Corning® PureCoat™ ECM Mimetic Cultureware: Animal-free, Synthetic Surfaces for Serum-free Culture of Adherent Cells.

Himabindu Nandivada, Jeff Partridge, Paula Flaherty, and Deepa Saxena

Corning Incorporated, Tewksbury, MA, USA

Application Note

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Introduction

Culture of adherent cells typically requires the use of animal-derived components such as serum and extracellular matrix (ECM) proteins, which promote cell adhesion via cell-surface adhesion receptors[1]. However, concerns about traceability and sourcing of these naturally-derived materials have led to the search for animal-free, serum-free defined culture environments, while still maintaining or improving growth and productivity of the cells.

Peptide sequences derived from ECM proteins have been shown to mimic cell-binding activity of the protein, and incorporation of such peptide sequences on a cell culture surface can render it bioactive[2,3]. Compared to native proteins, peptide fragments can be chemically synthesized in a defined manner and are more stable[4].

Herein, we present synthetic Corning PureCoat ECM Mimetic Cultureware for culture of cell lines relevant for biomanufacturing. Corning PureCoat ECM Mimetic Cultureware Fibronectin Peptide and Corning PureCoat ECM Mimetic Cultureware Collagen I Peptide are synthetic xeno-free, and animal-free (free of human or animal origin components) cell culture surfaces containing covalently-immobilized Fibronectin and Collagen peptide fragments respectively. Collagen I peptide consists of the GFOGER amino acid sequence that facilitates attachment of $\alpha 2$ integrin positive cells on the surface, and the Fibronectin peptide contains RGD to support cells expressing $\alpha 5$ integrin. These surfaces present the peptide fragments in a functional manner, providing a unique culture environment that promotes cell attachment and proliferation. Products are room temperature stable and ready to use. In this study, both Corning PureCoat ECM Mimetic Cultureware Fibronectin peptide and Corning PureCoat ECM Mimetic Cultureware Collagen peptide, supported enhanced proliferation of Vero cells in serum-free, low-protein medium when compared to a tissue culture (TC)-treated surface. In addition, Corning PureCoat ECM Mimetic Cultureware Fibronectin peptide enabled improved attachment and growth of CHO-K1 cells in serum-free medium when compared to a TC-treated surface.

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Materials and Methods

Cell Culture

For the cell culture experiments, Vero cells (African green monkey kidney epithelial cells, ATCC Cat. No. CCL-81) were cultured in serum-free, low-protein OptiPro™ SFM (Invitrogen Cat. No. 12309-019) and CHO-K1 cells (Chinese hamster ovary cells, ATCC Cat. No. CCL-61) were cultured in serum-free, protein-free HyClone™ SFM4CHO cell culture medium (ThermoFisher Cat. No. SH3082001). According to manufacturers' protocols, media were supplemented with L-glutamine (Invitrogen) before use.

Cells were thawed and seeded onto 6-well Corning PureCoat ECM Mimetic Cultureware Fibronectin Peptide (Cat. No. 356240), Corning PureCoat ECM Mimetic Cultureware Collagen Peptide (Cat. No. 356241) and TC-treated surface (Cat. No. 353224) at a density of 5000 viable cells/cm^2 in 2 mL of media. Cells were maintained at 37°C and 5% CO_2, and culture medium was changed on the day after cell seeding.

Cells were subcultured using 0.25% Trypsin-EDTA (1 mL per well, Invitrogen Cat. No. 25200-056) for 3-5 mins and neutralized with defined trypsin inhibitor (2 mL per well, Invitrogen Cat. No. R007-100). Two aliquots of the cell suspension were removed and counted using the Vi-CELL™ automated cell counter (Beckman Coulter). For each surface, cells from the wells were pooled and passaged onto the corresponding surfaces.

Cell Confluence Measurements

Cell confluence was quantified by collecting phase contrast images over time using IncuCyte™ (Essen BioScience). Cell confluence measurements were taken from 25 regions within each well, and values were averaged to calculate mean confluence/well.

