Isolation, characterization and preservation of extracellular vesicles in human first-void urine samples to facilitate self-collection

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Abstract

Introduction

Urinary extracellular vesicles (EVs) are the potential source of biomarkers to detect urogenital tract diseases, systemic neurological disorders, and cancer types. Despite significant advances, the issue of EV preservation has yet to be extensively studied. This is of particular concern for at-home self-collection, and multi-site urinary sample collections are challenging for large-scale recruitment, leading to variability in the time between collection and processing. In this study, we developed and evaluated a novel non-lytic formulation (UAS™) to preserve EVs to enable self-collection of urine samples.

Methods

First-void urine (FVU) samples were collected from healthy male and female donors using Colli-Pee® FV-5040 devices to generate male-pooled and female-pooled urine samples, respectively. Firstly, we compared a commercial EV extraction kit and an in-house ultrafiltration (UF) method using Western blot analysis for CD9 and TSG101 proteins. In one of the studies, EVs extracted from male-pooled urine sample using the UF method were characterized for size and concentration using nanoparticle tracking analysis and Western blot analysis (CD9 and TSG101 proteins). The extracted EVs were spiked into female-pooled urine sample (which lacked endogenous exosomal markers) with and without UAS preservative. Samples were then held at room temperature (RT) for up to 14 days and analyzed for EV content. In another study, FVU samples from healthy male donors were collected in Colli-Pee devices pre-filled with UAS preservative (Colli-Pee UAS), held at RT for up to 14 days, and analyzed for EV proteins (CD9 and TSG101) and RNA (GAPDH, KLK3) content.

Results

EVs prepared using the UF method showed efficient detection of CD9 and TSG101 proteins, while the commercial extraction kit failed to do so. Male FVU samples showed consistent and higher recovery of EV protein markers relative to female FVU samples. Unpreserved spiked urine samples showed reduced detection of EV protein markers when held at RT for up to 14 days, unlike samples containing UAS preservative, which showed improved detection under similar storage conditions. EV proteins and RNA content were found to be preserved in the urine samples collected in Colli-Pee UAS and stored at RT for up to 14 days.

Summary

Our studies demonstrate that there are gender-specific and extraction method-based differences in the expression and detection of EV markers, highlighting their heterogeneity in urine samples. Our results underscore the need for urine preservation and demonstrate UAS-based preservation of urinary EVs and their cargo, which could enable at-home self-collection solutions.

Results

Detection of exosome-specific markers seems to be gender, sample and extraction method dependent.

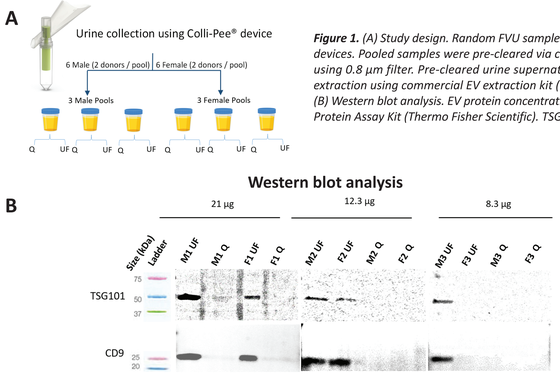

Figure 1. *(A) Study design. Random FVU samples were collected using neat Colli-Pee devices. Pooled samples were pre-cleared via centrifugation followed by filtration using 0.8 µm filter. Pre-cleared urine supernatants were stored at -80˚C until extraction using commercial EV extraction kit (Q) and ultrafiltration (UF) method. (B) Western blot analysis. EV protein concentration was determined using Micro BCA™ Protein Assay Kit (Thermo Fisher Scientific). TSG101 MW ~50 kDa; CD9 MW ~25 kDa.*

Unpreserved FVU sample showed loss of spiked EV protein markers, when held at RT for up to 14 days, unlike samples containing UAS preservative which showed improved detection.

