Microstructured Peptide-Functionalised Surfaces by Electrochemical Polymerisation

Peter Heiduschka,* Wolfgang Göpel, Werner Beck, Wolfgang Kraas, Stefan Kienle and Günther Jung

Dedicated to Professor Ivar Ugi on the occasion of his 65th birthday

Abstract: For the first time, antigenic peptides have been immobilised by electrochemical polymerisation after having been modified with a polymerisable functional group. 3-Hydroxyphenylacetic acid was chosen as the novel polymerisable group. The synthetic peptides represent epitopes of the bovine foot and mouth disease virus and of the sodium channel of the cardiac muscle. The polymerisation was performed by applying a constant anodic potential or by cyclic voltammetry. A combination of these two methods was also employed, that is, cyclic voltammetry with a delay at the anodic vertex potential. No additional free phenolic monomer was required for the polymerisation. The layers formed by the polymerisation were recognised by specific antibodies. The specific binding of the antibodies to the polymer film could be demonstrated by ELISA, an enzyme-linked amperometric immunoassay, and electrochemical impedance measurements, as well as by fluorescence-labelled antibodies. A peptide derived from laminine was also immobilised by electrochemical polymerisation. It could be shown that neuroblastoma cells adhere to this layer.

Keywords
binding assays · immunosensors · electrochemical polymerisations · peptide derivatives · peptide immobilisation

Immobilisation of molecules on surfaces is required for a broad range of applications. Examples include the immobilisation of libraries in the field of combinatorial chemistry, investigations into the binding of molecules to immobilised DNA and the screening of substances in order to find inhibitors or to analyse interactions between agonists or antagonists and receptors. Furthermore, immobilisation techniques are needed for the analysis of surface structures by means of various spectroscopic techniques, for the self-assembly of molecules on microstructures and for the formation of matrix-assisted lipid double layers, which are a prerequisite for the reliable and stable incorporation of receptor systems. Surfaces modified with specific peptides are also needed for the adhesion and directed growth of cells (e.g., neurons) on artificial substrates. For this purpose, surface-bound growth promoters are more important than soluble ones. A durable immobilisation of biological recognition units is crucial for the functioning and reliability of biosensors, which are becoming increasingly important in different areas of clinical, environmental and biotechnological analysis.[1]

Immobilisation techniques have to be compatible with other procedures applied in the production of microelectronic devices. Examples include adsorption, entrapment in gels, membranes or polymers, covalent binding to the substrate or to a polymer, cross-linking, etc.[2] Thin-layer systems, for example, self-assembled monolayers, lipid double layers and Langmuir–Blodgett films, are increasingly common.[3] Self-assembled monolayers have also been used in our laboratory to immobilise peptides modified by alkanethiols on gold electrodes.[4] Microstructured modification of surfaces can also be achieved by masking techniques,[5] as is the case in light-addressed peptide synthesis.[6] In this procedure, peptides are built up on surfaces by using photolabile protective groups. It is, however, time-consuming and requires expensive equipment.

It would be much more convenient if peptides synthesised by optimised methods and characterised in detail with respect to structure and purity could be immobilised directly. Such a method should also allow the immobilisation of libraries of peptides, mimetics or other compounds to produce durable films with good spatial resolution.

Electrochemical polymerisation: An elegant method of achieving this last goal is electrochemical polymerisation. The advantage of electrochemical polymerisation is that films can be prepared in a one-step procedure directly at the surface of an electrode without additional chemicals that could affect the molecule to be immobilised. No further equipment is needed except an elec-

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trochemical potentiostat used routinely by electrochemists. This method allows electrodes of an electrode array to be coated selectively simply by connecting single electrodes. Within the range of the diffusion layer, the polymer film formed by electrochemical polymerisation has the shape of the underlying electrode; no masking techniques are therefore necessary. The preparation of the polymer film is a fairly simple process: The monomer is dissolved in the electrolyte and an appropriate potential is applied to the electrode to be coated. The properties and the thickness of the polymer film are determined by the choice of the monomer, by the magnitude and the time-dependence of the potential applied to the working electrode, as well as by the choice of the electrolyte, the flowing current or the flowing charge.[7] The thickness of the polymer film can be influenced particularly easily when conducting polymers are formed. If nonconducting polymers are formed, the film thickness does not increase further when the surface of the electrode is completely covered by the polymer.

