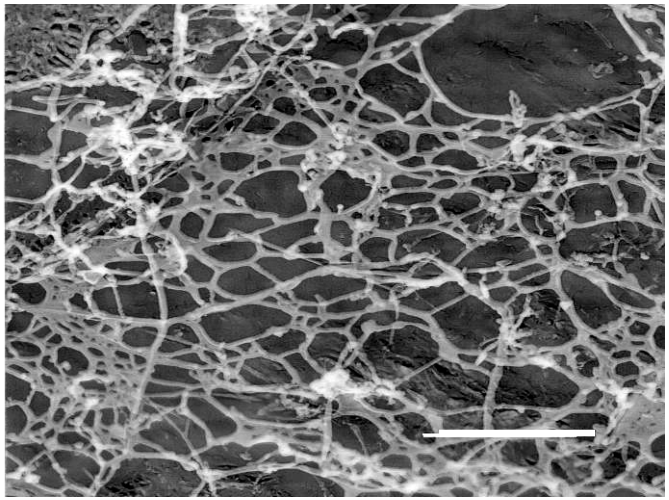
BD PuraMatrix Peptide Hydrogel

A Synthetic Biomaterial for Optimizing 3D Cell Culture Environments



Electron micrograph of BD PuraMatrix Peptide Hydrogel [bar, 100 nm].

- Material developed at Massachusetts Institute of Technology, Cambridge, MA
- Composed of synthetic peptide (1% w/v) and 99% water
- Salt-mediated peptide assembly into 3D hydrogel with average pore size of 50-200 nm
- Promotes attachment of many primary and transformed cell types
- Supports differentiation of cell types such as primary hepatocytes, neurons, endothelial cells, chondrocytes, neural stem cells, and mesenchymal stem cells
- Devoid of animal-derived materials and pathogens

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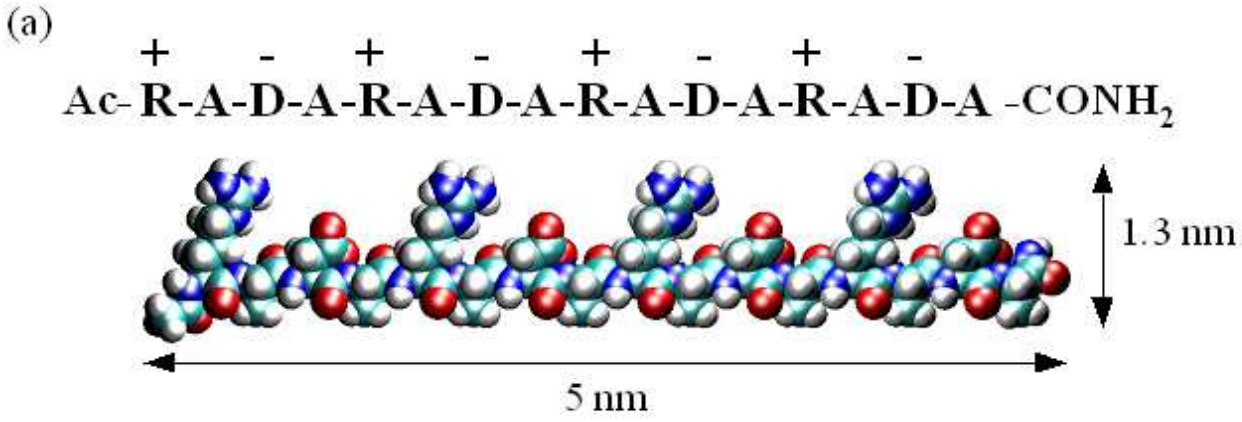
Synthetic Peptide Composition

- 16 amino acid sequence; 1% peptide solution (w/v)
- Alternating hydrophilic and hydrophobic side chains
- Promotes cell attachment, but does not activate RGD-dependent integrin signaling



Images of molecular models kindly provided by Dr. Shuguang Zhang, Center for Biomedical Engineering, Massachusetts Institute of Technology, Cambridge, MA

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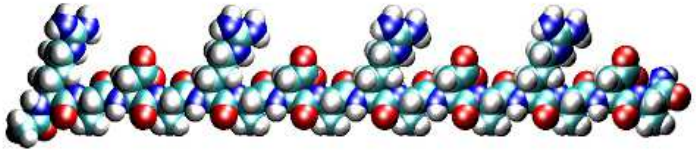
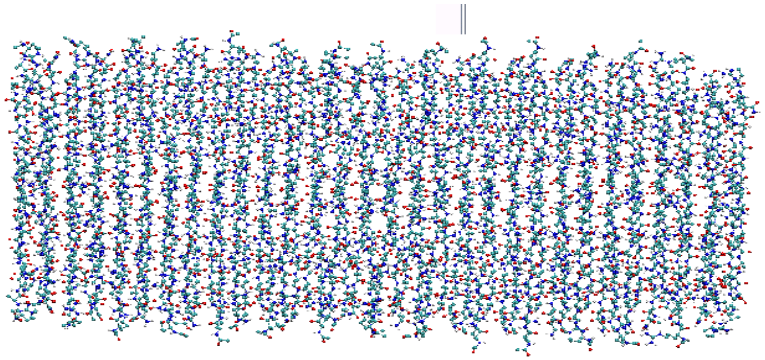
Salt-Mediated Molecular Self-Assembly into Nanofiber Structure

