Feasibility study of the modification of PLGA thin layers

Semester thesis SS 00

Swiss Federal Institute of Technology Zürich

Department of Material Science

Laboratory for Surface Science and Technology

Feasibility Study of the Modification of PLGA Thin Layers

Semester Thesis

by

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1 Introduction

This work was done in the Biomaterial and Biosensor Surface Group of the Laboratory of Surface Science and Technology at the ETH Zürich by Matthias Graf. The supervising person was Dr. Janos Vörös. The aim of the project was to show the feasibility of using Poly(L-lysine)-*g*-poly(ethylene glycol) (PLL-*g*-PEG) thin layers to modify the surface of Poly(lactide-co-glycolide) (PLGA) coated model substrates. It involved the development of spincoating PLGA onto waveguides with a controlled thickness and the characterization of the layer properties.

A simple set-up was used for the spincoating process and the layer thickness was varied by adjusting the concentration of PLGA using different solvents. OWLS was used for the primary control of the spincoating process, for the measurement of the PLL-*g*-PEG adsorption onto the PLGA and for testing the adsorption of proteins onto the modified PLGA surface.

X-ray Photoelectron Spectroscopy (XPS), Atomic Force Microscopy (AFM), Microdroplet Density (μDD) and Contact Angle (CA) measurements were used for the surface characterization. These techniques provide information on layer thickness layer homogeneity, wettability and surface constitution.

1.1 Medical relevance

PLGA can be used to produce microspheres. It is resorbable and it can be taken up by cells. It is also possible to load the PLGA microspheres with drugs (e.g. DNA strings) for controlled drug delivery. These drugs can establish specific functions in the targeted cells, e.g. kill them in the context of cancer therapy. This process is known as targeted drug delivery, which has the advantage that only a small amount of drug is used and only in places where it is needed without, incubation of the rest of the body. A common problem is that the PLGA microspheres are recognized as foreign bodies. This is probably due to an unfolded protein layer adsorbed onto the microspheres. Applying a layer of a protein-repellent polymer at the surface of the PLGA microspheres might reduce or eliminate the foreign body reaction. Another issue is that the recognition groups on the microspheres may become covered by an unfolded protein layer and thereby lose their ability to bind to the targeted cells. In this study PLL–*g*–PEG is used as a protein-repellent surface modification platform. To enable biological recognition, this polymer can be modified and specific targeting to cells becomes feasible.

1.2 Collaboration

The targeted drug delivery has three major issues to be solved:

- The targeting to the cells
- The surface modification
- The DNA string constitution

The Biomaterials and Biosensor Surface Group of the LSST is responsible for the surface modification, the Drug Formulation and Drug Delivery group of the Institute of Pharmaceutical Sciences for the medical aspects. [1]

2 Materials and Methods

2.1 Materials

2.1.1 TiO_2 coated glass slides

For the XPS analysis of the spincoating process, 130 µm thick glass slides with a 20 nm TiO_2 coating were used. The cleaning and the spincoating process were identical to the one used with the waveguides and described in sections 2.2.2 and 2.2.3.

2.1.2 $Si_{0.4}Ti_{0.6}O_2$ waveguides

The waveguides consist of a 200 nm thin layer of $Si_{0.4}Ti_{0.6}O_2$ with an optical grating on a glass support. All the waveguides are delivered from Microvacuum, Ltd (Budapest, Hungary). The geometry of the waveguides is rectangular with the dimension 8 mm × 12 mm and a thickness of 1mm. The surface roughness (R_a) is 0.2 nm if measured in an area of 1 µm × 1 µm.

2.1.3 Solvents

Two different solvents were used for the spincoating: acetone and dichloromethane. HEPES Z1 was used as a buffer for the flow cell measurements and as a solvent for PLL–*g*–PEG and human serum albumin (HSA).
Acetone: This solvent was used at first for the PLGA. PLGA is well soluble in acetone and the handling is very easy. The disadvantage is the fast evaporation, which hindered the formation of a homogenous thin layer during the spincoating process; the solvent evaporated too fast in comparison to the time needed for the formation of a homogenous layer.
Dichloromethane: Good solvent for PLGA, evaporates not so fast as acetone, but still fast enough to receive a PLGA layer without solvent within 30 minutes. More attention had to be paid during handling due to its cancerogeneity.
HEPES Z1: A 10 mM solution of 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid adjusted to pH 7.4 with 6 M NaOH.
(Ministil) ultra pure water was used for the buffer preparation and for the sample rinsing.

2.1.4 PLGA

PLGA is a resomer of Boehringer Ingelheim. Its name is RESOMER RG502H with a 50/50 percentage of lactide and glycolide components. It is capable to form microspheres and to resorb in the human body.

The technical specifications are:

Color	Nearly white
Shape	Fine powder
Odor	Nearly odorless
Identity	Conformed by NMR Spectrum
Polymer composition D,L-Lactide Glycolide	 51 mol% 49 mol%
Inherent viscosity 0,1 % Chloroform, 25°C	 0.17 dl/g
Water	<0.5 %
Residual monomer D,L-Lactide Glycolide	 <0.5 % <0.5 %
Residual solvent Acetone	 <0.1 %
Tin	105 ppm
Heavy metals	< 10 ppm
Sulphated ash	<0.1 %
Acid number	12.2 mg KOH/g

2.1.5 PLL–g–PEG

The used PLL–*g*–PEG had the following molecular weight distribution: PLL 20 kDalton, PEG 2 kDalton and the grafting ratio was 3.5.
Structure: Figure 1 shows the molecular structure with a PLL backbone and PEG side-chains. The PEG side-chains are responsible for the protein resistance. Modification of the side-chains gives the possibility for specific recognition.

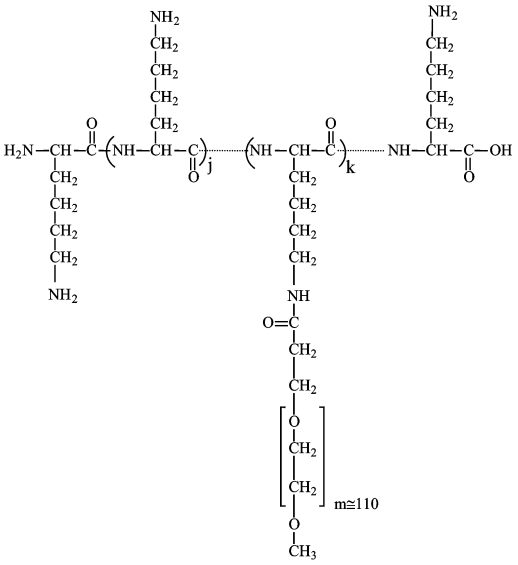


Figure 1:
Chemical structure of the PLL-*g*-PEG, with the PLL backbone and the protein repellent PEG side-chains.

2.2 Methods

2.2.1 Timetable

To give an overview of the activities, a timetable is shown in Table 1.

