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Short communication

Biosensors coated with sulfated polysaccharides for the detection of hepatocyte growth factor/scatter factor in cell culture medium

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ABSTRACT

Process control methods for cell culture bioreactors include on-line monitoring of protein concentrations. Bioreactor samples typically contain high amounts of different proteins. The direct detection of a single protein in this complex medium is a challenging task within the development of biosensors with label-free detection. We introduce the development of a mass-sensitive biosensor based on surface acoustic waves (SAW) for the detection of hepatocyte growth factor/scatter factor (HGF/SF) in the serum containing medium of a miniaturized bioreactor for culturing hepatocytes. The specificity of the biosensor was obtained following two approaches. In the first approach, antibodies against HGF (anti-HGF) were immobilized covalently via an intermediate layer of dicarboxy polyethylene glycol on the biosensor surface. In the second approach, dextran sulfate and fucoidan were used as sensor coatings exploiting the fact that HGF binds specifically to those sulfated polysaccharides. Performing HGF assays, similar results were obtained using biosensors coated with dextran sulfate and biosensors coated with anti-HGF. Even higher sensor signals were obtained using biosensors coated with fucoidan, particularly at 37 °C. Therefore, biosensor coatings based on biospecific sulfated polysaccharides offer a simple and cost-saving alternative compared to the commonly used coating with antibodies.

1. Introduction

Bioreactors for cell culture are increasingly investigated as a tool for ex vivo engineering of living tissues. Due to the complexity of growing functional tissues, monitoring of physical, chemical or biological parameters is essential (Martin et al., 2004). Particularly the control of the latter is considered to be beneficial for monitoring cell proliferation and differentiation, e.g., by monitoring concentrations of proteins involved in cell metabolism. A suitable method for the rapid determination of protein concentrations is offered by biosensors working with label-free detection. If these biosensors are operated in a flow through system they can be connected directly to the bioreactor allowing on-line monitoring and control of the cell culture process (Becker et al., 2007).

The detection of proteins with a biosensor is typically obtained by immobilizing the corresponding capture molecules, such as antibodies, on the biosensor surface. To prevent unspecific binding of non-analyte components contained in the sample medium, sensing layers of biosensors based on label-free detection usually require intermediate hydrogel layers, such as dextrans or polyethylene glycols, as they are known to effectively reduce unspecific protein binding on the biosensor surface (Cooper, 2003). Furthermore, a high binding capacity of the sensing layer often is advantageous, as this supports a diffusion-limited (or: mass-transport limited) binding regime of the analyte molecules to the biosensor surface. In a flow through system, this results in linear signal response curves, the slopes of which are proportional to the respective analyte concentrations. Consequently, plotting these slopes against the corresponding concentrations results in a linear calibration curve (Piehler et al., 1997). This calibration line permits the easy determination of further analyte concentrations, provided that these concentrations are within the calibration range and the flow conditions are kept constant. Additionally, a sufficiently high binding capacity allows the subsequent determination of several analyte concentrations on the same sensor without requiring intermediate regeneration steps. This would reduce the risk of process failure due to contamination of the cell culture (Becker et al., 2007).

In this work, a biosensor was to be developed that allows the monitoring of hepatocyte proliferation and differentiation (e.g., hepatoma cell line HepG2) in a miniaturized bioreactor (Altmann et al., 2008) based on the so-called KITChip culture system (Altmann et al., 2009). The biosensor was targeted on monitoring the concentration of hepatocyte growth factor/scatter factor (HGF/SF) in the bioreactor medium. This growth factor features a multitude of mitogenic and morphogenic functions (Trusolino et al., 1998) and hence could be a potential indicator for the current state of the hep-

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atocytes. Biosensors based on surface acoustic waves (SAW) were used. They enable direct and label-free detection of biomolecules in real-time and can be operated in flow-through systems. The binding of analyte molecules on the mass-sensitive SAW biosensor results in a change of the propagation velocity of the SAW and was read out as frequency change (Länge et al., 2008).

