

Ophthalmology Research: An International Journal

14(1): 40-57, 2021; Article no.OR.63505 ISSN: 2321-7227

Facial Reanimation of the Eye Using Neurovascularised Flap of Platysma

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Authors' contributions

This work was carried out in collaboration among all authors with equal contribution from co-first authors GHCL and KM. Authors GHCL and KM designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Author JHYL managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/OR/2021/v14i130185 <u>Editor(s):</u> (1) Dr. Stephen G. Schwartz, University of Miami Miller School of Medicine, USA. <u>Reviewers:</u> (1) Mehmet Erkan Kaplama, University of Health Sciences, Turkey. (2) Ana Luisa González Méndez, Peru. Complete Peer review History: <u>http://www.sdiarticle4.com/review-history/63505</u>

Original Research Article

Received 05 October 2020 Accepted 10 December 2020 Published 06 March 2021

ABSTRACT

Background: Dynamic reanimation is usually required to restore blink function in patients with chronic facial paralysis. In which case, platysma myocutaneous flap (PMF) is a good choice compared to gracilis flap. Platysma is a thin, pliable flap with matching skin colour to the eyelids. Despite this, it is underused.

Aim: To investigate the neurovasculature of platysma in order to find a common "window" containing nerves and blood vessels supply which is present in every individual. This will aid the plastic surgeons to reconstruct the neurovasculature of the flap for grafting onto the eyelids.

Methods: 3 fresh cadaver necks were dissected from 1 males and 2 females, aged 75-88 years old; (n=6 platysmas). 43 squared specimens (measuring 1.5cm x 1.5cm) surrounding any potential neurovascular structures were cut out, processed and analysed under high power microscope to confirm the presence of nerves and blood vessels. We also reviewed literature dated from 1999 to 2011.

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Results: From the literature reviewed, the authors concluded that PMF provided excellent functional and aesthetic outcome. In this study, we discovered a "window" flap (ranging from "2.5cm x 3cm" to "8cm x 10cm") bilaterally on each cadaver. This window is supplied by submental branch of facial artery, drained by facial vein, anterior and external jugular veins, and extensively innervated by cervical branch of facial nerve.

Conclusion: We strongly advocate the use of PMF "window" by plastic surgeons in dynamic eyelids reanimation.

Keywords: Platysma; blink function; facial reanimation; neurovasculature.

1. INTRODUCTION

What is facial paralysis? It is complete loss of voluntary facial muscle movements due to facial nerve (CN VII) palsy (Changing Faces, 2007). Facial paralysis can be caused by various aetiologies ranging from congenital, idiopathic, trauma, infections, neoplastic to iatrogenic [1].

In 2007, "Changing Faces" discovered that 100, 000 people in the UK have disfigurement due to facial paralysis (Changing Faces Research Council survey, 2007), 75% of these cases are caused by idiopathic Bell's palsy. Annual incidence of Bell's palsy in the UK is 1 in 5000 people and it most commonly affects patients aged between 10 to 30 years [1-4] strongly suggested that herpes simplex viral infection is the cause of Bell's palsy. Vascular ischaemia, autoimmune inflammatory disorders and heredity conditions have also been proposed as the underlying cause by [2] and [4] 85% or two thirds of patients who received no treatment will recover spontaneously within 9 months of onset. The remaining 15% have residual paralysis with synkinesis [5]. The most effective reported treatment for patients (aged over 16) suffering from idiopathic Bell's palsy is the administration of steroids within the first 72 hours of symptomatic onset (using prednisolone 25 milligram, twice daily) [6,7]. This has also been advocated by many current studies [5,8]. However, the effectiveness of this treatment is still questionable and inconclusive as Bell's palsy often resolves spontaneously [5].

Besides this, head trauma is also a common source of facial paralysis accounting for 20% of the cases [9] The site of trauma can be anatomically subcategorised into intracranial and extracranial. In extracranial trauma, skull base and temporal fractures are often missed whilst treating more life-threatening injuries. These fractures and cerebrospinal fluid fistulae could injure cranial nerves and lead to facial paralysis, blindness, deafness or meningitis. Early

detection and management of these injuries would decrease the severity of facial nerve palsy and other morbidities [9]. Whereas intracranial trauma caused by for example the resection of vestibular Schwannoma (i.e. benign primary tumour of the vestibulocochlear nerve) will result in delayed onset of facial paralysis (Arriaga et al., 1993).

The remaining 5% of the cases arise as a result of rarer aetiologies. One of them is resection of malignant head and neck tumour (e.g. meningioma, facial nerve tumour, etc) [10] Besides that, iatrogenic causes such as malignant parotid tumour ablation and influenza A vaccination could damage the facial nerve [11,12]. Occasionally, Möebius syndrome can also lead to facial paralysis (Westin and Zuker, 2003). It is manifested by paralysis of the eyelids and reduced facial muscle functions resulting from congenital facial and abducens nerves palsies [13]. In addition, Lyme disease is the commonest infectious cause of facial paralysis caused by Borrelia burgdorferi spirochetes [14]. Patients will present early with a localised skin lesion with erythema migrans and paralysis of cranial nerves especially facial nerve [15].

