Technical Note

Functionalized microfluidic devices without impact on DNA integrity

Abstract

HEIDENHAIN microfluidic devices are made from glass and are fabricated with a large variety of patterned surface modifications and customized designs depending on the intended use and experimental needs. The manufacturing process of these highly customized devices includes not only the structuring of glass, but also steps such as UV bonding and patterning of chemical surface functionalization (see Figure 1). With respect to applications such as DNA amplification (polymerase chain reaction, PCR), molecular diagnostics based on nucleic acids, or novel sequencing technologies (i.e., next generation sequencing, NGS), it is critical that all components of the microfluidic devices have no influence on DNA integrity.

In this Technical Note, we show DNA integrity test results of chemical surface coatings and assembly materials. The testing procedure ensures that the materials and coatings used in the HEIDENHAIN microfluidic manufacturing process are fully compatible with DNA-related applications.

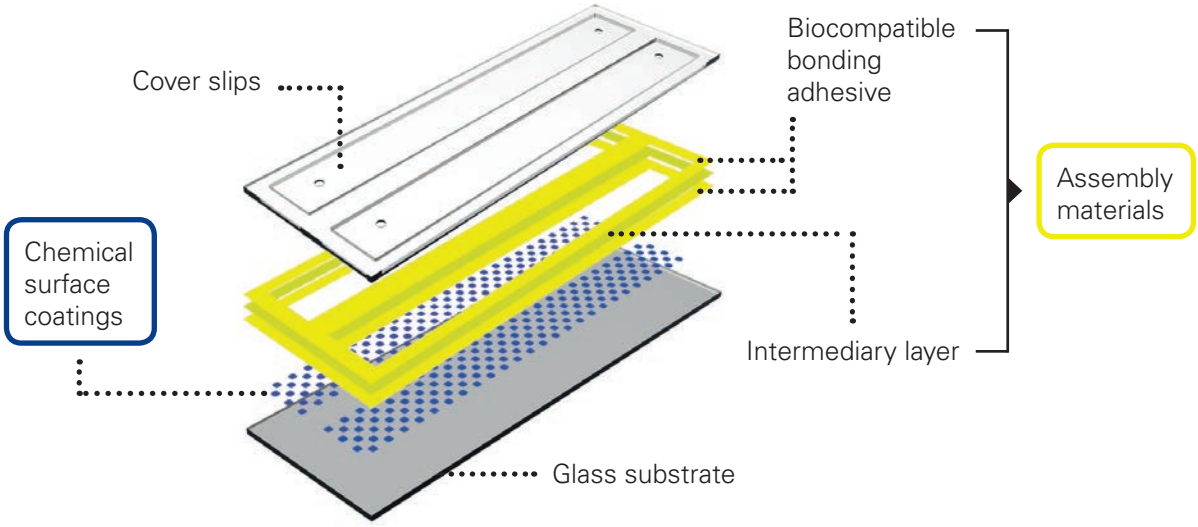

Figure 1. Exemplary illustration of the HEIDENHAIN microfluidic manufacturing process. The HEIDENHAIN microfluidic manufacturing process includes patterned chemical surface coatings of glass substrates. Furthermore, UV bonding is used in order to generate enclosed microfluidic flow cells (for further information, see the HEIDENHAIN Technical Note *"Biocompatible and room temperature bonding solution for microfluidic devices"*). These production steps together with other flexible process offerings enable highly customized microfluidic solutions. According to the high quality standards of HEIDENHAIN, none of the utilized surface coatings (blue) nor assembly materials (yellow)—such as SU-8 photoresist as intermediary layer or the epoxy UV adhesive for bonding—have an impact on DNA integrity. Thus, it is ensured that HEIDENHAIN microfluidic devices are highly suitable for DNA-related applications.

Results

In order to identify the influence of chemical surface coatings and assembly materials on DNA integrity, coated glass substrates were incubated with genomic DNA (isolated from HeLa cells). D 263® T eco glass that serves as standard substrate for HEIDENHAIN microfluidic devices was used as a negative control. As glass is chemically inert, it does not have any degrading or damaging properties to DNA. Copper-coated glass served as positive control, as it has strong DNA-damaging properties. The results of the tested materials were compared to the results of the controls (see Figure 2).

