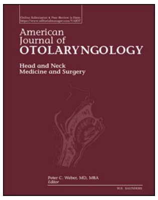
American Journal of Otolaryngology–Head and Neck Medicine and Surgery 44 (2023) 103822

Contents lists available at ScienceDirect

American Journal of Otolaryngology–Head and Neck Medicine and Surgery

journal homepage: www.elsevier.com/locate/amjoto



The evolution of facial reanimation techniques

Debbie R. Pan [a], Nicholas W. Clark [a], Harry Chiang [a], Russel R. Kahmke [a], Brett T. Phillips [b], Dane M. Barrett [a,*]

[a] *Department of Head and Neck Surgery & Communication Sciences, Duke University, Durham, NC, United States of America*
[b] *Division of Plastic, Maxillofacial, and Oral Surgery, Department of Surgery, Duke University, Durham, NC, United States of America*

ARTICLE INFO

Keywords:
Facial reanimation
Facial paralysis
Free muscle transfer

ABSTRACT

This review article provides an updated discussion on evidence-based practices related to the evaluation and management of facial paralysis. Ultimately, the goals of facial reanimation include obtaining facial symmetry at rest, providing corneal protection, restoring smile symmetry and facial movement for functional and aesthetic purposes. The treatment of facial nerve injury is highly individualized, especially given the wide heterogeneity regarding the degree of initial neuronal insult and eventual functional outcome. Recent advancements in facial reanimation techniques have better equipped clinicians to approach challenging patient scenarios with reliable, effective strategies. We discuss how technology such as machine learning software has revolutionized pre- and post-intervention assessments and provide an overview of current controversies including timing of intervention, choice of donor nerve, and management of nonflaccid facial palsy with synkinesis. We highlight novel considerations to mainstay conservative management strategies and examine innovations in modern surgical techniques with a focus on gracilis free muscle transfer. Innervation sources, procedural staging, coaptation patterns, and multi-vector and multi-muscle paddle design are modifications that have significantly evolved over the past decade.

1. Introduction

Facial animation is a critical element of human function, with influence over fundamental domains such as communication, expression, and deglutition; loss of facial movement can result in devastating psychosocial effects. Potential aesthetic and functional sequelae include facial asymmetry, smile distortion, articulation difficulties, exposure keratopathy, and oral incompetence, all of which can directly worsen quality of life [1]. Restoration of facial function through facial reanimation is an area of growing scientific and clinical interest, and management techniques have adapted to an improved understanding of the mechanisms of neural injury and subsequent repair.

The aim of this review is to provide an update on facial reanimation techniques, primarily focusing on the evolution of surgical techniques over the past decade as well as ongoing controversies. We discuss modern technologies used for patient evaluation, considerations regarding the timing of intervention, selection of donor innervation, and modifications to both conservative management such as botulinum injections with neuromuscular retraining therapy as well as surgical management. Innovations to the free gracilis muscle transfer will be highlighted which include a discussion on innervation sources, procedural staging, coaptation patterns, and multi-vector and multi-muscle paddle design.

2. Patient evaluation and developing assessment tools

Evaluation of a patient with facial paralysis relies foremost on the clinical history and physical examination. There should be a focus on the mechanism or etiology of injury, onset and progression of symptoms, prior surgical history, baseline functional status, and full neurological examination. Photographs and videos of resting and dynamic facial movements should be documented at the time of initial visit and all subsequent follow-up visits to monitor clinical progression and provide comparative information between pre- and post-intervention states (Fig. 1). Facial expressions to monitor, from superior to inferior zones of the face, usually include the face at rest, brow elevation, light-effort and full-effort eye closure, nose wrinkling, lip puckering, light-effort soft smile, full-effort smile with dental show, and lower lip depression [2].

---

[*] Corresponding author at: Department of Head and Neck Surgery and Communication Sciences, Duke South Yellow Zone 4000, DUMC Box 3805, Durham, NC 27710, United States of America.

E-mail address: dane.barrett@duke.edu (D.M. Barrett).

https://doi.org/10.1016/j.amjoto.2023.103822
Received 5 February 2023;
Available online 1 March 2023

Fig. 1. Facial assessment with resting and dynamic movements.

Table 1
Facial paralysis clinical assessment tools.

Assessment tool	Scale	Advantages	Disadvantages
House-Brackmann	Grade from I–VI	Well established, straightforward to use	Subjective, combined scoring obscures zonal differences
Sunnybrook Facial Grading System	Score from 1 to 100	Accounts for secondary defects, finer scoring scale	Subjective, prone to inter-observer variability
Burres-Fisch System	Continuous scale	Based on objective measurements	Time consuming measurements, does not account for secondary defects
Facial Clima	Optical facial plot system	Quantitative analysis of facial motion	Requires specialized software and equipment
Electronic Facial Paralysis Assessment (eFACE)	Software scoring system	Accounts for secondary defects, high interrater reliability	Only for unilateral paralysis, requires specialized software
Auto-eFACE	Automated scoring system	Removes subjectivity due to computerized evaluation	Requires specialized software and equipment

The most common forms of assessment are dependent on subjective measures such as clinical observation and patient-reported quality of life questionnaires [3,4].

While there is no standardized assessment tool for evaluating facial function, several well-known scales are traditionally used in clinical practice (Table 1). The universal tool is the House-Brackmann Scale which ranges from grade I (normal) to grade VI (total facial paralysis), factoring contributions from rest, forehead innervation, eyelid closure, and mouth innervation [5]. However, the House-Brackmann scale has been criticized due to high interobserver variability and a composite scoring system which fails to account for finer, zonal differences [6]. Another commonly used grading system is the Sunnybrook Facial Grading System which ranges from 0 (total facial paralysis) to 100 (normal) and is based on the evaluation of symmetry at rest as well as symmetry of voluntary, synkinetic, or involuntary movements associated with different facial expressions [7]. Although this classification is more sensitive to finer differences and incorporates synkinesis into its scale, it is susceptible to the same interobserver variability as the House-Brackmann Scale. The Burres-Fisch system was developed to provide a scale based exclusively on objective measurements. However, the Burres-Fisch method requires time consuming calculation and thus has seldom been used in clinical practice [6]. The desire to develop an efficient, standardized, objective assessment tool has inspired significant advancements over the years which utilize growing technology like three-dimensional modeling and artificial intelligence.

Hontanilla et al. developed an automatic, three-dimensional optical motion system called Facial Clima, which measures dynamic facial movements with a demonstrated reliability of 99 % [8]. Special reflecting dots are placed on a patient's face and infrared-light video cameras capture facial movements. This system has several advantages including short calibration time, measurement of information beyond distances such as oral commissural contraction velocities, and the ability

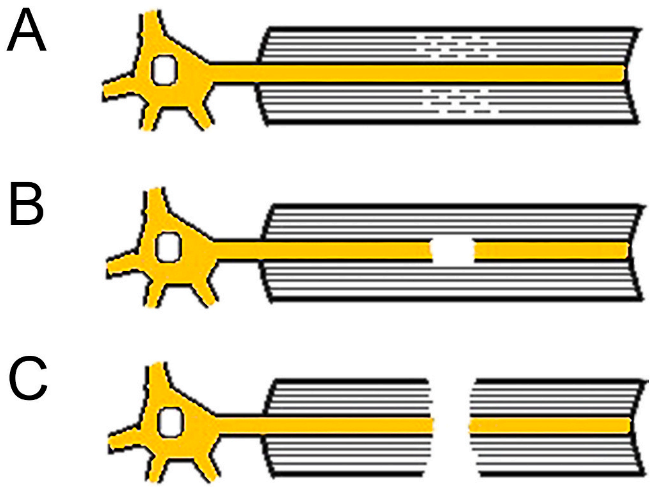


Fig. 2. Continuum of facial nerve injury: (A) neuropraxia, (B) axonotmesis, and (C) neurotmesis.

to compare results across different techniques and surgical centers [8,9]. Other groups have developed similar three-dimensional, quantitative, dynamic facial motion assessment tools, but widespread use is limited by the accessibility of software and equipment [10–12].

