• Basic Research •

Suitability for lacrimal duct research: anatomical and histological evidence favors the rabbit model over the rat

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Abstract

• **AIM:** To investigate the histological and anatomical characteristics of the lacrimal duct system (LDS) in rabbits and rats and compare with that in humans to establish a suitable animal for LDS studies.

• **METHODS:** New Zealand white rabbits (1.8–2 kg) and SD rats (250–300 g) were selected. Following tests were executed on their LDS, including anatomical measurements; computed tomographic dacryocystography; hematoxylin and eosin, Masson's trichrome, Periodic Acid-Schiff staining, and scanning electron microscope.

• **RESULTS:** Although there were anatomical similarities between the rats and humans in LDS, the size of LDS was too small and the epithelial types differed from that of humans in LDS. The rabbit only had a lower lacrimal punctum and a lacrimal canaliculus, while they had the same types of lacrimal epithelium as human's and the size was suitable for clinical probing and intubation.

• **CONCLUSION:** Histologically, the lacrimal canaliculi of both rabbits and rats resemble those of humans, making both species applicable for biological studies targeting the lacrimal canaliculi. Nevertheless, the epithelial types of the lacrimal sac and nasolacrimal duct in rats differ significantly from humans, whereas those in rabbits are consistent with humans. Therefore, rabbits are more appropriate for biological research involving the nasolacrimal duct and lacrimal sac.

• **KEYWORDS:** anatomy; computed tomographic dacryocystography; histology; lacrimal duct system; scanning electron microscope
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INTRODUCTION

Lacrimal duct obstruction diseases are common diseases in ophthalmology. It is generally believed that lacrimal duct obstruction diseases result from inflammatory changes followed by secondary scar tissue formation and adhesions. The source of inflammation is thought to be either ascending from the nasal cavity or descending from the ocular surface. However, the exact pathological mechanism remains poorly understood[1-4]. Although current surgical interventions can successfully resolve obstructions in most patients[5-6], postoperative recurrence due to mucosal adhesions remains a concern, and some cases, such as proximal lacrimal obstructions, continue to pose significant therapeutic challenges[7-8]. Therefore, further investigation into the mechanisms underlying lacrimal duct system (LDS) regeneration and repair is essential. The selection of appropriate animal models will facilitate progress in this field.

Previous studies have described the structural features of the nasolacrimal ducts (NLDs) in human, rabbits, rats, pigs, and apes[9-10]. The NLDs of rabbits, pigs, and apes are lined with a pseudostratified columnar epithelium, consisting of a basal cell layer and a superficial columnar layer, and lack goblet cells. These species also possess a surrounding cavernous system of blood vessels. In contrast, the rat NLD is lined with squamous epithelium containing goblet cells and lacks an associated cavernous vascular network. Among these animals, rabbits and rats are the most economical and widely used in experimental settings, and both have been employed in lacrimal duct studies[9,11]. However, the comprehensive morphology of the entire LDS—including the lacrimal punctum (LP), lacrimal canaliculus (LC), lacrimal sac (LS), and NLD—has not been

thoroughly described in these species. To provide a more detailed understanding of the LDS in rabbits and rats, this study presents a comparative analysis of their anatomical, radiological, and histological characteristics.

MATERIALS AND METHODS

Ethical Approval All animal handling procedures were in accordance with the Animal Management and Ethics Committee of Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology (TJH-202310008).

Animals Experimental animals were supplied by the Animal Center of Huazhong University of Science and Technology. New Zealand rabbits (weighing 1.8–2 kg) and Sprague-Dawley rats (weighing 250–300 g) were selected. Both eyes of each animal were utilized as experimental eyes. All animals were screened to exclude pre-existing ophthalmic conditions such as conjunctivitis, cataract, ocular tumors, or lacrimal duct obstruction. Lacrimal duct patency was assessed *via* irrigation using a 5 mL syringe connected to a sterile irrigator needle (Weigao, Shandong, China). The needle was inserted 2 mm into the lacrimal canaliculus through the punctum. Patency was confirmed when irrigation fluid flowed freely from the nasal cavity without reflux.

Anatomy of Rabbits and Rats Three rabbits and three rats were anesthetized *via* intraperitoneal injection of ethyl carbamate (Kerui, China) at a dose of 1000 mg/kg, administered as a 20% solution. Under microscopic guidance, the LP of both species were first identified and then measured using a gauge. Following measurement, the puncta were dilated with a specialized dilator. A sterile irrigator needle was introduced into the LC to serve as a guide. Subsequently, a skin incision was made, and subcutaneous tissues were carefully dissected to expose the LC and LS. The length of the LC, as well as the length and width of the LS, were precisely measured with the gauge.

