Bioinert – A Non-degradable Surface Passivation Without any Cell and Biomolecule Adhesion

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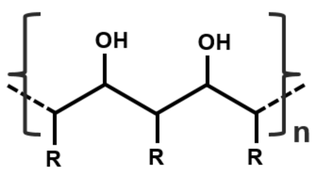
Abstract

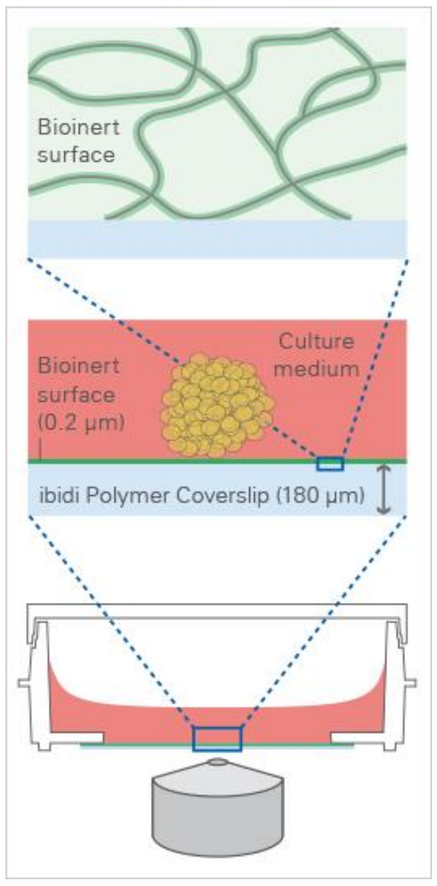
Tumor spheroids, embryoid bodies (EBs) and other 3D tissue and cell culture models demand defined cell-cell and cell-matrix interactions, usually without cell attachment to any hard 2D surface. Traditional Ultra-Low Attachment (ULA) surfaces exhibit only “ultra-low” protein binding and are not suitable for high resolution fluorescence microscopy.

The Bioinert technology is bridging this gap. It allows long-term experiments without any adhesion of proteins or cells. This stable passivation of the surface enables a variety of 3D cell culture assays for culturing tumor spheroids models, organoids, embryoid bodies or suspension cells. Unlike standard ULA surfaces on polystyrene, the Bioinert technology is ideal for fluorescence microscopy even with oil immersion and high resolution confocal microscopes.

Bioinert is a Polyol-based hydrogel with 200 nm thickness on a polymer coverslip. It is covalently bound onto the surface, hydrophilic, dry-stable and mechanically stable. The Bioinert surface is ready-to-use. There is no pre-hydration step necessary. Bioinert is non-degradable thus allowing long-term assays with aggressively adherent cell cultures and high ECM proteins in the culture medium. Being free of autofluorescence and even DIC compatible the Bioinert surface enables multiple readouts based on fluorescence microscopy techniques.

The Bioinert Technology



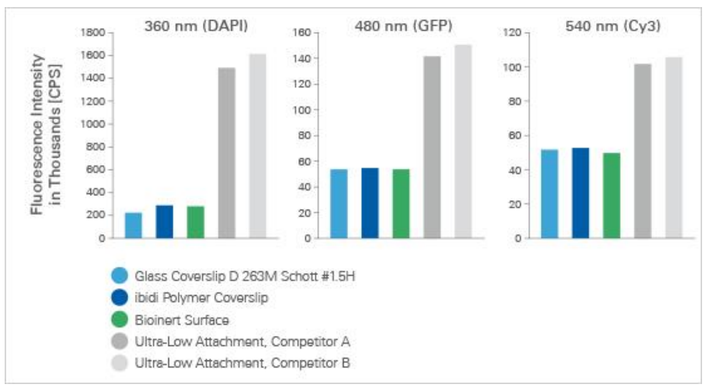


No Cell Attachment
The Bioinert surface is a thin polyol hydrogel layer that is covalently bound to the ibidi Polymer Coverslip #1.5. In contrast to standard ultra-low attachment (ULA) coatings, Bioinert is completely non-adherent and allows no binding of any biomolecule, even in long-term experiments. Therefore, the Bioinert technology provides a stable passivation in cell-based assays for several days or even weeks.

