Refining urine collection in mice: development of an innovative urine collection device.

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ABSTRACT

Urine collection can be challenging in studies involving small rodents like mice, as the actual methods of collection are anxiogenic and constrain animal welfare while having high variability in the volume of urine collected. To improve the current methods and eventually reduce the impact on mice's well-being, we developed an innovative 3D-printed urine collection device (UCD). This two-compartment UCD is shaped to fit in classical husbandry cages and allows urine collection by spontaneous urination from two mice housed in their own cage without cross-contamination while enabling potential social interactions. We used our UCD to study the evolution of urinary parameters related to renal functions in a model of antibody-mediated chronic kidney disease (AMCKD). Overall, we report here a time-saving and affordable method for urine collection providing a large amount of uncontaminated urine and which we believe may improve animal welfare in comparison to other methods.

Introduction

Studying mouse models of kidney diseases requires urine sample collection to accurately measure the evolution of the disease. However, ensuring the collection of a sufficient volume of uncontaminated urine with ease and at low cost, while maintaining the animal welfare, remains tedious.

Metabolic cages (MCs) allow the recording of multiple parameters, and ensure the collection of uncontaminated urine from mice(1). However, MCs require the isolation of mice for long periods on a small floor area, which does not meet European regulations(2). Housing in MCs leads to high and prolonged stress levels(3), which can be reduced by grouped housing(4). In addition, MCs are expensive and require a suitable rack in the animal facility.

Recently, more affordable methods were described, such as the use of hydrophobic sands (5) or stimulation of natural micturition. These methods require physical restraint or complete isolation of an animal and volumes of urine that are collected vary greatly. All these methods have variable efficiencies regarding the quality and quantity of urine collected and affect the well-being of the animal. To respect the principle of the 3Rs and particularly to refine urine collection, we developed an affordable, easy-to-use, and innovative UCD using 3D printing, also adaptable in aluminum.

Results

Our 3D-printed UCD contains two compartments, fits to the husbandry cages from Allentown (NexGen Mouse 500) or Techniplast (Green line GM500) and maintains the airflow in the cage through its perforated walls, when using ventilated racks (Figure 1A). The UCD permits urine collection from two

separated mice without any cross-contamination (Figure 1B-D; files for 3D printing available in Supplemental materials). Each compartment can receive a disposable, sterile, 96-well plate (polystyrene) fitting at the bottom of the UCD constituting the floor and spanning across 1/3 of the cage (140 cm^2/mouse; Figure 1A-D).

All animal housing and procedures followed the guidelines of the Directive 2010/63/EU of the European Parliament, and were reviewed and approved by our local ethical committee (Comité d'Ethique Paris Nord 121; APAFIS n°17819). To assess the efficacy of our device, we used adult 129S6/SvEvTac mice (10-11 weeks old) from both sexes, bred in-house. Mice are housed in standard cages in ventilated racks with bedding (litter and cottons), enrichments (cardboard roll) and free access to food and water. For urine collection, mice are placed in the UCD installed in their cage, overnight (<12 hours). The time of the day and duration for collection have to be adjusted to the species, strain and sex of the animals. In the UCD, animals have a free access to food/water as a hydrated gel in a cup (5 g/collection period; DietGel® 76A Clear H2O). At the end of the procedure, urine is easily collected, only from uncontaminated wells of the 96-well plates using a pipette, from each compartment. Next, the plates are discarded, the UCD is removed from the cage and completely disinfected with 70% ethanol, and mice are placed back in the cage with free access to food, water, bedding, and nesting.

Initially, our 3D-printed UCD (Figure 1B-C) was made out of a temperature- and pressure-sensitive polylactic acid and could not be autoclaved. Based on the blueprints of the 3D-printed UCD, we manufactured an easy to sterilize (autoclave) and affordable (75 €/device and long-lasting lifespan) aluminum-based UCD (Figure 1E). Both 3D-printed and aluminum-based UCDs are loaded with 96-well plates and showed similar efficiency for urine collection. The next experiments were conducted with aluminum-based UCDs.

To reduce anxiety, mice were placed in the UCD overnight, every day for a week, to get accustomed to the device and to the food (Figure 2A). During this "habituation period", uncontaminated urine samples were collected to measure baseline parameters (Figure 2B). Mice lost about 4% of their weight after the first night ($p<0.05$, One-way ANOVA, Prism software) in the UCD, most likely due to the change in the diet and to a slight environment-induced anxiety. However, weights remained steady on the following days (Figure 2C). The habituation period is essential, and we believe that the prolonged use of this device does not overstress mice. Using our UCD, we collected 50 to 700 µl of uncontaminated urine, per mouse, per collection period.

To validate this method of urine collection, we used the UCD to analyze renal functions in a model of AMCKD induced by the injection of nephrotoxic serum (NTS) (Probetex, reference PTX-001S-Ms)(6). Mice were monitored daily for 10 days following the intravenous injection of NTS (50 µl, day 0) and euthanized at the end of the experiment (Figure 2A). Mice were not subjected to other interventions. The collected urine samples allowed the efficient measurement of the urinary albumin/creatinine ratio, of the excretion of urea and of the urinary ionogram during AMCKD (Figure 2D-F) using an automated biochemical analyzer, Respons®920 (Diasys) (6) and its reagents (references: 102429910921; 117599910920; 131019910920; 136009910921; 960430). Results from all the animals are presented (no exclusion).

Conclusion

To conclude, our UCD (3D-printed and aluminum-based) is very efficient and easily allows urine collection without invasive intervention on mice. The UCD keeps mice in their own visual and olfactive environment, and possibly maintains social interactions. The physical isolation in each compartment guarantees the quality of the samples without cross-contamination. These advantages and the adaptability of this device to any lab, cage (shape, material), and protocols, are what sets this device apart from the other methods of urine collection.

However, the UCD also has some limitations encountered with other methods of urine collection. To avoid urine contamination by food, mice face a new diet (jellified) that requires a period of habituation. Additionally, this diet can impact urinary parameters, so using the same diet for all the experimental groups is essential. Moreover, the UCD is not a metabolic cage and it does not: 1-allow refrigerated urine collection. 2-determine precisely the volume of urine produced. 3-monitor the precise food/water intake over a period of collection. Finally, Van Loo et al.(7) suggested that “living apart together” can also affect mice well-being, and this aspect of the UCD will require further studies.

As it is challenging to maintain the balance between animal welfare and collection of urine, this innovative UCD represents an affordable and good alternative to efficiently collect large amount of uncontaminated urine suitable for the biochemical analysis of renal functions, while potentially creating a less stressful and a refined environment for the animals.

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