Biofouling on Protective Coatings for Implantable MEMS

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Abstract— **Protective coatings can replace traditional packaging methods, which are often voluminous and may spoil the otherwise excellent opportunity for miniaturized implantable medical MEMS. The bio-growth on a selection of biocompatible protective coatings (TiO_2, DLC and Parylene) was investigated. The model system for evaluation was a diaphragm based acoustic resonator primary designed for fish identification. By detecting the shift in resonance frequency, we wanted to highlight the following; i) does the amount of biological growth vary for the different coatings? ii) if biofouling occurs, is the growth devastating for the device characteristics? We found that the resonance frequency did not change significantly. From this we conclude that the stiffness, represented by the spring constant for the resonating structure, was not affected. This result is of major importance also for other diaphragm based *in vivo* devices to be, e.g. pressure sensors, ultrasonic imaging devices, and dosage pumps.**

I. INTRODUCTION

Small-sized, lightweight, and low power-consuming sensors open new avenues for monitoring of physiological parameters inside the human body. According to the international standard ISO 10993-1; Biological evaluation of medical devices, such devices shall be categorized according to the nature and duration of body contact. When long-term use or contact exceeds 30 days the device is categorized as type C; permanent contact. Implantable devices intended for use in the body for several years definitely belongs to this category. *In vivo* sensors are now being used for immediate inspection, or during surgery or for a short period after surgery. To our knowledge all with the exception of one device which will be left behind in the aneurism sac for possible post surgical pressure measurements [1], these devices are not meant for permanent implantation.

For permanent implantation in the human body biocompatibility - the impact of the sensor on the body as well as the body's reaction to the implanted sensor - is one major obstacle for the success [2]. Devices contacting tissue, tissue fluid, and blood all experience a harsh environment. For example, the cerebrospinal fluid holds 37-38°C and contains ions of Na^+, Cl^-, H^+ Ca^{2+}, K^+. The composition of blood plasma is similar, but with more proteins and with a different ion concentration. For sensors used *in vivo*, this harsh environment may affect the sensor stability and reliability. The use of a biocompatible protective coating may overcome these problems by reducing i) the microbially influenced corrosion and ii) biofouling, i.e. the accumulation of proteins/cells on the sensor surface. However, a protective coating may in itself affect the sensor characteristics dramatically.

The objective of the work presented in this paper was to investigate if the amount of biological growth varied for a selection of different coatings and, in case of such growth, to examine if the accumulation of biological matter degraded the device characteristics. The investigated device was a diaphragm-based resonator (Figure 1). Such a diaphragm-based configuration is also commonly used for pressure sensors.

II. MATERIAL AND METHODS

A. Biocompatible coatings

The generally accepted view is that surfaces that strongly adsorb proteins will generally bind cells, and that surfaces that resist protein adsorption will also resist cell adhesion. It is also

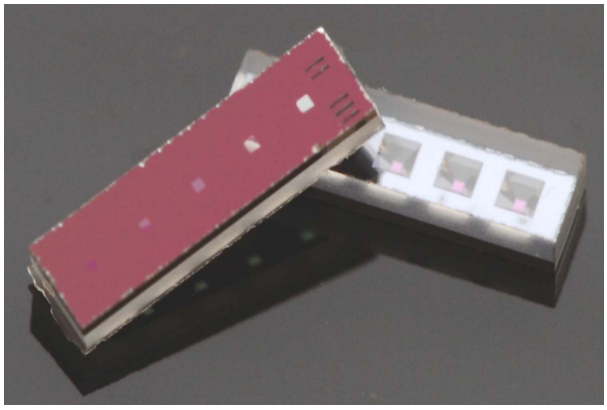


Figure 1 Top and bottom view of the acoustic resonators. Five silicon nitride diaphragms suspended over separate evacuated cavities and anodic bonded to a glass wafer.

generally recognized that hydrophilic surfaces are more likely to resist protein adsorption, and that hydrophobic surfaces usually will adsorb a monolayer of tightly adsorbed proteins [6]. However, the nature of body contact determines the biological response and hydrophilic coatings seem not to be favorable for blood interfacing applications [7],[8],[9]. In the presented work, coatings generally accepted as biocompatible materials i.e. TiO_2, DLC and Parylene, have been investigated, hydrophobic as well as hydrophilic. Based on the assumption that the active components in the body's immune system experience the outer surface of the implanted device only, film thickness in the range 10 – 20 nm was targeted, as a thinner film was thought to influence MEMS characteristics less than a thicker film. Justification of the choice of coatings is given in [3].

