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Protocol for randomized controlled trial of brief intraoperative electrical stimulation across cross-face nerve grafts in patients with facial paralysis

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Abstract

Brief Intraoperative Electrical Stimulation (BIES) can enhance axonal regeneration across axotomy sites. Cross-Face Nerve Grafts (CFNG) are typically performed in two stages, with a 6–12-month interval to allow for axonal growth. This provides an opportunity to assess axonal regeneration via biopsy. We describe a protocol to evaluate the effects of a 10-minute BIES on axonal regeneration across CFNG. This is a protocol for a multicenter, double-blind, randomized controlled trial in patients with unilateral facial paralysis undergoing two-stage CFNG. After nerve coaptation, the intervention group will receive 10 minutes of BIES (biphasic current, 2mA amplitude, 100 μsec pulse duration, 16 Hertz) applied proximal to the donor facial to sural nerve coaptation. Controls will undergo standard surgery without stimulation. The primary outcome is the histological count of regenerated myelinated axons from a biopsy obtained during the second stage surgery. Secondary outcomes include patient reported quality of life and objective facial reanimation based on photo analysis. We aim to recruit 20 participants. This protocol evaluates the impact of BIES on histological, patient reported, and objective facial reanimation outcomes in CFNG.

Key Words: electrical stimulation, facial paralysis, cross-face nerve grafting, clinical trial.

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People with facial paralysis experience substantial functional, aesthetic, and psychosocial repercussions.[1-3] They may struggle to smile, articulate speech, drink, or close their eyelids fully.[4] For patients where spontaneous recovery is unlikely, facial reanimation surgery may be pursued.[5] Cross-Face Nerve Grafting (CFNG), consisting of long autologous cable grafts that transfer axons from the unaffected side of the face to the paralyzed side, is a frequently used intervention in such surgeries. This technique has the advantage of enabling spontaneous, emotionally driven facial movement controlled by the unaffected side.[6]

While the CFNG can restore spontaneous facial movement, axonal growth through the graft can be disappointing. CFNGs can be over 20 centimeters (cm) long, and it typically takes six to twelve months for axons to traverse the full graft.[7] Delays in this reinnervation process and advanced age can limit axonal reach to the motor endplate, reducing the effectiveness of reinnervation and muscle activation.[8,9] Previous studies report that only 20% to 50% of donor axons reach the distal end of the CFNG,[10,11] contributing to high failure rate ranging from 16.7% to 47.2% for this long nerve grafting procedure.[12-15] Consequently, even with optimal surgical intervention, patients undergoing CFNG surgery often recover with suboptimal functional outcomes. Despite these challenges, no therapies currently

exist to enhance the efficiency of nerve regeneration across CFNGs.

Unlike conventional cable grafts, CFNG are performed in two stages, offering a unique opportunity to assess axonal regeneration without adding to patient morbidity.[16] In the first stage, the donor facial branch is connected to the proximal end of the CFNG, and axons are given six to twelve months to grow through the graft. Following this waiting period, the second stage, in which the distal CFNG is connected to a target recipient nerve is performed, allowing for reinnervation. Potential targets include recipient nerves of free muscle grafts or local facial nerve branches on the paralyzed side of the face.[17] During this second surgery, the distal end of the graft can be biopsied to assess axonal growth before performing the coaptation.

Despite research efforts to develop strategies for enhancing peripheral nerve regeneration, few effective and clinically feasible options have been found. Inherent biological constraints, such as distal Schwann cell atrophy and diminished neurotrophic growth factor signaling, present significant obstacles to successful nerve regeneration.[18] Electrical stimulation enhances peripheral axonal regeneration by modulating intracellular signaling pathways that promote Schwann cell proliferation, phenotypic programming, and increased myelin-associated gene expression.[19] Intraoperative Electric Stimulation (IES) has shown promising results. In murine models of sciatic nerve injury, IES accelerates axonal regeneration and improves functional outcomes.[20,21]

In humans, several randomized controlled trials of Brief Intraoperative Electrical Stimulation (BIES) have demonstrated improved functional outcomes following peripheral nerve stimulation in carpal tunnel, ulnar, and digital nerve surgeries.[22,23] While it is currently unclear whether electrical stimulation promotes facial nerve regeneration through mechanisms similar to those observed in other peripheral nerves, animal models of facial nerve injury have demonstrated that 30 to 60 minutes of intraoperative electrical stimulation accelerates axonal regeneration and improves facial nerve function.[24,25] Recent studies suggest that a single 10-minute session of BIES may result in similar functional recovery benefits as the standard 60-minute stimulation.[26,27] This approach reduced operative time and decreased anesthesia exposure which also makes adoption of the intervention more feasible.[27,28]

Here, we describe a protocol for a multicenter, double-blind, randomized, controlled clinical trial to evaluate the effects of BIES on enhancing axonal regeneration across CFNGs. This protocol aims to generate preliminary data on the safety and efficacy of intraoperative BIES to improve axonal growth and functional outcomes in CFNG used for facial reanimation surgery.