The introduction of the electronic facial paralysis assessment tool (eFACE) in 2015 marked a shift toward digital, user-friendly tools. eFACE is a validated 16-item clinician grading system that is designed to incorporate static, dynamic, and synkinetic facial movements with subregional scoring on a continuum from 1 (extreme disfigurement) to 100 (normal) [13]. The software also has the capability of generating a graphic display of score subsets to allow for comparisons across serial examinations. This assessment tool has been widely assessed and incorporated into facial paralysis literature since its development; however, it has not been universally accepted as it remains subjective in nature [14].

Efforts to standardize facial function assessment tools to eliminate the inherent subjectivity of clinician graded scales are ongoing. The use of objective, machine learning software has been incorporated to enhance these grading scales. Auto-eFACE is an automated scoring system utilizing a free facial landmark recognition software called Emotrics combined with machine learning technology. This system has demonstrated a robust ability to differentiate asymmetries between normal faces and those with flaccid facial palsy and severe synkinesis [15,16]. A recent study by Dusseldorp et al. utilized automated software to evaluate patients with unilateral facial palsy to compare results of gracilis free muscle transfer and found statistically significant results in the detection of quantified smile spontaneity after reanimation [17]. The growing focus on developing dependable, validated, and objective tools to assess patient facial function before and after intervention has the potential to powerfully shift the practice paradigm for facial paralysis patients as these tools improve our ability to objectively evaluate our interventions across providers and institutions.

3. Principles and controversies of management

3.1. *Facial nerve injury and repair*

The facial nerve is a complex nerve with multiple sites of potential injury from a host of etiologies. The basis for appropriate treatment planning depends on an adequate knowledge of neural physiology as well as the etiology, degree, and duration of injury. Neural injury presents on a continuum from neuropraxia (traction injury without axonal disruption) to axonotmesis (axonal disruption with an intact myelin sheath) and eventually neurotmesis (injury to endoneurium, perineurium, or epineurium) (Fig. 2). Upon axonal injury, the distal portion of the nerve undergoes Wallerian degeneration and the end-organ motor foot plate, which is maintained by intact axonal secretory factors, suffers irreversible damage after about 18–24 months if innervation of the mimetic musculature is not reestablished [18,19].

Electrodiagnostics including electroneurography (ENoG) and electromyography (EMG) can be useful adjunctive testing to better prognosticate facial nerve injury. ENoG records the amplitude of electrically evoked action potentials to measure facial nerve integrity of the injured side compared to the contralateral side. ENoG is most useful when utilized between 3 and 21 days after onset, as it is estimated to take approximately 3 days before Wallerian degeneration traverses to the most distal aspect of the injured nerve [20,21]. EMG is used to evaluate functional motor units without electrostimulation and is most useful between 2 and 12 weeks after onset. The presence of fibrillation potentials signals nerve degeneration and portends a poor prognosis for recovery of facial function [20]. Combined, these tests can provide valuable information regarding the functional status of both the facial nerve and the facial musculature to guide treatment decisions.

Strategies for repair are reliant on how intact the facial nerve is believed to be based on the etiology of injury and how reversible the injury is. In general, facial reanimation techniques aim to establish an early, tension-free repair, using techniques such as direct nerve neurorrhaphy, nerve grafting, or nerve transfer depending on the status of the facial nerve [22]. When appropriate, direct nerve neurorrhaphy is preferred, but nerve grafting is used when tension-free repair cannot be performed. The use of another nerve, most commonly the sural or great auricular nerves, allows bridging of the gap between the nerve ends [1]. When the proximal end of the facial nerve is not available from either retraction or location of injury proximal to the stylomastoid foramen, nerve transfer provides a means by which a nerve substitute is utilized to reestablish innervation to facial muscles [1]. Static surgical techniques and regional or free muscle transfer can be utilized to restore a degree of facial symmetry even when reinnervation cannot be achieved in the setting of irreversible facial paralysis.

3.2. *Timing of intervention*

The timing of intervention in facial paralysis has been a longstanding topic of debate. Although it is generally agreed upon that immediate or early nerve repair physiologically confers the best chance to achieve optimal functional outcomes in cases of facial nerve transection, there continues to be a lack of consensus on timing of intervention when the nerve is presumed to be anatomically intact [1,19,23]. Traditionally, surgical intervention had been deferred until 12 months after onset in order to allow for possible spontaneous recovery of facial function [19,23].

In a study by Rivas et al., 281 patients with facial nerve weakness, despite anatomic preservation of the nerve, after vestibular schwannoma resection surgery were followed postoperatively [24]. It was determined that the recovery rate within 12 months for patients with initial House-Brackmann grades V–VI was able to predict poor functional outcomes reliably and independently as early as 7 months with 97 % sensitivity and specificity [24]. Thus, earlier facial reinnervation was suggested for patients with House-Brackmann grades V–VI without clinical signs of improvement. There has been a shift in contemporary clinical practice toward considering surgical intervention after approximately 6 months, instead of waiting 12 months [1,23]. This recommendation is supported by Albathi et al. who demonstrated shorter times to functional improvements after reanimation surgery. In this study, patients with facial paralysis and an anatomically intact facial nerve after cerebellopontine angle tumor resection were offered reanimation after 6 months. These patients showed functional improvement compared to no clinical improvements in patients that chose to continue observation [25].

In contrast, comparable degrees of long-term improvements with

Table 2
Summary of donor nerve selections.

Donor nerve	Advantages	Disadvantages
Contralateral facial nerve	Allows for true spontaneous smile	May require interposition nerve graft, increased time for regeneration, success may be age dependent
Masseteric nerve	Minimal donor site morbidity, anatomic proximity, similar nerve caliber	Requires retraining
Hypoglossal nerve	Straightforward dissection	Significant donor site morbidity, requires retraining

conservative management in the setting of anatomically intact facial nerves are also observed in the current literature. Shoakazemi et al. examined a cohort of 18 patients following vestibular schwannoma resection surgery with anatomically intact facial nerves but complete conduction blocks with House-Brackmann grades V–VI; 39 % of patients improved to grades II–III at 1 year and 61 % improved to grades II–III on extended follow-up (mean of 34.28 months) without reanimation surgery [26]. Ultimately, the timing of repair for an anatomically intact but injured facial nerve continues to be a topic of debate and relies on thoughtful patient-centered conversations to optimize individualized goals.