	October		November				December				January					February
Week		45	46	47	48	49	50	51	52	53	1	2	3	4	5	6
Specials																
No lab work	Literature									Writing						
PLGA		Spincoating	Characterization of the PLGA layer													
PLL-g-PEG			Adsorption on the PLGA layer													
					Protein resistance											
Additional work									AFM			AFM				

Table 1:
Timetable of the project

2.2.2 Sample cleaning

The waveguides were cleaned as follows: First the waveguides were washed and rinsed with 0.1 M HCl. Then the waveguides were put into an ultra-sonic bath (Telesonic TEC 15) for 10 min in 0.1 M HCl. The wet waveguides were rinsed extensively with ultra-pure water and dried with nitrogen. Finally the waveguides were exposed to oxygen plasma (Harrick PlasmaCleaner/Sterilizer PDC-32G, Ossining, NY, USA) for 2 minutes. The instrument was operated at 100 W and the chamber had a pressure of about 0.02 mbar.

2.2.3 Spin coating

The waveguides were spincoated with Duss SB13/2RLE driller. The rpm was set to 1850, the maximum value possible with the spincoater. The drilling machine was mounted vertical with the tip upwards. On the tip an insert was mounted to attach the waveguide holder. This served as a support for the waveguide. After dropping 200 µl of the solution onto the surface of the waveguide, the spinning was immediately started.

Using acetone as solvent, the spinning time was 60 seconds. This was insufficient when using CH_2Cl_2. The layer did not dry within this time, so a spinning time of 120 seconds was used with CH_2Cl_2. All waveguides appeared to be dry after spincoating and the solvent was evaporated or spun off.

2.2.4 Optical Waveguide Lightmode Spectroscopy (OWLS)

Optical waveguide lightmode spectroscopy (OWLS) gives the possibility to measure adsorption to a surface in real time. A laser is coupled into a waveguide at a well-defined

incident angle (Figure 2). This angle is sensitive to the deposition of thin layers onto the surface. Measuring the changes in the incoupling angle allows for the determination of layer thickness and for the monitoring of the adsorption of macromolecules. Reference [2] provides an overview of this method and the underlying theory. OWLS was used to get control over the spincoating process, to monitor the adsorption of the PLL-*g*-PEG onto the PLGA and to test the protein resistance of the modified PLGA layer. Human serum albumin (HSA) was adsorbed onto PLGA layers as a control measurement. The method is sensitive up to ca. 100 nm above the surface of the waveguide. The real-time monitoring allows also the study of swelling of the spincoated PLGA surface and of the kinetics of adsorption processes. The measurements can be done in air or under liquid.

Two BIOS-1 instruments (ASI AG, Switzerland) were used for the measurements each controlled by a single computer.

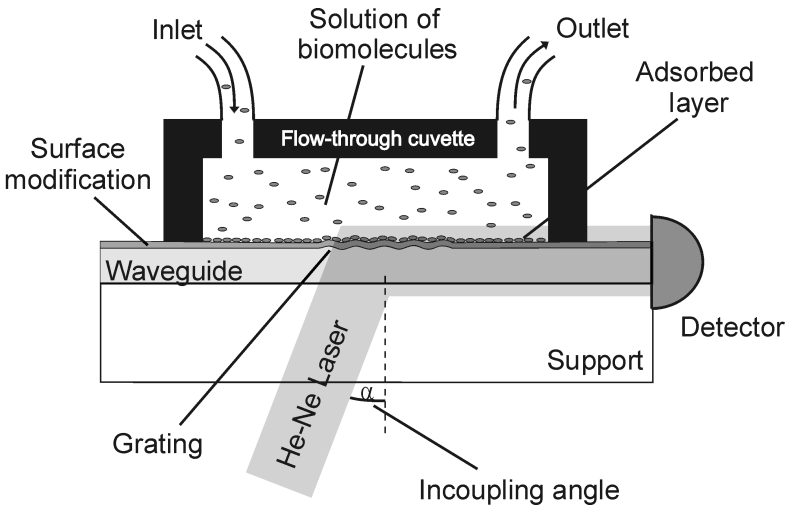


Figure 2:
Schematics of the OWLS instrument. [3]

The experiments were carried out in the following order: First the thickness was measured with the OWLS in air. For further experiments a flow-cell was placed onto the waveguides. HEPES Z1 was injected by a syringe. Swelling of the PLGA layer was observed when exposed to buffer.
For the PLL-*g*-PEG adsorption onto PLGA experiment, 1mg per ml PLL-*g*-PEG per ml HEPES Z1 was injected into the flow-cell and the adsorption was monitored with the OWLS. The stability of the adsorbed PLL-*g*-PEG layer was tested by washing the surface with pure HEPES Z1.
For the HSA adsorption, 5 mg per ml HSA solution in HEPES Z1 was injected into the flow-cell and the adsorption was monitored with the OWLS instrument. This was done using waveguides with PLL-*g*-PEG modified PLGA layers and waveguides with only PLGA layers for control and as a reference.

2.2.5 Contact angle measurements

Surface wettability was investigated by measuring advancing and receding contact angles in a sessile water drop type of experiment (Contact Angle Measuring System, G2/G40 2.05-D, Krüss GmbH, Hamburg, Germany). The measurements were performed in an automated way by stepwise increasing and decreasing, respectively, the water drop size. The average was taken for all advancing and all receding points. The measured advancing and receding contact angles are good measures of the wettability of the surface.

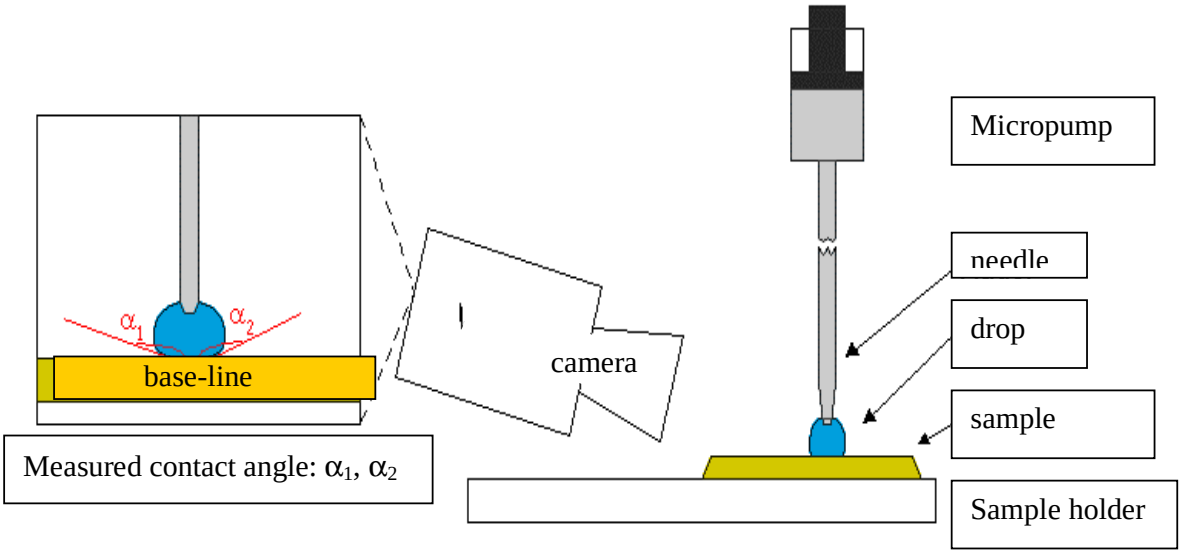


Figure 3:
Contact angle (CA) set up, with camera and measured angles.

