Medial and lateral canthal ligaments shown in P45 sheet plastination and dissection

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Purpose: Ocular suspensory ligament is an important part of the lower eyelid retractors. However, there is a scarcity of studies examining detailed en-block histologies of ocular suspensory ligaments. Methods: In this study, we included the cadavers of Chinese adults as subjects. These cadavers of Chinese adults were processed using P45 plastination techniques. The polymer resulted in transparent plastination, and the P45 sheet-plastinated sections of the lower eyelid were observed. The gross anatomy results of three Chinese adult heads (six hemifaces) were included as gross dissection data. All photographic documentation was performed via a Canon EOS 7D Mark camera. Results: The results showed that the inferior rectus muscle, inferior oblique muscle, ocular suspensory ligament, and its arcuate expansion are under the eyeball. The medial and lateral parts of the ocular suspensory ligament end at the medial and lateral canthal ligament. The middle part, a hammock-like shape, is slightly lower. The ocular suspensory ligament holds up the inferior oblique muscle, inferior rectus muscle, and the eyeball. As the inferior oblique muscle passes through the sheath of the inferior rectus, the fascia is thickened, forming the ocular suspensory ligament. The ocular suspensory ligament connects to the intermuscular septum, the inferior tarsal muscle, and the medial and lateral check ligaments. Conclusion: This study observed the ocular suspensory ligament and arcuate expansion through P45 sheet plastination for the first time and identified the distribution of the lower eyelid ligaments, thus laying the foundation for further research.

Key words: Lower eyelid retractors, ocular suspensory ligament, P45 plastination techniques

The ocular suspensory ligament is an important part of the lower eyelid retractors.¹ Gray’s Anatomy describes the sheath of the inferior rectus as thickened on its underside and blending with the sheath of the inferior oblique. These two, in turn, are continuous with the sheaths of the medial and lateral recti. Since the latter is attached to the orbital walls by check ligaments, a continuous fascial band, the suspensory ligament of the eye, is slung like a hammock below the eye, providing sufficient support.² It is connected from the medial canthal ligament to the lateral canthal ligament on the horizontal plane and has the function of keeping eyeballs in a normal position.³,⁴

A study reported that the ocular suspensory ligament can cause fat relaxation in the lower eyelid orbital septum due to the aging process.⁵ Another study introduced suspending the orbicularis oculi on the ocular suspensory ligament during lower blepharoplasty without removing the fat.⁶ Despite the importance of the ocular suspensory ligament in lower eyelid surgeries, few studies have shown detailed en-block histology of ocular suspensory ligaments.

Therefore, we conducted this study to elucidate a precise systematic and comprehensive anatomical study of the ocular suspensory ligament of Chinese people using P45 sheet plastination and gross dissection.

Methods

Subjects

In this study, we included cadavers of Chinese adults as the subjects, processed using the P45 plastination techniques. The polymer resulted in transparent plastination, enabling the P45 sheet-plastinated sections of the lower eyelid to be observed. This study was conducted in accordance with the Declaration of Helsinki and approved by the institutional ethics committee of our university.

Gross dissection

After removal of the skin from the heads, the submuscular fibroadipose layer of the orbicularis oculi muscle in the lower eyelid region was exposed. The preseptal fat was removed from its region, exposing the orbital structures. The types of relationship between the ocular suspensory ligament and the capsulopalpebral fascia were observed and marked. Photographic documentation was carried out using a Canon EOS 7D Mark camera.

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P45 sheet plastination

**Slicing**
The embalmed specimens of the head and neck were frozen at 
-70°C for 2 weeks, and then embedded in polyurethane foam 
and frozen at -20°C, again for 2 days. After freezing, sagittal slices 
were made at 3 mm from side to side using a high-speed band 
saw. The volume of residual dust was 1 mm. The slices were 
placed in an orderly fashion on polyethylene grids with a piece 
of fly screen. A light stream of running water was used to remove 
any dust. The grids were then stacked on top of each other and 
tied using twine to hold the grids together as one unit. All the 
units were then labeled and put into square polyethylene pails.

