High-Q polymer-coated microspheres for immunosensing applications

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Abstract: Homogeneous polymeric thin layers have been used as functionalizing agents on silica microspherical resonators in view of the implementation of an immunosensor. We have characterized the microspheres functionalised with poly-L-lactic acid and Eudragit® L100, as an alternative to the commonly used 3-Aminopropyltrimethoxysilane. It is shown that polymeric functionalization does not affect the high quality factor (Q greater than 10^7) of the silica microspheres, and that the Q factor is about $3x10^5$ after chemical activation and covalent binding of immunogammaglobulin (IgG). This functionalizing process of the microresonator constitutes a promising step towards the achievement of an ultra sensitive immunosensor.

OCIS codes: (350.3950) Micro-optics; (310. 0310) Thin films; (280.4788) Optical sensors and sensors.

References and links

1. J. L. Nadeau, V. S. Ilchenko, D. Kossakovski, G. H. Bearman, and L. Maleki, "High-Q whispering-gallery mode sensor in liquids," Proc. SPIE **4629**, 172–180 (2002).
2. F. Vollmer, D. Braun, A. Libchaber, M. Khoshsima, I. Teraoka, and S. Arnold, "Protein detection by optical shift of a resonant microcavity," Appl. Phys. Lett. **80**(21), 4057–4059 (2002).
3. F. Vollmer, and S. Arnold, "Whispering-gallery-mode biosensing: label-free detection down to single molecules," Nat. Methods **5**(7), 591–596 (2008).
4. V. S. Ilchenko, and A. B. Matsko, "Optical Resonators with Whispering-Gallery-Modes-Part II: Applications," IEEE J. Sel. Top. Quantum Electron. **12**(1), 15–32 (2006).
5. F. Vollmer, S. Arnold, and D. Keng, "Single virus detection from the reactive shift of a whispering-gallery mode," Proc. Natl. Acad. Sci. U.S.A. **105**(52), 20701–20704 (2008).
6. A. M. Armani, R. P. Kulkarni, S. E. Fraser, R. C. Flagan, and K. J. Vahala, "Label-free, single-molecule detection with optical microcavities," Science **317**(5839), 783–787 (2007), doi:10.1126/science.1145002.
7. F. Baldini, A. Carloni, A. Giannetti, G. Porro, and C. Trono, "A new optical platform for biosensing based on fluorescence anisotropy," Anal. Bioanal. Chem. **391**(5), 1837–1844 (2008).
8. J. R. Schwesyg, T. Beckmann, A. S. Zimmermann, K. Buse, and D. Haertle, K. Buse and D. Haertle, "Fabrication and characterisation of whispering-gallery-mode resonators made of polymers," Opt. Express **17**(4), 2573–2578 (2009), http://www.opticsinfobase.org/abstract.cfm?uri=oe-17-4-2573.
9. J. Lutti, W. Langbein, and P. Borri, "High Q optical resonances of polystyrene microspheres in water controlled by optical tweezers," Appl. Phys. Lett. **91**(14), 141116 (2007).
10. F. Baldini, and S. Bracci, "Optical-fiber sensors by silylation techniques," Sens. Actuators B Chem. **11**(1-3), 353–360 (1993).
11. O. Gaathon, J. Culic-Viskota, M. Mihnev, I. Teraoka, and S. Arnold, "Enhanced sensitivity of a whispering gallery mode biosensor with subwavelength confinement," Appl. Phys. Lett. **89**(22), 223901 (2006).
12. A. T. Rosenberg, E. B. Dale, D. Ganta, and J. P. Rezac, "Investigating properties of surfaces and thin films using microsphere whispering-gallery modes", Proc. SPIE **6872**, 68720U–1-68720U–9 (2008).
13. M. Brenci, R. Calzolai, F. Cosi, G. Nunzi Conti, S. Pelli, and G. C. Righini, "Microspherical resonators for biophotonic sensors" Proceedings of SPIE, **6158**, 61580S1–61580S9 (2006).
14. J. Kobayashi, T. Asahi, M. Ichiki, A. Oikawa, H. Suzuki, T. Watanabe, E. Fukada, and Y. Shikinami, "Structural and optical properties of poly lactic acids," J. Appl. Phys. **77**(7), 2957–2973 (1995).
15. Y. Lin, V. Ilchenko, J. Nadeau, and L. Maleki, "Biochemical detection with optical whispering-gallery resonators", Proceedings of SPIE **6452,** 64520U1–64520U8 (2007).

