

The Use of P45 Plastination Technique to Study the Distribution of Preseptal and Preaponeurotic Fat Tissues in Asian Eyelids

Pu Chun · Shengbo Yu · Hongzhi Qin ·
Hongjin Sui

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Abstract This study evaluated the anatomical features of preseptal and preaponeurotic fat tissues in the upper eyelids of individuals of Asian heritage. Specifically, we attempted to elucidate the role of these tissues in the formation of sunken upper eyelids and devise an easy and feasible approach to rectify this. Sixteen heads (32 facial halves) from fresh adult cadavers were processed using the P45 plastination techniques. The polymer resulted in transparent plastination, and the sagittal median section of the eyeballs was dissected. Gross anatomy results of 12 adult cadaveric heads (24 facial halves) were included as supplementary data. The orbital septum was observed on sagittal section slides prepared with P45 sheet plastination. Based on the amount of fat distribution, the upper eyelid was classified into three groups: preseptal fat predominant type, preaponeurotic fat predominant type, and orbital septum equilibrium type (relative distribution of 31.3, 12.5, and 56.3 % in plastinated slices, and 29.2, 16.7, and 54.2 % in gross anatomical studies). Major tissues on P45 sheet plastination slices in the supraorbital region were preseptal fat, preaponeurotic fat, frontalis muscle and frontalis muscle aponeurosis, and partial orbicularis oculi muscle. The muscle fibers of the frontalis muscle and orbicularis oculi were interconnected and extended backward to connect with the

orbital septum through preseptal fat. In conclusion, the morphology and external appearance of the upper eyelids depend on the relative relationship between preseptal and preaponeurotic fat tissues. Mildly or moderately sunken upper eyelids can be corrected by modifying the soft tissue in the supraorbital margin.

Keywords Preseptal fat · Preaponeurotic fat · Adipose tissue · Sunken upper eyelid · Plastination · P45 plastination

Introduction

An in-depth understanding of the anatomical features of circumocular soft tissue distribution is critical for surgical correction of the upper eyelid deformity. The degree of fullness of the upper eyelids in individuals of Asian heritage is associated with the following four groups of fat tissue: subcutaneous fat, preseptal fat, orbital fat, and pretarsal adipose tissue [1]. Orbital fat has been in the focus of previous studies. It is known that two fat pads are formed by separate medial and lateral fat tissues, also known as preaponeurotic fat [2, 3]. Shen et al. [4] demonstrated prominent fat tissue in the medial pretarsal adipose tissue. Still, detailed studies on preaponeurotic fat, and particularly on preseptal fat, are lacking. It is generally accepted that preseptal fat represents an extension of eyebrow fat and is regarded as suborbicularis fibroadipose tissue. Preseptal fat is very thin and is distributed beneath the orbicularis oculi in Caucasians [5]. However, its distribution in the upper eyelids of Asians varies considerably among individuals, and no systematic exploration of this tissue has been done to date. Both preseptal and preaponeurotic fat tissues act to support eyelid fullness. Their association with each other remains

P. Chun · S. Yu · H. Sui (✉)
Department of Anatomy, College of Basic Medicine, Dalian
Medical University, 9 West Section, Lushun South Road,
Dalian 116027, China
e-mail: drsuihongjin@163.com

H. Qin (✉)
Department of Plastic and Reconstructive Surgery, The First
Affiliated Hospital of Dalian Medical University, 787 Huanghe
Road, Dalian 116011, China
e-mail: drqinhongzhi@126.com

unclear. Surgical removal of upper eyelid fat is a key procedure in blepharoplasty. Therefore, heightened awareness of anatomical relationship between preseptal and preaponeurotic fat tissues could help improve outcomes of the surgeries to correct the sunken upper eyelid.

In the present study, P45 plastination technique was utilized to explore the anatomical features of the upper eyelid. These anatomical findings were utilized as supplemental evidence to discriminate between the distribution of preseptal and preaponeurotic fat to assess their respective impacts for periorbital surgery.

Materials and Methods

P45 Sheet Plastination

Sixteen adult specimens were selected for the study. Their faces were sliced in the sagittal direction. The P45 sheet plastination technique [6] was applied as follows:

Slicing

Embalmed head face specimens were frozen at -70°C for 2 weeks. Then, 3-mm-thick sagittal slices were prepared with a high-speed band saw.

Bleaching

All slices were rinsed with running water for 6–8 h and immersed in 5 % hydrogen peroxide overnight for bleaching.