2.2.6 Microdroplet density (μDD)

Microdroplet density data from condensation figures were obtained by using an apparatus consisting of a metal table placed in a transparent humidity chamber and a CCD-camera (Panasonic, model WV-BP 310/6, Matsushita Communication Deutschland GmbH, Germany) fixed on a microscope stage (Zeiss, Carl Zeiss AG, Switzerland). Images of the pattern of growing droplets on the surfaces were made after cooling the samples, which were placed on the metal table in the humidity chamber.

The microdroplet density (μDD) provides information on the homogeneity of the surface and the presence of macroscopic faults in the layer such as holes or uncoated spaces. Spincoated waveguides were put under a microscope and cooled down with ice-cold water. The humidity of the air reacts to this temperature change of the surface and drops of water condense on the surface. The drops appear at places with structural inhomogeneities. If the surface has a structure then this structure becomes visible by the formation of small drops at the surface. The set-up is very easy and the experiment is fast. The drops are growing with time and fuse into larger drops. With a camera coupled to the microscope and connected to a computer, pictures can be taken at a given time point.

2.2.7 X-Ray photoelectron spectroscopy (XPS)

XPS was used to get information on the chemical composition of the surface. The XPS is sensitive to the different atoms, present at or close to the surface and to their binding states. It provides information only from a restricted depth of the substrate. The sampling depth is about 10 nm for polymers. Thin layers within this thickness range can be examined. PLGA can be identified as well as contaminations from the spincoating.

XPS spectra were recorded with a SAGE 100 system (Specs, Berlin, Germany) using non-monochromatized Mg Kα radiation at 240 W (12 kV) and an electron energy analyzer pass energy of 50 eV for low resolution survey and of 14 eV for high-resolution detail scans. The area of information is 2x3 mm^2, the results therefore represent a laterally averaged chemical

composition. All bindings energies are referenced relative to the hydrocarbon set at the binding energy of 285.0 eV.

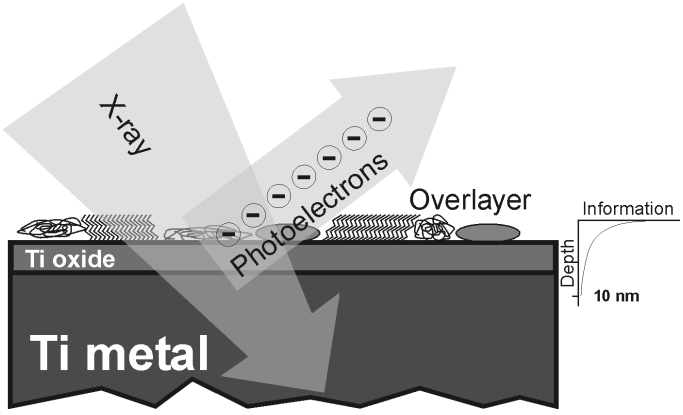


Figure 4:
Schematics of the XPS principle: Photoelectrons are emitted due to an X-ray radiation. The photoelectrons, which are emitted close to the surface, are collected and their kinetic energy is analyzed. [3]

Four TiO_2 coated glass slides spincoated using solutions of different concentrations were prepared:

- 10 mg PLGA per ml CH_2Cl_2
- 1 mg PLGA per ml CH_2Cl_2
- 0.1 mg PLGA per ml CH_2Cl_2
- A reference sample without spincoating, but with the same cleaning procedure as the spincoated.

Detailed spectra of the carbon and oxygen peaks were collected for the polymer identification (Appendix, Figures 6 to 13).

2.2.8 Atomic force microscopy (AFM)

In an atomic force microscope a nanoscale pyramid (tip) is guided over the surface by piezoelements. The tip is fixed to a thin cantilever on which a laser beam is focused. Any forces acting on the tip bend the cantilever and result in the deflection of the laser-beam, which is detected by photo-detectors. By scanning the tip, a topographical image of the surface is obtained.
AFM is useful to gain information on the nanostructure of the adlayer. The waveguides were examined directly after spincoating. The AFM measurements were done in water.

The resolution of the AFM is much higher than in the μDD experiment and all kind of structural defects and ordered structures at the surface can be detected.

3 Results

3.1 Optimizing PLGA coating thickness

At first acetone was used to dissolve the PLGA for the spincoating. The solubility was very good and the solution was clear. However it was possible to see by eye a ring structure after the spincoating. The same experiments with CH_2Cl_2 resulted in a clear adlayer and the surface was homogeneous with no visible rings. This can be attributed to the difference in the vapor pressures of the two solvents: acetone evaporates too fast, faster than the adlayer forms, which resulted in an inhomogeneous surface with visible white opaque color and rings.

The PLGA was spincoated onto the waveguides with the following adjustable parameters: solvent (acetone and dichloromethane) and concentration level. The thickness shows a linear dependency on the concentration [4] (Figure 20, Table 3). This linear behavior can be reduced to two factors: Solvent and concentration:

$d = s \times c$

Where: d is the thickness
s is a parameter describing the solvent
c is the concentration

The experiments have been done over a range of concentrations, most of them between of 0.1 and 0.4 mg per ml. This results in a thickness suitable for the OWLS examination. Because of the large standard deviations of the data with acetone another solvent was also tested: dichloromethane. It was chosen because of its smaller rate of evaporation and the thickness vs. concentration curve was measured again. The concentration for an optimum adlayer thickness was higher than with acetone, at about 2 mg per ml. (Fig. 5) The concentration of 2 mg PLGA per ml CH_2Cl_2 gives an adlayer thickness of 12 ± 2.6 nm. (Acetone shows for a concentration of 1mg per ml a thickness of 38 ± 31 nm.) With dichloromethane, the visible rings as observed during spincoating with acetone were no longer present. The obtained refractive index is representative for the polymer (Figure 9).

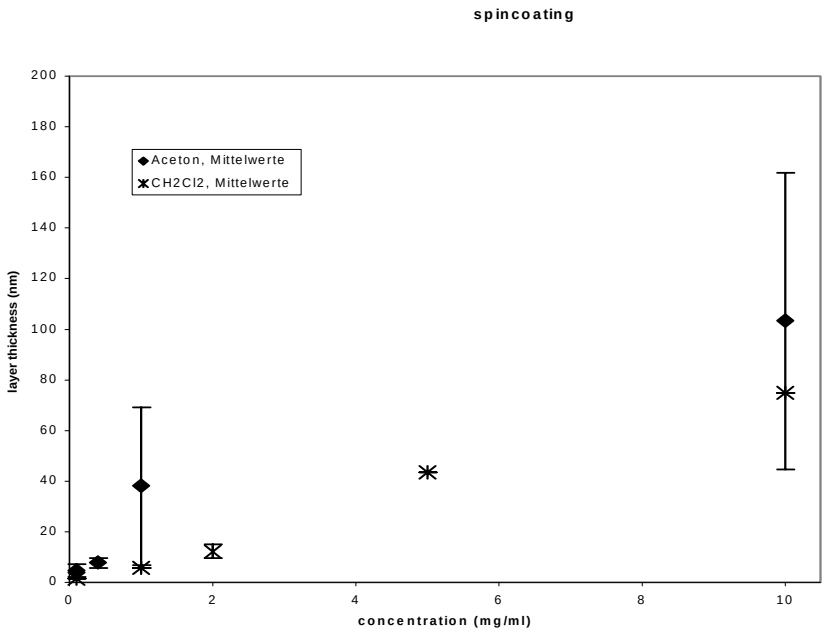


Figure 5:
The thickness of the spincoated PLGA layer in the function of the concentration in solution as measured by OWLS.