Bard et al. made one of the first attempts to immobilise functional units by electrochemical polymerisation of pyrrole in aqueous solution.[8] Tetrasulfonated iron phthalocyanine was entrapped into the growing polymer layer, and it thus retained its ability to reduce oxygen. Chiral glucose derivatives were immobilised by first coupling them covalently to pyrrole and then polymerising the resulting compound electrochemically.[9] It could be shown that the resulting film exhibits chiral properties and enantioselective recognition. Crown ethers that selectively bind different alkali metal ions were coupled with thiophene derivatives and immobilised by electrochemical polymerisation.[10] The crown ethers also maintained their specifity towards the metal ions. Electrodes modified with immunoglobulins have been used in a competitive immunosensor.[11] A layer that is sensitive towards human serum albumin (HSA) was formed by physical entrapment of anti-HSA antibodies into a layer of polypyrrole.[12] Recently, a polymer was formed from pyrrole derivatised with the dipeptide cystinylcholamide.[13] It could be shown that the polymer film forms a stable complex with $\{Fe_4S_4\}^{2+}$ clusters and exhibits ion-exchange functions.

Enzymes such as the glucose oxidase (GOD) have been immobilised on or in electrochemically deposited polymers.[14] Polymers used for the immobilisation include polypyrrole and its derivatives, poly(dithienylpyrrole), polyphenol, polyaniline, polyindole, polyazulene and poly(phenylenediamine). In most cases, immobilisation was achieved by simple entrapment in the polymer film, sometimes also by covalent coupling to the film after polymerisation. Lowe[15] and Schuhmann[16] covalently coupled pyrrole derivatives to GOD and immobilised the enzyme by polymerising the pyrrole electrochemically. However, a certain amount of nonderivatised pyrrole had to be added to the polymerisation solution. It is most likely that a large proportion of the enzyme molecules was immobilised by simple entrapment. In general, most of the approaches of entrapment cited above suffer from some leakage of the entrapped molecules.

Our approach: The goal of our work in the field of electrochemical polymerisation of peptides, which started at the end of 1992, is the immobilisation of ligands on surfaces by electrochemical polymerisation. As a target that is important for a variety of applications and demonstrates the feasibility of this approach, we chose surfaces functionalised with peptides that are recognised by specific antibodies. Topographic regions of natural antigens (e.g., envelope proteins of viruses) that are recognised by specific antibodies are called epitopes. Epitopes consisting of a continuous sequence of amino acids can be produced by peptide synthesis. Compared to viral proteins, peptides have the advantage that they are more stable, safer to handle and more widely available. This is why synthetic peptides are used in a wide variety of enzyme-linked immunosorbent assays (ELISA) in human diagnostics.

The immobilisation of the peptides is carried out with the help of an attached functional group that can be polymerised electrochemically. Due to the formation of polymer chains at the electrode, the peptide derivatives are deposited onto the electrode (Fig. 1). We used the phenol derivative 3-hydroxyphenylacetic

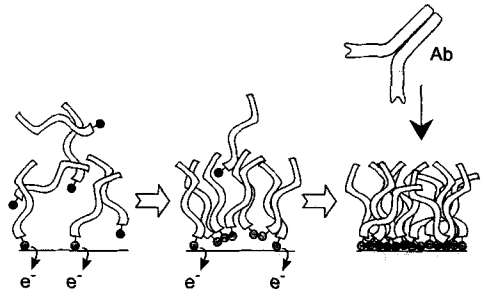
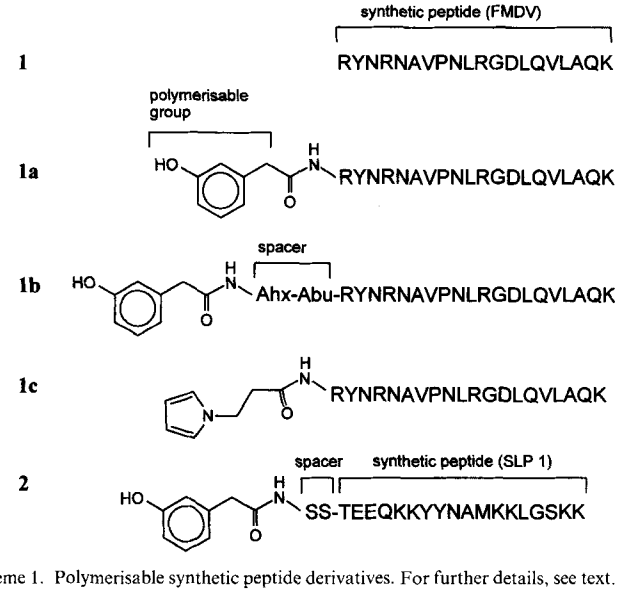