Dehydration

The slices were dehydrated using the freeze substitution method [7]. In brief, the slices were initially cooled to 5°C . Then, they were kept in the bath with 100 % acetone at -25°C for 1 week. Slices were then transferred to a second bath of 100 % acetone and incubated at -15°C for 10 days. Finally, the slices were immersed into 100 % acetone and kept at room temperature for 1 week. Following these dehydration steps, the slices were submerged in fresh 100 % acetone at room temperature for a week. Afterward, the slices were used for impregnation.

Casting and Forced Impregnation

For casting and forced impregnation, the flat chamber method was utilized. The flat chamber consisted of two plates of 5-mm tempered glass, a flexible latex tubing, and several large fold back clamps [8, 9]. The slices were placed between two glass plates. Then, the molds were filled with P45 polyester (Hoffen polyester P45, China) via a funnel

[10]. The components of Hoffen polyester P45 were mixed using 1000 ml of polyester P45 monomer, 10 g of P45a, 30 ml of P45b, and 5 g of P45c, where P45a and P45c served as plasticizers, and P45b as a hardener for sheet plastination.

After casting, the filled mold was placed upright into a vacuum chamber at room temperature for impregnation. The absolute pressure was gradually decreased to 20, 10, 5, and 0 mm Hg, following the release of bubbles from the tissue slices. The pressure of 0 mm Hg was maintained until bubbling ceased. The duration of impregnation exceeded 8 h.

Curing

After vacuum release, the top of the mold was clamped with large fold back clamps, and the sheet was ready for curing. The sheets were placed in an upright position and cured in a heated water bath at 40°C for 3 days.

Cutting and Sanding the Molds

After curing, the slices were taken out from flat chamber and were covered with adhesive plastic wrap for protection. A small band saw was used to cut and trim the plastic along the outside edges of the slices at a distance of approximately 1 mm from the tissue. To cut out the sharp edges of the slices, a wool sander was used. The sheets were then ready for use.

Gross Anatomy: Dissection of the Upper Eyelid

Dissection was performed on 12 donated adult cadaver heads (four male and eight female individuals). After removal of the skin from the heads, the submuscular fibroadipose layer (preseptal fat) of the orbicularis oculi muscle in upper eyelid region was exposed. The preseptal fat was removed from its region, exposing the structures of orbital and preaponeurotic fat. The types of relationship between preseptal fat and preaponeurotic fat were observed and marked. Photographic documentation was done using a Canon EOS 450D camera.

Results

P45 Sheet-Plastinated Upper Eyelid Sections

The P45 sheet-plastinated upper eyelid sections from 32 facial halves were transparent, and frontalis muscle, orbicularis oculi, orbital septum, preseptal fat, preaponeurotic fat, levator aponeurosis, Muller's muscle connected to the tarsus, and eyebrow fat pads posterior to the frontalis muscle could be observed in the median sagittal section of the P45 plastinated upper eyelids (Fig. 1).

We observed that preseptal fat is localized inferior to the supraorbital margin, posterior to orbicularis oculi, and closely attached to the orbital septum. It reaches the eyebrow fat pads and extends downward to the tarsus. Preaponeurotic fat is localized posterior to the orbital septum and anterior to levator aponeurosis, extending upward to the orbital bone and downward to the fat tissue anterior to levator aponeurosis.

The three types of preseptal and preaponeurotic fat are described according to their preponderant localization. Preseptal fat is predominant in 31.3 % of individuals. As shown Fig. 2, this fat type contains a prominent amount of extruding fat and fascia fibers, as compared with preaponeurotic fat. There is relatively low amount of preaponeurotic fat, which mildly extrudes toward the supraorbital margin. The orbital septum is the boundary. This type will henceforth be referred to as the “preseptal fat predominant type”. Preaponeurotic fat is predominant in 12.5 % of individuals. As shown in Fig. 3, in these individuals, preseptal fat tissue is extremely thin and slender, despite containing abundant fascia fibers. This tissue is located anterior to the supraorbital margin and extrudes forward. In contrast, preaponeurotic fat in these individuals is more abundant than preseptal fat and fills the entire upper eyelid area, extruding inferiorly–anteriorly. Again, the orbital septum is the boundary. This type is referred to as “preaponeurotic fat predominant type”.

