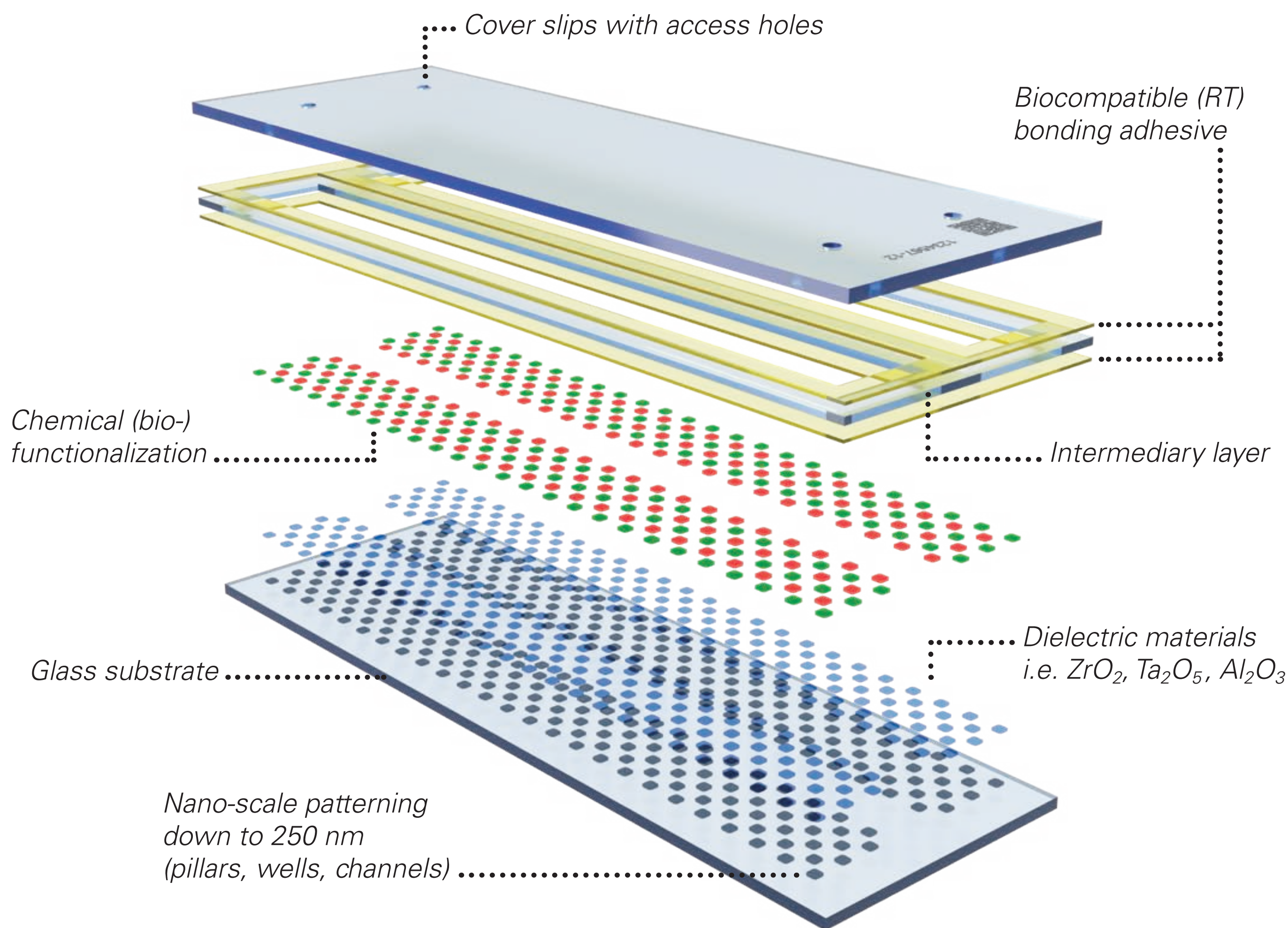
Application Note



Microfluidic materials with confirmed biocompatible performance

HEIDENHAIN | IMT

Introduction

According to the IUPAC definition, biocompatibility is the ability to be in contact with a living system without producing an adverse effect. Thus, it is an important quality property of devices and materials that are in direct or indirect contact with biomaterials such as living cells.

