Technical Note

Biocompatible and room-temperature bonding solution for microfluidic devices

Abstract

Microfluidic devices rely on an efficient bonding process to generate enclosed microfluidic flow cells. The right choice of connection technology and bonding material plays a crucial role. HEIDENHAIN offers a complete solution for the production of flow cells for life science applications, including quality control. In this technical note, we present a room-temperature ultraviolet-A (UV-A) cured adhesive technology that meets all requirements necessary for robust bonding of glass components for bioanalytical applications. Here we show the results of relevant physical and biological quality controls. The low-temperature bonding process enables a tight sealing of microfluidic devices, which remains well preserved without leakage even under high pressure conditions. The bonding strength remains highly stable even when exposed to wide and alternating temperature ranges, allowing temperature-critical processes like sterilization or polymerase chain reaction (PCR) techniques. The UV-cured adhesive bonding material is compatible with biological and life science applications. This was demonstrated by a cytotoxicity test according to DIN EN ISO 10993-5:2009 and a DNA integrity test. Furthermore, biochemical pre-patterning or chemically functionalized lattice structures on glass components remain functional after the bonding process, shown by pre-immobilization of oligonucleotides.

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Results

In order to demonstrate the durable and biocompatible bonding achieved by the UV epoxy adhesive, a microfluidic set of physical and biological studies was designed and performed. The fortitude of the epoxy adhesive bonding strength was evaluated in standard as well as extreme microfluidic application conditions. Burst pressure, leakage, and thermal stability tests were performed under standard operating conditions, using a HEIDENHAIN designed flow cell (see Figure 1). The respective UV-cured epoxy material was tested in a cell viability assay according to DIN EN ISO 10993-5:2009 to exclude cytotoxic effects. Two sets of UV epoxy-specific compatibility tests for DNA applications were included in the studies. Following a direct contact test with the UV-cured adhesive material, DNA integrity was verified and genomic DNA was amplified in a microfluidic-based PCR. To ensure bio-functionality of pre-immobilized oligonucleotides after the bonding process, a hybridization experiment was conducted.

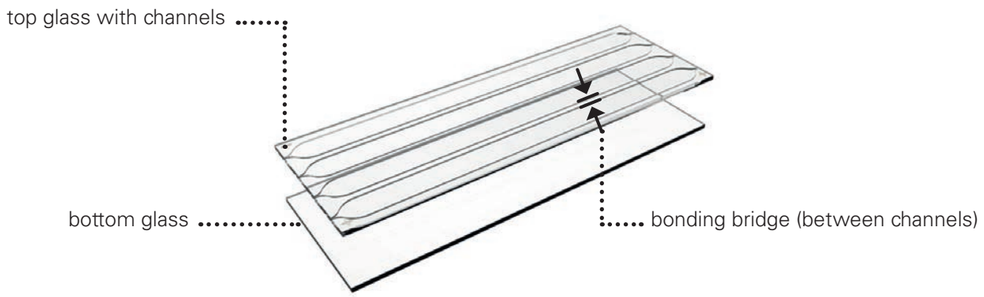

Figure 1. Exploded view drawing of a HEIDENHAIN designed and UV low-temperature bonded microfluidic flow cell. The design was used to perform physical and thermal stability tests of the epoxy UV-bonded microfluidic glass devices. The dimensions of the glass device (D 263® T eco glass) were 75.5 x 25.5 x 0.7 mm with a bonding bridge of 500 µm.

Burst pressure and leakage testing before and after thermal treatment

Microfluidic applications are typically integrated into systems where the reaction fluids are processed under varying pressure settings. The bonding needs to be sturdy for the specific maximum pressure conditions as well as for varying pressures throughout an analysis run. The burst pressure of microfluidic glass devices highly depends on the bonding bridge dimensions (see Figure 1). Pressurizing the microfluidic channel with air revealed a highly resistant UV epoxy bonding within the glass device, since the UV-bonded microfluidic device could operate up to a pressure value of 7 bar without exhibiting any breaking or leakage.

For testing the thermal stability of the epoxy UV bonding, the microfluidic device was exposed to the following thermal conditions:

- Sterilization process at 121 °C for 20 minutes
- Long-time high-temperature incubation at 100 °C for 60 minutes (representative as a temperature maximum for most PCR applications)

The experiments were performed to study the effect of high-temperature working conditions on the epoxy UV-cured bonding. A subsequent burst pressure analysis showed no decline in the compressive strength after thermal treatment at up to 7 bar air pressure. The impact of high temperatures on the bond strength was considered minor. Because no bonding leakage in the bond interface occurred after heat exposure, the bond strength is considered sufficient for biological applications and assays.

