Corning® CellBIND® Surface: An Improved Surface for Enhanced Cell Attachment

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Application Note

Ana Maria P. Pardo, Marie Bryhan, Helen Krasnow, Nicole Hardin, Mary Riddle, Ollie LaChance, Paul Gagnon, Todd Upton, and Debra S. Hoover
Corning Life Sciences

Introduction

Here we describe the Corning CellBIND surface, a proprietary plasma surface treatment for tissue culture vessels. This optimized tissue culture surface treatment increases the oxygen content of the polymer surface resulting in improved hydrophilicity and wettability, which is known to improve cell spreading and attachment[1,2]. In particular, this surface treatment has been shown to improve attachment of cells that may adhere poorly due to cell phenotype, stressful culture conditions (such as may occur in low serum conditions) or those which normally require additional biological coatings for attachment. The Corning CellBIND surface is particularly suited for a production environment where prolonged cell attachment and low serum concentrations require an optimized surface. The Corning CellBIND surface does not interfere with protein production and approvals by the Food and Drug Administration for production have required only minimal revalidation. The Corning CellBIND surface treatment results in faster and more robust attachment of cells onto tissue culture vessels and results in higher cell yields.

Biomaterials play a significant role in cell performance and downstream applications. Tissue culture vessels consist of high grade polystyrene which is molded and subsequently treated with a combination of energy and gas to create the surface roughness and hydrophilicity that is necessary for protein adsorption and cell attachment. These treatments can be broadly categorized into two types of commercial treatments. First, is an atmospheric plasma treatment in which an electrical energy source is combined with atmospheric gases (standard corona discharge treatment) to create a reactive plasma. The second class of treatments includes vacuum plasma treatment in which an electrical or a radio frequency (RF) energy source is used in combination with a vacuum chamber and pressurized oxygen or nitrogen/oxygen containing gases, to create a reactive plasma.

The Corning CellBIND surface treatment is a unique process which integrates a high energy microwave source, a vacuum chamber and gas mixtures to generate highly reactive plasma which modifies the chemical nature of the polystyrene backbone (Figure 1).

Corning CellBIND Surface

The treatment of pure polystyrene with the highly reactive Corning CellBIND surface plasma reduces the aromatic groups and increases the oxygen containing functional groups of the polystyrene backbone.

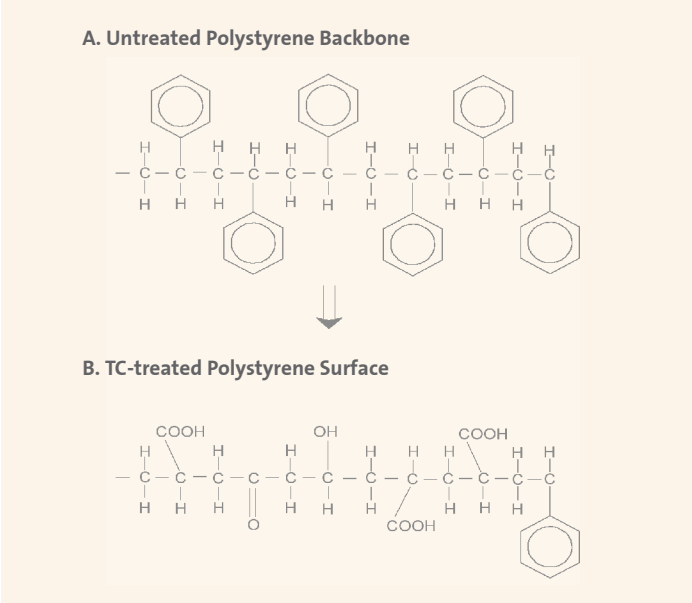

Figure 1. Corning CellBIND surface treatment derivatizes the core polymer chain and increases oxygen containing functional groups.

The Corning CellBIND surface has been analyzed using several analytical methods. The primary characterization of the surface composition was performed using ESCA (electron spectroscopy for chemical analysis or X-Ray Photoelectron Spectroscopy (XPS). Wettability was evaluated by measuring contact angle using a goniometer and functional testing was performed by evaluating cell growth and attachment/detachment.