Facial nerve palsy may occur unilaterally, bilaterally or even appear in the form of partial impairment - 'paresis' [15]. Like any other medical condition, a thorough medical history is essential to diagnose the underlying cause which should include information about the number of episodes, time of onset, concurrent viral or upper respiratory tract infection, pain behind the ears, facial weakness, appearance of vesicles, influenza A vaccination, ageusia (i.e. loss or change of taste) and past medical history of facial paralysis [11,12]. [12] have suggested Guillain Barre syndrome or Lyme disease to be considered in bilateral paralysis; and lymphoma, sarcoidosis or lyme disease in recurrent paralysis. However, the primary concern for facial paralysis in children is either Lyme or middle ear disease [12,16]. It is also imperative

to differentiate between the upper and lower motor neuron lesions, which can be clinically tested by asking the patient to raise their eyebrows. If the eyebrows are able to move normally but the lower face is paralysed, this means that the facial nerve in the regions within the brain is injured as a result of an upper motor neuron lesion. Lower motor neuron lesion affecting facial nerve would cause complete facial paralysis [17,18].

Facial paralysis manifests in different ways ranging from mouth sagging, ptosis. lagophthalmos (i.e. incomplete eye closure), dry eyes, flattened nasolabial fold, difficulty in articulating speech to drooling. This could impose debilitating consequences on the psychosocial aspect of patients' life leading to social isolation [19]. Other common symptoms accompanying facial weakness are synkinesis (i.e. involuntary movement of muscles with every voluntary movement), hyperacusis and facial pain (particularly behind ears) [20-22]. Patients also lack facial expression which is an integral part of everyday communication. As a result, they may face severe psychological stigma imposed by the society [22]. The worst case scenario could potentially be permanent blindness due to increased corneal exposure to insults, reduced tears production and corneal ulceration [23,19]. Hence, treatment should aim at limiting eye exposure through static or dynamic reanimation of the eyelids.

Static reanimation helps in controlling the aforementioned symptoms of both temporary and long-term facial paralysis. Static procedures may be combined with dynamic reanimation to improve the outcome in long-term paralysis [24]. Static therapy can re-establish the facial symmetry and protect the cornea but the natural facial expression will not be restored to normal. This technique can be approached from various ways: 1) Brow ptosis correction, 2) nasolabial fold alteration, 3) static facial sling support, and 4) strengthening of the external nasal valve [24-26]. A few static reanimation methods have been identified to specifically correct the paralysed evelids. For example, lateral tarsorrhaphy can be a permanent measure to bring the upper and lower lid tarsal plates together using mattress sutures. This reversible method is commonly used in cases of corneal keratitis or loss of corneal sensation and lagophthalmos [22]. In addition, thin profile platinum weights are more frequently opted for lagophthalmos repair (i.e. inserted into upper eyelid) compared to gold

implants because of its better cosmetic results and lower allergy risk [27]. Whereas for lower lid ectropion (i.e. drooping of lower eyelid), the lower tarsus can be trimmed and sutured to the lateral orbital rim periosteum. This technique is called lateral canthotomy and inferior crus cantholysis. On the medial end, medial canthopexy can be carried out by suturing the medial tarsus to the periosteum of lamina papyracea [28,23]. In short, static facial reanimation helps to restore defected visual fields, prevent cornea ulceration, reduce synkinesis, relief nasal obstruction and oral dysfuntions. Although quality of life is improved, the underlying problem still exists. Patients still could not move their facial muscles voluntarily to express themselves as the muscles were fixed in a position [27].

Dynamic reanimation is the key to restore voluntary facial movements. It is done by neurotisation (i.e. direct nerve repair/primary neuroraphy, cable nerve grafting and crossover nerve transfer), muscle transposition or free muscle flap transfer. Application of these treatment modalities depend on the duration of facial paralysis [7,29,30]. Acute facial paralysis occurring less than 3 weeks after onset may be treated with primary neuroraphy or cable nerve grafting if the static therapy failed [31]. However, if this method does not work out well or the proximal facial nerve segment is damaged near the brainstem, cable nerve grafting would be the next best option in minimising functional deficit. The great auricular nerve is frequently chosen for nerve grafting due to its close proximity to the facial nerve [2]. Nonetheless, both methods of facial nerve repair produce the same functional outcome - House-Brackmann Grade 3 [2,29]. Crossover nerve technique is recommended if facial paralysis occurs after surgical removal of a neuroma. In this case, the facial nerve is overstretched with subsequent slow recovery period (between 3 weeks and 2 years) [32]. There is a general consensus that the hypoglossal nerve to facial nerve crossover transfer is the most preferred option compared to other nerves [33], Hadlock et al., 2005) because of low probability of having post-op complications [7]. When the severity of facial paralysis does not fit the criteria of neural treatments, muscle transposition technique is the choice of procedure. This technique is used in a) absence of facial neuromuscular system (seen in congenital Moebius syndrome), b) long facial nerve recovery period (i.e. more than 3 years), c) destruction of motor end plates, and d) when no other cranial nerve is available for crossover

To overcome the shortcomings of muscle transposition, free muscle flap transfer technique can be employed. Normally, this method is approached to treat chronic facial paralysis lasting more than 6 months coupled with minute hope of nerve functional recovery [34]. There are a few muscles of choice like gracilis, pectoralis minor, serratus anterior, latissimus dorsi, and extensor digitorum brevis [35]. Many authors recommended the use of gracilis as the first choice for facial reanimation as it is a long, thin muscle. Free gracilis muscle flap has been used to reanimate the eyelid and subsequently portrayed good results [30]. For unilateral facial paralysis, dynamic reanimation is carried out in two stages. Stage one is the crossover of facialsural nerve where sural graft is harvested and coapted with the contralateral normal facial nerve. Six to twelve months post-neurotisation. stage two is carried out where the gracilis muscle is harvested and sutured to the paralysed side of face. Arteries and veins are anastomosed to the facial vessels or superficial temporal vessels. The obturator nerve innervating gracilis muscle flap is connected to the distal end of sural nerve graft which was coapted at the proximal end of facial nerve during the first stage. Normally, facial muscle functions are expected to return in 6 months time but it may take longer in some cases [36]. On the other hand, bilateral facial paralysis (e.g. congenital Moebius syndrome) can be treated in one stage. In this case, the masseteric branch of the trigeminal nerve must function normally so that it can be used to innervate the gracilis muscle [36,37].