The TiO_2 coating of anatase structure was made by Baldur Coatings AS by the atomic layer deposition (ALD) technique using $TiCl_4$ (99,9% Aldrich) and distilled water as precursors. Both precursors were supplied at room temperature, and the film was grown at 250°C using a total of 60 cycles targeting in a thickness of 10 nm. The film thickness of TiO_2 was later determined to ~ 12 nm by ellipsometry.

To deposit a thin DLC coating, a radio frequency plasma (r.f.) discharge technique was applied, producing an amorphous hydrogenated carbon film. The deposition was done by VTT Technical Research Centre of Finland. The a-C:H film was deposited with r. f. plasma using a mixture of argon and methane as the process gas. The r. f. power used was 100 W and the deposition time was 5 min, resulting in a film thickness of about 20 nm. The temperature during deposition was in the range 100°C to 150°C. The deposition equipment was situated in the normal laboratory environment.

Parylene HT was applied via a Vapor Deposition Polymerization (VDP) process provided by Specialty Coating Systems (SCS), Indianapolis, US. Through the VDP process the solid dimer molecules sublime at approximately 150°C. The next step is quantitative cleavage of the dimer vapor to a monomer vapor at about 680°C. Finally the monomer vapor is routed to a room temperature deposition chamber where it polymerizes on the substrate. The acoustic passive ID tags were coated as received. The final film thickness was measured on Si dummy chips that were coated together with the ID tags. By spectral reflectance measurements the Parylene HT thickness was determined to 197 nm by SCS.

B. Acoustic resonators

The MEMS device under investigation was an acoustic resonator composed of five 500 nm thick silicon nitride diaphragm suspended over separate evacuated cavities anodic bonded to a glass wafer [4], [5] (Figure 1). The outer dimensions of the tags are about 5 mm x 1.5 mm x 0.8 mm, and the diaphragms have quadratic form with side edge varying in size from 126 µm to 195 µm. The tag responds with a combination of specific resonance peaks to an interrogative ultrasound signal in the 200 kHz to 400 kHz range. The device was originally designed to be injected into the fish by means of an injector needle and to be read remotely.

C. Protein solution

Fish tags coated with TiO_2, DLC, and Parylene HT, were together with uncoated fish tags incubated in 5 mg/ ml of human liver extract at 37ºC. The incubation period was 2 hours, 7 days and 6 weeks and the tags were measured after each incubation period. The chips were washed in excess of distilled water after each measurement to remove possible contamination from the water tank before they were reinserted in the lever extract.

D. Experimental setup

The experimental setup for the acoustical resonators is described in full in [3]. The main parts are for the reader's convenience listed below:

- A waveform generator Agilent model 33220A
- an oscilloscope Agilent model DOS6014A
- two transducers GE Panametrix V1012
- a pre-amplifier set to 40dB amplification
- a water tank 75cm x 75cm x 45cm

A schematic drawing of the setup is shown in Figure 2. The temperature in the water was about 25°C. A positioning system in the water tank allowed accurate positioning of the transducers and the target. The fish tags were taped on a fish line held in place by two vertical poles and tested one by one. The poles were fixed far apart as not to disturb the acoustic field.

The frequency response was measured 3-5 times for each sample. The average of all 3-5 spectra was plotted and the peak for each resonance frequency was manually found.

III. RESULTS

A. Shift in resonance frequency on coated tags

In preliminary experiments intended to reveal the effect of coating on device characteristics, the frequency response of fish tags coated with TiO_2, DLC and Parylene HT together with uncoated control tags were measured before and after coating (Figure 3). The resonance frequencies were found to *increase* slightly (about 4-6 kHz or 2%) for fish tags coated with TiO_2 [3]. For tags coated with DLC a *decrease* in resonance frequencies of about 10-25 kHz (4-9%) was observed, while for tags coated with Parylene HT the resonance frequencies remained *unchanged.* The uncertainty in the measurements was estimated to ± 2%.

