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Plastic and Reconstructive Problems

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Home Study Course

Home Study Course

Section 4
April 2016

AMERICAN ACADEMY OF OTOLARYNGOLOGY–HEAD AND NECK SURGERY FOUNDATION

THE
HOME STUDY COURSE
IN
OTOLARYNGOLOGY -- HEAD AND NECK SURGERY

SECTION 4

Plastic and Reconstructive Problems

April 2016

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American Academy of Otolaryngology - Head and Neck Surgery Foundation

Section 4 suggested exam deadline: June 10, 2016
Expiration Date: August 5, 2016; CME credit not available after that date

Introduction
The Home Study Course is designed to provide relevant and timely clinical information for physicians in training and current practitioners in otolaryngology - head and neck surgery. The course, spanning four sections, allows participants the opportunity to explore current and cutting edge perspectives within each of the core specialty areas of otolaryngology.

The **Selected Recent Material** represents primary fundamentals, evidence-based research, and state of the art technologies in plastic and reconstructive problems. The scientific literature included in this activity forms the basis of the assessment examination.

The number and length of articles selected are limited by editorial production schedules and copyright permission issues, and should not be considered an exhaustive compilation of knowledge of plastic and reconstructive problems.

The **Additional Reference Material** is provided as an educational supplement to guide individual learning. This material is not included in the course examination and reprints are not provided.

Needs Assessment
AAO-HNSF's education activities are designed to improve healthcare provider competence through lifelong learning. The Foundation focuses its education activities on the needs of providers within the specialized scope of practice of otolaryngologists. Emphasis is placed on practice gaps and education needs identified within eight subspecialties. The *Home Study Course* selects content that addresses these gaps and needs within all subspecialties.

Target Audience
The primary audience for this activity is physicians and physicians-in-training who specialize in otolaryngology-head and neck surgery.

Outcomes Objectives

The participant who has successfully completed this section should be able to:

1. Identify the advances in soft tissue engineering, bioscaffolds, and stem cells in the head and neck
2. Describe the available treatment options of nasal valve collapse
3. Identify cosmetic nasal deformities and novel interventions to correct them
4. Describe therapeutic laser interventions and their indications
5. Relate the complications of blepharoplasty and facelift surgery and prevention options
6. Recognize the current options for botulinum toxin treatments and potential complications
7. Explain the current concepts in management of keloids and hypertrophic scars
8. Summarize the options for local and regional reconstruction using flaps and grafts
9. Discuss the treatment options related to facial paralysis
10. Describe the best evidence for mandible and maxillary fracture repair
11. Explain the use of propranolol for treatment of facial hemangiomas
12. Describe the terminology for pediatric vascular lesions

Medium Used
The Home Study Course is available as printed text. The activity includes a review of outcomes objectives, selected scientific literature, and a self-assessment examination.

Method of Physician Participation in the Learning Process
The physician learner will read the selected scientific literature, reflect on what they have read, and complete the self-assessment exam. After completing this section, participants should have a greater understanding of plastic and reconstructive problems as they affect the head and neck area, as well as useful information for clinical application.

Estimated time to complete this activity: 40.0 hours

Accreditation Statement
The American Academy of Otolaryngology—Head and Neck Surgery Foundation (AAO-HNSF) is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

Credit Designation
The AAO-HNSF designates this enduring material for a maximum of 40.0 *AMA PRA Category 1 Credit(s)*™. Physicians should claim credit commensurate with the extent of their participation in the activity.

ALL PARTICIPANTS must achieve a post-test score of 70% or higher for a passing completion to be recorded and a transcript to be produced. Residents' results will be provided to the Training Program Director.

PHYSICIANS ONLY: In order to receive *Credit* for this activity a post-test score of 70% or higher is required. Two retest opportunities will automatically be available if a minimum of 70% is not achieved.

Disclosure
The American Academy of Otolaryngology Head and Neck Surgery/Foundation (AAO-HNS/F) supports fair and unbiased participation of our volunteers in Academy/Foundation activities. All individuals who may be in a position to control an activity's content must disclose all relevant financial relationships or disclose that no relevant financial relationships exist. All relevant financial relationships with commercial interests[1] that directly impact and/or might conflict with Academy/Foundation activities must be disclosed. Any real or potential conflicts of interest[2] must be identified, managed, and disclosed to the learners. In addition, disclosure must be made of presentations on drugs or devices, or uses of drugs or devices that have not been approved by the Food and Drug Administration. This policy is intended to openly identify any potential conflict so that participants in an activity are able to form their own judgments about the presentation.

[1] A "Commercial interest" is any entity producing, marketing, re-selling, or distributing health care goods or services consumed by, or used on, patients.

[2] "Conflict of interest" is defined as any real or potential situation that has competing professional or personal interests that would make it difficult to be unbiased. Conflicts of interest occur when an individual has an opportunity to affect education content about products or services of a commercial interest with which they have a financial relationship. **A conflict of interest depends on the situation and not on the character of the individual.**

2016 Section 4 PLASTIC AND RECONSTRUCTIVE PROBLEMS

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This 2016 Home Study Course Section includes discussion of the following drugs and devices that have not been approved by the United States Food and Drug Administration:

Name of drug or device	Nature of off-label Discussion
Neuromodulators	Reconstitution and storage

Disclaimer
The information contained in this activity represents the views of those who created it and does not necessarily represent the official view or recommendations of the American Academy of Otolaryngology – Head and Neck Surgery Foundation.

June 10, 2016: Section 4 suggested exam deadline to be received.
August 5, 2016, midnight EST: deadline for *all exams* to be submitted.

EVIDENCE BASED MEDICINE

The AAO-HNSF Education Advisory Committee approved the assignment of the appropriate level of evidence to support each clinical and/or scientific journal reference used to authenticate a continuing medical education activity. Noted at the end of each reference, the level of evidence is displayed in this format: **[EBM Level 3]**.

Oxford Centre for Evidence-based Medicine Levels of Evidence (May 2001)	
Level 1	Randomized[1] controlled trials[2] or a systematic review[3] (meta-analysis[4]) of randomized controlled trials[5].
Level 2	Prospective (cohort[6] or outcomes) study[7] with an internal control group or a systematic review of prospective, controlled trials.
Level 3	Retrospective (case-control[8]) study[9] with an internal control group or a systematic review of retrospective, controlled trials.
Level 4	Case series[10] without an internal control group (retrospective reviews; uncontrolled cohort or outcome studies).
Level 5	Expert opinion without explicit critical appraisal, or recommendation based on physiology/bench research.

Two *additional ratings* to be used for articles that do not fall into the above scale. Articles that are informational only can be rated N/A , and articles that are a review of an article can be rated as Review. All definitions adapted from Glossary of Terms, Evidence Based Emergency Medicine at New York Academy of Medicine at www.ebem.org.

[1] A technique which gives every patient an equal chance of being assigned to any particular arm of a controlled clinical trial.
[2] Any study which compares two groups by virtue of different therapies or exposures fulfills this definition.
[3] A formal review of a focused clinical question based on a comprehensive search strategy and structure critical appraisal.
[4] A review of a focused clinical question following rigorous methodological criteria and employing statistical techniques to combine data from independently performed studies on that question.
[5] A controlled clinical trial in which the study groups are created through randomizations.
[6] This design follows a group of patients, called a "cohort", over time to determine general outcomes as well as outcomes of different subgroups.
[7] Any study done forward in time. This is particularly important in studies on therapy, prognosis or harm, where retrospective studies make hidden biases very likely.
[8] This might be considered a randomized controlled trial played backwards. People who get sick or have a bad outcome are identified and "matched" with people who did better. Then, the effects of the therapy or harmful exposure which might have been administered at the start of the trial are evaluated.
[9] Any study in which the outcomes have already occurred before the study has begun.
[10] This includes single case reports and published case series.

OUTLINE

April 2016 Section 4

Plastic and Reconstructive Problems

I. Cosmetic
- **A. Tissue Engineering for Facial Reconstruction**
- **B. Cosmetic Rhinoplasty**
- **C. Laser Therapy for Rejuvenation**
- **D. Fillers and Chemodenervation**
- **E. Rhytidectomy**
- **F. Blepharoplasty**

II. Reconstruction
- **A. Functional Rhinoplasty**
- **B. Treatment of Hypertrophic Scars and Keloids**
- **C. Flaps and Grafts**
- **D. Facial Paralysis**
- **E. Facial Fractures**

III. Congenital
- **A. Craniofacial Deformities**
- **B. Vascular Malformations**

TABLE OF CONTENTS
Selected Recent Materials - Reproduced in this Study Guide

2016 SECTION 4 PLASTIC AND RECONSTRUCTIVE PROBLEMS

ADDITIONAL REFERENCE MATERIAL…….

I. Cosmetic

A. Tissue Engineering for Facial Reconstruction

Ribeiro L, Castro E, Ferreira M, et al. The concepts and applications of tissue engineering in otorhinolaryngology. *Acta Otorrinolaringol Esp*. 2015; 66(1):43-48. EBM level 4

Summary: Ribeiro et al provide an excellent baseline explanation of the three components of tissue engineering—cell, regulators, and scaffolds—is provided, along with examples for how the process can work in laryngology, facial reconstruction, head and neck surgery, and otology.

Rajan A, Eubanks E, Edwards S, et al. Optimized cell survival and seeding efficiency for craniofacial tissue engineering using clinical stem cell therapy. *Stem Cells Transl Med*. 2014; 3(12):1495-1503. EBM level 2

Summary: The study describes an experiment that presents basic science principles to maximize cell viability at different time points of incubation. Once maximized, these cells are then used to reconstruct a maxillary bony defect with implants to obtain a good result. This is an excellent example of bench-top progress to improve clinical care and outcome.

B. Cosmetic Rhinoplasty

Baker SR. Diced cartilage augmentation: early experience with the Tasman technique. *Arch Facial Plast Surg*. 2012; 14(6):451-455. EBM level 4

Summary: This paper presents a retrospective review of diced cartilage grafts in rhinoplasty and offers helpful tips on use of this technique.

Bitik O, Uzun H, Kamburoğlu HO, et al. Revisiting the role of columellar strut graft in primary open approach rhinoplasty. *Plast Reconstr Surg*. 2015; 135(4):987-997. EBM level 4

Summary: This paper examines the role of the columellar strut and its role in tip support. It questions the standard thinking about the necessity of columellar strut placement in external rhinoplasty.

Ilhan AE, Saribas B, Caypinar B. Aesthetic and functional results of lateral crural repositioning. *JAMA Facial Plast Surg*. 2015; 17(4):286-292. EBM level 3

Summary: This paper describes the functional and cosmetic improvement in 71 patients who underwent lateral crural positioning using the NOSE and ROE measures.

C. Laser Therapy for Rejuvenation

Holcomb JD. Thermally confined micropulsed 1444-nm Nd:YAG interstitial fiber laser in the aging face and neck: an update. *Facial Plast Surg Clin North Am*. 2014; 22(2):217-

229. EBM level 4

Summary: This article reviews the use of the 1444-nm Nd:YAG interstitial fiber laser in in precision contouring of the face and neck in both nonsurgical and surgical uses. It addresses important considerations in maintaining safe clinical thermal parameters during the procedure.

Richter AL, Barrera J, Markus RF, Brissett A. Laser skin treatment in non-Caucasian patients. *Facial Plast Surg Clin North Am*. 2014; 22(3):439-446. EBM level 4

Summary: Laser treatments in ethnic skin pose unique challenges regarding technique and postprocedural care. This article reviews important factors in laser selection and preprocedural planning in order to avoid laser complications in ethnic skin.

D. Fillers and Chemodenervation

Trindade de Almeida AR, Secco LC, Carruthers A. Handling botulinum toxins: an updated literature review. *Dermatol Surg*. 2011; 37(11):1553-1565. EBM level 5

Summary: This article is an extensive review of the current literature on the storage, reconstitution, and handling of botulinum toxins as it relates to clinical medicine. Recommendations are made for the use of bacteriostatic saline for reconstitution as it is less painful, and for the refrigerated storage and use of reconstituted toxin for up to 3 weeks.

Acta Otorrinolaringol Esp. 2015;**66(1)**:43–48

Acta Otorrinolaringológica Española

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REVIEW ARTICLE

The Concepts and Applications of Tissue Engineering in Otorhinolaryngology☆

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KEYWORDS
Tissue engineering;
Vocal cords;
Ear pinna;
Rhinoplasty;
Trachea;
Tympanic membrane perforation

Abstract
Introduction: Tissue engineering is a rapidly developing field that, making biological substitutes for the repair and regeneration of damaged tissues, will play an important role in the future of otorhinolaryngology.
Objective: In this article we explain the principles of regenerative medicine and its potential applications in Otorhinolaryngology.
Materials and methods: The authors searched the published literature on this topic, chose relevant references, and extracted and systematized the data.
Results and conclusion: There are some exciting possibilities for future treatments in otorhinolaryngology applying the concepts of tissue engineering.

