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Photolithographically patternable modified poly(HEMA) hydrogel membrane

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Summary

Polymer with pendant methacryloyl group was synthesized by the substitution reaction of poly(2-hydroxyethyl methacrylate) [poly(HEMA)] with methacryloyl chloride. This photocurable polymer was characterized using ^{1}H-NMR and bromine titration, and the results were reasonably well consistent. The photocrosslinking of this modified poly(HEMA) was carried out by the addition of a low molecular weight sensitizer without adding functional monomer and crosslinker. These precursors showed a high photosensitivity. Polymers with a degree of substitution as low as 2 mol-% methacryloyl group could be efficiently crosslinked within few seconds of UV exposure to give water insoluble transparent hydrogel membranes. By irradiation of a film of the photocurable polymer precursor containing lacatate oxidase (LOD), a rather unstable enzyme, the enzyme was gently immobilized by physical entrapment.

Introduction

Photoinitiated crosslinking of hydrophilic polymers has been attracting current interest for its application to immobilize bioactive materials, such as drugs, enzymes, organelles, and microbial cells, and for the production of hydrogel coatings to increase biocompatibility. The principle most widely used is based on crosslinking via photo-dimerization of photosensitive groups such as stilbazolium or cinnamate groups[1, 2] which are attached to side chains of hydrophilic polymers. However, the long illumination time required for the effective crosslinking of these polymers, makes their application not very attractive.
Despite these polymers, most of the photopatternable hydrogel membranes are based on photopolymerization/crosslinking of monomer plus crosslinker systems which contain polymers only in order to increase the viscosity of the preparation and to alter the physicochemical properties of the formed hydrogel. Such systems have been successfully applied to the microfabrication of chemical sensors[3-7]. Actually, the ease and reliability with which these membranes can be prepared, makes them particularly attractive to develop cheap, small and multifunctional sensors, since they can be applied by a process compatible to semiconductor technology. However, although the illumination time was already reduced to minutes with these systems when exposed on conventional mask aligners used in microfabrication processes, there are still serious drawbacks of these systems. They are not solid but liquid before crosslinking and the evaporation of monomer from the applied thin film can significantly alter precursor composition and membrane performance.

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To overcome these drawbacks, in this work, we developed a modified poly(HEMA) precursor system which efficiently crosslinks within few seconds without the need to incorporate monomer and crosslinker. The modified poly(HEMA) was synthesized by the substitution reaction of poly(HEMA) with methacryloyl chloride in the presence of calcium, and this photocurable hydrophilic polymer with pendant methacryloyl group was quantitatively characterized using ^{1}H-NMR and bromine addition. The optimum conditions for preparing a patterned enzyme membrane and its lithographic performance were described.

Experimental

Materials

HEMA monomer was obtained from Fluka (Switzerland) and was further purified prior to use according to the following procedure [8]. First, the monomer was stirred in the presence of sodium carbonate and then filtered to remove methacrylic acid. The monomer was then dissolved in distilled water (25% solution) and washed with a 1/1 (volume) mixture of cyclohexane and carbon tetrachloride to remove the naturally occurring crosslinker ethylene glycol dimethacrylate. The HEMA was next salted out with sodium chloride. Lactate oxidase ex. aerococcus viridans (LOD) was purchased from Genzyme (UK). All solvents and other reagents were obtained commercially, and were used as received.

Synthesis

A mixture of purified HEMA (10ml) and N-methyl-2-pyrrolidon (NMP) (30ml) was purged with nitrogen at 70°C for 30 minutes, and then 100 mg AIBN dissolved in 1 ml NMP was added. This solution was continuously heated (whilst stirring) at 70°C under nitrogen atmosphere for 2 hours. The resulting poly(HEMA) was isolated by dissolving in methanol and was precipitated in water two times, finally it was precipitated again in diethyl ether two times and dried in vacuum. The yield is 75%.

