DEVELOPING BIOCOMPATIBLE THIN FILMS FOR COATING AND ENCAPSULATION OF IMPLANTABLE MICROSYSTEMS

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Abstract: The barrier-effect as a thin film property is of vital importance for medical applications. Therefore we have developed and tested several biocompatible coatimngs for implantable microsystems.

Introduction

Long-term implantable microsystems based on silicon (e.g. transponder for pressure or acceleration) are composed of many different basic materials. By encapsulation and coating of the micro implants the tissue compatibility and the option of sterilisation is obtained [1]. In addition thin films have to fulfil additional requirements regarding adhesion, abrasion resistance, elasticity and electrical isolation without restricting the function of the entire system. Materials fulfilling all these requirements for med. applications are mainly polymers as well as some metals, oxides and ceramics. Practical test options of thin layers are difficult and the amount of tests is restricted. Where a material is suited by virtue of its chemical and physical properties for use as an implant material, its behaviour in contact with tissue is of very great importance for clinical use. For this reason, extensive in vitro und in vivo test procedures are necessary in order to asses their biocompatibility before implant materials are used clinically.

Materials and methods

We developed, analysed and tested different coating systems for dip coating of micro-systems [2,3]. The polymers are dissolved in advance in suitable solvents.

- acrylates (AC1, AC7), UV-cross linking
- halogen polymers FL1, FL2, FL4
- epoxy resin (EP3)
- thermoplastic PUR and silicone coating (SIL5), (SIL8) as well as a fluoro-silicone (SILF) due to the high elasticity
- lacquers based on high temperature resistance polymers (HAT1, HAT2)

The acrylates AC1 and AC7 are formed from a photonetworking polymer which is hardened with UV light and becomes “tack free” on the surface. Its pot life when stored in the dark is over a year. It has high transparency and good wetting.

Of the halogen polymers, fluorterpolymer, polyvinylidene fluoride and polyvinylidene chloride were used. The fluorine compounds have high chemical bonding properties and therefore great stability. The pot life of FL1 is over half a year, while that of FL4 over three months. The pot life of FL2, however, is only one day. FL2 is very slightly milky, while FL4 has a slightly uneven surface. The wetting of the substrates is unproblematic.

Among the epoxy resins, the UV hardening types proved to be unsuitable. Among the heat hardening types there were wetting problems with glass. Further investigation of EP2 was therefore discontinued. EP3 wets better and forms a thicker, slightly structured, lightly clouded layer. Its pot life is around half a day. Working is hampered by relatively high viscosity.

The PUR coating lacquer is a thermoplastic PUR with a pot life of over one year. It has to be worked in a dry atmosphere. Wetting and spreading are acceptable. The surface of the hardened lacquer is slightly structured.

The SILF coating is a fluoro-silicone. It was selected for its high resistance, for the reason mentioned above. Its workability and spreading are acceptable. However, the isolated layer is opaque or very milky. The pot life is over a year.

Two further silicone lacquers were also investigated, for comparison (SIL5, SIL8). They both display good spreading and wettability. Their optical quality is good, although they are slightly milky. All silicone lacquers have to be worked in a dry atmosphere.

Two further lacquers were developed based on highly temperature resistant polymers (HT1 and HT2).

The obtainable layer thickness depends on content of solids in the solution and on the dip velocity. For specific requirements, they can potentially be altered so that thicker or thinner coatings are achieved. In addition, the dip velocity has an influence on the coating thickness. The dip equipment used was accommodated in a sealed space with air drying and clean room filtering

The cleaning of substrate surfaces was done before by ultrasonic in isopropyl alcohol and afterwards multiple washed in aqua dest. (DAB quality) by monitoring the electrolytic conductivity.

The samples were several times suspended in the water at 40°C for an hour and the conductivity of the water was then measured. The ratio of the surface of the

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sample to the volume of water was kept constant. In each case, preliminary cleaning was carried out in isopropyl alcohol with ultrasound support.

The quality of the film surfaces were examined using a scanning electron microscope at 100 kV and at magnification of more than 1000.