The orbital septum equilibrium is present in 56.3 % of individuals. As shown in Fig. 4, preseptal fat is evenly

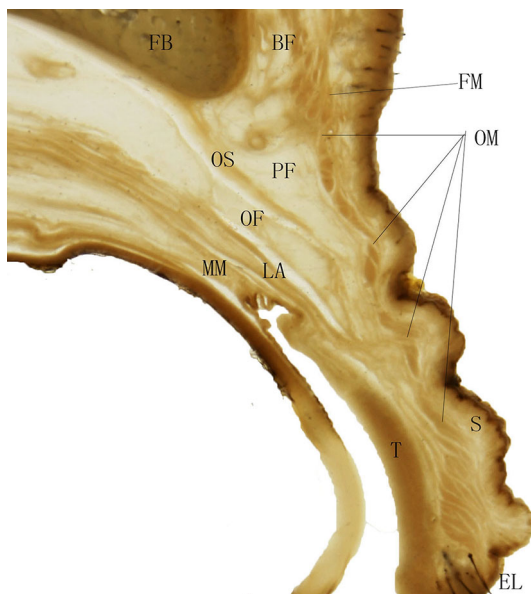


Fig. 1 Median sagittal section of the P45 plastinated upper eyelids. Preseptal fat containing fascia fibers posterior to the orbicularis oculi, and preaponeurotic fat between the orbital septum and levator aponeurosis. *FM* frontal muscle, *OM* orbicularis oculi muscle, *PF* preseptal fat, *OS* orbital septum, *OF* preaponeurotic fat, *LA* levator aponeurosis, *MM* muller’s muscle, *T* tarsus, *EL* eyelash, *S* skin

distributed anteriorly and posteriorly to the orbital septum, and contains equivalent amounts of preaponeurotic fat. Preseptal and preaponeurotic fat are equally abundant in these individuals. This type is referred to as the “orbital septum equilibrium type”.

Gross Anatomical Observation

The gross anatomy findings regarding the distribution of preseptal and preaponeurotic fat were consistent with those on the P45 sheet-plastinated sagittal sections.

The “preseptal fat predominant type” was present in 29.2 % of the individuals (Fig. 5). A prominent amount of fat and fascia fibers in preseptal fat is markedly extruded. In these individuals, fat tissue was mainly distributed in the superior and lateral-superior segments of the upper eyelid.

The “preaponeurotic fat predominant type” was seen in 16.7 % of the individuals (Fig. 6). The preseptal fat tissue was extremely thin and slender, and nearly invisible, while preaponeurotic fat was mainly located in the middle inferior and medial segments of the upper eyelid.

The “orbital septum equilibrium type” was present in 54.2 % of individuals (Fig. 7). Preseptal fat was less abundant than in individuals who had the “preseptal fat predominant type” and more abundant than in the “preaponeurotic fat predominant type”, and was evenly distributed around the medial, middle, and lateral segments of the upper eyelid, with predominant distribution in the superior middle segment. Preaponeurotic fat was abundant as well.

Tissue Distribution in the Supraorbital Region

Eyebrow fat is the major component in the supraorbital region, extending inferiorly to the preseptal fat, with a large amount of fascia fibers at the boundary. The frontalis muscle, frontalis muscle aponeurosis, and orbicularis oculi muscle are interconnected and localized anteriorly, while both orbital septum and partial preaponeurotic fat are localized posteriorly.

The frontalis muscle fibers in the supraorbital region cross with orbicularis oculi muscle fibers, extend posteriorly, and connect with the orbital septum through the preseptal fat. Preaponeurotic fat remains posterior to the orbital septum (Fig. 8).

Discussion

Plastination Technology

Traditional gross anatomy is a destructive anatomical observation technique which inevitably leads to damages of subtle structures and is incapable of keeping tissue integrity.

