CORNING

Life Sciences

Novel ECM Mimetic Surfaces for Cell Expansion and Differentiation

ASCB

December 17, 2012

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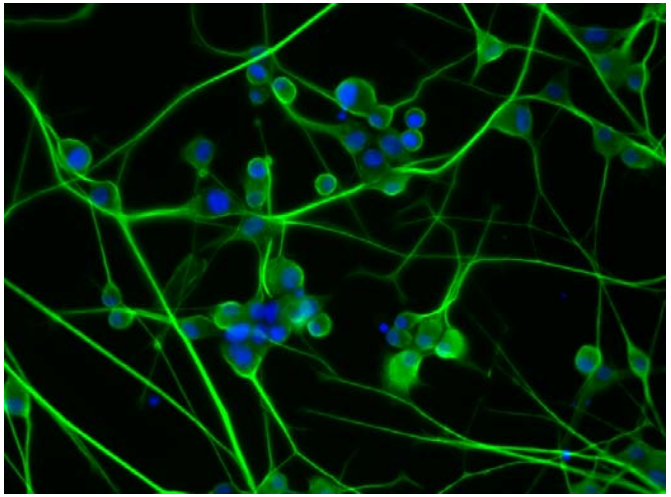
Technical Support Manager

Topics for Discussion

- Introduction
- Overview of Cell Culture Surfaces:
  Falcon®, Primaria®, BioCoat®
- PureCoat™ ECM Mimetic Surfaces
  - Chemically-defined, animal-free peptides
  - Key applications:
    - Mesenchymal stem cell expansion
    - Endothelial progenitor cell expansion and differentiation
    - Primary keratinocyte expansion and differentiation

Key Factors that Influence Cell Behavior

- Cell Source (tissue/organ type)
  - Initial growth conditions
- Cell characterization
  - Age, karyotype
- Medium Composition
- Growth Substrate (chemical groups vs. ECM; 2D vs. 3D)
  - Cell morphology
  - Functionality



PC-12 neurite outgrowth in the presence of NFG (200 ng/ml). Image captured using the **BD Pathway™ 855**

Cell Behaviors Influenced by Cues in the Microenvironment

- Cell morphology (structure, polarity, **phenotype**)
- Growth (proliferation)
- Cell motility (migration, invasion)
- Neurite outgrowth
- Signal transduction (receptor function)
- Gene and protein expression (different cell types can express different genes/proteins; liver vs. heart vs. brain)
- Biochemical activities (proteins, enzymes)

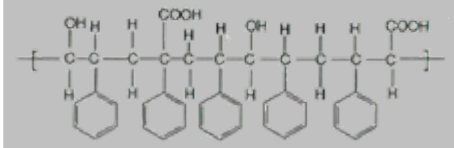
Surfaces for Culturing Adherent Cells

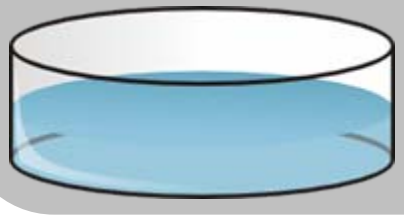
- Falcon® Tissue Culture (TC) treated (net [-] charge)
  - Primaria® (mix of [+] and [-] charge)
    - BioCoat® (variety of ECMs, poly-lysine)
      - PureCoat™ Surfaces
        - Amine [+], Carboxyl [-] (serum, low serum, SF)
        - ECM Mimetic Surfaces (SF, **XF, AF**)

PureCoat™ ECM Mimetics: *Enhanced Surfaces for Cell Expansion & Differentiation*

Traditional Tissue Culture

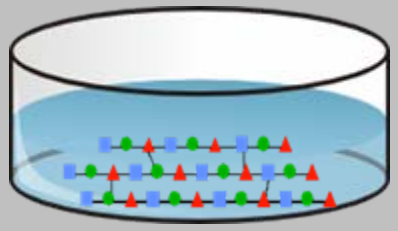
- Vacuum-Gas Plasma Treatment
- Hydrophilic





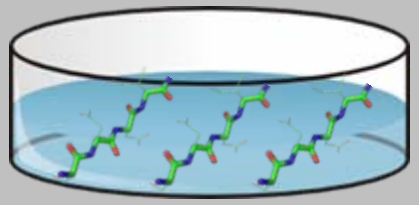
PureCoat™ (2009)

- Thin-Film Polymer Coating
- Chemically Defined; **AF**
- Surface Coatings:
  - **Amine (N_2) [+]**
  - **Carboxyl (O_2) [-]**
- Culture of primary, stem, transfected, transformed cells
- Serum, serum-reduced, SF



PureCoat™ ECM Mimetics

- Synthetic Peptide Coating; **AF**
- Cell adhesion promoting peptides
- Functional surface mimics native protein environment



- **Fibronectin Mimetic surface**
  - MSCs, endothelial progenitor cells
- **Collagen I Mimetic surface**
  - keratinocytes, epithelial cells
- SF, **XF, AF** media conditions

Biological Functionality

Serum Requirements

PureCoat™ ECM Mimetic Cultureware
Rationale

1. Functional peptides derived from full-length ECM proteins have been shown to interact with integrin receptors and influence cell behavior (*e.g., regulation of cell growth and differentiation*)

2. Cultureware surfaces with conjugated peptides derived from Fibronectin and Collagen I provide functionalized surfaces suitable for culturing a variety of cell types that exhibit FN/Col I dependent attachment, growth, and functionality

3. Utilizing such peptides, PureCoat ECM Mimetic cultureware provides synthetic, chemically defined, and 'animal-free' surfaces that support the expansion (differentiation) of cells with clinical potential (stem cells, primary cells)

Properties of Fibronectin

- Large dimeric protein (multiple isoforms)
- Contributes to matrix organization
- Cell receptors (integrins) bind to FN 'RGD motif'
- Promotes cell differentiation and functionality (e.g., cell migration, integrin signaling, gene expression)

