Reactive Hydrogels Grafted on Gold Surfaces

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Summary: Three different strategies for the fixation of stable hydrogel coatings on gold surfaces, by the grafting-to and grafting-from techniques, are described. Monomeric or polymeric disulfide initiators are employed to start the photopolymerization of N-[tris(hydroxymethyl)methyl]acrylamide **1** via decomposition of azo compounds or via a photoredox system. Different approaches, based on the post functionalization of the **poly(1)** films or on copolymerization of reactive monomers with **1**, are employed to bind potential receptor molecules bearing amino or thiol groups as anchor to the hydrogel. Thus, the functionalized coatings should allow the selective binding of particular analyzates. The overall goal is focused on hydrogel films for the preparation of biochips used in analytical devices such as Surface Plasmon Resonance (SPR), though our approach seems to be generally useful for related purposes.

Hydrogel coated surfaces

Biological fluids are typically complex mixtures of various components in an aqueous solvent, containing for instance salts, sugars, or proteins. The sophisticated functioning of biological systems requires delicate balances between such compounds in highly specialized environments. In consequence, biological molecules are rather sensitive to contact to foreign material on the one hand, but can be very aggressive to

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foreign material themselves, on the other hand, too. The use of materials in contact with biological fluids is therefore, independently of the purpose the material is asked to fulfill, complicated by the inherent interaction of these ingredients with the materials' surface. This implies the need to control such interactions, in order to avoid either damage of the material, or inversely damage of the biological compounds. This difficulty is not only encountered for the use of synthetic materials in vivo, but also in vitro.

One way of dealing with this general problem consists in covering the materials' surface with an inert coating which is typically a thin hydrogel layer. In order to be efficient, the hydrogel must not only provide biological inertness, but should be chemically and physically stable, adhere well to the support, and optionally, allow for a functionalization of the material if needed. More detailed specifications depend of course on the individual case.

In this context, we have explored strategies to fabricate hydrogels on noble metal surfaces, in particular on gold. Emphasis was given to hydrogel coatings for the use in biochips used in analytical devices based on Surface Plasmon Resonance (SPR),[1,2,3] though the approaches presented should be generally useful for related purposes. SPR devices are based on the detection of refractive index changes in a thin zone on top of a gold surface, probed by the evanescent field of a laser probe beam. The method is non-destructive, does not require labelling, is suited for solid-air or solid-liquid interfaces, including non-transparent media (as run in reflection mode), and allows for the analysis of changes in the probed zone in real-time. Typical applications are detection of the presence of analyzates by selective binding, the monitoring of modifications taking place on the chip surface, or investigation of receptor-ligand interactions. Consequently, useful hydrogels ask for several specifications, as they :

- should be rather thin (not thicker than 100nm),
- need to dispose of anchor groups binding selectively to gold,
- should be resistant to hydrolysis as well as biological degradation, as by enzymes or micro-organisms,
- must minimize unspecific adsorption (in particular of biological macromolecules),
- allow the fixation of a sufficient quantity of selective binding groups for direct or indirect functionalization.

Preferentially, the hydrogels should also allow easy handling and storage, as well as the regeneration of the chip surface.

To satisfy these specifications, we decided to employ polymeric hydrogels, applying two different strategies: either the fixation of prefabricated polymers to the gold surface ("grafting-to"), or the fixation of initiators with formation of the hydrogel in-situ via polymerization of suited monomers ("grafting-from"). In order to ensure stable fixation, the hydrogels were bound specifically to gold, via the reaction with disulfide anchors. In a second step, unsymmetrical bifunctional linker groups were fixed to the hydrogels, in order to enable the fixation of molecules for specific recognition of analytes etc., as outlined in Figure 1.