10 g such prepared poly(HEMA) was dissolved in NMP at 45°C to give a 25% solution. Then 100 mg Calcium and the desired amount of methacryloyl chloride were added. After four hours the reaction mixture was 1.0 μm filtered and precipitated according to the above procedure. The degree of substitution (DS) was determined by bromine addition and by ^{1}H-NMR.

Methods

(1) Bromine titration [9]:

300 mg modified poly(HEMA) was dissolved in 25 ml methanol and 5 ml water, then 5 ml 0.2 N bromine solution (0.8 g Br_2 and 6 g KBr in 50 ml water) was added and the mixture was allowed to react in the dark for 2 hours. 5 ml of 10% KI was added and the iodine formed from unused bromine was titrated with 0.1N sodium thiosulphate solution. A blank experiment without sample was carried out at the same time.

(2) ^{1}H-NMR spectra were obtained on a Bruker 200 FS FT-NMR (200 MHz) spectrometer. All NMR spectra were recorded in CD_3OD without using additional internal standard.

(3) Preparation of polymer membrane by photolithography:

Precursor solution, composed of 20 wt-% modified poly(HEMA) (2.1% methacryloyl content by ^{1}H-NMR), 40 wt-% ethylenglycol, 35 wt-% water, 5 wt-% photoiniator 2,2-dimethoxy-2-phenylacetophenone (IRGACURE 651) and 0.5 wt-% hydrochinon was cast over a polyimide wafer at room temperature in air and spin coated at 4000 RPM for 15 sec. After drying at room temperature for 1 hour, the film was photolithographically

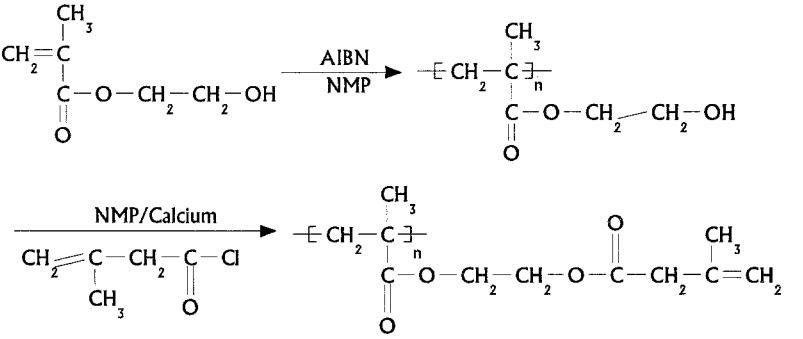
patterned under argon flushing on a mask aligner (Karl Suss MJB3 Standard) equipped with a high pressure Hg lamp. The lithographic mask used absorbs light $\lambda<320$ nm. Light intensity is in the order of 10 mW/cm^2. Exposure times ranging from 1 s to 90 s were used. After irradiation, the film was immersed into ethanol for three minutes in order to remove the unexposed part of the polymer and to develop the image. After rinsing with water the films were blown dry with nitrogen.

(4) Profiles and micrographs of the patterned membranes were obtained using a Sloan DEKTAK 3030ST profilometer and a conventional light microscope.

(5) Immobilization of Lactate oxidase and activity measurement
The desired amount of LOD was dissolved in the membrane precursor. This solution was dropped on to a platinum electrode of 0.8 mm^2 area which was insulated with a 50 μm thick dry film resist. Subsequently a 0.1 mm thick microscopic cover slide was put on the electrode in order to remove excess precursor. In this way a very well defined volume of precursor is applied to the electrode. After exposure to UV light on a mask aligner, the glass cover was removed and the electrode was immersed in a ethylenglycol-water mixture in order to remove non crosslinked material from the membrane.
A rough estimation of enzymatic activity was made by polarizing the electrode at +600 mV vs. Ag/AgCl reference electrode by means of a home made potentiostat. The current arising from the oxidation of hydrogen peroxide, which is produced by the enzyme in the reaction with L-lactate, was recorded. These measurements were done in phosphate buffered saline, which contained 5 mmol/l L-lactate, without stirring.