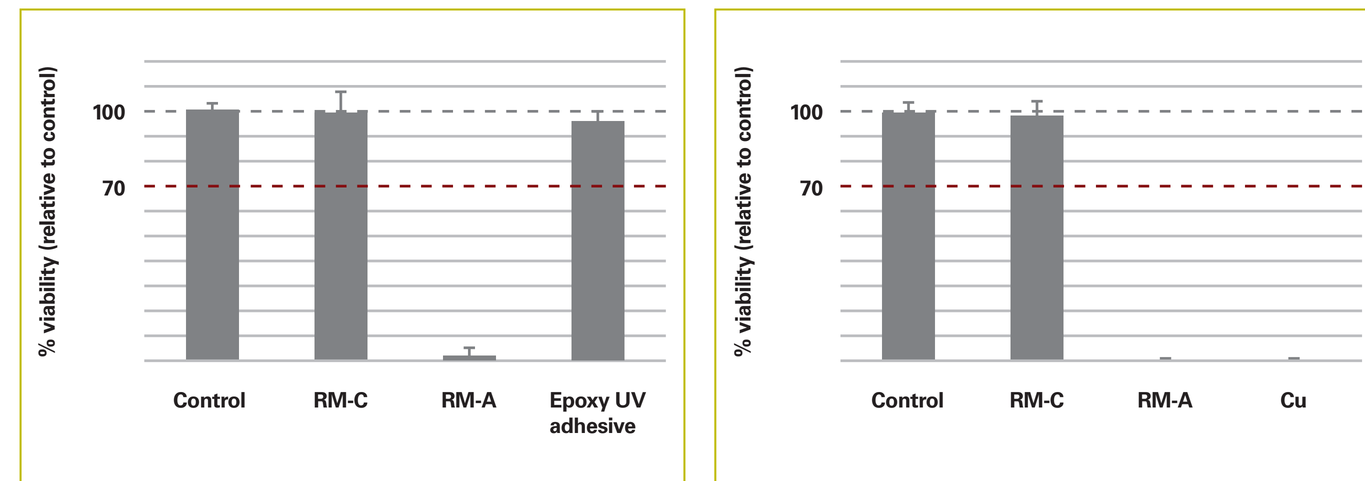
DIN EN ISO 10993-5:2009 describes a systematic procedure for in vitro cytotoxicity tests to ensure the biocompatibility of medical devices.

Here we describe a biocompatibility quality test procedure for materials that are utilized for the production of microfluidic devices. Our in-house testing ensures that all materials used in the HEIDENHAIN manufacturing processes are fully compatible with biological applications utilizing cells.

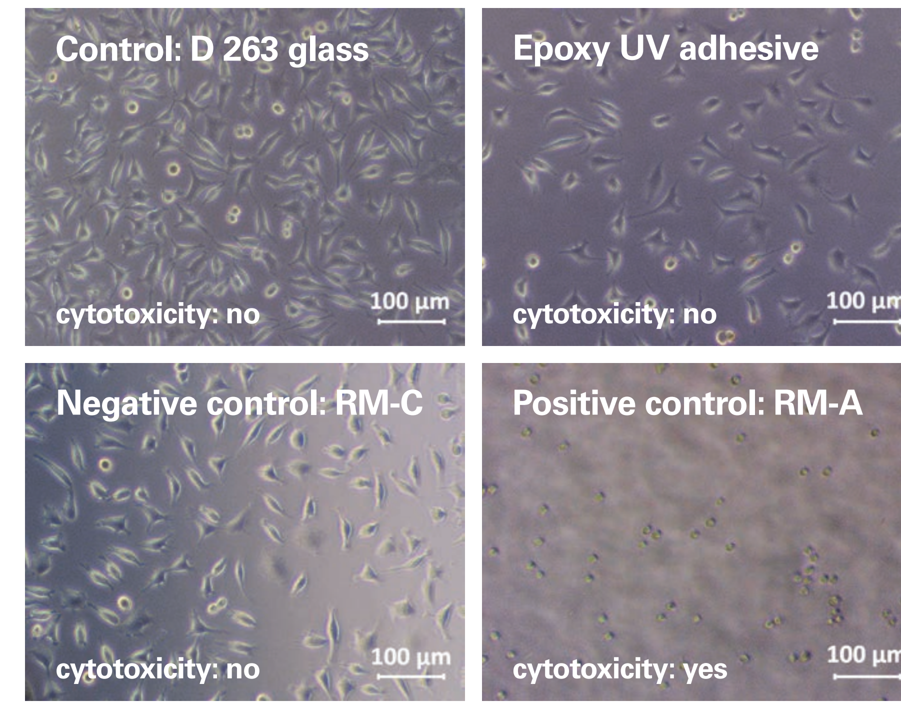
Our results show that direct contact with the microfluidic device materials will not reduce cell viability. For the Direct Contact Tests cells were directly seeded on tested material and control slides at the same density. Furthermore, the Extract Test demonstrates that materials within the microfluidic devices do not release cytotoxic substances into the fluid stream, which may cause downstream contamination or influence cell viability. For the Extract Tests cells were cultured together with extracts of control slides and test materials that were tested positive for cytotoxicity in direct contact tests.