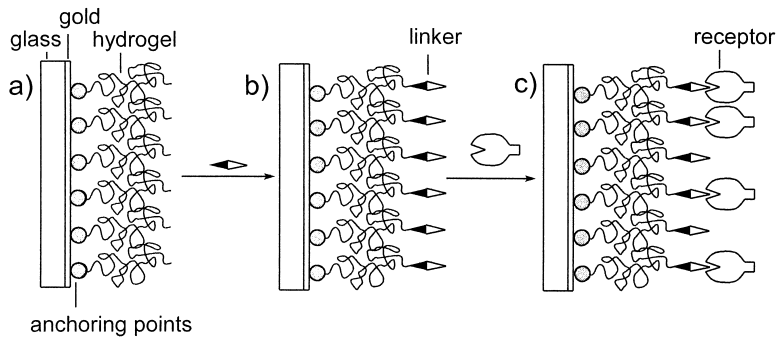


Figure 1. Scheme of fabrication of a biochip
a) fixation of the hydrogel; b) activation of the hydrogel by unsymmetrical bifunctional linker groups; c) functionalization of the hydrogel by receptors

The studies presented here are focused on hydrogels based on the monomer N-[tris(hydroxymethyl)methyl]acrylamide **1** (Fig. 2), but should be easily transferable to other hydrophilic monomers.

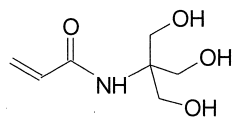


Figure 2. Monomer N-[tris(hydroxymethyl)methyl]acrylamide **1**

Monomer **1** was chosen because its polymer is non-ionic, very hydrophilic,[4,5] biocompatible,[6,7] and inert to biological fouling. Moreover, it disposes of a high number of primary hydroxyl groups which are well suited for post-functionalization (cf. Fig. 1b).

In the following, we will exemplify different strategies to obtain functional hydrogels on gold using **poly-1**.

Experimental section

The water was deionized and purified by an Elgastat Maxima system (resistance : 18.2 MΩ). Monomer N-[tris(hydroxymethyl)methyl]acrylamide (98%) was purchased from Aldrich, monomer **2** was prepared according the literature.[8] NMR spectra were recorded on Varian Gemini 200 running at 200 MHz for ^{1}H-spectra and 50.32 MHz for ^{13}C-spectra and on a Varian Gemini 300 running at 300 MHz for ^{1}H-spectra and 75 MHz for ^{13}C-spectra. Chemical shifts are given in ppm and coupling constant values in Hertz. Ellipsometry used a commercially available ellipsometer (Jobin-Yvon, Ellisel) operating in the fixed polarizer/rotating compensator/fixed analyzer configuration. Every sample was measured 5 times on different places. The thickness is estimated by homemade software, fitting the data with a model of a homogeneous film of a refractive index of 1.500, on a silicon substrate of a refractive index 3.882 +j 0.019 (j is the square root of 1). Upper and lower limits of the thickness are calculated using refractive indexes of 1.45 and of 1.7, since polymers in general have a refractive index between these values. SPR measurements were recorded on a SPR prototype (BioTul, Munich) with a diode laser (λ = 784 nm) as a light source, angular resolution f = 0.005°. The monochromatic light directed to a prism was reflected onto a photodiode. The intensity of the reflected light was measured as a function of external incidence angle. Every experiment was computer controlled. Thickness of the films was estimated by applying the standard Fresnel equations, assuming a refractive index of 1.478. Infrared spectra (GIR) were performed using a Bruker Equinox 55 FTIR spectrometer, using a low noise DGTS detector and a Spectratech FT-80 horizontal grazing angle accessory (80°) fitted with a homemade sample changer. A Graseby KRS-5 polarizer was used to polarize the electric field parallel to the surface normal. The spectra were obtained by averaging 5 measurements of 256 pulses each.

♦ Poly(1) grafted-from gold surface using **4** as initiator.

Initiator **4** was prepared as described in literature[9] and was purified by size exclusion chromatography using a LH 20 Sephadex™ gel and a mixture of EtOH / CH_2Cl_2 (1/1) as

eluent. The immobilization of the initiator **4** onto gold chips was carried out in degassed DMSO at room temperature (concentration approximately 5 mmol/l). After 15 h of adsorption, non-attached initiator was removed by washing with DMSO and EtOH. The chips were transferred in a flask, which was subsequently filled with the monomer **1** (1.50 g, 8.5 mmol, 15 % w/v) and a degassed mixture of DMSO/water (1v/9v, 10 mL). After removal of oxygen traces from the solution by bubbling argon for 30 minutes, the flask was irradiated at 350 nm for 5 h. The gold chips were removed from the flask and washed extensively with ultra pure water and stored in water.

♦ Synthesis of the polyamide **5**.