Fig. 1. Scheme of the polymerisation of the peptide and the formation of the polymer film on the surface of an electrode. The circles represent the polymerisable groups attached to the peptide chain. The specific antibody (Ab) binds to the peptide-functionalised surface.

acid (3-HPA) as the polymerisable group and linker to the surface, because here both the *ortho* and the *para* positions are free to couple during the polymerisation. Phenols are known to polymerise at the anode and to form a very thin insulating film at the electrode. It is the first time that 3-HPA has been used for electrochemical polymerisation. We also synthesised a peptide conjugate using an *N*-substituted pyrrole as the polymerisable group by coupling it to the *N*-terminal end of the peptide. The peptide derivatives suitable for electrochemical polymerisation are listed in Scheme 1.

The amino acid sequence of compounds **1a**, **1b** and **1c** is derived from an immunologically dominant epitope of the foot and mouth disease virus (FMDV, sequence 135–154 of the envelope protein VP1).[17] Compound **2** is derived from an epi-



Scheme 1. Polymerisable synthetic peptide derivatives. For further details, see text. Ahx: ε-aminocaproic acid; Abu: 2-aminobutyric acid.

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tope of the sodium channel protein of rat cardiac muscle cells situated in the cytosolic loop between the domains III and IV (so called SLP1 sequence, amino acids 1491–1508).[18] Highly specific antibodies against both peptides were available from former investigations.

Results and Discussion

Electrochemical polymerisation of the derivatised peptides was performed by two techniques, based on constant and cyclic potentials (see Experimental Procedures).

In a first set of experiments, glassy carbon electrodes (GCE) were coated with pure phenol, with the peptide **1a** and with a mixture of phenol and **1a** by applying a constant potential of 1.0 V versus SCE for 8 min. In order to check whether the specific antibody was able to bind to the polymer film formed, an ELISA was performed with the electrodes. A nonspecific antibody was used as a control. The binding assays were performed in the presence or absence of bovine serum albumin (BSA), which was used to prevent nonspecific adsorption. The results obtained from three reproducible assays demonstrated that the specific antibody binds to electrodes with polymerised peptide derivative **1a** (Fig. 2a). The accessibility of the peptide is maintained in the polymer film. As expected for phenol-derived polymer films, the peptide layer is not washed out by the repeated washing steps during the ELISA procedure. The electrodes coated with a mixture of phenol and **1a** reveal a significantly lower extinction; this indicates that the polymer film formed on these electrodes contains less peptide owing to the faster deposition of phenol. The values obtained with the nonspecific antibody are below 5% of those obtained with the specific antibody; this shows that there is almost no nonspecific binding to the peptide layer. The nonspecific adsorption to the polyphenol layer is negligible, which is important for the application in binding assays.

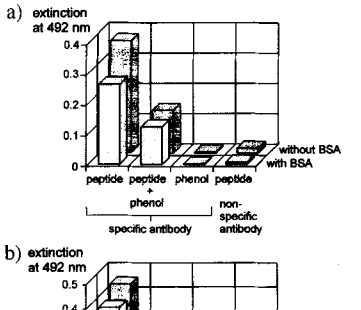
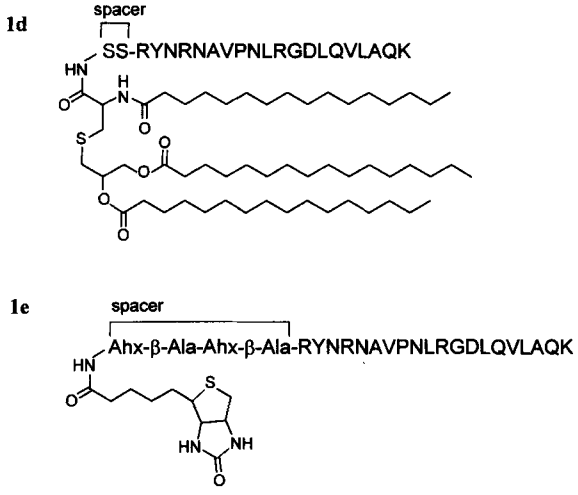


Fig. 2. a) Results of the ELISA with GCE coated with polymerised peptide **1a** in the presence and absence of BSA. b) Comparison of binding properties of polymer films produced by constant and cyclic potentials.