Results

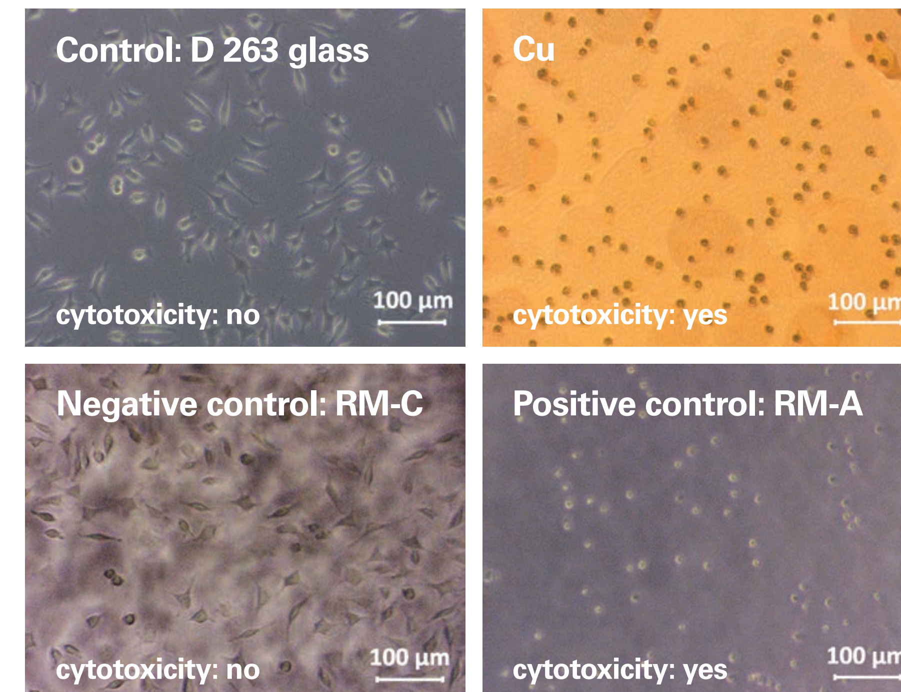
According to DIN EN ISO 10993-5:2009, materials do not exhibit cytotoxic potential if the quantitative measured cell viability is more than 70 % in comparison to control samples. Below, detailed result interpretations are shown using an example of a non-cytotoxic and a strongly cytotoxic material.



Detailed results for direct contact tests of a non-cytotoxic and a strongly cytotoxic material. The results of the biochemical assay according to DIN EN ISO 10993-5:2009 demonstrate that the epoxy UV adhesive shows no cytotoxic effect (left) whereas culturing cells with copper as expected led to complete cell death (right).



