A fabrication platform for electrically mediated optically active biofunctionalized sites in BioMEMS

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We report a new approach for microfluidic optical bioanalysis that is based on the electrically driven assembly of bio-components on a transparent sidewall and the optical detection of the assembled components using planar waveguides. This allows localized electrical signals for bio-assembly and optical signals for bio-detection that can easily be applied in MEMS systems. We demonstrate a BioMEMS design incorporating this scheme and its output signal when using fluorescent detection.

Introduction

Microfluidic channels and integrated planar waveguides have been gaining significant attention for the detection of biological species[1–3] due to the advantages microtechnology can provide over conventional analysis methods in terms of density, throughput, and cost.[4] Microfabrication is an inherently planar process, and despite recent advances in planar waveguide methods for fluorescence detection and soft-lithography[5] for surface biofunctionalization, there is a need for a biofunctionalization method that can use easily directed signals (such as electrical signals) and can be integrated with in-plane optical components for waveguide facet coupling. Soft-lithography (stamping) methods have proven to be effective means for the biofunctionalization of surfaces; however their application is restricted to the plane of a wafer. This limitation makes the technique inadequate for in-plane optics since the analyte must intersect the optical path. Delivery of the analyte in a fluid can overcome the geometrical limitations of stamping and has been used in other works.[6–9] Unfortunately, such fluid based methods necessitate the dispersion of the analyte in a large volume of solvent which diminishes the concentration of the analyte in the transducer area.

We propose a unique bioassembly and optical signal collection scheme by employing the electrically driven deposition of a reactive polysaccharide, chitosan, on microfluidic channel sidewalls. This work demonstrates the spatial localization of biofunctionalized sites and the capture of the fluorescent signal emanating from those sites using planar waveguides. The use of channel sidewalls is enabled by a transparent and conductive electrode material with a transparent biofunctionalization layer. We have demonstrated the suitability of indium tin oxide and chitosan for this purpose. Specific probe biospecies can be covalently coupled to chitosan to implement biofunctionality.[10] This new approach to probe

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assembly is expected to yield significant advantages in sensitivity, simplicity, and density for optical detection schemes involving fluorescence, absorbance, scattering, or other optical signatures. Our chitosan-based biofunctionalization scheme can be directed by electrical signals and performed *in-situ* with common laboratory equipment under biologically mild conditions. This facilitates operation with labile biological species in contrast to soft-lithography processing. In effect, the use of chitosan in our device provides the vital link between microfabrication and biology. Chitosan is particularly well suited to application in micro-fabricated biosensors and analytical systems due to its ability to be controlled by electrical signals which are germane to MEMS devices.

Sensor design

Our transducer cell design, shown in Fig. 1, follows from planar absorbance cells.[6–9] The alignment of the probe species on the waveguide facet maximizes the capture cross-section of the fluorescent emission. While beneficial in fluorescence mode, sidewall deposition is essential in absorbance applications to intercept a straight optical path. Unlike in other bioanalysis chips,[11] this planar optical architecture leaves the top and bottom boundaries of the channel open to additional forms of biochemical interrogation. The design utilizes surface micromachining methods on a Pyrex substrate due to its low index of refraction and chemical inertness. A single thick-film SU-8 polymer layer with higher refractive index defines the fluid channels and multi-mode
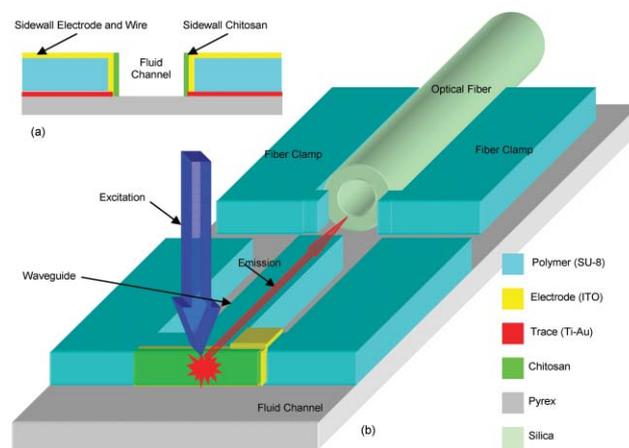