#112509 - $15.00 USD Received 8 Jun 2009; revised 7 Jul 2009; accepted 7 Jul 2009; published 4 Aug 2009

16. I. Horcas, R. Fernández, J. M. Gómez-Rodríguez, J. Colchero, J. Gómez-Herrero, and A. M. Baro, "WSXM: a software for scanning probe microscopy and a tool for nanotechnology," Rev. Sci. Instrum. **78**(1), 013705 (2007).
17. N. Hanumegowda, I. M. White, H. Oveys, and X. Fan, "Label-free protease sensors based on optical microsphere resonators," Sensor Lett. **3**(4), 315 (2005).
18. F. Vollmer, S. Arnold, D. Braun, I. Teraoka, and A. Libchaber, "Multiplexed DNA quantification by spectroscopic shift of two microsphere cavities," Biophys. J. **85**(3), 1974–1979 (2003).

1. Introduction

Optical microresonators have been recently proposed as an efficient tool for the realisation of optical biosensors [1–3], based on the measurement of the refractive index changes induced by the interaction of the investigated analyte with a selective layer immobilised on the microresonator surface. A crucial step for producing reliable biosensors is the surface functionalization, or chemical modification of the transducer surface in order to bind the biological recognition element on it. This functional layer, however, has to be very thin, 10 to 100 nm and homogeneous, in order to preserve the high quality of the transducer and the interaction with the sensing layer and the whispering gallery modes (WGM) [1,4]. Microresonator-based sensors are devices that aim at ultra-low detection of binding events (either biological or chemical) occurring at the surface, and quite recently have achieved the goal of single molecule detection [5,6].

Here we propose, characterise and compare polylactic-acid (PLLA, a crystalline polymer) and Eudragit® L100 (anionic copolymer made of metacrylic acid and methyl methacrylate) as functionalising materials [7] alternative to 3-Aminopropyltrimethoxysilane (APTS). Polymer coated silica microspheres are proposed as an alternative to polymeric microresonators which have low Q factors in the infrared regime [8] or possess high enough Q factors in the visible regime but need expensive and bulky experimental arrangements like optical tweezers [9]. Further advantage of polymeric coatings is their extremely easy protocol of preparation: once the polymer is dissolved in the solvent at the right concentration, a dip-coating of few seconds of the microsphere into this solution and then a short waiting-time for the solvent evaporation, are sufficient to have a functionalised surface. No magnetic-stirring of the solution or backing-time are requested for this procedure differently from what is needed for the silanisation protocol [10]. Regarding high refractive index polymer coating, they could increase the frequency shift sensitivity of WGM sensors [11] and reduce the thermal noise in high-Q silica microspheres [12].

The permanence of high Q values after the functionalization of the surface is an essential requirement in order to achieve highly sensitive devices. We investigated the effect of the mentioned materials on the silica microsphere Q factor by optical means and by atomic force microscope (AFM) scans.

2. Experimental methods

The experiments were done with fused silica microspheres functionalised with Eudragit® L100, PLLA and a mixture of Eudragit L100 and fluorescein. Fluorescein is a fluorescent marker and it remains trapped inside the polymeric mesh of Eudragit; it was chosen for an easy imaging of the microspheres by fluorescence microscopy (Nikon Ecclipse E600, 20x). The microspheres used in these experiments (R ~250 μm) were fabricated using a fibre fusion splicer [13], and stored under vacuum, in order to avoid contamination.

The transmission spectrum of the WGM resonator was observed using a tuneable external-cavity laser with linewidth of 300 kHz (Tunics Plus). The laser could be finely swept at very low frequency, around a resonance by a few GHz. The laser light is coupled to the WGM resonator by means of a tapered fibre of about 3 μm diameter, produced in-house too. The light transmitted through the coupler-WGM resonator system was monitored at the output of the taper using an amplified InGaAs photodiode detector connected to an oscilloscope, as shown in Fig. 1. The Q values (greater than 10^7) were obtained by measuring the resonance linewidth in air of the WGM modes around 1.55 μm. We used an open cell filled with 3 ml of a phosphate buffered saline (PBS) solution (137 mM NaCl, 10 mM Na_2HPO_4, 2.7 mM KCl,

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pH = 7.4) and we controlled the coupling by varying the gap between the taper and the microsphere.

Clean microspheres were immersed in different solutions: 1) 100, 10 and 1 mM Eudragit® L100 (Degussa, n_D^{20} = 1.39) in ethanol (Sigma); 2) 10 mM PLLA (DURECT Corporation, n = 1.46 [14]) in chloroform (Sigma); 3) 10 mM Eudragit® L100 in ethanol, containing different amounts (0.01-0.1-1 mM) of fluorescein (Sigma). After 1 minute of dip coating, the microspheres were let to dry until total solvent evaporation. No annealing was performed. All reactions were performed under a chemical hood. These coatings, as functionalising agents, were compared with silanised microspheres reported in literature [15].