single peptide → **nanofibers**





- Ionic and hydrogen bonding
- Hydrophobic and van der Waals interactions

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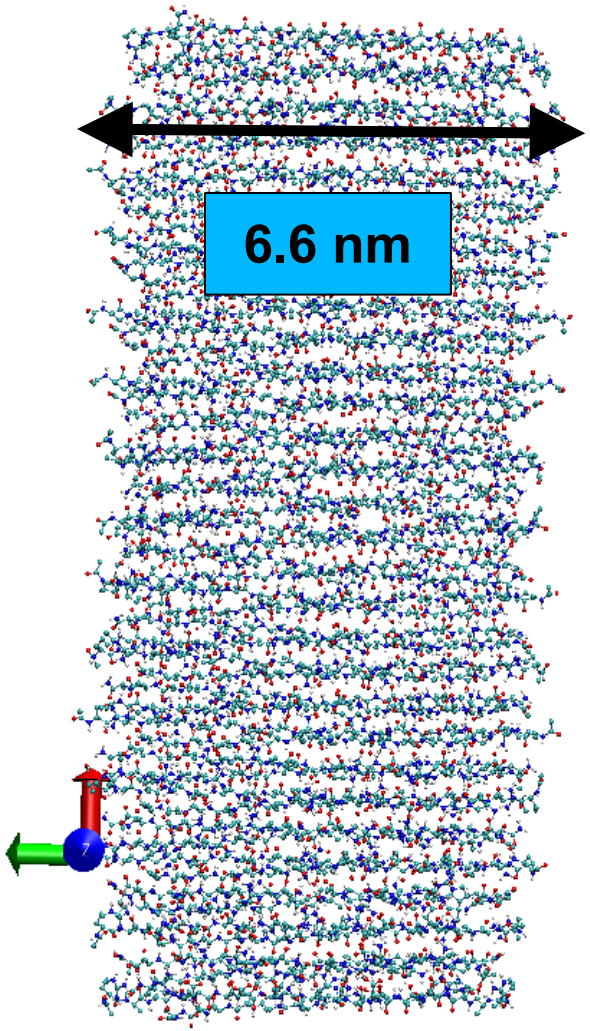
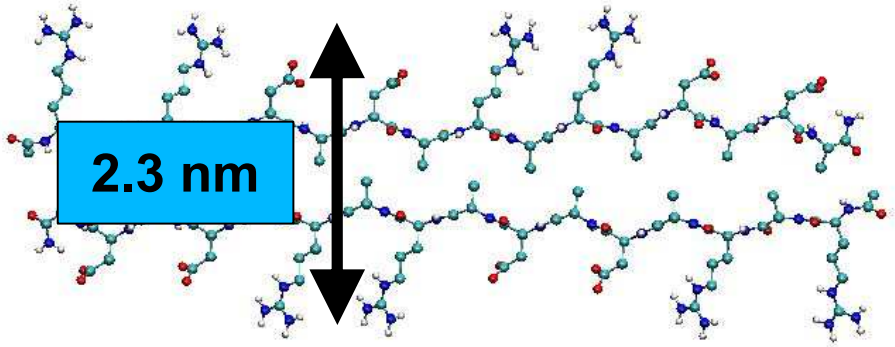


- β-sheet bilayer structure
- Filament dimensions:
  - Width = 6.6 nm
  - Height = 2.3 nm



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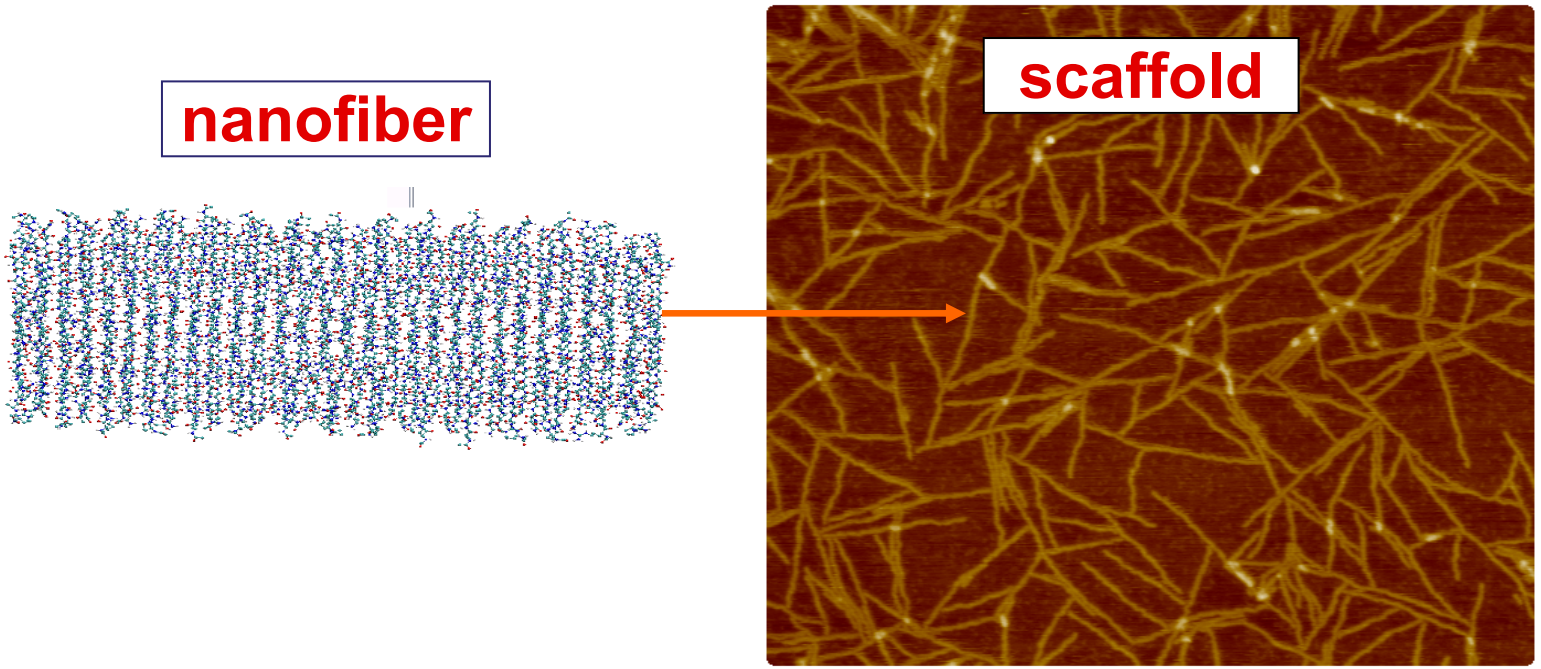
Nanofiber β-Sheets Further Organize into Macroscopic Scaffolds