In a second set of experiments, we compared the coating of the electrode achieved by constant (1.1 V vs. SCE, 10 min) and cyclic potentials (30 cycles between 0.0 and 1.1 V vs. SCE). In the ELISA, the electrodes were blocked with BSA (Fig. 2b). The amount of antibody binding to the polymer formed by cyclic voltammetry is comparable to that to the polymer formed by the constant potential. The only difference is that binding of nonspecific antibody seems to be higher when the film is formed by a cyclic potential. As expected, the extinction is higher when a higher antibody concentration is used. Polymerised 3-HPA (Fig. 2b) as well as phenol (Fig. 2a) reveal a very low nonspecific adsorption.

As the next step, the density of peptides deposited by electrochemical polymerisation and recognised by specific antibodies, that is, the density of binding sites, was compared with the density of binding sites available in the wells of commercial ELISA plates coated with peptides. For this purpose, three different methods for coating the wells with peptides were applied. Besides the free peptide, two additional peptide derivatives shown in Scheme 2 were used.



Scheme 2. Structures of the lipopeptide Pam_3Cys-Ser-Ser-[VP1-(135–154)] (**1d**) and the biotinylated peptide biotin-Ahx-β-Ala-Ahx-β-Ala-[VP1-(135–154)] (**1e**).

Free peptide **1** was allowed to adsorb onto the polystyrene material of the wells. The lipophilic peptide **1d** was immobilised by adsorption according to the protocol of the lipopeptide ELISA.[19] The biotinylated peptide **1e** was immobilised according to the protocol of the biotin ELISA in wells coated with streptavidin.[20] The ELISA technique based on the binding between biotin and streptavidin yields the best and most reproducible results. Nowadays, this technique is widely used in human immune diagnostics, for example, in the detection of HIV and HCV.[21]

The ELISA was performed simultaneously with the experiment shown in Figure 2b, that is, under identical conditions and with the same amount of solutions. In order to compare the results for the electrodes and the ELISA wells, the extinction values obtained had to be normalised to the surface of the electrodes because these have a smaller surface area (0.07 cm^2) than the wells of the ELISA plates (0.35 cm^2). It can be assumed that the surface of the electrode is coated with the peptide with maximum density because electrochemical polymerisation is performed until the surface is saturated. The quantity of peptide antigen necessary for maximum extinction in the ELISA was estimated (free peptide, 215 nmol; lipopeptide, 29 nmol; biotinylated peptide, 6.8 nmol), and the maximum extinction values were used for the comparison with the extinction values of the electrodes. The comparison demonstrates that the density of binding sites on the electrodes achieved with electrochemical polymerisation is comparable to the maximum density achievable on the streptavidin-modified wells (Fig. 3). However, assuming that adsorption and binding of antigenic peptides does not only occur at the bottom of the well but also at the side walls, the electrochemical polymerisation method could provide an even higher density of binding sites.

Two electrochemical methods were applied to detect the binding of specific antibodies to the polymerised peptides. First, we used secondary antibodies labelled with the enzyme glucose oxidase (GOD). This well-known enzyme catalyses reaction (1).

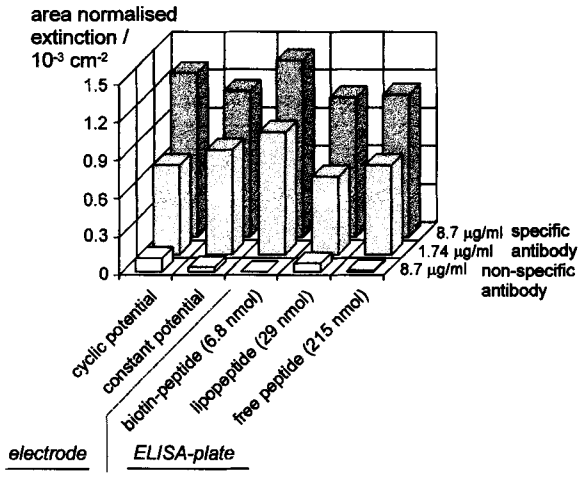


Fig. 3. Comparison of ELISA results for GCE coated with polymerised film and conventionally prepared wells of ELISA plates.