Table 1: Coating thickness of the dip coatings in µm according to the number of dip processes

Material	1.	2.	3.
PUR	36	85	135
SILF	61	119	174
SIL8	31	98	210
AC1	18	52	65
AC7	12	23	58
EP1	32	-	-
EP3	120	150	300
FL1	36	-	-
FL2	17	46	81
FL4	7	30	64

An adequate method for testing the adhesion is to apply a peeling test or a bolt pull test, commonly used for testing electroplated plastics. Because of the wide range of future applications the adhesion of all films was studied on glass, Au, Cu, metallic silicium and polyimid.

For the peeling test, an adhesive strip is attached to the coating to be tested and then removed with a bobbin. The traction necessary for this is a measure of the peeling resistance. In the present case, either a 3M adhesive tape or a 0.1 mm thick copper strip was attached to the samples to measure the peeling resistance. Since the evaluation of the test is not simple, a qualitative assessment was carried out.

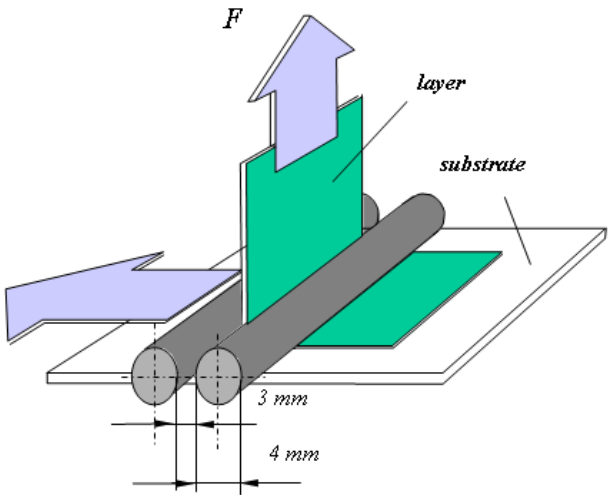

Figure 1: Principle of the peeling experiment

A further method of measuring the resistance of coatings is to attach bolts to the coating, which are then removed in an appliance. As with the previous test, the nature of the attachment has an influence on the conduct of the test. The method of conducting this experiment was based on EN 24 624 [4].

Furthermore a standard method [5] determining the abrasion resistance for optical glasses was used DIN 52 348 and EN 168. For this purpose, the surface to be tested was sprinkled with 3 kg of sand. Before and after this artificial scratching, the acute-angled scattered light was measured. The difference in the scattered light is a measure for the scratch resistance of the coating.

Electrical isolation was tested as mentioned in DIN/VDE 0303 Part 2. Brass plates 30 x 30 mm were used as the substrate. The electrode pairing was P20 / K10 (plate 20 mm thickness, ball of 10 mm diameter) with pressure of 3 N. A 20 kV DC current supply (Bertan) was used to provide the voltage. The current increase was 0.5 kV/sec.

To test the water absorption of the coatings, copper-clad EP plates were covered with the respective coatings and suspended in distilled water at 23 °C. For this purpose the capacity of the different coatings were recorded at different frequencies (1 kHz, 10 kHz, 100 kHz and 1 MHz) during storage in water at logarithmically staggered intervals. With a known geometry and separately measured coating thickness, the capacity of the coating changes, since the dielectricity constant ϵ_r changes with the proportion of water depending on frequency.

Microelectronics relies on copper and gold printed circuit boards. The diffusion of components of the coating itself as well as the diffusion of substances through the coating is of great interest. The release of ions from copper and gold printed circuit boards under physiological conditions was investigated. The appearance of soluble ions and colloidal copper and gold compounds is of importance to the extent that they have a cytotoxic potential. Copper or gold-based printed circuit boards coated with a polymer lacquer were extracted with a suitable buffer (phosphate-buffered physiological sodium chloride solution). Subsequently, the copper and gold concentration in the extract was determined by means of atom absorption spectrometry (AAS). The great selectivity of AAS is to be attributed to the use of a special hollow-cathode lamp for each element. AAS has developed into a widely used method in element trace analysis and is considered to be one of the most effective element trace determination methods.