Cytotoxicity testing

The results of an *in vitro* cytotoxicity and cell viability test according to DIN EN ISO 10993-5:2009 demonstrate that the tested UV-cured epoxy bonding compound does not exhibit cytotoxic effects on living cells. For further information, see the HEIDENHAIN Application Note: *"Microfluidic materials with confirmed biocompatible performance".*

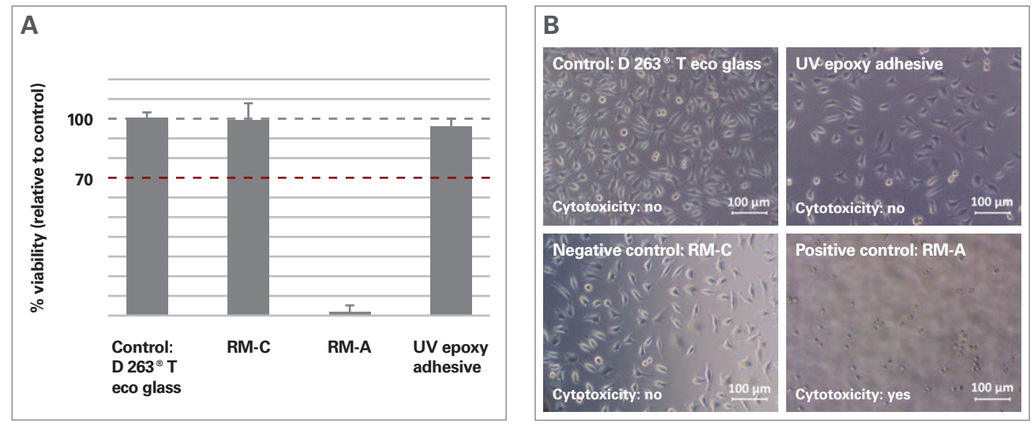

Figure 2. Cytotoxicity test of the UV-cured epoxy adhesive compound. **A.** The measured viability of cells cultured with UV-cured epoxy adhesive showed more than 70 % (which is the threshold for cytotoxicity according to DIN EN ISO 10993-5:2009) in comparison to the control sample. **B.** The quantitative cell viability assay was confirmed by qualitative microscopic images. Cells cultivated with epoxy UV-cured adhesive did not show any differences in morphology compared to cells on the uncoated control as well as cells cultivated with RM-C[1] , while cells cultivated with toxic RM-A[2] showed cell death as positive control.

[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.

DNA integrity testing
The integrity of the tested genomic HeLa DNA is still fully given after incubation with the UV-cured epoxy adhesive bonding compound. For further information, see the HEIDENHAIN Application Note: *"Functionalized microfluidic devices without impact on DNA integrity".*

