The integration of electrodes into microfluidic devices is a prerequisite for several key technologies such as electrophoresis, dielectrophoresis, electrowetting, or analysis and manipulation of biological cells in organ-on-a-chip applications. However, conventional sputtering or metal deposition methods result in electrodes on the top or the bottom of the microfluidic device leading to either an inhomogeneous electrical field or considerable restrictions regarding optical analysis methods. Here, a novel method for integrating electrodes into microfluidic devices based on the deposition of silver films on the sidewalls of microfluidic channels by Tollens reaction is presented. Aldehydes diffuse readily through polydimethylsiloxane walls and reduce noble metal ions such as silver, which in turn precipitate and deposit on the phase border as homogeneous thin layers. The process results in sidewall electrodes that adopt the channel’s geometry and offer a highly homogeneous electrical field in the case of a rectangular channel. The electrodes were analyzed by dissipative X-ray (EDX) and electrical impedance spectroscopy, while the electrical field was visualized by particle image velocimetry. The method can fabricate electrodes in different shapes rendering the process highly promising for a wide range of different electrochemical applications in the field of microfluidics, while simultaneously enable optical analysis methods.

1. Introduction

Microfluidic applications that require an electric field such as the investigation of electrochemical reactions,[1] electrical detection,[2] or electro-osmosis[3] must consider how electrodes can be integrated into microfluidic devices. The standard method for electrode integration is a combination of photolithography, sputtering, and lift-off processes. These techniques lead to a thin metal layer on the bottom of a microfluidic channel, which can serve as an electrode.[4–6] This well-established method has the drawback of limiting the visible area for microscopic analysis while also demanding specific cleanroom facilities for fabrication. Additionally, the resulting floor-electrodes lie in one plane generating an inhomogeneous electric field that exhibits an exponential decrease in field strength with increasing channel height.[4] Floor electrodes placed outside of the visual window may allow optical analysis but lead to substantial attenuation of the electric field, whereby separation methods lose efficiency and analysis methods lose precision.[7]

An alternative to the photolithographic fabrication method of planar electrodes is the integration of wall electrodes. These electrodes are positioned perpendicular to the visual field and possess superior properties concerning the resulting electrical field’s strength and homogeneity. The voltage drop is less pronounced, and the dielectrophoretic (DEP) force is independent of the channel height.[7,8] Additionally, the visual window is accessible for optical analysis, such as microscopic observation. Different methods for the introduction of wall electrodes were already presented in the literature. Common techniques include the insertion of metal wires into microfluidic devices[9] or the injection of conductive substances into spatially separated channels.[10] While wiring requires high precision regarding the correct electrode alignment, spatial separation substantially weakens the electric field due to voltage loss inside the wall. Even though alternative processes such as the doping of polydimethylsiloxane (PDMS) with conductive particles[11,12] or the penetration-based introduction of liquid bismuth alloys into microfluidic devices demonstrate feasible alternatives,[5] the development of a simple and reproducible manufacturing method for the integration of wall electrodes still constitutes a major challenge for current research.

Here, we present a novel and highly reproducible in situ method for the integration of wall electrodes within microfluidic PDMS devices, which neither require the doping of PDMS nor the use of an additional lithographic step. The procedure is based on the Tollens reagent, which was introduced by Bernhard Tollens in 1882 as a method for the detection of aldehydes in aqueous solutions and was later used to distinguish between reductive and non-reductive sugars.[13] The Tollens reagent applied for the reaction consists of silver nitrate, sodium
hydroxide, and ammonia, forming diamine silver complexes as the reactive species. When these complexes are exposed to the redox reactive aldehyde group (−CHO), the silver ions Ag⁺ are reduced to elemental silver, which precipitates on the walls of the reaction container.[14] The sum of reactions can be written as

\[ \text{RCHO} + 2\text{[Ag(NH₃)₂]⁺} + 2\text{OH}^- \rightarrow 2\text{Ag} + \text{RCOOH} + \text{H₂O} + 4\text{NH}_₃. \]

