Published in final edited form as:

Biomaterials. 2012 January ; 33(1): 225–236. doi:10.1016/j.biomaterials.2011.09.036.

Whispering Gallery Mode Biosensor Quantification of Fibronectin Adsorption Kinetics onto Alkylsilane Monolayers and Interpretation of Resultant Cellular Response

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Abstract

A Whispering Gallery Mode (WGM) biosensor was constructed to measure the adsorption of protein onto alkylsilane self-assembled monolayers (SAMs) at solution concentrations unattainable with other techniques. The high sensitivity was provided by a WGM resonance excited in a silica microsphere that was functionalized with alkylsilane SAMs and integrated in a microfluidic flow cell under laminar flow conditions. It was found that FN adsorbed at biologically relevant surface densities, however, the adsorption kinetics and concentration dependent saturation values varied significantly from work published utilizing alkanethiol SAMs. Mathematical models were applied to the experimental results to interpret the observed kinetics of FN adsorption. Embryonic hippocampal neurons and skeletal myoblasts were cultured on the modified surfaces, and a live-dead assay was used to determine the viability of the FN surfaces for cell culture, and major differences were noted in the biological response to the different SAMs. The high sensitivity and simplicity of the WGM biosensor, combined with its ability to quantify the adsorption of any dilute protein in a label-free assay, establishes the importance of this technology for the study of surface accretion and its effect on cellular function, which can affect biomaterials for both *in vivo* and *in vitro* applications.

Keywords

Biosensor; Biocompatibility; Computational fluid dynamics; Modeling; Protein adsorption; Surface modification

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1. INTRODUCTION

Non-specific binding (adsorption) of biomolecules at solid-liquid interfaces is a phenomenon that affects the function of materials and devices intended for use with physiological fluids and tissues. Therefore, understanding biomolecule adsorption is a crucial factor in determining detection limits, biocompatibility, and long-term efficacy of lab-on-chip, microfluidic, and Bio-MEMS devices due to loss of analyte by adsorption or fouling of microchannels and active sensors. Proteins are particularly notorious for their ability to non-specifically stick to materials [1]. Adsorption induced changes in the structure of a protein molecule is a critical factor in determining its subsequent function. However, the inherent variability of protein sequence and structure makes the prediction of protein adsorption from first principles an intractable problem [2]. Thus, it is necessary to devise experimental solutions for making quantitative observations that can be used to assess the biocompatibility of materials and create reduced-order models for predicting protein adsorption.

Many studies to date have focused on the adsorption behavior of proteins using alkanethiol SAMs as model surfaces, and multiple theoretical models with varying degrees of complexity have been proposed to describe the kinetics observed. These surfaces are convenient in that they are relatively easy to prepare, present highly ordered monolayers with well-defined composition, and are compatible with integrated electrodes and other sensor systems utilizing metal coated surfaces, such as surface plasmon resonance (SPR). In-depth discussions of protein adsorption on alkanethiol SAMs are easily found in the literature. Less attention, however, has been given to alkylsilane monolayers, perhaps because they lack the highly ordered packing formed by alkanethiol SAMs (resulting in less well defined surfaces), or perceived difficulty in the preparation of well-characterized alkylsilane surfaces. This is somewhat unfortunate as alkylsilanes represent a broad and useful class of compounds that are used in an increasing variety of biomedical and biotechnological applications. For this reason a biosensor system utilizing whispering gallery mode (WGM) technology, where the active sensor is typically a silica disk, ring, toroid, or sphere/spheroid, can provide new insights into the adsorption behavior of biomolecules onto alkylsilane-modified surfaces.

WGM biosensing is based on monitoring the frequency shift of an optical resonance excited inside a glass microsphere [3, 4]. Near-infrared light is evanescently coupled to a glass microsphere with a radius of 50–200 μm from a tapered optical fiber, which is connected to a tunable distributed feedback (DFB) laser at one end and a photodetector at the other. The laser and detector are used to precisely monitor changes in the resonant wavelength of the microsphere. As proteins or other material accrete at the surface of the microsphere, the effective radius of the sphere increases, resulting in a red shift of the resonant wavelength that can be quantified and used to calculate the average surface density of adsorbed material. Even with a simple experimental configuration [4] it has been shown that a detection limit of $\sim 1 \text{ pg/mm}^2$ can be readily achieved. This is ten times more sensitive than an SPR biosensor and theoretical calculations predict the ultimate detection limit of the method to be close to the single molecule level [5–8]. These qualities make WGM biosensing an ideal method for studying protein adsorption, as the dynamic range of the method allows measurements to be performed in concentration regimes that previously were unattainable. To date, optical resonators of this kind have been applied to a variety of biosensing applications with great effect. In addition to the inherent sensitivity of the method, standard CMOS technology can be applied to fabricate arrays of resonators on silicon wafers that provide a scalable multiplex sensing capability for detecting multiple biological or chemical markers from a single sample in parallel [9, 10]. Furthermore it has been shown that these measurements can be performed in complex samples such a blood plasma and serum [11]. This provides an

added level of complexity as “real-world” samples such as plasma and serum contain a mixture of hundreds of proteins, which may non-specifically bind to a sensor giving inaccurate readings or false positive measurements. For this reason it is critical to understand the underlying processes that govern the adsorption of biomolecules to silica substrates and the coatings used to functionalize them and their subsequent interactions with cells and tissue constructs. In this study the unique capabilities of the WGM sensor were utilized to quantify the kinetics of the adsorption of fibronectin (FN) onto engineered surfaces. Cell studies were then done to evaluate the biological activity of the adsorbed FN [12]. Fibronectin is an important protein in the extracellular matrix (ECM) that mediates the interaction of cells with surfaces, but its activity has been shown to be influenced by its surface structure [13, 14]. Since the adsorption of FN has been widely studied, the results from the WGM instrument could be compared to an extensive amount of data.

The glass WGM resonator was modified with alkylsilane monolayers presenting well-defined surface chemistries: 2-[Methoxypoly(ethyleneoxy)propyl]trimethoxysilane (SiPEG), (3-trimethoxysilyl propyl) diethyltriamine (DETA), and 1,1,2,2-perfluorooctyl trichlorosilane (13F). The WGM biosensor was then used to quantitatively study the adsorption of FN at varying concentrations onto SiPEG, DETA and 13F alkylsilane SAMs. The bench-top WGM biosensor incorporated a flow cell, which minimized the effect of transport limitations on protein adsorption, and this, along with the inherent sensitivity of the method, allowed the kinetics of adsorption of FN to be measured at concentrations lower than those that have previously been reported [14]. The kinetic data provided additional information about the mechanism of adsorption that is not available from equilibrium experiments. The well-known RSA model [15] was fitted to the measured kinetic curves, and the resulting parameters were used to assist in interpreting the results. In addition, a more sophisticated model featuring a post-adsorption transition was fitted to the experimental data [16, 17]. To maximize the accuracy of the kinetic parameters, computational fluid dynamics (CFD) simulations were used to study limitations in the transport of protein to the sensor surface. For determining the biological activity of the adsorbed FN, neuronal and skeletal muscle cells were cultured on the SiPEG, DETA, and 13F surfaces in a serum-free culture system [18, 19]. Glass and silicon oxide surfaces are much more common than metal surfaces in cell culture applications, so silane surface modification is of greater practical importance than thiol surface modification for tissue engineering. WGM biosensing provides a unique capability to quantify protein adsorption on silane-modified surfaces for the purpose of understanding the interactions between tissues and tailored interfaces.