**Bleaching**
All the slices were rinsed overnight in cold running water, 
after which the slices were immersed in 5% hydrogen peroxide 
overnight.

**Dehydration**
After bleaching, the slices were dehydrated using a 
freeze-substitution method. First, the slices were precooled at 
5°C to avoid the formation of ice crystals and shrinkage before 
being placed into cold acetone. The slices were then placed 
in the first bath of 100% acetone at -25°C for 1 week and then 
transferred into a second bath of 100% acetone at -15°C for 
10 days. Following these 10 days, they were then put into 100% 
acetone at room temperature for 1 week. The slices were finally 
submerged in fresh 100% acetone at room temperature. After 
1 week, the slices were taken out for impregnation. The purity 
of the acetone was monitored with an acetonometer daily. 
Provided the purity remained similar on three observations, 
the slices were moved to a fresh dehydrating solution.

**Casting and forced impregnation**
After dehydration, the casting mold was prepared. This was 
a vertical chamber consisting of two plates of 5-mm tempered 
glass, flexible 4-mm latex tubing, and several large fold-back 
clamps. The slices were lifted out of the acetone bath and 
placed between the two glass plates. The molds were then filled 
with polyester (Hoffen polyester P45, China) via a funnel. The 
components of Hoffen polyester P45 were mixed at a ratio of 
1000 mL of polyester P45 monomer to 10 g of P45a to 30 mL of 
P45b to 5 g of P45c. P45a and P45c were used as plasticizers, 
and P45b was used as a hardener for sheet plastination. After 
casting was completed, the filled mold was placed upright into 
a vacuum chamber at room temperature for impregnation. Larger 
bubbles were removed manually via a piece of 1-mm stainless 
steel wire. Absolute pressure was slowly decreased to 20 mmHg, 
10 mmHg, 5 mmHg, and 0 mmHg according to the release of 
bubbles. A pressure of 0 mmHg was maintained until bubbling 
ceased. The duration of impregnation was more than 8 h.

**Curing**
After the vacuum was released, the air bubbles within the 
sheets were checked and removed. The alignment of the slices 
was checked and corrected using the steel wire. The top of the 
mold was clamped with large fold-back clamps, and the sheet 
was then ready for curing. The sheets were cured using heated 
bathwater. The sheets were placed upright in the bathwater at 
40°C for 3 days. To equilibrate the temperature of the water, a 
small circulatory pump was used to circulate the water around 
the bath.

**Cutting and sanding the molds**
After curing, the sheets were removed from the bath and cooled 
to room temperature on a rack. The slices were then taken from 
the vertical chamber and were covered appropriately with 
adhesive plastic wrap for protection. A bend saw was used to 
cut and trim the plastic along the edges approximately 1 mm 
outside of the slices. Following this, a wool sander was used to 
to remove the sharp edges of the slices, and after sanding, the 
adhesive plastic wraps were removed and the slices were put 
into plastic wraps to avoid scratches.

The head and neck sheets were then observed and 
photographed using LED reading lights.

**Results**

**The general characteristics**
In this study, a total of 14 heads (28 hemifaces) cadavers 
of Chinese adults were used (22 hemifaces for P45 sheet 
plastination,[6] hemifaces for gross dissection).

**Gross dissection**

**Origin and insertion**
The medial and lateral parts of the ocular suspensory ligament 
end at the medial and lateral canthal ligaments. The middle 
part is slightly lower, appearing as a hammock-like shape, 
holding up the inferior oblique muscle, inferior rectus muscle, 
and the eyeball [Fig. 1].

**Adjacent structures**

**Muscle**
The structures below the eyeball are the inferior rectus muscle, 
the inferior oblique muscle, the ocular suspensory ligament, 
and its arcuate expansion. Below the inferior oblique muscle, 
there is a fascia structure that issues forward, forming the ocular 
suspensory ligament.

**Fat**
The inferior oblique muscle and arcuate expansion, respectively 
travel inward and downward. They divide the postseptal fat of 
the lower eyelid into three independent parts: lateral, middle, 
and medial.