Fig. 1 Cross section view along fluid channel and waveguide. (b) Abbreviated perspective view of devices with shortened waveguide to show fiber coupling.

waveguides, with transparent sidewall electrodes defining the addressable sites for biofunctionalization. In addition to optical coupling, our sidewall biofunctionalization design has two more advantages over current designs. First, the probe species is immobilized in the transducer area, thereby focusing the analyte–probe species interaction in that region. Other methods, such as electro-osmotic temperature gradient focusing,[12] involve greater complexity, fluid property specificity, and implementation challenges. Second, the probe species immobilization can result in a reduction of fluid control structures such as reservoirs, pumps, and valves by a significant factor because the probe species can be introduced and deposited independently of the analyte. The simplification of fluid control structures can lead to smaller devices and hence denser integration.

Materials and properties

Fig. 2(a) shows a fabricated transducer implementing the sidewall design described above. Fig. 2(b) shows a transducer following the electrically driven assembly of a fluorescein-labeled chitosan indicator on the microfluidic channel sidewall. The selection of materials and their related properties are of critical importance to device operation. The electrodeposition capabilities[13,14] of chitosan make it an attractive biological interface material for microsystems. Chitosan is an amine-rich biopolymer upon which a wide variety of molecules such as proteins[15] and oligonucleotides[10] can be easily conjugated to create an active, spatially-selective[16] surface. The conjugation can be performed while chitosan is in solution prior to deposition or on the patterned surface after deposition. Conjugation is accomplished by chemical/biochemical methods, including glutaraldehyde[10] and tyrosinase-mediated[15] coupling. The deposition of chitosan can be selectively and independently initiated at each transducer site by electrically driven

assembly when voltage is applied to the sidewall electrodes. Electrical current causes the evolution of hydrogen and a localized region of high pH[17] at the cathode. Since chitosan becomes insoluble at high pH, it forms a solid layer on the cathode. Furthermore, an array of specific sites can be programmed by varying the fluid in the channel at the time of voltage application on the electrodes. To provide a conductive and transparent sidewall for functionalization we selected indium tin oxide (ITO), a wide band-gap degenerately doped semiconductor whose N-type conductivity results from tin substitution and oxygen vacancies. The waveguides and fluid channel are defined in a single application of SU-8, a photodefinable epoxy resin. SU-8 is inexpensive and is well suited to this application due to its ability to form thick films and microchannels[18] while maintaining a high aspect ratio in a single coat,[19] inertness, and high refractive index. The optical loss in SU-8 waveguides on Pyrex substrates with air cladding was measured by the cut-back method, where the losses of waveguides of several lengths were determined. For waveguides with a 130 μm × 150 μm cross section we have measured losses of approximately 1.75 dB cm^{-1}.

