



# Raman spectral signature of urinary extracellular vesicles as a diagnostic biomarker for diabetic kidney disease

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## Introduction

Diabetic Kidney Disease (DKD) is a leading cause of chronic kidney disease. This complication of diabetes develops in about 10% of type 2 diabetes mellitus (T2DM) patients. Urine is a rich reservoir of extracellular vesicles (EVs) which are released by glomerular epithelial cells present in the urinary track. The alterations in the molecular content of EVs make them promising biomarkers for DKD.

## Study group

T2DM patients (n=24) with different stage of DKD and healthy subjects (n=7) were enrolled to the study. Patients were classified into 5 groups according to the eGFR level (ml/min/1.73cm<sup>2</sup>) calculated from CKD-EPI formula.

**G1** - > 90

**G2** - 60 – 89

**G3** - 30 – 59

**G4** - 15 – 29

**G5** - < 15

## Methods

UEVs were isolated using low vacuum filtration method (LVF) followed by ultracentrifugation (150 000 x g). Raman spectra of UEVs were analyzed using chemometric methods such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA).

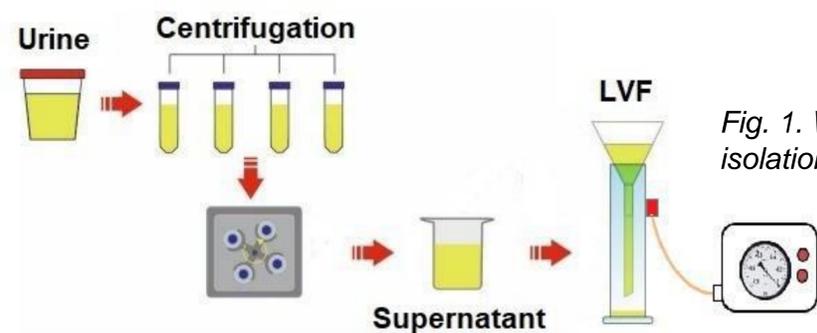


Fig. 1. Workflow of UEVs isolation procedure.

## Results

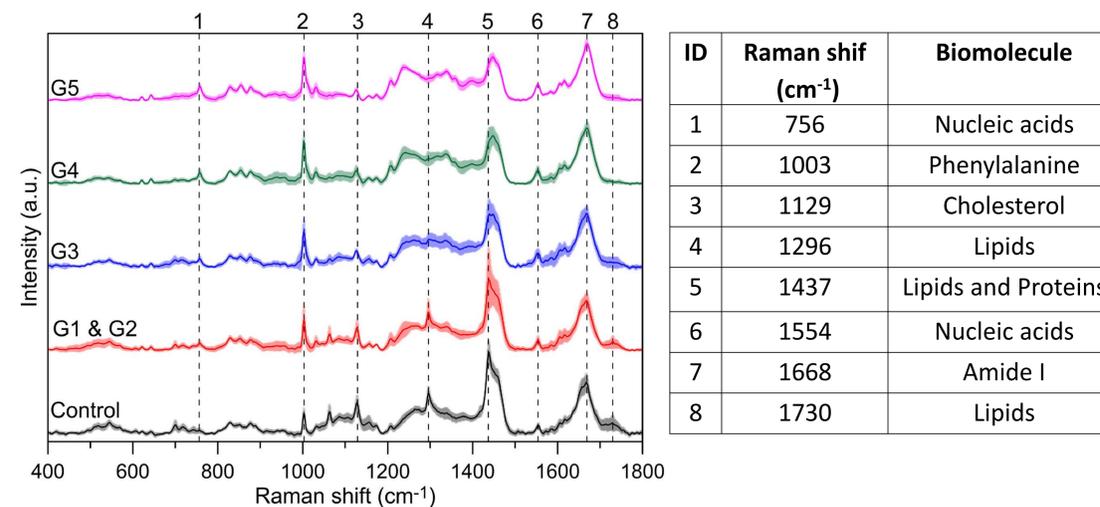


Fig. 2. Average Raman spectra of UEVs of diabetic patients with different stage of DKD.