2. MATERIALS AND METHODS

2.1. WGM Instrument Fabrication and Resonator Modification

A simplified schematic representation of the bench-top instrument used in these experiments is shown in Figure 1. The instrument can be broken down into three components: the fluidic system, the laser and detector and the data acquisition and analysis system. The fluidic system was comprised of a peristaltic pump for delivery of the buffer and protein solutions to the sensor, a flow cell for housing the resonator and waveguide and focusing the buffer and protein solutions onto the resonator, and a water bath for keeping solutions at a constant temperature. The laser and detection system consisted of a wavelength-tunable distributed feedback (DFB) diode laser, a function generator, a current and temperature control unit for controlling the DFB laser and an InGaAs photodetector. The data acquisition and analysis system was comprised of a computer with a data acquisition card and Labview software for acquiring the data, and data analysis software for calculating the surface concentration of adsorbed protein.

2.1.1. Laser and detection system—The laser and detection system was based on that described by Vollmer et al [4]. A Lucent D2304G DFB laser diode with a FC/PC connector, nominal wavelength of 1310 nm and maximum power output of 10 mW was used as the excitation source in all experiments. The laser was mounted on an LDM-4984 butterfly laser diode mount (ILX Lightwave Corp, Bozeman, MT) and controlled with a LDC 3724B single channel current and temperature controller (ILX Lightwave Corp, Bozeman, MT). The wavelength of the DFB laser was modulated using an HP 33120A arbitrary waveform generator. The waveform generator was connected to the modulation input of the laser controller and the transfer coefficient was set to 20 mA/V. Under these conditions, a change of 1 V in the modulation signal resulted in a 20 mA change in laser current. Thus, the wavelength emitted by the DFB laser was modulated in a current dependent manner (~0.009 nm/mA). For all experiments a saw-tooth function with a variable peak-to-peak height was used to modulate the laser at a frequency of 100 Hz. The laser output was coupled to an SMF-28e+ optical fiber (Corning Inc., Corning, NY), the end of which was connected to a Thorlabs model PDA10CF InGaAs photodetector. The photodetector was interfaced with a Labview M-series data acquisition card and the signal from the detector was analyzed using a virtual instrument (VI) written in Labview 7.0 (National Instruments, Austin, TX). The data acquisition VI tracked all resonant valleys in the acquired spectrum using a peak fitting algorithm that selected all valleys with a minimum FWHM value set within the VI and determined the position of the valley minimum using a Bessel function. The data acquisition was synchronized with the saw-tooth function created by the function generator such that acquisition began at the minimum and ended at the peak of the function. The position of each resonance over time was saved to a binary file to be analyzed later.

2.1.2 Microsphere and waveguide fabrication—A single mode optical fiber with a 250 μm acrylate polymer coating and 125 μm cladding with a 9 μm core (SMF-28e+, Corning Inc., Corning, NY) was used to fabricate the resonators [4]. The acrylate coating was first removed using a fiber optic stripper and the stripped region was cleaned with isopropyl alcohol (iPA) to remove any residual acrylate. The end of the stripped fiber was then placed in the flame of a nitrous-butane Microflame torch (Azuremoon trading company, Cordova, TN). A nitrous-butane flame was used due to the very high temperatures needed to melt the glass and form the resonator ($> 700^\circ\text{C}$). The tip of the fiber was placed in the flame until the glass glowed bright white and began to melt. The surface tension of the molten glass caused it to form into a spheroidal droplet. As the tip melted, the fiber was rotated to ensure that the resonator remained centered on the stalk of the fiber. Resonator radii used for these studies ranged from 125 to 175 μm .

A waveguide was fabricated by flame drawing a single mode optical fiber. First, a 1–2 cm section of the acrylate coating in the middle of a length of single mode fiber was stripped and rinsed with iPA. The stripped section of the fiber was mounted between the moving parts of a syringe pump (KD Scientific, Holliston, MA) such that the fiber could be stretched as the glass was softened by the flame from a nitrous-butane torch. The tapered region of the fiber was slowly pulled down to a final diameter of ~4 μm .

2.1.3. Surface modification of microspheres with silane monolayers—Glass microspheres, and glass coverslip controls for XPS and contact angle analysis, were placed in a Harrick model PDC-32G plasma cleaner (Harrick, Ithaca, NY). The chamber was evacuated to a pressure of 300 millitorr. Ultrapure oxygen was then purged into the system to a pressure of 800 millitorr, and evacuated again to 300 millitorr. An oxygen plasma was initiated by applying an RF field around the chamber. After initiation of the plasma, the pressure in the chamber was adjusted to ~550 millitorr. Cleaning was allowed to proceed for 20 minutes. After cleaning, the resonators and coverslips were removed for reaction with the

silanes. Prior to cleaning and coating, resonators were mounted on a block of PDMS bonded to a microscope slide to allow easy handling.

All silane solutions were prepared at a concentration of 0.1% (vol:vol) in distilled toluene in an MBraun glovebox (Stratham, NH) under anhydrous, low oxygen conditions. Storing and preparing solutions in this way prevented solution phase polymerization of the silane as nascent water vapor and atmospheric oxygen can react with the monomer. Preparation of 13F surfaces was performed in the glovebox due to the extreme reactivity of the monomer. Microspheres were immersed in the silane solution for 30 minutes. 5 minutes prior to completion of the reaction, the beaker was removed from the glovebox and placed in a chemical fume hood. Both the SiPEG and DETA surface modifications were performed in a chemical fume hood. The SiPEG coating was achieved by adding 0.8 ml/L of concentrated HCL to the silane solution then immersing the resonators and substrates for 1 hour in the resultant solutions. DETA coated substrates were immersed in the silane containing solution, gently heated to 65°C over 30 minutes and then allowed to cool for 15–20 minutes. Substrates were rinsed (3×) with fresh toluene and heated again to 65°C in fresh toluene for 30 minutes. Upon completion of the reactions, the microspheres and coverslips were washed 3× in dry toluene and stored in a desiccator until needed. Control coverslips were analyzed by XPS and contact angle goniometry and were consistent with previously published results [20].