Fabrication and testing

The fabrication procedure requires two lithography masks, one for defining the SU-8 polymer layer and the other for defining the electrodes. Processing begins by depositing 200 nm of ITO *via* radio frequency (RF) magnetron sputtering on a 100 mm diameter Pyrex wafer to form the first electrical contact layer. Photoresist is spun and patterned with the electrode mask using contact lithography. The ITO is etched using hydrochloric acid solution. SU-8 (SU-8 50, Microchem, Inc.) in gamma-butyrolactone solvent is spin-coated to a final thickness of 130 μm, exposed in a contact aligner, and developed. Adhesion between the SU-8 layer and the
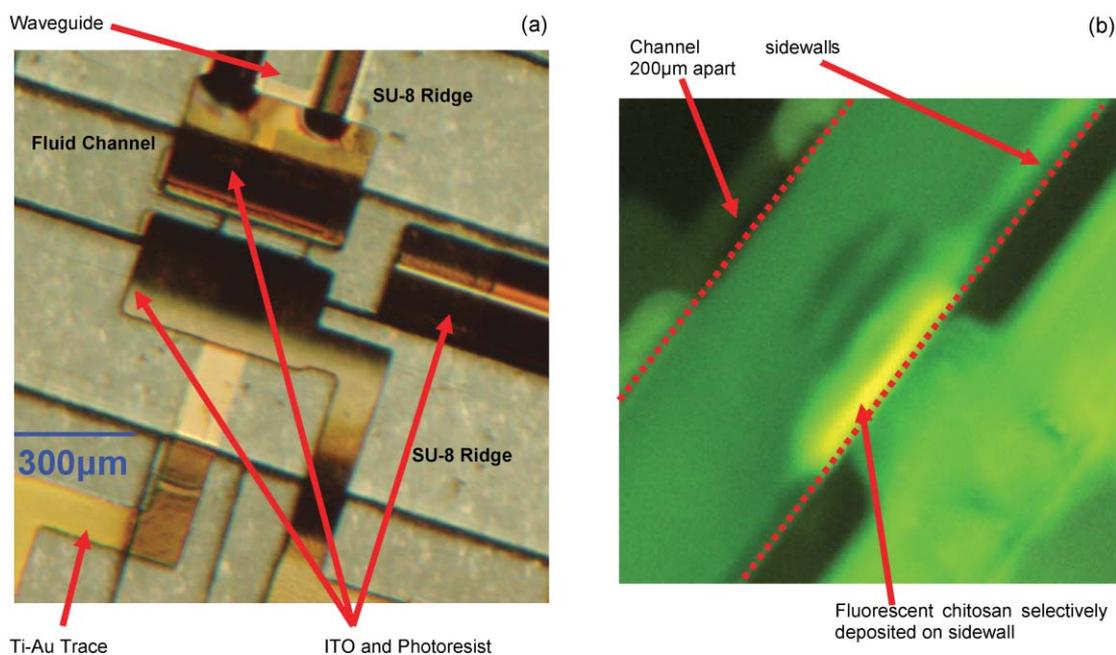

Fig. 2 (a) Left: Micrograph of transducer cell. Photoresist is left on ITO surfaces for clarity. (b) Right: Fluorescent micrograph with a green fluorescein dye chitosan conjugate indicating successful sidewall deposition. The active area on the sidewall appears yellow due to the high intensity of the fluorescence. Fluorescein dye was used in fabrication tests to gauge the efficacy of the sidewall deposition due to fluorescein's activity in the visible region.

Pyrex substrate was substantially improved by the use of an adhesion promoter, AP 300, from Silicon Resources, Inc. After patterning of the polymer is complete, a second layer of ITO is deposited by the same method. A high viscosity, low spin speed photoresist recipe was developed for coating the sidewalls of the 130 μm structures with a single spin of resist. This allowed for adequate coating of the sidewalls due to the surface tension of the resist, although the thickness was highly nonuniform and varied from 9.5 μm on planar surfaces to 130 μm on the sidewall coated areas. The exposure dose was developed for the 130 μm thickness. The resist is patterned using the electrode mask and etched accordingly. We use two identical metal layers to increase the probability of contact to the sidewall electrodes. The final step is to biofunctionalize the transducers by submerging the wafer in a bath of chitosan conjugate solution (Sigma-Aldrich, 0.4% w/v, 5.07 pH) and applying voltage to the electrodes. Layers with thickness in the micrometer range were deposited in 15 minutes with the application of 2 VDC. Chitosan forms a 3 dimensional polymeric network with significant water content, *e.g.* a "hydrogel" upon deposition. The wafer was then rinsed with water to ensure that no undesirable residues of the chitosan probe species conjugate remain.

Fig. 3 shows the spectrum of the output collected from the planar waveguide with and without electrodeposition of fluorescently-labeled chitosan. For fluorescence sensing, the device is interfaced by means of two optical fibers (62.5 μm core, 125 μm cladding). An output fiber is aligned to a waveguide with a "fiber clamp" structure shown in Fig. 4, and is secured by the application of UV-curable epoxy. The index-matching UV epoxy fills the gap between the fiber and the waveguide to eliminate reflective losses and cavity resonances. Our choice of waveguide dimensions minimizes losses due to surface scattering and optimizes for adhesion of the SU-8 to Pyrex. There is significant loss from the waveguide–fiber core size mismatch is acceptable for this demonstration but could be improved by using larger core fiber. The excitation light is from a 1.2 mW pigtail-coupled semi-conductor diode laser at 635 nm. The device is secured to an optical bench along with other optical components that fix the input fiber above the wafer and direct it normal to its surface. The spot size of the beam from the fiber is large in comparison to the optically active sites so good uniformity is maintained. We use normally directed illumination to reduce the amount of excitation light captured by the waveguides. Alternatively, a 2 × 2 directional fiber coupler could be used to deliver excitation light and collect
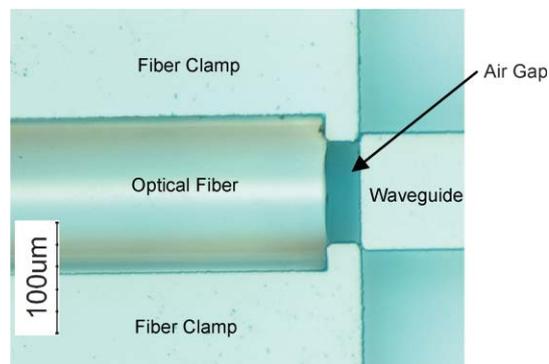
Fig. 4 Top-view micrograph of fiber alignment structure.