4,4'-Azobis(4-cyanopentanoic acid chloride) was prepared by a modified literature procedure.[10,11] 4,4'-Azobis(4-cyanopentanoic acid) (5.0 g, 17.8 mmol) was slowly added to a suspension of PCl_5 (14.9 g, 71.5 mmol) in 40 mL of CH_2Cl_2 with ice cooling under argon atmosphere. The mixture was stirred 2 h at 0°C and then 3 h at ambient temperature. The excess of PCl_5 was filtered off, and the solvent and $POCl_3$ were removed under reduced pressure. The crude product was triturated twice in a diethyl ether / pentane mixture (1v/3v), filtrated and dried in vacuo. The acid chloride was obtained as a white powder in 90 % yield (5.1 g, 16.0 mmol).

^{1}H NMR ($CDCl_3$, 200 MHz) : 3.30 - 2.92 (m, 4H, C*H*$_2$CO), 2.74 - 2.42 (m, 4H, C*H*$_2$C), 1.74 (s, 3H, C*H*$_3$), 1.70 (s, 3H, C*H*$_3$).

^{13}C NMR ($CDCl_3$, 50 MHz) : 172.9 (2C, CO), 117.5 (2C, CN), 72.1 (2C, CH_2C), 42.5 (2C, *C*H$_2$CO), 33.7 (2C, *C*H$_2$C), 24.5 (2C, *C*H$_3$).

Cystamine hydrochloride (0.7 g, 3.1 mmol) was stirred in 8 mL of a NaOH (0.5 g, 12.5 mmol) aqueous solution cooled to 0°C. A solution of 4,4'-azobis(4-cyanopentanoic acid chloride) (1.0 g, 3.1 mmol) in 30 mL of CH_2Cl_2 was added and the reaction mixture was left under rapid stirring for 3 h. The white solid was filtered off, rinsed with water, and dissolved in 15 mL of a mixture of CH_3CN / ethylacetate (2v/1v). The mixture was dried on Na_2SO_4 and the polymer was precipitated into a large volume of diethyl ether. Reprecipitation from a DMF / acetone (8v/2v) mixture into diethyl ether produced a white solid. Yield: 1.0 g

^{1}H NMR (DMSO, 200 MHz) : 8.3 (m, 2H, NH), 3.6 - 3.2 (m, 8H, C*H*$_2$CO and C*H*$_2$NH), 3.0 (m, 4H, C*H*$_2$S), 2.7 - 2.5 (m, 4H, C*H*$_2$C), 1.9 - 1.7 (m, 6H, C*H*$_3$).

^{13}C NMR (DMSO, 50 MHz) : 170.1 (2C, CO), 119.1 (2C, CN), 71.7 (2C, C^{IV}), 37.1 (2C, CH_2NH), 33.0 (2C, CH_2CO), 32.8 (2C, CH_2C), 29.6 (CH_2S), 23.2 -22.2 (2C, CH_3).

♦ **Poly(1)** grafted-from gold surface using **5** as initiator.

The same general procedure was employed for the adsorption of **5** (5 g/l in DMSO) onto gold chips and the photopolymerization as described for **4**.

♦ Synthesis of poly(oxy-1,2-dithiane 4,5-diyl - oxycarbonyliminohexamethylene imino carbonyl)-co-(oxy dimethylene N-phenylimino-dimethylene - oxycarbonyl iminohexa methylene iminocarbonyl) **6**.

A solution of 1,2-dithiane 4,5-diol (0.19 g, 1.27 mmol) and N-phenyldiethanolamine (0.23 g, 1.27 mmol) in 15 mL of dry THF was added under stirring to a solution of hexamethylene diisocyanate (0.43 g, 2.54 mmol) in 5 mL of dry THF. The mixture was heated for 30 minutes at 50°C and left at room temperature overnight. The solvent was removed under reduced pressure. The viscous solid was dissolved in 10 mL of DMSO and **6** was precipated into a large volume of water, and repricipitated from a $CHCl_3$ / DMF (1v/9v) mixture into a mixture of EtOH / water (1v/2v). Yield : 0.5 g.