2.1.4. Flow-cell fabrication and assembly—A flow-cell was fabricated for protein adsorption experiments using polycarbonate blocks (l = 50 mm, w = 50 mm, h = 6 mm). The final device configuration can be seen in Figure 2. The body of the flow cell was fabricated by milling out a fluid channel (l = 30 mm, w = 2 mm, h = 2 mm) in one of the polycarbonate blocks. A channel for mounting the resonator (l = 10 mm, w = 2 mm, h = 1 mm) was established at one end of the main channel, and another channel for mounting the waveguide (l = 50 mm, w = 2 mm, h = 1 mm) was also created. A lid was fabricated using a similar polycarbonate block with 6–32 tapped holes aligned to the ends of the main channel, which served as the inlet and outlet ports. Female Luer connectors with 6–32 tapped ends were threaded into the inlet and outlet ports and sealed with hot glue.

The cross-channel of the flow cell was aligned to the waveguide, ensuring the narrowest region of the waveguide was centered in the main fluidic channel. The flow cell was raised into place using a 3-axis micromanipulator (Newport, Irvine, CA) such that the waveguide rested on the channel bottom. The waveguide was sealed in place with Kwik-sil silicon elastomer adhesive (World Precision Instruments Inc., Sarasota, FL). After the adhesive was set (approximately 10 minutes) the waveguide was connected to the laser and detection system. The polymer coating on the free ends of the waveguide was stripped using a fiber optic stripper and cleaned with iPA. The ends of the waveguide were then cleaved and mechanical fiber optic splices (Fiber Instrument Sales, Oriskany, NY) were used to connect the waveguide to the DFB laser and the detector.

A silane coated microsphere was then mounted into the flow cell. A microsphere was taped to a 3-axis micrometer and aligned over the waveguide. The microsphere was slowly lowered into contact with the tapered section of the waveguide. Upon contact with the waveguide, resonances could be observed in the resulting spectrum on the data acquisition VI. The microsphere was aligned so that it was centered in the fluid channel and the stalk of the fiber rested on the bottom of the microsphere channel. The microsphere was then secured into place using Kwik-sil elastomer adhesive as with the waveguide. The gain on the DAQ card and detector were adjusted to optimize the signal to noise ratio. After the adhesive had set the channels were primed with 50 mM PBS (pH 7.4). Priming the channels ensured that no bubbles would be present in the flow cell when it was sealed.

A 1 mm thick PDMS gasket was placed between the lid and the body of the flow cell and the lid was sealed to the flow cell using wing nuts to apply even compression across the lid. More PBS was added through the inlet port using a hypodermic needle and syringe to displace the air remaining in the flow cell. Silastic tubing (1.6 mm O.D., 0.76 mm I.D., Dow Corning) and Luer connectors (Harvard Apparatus, Cambridge, MA) were used to connect the flow cell with containers for the buffer and protein solution. Buffer and protein solution were re-circulated in a closed loop using a peristaltic pump and 3-way stopcocks were used to switch between the two solutions. Using this system it was possible to switch between the buffer and protein lines without significantly disturbing the flow field.

2.2 Fibronectin adsorption experiments

FN adsorption was measured on resonators modified with DETA, 13F, and SiPEG. Fibronectin (M.W. 450kD) from bovine plasma (Sigma-Aldrich, St. Louis, MO) was diluted to 10 µg/ml, 1 µg/ml, 0.5 µg/ml, and 0.25 µg/ml in 50mM PBS (pH 7.4) for the experiments. The tubing was primed with buffer to ensure no bubbles were present in the line and buffer was flowed at a rate of 150 ml/hr to thermally equilibrate the system for at least 15 minutes. After a stable baseline had been achieved the protein solution was introduced. Data was collected until a stable equilibrium had been achieved and no further shift in the resonant wavelength was quantifiable. Data analysis software was written using the Python programming language. The binary file from each experiment was loaded into the software and the spectral location (in nm) of each resonance was reconstructed over time from the raw data. One resonance, with a continuous trace and the lowest FWHM value, was chosen for further analysis. A linear baseline subtraction was applied to correct for baseline drift. A method based on first order perturbation theory [4, 6] was used to calculation of the surface concentration of the adsorbed species, σ_s , based on the measured change in resonant wavelength, $\Delta\lambda$:

$$\frac{\Delta\lambda}{\lambda} = \frac{\alpha_{ex}\sigma_s}{\epsilon_0(n_s^2 - n_m^2)R}$$

where λ is the nominal wavelength of the resonance, $\Delta\lambda$ is the wavelength shift of the resonance, n_s is the refractive index of the sphere (1.46), n_m is the refractive index of the medium surrounding the sphere (1.3357), α_{ex} is the excess polarizability of the protein molecule (0.184 cm³/g), ϵ_0 is the permittivity of free space and R is the radius of the spheroid. Spheroid radii were measured from images taken using brightfield microscopy. Two runs for each concentration were averaged for the DETA and 13F surfaces, and a single run was used for each concentration on the SiPEG surface.

2.3 Cell culture studies

To determine the biological activity of the protein adsorbed on the various silanes, cell culture experiments were performed on silane-coated coverslips that had been treated with 1µg/ml of FN in PBS (pH 7.4). Embryonic hippocampal neurons and skeletal myoblasts were used. After plating, cultures were maintained in a water-jacketed incubator at 37°C (85% relative humidity) and 5% CO₂ for seven days. Phase-contrast microscopy images were taken during the course of the culture to document the morphology of the cells and, in the case of skeletal myoblasts, the differentiation of the cells into functional myotubes.

2.3.1 Embryonic skeletal muscle culture—Skeletal muscle was dissected from the hind limb thighs of a rat fetus at embryonic day 18 (Charles River Laboratories, Wilmington, MA) according to a previously published protocol [19] with some modification. Tissue samples were collected in a sterile 15-ml centrifuge tube containing 1

ml of calcium and magnesium free phosphate buffered saline (PBS). Tissue samples were enzymatically disassociated using 3 ml of 0.05% of trypsin-EDTA (Invitrogen, Carlsbad, CA) solution for 60 min in a 37°C water bath with agitation of 100 rpm. After 60 min, the trypsin solution was removed and 6 ml of L15 media (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (FBS) was added to terminate the trypsin action. The tissue was then mechanically triturated using a sterile narrow bore Pasteur pipette, allowed to settle for 3 min, and transferred to a 15-ml centrifuge tube. This was repeated three times. The dissociated tissue was then centrifuged at 300g for 10 minutes at 4°C on 6 ml of a 4% (wt/vol) cushion of bovine serum albumin (BSA). The pellet was resuspended in 10 ml L15 + 10% FBS and plated in uncoated 100-mm Petri dishes for 20–30 min depending on the amount of tissue, to allow contaminating fibroblasts to settle out. After 20–30 minutes the supernatant was layered on 6 ml of a 4% BSA cushion, and centrifuged at 300g for 10 min at 4°C. The pellet was resuspended in 1.5 ml of medium. Purified myocytes were plated at a density of 500–800 cells per square millimeter onto FN-coated coverslips. Myocytes were allowed to attach for 1 hour after which time 3 ml of culture medium (Neurobasal media containing B-27 [Invitrogen, Carlsbad, CA], Glutamax [Invitrogen, Carlsbad, CA], and Pencillin/Streptavidin) was added. Culture medium was exchanged every 4 days.