To obtain biosensor surfaces specific to HGF, we first used the conventional approach based on immobilized antibodies against HGF (anti-HGF). Another approach used the property of HGF to bind to sulfated polysaccharides, such as heparin, dextran sulfate and fucoidan. HGF is known to provide a heparin binding domain (Trusolino et al., 1998). Furthermore, heparin, dextran sulfate and fucoidan were found to inhibit binding of HGF to sulfoglycolipids (Kobayashi et al., 1994) and hence could be considered to bind to HGF themselves. Binding inhibition via dextran sulfate and fucoidan was reported to be most effective suggesting higher binding affinities compared to heparin, hence, they were chosen as sensor coatings in our study. SAW biosensor measurements were performed to detect HGF in cell culture medium. The results obtained with the novel biosensor coatings based on sulfated polysaccharides were compared to the results obtained with a conventional biosensor coating based on anti-HGF.

2. Material and methods

2.1. SAW device and instrumentation, fluidic setup

A shear horizontal SAW resonator based on a 36°YX-$LiTaO_3$ device (4 mm × 4 mm) with gold transducers and a frequency of operation of 428.5 MHz was used. SAW measurements were performed in an oscillator circuit developed in-house with the SAW resonator as frequency-determining element. Difference frequencies relative to a permanently oscillating reference oscillator, featuring a constant frequency in the range of 434 MHz, were used as signal output. The frequency resolution was 1 Hz. Details of the instrumentation have been described earlier (Länge et al., 2003; Rapp et al., 2000). Experiments were performed using a flow injection analysis (FIA) system equipped with two peristaltic pumps (Ismatec, Wertheim, Germany) and an injection valve (Besta-Technik, Wilhelmsfeld, Germany). PTFE tubes served as connections between single units and as sample loop. The SAW resonator was included by means of a flow cell which was designed as part of an electronic circuit board integrated in the oscillator unit. The SAW device was mounted upside down onto isolated contact pads on the electronic board and coupled capacitively (Bender et al., 2004a). The milled flow channel in between the contact pads allowed the fluid to pass along the SAW path. The dimensions of the flow channel at the sensor position were 0.6 mm (height) × 0.8 mm (width) × 4 mm (length) leading to a volume of 1.92 μl.

2.2. Preparation of the biosensor surfaces

Unless otherwise noted, solvents were obtained from Merck, Germany, and other chemicals and biochemicals from Sigma–Aldrich, Germany.

2.2.1. Parylene coating and activation

All SAW devices were coated with 0.1 μm parylene C (poly(2-chloro-p-xylylene)) using a commercial parylene deposition system (Labcoter 1, PDS 2010; Speedline Technologies, IN, USA) (Bender et al., 2004b). The parylene surfaces were activated by oxidation via plasma treatment using a commercial plasma cleaner (PDC-32G, Harrick Plasma, NY, USA) and subsequent silanization with (3-glycidyloxypropyl)trimethoxysilane (Länge et al., 2007).

2.2.2. Coating with dicarboxy polyethylene glycol and anti-HGF

Activated parylene surfaces were treated overnight with 5 μl of an aqueous solution of dicarboxy polyethylene glycol (DC-PEG), M_r 2000 (Rapp-Polymere, Germany), c = 2 mg/ml, rinsed thoroughly with bidistilled water and dried. Anti-HGF (monoclonal, anti-human, clone 24612.111) was immobilized on DC-PEG coated surfaces using the FIA system described above. Phosphate buffered saline (PBS), pH 7.4, containing 10 mM phosphate buffer, 137 mM NaCl and 2.7 mM KCl, was used as carrier stream. The flow rate was set to 0.03 ml/min. Solutions were loaded in the sample loop, then injected in the carrier stream via the injection valve and transported to the sensor. Injection intervals and rinsing times were set to 8 min each. First the surface was activated with a solution of 0.05 M N-hydroxysuccinimide and 0.2 M N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) in bidistilled water and rinsed with PBS. Then the surface was incubated with a solution of 1.5 μM anti-HGF in acetate buffer, pH 5, and rinsed with PBS. Next the surface was flushed with a solution of 1 M ethanolamine, pH 8.5, and rinsed with PBS. After that the sensor was immediately used for the HGF assay.