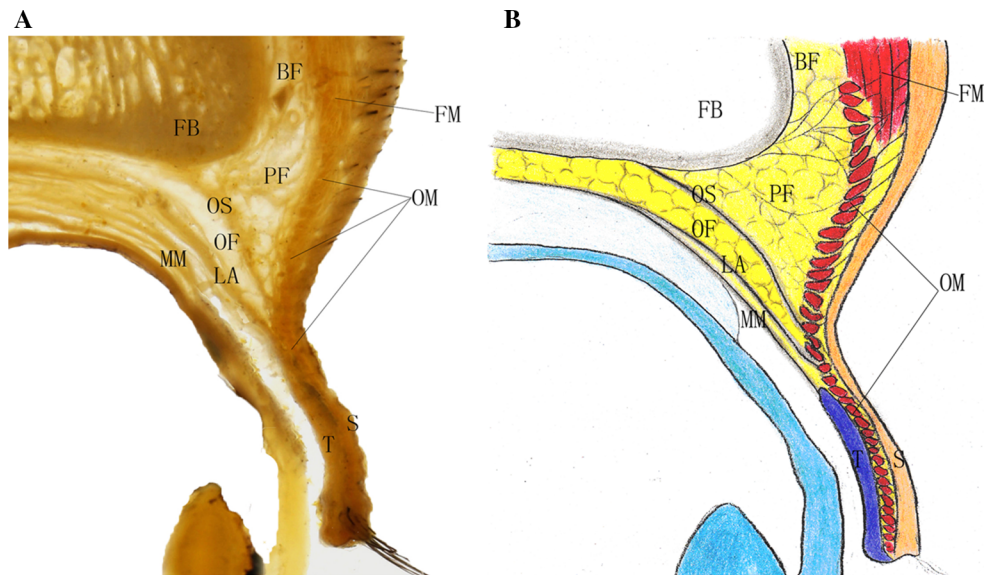


Fig. 2 Preseptal fat predominant type. **a** Preseptal fat is abundantly distributed anterior to the orbital septum, prominently extruded; while the fat posterior to orbital septum extrudes mildly inferiorly–anteriorly. **b** Represents a traced diagram. *OM* orbicularis oculi

muscle, *PF* preseptal fat, *OS* orbital septum, *OF* preaponeurotic fat, *LA* levator aponeurosis, *MM* Muller’s muscle, *T* tarsus, *BF* brow fat, *S* skin

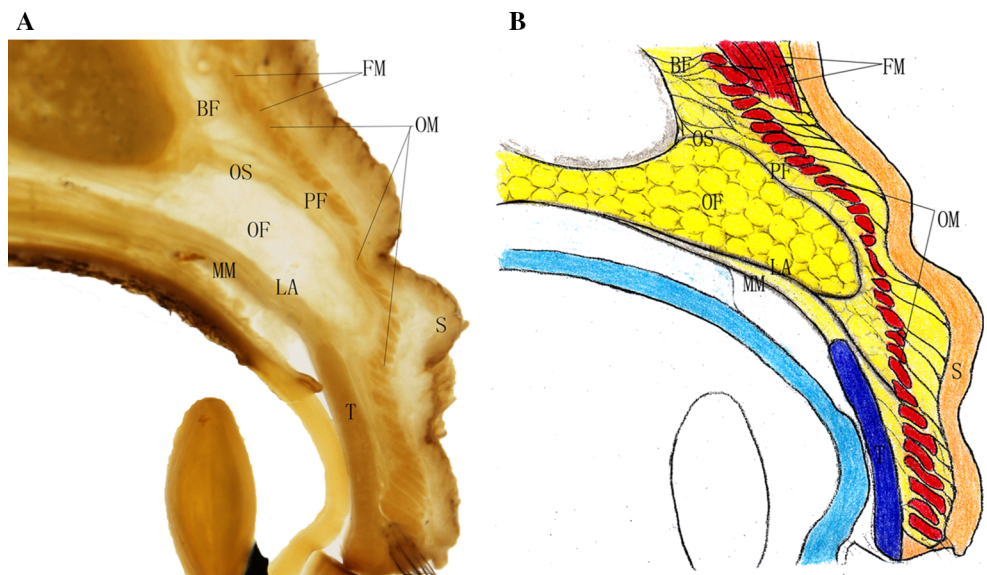


Fig. 3 Preaponeurotic fat predominant type. **a** long, narrow preseptal fat tissue occurring anterior to orbital septum, with abundant preaponeurotic fat markedly extruding inferiorly–anteriorly. **b** is a

Traced diagram. *OM* orbicularis oculi muscle, *PF* preseptal fat, *OS* orbital septum, *OF* preaponeurotic fat, *LA* levator aponeurosis, *MM* Muller’s muscle, *T* tarsus, *BF* eyebrow fat, *S* skin

The accuracy level of traditional gross anatomy can be greatly diminished because of inherent artificial operation factors. On the other hand, histological techniques suffer from the disadvantages of narrow range and limited observations field. With plastic surgery developing rapidly in recent years, past anatomical knowledge cannot warrant the requirements of minimally invasive facial surgery. Therefore, further advancement and refinement of anatomical

knowledge is necessary. The P45 plastination technique is one of such advanced techniques that maintain the tissue structure integrity with clear and transparent specimen characteristics. This technique addresses the limitations of gross anatomy and histological techniques [10] and allows an observation of soft tissue structure on a large area with higher clarity. The P45 plastination technique is also helpful for observing the correlation between tissue structures,