The hydrogen peroxide produced can be detected electrochemically by anodic oxidation [Eq. (2)]. This method was applied

$$\beta\text{-D-glucose} + O_2 \longrightarrow \text{D-gluconolactone} + H_2O_2 \quad (1)$$

$$H_2O_2 \longrightarrow 2H^+ + O_2 + 2e^- \quad (2)$$

in a straightforward manner to the same GCE previously coated with the peptide. In order to decrease the potential necessary for the oxidation of hydrogen peroxide, the GCE was coated with a thin layer of platinum particles before polymerisation of the peptide. After the binding of the specific antibody and the GOD-labelled secondary antibody, the GCE was transferred into an electrochemical cell. The current measured increased upon addition of glucose (Fig. 4).

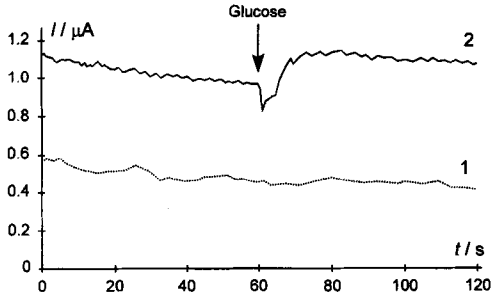


Fig. 4. Current measured at 0.65 V vs. Ag/AgCl at a GCE after incubation with specific antibody and subsequently with secondary antibody labelled with GOD. The arrow indicates addition of glucose: curve 1, bare GCE; curve 2, GCE coated with polymerised peptide **1b**.

The current generated by the oxidation of hydrogen peroxide was small compared with the noise. There are two possible reasons for the small current: First, given that the layer of GOD is monomolecular, there are only a few enzyme molecules available for hydrogen peroxide production. Second, the hydrogen peroxide is produced at a distance from the surface of the electrode, and most of it will diffuse into the bulk solution before it can be detected by the electrode.

The second electrochemical method was the measurement of changes in electrochemical impedance. When antibodies bind to the surface, the dielectric constant can change in the vicinity of the electrode surface. However, the impedance effect should be rather small because the hydrated antibody molecules are expected to cause only very small changes in the dielectric constant, and the space between the bound antibody and the electrode surface is filled with the polymer incorporating water molecules, which diminish the influence of the bound antibody.

For the impedance measurements, we used small platinum microband electrodes (5 µm × 1 mm), arranged in parallel with separations of 5 µm. Figure 5 shows the behaviour of the impedance at different frequencies. In order to avoid any non-specific effects, the measurements were carried out in the presence of 2% BSA. At a frequency of 1 kHz, a gradual increase of the impedance could be detected after addition of specific antibody.

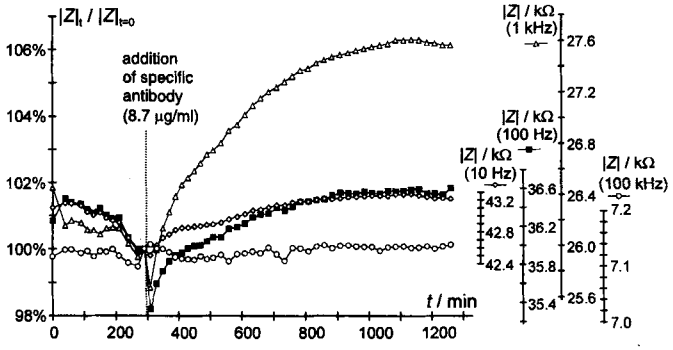


Fig. 5. Increase in the impedance at 1 kHz after addition of specific antibody. The surface is clearly completely saturated after 10 h.