Results and Discussion

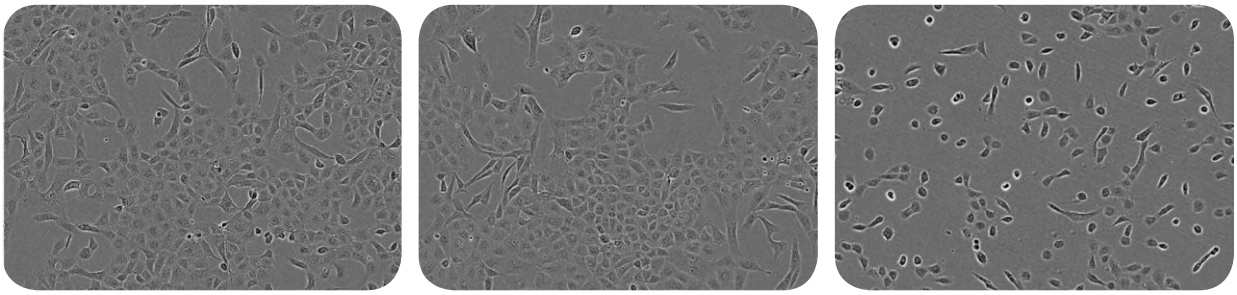
Cell Culture Experiments

In this study, we chose two mammalian continuous cell lines, CHO-K1 and Vero cells. Chinese Hamster Ovary (CHO) cells are commonly used in biomanufacturing (mainly for the production of recombinant proteins) and biomedical research. Vero cells are used for vaccine production, as they are amenable to bioreactors and large-scale culture conditions.

Vero Cell Culture

Collagen I mimetic and Fibronectin mimetic cultureware supported robust attachment and proliferation of Vero cells in the presence of serum-free, low protein medium (OptiPro™ SFM growth medium) for 5 passages. Vero cells from the frozen stocks were thawed and transferred to a tube containing 9 mL culture medium. Cells were spun at 200xg for 5 minutes, supernatant was removed, and the pellet was resusupended in culture medium. Cells were seeded directly onto Fibronectin mimetic, Collagen mimetic and TC-treated surfaces in serum-free, low protein growth medium. Vero cells attached to Fibronectin mimetic and Collagen I mimetic surfaces and did not require any adaptation. We observed comparable cell morphology for Vero cells growing on both Collagen I mimetic and Fibronectin mimetic surfaces as opposed to that for cells cultured on TC-treated surface (Figure 1). Cells on Corning PureCoat ECM mimetic cultureware surfaces appeared well attached and spread out, while Vero cells grown on TC-treated surfaces appeared clumpy and less spread out.

Figure 1. Vero cells (on day 4 post-seeding for passage 3) on (A) Corning PureCoat ECM Mimetic Cultureware Collagen I Peptide (B) Corning PureCoat ECM Mimetic Cultureware Fibronectin Peptide (C) TC-treated surface in serum-free, low protein growth medium.



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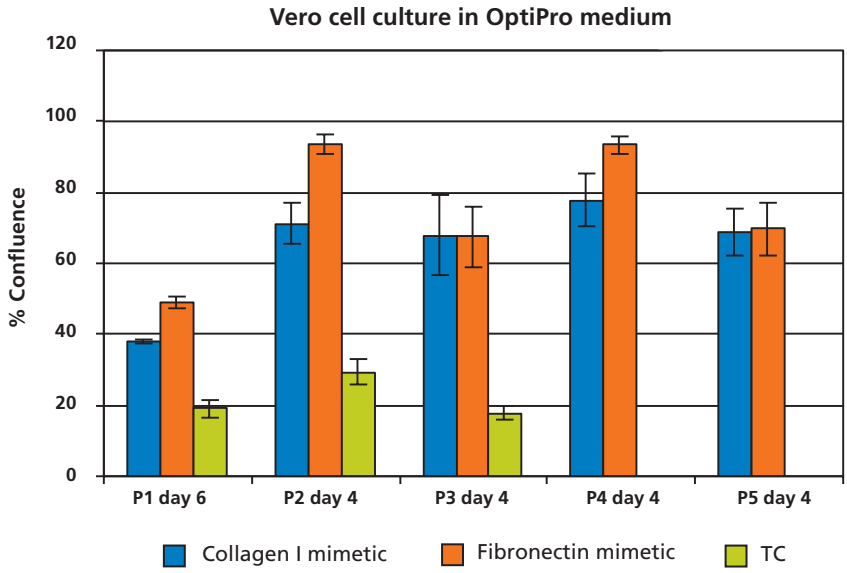
Compared to uncoated TC-treated surface (recommended for Vero cell culture with OptiPro™ SFM growth medium), Vero cell growth was faster on Fibronectin mimetic and Collagen I mimetic surfaces as demonstrated by higher confluence (Figure 2).

Figure 2. Percentage confluence of Vero cells cultured for 5 passages on Fibronectin mimetic, Collagen I mimetic and TC-treated surfaces in serum-free, low protein growth medium.



CHO-K1 Cell Culture

Corning PureCoat ECM Mimetic Cultureware Fibronectin peptide supported attachment and expansion of CHO-K1 cells in the presence of serum-free, protein-free media. For this study, defined protein-free medium, HyClone SFM4CHO cell culture medium, was used to evaluate cell growth for 5 passages (Figure 3). CHO-K1 cells were thawed and seeded (5000 cells/cm^2) directly onto the test surfaces.