^1H NMR (DMSO, 200 MHz) : 7.1 (m, 4H, C*H* Ar.), 6.8 (m, 4H, C*H* Ar.), 6.6 (m, 2H, C*H* Ar.), 5.7 (m, NH), 4.7 (m, 2H, C*H*O), 4.1 (m, 8 H, CH_2N), 3.5 (m, 8 H, CH_2O), 3.2 - 2.8 (m, 20H, CH_2NCO, CH_2S), 1.5 - 1.1 (m, 32 H, CH_2).

Elemental analysis :

found	53.4 % C	7.6 % H	11.6 % N	6.8 % S

According to the results of sulfur elemental analysis, a ratio of 1 to 2 was estimated for the incorporation of the disulfide diol relatively to N-phenyldiethanolamine.

♦ **Poly(1)** grafted-from gold surface using **6** as initiator.

The same general procedure was employed for the adsorption of **6** (5 g/l in DMSO) onto gold chips. The chips were transferred in a flask, which was subsequently filled with the monomer **1** (1.50 g, 8.5 mmol, 15 % w / v), the sensitizer **7** (0.60 g, 1.5 mmol) and a mixture of DMSO/water (1/9, 10 mL). After removal of oxygen traces from the solution under vacuum during repeated freeze-thaw cycles, the flask was irradiated at 420 nm for 5h. The gold chips were removed from the flask and washed extensively with ultra pure water and stored in water.

♦ **Copoly(1-co-2)** using 4 as initiator.

Initiator 4 (0.054g, 0.07 mmol, 1%), monomer 1 (1.20g, 6.8 mmol), and monomer 2 (0.031g, 0.14 mmol) were dissolved in 25 mL of DMSO. After removal of all oxygen traces from the solution under vacuum during repeated freeze/thaw cycles, the flask was heated to 65°C for 24 h. The polymer was purified by precipitation into a mixture of EtOH/diethylether (1/1) and by dialysis against water. Lyophilization yielded to 1.0 g of colorless, strongly hygroscopic powder.

^{1}H NMR (D_2O, 200 MHz) : 3.8 (s, CH_2), 3.2 - 3.1 (m, CH_2), 3.0 - 2.9 (m, CH_2), 2.4 - 2.1 (m, CH), 1.9 (s, CH_3), 1.8 - 1.4 (m, CH_2).

Disulfide based anchor groups

Two strategies, the grafting-to and the grafting-from, are well known for the chemisorption of compounds onto surfaces. Both approaches have their advantages and theirs drawbacks. The grafting-to technique is the most frequently used. An advantage of this method is that one can investigate and characterize the polymers by standard methods before grafting. However, the layers prepared by this technique tend to have low graft densities due to the hindrance by the already attached chains that create a diffusion barrier (limitation of the adsorbed materials by steric and entropic forces).[12,13] To overcome this limitation, the grafting-from technique can be applied. Fixing first an initiator, the hydrogel is formed in-situ via polymerization of suited monomers. Higher grafting densities are expected because of the easy access of monomers to the active center. Therefore in recent years, this technique has become more popular.[14-18] The grafting of hydrophobic monomers from bulk or from concentrated solutions led to films with thicknesses in the range of ten to hundreds of nanometers. In particular, various systems have been employed for the fixation of the initiators and for a free radical polymerization process, but in general, free chains were produced in solution and required extraction of the adsorbed chains from the covalently bound polymer.[10]

Monomeric disulfide anchor groups

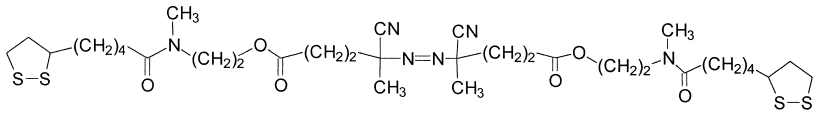


Figure 3. Symmetric azo initiator **4**

As the grafting-from technique seemed to be the more appropriate to prepare thin uniform hydrogel with our specifications, we prepared a symmetric azo initiator (**4**)[9] bearing at each side disulfide groups (Fig. 3), which should minimize the formation of free chains in solution. In such systems, azo initiators seemed to be the most appropriate ones. Peroxide initiators[19] were avoided due to their ability to oxidize sulfides, thus destroying the anchoring points. Equally, Atom Transfer Radical Polymerization (ATRP) technique was avoided because of the contamination of the system by heavy metals and, as in the case of the nitroxide controlled polymerization technique, because of the low stability of the Au-S bond at the high temperatures required.[20,21] In fact, grafting of initiator **4** should lead after the polymerization step to stable films against washing, moderate temperature and substitution by other functional compounds due to the presence of two sulfur atoms as anchoring point to the polymer chains. Also, we aimed at minimization of transfer reactions between the disulfide group and the radical site[22] by introducing a spacer arm.