2.3.2 Embryonic hippocampal neuron culture—Embryonic hippocampal neurons were prepared according to previously published protocols with some modification [18, 19, 21–24]. Rat pups at embryonic day 18 were dissected from timed pregnant rats that were euthanized using CO₂ asphyxiation. Embryos were collected in ice cold Hibernate E/ B27/ Glutamax™/ Antibiotic-Antimycotic. The hippocampi were isolated from the embryonic brain and collected in a tube containing 1ml of Hibernate E/ B27/ Glutamax™/ Antibiotic-Antimycotic. The embryonic hippocampal neurons were obtained by triturating the tissue using a Pasteur pipette. The 1ml cell suspension was layered over a 4 ml step gradient (Optipep diluted 1:1 (vol:vol) with Hibernate E/ GlutaMAX™ / antibiotic-antimycotic/ B27 and then made to 15%, 20%, 25% and 35% (vol:vol) in Hibernate E/ GlutaMAX™/ antibiotic-antimycotic/ B27) followed by centrifugation for 15 min, using 800g, at 4°C. After centrifugation, pyramidal hippocampal neurons with large somas formed one strong band at the top. The cells were resuspended in culture medium (Neurobasal / B27 / Glutamax™ / Antibiotic-antimycotic) and plated at a density of 75 cells/mm². After plating, 3 ml of culture media was added. Half of the medium was changed every 3–4 days.

2.3.3 Live-dead assay evaluation—A live-dead assay (Invitrogen, Carlsbad, CA) was performed at day 7 to determine the amount of living versus dead cells on the coverslips. Briefly, a solution containing 5 µM of casein and 20 µM ethidium bromide was prepared in 50 mM PBS. Cells were washed (3×) in PBS and incubated in the live-dead solution for 30 minutes. Random images were taken on an epifluorescence microscope (Zeiss) and the number of live cells (green fluorescence) and dead cells (red fluorescence) were counted. For the myocyte cultures the extent of myotube formation was also evaluated.

2.4. Theoretical and Simulation-based Methodology

2.4.1. Transport model—Computational fluid dynamics (CFD) simulations were run with a CFD-ACE+ multiphysics solver (ESI Software, Huntsville, AL) to determine the influence of transport on the adsorption of protein on the resonator surface. Before beginning, a Reynolds number calculation was performed to confirm that flow in the device would be entirely laminar. For all CFD simulations, the size of the mesh and the time step were refined until the simulation results did not change significantly. Upwind differencing was used to approximate velocity derivatives, a 2nd order limiter was used to approximate concentration derivatives, and the Euler method was used for time stepping in transient simulations. A multi-scale approach was used to obtain high-resolution results while

keeping the simulation run time reasonable. A large-scale model of the flow system, including the 40 cm of tubing between the three-way stopcock and the flow cell, was created using CFD-GEOM and discretized using a structured mesh. This model did not include the resonator and waveguide, as they have a minimal effect on the overall flow in the channel. A steady-state simulation was performed first to determine the flow field. Transient simulations were then performed in which the concentration at the inlet was suddenly increased from zero to the target concentration. The concentration of protein was monitored at a point in the flow cell at the location of the resonator, and the simulation was run until the concentration at this point reached the target value. It was assumed that the low concentrations of protein used in these experiments did not affect the flow field significantly, so the transient simulations utilized the flow field from the steady-state simulation to reduce computation time.

Two additional CFD models were created to simulate the transport of protein near the resonator. A detailed three-dimensional model was used to model the flow field around the resonator and the waveguide. This model ran too slowly to be used for transient simulations of adsorption, so a simplified model was created with a two-dimensional axisymmetric geometry. Because the axisymmetric geometry models a channel with a circular cross section while the actual channel has a square cross section, the flow velocity at the inlet was adjusted so the velocity near the resonator matched the results from the three-dimensional simulation. Data from the simulation of the whole flow cell was used to set the concentration over time at the inlet of the axisymmetric model. A surface reaction was defined on the surface of the resonator using the Langmuir model built in to the Biochemistry module of ACE+. The purpose of this reaction was to deplete the protein near the resonator at an appropriate rate, rather than to model the actual chemistry of adsorption. An association rate constant of $1.44 \times 10^6 \text{ L mol}^{-1} \text{ s}^{-1}$, a dissociation rate constant of $8.9 \times 10^{-4} \text{ s}^{-1}$, and a maximum density of adsorption sites of $4.4 \times 10^{-9} \text{ mol/m}^2$ were used to match the maximum adsorption rate that was observed in the experimental data for DETA and 13F. A steady-state simulation was used to establish the flow field and a transient simulation was used to determine the concentration of protein near the surface of the resonator. The adsorption simulation mandated a fairly small time step (on the order of 10^{-4} sec), so it was impractical to run the simulation to equilibrium due to the large number of time steps required. The system was simulated for 150–300 seconds (of simulated time). It was assumed that the near-surface concentration increased linearly from the end of the CFD simulation to the target concentration.

2.4.2. Adsorption kinetics models—The adsorption of protein was modeled using two methods. The Random Sequential Adsorption (RSA) model used to fit the experimental data is well known [15]. The surface concentration of adsorbed protein is modeled by the differential equation:

$$\frac{d\theta}{dt} = k_a C \phi(\theta) - k_d \theta$$

where θ is the fraction of the surface covered by adsorbed protein, C is the concentration of protein in solution near the surface, k_a is the adsorption rate constant, and k_d is the desorption rate constant. The near-surface concentration C is often constant, but for this work it was allowed to vary over time as predicted by the CFD simulations. The surface exclusion effect function $\phi(\theta)$ describes how adsorbed particles block the adsorption of additional particles. This function can be accurately approximated by the empirical formula:

$$\phi(\theta) = \frac{(1-x)^3}{1 - 0.8120x + 0.2236x^2 + 0.0845x^3}$$

where $x = \theta/\theta_\infty$ and θ_∞ is the equilibrium fractional surface coverage for an RSA process, which is 0.547 for spherical particles [15]. The differential equation was solved numerically with the **odeint** routine from Scipy [25]. The surface concentration was computed from the fractional surface coverage using $\Gamma = \theta/\pi r^2$, where r is the radius of the adsorbed particle.