Materials and Methods

Table 1 shows a timeline of study-related visits and procedures.

Table 1. *Schedule of study-related visits and procedures.*

	Day 1 Enrollment	Week 1 Allocation to BIES or control	Month 1-6	Month 6-9*	Month 6[a]	Month 12[a]	Month 24[a]
Eligibility & Consent	X						
1st Stage Surgery		X Intervention: BIES *vs* control					
Intervention		To take place during surgery					
2nd Stage Surgery				X			
Nerve Biopsies		X During 1st stage surgery		X During 2nd stage surgery			
Photography	X				X	X	X
Questionnaires	X				X	X	X
AEs Inquiry			X		X	X	X

AEs, adverse effects; BIES, brief intraoperative electrical stimulation therapy.
**Exact month may vary following standard clinical protocols.*
[a]Following second-stage surgery.

The study was structured as a multicenter, double-blind, randomized controlled trial in patients with unilateral facial paralysis who are undergoing two-stage CFNG for facial reanimation surgery.

Inclusion and exclusion criteria

Patients aged 1 month to 80 years with unilateral facial paralysis who are candidates for two-stage CFNG facial reanimation surgery, including CFNG-driven free gracilis muscle transfer (FGMT), dual innervation FGMT (CFNG + masseteric nerve), or direct facial nerve coaptation after babysitter innervation with masseteric or hypoglossal nerve, are eligible for this study (Table 2). Patients outside the specified age range and patients with bilateral facial paralysis will be excluded.

Screening

Patients identified as candidates for two-stage CFNG by surgeons at the facial nerve center who meet the inclusion criteria outlined above will be considered for potential recruitment.

Enrollment (Day 1)

Consent discussion will occur prior to enrollment. Study procedures, potential risks, and benefits will be clearly explained to participants or their parents, ensuring they understand that participation is entirely voluntary and free from any undue pressure. Those who consent to participate will then proceed to randomization and group allocation.

Randomization and blinding

Participants will be randomized and allocated to either control (standard surgical intervention) or active intervention (standard surgical intervention combined with BIES) groups in a 1:1 ratio. Participants will be block randomized by study site. Outcome evaluators and participants will be blinded to group allocation. The participants will be unblinded at study completion.

Surgical interventions

Both the control and active intervention groups will undergo a two-stage CFNG surgical procedure following standard clinical practice.

First stage

Per standard clinical practice, the first stage involves harvesting, preparing, placing, and coapting a sural nerve graft to an identified donor facial nerve branch. Biopsies of the distal donor facial nerve branch and proximal and distal sural nerve graft, measuring approximately 3 to 5 millimeters (mm) in width, will be taken in both groups prior to coaptation of the donor nerve to the graft. The total length of the CFNG will be recorded. No local or regional nerve blocks will be used to ensure nerve conduction from the peripheral nerve to the brainstem. No ink will be used to mark the nerves as it can distort specimen processing.

Second stage

The second-stage surgery will take place after 12 months and before 16 months from the initial procedure. During the second stage surgery, the distal end of the CFNG will be coapted to the intended recipient nerve. A 3-5 mm biopsy of the distal end of the CFNG will be collected at this stage prior to coaptation. When a FGMT is being performed the distal end of CFNG is coapted to the nerve to the gracilis muscle (*i.e.* anterior obturator nerve). This procedure may also involve coaptation of the nerve to the gracilis to the masseteric nerve (*i.e.* dual innervated FGMT). When direct facial nerve coaptation after babysitter innervation with masseteric or hypoglossal nerve is being performed, the distal end of the CFNG will be coapted to a facial nerve branch on the affected side. The exact procedure performed at this stage will be recorded for all participants.

Biopsy processing

Biopsies will be processed and manually counted using high-powered and electron microscopy by a team of neu-

Table 2. *General inclusion and exclusion criteria*

Inclusion criteria
Patients 1 month to 80 years old
Unilateral facial paralysis
AND
Two-stage CFNG surgery
Exclusion criteria
Age <1 month or >80 years of age
Bilateral facial paralysis
Comorbid medical condition preventing two-stage CFNG surgery

CFNG, cross-face nerve graft.

roscientists following the protocol outlined by Terzis *et al.*[11] Additional details regarding sample processing are provided in the supplementary material section of this protocol.