PALABRAS CLAVE
Ingeniería de tejidos;
Cuerdas vocales;
Oreja;
Rinoplastia;
Tráquea;
Perforación de la membrana timpánica

Conceptos y aplicaciones de la ingeniería tisular en Otorrinolaringología

Resumen
Introducción: La ingeniería de tejidos es un campo en rápido desarrollo, haciendo unos sustitutos biológicos para la reparación y regeneración de tejidos dañados, jugará un papel importante en el futuro de la otorrinolaringología.
Objetivo: En este artículo se explican los principios de la medicina regenerativa y sus posibles aplicaciones en Otorrinolaringología.
Materiales y métodos: Los autores han buscado en la literatura publicada sobre el tema, eligió referencias pertinentes y se extrajo y sistematizado de la fecha.

☆ Please cite this article as: Ribeiro L, Castro E, Ferreira M, Helena D, Robles R, Faria e Almeida A, et al. Conceptos y aplicaciones de la ingeniería tisular en Otorrinolaringología. Acta Otorrinolaringol Esp. 2015;66:43–8.

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Resultados y conclusiones: Hay posibilidades muy interesantes para futuros tratamientos en otorrinolaringología que aplican los conceptos de la ingeniería de tejidos.

Introduction

In the current era the paradigm of medicine is constantly changing, as new concepts and methods of life support and disease control arise. Tissue engineering is becoming one of the most promising weapons in medical practice. Based on highly advanced technological procedures, tissue and organ reconstruction may, within a short time, become gold-standard treatments for a rising number of medical conditions where classical pharmacological or surgical interventions have limited effectiveness. In fact, recent developments in the area clearly show impressive results in the rehabilitation of functionally or structurally committed organs and tissues.[1]

Otorhinolaryngology (ORL), as a medical specialty with a wide range of medical and surgical interventions, naturally assumes a position of leadership in the application of tissue engineering techniques.

The aim of this review is the description of the fundamentals of regenerative medicine and its potential applications in ORL.

Fundamentals

The main goal of tissue engineering is restoring functional or structural tissue by using living elements that will later be integrated in patients.[1] In this process, 3 basic components are generally present: cells, regulators/growth factors and scaffolds, which may or may not be used simultaneously.[2,3]

Cells

Most papers published within the past 20 years have focused mainly on cell therapy,[1] which consists in the deposit of selected living cells in an appropriate scaffold, that, when exposed to a specific microenvironment, will multiply and differentiate into the desired structure. Different cell subtypes may be used: stem cells and adult cells.[1–3]

Stem cells are characterized not only by their ability of continuous and unlimited self-renewal, but also by the possibility of differentiation into any cellular phenotype.[2] Stem cells are assumed as having the highest potential in regenerative medicine, although their use is limited by ethical issues and the potential risk of neoplastic transformation. Stem cells can be obtained from embryonic or mature tissues. Embryonic stem cells are derived from blastocysts, and therefore can differentiate into any mature cell type of the 3 germ layers.[4] On the other hand, adult stem cells can be collected from certain niches in the body, namely bone marrow, adipose tissue or blood,[4] and are not totally pluripotent as they are positioned in a later stage of the differentiation line, having a finite capacity to multiply depending on the origin of the tissue.

Adult cells can be obtained from a biopsy specimen of the tissue to be regenerated, and their replication is induced *in vitro* before transplantation. Being phylogenetically more advanced, adult cells do not have the ability to replicate endlessly or to transform into different cell types. These features, combined with the possibility of perpetuation of pre-existing pathological changes in the donor organ or tissue, represent important limitations in their use.

Regulators/Growth Factors

Growth factors are molecules that regulate proliferation, differentiation and cell function, and therefore may induce, accelerate or inhibit those cellular processes. They are an essential element in regenerative medicine. Depending on the technique used, these molecules can be included in a scaffold, which serves as a means for their controlled release, which will influence and control cell growth.[3]

Scaffolds

Scaffolds are porous 3-dimensional structures that provide mechanical support and physical protection to cells and growth factors.[2] These should be composed of a biocompatible and reabsorbable matrix,[1,2,5] allowing for complete tissue regeneration. Collagen and fibrin are among the most commonly used materials, and are generally obtained from natural sources; polyglycolic acid, a synthetic polymer, may also be used.[2]

Applications of Tissue Engineering in Otorhinolaryngology

Laryngology

The vocal folds are able to vibrate at a frequency up to 1000 Hz[3], due to their microstructure consisting of epithelium, lamina propria and the vocal muscle. The lamina propria is composed by a superficial layer (Reinke space), an intermediate layer and a deeper layer, each of these having specific cellular components, which are ultimately related to the organ function.

The stratified epithelium covers the entire surface of the vocal folds, and represents a barrier against physical, chemi-

cal and microbiological aggression.[5] Fibroblasts are the main cell type in the lamina propria. These cells are produced and embedded in an extracellular matrix. The extracellular matrix supports all tissue cells and plays an important role in the regulation of cell migration, proliferation and differentiation.[6,7] Hyaluronic acid abounds particularly in the superficial layer of the lamina propria, hydrating the vocal folds and making them compressible. The remaining layers are mainly composed of collagen and elastin, which are responsible, respectively, for tensile and elastic resistance of the vocal folds.[5,6]

When the ultrastructure of the extracellular matrix is altered, either by surgical interventions or factors such as infection, trauma, or radiation, a disruption of the vibrating function of the vocal folds may result.[6,8,9] In these situations there is usually an aberrant healing process that favors collagen deposition and decreased production of hyaluronic acid and elastin fibers, which ultimately, leads to scar formation, responsible for dysphonia. A number of therapeutic interventions have been described to prevent and/or treat vocal fold scars or atrophy, invariably with limited success due to the difficulty of restoring the mucosal wave. Thus, the goal of regenerative medicine concerning vocal folds is restoring the vibratory and respiratory functions of the larynx through the reconstruction of the lamina propria's extracellular matrix using the elements described below.

Regulators/Growth Factors

Growth factors are the only elements that, to date, have been successfully applied in vocal fold bioengineering.[5,10] Within these, fibroblast growth factor assumes a prominent position since it has an important role in the regulation of scar formation. Hirano showed that fibroblast growth factor enhanced the production of hyaluronic acid by fibroblasts of the vocal folds, while inhibiting local deposition of collagen.[11,12] He described a clinical case in which fibroblast growth factor was used in the treatment of atrophic vocal folds of a 63-year-old male, with clear improvement of acoustic parameters only 1 week after surgery.[12]

Another cytokine that has been increasingly studied in the treatment of vocal scars is the hepatocyte growth factor, mainly due to its anti-fibrotic and angiogenic properties.[5] Similar to the fibroblast growth factor, hepatocyte growth factor also stimulates the production of hyaluronic acid and elastin and inhibits collagen synthesis.[13,14] Hirano made another experience where he injected hydrogel impregnated with hepatocyte growth factor in a previously injured canine vocal fold. Here, a structural regeneration of the vocal fold with improvement of the mucosal wave was observed.[3,5]

Despite the promising results, the safety profile of these newly used growth factors has not been completely defined, and the risk of malignant transformation has been considered as a major limitation to their clinical application.[5]

Scaffolds

Any scaffolds used in laryngology should have structural features, chemical composition and mechanical properties similar to those of the lamina propria. These should be constituted by biocompatible and re-absorbable material that promote adhesion, proliferation and cell differentiation, in order to allow a successful restoration of the extracellular matrix.[5,15]

A wide range of materials has been used, such as hydrogels based on hyaluronic acid (Carbylan-SX and Carbylan-GSX).[12] These biomaterials, when applied to an injured vocal fold (such as a deep biopsy), modulate scar formation and consequently preserve the vocal fold viscoelastic properties.[9]

Acellular biological scaffolds have also been used as an alternative to hydrogels. These structures are derived from porcine intestinal mucosa, lamina propria of bovine vocal folds or human umbilical vein. They are then submitted to decellularization procedures based on the immune response triggered by contact with human vocal fold fibroblasts.[16] Kishimoto et al.[17] conducted a study in 6 patients with a vocal fold scar or sulcus, who were submitted to the placement of such scaffolds in a subepithelial bag after excision of scar tissue. In the 6 months following surgery, a significant restoration of the extracellular matrix was observed, with improvement of acoustic parameters, and total degradation of the scaffold material. Therefore, it is a promising medical device.

Cells

Cell therapy in laryngology is based on the injection of cells that will produce extracellular matrix elements, resulting in the reconstruction of the vocal fold microstructure. These cells are generally fibroblasts,[18] bone marrow or adipose tissue stem cells[19] (which are capable of producing, *in vitro*, all the elements of the vocal fold),[20] and embryonic stem cells.[21] In all cell therapy-based studies, a clear improvement of vocal fold fibroelastic properties has been observed.[18–21] However, the potential risk for neoplastic transformation of targeted tissues[3,5] and many ethical issues concerning the manipulation of embryonic cells have limited its use.

Plastic and Reconstructive Surgery

Many head and neck surgical procedures, such as rhinoplasty, septoplasty, correction of septal perforation, or pinna reconstruction involve the use of autologous cartilage which is collected from the nasal septum, ribs, or concha.[2] Despite being a limited resource concerning its finite extension and particular geometrical configuration, septal cartilage has been the most commonly used, due to its structural features and accessibility.[22,23] Tissue engineering may, therefore, provide an alternative, with the possibility of originating a higher amount of cartilage with the desired shape.

The first and most popular work in this area was published in 1997 by Cao et al.[24] The production of an ear-shaped cartilage matrix for treating a 3-year-old child was described, using bovine chondrocytes added to a previously molded polyglycolic acid matrix (scaffold). These elements were implanted in the back of a laboratory mouse, and new replicating chondrocytes were observed within 12 weeks.

More recently, Yanaga et al.[25] published a series of 4 cases in which the surgical technique for reconstruction of

congenital microtia was based on bioengineering. In these cases, chondrocytes were obtained from conchal cartilage and expanded *in vitro* to form a matrix to which fibroblast growth factor was added. The resulting tissue was then implanted subcutaneously overlying the abdominal fascia for about 6 months, originating a large amount of mature cartilage that was consequently shaped and transplanted into the temporal skin in order to reconstruct the pinna. These patients were followed for 5 years, with good results. In particular, no reabsorption of cartilaginous tissue was observed.

The same team also applied this procedure to rhinoplasty.[26] Similarly, a gelatinous matrix obtained from chondrocytes collected in conchal cartilage was injected subcutaneously in the nasal dorsum, originating a solid neocartilage in 2 weeks. In this study, 75 patients were submitted to a 6-year follow-up after surgical procedure, yielding promising results.

These procedures can therefore be an alternative to the use of other materials, particularly hyaluronic acid, which is considerably reabsorbed over time.

Head and Neck Surgery

The greatest success of bioengineering in head and neck surgery has been observed in the treatment of tracheal stenosis. This condition frequently follows prolonged endotracheal intubation or surgical/percutaneous tracheostomy, but may also be due to factors such as trauma, radiation, cancer, or chronic inflammatory diseases (amyloidosis, sarcoidosis, relapsing polychondritis).[27,28] Among different treatment options, the one considered most effective involves the resection of the stenosed segment followed by anastomosis,[29] although this procedure is not applicable when the condition affects a large tracheal segment or the cricoid cartilage.[29]

In 2005, Omori et al.[30] described the first successful reconstruction of a large segment of a 78-year-old male trachea, which had been previously destroyed by a thyroid carcinoma. The authors used a matrix of polypropylene as scaffold, coated with collagen collected from porcine dermis, which was locally implanted, after resection of the damaged tracheal segment. Two weeks after the procedure, the implanted tissue was fully integrated in the neighboring structures, and complete regeneration of respiratory epithelium was observed in 2 years, without complications.

A different technique was suggested by Macchiarini et al. in 2008.[31] In this paper the authors describe the bronchial reconstruction in a 30-year-old patient suffering from advanced bonchomalacia. A cadaverous bronchus, previously submitted to decellularization procedures, was used as a scaffold, to which epithelial cells and chondrocytes collected from healthy bronchus were added. The obtained material was anastomosed with the affected bronchus, with immediate symptom relief. The patient was discharged 10 days after surgery.

These works illustrate the possibility of rebuilding compromised airway segments, using recent bioengineering techniques.

