THE JOURNAL OF
SEXUAL MEDICINE

ORIGINAL RESEARCH

BASIC SCIENCE

Novel Application of Micro-Computerized Tomography for Morphologic Characterization of the Murine Penis

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ABSTRACT

Background: The murine penis model has enriched our understanding of anomalous penile development. The morphologic characterization of the murine penis using conventional serial sectioning methods is labor intensive and prone to errors.

Aim: To develop a novel application of micro-computerized tomography (micro-CT) with iodine staining for rapid, non-destructive morphologic study of murine penis structure.

Methods: Penises were dissected from 10 adult wild-type mice and imaged using micro-CT with iodine staining. Images were acquired at 5-μm spatial resolution on a Bruker SkyScan 1272 micro-CT system. After images were acquired, the specimens were washed of any remaining iodine and embedded in paraffin for conventional histologic examination. Histologic and micro-CT measurements for all specimens were made by 2 independent observers.

Outcomes: Measurements of penile structures were made on virtual micro-CT sections and histologic slides.

Results: The Lin concordance correlation coefficient demonstrated almost perfect strength of agreement for interobserver variability for histologic section (0.9995, 95% CI = 0.9990−0.9997) and micro-CT section (0.9982, 95% CI = 0.9963−0.9991) measurements. Bland-Altman analysis for agreement between the 2 modalities of measurement demonstrated mean differences of −0.029, 0.022, and −0.068 mm for male urogenital mating protuberance, baculum, and penile glans length, respectively. There did not appear to be a bias for overestimation or underestimation of measured lengths and limits of agreement were narrow.

Clinical Translation: The enhanced ability offered by micro-CT to phenotype the murine penis has the potential to improve translational studies examining the molecular pathways contributing to anomalous penile development.

Strengths and Limitations: The present study describes the first reported use of micro-CT with iodine staining for imaging the murine penis. Producing repeated histologic sections of identical orientation was limited by inherent imperfections in mounting and tissue sectioning, but this was compensated for by using micro-CT reconstructions to identify matching virtual sections.

Conclusion: This study demonstrates the successful use of micro-CT with iodine staining, which has the potential for submicron spatial resolution, as a non-destructive method of characterizing murine penile morphology.
O'Neill M, Huang GO, Lamb DJ. Novel Application of Micro-Computerized Tomography for Morphologic Characterization of the Murine Penis. J Sex Med 2017;14:1533−1539.

Key Words: Micro-Computed Tomography; Mouse Model; Hypospadias; Penile Anomalies

Received June 16, 2017. Accepted October 19, 2017.

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https://doi.org/10.1016/j.jsxm.2017.10.065

INTRODUCTION

Hypospadias, the most common manifestation of congenital penile anomalies, occurs in approximately 1 of 200 live male births.[1] The correction of severe and even sometimes mild forms of hypospadias can pose a significant challenge for surgeons.[2,3] Surgical complications, which require additional procedures, can lead to functional, psychologic, and esthetic problems that contribute to health care expenditures and patient suffering. There is growing interest in identifying genetic and

environmental factors contributing to the development of hypospadias, with the overarching goal of preventing its occurrence.[4] Notably, Cunha et al[5] made significant advances in understanding the similarities and differences in urethral development and hypospadias formation in the murine model compared with humans. By creating diethylstilbestrol- or flutamide-induced hypospadias in the murine model, their work has characterized the morphologic criteria specific to this malformation in the mouse and rat.

Current methods for evaluation of penile anomalies in the murine model include macrophotography or scanning electron microscopy and techniques involving 3-dimensional (3D) visualization of the penile specimen.[5] Although macrophotography and scanning electron microscopy allow for examination of the outer surface, they do not allow for morphologic assessment of internal penile structures. 3D reconstruction of specimens can be achieved using destructive (serial sectioning) and non-destructive (whole-volume imaging) approaches.[6] Because conventional histology and serial sectioning are labor intensive and prone to artifacts and error, non-destructive whole-volume imaging using optical projection tomography, micro-computerized tomography (micro-CT), and micro-magnetic resonance imaging (micro-MRI) for small specimens has seen increasing use. Despite the ability to achieve resolution on the order of a few microns with optical projection tomography, it is limited by sample thicknesses greater than 10 mm and opaque tissues.[7] Micro-MRI is well suited for soft tissue imaging but is limited by lengthy acquisition times and a relatively low spatial resolution of 25 μm.