Contrary to the original reaction, however, the reactants in our case are spatially separated by a semipermeable PDMS membrane restricting the reduction reaction to the phase border. Aldehydes can diffuse readily through PDMS, causing the deposition of thin film silver electrodes on the channel walls. The diffusion with a constant concentration source can be described by

\[ D = \frac{x^2}{2t} \]  

where \( D \) is the diffusion coefficient in m² s⁻¹, \( x \) the diffusion length in m and \( t \) the time in s.[15] This relationship suggests that the time required for a species to travel a certain distance \( x \) by diffusion is proportional to the square of the distance itself. As a result, the initiation time of a diffusion-limited interfacial reaction depends on the distance of the source to the interface. This principle can be harnessed to gain control over the site of metal deposition in the context of electrode integration by an interfacial reduction reaction. By increasing the central channel’s height in relation to the side channels, the reduction agent requires more time to diffuse to the channel ceiling than to the wall of the microfluidic channel. A well-chosen termination time finally results in thin film wall electrodes directly located within the channel of interest.

2. Results and Discussions

The basis for integrating silver film electrodes into microfluidic PDMS chips by Tollens reaction is a specific channel layout featuring three parallel channels in close proximity (Figure 1). In this configuration, the Tollens reagent is supplied via the central channel, while the side channels are used for aldehyde injection (Figure 1A). Considering that PDMS is highly permeable for aldehydes, these molecules will readily sorb into the surrounding PDMS matrix and non-directionally diffuse along the concentration gradient. Aldehydes that reach the central channel and desorb into the Tollens reagent reduce diamine silver complexes to elementary silver, which in turn deposits on the microfluidic channel walls (Figure 1B,C). Since aldehyde molecules at the phase border are continuously converted by the Tollens reaction and excess molecules are removed by continuous fluid motion, the concentration gradient between the channels is maintained. As a result, silver film electrodes slowly grow from the glass bottom upward to the channel ceiling and suppresses premature electrode fusion.[16] As a result, the silver film electrodes slowly grow from the glass bottom upward to the channel ceiling. A well-chosen termination time for the reaction inhibits the wall electrodes from fusing. In the case of 4:1 height ratio, time limits between 1 and 10 min were determined to be optimal.

Figure 1 demonstrates the results of the coating procedure for the corresponding aldehydes as a function of time, where the first row of images presents the top view of one half of the microfluidic chip at 1 min, while the second row shows the identical section at 3 min. During the experiment, all fluids were in continuous motion, and parameter variation was restricted to the type of aldehyde. Microscopic analysis revealed that after 1 min, all microfluidic setups show noticeable optical changes. In the case of formaldehyde and benzaldehyde, a moderate darkening of the central channels’ wall can be observed, indicating the onset of a successful silver deposition. In contrast, the introduction of glutaraldehyde leads to integral changes in the
PDMS membrane. The wall swells up to 50% compared to the non-wetted state and turns completely opaque, suggesting the accumulation of by-products within the membrane. For glutaraldehyde, this swelling increases steadily up to the 3 min mark, at which point the swelling reaches factors of 2 to 2.5 and by-product precipitations expands into the side channels. Similar to glutaraldehyde, the membrane also swells significantly after prolonged contact with formaldehyde. The swelling causes increases in wall diameter of up to 200% (factor of 3) and results in major membrane corrugations. In the case of benzaldehyde, even after 3 min of reaction, no substantial membrane alterations can be detected. Substance deposition in the vicinity of the central channels’ walls intensifies, while minor precipitation of transparent crystals can be observed in the aldehyde channels. This result agrees well with the fact that aromatic aldehydes are less reactive partners for the Tollens reagent,[14] which in turn improves process control. Therefore, subsequent investigations on the integration of wall electrodes were limited to benzaldehyde as a reducing agent as it demonstrates the best performance in terms of unwanted swelling, permeability, and silver reduction.