Otology

Chronic tympanic perforation is a common condition, frequently resulting from ear infections, trauma, or tympanostomy tube extrusion.[32] Spontaneous closure, occurring in up to 90% of acute perforations, occurs by epithelial migration. This may lead to the formation of a neomembrane lacking the intermediate layer, which is susceptible to not only new perforations due to its reduced thickness but also the formation of retraction pockets.[32,33] Tympanoplasty with temporal fascia or tragal perichondrium remains the treatment of choice, but usually with considerable surgical morbidity. For this reason, large efforts have recently been made in order to find alternative biomaterials that allow easier and more effective procedures.[34,35]

As described for vocal folds, bioengineering applied to surgical treatment of chronic tympanic perforation involves the following elements:

Regulators/Growth Factors

Hyaluronic acid assumes, once again, a prominent position in the treatment of tympanic membrane perforations. Its esterified form (Merogel) was tested by Ozturk[36] for treating induced tympanic membrane perforation in laboratory mice. The results were compared with the contralateral perforated tympanic membranes after local application of a placebo. After 7 days, the authors observed that tympanic membrane treated with Merogel had a higher closure rate than tympanic membrane treated with placebo (91.7% *versus* 70.85%) and a relatively higher amount of fibroblast growth factor and vascular endothelial growth factor on immunohistochemistry analysis.

Fibroblast growth factor seems to be another key growth factor that has been intensively studied. Kanemaru et al.[37] conducted a study that consisted in the application of a gelatin sponge impregnated with fibroblast growth factor in chronically perforated tympanic membranes after scarification of wound edges, and compared the results with control tympanic membranes submitted to the same procedure, but lacking fibroblast growth factor. As predicted, the occlusion rate was significantly higher in the group treated with fibroblast growth factor, with no evidence of side effects, which is in concordance with Ozturk's results.[36]

Pentoxifylline is a vasodilator drug that maximizes the oxygen tension in peripheral tissues.[32] Ramalho et al.[38] studied its effects by using its oral form in combination with topical endothelial growth factor in chinchillas with subacute tympanic perforations. In the described protocol, endothelial growth factor was applied every 70 h, and pentoxifylline was used in a daily dose of 20 mg/kg for 10 days. A sponge was used in every perforated tympanic membrane as a scaffold. About 1 month after treatment, the closure rate was 8.7% in the placebo group, 3.6% in the group treated with pentoxifylline alone, 30.3% in the group treated with endothelial growth factor alone and 16.5% in the group that was submitted to pentoxifylline and endothelial growth factor. Given these results, the authors concluded that endothelial growth factor promotes the closure of perforated tympanic membranes, contrary to pentoxifylline alone or in association.

Despite these promising studies, there are many issues that still need to be clarified. Most important of all, the ideal dose and duration of the treatment must be determined and special attention must be given to the definition of potential risks that may arise with the use of these factors, such as cholesteatoma formation.

Scaffolds

A range of different materials have been studied in the reconstruction of the tympanic membrane, namely the components of extracellular matrix and calcium alginate.

The components of extracellular matrix are derived from natural sources (acellular dermis and dura mater) and used as templates for tissue reconstruction based on their ultrastructure, particularly the presence of functional proteins such as collagen and proteoglycans.[32] The extracellular matrix extracted from porcine dermis and dura mater and submitted to decellularization processes were used in a study by Deng et al.[39] In this work, fibroblasts isolated from guinea pig's tympanic membrane were added to the described biomaterial and then placed on a chronically perforated tympanic membrane using the tympanoplasty underlay technique. Subsequent microscopic analysis revealed progressive reconstruction of a characteristic 3-layered tympanic membrane, associated with improvement of hearing thresholds in the auditory evoked potential examination.

On the other hand, alginate is a natural polymer originated from seaweed, which has been used as a scaffold in tissue engineering due to its positive effects on cellular proliferation.[40] When cross-linked with calcium salts, its properties are significantly enhanced, particularly in what concerns handling and resilience,[40] as observed in a study performed by Weber et al.[41] comparing it with the paper patch technique used in myringoplasties on chinchilla with induced chronic tympanic perforations. At the end of the study, perforated tympanic membrane treated with calcium alginate had a higher occlusion rate when compared to controls, while auditory potentials confirmed the absence of toxic effects.

Despite these promising results, these materials must be extensively evaluated concerning the potential risks of its use compared with autologous materials currently used in common practice, with very satisfactory results but with considerable morbidity.

Conclusion

With the increased knowledge and establishment of the concepts of regenerative medicine, as well as the constant development of new biomaterials, the paradigm of medicine will soon change. In the future, the doctor, and particularly the otolaryngologist will assume a role in the process that includes not only the diagnosis but in the restoration of compromised biological functions, being part of a multidisciplinary team which will soon include engineers, biologists and other related professionals.

Again, further studies are clearly needed to regulate inherent ethical issues, particularly regarding the use of embryonic stem cells, and to clarify long-term safety profiles of these promising biomaterials.

Conflict of Interest

The authors declares no disclosures.

Acknowledgments

The 3B's Research Group (Biomaterials, Biodegradables and Biomimetics).

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STEM CELLS TRANSLATIONAL MEDICINE®

TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Optimized Cell Survival and Seeding Efficiency for Craniofacial Tissue Engineering Using Clinical Stem Cell Therapy

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ABSTRACT

Traumatic injuries involving the face are very common, yet the clinical management of the resulting craniofacial deficiencies is challenging. These injuries are commonly associated with missing teeth, for which replacement is compromised due to inadequate jawbone support. Using cell therapy, we report the upper jaw reconstruction of a patient who lost teeth and 75% of the supporting jawbone following injury. A mixed population of bone marrow-derived autologous stem and progenitor cells was seeded onto β-tricalcium phosphate (β-TCP), which served as a scaffold to deliver cells directly to the defect. Conditions (temperature, incubation time) to achieve the highest cell survival and seeding efficiency were optimized. Four months after cell therapy, cone beam computed tomography and a bone biopsy were performed, and oral implants were placed to support an engineered dental prosthesis. Cell seeding efficiency (>81%) of the β-TCP and survival during the seeding process (94%) were highest when cells were incubated with β-TCP for 30 minutes, regardless of incubation temperature; however, at 1 hour, cell survival was highest when incubated at 4°C. Clinical, radiographic, and histological analyses confirmed that by 4 months, the cell therapy regenerated 80% of the original jawbone deficiency with vascularized, mineralized bone sufficient to stably place oral implants. Functional and aesthetic rehabilitation of the patient was successfully completed with installation of a dental prosthesis 6 months following implant placement. This proof-of-concept clinical report used an evidence-based approach for the cell transplantation protocol used and is the first to describe a cell therapy for craniofacial trauma reconstruction. STEM CELLS TRANSLATIONAL MEDICINE *2014;3:1495–1503*

INTRODUCTION

In addition to bruises, hematomas, and lacerations, dentoalveolar injuries are the most common injuries that occur in the facial region, accounting for 50% of the injuries for those seeking emergency treatment for head and neck injuries [1–3]. The resulting functional and aesthetic deficiencies from the loss of teeth and associated jawbone support due to these injuries are debilitating and very difficult to treat. The current standard-of-care protocol for advanced craniofacial reconstruction involving the oral cavity involves the use of large autogenous "block" bone grafts, whereby the donor bone blocks of bone are harvested from intraoral sites (mandibular ramus or symphysis) or extraoral sites (iliac crest, tibia) [4–7]. Although advanced grafting procedures have historically demonstrated varying degrees of success, major limitations are that they require two surgical sites (donor and recipient) and are often associated with long postoperative healing periods, moderate to severe discomfort during healing, tissue morbidity in the donor site, and prolonged sensory disturbances in the donor site.

Stem cell therapy is an emerging strategy that can potentially be used for the reconstruction of craniofacial deficiencies [8, 9]. Because cell-therapy approaches often involve the use of a polymer material to deliver cells to the defect area, the success of these approaches is heavily dependent not only on the polymer and cells used but also the conditions under which they are used. Despite many in vitro and in vivo studies designed to evaluate and optimize the cell attachment and biocompatibility of different materials, there is no clinical evidence of efficacy to support these data. In contrast, in the limited clinical reports investigating a cell-transplantation approach to regenerating craniofacial tissue, the clinical protocols and conditions used to deliver the cells are either not well described or not well justified [10–14].

In a randomized controlled clinical trial, our group recently reported the use of a gelatin sponge to deliver stem cells into small, localized, oral bone defects created following tooth removal [15]. Although results were favorable, the use of this sponge material as a cell carrier is not suitable for regeneration of large oral and

craniofacial defects. β-Tricalcium phosphate (β-TCP) has more ideal properties as a cell carrier for addressing larger, more severe bone defects because it has rigid structural properties and is osteoconductive, which facilitates bone growth [16, 17]. Clinically, it has been used as a bone-graft substitute material in very limited orthopedic indications and in small, localized bone deficiencies around teeth [18, 19]. Recently, its use as a cell carrier for autologous adipose-derived stem cells has also been reported with the combined use of recombinant human bone morphogenetic protein-2 to treat craniofacial defects [14]. Nonetheless, to date, there has been no reported clinical investigation of its use as a scaffold for a stand-alone cell therapy in the treatment of large craniofacial deficiencies.

We report a stem cell therapy to reconstruct the upper jaw of a patient who lost front teeth and associated bone tissue following a severe traumatic injury to the face. β-TCP was used as a scaffold to deliver the cells to the jawbone defect, and following 4 months of healing, sufficient bone was regenerated to insert oral implants and restore them with dental prosthetics. In addition, the clinical conditions for cell attachment and survival were optimized for this cell-transplantation approach.

Materials and Methods

Patient

Following U.S. Food and Drug Administration and University of Michigan institutional review board approval to conduct a cell-therapy study for the oral reconstruction of traumatic craniofacial injuries, a 45-year-old woman presented to the clinic following an injury in which she suffered a traumatic blow to the face. The injury occurred 5 years prior to her initial presentation. and as a result of the injury, seven teeth (four in the anterior segment of the upper jaw and three in the anterior segment of the lower jaw) were avulsed and lost. Moreover, 75% of the supporting jawbone and soft tissue surrounding these teeth were also lost as a result of the injury. Consequently, the patient had severe oral-facial functional and aesthetic deficiency. Due to inadequate alveolar bone as a result of the injury, the patient was not a candidate for rehabilitation with oral implant therapy without advanced reconstructive bone-grafting procedures being performed. The patient was wearing an ill-fitting removable dental prosthesis on initial presentation and was deemed eligible for participation in the study.

Cell-Seeding Efficiency and Viability Studies

The production of ixmyelocel-T (tissue repair cells or ixmyelocel-T; Aastrom Biosciences, Ann Arbor, MI, http://www.aastrom.com) has been described previously [20]. Briefly, a bone marrow aspiration of the posterior ilium was performed under conscious sedation and local anesthetic. Collected marrow was transferred to a sterile blood bag, and bone marrow mononuclear cells (BMMNCs) were purified by Ficoll density gradient centrifugation. BMMNCs were then inoculated into a bioreactor, which is a proprietary computer-controlled, automated cell processing unit (Aastrom Replicell system; Aastrom Biosciences). This system incorporates single-pass perfusion in which fresh medium flows slowly over cells without retention of waste metabolites or differentiating cytokines. The culture medium consists of Iscove's modified Dulbecco's medium, 10% fetal bovine serum, 10% horse serum, and 5 μM hydrocortisone. After cultivation for 12 days at 37°C in 5% CO_2 with a ramped continuous medium perfusion schedule, the ixmyelocel-T product was harvested by trypsinization, washed in a physiologic buffer, and collected into a sterile bag for storage until the time of transplantation. The cells were composed of a mixture of bone marrow-derived cells including expanded $CD90^+$ mesenchymal stem cells, $CD14^+$ monocytes/macrophages, and mononuclear cells from the original bone marrow aspirate [21, 22]. The cell population from this patient consisted of 26% $CD90^+$ cells and 15% $CD14^+$ monocytes and had a final concentration of 14.1 million cells per milliliter with cell viability of 91%. The primary purpose for obtaining these cells was their use in the clinical treatment of the bone defect; however, a specific section of the informed consent document obtained the patient's permission to use and/or store "excess" cells and/or bone marrow (if available) for additional laboratory or preclinical studies.

For the cell-seeding and viability studies, T -150 flasks containing 90% confluent cell populations of ixmyelocel-T were trypsinized and counted. Cells were seeded onto equal volumes of β-TCP (Cerasorb; Curasan AG, Kleinostheim, Germany, http://www.curasan.de) particles (1:1 ratio of cell suspension to volume β-TCP) and allowed to incubate at either room temperature (RT) or on ice (4°C). After 15, 30, and 60 minutes, the residual cell suspension from the respective condition was collected, and the number of cells remaining was counted. The cell-seeding efficiency was an indirect measure of the number of cells that attached to the β-TCP particles. It was calculated through an assumption of a constant number of cells for seeding and deduction of the floating cells from this constant number to get the number of seeded cells (efficiency). Cell viability was measured as cell survival, determined through the dye exclusion method of trypan blue staining of the remaining or floating cells following incubation with the β-TCP and counting this proportion of cells relative to the total number of floating cells during the three respective time frames at RT and at 4°C.