3.2 Characterization of the PLGA layer

3.2.1 Wetting properties

The wetting properties give a first impression whether the surface is covered and whether contamination is present on the surface. The two methods used in this project were CA and µDD. Table 2 summarizes the obtained CA results.

Contact angle	PLGA (measured)	TiO_2 (clean)	PLGA (literature)[1]
Advancing	69.6 ± 4.3	< 1	65-66
Receding	26.7 ± 5.8	< 1	31-36

Table 2:
Measured contact angles of the spincoated PLGA layer. The experimental values are in good agreement with the literature values.

The homogeneity of the spincoated layer was tested with µDD (Fig 6 and 7). The images have magnification factors of 2.5 and 6. The pictures were taken as soon as the drops showed a fine structure of the surface. We were interested to see whether rings from the spincoating process are visible or if the layer shows structural inhomogeneities.

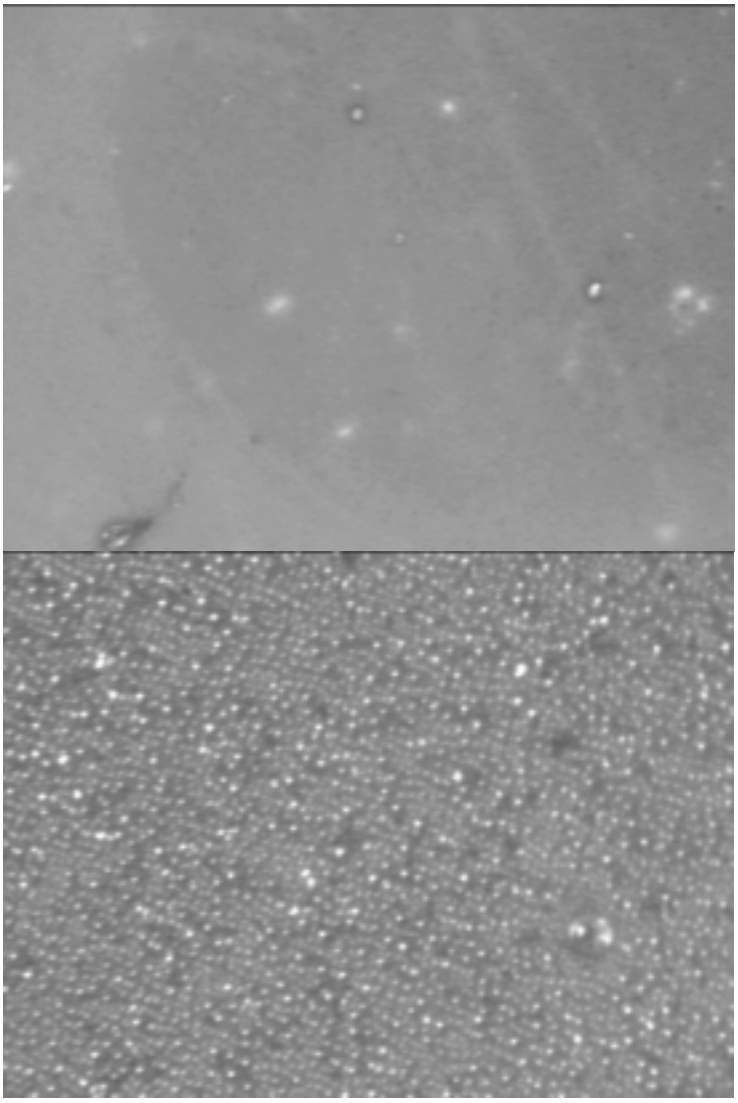


Figure 6:
µDD, 2x magnification, size 1.5 mm * 1.5 mm; No structural defects are visible, the surface is homogenous on this magnification level.

Figure 7:
µDD, 6x magnification, size 0.5 mm * 0.5 mm; single drops are visible and form homogenous structure. No defects are visible on the microscale.

The images show no structural defects on this level. The droplets are homogeneously distributed on the surface.