The complete coating procedure using benzaldehyde is depicted in Figure 3 as a time series of microscopy pictures, where Figure 3A) shows the chronological sequence of the Tollens reaction, while Figure 3B) demonstrates the cleaning protocol for the removal of the crystalline by-products from the PDMS walls and side channels. At the time \( t = 0 \) min, the two reagents are fed into the corresponding channels to initiate the reaction. At this stage, the microfluidic channels are entirely transparent, and no visual changes can be detected. After 3 min, the previously described phenomena occur. Silver is deposited in close proximity to the wall along the central channel, and transparent crystals form within the side channels. Both effects steadily intensify over time until the aldehyde channels are finally severely obstructed by side-product, and the onset of silver deposition can be detected as a minor discoloration at the ceiling of the central channel. To prevent falsification of experimental data applying the silver film electrodes, the crystalline by-product has to be removed from the microfluidic setup. For this purpose, ethanol has proven to be a suitable solvent to dissolve the precipitated crystals, as showcased in Figure 3B.

Moreover, it should be mentioned that continuous fluid motion above a certain velocity threshold constitutes a major necessity of the electrode coating process. In this context, experiments could demonstrate that a dormant or low-velocity Tollens reagent leads to the precipitation of a crystalline substance within the central channel (Figure S2, Supporting Information). This effect seems to be related to the removal of ammonia from the Tollens reagent by diffusion into the surrounding PMDS matrix and occurs at Tollens reagent velocity below 0.5 cm s\(^{-1}\). The decrease of ammonia can lead to the destabilization of silver diamine complexes, resulting in the precipitation of crystalline silver oxide. The possibility of redisolution by an increase in reactant flow rate or exposure to ammonia supports this hypothesis.

Considering that even the best electrode cannot be operated without an appropriate electrical connection, the basic three-channel layout of the microfluidic chip had to be extended by two contacting chambers (Figure 4A). These two 914 (W) \( \times \) 200 (H) \( \mu \)m geometries enable the introduction of liquid contacting substances such as silver conductive lacquer by capillary force. The contacting chambers are attached to the 500 (W) \( \times \) 200 (H) \( \mu \)m central channel by two 57 (W) \( \times \) 200 (H) \( \mu \)m junctions. These channel constrictions were implemented to substantially increase the flow resistance and allow for more precise control over the contacting substance’s penetration depth. To establish a reliable electrical contact to the silver film electrodes, the 50 (W) \( \times \) 50 (H) \( \mu \)m aldehyde side channels were not only guided in parallel to the desired electrode position but also to the junction channels as well as the inner section of the contacting chambers at a distance of 40 \( \mu \)m. Silver deposition within the respective parts provided an extensive contacting area for the silver conductive lacquer outside the

Figure 2. Microscopic analysis of the suitability of different aldehydes with regard to silver deposition by Tollens reaction within a microfluidic PDMS chip. Formaldehyde concentration: 12.5 \%wt, glutaraldehyde concentration: 50 \%wt, benzaldehyde concentration: 99.5 \%wt. Aldehyde flow rate: 10 cm s\(^{-1}\), Tollens reagent flow rate: 1.67 cm s\(^{-1}\).
central channel (Figure S3, Supporting Information). This intricate layout design enabled the integration of electrical connections to the silver film electrodes by means of Tollens reaction already during the coating process. To minimize the number of syringe pumps necessary for the setup operation, the side channels were interconnected by tubing according to the numerical sequence indicated in Figure 4A). The aldehyde was initially injected into position 0 from where it flowed along the respective channel toward position 1. Position 1 was subsequently connected to position 2 via a Smith Medical Portex Fine Bore LDPE Tubing (0.38 mm ID, 1.09 mm OD) and finally excited at position 3, where it was collected in a waste container. This interconnection of the aldehyde channels was repeated on the opposite side of the chip. The general flow direction of all reactants is indicated in Figure 4A. An overview of the final product featuring coated silver film electrodes and fully functional electrical connections integrated into the contacting chambers via silver conductive lacquer is presented in Figure 4B.