AFM image provided by Dr. Shuguang Zhang, MIT

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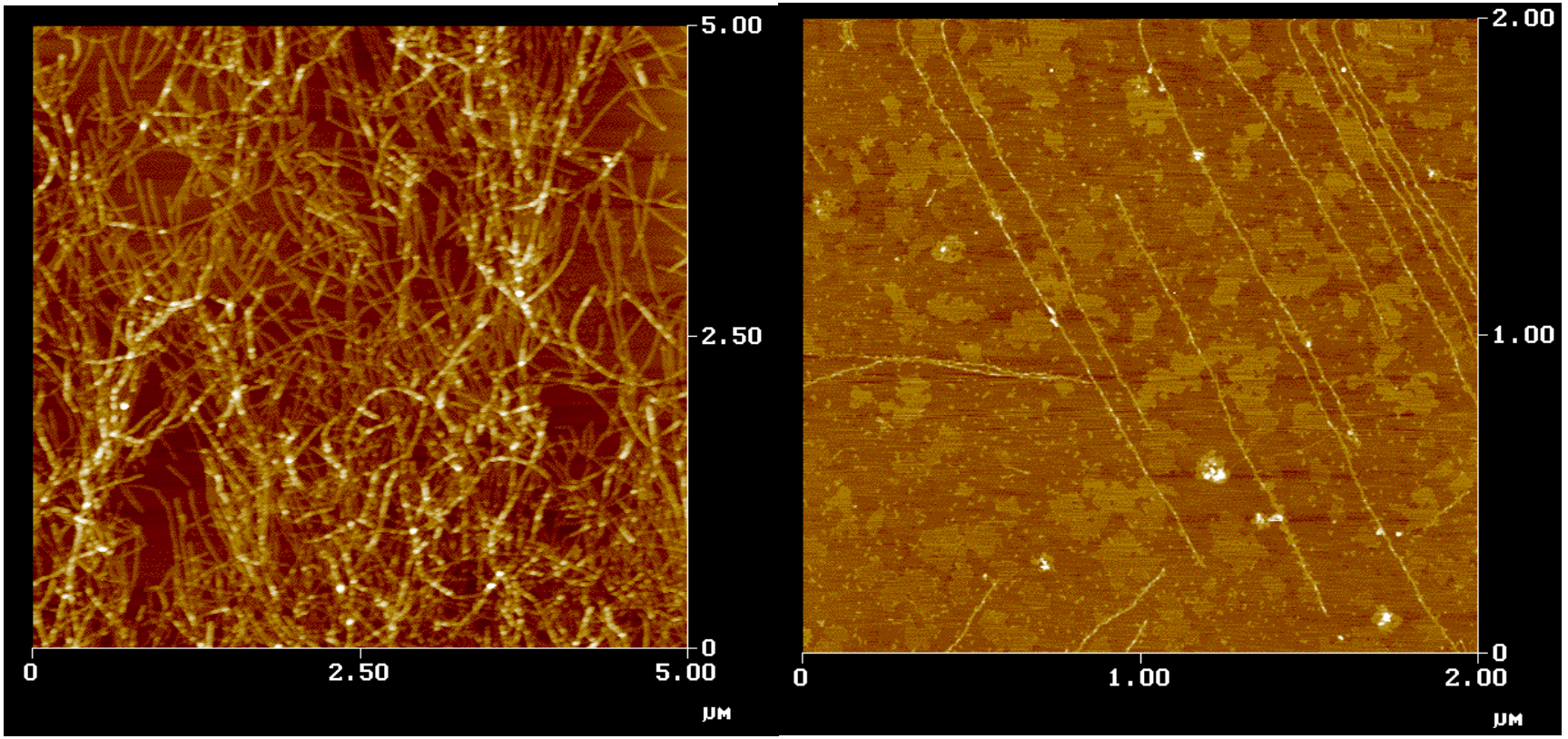
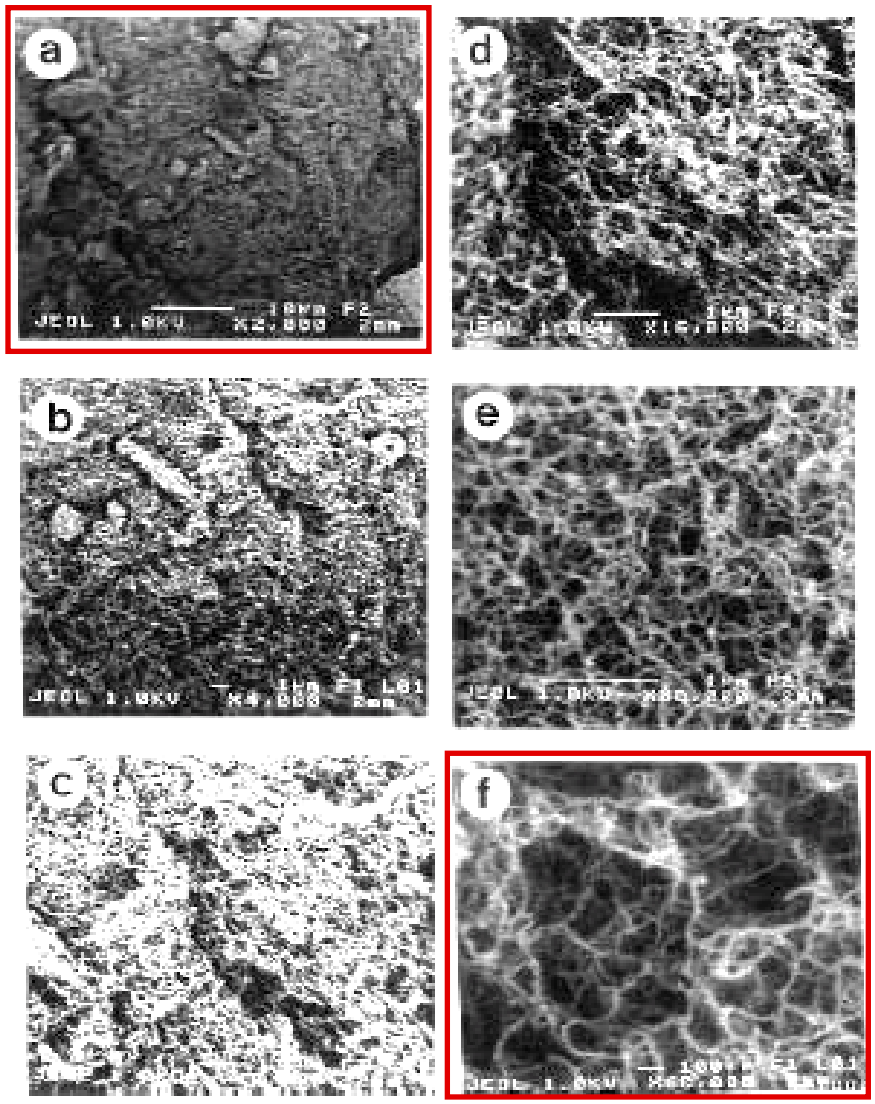
AFM Images of BD™ PuraMatrix™ Nanofibers



SEM Images of Macroscopic Structure



Images of BD PuraMatrix Peptide Hydrogel at increasing magnification

- At lower magnification, the scaffold exhibits felt-like appearance (**a**).
- Higher magnification reveals interwoven peptide fibers approximately 10-20 nm in diameter (**f**).