ESCA measurements are highly quantitative and define the atomic composition of the surface. These measurements are performed by bombarding the surface *in vacuo* with monoenergetic soft x-rays and the resulting photoelectrons are analyzed. Each element has a unique spectrum, allowing elemental identification, and information about the chemical state of the element to be obtained. Photoelectrons originating from atoms deeper than the top few atomic layers are absorbed by the solid making XPS extremely surface sensitive. The results of the ESCA measurements (Table 1) indicate a reduction in the percent carbon and a concomitant increase in the oxygen composition in all treated surfaces relative to the untreated polystyrene. Importantly, the Corning® CellBIND® surface contained 68% greater oxygen levels than standard Tissue Culture (TC)-treated surface and 105% more than a competitive amine surface. The Corning CellBIND surface treatment yields surfaces with more

cyclic C-O-C, C=O, and COOH/R functionalities than either the RF or Corona treatment. The reduction in carbon is consistent with the loss of aromatic compounds that was observed on the Corning CellBIND surface.

Importantly, both increased adsorption of extracellular matrix (ECM), which is fundamental to cell attachment, and cell proliferation have been correlated with increased levels of surface oxygen[3]. Additionally, assembled fibronectin matrix has been shown to give better surface coverage on surfaces with increased oxygen containing functional groups[4]. This suggests the increased oxygen content of the Corning CellBIND surface can improve cell attachment.

More Wettability

Wettability has been shown to be an important attribute for cell attachment and downstream cell performance. To investigate the impact of the Corning CellBIND surface treatment and the increased surface oxygen on wettability, contact angle measurements were taken. Measurements were performed on multiple sizes of flasks and standard roller bottles as well as expanded surface roller bottles (ESRB). Measurements were performed with a goniometer and indicated the contact angle of the Corning CellBIND surface was significantly less than the standard TC-treated and a competitive amine treated surfaces (Table 2). The contact angles for the Corning CellBIND surface ranged from 12° to 16.3°, while the TC-treated surface ranged from 56° to 64° and the contact angle of the competitive amine flask was 74.9°. This demonstrated that the Corning CellBIND surface wets more efficiently than the standard TC-treated flask or the competitive amine surface. The increased hydrophilicity is likely the result of the reduction in hydrophobic aromatic groups and the increase in negatively charged oxygen groups observed by ESCA measurements.

Better Cell Attachment

Modified surfaces have been reported to improve cell proliferation, increase cell adhesion and improve cell yields[3]. We have evaluated the effects of the Corning CellBIND surface treatment

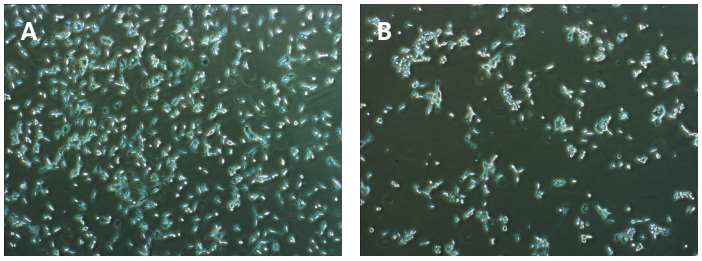

Figure 2. Twenty-four hour thaw LNCaP cells on Corning CellBIND surface (A) and standard TC-treated (B), T-25 flasks. Random field viewed by light microscopy (10X magnification).

Table 1. Surface composition of various polystyrene surfaces as measured by ESCA (in atomic percent) (n≥3).

Flasks	TOA (degrees)	% C	% O	% N	% Others
Polystyrene	45	98.2	1.8	0	0
TC-treated, polystyrene	45	82.4	17.2	0.2	0.2
Corning CellBIND surface	45	70.4	29.0	0.6	<0.01
Competitive Amine surface	55*	74.6	14.1	11.1	0.2

*Previously published data[2].

Table 2. Contact angle measurements of Corning CellBIND surface compared to standard tissue culture vessels (all vessels were incubated for 5 days at 52°C).