The connection to the electrode assembly was probed with electrical impedance spectroscopy (EIS). The Bode plot of the EIS spectra in Figure 5 shows the modulus and phase shift of the impedance above the frequency. In the high-frequency range (above $10^4$ Hz), no significant phase-shift between voltage and current can be observed, representing the electrode and electrolyte’s resistance. Below $10^4$ Hz the forming ionic double-layer on the electrode–electrolyte interfaces increase the impedance with decreasing frequency. The electrical double-layer can be modeled with a constant phase element (CPE). Since both electrodes are from the same material, both electrode–electrolyte interfaces can be modeled with one CPE.

Figure 4. The layout of the microfluidic chip. A) Scheme of the general chip structure as CAD top-view; green: Tollens reagent channel and contacting chambers; yellow: aldehyde channels; circles: Tubing insertion points. B) Microscopy image of a complete chip coated with silver film electrodes.
To determine whether the deposit within the central channel is silver, Tollens-reaction based coating was conducted for 1 min, and the resulting microfluidic chip was examined by SEM and EDX. Figure 6A,B demonstrates the PDMS part of the microfluidic device rotated 180°. Since the applied electron microscope features a hybrid detector allowing for both the detection of secondary and backscattered electrons, brightness differences can be directly correlated to detecting different elements.[18] Heavy elements of high atomic number backscatter more intensively than light elements of low atomic number and appear brighter in the resulting image. In Figure 6A the channel wall appears considerably brighter than the channel ceiling, providing the first indication of a successful spatially selective coating. Moreover, this result is supported by the EDX analysis in Figure 6B, which presents the distribution of silver across the microfluidic channel in red. Figure 6C depicts site-specific EDX data of the channel ceiling (black) and the channel wall (blue) for carbon, oxygen, silicon, and silver. Analysis of the data reveals that the channel ceiling merely presents a silver content of 3.0 wt%, while the side wall exhibits 39.5 wt% silver.

Particle image velocimetry (PIV) enables the in-depth optical analysis of particle movement within optically transparent fluids. Figure 7 presents fluorescently labeled polystyrene beads located within the central channel of the microfluidic device in absence (Figure 7A) and presence (Figure 7B) of an applied electrical field, where each image constitutes the sum of 20 consecutive video frames. Considering the usage of spherical beads and the fact that the particle position is traced over the duration of 20 frames, particle motion become visible as continuous white lines, while dormant beads are observable as white circular dots. The alternating electrical field evokes a migration of the negatively charged tracer particles toward the counter-charged electrode until the electric double-layer is formed, which leads to a strong potential drop in the boundary layer. The fact that

Figure 5. Bode plot presentation of the EIS measurement of the wall electrodes in 50 mmol L⁻¹ NaCl solution. The ionic double-layer on the electrode-electrolyte interface is fitted with a constant phase element, while the electrolyte as well as connectors with a resistor in series. The fitting results in $R = 1750 \Omega$; $\text{CPE-Q} = 1.42 \times 10^{-6} \text{s}^{-1}$; $\text{CPE-n} = 0.7693$.

Figure 6. Verification and quantification of in-chip silver deposition. A) SEM cross-section image of the microfluidic chip after the coating process. B) Visual representation of the silver distribution within the microfluidic channel, where silver is depicted in red. C) EDX analysis of channel ceiling (black) and channel wall (blue).
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Figure 7. Visualization of the electrical field orientation between thin film silver electrodes inside a microfluidic channel. Each image constitutes the sum of 20 consecutive video frames. A) Fluorescent tracer particles (white) between thin film silver electrodes in the absence of an electrical field. B) Fluorescent tracer particles (white) between thin film silver electrodes in the presence of an electrical field.

Particle movement occurs exclusively upon the application of voltage proves the presence of fully functional electrodes, while the highly specific direction of motion perpendicular to the electrodes emphasizes the homogeneity of the electric field in the case of rectangular channel geometries. The particle oscillation can be seen in Video S4, Supporting Information.