Originally described in ***Holmes, T.C., et al. (2000) PNAS, Vol 97, pp 6728-6733.***

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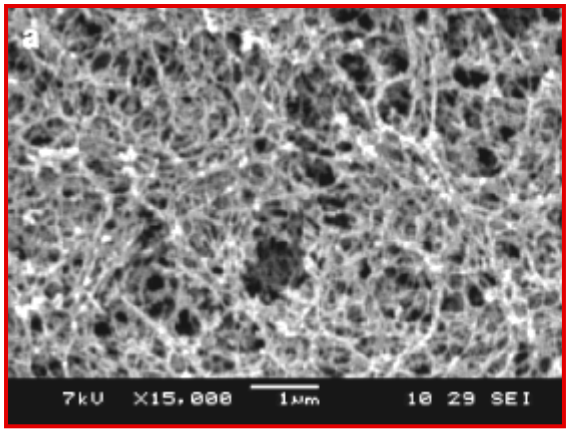
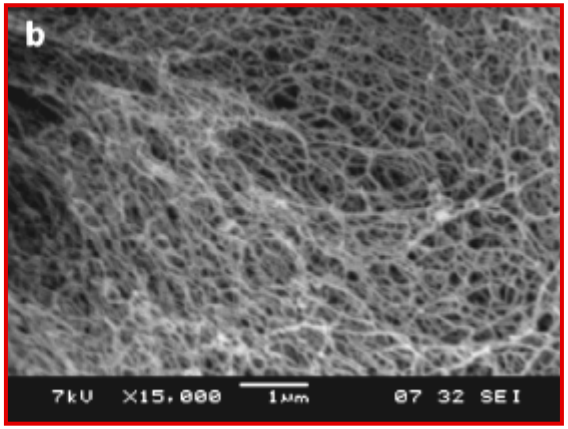
SEM Analysis of Macroscopic Structure: *BD PuraMatrix vs. BD Matrigel Matrix*



BD Matrigel matrix



BD PuraMatrix peptide hyrdogel

Data kindly provided by Dr. Shuguang Zhang and originally described in **Gelain, F., et al. (2006) *PLoS ONE* 1(1):e119.**

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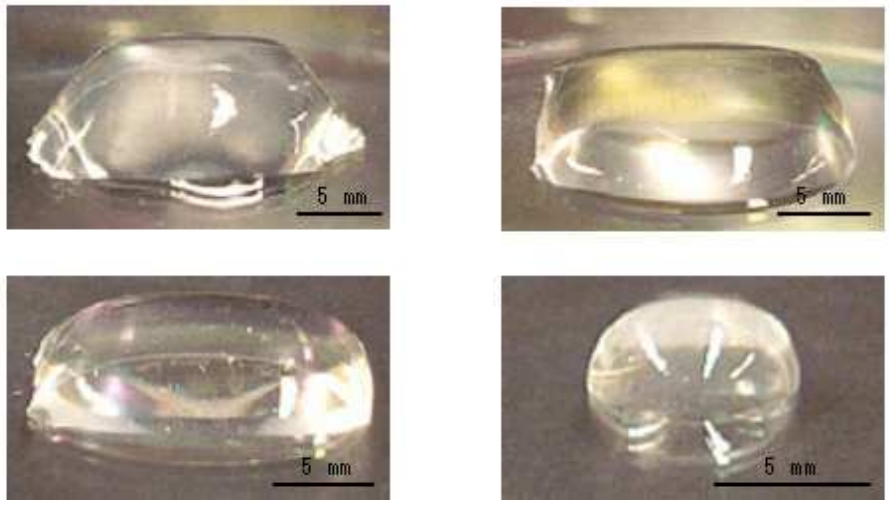
The Assembled Hydrogel is Comprised of ≥ 99% water



The hydrated 'hydrogel' environment promotes effective diffusion of nutrients and macromolecules