Cell Therapy, Regenerative Analyses, and Oral Reconstruction

A cone beam computed tomography (CBCT) radiographic scan was performed to volumetrically evaluate the upper jawbone deficiency and generate three-dimensional reconstructions of the upper jaw. Under conscious sedation and local anesthesia, an intraoral full-thickness mucoperiosteal flap was elevated to expose the margins of the bony defect in the upper jaw. In the operating room, approximately 10^7 cells in suspension were incubated with β-TCP at RT 30 minutes prior to being administered to the defect site. Following clinical open bone measurements of the width of the alveolar bone, the defect site was prepared to receive the graft by creating small osteotomies penetrating through the outer cortical layer of bone to facilitate vascular infusion of the graft during healing. Four 8-mm "tenting" screws were used to help stabilize the β-TCP particles, and the β-TCP was then placed and covered with a resorbable collagen membrane (Conform collagen; Ace Surgical Supply, Inc., Brockton, MA, http://www.acesurgical.com) to help contain the grafted β-TCP/cell construct. In addition, 4-0 sutures were used to approximate the tissues, and the area was allowed to heal for 4 months. A second CBCT scan was performed immediately following grafting. Postoperative

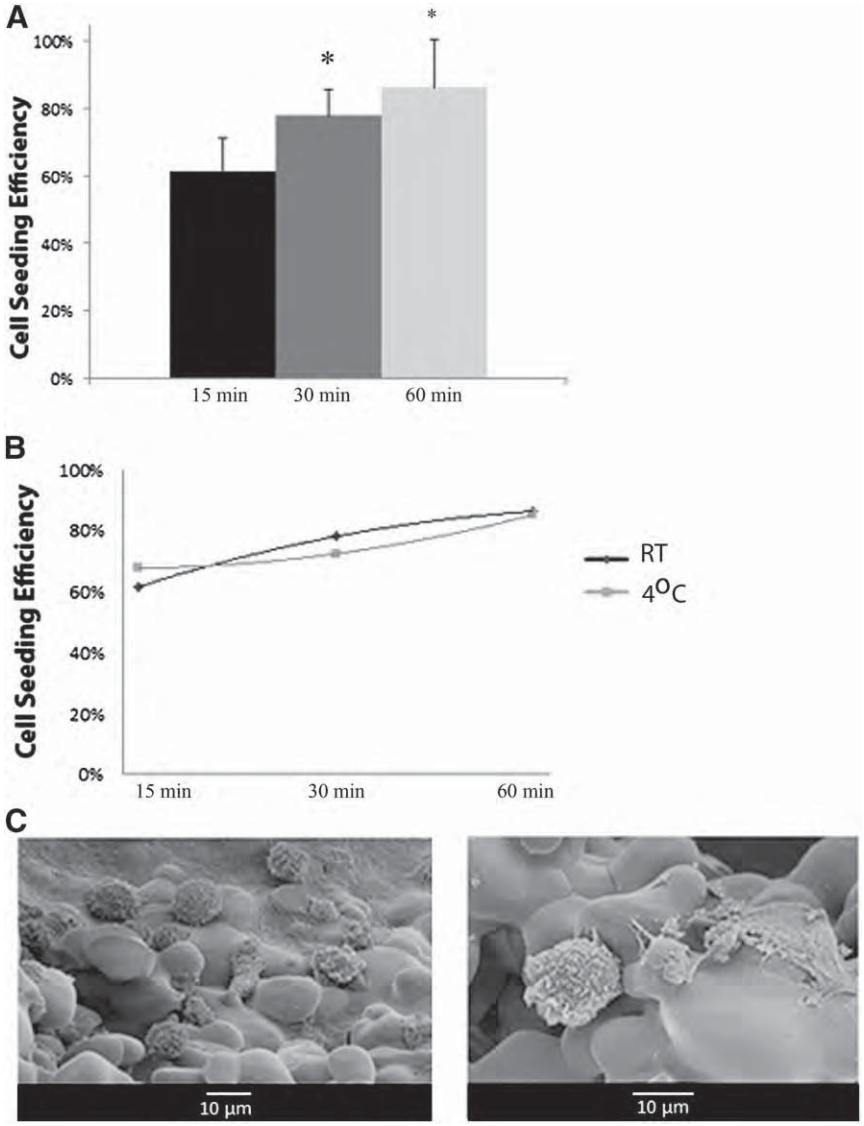

Figure 1. Cell-seeding efficiency of β-tricalcium phosphate (β-TCP). **(A):** The overall cell-seeding efficiency is shown at different time intervals (15, 30, and 60 minutes) following seeding of the scaffold with cells. **(B):** The cell-seeding efficiency at the different time intervals was stratified by the temperature at which the cells were allowed to incubate with the scaffold. **(C):** SEM images at low and high magnification showing the distribution of cells and cell attachment to one β-TCP particle (individual particle sizes ranged from 500 to 1,000 μm). $*$, $p < .05$ relative to the 15-minute condition. Abbreviation: RT, room temperature.

medications included an antibiotic, a corticosteroid, and an analgesic for pain management.

Four months following placement of the graft, a third CBCT was performed to evaluate the presence of mineralized tissue in the grafted area prior to implant placement. Following the imaging, the area was surgically re-entered for the placement of oral implants. During re-entry, clinical bone measurements were recorded, and a bone biopsy was performed in an area of the previously grafted region. This tissue biopsy was processed for micro-computed tomography (micro-CT) and histological analysis, as described previously [23]. Oral implants were placed in the grafted sites and allowed to heal underneath the gingival tissue for 6 months. At 6 months, the implants were uncovered, and a dental prosthesis was engineered and installed to connect to the oral implants. This study is registered with ClinicalTrials.gov identifier CT00755911.

Statistical Analysis

Statistical analysis was performed with the use of InStat software (GraphPad Software, Inc., San Diego, CA, http://www.graphpad.com). All data were plotted as mean $\pm$ SEM unless otherwise noted. One-way analysis of variance was performed for the cell-seeding efficiency and cell-viability studies with Bonferroni-corrected, post hoc, two-tailed *t* tests to determine statistically significant differences between groups. Statistical significance was defined as $p < .05$.

Results

Cell-Seeding Efficiency

The time frame needed to achieve the highest cell attachment to β-TCP was determined in our cell-seeding efficiency studies

(Fig. 1A). Cell-seeding efficiency of β-TCP following 15 minutes of incubation with cells was 60%, with a significant increase to 81% following 30 minutes of incubation ($p < .05$). There was no difference in the seeding efficiency between 30 minutes and 1 hour of incubation. In addition, when evaluating the effect of temperature on cell-seeding efficiency, there was no difference in seeding efficiency at 4°C relative to room temperature at the three time points evaluated (Fig. 1B). SEM images show diffuse distribution and attachment of the cells to one particle (500- to 1,000-μm particle sizes) of the graft material following 30 minutes of incubation at room temperature (Fig. 1C).

Cell Viability During Cell Seeding

Another important variable in the context of cell therapy is the cell viability throughout the process of cell seeding and transplantation. Cell viability was evaluated in a similar manner to cell-seeding efficiency, at three different time points (15, 30, and 60 minutes) and two temperatures (RT, 4°C). Between the three time points evaluated, cell survival was no different, between 88% and 94% (Fig. 2A). However, when stratifying for temperature, there was a significant decrease ($p < .05$) in cell survival when incubated at RT for 1 hour relative to incubation at RT for 30 minutes or when incubated at 4°C for 1 hour (Fig. 2B). When at 4°C, the time frame of incubation did not affect cell survival. Overall, the optimum conditions for cell survival were 30-minute incubations at RT or 4°C or a 60-minute incubation period at 4°C.

Clinical Cell Transplantation

The protocol for transplantation of the cells used the optimized attachment and survival conditions, which were to maintain the cells on ice (4°C) until 30 minutes prior to transplantation, at which time they were incubated with the β-TCP at RT. During this period in which the cells were incubating, the gingival flap was reflected to expose the underlying bone, and measuring instruments were used to measure the horizontal dimension of the alveolar ridge, which was 3 mm (Fig. 3A–3D). In a healthy dentition, horizontal ridge width of this area of the maxilla normally ranges from 8 to 12 mm, and to securely place and stabilize a dental implant, 7–8 mm is the minimum width required. Tenting screws were placed in the area to receive the graft and were used to help consolidate the graft material and prevent collapse of the overlying collagen membrane and soft tissue following closure of the flap (Fig. 3E, 3F). The graft was applied to the deficient area, and an additional 0.5 mL of the cell suspension was added following placement of the graft into the site (Fig. 3G, 3H). A barrier membrane was placed over the graft to prevent soft tissue infiltration into the graft during the early stage of healing (Fig. 3I), and the tissues were approximated completely (Fig. 3J).

Radiographic, Clinical, and Histological Analyses of Jawbone Reconstruction

The 75% horizontal bone deficiency in the upper jaw in the area of the missing teeth was clearly evident radiographically and using volumetric evaluation of three-dimensional reconstructed CBCT images prior to treatment (Fig. 4A). Immediately after grafting, a second CBCT was performed and showed a 10- to 12-mm increase in horizontal width of the jawbone (Fig. 4B). Four months after grafting and immediately before implant placement, a third CBCT was performed and showed that, compared with immediately following grafting, there was an overall 25% reduction of the initial grafted width (Fig. 4B, 4C). However, relative to the original jawbone deficiency, there was a net 5- to 6-mm horizontal gain in width of the jawbone, resulting in 80% regeneration of the original jawbone deficiency (Fig. 4A, 4C).

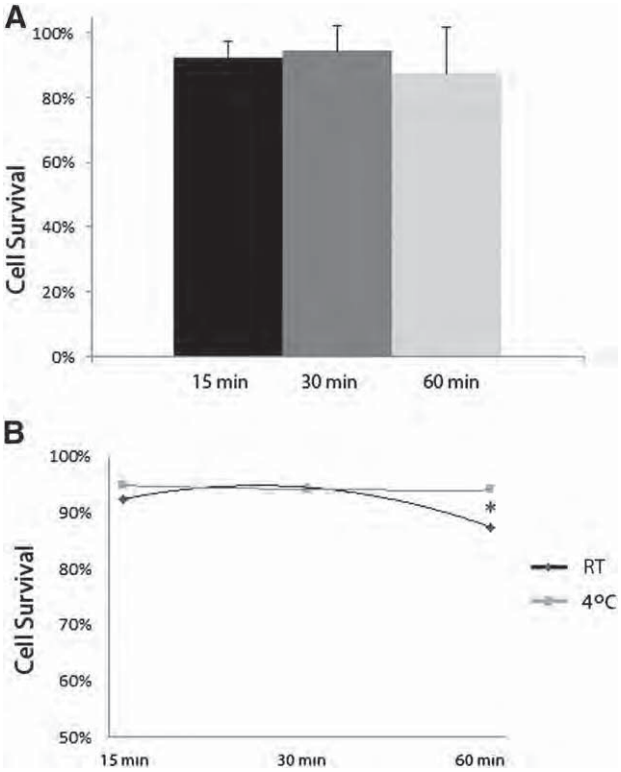

Figure 2. Cell viability following seeding on β-tricalcium phosphate. **(A):** Cell survival at different time intervals following loading of the scaffold is shown. **(B):** Cell survival at the different time intervals was stratified by the temperature at which the cells were maintained during the respective time intervals during which the cells were allowed to incubate with the scaffold. *, $p < .05$ between conditions. Abbreviation: RT, room temperature.

Four months following healing, the grafted site was re-entered for oral implant placement, and there was clinically apparent evidence of bone regeneration with a new horizontal ridge width of 8–9 mm (Fig. 5A, 5B). Oral implants were then stably placed in the previously grafted sites and biomechanically torqued to standard-of-care guidelines of 35 newton centimeters (Fig. 5C, 5D). Implants were left submerged under the gingival tissue (Fig. 5E, 5F) for 6 months of healing. Micro-CT and histological evaluation of the bone biopsy harvested from the area of the grafted region revealed highly vascularized, mineralized tissue indicative of bone formation and 80% of the β-TCP matrix resorbed (Fig. 5G, 5H). Full functional and aesthetic restoration of the area was completed 6 months following implant placement, with the engineering and placement of an oral implant-supported dental prosthesis (Fig. 6).