2.2.3. Coating with sulfated polysaccharide

Activated parylene surfaces were treated overnight with 5 μl of an aqueous solution of sulfated polysaccharide, c = 50 mg/ml, rinsed thoroughly with bidistilled water and dried. Sulfated polysaccharides were dextran sulfate, M_r 100,000, and fucoidan, M_r 100,000–150,000. Sensors coated with sulfated polysaccharide could be stored at +4 °C, they were used for HGF detection within 1 month.

2.3. SAW biosensor measurements: HGF assay

HGF (human, recombinant) assays were performed using the FIA system described above. Cell culture medium composed of Eagle's Minimum Essential Medium (LGC Standards, Germany) containing 10% foetal calf serum (LGC Standards, Germany) and 1% penicillin/streptomycin (PAA Laboratories, Germany) was used as carrier stream. The flow rate was set to 0.05 ml/min and kept constant during the measurements. The sensor surface was pretreated by rinsing with carrier stream for 1 h. After this blocking step, five samples with increasing concentrations of HGF dissolved in cell culture medium, c = 0 (0.126, 0.25, 0.5, 1) μg/ml, were applied subsequently. Samples first were loaded in the sample loop, then injected in the carrier stream via the injection valve and transported to the sensor. The injection interval was set to 60–300 s. After each sample injection, the sensor was rinsed in the carrier stream for 5 min. Measurements were generally performed at room temperature, for measurements at T = 37 °C the complete experimental setup was put into an incubator.

3. Results and discussion

Sulfated polysaccharides were investigated as biosensor coatings for the detection of HGF in cell culture medium. HGF assays were performed using SAW biosensors coated with dextran sulfate and fucoidan, respectively. The results were compared to HGF assays performed with biosensors coated with the corresponding antibody, anti-HGF.

Fig. 1 shows typical signal responses obtained with a HGF assay using a SAW biosensor coated with dextran sulfate. Five samples containing increasing concentrations of HGF dissolved in cell culture medium were injected subsequently in the cell culture medium carrier stream. The signal response curves did not decrease after the injection interval, which means that the binding of HGF to the dextran sulfate is irreversible. Still, and despite the additional fact that no regeneration steps were performed in between

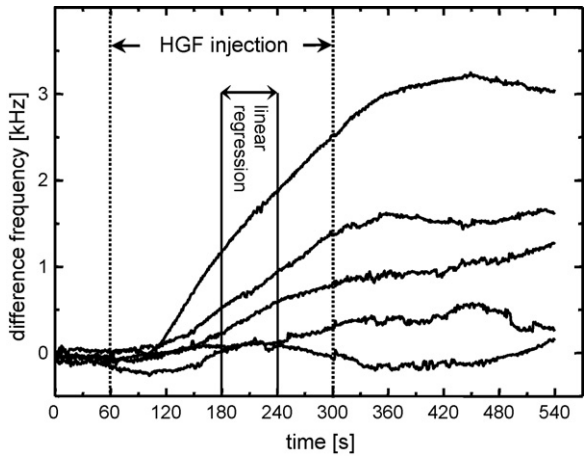

Fig. 1. Signal response curves obtained with a HGF assay using a SAW biosensor coated with dextran sulfate. Five samples containing HGF dissolved in cell culture medium, *c*(HGF) from bottom to top: 0 (0.126, 0.25, 0.5, 1) μg/ml, were injected subsequently in a cell culture medium carrier stream. Injection interval: 60–300 s. Linear regression range for calibration curve (details see text): 180–240 s.