Images provided by Dr. Shuguang Zhang, MIT

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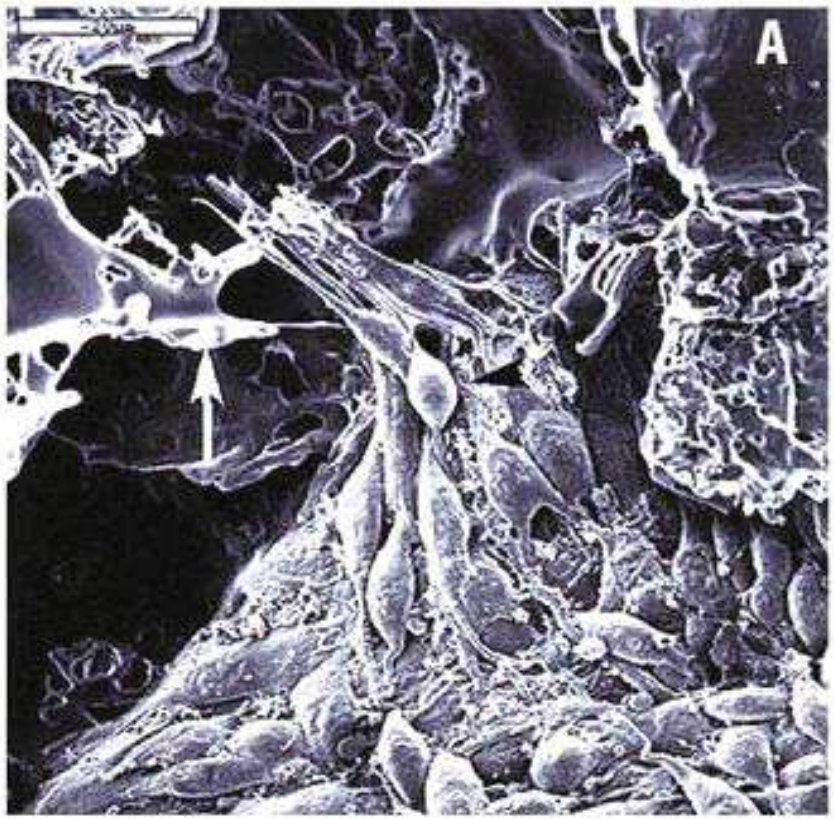
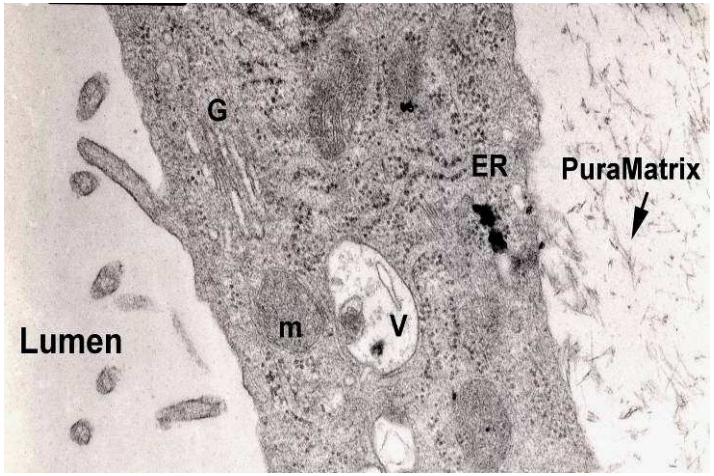
BD PuraMatrix Peptide Hydrogel vs. Macroscaffolds



- Nanofiber structure comparable to physical scale of ECM *in vivo*
- Macroscaffold pores much larger than cells, which may be sub-optimal key cell functions



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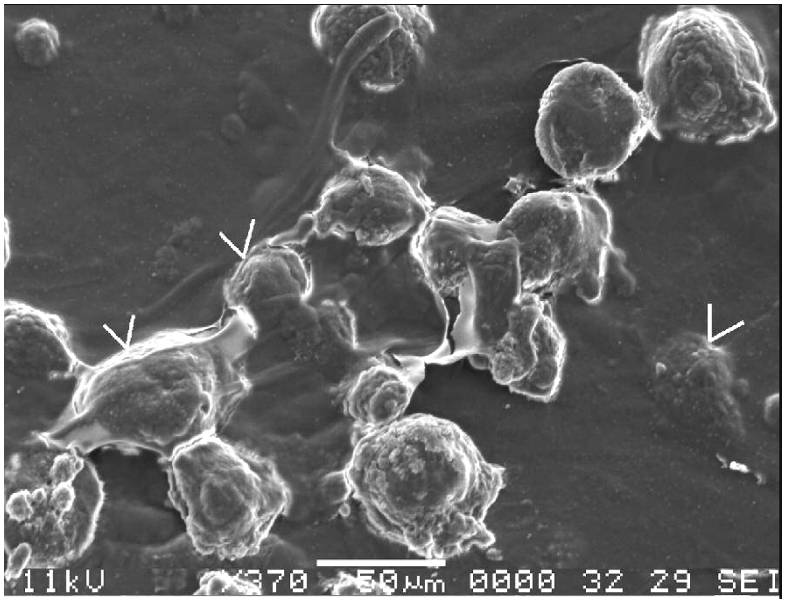
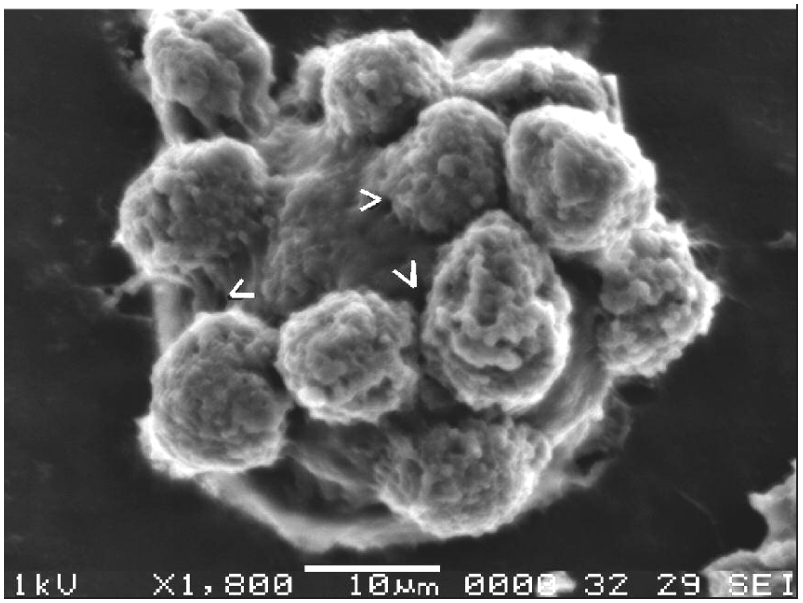
BD PuraMatrix Peptide Hydrogel: *Methodology*

- 3D cell suspension and encapsulation
- Surface plating of adherent cells on a BD Falcon™ Cell Culture Dish or Plate
- Surface plating of adherent cells or tissue slices on BD™ Cell Culture Inserts

Scanning Electron Microscopy of Rat Primary Hepatocyte Spheroids on BD PuraMatrix Peptide Hydrogel



Spheroids attached on the surface and embedded within the hydrogel (white arrows)



High magnification view of a spheriod

Data kindly provided by Sihong Wang and originally described in **Wang, S., et al. (2008)** ***Tissue Engineering Part A*** **14(2):227.**