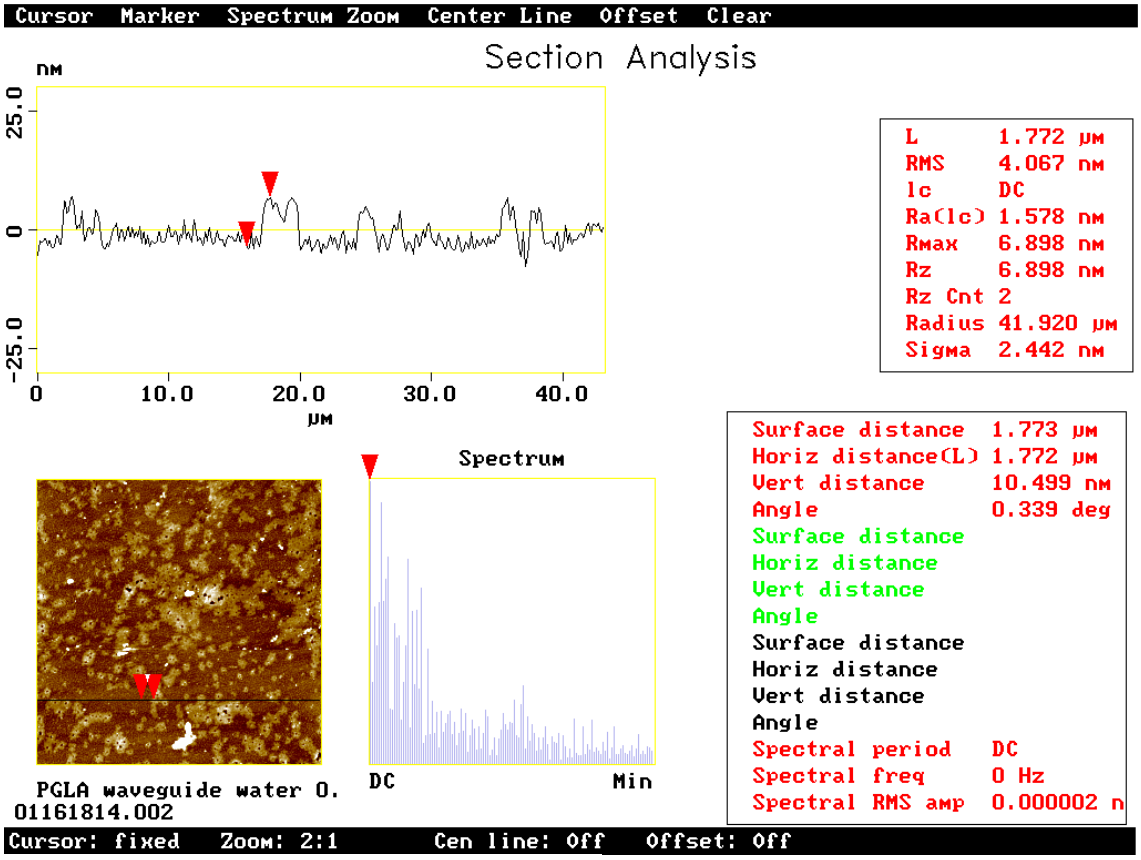
3.2.2 XPS

Photoelectrons from carbon C 1s, oxygen O 1s and from titanium Ti 2p were detected. For the high concentrations, the mean free path of the photoelectrons is lower than the thickness of the spincoated adlayer therefore no titanium was detected. The detailed spectra of the oxygen and carbon peaks were deconvoluted. The measured peaks are compared to the PLA, PGA and TiO_2 reference peaks. The different peak components can be attributed to the different carbon and oxygen binding states, reflecting the copolymer state of PLGA, consisting of poly-lactide and poly-glycolide. The reference sample shows the amount of contamination on the surface. The data are summarized in Table 4 and 5. Due to the charging of the samples the measured peaks are shifted compared to the theoretical values. As a reference the carbon peak of aliphatic hydrocarbons (285 eV) was used to calculate the shift for all samples (Table 4 and 5).

The identification of the peaks is reasonable if the charging is compensated and the identification of the individual carbon and oxygen peaks is straightforward. (Figures 13 to 20, table 4 and 5)
The reference shows the presence of advantageous hydrocarbon contamination on the surface. The sample coated with PLGA in a concentration of 0.1 mg per ml does not show the expected polymer peaks of carbon and oxygen but just the same peaks as the uncoated reference, but the values are higher. The O2 and O3 oxygen peaks of the samples coated with PLGA, using 1 and 10 mg per ml concentrations, show good agreement with the expected values of the PLGA. The deconvoluted carbon peaks are also in a good agreement with the PLGA reference peaks.

3.2.3 AFM

The AFM images do not show spincoating rings or coarse structural defects, which is in agreement with the µDD information. The surface is flat, although the polymer builds up a basic layer with single spots on top. The height of the visible spots is small compared to their longitude. The polymer here does not build up microspheres, but builds a layer with drops on it.



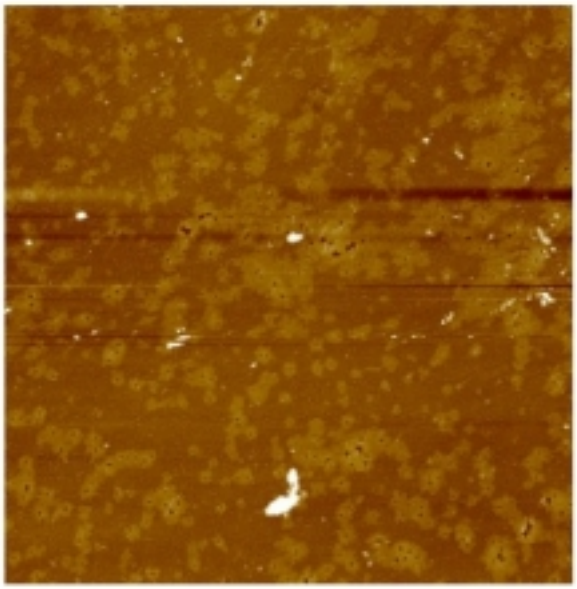


Figure 8:
AFM image of a spincoated waveguide. The surface is flat but discontinuities are visible. Their height 5 nm is small compared to their longitude (5 µm)

Scan size 50 µm, 0.06N/m cantilever in H_2O.

3.3 OWLS Experiments with PLL–*g*–PEG

3.3.1 Adsorption of PLL-g-PEG on PLGA

Prior to the experiment the thickness and the refractive index of the spincoated PLGA layer on the waveguides were measured in the OWLS (Figure 9). In HEPES Z1 there was only a minor swelling observed and the PLGA layer was stable over 18 hours in this buffer.

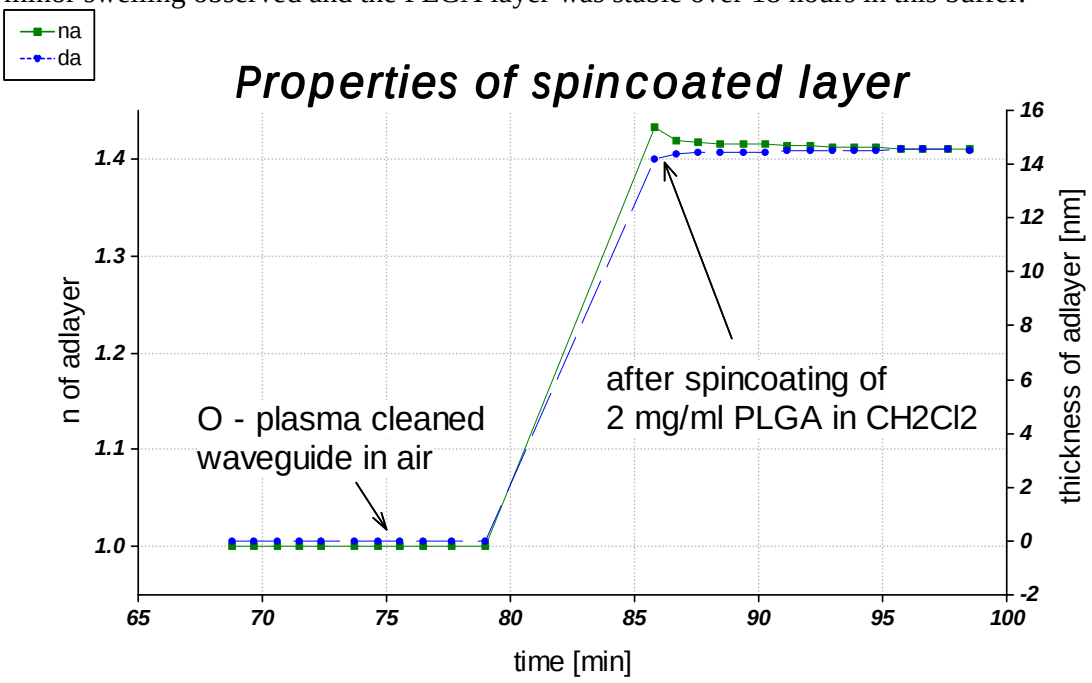


Figure 9:
Adlayer thickness and refractive index as measured by OWLS, a typical example for the spincoating of PLGA onto a waveguide. Here adlayer thickness is 14 nm. The solvent was dichloromethane.

PLL-*g*-PEG adsorbed onto the PLGA layer (Figure 10). The initial amount of adsorption was 140 ng/cm^2, but the total mass was slowly decreasing during the adsorption process.