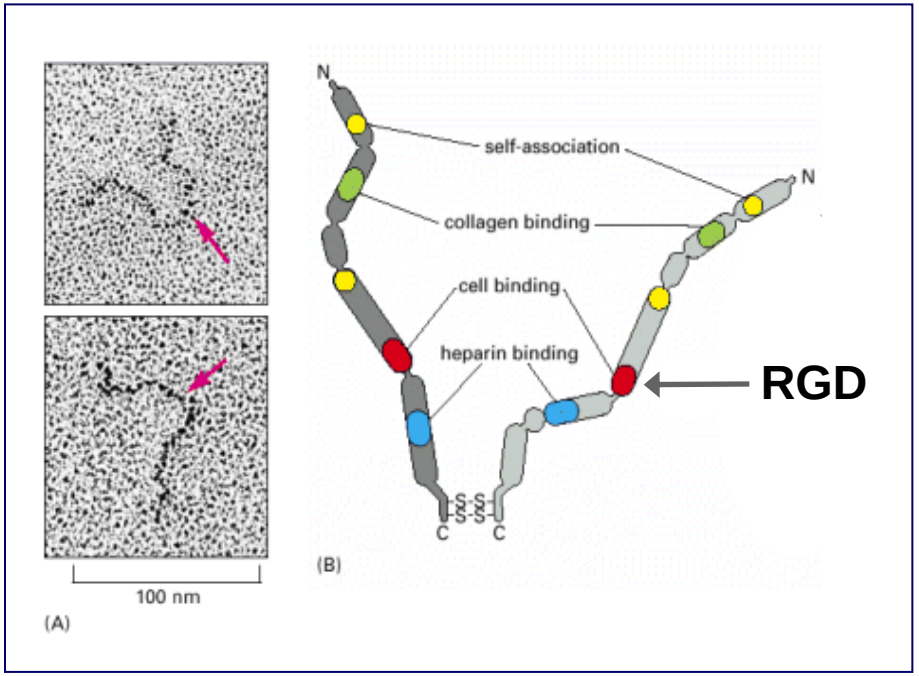


Figure: Adapted from *Molecular Biology of the Cell (4th Edition)*

PureCoat™ ECM Mimetic Cultureware
Fibronectin Peptide

- Chemically defined, animal-free surface
- Properties of fibronectin-derived peptide:
  - ➢ Rationally designed to mimic cell attachment regions present in full-length (native) fibronectin protein
  - ➢ Contains **RGD** amino acid sequence (motif), which supports attachment of cells expressing the **α5 integrin** subunit

    R = Arginine
    G = Glycine
    D = Aspartate

  - ➢ Peptide is covalently immobilized on a proprietary surface, and presented in a functionally active orientation

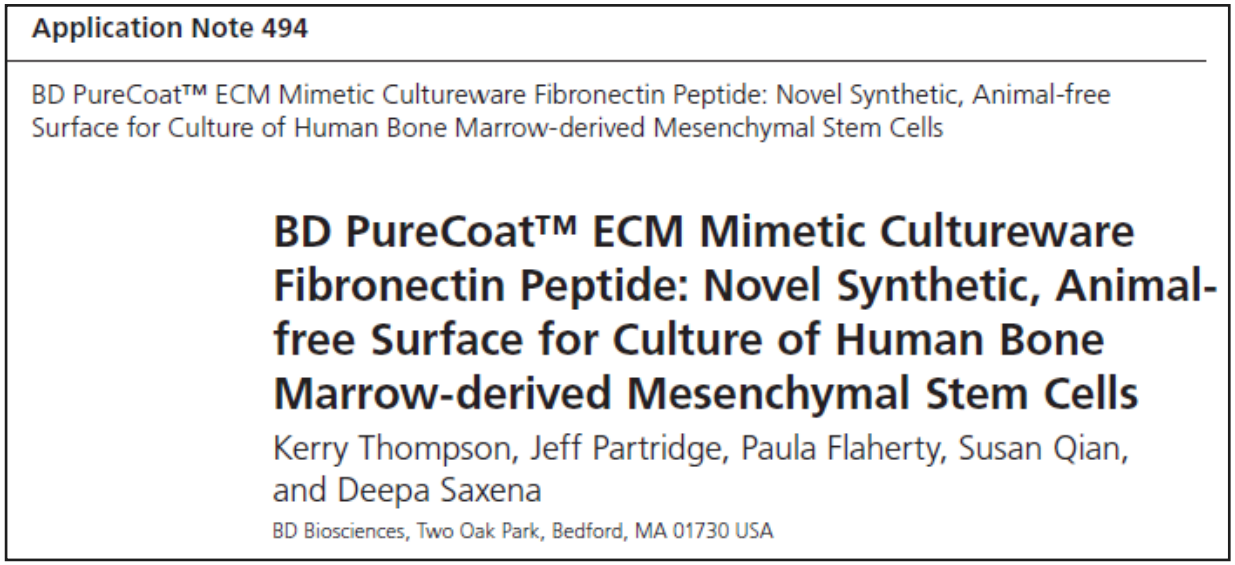
PureCoat™ ECM Mimetic Cultureware
Fibronectin Peptide

Key cell types and applications

- Human mesenchymal stem cells (MSCs)
  - ➢ cell attachment and expansion under xeno-free conditions
  - ➢ MSCs retain multipotent differentiation potential
- Endothelial colony forming cells (ECFCs)
  - ➢ increased population doublings compared to native protein-coated surface (low serum)
  - ➢ cell differentiation

PureCoat™ ECM Mimetic Cultureware Fibronectin Peptide

Human Mesenchymal Stem Cells



Application Note 494

BD PureCoat™ ECM Mimetic Cultureware Fibronectin Peptide: Novel Synthetic, Animal-free Surface for Culture of Human Bone Marrow-derived Mesenchymal Stem Cells

BD PureCoat™ ECM Mimetic Cultureware Fibronectin Peptide: Novel Synthetic, Animal-free Surface for Culture of Human Bone Marrow-derived Mesenchymal Stem Cells