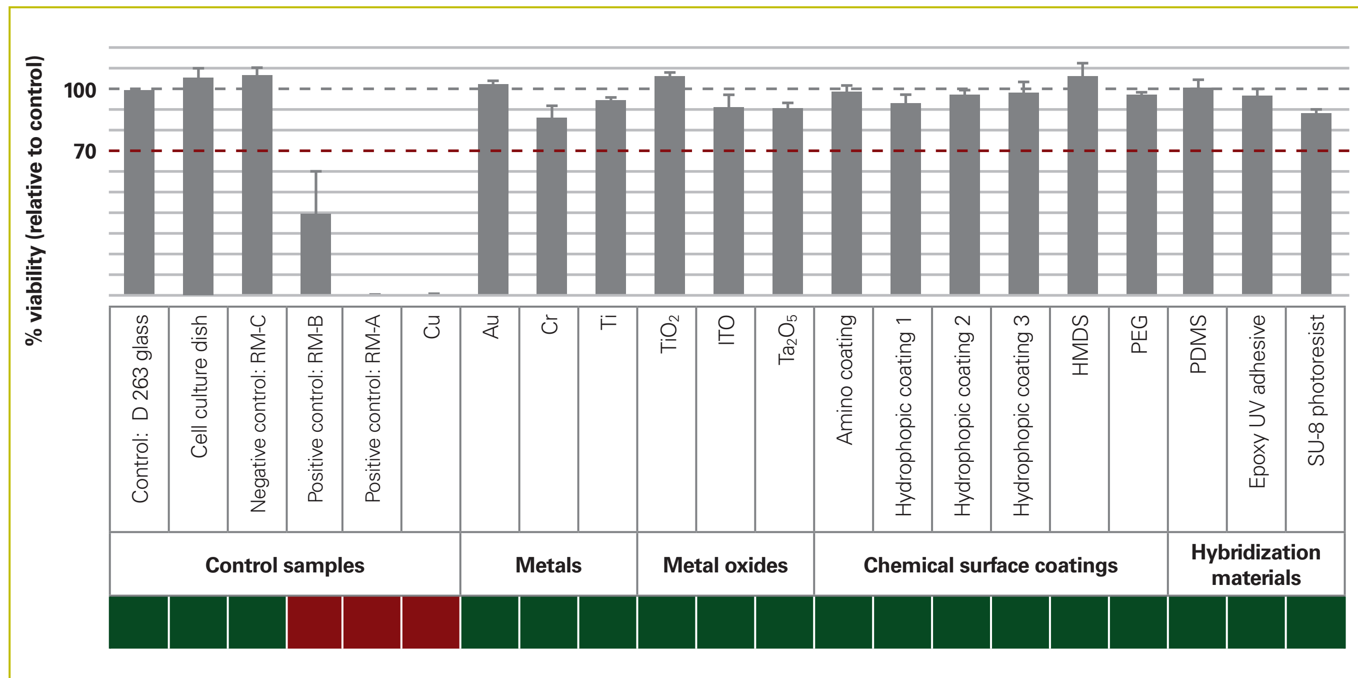
Optical inspection of direct contact test for a non-cytotoxic material. The optical inspection confirms the results of the biochemical assay. Cells cultured on the control slide, the epoxy UV adhesive, and cells cultured with the negative control RM-C[1)] show unremarkable morphology whereas culturing cells with the positive control RM-A[2)] as expected led to morphological changes and cell death.



Optical inspection of direct contact test for a cytotoxic material. In comparison to controls, metallic copper exhibits strong cytotoxic effects as expected. Control cells and cells cultured with RM-C[1)] showed normal cell growth and morphology while culturing cells on metallic copper led to cell death as well as culturing them together with the positive control RM-A[2)].

[1)] RM-C is a polyethylene film that is inert to living cells and thus does not exhibit a cytotoxic effect.

