Heinz Nixdorf-Lehrstuhl für Biomedizinische Elektronik
Fakultät für Elektrotechnik und Informationstechnik
Technische Universität München

Magnetically Enhanced Microflow Cytometer for Bead- and Cell-based Immunoaffinity Measurements in Whole Blood Samples

Scientific thesis for the attainment of the academic degree
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Supervised by Dr.-Ing. Mathias Reisbeck
Prof. Dr. rer. nat. Oliver Hayden

Submitted by Johann Alexander Brenner
Weisbergerstraße 5a
85053 Ingolstadt
03662733

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conservation of mass, momentum reynolds number

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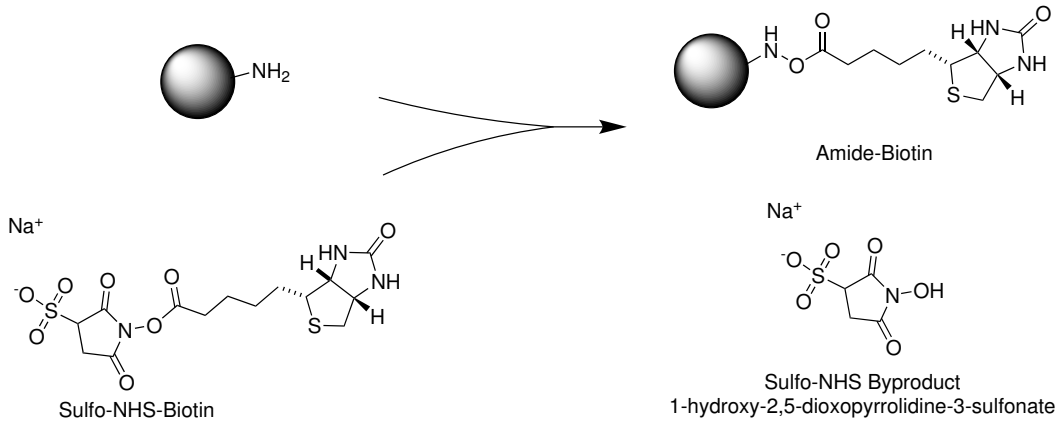

Figure 1 TestSvg

2.2.3. Microscopic Particle Surface Physics

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Short intro over MRCyte Foto of setup with arrows to necessary parts Microscope Stages PEEK holder Helmholtz coils Kepco MFLI DAQ

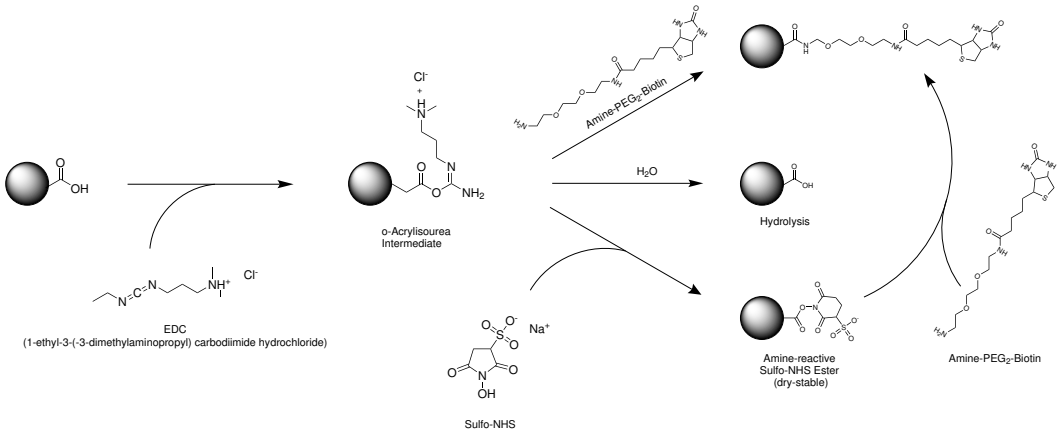

Figure 2 TestSvg

2.3.1. Focusing Structures

test,test Loss because of reduced velocity and magnetic drag

2.3.2. GMR

Different produced GMR stacks Wheatstone Bridge setup Magnet alignment

2.3.3. Electrical Circuit

Ground PCB Stacked PCBs with spacer

2.3.4. Electronic Readout

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Hysteresis Alignment

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Single GMR

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Dual GMR

one MFLI supplies both at same freuqency. Aux Trigger tested, but no advantage.