Kerry Thompson, Jeff Partridge, Paula Flaherty, Susan Qian, and Deepa Saxena

BD Biosciences, Two Oak Park, Bedford, MA 01730 USA

Mesenchymal Stem (Stromal) Cells

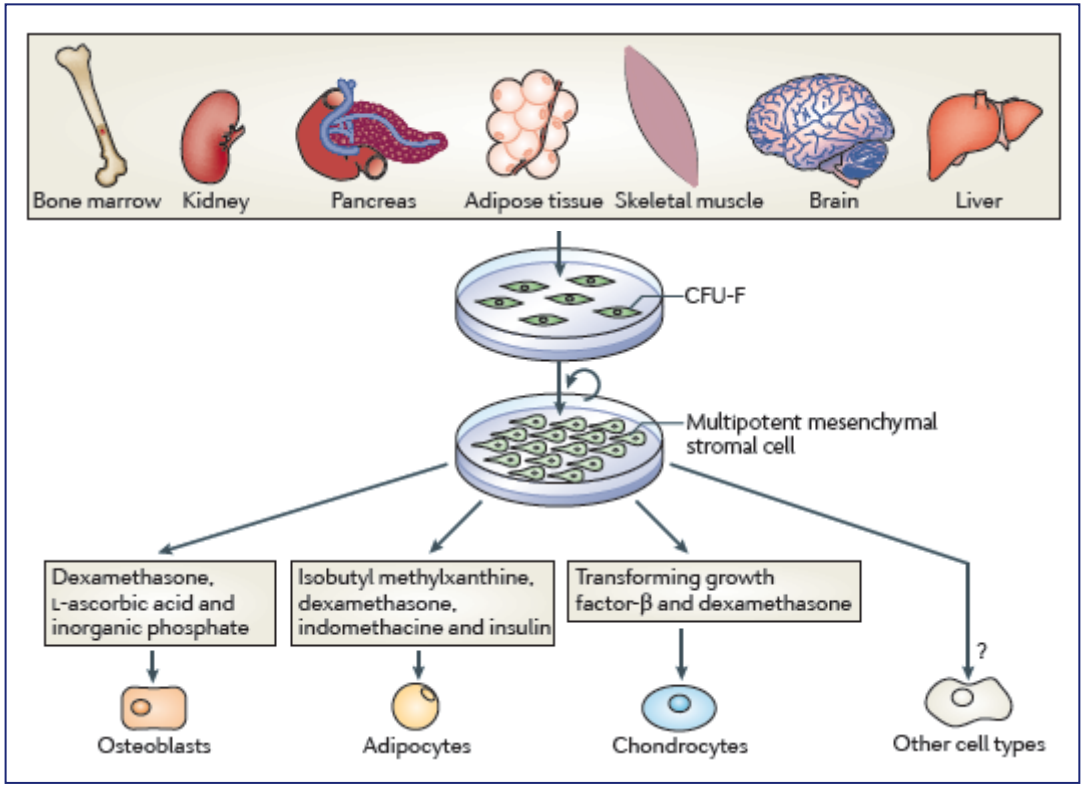


Figure adapted from Nombela-Arrieta *et al.* (2011) Nature Reviews Molecular Cell Biology 12:126

- MSCs are multipotent stem cells and have the ability to self-renew
- MSCs can differentiate into adipocytes [fat], osteocytes [bone] and chondrocytes [cartilage]
- MSCs have been shown to regulate immune cells (immunosuppressive)
- Clinical significance in a range of disease areas

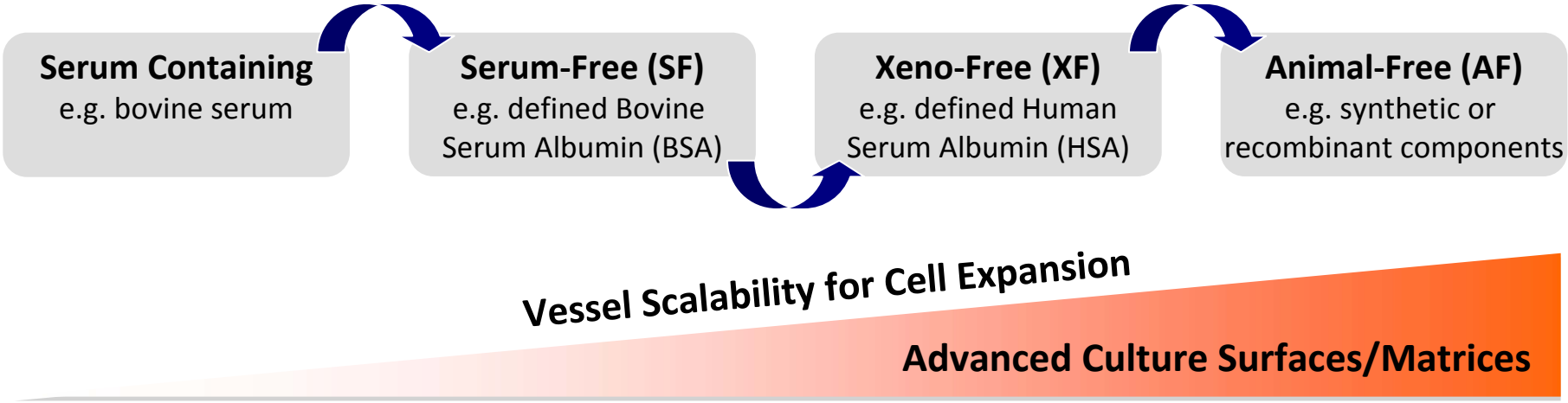
Mesenchymal Stem (Stromal) Cells
Representative Clinical Trials

- Cardiovasular diseases (e.g., myocardial ischemia, heart failure)
- Neurological disorders (e.g., stroke, spinal cord injury)
- Diabetes
- Hepatic cirrhosis
- Bone/cartilage defects, bone fusion
- Skin disease (e.g., diabetic wound)
- Autoimmune diseases (e.g., sclerosis)

Review Article: Si, Y-L, et al. (2011) Ageing Research Reviews, Vol 10, pp. 93-103.

MSC Expansion – Technical Needs

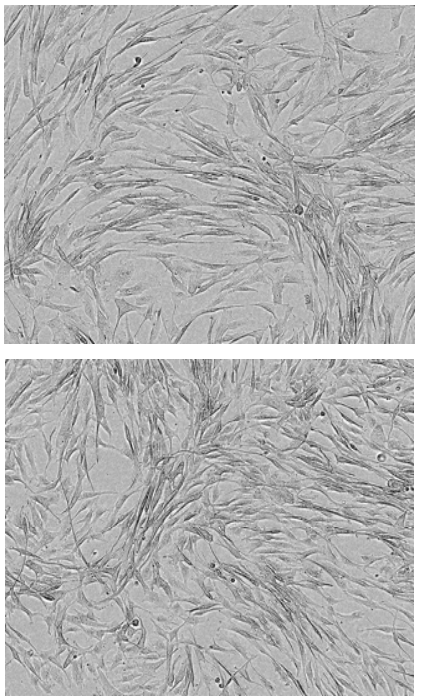
- Scalable, reproducible cell culture platforms for large-scale expansion through multiple passages
- Maintenance of the MSC phenotype (surface marker expression) and differentiation capability
- Consistency of quality/performance/cell yield
- Availability of chemically defined systems (vessel surface, medium)



PureCoat™ ECM Mimetic Cultureware Fibronectin Peptide Supports the Attachment and Growth of Human MSCs Under Xeno-Free Conditions

Protocol:

1. Human MSCs (Lonza) were seeded onto 6 well plates at 4000 cells/cm^2 (2 mls/well)
2. Cells were cultured in **MesenCult-XF Medium** (STEMCELL Tech)
3. Plates incubated at 37^{o}C
4. Cell were passaged at 60-70% confluence
5. At passages 1-5, population doubling determined (based on cell yield) and images captured.