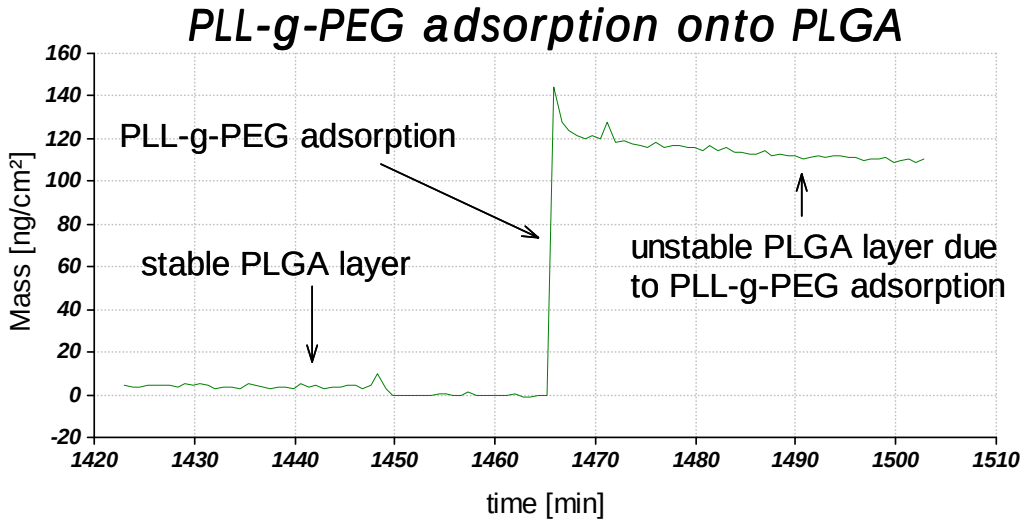


Figure 10:
PLL-g-PEG adsorption onto PLGA measured by OWLS. After adsorption the total mass is decreasing. The PLGA adlayer is instable, which is maybe due to aminolysis.

3.3.2 Protein resistance

The adsorption of HSA onto PLGA is 160 ng/cm^2 after rinsing. This corresponds to a monolayer of proteins on the surface (Figure 11). A comparison was made to HSA adsorption onto the PLL-*g*-PEG modified PLGA, which resulted in a value of 40 ng/cm^2 after washing with HEPES Z1 (Figure 12).

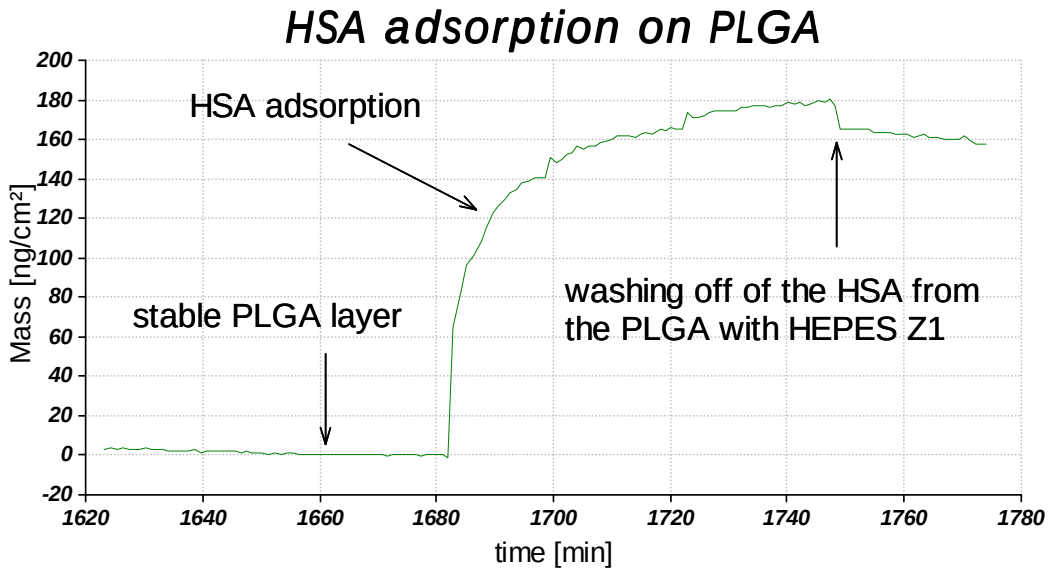


Figure 11:
OWLS, HSA adsorption onto PLGA, reference measurement.

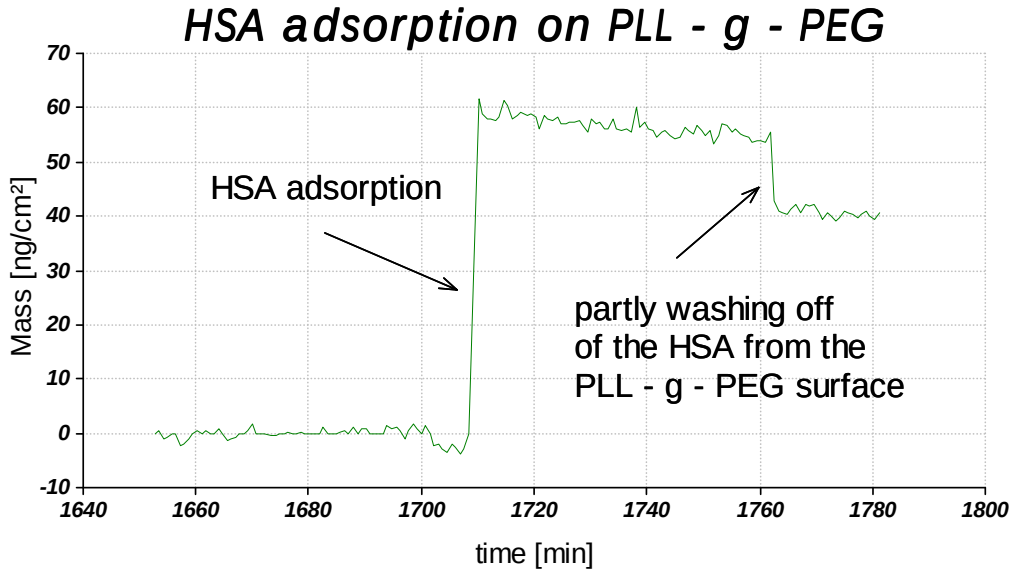


Figure 12:
OWLS, HSA adsorption onto PLL-g-PEG modified PLGA adlayer; the adsorbed amount is high compared to HSA adsorption onto PLL-*g*-PEG modified TiO_2 layers, but still less than HSA adsorbed onto PLGA.

4 Discussion of the results

The optimization of the spincoating was straightforward. Good agreement with theory was found for the relation of coating thickness on concentration. Using acetone as solvent produced less reproducible layers than using Cl_2CH_2 which can be attributed to the differences in their evaporation rate. Dichloromethane is proved to be better for spincoating because during the process drops for the solution fly out of the machine, which is necessary for an optimal spincoating process.

The contact angle measurements were in very good agreement with the literature value for PLGA and the layer was found to be homogenous in the micrometer scale by μDD.

The AFM picture confirms that the PLGA layer has no large structural defects, however small but flat islands are visible. This may be a reason for the instability of the PLGA layer due to the PLL-*g*-PEG adsorption and those regions may become detached during further exposure to the PLL-*g*-PEG solution or buffer.

The XPS analysis of the carbon peaks shows three peaks as expected. The carbon peaks help to distinguish between contamination and spincoated polymer. The reference surface shows the presence of hydrocarbon contamination. The three carbon peaks of the spin-coated layer correspond to the three binding states of carbon in PLGA.