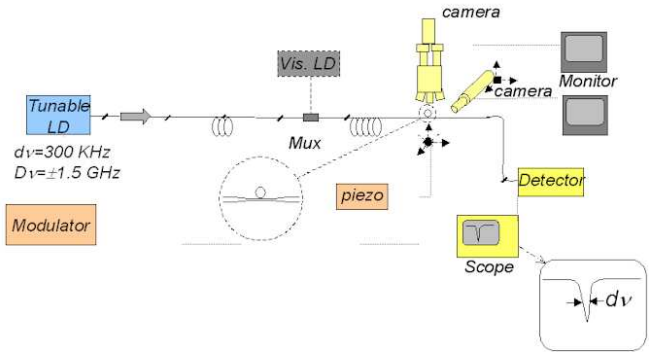

Fig. 1. A schematic diagram of the experimental arrangement.

3. Experimental Results

3.1 Atomic force microscopy characterisation

Atomic force microscope measurements have been performed by using an instrument developed in-house, based on a SPMagic R2 controller from Elbatech srl, Livorno- Italy, and capable of operating in contact or dynamic mode. We used a non-contact cantilever (NSG01- from NT-MDT Moscow, Russia) with a nominal resonance frequency of 150 KHz and a typical elastic constant of 5 N/m.

A selected sample (a microsphere, coated using a solution of 10 mM Eudragit® L100) was attached to a metallic disc and positioned on the magnetic sample holder with the fibre axis oriented in the scanning direction. A first image of the surface under investigation was acquired in non-contact mode. Then the AFM is switched to contact mode keeping the same hard cantilever as probe. The feedback set point was increased in order to apply adequate force to engrave the soft coating on the surface of the glass microsphere. As a result of a square scan of 3 µm x 3 µm, at about 1 line/sec scan speed, the surface was peeled in a small area approximately of the same square size. Finally the microscope was switched back to non-contact operating mode and a new image, on a larger area, was acquired to investigate the thickness of the engraved coating. Figure 2 shows the step at the edge of the scratched area.

All images were acquired at 256x256 pixel resolution. Data processing was performed on the topographic images with the free software WSxM [16]. In particular, we used a smoothing filter and the root mean square (RMS) roughness and the profile tools to extract the information of interest. Figure 3 (a) shows an AFM image of an equatorial cap of the coated microsphere and Fig. 3 (b) the measured profile, its waviness and RMS roughness. As it can be seen, the surface texture is good enough to preserve a high Q. The measured step height of the Eudragit film is about 30 ± 6 nm and the RMS roughness is about 6 nm.

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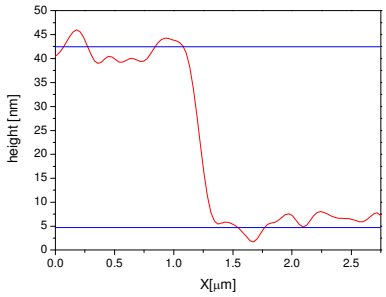

Fig. 2. AFM profile of the edge of the scratched area of a microsphere functionalised with Eudragit® L100 (10 mM). The blue lines are guides to the eye and mark an average value: 42 ± 2 nm for the upper one and 6 ± 2 nm for the lower line.

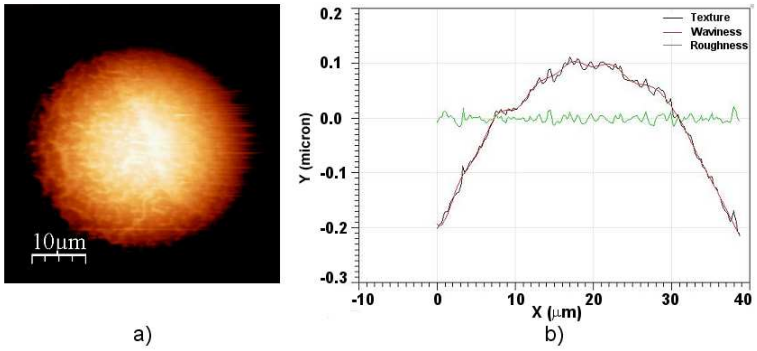

Fig. 3. a) AFM image of a portion of a silica microsphere functionalised with Eudragit® L100 (10 mM); b) Vertical profile (black), waviness (red) and the corresponding RMS (green).

3.2 Optical characterisation

We have checked the smoothness of the PLLA films by optical microscope. The images showed a homogeneous film, despite the fact that PLLA is a crystalline polymer. It is likely that the domains were either small or not formed. The measured Q factor in air is about $2.6 \cdot 10^7$ for the PLLA coated microsphere (not shown here).

Figure 4 (a) shows an image of a microsphere coated with a 10mM solution of Eudragit and 1mM solution of fluorescein obtained by the fluorescence microscope. For lower concentrations of fluorescein, a reduction of the homogeneity and of the Q factor of the surface could be observed. The measured Q factor in air is about $5.6 \cdot 10^6$ for the Eudragit-fluorescein coated microsphere (Fig. 4.b), and $1.7 \cdot 10^7$ for 10 mM Eudragit coated microsphere. Lower and higher polymer concentrations lead to the formation of an inhomogeneous coating, with the presence of clusters. No resonances were observed in these cases.