Histology Examination of Lacrimal Duct System Following the anatomical measurements, tissues from the LC and LS were carefully harvested under microscopic guidance. The anterolateral bony wall of the NLD was then removed using bone-biting forceps to allow extraction of the NLD tissue. Each collected tissue sample was separately placed in specimen vials and fixed in 4% paraformaldehyde for 24h. Subsequently, the tissues were processed by routine paraffin embedding, sectioned into slices, and subjected to histological staining including hematoxylin and eosin, Masson's trichrome, and Periodic Acid-Schiff. Stained sections were observed under an optical microscope (Olympus BX53, Japan) for further analysis.

Computed Tomographic Dacryocystography Three rabbits and three rats were anesthetized *via* intraperitoneal injection of ethyl carbamate (Kerui, China) at a dose of 1000 mg/kg, administered as a 20% solution. A viscous contrast mixture was prepared by combining medical-grade sodium hyaluronate gel (Haohai Biological Technology, China) with the contrast agent iohexol (Yangtze River Pharmaceutical Group, China) at a 1:1 volume ratio. The mixture was drawn into a 5 mL disposable syringe fitted with a 0.45 mm diameter lacrimal irrigator needle and rapidly injected into the lacrimal passage *via* the LP until efflux from the nasal orifice was observed. Immediately after injection, the animals were scanned using a LightSpeed 16-slice spiral computed tomographic (CT) scanner. Scanning was performed in the prone position, with axial acquisition extending from the anterior nasal cavity to the neck at a slice thickness of 0.625 mm. The acquired images were reconstructed three-dimensionally using maximum intensity projection.

Scanning Electron Microscope Under microscopic guidance, the skin and subcutaneous tissues were incised, followed by sequential dissection and excision of epithelial tissues from the LC, LS, and NLD. Tissue blocks measuring approximately 2–3 mm^3 were gently rinsed with phosphate-buffered saline to remove blood and other contaminants. The entire procedure was completed within 3min. Subsequently, the processed tissues were immersed in electron microscopy fixative (Servicebio, Wuhan; Product No.G1102-100ML) and fixed at room temperature for 2h, after which they were transferred to 4°C for storage.

Statistical Analysis Statistical analyses were performed with SPSS statistical software version 22.0 (SPSS Inc., Chicago, IL, USA). All measurements were repeated three times, and measurements were expressed as mean±standard deviation (SD).

RESULTS

Anatomy In rabbits (Figure 1), the LP appeared as fissure-like structures located in the lower conjunctiva, covered by the third eyelid, with a length of 2.00±0.26 mm and a width of 0.30±0.1 mm. The distance from the puncta to the eyelid margin was 4.27±0.29 mm, and to the canthus was 5.97±0.71 mm. The LC had a length of 4.60±0.40 mm. The LS measured 5.07±0.55 mm in length and 2.67±0.90 mm in width. In rats (Figure 1), both upper and lower puncta were round in shape, with a diameter of 0.37±0.06 mm. These puncta were located 0.8±0.06 mm from the eyelid margin and 1.83±0.06 mm from the canthus. The LC measured 2.43±0.49 mm in length, while the LS were 2.57±0.67 mm in length and 2.27±0.55 mm in width (Table 1).

Computed Tomographic Dacryocystography In rabbits, NLD exhibited three distinct turns (Figure 2). The first turn was situated at the entrance of the NLD, corresponding to the junction between LS and the upper segment of the NLD,