Platysma flap is an alternative choice to all the muscles mentioned above. The application of PMF is first proposed by Harii in 1976 (Harii et al., 1976). Since its introduction, it has been extensively used for intraoral reconstruction to replace buccal mucosa, floor of the mouth and pharyngeal wall (Szudek and Taylor, 2007), [38]. recommended the use of platysma flap in restoration of blink response as it is a thin, pliable flap and a perfect match of the eyelid's skin Despite all its colour. of favourable characteristics, its use is not commonly practiced for blink restoration (Koch et al., 2002).

There are three types of platysma flaps: (1) superiorly based flap with good arterial supply by submental artery (which is the largest branch of facial artery), innervated by cervical branch of facial nerve, but poorly drained by the submental vein into internal jugular vein; (2) posteriorly based flap with good venous drainage into external jugular vein, but poor arterial supply by superior thyroid artery, occipital artery and posterior auricular artery, and without innervation; (3) inferiorly based flap which is perfused by transverse cervical artery but has no role in facial reconstruction [39-41]. The superiorly based platysma flap is thought to be the best for orbicularis oculi dynamic reconstruction as it has good submental arterial supply and well preserved motor innervation [41]. Although its venous drainage through the submental vein is poor, it still has all three components compared to the denervated posterior flap [39,Baul et al., 2002, [41].

Free muscle flap transfer has some complications such as loss of distal skin flap at the donor site, postoperative fistulas, and flap venous congestion. The most disconcerting complication of free platysma flap transfer is skin sloughing at the recipient site. But, there is no significant harm done to the underlying muscle when used in intraoral reconstruction as it remains viable and epitheliasation occurs [39,40,Agarwal et al., 2004). These complications can be conservatively managed to enhance the functional and cosmetic results. For instance, hyperbaric oxygen and leeching treatment of venous congested flap will significantly increase flap survival rate (Lozano et al., 1999). Wayne et al. (2009) reported that the neurovascularied PMF was an underused but advantageous alternative in reconstruction of head and neck defects. [41] also recommended PMF for its thinness, pliability, low donor and recipient site morbidity rates and easy access to the donor site.

Therefore, this project aims to find a "window" on the platysma flap which contains nerve and blood vessels with an intention of aiding plastic reconstruction surgeons in of their neurovasculature pedicle to restore blink response. Further investigations are required to test the reliability of this "window" for eyelid reanimation in a clinical setting, to measure the functional outcome using the gold standard House-Brackmann scale (House and Brackmann, 1985) and to evaluate the aesthetic results by comparing pre-operative and postoperative digital photos.

2. MATERIALS AND METHODS

2.1 Materials

All the cadaveric materials used in this research were provided by the Laboratory of Human Anatomy, University of Glasgow.

2.1.1 Cadavers preparation

We dissected a total of 3 embalmed cadavers (1 male, 2 females) and obtained 6 platysma flaps (n=6).

Cadaver 1: This was a female of 88 years of age when died from cardiogenic shock. The inferior half of the cadaver's right neck had been operated by a cardiothoracic surgeon for cardiovascular research. So, the inferior half of the right platysma and the neurovascular structures of the neck from that level to the clavicle were damaged. Therefore, only the superior half of the right platysma was available for this project. A midline incision from the suprasternal notch to the xiphoid process had been introduced and sutured by the surgeon as well.

Cadaver 2 and 3: Cadaver 2 was a 75 year-old male who died from pneumonia; Cadaver 3 was an 87 year-old female whose cause of death was bronchopneumonia. The condition of their platysma flaps and neurovascular structures of the necks before dissections were intact. No other craniofacial or neck abnormalities were noted in the cadavers.

The cadavers were embalmed by qualified anatomists according to protocol. For reference, refer to Appendix 1.

2.1.2 Dissection instruments

For dissection, we used size 4 scalpel with size 24 blade, size 3 scalpel with size 11 blade, blunt and fine forceps, small dissecting scissors and different colours of fine threads.

2.1.3 Histological processes

We employed Leica tissue processor 1020, Raymond A Lamb blockmaster II, Leica RM 2035 microtome, thermostat controlled heated water bath, standard protocol for Masson's staining (refer to Appendix 3), AxioScope Zeiss microscope with camera attached, fluorescence filter, brightfield filter and AxioVision Release 4.8 software to process the images captured.

2.1.4 Documentation of results

Pictures of the specimens at different stages of the research were captured using Canon EOS 550D Digital SLR and Nikon Coolpix 4500. The photos were uploaded to Adobe Photoshop Elements 7.0, Image J and Microsoft Word 2003 for further analysis.

