alkanethiols of appropriate lengths form densely packed hydrophilic layers tilted at about 30° to the surface [10].

Speci f c HSA binding capacity

To assess the speciŽcity of the HSA detection a reference surface was prepared too, o x ine, by immobilizing HSA-speciŽc as well as BrdU-speciŽc antibodies by inverse micro-contact printing in the 17-channel connection plate [5], using 5 µL of the antibody solutions and the amine coupling kit according to the manufacturer's instructions. HXe

Immobilization of the antibodies

Another requirement for the mixed SAM is stable immobilization of an appropriate antibody (via covalent bonding to the displayed COOH groups of the anchoring thiols) to enable continuous speciŽc monitoring of HSA levels during cultivations. To compare the antibody immobilization capacity of the SAMs with each alkanethiol ratio we determined amounts of the HSA-speciŽc antibody immobilized from a 1.4 µM solution (Figure 2).

Figure 2: Immobilization levels (via amine coupling) of HSA-speciŽc antibody from a 1.4 µM solution to SAMs with indicated alkanethiol ratios (means from 1280 monitored spots across a 9×0.8 mm measuring area; error bars indicate corresponding standard deviations). Chips 1 and 2 refer to results from two independent experiments.

HXe immobilization level of the antibody increased with increases in the anchoring thiol content up to 50 %, at which the surface seems to be saturated. Previous analysts have even detected lower antibody immobilization levels when using a SAM consisting solely of anchoring thiol than when using one with 10 % anchoring thiol [21], probably because a SAM with a single chain length hinders access to the activated carboxyl groups. Accordingly, in our experiments the diwerent alkanethiol chain lengths of the mixed SAM appeared to enhance accessibility of the anchor group. HXe observed immobilization levels were also within the range recorded for dense antibody monolayers, giving 1300 - 6500 RU signals [26]. It should be noted that high antibody concentrations are desirable for concentration measurements [27], but too dense packing can hinder access to the antigen binding sites [28].

Speci f c HSA binding capacity

To identify the optimal composition of the SAM, the target binding capacity must also be considered. HXerefore, the speciŽc binding of HSA was determined by injecting HepaRG medium spiked with 1.5 µM HSA (Figure 3). To ensure that positive results were not false-positives due to interfering sample components we also measured HSA binding to a reference surface with immobilized bromodeoxyuridine (BrdU)-speciŽc antibody. BrdU is a synthetic analog of thymidine used for labeling nucleic acids. HXus, it has no relation to HSA or any other sample components.

Figure 3: Binding levels of HSA from a 0 µM and a 1.5 µM solution to antibodies immobilized on SAMs with indicated alkanethiol ratios (means from two independent experiments, with four channels per experiment; error bars indicate corresponding deviations). Signals obtained with the reference (BrdU-speciŽc) antibody were subtracted from those obtained with the HSA-speciŽc antibody.

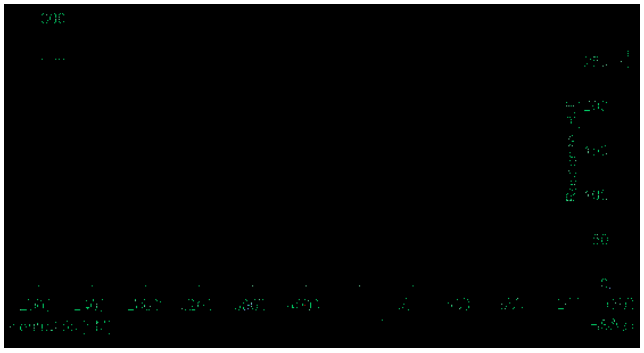

Figure 4: Concentration series obtained from sequential injections of HSA in HepaRG medium at concentrations, from bottom to top, of 0.8, 1.5, 3.0, 6.0, 12.0, 24.1, 48.1, 96.2, 192.5 and 385.0 nM (means from six channels; error bars indicate corresponding standard deviations; signals obtained using the reference BrdU-speciẐc antibody were subtracted from those obtained using the HSA-speciẐc antibody).

As shown in Figure 4, a typical saturation curve was recorded, with a limit of detection (LOD) of the proposed assay of 59 RU (mean signal from a pure HepaRG media injection plus three times the standard deviation of measurements from six channels). HŸus, by using the 70 % anchor thiol SAM and the proposed SPR assay it is possible to detect as little as 0.8 nM HSA speciẐcally in cell culture media containing up to 10 % fetal calf serum.

HŸis assay was used to generate a calibration curve, similar to the SPR-generated curve, using samples of HSA spiked in HepaRG medium at concentrations ranging from 0.1 to 7.5 nM. In both cases, to determine unknown HSA concentrations in cell culture media the log-linear ranges below the saturation levels of the curves should be used, as illustrated in Figure 5.

HŸe regression equation and correlation coeẌcient obtained from the ELISA were Δabsorption = 0.2167 × ln(HSA) + 0.3329 and 0.9912, respectively, for HSA concentrations ranging from 0.4 to 7.5 nM. HŸe regression equation and correlation coeẌcient obtained using the SPR assay were ΔRU = 38.65 × ln[HSA] + 75.539 and 0.9923, respectively, for concentrations ranging from 0.8 to 96.2 nM HSA.

Minimal HSA concentrations in media when hepatocytes purely and together with skin cells cultivated in the multi-organ-chip system mentioned above reportedly range from ca. 3.3 to 170 nM [16]. HŸis means, cell culture samples containing HSA concentrations above 96.2 nM (e.g. from pure hepatocyte cultures or advanced cultures) have to be diluted at least 1 to 30 for the SPR assay in a deẐned manner. HŸis is necessary due to the saturation of binding signals above 96.2 nM.

However, unlike the SPR assay the ELISA relies on enzyme-based signal enhancement, the additional recognition steps increase costs and operation times, and of course a labeled counterpart of the target is needed. Furthermore, ELISAs consume time and samples, while in marked contrast the ^{li}SPR instrument aẇords high-sensitivity with minimal sample requirements and real-time detection.

Conclusion

Previously published methods for functionalizing SPR chips cannot be applied to ^{li}SPR chips as they have no adhesive layer to stabilize the

medium of the multi-organ chip. HXis work was Ẑnancially supported by the European Union and the Free State of Saxony (SAB project UNILOC).

References

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