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

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Application Note

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Introduction

Keratinocytes are the most common cell type of the skin constituting ~95% of the cells. The most important application of keratinocytes is protection of the body by forming a barrier against pathogens and environmental factors such as UV rays[1]. These cells are very important in creating epithelial sheets for skin grafting on patients suffering with wounds and burns[2,3]. Keratinocyte accessibility, proliferation potential and ease of culture has enabled use of these cells in regenerative medicine. *Ex vivo* expansion of keratinocytes requires either coating of the culture vessel with human or animal-derived extracellular matrix protein or a growth medium with bovine serum or animal-derived components. Growing concerns about introducing human and animal-derived pathogens into the culture system necessitate the need for an animal-free (xeno-free and human origin components-free) culture system environment. Also, media components and coating matrices of biological origin may have batch to batch variability and can be undefined. Self-coating requires additional time resulting in coated vessels having a limited shelf-life.

Here, we report Corning PureCoat ECM Mimetic Cultureware Collagen I Peptide for culture of cell types that require collagen I coating. Corning PureCoat ECM Mimetic Cultureware collagen I peptide is a pre-coated, synthetic, xeno-free, animal-free, and room temperature stable product. Collagen I peptide is covalently immobilized on a surface so that it is presented in functionally active orientation to the cells. The peptide consists of GFOGER amino acid sequence that facilitates attachment of α2 integrin positive cells on the surface[4].

In this study human neonatal keratinocyte (HKN) cells were cultured on the collagen I mimetic surface for multiple passages in defined and xeno-free medium. Cell growth and morphology on the collagen mimetic surface were comparable to cells grown on a native Collagen I-coated surface. Cell functionality was tested following multiple passages using an *in vitro* wound healing model. Keratinocytes were division-arrested and a scratch was made on the monolayer. Cells migrated to the scratched region, healing the wound. The collagen I mimetic surface also supported attachment of HKN in serum-free medium. The Corning PureCoat ECM Mimetic Cultureware Collagen I Peptide provides a ready to use alternative to native Collagen I coating for cell culture with comparable cell attachment and functionality.

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Materials and Methods

Reagents

Human neonatal keratinocytes (Cat. No. C-001-5C), culture media, and dissociation reagents were purchased from Invitrogen. Cells were cultured either in a defined and xeno-free culture medium comprised of Epilife® medium (Cat. No. MEPI500CA) supplemented with S7 supplements (Cat. No. S0175) or in a serum-free medium, i.e., Epilife with EDGS supplements (Cat. No. S-012-5). Corning PureCoat ECM Mimetic Cultureware Collagen I Peptide 6-well plate (Corning Cat. No. 356270) was used for expansion of cells. Recombinant Collagen coating matrix (CM Invitrogen, Cat. No. R011K) was coated on Falcon® 6-well cell culture plates (Corning Cat. No. 353224). Rat tail Collagen I-coated vessels (Corning Cat. No. 354236) were also included in the experiment for comparative studies with the Collagen I mimetic vessel. Cells were passaged by enzymatic dissociation using Trypsin/EDTA solution (Cat. No. R-001-100), and Trypsin was neutralized by adding Trypsin inhibitor (Cat. No. R-007-100).

HKN Culture

Corning PureCoat ECM Mimetic Cultureware Collagen I Peptide are pre-coated ready to use vessels. Falcon 6-well cell culture plates self-coated with CM served as a control. HKN were thawed from the frozen stock, diluted in culture medium, counted using the Vi-CELL™ cell counter (Beckman Coulter) and seeded at a density of 2500 cells/cm^2 in 2 mL culture medium/ well in 6-well plates. Cells were allowed to grow in a humidified incubator at 37°C with 5% CO_2. Confluence was monitored under the microscope and cells were passaged at ~60-70% confluence. Cell confluence was also quantified by collecting phase contrast images over time using the IncuCyte™ instrument (Essen BioScience). Cell confluence measurements were taken from 25 regions within each well, and values were averaged to calculate mean confluence/well. At each passage, confluence was also recorded (IncuCyte, Essen) and percent confluence was plotted for each culture surface. For passaging, culture media was aspirated and cells were washed once with DPBS, 0.5 mL Trypsin/EDTA solution was added to each well (6-well format), the cells were examined under the microscope, and Trypsin solution was neutralized by adding 1.5 mL of Trypsin inhibitor when the cells were detached from the surface. Cells were transferred to a polystyrene Falcon tube (Corning Cat No. 352097). Wells were rinsed with 1 mL culture medium to recover remaining cells. Cells were counted at the end of each passage and population doubling was determined. For expansion, cells were centrifuged at 150 x g for 5 minutes, the supernatant was removed, and cells were resuspended in culture medium. Cells were counted, seeded in the vessels and incubated in a humidified incubator at 37°C with 5% CO_2. Cell attachment and growth after culture in EDGS supplemented media was quantified by MTS assay using a CellTiter 96® AQUEOUS Non-Radioactive Cell Proliferation Assay Kit (Promega, Cat. No. G5421).