the HGF sample injections, linear binding curves were obtained up to the last sample with the highest concentration, *c*(HGF) = 1 μg/ml. This implies a high binding capacity of the sensing layer promoting a diffusion-limited binding regime of HGF to the sensor surface (Piehler et al., 1997). The behaviour of the SAW signal responses using biosensors coated with fucoidan and anti-HGF, respectively, was similar, i.e., diffusion-limited binding of HGF could be observed as well. Therefore, calibration lines could be determined by plotting the slopes of the linear part of the signal response curves against the respective concentrations.

Fig. 2 shows calibration lines obtained from HGF assays using the conventional biosensor coating based on anti-HGF and using dextran sulfate coated biosensors, respectively. The slopes of the calibration lines were (10.5 ± 0.8) (Hz/s)/(μg/ml) for the biosensors coated with anti-HGF and (8.0 ± 1.1) (Hz/s)/(μg/ml) for the biosensors coated with dextran sulfate, i.e., the sensitivity achieved with the latter was slightly lower. On the other hand, a significantly higher sensitivity could be obtained using fucoidan coated biosensors as shown in Fig. 3. In this case the slope of the calibration line was (15.7 ± 1.0) (Hz/s)/(μg/ml).

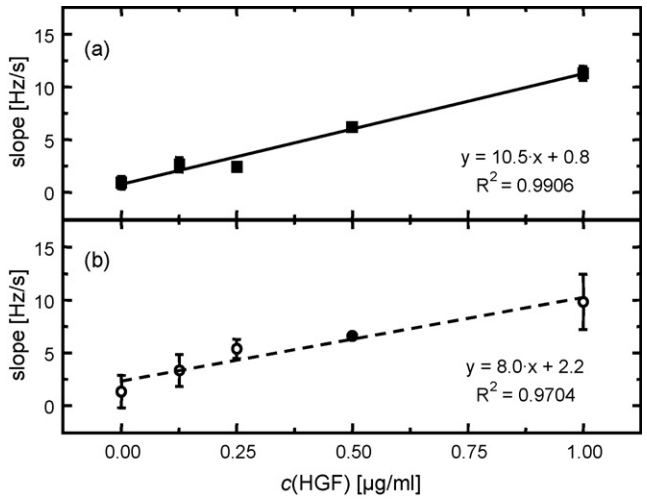

Fig. 2. Calibration lines obtained with HGF assays using SAW biosensors coated with (a) anti-HGF and (b) dextran sulfate. Slopes were calculated from the SAW signal response curves via linear regression in the range 180–240 s (compare Fig. 1). For each sensor coating, each HGF concentration was measured once in a series of measurement. Three series of measurement were taken using a separate sensor for each. Symbols represent the means, error bars represent the standard deviations.

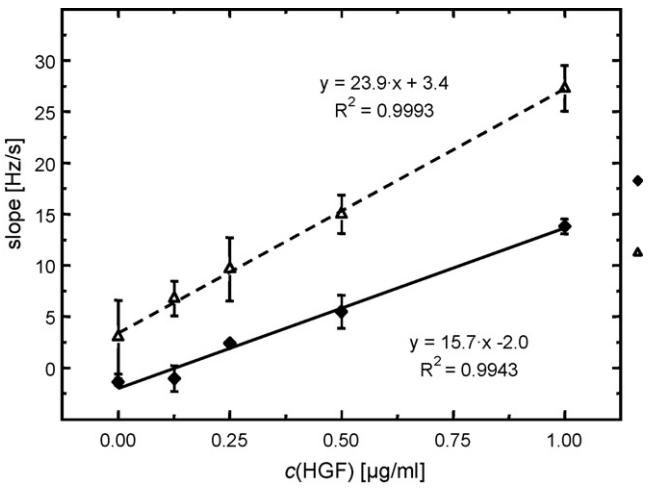

Fig. 3. Calibration lines obtained with HGF assays using SAW biosensors coated with fucoidan. Slopes were calculated from the SAW signal response curves via linear regression in the range 180–240 s (compare Fig. 1). Experiments were performed at room temperature (rhombic symbols) and at *T* = 37 °C (triangular symbols). In each experiment, each HGF concentration was measured once in a series of measurements. Three series of measurement were taken using a separate sensor for each. Symbols represent the means, error bars represent the standard deviations.