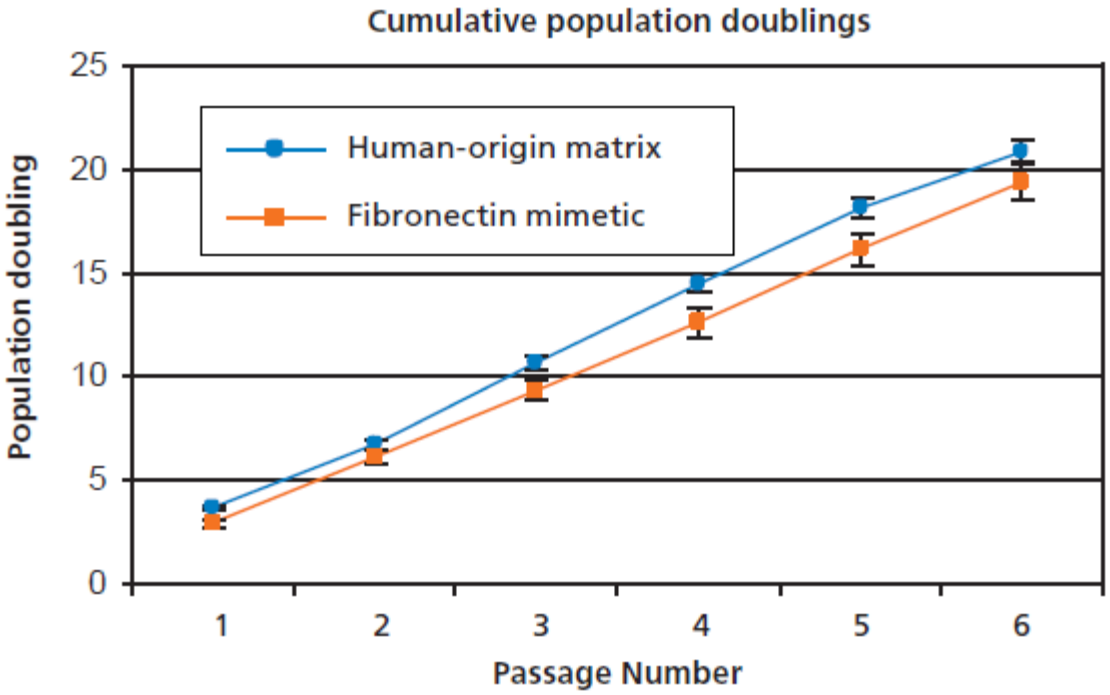
Fibronectin Mimetic

Human-Origin Matrix

P3 P5

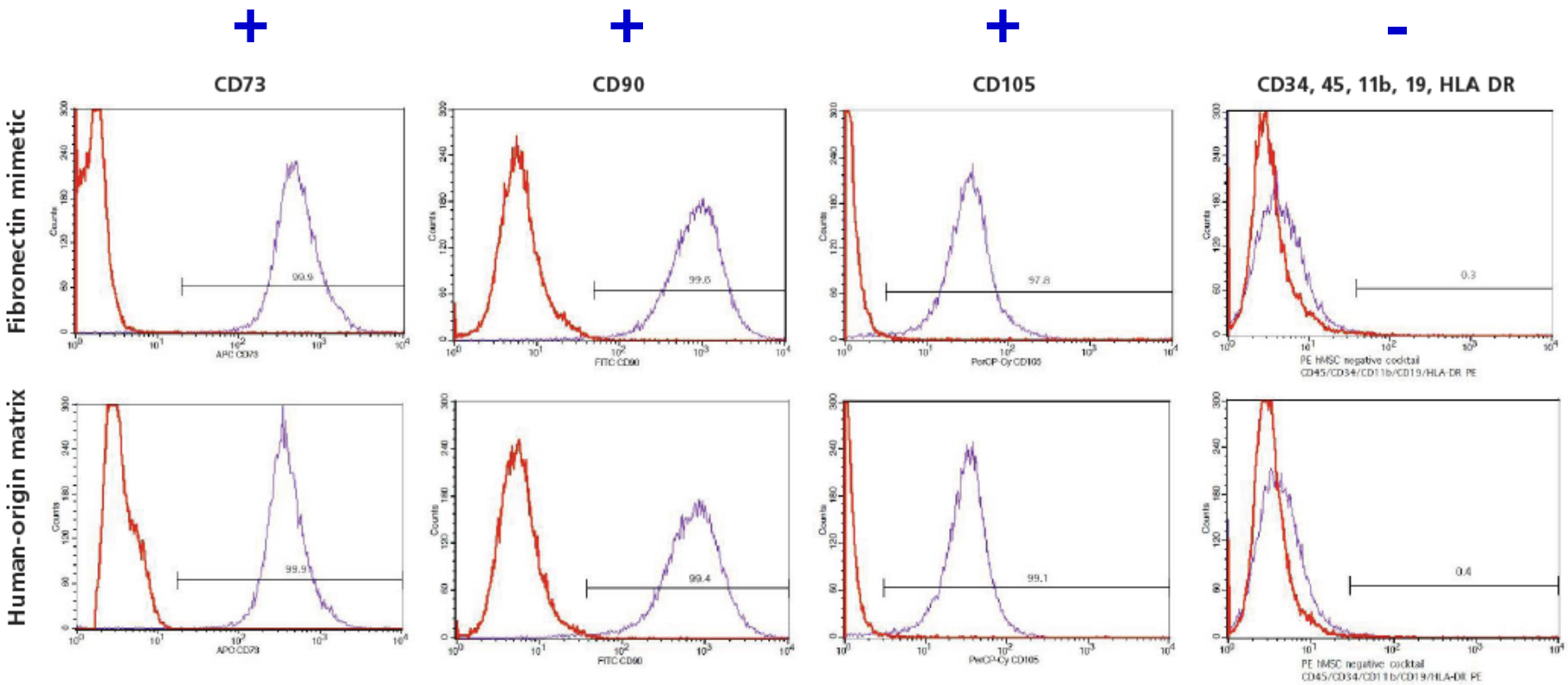
Human MSCs exhibit comparable growth properties when cultured on the fibronectin mimetic and human-origin matrix under xeno-free conditions.