2.2 Methods

2.2.1 Platysma dissection

The cadavers were lying supine with their neck in an extended position. Fig. 1. outlines the incisions (black dotted lines) made to expose the neurovascular structures underneath the platysma. Firstly, an incision as deep as skin layer was made along the midline from the mental protuberance to suprasternal notch using a size 4 scalpel. On both sides of the neck, horizontal incisions were extended laterally along the lower border of body of mandible from the mental protuberance to the angle of mandible. Another horizontal incision was performed bilaterally starting from the suprasternal notch, and running along the superior border of the clavicles as shown in Fig. 1. Subsequently, the lower edge of the skin was reflected upwards and anteriorly from the suprasternal notch using size 3 scalpel and fine forceps. In this process, fascia and adipose tissues immediately inferior to the skin were separated following the outlined incisions. Upon reflecting the skin, the platysma muscle could be appreciated. In order to define borders of platvsma, size 4 scalpel was used to remove off excess adipose tissue covering it. Once the platysma muscles were fully exposed on both sides, the lateral and medial border were demarcated. Again starting from the suprasternal notch, blunt dissection technique (using fine forceps, blunt forceps and small dissecting scissors) was employed to reflect the platysma upwards and anteriorly from the suprasternal notch. While carefully reflecting the platysma, nerves or blood vessels found were cut using small dissecting scissors. The severed nerves or blood vessels were tied proximally and distally to the cut with different colours of threads. This step was very important to identify and trace the origin of neurovascular structures once histological confirmation was made.

Once platysma was reflected up to the mandible level, a size 4 scalpel was used to make a horizontal incision (as deep as the platysma layer) from its medial border at the mental protuberance, across the body of the mandible to



Fig. 1. Skin and platysma layers were separated and reflected anteriorly. A: Angle of mandible, B: Body of mandible, M: Mental protuberance, S: Suprasternal notch and C: Clavicle

the angle of mandible. At this point, a photo was taken with a scale placed next to it for measurement of the window. Next, the platysma flap was cut out completely along its lateral border from the angle of mandible to the clavicle. This isolated platysma flap was placed on a piece of white A4 paper with its posterior aspect facing anteriorly. All the neurovascular structures and outline of the flap were traced onto the paper using pencil. Another photo is taken.

2.2.2 Histology

From the flap, a size 4 scalpel was used to cut out 1.5cm x 1.5cm squares around the entry point of every potential neurovascular structures that were tied off with threads. These squared specimens were processed over 24 hours (refer to Appendix 2), embedded in paraffin wax (Appendix 2) and stained using Masson's trichrome dye (see Appendix 3) according to their respective protocols. Following this, the mounted slides were viewed under AxioScope Zeiss microscope with attached camera and connected to the computer (with uploaded AxioVision Release 4.8 software). Photos of the blood vessels and nerves were taken under low magnification (i.e. 2.5x) and then at a higher magnification (i.e. 20x) to differentiate between an artery and vein. Then, a fluorescence filter was used to replace the brightfield filter on the

microscope. The fluorescence filter was also used to highlight the internal elastic lamina.

2.2.3 Tracing of the blood vessels and nerve

The origins of a few arteries were not certain. In order to establish the identity of the arteries (i.e. superior thyroid, facial or submental arteries), a small dissecting scissors and a fine forcep were used to dissect bluntly from the platysma flap and were traced back to their origin.

A single main vessel (i.e. common facial artery) was identified halfway lateral to the submandibular gland (inferior to the body of mandible). This vessel branched out medially into two smaller vessels (i.e. facial and submental arteries). As the facial artery travels superiorly to the body of mandible, it gives off a few branches to supply the platysma. The other branch (i.e. submental artery) gives off branches to supply the platysma whilst running medially, into and out of the submandibular gland.

The veins draining from the platysma flap were quite superficial and easily identified. In order to identify the cervical branch of facial nerve, the dissection was started from the parotid gland and traced downwards to the neck. This was done with extreme care not to damage the nerves in the parotid gland while removing the gland bit by bit to find the delicate cervical branch.

2.2.4 Documentation of results

All the stages of tracing the neurovascular structures, position of the flap and the cut out squares were photographed using Canon EOS 550D Digital SLR and Nikon Coolpix 4500. The photos were utilised in this dissertation by uploading them onto Adobe Photoshop Elements 7.0 and Microsoft Word 2003. The true interpretations of the results were not distorted in the process. Image J software was used to measure the size of the "window" flap and its distance from the landmarks (i.e. superiorly lower border of mandible body, inferiorly - one third of the clavicle's medial shaft, medially midline of the neck from mental protuberance to the suprasternal notch) were marked in this project.

2.2.5 Clinical application

We collected evidence of PMFs' success rate, complication rate, donor site morbidities rates and advantages in reconstructive surgeries from retrospective literature dated from 1999 to 2011.

3. RESULTS

3.1 Tracing of the Blood Vessels and Nerve

Fig. 2 displays a posterior view of a dissected left platysma flap (from cadaver 1) with its arteries, veins and nerve exposed. These structures were tied off distally with different coloured threads at the entry point before the platysma and highlighted in this picture with dotted circles (– red circles representing arteries, light blue encircles veins and yellow circles surround the nerves)

As a result of tracing the origin of the neurovascular structures in the neck, three arteries were found to supply the platysma (i.e. facial, submental and superior thyroid arteries). The facial and submental arteries supply the flap superiorly, whilst the superior thyroid artery supplies infero-medially. Tributaries of three veins were also discovered to drain from the platysma (i.e. facial and anterior jugular veins drain midsuperior flap, whereas external jugular vein drain infero-medially). Most importantly, the cervical branch of facial nerve was identified to he innervating the mid-superior and supero-medial section of platysma myocutaneous flap.