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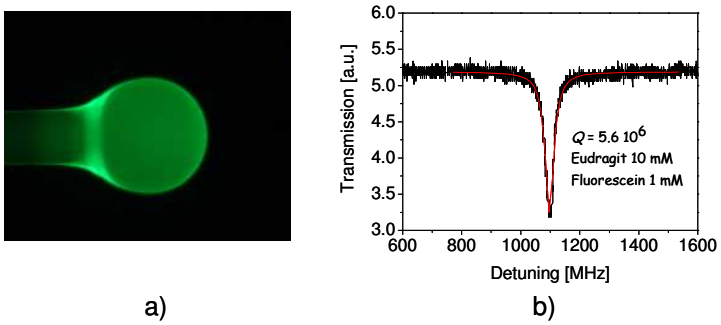

Fig. 4. a) Fluorescent image of a silica microsphere functionalised with Eudragit® L100 (10 mM) and fluorescein (1 mM); b) Q factor of the microsphere shown in Fig. 4a, measured in air. The red line is the Lorentzian fit of the resonance. The measurement was done in air.

After these preliminary tests with fluorescein, a fluorescent-labelled IgG was immobilised. 0.1 mg/ml fluorescein-IgG (Zymed Laboratories, Invitrogen Immunodetection) in PBS was used on functionalised microspheres with 10 mM Eudragit,. The surface was activated for the immobilisation with a solution of 2 mM 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and 5 mM N-hydroxysuccinimide (NHS) (Pierce) for 30 minutes and then let to react with the labelled IgG solution for 1 hour. A final washing step in PBST (PBS and 0.1% Tween 20 – from Sigma) was performed.

Figure 5 shows the measured Q factor in liquid environment (PBS) of a fluorescent-labelled IgG immobilised microsphere; the typical Q of these coated microresonators is close to $3 \cdot 10^5$. It has to be considered that the Q is also lowered by water overtone absorption. This Q value achieved with 0.1mg/ml of IgG demonstrates that the formation of the active layer necessary for the further development of the immunoassay still provides sufficient resonant properties to the microsphere for biosensing.

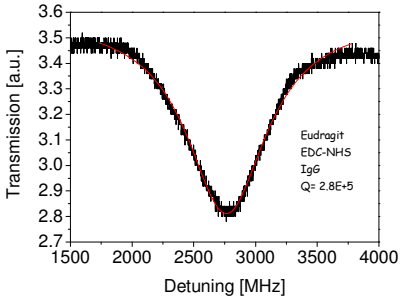

Fig. 5. Measured Q factor of the Eudragit coated microsphere after chemical activation with EDC-NHS and covalent binding of fluorescent labelled IgG. The measurement was done in liquid environment (PBS).

Table 1 shows the measured Q factors both in air and in liquid environment (PBS) for the different coating layers we have used in our study.

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Table 1. Comparison of the *Q* factor measured in air or in liquid environment (PBS)

	Air	PBS
Coating Material	*Q*	*Q*
none	$2\ 10^8$	$> 10^7$
Eudragit	$1.7\ 10^7$	$>10^6$
Eudragit-Fluorescein	$6\ 10^6$	$> 10^5$
Eudragit-EDC/NHS-IgG	NA	$2.8\ 10^5$

Table 2 shows the measured *Q* factors in liquid environment (PBS) for the different coating layers found in the literature, for similar experimental conditions, i.e. microsphere size and laser wavelength in the near infrared. Thus, our new and simple surface functionalization procedure gives *Q* values that are comparable with those reported for more complex procedures.

Table 2. *Q* factor measured in liquid environment.

	PBS
Coating Material	*Q*
3-Aminopropyltrimethoxysilane [2]	$2\ 10^6$
3- Mercaptopropyltrimethoxysilane [17]	$\sim 2\ 10^5$
Dextran-biotin hydrogel [18]	$5\ 10^5$

4. Conclusion

We have studied and developed a very simple and efficient chemical protocol to obtain a homogeneous thin polymeric layer for the functionalization of a microspherical resonator. Our experiments indicate that the best concentration of Eudragit® L100 is of about 10 mM, with a thickness of the layer of about 31 nm and a RMS roughness of about 6 nm. Lower and higher polymer concentrations lead to the formation of an inhomogeneous coating, with the presence of clusters. Subsequent chemical activation and covalent binding of globular proteins like IgG preserve a value of *Q* high enough for immunosensing applications. We also verified that PLLA layers are homogeneous as well and allowed us to obtain high *Q* factors, comparable with the values achieved with Eudragit® L100, showing that both these materials are a valid alternative to silanization.

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