Results and Discussion

Preparation of modified poly(HEMA) and its characterization
Poly(HEMA) was prepared by free radical solution polymerization. The photocurable methacryloyl-bearing polymers with various degrees of methacryloyl content were prepared by the esterification of poly(HEMA) with methacryloyl chloride, as shown in Scheme 1.



Scheme 1

Figure 1 shows the 200 MHz ^{1}H-NMR spectra of the poly(HEMA) and modified poly(HEMA) (degree of substitution of methacryloyl moiety, 2.1 mol-% by NMR) in CD_3OD. The content of the methacryloyl unit was calculated from the integral ratio between the vinyl protons (δ 6.15 ppm) and ethyl protons (δ=3.5 - 4.5 ppm) of modified poly(HEMA) [Fig. 1(b)]. After the reaction of methacryloyl chloride with poly(HEMA), the ^{1}H-NMR spectrum showed that the peaks ascribed to the vinyl protons (δ 5.7 and 6.15

ppm) appear, whereas the OH- proton (δ 4.8 ppm) was still observed [comparing Fig. 1(a) and Fig. 1(b)] because CD_3OD has to be chosen as solvent taking into consideration of the poor solubility of such modified poly(HEMA) in other solvent like DMSO. The DS was calculated by two alternative methods. One method calculated the value from the integral ratio between these vinyl protons and the unchanging ethyl protons. For validation of these values a chemical titration method was also done.

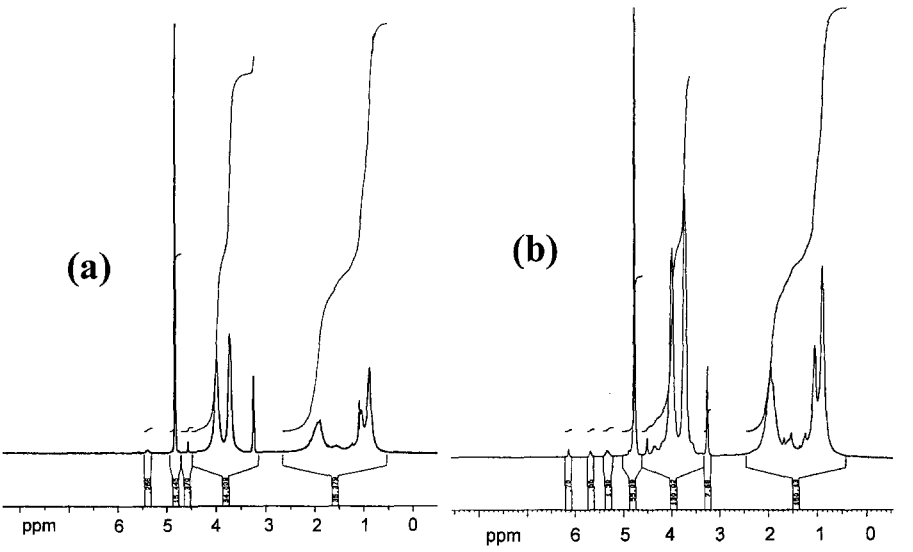


Fig.1. ^{1}H-NMR spectra (200 MHz) of poly(HEMA) (a) and modified poly(HEMA) (b) in CD_3OD.

Table 1 summarizes the preparation and methacryloyl contents of modified poly(HEMA). The DS values determined from ^{1}H-NMR spectra were reasonably consistent with those calculated from titration method. The methacryloyl content greatly effects the photosensitivity of modified poly(HEMA) and its solubility in the polymer precursor solution (an ethyleneglycol/water mixture). The higher the content of methacryloyl in the modified poly(HEMA) is, the higher the photosensitivity (corresponding to a short exposure time, see Fig. 4) and the lower the solubility is. The optimum DS value for obtaining a good photosensitivity and solubility in the polymer precursor is approximately 2 mol-%.