Discussion

Regenerative medicine aims to use tissue engineering and biomimetic strategies to functionally restore and replace damaged and lost tissue [24]. In this report, we describe a cell therapy for the oral reconstruction of a patient who lost teeth and supporting

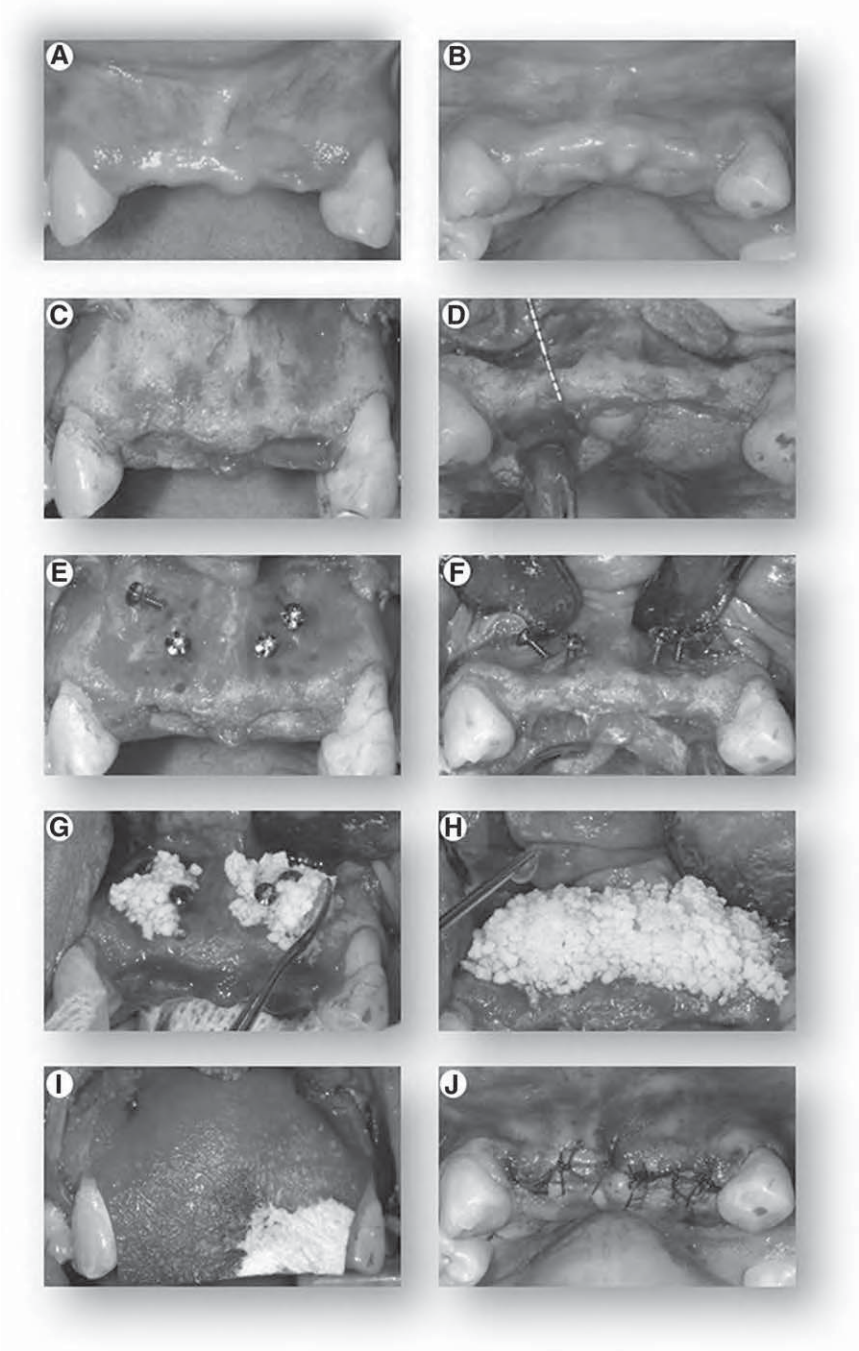

Figure 3. Cell transplantation procedure. Front view **(A)** and top view **(B)** of the initial clinical presentation showing severe hard and soft tissue alveolar ridge defects of the upper jaw. Following elevation of a full-thickness gingival flap, the images show front view **(C)** and top view **(D)** of the severely deficient alveolar ridge, clinically measuring a width of only 2–4 mm. Front view **(E)** and top view **(F)** of the placement of "tenting" screws in preparation of the bony site to receive the graft. Placement of the β-tricalcium phosphate (seeded with the cells 30 minutes prior to placement at room temperature) into the defect **(G)**, with additional application of the cell suspension following placement of the graft in the recipient site **(H)**. Placement of a resorbable barrier membrane **(I)** to stabilize and contain the graft within the recipient site, and top view **(J)** of primary closure of the flap.

jawbone tissue as a result of a traumatic injury to the face. In addition, optimized parameters for cell attachment and survival were defined for the cell transplantation protocol used in this approach. To date, this study represents the most advanced craniofacial trauma reconstruction using a stem cell-based therapy for oral rehabilitation involving oral implants.

Important considerations in regenerative medicine involving cell-transplantation protocols are the conditions under which cells are delivered [9, 25]. These parameters are of even greater importance if biomaterials are used for delivery of cells. β-TCP has been used as a bone graft substitute material to fill in small, localized bone deficiencies around teeth and in very limited

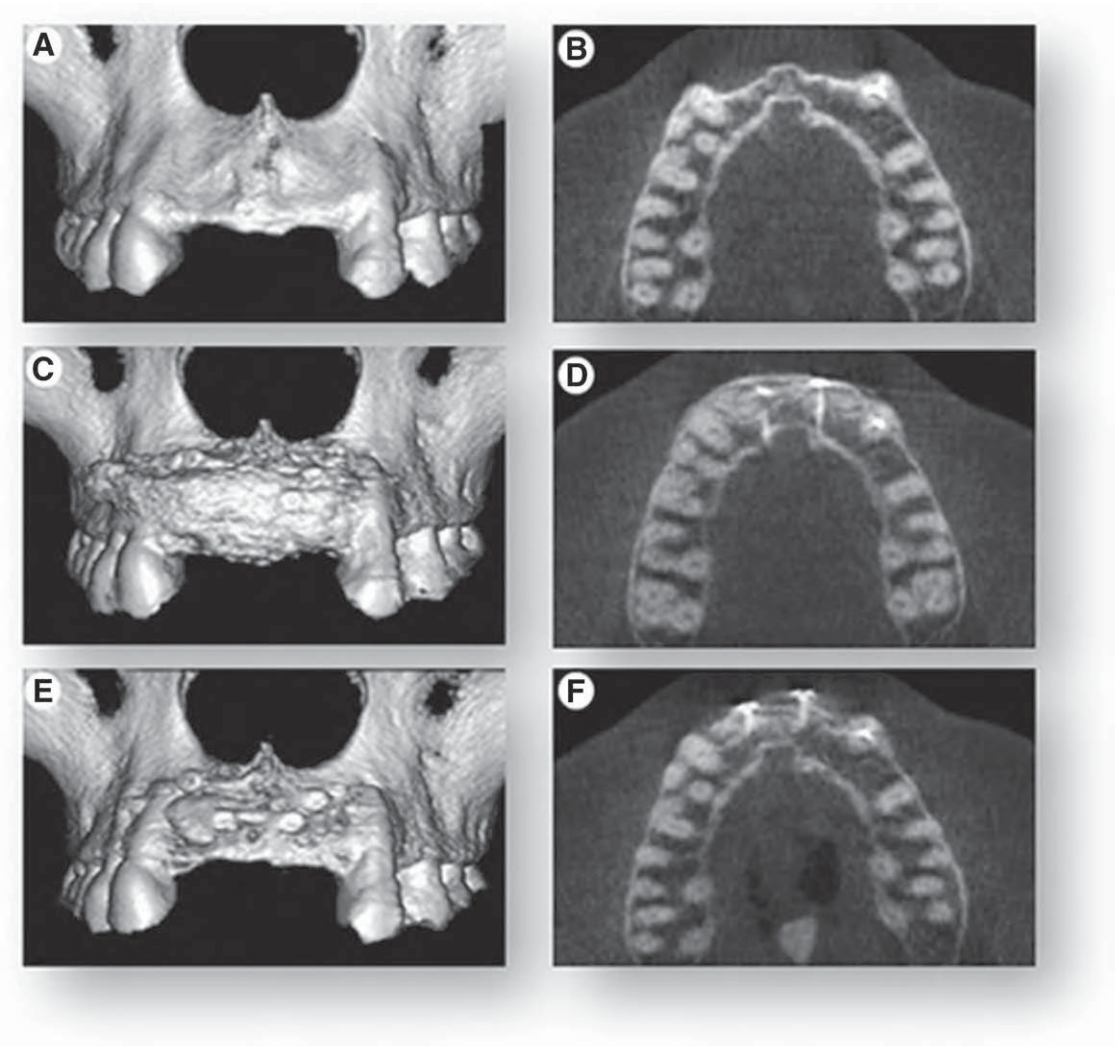

Figure 4. Cone-beam computed tomography (CBCT) scans. CBCT scans were used to render three-dimensional reconstructions of the anterior segment of the upper jaw and cross-sectional (top view) radiographic images to show volumetric changes of the upper jaw at three time points. **(A, B):** The initial clinical presentation shows 75% jawbone width deficiency. **(C, D):** Immediately following cell therapy grafting, there is full restoration of jawbone width. **(E, F):** Images show 25% resorption of graft at 4 months and overall net 80% regeneration of the original ridge-width deficiency.

orthopedic indications. However, there have been no reported clinical investigations of its use as a scaffold for a stand-alone cell therapy to treat large craniofacial deficiencies. In a case series, Sandor and colleagues recently reported its use as a scaffold to deliver adipose-derived stem cells to jawbone defects in combination with large doses of BMP-2 [14]. These defects were secondary to tumor resective surgery, and although radiographic outcomes were deemed favorable, limited data were presented relative to the clinical, functional, and histological integrity of the regenerated jawbone tissue. We used β-TCP in our study as a carrier to deliver the cells because tricalcium phosphates are highly biocompatible, have been shown to support osteogenic activity of mesenchymal stem cells, and have been used as a delivery vehicle in a number of animal studies in which cell transplantation has been used [16, 26–28]. Krebsbach and colleagues reported that relative to other biomaterials commonly used clinically, such as gelatin sponges and demineralized bone matrix, tricalcium phosphates most consistently yield bone formation in vivo when used as a delivery vehicle for mesenchymal stem cells [27]. Although this material has favorable characteristics for cell proliferation, differentiation, and in vivo bone formation, studies have not evaluated or reported the cell-seeding efficiency (i.e., how efficiently cells attach to β-TCP) of the cells when β-TCP is used as a delivery vehicle. Cell attachment and seeding efficiency can have significant influence on the regenerative response in determining the number of cells that reach the regenerative site [29]. Our study determined that the minimum time needed for incubation of the cells to allow the greatest cell attachment ($>$81%) was 30 minutes. We did not evaluate time points longer than 60 minutes because cell seeding occurs at the time of surgical application of the cells. Hence, incubation times greater than 60 minutes would affect the clinical protocol and prolong the surgical procedure, which could have adverse consequences on outcomes (e.g., increased risk of infection, increased bleeding, increased inflammation). Another important clinical consideration for cell transplantation, particularly if there is an incubation period prior to delivery of the cells, is the incubation temperature. During the incubation time frames of 15, 30, and 60 minutes, it was determined

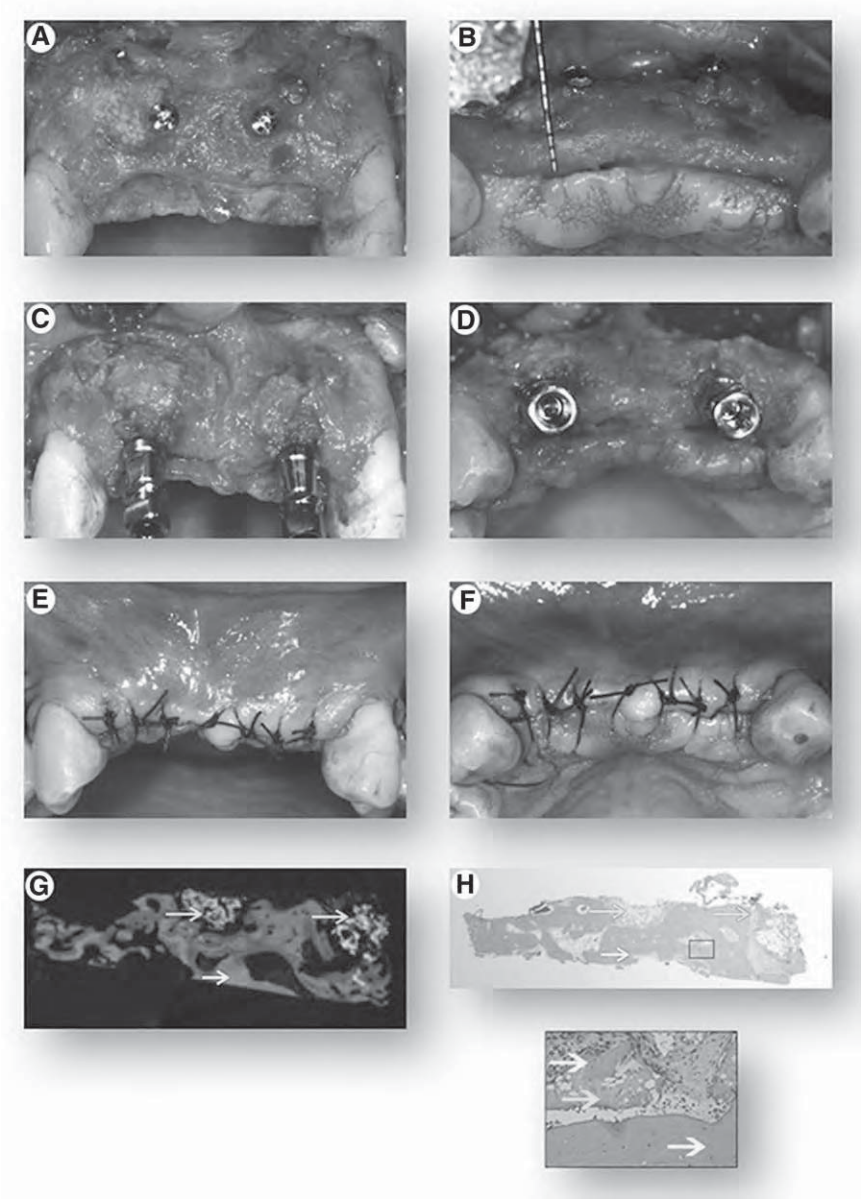

Figure 5. Surgical re-entry of the grafted site and implant placement. Following elevation of a full-thickness gingival flap, front view **(A)** and top view **(B)** of the treated site reveal regenerated tissue and a reconstructed alveolar ridge clinically measuring a width of 8–10 mm. Front view **(C)** and top view **(D)** of the placement of dental implants in the regenerated sites. **(E, F):** Primary closure of the site. A bone core biopsy was retrieved from one of the regenerated sites to determine the presence of mineralized tissue with micro-computed tomography analysis **(G)** and to confirm the histomorphometric appearance of bone tissue histologically **(H)** with hematoxylin and eosin staining (green arrows highlight residual β-tricalcium phosphate, yellow arrows highlight bone tissue; magnification: $\times$40 and $\times$100).

that the cell-seeding efficiency was no different if cells were incubated at 4°C or at room temperature.