		Flasks	Roller Bottles	
		75 cm²	Standard	ESRB
Corning CellBIND surface	Mean	13.4°	16.3°	12.3°
	SD	4	2	2.4
	N	9	30	6
TC-treated	Mean	55.75°	56.3°	63.5°
	SD	2.1	7.9	0.7
	N	4	9	2
Competitive Amine surface	Mean	74.89°	N/A	N/A
	SD	1.54	N/A	N/A
	N	3	N/A	N/A

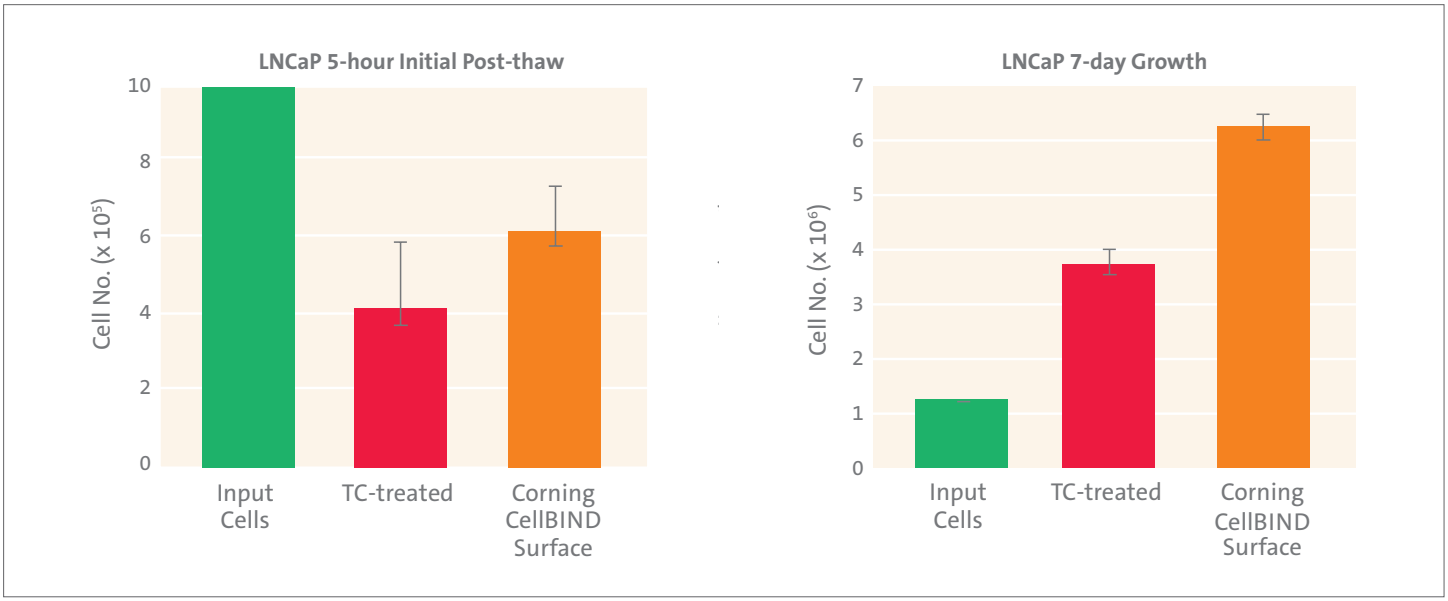

Figure 3. Total cell yield at 24- and 168- hour incubation post-thaw under standard conditions. Seeding density 1.0 x 10^6 cell/flask. 24-hour data represents average count + SE from three independent experiments. 7-day growth data represents average count + SE from four flasks.

on cell growth and attachment. The Corning CellBIND surface has a beneficial effect on three aspects of cell culture: 1) the rate of attachment and initiation of cell doubling; 2) the robustness of attachment or the reduction of detachment for cell growth as long as 21 days after seeding and in serum-free media; and 3) the consistency of cell growth as a result of more uniform surface treatment resulting in higher yields for problematic cell types.