PureCoat™ ECM Mimetic Cultureware Fibronectin Peptide Supports the Attachment and Growth of Human MSCs Under Xeno-Free Conditions



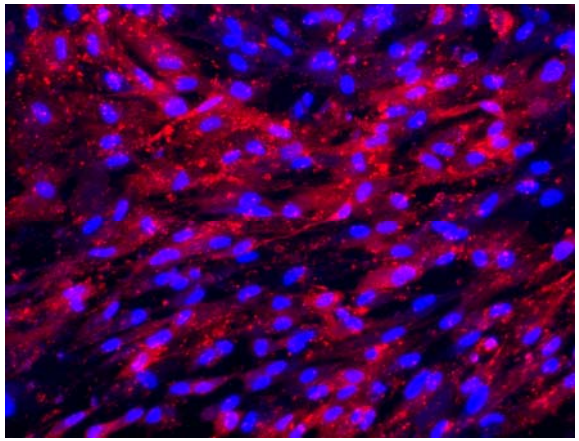
Human MSCs exhibit comparable population doublings when cultured for 6 passages on the fibronectin mimetic and human-origin matrix under xeno-free conditions.

PureCoat™ ECM Mimetic Cultureware Fibronectin Peptide Supports the Expression of Human MSC Surface Markers

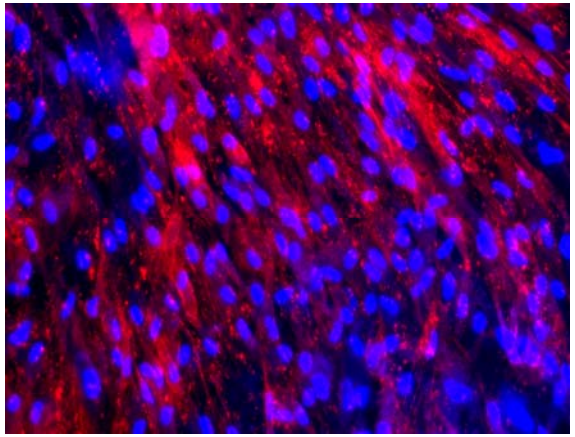


Human MSCs exhibit characteristic surface markers when cultured for 3 passages on the fibronectin mimetic and human-origin matrix under xeno-free conditions.

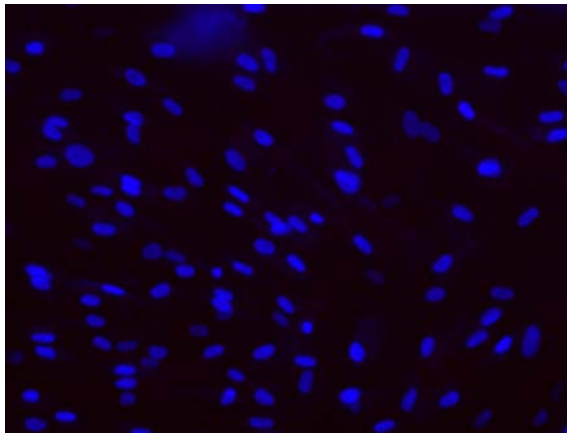
Human MSCs Cultured on PureCoat™ ECM Mimetic Cultureware Fibronectin Peptide Retain Capacity for Osteogenic Differentiation



Fibronectin Mimetic



Human-Origin Matrix



Uninduced Control

Protocol:

1. Human MSCs were cultured on the FN mimetic or human-origin matrix for 3 passages.
2. Cells were then cultured on BioCoat® Collagen I 24 well plate for 24 hours [3.1 x 10^3 cells/cm^2 in 0.5 ml **MSCGM medium** (Lonza)]
3. The medium was then replaced with **Osteogenic Induction Medium** (Lonza), and MSCs were induced for 2-3 weeks (fed every 3-4 days). Uninduced controls were cultured in MSCGM medium.
4. Cells were fixed and stained for alkaline phosphatase activity (red), and counterstained with DAPI (nuclear dye, blue)

Properties of Collagen

- Most ubiquitous ECM molecules (at least 16 types)
  - ➢ subunits of collagen 'trimer' encoded by multiple genes
- Fibrous proteins that provide structure and resiliency to tissues
- Major component of skin and bone
- Most abundant protein in mammals (~ 25% of total protein mass)
- Interact with $\alpha 1\beta 1$, $\alpha 2\beta 1$ integrins and promote signaling

	Type	Tissue Distribution
Fibrillar (polymerized fibrils)	**I, V**	**bone, skin, tendon, cornea, internal organs**
	II	**cartilage, notochord**
	III	**skin, muscle, blood vessels**
Network-Forming	**IV**	**all basal laminaes**

PureCoat™ ECM Mimetic Cultureware
Collagen I Peptide

- Chemically defined, animal-free surface
- Properties of collagen type I-derived peptide:
  - ➢ Rationally designed to mimic cell attachment regions present in full-length (native) collagen I protein
  - ➢ Contains **GFOGER** amino acid sequence (motif), which supports attachment of cells expressing the **α2 integrin** subunit

    G = Glycine
    F = Phenylalanine
    O = Hydroxyproline
    G = Glycine
    E = Glutamate
    R = Arginine

  - ➢ Peptide is covalently immobilized on a proprietary surface, and presented in a functionally active orientation

PureCoat™ ECM Mimetic Cultureware
Collagen I Peptide

Key Cell Types and Applications

- Human endothelial colony forming cells (ECFCs)
  - ➢ increased population doublings compared to native protein-coated surface
  - ➢ cell differentiation
- Human keratinocytes
  - ➢ cell attachment, growth, & functionality (migration)

PureCoat™ ECM Mimetic Cultureware Fibronectin and Collagen I Peptides

Human Endothelial Progenitor Cells

Application Note 492

BD PureCoat™ ECM Mimetic Cultureware: Novel Synthetic, Animal-free Surfaces for Human Endothelial Colony Forming Cell Expansion

BD PureCoat™ ECM Mimetic Cultureware: Novel Synthetic, Animal-Free Surfaces for Human Endothelial Colony Forming Cell Expansion

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Endothelial Progenitor Cells
Clinical Relevance