Regardless of the material or modality used to deliver cells during the process of cell transplantation, it is clear that cell-seeding efficiency is an important determinant of the number of cells that reach a regenerative site. Despite the impact of seeding efficiency on successful regenerative outcomes, the viability of the cells that are delivered is even more critical to the outcomes achieved with this approach [30]. Although temperature did not affect cell-seeding efficiency in our study, it is well established that temperature can have a profound effect on cell viability. In various tissue-grafting and organ-transplantation protocols, it is often highly desirable to maintain tissue specimens at 4°C (on ice) until ready for application or placement in the recipient site [31–34]. In regenerative cell-transplantation strategies involving stem cells, although important, this parameter has not been thoroughly examined. In our study, we found that if cells were incubated with β-TCP for 30 minutes or less, survival was not affected by the incubation temperature (room temperature vs. 4°C). If cells were incubated for 1 hour, cell survival was significantly greater when the cells were incubated at 4°C relative to when incubation occurred at room temperature. Beyond 30 minutes, it was determined that cells should be maintained at 4°C prior to delivery to achieve the greatest cell viability.

Using a different biomaterial for cell delivery, our group recently completed a randomized controlled clinical trial investigating

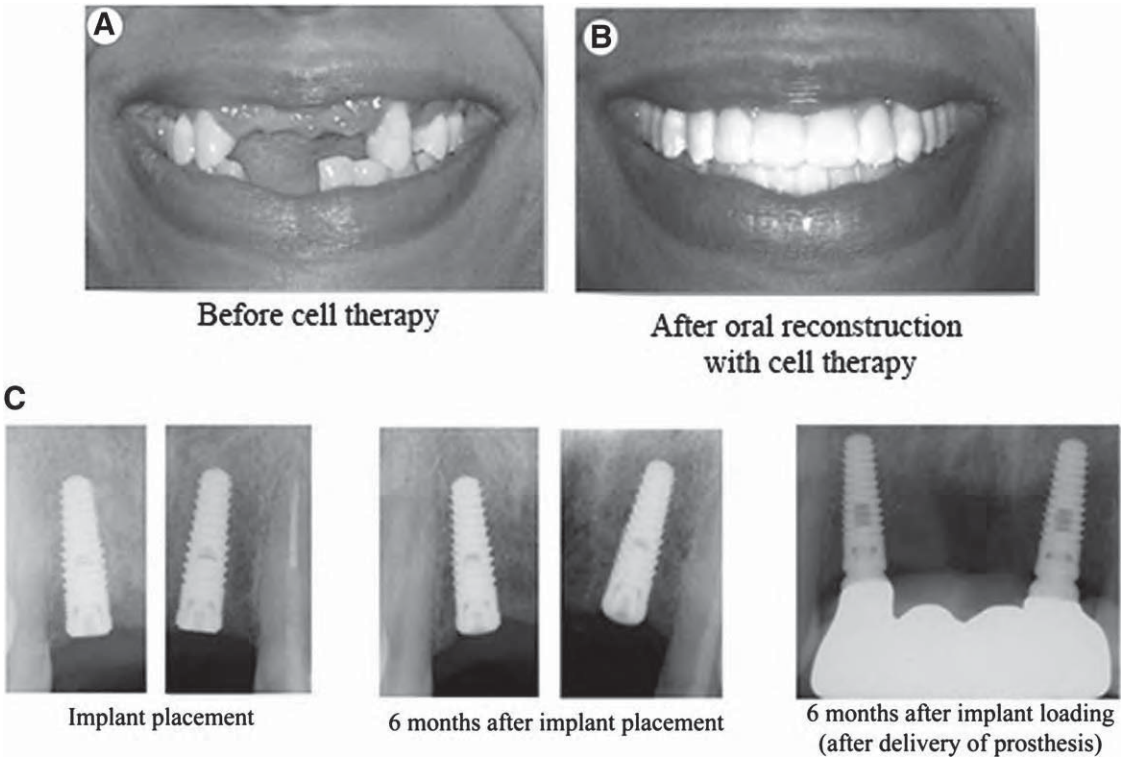

Figure 6. Complete oral rehabilitation. Clinical presentation of the patient prior to initiation of treatment **(A)** and following completed oral reconstruction **(B)**. **(C):** Periapical radiographs of oral implants showing osseointegration of implants and stable bone levels at the time of placement, 6 months following placement, and 6 months following functional restoration and biomechanical loading of implants with a dental prosthesis.

a similar cell-therapy approach in the treatment of small, localized, alveolar bone defects created following tooth removal [15]. The extraction socket defect created in alveolar bone following tooth removal heals, to an extent, without intervention and thus serves as a natural clinical model of bone healing [35]. In our previous study, ixmyelocel-T was administered to these extraction socket defects at the time of tooth removal, and delivery of the cells resulted in acceleration of the innate bone regeneration within the localized defect. Despite these promising results, because there is innate bone regeneration following tooth removal, this model does not serve as an ideal model for evaluating de novo bone regeneration. In addition, this study was performed as a proof of concept to demonstrate safety and efficacy of the approach; however, from a feasibility standpoint, cell therapy would not be used in such a localized defect following removal of a tooth. The more appropriate application for this cell therapy would be in more complex and severe craniofacial defects, as often occur following oral-facial trauma. A severe defect resulting from a traumatic injury would not naturally resolve without significant intervention and also results in significant functional and aesthetic deficiencies. As such, these defects typically require advanced bone-grafting procedures with autogenous blocks of bone or guided bone regenerative (GBR) procedures [36]. Similar to our surgical procedure, the GBR approach uses a protective barrier membrane to cover the allogeneic or alloplastic graft material during healing. However, following GBR for large reconstructions of alveolar bone, most protocols allow a healing period, minimally, of 6–8 months before re-entry for oral implant placement [37]. Through delivery of 100 million cells using a tissue engineering cell-therapy approach, in only 4 months we were able to regenerate 80% of the original jawbone deficiency, which was sufficient to stably place oral implants to biomechanically support a dental prosthesis.

Conclusion

Cell survival and seeding efficiency in the context of tissue engineering and cell-therapy strategies are critical parameters for success that have not been rigorously examined in a clinical context. This study defined optimized conditions for these parameters using an autologous stem cell therapy to successfully treat a patient who had a debilitating craniofacial traumatic deficiency. To our knowledge, there have been no other clinical reports of cell therapy for the treatment of craniofacial trauma defects. This clinical report serves as solid foundation on which to develop more expanded studies using this approach for the treatment of larger numbers of patients with other debilitating conditions (e.g., congenital disorders) to further evaluate efficacy and feasibility.

Acknowledgments

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Author Contributions

A.R.: conception and design, provision of study materials or patients, data analysis and interpretation, manuscript writing, final approval of manuscript; E.E.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; S.E.: conception and design, financial support, data analysis and interpretation, final approval of manuscript; S.A.: conception and design, data analysis and interpretation, final approval of manuscript; S.T.: provision of study materials or patients, data analysis and interpretation, final approval of manuscript; I.R., F.W., and A.L.: collection and/or assembly of data, data analysis and interpretation, final approval of manuscript; D.K.: conception and design, financial support, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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SURGICAL TECHNIQUE

Diced Cartilage Augmentation

Early Experience With the Tasman Technique

Shan R. Baker, MD

Numerous methods have been used for dorsal augmentation in reconstructive and aesthetic rhinoplasty. The Tasman technique is a method for dorsal augmentation using diced cartilage solidified by tissue sealant. This article describes the author's early experience using the Tasman technique and offers some helpful suggestions to surgeons who might wish to use this unique method of preparing a cartilaginous graft.

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Numerous methods have been used for dorsal augmentation in reconstructive and aesthetic rhinoplasty. Septal cartilage is the preferred material for dorsal augmentation. Septal cartilage grafts tend not to warp or resorb and are easy to shape and fixate. However, septal cartilage is not always of adequate amount or quality in revision rhinoplasty.

REVIEW OF DORSAL GRAFTING MATERIAL

Auricular cartilage harvested from the concha cavum and concha cymba can be partially incised along the graft's vertical axis and folded, and the 2 segments can be sutured together. Folding it over and suturing the 2 opposing concave surfaces together creates a smooth cylindrical dorsal graft measuring 3.0 cm long and 0.7 to 0.9 cm wide. This technique has the disadvantage that it cannot be adapted to differing height requirements for greater or less dorsal projection. If used as a single unfolded graft, auricular cartilage is subject to warping and distortion as the soft tissue heals over it.

Costal cartilage typically is the preferred dorsal grafting material when there is insufficient septal cartilage available for necessary dorsal augmentation. Costal cartilage has the advantage of an ample supply. However, when the rib graft is cut to size for precise dorsal augmentation, it may warp, becoming twisted and deformed. Often the grafts are too thick and result in palpable and sometimes visible edges, particularly when placed under a thin-skinned envelope. This problem led Sheen[1] to cut small strips of costal cartilage and morselize them into longitudinal strips for all dorsal reconstructions using costal cartilage.

A solid autogenous graft, whether it is septal, auricular, or costal cartilage, has the disadvantage that it must be precisely sculptured to create the ideal size and shape necessary for the ideal dorsum augmentation. A solid graft by its very nature requires a larger quantity of tissue because it is denser than if the same material is diced and used for a similar degree of dorsum augmentation. This is because diced cartilage is less dense, with space interspersed between the particles of cartilage. This space eventually is filled with connective tissue as the graft is successfully integrated. Diced cartilage also has the advantage of being easily molded and shaped compared with solid or morselized cartilage.

ADVANTAGES OF DICED CARTILAGE

The advantages of diced cartilage for dorsum augmentation have led several surgeons to advocate its use. In 1999, Erol[2]

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described the "Turkish Delight," in which he used oxidized regenerated cellulose (Surgicel; Ethicon Inc) to wrap and contain diced cartilage. He used these as grafts in 2365 patients over a 10-year period. He used septal, alar, conchal, and sometimes costal cartilage cut into pieces of 0.5 to 1.0 mm, wrapped in 1 layer of Surgicel moistened with an antibiotic solution. The graft was then made into a cylindrical form and inserted under the dorsal nasal skin. He even used this technique to correct recurrent deviation of the nasal bridge when augmentation was not required. A few of his cases required revision surgery because of overcorrection. This allowed an opportunity to histologically examine the diced cartilage removed at the time of revision surgery 3 and 12 months postoperatively. Such grafts showed a mosaic-type alignment of graft cartilage dispersed within fibrous connective tissue.

In 2003, Elahi and colleagues[3] performed a retrospective review of 40 consecutive primary and revision rhinoplasty patients in whom the authors used Surgicel-wrapped diced auricular and septal cartilage for dorsal augmentation. When performing dorsal augmentation, they recommended overcorrection of approximately 20%, while not deforming the aesthetic appearance of the nose. They diced the cartilage into 2.0-mm pieces and then crushed and morselized the cartilage in a Cottle cartilage crusher. The material was then wrapped in a double layer of Surgicel. The mean follow-up time for the patients treated was 13.7 months. Only 1 patient experienced resorption of the graft, presumably caused by a postoperative infection.