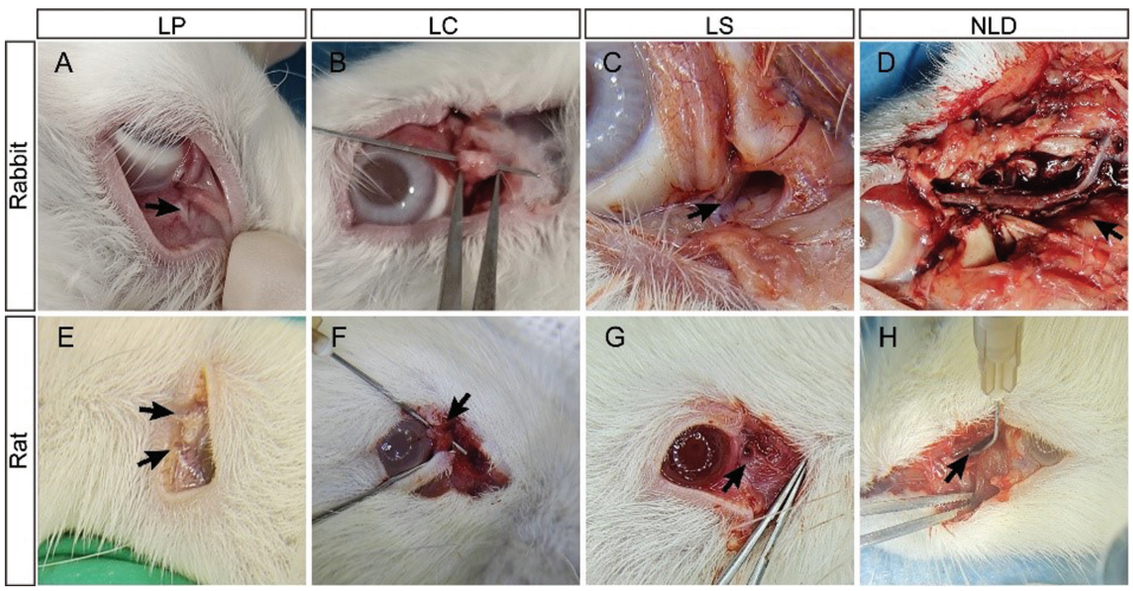

Figure 1 Anatomy of the lacrimal duct in rabbits and rats A: LP in the shape of a fissure (arrow) in rabbit; B: LC that the probe passes through in rabbit; C: The round-like LS (arrow) in rabbit; D: NLD with a marked curve in the inferior part (arrow) in rabbit; E: The upper and lower LP (arrows) in rat; F: LC that the probe passes through in rat; G: The round-like LS (arrow) in rat; H: NLD that the probe passes in (arrow) in rat. LP: Lacrimal punctum; LC: Lacrimal canaliculus; LS: Lacrimal sac; NLD: Nasolacrimal duct.

Table 1 Comparison of the anatomy of the lacrimal duct in rabbits and rats mean±SD, mm

Species	LP				LC	LS	
	Length	Width	Distance to eyelid margin	Distance to the canthus	Length	Length	Width
Rabbit	2.00±0.26	0.30±0.1	4.27±0.29	5.97±0.71	4.60±0.40	5.07±0.55	2.67±0.90
Rat	Diameter 0.37±0.06		0.8±0.06	1.83±0.06	2.43±0.49	2.57±0.67	2.27±0.55

LP: Lacrimal punctum; LC: Lacrimal canaliculus; LS: Lacrimal sac; SD: Standard deviation.

Table 2 Comparison of CT-DCG of the lacrimal duct in rabbits and rats mean±SD

NLD	Turns, degree			Diameter, mm		Segments, mm		
	The first	The second	The third	Maximum	Minimum	The first	The second	The third
Rabbit	100.29±5.68	126.43±5.03	137.43±6.29	1.60±0.24	0.77±0.18	22.83±2.26	6.57±0.45	4.59±0.72
Rat	134.00±3.00	102.67±8.96	148.67±5.69	1.69±0.07	0.49±0.05	4.44±0.37	10.29±0.54	3.41±0.15

CT-DCG: Computed tomographic dacryocystography; NLD: Nasolacrimal duct; SD: Standard deviation.

with an angle of 100.29°±5.68°. The second turn occurred at the inferior endosteal segment of the NLD, measuring 126.43°±5.03°. The third turn, located at the junction between the endonasal and endosteal segments, had an angle of 137.43°±6.29°. The maximum and minimum diameters of the NLD in rabbits were 1.60±0.24 and 0.77±0.18 mm, respectively. The NLD was divided into three segments for measurement: the first segment measured 22.83±2.26 mm, the second 6.57±0.45 mm, and the third 4.59±0.72 mm in length (Table 2).

Similarly, the NLD in rats also presented three turns (Figure 2). The first turn was located at the junction between the LS and the vertical portion of the NLD, with an angle of 134.00°±3.00°. The second turn occurred at the junction between the vertical and horizontal segments of the NLD, measuring 102.67°±8.96°. The third turn was found at the junction of the endosteal and endonasal segments, with an angle of 148.67°±5.69°. The maximum and minimum diameters of the NLD in rats were 1.69±0.07 and 0.49±0.05 mm, respectively. The first segment of the NLD measured 4.44±0.37 mm in length, while the second segment measured 10.29±0.54 mm, The third part of the NLD was 3.41±0.15 mm (Table 2).

Histology Under light microscopy, LC in rabbits was lined by stratified squamous epithelium (Figure 3A) and associated with relatively dense collagen fibers in the underlying tissue. Subepithelial glands opening into the epithelial layer were observed (Figure 3B). LS and NLD were both lined by a pseudostratified columnar epithelium, featuring superficial columnar cells with microvilli and a distinct basal cell layer (Figure 3D, 3G). Beneath the epithelium lay loose connective tissue and muscle fibers. Sparse blood vessels were observed within the LS (Figure 3E), while the NLD was encircled by a vascular-rich cavernous plexus (Figure 3H). No goblet cells