LNCaP cells (ATCC CRL-1740) are a prostate cancer cell line that have been reported to attach poorly to standard TC-treated vessels (personal communication with customer). In the experiment below, LNCaP cells were removed from cryostorage, seeded and monitored for attachment to standard TC-treated or Corning CellBIND surface (Figures 2 and 3). After 24 hours, the supernatant was removed and unattached cells were counted and viability was performed. The data demonstrates that the cells attach faster to the Corning® CellBIND® surface and results in an average of 60% higher yields at harvest. Additionally, cells floating in the supernatant were 70% viable, indicating that cell death was not the cause of poor attachment to the standard surface.

Serum-free growth has become an increasingly important requirement for many cell culture related applications from transfections to industrial protein production. We have found, for some cell types, that the Corning CellBIND surface supports more robust attachment in low or no serum. Figure 4 demonstrates that WS1 cells (a Human Embryonic Kidney cell stably transfected with GABA receptor) resulted in 57% more cells when grown on the Corning CellBIND surface flasks than standard TC-treated flasks in 1% serum. The cells also showed less detachment during handling on the Corning CellBIND surface. In a separate experiment, cell attachment to both standard TC-treated and Corning CellBIND surface roller bottles was evaluated (Figure 5).

Chinese Hamster Ovary cells expressing recombinant protein were evaluated for their ability to withstand vigorous handling in serum-free conditions. Only the Corning CellBIND surface exhibited no cell sloughing after 14 days of growth. Additionally, medium from cells grown on the Corning CellBIND surface was recovered out to 21 days (data not shown) without sloughing of cells from the bottle walls. In these experiments pH, lactate production, glucose utilization and protein production were all similar to levels found in cells grown on a standard TC-treated surface indicating there were no differences in cell metabolism when grown on the Corning CellBIND surface (data not shown).

Applications which require maximum yields of cells such as protein production or scale up for high throughput screening require consistent surface treatment to enable optimum yields. Figure 6 demonstrates that the Corning® CellBIND® surface results in cell growth that exhibits highly uniform cell attachment and increased yields as compared to standard TC-treated surfaces. In this experiment, HEK-293 cell growth was compared on standard TC-treated, Corning CellBIND surface, and Poly-D-Lysine (PDL)-coated roller bottles in 10% serum. (Cells were quantified as unattached [floating], detached [those cells removed during a PBS wash], and attached [those cells removed by trypsinization]). It is evident that cell attachment was more robust on the Corning CellBIND surface and PDL surface than the standard TC-treated bottle. The Corning CellBIND surface produced 28% more cells and in a production environment, this can have a big impact on total cells and protein yield.

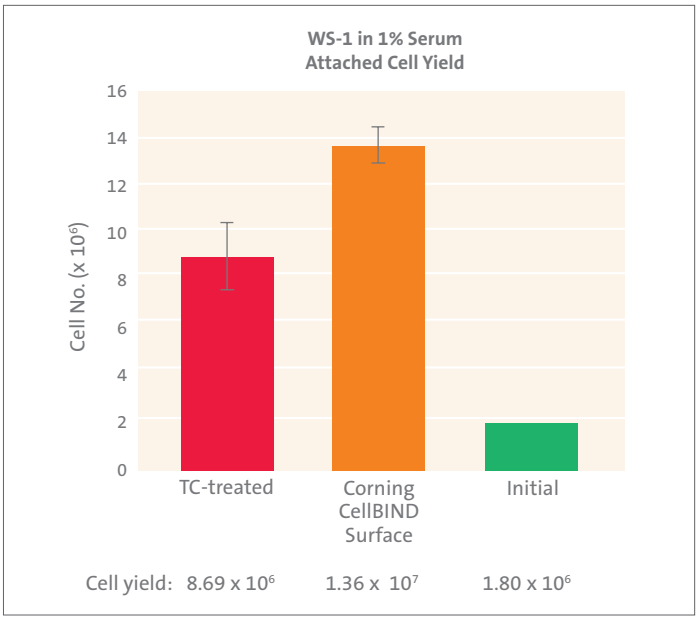

Figure 4. WS-1 5 days growth in 1% serum media on Corning CellBIND surface and standard TC-treated 75 cm^2 flask. Initial seeding density at 1.8 x 10^6 cells/flask. Data represents average count ± SE from 6 flasks from 2 independent experiments.