A trigger for inflammation reactions is physical, chemical and/or toxic damage to tissues from bio-incompatible materials. The biocompatibility was tested in co-operation with IBMT Rostock. The specimens have dimensions of 10 x 10 x 1 mm.

Leucocytes and four plasma enzyme systems (the clotting system, the fibrinolytic system, the kinin system and the complement system) represent significant components of an inflammation reaction. In addition to

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the leukocytes, thrombocytes, endothelial cells and fibroblasts also play an important role in the inflammation process.

The inflammation cells secrete a substantial number of mediators, which serve both to combat the noxa which have entered and to facilitate signal exchange between the cells, and thus control the activation and deactivation of these cells. The mechanisms by which inflammations are triggered and by which they are maintained also take place in vitro with the help of various cellular and plasmatic blood components, with the consequence that they can be used to assess the hemocompatibility of implant materials.

Fresh human serum serves as a complementary source which can be obtained from whole blood of healthy donors. Fresh, thrombocyte-rich human citrate plasma serves as a source of thrombocytes. As a source of prothrombins, fresh heparin plasma is used. The concentrations of C3a-des Arg, platelet factor 4, and prothrombin fragment 1+2 are determined by means of ELISA.

The following are used as reference substances:

- inulin from dahlia tubers (complement activitation)
- collagen type III (activation of cellular clotting)
- kaolinite (activation of plasma clotting).

For the *in vitro* assessment of the hemocompatibility with the aid of complement activation by the alternative route, the specimens were placed in polypropylene containers with 1 ml fresh human serum and incubated for one and two hours at 0 °C with occasional agitation. One serum sample without material (negative control) and one serum sample with 50 µl inulin suspension (positive control), produced from 50 mg inulin in 1 ml water, were also subjected to the same process, in the same conditions. After the incubation, the serum was pipetted off and the C3a-desArg content in the serum was determined with the aid of an enzyme immunoassay (PROGEN Biotechnik GmbH, Heidelberg).

The investigation of the concentration of C3a-desArg was carried out in repeat determinations. In each case, 100 µl blank sample, standards, control and serum samples thinned 1:100 with sample buffer were introduced into the depressions of the microtitration plate, which were coated with monoclonal mouse antibodies with specificity for C3a-desArg. The sample incubation took place over one hour at room temperature (20 - 25 °C). After washing out unbound components, incubation with 100 µl each of a monoclonal mouse anti-C3a peroxidase conjugate took place for one hour at room temperature. Following a further washing step, the substrate solution, 3.3', 5.5' tetramethylbenzidine in water was added. After 10 to 15 minutes, the enzyme/substrate reaction was stopped by adding 100 µl in sulphuric acid. Following this, the optical density was determined in a microtitration plate photometer at 450 nm measurement wavelength.

To activate the cellular hemostasis, the specimens were placed in 24-hole microtitration plates with 1 ml human thrombocyte-rich citrate plasma and incubated for ½ h at room temperature. One plasma sample without material and one plasma sample with collagen (thrombocyte activator) were also subjected to the same process, in the same conditions. After the incubation, the thrombocyte-rich plasma was transferred to test tubes which contained CTAD (citrate, theophylline, adenosine, dipyridamole) for stabilisation and centrifuged at 2000 g at 2 °C for 30 min. Subsequently the concentration of ß-Thromboglobulin or of platelet factor 4 was established in repeat determinations with the aid of sandwich enzyme immunoassays.

In each case, 200 µl blank sample, standards, control and samples were introduced into the depressions of the microtitration plate, which were coated with specific antibodies against ß -TG. The sample incubation took place over one hour at room temperature (20 - 25 °C). After washing out unbound components, incubation with 200 µl each of anti-ß-TG peroxidase conjugate took place for one hour at room temperature. Following a further washing step, the substrate solution, H_2O_2 and o-phenylendiamine in water was added. After 3 minutes, the enzyme/substrate reaction was stopped by adding 50 µl sulphuric acid (3 mol/l). After ten minutes incubation time, the optical density was determined in a microtitration plate photometer at 492 nm measurement wavelength within two hours.