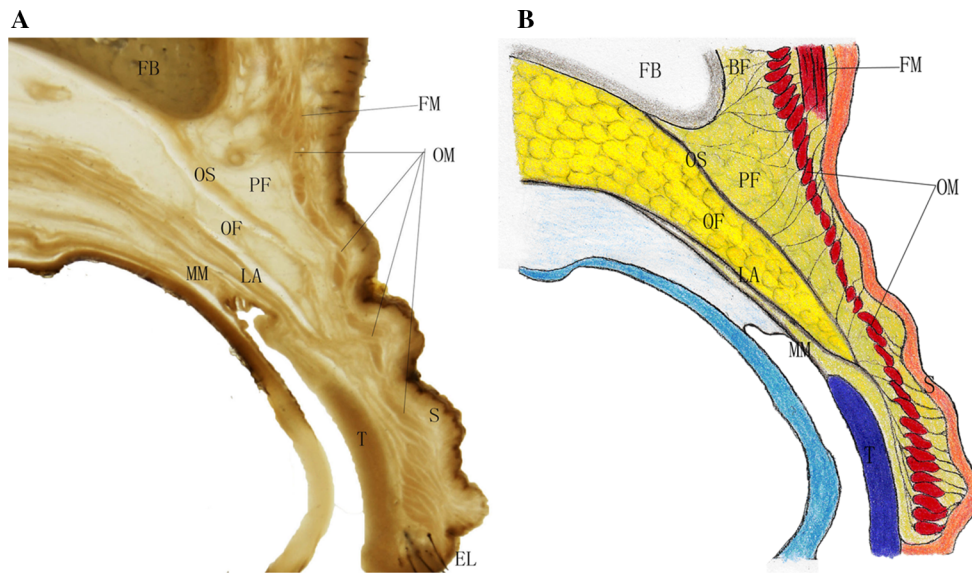


Fig. 4 Orbital septum equilibrium type. **a** Preseptal and preaponeurotic fat are equally distributed on both sides of the orbital septum and have a well-balanced ratio. **b** Represents a traced diagram. *OM*

orbicularis oculi muscle, *PF* preseptal fat, *OS* orbital septum, *OF* preaponeurotic fat, *LA* levator aponeurosis, *MM* Muller's muscle, *T* tarsus, *BF* eyebrow fat, *S* skin

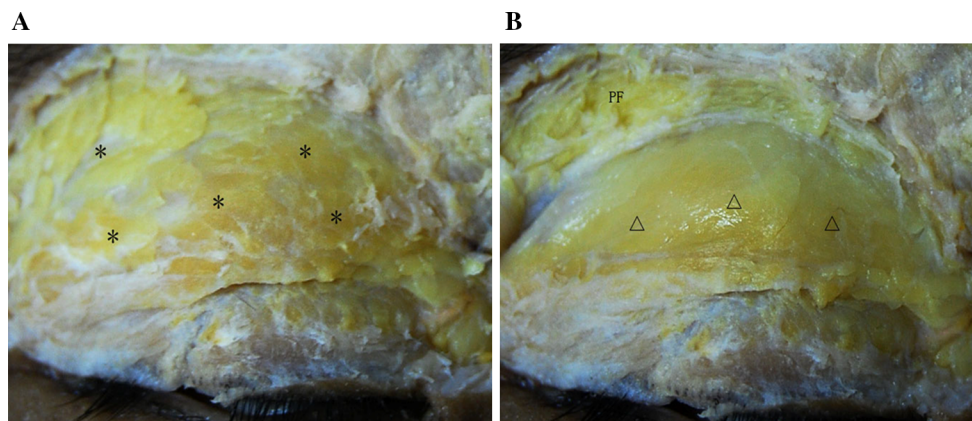


Fig. 5 Gross anatomy of preseptal fat predominant type upper eyelid. **a** Preseptal fat is marked with an *asterisk*. **b** Preaponeurotic fat pad is marked with a *triangle*. *PF* preseptal fat

especially fibrous tissues. Thus, in our study, we used P45 plastination technology to study facial anatomy.

Composition of the Upper Eyelid Adipose Tissue

The soft tissue structure of the upper eyelid varies considerably among individuals. The fullness of upper eyelids is maintained by both preseptal and preaponeurotic fat.

Preseptal fat is located between the deep layers of the orbicularis oculi and orbital septum, and originates from the eyebrow fat pad, reaching the supraorbital nerve medially and laterally above the orbital bone [5, 11–13]. Seiff et al. and Ichinose et al. [14, 15] reported that a very slender piece of fat tissue anterior to the orbital septum was broadly

distributed, reaching the superior area of tarsus. Although preseptal fat is thicker in the eyelids of Asian individuals [16, 17], its explicit nature has not yet been studied. Using 32 P45 plastinated slices, we confirmed that preseptal fat is an independent tissue and contains a relatively high amount of fascia tissue fibers. Preseptal fat extends upward to connect with the eyebrow fat, but has ambiguous boundaries. We further found that the thickness of preseptal fat varies among individuals and can be classified into three categories based on its comparison with preaponeurotic fat. These three categories are more abundant (31.3 %), less abundant (12.5 %), and equivalent (56.3 %) to the amount of preaponeurotic fat. Therefore, plastination of the upper eyelid area differed according to the preseptal fat category.