3.3. *Choice of donor nerve for facial reinnervation*

The most commonly used donor nerves for nerve transfer procedures include the contralateral facial, ipsilateral masseteric, and ipsilateral hypoglossal nerves. While the choice of donor nerve can be influenced by individual surgeon comfort or training bias, the decision for which donor nerve to use largely depends on an understanding of the advantages and limitations of each technique (Table 2). The contralateral facial nerve is utilized as a cross-facial nerve graft (CFNG) and is the only technique that allows a true, spontaneous smile. However, there are several limitations regarding operative considerations, axonal regeneration rate, number of available axons, and time to reinnervation. Given the physical distance between the contralateral facial nerve and the affected facial nerve, an interposition nerve graft to bridge the gap, commonly the sural nerve or great auricular nerve, is necessary to harvest for the CFNG technique to work. This increases the number of coaptation sites that the contralateral facial nerve input must cross and can affect time until return of function. Axons regenerate at approximately 1–2 mm/day which confers a recovery rate in the order of at least 6 months to cross an average 15–20 cm CFNG [1,21,22]. There has also been a statistically significant negative correlation between age and axonal load for CFNGs that impacts how factors such as patient age play a role in patient selection for this technique [27].

The masseteric nerve offers the advantages of minimal donor site morbidity, anatomic proximity, and ease of adaptation. Masseteric nerve dissection has been found to fall reliably within the landmarks of the “subzygomatic triangle,” between the zygomatic arch, temporomandibular joint, and frontal branch of the facial nerve approximately 10–15 mm deep to the parotidomasseteric fascia as described by Collar et al. [28]. The caliber of the masseteric nerve is similar to the facial nerve and direct coaptation can be readily performed between these nerves. Although the resting tone of the masseteric nerve is lower compared to the other commonly used donor nerves, tone and movement following masseter to facial nerve transfers can be seen usually by 4–6 months after surgery [29]. The masseteric nerve also has high axonal density which can produce powerful oral commissure excursion [30]. While this technique offers excellent smile restoration, there is a period of retraining where patients need to relearn the effortless smile after initially relying on a smile provoked by teeth clenching. Several groups have demonstrated successful restoration of smile vector and strength comparable to the normal side after masseter-to-facial nerve transfer, with up to 85 % of patients able to achieve an effortless smile [30,31].

In comparison to the previous techniques, the hypoglossal nerve transfer is perhaps least utilized given its functional implications from significant donor site morbidity. Numerous complications have been described in literature including partial tongue trophy, dysphagia, and dysarthria [30,32]. Techniques to reduce morbidity including isolating the post-descendens hypoglossal nerve to preserve C1 fibers, end-to-side anastomosis, or use of interposition grafting have been suggested [1,30,33]. According to Samii et al., a comparative study between end-to-end and end-to-side hypoglossal nerve to facial nerve coaptation techniques in 26 patients, although there was a lower rate of lingual morbidities (tongue atrophy 100 % vs 5.8 %, dysphagia 55 % vs 11.7 %, dysarthria 33 % vs 0 %) in the end-to-side group, there was no significant difference in recovery rate of the facial nerve [33]. Despite modifications to the traditional technique, the possibility of severe hemiglossal dysfunction still presents as a hindrance to the routine use of the hypoglossal nerve when compared to the CFNG or masseteric nerve overall.

While these various donor nerves are described here as stand-alone procedures, in reality, there has been a trend toward using dual innervation with “babysitter” grafts. This concept employs the use of multiple nerves whereby the masseteric or hypoglossal nerve provides the injured facial nerve with neural input, like a “babysitter,” while the axons from the CFNG traverse the interposition graft [34]. There are many innervation patterns that have been explored and are preferably performed whenever possible without the use of free muscle or tissue transfer. However, these combined techniques will be discussed further in Section 4.2.

3.4. *Management of nonflaccid facial palsy*

In situations where patients have nonflaccid facial palsy with some degree of recovered facial mimetic motion, management becomes challenging. Clinically, these patients often have resting tone and may have developed some degree of synkinesis. Synkinesis is thought to result from aberrant regeneration of facial nerve fibers leading to involuntary, aberrant co-contraction of muscles in 15–55 % of patients approximately 6 months after facial nerve injury [2,35]. The most common types of synkinesis include ocular-oral, ocular-chin, ocular-nasal, and platysmal synkinesis [35]. The mainstay treatments for nonflaccid facial palsy largely include nonsurgical interventions such as botulinum toxin injections and volumizing fillers as well as physical therapy [1,36]. However, there has been increasing interest in developing and implementing procedural solutions, from minimally invasive procedures to novel surgical reanimation techniques.

One such low-risk procedure is an ipsilateral depressor anguli oris (DAO) resection and is routinely accomplished under local anesthesia. The DAO is a smile antagonist and outcome studies have demonstrated significant improvements in smile dynamics and perceived emotional expression with intentional weakening of this muscle on the affected side [37,38]. In a study by Derakhshan et al., DAO resection in 43 patients led to a statistically significant average increase in oral commissure median excursion of 3.02 mm and dental show of 2.36 mm [38]. Similarly, platysmectomy has been described to meaningfully improve quality of life scores for patients with platysmal synkinesis under local anesthesia [39].

Another procedure designed to address synkinesis is DAO resection with transfer to the ipsilateral depressor labii inferioris (DLI). Concurrent DAO hypertonicity and DLI hypotonicity has been implicated as a contributor to the asymmetry experienced by patients with facial synkinesis [40]. This repositioning technique aims to reduce the negative vector limiting commissure excursion while overall improving lower lip depression. Halani et al. designed a prospective, single-center study to compare the efficacy of DAO resection vs DAO to DLI transfer and reported similar results in postoperative modiolus resting position, angle, excursion and dental show [41]. However, DAO to DLI transfer lacked a significant increase in modiolus excursion compared to pure DAO resection [41].

Instead of targeting muscle itself, techniques to target dysfunctional nerve endings were also developed as a means of eliminating unwanted facial hyperactivity. The surgical technique for modified selective neurectomy was described by Azizzadeh et al. in 2018 in which careful dissection and selective transection of multiple distal facial nerve branches is performed using a standard rhytidectomy incision approach under general anesthesia with nerve monitoring [42]. Specifically, cervical, and buccal branches that cause platysmal tightening or downward and lateral excursion of oral commissure are transected. A study by the same group later reported that of the 65 patients who had undergone modified selective neurectomy surgery performed over 4.5 years, 98 % had reported satisfaction with the procedure and all eFACE scoring metrics except for two categories (DLI lower lip movement, oral commissure position at rest) showed statistically significant improvement [43]. Most patients had simultaneous procedures performed including rhytidectomy, CFNG, and rerouting of transected buccal branches into other branches [43]. Further combination techniques have been explored including simultaneous modified selective neurectomy and masseteric-to-facial nerve transfer with reported improvements in smaller cohorts regarding synkinesis and smile symmetry [44,45]. Despite these advancements in techniques, nonflaccid facial palsy management continues to rely on a combination of both conservative and procedural approaches.