Thus using **4**, the in situ initiation of polymer chains from the surface using monomer **1** should produce homogenous hydrogels without formation of free polymer chains in solution.

Polymerizations on modified gold substrates were started by irradiation at 350 nm with dilute or moderate concentrations of monomer **1**. Thermal polymerization experiments have been used as well, but required longer reaction times and provided less dense films, so that photoinitiation was preferred. Moreover, as the strength of the Au-S bond is only of 44 kcal/mol[21,23] and as no additional interactions, such as semi-crystalline alkyl chains or hydrogen bonds, contribute to the stabilization of the initiator layer onto the surface, elevated temperatures were avoided. Reaction mixtures were degased for photopolymerization to avoid the inhibition of the propagating radical by O_2, and to prevent the photoxidation of the sulfide bonds.

The formation of hydrogels on gold plates bearing **4** via the grafting-from technique was investigated by measuring the contact angles of the clean and modified substrate (Table 1). The properties of the modified gold surfaces changed markedly, as can be seen by the shift towards lower contact angles of 30 to 55° depending of the monomer concentration (2 % to 20 % (w/v), respectively). In agreement, SPR and ellipsometry measurements indicated that the thickness of the hydrogel depends of the concentration of monomers **1**.

Table 1. Modification and characterization of the gold chips (a DMSO / water mixture (1v/9v) was employed for the polymerization. Reaction mixtures were irradiated at 350 nm by 2 x 2 W lamps placed at 4 cm from the gold plates, except for initiator **6** which was irradiated at 420 nm).

Sample	Initiator	Concentration of monomer **1** (w/v)	Contact Angle (water drop)	Thickness (SPR)	Thickness (ellipsometry)
S0	no	-	80°		
S1	**4**	5 %	55°		2 nm (± 0.2)
S2	**4**	20 %	30°	10 nm	
S3	**5**	10 %	55°		4 nm (± 0.4)
S4	**6**	15 %	30°		9 nm (± 0.3)

From concentrations of 2 % up to 20 % of monomers (w/v), layer thickness increases by a factor of five (2 nm to 10 nm respectively) which exemplifies the versatility of the method. SPR investigations indicated that hydrogel thicknesses of about 10 nm are efficient to avoid unspecific adsorption of Bovine Serum Albumine (BSA), thus demonstrating the validity of the approach.

Polymeric disulfide anchor groups which depolymerize upon binding

In the example given above, a low molar mass initiator was chemisorbed. We hypothesized that the grafting of polymeric initiators, which depolymerize in single initiator unit during the chemisorption process (Fig. 4), could improve the adsorption step and the homogeneity of the final hydrogel coating. Moreover, the polymer chains should be linked to the sulfur groups through hydrolysis resistant bonds, such as amides, in order to improve the stability of the chips during long time storage and basic or acid conditions.

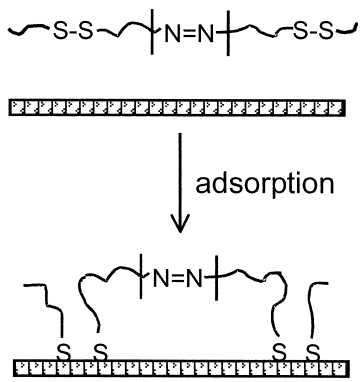
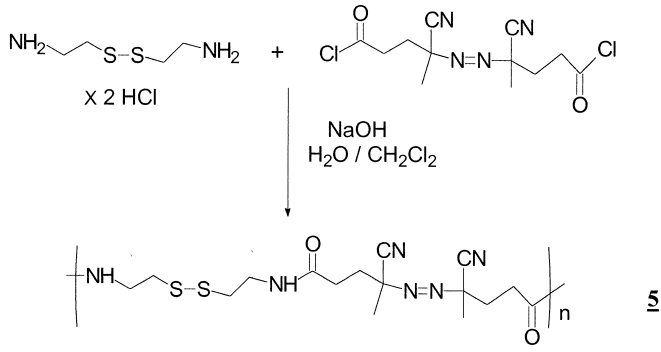


Figure 4. Model of the adsorption of the polymeric disulfide **5** on gold.