For each HGF concentration the respective mass loading due to HGF binding could be considered to be the same for all coatings, because a diffusion-limited binding regime was observed in all HGF binding intervals, and flow conditions were kept constant. Therefore, the reason for the different sensitivities obtained with different sensor coatings could be traced back to the fact that it is not only mass loading but also change of viscoelastic properties of the sensing layer during analyte binding which influences the signal response of a SAW biosensor. This effect mostly depends on the morphology of the sensing layer and is difficult to predict (Länge and Rapp, 2008, 2009).

In case of biosensors coated with anti-HGF via an intermediate DC-PEG layer the effect of viscoelasticity change during analyte binding is minimized owing to the two-dimensional (2D) scaffold of DC-PEG. This means that functional groups for antibody coupling, including subsequent analyte binding, occur on top of the sensing layer only. In contrast to that, in case of biosensors coated with dextran sulfate and fucoidan, respectively, both providing a three-dimensional (3D) scaffold, analyte binding may occur within the sensing layer leading to a distinct change in the effect of viscoelasticity. It has been shown before that this viscoelasticity change may add both positively or negatively to the effect of mass loading, i.e., leading to increase or decrease in the SAW signal response. In our experiments both effects could be observed. Using dextran sulfate, up to a concentration of 0.5 μg/ml HGF the signal response curves, implying the respective slopes in the linear range, were slightly higher than those obtained with the 2D sensing layer based on DC-PEG and anti-HGF. On the other hand, using a concentration of 1 μg/ml HGF, the SAW signal responses were slightly smaller. In consequence, the slope of the calibration curve obtained with dextran sulfate was slightly lower than that obtained with DC-PEG and anti-HGF. Using fucoidan, the opposite effect was observed. The highest signal response curves were obtained using the highest concentration of HGF, 1 μg/ml. Using smaller concentrations of HGF, the signal responses were slightly lower than that obtained with the 2D sensing layer based on DC-PEG and anti-HGF. For the lowest concentrations of HGF, 0 μg/ml and 0.126 μg/ml, even negative slopes of the signal response curves were obtained. We think

that this can be traced back to morphology changes of the fucoidan layer due to the sample matrix. Still, the resulting calibration curve is linear and its slope is significantly higher than that obtained with DC-PEG and anti-HGF, i.e., the morphology of this sensing layer is better suited for HGF detection.

The motivation of this work was the development of a HGF biosensor suitable for application as an on-line monitoring system in the medium of a bioreactor for culturing hepatocytes. This includes HGF detection at the operating temperature of the bioreactor, i.e., $T = 37\,^{\circ}C$. Therefore, HGF assays were performed at 37 °C using the sulfated polysaccharide as biosensor coating which was the most promising sensor coating at room temperature: fucoidan. Again, a diffusion-limited binding regime could be observed. The resulting calibration line is shown in Fig. 3, the slope was (23.9 ± 0.5) (Hz/s)/(μg/ml), i.e., it was significantly higher than the slope obtained at room temperature.

As the diffusion coefficient of HGF was expected to be increased by the enhanced assay temperature (Cussler, 1997) leading to increased slopes of the SAW signal response curves, the higher slope of the calibration line at 37 °C was in line with our expectations. A potential effect of the enhanced assay temperature on the morphology of the fucoidan layer could not be distinguished from its prevailing effect on the diffusion coefficient of HGF. However, it is safe to say that the higher temperature did not affect the fucoidan sensing layer in a way that the positive effect resulting from the higher diffusion coefficient was counteracted completely.