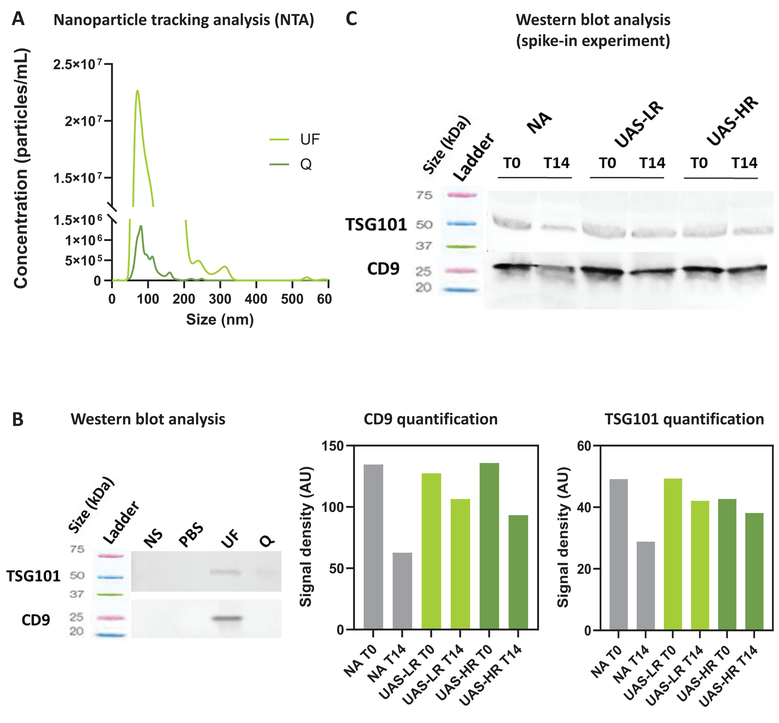

Figure 2. *Characterization of EVs isolated from male-pooled FVU sample using UF method and their comparison with commercially available urinary EVs (Q). (A) For nanoparticle tracking analysis (NTA), EV samples were labeled with ExoGlow™-NTA Fluorescent Labeling Kit (System Biosciences) for tracking and visualization. (B) Western blot analysis. Isolated (UF) and commercial (Q) EVs were spiked in female-pooled FVU sample (lacking endogenous signal for CD9 and TSG101). NS stands for non-spiked sample. PBS stands for FVU sample spiked with 1x phosphate-buffered saline alone. (C) Western blot analysis of spiked EVs in unpreserved (NA) and UAS preservative containing urine samples. Lower ratio (LR) represents FVU:UAS ratio of 1:0.2. Higher ratio (HR) represents FVU:UAS ratio of 1:0.43. Bands were imaged with Syngene, G:BOX F3 gel doc system and quantified by densitometry analysis using Image-J software National Institutes of Health (NIH), Bethesda, Maryland, USA.*

Colli-Pee UAS device preserves exosomal protein markers and RNA cargo in FVU samples held at RT for up to 14 days.

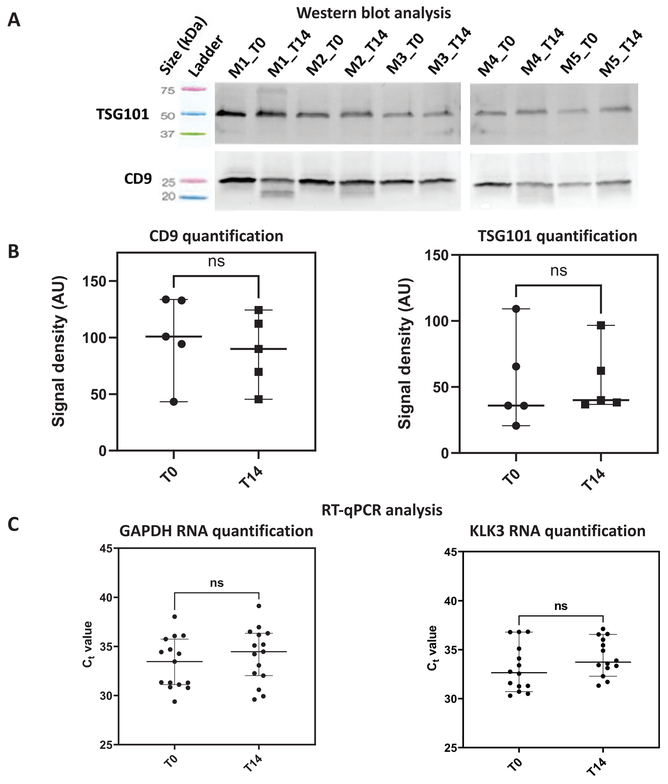

Figure 3. *(A) Representative Western blot data on EVs isolated from male FVU samples collected in the Colli-Pee UAS devices. T0 and T14 stand for day 0 and day 14 time points, respectively. (B) Western blot band quantification by densitometry analysis using Image-J software. (C) RT-qPCR analysis on EV RNA extracted from male FVU samples (n = 15) using commercially available TaqMan™ assays (Thermo Fisher Scientific) for GAPDH (Cat. No. Hs00266705_g1), KLK3 (Cat. No. Hs02576345_m1) RNA. Isolated EVs were subjected to RNase treatment prior to RNA extraction. Statistical analysis was performed using paired t-test utilizing GraphPad Prism 9.2 Software. NS represents non-significant p-values of 0.39 (CD9 protein), 0.79 (TSG101 protein), 0.23 (GAPDH RNA) and 0.10 (KLK3 RNA). Horizontal bars represent median with 95% confidence interval (CI).*

Conclusions

- We propose a workflow for urinary EV collection, preservation, extraction and characterization.
- FVU samples collected in Colli-Pee UAS devices showed preservation of EV proteins, EV RNA cargo and reduced EV degradation over time.
- Our results highlight the need for urine preservation and demonstrate Colli-Pee UAS-based preservation of urinary EVs, which could enable at-home self-collection solutions.

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