Table 1. Preparation of photocurable modified poly(HEMA)

Methacryloyl chloride (wt-%)	Methacryloyl content (mol-%)		Photosensitivity (t=30 seconds)	Solubility
	Titration	^{1}H-NMR		
5	0.96	1.08	not enough	very good
10	1.87	2.11	enough	good
20	4.78	5.24	high	very poor

Properties of modified poly(HEMA)
The modified polymers with a DS of 1.1 mol-% were first tested with an exposure time from 1 to 90 second to obtain photolithographically-patterned membranes. A patterned membrane could be formed at 30 to 90 seconds, but the membrane obtained often peeled off partially from the surface. To overcome this problem, a higher DS value (2.1 mol-%) was examined and found to be suitable for obtaining a well resoluted and homogeneous patterned membrane at an exposure time from 10 to 60 seconds. Using a light microscope, a typical patterned membrane such as shown in Fig. 2 is obtained.

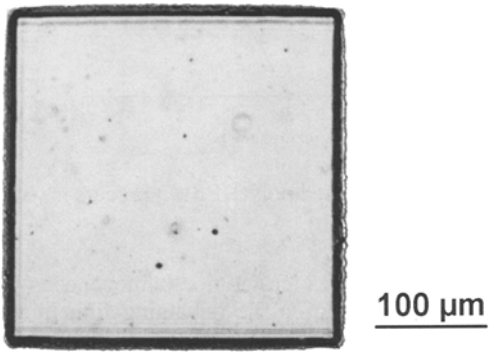


Fig.2. Photo micrograph of a patterned hydrogel membrane.

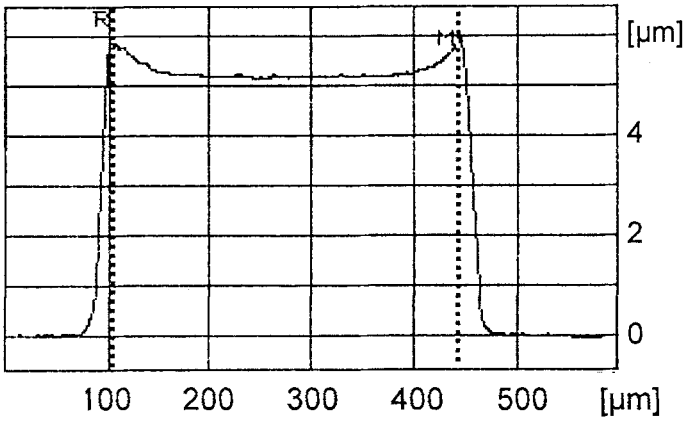


Fig.3. Surface profile of a patterned hydrogel membrane.

This film is uniformly thick expect at the edges where it is considerably thicker. Fig. 3 is a height profile of a membrane illustrating this effect. This improved adhesion is thought to be due to the higher crosslinking density of the membrane given by the chemical crosslinking reaction of vinyl group in polymer. When the membrane was prepared by polymer of 5 mol-% methacryloyl content, even membranes which had not been exposed

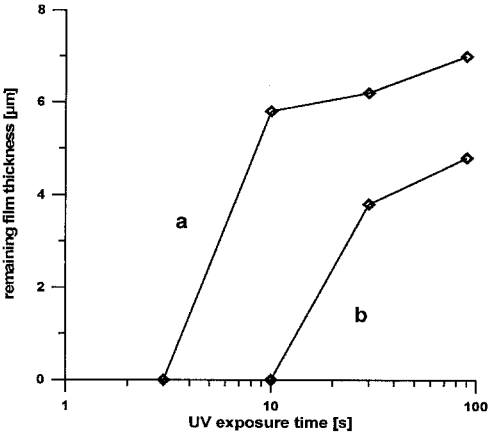


Fig.4. Contrast curve of modified poly(HEMA) precursor with DS value of 2.1 mol-% (a) and 1.1 mol-% (b).