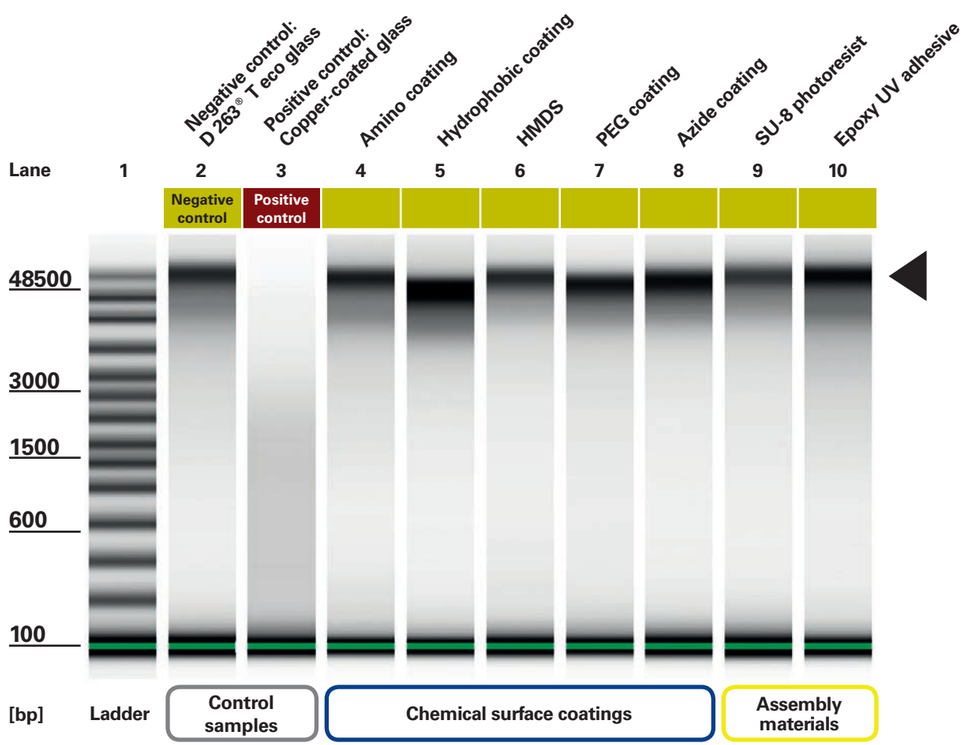

Figure 2. Influence of all tested materials on DNA integrity. The DNA bands are indicated with the arrow, intact DNA is marked in green and degraded DNA is marked in red. As expected, the DNA incubated with the positive control (copper-coated glass) shows a highly blurred band (Lane 3), indicating a strong DNA-degrading effect. As the DNA integrity is not reduced for the tested materials (Lane 4 to 10) when compared to the negative control D 263® T eco glass (Lane 2), the results demonstrate that the materials utilized in the HEIDENHAIN manufacturing process have no DNA-damaging or -degrading effect.

Overview Table of Test Results

Material	Category	Description	Impact on DNA integrity
Negative control: D 263® T eco glass	Control samples	Uncoated D 263® T eco glass: negative control	no
Positive control: Copper-coated glass		Copper-coated glass: positive control	yes
Amino coating	Chemical surface coatings	Coupling group for biomolecule immobilization	no
Hydrophobic coating		Organic low surface energy coating	no
HMDS		Hexamethyldisilazane: no functionality, adhesion promoter	no
Azide coating		Coupling group for other molecules	no
PEG coating		Polyethylene glycol: anti-fouling/anti-adhesive organic polymer	no
SU-8 photoresist	Assembly materials	Negative photoresist used as an intermediary layer	no
Epoxy UV adhesive		UV-cured epoxy resin (glue) for bonding of wafers	no

Conclusion

The established test procedure for the HEIDENHAIN microfluidic manufacturing process demonstrates

- no effect on DNA integrity
- no DNA-damaging properties

of all tested chemical surface coatings and assembly materials.

The HEIDENHAIN microfluidics product line 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. For more information, please visit our life-science website **www.heidenhain.us/lifesciences.**

Materials & Methods

The cervix carcinoma cell line HeLa (Sigma-Aldrich Corp., USA) was cultured in minimum essential medium (supplemented with 10 % Fetal Bovine Serum, FBS) in a humidified atmosphere at 37 °C and 5 % CO_2. The cells were harvested and genomic DNA was isolated with QIAamp DNA Micro Kit (QiaGEN, Netherlands) according to the manufacturer's instructions.

All tested materials were provided as flat surface coating on D 263® T eco glass (Schott AG, Germany). Copper-coated D 263® T eco glass was used as a positive control because copper has strong DNA-damaging properties. The presence of copper ions increases the presence of reactive oxygen species (ROS) and thus leads to DNA degradation (Cervantes-Cervantes *et al.,* 2005). The substrate (D 263® T eco glass) was used as negative control as glass is chemically inert and thus reliably has no influence on the integrity of DNA.

Chambers were adhered to control and test surfaces and HeLa DNA solution (40 ng/µL) was filled into wells. All tested materials were incubated for ~ 24 hours at room temperature. Afterward, the integrity of the incubated DNA was determined with the help of an automated electrophoresis system (Agilent 4200 TapeStation system, Agilent Technologies Inc., USA). The result of each test procedure was a digital gel image. DNA integrity was determined by optical inspection of the resulting DNA bands.

References

2020, HEIDENHAIN, Technical Note: Biocompatible and room temperature bonding solution for microfluidic devices

2005, Cervantes-Cervantes *et al.,* Copper increases the damage to DNA and proteins caused by reactive oxygen species, Biological Trace Element Research, 103(3), 229-48.

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