Micro-CT was first described for preclinical imaging in small animals in 1980 and has rapidly gained popularity in the past 2 decades.[8] Compared with the ubiquitous hospital-based CT scanner, which can achieve resolutions as high as 40 μm, micro-CT scanners can achieve spatial resolution to the submicron level.[9] Image acquisition times are faster than those for micro-MRI. Because soft tissues yield little to no contrast when imaged with CT, initial usage in preclinical imaging was limited to highly radiopaque specimens, such as fossils and calcified bones. However, the recent introduction of staining methods for contrast-enhanced imaging has allowed for improved soft tissue visualization.

To address the shortcomings of conventional histology and serial sectioning, we report the novel use of micro-CT with iodine staining in characterizing murine penile morphology. Furthermore, we demonstrate that the ability to perform serial sectioning with histology is preserved even after processing for micro-CT.

METHODS

Experimental Design

The study was carried out in accordance to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All animal research was conducted under the oversight of the Baylor College of Medicine institutional animal care and use committee approved protocols.

Sample Preparation

Penises were dissected from C57BL6/J adult wild-type mice and fixed overnight in 10% formalin. The fixed tissue was washed twice with 70% ethanol, followed by dehydration and storage in 70% ethanol. 24 hours before micro-CT scanning, iodine staining was achieved by submerging the samples in a 0.1 N iodine solution (Sigma-Aldrich, St Louis, MO, USA) on a shaker at 4°C. Before scanning, the samples were rinsed with 1× phosphate buffered saline and suspended in a sample tube with 1% agarose.

Micro-CT Image Acquisition and Reconstruction

Scans were acquired at the Optical Imaging and Vital Microscopy Core at Baylor College of Medicine using the Bruker SkyScan 1272 micro-CT system (Bruker, Kontich, Belgium). The x-ray source was set at 60 kV and 166 μA with a 0.5-mm aluminum attenuation filter. Sequential projection images at 2,016 × 1,344 pixels and 5-μm resolution were obtained as the sample was rotated 180° at 0.3° increments in the anteroposterior axis. Acquisition time for each specimen was 120 minutes and batch acquisition was feasible with up to 6 specimens suspended in a single sample tube.

The raw projection image files were reconstructed using the Fledkamp cone-beam algorithm in NRecon Reconstruction (Bruker) software.[10] Reconstructed images were cropped and processed using HARP (Harwell Automated Reconstruction Processor, Medical Research Council Harwell, Harwell, Oxfordshire, UK) software to create nearly raw raster data files, a file format designed for scientific visualization and image processing. 3D volume rendering and virtual sectioning in the coronal, sagittal, and transverse planes were achieved using 3D Slicer (https://www.slicer.org), an open-source platform for image processing and visualization. Measurements in 3D Slicer were made by 2 independent observers after identifying the virtual section that matched with the corresponding histologic slide.

Histology

After image acquisition, the samples were removed from agarose gel and placed in 10% sodium thiosulfate pentahydrate (Sigma-Aldrich) for 4 hours while shaking at room temperature. This reduced the iodine to water-soluble iodide, which removed the iodine staining from the specimen. The samples were washed in 70% ethanol and demineralized in 3% nitric acid (EMD Millipore, Billerica, MA, USA) at room temperature overnight. Demineralization of the baculum facilitates serial sectioning. The following day, the samples were serially dehydrated and embedded in paraffin. 7-μm serial sections were created using a rotary microtome, mounted, and stained using hematoxylin and eosin. The slides were examined under a SZX-1000 microscope (Olympus, Center Valley, PA, USA) and digital images were