Spincoating with the low concentration 0.1 mg per ml does not give a confluent layer. The three typical carbon peaks are not visible, indicating that there is no, or at least not much polymer on the surface, while the contamination peak is high. This is probably due to the spincoating process, which raises the amount of contamination. Therefore, the sample spincoated with 0.1 mg per ml PLGA is probably a better reference than the clean TiO_2 for the XPS analysis. The carbon peaks of the samples coated with 1mg per ml and 10 mg per ml

PLGA solutions look similar. The higher concentration gives higher peaks. The theoretical ratio of the three peaks should be 2:2:1, but in both cases the peak with the lowest binding energy is much higher than the two others. This is due to the large amount of contamination present on the surface. If we use the 0.1 mg per ml spectrum as reference and subtract the contamination peak from the third peak, the ratio of the three peaks is close to the theoretical level.

The oxygen peaks can be used as indicators of the adlayer thickness. The oxygen peak of TiO_2 will appear as soon as the layer is thinner than 10 nm. The O1 (titanium oxide) peak dominates the XPS spectra in the case of the sample coated with 0.1 mg per ml PLGA and also in the case of the reference spectrum. It is still visible in the spectrum of the 1 mg per ml sample, but disappears for the 10mg per ml adlayer. The O1 peak for the 0.1 mg per ml adlayer is not as strong as the one of the reference. This might be due to the carbon contamination layer added by the spincoating process. The polymer has two oxygen peaks. The ratio is 50:50. The peaks are the C=O and the C-O binding states in the PLA and the PGA copolymer chains. The 50:50 ratio is closely reflected by the XPS analysis and is not disturbed by the presence of contamination as the carbon peaks. The oxygen peaks suggest that the polymer is present on the surface after spincoating in the correct ratio of PLA and PGA. The peaks are identified after the correction with the shift of the hydrocarbon (285 eV) peak. The correlation between theoretical and shifted peaks is given in Figure 16. The shift for the thickest layer is the lowest.

The PLL-*g*-PEG adsorption experiments show that the PLL-*g*-PEG adsorbs to the PLGA. This adlayer makes the PLGA partially protein resistant. The amount of adsorbed HSA on the PLL-*g*-PEG modified PLGA is 40 ng/cm^2, i.e. is only 25% of the HSA amount that adsorbs to the unmodified PLGA (160 ng/cm^2). However this is still high compared to HSA on the PLL-*g*-PEG modified TiO_2 surface (< 1 ng/cm^2). Therefore, the PLL-*g*-PEG adlayers have different structures, or the PLGA is not completely covered with PLL-*g*-PEG.
It is also observed that the PLL-*g*-PEG destabilizes the PLGA layer: the mass of the PLGA+PLL-*g*-PEG layer is continuously decreasing. This makes the protein adsorption measurements less reliable compared to TiO_2 (the values have large standard deviations). The mechanism of the destabilization is not yet clear. It may be due to aminolysis of PLGA by the high concentration of amino groups present in PLL. Further investigation will be needed to solve this problem.

5 Summary and Outlook

The PLGA spincoating onto the waveguides works well and follows the predicted linear behavior as shown in [4]. The thickness control during spincoating is possible when the solvent has the right physical properties. CH_2Cl_2 was found to be a good solvent for the spincoating of PLGA. The XPS analysis showed the presence of a thin PLGA layer on the surface. The µDD pictures showed a homogeneous layer at the microscale, but nanoscale inhomogeneities were found by the AFM. The PLGA layer is stable in HEPES Z1, but the adsorption of PLL-*g*-PEG seems to destabilize the adlayer. Therefore HSA adsorption on the PLL-*g*-PEG modified PLGA could not be measured reliably but it is estimated to be still much less than on pure PLGA. A reduction from 160 ng/cm^2 to 40 ng/cm^2 was determined experimentally. However, this value is higher than found for the HSA adsorption on PLL-g-PEG modified TiO_2 surface.

It is not yet clear in which way the PLL-*g*-PEG destabilizes the PLGA adlayer and whether this is a problem for the microsphere application or not. It is also not clear whether the HSA can be used as a representative of the protein repellent properties, other proteins such as IgG need to be tested in order to explore the details of the protein resistance of the modified layers.

The stability of the PLL-*g*-PEG modified PLGA layer needs further investigations. Modification of the PLGA micelles with PLL-*g*-PEG and the performance of the functionalized PLL-*g*-PEG on the PLGA need to be tested.

6 Appendix

- Figures 13 to 20 (XPS carbon and oxygen peaks)
- Table 3 to 5

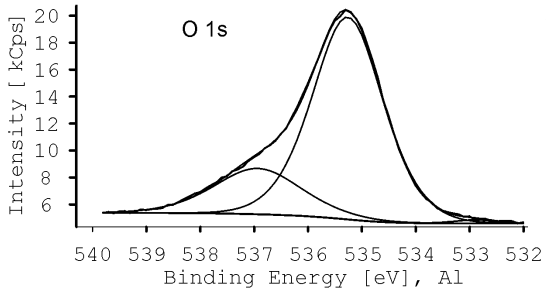
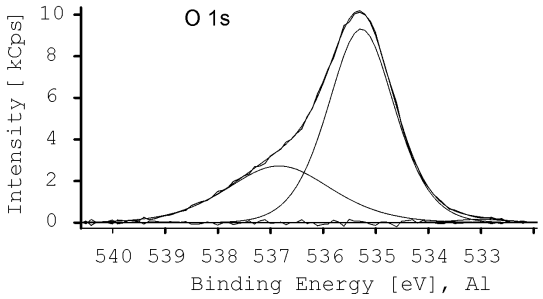


Figure 13:
Deconvolution of the O 1s spectra of the clean TiO_2 substrate, used as a reference.

Figure 14:
XPS O 1s-peak of TiO2, spincoated with a 0.1 mg/ml PLGA solution. No polymer O-peaks are visible, only the TiO_2 peak.



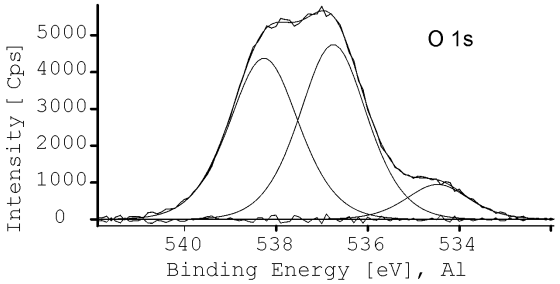
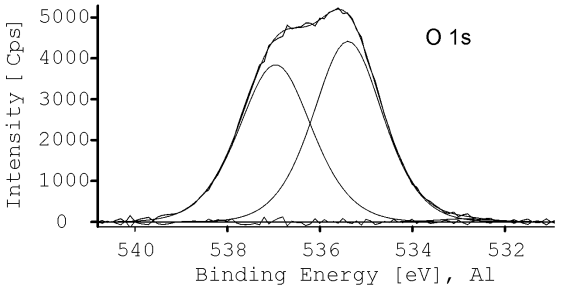


Figure 15:
XPS O 1s-peak of TiO_2, spincoated with a 1.0 mg/ml PLGA solution. Typical polymer O peaks and TiO_2 peak are visible

Figure 16:
XPS O 1s-peak of TiO_2, spincoated with a 10 mg/ml PLGA solution. Only polymer peaks are visible.