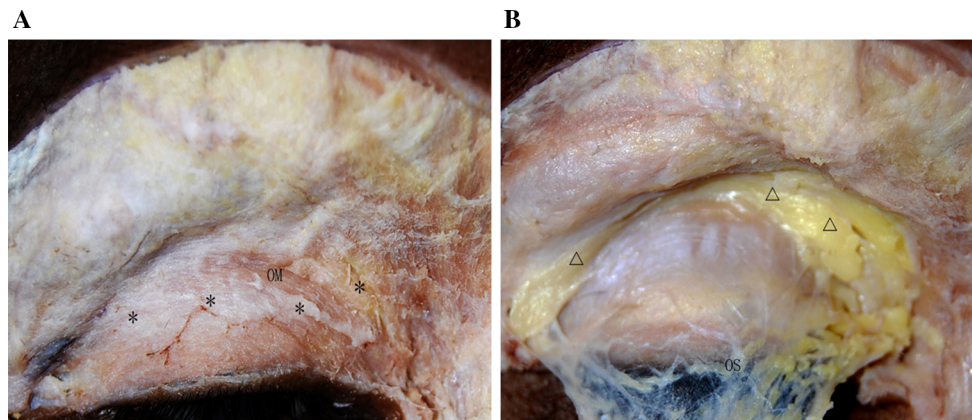


Fig. 6 Gross anatomy of preaponeurotic fat predominant type upper eyelid. **a** Preseptal fat is marked with an *asterisk*. *OM* orbicularis oculi muscle. **b** Preaponeurotic fat pad is marked with a *triangle*

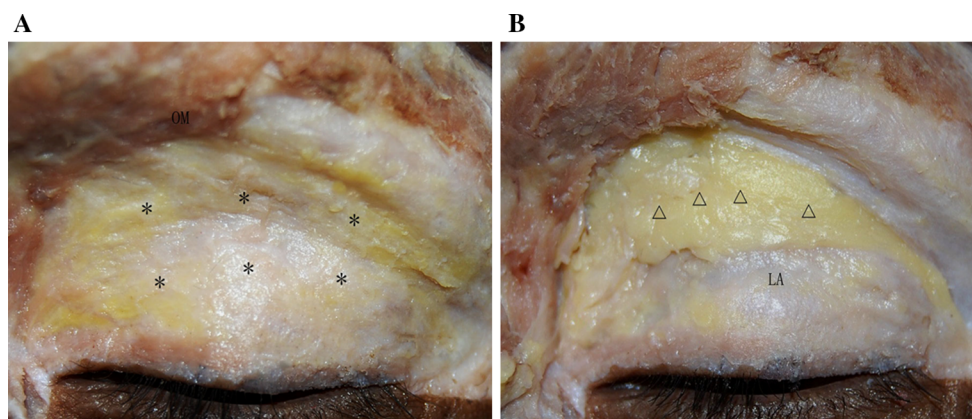


Fig. 7 Gross anatomy of orbital septum equilibrium type upper eyelid. **a** Preseptal fat is marked with an *asterisk*. *OM* orbicularis oculi muscle. **b** Preaponeurotic fat pad is marked with a *triangle*. *LA* levator aponeurosis

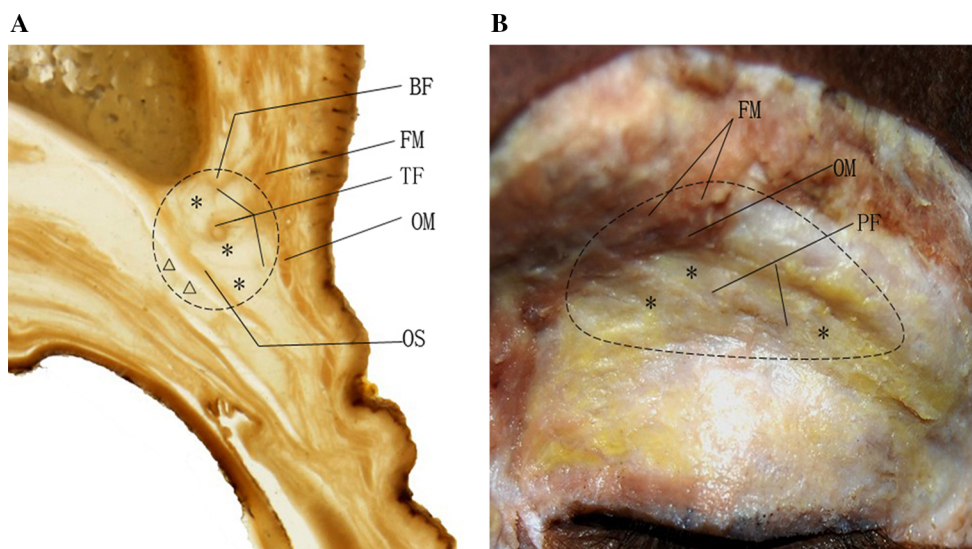


Fig. 8 **a** Specimen picture of a P45 plastinated slice. **b** Picture of gross anatomy specimen. *FM* frontal muscle, *OM* orbicularis oculi muscle, *BF* eyebrow fat pad, *OS* orbital septum. *Asterisk* preseptal fat; *triangle* preaponeurotic fat; *TF* fascial tissue