4. Current advancements in management

4.1. Conservative management

Botulinum toxin is a powerful therapeutic agent used to treat asymmetries caused by facial paralysis and serves as the backbone of conservative management for this condition. Currently, there are several botulinum toxin type A formulations approved for use in both the aesthetic and therapeutic realms including Onabotulinum-toxin A (ONA), Abobotulinum-toxin A (ABO), and Incobotulinum-toxin A (INCO) [35]. Although these formulations are all commonly used, INCO is uniquely manufactured to isolate pure neurotoxin without clostridial proteins which offers the advantage of low immunogenicity rates and increased therapeutic efficacy especially in the setting of multiple anticipated treatments [46]. In order to achieve improved facial symmetry, botulinum toxin is injected mainly into the contralateral, non-affected side of the face to address compensatory hyperkinesia but targeted injection into specific muscles on the ipsilateral, affected side can be useful in cases of synkinesis [35]. Temporary chemical denervation with botulinum toxin on the contralateral, non-affected side has been shown to increase ipsilateral facial strength and symmetry [47–49]. In a rat study by Guntinas-Lichius with 65 rats with hemifacial paralysis, it was shown that enhanced reinnervation of the lesioned buccal branch of the facial nerve and functional recovery of the whisker pads were appreciated after contralateral but not ipsilateral injection of botulinum toxin [50]. This effect is attributed to what is known as the “neuroplasticity phenomenon” or “strength redistribution phenomenon” in which central cortical reorganization allows the paralyzed side to become more active [47,49,50].

During the first treatment session, it is recommended that lower-than-normal amounts are injected followed by a close, two-week follow-up visit at which point discussions for additional injections or verification of appropriate dose ranges can be made [35]. The selected injection sites are based upon clinical comparison between the facial muscle movements of the affected and non-affected sides. While there is variability in the exact injection sites for each patient, sites are chosen to achieve resting and dynamic symmetry based on an understanding of the functional muscles of the periocular, midface, and perioral regions. Typically, 4 units per point can be used for the frontalis to counteract brow elevation and at points around the corrugator supercilli, procerus, depressor supercilli, and orbicularis oculi to counteract depressor action [2]. Approximately 1–2 units per point can be used around the perioral muscles for the elevators (levator labii superioris, levator anguli oris, major and minor zygomatic muscles, and levator labii superioris alaeque nasi) and depressors (DAO, DLI) [2]. The mentalis and platysma are also lower lip depressors and may require higher doses of 4 units per point for effect [2].

In addition to botulinum toxin injection, a specialized form of physical therapy known as targeted facial neuromuscular retraining (NMR) therapy is another critical component of conservative management for facial paralysis [51]. New precise motor behaviors are taught through NMR to stimulate neural plasticity [2]. NMR utilizes sensory input, surface proprioception, and mirror feedback to functionally retrain the paralyzed face [52]. The classic mirror biofeedback exercise during which a patient would perform therapy exercises while looking into a mirror evolved into a new method known as half-mirror biofeedback where the normal half was covered to better allow the patient to compare sides without sensing movement from the non-paralyzed side [53]. In a prospective study in 17 patients with unilateral facial paralysis, a combination treatment strategy of three botulinum toxin injections spaced 6–8 months apart and daily half-mirror biofeedback rehabilitation management starting from first injection was observed for two years with significant improvements in facial synkinesis and facial symmetry as evidenced by notable increases in Sunnybrook scores (11.4 point increase after first injection, 14.6 point increase after second injection, and 15.6 point increase after third injection) [53]. According to a meta-analysis by Pereira et al. that examined the effectiveness of facial exercise therapy for facial palsy, only one randomized control trial had sufficient data to perform the meta-analysis but facial functionality in the group to have received NMR was statistically significantly higher than in the non-intervention group [54]. The experimental group of 25 patients received therapy for ten weekly 45-min sessions with daily homework compared to 25 control patients and there were appreciated differences noted in the physical and social indices of the Facial Disability Index, a scale used for outcomes reporting in this study [55]. Despite evidence to support NMR as a standalone therapy, it is rarely conducted in clinical practice without concomitant botulinum toxin injections. However, an initial 6 months of NMR is suggested before botulinum toxin therapy in order to better consolidate synkinesis inhibition techniques which would not be as easy to learn if botulinum toxin were administered before NMR [56].

A novel technique which is currently being explored in the facial paralysis arena is thread lifting and its possible application as thread embedded acupuncture. Thread lifting has existed since the early 1990s for cosmetic purposes. The minimally invasive nature of this procedure and the ability to noticeably elevate facial musculature for 6–18 months on average makes its potential for treatment of facial paralysis an option that is readily investigated [57–59]. Thread lifting procedures are routinely performed under local anesthesia and place cogged threads, commonly polydioxanone threads, into the subcutaneous plane along an intended facial trajectory. In a study by Choe et al., 34 patients were treated with ipsilateral, affected side thread lifting and contralateral botulinum toxin injections with a range of 6–35 pieces of thread used for each facial zone [58]. Average facial function by the Sunnybrook score and dynamic facial symmetry ratios demonstrated statistically significant improvements from pre- to post-procedural measures [58]. More recently, acupuncture has been proposed as a treatment for facial paralysis based on the premise that facial regeneration is optimized in the setting of decreased inflammation and enhanced vascular circulation [60]. A recent retrospective study by Pu et al. demonstrated increased quality of life measures such as reduced pain, improved facial function, and high satisfaction survey scores with iatrogenic facial nerve palsy patients following vestibular schwannoma treatment [60]. A current Chinese randomized control clinical trial protocol was recently

Table 3
Summary points for advancements in free gracilis muscle transfer techniques.

Innervation type
▪ Types include one-stage single innervation, one-stage dual innervation, and two-stage dual innervation.
▪ Masseteric nerve marginally favored over CFNG for one-stage procedures due to superior smile excursion results.
▪ Dual innervation techniques (mainly with the masseteric nerve and CFNG) predominate contemporary practice.
▪ No clear consensus for one vs two-stage outcomes due to variability in coaptation patterns and innervation choices.
Coaptation pattern
▪ Patterns include end-to-end, end-to-side, Y-shaped, and Y-shaped, and Y-shaped with interfascicular split.
▪ Most common coaptation pattern includes dual innervation of end-to-end masseteric nerve to obturator nerve coaptation and end-to-side CFNG to obturator nerve coaptation.
▪ No significant outcome differences noted between obturator nerve split patterns for coaptation with the masseteric nerve and CFNG nerve.
Vector design
▪ Designs include multi-vector and multi-paddle.
▪ Secondary vectors enhance periorbital wink, oral commissure symmetry, and dental show.
▪ Multi-paddle with tendinous split of the harvested gracilis has improved smile angle, lip animation, and oral commissure excursion.

published (Chinese Clinical Trial Registry, ChiCTR1900027170). This study will investigate the safety and efficacy of thread embedded acupuncture for facial paralysis across 8 weeks. The stated outcome measures were defined as facial expression muscle thickness ratio of affected/unaffected side, House-Brackmann grades, and lip mobility scores at different time points of intervention [61]. To date, this trial is ongoing. While further clinical studies will be needed before there is widespread adoption of this or any other novel technique, the momentum with which advancements are being made in this field shows a promising future for the treatment of facial paralysis.