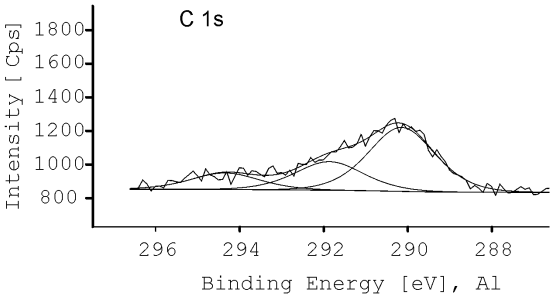
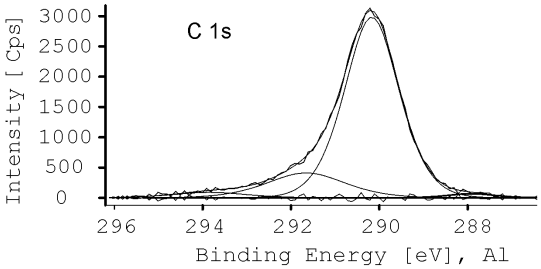


Figure 17:
Deconvolution of the C 1s spectra of the clean TiO_2 substrate, used as a reference. Hydrocarbon peaks resulting from contamination.

Figure 18:
XPS C 1s-peak of a TiO_2 coated glass slide, spincoated with a 0.1 mg/ml PLGA solution. No typical polymer peaks are visible but only a higher contamination due to the spincoating process.



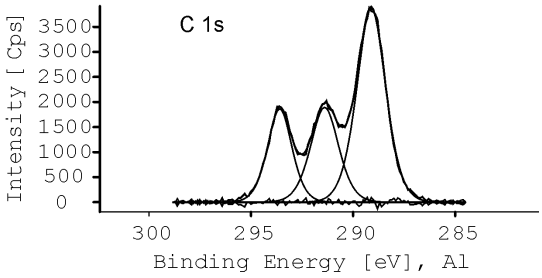
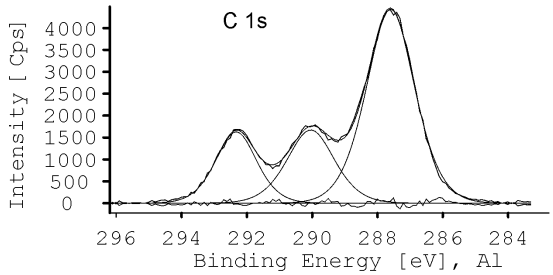


Figure 19:
XPS C 1s-peak of a TiO_2 coated glass slide, spincoated with a 1.0 mg/ml PLGA solution. Typical PLGA carbon peaks are visible.

Figure 20:
XPS C 1s-peak of a TiO_2 coated glass slide, spincoated with a 10 mg/ml PLGA solution. Typical PLGA carbon peaks are visible, but with a lower shift because of a thicker adlayer.



	concentration	layer thickness	standard deviation
Acetone	0.1	4.5	2.5
	0.4	7.7	1.8
	1.0	38.0	31.1
	10.0	103.3	58.6
	100.0	1000.0	0.0
CH_2Cl_2	0.1	1.6	0.0
	1.0	5.7	0.0
	2.0	12.2	2.6
	5.0	43.5	0.0
	10.0	75.0	0.0

Table 3:
Thickness values of the spincoated waveguides

slide	C	O	Ti	Si	S
reference	8.1%	66.6%	24.0%	0.0%	1.3%
0.1mg per ml	35.0%	49.5%	14.3%	0.0%	1.3%
1.0 mg per ml	62.9%	34.5%	1.2%	1.4%	0.0%
10 mg per ml	60.9%	36.2%	0.0%	2.9%	0.0%

Table 4:
Normalized XPS intensities

		O1(TiO2)				O2 (C-O)				O3 (C=O)				shift
		Energy		atm%	Integral	Energy		atm%	Integral	Energy		atm%	Integral	
		measured	corrected			measured	corrected			measured	corrected			
measured	reference	535.3	530.2	78.2	25842	536.9	531.8	21.8	7205	0.0	0.0	0.0	0	5.1
	0.1	535.3	530.1	68.5	15390	536.8	531.6	31.5	7080	0.0	0.0	0.0	0	5.2
	1	534.5	530.4	8.7	1683	536.8	532.7	46.8	9102	538.3	534.2	44.5	8656	4.1
	10	532.9	530.3	0.7	112	535.4	532.8	51.9	8638	537.0	534.4	47.4	7878	2.6
theoretical	PLA					533.7	533.7	0.5		532.3	532.3	0.5		0.0
	PGA					533.6	533.6	0.5		532.1	532.1	0.5		0.0
	TiO2		529.9	100.0										0.0

		C1(CH3)				C2 (CH)				C3 (C=O)				shift
		Energy		atm%	Integral	Energy		atm%	Integral	Energy		atm%	Integral	
		measured	corrected			measured	corrected			measured	corrected			
measured	reference	290.1	285.0	58.6	381	291.9	286.8	26.2	170	294.3	289.2	15.2	99	5.1
	0.1	290.2	285.0	85.6	2983	291.7	286.5	11.8	411	293.8	288.6	2.6	90	5.2
	1	289.1	285.0	50.8	3877	291.4	287.3	24.8	1893	293.6	289.5	24.4	1863	4.1
	10	287.6	285.0	57.3	4437	290.0	287.4	21.6	1672	292.3	289.7	21.1	1631	2.6
theoretical	PLA		285.0	36.0			287.0	33.0			289.1	31.0		0.0
	PGA						286.6	52.0			289.0	48.0		0.0

Table 5:
Summary of the deconvoluted Oxygen and carbon peaks. Comparison with literature values is also shown.

7 References

[1] *E. Walter, D. Dreher, M. Kok, L. Thiele, St. G. Kiama, P. Gehr, H. P. Merkle*; Interactions between DNA-loaded poly(DL-lactide-co-glycolide) microspheres and human antigen-presenting cells; Department of Applied BioSciences ETH Zürich; **2000**;

[2] *J. Vörös, J.J. Ramsden, G. Csus, I. Szendro, M. Textor, N. D. Spencer*; Optical Grating Coupler Biosensors; J. Mol. Cell. Biol.; submitted **2000**;

[3] *M. Textor eds.*, Titanium in Medicine, Elsevier, **2001**.

[4*] St. M. Butler, M. A. Tracy, R. Tilton*; Adsorption of serum albumin to thin films of poly(lactide-co-glycolide); Journal of Controlled Release 58 ; **1999**;

[5] *G. L. Kenausis, J. Vörös; D. L. Elbert, N.-P. Huang, R. Hofer L. Ruiz-Taylor, M. Textor, J. A. Hubbell, N. Spencer*; Poly(L-Lysine)-g-Poly(ethylene glycolide) Layers on Metal Oxide Surfaces: Attachment Mechanism and Effects of Polymer Architecture on Resistance to Protein Adsorption; J. Phys. Chem. B,104; **2000**;

[6] *E. Walter, K. Moelling, J. Pavlovic, H. P. Merkle*; Microencapsulation of DNA using poly(DL-lactide-co-glycolide): stability issues and release characteristics; Controlled Release 61 (3),361-374 **1999**;

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