The location of upper eyelid fillings also varies among individuals. Preseptal fat plays a major role in maintaining the external appearance of the upper eyelid in individuals with the “preseptal fat predominant type”, while both preseptal and preaponeurotic fat contribute equally to the fullness of the upper eyelid in individuals with the orbital septum equilibrium type. Preseptal fat tissue is slender and narrower in the “preaponeurotic fat predominant type”.

Atrophy of preaponeurotic fat can result in a deeper orbita, suggesting a possible role of preaponeurotic fat in the fullness of the upper eyelid [18]. The results of our study show that the amount of preaponeurotic fat was significantly less than that of preseptal fat in individuals of the “preseptal fat predominant type”. The extrusion of preaponeurotic fat was not prominent even in individuals with the “orbital septum equilibrium type”. It mainly extended downward and parallel to preseptal fat, and filled the lower two-thirds of the upper eyelids. Individuals with the “preaponeurotic fat predominant type” had significantly more preaponeurotic fat than preseptal fat. Moreover, it extruded prominently and filled the lower two-thirds of the upper eyelid. In a previous study [1], the factors influencing the fullness of the upper eyelids were the upper eyelid subcutaneous fat, preseptal fat, preaponeurotic fat, and pretarsal fat. Unfortunately, this description fails to effectively distinguish the relative contribution of these factors to a plastic surgery design regarding the location and degree of fullness. It is also difficult to classify upper eyelids based solely on fat distribution. Our findings suggest that the upper eyelid fullness was associated, with a degree of regularity, with the amount of preaponeurotic fat. Preaponeurotic fat contributes more to the lower two-thirds of the upper eyelid in individuals with either the “preaponeurotic fat predominant type” or the “orbital septum equilibrium type”, whereas preseptal fat was predominant in the upper one-third of the upper eyelid in all individuals.

Relationship Between the Type of Adipose Tissue in the Upper Eyelid and Its Sunken Appearance

Previously, it was found that eyeball repositioning and upper eyelid sinking were two causes of sunken upper eyelids [19]. Regensburg et al. [20] measured the fat, orbital bone cavity, and muscle volumes with the software program Mimics in 160 Caucasians. The results showed that fat volume is related to aging. As age progresses, orbital fat volume increases, given a more swollen eyelid appearance. Park et al. [21] studied 50 Asian individuals with sunken upper eyelids by injecting a filling into preaponeurotic fat. The results indirectly proved that the orbital septum degenerates and undergoes atrophy with age, and that it can shift back along the orbital track,

increasing the extrusion of the orbital bone and making people appear more fatigued and aged. Further, these observations confirmed the effect of preaponeurotic fat on the formation of the sunken upper eyelid.

It was reported that there exists fascial tissue between orbicularis oculi muscle and orbital septum in the upper eyelid [22, 23]. In contrast, in the lower eyelid, the presence of fewer fascia in the preseptal fat and less amount of adipose tissue may lead to fragile nature, where the projection of orbital fat easily occurs [24]. It is suggested that this tissue acts as a protective shield to stabilize the orbicularis oculi and orbital septum, with adipose tissue in between, to prevent preaponeurotic fat extrusion. Our findings indicate that individuals with the “preseptal fat predominant type” have relatively more preseptal fat and fascia tissue fibers compared with other types. One could speculate that preseptal fat effectively replenishes the sunken appearance of the upper eyelid after the age-related decrease of preaponeurotic fat. Markedly sunken upper eyelids are most easily found in older individuals of the eyelid adipose tissue of the “orbital septum equilibrium type”. This is because of a lack of complementary effects from preseptal fat after orbital septum atrophy. However, in individuals with the “preaponeurotic fat predominant type”, the upper eyelid may appear bulkier with age for other reasons. First, the prominent amount of preaponeurotic fat, extruding inferiorly–anteriorly, fills the lower part of the upper eyelid. Second, location of preaponeurotic fat was relatively lower and more extruding in Asians compared with the situation in Caucasian individuals. This is because Asians usually have single-fold eyelids, and the junction between orbital septum and levator aponeurosis is lower [25]. Third, the amount of preaponeurotic fat may increase with age [20]. On the other hand, lacking preseptal fat and fascia tissue fails to prevent preaponeurotic fat from extruding forward and cannot replenish the supraorbital region. This factor also leads to sunken upper eyelids, sometimes accompanied by the upper eyelid ptosis.

