Introduction
Urinary incontinence, caused by either injury of pudendal nerve or external urethral sphincter muscle, is a popular medical condition that primarily affects postpartum women and the aging population.

Recent studies examining morphological, physiological or pharmacological aspects of the urinary incontinence concentrated on urinary bladder instead of the urethra. To gain insight into the structural changes associated with the urinary incontinence, we first studied the detailed structure of normal female rat urethra. Distribution of striated muscles in all segments of the urethra was reconstructed in three dimensions. These data will serve as the basis for later morphological, physiological, pharmacological and biomedical engineering studies on the changes associated with nerve injury-induced urine incontinence.

Materials and Methods
Female SD rats (250g ~ 300g) were perfused intracardially with 4% paraformaldehyde after deep anesthesia with 7% chloral hydrate. Urethras were harvested and post-fixed in the same fixative overnight. After being cross-sectioned in 36% sucrose, each urethra was cut into three, proximal, middle and distal segments. Each segment of the urethra was sectioned in the cross-sectional plane into 8 μm-thick sections with a cryostat. HE staining, ABC histochemistry and immunohistochemistry of the fast type MHC, slow type MHC and α–actin were performed on these sections to visualize cytoarchitecture, motor end-plates, striated muscle (fast), striated muscle (slow) and smooth muscle, respectively.

Discussion
Our studies showed that smooth muscle and fast MHC striated muscles were relatively abundant in the middle region of the urethra and this is associated with a higher motor end plate density in the area. These suggest that the main functional unit of the urethra probably lay in the middle part of the urethra. This result is consistent with previous studies describing that striated muscle, believed to be the external urethral sphincter muscle, is a popular medical clue for the site of incontinence [1, 2].

Another interesting finding of the present study is that we observed two longitudinal bundles of striated muscles, which resemble the urethrovaginal sphincter muscles of human [4]. This may also be important to urinary continence. These two striated muscle bundles could work as a rope syng to the urethra as a tube. This also explains that striated muscles are the thickest in the middle segment of dorsal urethra.

In addition to the co-localization of fast and slow type MHC striated muscles, fast MHC striated muscles which constituted the bulk of the inner circular layer of striated muscles dominated over the slow MHC in a ratio of 3 to 1. Our quantitative data are consistent with previous qualitative descriptions [2].

Conclusion
We illustrated in detail quantitatively the morphological features of normal female rat urethra and reconstructed these anatomical features of the urethra in the three-dimensional plane. These will be critical to researches on the morphological, physiological, pharmacological and biomedical engineering aspects of the studies on this on injury-induced urine incontinence model.

References

Results

Fig. 1 Photographs showing cross sections of the proximal (A), middle (B) and distal (C) segment and longitudinal (D) section of urethra. 3D-structures of smooth muscle (E) and striated muscle (F) layers were reconstructed using 3D Doctor software. Arrows indicate the urethra, V vagina, EVM external vaginal muscle. Scale bar: A, B, C = 300 μm; D = 30 μm.

Fig. 2 Immunohistochemical staining of α–actin to reveal the normal distribution of smooth muscle in the proximal (A), middle (B) and distal (C) region of urethra. D, the percentage of smooth muscle measured using the ratio of immunoreactive area to total urethral cross area. The smooth muscle distribution was distributed in the distal region. Scale bar: A, B, C = 300 μm; P < 0.05, t-test.

Fig. 3 Micrographs showing the striated muscle composition of the proximal, middle and distal parts of the urethra. The distribution of fast MHC muscle fiber is shown in the left column (A, B, C) and slow MHC muscle fiber in the right column (A’, B’, C’). D, the percentage of fast and slow MHC muscles fibers was derived from the ratio of immunoreactive area to total urethral cross area. Fast MHC muscle fiber predominated in the middle urethra and the slow MHC muscle fiber was most prominent in the distal region. Note that fast MHC muscle fiber was far numerous than slow type MHC muscle fiber. Scale bar = 300 μm for all micrographs; P < 0.05, unpaired t-test.

Fig. 4 High-power micrographs showing the co-localization of fast (A) and slow (B) MHC muscle fiber in the proximal, middle and distal segments of urethra. A, fast type MHC muscle fiber; B, slow type MHC muscle fiber. C and C’, arrow indicates the reference structure between these sections. The co-expression of fast and slow MHC muscles fiber was marked with white arrows. Red arrows indicated fibers expressing only slow MHC. Scale bar = 20 μm in A and B.

Fig. 5 Photograph showing the extension of urethral striated muscle fiber bidirectional on the surface of the vagina. Longitudinal bundles of striated muscles, resembling the urethrovaginal sphincter muscle of human, appeared to originate from the crural side of the middle urethra, extended toward the dorsal surface crossed to the other side and terminated on the lateral surface of the vagina. Scale bar = 300 μm.

Fig. 6 ACHE-histochemistry demonstrates the motor end-plates (A) on striated muscle fibers. B plotted the distribution of motor end-plates in the proximal, middle and distal portions of urethra. Motor end-plates are most abundant in the middle urethra. Scale bar = 20 μm in A.

Table 1. The thickness of urethral laminae (μm)

<table>
<thead>
<tr>
<th>Layer</th>
<th>Pro.</th>
<th>Mid.</th>
<th>Dist.</th>
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<tbody>
<tr>
<td>Mucosa</td>
<td>71.8 ± 3.2</td>
<td>81.9 ± 4.3</td>
<td>87.7 ± 5.3</td>
</tr>
<tr>
<td>Submucosa</td>
<td>65.0 ± 3.8</td>
<td>82.4 ± 4.9</td>
<td>87.7 ± 3.1</td>
</tr>
<tr>
<td>Lamina propria</td>
<td>29.5 ± 2.6</td>
<td>34.2 ± 3.8</td>
<td>39.3 ± 4.6</td>
</tr>
</tbody>
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A table of all the parameters examined. *, significantly difference in thickness within a region, ±, significant difference in thickness within a given segment of the urethra. All data are presented as the mean ± SEM, and P < 0.05.