4.2. Surgical management

While decision-making tenets and current controversies of surgical management for facial paralysis were discussed in Section 3, further discussion on recent advancements in reanimation surgical techniques is warranted. The focus in this section will be on innovations with free gracilis muscle transfer techniques (Table 3).

The gracilis muscle is the most common donor and is considered the gold standard. It was first introduced as a viable donor in 1976 by Harii et al. as a method to address cases of longstanding, irreversible facial paralysis [1,23,62]. Among other donor muscles like the latissimus dorsi or rectus abdominis, the gracilis muscle is ideal. The gracilis muscle is easy to access with minimal donor site morbidity, has adequate length and ability to tailor to facial contour by debulking, and has a low flap failure rate when used in facial reanimation [63]. In the literature, the masseteric nerve is marginally favored over the CFNG as the source of motor innervation for one-stage gracilis free muscle transfer due to more robust axonal density at the site of neurorrhaphy which produces higher rates of restored smile excursion [64–67]. In a recent meta-analysis by Vila et al., 3 studies with 108 patients undergoing free gracilis muscle transfer (56 masseteric nerve vs 52 CFNG) were analyzed and excursion was superior with the masseteric nerve compared with CFNG [68]. Comparing available data in 2 retrospective studies (51 masseteric nerve vs 47 CFNG), the smile symmetry at rest and with dynamic effort were also better with the masseteric nerve although this difference was not statistically significant [68].

However, results in current literature are supporting the use of the masseteric nerve and CFNG together as opposed to either alone as the sole donor nerve for neurotization of the gracilis muscle transfer [68–70]. Dual innervation free gracilis muscle transfer was introduced in 2012 by Biglioli et al. with an end-to-end coaptation between the masseteric and obturator nerves and an end-to-side coaptation of the CFNG to the obturator nerve inserting distal to the masseteric neurorrhaphy site in a one-stage procedure [70]. The rationale for this technique was based on the advantages and disadvantages of the two most commonly utilized donor nerves, the masseteric nerve and CFNG, as discussed earlier in Section 3.3. The masseteric nerve, with greater axonal load, is able to provide neural input to the ipsilateral, affected facial nerve as a "babysitter" graft to augment smile excursion and prevent chronic denervation while the CFNG is maturing in order to provide a spontaneous smile. In contemporary practice, a two-stage, dual innervated free gracilis muscle transfer is gaining momentum as a means by which optimal and reliable facial reanimation results are achieved [27,71,72]. In Cardenas-Mejia et al., a novel two-stage technique was described in 2015 involving dual innervation of a free gracilis muscle transfer with a first-stage CFNG followed by a second-stage muscle flap inset 3–4 months later with coaptation to both the CFNG and masseteric nerve with an initial clinical series of 9 patients [72]. The coaptation pattern was reported to be end-to-end between the CFNG and the obturator nerve and end-to-side between the masseteric and obturator nerves with a mean reinnervation time of 8.78 weeks [72]. These principles are illustrated in Figs. 3 and 4A.

Despite the evolution of practice toward dual innervation, there continues to be high variability in reported techniques for facial reanimation using free gracilis muscle transfer which make direct comparisons in outcomes difficult to attribute to unique variables such as number of staged procedures or coaptation techniques. According to a comparative study by Kim et al., a total of 49 patients were assessed from a single institution and compared between a two-stage CFNG free gracilis muscle transfer group and a one-stage dual innervated (end-to-end masseteric nerve neurorrhaphy, end-to-side CFNG neurorrhaphy) free gracilis muscle transfer group [71]. While there was a higher reported symmetry index in the latter group, the two groups did not show a significant difference in functional aspects [71].

With respect to coaptation techniques, there are varying perspectives on the choice of either end-to-end or end-to-side anastomoses in dually

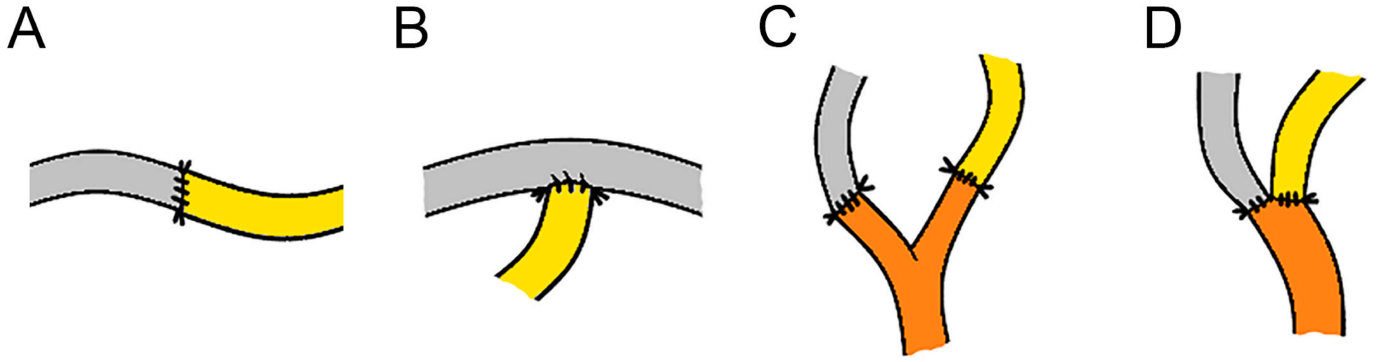


Fig. 3. Nerve coaptation patterns: (A) end-to-end, (B) end-to-side, (C) Y-shaped, and (D) Y-shaped with interfascicular split.