3. Materials and Methods

3.1. Microfluidic Fabrication

3.1.1. Development of Layout
3.1.2. Patterning of Photoresist
3.1.3. Soft Lithography

The fabricated wafer was placed the center of a 90 cm petri dish. A PDMS (Poly(dimethyl siloxane)) mold was created by vigorous mixing of the pre-polymer base with its curing agent (Sygard 184, Dowsil) in a ratio of 10:1 (w/w). For 3" wafers, thin channels were casted from 15 g, normal channels from 20 g PDMS in the petri dish. Gas bubbles were removed from the mixture in a desiccator for 20 min at 2 hPa , and the clear PDMS was cured in an oven (Um, Memmert) for 1 h at 60 °C. After curing, the PDMS mold was released from the petri dish carefully, taken off the wafer and stored in a clean petri dish upon further processing.

3.1.4. Bonding of Microfluidic

Under laminar flow, crosslinked molds were cut into pieces with the respecting single µF (Microfluidic) with a razor blade. Holes for in- and outlet were punched through the containing channels with a biopsy puncher (ID 0.5 mm, WellTech). The substrates and µFs were sonicated in acetone and diH_2O (deionized water) for 5 min and dried with filtered N_2 (Nitrogen Gas) completely. For the bonding of PDMS to various substrates different protocols have been established:

PDMS Glueing

Here, a micron-height layer of uncured PDMS was used as an adhesive layer between µF and substrate. Approx. 3 mL were poured onto a 3" wafer and spun down for 5 min at 6000 min^{-1}. The microchannel was placed on the substrate by visual control of a stereo microscope (SMZ800, Nikon) with 8-fold magnification. Subsequently, the bonding process could be finished by a 1 h bake at 60 °C or over-night at room temperature.

Plasma Bonding

Due to the chemical nature of glass (or more generally oxides) and PDMS, the respective parts can be activated by the exposure to a controlled oxygen plasma which generates additional silanol (Si-OH) groups on their surfaces and removes impurities at the

same time. Bringing the activated surfaces in contact triggers the formation of covalent bonds almost immediately. First, the acetone-wiped substrates and the microchannels were centered inside the plasma cleaner (Zepto, Diener). Second, vacuum was applied to a final pressure <0.2 hPa. Third, the chamber was flushed with pure O_2 (Oxygen Gas) until a chamber pressure from 0.7 hPa to 0.8 hPa had been stabilized. Fourth, the plasma process was executed with 30 W (Power-Poti: 100) for 45 s to 60 s (Time-Poti: 15-20). Upon finish, the chamber was flushed for 5 s and ventilated. Immediately, the corresponding workpieces were brought into contact and pressed together gently. To ensure a durable bond, the assembled workpieces were baked for 1 h at 60 °C.

$$Heregoesthemassflowequation \tag{3.1}$$

Reversible Bonding
To bond the µF to a substrate reversibly and without residues, the channel can be brought into contact with the bottom part without any adhesinon agent. For low-pressure as well as vacuum driven flows, this method is preferrable due to its time and work efficiency.

3.2. Surface Bio-Functionalization

3.2.1. Surface Activation

To functionalize any silicon containing surface with $Si-OH$ groups which the utilized silane could interact with, multiple surface activation pathways were explored. First, substrates were cleaned in HCl (Hydrochloric Acid):MeOH (Methanol) and H_2SO_4 (Sulfuric Acid) before they were immersed in boiling water. Second, surface silanol groups were achieved by Piranha (H_2O_2:H_2SO_4) immersion and third by a HF (Hydrofluoric Acid) dip.

For all methods, the following reagents were used: diH_2O (0.054 µS, Merck MilliQ)), acetone ($>$99.9 %, VWR), EtOH (Ethanol) (absolute, VWR), MeOH (99.8 %, VWR), AcOH (Acetic Acid) (glacial, VWR), HCl (37 %, Sigma-Aldrich), H_2SO_4 (95 % to 98 %, VWR), H_2O_2 (Hydrogen Peroxide) (30 % (w/w), Sigma-Aldrich), HF (5 %, VWR)

Work Safety Remarks
Before the work with one of the acid solutions was carried out, serveral safety measures were implemented. As any diluted acid solution becomes very hot immediately due to the exothermic reaction, every container should be placed inside a cooled water or ice bath. Additionally, the beaker as well as concentrated acid flasks should be gripped firmly by a laboratory stand to avoid a tip over. As the reactivity of chemicals is highly temperature-dependent, the solutions was processed further when they had been cooled to $\leq$70 °C. It should be also noted that - as in every chemical reaction, but especially ones with H_2SO_4 - the acid was always poured into the other reactant to avoid splashing and boiling.