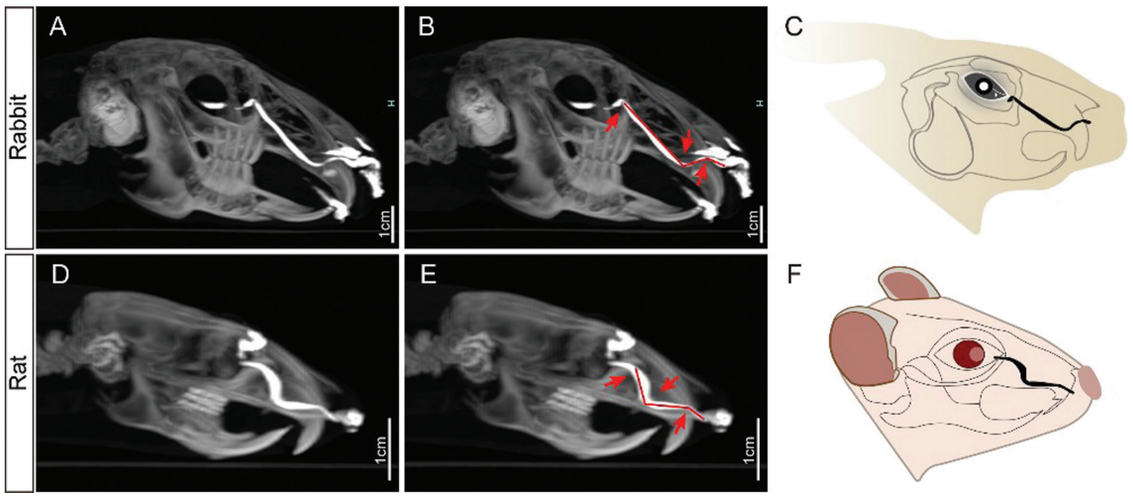

Figure 2 CT-DCG images of LDS in rabbits and rats A, B: There are three turns at the junction of LS and NLD, the inferior endosteal segment of NLD, and the junction of the endonasal and endosteal segment (arrows) in rabbit; C: Schematic illustration of LDS in rabbit; D, E: There are three turns at the junction of LS and the vertical NLD, the junction of the vertical NLD and the horizontal NLD, and the junction of the endosteal and endonasal segments (arrows) in rat; F: Schematic illustration of the LDS in rats. CT-DCG: Computed tomographic dacryocystography; LDS: Lacrimal duct system; LS: Lacrimal sac; NLD: Nasolacrimal duct.