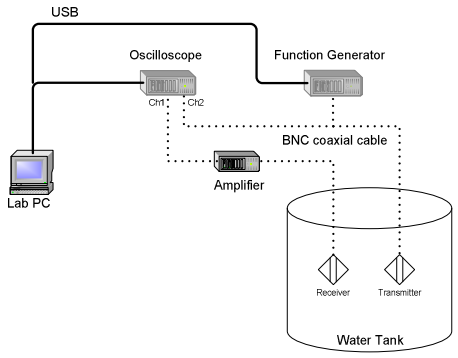
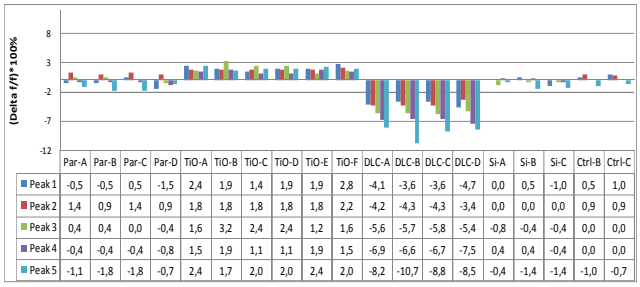


Figure 2 Schematic diagram of the measurement setup



	Par-A	Par-B	Par-C	Par-D	TiO-A	TiO-B	TiO-C	TiO-D	TiO-E	TiO-F	DLC-A	DLC-B	DLC-C	DLC-D	Si-A	Si-B	Si-C	Ctrl-B	Ctrl-C
Peak 1	-0,5	-0,5	0,5	-1,5	2,4	1,9	1,4	1,9	1,9	2,8	-4,1	-3,6	-3,6	-4,7	0,0	0,5	-1,0	0,5	1,0
Peak 2	1,4	0,9	1,4	0,9	1,8	1,8	1,8	1,8	1,8	2,2	-4,2	-4,3	-4,3	-3,4	0,0	0,0	0,0	0,9	0,9
Peak 3	0,4	0,4	0,0	-0,4	1,6	3,2	2,4	2,4	1,2	1,6	-5,6	-5,7	-5,8	-5,4	-0,8	-0,4	-0,4	0,0	0,0
Peak 4	-0,4	-0,4	-0,4	-0,8	1,5	1,9	1,1	1,1	1,9	1,5	-6,9	-6,6	-6,7	-7,5	0,4	0,4	-0,4	0,0	0,0
Peak 5	-1,1	-1,8	-1,8	-0,7	2,4	1,7	2,0	2,0	2,4	2,0	-8,2	-10,7	-8,8	-8,5	-0,4	-1,4	-1,4	-1,0	-0,7

Figure 3 Relative shift in resonance frequencies for the resonators as a function of coating material (After coating – Before coating). Tags coated with TiO_2 are denoted TiO_2-A to TiO_2-F, tags with DLC are denoted DLC-A to DLC-D, and tags with Parylene HT are denoted Par-A to Par-D. Uncoated fish tags are denoted Si-A to Si-C and CTRL-A to CTRL-C.

B. Biofouling experiments

The biofouling experiments were carried out on the same fish tags as used for the experiments above. The results are given in Figure 4 to Figure 6. The figures show the relative shift in resonance frequencies for the resonators as a function of incubation time in human lever extract for 2 hours (Figure 4), 7 days (Figure 5), and 6 weeks (Figure 6), respectively. Tags coated with TiO_2 are denoted TiO_2-A to TiO_2-F, tags coated with DLC are denoted DLC-A to DLC-D, and tags coated with Parylene HT are denoted Par-A to Par-D. Uncoated fish tags that *were incubated* are denoted Si-A to Si-C, and uncoated fish tags that *were <u>not</u> incubated* are denoted CTRL-A to CTRL-C. Not all samples were measured each time, this was done to control that the reinsertion of the fish tags in the lever extract (after a measurement) did not cause severe stress.

Significant changes in the resonance frequency response were not detected during any of the different periods. The variation in frequency response was for most samples less than the estimated measurement uncertainty of 2 %.