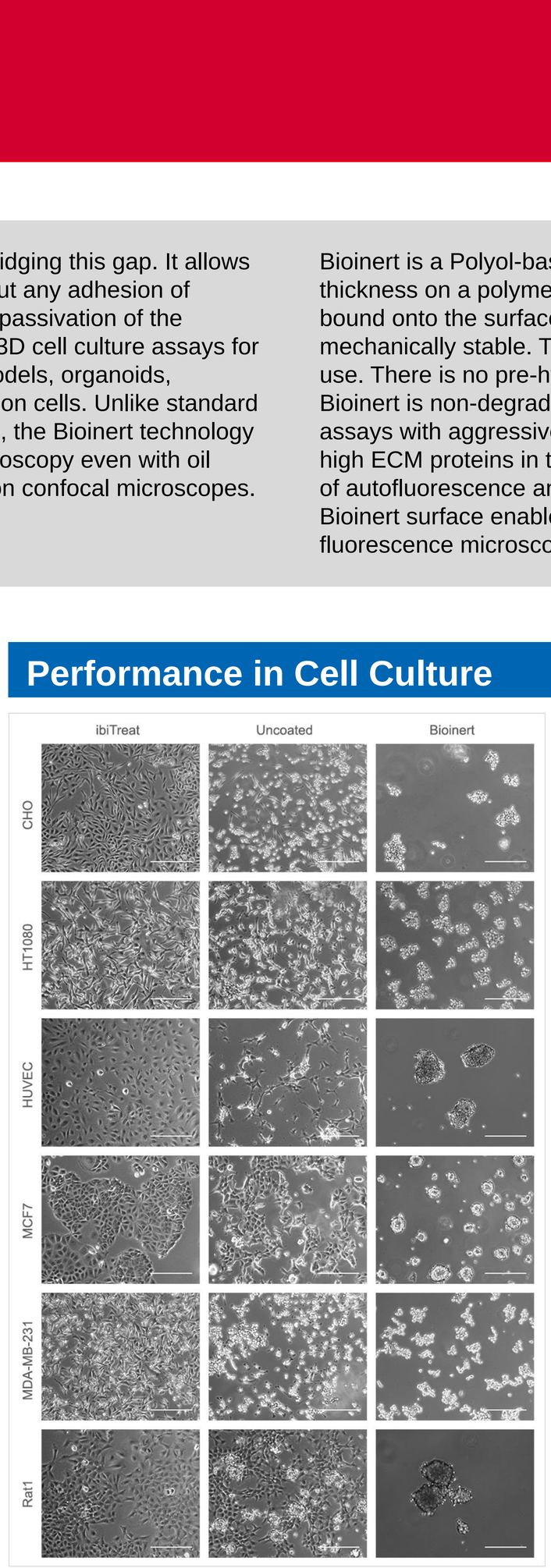
No Cell-Substrate Interactions
Bioinert creates an environment in which cell-cell interactions dominate over cell-substrate interactions. In fact, the latter are completely blocked. The stability of the Bioinert surface allows for long-term experiments on the very same dish—for several days and even weeks—without the adhesion of any proteins. Even if your cells require medium with a high fetal calf serum concentration, Bioinert prevents any cell or protein from adhering to the surface.

No Prior Preparation
The Bioinert surface is ready-to-use. There is no pre-hydration step necessary. The surface will swell by itself once wetted with buffer or cell medium.

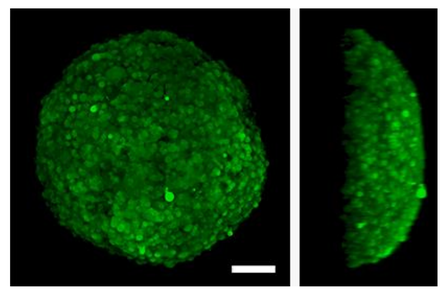
No autofluorescence
Both the flat, thin bottom material and the excellent optical quality of the ibidi Polymer Coverslip enable high-resolution microscopy without any disruptive autofluorescence.



