Application Note

Formation of spheroids and suppression of adhesion by adherent growing cells in inertGrade™ microplates

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Introduction

Suppression of the cell adhesion that is mediated by integrin is of significant importance in many experimental procedures, such as the production of embryonic bodies, self-assembled 3D cultures, and dose-response relationships in cultivated tumor spheroids. BRAND*plates*® inertGrade™ surfaces suppress the adhesion and spreading of adherent growing cells. Subsequently, cell migration and preferential biological adaptation of the cells through cadherin-facilitated cell-cell contacts leads to the spontaneous formation of spheroids. The size and density of these spheroids increases over the course of the cultivation period through proliferation.

The present study indicates that inertGrade™ surfaces suppress adhesion in a variety of adherent growing cell lines (HeLa, CHO-K1, L929, MCF-7). The proliferative capacity is retained, and spheroids are formed with uniform distribution throughout the well.

Materials and methods

In this comparative study, HeLa, CHO, L929 and MCF-7 cells (DSMZ Braunschweig) were cultivated in BRAND*plates*® inertGrade™ plates (F-bottom, 781902) and in competitor cell-repellent plates, while BRAND*plates*® cellGrade™ plates (F-bottom, 781962) were used as a control.

In each case, the cells were seeded at a density of 4000 cells/well in 200 µl DMEM (PAA Laboratories, E15-810) + 5 % FCS (PAA Laboratories, A11-101), and incubated under a 5 % CO_2 atmosphere at 37 °C in a C200 incubator (Labotect Labor-Technik Göttingen GmbH). The cell morphology and distribution were evaluated after 24, 48, 72, and 96 h using a microscope (IX81, Olympus Europe GmbH, with F-View II camera and cell^P software), and proliferation was determined via indirect cell count measurements using a resazurin assay (Gemini EM fluorescence plate reader, Molecular Devices GmbH).

Results

On the BRAND*plates*® cellGrade™ surfaces, all four cell lines showed rapid adherence and formation of a closed (HeLa, CHO, L929) or semi-confluent monolayer (MCF-7) within 96 h (Figure 1).

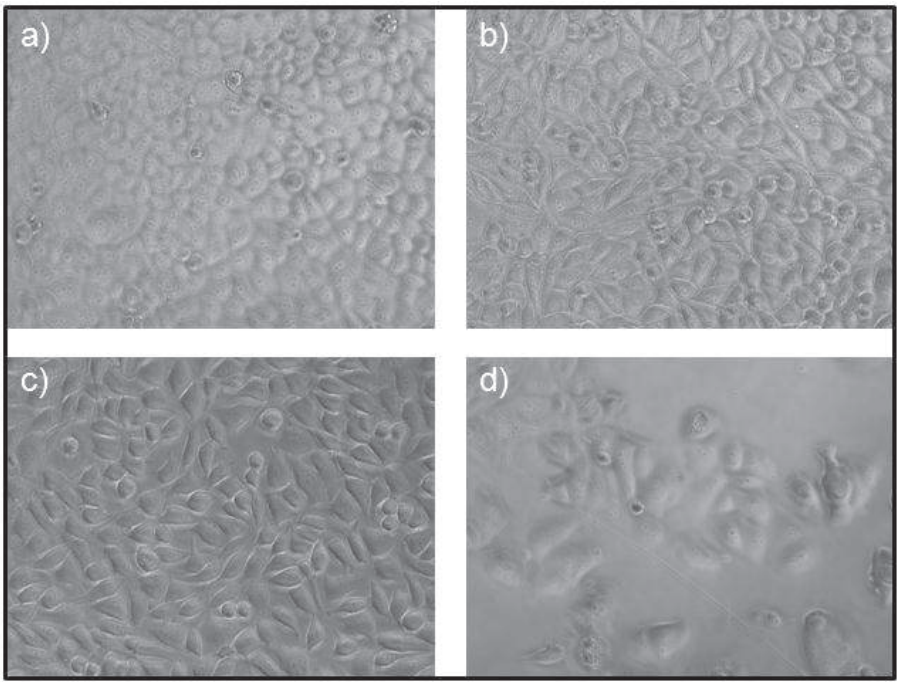

Figure 1: Cell morphology after cell culture for 96 h on cellGrade™ surfaces. Shown here are phase-contrast photomicrographs of cells: a) HeLa, b) CHO, c) L929 and d) MCF-7 (200× magnification).

The inertGrade™ and competitor cell-repellent surfaces reliably and reproducibly inhibit cell binding. However, there are significant differences between the two surfaces with respect to the morphology and distribution of the resulting cell aggregates and spheroids. While the cell aggregates in the competitor plates formed rapidly, were larger, fewer, and densely packed, and exhibited a preference for accumulating on the outer edges of

the wells (Figure 2), a trend of smaller aggregates and spheroids was observed with the inertGrade™ surfaces, with a more uniform distribution over the well surfaces (Figure 3).