Fig.3 is a dissection photo of the left neck of Cadaver 1 with the neurovascular structures (highlighted in circles corresponding to Fig. 2) beneath the platysma uncovered. This is a result of reflecting the overlying skin layer and platysma flap.

As mentioned earlier, the vessels and nerve were traced back proximal to the origin. In this picture, branches of three arteries were noted to supply the platysma flap – submental, facial and superior thyroid arteries [42-45]. The submental artery is a branch of facial artery. Before entering the submandibular gland, submental artery branches out from the facial artery and gives off small branches to supply the platysma. Superior thyroid artery arises from the external carotid artery at the level of hyoid bone and descends towards the thyroid gland at mid-medial section of the anterior neck. Tributaries draining from the platysma flap into the external jugular, anterior jugular and facial veins are highlighted in this figure. The anterior jugular vein starts draining from the inferior aspect of the anterior neck and travel on the anterior surface of the platysma. Then, it branches into two at the mid-medial section of the posterior platysma flap - (1) one branch drains into the external jugular vein (i.e. lateral to the anterior jugular vein), and (2) the other branch pierces through the flap to the underlying surface, continues superiorly and anastomoses with the common facial vein to drain into the internal iugular vein. At the anterior triangle of the neck, the cervical branches of facial nerve overlie the sternocleidomastoid and extend towards the midline [46-48].

All the other specimens were documented in the same approach as the results portrayed in Fig. 2 and 3. Following this, 1.5cm x 1.5cm squares were marked around suspected neurovascular structures. A total of 43 squares were obtained for histological confirmation. Following this, all of these blocks were confirmed to have either blood vessels, nerves or both in the platysma flap. 24 of these had nerve and arterial branches, 14 nerve squares contained branches and tributaries to veins, whereas the remaining 7 blocks had all the neurovascular structures. From all these specimens, the associated arteries, veins and nerve with platysma were identified and tabulated in Table 1.

3.2 Histological Confirmation

The longitudinal section of cervical branch of facial nerve can be easily identified (shown in Figs. 4. and 5). Its outermost layer (epineurium)

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was rich in connective tissue. Therefore, it stained green or deep blue by Masson's dye (Appendix 3). The next inner layer (i.e. the perineurium surrounding each individual fascicle) formed a grey coloured epithelial layer. In this sheath, there was an endoneurium layer made up of loose connective tissue which enveloped stacks of axons, flattened Schwann cells, small arterioles and venules and type 1 collagen fibres (Stevens & Lowe, 2004). The connective tissues and collagen fibres in this layer were stained green, while the axons and Schwann cells were stained red (Sasaki et al., 2009). On the other end, the skeletal muscles were also stained red.







Fig. 3. Illustrates the anterior view of left neck with reflected skin and platysma. All the blood vessels and nerves identified were tied off proximally in this figure and labelled – A: tributaries to Anterior Jugular Vein, C: branches of Cervical branch of Facial Nerve, CF: common facial artery, E: tributaries to External Jugular Vein, F: branches of Facial Artery, S: branches of Submental Artery, T: branch of Superior Thyroid Artery, V: tributaries to Facial Vein

Specimens Structures	1		2		3	
Sides	Right(R)	Left(L)	R	L	R	L
ARTERIES	u ()					
Submental/ Facial	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
Superior thyroid	×	\checkmark	\checkmark	\checkmark	×	×
VEINS						
Facial	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
External Jugular	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
Anterior Jugular	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
NERVE						
Cervical branch of facial nerve	\checkmark	\checkmark	√	\checkmark	\checkmark	\checkmark

Table 1. Shows the arteries, veins and nerve associated with platysma flap

x = No supply to the platysma

✓ = Supplies the platysma



Fig. 4. Histological section of branches from submental artery, tributaries to external jugular vein and a branch of cervical nerve shown at low magnification (2.5x). Abbreviations: A: arterial branch, V: tributary to vein, N: nerve branch, & S: Skeletal muscles

In Fig. 5. the structures of artery and vein were differentiated using a brightfield microscopic image at high magnification (20x). The arterial wall was more muscular and thicker compared to vein. This was portrayed much clearer in Figure 6 using a fluorescence microscopic image at high magnification (20x). The fluorescence filter highlighted the presence of an internal elastic lamina (IEL) in the arterial wall, but this was absent in the vein as shown in Fig. 6.

3.3 Location of the "Window" Flap

Pictures of all three cadavers with their individual repositioned platysma flaps on the anterior neck were taken and uploaded onto Image J in order to measure the windows' sizes and distances from the landmarks marked in red dots (shown in Fig. 7. & Table 2). The average size of the smallest window measured using Image J was 2.5cm x 3.0cm. This window could be extended up to 8.0cm x 10.0cm. These "windows" were richly supplied by submental artery, drained by anterior jugular vein and extensively innervated by cervical branch of facial nerve.