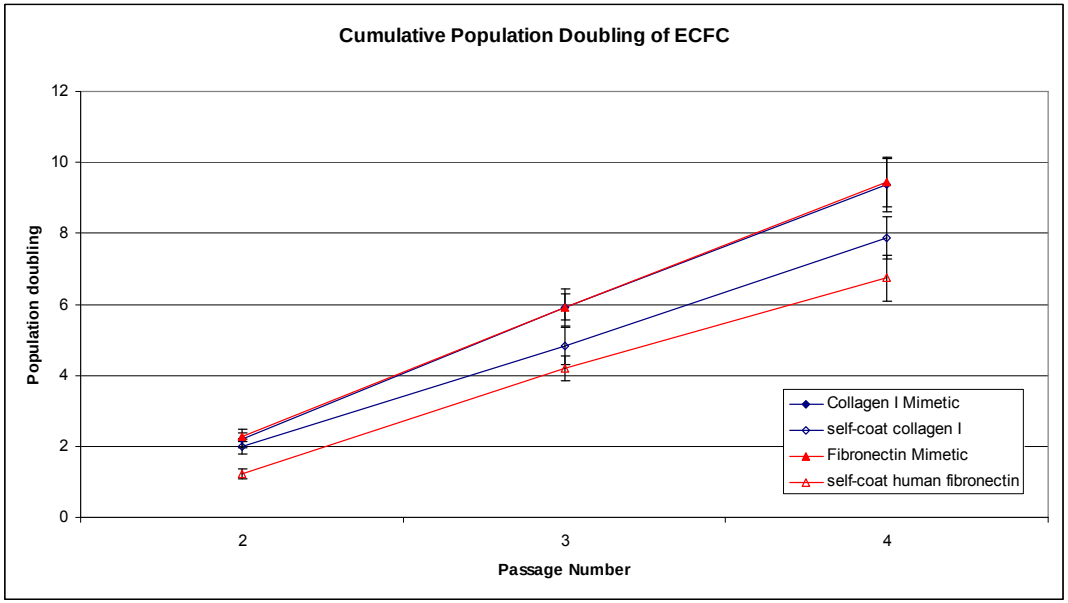
Clinical application areas currently under investigation:

- regenerate/repair blood vessels (angiogenesis)
- expansion and generation of differentiated endothelial cells for tissue engineering applications (e.g., blood vessel engineering and implantation)
- bone fracture healing (combination of angiogenesis and osteogenesis)

PureCoat™ ECM Mimetic Cultureware Fibronectin and Collagen I Peptides

Protocol:

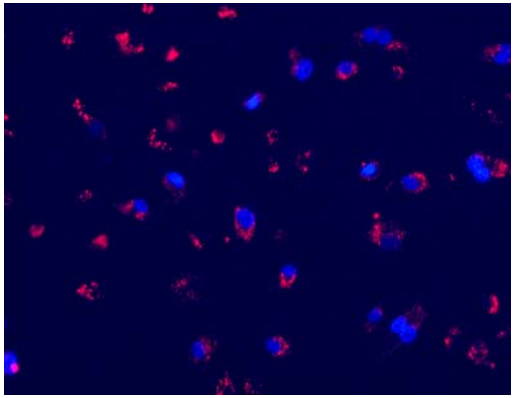
1. Human ECFCs (Lonza) were seeded onto 6 well plates at 100K cells/well
2. Cells were cultured using **EGM-2 BulletKit** containing 2% FBS (Lonza)
3. Plates incubated at 37°C
4. Cell were passaged every 2-3 days
5. At passages 2-4, population doubling was determined (based on cell yield).

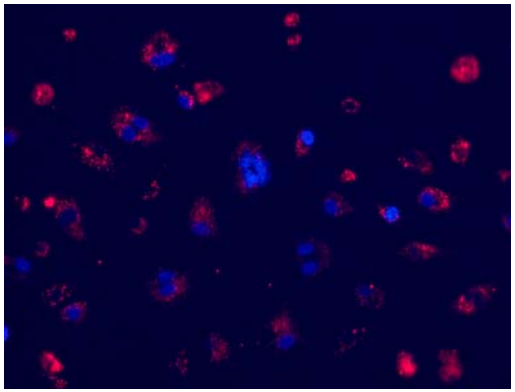


Human ECFCs exhibit a comparable growth rate when cultured for 2-4 passages on the FN and Col I Mimetic surfaces compared to self-coated FN and col I under low-serum conditions.

Human ECFCs cultured on PureCoat™ ECM Mimetic Cultureware Fibronectin and Collagen I Peptides Exhibit Properties of Differentiated Endothelial Cells

Uptake of Acetylated-LDL

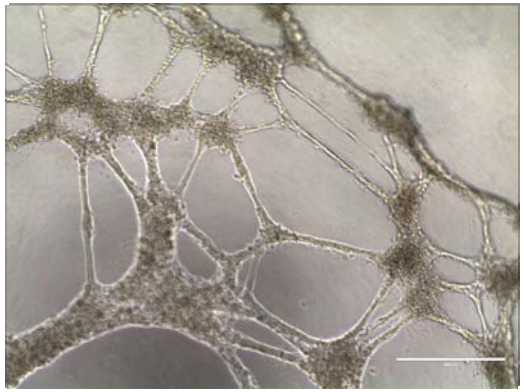


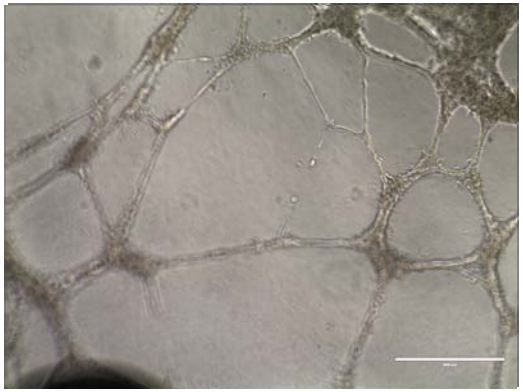


FN Mimetic

Col I Mimetic

Tubulogenesis





Cells stained with 10-20 μg/ml **DiI Ac-LDL** (Life Tech) for 4 hrs at 37ºC, and then counterstained with DAPI. Images captured with Olympus IX70 Fluorescence Microscope.

Cells plated on *BioCoat® Angiogenesis System: Endo Tube Formation* at 20-80K cells/well overnight at 37ºC. Images captured with Olympus IMT-2 Fluorescence Microscope.