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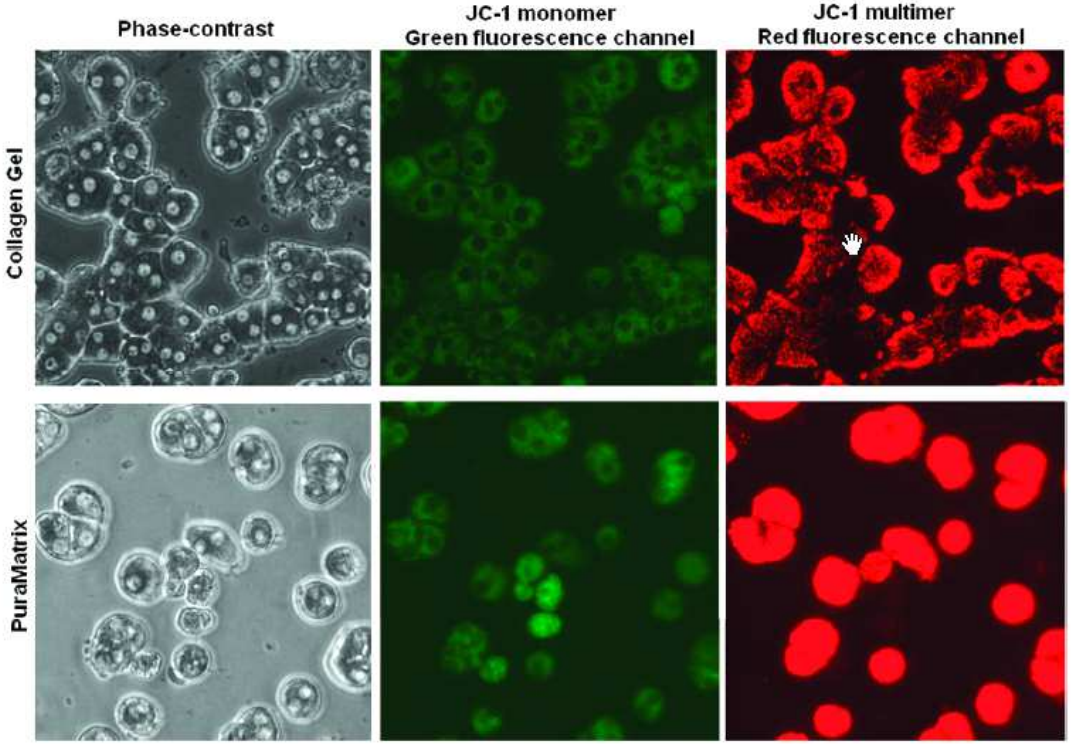
Analysis of Mitochondrial Membrane Potential in Rat Primary Hepatocytes: *BD™ PuraMatrix™ Peptide Hydrogel vs. Collagen Gel*



- JC-1 staining used to assess mitochondrial membrane potential
- JC-1 red staining on BD PuraMatrix ~2X greater than that on collagen gel, suggesting that membrane potential is higher on BD Puramatrix peptide hydrogel

Data kindly provided by Dr. Sihong Wang and originally described in **Wang, S., et al. (2008)** ***Tissue Engineering Part A*** **14(2):227.**

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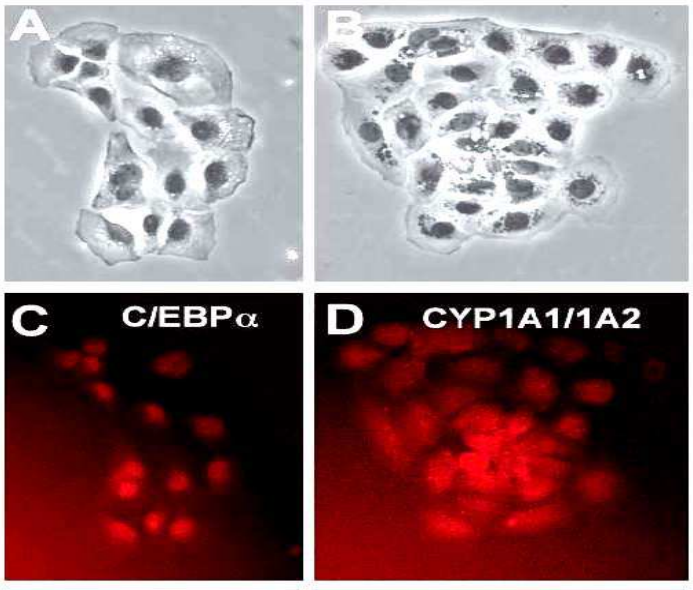
BD PuraMatrix Peptide Hydrogel: *Hepatocyte Progenitor Cells*



Hepatocyte progenitor cell colony formation and tissue-specific gene expression. All cells in spheroid colonies express markers of differentiation.

Data provided by 3DM, Inc. and originally described in **Semino, CE, et al. (2003) *Differentiation* 71:262-270.**

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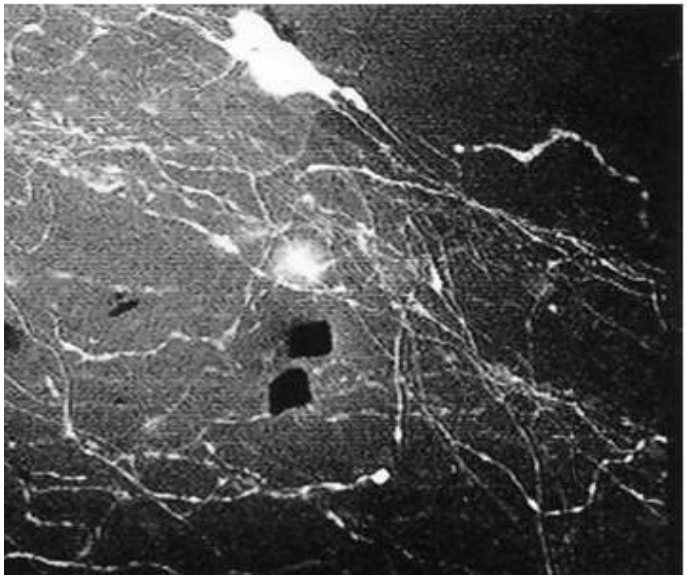
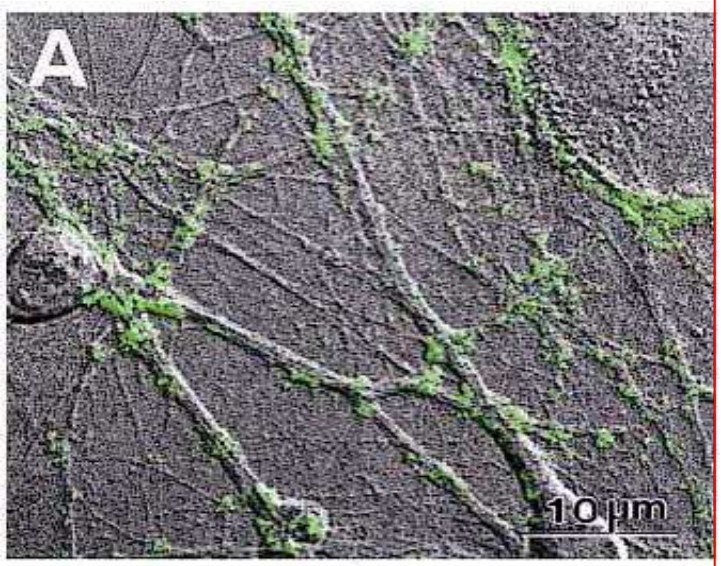
BD PuraMatrix Peptide Hydrogel: *Neuronal Cell Differentiation*



