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Commentary on: JPHYSIOL/2013/264069 Quantification of human urinary exosomes by nanoparticle tracking analysis

Title: Nanotechnology tracks to the renal ward

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The phospholipid bilayer is a highly dynamic structure. Approximately 2% is recycled every 5 to 10 minutes, so the whole membrane is recycled every one to two hours. The process involves constant formation of endosomes that bud off by endocytosis from the plasma membrane and become internalised into the cytoplasm. These endosomes, together with their associated intra-membranous proteins, represent a snapshot of that cell's plasma membrane composition. Further rounds of endocytosis within the endosomes themselves generate intracellular multivesicular bodies. Upon fusing with the plasma membrane, these endosomes release their contents into the circulation (they were first identified in the maturing mammalian reticulocyte) or, in the case of renal tubular epithelial cells, into the urine. The resultant urinary 'exosome' may be characterised by their size (generally between 20-100 nm) and density (1.10-1.19 g/ml). They are representative of the plasma membrane from which they originated and therefore offer a potential window into the pathophysiology of the kidney, providing information about changes in membrane or cytosolic composition from specific segments of the nephron.

Chronic kidney disease (CKD) is highly prevalent and is expected to increase further in the next 5-10 years because of the rising prevalence of obesity and diabetes. Acute kidney injury (AKI), the loss of kidney function over hours to days, is also very common, being seen in up to 20% of acute hospital admissions. There are often delays in detection of both AKI and CKD, which can lead to worse clinical outcomes. The use of urinary biomarkers is key to the early detection of kidney disease (and also to some systemic conditions that might lead to changes in renal epithelial composition). Urine is an excellent fluid for biomarker discovery and development, having sufficient quantities of measurable peptides and/or proteins and being relatively easy to non-invasively obtain in reasonable quantities (assuming the patient is not oligouric). Hence, some urinary biomarkers such as albumin:creatinine ratio (ACR) are already used in routine clinical practice. Detection of increased ACR might, for example, be the first sign of diabetic nephropathy. AKI is currently defined by an increase in serum creatinine or a fall in urine output, but these changes can occur relatively late with respect to the renal injury, potentially leading to delays in treatment. Urinary biomarkers such as neutrophil gelatinase associated lipocalin (NGAL) and kidney injury molecule 1 (KIM-1) have shown promise as novel early biomarkers of AKI. Biomarker discovery and development is an active field of basic and clinical research. More detailed examination of the urinary proteome, including analysis of exosomes, creates further opportunity for discovery of clinically useful early biomarkers of disease. Putative exosomal biomarkers of AKI have been reported, such as the Na⁺/H⁺ exchanger isoform 3 (NHE3) (du Cheyron *et al.*, 2003) and Fetuin-A (Zhou *et al.*, 2006) but with improved technology for exosome analysis, more sensitive and specific biomarkers might be discovered.

To date, the difficulty with determining and quantifying urinary exosomes has been their lability, small size and particular density. Accurate and reproducible identification has been labour intensive, expensive and has required specific laboratory equipment and skills (for example, Western blotting). A new study published in the current issue of the Journal suggests a novel approach that may change this situation. Using nanoparticle tracking analysis (NTA) Oosthuyzen *et al* successfully identified a range of particle sizes in urine, including those classified typically as exosomes. They validated the technique by fluorescently tagging known exosomal proteins such as CD24 (a cell surface marker) and aquaporin 2 (AQP2) and co-localising their fluorescent read-out in the range of particle sizes typically defining exosomes (20-100nm). They prospectively identified an increase in the output of urinary exosomes tagged with AQP2 under known stimulatory conditions

(treatment with the arginine vasopressin analogue, desmopressin). The authors conducted their studies in a cell line, then in an animal model and finally in 5 healthy volunteers and a patient with central diabetes insipidus treated with desmopressin. The authors also established optimal conditions for urine storage for potential use in biomarker discovery studies using NTA. So what exactly is NTA?

NTA was invented in the UK by Dr Bob Carr who subsequently founded Nanosight Ltd (<http://www.nanosight.com/>) in 2003. NTA is used to observe (in conjunction with a high-powered microscope) and analyse (using specialised software) particle movement within a solution. The rate of movement of these particles (Brownian motion) is determined by a number of factors including, particle size, viscosity and temperature of the liquid but is not affected by particle density or refractive index. Thus, using NTA, a size distribution profile of small (10-1000 nm) particles in solution (e.g. urine) can be produced with minimal sample preparation and hence time associated with the procedure. With further development, refinements and validation it is possible in time that the analysis may be done in real-time with no preparation. However, given the complexity of the equipment required, a simple point of care ('bedside') test would appear to be some time off. Nevertheless, by elucidating optimal handling of samples for NTA analysis of urinary exosomes, the authors have contributed to bringing this exciting technique a step closer to routine clinical use. No doubt researchers in acute and chronic kidney disease, interested in identification of disease biomarkers, will take note.

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