As proteins may denature on the surface after adsorption, a more complex model was used to model adsorption with a post-adsorption transition and used for comparison to the RSA model. The model described in [16] is summarized here for convenience. Protein initially adsorbs on the surface in a reversible state with an effective radius of r_α and area A_α . Adsorbed protein then undergoes a transition to an irreversibly bound state with an effective radius of r_β and area A_β . The dimensionless spreading magnitude is $\Sigma = r_\beta/r_\alpha$. The protein is assumed to adsorb in a single layer, and adsorbed protein molecules are not allowed to overlap. This model is described by the following equations:

$$\frac{\partial \theta_\alpha}{\partial t} = k_a A_\alpha c \Phi_\alpha(t; \Sigma, k_s, k_d) - k_s \theta_\alpha \Psi_{\alpha\beta}(t; \Sigma, k_s, k_d) - k_d \theta_\alpha$$

$$\frac{\partial \theta_\beta}{\partial t} = k_s \Sigma^2 \theta_\alpha \Psi_{\alpha\beta}(t; \Sigma, k_s, k_d)$$

The adsorption rate constant is k_a , the desorption rate constant is k_d , and the transition rate constant is k_s . The fractions of the surface covered by protein in states α and β are represented by θ_α and θ_β , respectively, and $\theta = \theta_\alpha + \theta_\beta$. The following functions were derived from scaled particle theory and represent the probability of finding a cavity on the surface large enough to accommodate an additional molecule.

$$\Phi_\alpha = (1 - \theta) \exp \left[\frac{-2(\bar{\rho}_\alpha + \Sigma \bar{\rho}_\beta)}{1 - \theta} - \frac{\bar{\rho}_\alpha + \bar{\rho}_\beta + (\Sigma - 1)^2 \bar{\rho}_\alpha \bar{\rho}_\beta}{(1 - \theta)^2} \right]$$

$$\Phi_\beta = \exp \left[\frac{-2(\Sigma - 1)(\bar{\rho}_\alpha + \Sigma \bar{\rho}_\beta)}{1 - \theta} - \frac{(\Sigma^2 - 1)[\bar{\rho}_\alpha + \bar{\rho}_\beta + (\Sigma - 1)^2 \bar{\rho}_\alpha \bar{\rho}_\beta]}{(1 - \theta)^2} \right]$$

The cavity functions are defined in terms of the dimensionless surface densities $\bar{\rho}_\alpha = \theta_\alpha$ and $\bar{\rho}_\beta = \theta_\beta/\Sigma^2$.

The modeled adsorption curves were fitted to the experimentally measured curve using the least squares fitting routine from Scipy, which uses a modified version of the Levenberg-Marquardt algorithm [25]. For each surface, the parameters k_a , k_d , k_s , r_α , and Σ were fitted simultaneously for all of the available concentrations. The sum of squared errors (SSE) was

computed for each type of surface and normalized by dividing by the total number of data points in each data set so that the SSE values from different data sets could be compared.

3. RESULTS

3.1. Analysis of transport in the flow cell

The steady-state velocity magnitude predicted by the model of the whole flow cell is shown in Figure 3, along with a close-up view of the simulated flow field near the junction of the resonator and waveguide. It can be seen that the flow field around the resonator was symmetric about the long axis of the resonator, as intended. This configuration also ensured that the shear rate was constant in the region where the evanescent wave was excited, minimizing any shear rate effects on the adsorption of protein from solution. Also, it should be noted that the waveguide coupled to the resonator created a negligible disturbance of the flow field.

The evolution of bulk concentration in the flow cell over time is shown in Supplemental Figure 1a. The solution concentration in the flow cell in the vicinity of the resonator required less than 60 seconds to reach the bulk solution concentration. The near-surface concentration predicted by the high-resolution CFD model is shown in Supplemental Figure 1b. The evolution of the protein concentration near the surface of the resonator required considerably more time. The effects of this difference in near surface concentration with respect to the bulk were taken into account when fitting the model to the experimental data.

3.2. Fibronectin adsorption onto alkylsilane monolayers

Averaged sensograms from FN adsorption experiments on resonators coated with DETA, 13F, and SiPEG are shown in Figure 4. Over the range of concentrations tested, the amount of protein adsorbed to the different silanes followed the trend 13F = DETA $\gg$ PEG. While it was not wholly unexpected that similar amounts of protein adsorbed to DETA and 13F, the fact that the saturation values at solution concentrations of 1 μ g/ml and below were so high was not anticipated. These results stand in contrast to much of the published literature as shown in Table 1. To facilitate comparison, the surfaces types were grouped according to their wetting properties as not all groups used exactly the same surface modification. Thus, the surfaces were considered as hydrophobic (13F, and -CH₃ terminated SAMs), hydrophilic charged (DETA, and other -NH₃ terminated SAMs), or hydrophilic (SiPEG, or -OH terminated SAMs).

As can be seen in Table 1, the saturation values measured for FN on hydrophobic surfaces from solution concentrations of 10 μ g/ml were generally in agreement with the lowest saturation value at 110 ng/cm², the highest value at 200 ng/cm² (measured with the WGM biosensor), and the remaining values closely grouped at $\sim$ 160 ng/cm². Similarly, for charged hydrophilic surfaces, the majority of the reported values ranged from 140–190 ng/cm², with one report as low as 70 ng/cm². Once again the measurements provided by the WGM biosensor constituted the high end of the saturation values for these particular surfaces. For hydrophilic surfaces, however, WGM biosensor measurements were in line with published data. The range of published results was considerably more consistent with a range of 25–30 ng/cm², with one report of a saturation value of 50 ng/cm². In general, the results obtained by the WGM biosensor at higher concentrations of FN were in agreement with the literature.

The results for FN adsorption from lower solution concentrations were strikingly different from previously published results with the WGM biosensor. Saturation values from the literature were compared to measurements for FN solution concentrations of 1 μ g/ml. Generally, comparison of kinetics and saturation values from solutions below 1 μ g/ml in concentration is difficult, as other methods are typically limited by sensitivity constraints

and very little published data exists in this range. As can be seen in Table 1, for hydrophobic surfaces, results in the literature ranged from 10–30 ng/cm². The average saturation value measured with the WGM biosensor was ~135 ng/cm². Similarly, with charged hydrophilic surfaces saturation values in the literature ranged from 10–20 ng/cm², but values measured with the WGM biosensor were ~137 ng/cm². These results were significantly higher than what has been previously published, and raised questions as to the source of this variation. However, when one compares saturation values on hydrophilic surfaces it can be seen that the WGM measurements were once again in line with published results. The saturation values measured in this study ranged from 5–14 ng/cm². This observation lends credibility to the higher saturation values on hydrophobic and charged hydrophilic surfaces as being due to differences in surface chemistry between the silane and thiol monolayers rather than a systematic error introduced by the method.

3.3. Quantitative analysis of fibronectin adsorption

The random sequential adsorption (RSA) model and the model of adsorption with a post-adsorption transition were fitted to the experimental data. The fitted curves for the RSA model are shown in Figure 5, and fitted curves for the two-stage adsorption model are shown in Figure 6. The quality of fit was quantified by the sum of squared errors (SSE). The fitted parameters and SSE for the RSA model are shown in Table 2, and the fitted parameters and SSE for the two-stage model are shown in Table 3. The RSA model matched the data for FN on DETA reasonably well, with the exception of the saturation surface concentration for the concentration of 5 µg/ml. The two-stage adsorption model offered little improvement, which was reflected by the small difference in the SSE values for the two models. Qualitatively, it can be seen that the two-stage model fitted the data for FN on 13F better than the RSA model. The RSA model predicted that the surface concentration approached a horizontal asymptote at saturation, but the experimental data indicated that the surface concentration increased in a linear fashion. The two-stage adsorption model emulated this behavior more effectively. This was reflected in the SSE value for the two-stage adsorption model fitted to FN on 13F, which was significantly lower than the SSE for the RSA model fitted to the same data. Although both the RSA model and the two-stage adsorption model fitted the SiPEG data fairly well, the SSE for the two-stage model showed an improvement over the RSA model.