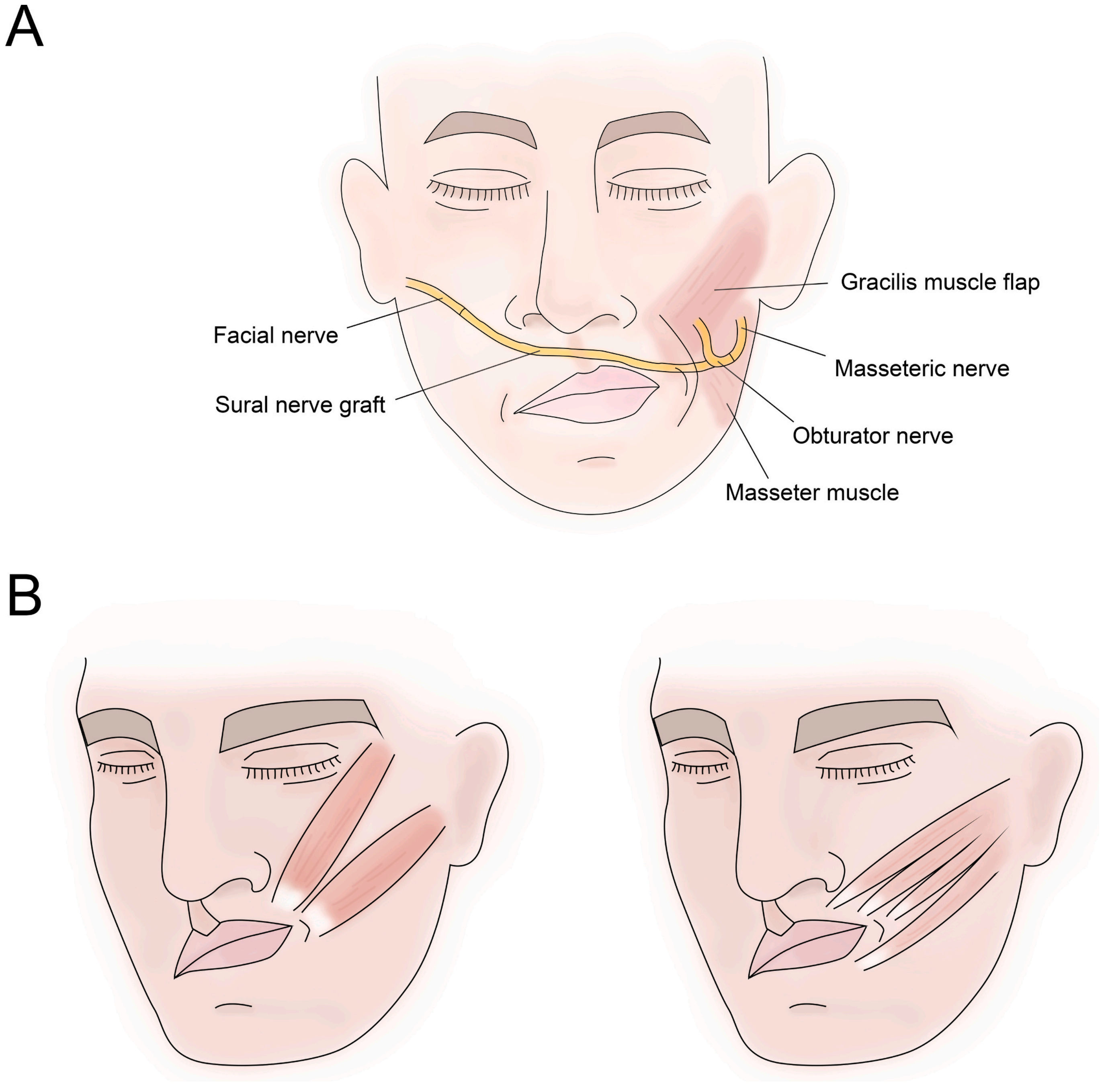


Fig. 4. Illustration of free gracilis muscle transfer techniques. (A) One-stage dual innervation utilizing an end-to-end masseteric nerve to obturator nerve coaptation and end-to-side cross facial nerve graft to obturator nerve coaptation. This is the most commonly performed innervation technique. (B) Vector design techniques. Left shows a multi-vector approach; right shows a multi-paddle approach.

innervated free gracilis muscle transfers. These techniques derive their utility from the coexisting mechanisms of collateral and terminal sprouting in axonal regeneration. Axonal regeneration occurs along the length of an axon in collateral sprouting while regeneration occurs along the most distal aspect of an axon in terminal sprouting [73]. Cardenas-Mejia and colleagues believe that end-to-end CFNG to obturator nerve provides the greatest chance for major motor unit recruitment in the gracilis muscle which will allow for more symmetrical and spontaneous smile [72]. Other coaptation patterns have been described including Y-shaped with or without interfascicular split [74–76] (Fig. 3). In Dusseldorp et al., three different neurorrhaphy patterns were compared: interfascicular split of the obturator nerve with end-to-end anastomosis with both the masseteric nerve and CFNG, Y-shaped end-to-end anastomosis of obturator nerve with both the masseteric nerve and CFNG without interfascicular split, and end-to-end masseteric nerve to obturator nerve neurorrhaphy with end-to-side CFNG to obturator nerve neurorrhaphy [75]. There were no significant differences in eFACE scores, or oral commissure excursion noted between these patterns [75]. Anecdotally, Dusseldorp and colleagues reported transitioning their dual innervation techniques to end-to-end masseteric nerve to obturator nerve and end-to-side CFNG to obturator nerve coaptation pattern based on the experience of others per personal communications [75]. Ephaptic coupling, whereby primary nerve firing can induce secondary excitation firing of an adjacent nerve, is an alternate form of nerve conduction which has been suggested to explain why there may not be substantial differences in coaptation techniques [77]. According to a database literature search by Boonipat et al., the majority of dual innervation free gracilis muscle transfers reported were performed as a one-stage dual innervation end-to-end masseteric nerve to obturator nerve and end-to-side CFNG to obturator nerve coaptation [78].

Advancements in gracilis muscle flap design have also yielded positive results in recent literature (Fig. 4B). In 2018, Boahene et al. proposed a multi-vector gracilis flap design in a prospective study in which a secondary muscle paddle would insert to elevate the medial upper lip in addition to the primary muscle paddle simulating outward contraction of the zygomaticus major muscle [79]. This technique enhanced the natural smile with improved oral commissure symmetry, dental show, and dynamic periorbital wink with contraction along the secondary vector. [79]. Tzafetta et al. also investigated a multi-vector, multi-paddle design with sectioning of the gracilis muscle tendon into four strips in a two-stage dual innervation approach [80]. One strip is inset at the oral commissure, one is inset at a location one-third of the horizontal distance between the philtrum and the commissure, another at two-thirds of the aforementioned distance, and the last strip is secured at the lower lip approximately 2 cm below the oral commissure [80]. Voluntary, spontaneous smiles without teeth clenching were achieved in all 11 patients by 18 months after surgery with statistically significant improvements in smile angle, upper lip animation, commissural excursion post-operatively [80]. Future, larger, multi-institutional studies will guide continued innovation in the field of facial reanimation.

5. Conclusion

Facial paralysis can be a devastating condition with implications in functional, aesthetic, and quality of life contexts for affected patients. The treatment of facial paralysis, while complex, can be approached generally in a stepwise fashion with most patients receiving benefit from conservative strategies such as botulinum toxin injections and neuromuscular retraining therapy preceding and/or alongside surgical intervention. Decisions around management are challenging and depend on a comprehensive consideration of several factors including patient goals, timing, and etiology of onset, as well as predicted prognosis. The evolution of techniques, especially marked by numerous technical modifications and advancements over the past decade, points toward a promising future whereby more effective and optimal options can be offered to treat any individual with facial paralysis.

CRediT authorship contribution statement

D.R.P: Conceptualization, Writing – original draft preparation, Creation of tables and figs.

N.W·C: Writing – review and editing, Creation of tables and figs.

H·C: Writing – review and editing, Creation of tables and figs.

R.R.K: Writing – review & editing.

B.T.P: Writing – review & editing.

D.M.B: Conceptualization, Writing – review & editing, Creation of tables and figures.