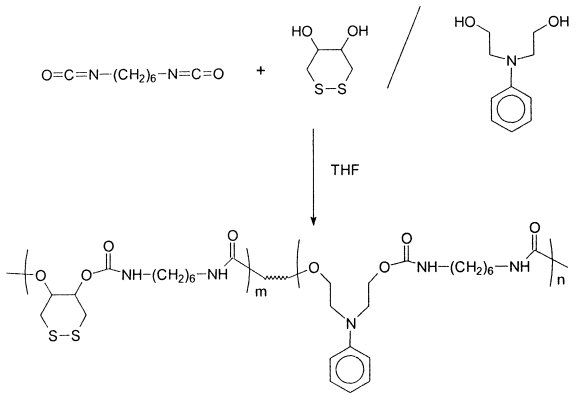
Hence, macroinitiator **5** was prepared by straightforward chemistry as illustrated in scheme 1. The convenient polycondensation of cystamine hydrochloride with the 4,4'-azobis(4-cyanovaleric acid chloride) in a biphasic system led directly to a functionalized macroinitiator bearing disulfide and initiator moieties.



Scheme 1. Synthesis of macroinitiator **5**.

Chemisorption of **5** on gold plates and subsequent photopolymerization in a mixture DMSO / water were realized with monomer **1**. As indicated by contact angle and ellipsometry measurements (Table 1), a hydrophilic film with a thickness of 4 nm was fixed on gold.

Stable polymeric disulfide anchor groups



Scheme 2. Synthesis of the macroinitiator **6**.

A further extension of this approach is the preparation of macroinitiators that chemisorb onto gold through a large number of anchoring points. In this way, we aimed at minimizing the eventual desorption of the initiator (or of the final film) by virtue of multiple anchoring. In this context, the polyurethane photoredox initiator **6** was synthesized (Scheme 2) by copolycondensation of hexamethylene diisocyanate with two kinds of diol: 1,2-dithiane-4,5-diol units for the covalent binding, and N-phenyldiethanolamine units as reactive groups with respect to the photoredox system. With **6**, the anchoring and initiating sites were located on the side chains of the polymer backbone and the compound was not thermally sensitive, such as the azo initiator **4** and **5**. This structure should be rather inert toward hydrolysis during the storage or the use of moderated acid or basic solutions. Moreover, to render the system more easy to handle, and to avoid low initiator efficiency due to the pronounced cage effect, favoring the recombination of the primary radicals, we replaced the azo initiator by a photoredox system.[24] Thus, termination by disproportionation and recombination are avoided, as along the two radical generated in the medium, only the radical derived from **6** is reactive enough to initiate polymerization. The stabilized radical coming from the thioxanthone sensitizer will deactivate otherwise.[24] This photoredox system allows to choose the wavelength in large limits by adapting the sensitizer.

In our experiments, we employed the water-soluble thioxanthone **7** (Fig. 5) that acts by hydrogen atom abstraction in α-position of secondary amines and thioethers under irradiation at 420 nm.[24] In particular, alkylated anilines, as in **6**, are well-suited partners.