3.4 Clinical Application

A surgical technique has been proposed by [41] in intraoral reconstruction. An elliptical skin area (i.e. at least "4 x 2 cm in diameter") in the middle of a hyperextended anterior neck, away from the midline was outlined. Then, a horizontal incision (of platysma including superficial fascia colli) 1cm below the inferior edge of the outlined elliptical skin area was made and extended in two directions (i.e. towards the midline and the anterior border of sternocleidomastoid respectively). This was followed by reflection of the pedicle inferiorly down to 2-3cm above the clavicle (exposing the rest of the platysma muscle) and then superiorly with adequate length to fold over the mandible for intraoral cavity access. During the reflecting process, the facial artery and vein were ligated and the submandibular gland was removed [41]. This technique has been proven to produce equally excellent aesthetic results and near-normal function (i.e. House-Brackmann Grade III) of the paralysed eyelids and orbicularis oris compared to free gracilis flap [40, Nikolaos et al., 2007).

The clinical application of the platysma flap "window" was discussed with Mr S. Morley, consultant plastic surgeon at the Canniesburn Plastic Surgery Unit, Glasgow Royal Infirmary about the surgical method for eyelids reconstruction. He proposed a different method for it. This was performed in two stages. In the first stage, a segment of great auricular nerve lying in close proximity to the damaged facial nerve was isolated and harvested. Then, it was cross-grafted to the healthy proximal facial nerve segment and rested near the orbit. Following this, a period of at least 6 months was allowed to lapse for neurotisation to occur. Subsequently, moving onto the second stage, a pre-auricular incision was done and extended superiorly along the hairline [49-52]. The incision was then continued inferiorly downwards, several inches into the anterior neck and crossed the neck horizontally along the crease towards the midline. This would result in less scarring and faster healing [53,44]. The skin above the incision at the neck was reflected anteriorly. Following this, the surgeon would have to look for the nerve, artery and vein supplying a 4cm x 4cm elliptical area on platysma. Before incising and harvesting this demarcated elliptical area of platysma flap, the vessels and nerve branches would be ligated and severed. This flap would be shaped into a trousers graft. Following this, the skin and connective tissues covering the orbit was raised. The platysma flap was inserted through the pre-auricular incision. Each end was attached to the superior and inferior region of orbicularis oculi respectively. This was also held by the temporalis fascia so that the muscle would contract and pull the eyelids backwards leading to eye closure. A few more incisions would be needed on the medial eyelids to pull the trousers graft through. Finally, the ligated blood vessels were attached to the superficial temporal branches, whilst the nerve was attached to the cross-graft.



Fig. 5. Histological section of Fig. 4 (focusing at the dashed red box) at a higher magnification (20x). It contains an artery, vein and nerve. Abbreviations: same as Fig. 4

Fig. 6. Histological section of Fig. 5 at the same magnification (20x) under fluorescence filter. Abbreviations: IEL – internal elastic lamina, A – artery, V – vein & N – nerve



Fig. 7. Shows the location of the "window" flap outlined by dashed red box. Abbreviations: MP – mental protuberance, AM – angle of mandible, C – one third length of clavicle, SN – suprasternal notch

	Distance of the "window" from the landmarks (cm)						Average distance	
Cadavers	1		2		3		(cm)	
Landmarks								
Sides	Right(R)	Left(L)	R	L	R	L	R	L
Superior								
Body of mandible	2.0	3.0	4.0	4.0	4.0	3.5	3.3	3.5
Medial								
Midline of the body(i.e. along mid-	3.0	2.0	2.0	2.0	2.0	3.0	2.3	2.3
mental protuberance to mid-								
suprasternal notch)								
Inferior								
One third of the medial clavicle shaft	7.0	6.0	5.0	5.0	5.5	7.0	5.8	6.0

Table 2. Distance of the "window" from demarcated landmarks

4. DISCUSSION

Blink reflex is an essential involuntary movement of the orbicularis oculi that protects the cornea from insults like intense lights and foreign objects [24]. This reflex is normally absent in facial paralysis. In acute facial paralysis lasting less than 3 weeks after the onset, authors recommended direct neurotisation and static procedures (e.g. platinum weight lid-loading and lateral tarsorraphy). But, these treatments could only achieve a functional outcome of House Brackmann scale 3 (i.e. good symmetry at rest and strong eye closure but no voluntary movement can be achieved) [25,31]. If there is residual paralysis that progressed to longer than 3 years, static procedures and neurotisation are insufficient to relief the symptoms of corneal keratopathy and may lead to permanent blindness [38]. Hence, direct neurotisation can no longer be an option. This is due to degenerative changes of the damaged facial nerve, orbicularis oculi atrophy near the lesion and silent electromyography. Most importantly, patients expect to recover their blink response especially in the younger folks as this will enhance their quality of life (Ylikoski et al., 1981). In this case, cross-facial nerve grafting followed by free muscle flap transfer procedure is indicated. Therefore, treatment should be tailored patients to individual and planning should also into surgical take consideration of patients' age, health conditions, expectations. concerns, pre-operative electromyographic and electroneurographic results (Bienstock et al., 2009; [24].

For most reconstruction surgeries of intraoral and oropharyngeal defects using free muscle transfer technique, many authors would recommend gracilis, pectoralis minor or temporalis muscles. However, we found that the platysma flap is most suitable for eyelids sphincter substitution because platysma originates from the same second branchial arch of the embryologic mesoderm as the frontalis and orbicularis oculi [24].