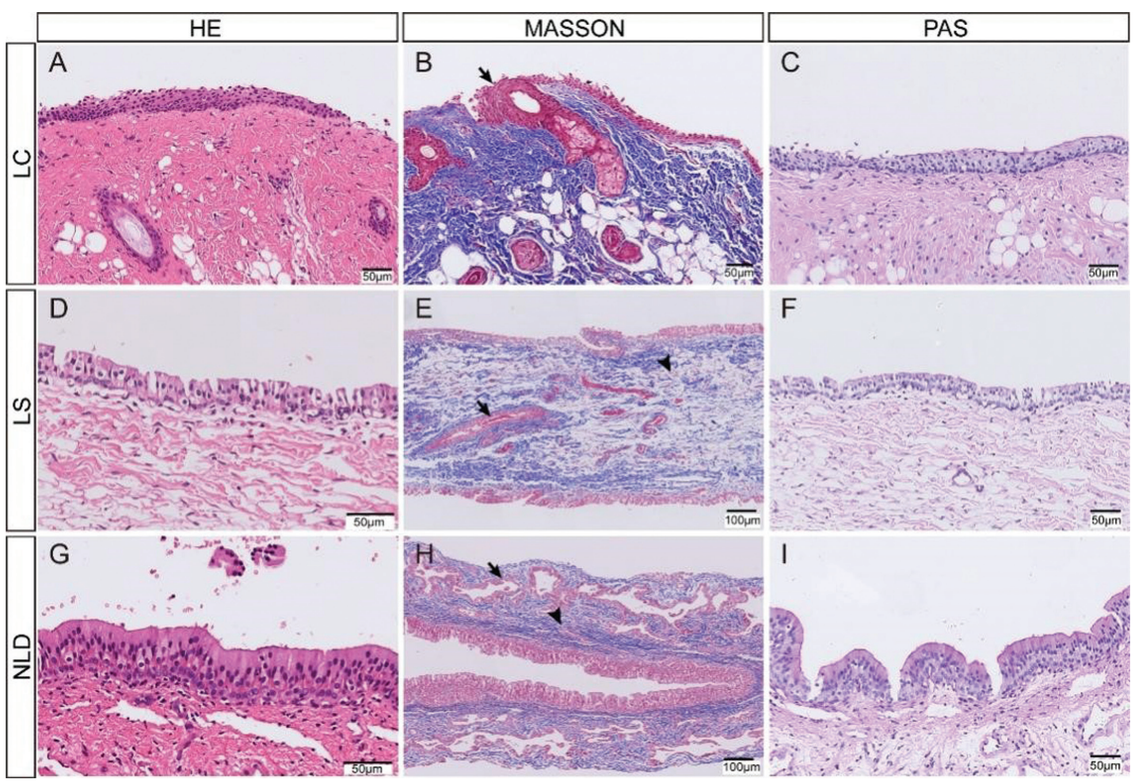

Figure 3 Tissue staining of rabbits A: Multilayered squamous epithelium in LC (HE); B: The gland opening to the epithelial surface (arrow) of LC (MASSON); D: Pseudostratified columnar epithelium in LS (HE); E: A small number of blood vessels (arrow) and muscle fibers (arrowhead) in the stomal layer of LS (MASSON); G: Pseudostratified columnar epithelium in NLD (HE); H: A large number of blood vessels (arrow) and a small number of muscle fibers in the stomal layer (arrowhead) of NLD (MASSON); C, F, I: Goblet cells were not found (PAS). LC: Lacrimal canaliculus; LS: Lacrimal sac; NLD: Nasolacrimal duct; HE: Hematoxylin and eosin staining; MASSON: Masson's trichrome staining; PAS: Periodic Acid-Schiff.

were detected throughout the LDS of rabbits (Figure 3C, 3F, 3I). In rats, the entire LDS was lined by stratified squamous epithelium (Figure 4A, 4D, 4G). Abundant collagen fibers were present in the stromal tissue of both the LC and LS (Figure 4B, 4E), and numerous glandular structures were identified within the LC (Figure 4B). The NLD was surrounded by numerous muscle fibers, with scant collagen fibers situated between the muscle layer and the epithelium (Figure 4H). Similarly, no goblet cells were observed within the epithelial lining of the LDS in rats (Figure 4C, 4F, 4I).

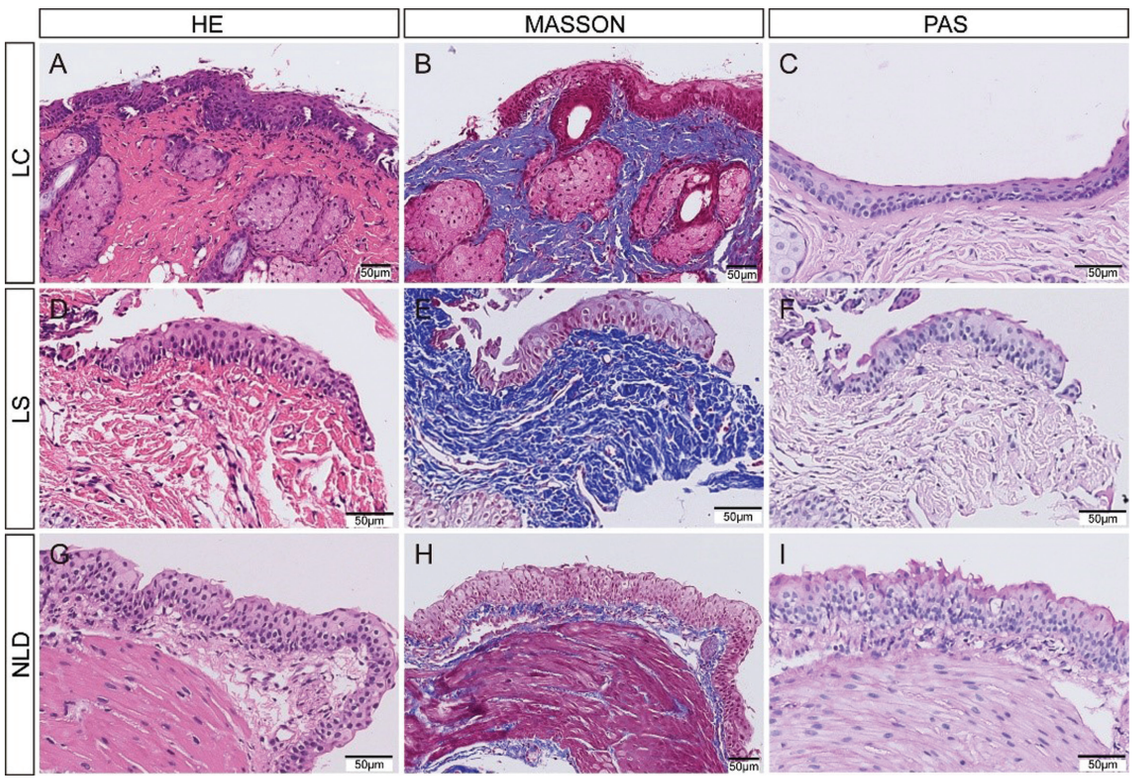

Figure 4 Tissue staining of rats A: Multilayered squamous epithelium in LC (HE); B: The stromal layer showed dense collagen fibers in which a large number of glands can be seen (arrows) of LC (MASSON); D: Multilayered squamous epithelium in LS (HE); E: A large number of collagenous fibers was under the epithelium of LS (MASSON); G: Multilayered squamous epithelium in NLD (HE); H: Muscle fibers were under the epithelium of NLD; C, F, I: Goblet cells were not found (PAS). LC: Lacrimal canaliculus; LS: Lacrimal sac; NLD: Nasolacrimal duct; HE: Hematoxylin and eosin staining; MASSON: Masson's trichrome staining; PAS: Periodic Acid-Schiff.