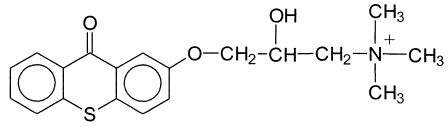


Figure 5. Thioxanthone sensitizer **7**

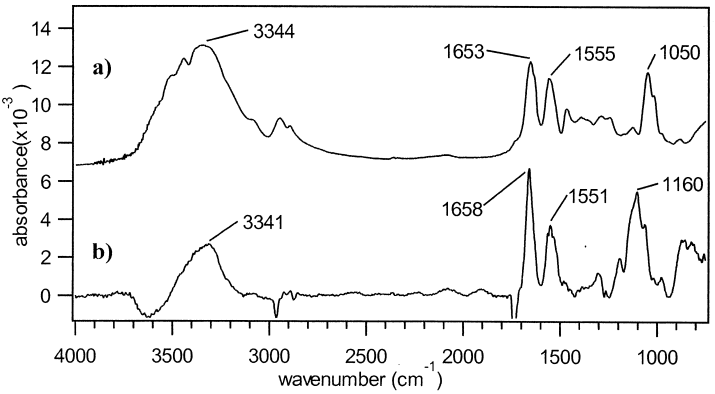


Figure 6. FTIR spectra of a) **poly(1)** initiated with **6**, as KBr pellet, b) gold plates with **poly(1)** grafted-from **6** modified surface.

After the polymerization step, modified gold plates with **6** were coated by stable hydrogels with typical thicknesses of about 10 nm (Table 1). Grazing Incidence Reflexion IR spectroscopy (GIR-FTIR) confirmed the grafting of **poly(1)** on gold. As shown in the spectrum depicted on Figure 6, the photopolymerized plate bearing initiator **6** shows the typical signals for **poly(1)** surfaces, as can be seen from the reference spectrum (top, Figure 6).

Incorporation of linker groups for the fixation of receptor groups

Having examplified different strategies to obtain hydrogel on gold in the first part, we wish to illustrate different ways to obtain functional hydrogels in the following.

Incorporation by post-functionalization

In initial studies, hydrogel of **poly(1)** grafted on gold plates were functionalized by using classical methods employed for polysaccharide hydrogels, such as dextran gels.[25,26] These methods are based on the incorporation of carboxylic groups by reacting the hydroxyl groups of the hydrogel with excess of bromoacetic acid, and to convert it in activated esters using the EDC / NHS systems. SPR measurements using protein A as the linked receptor and anti-BSA as the specific ligand, showed that **poly(1)** hydrogel can be functionalized and that selective and efficient sensing can be achieved.[27] Nevertheless, this method is lengthy and asks for a three-step process. Thus, we looked for more convenient methods for the functionalization of the hydrogel matrixes by compounds of interest bearing amino or thiol groups, employing unsymmetrical reactive linkers that can react in aqueous media. For example, the use of N,N'-disuccinimidyl carbonate (DSC) seemed attractive to us, because one can discriminate between hydroxyl and amine groups in water: only one activated ester is sufficiently reactive to couple to a hydroxyl group, whereas the second NHS ester can couple only with amino or thiol groups.[28,29]

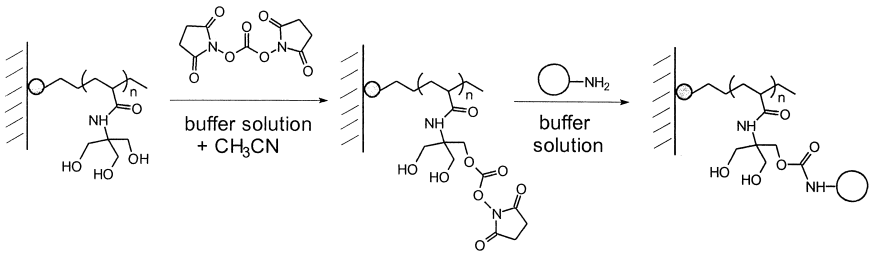
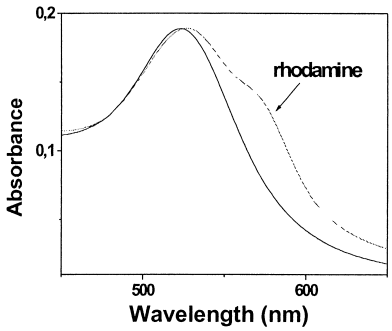


Figure 7. Activation of the hydrogel by DSC and fixation of receptors.

Using a mixture of acetonitrile/water (1/9) at pH 8, activated esters were incorporated directly onto the surface and allowed the introduction of compounds bearing amino groups after a simple rinsing step (Fig. 7).

Such modifications of gold surfaces are not only possible with planar supports, but also with colloids. For instance, gold colloids were labelled by Lissamine™ rhodamine B ethylenediamine **8** using the DSC strategy (Fig. 8), illustrating the large potential of this approach. In this example, **8** acted as a spectroscopic probe. But as specific antibodies of rhodamine are known, Lissamine™ can be used as a receptor molecule for a sensor device, alternatively.