**P45 Sheet-plastinated sections**
The head of the capsulopalpebral fascia (CPF) splits open 
superiorly and inferiorly, wrapping around the inferior oblique 
muscle and meeting anteriorly. As the inferior oblique muscle 
passes through the sheath of the inferior rectus, the fascia is 
thickened, forming the ocular suspensory ligament. The CPF 
enters the inferior border of the tarsus, merging with the anterior 
border of the inferior tarsal muscles. The intermuscular septum, 
the inferior tarsal muscle, and the medial and lateral check 
ligaments are connected to the ocular suspensory ligament.

**Sagittal plane: (10 hemifaces observed)**

**Lateral part** [Fig. 2]
The CPF is a layer of dense fiber that enters the inferior border 
of the tarsus, merging with the anterior border of the inferior 
tarsal muscles. The head of the CPF splits open superiorly and 
inferiorly, wrapping around the inferior oblique muscle and 
meets anteriorly. As the inferior oblique muscle passes 
through the sheath of the inferior rectus, the fascia is thickened 
and forms the ocular suspensory ligament. A fibrous structure 
can be observed in the lower border of the ocular suspensory 
ligament, which is postseptal fat.

**Midpupillary part** [Fig. 3]
The inferior oblique muscle becomes smaller than the lateral 
part. All cases indicate that the fascia between the inferior 
oblique muscle and the inferior rectus muscle is thickened and 
forms the ocular suspensory ligament.
Medial part [Fig. 4]
The inferior oblique muscle becomes smaller than the midpupillary part. The fascia between the inferior oblique muscle and the inferior rectus muscle forms the ocular suspensory ligament, and the ocular suspensory ligament is thinner than the midpupillary part, connecting the inferior oblique muscle and the inferior rectus muscle. The upper border of the ocular suspensory ligament is loose tissue, and the lower border is postseptal fat.

Coronal plane: Six hemifaces observed [Fig. 5]
Lateral part
A single layer of dense fiber can be observed around the outer border of the inferior part of the eyeball, which is the ocular suspensory ligament in the coronal plane. It can be observed in the postseptal fat, issuing dense fibers and traveling laterally and inferiorly. The lateral check ligaments are connected to the ocular suspensory ligament.

Midpupillary part
A single layer of dense fiber can be observed around the outer border of the inferior part of the eyeball, which is the ocular suspensory ligament in the coronal plane. It can be observed in the postseptal fat, the inferior oblique muscle tissues from the lower part of the eyelid and travels medially and inferiorly.

Medial part
A single layer of dense fiber can be observed around the outer border of the inferior part of the eyeball. It can be observed in the postseptal fat, and the inferior oblique muscle continues to travel medially and inferiorly, stopping at the periorbital in a 7 o’clock direction. The medial check ligaments were connected to the ocular suspensory ligament.

Horizontal plane: Six hemifaces observed [Fig. 6]
Lateral part
A layer of dense fiber, which runs along the outer edge of the anterior side of the eyeball from the lateral canthal ligament, is the ocular suspensory ligament. There is also a layer of dense fiber running on the outer side of the ocular suspensory ligament, which merges with the ocular suspensory ligament. It is an arcuate expansion of the ocular suspensory ligament.

Midpupillary part
There is a layer of dense fiber on the outer edge of the anterior side of the eyeball, which is the ocular suspensory ligament. No branch was observed on the middle side of the ocular suspensory ligament.

Medial part
A layer of dense fiber, which runs along the outer edge of the anterior side of the eyeball and ends at the medial canthral ligament, is the ocular suspensory ligament. No branch was observed on the medial side of the ocular suspensory ligament.

Discussion
In this study, we first observed the ocular suspensory ligament using P45 plastination technology, which is suitable for the preservation and transparency of large and thin fault specimens. In the lower eyelid, the only ligament currently recognized is the ocular suspensory ligament.5,8

According to Whitnall, distal to the point where the inferior oblique crosses the inferior rectus, their blended sheaths send forwards thin lamellae, one of which follows the tendon of the latter muscle to help form the anterior part of the capsule of Tenon, while the other can be traced with difficulty into the lower eyelid. The sheaths of the muscles become confluent with each other by their adjacent margins immediately before the fusion with the capsule; the sheath of the inferior rectus is no exception to the rule, and the thickening of its sheath can be traced laterally and medially up to the sheaths of the lateral and medial recti, and since these are fixed to the orbital walls by expansions there is the formation of a continuous band about one-tenth of an inch beneath the globe, supporting it as in a hammock (ocular suspensory ligament of Lockwood) [Table 1].5,9