For screening assays with libraries of peptides and other compounds, a miniaturisation or microstructuring is necessary. In order to demonstrate that this is possible, interdigitated platinum "comb" electrodes were used (Fig. 6). The goal was to

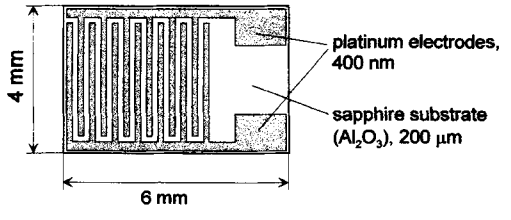


Fig. 6. Structure of the interdigitated platinum "comb" electrodes.

visualise the binding of the specific antibody to the peptides by fluorescent dyes. The platinum electrodes were platinised to minimise the inherent reflectivity and to increase the effective surface. The electrodes were then coated by electrochemical polymerisation of the peptide derivatives **1a**, **1b**, **1c** and **2**.

The polymerisation was performed by applying a combination of the constant potential procedure with cyclic voltammetry: 20 cycles between 0.1 and 1.2 V (vs. SCE) were applied with a scan rate of 0.1 Vs^{-1}, and at the anodic vertex point the potential of 1.2 V (vs. SCE) was held for 20 s. After the polymerisation, the electrode was incubated first with the specific antibody in the presence of 1% BSA, then with the secondary antibody labelled with a fluorescent dye. The fluorescence of the electrodes coated with the peptide is demonstrated clearly in Figures 7a–d. Figures 7c and 7d show an interdigital structure where only one electrode has been coated. Consequently, only this particular electrode shows fluorescence.

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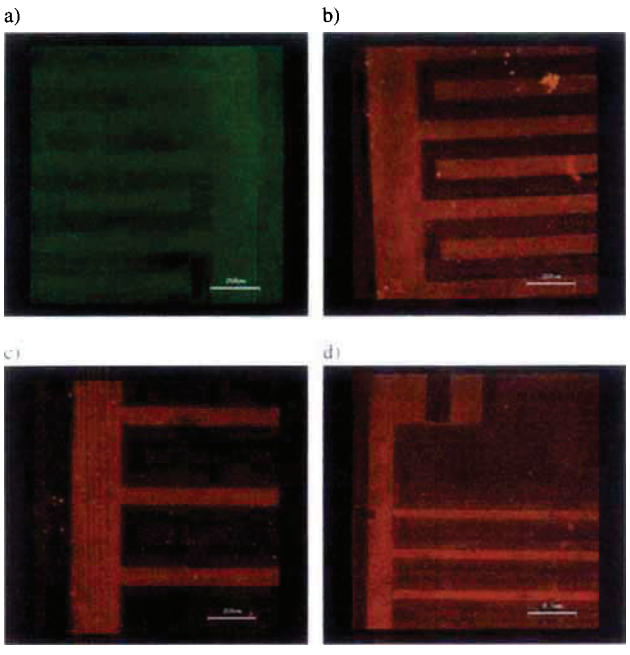

Fig. 7. a) Detailed view of the interdigitated structure with polymerised **1c**. b) Detailed view of the interdigitated structure with polymerised **2**. c), d) Detailed views of the interdigitated structure with only one electrode coated with **2**. The fluorescence of the FITC label (a) and the TRITC label (b–d) is clearly visible. The bar corresponds to 0.25 mm in a)–c) and 0.5 mm in d).

Final Remarks

It was shown that functional peptides can be immobilised on surfaces of electrodes by electrochemical polymerisation after modifying them with a polymerisable group. To the best of our knowledge, it is the first time that small peptides have been immobilised in this way, and that the phenol derivative 3-HPA has been used for polymerisation. In contrast to other methods,[15, 16] no free monomer was necessary for the polymerisation. The peptides in the polymer film could be recognised by specific antibodies.

There are many other types of binding assay that could be carried out after functionalisation of surfaces by the technique described above. A gold layer placed on the back side of a prism could be coated by electrochemical polymerisation; this would allow measurements by surface plasmon resonance (SPR). Electrodes on a quartz crystal microbalance could also be coated. Binding of antibodies would cause mass changes at the surfaces, which could be detected by the decrease in resonance frequency.

One advantage of the method of electrochemical polymerisation is that it does not depend on the electrode material. The substrate only has to be an electrical conductor. By coating surfaces made from indium–tin oxide (ITO), a large variety of optical methods can be applied.