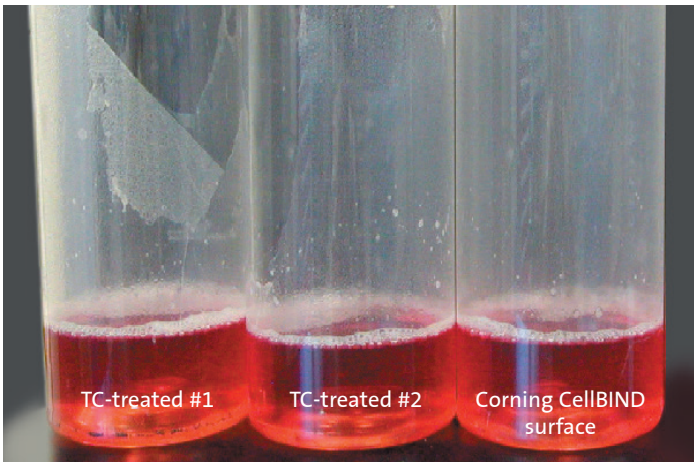

Figure 5. Corning CellBIND surface resists cell sloughing in a production environment. Engineered CHO cell line, 2-week growth. Bottles photographed after 1 inversion.

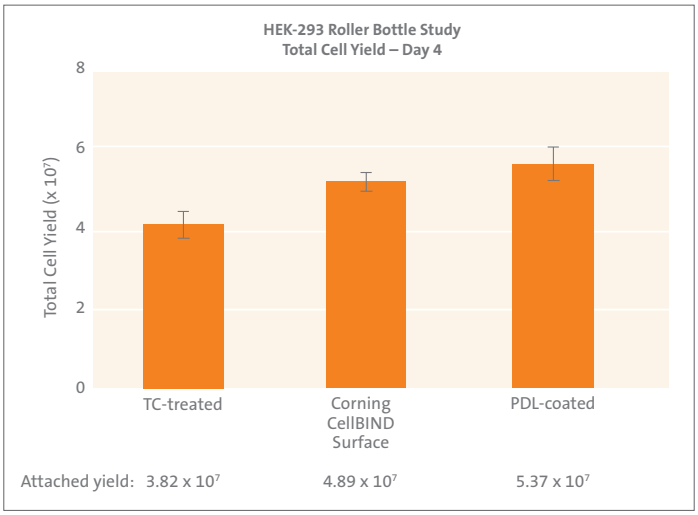

Figure 6. Attached cell yields from HEK-293 cells on TC-treated, Corning CellBIND surface, and PDL-coated 850 cm^2 roller bottles. Initial seeding density of 17 x 10^6 cells/bottle. Data represents average yield ± SE from 2 independent studies.

Conclusions

Here we demonstrated the performance of the Corning CellBIND surface, an innovative surface treatment for tissue culture vessels. This surface is not a biological coating yet it offers a significant advantage over traditional cell culture surfaces. The increase in oxygen containing functional groups results in increased wettability and hydrophilicity and can impact the ability of ECM proteins to adsorb to the surface[3]. It is likely that these findings are related to the increased attachment and recovery of cells grown on the Corning CellBIND surface.

The Corning CellBIND surface:

- Increases adherence and cell yields of fastidious cell lines
- Enables better cell recovery of primary cell isolates
- Can eliminate the need for biological coatings for cell attachment
- Enables growth and protein production in low serum
- Enables rigorous handling/automation
- Does not require special storage or handling
- Requires minimal revalidation by the FDA

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EUROPE
CSEurope@corning.com
France
t 0800 916 882
Germany
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The Netherlands
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United Kingdom
t 0800 376 8660
All Other European Countries
t +31 (0) 206 59 60 51

LATIN AMERICA
grupoLA@corning.com
Brazil
t 55 (11) 3089-7400
Mexico
t (52-81) 8158-8400