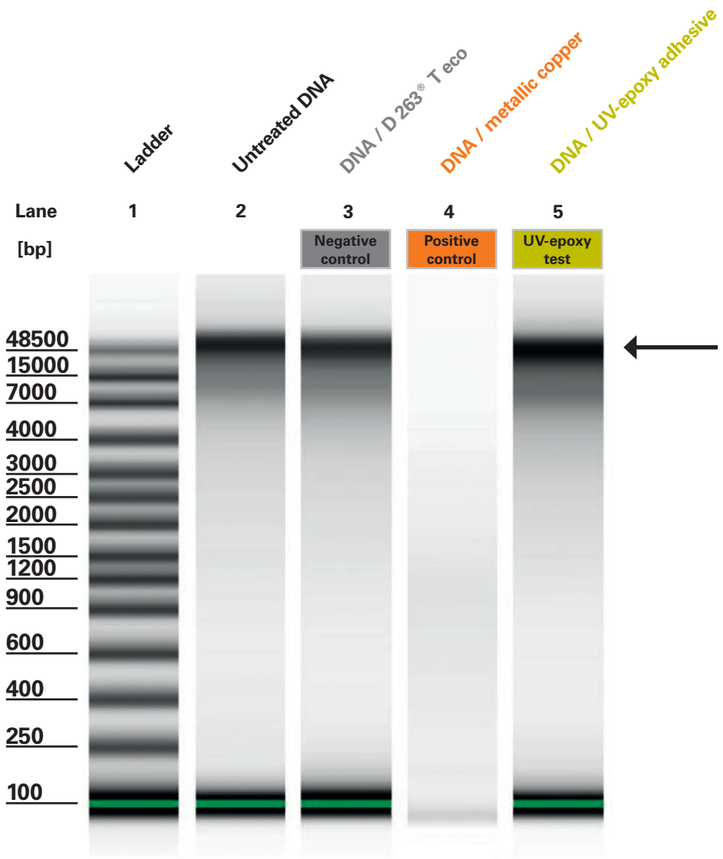

Figure 3. DNA integrity before and after direct contact with the UV-cured bonding compound. A base pair specific analysis and comparison between untreated genomic HeLa DNA (length ~48500 base pairs) and UV-cured epoxy-treated genomic HeLa DNA revealed no loss of DNA integrity after incubation with the UV-cured epoxy bonding material (indicated by the arrow). The treated DNA sample, shown in lane 5, is almost identical to the untreated DNA sample, shown in lane 2. After incubation of the input DNA with non-damaging D 263® T eco glass as negative control, the DNA is highly intact, which is displayed in lane 3. DNA incubated with damaging metallic copper (DNA / copper-coated D 263® T eco) appears as a smear indicating highly degraded DNA (see lane 4). The metallic copper exhibits a strong toxic effect on DNA.

Life science application: DNA amplification
In order to demonstrate both the biocompatibility and the exceptional strength of the bonding, an all-glass flow cell device bonded by UV adhesive was successfully used to perform an on-chip PCR under standard operating parameters.

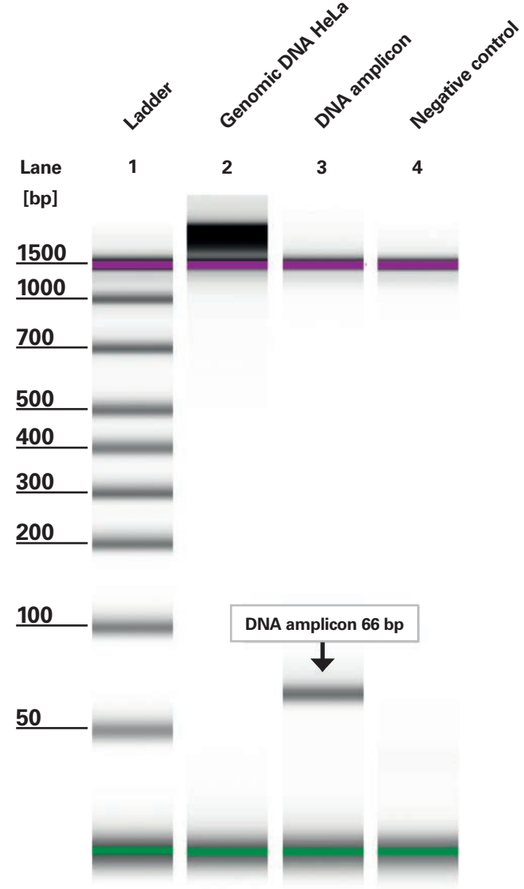

Figure 4. DNA amplification inside the channel of a low-temperature bonded device. The amplification of genomic HeLa DNA and the verification of the selected 66 bp amplicon (see lane 3) validated the UV epoxy adhesive bonding compound as compatible for biomedical applications, even under standard life science application operating conditions. The image displays the DNA ladder in lane 1 and the genomic DNA of the HeLa cell line as input for the amplification in lane 2. Since genomic HeLa DNA has considerably more than 1000 base pairs, it is out of range relative to the selected assay. The synthesized PCR product after amplification is shown in lane 3 and the negative control in lane 4.

Life science application: low-temperature bonding after pre-immobilization of target oligonucleotides
In addition to the presented set of physical and biological testing procedures, the described UV adhesive material has already been successfully applied in life science applications for an integration of structured bio-functionalization into microfluidic channels utilizing UV bonding. The specialized low-temperature UV-cured bonding method enables sealing of microfluidic glass devices in the presence of pre-immobilized oligonucleotides and other bio-functionalization, maintaining their integrity during the fabrication process. For further information, see the CSEM Scientific and Technical Report 2019: *"Disposable Glass Microfluidics for Nucleic Acids Bioassays",* CSEM SA and IMT AG, Switzerland[3].