Cell Migration Assay

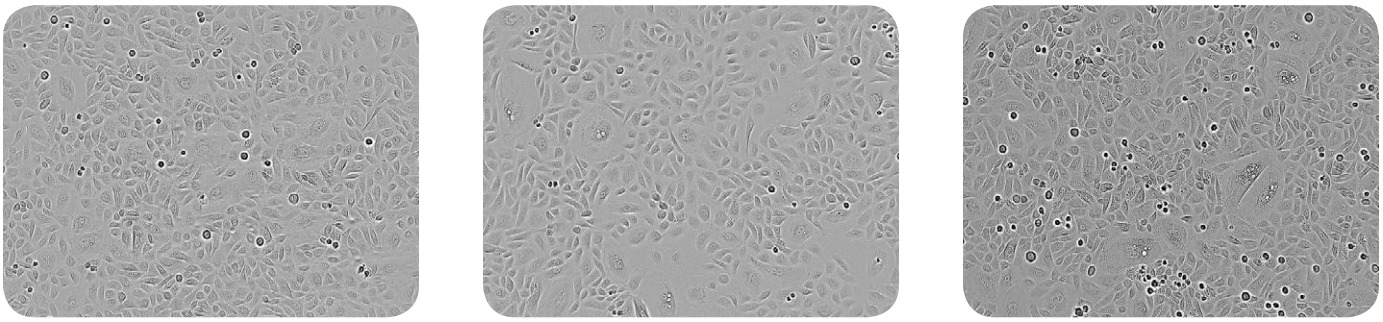
After 3 passages, cells were division-arrested by Mitomycin C treatment, and cell migration assay was performed. Culture media was removed and cells were treated with 4 µg/mL Mitomycin C for 2 hr[5]. Cells were washed with DPBS to remove Mitomycin C and a scratch was made across the well with a pipet tip to create a wound in the monolayer. Culture media was added to the wells, cells were incubated as described above, and images were then captured every hour using the IncuCyte instrument.

Results and Discussion

HKN Culture and Population Doubling

HKN from the frozen stock were seeded on the CM and collagen I mimetic surfaces. Cells did not require any adaptation on the Collagen I mimetic surface; cell attachment was comparable to the CM control. Cells were cultured for 5 passages in defined and xeno-free media. Cell morphology on the Collagen I mimetic surface was similar to cells cultured on the CM surface (Figure 1). Cells were counted after each passage and population doubling was determined. Growth on the two surfaces was compared by cumulative population doubling over the course of the culture. As shown in Figure 2a, population doubling did not reveal differences in cell growth on Collagen I mimetic and recombinant protein-coated vessel. Confluence monitored over 5 passages also showed cell attachment and growth comparable to recombinant extracellular matrix protein (CM), as shown in Figure 2b. Cell morphology (Figure 1) and growth (Figure 3) on the Collagen I mimetic surface was also comparable to rat tail Collagen I-coated vessels. Thus, Collagen I mimetic surface supported cell attachment and growth for multiple passages demonstrating suitability for kerotinocyte culture in defined and xeno-free media.

Figure 1. Phase contrast images of human neonatal keratinocytes on Collagen I mimetic surface, (recombinant Collagen I (CM) and rat tail Collagen I. Images were captured using 10X objective.



Collagen I Mimetic **Coating Matrix** **Rat tail Collagen I**

Figure 2a. Cumulative population doubling of human keratinocytes over 5 passages in defined and xeno-free media. Collagen I mimetic surface supported HKN attachment and growth comparable to freshly coated recombinant Collagen I.