Soft Tissue in Supraorbital Region

Our findings indicate that both preseptal and preaponeurotic fat work together to maintain the fullness of the upper eyelid. The distribution of preseptal fat focused on the upper segment of the upper eyelid, near the inferior region to the supraorbital region. Part of the preaponeurotic fat was also located in a similar region. Therefore, there is a large amount of preseptal fat, a relatively large amount of preaponeurotic fat, frontalis muscle, and orbicularis oculi colocalized in the supraorbital region and called supraorbital soft tissue (circle imaginary line area of Fig. 8). The movement activity in the supraorbital area increased from the combined action of frontal muscle and orbicularis oculi

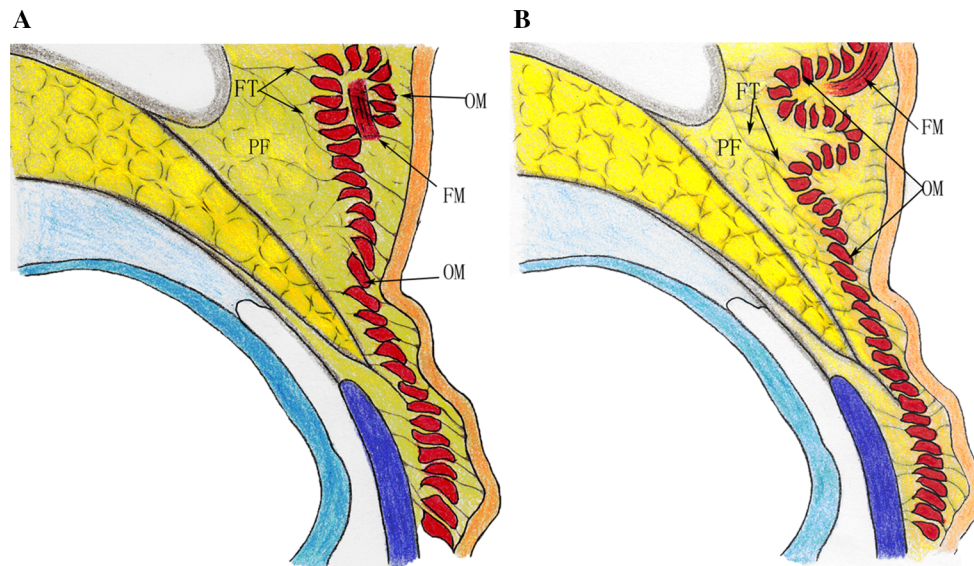


Fig. 9 Map of muscle–fascia–fat flap treatment of sunken upper eyelids. **a** The incision was made inferior to the eyebrow, and subcutaneous tissue was peeled down. The incision was to the preseptal fat to form a muscle–fascia–fat flap, which was folded to fill

the preseptal fat and supraorbital area. **b** The incision and peel were the same on **a**. The padding was used to directly fill the preseptal fat and supraorbital areas. *FM* frontal muscle, *OM* orbicularis oculi muscle, *PF* preseptal fat, *TF* fascia tissue

[26]. The abundant fascial tissue in the preseptal fat protected the preaponeurotic fat and stabilized the morphology of upper eyelid in supraorbital region.

Finally, mildly or moderately sunken upper eyelids can be corrected by modifying the soft tissue in the supraorbital margin.

Guiding Significance for Sunken Upper Eyelids

Previous surgical treatments for a sunken upper eyelid mainly utilized fat granule transplantation [27] and orbital fat release method [28]. We demonstrate an interconnection of the frontalis muscle and orbicularis oculi in the supraorbital area, which was thickened, with a large amount of fascial tissue fibers that prevent preaponeurotic fat from shifting. Therefore, this advantageous supraorbital soft tissue in Asians could be used for the treatment of mild-to-moderate sunken upper eyelids. When eyebrows are surgically lifted, the subcutaneous tissue could be peeled down along the eyebrow incision to form a muscle–fascia–fat flap in the upper eyelid. The padding could be filled directly, or the upper eyelid folded as filler, to successfully treat mild-to-moderate sunken upper eyelids in Asians (Fig. 9).

Conclusion

The P45 plastination is a new technique used to study circumocular soft tissue. The morphology and external appearance of the upper eyelid depend on the distribution relationship between preseptal and preaponeurotic fat.

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