Fink and Duke-Elder thought that the ocular suspensory ligament is a blending of the sheaths of the inferior oblique and inferior rectus muscles extending upward and laterally to join the sheath of the lateral rectus and upwards and medially to join that of the medial rectus. There are also connections between this ligament and the intracapsular ligaments of the capsule of Tenon at the sites where the lateral and medial rectus muscles pass through the capsule.10-13 The fibers at the lateral and medial ends of the ligament converge to their insertion, but, centrally, the fibers diverge to form a suspensory hammock which supports the eyeball. Fink believed that the check ligament of the inferior oblique may be regarded as part of the ocular suspensory ligament, and it throws out extensions into the lower lid where it is attached anteriorly to the orbital septum, the tarsal plate, and the cul-de-sac formed by the inferior fornix. Meanwhile, the fascicular bands pass from it to the skin, the intermuscular tissue of the lower lid, and the periorbital of the orbital margin and floor [Table 1].14,15

Beard and Hornblass described the ocular suspensory ligament as having few attachments to the medial and lateral retinaclu, with major support coming from these retinacula. The fascial confluences that occur as the inferior oblique muscle passes through the capsulopalpebral head contribute significantly to the ocular suspensory ligament. Besides, the ocular suspensory ligament has contributions from the intermuscular septum, the inferior tarsal muscle, and the medial and lateral check ligaments [Table 1].10

Our observations concord well with studies by Whitnall, Fink, Duke-Elder, Beard, and Hornblass. The previous studies were mainly gross dissections and histologies of a part of an orbit. In our study, our serial cross-sections included whole hemifaces; consequently, we could observe the orbital bone as well as the soft tissue structure adjacent to the ocular suspensory ligament.

Hawes and Dorzbach et al. termed the fascial tissue anterior to the ocular suspensory ligament of Lockwood as “capsulopalpebral fascia.”17 In our observation, the head of the CPF split open superiorly and inferiorly, wrapping around the inferior oblique muscle and meeting anteriorly. As the inferior oblique muscle passes through the sheath of the inferior rectus, the fascia is thickened and forms the ocular suspensory ligament.

Kakizaki et al.5 insisted that they observed the ocular suspensory ligament as having two layers; the other layer is not a muscle but does include some smooth muscle cells. However, other studies do not agree that these are unique structures in the lower eyelids.18 Our research, both via Hornblass’s book and our previous and present studies, have not revealed two layers of the ocular suspensory ligament. Kakizaki et al. also observed two branches of ocular suspensory ligaments (superior ligament and inferior ligament). However, we could not find these branches in any serial cross-sections [Table 2].

In this study, we identified the distribution of the lower eyelid ligaments. We observed the ocular suspensory...
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Figure 1: Gross anatomy of the lower eyelid. CPF: capsulopalpebral fascia, PSF: postseptal fat, asterisk: ocular suspensory ligament

Figure 3: Sagittal section of the midpupillary part of the lower eyelid. CPF: capsulopalpebral fascia, IO: inferior oblique muscle, IR: inferior rectus muscle, OSL: ocular suspensory ligament, S: orbital septum, T: tarsal plate

Figure 5: Coronal section of the lower eyelid. AE: arcuate expansion, IO: inferior oblique muscle, IR: inferior rectus muscle, PSF: postseptal fat, asterisk: ocular suspensory ligament

Figure 2: Sagittal section of the lateral part of the lower eyelid. CPF: capsulopalpebral fascia, IO: inferior oblique muscle, IR: inferior rectus muscle, OSL: ocular suspensory ligament, S: orbital septum, T: tarsal plate

Figure 4: Sagittal section of the medial part of the lower eyelid. CPF: capsulopalpebral fascia, IO: inferior oblique muscle, IR: inferior rectus muscle, OSL: ocular suspensory ligament, S: orbital septum, T: tarsal plate