Currently, there only few are а literature addressing the use of free platysma flap in dynamic reanimation of eyelids [54,55,Koch et al., 2002,24]. The use of platysma as a free flap for muscle transfer was first introduced by Futrell et al. in 1978. Ever since then, the popularity of platysma flap is limited and it is also an underused option although platysma flap has been used in reconstructive surgeries for over 20 years (Willer et al., 1992; Koch et al., 2002). However, a few authors went on to further investigate the neurovasculature of platysma and promote the use and advantages of free platysma flap in dynamic facial reanimation (i.e. thin, pliable, large enough to close 70cm² defects, minimal donor site morbidities. etc) (Koch et al.. 2002: Ariyan, 2002; 40; Nitzan et al., 2005; 41; Szudek et al., 2007 and 24, Koch et al. (2002) retrospective review performed а of medical records dated from 1987 to 2001 which involved 34 patients aged 24 to 81 years old. They had undergone intraoral reconstruction using pedicled platysma flap. This resulted in low post-operative complication rate of 10%-20% and mostly self-resolved. There is 100% flap survival rate, modest cost and no complication sequelae of neck function from this trial (Koch et al., 2002). [24] performed two trials in 2010 consisting of 20 adults (mean age of 34.9 years) and 14 children (mean age of 7.5 years) respectively, who underwent dynamic blink restoration using free platysma flap. [24] advocated that platysma flap is a viable alternative for muscle transfer.

4.1 Results of Findings

Neurovascular bundles supplying the platysma flaps are shown in Table 1. There is a slight variability in the arterial supply, whereas the rest are quite consistent in our findings.

The main artery supplying the all platysma flaps is submental artery, followed by facial artery and occasionally, the superior thyroid artery. [39] supports this result where majority of platysma flaps are supplied by submental artery. The platysma also received dominant supply from facial artery (Cartier et al., 2009). Generally, these arteries supply the supero-medial area of the platysma flap, whereas the infero-medial section is maintained by superior thyroid artery, transverse cervical artery and supraclavicular artery (Kocer et al., 2005). Less commonly, the posterior auricular artery supplies the lateral half of the platysma flap [39]. All in all, our main results are consistent with the current studies but some of the minor blood supplies (i.e. transverse cervical and supraclavicular arteries) to the platysma were not found in our study. This may be due to human error and inexperienced dissection, damaged blood vessels by other researches before us, or less anatomical variation in our cadavers due to small sample size. These reasons are the same for finding the venous drainage as well.

Across all our specimens, most of the tributaries were found to drain into the external jugular vein and anterior jugular vein. Again, this is quite consistent with current literature [39-41]. The external jugular vein mainly drains from the lateral aspect of platysma flap, whereas the common facial, anterior jugular and superior thyroid veins drain the supero-medial area (Agarwal et al., 2004). However, this does not concur with our results.

The cervical branch of facial nerve is the only source of innervation found in all our cadavers that supplies the platysma flap from sternocleidomastoid upwards and medially. Socolovsky et al. (2008) and [41] discovered that apart from cervical branch of facial nerve, a few branches of the marginal mandibular branch (of facial nerve) and tranverse cervical plexus (C2, C3 & C4) also innervate the platysma flap on the lateral and inferior aspects.

4.2 Study Limitations

Our research sample size (n=6) is small due to time constraint and limited resources. In addition,

as aforementioned the inferior half of one of the right platysmas has been used for another research by a cardiothoracic surgeon. Therefore, there are fewer samples taken from that area. This may affect our findings of locating the "window" which tends to be positioned at the supero-medial aspect of the platysma flap. Hence, there is a need to increase our sample size in future research in order to eliminate any anatomical variations in terms of age and gender.

We may have missed many blood vessels and nerves that were found in other literature and not in this study [56-58]. This could be due to inexperienced dissection. In order to increase the accuracy, I suggest injecting a latex dye into the blood vessels of specimens in our future research work.

Apart from that, we did not follow up on any prospective surgical cases involving the use of free platysma flap for dynamic eyelids reanimation because no such cases were available during the research period. Alternatively, we reviewed the success rate of blink restoration from previous studies dated from 1999 to 2011 and found a general consensus that the platysma flap is a good alternative for dynamic evelids reanimation. Nonetheless, for more validated results, further research using the "window" flap in blink restoration needs to be carried out.

In support of that, a systematic review of literature (from 1982 to 2002) consisting of 190 patients who had head and neck defects reconstruction using platysma flap, confirmed that the post-operative complication rates (between 10%-40%) are low (Szudek et al., 2007). Szudek et al. (2007) also concluded that the complication rates were not affected by age or sex and the platysma flap is best suited to repair small defects compared to other free muscle flaps.

4.3 Study Advantages

The results of this research would add onto the data of current research about the neurovasculature of platysma flap (i.e. arterial supply by submental or superior thyroid arteries; venous drainage by common facial, anterior jugular or external jugular veins; and innervations by cervical branch of facial nerve). These blood vessels and nerve were confirmed using histology and high powered microscopic analysis

(with fluorescence filter). Hence, this will further enhance the accuracy of our results by excluding false positive results (i.e. misjudgement in differentiating between arteries and veins). During dissection, the neurovascular structures identified were tied off proximally and distally to the incision using different colours of threads [59-61]. Therefore, this eases the process of identifying the origin of the blood vessels and nerves in the neck which are supplying the platysma. This method is reliable and reproducible. For documentation and future reference, photographic evidence of the different stages throughout this experiment was taken using high resolution cameras. Although this is subjected to human error, it can be reduced by using analytical software (Image J) and mini measurement ruler (placed on cadavers when photos were captured).