PureCoat™ ECM Mimetic Cultureware Collagen I Peptide

Human Neonatal Keratinocytes

Application Note 493

BD PureCoat™ ECM Mimetic Cultureware Collagen I Peptide: Novel Synthetic, Animal-free Surface for Culture of Human Keratinocytes

BD PureCoat™ ECM Mimetic Cultureware Collagen I Peptide: Novel Synthetic, Animal-free Surface for Culture of Human Keratinocytes

Kerry Thompson, Jeff Partridge, Elizabeth Abraham, Paula Flaherty, Susan Qian, and Deepa Saxena

BD Biosciences, Two Oak Park, Bedford, MA 01730 USA

Human Keratinocytes
Clinical Relevance

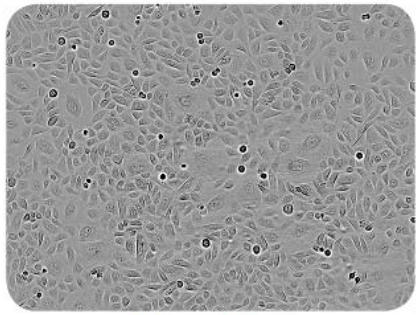
Representative clinical trials (NIH, *clinicaltrials.gov*):

- wound healing (skin grafts)
- wounds associated with Epidermolysis Bullosa (skin blisters)
- repair/regeneration of acute cutaneous wounds
- treatment of ulcers & burns (grafting)

PureCoat™ ECM Mimetic Cultureware Fibronectin Peptide Supports the Attachment and Growth of Human Keratinocytes Under Animal-Free Conditions

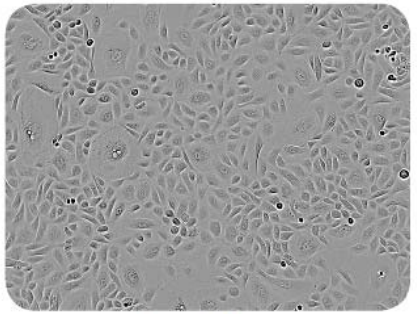
Protocol:

1. Human keratinocytes (Invitrogen) were seeded onto 6 well plates at 2500 cells/cm^2 (2 mls/well)
2. Cells were cultured in **EpiLife AOF Medium** with S7 supplements (Invitrogen)
3. Plates incubated at 37^{o}C
4. Cell were passaged at 60-70% confluence
5. At passages 1-4, population doubling determined (based on cell yield) and images captured.



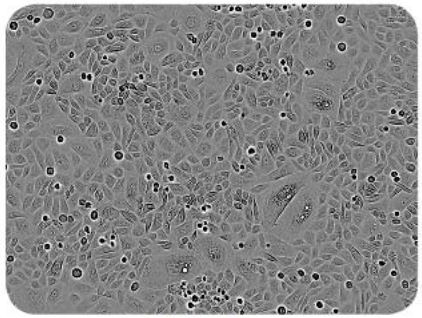
Collagen I Mimetic

AF



Coating Matrix

AF

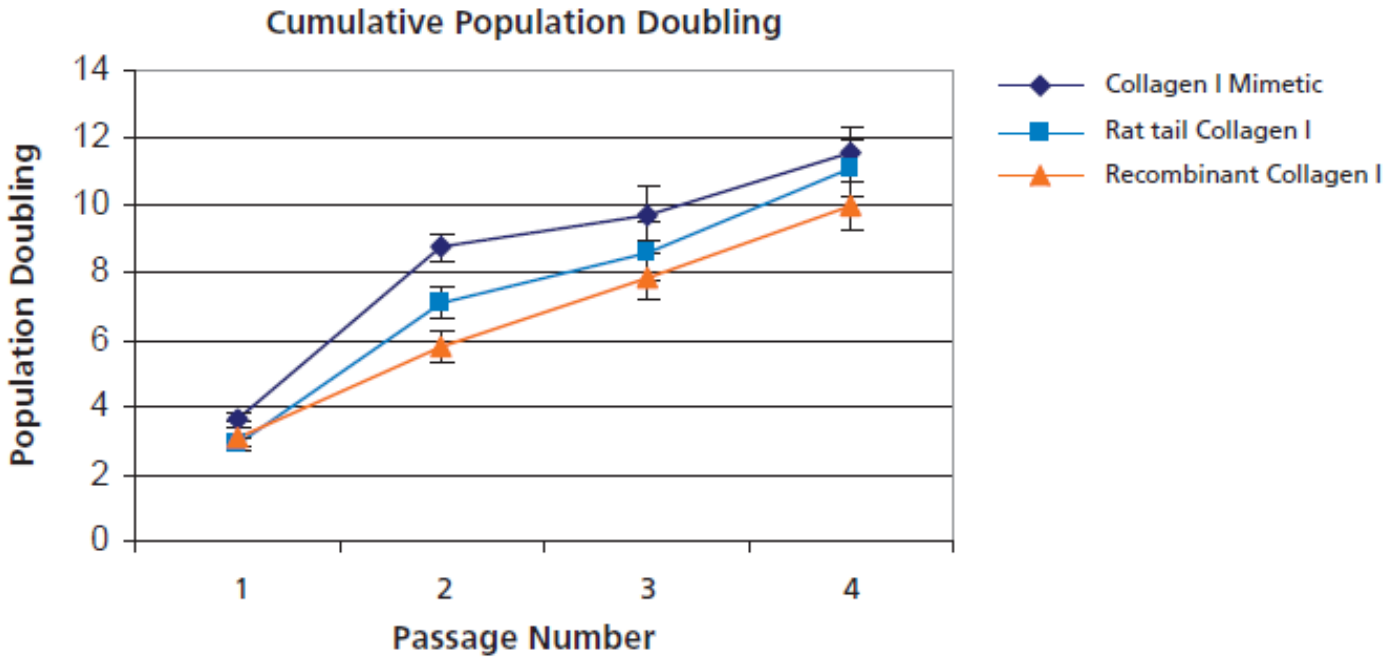


Rat tail Collagen I

SF

Human Keratinocytes exhibit comparable attachment and growth when cultured on the Col I Mimetic and Human Recombinant Col I (Coating Matrix) under AF conditions, and Rat Tail Col I under SF conditions.