In the activation of the plasmatic hemostasis, the specimens were placed in 24-hole microtitration plates with 1 ml human heparin plasma and incubated for ½ hour and one hour at room temperature. One test application without material and one with kaolinite (activator of the intravascular hemostasis) were also subjected to the same process, in the same conditions. Following this, the concentration of F 1+2 was established in repeat determinations with the aid of a sandwich enzyme immunoassay. In each case, 50 µl sample buffer was first introduced and 50 µl each of blank sample, standards, control and samples were added by pipette into the depressions of the microtitration plate, which were coated with rabbit antibodies against human prothrombin fragment 1+2. The sample incubation took place for 30 minutes at 37 °C. After washing out unbound components, incubation with 100 µl each of anti-human prothrombin-peroxidase conjugate for 15 min at 37 °C. Following a further washing step, 100 µl of the freshly applied substrate solution, H2O2 and o-phenylendiamine in water were added. After fifteen minutes incubation protected from light at 20 - 25 °C the enzyme/substrate reaction was stopped by adding 100 µl 0.5 n sulphuric acid in each case. Within two hours, the optical density was determined in a microtitration plate photometer at 492 nm measurement wavelength.

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Results

In addition to the viscosity of the solution, as long a pot life as possible is to be attained for automated coating. EP3 is therefore suitable only for an experimental trial.

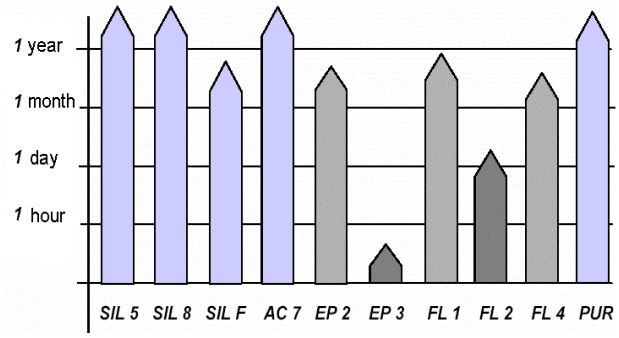

Figure 2: Pot life of the solutions used

When cleaning the substrates in distilled water, it was possible to detect substantial impurities of the surfaces in spite of pre-cleaning in isopropyl alcohol with ultra-sound support. Two essentially different behaviours manifested themselves, which can be described as cleaning and washing out.

For most substrates, no further change in conductivity could be detected at the latest after the third deposition in distilled water. For those substrates for which washing out took place, the conductivity of the elution initially declined more strongly (interaction of cleaning and washing out) and then settled down at a level above the conductivity of the water. It is possible to establish by this means that the dip lacquer AC1 was not fully hardened. The measurements on the EP resins show that residual monomers may have been present on the surface, which it was, however, possible to wash off.

The investigation of the coated surfaces with raster electron microscopy gave rise to no special features or disturbances of the surfaces could be observed. Only with the SILF was it possible to observe particles in the coating which probably derive from a filling material which also leads to the white colouring.

The investigations of the water absorption of the coatings showed that none of the coatings was watertight. The silicones SIL5 and SIL8, against expectations, showed the lowest increases, while SILF in contrast displayed the greatest increase. The following coatings show a moderate constant increase: SIL8, FL1, FL2, PUR, HT1, HT2 and AC7.

Films like FL1, FL4, AC1 and AC7 show an electrical insulation of 5 kV at layer thickness of less than 0.05 mm. The EP3 however has to have minimum thickness of 0.15 mm.

In the testing of the peeling resistance, it should be noted that in most cases the failure took the form of a breach of adhesion in the bonding: that is, the real resistances are greater than the values measured. The qualitative results are given in the overview table.

In the testing of shear resistance of elastic silicone coatings, problems appeared in the bonding: here the breach occurred predominantly in the adhesive joint to the stamp. The real resistance was greater than the values measured. Since the coatings are not adhesives and most coatings are also not exposed to great shear stresses, the conclusion can be drawn that – from the point of view of mechanical rigidity – most coatings are suitable, although FL2 and PUR may not suffice for all applications.