Declaration of competing interest

There are no declarations of interest or competing interests to disclose. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] Kim L, Byrne PJ. Controversies in contemporary facial reanimation. Facial Plast Surg Clin North Am 2016;24(3):275–97.
[2] Heydenrych I. The treatment of facial asymmetry with botulinum toxin: current concepts, guidelines, and future trends. Indian J Plast Surg 2020;53(2):219–29.
[3] Kahn JB, et al. Validation of a patient-graded instrument for facial nerve paralysis: the FaCE scale. Laryngoscope 2001;111(3):387–98.
[4] VanSwearingen JM, Brach JS. The Facial Disability Index: reliability and validity of a disability assessment instrument for disorders of the facial neuromuscular system. Phys Ther 1996;76(12):1288–98. discussion 1298-300.
[5] House JW, Brackmann DE. Facial nerve grading system. Otolaryngol Head Neck Surg 1985;93(2):146–7.
[6] Kang TS, et al. Facial nerve grading systems (1985–2002): beyond the House-Brackmann scale. Otol Neurotol 2002;23(5):767–71.
[7] Ross BG, Fradet G, Nedzelski JM. Development of a sensitive clinical facial grading system. Otolaryngol Head Neck Surg 1996;114(3):380–6.
[8] Hontanilla B, Aubá C. Automatic three-dimensional quantitative analysis for evaluation of facial movement. J Plast Reconstr Aesthet Surg 2008;61(1):18–30.
[9] Hontanilla B, Marré D. Comparison of hemihypoglossal nerve versus masseteric nerve transpositions in the rehabilitation of short-term facial paralysis using the Facial Clima evaluating system. Plast Reconstr Surg 2012;130(5):662e–72e.
[10] Sforza C, et al. Three-dimensional superimposition for patients with facial palsy: an innovative method for assessing the success of facial reanimation procedures. Br J Oral Maxillofac Surg 2018;56(1):3–7.
[11] Feng G, et al. A new 3-dimensional dynamic quantitative analysis system of facial motion: an establishment and reliability test. PLoS One 2014;9(11):e113115.
[12] Kim J, et al. Numerical approach to facial palsy using a novel registration method with 3D facial landmark. Sensors 2022;22(17) (Basel).
[13] Banks CA, et al. Clinician-graded electronic facial paralysis assessment: the eFACE. Plast Reconstr Surg 2015;136(2):223e–30e.
[14] Banks CA, et al. Worldwide testing of the eFACE facial nerve clinician-graded scale. Plast Reconstr Surg 2017;139(2):491e–8e.
[15] Guarin DL, et al. A machine learning approach for automated facial measurements in facial palsy. JAMA Facial Plast Surg 2018;20(4):335–7.
[16] Miller MQ, et al. The auto-eFACE: machine learning-enhanced program yields automated facial palsy assessment tool. Plast Reconstr Surg 2021;147(2):467–74.
[17] Dusseldorp JR, et al. Automated spontaneity assessment after smile reanimation: a machine learning approach. Plast Reconstr Surg 2022;149(6):1393–402.
[18] Biglioli F, et al. Mixed facial reanimation technique to treat paralysis in medium-term cases. J Craniomaxillofac Surg 2018;46(5):868–74.
[19] Dougherty W, Liebman R, Loyo M. Contemporary techniques for nerve transfer in facial reanimation. PlastAesthetRes 2021;2021.
[20] Guntinas-Lichius O, et al. Facial nerve electrodiagnostics for patients with facial palsy: a clinical practice guideline. Eur Arch Otorhinolaryngol 2020;277(7): 1855–74.
[21] Jandali D, Revenaugh PC. Facial reanimation: an update on nerve transfers in facial paralysis. Curr Opin Otolaryngol Head Neck Surg 2019;27(4):231–6.
[22] Kim J. Neural reanimation advances and new technologies. Facial Plast Surg Clin North Am 2016;24(1):71–84.
[23] Davis ME, Greene JJ. Advances and future directions in the care of patients with facial paralysis. Oper Tech Otolaryngol Head Neck Surg 2022;33(1):60–71.
[24] Rivas A, et al. A model for early prediction of facial nerve recovery after vestibular schwannoma surgery. Otol Neurotol 2011;32(5):826–33.
[25] Albathi M, et al. Early nerve grafting for facial paralysis after cerebellopontine angle tumor resection with preserved facial nerve continuity. JAMA Facial Plast Surg 2016;18(1):54–60.
[26] Shoakazemi A, et al. Long-term outcomes of the electrically unresponsive, anatomically intact facial nerve following vestibular schwannoma surgery. J Neurol Surg B Skull Base 2022;83(4):367–73.
[27] Hembd A, et al. Correlation between facial nerve axonal load and age and its relevance to facial reanimation. Plast Reconstr Surg 2017;139(6):1459–64.
[28] Collar RM, Byrne PJ, Boahene KDO. The subzygomatic triangle: rapid, minimally invasive identification of the masseteric nerve for facial reanimation. Plast Reconstr Surg 2013;132(1):183–8.
[29] Boahene K. Reanimating the paralyzed face. F1000Prime Rep 2013;5:49.
[30] Klebuc MJA. Facial reanimation using the masseter-to-facial nerve transfer. Plast Reconstr Surg 2011;127(5):1909–15.
[31] Manktelow RT, et al. Smile reconstruction in adults with free muscle transfer innervated by the masseter motor nerve: effectiveness and cerebral adaptation. Plast Reconstr Surg 2006;118(4):885–99.
[32] Elkatatny A, et al. Hypoglossal facial nerve anastomosis for post-operative and post-traumatic complete facial nerve paralysis. Open Access Maced J Med Sci 2019;7(23):3984–96.
[33] Samii M, et al. Comparison of direct side-to-end and end-to-end hypoglossal-facial anastomosis for facial nerve repair. World Neurosurg 2015;84(2):368–75.
[34] Terzis JK, Tzafetta K. The "babysitter" procedure: minihypoglossal to facial nerve transfer and cross-facial nerve grafting. Plast Reconstr Surg 2009;123(3):865–76.
[35] de Sanctis Pecora C, Shitara D. Botulinum toxin type a to improve facial symmetry in facial palsy: a practical guideline and clinical experience. Toxins 2021;13(2) (Basel).
[36] Miller MQ, Hadlock TA. Beyond botox: contemporary management of nonflaccid facial palsy. Facial Plast Surg Aesthet Med 2020;22(2):65–70.
[37] Jowett N, Malka R, Hadlock TA. Effect of weakening of ipsilateral depressor anguli oris on smile symmetry in postparalysis facial palsy. JAMA Facial Plast Surg 2017; 19(1):29–33.
[38] Derakhshan A, et al. Releasing the smile: depressor anguli oris excision in the context of managing nonflaccid facial palsy. Plast Reconstr Surg 2022;149(2): 261e–9e.
[39] Henstrom DK, et al. Platysmectomy: an effective intervention for facial synkinesis and hypertonicity. Arch Facial Plast Surg 2011;13(4):239–43.
[40] Krag AE, et al. Topographic and neural anatomy of the depressor anguli oris muscle and implications for treatment of synkinetic facial paralysis. Plast Reconstr Surg 2021;147(2):268e–78e.

[41] Halani SH, et al. Depressor anguli oris myectomy versus transfer to depressor labii inferioris for facial symmetry in synkinetic facial paralysis. J Reconstr Microsurg 2022;38(4):328–34.

[42] Azizzadeh B, Frisenda JL. Surgical management of postparalysis facial palsy and synkinesis. Otolaryngol Clin North Am 2018;51(6):1169–78.

[43] Azizzadeh B, et al. Modified selective neurectomy for the treatment of post-facial paralysis synkinesis. Plast Reconstr Surg 2019;143(5):1483–96.

[44] Vincent AG, et al. Masseteric-to-facial nerve transfer and selective neurectomy for rehabilitation of the synkinetic smile. JAMA Facial Plast Surg 2019;21(6):504–10.