Daniel and Calvert[4] performed a prospective study of 50 primary and secondary aesthetic rhinoplasties using diced cartilage wrapped in Surgicel or in temporalis fascia. A third group had diced cartilage grafts as free bits of cartilage without an envelope of Surgicel or fascia. In patients with a minimum follow-up of 1 year, all 22 patients receiving the Surgicel-wrapped grafts experienced resorption of the grafts, while none of the free or fascia-wrapped grafts underwent resorption. This was true of radix, dorsum, and full-length grafts. The authors revised 5 patients having had Surgicel-wrapped grafts and biopsied tissue obtained in the area of the grafting. Histological examination showed evidence of fibrosis and lymphocytic infiltrates. Remnants of cartilage were present but were metabolically inactive on the basis of a negative glial fibrillary acidic protein staining.

Six of the fascial-wrapped grafts were overcorrected by 20%. These cases required revision to reduce the overcorrected grafts because they did not resorb. Histological examination of these specimens demonstrated normal cartilage architecture and cellular activity. The diced cartilage grafts wrapped in fascia showed coalescence of the diced cartilage into a single cartilage mass with viable cartilage cells and normal metabolic activity on the basis of glial fibrillary acidic protein staining. The authors concluded that Surgicel has a deleterious effect on the viability of diced cartilage. The authors postulated that the foreign body reaction observed in the Surgicel-wrapped cartilage specimens probably accounts for the resorption of the grafts due to inflammatory reaction. These findings were confirmed by a more in depth histological analysis of this same grafting material and was reported by Calvert et al.[5]

Yilmaz et al[6] conducted an experiment using rabbits that compared diced cartilage grafts as free bits of cartilage and as diced cartilage wrapped in Surgicel. Similar to studies by Daniel and Calvert[4] and Calvert et al,[5] the authors found that all Surgicel-wrapped grafts were glial fibrillary acidic protein negative, indicating negative regenerative capacity.

Brenner et al[7] looked at diced septal cartilage wrapped in deep temporal fascia and in Surgicel implanted in nude rats. They found that diced cartilage wrapped in Surgicel yielded the lowest percentage viability of graft material compared with fascial-wrapped grafts. For over 2 years, Kelly et al[8] followed 20 patients undergoing diced cartilage wrapped in fascia used to augment the nasal dorsum. Apart from 1 infection, all of the diced cartilage grafts wrapped in fascia retained their original volume and did not resorb by a mean time of 16 months postoperatively.

The studies cited strongly suggest that diced cartilage survives very well when used as a dorsal nasal graft, whether it is contained within a fascial sheath or implanted without an enclosing envelope of tissue. The primary reason deep temporal fascia is used as a conduit for the diced cartilage is to maintain shape and contour to the malleable implant. It can be easily molded once the graft is in place. The fascial tube is necessary to prevent the diced cartilage from spilling from its tissue envelope when the graft is molded over the nasal dorsum once the graft has been inserted beneath the nasal skin. I have been using this technique for the past 5 years and agree with the findings that the graft does not resorb and that overcorrection is not necessary. I have found that unintentional over or undercorrection is possible because the bulk of the fascial graft makes it somewhat difficult to accurately assess the dorsal height during grafting. There is also a tendency to overgraft the radix area because the diced cartilage spills into this space when the dorsum is compressed during molding of the graft under the nasal skin as part of the completion of the dorsal grafting procedure. Kelly and colleagues[8] have attempted to prevent this from happening by sewing off the component of the fascial tube extending to the radix in patients undergoing full-length dorsal augmentation.

TASMAN TECHNIQUE: EARLY EXPERIENCE AND SUGGESTIONS

So why not use the diced cartilage as a free graft rather than a fascial-wrapped graft? Until recently, the fascial tube was necessary to maintain the integrity and continuity of the diced cartilage. I was honored to be the moderator of a session for Advances in Rhinoplasty sponsored by the American Academy of Facial Plastic and Reconstructive Surgery in May 2011, in Chicago, Illinois. As part of the session, Abel-Jan Tasman, MD, presented an intriguing new technique for using diced

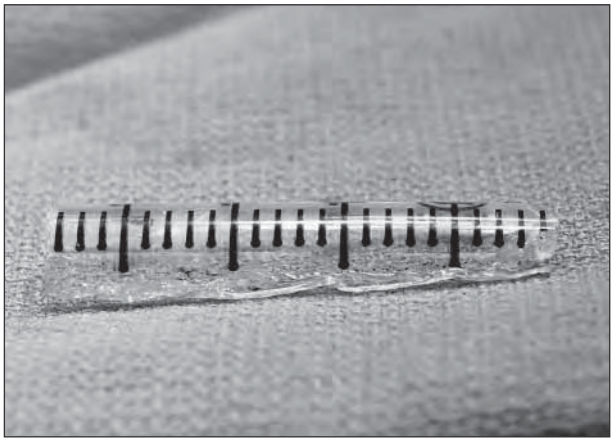


Figure 1. A 3-mL syringe cut in a taper along the long axis creates a trough that serves as a mold for diced cartilage.

cartilage to augment the dorsum as a free graft without the use of fascia.[9] He used a modified 3-mL syringe to serve as a trough in which he placed diced cartilage cut to 0.5 mm or smaller. The syringe was cut in a diagonal fashion along its long axis to create a sloping trough measuring approximately 1.0 cm wide and 3.0 to 4.0 cm long. He solidified the diced cartilage by using a few drops of human tissue sealant. This created a graft that was sufficiently rigid to enable transferring the graft from its plastic mold to the nasal dorsum.

I was fascinated with this unique method of preparing a cartilaginous graft and decided to use it in patients who required augmentation of the dorsum of greater than 3 mm. I would like to share my early experience and some helpful suggestions for surgeons who might wish to use this technique. I have used the Tasman technique for dorsal augmentation using costal cartilage as well as septal and auricular cartilage. First, it is difficult to use a scalpel to diagonally cut a plastic 3-mL syringe longitudinally. A handheld battery-operated heat cautery device used for ophthalmological surgery is more effective in cutting the syringe than using a scalpel (**Figure 1**). However, I am in the process of working with a surgical instrument company to develop variable sizes of metal troughs that can be used as a mold for the diced cartilage. Recently, I used the trough of an Aufricht retractor (10 mm×45 mm, solid blade) as the mold for the diced cartilage. Another instrument that could be used is a Cobb gauge (9.5 mm).

Cartilage used for the graft should be cut into 0.5-mm pieces or smaller. This is most effectively accomplished using a number 10 scalpel blade and a cutting block. The diced cartilage is then placed within the plastic mold and compacted using a freer elevator so that the cartilage assumes the shape of the trough with an outer curvature equal to the circumference of the trough (**Figure 2**A). This is approximately 1 cm and corresponds nicely with the ideal width of the nasal dorsum. In the center along the long axis of the trough filled with diced cartilage, a shallow groove is created if the recipient site on the dorsum has a convex contour. This groove will accommodate the convexity and possibly help prevent side-to-side movement of the graft once it is placed over the dorsum. If the recipient dorsum is a flat plateau, the plastic trough is completely filled with diced cartilage and no groove is created. I use a 2-mL package of Evicel (Omrix Biopharmaceuticals Ltd) tissue sealant as the solidifying agent when preparing the diced cartilage. This material is provided as a single-use kit consisting of 2 vials: one vial contains Thrombin, which is a sterile solution containing highly purified human thrombin and calcium chloride, and the other vial contains Biological Active Component 2 (BAC2), which consists mainly of a concentrate of human fibrinogen. Fibrinogen is a protein from human blood that forms a clot when combined with Thrombin. Each vial is transferred to an individual 3-mL syringe. The syringe is attached to a 21-gauge needle. Two or 3 drops of Thrombin are applied to the trough first, and then the diced cartilage is added. If the cartilage is too saturated with the Thrombin, the cartilage is held in place with a freer elevator while tilting the trough slightly to drain off excess Thrombin. The excess may also be eliminated by touching the graft material with nonadherent surgical dressing (Telfa; Covidien). The cartilage is then compacted and molded into the desired shape with a freer elevator. Then, 2 or 3 drops of fibrinogen (BAC2) are distributed evenly over the entire surface of the diced cartilage. The quantity of fibrinogen should be just sufficient to fill the spaces between the fragmented cartilage. It is gently massaged into the cartilage with a freer elevator. The fibrinogen rapidly diffuses through the pieces of cartilage to react with the Thrombin. Within 3 to 5 minutes, the diced cartilage is solidified into a semirigid graft conforming to the size and shape of the plastic trough (Figure 2B). It can be gently lifted

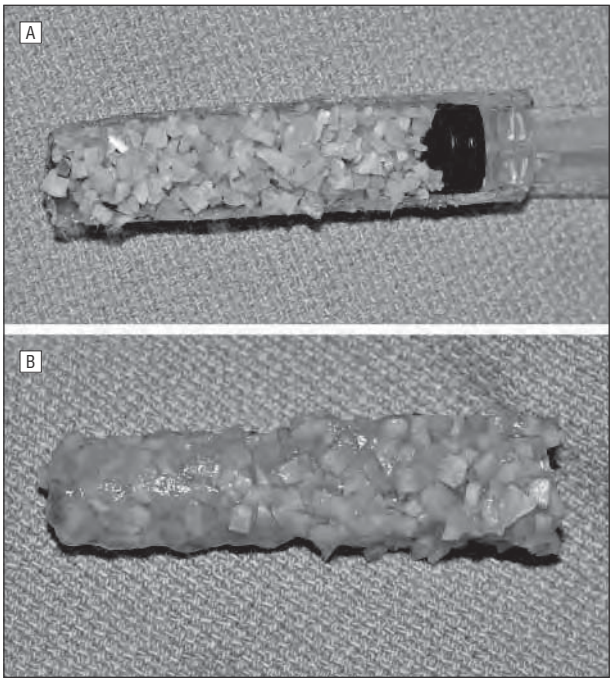


Figure 2. A, Diced costal cartilage compacted in syringe mold; B, diced costal cartilage graft solidified by tissue sealant.

from the trough and placed in the recipient site on the dorsum. The graft must be maneuvered very gently with smooth forceps because it is fragile and can be disrupted. The dorsal nasal skin must be widely elevated to create a space larger than the graft. This facilitates placement, since the graft is semirigid and can be fractured easily if excessive forces from a constricting soft-tissue pocket are exerted on the graft. Because the graft is fragile, it is best to place the graft on top of the skin of the dorsum and trim it to the appropriate size before placing the graft beneath the nasal skin (**Figure 3**). Once the graft is in place, the nasal skin is redraped over the graft. The tissue sealant mixed with diced cartilage produces a graft that has the consistency of very soft but solid silicone rubber. Because the graft is pliable, it can be molded by gentle compression through the nasal skin to adjust the graft for an ideal profile. However, because the graft material is malleable, overzealous compression may disrupt the graft resulting in loss of graft integrity.

Once the grafting procedure is complete and adjustments are made to create the ideal profile, incisions are closed, the nose is taped, and an external splint is applied. The splint should be applied with limited digital compression so the graft is not fractured or disrupted.

It would be difficult to use an endonasal approach for placement of the diced cartilage grafts. A wide surgical exposure of the dorsum with a large space created under the nasal skin to allow maneuvering and placement of the diced cartilage is necessary to prevent fracturing or distortion of the graft (**Figure 4**). Thus, an open rhinoplasty approach is the preferred method of performing this surgery. I believe that should an endonasal approach be used by the surgeon, diced cartilage placed within a temporal fascial tube is the preferred method of preparing the graft for augmentation of the dorsum. However, it is likely the fascial tube containing the diced cartilage will be difficult to insert and properly orient using the endonasal approach. Bullocks et al[10] recently reported using diced cartilage grafts with an endonasal approach. They created a malleable construct of autologous diced cartilage grafts stabilized with autologous tissue glue created from platelet-rich plasma (platelet gel) and platelet-poor plasma (fibrin glue). The authors combined the diced cartilage with the tissue glue and placed the mixture in a 5-mL syringe with the plunger removed and the distal beveled portion cut off. The plunger was then replaced, and the graft material was injected on the nasal dorsum. With this technique, the authors were able to graft the nasal dorsum with diced cartilage using the open as well as endonasal approach.

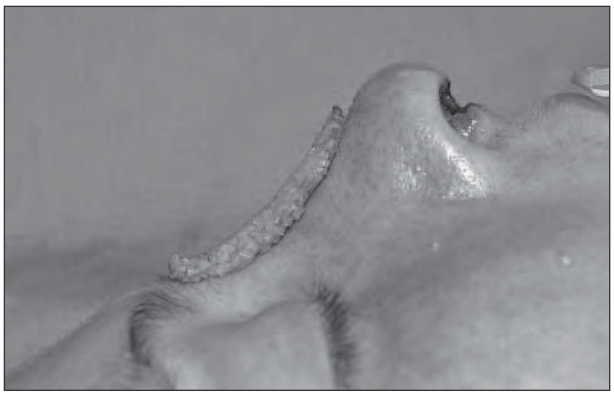


Figure 3. Diced cartilage graft is best modified for proper length and width by placing graft on the dorsal surface of the nasal skin rather than attempting to modify the graft once it has been placed beneath skin.