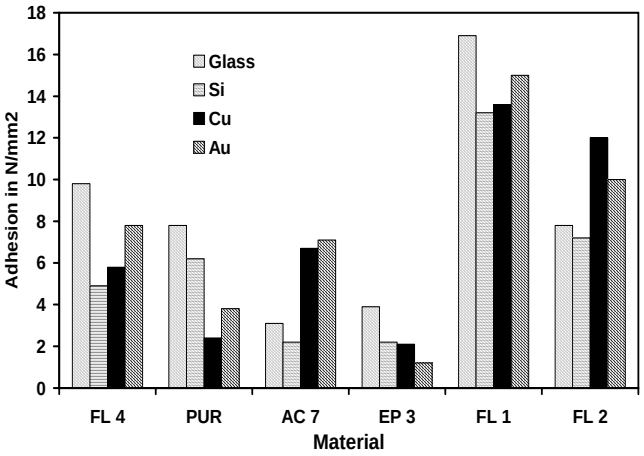

Figure 3: Adhesion of the films

The scratch resistance of the coatings was compared in the same experiment with the optical materials. It is known that elastic coatings such as those that are based on silicone display very good scratch resistance in this type of examination since the coating does not become damaged as a result of its elasticity. It's well known that soft coatings have a high scratch resistance. Acrylate coatings (like AC7) have a good stability, FL1 has lower scratch resistance.

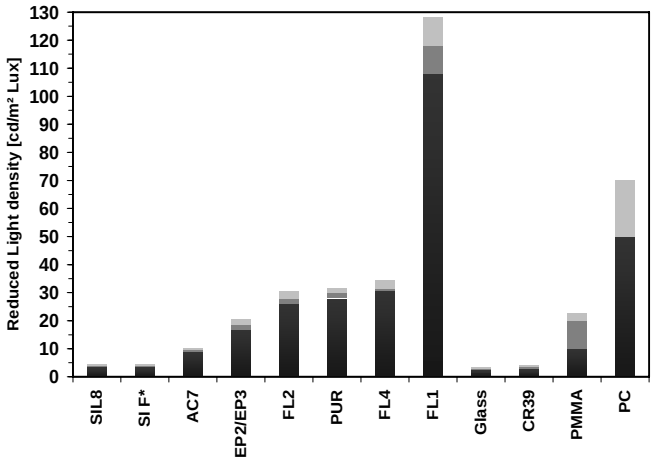

Figure 4: scratch resistance

The following summarises the various properties of the coatings examined.

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Table 2: Properties of the coatings

Material	Workability				General properties					Mech. and elec. properties				Initial material		
	Potfile	Working effort	Transparency	Spreading, wetting	Film thickness	Surface topolgy	Water absorption	Cu migration °	Durface cleanliness	Peel resistance	Shear resistance	Abrasion resistance	El. breakdown resist.	Hardness	Expansion	Tensile strength
SI 5	++	+	o	+	**	+	+	-	+	+	-	++	+	n	h	n
SI 8	++	+	o	+	**	+	+	-	+	+	-	++	+	n	h	n
SILF	++	+	--m	+	**	o	-	-	+	+	-	++	+	n	h	n
AC7	++	+	++	++	*	+	+	o	+	-	o	++	++	h	n	h
EP3	--	-	o	+	***	+	-	--	o	o	o	+	-	h	n	h
FL1	+	++	++	++	*	+	o	+	+	+	++	--	++	h	h	m
FL2	o	o	+	++	*	+	o	n	+	--	+	+	+	h	h	m
F 4	+	+	++	++	*	+	o	+	+	o	o	+	++	h	h	m
PUR	++	+	+	++	*	+	o	n	+	--	o	+	+	m	h	h
++ very good		o satisfactory			* thin			m milky						h high		
+ good		- moderate			** thick			n not tested/not testable						m medium		
		-- poor			° for gold-covered conductors									n low		

As was to be expected, none of the coatings examined received the best evaluation in all requirements. The FL2 coating is perhaps the coating to which recourse will not be made. The final selection will rather be determined by the specific requirements imposed on the coating. For this reason, mechanical properties of the pure polymers are given in the right column of Table 2. These can also be drawn on indirectly for the assessment of the coatings.