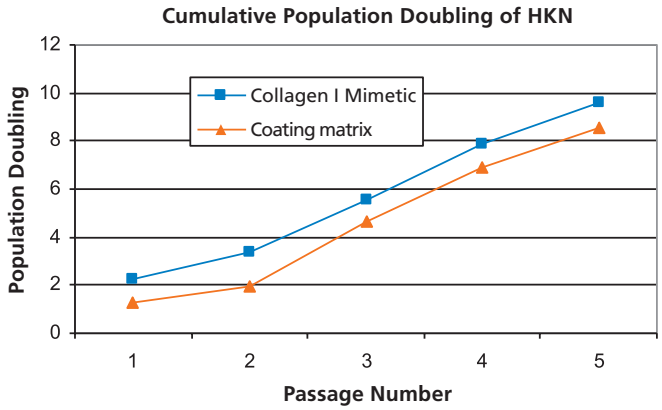


Figure 2b. Confluence of HKN measured for 5 passages. The cells exhibited comparable growth on both surfaces.

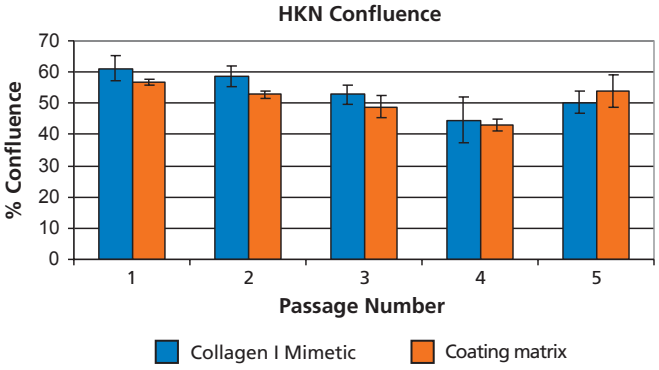
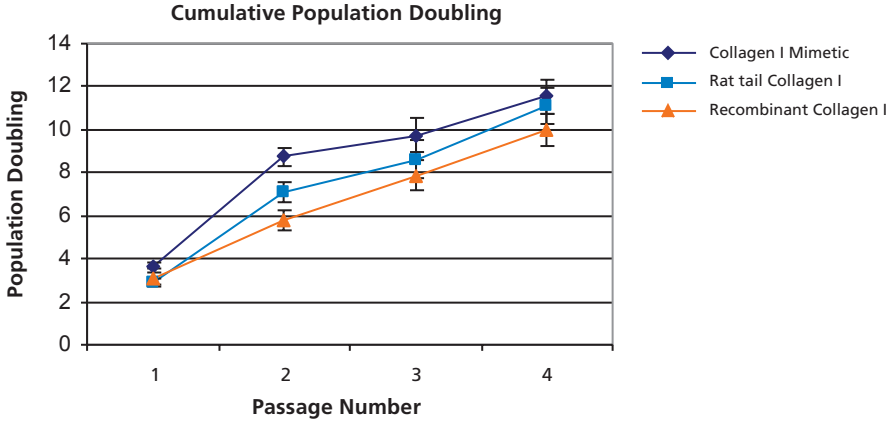


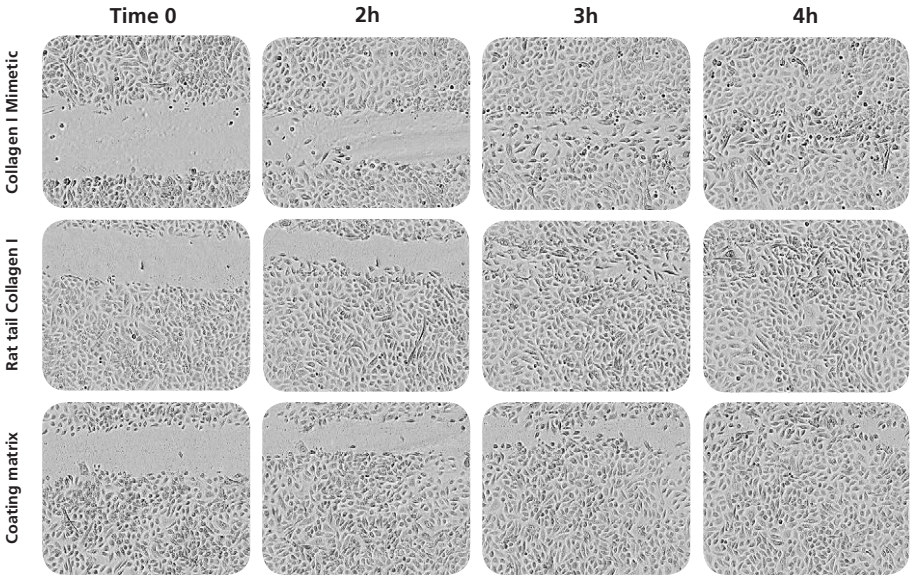
Figure 3. Cumulative population doubling of human keratinocytes in a defined and xeno-free media. HKN attachment and growth on the Collagen I mimetic surface are comparable to that on rat tail Collagen I and recombinant Collagen (CM).