Our present investigation deals with the polymerisation of peptides that are derived from the extracellular membrane protein laminin.[22] Surfaces modified with such peptides should be suitable substrates for nerve-cell adhesion and growth. In preliminary experiments, we have used the sequence CDPGYIGSR modified with 3-HPA for the polymerisation. This peptide had been shown to promote adhesion of neurons.[23] Neuroblastoma cells adhere to electrodes coated with a polymer of this peptide. An example is shown in Figure 8. Whereas neuroblastoma cells do not show any specific adhesion behaviour at an interdigitated structure without peptide coating, they adhere according to the shape of the electrodes after coating with polymerised peptide.

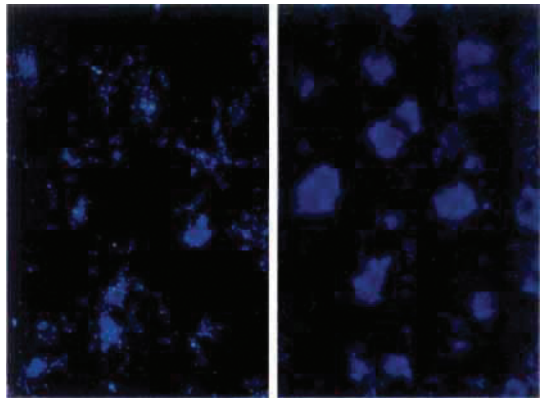

Fig. 8. Comparison of neuroblastoma cell growth on interdigitated platinum electrodes without (left) and with (right) electrochemically polymerised peptide [24].

In further studies, we will immobilise other compounds acting as ligands or antiligands and especially substance libraries by electrochemical polymerisation.

Experimental Procedures

Materials: All chemicals and solvents used, including acetonitrile and trifluoroacetic acid (TFA) for HPLC, amino acid derivatives, functionalised polystyrene resins, tetrafluoroborate, formic acid, and diisopropylcarbodiimide (DIC), were purchased with high purity from commercial sources and used without further purification. Ultrapure water (18 MΩcm) was prepared with a Millipore filtration device. Goat-anti-mouse antibodies (IgG and IgM) conjugated with horseradish peroxidase (HRP) were obtained from Dianova (Hamburg, Germany). Monoclonal antibodies (mouse) against the FMDV were supplied by Dr. E. Pfaff (Federal Research Institute for Animal's Virus Diseases, Tübingen, Germany).

Methods: HPLC was performed with a System-Gold device from Beckman, Scan Ramon (USA). Mixtures of TFA with water or acetonitrile served as eluents. Electrospray mass spectrometry (MS) was performed with an API III TAGA 6000E triple-quadrupole mass spectrometer from Sciex, Thornhill, Ontario (Canada). Automated solid-phase peptide synthesis was performed with the peptide synthesiser 430A (Applied Biosystems, Weiterstadt, Germany) with the Fmoc/*t*Bu technique and styrene–divinylbenzene copolymers as solid phase. These resins were modified with *p*-benzyloxybenzyl alcohol anchors (Novabiochem, Läufelfingen, Switzerland), which were loaded with the first Fmoc-protected amino acid. The peptides were cleaved from the resin over 3 h with a mixture of TFA, thioanisol, ethane dithiol, phenol and water. The peptides released in this way were precipitated three times with cold diethyl ether and lyophilised from a mixture of water and *tert*-butyl alcohol.

Preparation of N_α-3-HPA-[VP1-(135–154)]-OH (1a): [VP1-(135–154)] was synthesised with the automatic synthesiser. 3-HPA was dissolved in DMF containing 1-hydroxybenzotriazol and added to the resin with the attached peptide. Coupling was performed over 15 h by means of DIC dissolved in dichloromethane (DCM). The peptide was cleaved from the resin and purified. The mass spectrum revealed a relative molecular mass of $M_{r,exp} = 2458.98 \pm 0.52$ ($M_{r,theor} = 2459.8$). The product of the double coupling of 3-HPA onto the free hydroxyl group of 3-HPA could be observed with approximately 60% relative intensity: $M_{r,exp} = 2593.93 \pm 0.21$ ($M_{r,theor} = 2594.08$). The free hydroxyl group of 3-HPA could be protected in future syntheses by *tert*-butylation. However, the product of the double coupling of 3-HPA could be polymerised as well. There was therefore no need to protect the free hydroxyl group. Biotinylated peptides and lipopeptides were synthesised as described elsewhere [18].