3.4 Cell culture for fibronectin adsorbed on alkylsilane monolayers

It is well known that the surface composition has a strong influence on the viability of cells for both in vivo and in vitro model systems. Figure 7 shows fluorescent microphotographs of a live/dead assay for embryonic hippocampal neurons cultured on each silane. Panels A–C and D–F show phase contrast images of cells after 1 day and 7 days in culture. Significantly more embryonic hippocampal cells survived on the DETA surfaces than on the 13F or SiPEG surfaces. Table 4 indicates the number of live and dead embryonic hippocampal neurons on the three different surfaces examined in this study.

Figure 8 shows fluorescent microphotographs of live/dead assays performed on embryonic skeletal myocytes (ESM) 1 day and 7 days after plating. At day 1 in culture, dense myocyte adhesion can be seen on DETA coverslips coated with FN, while significantly fewer adhered are indicated on 13F and SiPEG. On the DETA coverslips, myocytes can be seen to be adopting a spindle shaped morphology. Cells on 13F and SiPEG maintain an unelongated morphology. After 7 days in culture, myocytes on DETA have begun to form long cylindrical myotubes. The myotubes could be seen to spontaneously twitch, indicating that they were functional. After 7 days no myotube formation had occurred on either the 13F or SiPEG coated substrates. Table 4 lists the cells counts from the live/dead assay for ESMs on the three surfaces.

4. DISCUSSION

The equilibrium surface concentration of FN, as measured by the WGM system, compares favorably with previously published results for a solution concentration of 10 $\mu\text{g/ml}$, as shown in Table 1. Although different measurement techniques and surface preparations were used, the surface concentrations on hydrophobic and hydrophilic surfaces were fairly similar among the various references (reference [26] being the only exception). The saturation surface concentration of FN on hydrophilic neutral surfaces measured by the WGM sensor also indicated excellent agreement with previously published results. However, the amount of adsorbed protein measured by the WGM system at lower concentrations was significantly greater than the amount reported in previously published results for hydrophobic and hydrophilic charged surfaces. In contrast, the surface concentration value for a neutral hydrophobic surface was in good agreement with previous results. Table 1 also shows the saturation surface concentration of FN at a solution concentration of 1 $\mu\text{g/ml}$. The discrepancy between the WGM sensor results and the other methods at low concentrations may be explained by differences in the measurement system, the surface chemistry, or the adsorption process. Since the limiting surface coverages measured by the WGM sensor agree well with other techniques for 10 $\mu\text{g/ml}$ and for neutral hydrophilic surfaces at 1 $\mu\text{g/ml}$, the results from the WGM sensor can be considered reliable. It is likely that if systematic errors were inherent to the WGM method, those errors would be reflected throughout all solution concentrations measured.

One possible interpretation of the higher saturation values measured at 1 $\mu\text{g/ml}$ and lower on 13F and DETA could be the relative packing order of silane monolayers compared to alkanethiol monolayers. Alkanethiol SAMs are known to create highly ordered monolayers due to their tight packing on highly ordered gold films [27]. Because of this only the terminal functional groups of the alkanethiol are presented at the surface, resulting in highly defined surface chemistries. Alkylsilane monolayers, which are formed on silica surfaces, are less tightly packed and therefore form less ordered monolayers, which can potentially present more than just the terminal functional group. It has been hypothesized that this may result from interaction of electron donating groups of the silane side chain with silanol groups at the surface resulting in reaction site-limited substrates [28]. This can lead to incomplete monolayers that allow interaction of protein with the unreacted substrate or allow sufficient degrees of freedom for the silane side-chains to adopt multiple conformations, creating less ordered monolayers that can rearrange to accommodate the native protein structure. At the high concentrations the amount of protein available to bind would swamp out these effects but they would be present at the lower concentrations. This could explain why the differences between the silane chemistry used in this work and the alkanethiol chemistry used in previous work [29] did not show up at 10 $\mu\text{g/ml}$. Thus, this additional degree of freedom would allow the long side chains to rearrange to accommodate greater protein interaction for structural stabilization and higher coverage or to expose new surface sites for increased protein adsorption. This would not be possible with the tightly packed alkane thiol monolayers. However, if the observation of high saturation values from low solution concentrations were due strictly to monolayer packing, this would be reflected in the literature. Thus, SAM surface structure differences are not seen as the only possible explanation, but it should be noted that this effect could also have major consequences for protein function, as described later.

Another significant difference between the WGM measurement system and previous work was that the protein was deposited on the WGM resonator under flow conditions and the measurement was continued until saturation was reached, while previous measurements were made after exposure to a static FN solution for a fixed amount of time (30–60 minutes.) The combination of high-affinity surfaces and low solution concentration is

conductive to transport-limited adsorption, which could explain the discrepancy between WGM and static experiments for hydrophobic and charged hydrophilic surfaces, but also give new insight into the reasons behind why silane monolayers seem to be better cell culture substrates than thiol monolayers. In contrast, neutral hydrophilic surfaces have a much lower affinity for protein, so depletion of protein near the surface would be much less of a factor, resulting in a good match between the WGM and static measurements.

Both the embryonic hippocampal neurons and myocytes showed significantly better survival on DETA surfaces than 13F surfaces. However, the amount of adsorbed protein measured on the 13F surfaces was comparable to that of DETA, indicating that the conformation of adsorbed FN, and its function, was just as important as the quantity of FN for cell survival. This is consistent with the postulate made above that the silane monolayers are able to rearrange to accommodate more protein and that the DETA surface, being charged and hydrophilic, could accommodate the functional conformation of the FN so little or no denaturation would occur. Conversely, the hydrophobic side chains of the 13F could rearrange to allow for the adsorption of more protein but would also promote the exposure of the protein's hydrophobic core, thus denaturing the protein and deactivating its biological activity as postulated previously for hydrophobic surfaces [29]. Results from the skeletal myocyte culture provided further information about the bioactivity of adsorbed FN. Skeletal myocytes are precursor cells that fuse and differentiate into contractile myotubes. This differentiation is mediated by, among other factors, the interaction of the $\alpha 5\beta 1$ integrin receptors on the surface of the myocytes with the cell binding domain of the FN molecule [13]. Without this interaction, myotubes do not form. The muscle cell culture on 13F indicated that while a significant number of cells survived, no myotubes formed. The number of dead cells was actually less than that of SiPEG or DETA, and the fact that so many cells survived on the 13F substrate indicates that there was enough protein adsorbed to the surface to promote adhesion. However, the lack of myotube formation indicates that FN adsorbed on 13F had reduced biological activity due to its denaturation and did not activate the $\alpha 5\beta 1$ integrin signaling pathways necessary for myotube differentiation. These proliferation and differentiation results are consistent with previously reported results [13]. The lack of survival of cells on SiPEG surfaces can be attributed to the small amount of adsorbed FN and the possibility that the protein was also denatured. Utilization of the WGM sensor enables, for the first time, quantitative analysis of protein adsorption on silane monolayers, which are more commonly used as substrates for cell culture than thiol monolayers. The sensitivity of this bench-top setup also can be readily enhanced by a number of methods, such as fabricating smaller microspheres and using a laser with a shorter wavelength [8] or coating the glass microsphere with a high-index waveguiding layer [30].