to UV irradiation became insoluble, and a non-homogeneous thin membrane was obtained. The effect of exposure time on the remaining film thickness is presented in Figure 4 for two polymers of different DS. As expected, the polymer with a DS of 2.1 mol-% (a) shows a considerable higher photosensitivity than the polymer with a DS of 1.1 mol-% (b). Of course, the molecular weight of the modified polymer also greatly affects the photosensitivity of such precursors. A commercially available high molecular weight ploy(HEMA) with a DS of 2 mol-% effectively crosslinked at an exposure time of only three seconds.

The enzyme membrane

Lactate oxidase was chosen as the test enzyme since it is well known for its instability. Contrary to glucose oxidase which is an extraordinary stable enzyme and therefore withstands even the roughest immobilization procedures, LOD is rapidly denatured under

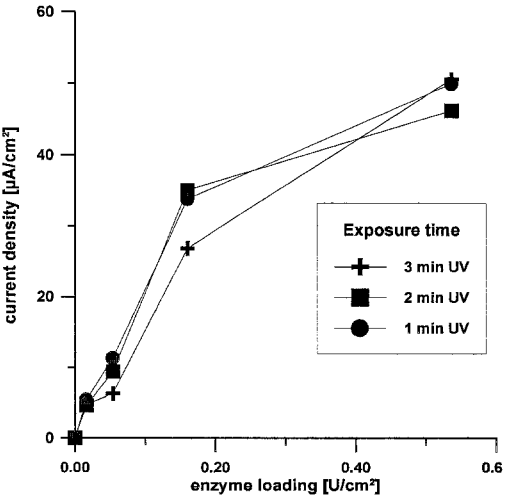


Fig.5. The plot of enzyme loading vs. current density at different exposure times.

harsh conditions. Fig. 5 shows the results of a loading study done with LOD and a precursor containing modified poly(HEMA) with a DS of 2.1 mol-%. In order to study the effect of exposure time on the activity retention of the enzyme, membranes with one, two, and three minutes exposure time were made. One minute is the minimum necessary exposure time to make stable enzyme membranes. For high enzyme loading, the current due to the oxidation of enzymatically produced hydrogen peroxide is running into saturation, since in the enzymatic oxidation of lactate with LOD, molecular oxygen is consumed, whose supply is limited by diffusion. For small levels of enzyme loading it is assumed that the signal is only limited by the enzyme activity itself, in this way giving us the opportunity to calculate the amount of activity retained. First of all, the signals of the membranes made with different exposure time are practically identical. This means that the enzyme denaturation is independent of exposure time. Per definition one unit of LOD produces one μmol hydrogen peroxide per minute. Conversion to current by application of the Faraday constant gives us a theoretical value of 3.3 mA/U for the case of 100 % activity retention. From the initial slopes of the graphs in Fig. 5 we calculate a practical value of 0.6 mA/U. So activity retention for the immobilization of LOD by physical entrapment with such photopatterned hydrogel membranes is at least 20 %. This value is possibly even higher, since as Zhang [10] has recently shown, the effectiveness of hydrogen peroxide oxidation on platinum electrodes can be far below 100 %.

Conclusions

It has been demonstrated that incorporation of very low content of methacryloyl moiety into poly(HEMA) back-bone, gives a modified polymer which can be favorably used for the photolithographic production of hydrogel and enzymatic membranes. The lactate oxidase, a rather unstable enzyme, could be gently immobilized in this photopatterned hydrogel membrane. Since the modified poly(HEMA) membrane is formed in situ on the device by photopolymerization, this technique is compatible with microelectronic technologies and therefore is especially applicable in integrated micro-biosensor fabrication. Studies on glucose micro biosensors using this modified poly(HEMA) hydrogel are now in progress.

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