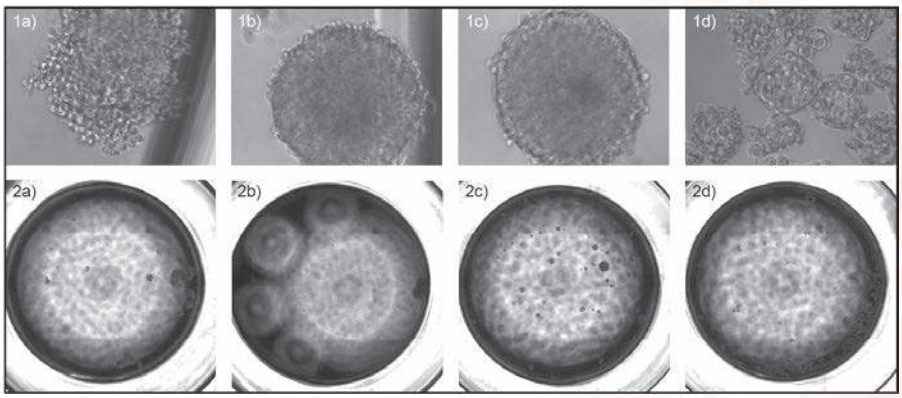

Figure 2: Cell morphology after cell culture for 96 h on the cell-repellent competitor surface. Shown here are 1) microscopic close-ups (200× magnification) and 2) well scan photographs (40× magnification) of a) HeLa, b) CHO, c) L929 and d) MCF-7 cells in phase contrast images.

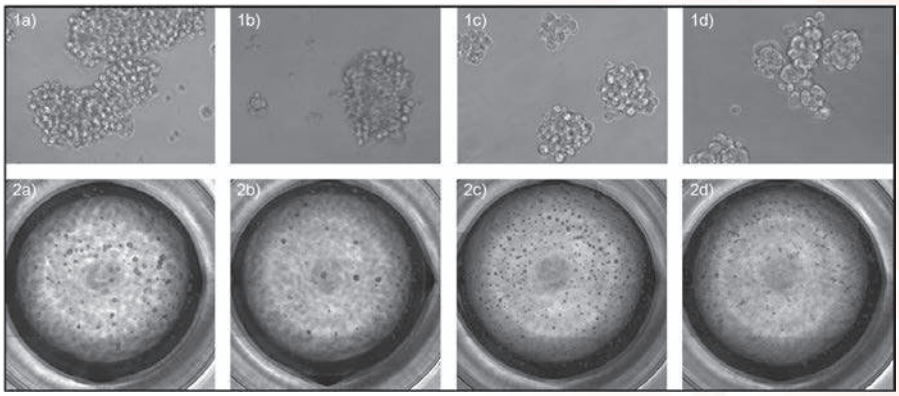

Figure 3: Cell morphology after cell culture for 96 h on cell-repellent BRAND*plates*® inertGrade™ surfaces. Shown here are 1) microscopic close-ups (200× magnification) and 2) well scan photographs (40× magnification) of a) HeLa, b) CHO, c) L929 and d) MCF-7 cells in phase contrast images.

As expected, when proliferation in all cell lines was evaluated using indirect cell count measurements (Figure 4), the monolayer cultures exhibited the highest growth rate. Due to the smaller spheroids and higher metabolic activity, the rates of proliferation when the cells were cultivated on inertGrade™ plates were comparable (CHO) or higher (HeLa, L929, and MCF-7) than those for culturing on the competitor cell-repellent surfaces.

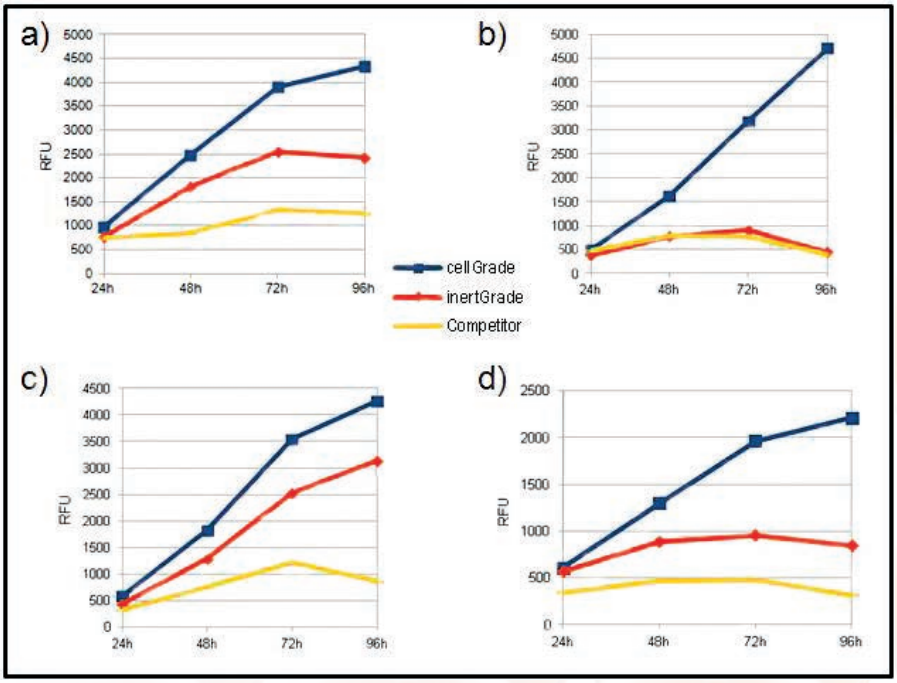

Figure 4: Comparison of proliferation rates on the cellGrade™, inertGrade™, and competitor cell-repellent surfaces using indirect cell count measurements. Shown here are the absolute values of the fluorescence intensities for resorufin liberated from the a) HeLa, b) CHO, c) L929 and d) MCF-7 cells in the resazurin assay.

Discussion

BRAND*plates*® inertGrade™ surfaces exhibit reliable suppression of adhesion in the growth of HeLa, CHO, L929 and MCF-7 cell lines. This suppression of cell adhesion leads to the formation of aggregates, the size and density of which increases over the course of the cultivation period. Compared to a competitor cell-repellent surface, larger numbers of smaller aggregates form and are uniformly distributed over the entire well surface. The proliferation capacity is retained, although as expected, it is diminished with respect to monolayer culture.

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