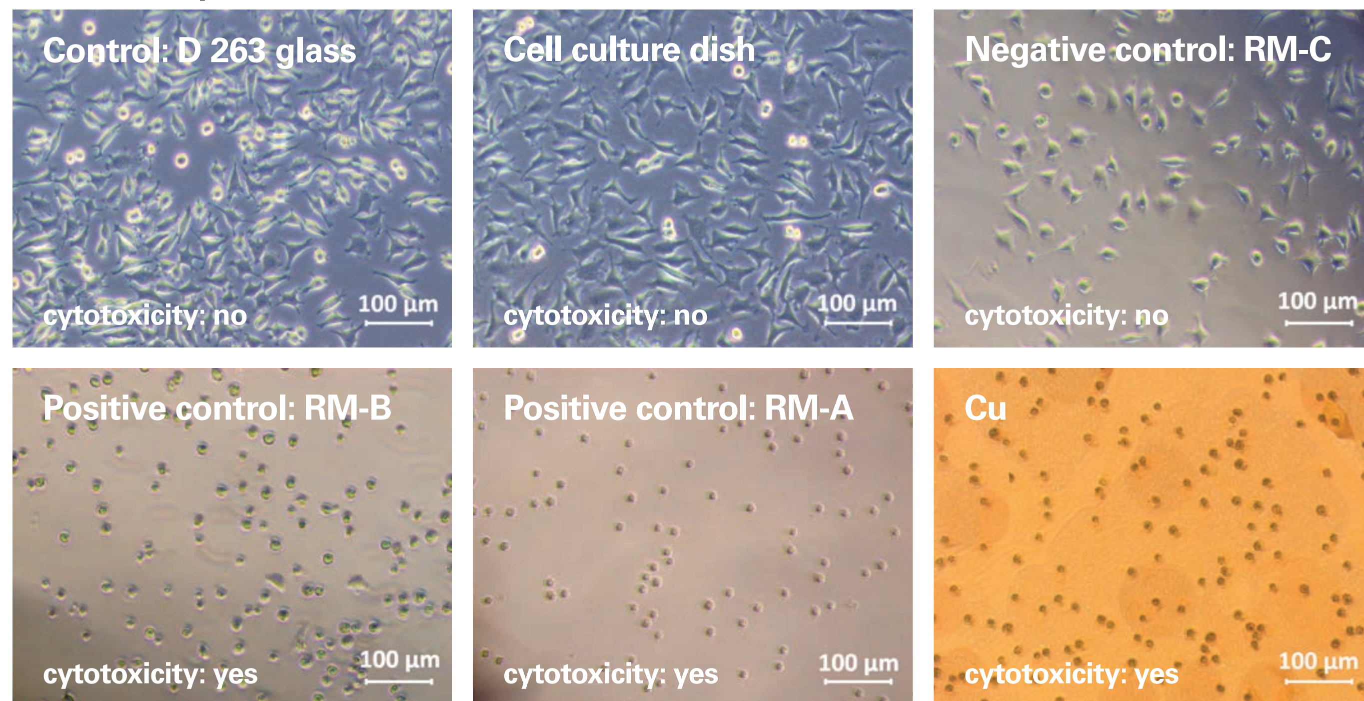
[2)] RM-A is a polyurethane film containing 0.1% zinc diethyldithiocarbamate. Dithiocarbamates are, for example, used as fungicides and work as heavy metal chelators. The cytotoxic effect is caused by inhibition of enzymes due to binding of the chelating agent to their metal ion.



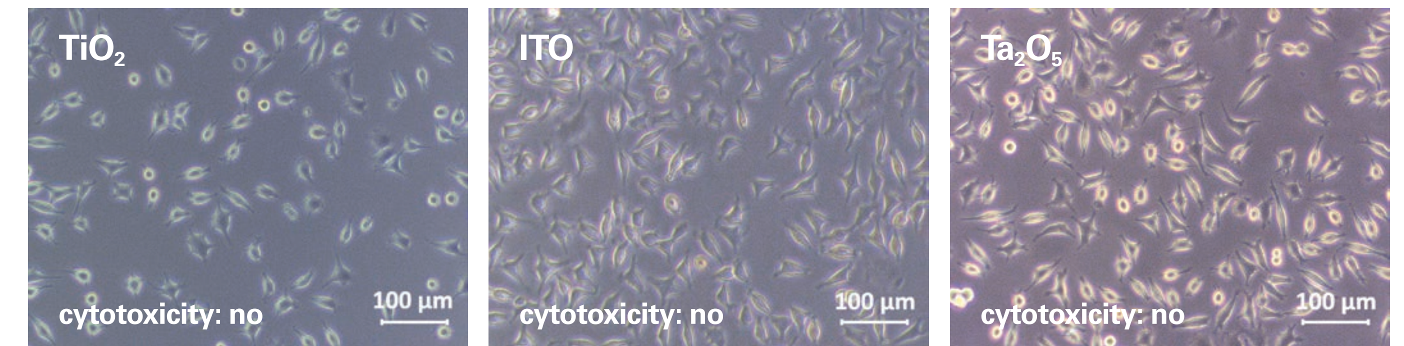
Overview graph of direct contact tests for all tested materials. The use of a quantitative biochemical assay to demonstrate cell viability indicates all tested materials do not have cytotoxic effects (marked green) with the exception of reference material A and reference material B[3] and metallic copper (marked red) that was used as example for a material that expectedly exhibits strong cytotoxicity and that is processed the same way as other metallic/dielectric coatings.

[3] RM-B is a polyurethane film containing 0.25 % zinc dibutyldithiocarbamate that can be used as control for moderate cytotoxicity.