Scanning Electron Microscope In rabbits, LC was lined with squamous epithelial cells exhibiting a polygonal morphology and a relatively smooth surface. These cells were interconnected with visible folds and intercellular spaces (Figure 5A, 5B). Under higher magnification, secretory activity was observed (Figure 5C). LS was covered with a layer of columnar epithelial cells (Figure 5D, 5E), among which goblet-like cells were occasionally identified (Figure 5F). The surface of NLD was densely covered with microvilli, and evidence of secretory products was apparent (Figure 5J, 5H, 5I). In rats, the LC, LS, and NLD were all lined with squamous epithelial cells that exhibited irregular shapes, smooth but occasionally wrinkled surfaces, and distinct intercellular spaces (Figure 6). Secretions were observed on the surfaces of both the LS and NLD (Figure 6F, 6I).

DISCUSSION

Rats, which share approximately 95% genetic homology with humans[12], are widely employed in medical research due to their considerable anatomical and physiological similarities to humans. Rabbits have also been frequently used as models for studying LDS. However, rabbits possess only a single lacrimal punctum and canaliculus, which differs from the human LDS. Moreover, detailed reports on the structural characteristics of the rabbit LDS remain limited. To better understand and compare the LDS in these two species, this study investigated and contrasted their anatomical features, imaging manifestations, and histological structures.

Anatomically, in humans, LP—which serves as the opening of LDS—is located on the eyelid margin approximately 6.0–6.5 mm from the inner canthus, with a diameter of 0.2–0.3 mm. There is one punctum in both the upper and lower eyelids[1]. In rabbits, however, LP is fissure-shaped and notably larger than the round punctum of humans. It is situated in the conjunctiva just beneath the third eyelid. In contrast, LP of rats more closely resemble those of humans in both shape and size. However, the rat punctum is not located on the eyelid margin, but rather on the inner conjunctiva of the upper and lower eyelids, approximately 0.8 mm from the margin. In humans, the upper and lower puncta extend inward to form the canaliculi. More than 95% of individuals have a common canaliculus (approximately 3 mm in length) formed by the convergence of the superior and inferior canaliculi, which then connects to LS[1]. Rabbits possess only a single LC and lack a common canaliculus. Although rats have both upper and lower puncta, no common canaliculus was observed in the animals examined in this study. In humans, LS has a vertical length of

