**Introduction**

Current biomarkers for acute kidney injury (AKI) and diabetic nephropathy (DN) have limited ability to classify disease and stratify patients with respect to disease progression. Using RT-qPCR-based methods, we have identified distinct urinary microRNA (miRNA) expression profiles specific for AKI and DN. To optimise tests to identify these profiles in the point-of-care environment, we describe here the development of electrochemistry-based methods to detect urinary miRNAs.

**Methods**

Firstly we generated a miRNA biosensor by electrochemical deposition of a naphthalene sulfonic acid derivative onto a glassy carbon electrode (GCE). This process was then used to generate sets of disposable screen printed carbon electrodes (SPCEs) designed to detect miRNAs in the point-of-care environment. Following modification of the sulfonic acid group to a sulfonyl chloride, a single-stranded amino-labelled DNA probe complementary to the target miRNA was then covalently bound to the base electrode surface. Sensor miRNA detection was tested using ferri/ferrocyanide based electrochemical measurements including chronocoulometry and electrochemical impedance spectroscopy.

**Results**

Our GCE miR-21 probe detected synthetic miR-21 in buffered solutions to a sensitivity of 20 fM (2 × 10−14 M). This probe was selective when compared to probe sequences with 1 (37% residual signal), 2 (23%) and 3 (14%) nucleotide changes. Optimised urinary miRNA detection using this probe compared favourably with RT-qPCR detection. Adapting this methodology for single SPCEs, we detected miR-21 to sensitivities of 715 fM (2 mm diameter SPCEs) and 1.8 fM (3 mm). We then designed a sensor with 3 x 3 mm electrode surfaces for multiplex miRNA detection, and carried out initial testing using this SPCE to generate target urinary miRNA data in triplicate. Comparing DN patient and control urine samples (n = 5), our novel triplex electrode successfully detected significant differences for miR-126 (p = 0.006) and miR-192 (p = 0.034) established previously using RT-qPCR.

**Conclusion**

We have modified GCEs and SPCEs for direct detection of urinary miRNAs. Our triplex SPCE identified significant differences in urinary miR-126 and miR-192 detection between DN patients and control subjects. Ongoing studies are focused on optimising multiplex urinary miRNA detection in preparation for use at point-of-care / adoption into existing care pathways.