back-scattered fluorescence through the input waveguide. The free end of the output fiber is connected to a grating spectrometer. When no voltage is applied and no deposition is present, only the laser emission at 635 nm is observed due to scattering captured by the waveguide as shown by the sharp peak in Fig. 3(a). When chitosan conjugated with Alexa Fluor 633 is assembled in a transducer, fluorescent emission with a peak near 650 nm is clearly evident as shown in Fig. 3(b). This result confirms the feasibility of the optics design, microfabrication, and biofunctionalization method for a fluorescence based detection system using a sidewall biofunctionalization scheme.

Conclusion

We have demonstrated for the first time the electrically driven assembly of fluorescently labeled chitosan on the sidewall of a microfluidic channel and presented its unique application with fluorescent signal capture using planar waveguides. Future systems can be adapted to a wide variety of biomolecules and more complicated biophotonic interactions utilizing this versatile chitosan platform. Currently, off-chip optical detectors and sources are being used; however this design is open to enhancement by monolithic integration of photo-active components. With easily directed electrical signals, we combine biological material and microfabricated components to facilitate the application of MEMS as biological analysis systems. Our chitosan based sensor design and fabrication process is a significant advance toward high-throughput and user-configurable biosensors that are comprised of multifaceted sensing elements arrayed in complex
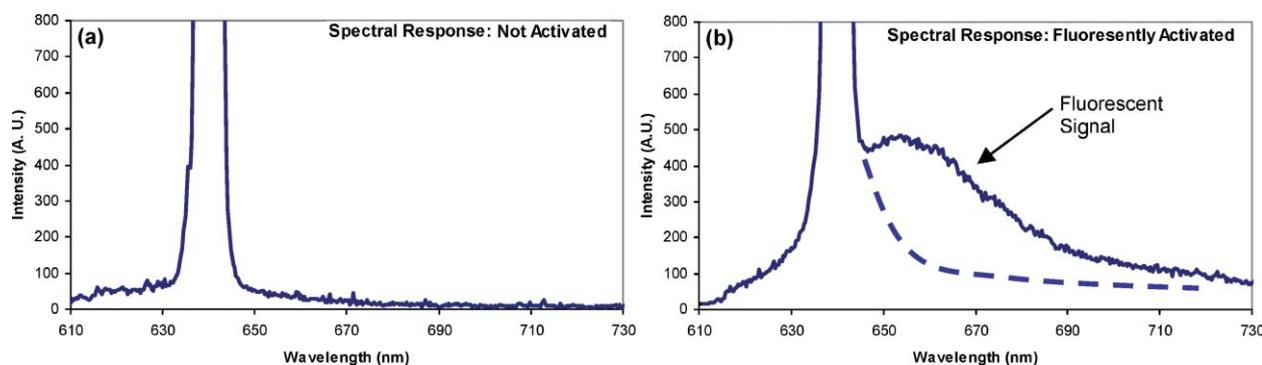

Fig. 3 Output spectrums from collector waveguide (a) Left: Spectral output of collector waveguide without chitosan deposition. (b) Right: Spectral output of a collector waveguide with Alexa Fluor 633 chitosan conjugate deposition.

geometries for the simultaneous analysis of multiple analytes, enabling the next generation of micro-biophotonic systems.

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