Rat PC12 cell neurite outgrowth. The image is a merged stack of multiple confocal optical sections.



Primary rat hippocampal neurons form active synapses. Confocal image of synaptically active neuronal membranes

Data provided by 3DM, Inc. and originally described in **Holmes, TC, et al. (2000) *PNAS USA* 97:6728-6733.**

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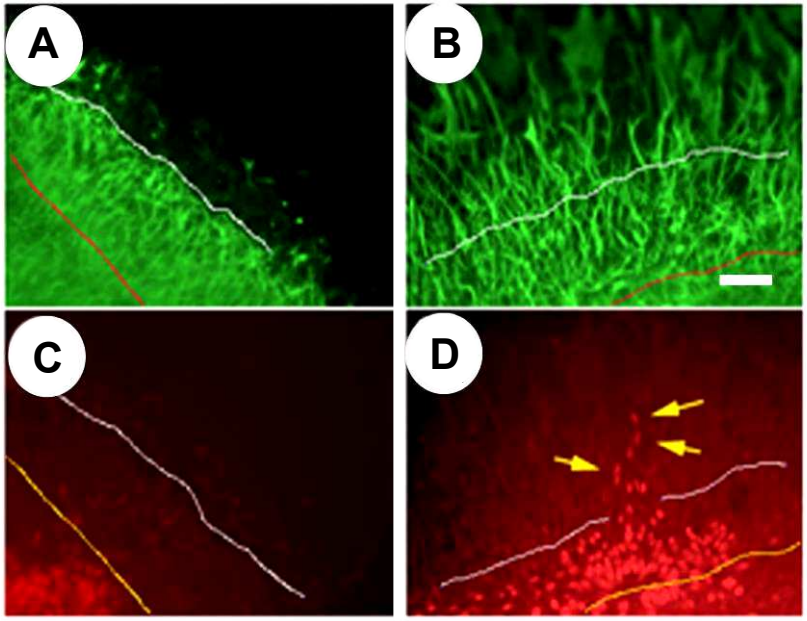
BD PuraMatrix Peptide Hydrogel: *Hippocampal Tissue Slices*



Control

BD PuraMatrix peptide hydrogel

- Hippocampal tissue slices from post-natal rats cultured on cell culture inserts
- Inserts uncoated controls (**A**,**C**) or coated with BD PuraMatrix peptide hydrogel (**B**,**D**)
- Glial (**B**) and neuronal (**D**) progenitor cell migration into the hydrogel layer, but not in control samples (**A**,**C**)

Data provided by 3DM, Inc. and originally described in **Semino, CE, et al. (2004) *Tissue Engineering* 10:643-655.**

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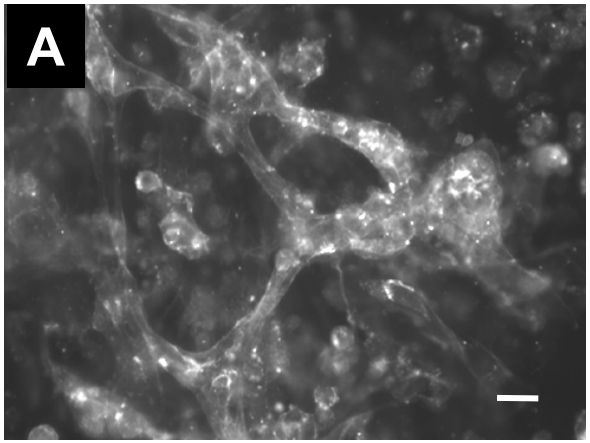
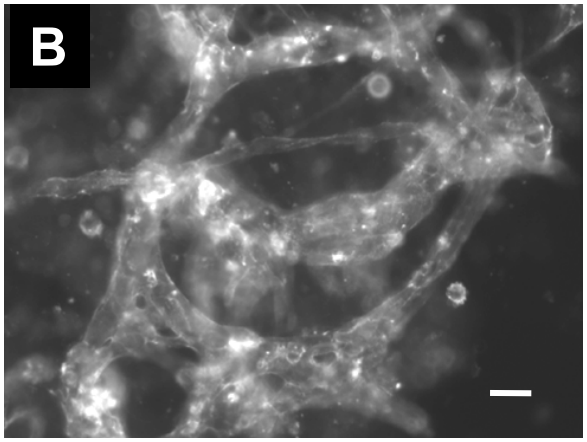
BD™ PuraMatrix™ Peptide Hydrogel: *Human Umbilical Vein Endothelial Cells*





- HUVECs encapsulated within BD PuraMatrix hydrogel elongate and form interconnected microvascular networks
- Cells cultured for 2 days in endothelial cell growth medium supplemented with 50 ng/ml VEGF and 50 ng/ml PMA. **(A)** 0.74 mg/ml BD PuraMatrix hydrogel; **(B)** 1.3 mg/ml BD PuraMatrix hydrogel