[45] Leader B, et al. End-to-trunk masseteric to facial nerve transfer with selective neurectomy for facial reanimation. J Craniofac Surg 2021;32(8):2864–6.

[46] Kerscher M, et al. IncobotulinumtoxinA: a highly purified and precisely manufactured botulinum neurotoxin type A. J Drugs Dermatol 2019;18(1):52–7.

[47] Choi KH, et al. Botulinum toxin injection of both sides of the face to treat post-paralytic facial synkinesis. J Plast Reconstr Aesthet Surg 2013;66(8):1058–63.

[48] Kim J. Contralateral botulinum toxin injection to improve facial asymmetry after acute facial paralysis. Otol Neurotol 2013;34(2):319–24.

[49] Hamidian Jahromi A, Konofaos P. Contralateral facial botulinum toxin injection in cases with acute facial paralysis may improve the functional recovery: where we stand and the future direction. World J Plast Surg 2021;10(2):89–92.

[50] Guntinas-Lichius O, et al. Improved functional recovery after facial nerve reconstruction by temporary denervation of the contralateral mimic musculature with botulinum toxin in rats. Neurorehabil Neural Repair 2011;25(1):15–23.

[51] Robinson MW, Baiungo J. Facial rehabilitation: evaluation and treatment strategies for the patient with facial palsy. Otolaryngol Clin North Am 2018;51(6):1151–67.

[52] Azuma T, et al. Mirror biofeedback rehabilitation after administration of single-dose botulinum toxin for treatment of facial synkinesis. Otolaryngol Head Neck Surg 2012;146(1):40–5.

[53] Lee JM, et al. Half-mirror biofeedback exercise in combination with three botulinum toxin A injections for long-lasting treatment of facial sequelae after facial paralysis. J Plast Reconstr Aesthet Surg 2015;68(1):71–8.

[54] Pereira LM, et al. Facial exercise therapy for facial palsy: systematic review and meta-analysis. Clin Rehabil 2011;25(7):649–58.

[55] Beurskens CH, Heymans PG. Positive effects of mime therapy on sequelae of facial paralysis: stiffness, lip mobility, and social and physical aspects of facial disability. Otol Neurotol 2003;24(4):677–81.

[56] Mehdizadeh OB, Diels J, White WM. Botulinum toxin in the treatment of facial paralysis. Facial Plast Surg Clin North Am 2016;24(1):11–20.

[57] Halepas S, Chen XJ, Ferneini EM. Thread-lift sutures: anatomy, technique, and review of current literature. J Oral Maxillofac Surg 2020;78(5):813–20.

[58] Choe WJ, et al. Thread lifting: a minimally invasive surgical technique for long-standing facial paralysis. HNO 2017;65(11):910–5.

[59] Bhatnagar A, et al. Safety and efficacy of restoring facial symmetry using polydioxanone thread face lift technique in patients with facial palsy. J Clin Aesthet Dermatol 2022;15(2):26–9.

[60] Pu JK, et al. Acupuncture as part of iatrogenic facial nerve palsy rehabilitation-first report. World Neurosurg 2020;140:e343–7.

[61] Yu B, et al. Efficacy and safety of thread embedding acupuncture for facial expression muscles atrophy after peripheral facial paralysis: study protocol for a randomized controlled trial. Trials 2021;22(1):755.

[62] Harii K, Ohmori K, Torii S. Free gracilis muscle transplantation, with microneurovascular anastomoses for the treatment of facial paralysis.A preliminary report. Plast Reconstr Surg 1976;57(2):133–43.

[63] Roy M, et al. Effectiveness and safety of the use of gracilis muscle for dynamic smile restoration in facial paralysis: a systematic review and meta-analysis. J Plast Reconstr Aesthet Surg 2019;72(8):1254–64.

[64] Bhama PK, et al. Objective outcomes analysis following microvascular gracilis transfer for facial reanimation: a review of 10 years' experience. JAMA Facial Plast Surg 2014;16(2):85–92.

[65] Lenz Y, et al. Pre-operative masseter muscle EMG activation during smile predicts synchronicity of smile development in facial palsy patients undergoing reanimation with the masseter nerve: a prospective cohort study☆. J Plast Reconstr Aesthet Surg 2019;72(3):505–12.

[66] Hontanilla B, et al. Cross-face nerve grafting versus masseteric-to-facial nerve transposition for reanimation of incomplete facial paralysis: a comparative study using the FACIAL CLIMA evaluating system. Plast Reconstr Surg 2018;142(2):179e–91e.

[67] Singh Kalra G, Kalra S, Gupta S. Facial reanimation using free functional muscle transfer: lessons learnt from a long term experience comparing innervation with cross facial nerve graft and masseter nerve. J Craniofac Surg 2022;33(8):e791–6.

[68] Vila PM, et al. Powering the gracilis for facial reanimation: a systematic review and meta-analysis of outcomes based on donor nerve. JAMA Otolaryngol Head Neck Surg 2020;146(5):429–36.

[69] Faria JC, Scopel GP, Ferreira MC. Facial reanimation with masseteric nerve: babysitter or permanent procedure?Preliminary results. Ann Plast Surg 2010;64(1):31–4.

[70] Biglioli F, et al. Double innervation in free-flap surgery for long-standing facial paralysis. J Plast Reconstr Aesthet Surg 2012;65(10):1343–9.

[71] Kim MJ, et al. Comparative study of 2 different innervation techniques in facial reanimation: cross-face nerve graft-innervated versus double-innervated free gracilis muscle transfer. Ann Plast Surg 2020;84(2):188–95.

[72] Cardenas-Mejia A, et al. Double innervated free functional muscle transfer for facial reanimation. J Plast Surg Hand Surg 2015;49(3):183–8.

[73] Konofaos P, Bassilios Habre S, Wallace RD. End-to-side nerve repair: current concepts and future perspectives. Ann Plast Surg 2018;81(6):736–40.

[74] Miller MQ, Hadlock TA. Lessons from gracilis free tissue transfer for facial paralysis: now versus 10 years ago. Facial Plast Surg Clin North Am 2021;29(3):415–22.

[75] Dusseldorp JR, et al. Spontaneity assessment in dually innervated gracilis smile reanimation surgery. JAMA Facial Plast Surg 2019;21(6):551–7.

[76] Baccarani A, et al. Obturator nerve split for gracilis free-flap double reinnervation in facial paralysis. Plast Reconstr Surg Glob Open 2019;7(6):e2106.

[77] Klebuc MJ, Xue AS, Doval AF. Dual innervation of free functional muscle flaps in facial paralysis. Facial Plast Surg Clin North Am 2021;29(3):431–8.

[78] Boonipat T, et al. Dual innervation of free gracilis muscle for facial reanimation: what we know so far. J Plast Reconstr Aesthet Surg 2020;73(12):2196–209.

[79] Boahene KO, et al. The multivector gracilis free functional muscle flap for facial reanimation. JAMA Facial Plast Surg 2018;20(4):300–6.

[80] Tzafetta K, et al. Long-term outcomes of dual innervation in functional muscle transfers for facial palsy. J Plast Reconstr Aesthet Surg 2021;74(10):2664–73.