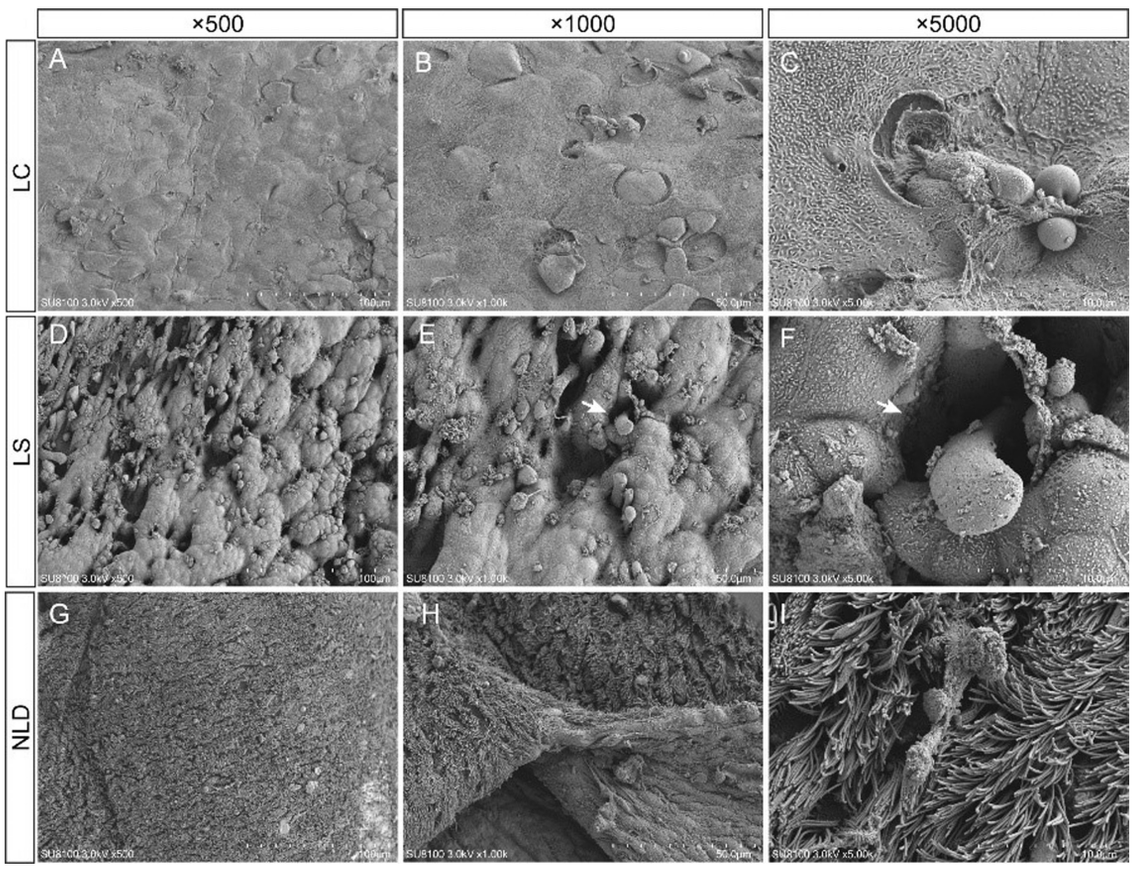

Figure 5 SEM of rabbits A–C: LC images show secretion was drained from the pore (A: 500×, B: 1000×, C: 5000×); D–F: LS and higher magnification view of a goblet-cell-like cell (arrow; D: 500×, E: 1000×, F: 5000×); G–I: A large number of microvilli in cross-section with epithelium and secretion on the microvilli of NLD (G: 500×, H: 1000×, I: 5000×). LC: Lacrimal canaliculus; LS: Lacrimal sac; NLD: Nasolacrimal duct; SEM: Scanning electron microscope.

10–12 mm[1] and an average width of 5.6 mm[13]. In humans, NLD measures 12–18 mm in length[1,14-15] and 2–7 mm in width[16]. While LC and LS in rabbits are smaller than those in humans, NLD is longer. Additionally, the diameter of LC in rabbits is larger than in humans, whereas the LS and NLD are narrower. In rats, both the length and diameter of the entire LDS are smaller compared to humans. The human NLD follows an almost vertical trajectory, which facilitates clinical procedures such as probing and retrograde catheterization. In contrast, the NLD in both rabbits and rats contains several pronounced turns, making cannulation or retrograde catheterization considerably more challenging. Specifically, the rabbit NLD is highly tortuous and contains two naturally narrow segments. NLD in rats follows a relatively straight course; however, its junction between the endosteal and endonasal segments is narrow and tortuous—though slightly wider than that observed in rabbits. The horizontal segment of the rat NLD approximates the length of the corresponding human segment.

In summary, the anatomical features of LDS in both rabbits and rats exhibit considerable differences from those in humans. Nevertheless, the relatively larger diameter of the upper LDS in rabbits allows for the insertion of both human lacrimal probes and endoscopes, facilitating easier manipulation and observation during experimental procedures.

Histologically, the epithelium of LC is homologous in humans, rabbits, and rats, consisting of stratified squamous epithelium underlaid by relatively dense collagen fibers. Subepithelial glands are present in the LC of both rabbits and rats. While LS and NLD in both humans and rabbits are lined with columnar epithelium, goblet cells are distributed within this epithelial layer in humans but are absent in rabbits[17]. In this study, Periodic Acid-Schiff staining in rabbits revealed no goblet cells; however, goblet-like cells were observed in LS under scanning electron microscope. In contrast, a study by Paulsen *et al*[9] reported the presence of goblet cells within the multilayered squamous epithelium of the rat NLD. In this study, the NLD epithelium of the rat was identified as stratified squamous epithelium; however, no goblet cells were detected by Periodic Acid-Schiff staining, a finding that contrasts with previous reports. Paulsen *et al*[9] employed Goldner's staining to identify the presence of goblet cells, a method primarily used for demonstrating bone tissue and collagen fibers. In contrast, Periodic Acid-Schiff staining is considered the gold standard