Fitting models to the experimental data provided additional insight about the process of adsorption. It was assumed that the kinetic constants did not vary across the limited concentration range in this study. Therefore, a single set of parameters was fitted to multiple concentrations for a single surface. Fitting more concentrations, while holding the number of parameters constant, increased the possibility of finding a unique combination of parameters that minimized the SSE. A model with too many parameters can have multiple parameter sets with equivalent optimal fits, much like an under-determined system of linear equations. Although a lower SSE could have been achieved by fitting each concentration individually, the likelihood of finding non-unique parameters would have increased.

The fitted parameters of the RSA model were very similar for FN adsorption on DETA and 13F surfaces, reflecting the similar shapes of the experimental curves. The RSA model fitted the DETA data well, indicating that the assumptions of the RSA model were valid for the process of adsorption on DETA. This result was confirmed by the fitting results for the two-

stage adsorption model. The sum of squared errors was only slightly lower than the SSE value for the fitted RSA model. We concluded that fibronectin adsorbed on DETA with a well-defined footprint, which does not change significantly after adsorption. This result is consistent with the well-established theory that proteins generally experience less denaturation on a hydrophilic surface than on a hydrophobic surface [31]. The experimental results for FN on 13F showed significant deviations from the RSA model in the saturation region, especially at higher solution concentrations. The two-stage model allows particles to change size after adsorption, which significantly improved the fit of the model to the data for FN on 13F. The fitted values for the association constant were quite similar for DETA and 13F, but the transition rate constant k_s for the 13F surface was an order of magnitude larger than k_s for the DETA surface. The results from the fitting process indicated that FN denatured after adsorption on 13F, which had been previously postulated for certain hydrophobic surfaces [14, 32] and this was also consistent with our cell culture results.

The RSA model fitted the SiPEG data well. The k_a value for FN adsorption on SiPEG was lower than the k_a values for DETA and 13F while the dissociation rate constant was higher, which is expected for a protein-resistant surface. This result is consistent with findings that SiPEG is an electrostatically neutral surface that does not exhibit coulombic attraction for proteins in solution. Surprisingly, the fitted radius of FN adsorbed on SiPEG was more than twice the fitted radius of FN adsorbed on DETA or 13F. For the two-stage model, the transition rate constant for adsorption on SiPEG was significantly higher than for the other surfaces. The fitted pre-transition radius and post-transition radius of adsorbed FN were also larger for SiPEG than DETA or 13F. The large radius predicted by the RSA model and the significant transition predicted by the two-stage model seemed to indicate that FN denatures after it adsorbs to PEG. This prediction was not consistent with the well-known observation that proteins in contact with hydrophobic surfaces tend to denature, while proteins in contact with hydrophilic, charged surfaces tend to retain their native conformations. However, it also may indicate that the SiPEG surface could be promoting the denaturation of adsorbed proteins, which could explain why it is a cell-resistant surface despite being hydrophilic.

Although the SSE of the fitted two-stage model was about 30% lower than the SSE for the RSA model, the absolute change in SSE was relatively small, and may not be significant. It is possible that the two extra variable parameters (transition rate constant and post-transition radius) are redundant for the SiPEG surface, in which case their fitted values should not be considered significant. It is also possible that the radius predicted by the fitting process for SiPEG is an artifact caused by fitting the data with a model that is not well suited to the surface chemistry. Given the assumptions of the RSA model, surface coverage can reach saturation in only two ways: either the rate of desorption equals the rate of adsorption, or there is no space left on the surface for another protein to adsorb. The second case may not apply to an adsorption-resistant surface like SiPEG. However, combinations of parameters k_a and k_d that fitted the initial adsorption kinetics did not predict the low saturation level of protein observed in our experiments. One possible explanation is that FN adsorbed to a small number of defects in the SiPEG monolayer, which could explain both the rapid initial adsorption and the small amount of adsorbed protein when the surface is saturated. If this were the case, a site-limited adsorption model like the Langmuir model may be better for modeling adsorption on SiPEG. Our prototype instrument did not have the sensitivity to perform a more thorough study of adsorption on SiPEG at low solution concentrations. Future systems based on whispering gallery mode technology have the potential to study the adsorption of proteins on SiPEG surfaces in greater detail, which could lead to greater understanding as to why SiPEG resists protein adsorption.

Although the flow cell was designed to minimize transport limitations, CFD analysis indicated that transport had some influence on the rate of adsorption on DETA and 13F.

Initially, transport was limited by the large amount of buffer that had to be displaced from the flow system before the protein solution could reach the resonator at full concentration. This limitation was due to the prototype nature of the system, and can be easily eliminated in future systems. After the protein solution in the channel reached its target concentration, transport was limited by the rate of diffusion across a depletion layer that formed at the surface of the resonator. This type of limitation is virtually unavoidable in microfluidic systems when the rate of adsorption is high and the solution concentration is low. The flow of water is laminar at small length scales, and the no-slip boundary condition at the resonator surface means that transport to the surface is almost entirely diffusive. Our modeling method was approximate in that the evolution of the near-surface concentration over time was computed once, based on the estimated adsorption rate, and was not modified during the curve-fitting procedure. A more accurate method would be to incorporate the CFD model into the fitting routine, so that the near-surface concentration would be updated as the adsorption rate changed [33]. However, this method is only practical when the CFD simulation is simple enough to run very quickly. It was also assumed that the near-surface concentration increased linearly from sixty seconds until the target concentration was reached. Although this probably had some impact on the modeled kinetics, it was better than assuming that the concentration near the resonator remained constant. The near-surface concentration can have a large effect on the kinetics of protein adsorption, and must be taken into account when fitting kinetic parameters.