5. CONCLUSION

Normally, surgeons would have to explore the platysma flap during surgery in search of neurovascular bundles [41]. As a result, this would prolong the surgery period, inadequate finding of blood vessels or nerve supply and impose post-operative complications such as distal skin flap loss, post-surgical flap venous congestion, etc. So far, there is no single literature that quantified the existence of a "window" on the platysma flap which consists of blood vessels and nerves all the time. Therefore, this study was undertaken and we were able to locate the "window" (measuring as small as 2.5cm x 3.0cm and could extend as large as 8cm x 10cm) on the platysma flap. Bearing in mind, this would aid plastic surgeons in reconstruction of their neurovasculature pedicles (that requires a minimum flap size of 3cm x 3cm while the maximum size needed is 6cm x 10cm) for eyelids reanimation. We anticipate that discovery of the "window" flap would aid plastic surgeons by reducing the operation time and providing adequate blood vessels and nerve supply. Thus, further decreases the post-operative complications rates. Further investigation of this study is needed by using the newly discovered free platysma "window" flap for reanimation of the eyelids in a clinical setting whilst comparing it with other muscles (e.g. pectoralis minor, temporalis) and best current treatment for chronic facial paralysis (i.e. free gracilis or occipitalis muscle flap transfer as a control). Then, measure the functional outcome using the gold standard House-Brackmann scale whereas the aesthetic results can be measured by

comparing the pre-operative and post-operative digital photos or video-graphs.

CONSENT AND ETHICAL APPROVAL

As per university standard guideline participant consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX 1

Glasgow University Embalming Protocol

- Cadavers presented to the anatomy department for dissection are initially embedded using arterial injections and completed by subcutaneous spot injection.
- All name tags, hospital gown, jewellery and hair are removed.
- Cadaver is tagged with a year / body number as required by the anatomy act (1984, amended 2006).
- Mouth is sutured and eye caps placed between eye and eyelid.
- Incision made above the clacile and the carotid artery is raised and opened to accept a large bore cannula.
- Three litres of industrial methylated spirits (IMS) is injected to stop rapid fixation of blood by formaldehyde (and subsequent blocking of the arteries)
- 25-30 litres of embalming fluid* is then injected using a 'PORTIBOY' markV high pressure injection pump at between 30 and 50psi
- Cadaver left overnight to allow fixative to settle
- Any area of poor fixation remaining is subcutaneously spot inject using an open ended cannulla connected to embalming pump.
- Carotid incision is sutured, body washed down and bagged in a clear plastic body bag, tagger with a label, placed in a four degree fridge and left for 6 weeks before use.

* Cambridge Formulation Embalming fluid (from Vickers Laboratories, Pudsey, West Yorkshire, LS28 6QW) was used. 1 litre of embalming fluid contains:

IMS	625ml
Phenol 80%	125ml
Formaldehyde 37%	75ml
Glycerol	175ml
5ml of phenoexetol is	added as a mould growth inhibitor.

APPENDIX 2

A. Automated tissue processing schedule for 5-6mm thick specimens

- 70% methyl alcohol 2-4 hours
- 90% methyl alcohol 2-4 hours
- 2% celloiden in 100% ethyl alcohol 2-4 hours
- 2% celloiden in 100% ethyl alcohol 2-4 hours
- 2% celloiden in 100% ethyl alcohol 2-4 hours
- 1st amyl acetate 2-4 hours
- 2nd amyl acetate 2-4 hours
- 3rd amyl acetate 2-4 hours
- 1st molten wax 4-6 hours
- 2nd molten wax 4-6 hours

B. Histology

- Cassette mould filled with molten wax (i.e. paraffin wax with a melting point around 56 degrees centigrade) using Raymond A Lamb blockmaster II and specimen added.
- Cassette placed on cold plate and left to solidify.
- Cassette attached to Leica RM 2035 microtome and sligned such that it is parallel to the knife
 of the microtome.
- 7microns thick slices are then taken. Slices stretched in a thermostat controlled heated water bath (to 40 degrees Celsius)
- Slices placed on slide and left in oven at 37 degrees Celsius overnight (12 hours minimum)
- Staining of samples occurs and then slide is mounted.

APPENDIX 3

C. Staining protocol (Haematoxylin and Eosin)

•	Dewax slides in histoclear	10-15 mins
Hydr	ation	
• • •	1 st absolute alcohol 2 nd absolute alcohol 90% alcohol 70% alcohol Wash in water x 2 changes	30 secs 30 secs 30 secs 1 min 2 mins
Stair	1	
• • • •	Place slides in Mayer's Wash in water till sections turn blue Stain in 0.5% Ponceau Fuschin 1% acetic acid Rinse in water rapidly [dip] Mordant in 1% aqueous, Phosphomolybdic acid Drain excess acid and stain in 2% light green in 2% acetic acid Wash in water x 2 changes	8-10 mins 2 mins 30 sec. 15 mins 3 mins 2 mins
Dehy	/drate	
• • • •	70% alcohol [quick dip] 90% alcohol [quick dip] Absolute alcohol Absolute alcohol Clear in histoclear Mount slides in histomount	1 min 2 mins 10 – 15 mins

Results: *Nuclei*: Blue/Black; *Cytoplasm, muscle and acidophil granules*: Red; *Collagen, cartilage, mucin and basophil granules*: Green.

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Peer-review history: The peer review history for this paper can be accessed here: http://www.sdiarticle4.com/review-history/63505