The linearity of all calibration curves also implies the specificity of the biosensor coatings, because additional components in the cell culture medium binding on the biosensor surface would interfere with the sensor response leading to a non-linear behaviour of those calibration curves. Therefore, fucoidan based sensing layers can be seen as cost-saving alternative to sensing layers based on antibodies against HGF.

4. Conclusion

We investigated the use of the sulfated polysaccharides dextran sulfate and fucoidan as SAW biosensor coatings for the detection of HGF in the medium of a cell culture bioreactor. The results obtained with biosensors coated with sulfated polysaccharides were compared to those obtained with conventionally coated biosensors using an antibody against HGF. It could be shown that fucoidan coated biosensors feature the highest sensitivity to HGF, hence offering a simple and cost-saving alternative to the use of antibodies as biorecognition element for HGF detection. The fucoidan coating can also be applied at $T = 37\,^{\circ}C$, the typical temperature of a mammalian cell culture setup. The high binding capacity of the fucoidan coated biosensors allows the determination of several HGF concentrations subsequently, i.e., without the need of intermediate regeneration steps or changing the sensor. This reduces potential interferences with the cell culture process. Furthermore, the measurement setup provides the biosensor in a flow cell allowing connection to other fluidic systems as well as continuous flow measurements. This makes the fucoidan coated SAW biosensors particularly suitable for monitoring purposes of a bioreactor for culturing hepatocytes.

References

Altmann, B., Giselbrecht, S., Weibezahn, K.F., Welle, A., Gottwald, E., 2008. Biomed. Mater. 3, Article No. 034120.
Altmann, B., Welle, A., Giselbrecht, S., Truckenmüller, R., Gottwald, E., 2009. World J. Stem Cells 31, 43–48.
Becker, T., Hitzmann, B., Muffler, K., Pörtner, R., Reardon, K.E., Stahl, F., Ulber, R., 2007. Adv. Biochem. Eng. Biotechnol. 105, 249–293.
Bender, F., Länge, K., Voigt, A., Rapp, M., 2004a. Anal. Chem. 76, 3837–3840.
Bender, F., Länge, K., Barié, N., Kondoh, J., Rapp, M., 2004b. Langmuir 20, 2315–2319.
Cooper, M.A., 2003. Anal. Bioanal. Chem. 377, 834–842.
Cussler, E.L., 1997. Diffusion: Mass Transfer in Fluid Systems, second ed. Cambridge University Press, Cambridge.
Kobayashi, T., Honke, K., Miyazaki, T., Matsumoto, K., Nakamura, T., Ishizuka, I., Makita, A., 1994. J. Biol. Chem. 269, 9817–9821.
Länge, K., Rapp, M., 2008. Anal. Biochem. 377, 170–175.
Länge, K., Rapp, M., 2009. Sens. Actuators B: Chem. 142, 39–43.
Länge, K., Bender, F., Voigt, A., Gao, H., Rapp, M., 2003. Anal. Chem. 75, 5561–5566.
Länge, K., Grimm, S., Rapp, M., 2007. Sens. Actuators B: Chem. 125, 441–446.
Länge, K., Rapp, B.E., Rapp, M., 2008. Anal. Bioanal. Chem. 391, 1509–1519.
Martin, I., Wendt, D., Heberer, M., 2004. Trends Biotechnol. 22, 80–86.
Piehler, J., Brecht, A., Giersch, T., Hock, B., Gauglitz, G., 1997. J. Immunol. Methods 201, 189–206.
Rapp, M., Reibel, J., Voigt, A., Balzer, M., Bülow, O., 2000. Sens. Actuators B: Chem. 65, 169–172.
Trusolino, L., Pugliese, L., Comoglio, P.M., 1998. FASEB J. 12, 1267–1280.