Electrochemical Biomarker Detection in Common Metabolic Disorders

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Introduction

- Metabolic control is of utmost importance for a number of common metabolic diseases, such as diabetes mellitus (DM) and insulin resistance.

Parameters like fasting glucose in blood are sometimes difficult to obtain (children, local structures) and might be only a short-cut in overall disease monitoring, whereas HbA1c tests might be too wide-meshed.

Using a new electrochemical biomarker approach via urinary molecules, might be a potential diagnostic and monitoring tool for diseases such as DM or other metabolic disorders.

- To create an intermediate non-invasive surrogate biomarker for various applications, urinary proteins have to be investigated outside the actual laboratory- and hospital-bound methods and might involve patients at their homes.

A number of potential biomarkers using a stable device for various measurements in patients e.g. at risk for diabetic nephropathy might be established via this technology in future.

Methods

- The method of detection is the use of gold nanoparticles (AuNP) on an gold working electrode. This AuNPs are linked with EDC/NHS (see below) which are bound to antibodies.

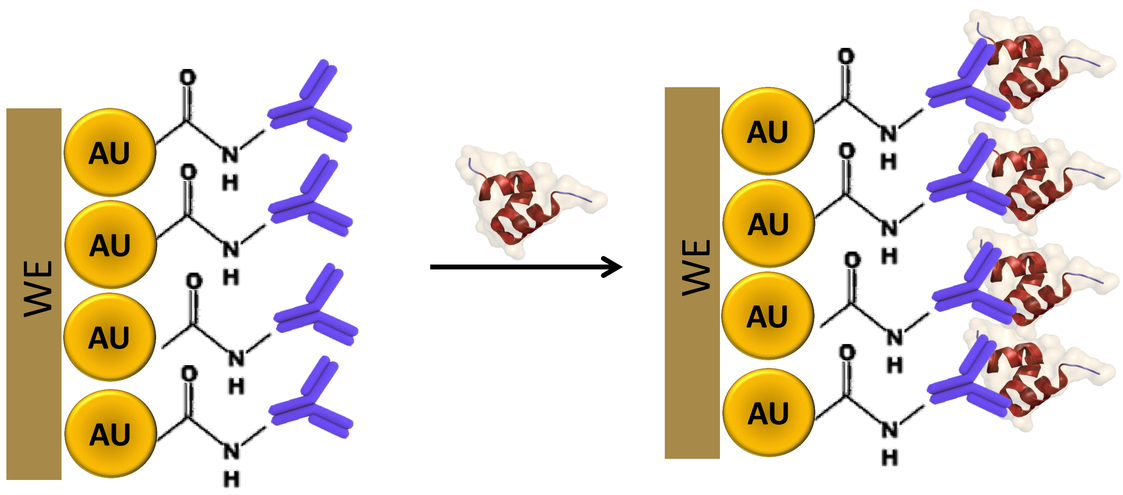

Fig.1: After linking the antibodies to the AuNP using N-Ethylcarbodiimide and N-Hydroxysulfosuccinimide (EDC/NHS), the samples are ready to be measured. The binding of the protein to the antibody on the working electrode (WE) decreases the current, as measured by differential pulse voltammetry.

Results

- Cleaning of the surface of the gold electrode using harsh reagents including HNO_3, the material of the sensor and gold thickness of the electrode needed technical adjustments.

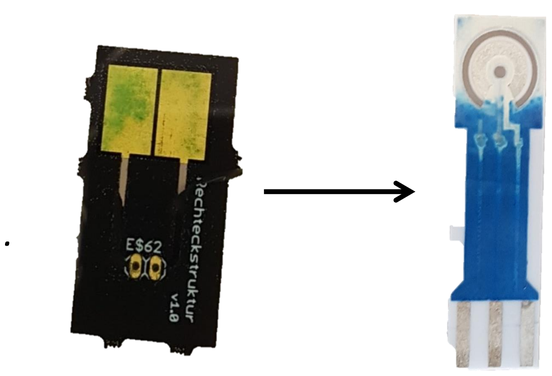

Fig.2: First version of the sensor (left) with a gold surface turning green and a destroyed isolation layer. This happened due to the oxidation of the included copper layer. Therefore we changed to a gold electrode printed on a ceramide surface (right) during development of the protocol

- We developed a list of potential biomarkers which should be used in a multiplex approach on the sensor for clinical predictions from urine. For the validation of these biomarkers, a cohort of >300 new patients was recruited for the project.
- The Graz BioPersMed cohort database >1000 volunteers with deep clinical phenotyping was adapted and cleared (Fig.3).

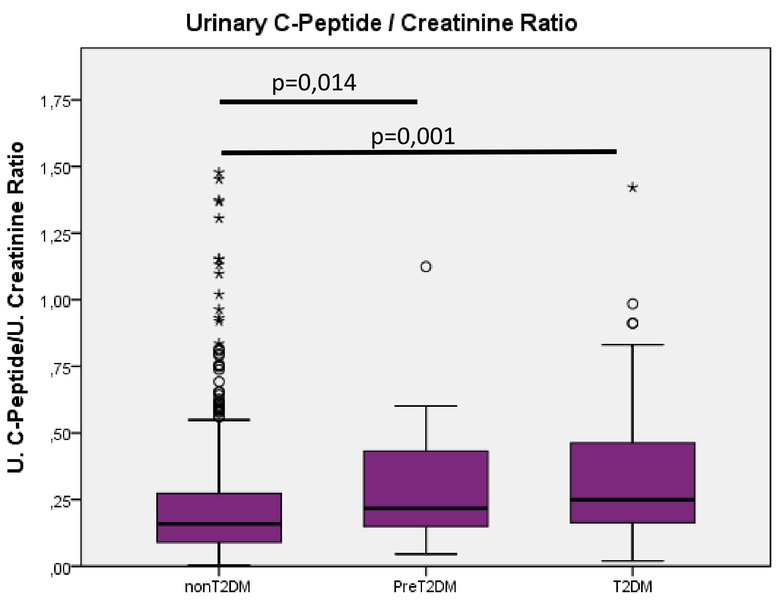

Fig.3: Using this cohort, we found that the urinary C-peptide/creatinine ratios are increased very early in T2DM - not only in T2DM patients but also in pre-T2DM compared to healthy patients.

Conclusions

- Although the electrochemical detection of proteins in urine is still challenging, we did already successful steps in the development process.

- Changing the method from antibodies on gold surface to a more structured approach using AuNPs in combination with a linker allows for a decrease of the LOD down to low pg/mL values as well as a high reproducibility of the analyte. (Khashayar P. et al. Biotech 2017)

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1. Khashayar P. et al B

funded by:

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