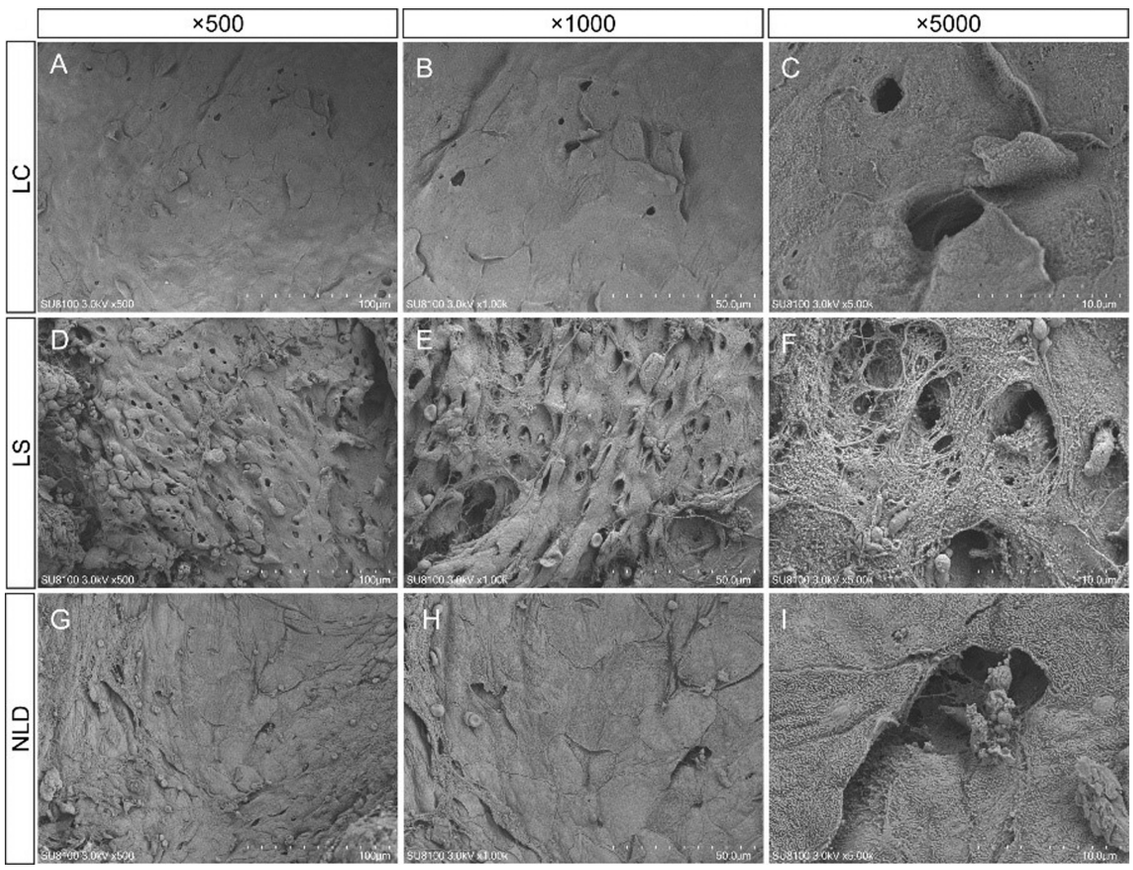

Figure 6 SEM of rats A–C: The squamous epithelial of LC (A: 500×, B: 1000×, C: 5000×); D–F: The squamous epithelium of LS and secretions are visible in the pores (D: 500×, E: 1000×, F: 5000×); G–I: The squamous epithelial surface of NLD and discharge of secretion (G: 500×, H: 1000×, I: 5000×). LC: Lacrimal canaliculus; LS: Lacrimal sac; NLD: Nasolacrimal duct; SEM: Scanning electron microscope.

for detecting goblet cells. This discrepancy may be attributed to differences in experimental methodologies. The contribution of the LS to lacrimal pump is subject to debate. Some researchers minimize or regard the function of the LS in the lacrimal pump mechanism as passive, while others argue that it is a crucial component[18]. Our study revealed the presence of muscle fibers in the LS stroma of rabbits, which exhibited a similar distribution pattern to that of humans. In the future, rabbits could be considered for studies on the lacrimal pump. In both rabbits and humans, the NLD epithelium is covered with abundant microvilli and surrounded by extensive cavernous tissue. These cavernous bodies facilitate tear drainage through swelling and contraction, which effectively close and open the duct lumen[19]. At the same time, the tear composition may have changed as the tears travel from eyes to nasal cavities[20]. Paulsen *et al*[9] found that the absorption of lipophilic substance could occur in NLD. Our findings indicate a high degree of homology between the epithelia and surrounding cavernous bodies in rabbits and humans, with the exception of goblet cell distribution. In contrast, the NLD epithelium in rats consisted of stratified squamous epithelium and was surrounded by abundant muscle fibers rather than cavernous bodies, which may affect the absorption. Furthermore, previous research on the absorption of iodinated albumin in rats demonstrated that this protein cannot be absorbed through LDS[10]. In a word, the rat may not be a suitable animal model in studies of lacrimal duct functions.

Combined with previous research, the lacrimal duct system of the rabbit exhibits a closer histological resemblance to that of humans compared to the rat, suggesting that rabbits may serve as a potential animal model for studying lacrimal drug absorption. In contrast, the LDS of the rat differs considerably from that of humans.

Limitations of this study: 1) Limited number of animals. The results of anatomy and computed tomographic dacryocystography are preliminary and require further validation. 2) Due to current limited investigations and the preliminary nature of this study on the lacrimal ducts of rabbits and rats, we have not yet conducted an in-depth investigation into tear drainage and pump function.

Both rabbits and rats possess the anatomical structures of the puncta, LC, LS, and NLD. However, certain anatomical differences exist between their lacrimal systems and the human lacrimal system. Rabbits are more suitable in terms of size for procedural intervention and observation. Histologically, the LC of both rabbits and rats resemble those of humans,

making both species applicable for biological studies targeting the LC. Nevertheless, the epithelial types of the LS and NLD in rats differ significantly from humans, whereas those in rabbits are consistent with humans. Therefore, rabbits are more appropriate for biological research involving the NLD and LS.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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