PureCoat™ ECM Mimetic Cultureware Fibronectin Peptide Supports the Attachment and Growth of Human Keratinocytes Under Animal-Free Conditions

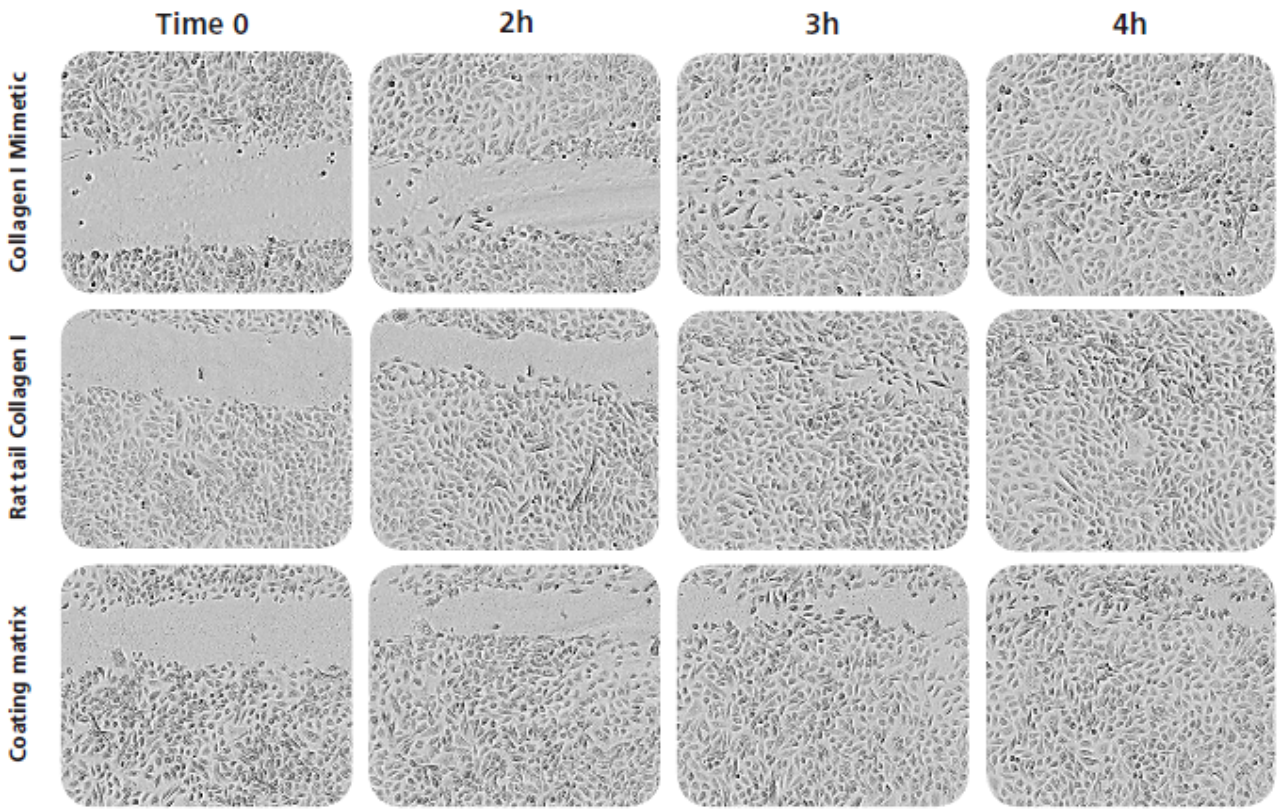


Human Keratinocytes exhibit comparable population doublings when cultured for 4 passages on the Col I Mimetic, Rat Tail Col I, and Human Recombinant Col I.

Human Keratinocytes Cultured on PureCoat™ ECM Mimetic Cultureware Collagen I Peptide Exhibit Migratory Function

Wound Healing Assay:

1. Culture cells for 3 passages
2. Treat cells with **4 µg/ml mitomycin C** for 2 hrs to establish growth arrest
3. Wash with DPBS
4. Create 'wound' by scratching across well with pipet tip
5. Add culture medium and incubate at 37ºC for 4 hours
6. Capture images at hourly intervals using the IncuCyte instrument

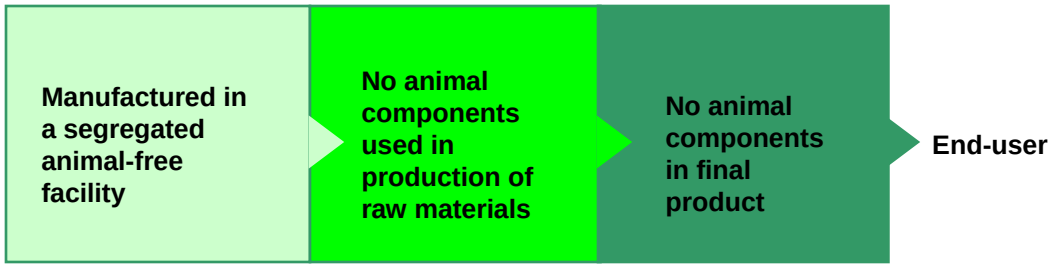


HKN migration #1 4 per sec 1136-536_A4_2.wmv

PureCoat™ ECM Mimetic Cultureware

Manufacturing & Quality

- PureCoat ECM Mimetic Cultureware Collagen I and Fibronectin Peptides are produced at an ISO 13485:2003, ISO 9001:2000 and ISO 14001:2004 certified manufacturing facility.
- Manufactured in a GMP compliant facility under the requirements of FDA part 21CFR820
- ECM Mimetic Cultureware is certified animal-free, manufactured in an animal-free segregated facility



PureCoat™ ECM Mimetic Cultureware

Manufacturing & Quality

- **Quality control confirms the following:**
  - Non-pyrogenic
  - Non-cytotoxic
  - Functional activity
  - Sterility (SAL 10^{-3})

- **Regulatory documentation available**
  - Certificate of Compliance
    - Contains AF statement, QC and manufacturing information
  - Certificate of Analysis
    - Lot-specific QC results

Summary

- **PureCoat™ ECM Mimetic Cultureware Fibronectin Peptide** surface supports xeno-free (**XF**) expansion of human MSCs, and maintains capacity for cell differentiation:
  - MesenCult™-XF Medium (STEMCELL Tech.) [Application Note 494]
  - StemPro MSC SFM Xeno-Free Medium (Life Tech.) [Application Note 496]

- Human endothelial progenitor cells exhibit an enhanced growth rate and maintain differentiation potential when cultured on the **Fibronectin and Collagen I Mimetic surfaces** under low serum conditions [Application Note 492]

- Primary human keratinocytes exhibit cell attachment, growth, and functionality (cell migration) when cultured on the **Collagen I Mimetic surface** under animal-free (**AF**) conditions [Application Note 493]

Contact Information

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or

Technical Support

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