Cell Migration Assay

After 3 passages, the functionality of the keratinocytes was evaluated by cell migration assay, a commonly used wound healing model. A wound was made by scratching the monolayer of division arrested cells, and healing was monitored by collecting phase contrast images over time using the IncuCyte™ instrument. Cells migrated into the wounded site, resulting in narrowing of the region within two hours (Figure 4). Cell migration continued and the wound was completely sealed by 4 hours.

Figure 4. Wound healing assay: HKN migrated to seal the scratch in the cell monolayer. Cell migration function was demonstrated on all the three surfaces including Collagen I mimetic, rat tail Collagen I and coating matrix.



HKN Culture in Serum-free Media

Keratinocyte attachment and culture was also evaluated in serum-free medium. HKN from the frozen stock were seeded on the CM, rat tail Collagen I-coated surface, and Collagen I mimetic surface. Cell attachment and growth was measured using the MTS assay after 5 days. As shown in Figure 5a, cell attachment and growth on the ECM mimetic surface was comparable to rat tail Collagen I-coated surface. Cells also exhibited comparable morphology on the Collagen I mimetic surface and rat tail Collagen I surface. (Figure 5b).

Figure 5a. Human keratinocyte attachment and growth in serum-free medium. Cell attachment and growth is comparable on Collagen I mimetic and rat tail Collagen I-coated surfaces. Tissue culture surface (TC).

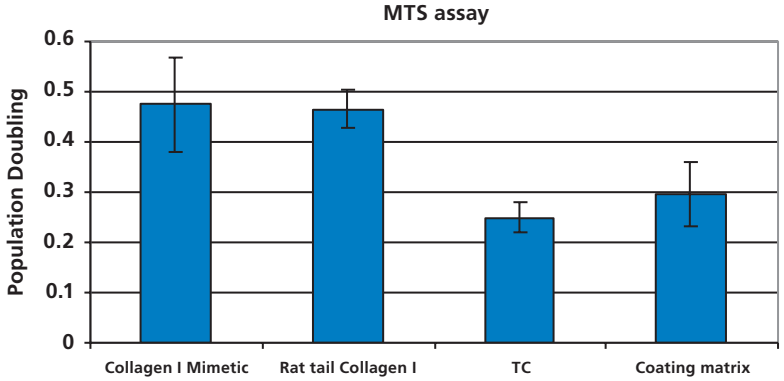
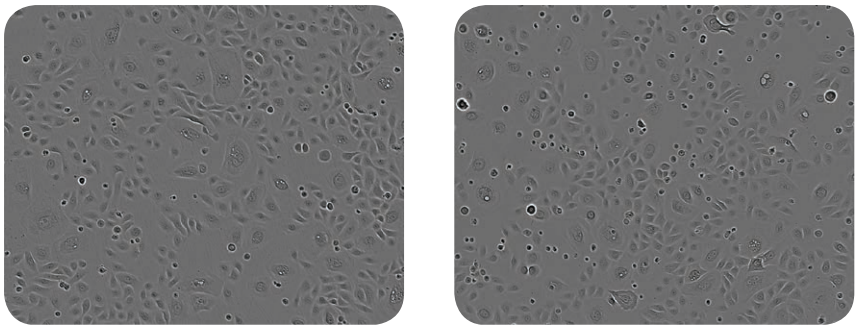


Figure 5b. Morphology of human keratinocytes on rat tail Collagen I and Collagen I mimetic surfaces: cells exhibited comparable morphology on both the surfaces.



Rat tail Collagen I **Collagen I Mimetic**

Conclusions

- Corning PureCoat ECM Mimetic Cultureware Collagen I Peptide supported human keratinocyte attachment and growth for multiple passages in xeno-free and serum-free media.
- Keratinocytes retained their functionality after several passages on the Collagen I mimetic surface as demonstrated by a wound healing assay.
- The Corning PureCoat ECM Mimetic Cultureware Collagen I Peptide offers distinct advantages of being pre-coated, synthetic, animal-free, and xeno-free vessels for culture of collagen I-dependent cells.

References

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Corning acquired the Discovery Labware Business including the Falcon® and PureCoat™ brands. For information, visit www.corning.com/discoverylabware.

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