Table 3: Diffusion of copper and gold

Coating	Cu [µg/l] from Cu plates	Au [µg/l] from gold-coated Cu plates
PBS	- *	- *
PUR	163.98	- *
FI1	473.56	- *
FI2	381.20	- *
FI4	495.60	- *
EP3	155.04	- *
AC7	1,534.60	- *
SILF	269.40	- *
SIL5	247.83	- *
SIL8	596.65	- *
HT1	2,737.40	- *
HT2	4,731.20	- *

* below the detection limit of 5 µg/l

The copper and gold concentrations in PBS extracts of polymer coated copper and gold layers were investigated in several series of tests. These investigations of diffusion showed that in the physiological buffer environment copper ions are washed out of the copper plates and make their way through the polymer coating into the extract. Here, they can be detected with AAS in different concentrations. Under the extraction conditions (physiological environment) no gold compounds become dissolved, with the result that none can be detected even with AAS.

It was examined whether the materials activate the complement and hemostatic systems when they come into contact with human blood components and whether they cause inflammation mediators to be released. In the following, the results of the *in vitro* test for assessing the hemocompatibility of the polymer coating lacquers are presented in table form.

The values represent the ratio of the concentration with the test material to the concentration without the test material. Where the relative concentrations after material contact are above 1.25 times the value of the concentrations without the test material, this indicates activation by the material samples.

The investigations carried out in vitro for the biological assessment of the coating lacquers and the material PES with the aid of human serum (complement source), thrombocyte-rich human citrate plasma (thrombocyte source) and human

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Table 4: Rel. Concentrations of C3a-des Arg after contact with fresh human serum

Material	Rel. concentration of C3a-desArg	
	1 h incubation	2 h incubation
without material	1.00	1.00
FI 1	1.11	1.19
FI 2	1.07	1.18
FI 4	0.99	1.19
EP 1	0.79	0.85
EO 2	1.03	1.20
EP 3	0.78	1.00
AC 7	0.30*	0.95
SIL F	0.95	1.12
SIL 8	0.80	0.26*
PUR	0.88	0.96
Inulin	22.20	49.01
The concentrations marked with * were not used for the evaluation.		

Table 5: Relative concentrations of PF4 after contact with thrombocyte-rich human citrate plasma

Material	Rel. concentration of platelets factor 4, 30 min incubation
without material	1.00
FI 1	1.15
FI 2	0.99
FI 4	0.87
EP 1	0.90
EO 2	1.01
EP 3	0.91
AC 7	0.95
SIL F	0.92
SIL 8	0.97
PUR	1.00
Collagen	2.68

Table 6: Relative concentrations of F 1+2 after contact with human heparin plasma

Material	Rel. concentration F 1+2	
	0.5 h incubation	1 h incubation
without material	1.00	1.00
FI 1	0.89	0.97
FI 2	0.87	0.94
FI 4	0.84	0.93
EP 1	0.98	0.95
EO 2	0.88	1.00
EP 3	0.96	0.96
AC 7	0.87	1.05
SIL F	0.96	1.03
SIL 8	0.86	0.95
PUR	0.91	0.92
Kaolinite	3.99	5.64

heparin plasma (prothrombin source) give rise to the expectation of good hemocompatibility.

In the three test systems, no material-induced increased concentrations of the inflammation marker C3a, platelet factor 4, ß-thromboglobulin or prothrombin fragment 1+2 after material contact were detected. The results of biological test series proved a good biocompatibility at all.

Conclusions

Most of our surface coatings have enough mechanical resistance for the micro sensor application field. Some are soft and elastic, others are rigid and tough. Obviously not all coatings suit for all purposes. All the investigated coatings passed the tests and did not show weakness [5]. The polymers which are discussed here need at last an authorisation by FDA/USP before being used in medical applications. In contrary to our expectations normal silicone coatings like SIL5 and SIL8 show less water absorption than the SILF.

The impermeability of used polymers should be as high as possible. The barrier-effect as a thin film property is of vital importance today. Long-term experiments lead us to the assumption that layers are never absolutely hermetic impermeable. Hence combination of different polymer films should be considered. This could prevent washing out of soluble copper and gold compounds. We see here an important starting point for further work.

Acknowledgements

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