Data kindly provided by Dr. Alisha Sieminski

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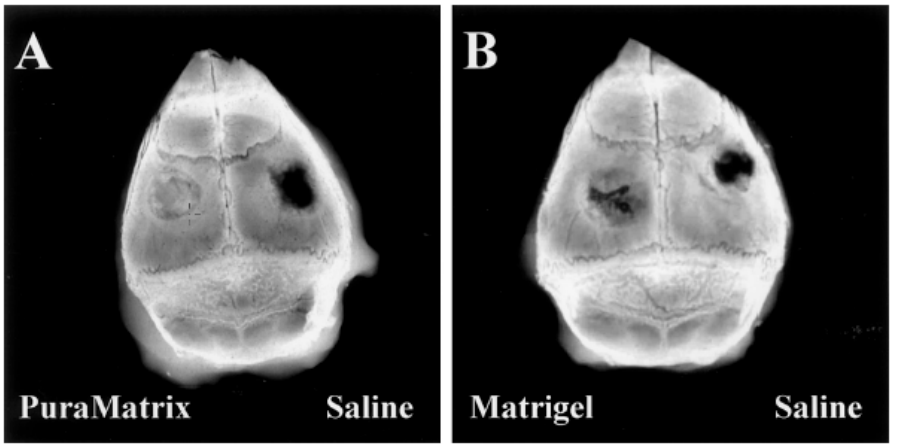
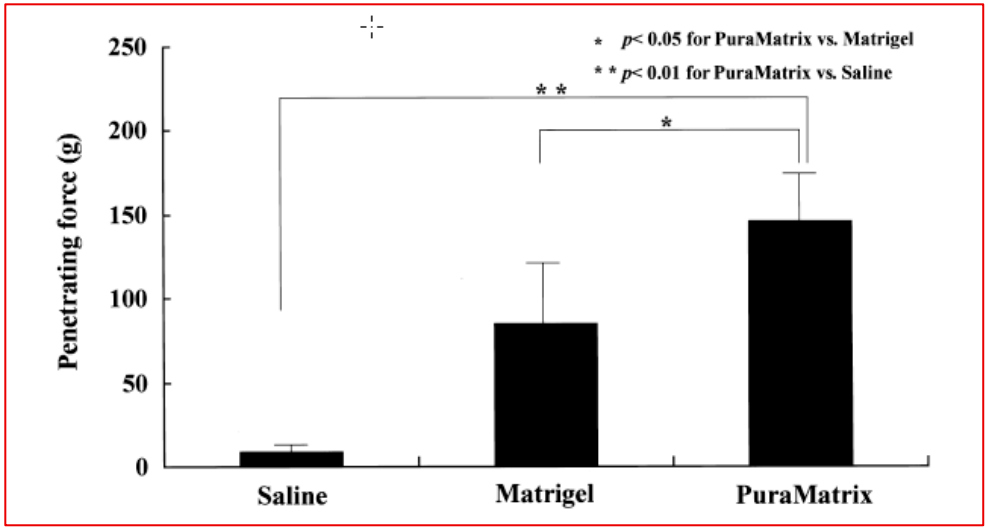
Repair of Bone Defect in Calvaria of SKID Mice





Misawa, H., et al. (2006) *Cell Transplantation* 15:903-910.

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Other Representative Applications

- Controlled release of functional proteins and cytokines
  - Koutsopoulos, S, et al. (2009) *PNAS* **106**:4623-4628. [**proteins**]
  - van Putten, SM, et al. (2009) *Biomaterials* **30**:730-735. [**IL-10**]
- 3D drop culture method for analysis of receptor-protein interactions
  - Yoshida, D and Teramoto, A (2007) *Cell Adhesion & Migration* **1**:92-98.
- Mesenchymal stem cell differentiation and bone/cartilage regeneration
  - Hamada, K, et al. (2008) *J Biomed Mater Res A* **84**:128-136. [**bone**]
  - Erickson, IE, et al. (2009) *Tissue Eng A* **15**:1041-1052. [**cartilage**]
- Disruption of prion accumulation and Scrapie disease process, resulting in extended animal survival
  - Hnasko, R and Bruederle, CE (2009) *PLoS ONE* **4**:e4440.

Summary

BD PuraMatrix peptide hydrogel provides a 'blank slate' scaffold for creating highly defined 3D culture environments

- Supports differentiation of a variety of primary and stem cell types
- Suitable for *in vivo* studies of tissue regeneration and repair
- *Other potential applications*: tumor cell biology, angiogenesis assays, co-culture, sandwich assays
- Easily mixed with cells and/or bioactive molecules prior to gelation
- Transparent; samples readily visualized with standard staining and microscopy
- Devoid of animal-derived material and pathogens
- Biocompatible

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References

Bone

1. Misawa, H., et al. (2006) PuraMatrix facilitates bone regeneration in bone defects of calvaria in mice. *Cell Transplant.* **15(10)**:903.
2. Garreta, E., et al. (2007) Fabrication of a three-dimensional nanostructured biomaterial for tissue engineering of bone. *Biomol Eng.* **24(1)**:75.

Cartilage

1. Tokunou, T., et al. (2008) Engineering insulin-like growth factor-1 for local delivery. *FASEB J.* **22(6)**:1886.
2. Yamaoka, H., et al. (2006) Cartilage tissue engineering using human auricular chondrocytes embedded in different hydrogel materials. *J. Biomed. Mater Res A.* **78(1)**:1.

Cardiac/Endothelial

1. Sieminski, AL, et al. (2008) Primary sequence of ionic self-assembling peptide gels affects endothelial cell adhesion and capillary morphogenesis. *J Biomed Mater Res A* **87**:494.
2. Hsieh, P.C., et al. (2006) Controlled delivery of PDGF-BB for myocardial protection using injectable self-assembling peptide nanofibers. *J Clin Invest.* **116(1)**:237.
3. Narmoneva, D.A., et al. (2005) Self-assembling short oligopeptides and the promotion of angiogenesis *Biomaterials* **26**:4837.
4. Davis, M.E., et al. (2005) Injectable self-assembling peptide nanofibers create intramyocardial microenvironments for endothelial cells. *Circulation* **111**:442.

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References

Hepatocytes

1. Wang, S., et al. (2008) Three-dimensional primary hepatocyte culture in synthetic self-assembling peptide hydrogel. *Tissue Engineering Part A* **14(2)**:227.
2. Navarro-Alvarez, N., et al. (2006) Self-assembling peptide nanofiber as a novel culture system for isolated porcine hepatocytes. *Cell Transplant.* **15(10)**:921.
3. Semino, C.E., et al. (2003) Functional differentiation of hepatocyte-like spheroid structures from putative liver progenitor cells in three dimensional peptide scaffolds. *Differentiation* **71(4-5)**:262.

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