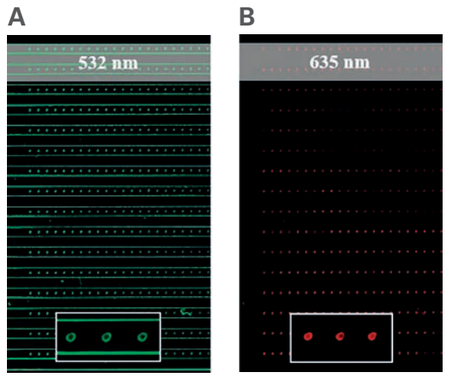

Figure 5. Hybridization assay of pre-spotted oligonucleotides in the sealed device. A. The glass device was characterized using fluorescently labelled oligonucleotides (preserved green target spots, magnified in insert) being immobilized in the microfluidic channel. **B.** A hybridization assay was performed in the sealed microfluidic device with an efficient pairing of the analyzed probe (red spots, magnified in insert) showing no non-specific binding.

[3] https://www.csem.ch/Doc.aspx?disp=yes&id=128412&name=CSEM-STR-2019-p56.pdf

Conclusion

The evaluated epoxy UV adhesive bonding method shows remarkable benefits compared to other bonding methods commonly used by the microfluidics community. The low-temperature bonding enables a tight sealing of microfluidic channels, which remains well preserved without leakage even under high pressure conditions up to 7 bar. Additionally, the bonding strength remains highly stable even when exposed to wide temperature ranges higher than 100 °C, allowing temperature-based sterilization processes and PCR cycling procedures. Characterizing the cytotoxic potential of the adhesive demonstrates it is a non-toxic material for living cells and other bio-materials. We have also demonstrated the UV-curing adhesive bonding solution to be compatible with life science applications by successfully performing an on-chip PCR, and bonding the channels in the presence of pre-immobilized oligonucleotides with no loss of integrity.

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

Burst pressure and leakage testing
Burst pressure tests and leakage tests of the device sealed by epoxy UV adhesive bonding were carried out using an MFCS™-EZ microfluidic control system (Fluigent, France). The microfluidic devices to be tested were connected to the control system and placed in a water bath. Pressure ranges from 0 mbar to 7000 mbar were applied and increased stepwise until a leakage or breakdown of the glass devices occurred or the maximum pressure was reached. For testing the thermal stability, the microfluidic glass devices were autoclaved at 121 °C for 20 min as well as incubated at 100 °C for 60 min. After heat exposure, burst pressure tests and leakage tests were repeated and compared to untreated glass devices.

Cytotoxicity testing
Cytotoxicity tests have been conducted using L929 mouse fibroblast cells recommended in DIN EN ISO 10993-5:2009 (Sigma-Aldrich Corp., USA) that were cultivated in direct contact with the material to be tested and the control slides at the same density. Cells on D 263® T eco glass served as 100 % viability control. All slides were incubated for ~24 hours. The epoxy UV bonding material was provided as flat surface coating on D 263® T eco 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). 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) was used according to § 8.5 of DIN EN ISO 10993-5:2009. After incubation, luminescence could be read in a plate reader (Cytation 5, BioTek Instruments GmbH, Germany) and relative viability was calculated. 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. 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 detailed information, see the HEIDENHAIN Application Note: *"Microfluidic materials with confirmed biocompatible performance".*

DNA integrity testing
After 24 h of incubation with the UV-cured adhesive at room-temperature, an analysis of the genomic DNA analyte (HeLa cell line, Sigma-Aldrich Corp., USA) was performed by an automated electrophoresis tool (Agilent 4200 TapeStation system, Agilent Technologies, USA). The system provides a reliable tool for the assessment of the integrity of the respective genomic DNA. A digital gel image is supplied allowing the visual determination of the DNA integrity. A negative control (DNA incubation with non-damaging D 263® T eco glass) as well as a positive control (DNA incubation with damaging metallic copper coated D 263® T eco glass) were included in the studies. For detailed information, see the HEIDENHAIN Application Note: *"Functionalized microfluidic devices without impact on DNA integrity".*

Life science application: DNA amplification
The genomic DNA of the human cell line HeLa (Sigma-Aldrich Corp., USA) was used as the DNA template and an assay targeting the XIST gene on human chromosome X was used to carry out the amplification of DNA in a 40 µl reaction volume. High and alternating temperature cycles from a standard PCR setting (denaturation temperature: 95 °C, annealing temperature: 59 °C, and elongation temperature: 72 °C for 40 cycles) were applied. The amplified PCR product was analyzed by the Agilent 4200 TapeStation system, together with the product of a negative control performed with no DNA template, following the same protocol.

Life science application: low-temperature bonding after pre-immobilization of target oligonucleotides
For detailed information, see the CSEM Scientific and Technical Report 2019: *"Disposable Glass Microfluidics for Nucleic Acids Bioassays",* CSEM SA and IMT AG, Switzerland.

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