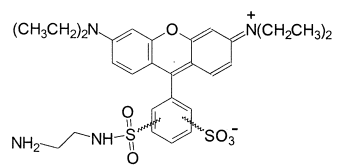


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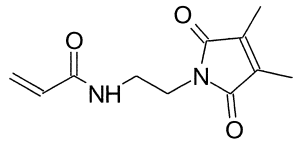
Figure 8. UV-visible spectra of a suspension of gold colloids in H_2O stabilized by **poly(1)** prepared with **4** as initiator before (left curve), and after (right curve) labelling with the functional rhodamine dye Lissamine™

Incorporation by copolymerization

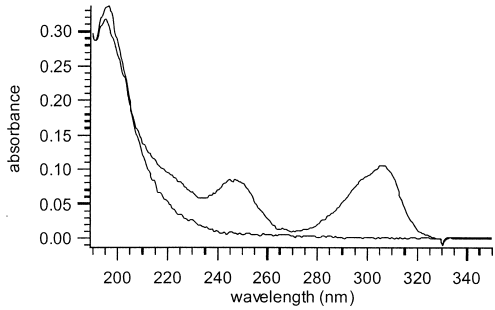
An alternative to the approach presented above consists in the direct copolymerization of monomer **1** with reactive monomers. In this way, the intermediate activation step (cf. Fig. 1b) is short-circuited. However, this strategy requires the compatibility of the activated group with the polymerization process. Again, activated esters are useful, allowing the binding of functional amines, but a number of others possibilities exist. For example, comonomer **2** (Fig. 9) allows not only the coupling of functional amines, but also of functional thiols, such as cysteine tagged proteins or FAB fragments.

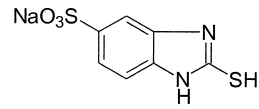


2

Figure 9. Maleimide functionalized monomer **2**

Actually, monomer **2** has been employed as a photocuring agent by [2+2] cycloaddition[30] to prepare gels. In our case, we were interested in the possible Michael-addition of thiol groups on the maleimide double bond. The two methyl substituents on the maleimide are necessary in order to avoid the copolymerization of the maleimide double bond, leading to crosslinking. This approach is illustrate by the binding of 2-mercapto-5-benzimidazolesulfonate **9** onto **poly(1-co-2)** in buffer solution which can be easily followed by UV-visible spectroscopy (Figure 10).





9

Figure 10. UV-visible spectra of aqueous solutions of **poly(1-co-2)** (lower curve), and of **poly(1-co-2)** modified with 2-mercapto-5-benzimidazolesulfonate **9** (upper curve).

Perspectives

In addition to the chemical modification of gold surfaces, we explored the possibility to prepare 'smart' surfaces by grafting thermally sensitive polymers onto gold plates by described strategies.[9,31] Varying the temperature, changes in the organization of the interface of the coating are induced. Above the Lower Critical Solution Temperature

(LCST), collapse of the films might allow for instance the masking of functional or sensitive groups from the aqueous solution, and should improve the stability of the sensing chips for long time storage. Using copolymers of **1** with N-isopropyl acrylamide ("NIPAM") prepared from initiator **4**, the character of the films grafted on gold can be reversibly switched from hydrophilic to hydrophobic by heating above 34°C. [32,33)]

Conclusions

Using disulfide initiator systems, it is possible to obtain stable and functional hydrogel coatings based on the monomer N-[tris(hydroxymethyl)methyl]acrylamide **1** onto gold surfaces, by the grafting-to and grafting-from strategies. Different kinds of "sticky" initiators such as low molar mass disulfides, polymeric disulfide which depolymerize upon binding, and stable polymeric disulfides were prepared and employed to elaborate thin hydrogels on gold surfaces. Two different approaches were developed to functionalize the hydrogel films. The first uses bifunctional coupling agents with differentiated reactivities; e. g. disuccinimidyl carbonate proved to be a versatile reagent to incorporate activated ester groups into the hydrogel film in a single step procedure, when used under appropriate conditions. Equally, copolymerization of reactive monomers with **1** produced hydrogel films to which specific substrates can be anchored without an intermediate activation step.

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