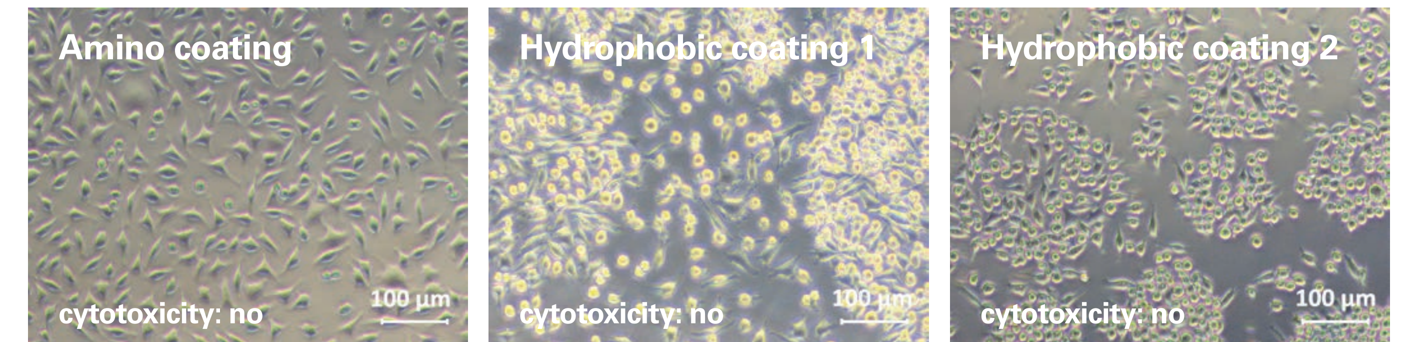
Control samples

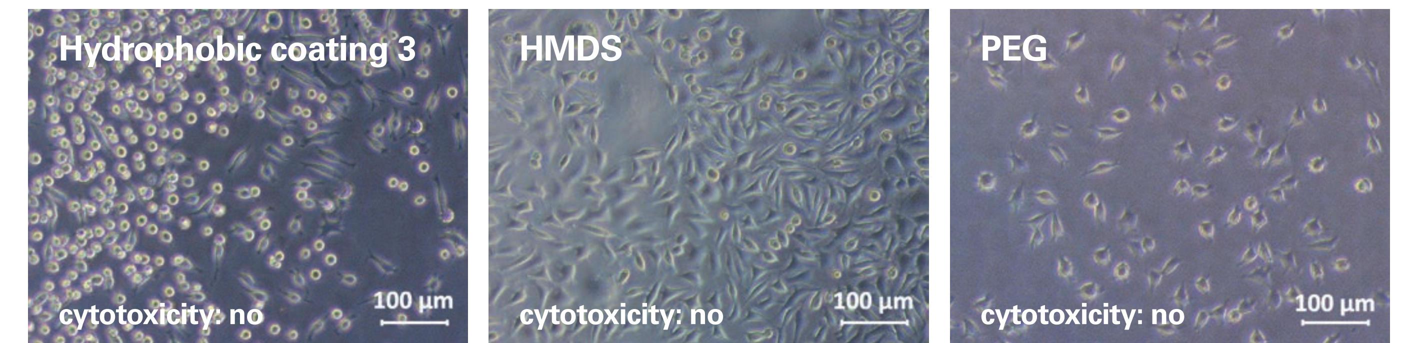


Metal oxides

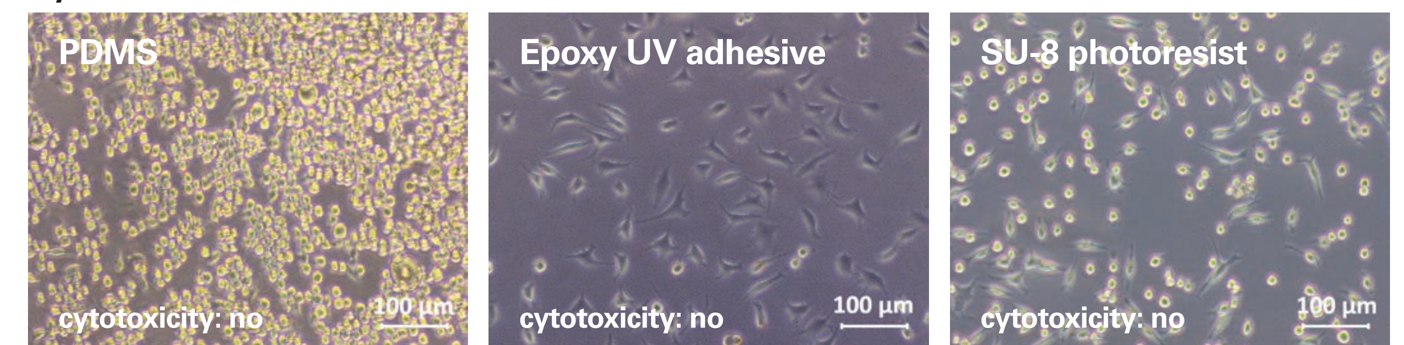


Chemical surface coatings

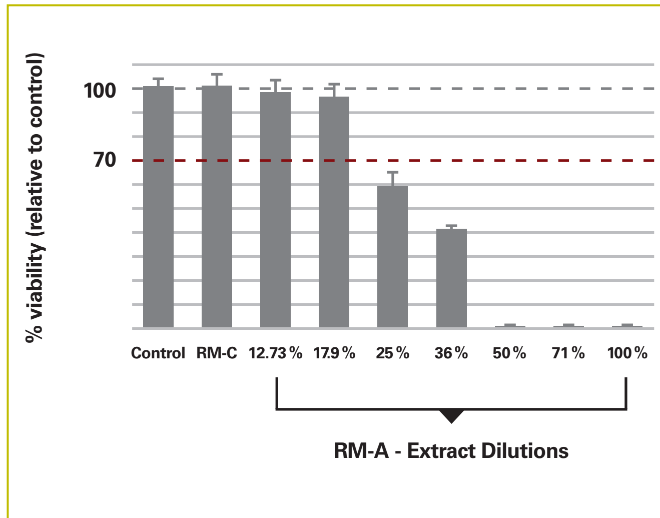


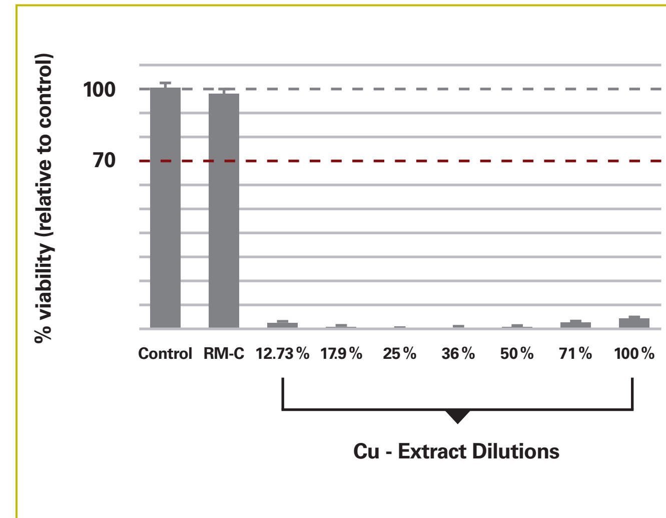


Hybridization materials



Optical inspection of all tested transparent materials. The qualitative optical inspection confirmed the results of the biochemical assay for transparent materials. Cells cultured on tested hydrophilic surfaces showed unremarkable cell growth and morphology. Cells cultured on hydrophobic surfaces were partly detached. However, the cells were not influenced by this detachment with respect to cell viability. Culturing cells with positive controls RM-A and RM-B as well as metallic copper as expected led to cell shrinkage and cell death.





Detailed results for extract test of a cytotoxic material. Extract tests were performed only for materials that tested positive for cytotoxicity in direct contact tests to determine if the material would also have downstream effects due to leaching or extraction of cytotoxic agents. In order to obtain knowledge about the relationship between extract concentration and cytotoxic effects, a serial dilution of positive control RM-A extracts (left) was compared to a serial dilution of test material extract, here using the example of metallic copper (right). Cytotoxic potential of RM-A extract dilutions decreases with decreasing concentration, while in the case of metallic copper, the highest dilution also exhibits a strong cytotoxic effect. The results indicate that copper ions pass into solution due to leakage and that they exhibit very strong cytotoxic potential even in low concentrations.

Conclusion

The established quality test procedure in accordance with biocompatibility testing of DIN EN ISO 10993-5:2009 for the HEIDENHAIN microfluidic manufacturing process demonstrates

- confirmed biocompatibility
- no interference with cell viability
- no contamination of the microfluidic application by cytotoxic substances

of all tested metals, metal oxides, chemical surface coatings, and hybridization materials.

The product line microfluidics includes customized micro- and nano-patterns and structures in glass, integration of electrodes, waveguides and structured functionalization for life science applications. We provide flexible offerings from design consultancy and prototyping to scalable manufacturing.

