BRAND*plates*® inertGrade™ surface supports neurosphere formation and maintenance

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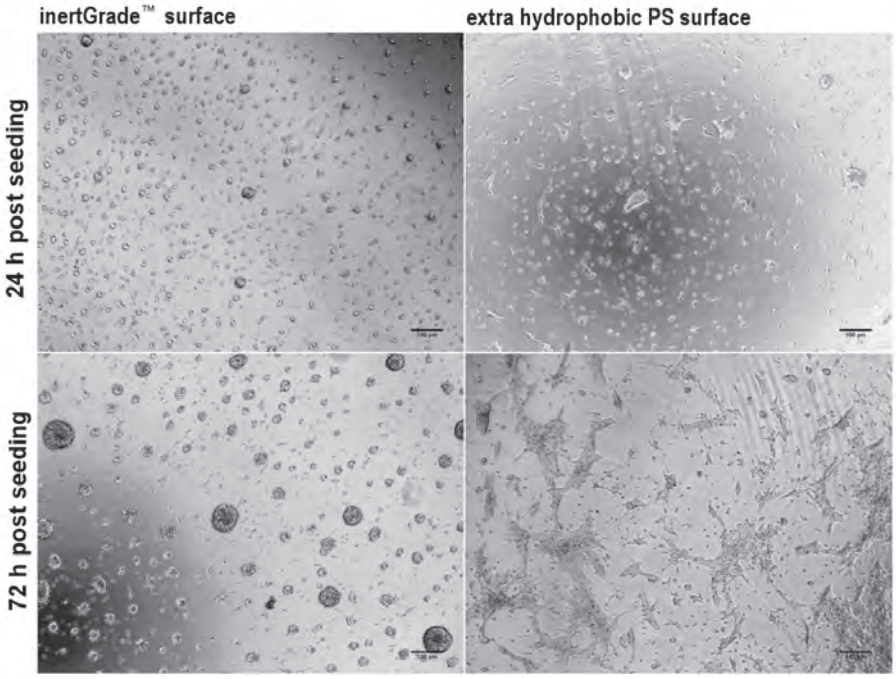
Disposable culture dishes and microplates are mainly manufactured from polystyrene (PS) due to its excellent optical clarity and the possibility to induce surface modifications for certain requirements. Tissue culture (TC) grade PS surfaces are usually achieved by the physicochemical incorporation of the charged carboxyl-, hydroxyl- and/or amino-groups on the PS surface. These charges in the PS structure promote serum and membrane protein mediated attachment of adherent cells. Conversely, suspension cultures of e.g. embryoid body derived neurospheres need culture surfaces with low binding capacity for proteins and cells. To meet such demands, some manufacturers increased the naturally high hydrophobic characteristics of PS culture plates, preventing interactions of water soluble substances and cells with the substrate. The BRAND GMBH + CO KG and other companies have developed super hydrophilic surfaces based on hydrogels. These hydrogels establish an isolating water film in aqueous solutions, which strongly suppress interactions of cells or proteins with the culture plastic. This technical note shows that the newly hydrogel derived inertGrade™ surface efficiently inhibits cell attachment and thereby prevents unintended differentiation processes of neurospheres.

Murine neuronal stem cells (NSCs) isolated from embryonic brain at embryonic day 13.5 were cultured according to "in vitro Proliferation and Differentiation of Mouse Neuronal Stem Cell (Neurospheres)" protocol (Stem cell technologies, #28704). After dissociation, an equal number of cells were seeded into 96 well microplates for suspension culture to compare the BRAND*plates*® inertGrade™ and an extra hydrophobic PS surface in supporting neurosphere formation and maintenance.



Neurosphere formation and growth on 96 well BRAND*plates*® with inertGrade™ surface (left panel) and on an extra hydrophobic surface (right panel) reveal strong repulsive effect of the hydrogel. Representative phase contrast images of neurospheres after 1 and 3 days in vitro show embryonic brain derived neurospheres. Scale bar 100 µm

Neurosphere formation and growth on 96 well BRAND*plates*® with inertGrade™ surface (left panel) and on an extra hydrophobic surface (right panel) reveal strong repulsive effect of the hydrogel. Representative phase contrast images of neurospheres after 1 and 3 days in vitro show embryonic brain derived neurospheres. Scale bar100 µm NSCs cultured on the inertGrade™ surface for 24 h show no detectable cell attachment (upper left) whereas an extra hydrophobic PS surface of a competitor microplate did not prevent NSCs attachment to the culture plate. After 72 h in culture, the inertGrade™ surface still kept neurospheres in suspension (lower left) leading to normal sphere formation. While on the extra hydrophobic PS surface nearly all cells attached to the culture plate and showed signs of differentiation and less sphere formation.

This result demonstrates that the BRAND*plates*® inertGrade™ surface efficiently impede cell attachment resulting in higher yields of neurospheres for further experimental processing.

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