Figure 6: Horizontal section of the lower eyelid. LPL: lateral canthal ligaments, MPL: medial canthal ligament, asterisk: ocular suspensory ligament, arrow: arcuate expansion

ligament and arcuate expansion through P45 sheet plastination for the first time, laying the foundation for
Table 1: Structure and function of the ocular suspensory ligament

| Author          | Year | Tissue                               | Lateral                                    | Medial                                     | Extension                                      | Function                          |
|-----------------|------|--------------------------------------|--------------------------------------------|--------------------------------------------|-----------------------------------------------|-----------------------------------|
| Lockwood        | 1886 | Band of fibrous tissue               | Zygoma on the lateral side of the orbit   | Lacrimal bone                             |                                               | Anchor the globe in position draw   |
| Whitnall        | 1932 | Sheath of IR 1/10 inch thick         | Sheath of LR to retinaculum oculi laterale | Sheath of MR to retinaculum oculi mediale | Fornix                                        | in fornix backward                |
| Fink            | 1948 | Blending of sheath of IO and IR      | Upward and laterally to sheath of LR       | Upward and medially to sheath of MR        | Check lig., orbital septum, tarsal plate, cul-de-sac fornix | Suspensory hammock                |
| Duke-Elder      | 1955 | Fascia of IO pass through CPH        |                                            |                                            |                                               |                                   |
| Beard           | 1977 |                                       |                                            |                                            |                                               |                                   |
| Hornblass       | 1988 |                                       |                                            |                                            |                                               |                                   |
| Present         | 2020 | Multiple layers of dense fibers in front of IO | Lateral palpebral lig. | Medial palpebral lig. | CPF, Arcuate expansion | Hammock holding IO, IR, and eyeball |

IO: inferior oblique muscle, IR: inferior rectus muscle, CPH: capsulopalpebral head, LR: lateral rectus, MR: medial rectus

Table 2: Ocular suspensory ligament (OSL)

| Branches      | Author         | Year | Tissue                                | Insertion                                      | Location                           | Kakizaki (2005) | Present (2020) |
|---------------|----------------|------|---------------------------------------|-----------------------------------------------|-----------------------------------|-----------------|----------------|
| Superior      | Kakizaki H,    | 1997 | Inferior ligament                     | Posterior aspect of the IO, Not clear in the lateral margin of the CPF | Superior to the OSL | No other branches found | Medial palpebral lig. |
| Ligament      | Zako M,        |      |                                       |                                               |                                   |                 |                 |
|               | Nakano T,      |      |                                       |                                               |                                   |                 |                 |
|               | Asamoto K,     |      |                                       |                                               |                                   |                 |                 |
|               | Miyagawa T,    |      |                                       |                                               |                                   |                 |                 |
|               | Iwaki M        |      |                                       |                                               |                                   |                 |                 |
|               |                |      |                                       |                                               |                                   |                 |                 |
|               |                |      |                                       |                                               |                                   |                 |                 |
|               |                |      |                                       |                                               |                                   |                 |                 |
| Medial        |                |      |                                       |                                               |                                   |                 |                 |
| Ligament      |                |      |                                       |                                               |                                   |                 |                 |
|               |                |      |                                       |                                               |                                   |                 |                 |
|               |                |      |                                       |                                               |                                   |                 |                 |
|               |                |      |                                       |                                               |                                   |                 |                 |
| Inferior      |                |      |                                       |                                               |                                   |                 |                 |
| Ligament      |                |      |                                       |                                               |                                   |                 |                 |
|               |                |      |                                       |                                               |                                   |                 |                 |
|               |                |      |                                       |                                               |                                   |                 |                 |
|               |                |      |                                       |                                               |                                   |                 |                 |

IO: inferior oblique muscle, CPF: capsulopalpebral fascia

further research. The ocular suspensory ligament should be approximated in place even after excision or fracture of the upper part of the jaw.

Conclusion

This study observed the ocular suspensory ligament and arcuate expansion through P45 sheet plastination for the first time and identified the distribution of the lower eyelid ligaments, laying the foundation for further research.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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