3. Conclusions

In this work, we present a facile and highly-reproducible method for the integration of versatile wall electrodes into microfluidic PDMS devices based on the site-specific reduction of metal ions by Tollens reaction. The linchpin of this procedure is the spatial separation of the reactants by a semipermeable PDMS membrane, which exclusively allows for diffusive exchange of species and hence delays the interaction between reducing aldehydes and metal ions. Considering that the length of the diffusion path dictates the arrival time of the reducing agent, the site of metal deposition can be controlled by a difference in channel heights. In this context we demonstrate that aromatic aldehydes such as benzaldehyde constitute the reducing agent of choice enabling the integration of functional film electrodes in less than 10 min. Furthermore, by applying our unique chip design, it is possible to integrate not only the electrodes, but also the corresponding electrical leads in just a single step. The success of our method was verified by EIS, EDX, and μPIV measurements. Since the electrodes adopt the channel wall geometry, the presented electrode integration method can also be used to generate inhomogeneous electric fields. For example, a meandered channel wall geometry would lead to a meandered electrode, which could be applied for dielectrophoresis. To our knowledge, this is the first approach using the permeability of PDMS toward hydrophilic substances as a possibility to bring a reducing agent to metal ions and using this metal as electrodes. We hope this will help researchers working in microfluidics to integrate electrodes serving different requirements.

4. Experimental Section

Microfluidic Chips by Soft-Lithography: Microfluidic chips were prepared via PDMS replica molding. The positive master mold necessary for the replica was 3D-printed using a multiphoton 3D printer (Nanoscribe, Photonic Professional GT). A detailed description of the master and chip fabrication procedure can be found in the following publication, ref. [19]. In short, the master used for the replica production was designed using the computer-aided design (CAD) software Autodesk Inventor 2019 and was 3D-printed via dip-in laser lithography on microscopy glass substrates (Sigma-Aldrich, 25 × 75 × 1 mm). Before printing, the glass substrates were cleaned in isopropanol (Sigma-Aldrich, ≥99.8) and acetone (Honeywell, ≥99), followed by a silanization in a 3(trimethoxysilyl)propyl acrylate (Sigma-Aldrich, 92 with 100 ppm BHT) solution. The silanized substrates were covered with several photoresist droplets (Nanoscribe, IP-5), and the microfluidic master mold was printed section-wise with rectangular splitting two-photon polymerization. Subsequently, the master mold was developed in propylene glycol methyl ether acetate (Sigma-Aldrich, ≥99.5), cleaned in isopropanol, and post-cured by exposure to an ultraviolet light source (302 nm, 8 W) for 12 h. For microfluidic chip fabrication, the resulting master mold was covered by PDMS (DowCorning, Sylgard284 plus curing agent 10:1 (w/w)) and cured in an oven overnight at 60 °C. Afterward, the solidified PDMS was separated from the master mold, and holes for the connecting tubings were punched using biopsy punches (EMS, Rapid-Core 0.75 mm). Finally, the PDMS slab was cleaned by sonication in isopropanol, dried in a desiccator at room temperature and plasma bonded to a microscope glass substrate (Sigma Aldrich, 25 × 75 × 1 mm) applying an absolute oxygen pressure of 0.3 mbar (40 mL min⁻¹) at 60 W for 30 s (TePla 100 Plasma System, PVA). Tollens Reagent: The alkaline silver nitrate solution stabilized by ammonia is also named Tollens reagent. In the first step, the silver ions were precipitated by mixing a 10 wt% silver nitrate (Sigma-Aldrich) solution and 1.0 mol L⁻¹ sodium hydroxide solution (Sigma Aldrich) in a molar ratio of 1. Subsequently, an ammonia solution (5 wt% in DI water) (Sigma Aldrich) was added dropwise until the brown sediment was completely dissolved. Considering that ammonia readily diffuses into PDMS 4 vol% of excess ammonia solution was finally added to prevent the precipitation of silver hydroxide or silver oxide within the microfluidic chip.