5. CONCLUSIONS

We have constructed a bench-top WGM biosensor system integrated with a flow cell to study the kinetics of protein adsorption on functionalized glass surfaces relevant for tissue culture. Assembly of the table-top biosensing setup does not require any specialized equipment, and the device components (DFB laser, photodetector, optical fiber, simple flow cell) are inexpensive as compared to commercial systems and can be readily assembled in any laboratory environment. Despite its simplicity, the sensitivity of the setup rivals that of a state-of-the-art surface plasmon resonance sensor ($\sim 1 \text{ pg/mm}^2$ mass loading), with the additional advantage that silica microspheres can be easily fabricated and conveniently modified with various silane surface coatings by exploiting established silanol surface chemistries. This is an alternative to other methods, such as SPR, that require coating the sensor with gold and are mostly limited to surface modification with thiols. It also allowed for the first time the detailed kinetic analysis of protein adsorption on silane monolayers and enabled some explanation of the differences between silane and thiol surface modifications. The WGM sensor was used to obtain the most comprehensive set of kinetic data that has been reported for the adsorption of fibronectin at low solution concentrations. Measuring adsorption kinetics at multiple concentrations allowed a single set of kinetic constants to be fitted for multiple concentrations, which increased the likelihood of obtaining a unique set of fitted parameters. The results of the model fitting and the cell culture experiments indicate that similar amounts of FN adsorb on hydrophobic surfaces and charged hydrophilic surfaces. When combined with antibody data from other studies, our data supports the conclusion that FN denatures after adsorption on hydrophobic surfaces, leading to a loss of biological activity. Thus, it demonstrates that a much more sophisticated analysis of how protein adsorption can affect cellular response to surfaces can be undertaken with this system. Further improvements to the sensitivity will help answer difficult questions in biomaterials research, such as improving our understanding of cell-surface interactions and surfaces that resist protein adsorption.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to acknowledge support from grant number R01EB005459 from the National Institutes of Health. Frank Vollmer would like to acknowledge financial support from the Rowland and from the Wyss Institute, both at Harvard University. Craig Finch would like to acknowledge support from the Institute for Simulation and Training (IST), the I2 Lab and the NanoScience Technology Center at the University of Central Florida. IST graciously donated computing time on the Stokes cluster.

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Figure 1. Schematic representation of a WGM biosensor system. The instrument was comprised of three components: a fluidic system for driving buffer and protein solutions (dashed lines), the laser and detection system (dotted lines), and the data acquisition and analysis system (dotted and dashed lines).

Figure 2.

Flow cell geometry and experimental setup. A) Side view of the CFD model of the channel in the flow cell. The shallow channel to the left of the outlet port was used for mounting the stalk of the glass microsphere. B) Top view. C) Top view showing the experimental configuration of WGM biosensor. A tapered waveguide was mounted in the transversal channel and sealed in place with silicone elastomer. The glass microsphere (not shown to scale) was then lowered into contact with the waveguide and its position adjusted to ensure good coupling and high Q resonances. The microsphere was also secured in place with elastomer.

Figure 3.

CFD results for a fluid flow simulation. A) Fluid flow through the entire flow cell achieved a laminar flow profile a few millimeters downstream of the inlet and remained laminar as sample passed over the sensor surface. B) High resolution view of fluid flow across the sensor surface. The fluid flow was evenly distributed across the active region of the sensor with no noticeable perturbations in the flow field.

Figure 4.

Experimentally measured adsorption of FN onto DETA (A), 13F (B), and SiPEG (C) coated microspheres. Dotted lines indicate ± 1 standard deviation.

Figure 5. RSA model fitted to experimental data for FN on DETA, 13F, and SiPEG surfaces. Fine lines show the experimental average, dotted lines indicate ± 1 standard deviation, and thick lines indicate the fitted model.

Figure 6.

Two stage model fitted to experimental data for FN on a) DETA, b) 13F, and c) SiPEG. Fine lines show the experimental average, dotted lines indicate ± 1 standard deviation, and thick lines indicate the fitted model.

Figure 7. Embryonic hippocampal cells cultured on DETA (A-D-G), 13F (B-E-H), and SiPEG (C-F-I) coated substrates. All scale bars represent 100 micrometers.

Figure 8.

Embryonic skeletal muscle cultured on DETA (A-D-G), 13F (B-E-H), and SiPEG (C-F-I) coated substrates. A–C) Cells adhered to substrates after 1 day in culture. D–F) After 7 days in culture only myocytes on DETA (D) had differentiated into functional myotubes. Myocytes on 13F and SiPEG (E and F, respectively), failed to fuse. A live-dead assay showed that myotubes grown on DETA were viable after 7 days. Myocytes grown on 13F survived up to 7 days, but did not differentiate into myotubes. On SiPEG coated coverslips, myocytes were unable to adhere at all and only dead cells were detected.

Table 1

Comparison of the saturation surface concentration of FN (ng/cm²) from this work and previous studies on three different types of surfaces.

Reference	Solution Concentration	Hydrophobic	Hydrophilic charged	Hydrophilic neutral	Notes
This work	10 µg/ml	200 (13F)	190	25	
[29]	10 µg/ml	170 (CH3)	170	30	
[34]	10 µg/ml	160 (CH3)		30	
[14]	10 µg/ml	140	140	50	FN III ₇₋₁₀ fragment
[26]	10 µg/ml	110	70	25	
[35]	10 µg/ml	~175	~175		Saturation value inferred
This work	1 µg/ml	135 (13F)	137	14	
[29]	1 µg/ml	20 (CH3)	20	10	
[34]	1 µg/ml	20 (CH3)		5	
[14]	1 µg/ml	30	25	10	FN III ₇₋₁₀ fragment
[26]	1 µg/ml	10	10	10	

Table 2

Fitted parameter values for the RSA model.

	DETA	13F	SiPEG	
K_a	2.05×10^{-6}	2.24×10^{-6}	9.25×10^{-7}	$\text{cm}^3/\text{ng/s}$
k_d	3.16×10^{-4}	2.49×10^{-4}	7.14×10^{-4}	$1/\text{s}$
r	7.61×10^{-9}	7.89×10^{-9}	1.99×10^{-8}	m
SSE	86.1	121	2.7	

Table 3

Fitted parameter values for the two-stage adsorption model.

	DETA	13F	SiPEG	
K_a	2.37×10^{-6}	1.99×10^{-6}	1.12×10^{-6}	$cm^3/ng/s$
K_s	1.14×10^{-4}	8.00×10^{-3}	1.16×10^{-2}	l/s
K_d	2.12×10^{-4}	9.95×10^{-5}	1.04×10^{-4}	l/s
r_a	8.03×10^{-9}	7.49×10^{-9}	1.99×10^{-4}	m
r_β	1.10×10^{-8}	9.82×10^{-9}	3.30×10^{-8}	m
SSE	86.0	85.5	1.86	

Functional assay results for embryonic hippocampal cells and embryonic skeletal muscle cells on silanes (N=9 or more for all conditions). All values are units of cells/mm².

Table 4

	Cell Type	Minimum N	DETA	13F	SiPEG
Live	Hippocampal	9	212±102	1±3	4±5
Dead	Hippocampal	9	340±75	218±81	265±86
Myotubes	Muscle	4	35±13	0±0	0±0
Live	Muscle	4	178±43	50±32	0±0
Dead	Muscle	4	63±66	18±14	111±59