Polymerisation: Electrochemical polymerisation was performed in two different ways: 1) A constant anodic potential in the range of 1.0 V vs. a saturated calomel electrode (SCE) was applied to the electrode. 2) A triangular change in potential, as is used in cyclic voltammetry, with a scan rate of 0.05 Vs^{-1} was applied to the electrode. The initial (and final) potential was 0.0 V vs. SCE, and the vertex potential was 1.0 vs. SCE. In order to improve the oxidation of the phenolic group and to enhance the rate of polymerisation, 1.2 V vs. SCE was later used instead of 1.0 V in both constant and cyclic potential modes. Polymerisation was carried out in Sørensen phosphate buffer (pH 7.4), with approximately 0.1 $mg\,mL^{-1}$ peptide.

ELISA: For the ELISA, phosphate buffer saline (PBS) (pH 7.4) was used. A solution (100 mL, pH 5) of *o*-diaminobenzene (4 mM), citric acid (20 mM), Na_2HPO_4 (50 mM) and hydrogen peroxide (0.004%) served as the substrate solution for the HRP. Hydrogen peroxide was added immediately before application of the solution to the samples with the secondary antibody labelled with HRP. After 30 min of enzymatic reaction, the extinction of the solution was measured at a wavelength of 492 nm. The ELISA was carried out three times, with a variation of less than 10%. The samples were washed between each incubation step with PBS/0.05% Tween 20.

Peptide and lipopeptide ELISA [19]: Compounds **1** and **1d** were dissolved in PBS and transferred into the wells of the ELISA plate. The wells were washed, blocked with PBS/1% BSA and washed again. Antibody diluted with PBS/1% BSA was added to the wells and incubated for 2 h. After repetitive washing, the secondary antibody labelled with HRP and diluted in PBS/1% BSA was incubated for 1.5 h. After washing, the substrate solution was added to the wells, and extinction was measured after 30 min.

Biotin ELISA [20]: Compound **1e** was dissolved in PBS and added to the wells coated with streptavidin. After 2 h of incubation, the wells were washed. Antibody diluted with PBS/1% BSA was incubated, and after washing, the procedure with the secondary antibody was performed as described for the lipopeptide ELISA.

ELISA with the GCE: Glassy carbon electrodes (GCE) were rinsed with distilled water after the electrochemical polymerisation and fixed in position with the electrode surface horizontal. The solutions for blocking and with the different antibodies were placed as 150 μL drops onto the surface, and the electrode was thus covered completely. After applying the washing procedure between each of the steps, remaining droplets were removed carefully with filter paper. To measure the extinction, 100 μL of the substrate solution on the electrodes with the dye produced by the enzymatic reaction was transferred with a pipette into the wells of an ELISA plate.

Electrochemical polymerisation and measurements were performed with a potentiostat model 273 coupled with a lock-in amplifier model 5210 (Princeton Applied Research, USA) and connected with a 486 computer through an IEEE-488 interface (National Instruments, USA). For the polymerisation procedure and the amperometric measurements, a program developed in our group was used, whereas the commercial program model 398 (Princeton Applied Research, USA) was used for the impedance measurements.

Immunoassay with fluorescent secondary antibodies: After the platinum interdigitated electrodes had been blocked with PBS/BSA, they were exposed for 1.5 h to solutions of antibodies specific for the FMDV sequence or for the SLP1 sequence, and then to the appropriate secondary antibody labelled with a fluorescent dye. Fluorescein isothiocyanate (FITC, anti-FMDV antibody) and tetramethylrhodamine isothiocyanate (TRITC, anti-SLP1 antibody) were used as fluorescent dyes. The measurements were performed with the computer-assisted laser-scan microscope LSM410 Invert (Carl Zeiss, Oberkochen) with a resolution of 512×512 pixels. For the excitation of rhodamine, a HeNe laser (543 nm, 0.5 mW, long pass filter 570 nm) was used. For the excitation of fluorescein, an Ar laser (488 nm, 15 mW, band pass filter 515–565 nm) was used. The maxima of the adsorption/emission are at 544 nm/570 nm for TRITC and at 495 nm/519 nm for FITC. No fluorescence was found when peptide was not polymerised on the electrodes.

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