	Par-A	Par-B	Par-C	Par-D	TiO-A	TiO-B	TiO-C	TiO-D	TiO-E	TiO-F	DLC-A	DLC-B	DLC-C	DLC-D	Si-A	Si-B	Si-C	Ctrl-B	Ctrl-C
Peak 1	0,5	0,3	0,5	1,8			0,5	-1,0	0,2	-0,7	0,0	0,0	-0,3	0,0	-0,5	-0,8	0,5	0,0	0,5
Peak 2	-0,5	-0,9	-0,2	0,2			0,4	-2,4		-0,2	0,0	1,0	0,7	0,0	-0,7	0,2	-0,2	-0,5	0,4
Peak 3	-0,2	-0,2	1,1	1,1			0,2	-1,4	0,0	-2,2	0,2	0,9	0,2	-0,2	-0,4	-0,2	0,2	0,4	0,4
Peak 4	0,0	-0,2	0,2	0,6			1,1	0,7	0,0	-3,1	0,6	0,2	0,2	0,4	-1,6	-0,4	0,8	0,8	0,4
Peak 5	0,0	-0,2	0,9	0,4			-0,3	-3,0	-1,2	-2,3	-0,4	1,9	0,4	-1,2	-0,7	0,4	-0,2	0,5	-0,2

Figure 4 Relative shift in resonance frequencies for the resonators as a function of incubation in human lever extract for 2 hours. (After Incubation – Before Incubation).

	Par-A	Par-B	Par-C	Par-D	TiO-A	TiO-B	TiO-C	TiO-D	TiO-E	TiO-F	DLC-A	DLC-B	DLC-C	DLC-D	Si-A	Si-B	Si-C	Ctrl-B	Ctrl-C
Peak 1	0,5	0,3	0,5	1,8			0,5	-1,0	0,2	-0,7	0,0	0,0	-0,3	0,0	-0,5	-0,8	0,5	0,0	0,5
Peak 2	-0,5	-0,9	-0,2	0,2			0,4	-2,4		-0,2	0,0	1,0	0,7	0,0	-0,7	0,2	-0,2	-0,5	0,4
Peak 3	-0,2	-0,2	1,1	1,1			0,2	-1,4	0,0	-2,2	0,2	0,9	0,2	-0,2	-0,4	-0,2	0,2	0,4	0,4
Peak 4	0,0	-0,2	0,2	0,6			1,1	0,7	0,0	-3,1	0,6	0,2	0,2	0,4	-1,6	-0,4	0,8	0,8	0,4
Peak 5	0,0	-0,2	0,9	0,4			-0,3	-3,0	-1,2	-2,3	-0,4	1,9	0,4	-1,2	-0,7	0,4	-0,2	0,5	-0,2

Figure 5 Relative shift in resonance frequencies for the resonators as a function of incubation in human lever extract for 7 days. (After Incubation – Before Incubation).

	Par-A	Par-B	Par-C	Par-D	TiO-A	TiO-B	TiO-C	TiO-D	TiO-E	TiO-F	DLC-A	DLC-B	DLC-C	DLC-D	Si-A	Si-B	Si-C	Ctrl-B	Ctrl-C
Peak 1	0,0	-1,8	-0,3	1,0	-1,0		-0,2	-0,2	0,2	-0,2	-0,3	-0,8	-1,0	-0,8	-1,5	-1,3	-0,2	0,5	0,5
Peak 2	0,0	-1,4	-0,5	0,5	-0,4	-1,8	0,2	0,4		-0,6	0,9	0,7	0,7	-0,5	-0,7	0,2	-1,8	-0,5	-0,9
Peak 3	-0,4	-1,1	0,4	1,1	-0,6	-1,6	0,0	-0,4	-0,4	-2,2	0,9	0,0	-0,2	-0,9	-0,8	-0,4			0,4
Peak 4	-0,2	-0,4	-0,2	0,6	0,0	0,0	1,1	0,9	0,4	-3,3	1,0	0,0	0,2	0,2	-1,6	-1,2			0,4
Peak 5	0,7	0,4	1,8	1,8	-0,2	-0,3	0,2	-1,2	-0,7	-2,2	1,3	0,4	-1,0	-1,9	-0,7	0,4	-0,2	0,9	0,5

Figure 6 Relative shift in resonance frequencies for the resonators as a function of incubation in human lever extract for 6 weeks. (After Incubation – Before Incubation).