Plasma Activation
Hier plasma protocolle raussuchen

Hydrochloric-Sulfuric Acid Activation
To degrease any glass or SiN (Silicon Nitride) surface, a protocol according to Dressick, Dulcey, Georger, *et al.* [1] was used. There, the surfaces were first sonicated in acetone and diH_2O for 5 min. Afterwards these were immersed in a 1:1 (v/v) solution of HCl:MeOH for $>$30 min, rinsed with diH_2O copiously and soaked in H_2SO_4 for $>$30 min as well. Then, the samples were rinsed again in deionized water. To form silanol groups on the activated surface, the surfaces were finally immersed in $>$90 °C heated (SuperNuova+, Thermo Scientific) diH_2O for at least 2 h.

Piranha Activation
In this method, activation was carried out in a 1:5 (v/v) Piranha (H_2O_2:H_2SO_4) solution at 70 °C for 30 min. After treatment, the samples were rinsed carefully with diH_2O three times.

Hydrofluoric Acid Activation

3.2.2. Chemical Surface Functionalization

Chemically activated surfaces were now coupled with APTES ((3-aminopropyl)triethoxysilane) covalently. Therefore an aqueous silane solution was prepared from EtOH with volume fractions of 5 % diH_2O, 0.5 % aqueous AcOH (pH 4.5) and 1 % APTES in this order. The samples were soaked immediately after their activation in the silane solution. The reaction was carried out for 2 h to 4 h at $>$40 °C. At finish, all specimens were rinsed three times or sonicated for 5 min in absolute EtOH.

Then, the amine terminated surface modification was enhanced by a carbodiimide conjugation with PAA (Poly(acrylic) Acid). As above, a reaction consisting of 0.5 M MES (2-(N-morpholino)ethanesulfonic Acid) buffer with 1 mg mL^{-1}, 6 mM EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) and 3 mM NHS (N-hydroxysuccinimide) was activated for 15 min on a magnetic stirrer. Subsequently, the prepared samples were immersed in the solution for 1 h on a rotation shaker (VWR). As final cleaning, the slides were rinsed or sonicated for 5 min in diH_2O and stored in fresh diH_2O at 4 °C up to 14 d upon further use.[2]

3.2.3. Surface Bioconjugation

3.3. Magnetic Beadometry

3.3.1. Standard Parameters
3.3.2. Concentration Measurement
3.3.3. Whole Blood Bead Spiking
3.3.4. Bead Capture Assay
3.3.5. Optical Particle Tracking

3.4. Tensiometry

4. Results

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4.1. Signal Similarity For Cells With Varying Bead Coverages

Cross-Correlation between single dipole with sum magentic moment and surface covered with randomly distributed magnetic particles

4.1.1. Single Cell Signal

4.1.2. Cell Aggregates

4.2. Reference Bead Surface Functionalization

4.2.1. Amine-Surface Biotinylation

Streptavidin-Atto488 reference calibration Anti-Biotin-PE working? BNF-Dextran-Streptavidin unspecific binding?

Magnetic Polystyrene Bead
Non-Magnetic Polystyrene Bead

4.2.2. Carboxy-Surface Biotinylation

4.3. Concentration Measurements in MRCyte

4.3.1. Count Stability

Measurement over 1h Measurement of Syringe Tubing Losses

4.3.2. Velocity Measurement

4.3.3. 2-Chip-Setup for Macro Measurements

Sensitivity Calibration
Concentration Measurements

4.4. Protein Immobilization On The Microfluidic Channel Bottom

4.4.1. Physisorption

Quantification in Plate Reader Trial with Neutravidin + Sensor (Esthis Versuch)

4.4.2. Covalent Attachment

Plasma-Based Approach

Water-Based Approach

Sonicate in Acetone and Water 5' 1:1 HCl:Methanol H_2SO_4 Treat for 30 min in light boiling water

5. Discussion

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6. Outlook

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Statement

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Munich, December 4th, 2020, Signature