Active intervention

Participants in the active intervention group will receive BIES during the first stage CFNG surgery after coaptation of the donor facial nerve to the sural nerve graft. Intraoperative electrical stimulation will be delivered proximal to the coaptation of the donor facial nerve to the sural nerve graft. Electrical stimulation setting will be as follows: biphasic waveform, 16 Hz pulse rate, 100 µsec pulse duration, 2 milliamps (mA) amplitude, and 10 minutes total duration. Stimulation will be administered using a Checkpoint Guardian™ nerve stimulator (Checkpoint Surgical, Inc., Independence, OH). Stimulation will be applied after the sutures have been placed and before fibrine sealant is applied. Stimulation will be confirmed by the flashing yellow Light-Emitting Diode (LED) indicator and periodically verified throughout the 10-minute therapy. During stimulation, the surgical team will focus on another aspect of the procedure to avoid extending operating room or anesthesia time for the active intervention group.

Control

Participants in the control group will undergo the standard surgical procedure and the same biopsies conducted in the active intervention group.

Outcomes

The primary objective of the study is to evaluate the efficacy of BIES to improve axonal regeneration across a CFNG (Tables 3 and 4).

Primary outcome

The primary outcome will be the count of myelinated axons during the second stage surgery, when the axons have grown across the CFNG.

Secondary outcomes

Histopathological analysis

Myelinated axon counts from the first (donor facial nerve branch and sural nerve) stage biopsies will be obtained.

Clinical recovery and facial reanimation outcome

Clinical recovery will be evaluated as a secondary outcome including clinician-graded ratings of facial function, objective photographic analysis and patient reported outcome measures. Clinical assessments will be performed at baseline, and 6, 12, and 24 months after the second stage surgery.

Facial video and photograph analysis: participants will have video and eight digital frontal face photographs taken by a member of the research team showing: i) rest, ii) gentle eye closure, ii) strong eye closure, iv) smile showing teeth, v) smile without teeth,vi) brow elevation, vii) lip pucker, and viii) patient saying 'eeee' to evaluate lower lip function.

Clinical graded facial function: blinded evaluators will rate participant facial function using a validated, electronic clinician-graded facial function scale (eFACE) with high intrarater and interrater reliability.[29] eFACE rates facial function and symmetry on a 0-100 scale from sixteen features of facial photographs and provides a global score and subscores for rest, dynamic, and synkinesis scores.

Objective photographic analysis of facial function: metrics of oral commissure excursion, smile angle, and dental show will be calculated. Emotrics (Massachusetts Eye and

Table 3. *Outcome parameters.*

Variable	Scale	Timeline
Regenerated myelinated axons	Axonal count	First surgery and second surgery
Clinician graded facial function	eFACE*	Baseline, 6, 12, and 24 months after 2nd stage surgery
Patient reported quality of life	FaCE	Baseline, 6, 12, and 24 months after 2nd stage surgery
Oral commissure excursion, smile angle, dental show	Emotrics*	Baseline, 6, 12, and 24 months after 2nd stage surgery
Adverse effects	Proportion	Baseline and throughout follow-up clinic visits

eFACE, electronic clinician-graded facial function scale; FaCE, facial clinimetric evaluation scale.
**Based on standardized facial photography.*

Ear, Boston, MA), which is a semi-automated, machine learning-based software that provides quantitative data of facial landmarks on patient photograph, will be used.[30]
Patient-Reported Outcome Measures (PROMs): to measure facial paralysis specific Quality Of Life (QOL), the Facial Clinimetric Evaluation scale (FaCE) will be used. FaCE is a validated QOL instrument with 15 items with 6 domains that include facial movement, facial comfort, oral function, eye comfort, lacrimal control, and social function. The total score ranges from 0 to 100, with lower scores indicating worse dysfunction.[31]
Adverse Effects (AEs): while we do not anticipate AEs to occur intraoperatively, we will query surgeons and document any AEs using a standardized form.

Statistical analysis

To compare the number of myelinated axons between the stimulation group and the non-stimulation group in CFNGs, we will perform an Analysis Of Covariance (ANCOVA). The primary outcome variable will be the number of myelinated axons in the nerve graft at the final time point. The independent variable will be group allocation (control *vs*. active intervention), and the original number of myelinated axons in the donor branch and cable graft will be included as covariates to control for baseline variability in axon counts.
Descriptive statistics will be presented as means (± standard deviation) and prevalence (%) values. For the secondary outcomes that consist of continuously scored instruments or objective measurements, t-tests or Wilcoxon tests will be performed, contingent on data distribution.