Overview Table

Material	Category	Description	Result - Cytotoxicity
Control	Control samples	Uncoated D 263 glass	no
Cell culture dish		Polystyrene culture dish for cell culture maintenance	no
RM-C (Negative control)		High density polyethylene film (PE)	no
RM-B (Positive control)		Polyurethane film containing 0.25 % zinc dibutyldithiocarbamate (ZDBC)	yes
RM-A (Positive control)		Polyurethane film containing 0.1 % zinc diethyldithiocarbamate (ZDEC)	yes
Cu		Copper: Positive control for metallic surface coatings	yes
Au	Metals	Gold: Bioinert surface coating used for e.g. electrodes, the adhesion of biological active cells, Surface Plasmon Reflectometry, microarrays or scanning probe microscopy	no
Cr		Chromium: Corrosion resistant surface coating used e.g. for electrodes, optical apertures or as adhesive layer for gold	no
Ti		Titanium: Electrode material or adhesive layer for gold	no
TiO_2	Metal oxides	Titanium dioxide: Electrode material	no
ITO		Indium tin oxide: Transparent electrode	no
Ta_2O_5		Tantalum pentoxide: Dielectric material used as waveguide	no
Amino coating	Chemical surface coatings	Coupling group for biomolecule immobilization	no
Hydrophobic coating 1		Organic low surface energy coatings	no
Hydrophobic coating 2			no
Hydrophobic coating 3			no
HMDS		Hexamethyldisilazane: No functionality, adhesion promotor	no
PEG		Polyethylene glycol: anti-fouling/anti-adhesive organic polymer	no
PDMS	Hybridization materials	Polydimethylsiloxane: Biocompatible silicon-based polymer used in academia for rapid prototyping of microfluidics	no
Epoxy UV adhesive		UV-cured epoxy resin (glue) for bonding of wafers	no
SU-8 photoresist		Negative photoresist used as an intermediary layer	no

Materials & Methods

Cytotoxicity tests have been conducted using L929 mouse fibroblast cells recommended in DIN EN ISO 10993-5:2009 (Sigma-Aldrich Corp., USA) that were either cultivated in direct contact with the materials to be tested or were exposed to extracts from the test materials. Cells were cultured in minimum essential medium (supplemented with 10 % FBS) in a humidified atmosphere at 37 °C and 5 % CO_2. All materials to be tested were provided as flat surface coating on D 263 glass (Schott AG, Germany). As positive control for the cytotoxicity tests, the reference material RM-A was used. Reference material RM-C was used as negative control (Hatano Research Institute, Japan). RM-A and RM-C were placed in the culture vessel on D 263 glass prior to the addition of cell suspension or extraction medium. The health/viability of the cells was determined with the help of a standardized biochemical assay. In this study the biochemical luminescence assay CellTiter-Glo® 2.0 (Promega Corp., USA) according to § 8.5 of DIN EN ISO 10993-5:2009 was used. CellTiter-Glo® 2.0 lyses cells and quantitates the present ATP level, which is directly proportional to cell viability. According to DIN EN ISO 10993-5:2009, materials do not exhibit cytotoxic potential if the measured cell viability is more than 70 % in comparison to control.

Direct Contact Tests

Cells were directly seeded on tested material and control slides at the same density. Cells on D 263 glass served as 100 % viability control. All slides were cultured for approximately 24 hours. In order to examine cells to identify changes in cell morphology of control and treated cells, a qualitative optical inspection was performed using a phase contrast microscope (AxioVert.A1, Carl Zeiss GmbH, Germany). For determination of cell viability, Cell Titer Glo 2.0® assay was added to each well. After incubation luminescence could be read in a plate reader (Cytation 5, BioTek Instruments GmbH, Germany) and relative viability was calculated.

Extract Tests from surfaces of test materials

In order to prepare extracts of test materials, eight-well ibidi sticky-Slides (ibidi GmbH, Germany) were stuck on D 263 glass surface coated with test material and cell culture medium was added to each well. The amount of added extraction medium was calculated from a defined surface to volume ratio (according to § 10.3.3 of DIN EN ISO 10993-12:2012). After 24 hours of test material incubation, cell culture medium of prepared L929 mouse fibroblast cells was replaced by the extraction medium. Before addition, extracts of RM-A and test materials were diluted serially with fresh cell culture medium. Cells that served as 100 % viability control were treated with fresh, pre-warmed medium. Treated cells were cultured for another approximately 24 hours. After that, cell viability was determined by means of CellTiter-Glo® 2.0 assay, according to the manufacturer's instructions.

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