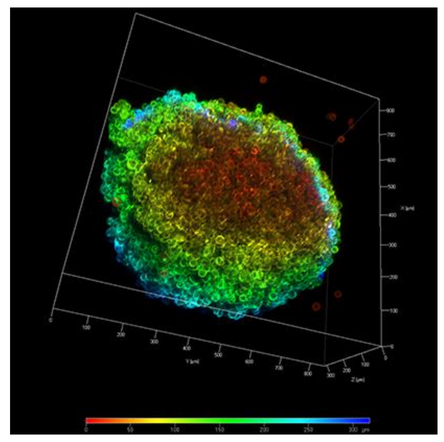
Performance in Cell Culture



The Bioinert surface allows cultivation of various cell types. On the ibiTreat surface, all adherent cell types show a strong cell adhesion. A weaker cell adhesion is observed on the Uncoated surface since there is only a serum protein coating. On the Bioinert surface, cells do not attach at all and spheroid formation can be observed in most of the cell types. Phase contrast microscopy, Scale bar is 300 µm.



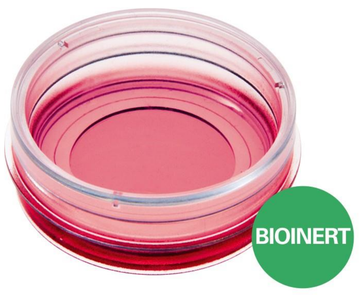
High resolution confocal microscopy on the Bioinert surface. Frontal view and sideview of an FDA-PI-stained MCF-7 cell spheroid. Confocal laser scanning images are displayed from an overlay of the serial confocal sections of the FDA signal. Scale bar is 100 µm.



High resolution confocal microscopy on the Bioinert surface. Confocal laser scanning image of an HT-1080 LifeAct spheroid. The image is made from a series of confocal sections. The rainbow color scale indicates the distance from the coverslip surface. Warm colors = close to the surface, cold colors = distant from the surface.

µ-Dish 35 mm, high Bioinert

Spheroid formation without cell attachment. The hydrophilic Bioinert surface hinders any protein attachment, thus inhibiting subsequent cell attachment.
The Bioinert surface is ideal for the culture and microscopy of suspension cells and cell aggregates.



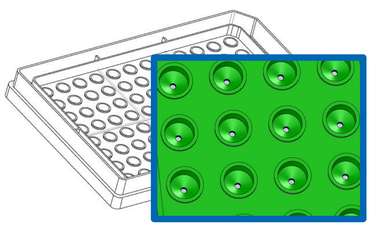
Bioinert in a Petri Dish for imaging. The µ-Dish 35 mm, high Bioinert combines the advantages of the Bioinert surface with the standard Petri Dish format of 35 mm.
Additional features:

- Lid with lock position, which minimizes evaporation
- Suitable for DIC, when used with the special DIC Lid
- Compatible with staining and fixation solvents

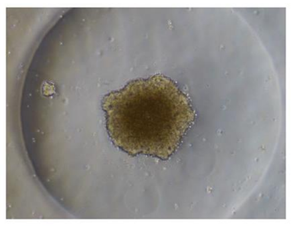
Future Applications

3D Spheroid Formation

Spheroid formation on Bioinert inside a conical well or a thermoformed cavity. Bioinert is the ideal platform for creating and analyzing 3D cell cultures at the same time. Conical wells of different sizes can be used to create one spheroid per well in a precise way. Thermoforming can also be used to create micro-cavities.



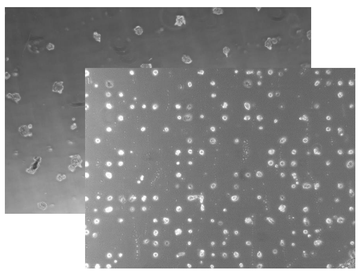
Conical wells by injection molding.



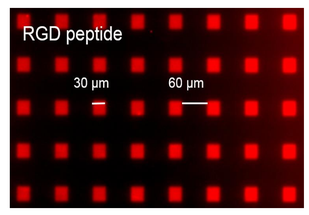
Thermoformed micro-cavity.

2D Patterning

2D patterning with passivated surrounding. Single cell arrays or single spheroid arrays can be created by leaving small spots with ECM proteins or covalently bound RDG peptide for cell adhesion. This can create single cell or single spheroid arrays in a defined manner.



Single spheroid or single cell arrays.



Specific protein arrays.