Power calculations

There are limited available data to perform a power analysis to determine the sample size required to detect a difference in axonal nerve growth. The published axonal counts through a CFNG for facial reanimation is 453 axons with a standard deviation of 265.[20] With 10 subjects in each group (total n = 20), the study is powered to detect a difference of at least 235 axons between the stimulated and non-stimulated groups with 80% power at a 5% significance level. Furthermore, the proposed cohort size (n=20) is consistent with previously published clinical studies evaluating brief electrical stimulation to promote nerve regeneration.[4,5,29]

Discussion

CFNG offer a unique surgical procedure to histologically evaluate the efficacy of intraoperative electric stimulation in enhancing growth through a nerve graft. Currently, CFNG procedures have unacceptably high failure rates in facial reanimation. The failure rate for CFNG to restore eye closure is as high as 42.9% and for smile 47.2%.[15] This is often attributed to staggered axonal growth initiation, length-dependent arrest, and intrinsic biological factors.[16] To date, there are no therapies available to enhance the efficiency of nerve regeneration across CFNG. Building on decades of experimental evidence in murine models, several recent, prospective, randomized controlled trials have affirmed BIES as a clinically translatable technique to enhance functional recovery in patients with peripheral nerve injuries requiring surgery.[22-25] This study protocol is designed to obtain preliminary safety and efficacy data on the adjunctive therapeutic use of BIES in a two-stage CFNG facial reanimation procedure.
This protocol has several strengths. It is a double-blind, randomized controlled trial with the objective measures of axonal counts as the primary outcome measure. Axonal counts are obtained both at baseline (during surgery) and at a subsequent time point, allowing for direct measurement of axonal growth through the nerve graft. Histological evaluation provides a quantifiable and objective assessment of axonal regeneration, offering advantages over clinical assessments of facial reanimation which are influenced by multiple confounding variables. Unlike clinical outcomes— which can be affected by factors such as variability in surgical technique, concurrent procedures, patient-specific differences in nerve regeneration, and subjectivity in outcome assessment—histological analysis enables a direct and standardized assessment of nerve regeneration at the cellular level. It also allows for objective measurement of axonal growth in response to stimulation.

Table 4. *Collected clinicodemographic information.*

Variable
Age
Sex
Race
Medical comorbidities (*e.g.* diabetes mellitus, congestive heart failure, tobacco/alcohol use)
Facial paralysis etiology
FGMT innervation pattern

FGMT, free gracilis muscle transfer.

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In addition to histological analysis, the protocol incorporates clinical outcome evaluation through facial movement assessment using standardized photo and video analysis. This dual approach enhances the study's ability to correlate structural nerve regeneration with functional recovery, providing a comprehensive evaluation of the intervention's efficacy. Secondarily, it allows for direct correlation between histological findings and both patient-reported outcomes and clinician-graded recovery.

This protocol is limited to evaluate the impact of a specific BIES intervention and will not evaluate the impact of IES delivered with other parameters or protocols. The parameters were chosen based on review of the literature and investigator consensus.[2-5]

Conclusions

This protocol is designed to evaluate the histological and clinical impact of a 10-minute dose of BIES to enhance axonal regeneration across CFNG. CFNG provides the unique opportunity to biopsy the distal graft end to evaluate the effect of BIES on axonal regeneration. We outline recommended intraoperative parameters and key outcomes for evaluation. We believe that publishing the protocol with predefined entry criteria, intervention details, and outcome measures enhances research transparency and improves reporting standards for future facial paralysis research endeavors.

List of abbreviations

BIES, Brief Intraoperative Electrical Stimulation
CFNG, Cross-Face Nerve Grafts
IES, Intraoperative Electric Stimulation
Free Gracilis Muscle Transfer (FGMT)
LED, Light-emitting diode
eFACE, electronic clinician-graded facial function scale
QOL, Quality Of Life
FaCE, Facial Clinimetric Evaluation scale
AEs, Adverse Effects
ANCOVA,Analysis Of Covariance

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Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical considerations

This study was approved by the Cleveland Clinic Foundation (22-926) and the Oregon Health & Science University Institutional Review Boards (26-501).

Contributions

Myriam Loyo, Dane J. Genther, Matthew Q. Miller, Patrick J. Byrne, conceptualization; Bastien A. Valencia-Sanchez, Myriam Loyo, Hana Rosen, Dane J. Genther, Michelle Cameron, Patrick J. Byrne, Hanna Rosen, methodology; Bastien A Valencia-Sanchez, Myriam Loyo, writing – original draft; Stephen Hadford, Dane J. Gentehr, Matthew Q. Miller, Michelle H. Cameron, Patrick J. Byrne, Hana Rosen, writing – review & editing.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

No new data were created or analyzed in this study. Data supporting the findings can be found in previously published sources cited in the manuscript.

Trial registration

Clinicaltrials.gov (NCT06335719).

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