CHO-K1 cells cultured on Fibronectin mimetic surface grew faster than those on the TC-treated surface as reflected by differences in confluence (Figure 4). Collagen I mimetic surface did not support much attachment and growth. This result is consistent with previously published literature which shows that CHO-K1 cells attach and proliferate on Fibronectin, but not on Collagen[5,6].

Figure 3. Percentage confluence of CHO-K1 cells cultured for 5 passages on Corning PureCoat ECM Mimetic Cultureware Collagen I peptide, Corning PureCoat ECM Mimetic Cultureware Fibronectin peptide and TC-treated surfaces in serum-free, protein-free cell culture medium.

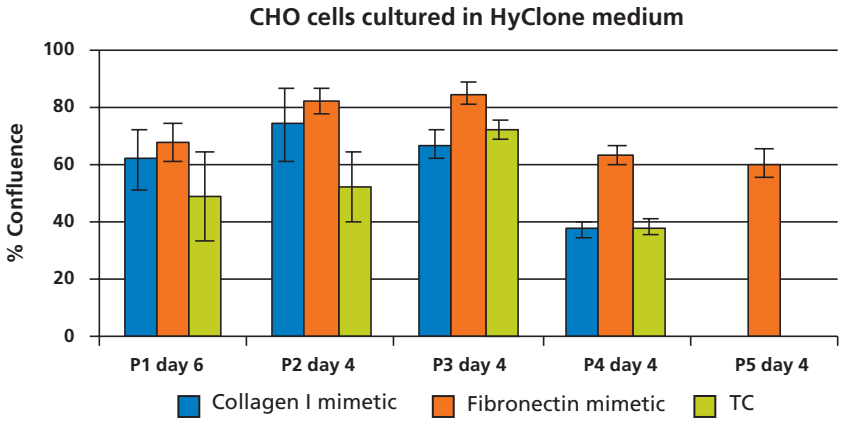
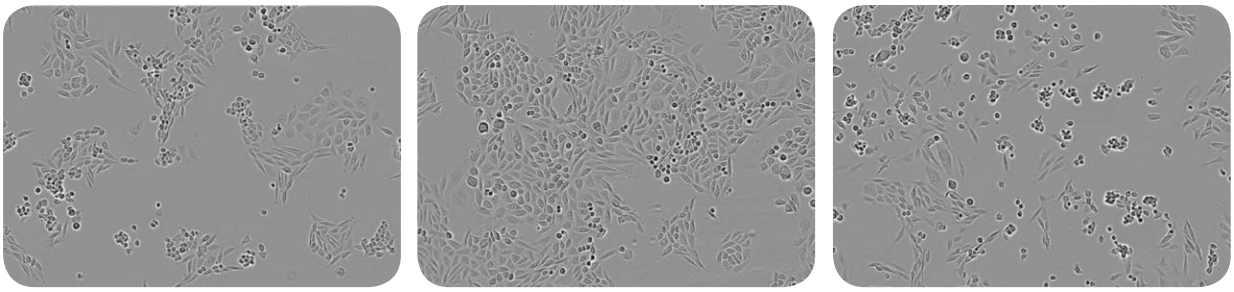


Figure 4. CHO-K1 cells (on day 3 post seeding for passage 4) on (A) Collagen I mimetic surface (B) Fibronectin mimetic surface (C) TC-treated surface in serum-free, protein-free cell culture medium.



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Conclusions

- Corning PureCoat ECM Mimetic Cultureware successfully supported culture of adherent cells in the presence of serum-free, low-protein media.
- Corning PureCoat ECM Mimetic Cultureware Fibronectin peptide supported improved growth of CHO-K1 cells for at least 5 passages in serum-free, protein-free media.
- Corning PureCoat ECM Mimetic Cultureware Collagen I peptide and Corning PureCoat ECM Mimetic Cultureware Fibronectin peptide supported enhanced attachment and proliferation of Vero cells for at least 5 passages in serum-free, low-protein medium.
- Corning PureCoat ECM mimetic cultureware are animal-free, synthetic, ready-to-use surfaces suitable for culture of cells in animal-free and defined environment.

References

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Corning acquired the Discovery Labware Business including the PureCoat™ brand. For information, visit www.corning.com/discoverylabware.

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Corning Incorporated
Life Sciences
836 North St.
Building 300, Suite 3401
Tewksbury, MA 01876
t 800.492.1110
t 978.442.2200
f 978.442.2476
www.corning.com/lifesciences