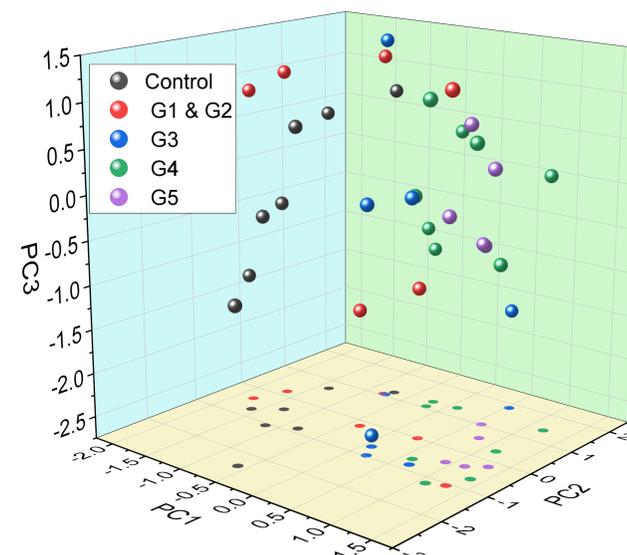


Fig. 3. PCA analysis of Raman spectra recorded from UEVs showed as 3D-score plot of the first three components.

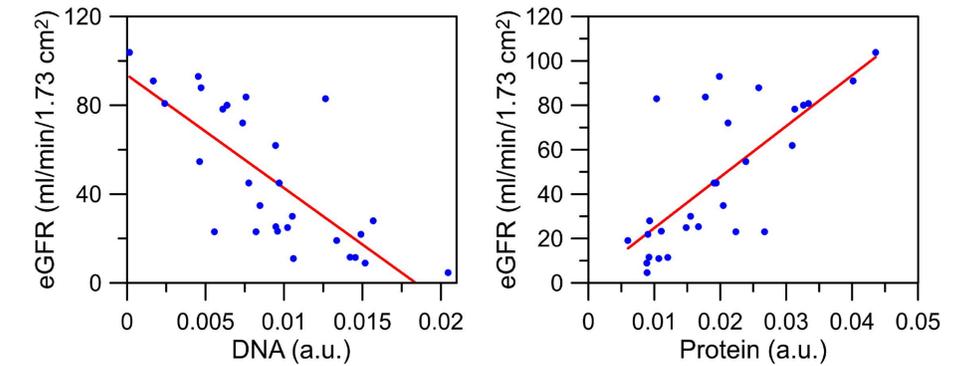


Fig. 4. Correlations between eGFR factor and area under DNA and protein bands.

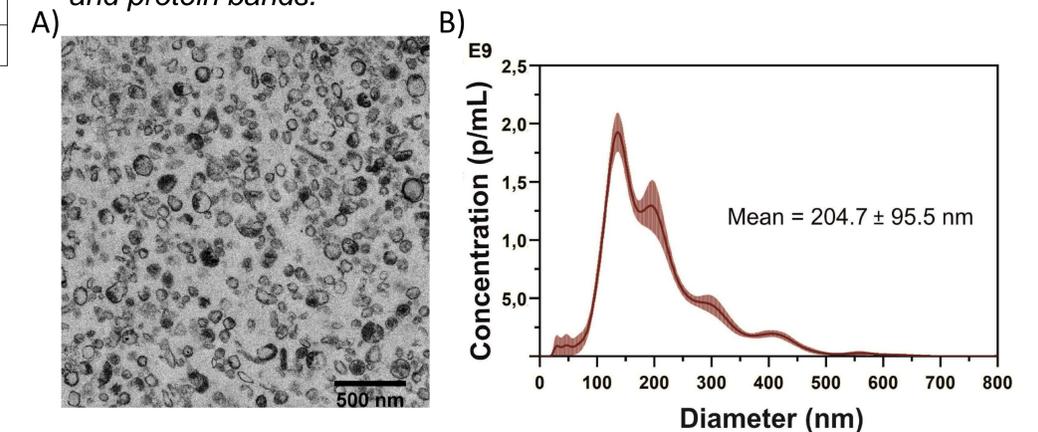


Fig. 5. TEM image of UEVs from control sample (A) and size distribution of UEVs obtained by NTA method (B).

## Conclusions

- Characteristic Raman spectra of UEVs are promising candidates for new, noninvasive biomarkers for DKD
- Raman spectra of UEVs can be used to differentiate DKD patients with different stage of kidney damage.

## Funding

This work was supported by the National Centre for Research and Development (Grant No. LIDER/9/0031/L-9/NCBR/2018)