IV. DISCUSSION

When looking upon the tags as diaphragm-based resonating structures, where the diaphragms are small compared to the wavelength, the resonance frequency is given by

$$f = \frac{1}{2\pi}\sqrt{\frac{k}{m}} = \frac{1}{2\pi}\sqrt{\frac{k}{m_d + m_f}}\,. \tag{1}$$

k is the spring constant, m_d the mass of the diaphragm, and m_f the mass of the surrounding fluid when the device is operated in water or body fluid (e.g. blood, cerebrospinal fluid or stomach acid). The effect of the fluid mass is quite marked, giving a decrease in the resonance frequency of the acoustic resonators compared to the resonance frequency when operating in air. The acoustic resonator operating in fluid can be looked upon as a resonating diaphragm with side edge L, loaded with a volume of fluid with density ρ, having a cross-sectional area L^2 and an effective height $4L/3\pi$ [4]:

$$m_f = \rho L^2\left(\frac{4L}{3\pi}\right). \tag{2}$$

The shift in resonance frequency is given by

$$\frac{\Delta f}{f} = \frac{1}{2}\frac{\Delta k}{k}. \tag{3}$$

For a uniformly loaded diaphragm in the small-deflections domain, k is expressed by

$$k = \left(C_r\sigma t + C_b\frac{Et^3}{\left(1-\nu^2\right)L^2}\right)\cdot c\ . \tag{4}$$

σ is in-plane stress, t is the thickness of the diaphragm, L is the side edge of the diaphragm, ν is Poisson's ratio, E is Young's modulus, c is the deflection, and C_r and C_b are numerical constants [10]. Normally, k depends on the stiffness term due to residual stress and on the bending term (the first and second term in (3), respectively), but two limiting cases exist; one for stress-dominated diaphragms and another for diaphragms dominated by the bending stiffness. The nitride diaphragms are stress-dominated prior to incubation in protein solution [3].

When vibrating in air, a change in thickness due to a thin coating would alter m_d and k equally and a modification of the resonance frequency would depend only on the sign of the in-plane stress of the added film. The resonance frequency would

increase if the additional coating is in tension and correspondingly decrease if it is in compression. However, as discussed above, for measurements in water or body fluid there will be an additional mass m_f which completely dominates m_d . Therefore the resonance frequency f will not longer depend only on the sign of the in-plane stress of the added film, but also on the thickness t.

Occurrence of protein adsorption on DLC and TiO_2 has been reported earlier [12]. It is therefore a reason to believe that proteins were absorbed on the surface after immersion in homogenized extract from human liver. Significant changes in resonance frequency response were however *not* detected for any coated (or uncoated) samples during a period of 6 weeks. The results indicate that the device characteristics were not influenced by the bio-growth.

The amount of protein was not detected in the reported experiments but is under investigation in ongoing *in vitro* tests. According to the discussion above the size of the resonance shift will depend on the thickness of the additional layer; here the expected protein growth. However, if internal stress in the additional layer is absent, a frequency shift will not happen. The achieved results indicate that neither the stress in the accumulated layer nor the thickness is decisive for the MEMS characteristics.

V. CONCLUSIONS

The purpose of the experiments reported here was to investigate the bio-growth on a selection of biocompatible protective coatings (TiO_2, DLC and Parylene). The frequency spectra of acoustic resonators were measured after incubation in homogenized extract from human liver for 2 hours, 7 days and 6 weeks. The observed frequency shift due to the expected accumulated layer of proteins was for most samples less than 2%, i.e. less than the estimated measurement uncertainty. Although coating with thin biocompatible films was not devastating for the investigated MEMS device, a more pronounced change in characteristics was caused by the coatings than by the protein layer. The lacking influence from biofouling indicate that neither the internal stress in the protein layer nor the thickness of accumulated proteins diminished the acoustic resonators after incubation in human liver extract for 6 weeks.

Biofouling has for long been of great concern for *in vivo* sensors. The result presented here is very promising for permanent implantable *in vivo* sensors in general and for diaphragm-based sensors in particular. Even if the biological response is expected to be dynamic, i.e. the thickness of the accumulated layer will vary by time, this might not present any problem if internal stress in the added layer is minor.

More research is however required. The interaction between the implanted device and the biological surroundings must also be evaluated by *in vivo* tests as other immunologic responses than depicted here may occur in a living organism, e.g. cell adhesion and encapsulation. If encapsulation occurs, the above picture will be changed. Each specific application must also be assessed as the nature of body contact determines the biological response.

This work is part of a series of experiments mapping out the effect of biocompatible protective coatings on characteristics of diaphragm-based devices, and their affinity for biological growth. Ongoing *in vitro* tests aim at identifying the accumulated proteins and to quantify the amount of protein bound to the different surfaces.

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