Silver Film Electrodes by Tollens Reaction: For the deposition of thin film silver electrodes on the walls of the microfluidic channels, benzaldehyde (99.5%, Sigma-Aldrich) and the Tollens reagent were introduced into the corresponding channels via a syringe pump (Chemxy, Fusion 4000) at flow rates of 0.02 and 0.1 mL min⁻¹, respectively. To limit the reaction to the channel walls, the initiation of the reactant flows was sequenced. The aldehyde flow was only initiated when the central channel was completely filled with Tollens reagent. The progression of the Tollens reaction was monitored via a bright field microscope (Leica Microsystems, DMIL LED Fluo). Based on the extent of silver deposition, the reaction was terminated after 1 to 10 min by replacing the aldehyde with an ethanol flow.
the Tollens reagent was removed by flushing the central channel with DI water preventing post-reactions at the channel walls due to residual aldehyde stored within the PDMS. The ethanol and DI-water flush was conducted for at least 60 min at flow rates of 0.02 and 0.1 mL min$^{-1}$, respectively. Finally, the microfluidic channels were dried by applying a constant airflow, and the chip was stored in a fume hood for 24 h to remove the remaining solvent residues by evaporation.

**Electrode Contacting:** To contact the silver film electrodes, the corresponding contacting chambers were opened by a perpendicular cut using a razor blade. Subsequently, silver conductive lacquer (Ferro) was applied to the openings with a brush, entering the chambers by capillary force (Figure S1, Supporting Information). To guarantee an appropriate contacting area, additional silver conductive lacquer was spread uniformly between the chamber openings and the edge of the microscopy slide. The chips were finally dried in atmospheric conditions for at least 1 week to ensure the silver conductive lacquer’s gentle curing.

**Electrical Impedance Spectroscopy:** To verify proper electrode contacting, electrical impedance spectroscopy (EIS) and subsequent equivalent electric circuit fitting were used. The EIS measurements were performed in potentiostatic mode with an amplitude of 10 mV and at a frequency range of 10 Hz to 100 kHz with ten probes per decade. A Reference 3000 potentiostat (Gamry Instruments) was used for the electrochemical characterization. The potentiostat’s inputs and outputs were connected to the silver conductive lacquer by a combination of silver plates and crocodile clips to ensure proper contact. The microfluidic channel was filled with 50 mmol L$^{-1}$ aqueous sodium chloride solution as an electrolyte. A CPE in series with a resistor was chosen as an equivalent electric circuit. The CPE represents the electrode/electrolyte interface, and the resistor represents the ohmic resistances of the electrode and electrolyte bulk resistance.

**SEM and EDX:** Morphology, position, and coverage of the deposited silver film electrodes were analyzed using scanning electron microscopy (SEM) in combination with energy dispersive X-ray (EDX) (Hitachi High Tech TM 3030Plus). To investigate the chips with SEM and EDX, the plasma bonded PDMS part of the microfluidic chips was gently separated from the microscopy slide with a razor blade.

**Particle Image Velocimetry (PIV):** A µPIV system (LaVision GmbH) was applied to demonstrate the electrical field’s homogeneity between thin film silver electrodes. Rhodamine B labeled polystyrene tracer particles (ThermoFisher Scientific, 0.86 μm) were diluted 1:5000 in 1 mmol L$^{-1}$ KCl solution and were flushed into the channel. To exclude the influence of fluid movement on the particles during observation, the in- and outlet of the microfluidic device were sealed after filling. The negatively charged tracer particles were subsequently excited by a potential of 0.4 V with 5 polarity changes per second. Images were taken with a microscope (STereo Discovery V20 Carl Zeiss Microscopy GmbH) using a 24× magnification resulting in a depth of field of 27 μm. Pulsing frequency of the Nd:YAG laser (Photonics Industries Inc. DM150-532) was set to 100 Hz. Laser current was adjusted at 16 A. The high speed cameras (Phantom VEO710L, Vision Research) recorded with 200 fps and an exposure time of 100 μs. Image processing was performed via DaVis 10.0.5 (LaVision GmbH) Software. For visualization of the particle movement, 20 consecutive frames were accumulated.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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3D electrodes, electrochemical, microfluidic, silver electrodes

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