

Silane-dextran chemistry on polymer chips for point of care diagnostics

Christina Jönsson^{1,2}, Magnus Aronsson¹, Gerd Rundström¹, Christer Pettersson¹,

Ib Mendel-Hartwig¹, Jimmy Bakker^{1,2}, Brian MacCraith², Ove Öhman¹, Jonas Melin^{1,2}

¹ Åmic AB, Uppsala, Sweden. ² Biomedical Diagnostics Institute, DCU, Dublin, Ireland.

INTRODUCTION: High performance point-of-care (POC) diagnostic devices, could substantially improve the prognosis for patients suffering from cardio vascular disease (CVD) by enabling early detection and treatment. Disposable plastic test chips, with integrated fluid handling and assay components, provide a platform for realizing such devices. The native hydrophobic nature of plastic surfaces does however require surface modifications to allow for proper fluid distribution and immobilization of affinity ligands.

We have developed a surface chemistry for cycloolefin polymer (COP) surfaces based on direct silanization of an oxidized plastic surface. The resultant amino terminated surface has further been functionalised with a surface-enlarging dextran matrix and used in an immunoassay for the inflammatory marker C-reactive protein (CRP).

METHODS: 4castchips were injection molded by Åmic AB (Uppsala, Sweden) in COP (Zeonor 1020R) and oxidized in oxygen plasma. The chips were immersed in a solution of 3% (3-Aminopropyl)Triethoxysilane (APTES) in 95:5 ethanol:water for 2h¹. The surface concentration of reactive amino groups was measured by confining an ~1 cm² area of the chip surface with a silicone frame. The cup defined by the chip surface and silicon walls was filled by 100 µl Alexa Fluor 647 succinimidyl ester dye (10 µg/ml, in 100 mM NaHCO₃) and incubated for 1 h. After extensive washing and sonication in 0.1% SDS and milliq-H₂O the signal was read out in a microarray scanner. APTES covered surfaces were immersed in 2% oxidized dextran solution for 2h and, rinsed in milliq-H₂O and further oxidized in 30 mM NaIO₄ for 2h. Capture Ab (αCRP, Mxxx,...) were spotted across the fluidic channel of the chip. CRP assays were carried out by sequential addition of: 15 µl of CRP-depleted serum spiked with a known concentration of CRP, 5 µl Alexa 647 labeled detection Ab (αCRP, Mxxx,...) in serum and finally wash with 15 µl CRP-depleted serum. The signal intensities were recorded in a prototype lineilluminating fluorescence reader.

RESULTS & DISCUSSION: Upon oxidation OH-groups are formed on the COP surface which enables silanization. Reactive amino groups were found on APTES treated chips only (fig. 1). The

fluorescence intensity corresponds to a surface concentration of approximately 10¹³ amino-groups/cm², which is comparable to what has been shown for glass². Immobilization of dextran to the amine surface resulted in a low contact angle (~30°), which allowed for easy fluid distribution, and provided a high capacity matrix for antibody immobilization.



Fig. 1: Reactive amino groups on APTES treated (left) chip and negative control (right).

CRP was assayed with a limit of detection of 2 ng/ml and a dynamic range of 10² (fig. 2). The relative precision was about 15%, calculated from triplicate experiments.

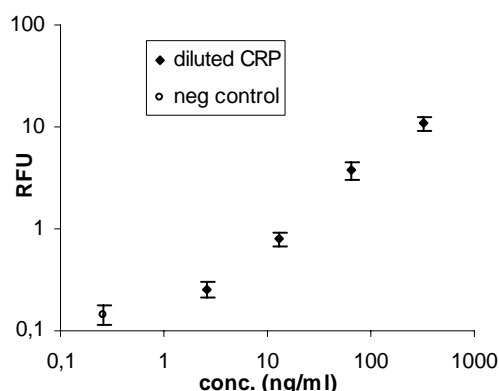


Fig. 2: CRP assay. Error bars indicate ±1SD.

CONCLUSIONS: We have demonstrated a straightforward and capable protocol for surface modifications of COP surfaces. The covalently formed amino layer functions as an anchor for covalent coupling of dextran and antibodies.

REFERENCES: ¹ Miksa, D. et al. (2006), *Biomacromolecules* **7**:557-564. ² Xing, Y., Borguet, E. *Langmuir* (2007) **23**:684-688.

ACKNOWLEDGEMENTS: This work was supported by Marie-Curie Fellowships and the Swedish Governmental Agency for Innovation Systems.