Using diced cartilage solidified by thrombin mixed with fibrinogen has the theoretical advantage of earlier and more rapid revascularization compared with diced cartilage that is surrounded by an avascular fascia graft. The fascia itself must be revascularized before the cartilage within the fascial tube undergoes ingrowth of vascular channels. Thus, it is likely that graft integration is delayed by the suboptimal porosity of the fascia. Diced cartilage and perichondrocyte coalesced with fibrin offers rapid imbibition through the interstitial matrix and optimal adherence of the graft to adjacent bone and cartilage.

In patients with severe saddle noses from loss of cartilaginous septal support, I use autogenous costal cartilage to construct an L-shaped strut to restore support to the nasal dorsum and tip. The strut also provides a foundation for further dorsal augmentation if required to achieve an ideal profile using diced cartilage solidified by tissue sealant (**Figure 5**).

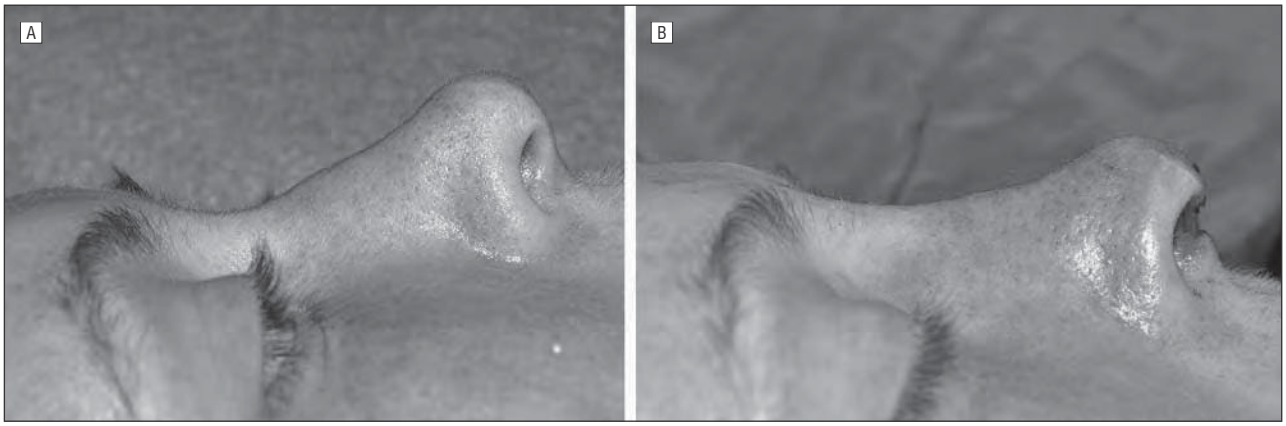
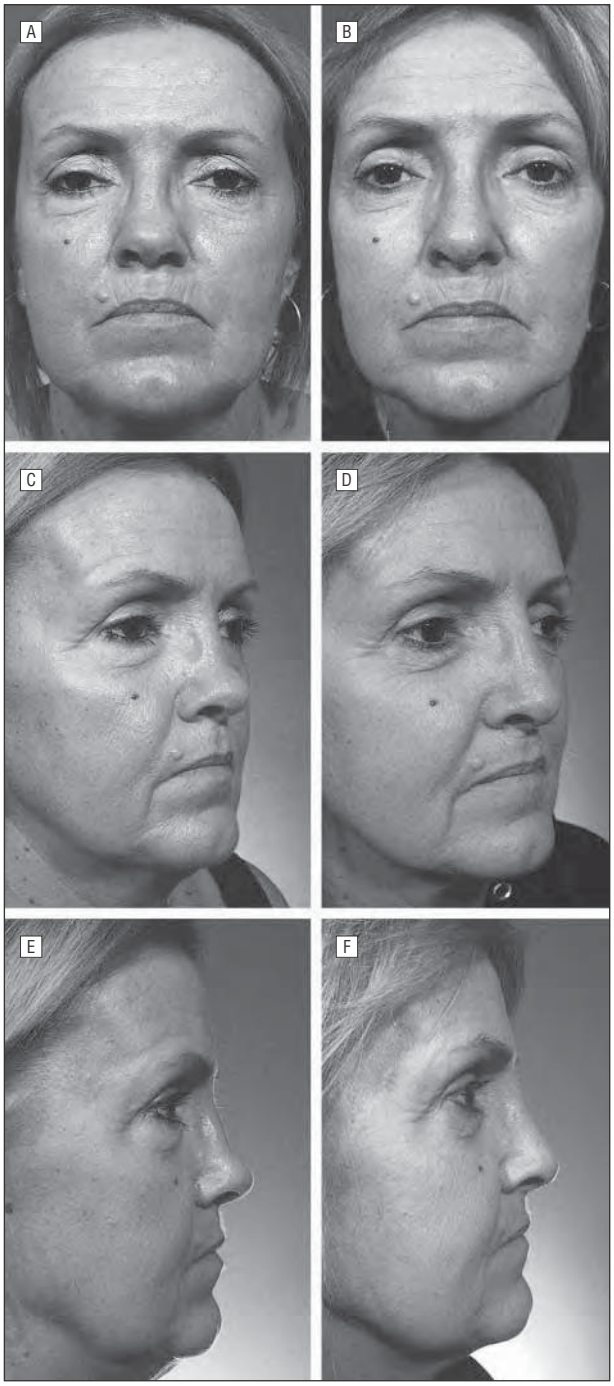


Figure 4. Same patient shown in Figure 3 before (A) and after (B) dorsal placement of dice cartilage graft.



Figures 5. Preoperative views (A, C, and E) and 9 months following (B, D, and F) costal cartilage L-strut reconstruction and diced cartilage dorsal graft.

Five patients have undergone dorsal augmentation using diced septal, auricular, or costal cartilage solidified using tissue sealant, and no resorption of the grafts have been observed, with the longest follow-up of 9 months. Compared with the patients I treated using diced cartilage contained within a fascial tube, the patients treated using diced cartilage and tissue sealant appear to have considerably less long-term edema of the nose. This may be related to a more rapid incorporation of the graft by early revascularization of the diced cartilage.

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Revisiting the Role of Columellar Strut Graft in Primary Open Approach Rhinoplasty

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Background: The effect of a columellar strut graft on final nasal tip position has been a subject of ongoing debate. The purpose of this study was to retrospectively analyze a series of 100 consecutive primary rhinoplasty cases performed without the use of columellar strut grafts, with a specific focus directed toward comparing preoperative, morphed, and actual postoperative changes in nasal tip position.

Methods: Data were collected from patient charts and digital images of 100 consecutive primary open rhinoplasty patients. Preoperative, morphed, and actual postoperative digital images were quantitatively analyzed using image-processing software to compare various anatomical features, including nasal tip projection, nasolabial angle, and Goode ratio. Patient satisfaction regarding long-term postoperative results was also surveyed.

Results: Primary rhinoplasty did not demonstrate a universal trend toward either an increase or a decrease in nasal tip projection. The planned changes in nasal tip projection, nasal tip rotation, and nasal profile proportions were obtained with statistically significant accuracy without the use of columellar strut grafts. The overall incidence of columellar contour irregularities was 3 percent.

Conclusion: In primary open approach rhinoplasty, if native anatomical support structures of the nasal tip are preserved or reconstructed, preoperative goals regarding nasal tip projection, nasal profile proportions, and columellar integrity can be consistently achieved without using columellar strut grafts. (*Plast. Reconstr. Surg.* 135: 987, 2015.)

CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, IV.

Our understanding of nasal tip support in primary rhinoplasty has evolved considerably in recent years. At the same time, so have the techniques used to stabilize the tip. Several theories of tip support have gained favor. Anderson's tripod concept has frequently been cited to support methods for altering the lower lateral cartilages. The publications by Anderson[1] and Janeke and Wright[2] on major and minor tip-supporting mechanisms have also become a widely accepted means of explaining tip dynamics.

More recently, Adamson et al. described an expanded version of the tripod concept. Their M-arch model addresses lower lateral cartilage dynamics in more detail. This model describes the conjoined medial and lateral crura as a continuous elastic spring, the curvature of which creates intrinsic tension in the structure. Considered together, these viewpoints have fueled the popularity of such surgical techniques as the lateral crural and columellar strut grafts, the aim of which is to reinforce the lower lateral cartilage construct.[3]

It is our contention that the Anderson/Adamson model overemphasizes the contribution of the lower lateral cartilage and underemphasizes the importance of the nasal septum with regard to tip support. A rival school of thought highlights the importance of caudal septum and its midline position to nasal tip stability. Adams et al. have quantitatively measured the impact of selected surgical maneuvers on nasal tip projection. In both open and closed rhinoplasty, septal removal was found to produce the greatest loss of nasal tip projection.[4]

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Beaty et al. have also quantitatively measured the impact of selected surgical maneuvers on nasal tip support. Their findings indicate that the nasal tip is cantilevered, almost suspended, at the anterior septal angle by the association of the septum with the nasal domes through central suspending ligaments. Disruption of this ligamentous structure alone resulted in a 35 percent decrease in tip support.[5] Gassner et al. and Westreich et al. have also found the anterior septal angle to be the most supportive structure in their quantitative nasal tip tissue resilience/cartilage elasticity studies.[6,7]

Finally, the cantilevered spring theory of Westreich and Lawson describes a spring-loaded tripod that gains its stability from a rigid abutment. In this model, the paired lower lateral cartilages have a single point of dominant fixation, usually along the caudal septum, around which the elastic tripod will rotate. Unlike preceding models, the rigid abutment is not located at the feet of the tripod but rather at the septum, the strongest midline element of the nose.[8]

One aim of primary open rhinoplasty is to reconstitute the ideal nasal anatomy when this anatomy is less than ideal. We therefore suggest that if (1) a proper anterior septal angle position is created, (2) a central suspending ligament repair is performed, and (3) the scroll area is preserved or reconstructed, the desired nasal tip position and stability can be consistently achieved without a columellar strut graft. We present a series of 100 consecutive primary open rhinoplasties performed with special attention to restoration of native tip support elements but without the use of columellar strut grafts.

PATIENTS AND METHODS

Medical records and digital images from 100 consecutive primary open rhinoplasty patients (84 female and 16 male patients) operated on by the first author (O.B.) from July of 2011 to October of 2012 were retrospectively reviewed for patient demographics, surgical techniques, complications, and postoperative columellar deformities (Table 1).

All patients underwent an open rhinoplasty approach without the use of a columellar strut, tongue-in-groove technique, septocolumellar sutures, or columellar/medial crural reinforcement of any kind. Nasal tip support and projection were provided by (1) precise adjustment of the anterior septal angle using a septal extension graft if necessary, (2) a median suspension suture between the distal insertion of the Pitanguy ligament and the middle vault, and (3) reconstruction of the scroll attachments by primary ligament repair or by using a horizontal mattress type lower lateral cartilage–to–upper lateral cartilage suspension suture (Fig. 1). Nasal tip was refined with suture techniques only; no tip grafts were used.

Table 1. Surgical Techniques

Characteristic	No.
Total no. of rhinoplasties	100
Septal surgery	
Septoplasty/septal harvest	88
Caudal trim	34
Dorsal reduction	80
Anterior septal jig-type extension without using a graft	6
Septal extension graft	4
Middle vault	
Unilateral spreader grafts	18
Bilateral spreader grafts	5
Upper lateral fold-in flap	79
Upper lateral caudal trim	36
Osteotomies	
Unilateral lateral	13
Bilateral lateral	76
No osteotomy	11
Dorsal camouflage	
Recycled dorsal hump	80
Temporal fascia	4
Lower lateral cartilages	
Cephalic trim	91
Horizontal repositioning	22
Lateral crural steal	4
Lateral crural overlay	4
Nasal tip	
Hemitransdomal sutures	100
Interdomal sutures	100
Medial crural transfixion sutures	84
Tip grafts	0
Columellar strut	0
Alar base reduction	6
Alar rim grafts	64

All 100 patients had a complete set of digital images, including (1) a preoperative series, (2) a preoperative morphed right profile image, and (3) a late postoperative (8 to 12 months) series. Digital photographs were obtained using a standardized setup with the patient seated at a fixed distance from the camera. Morphed right profile images were created using Virtual Plastic Surgery Software (Version 1.0; Kaeria EURL, Paris, France). Digital images were processed by using open-source GNU Image Manipulation Program, GNU Public License Version 2.8. Patient satisfaction regarding long-term postoperative nasal tip elasticity was assessed subjectively by means of an e-mail survey in December of 2013. Survey results were tabulated by an unbiased observer.

Analysis of Nasal Tip Projection

Nasal tip projection was measured on preoperative, morphed, and postoperative right

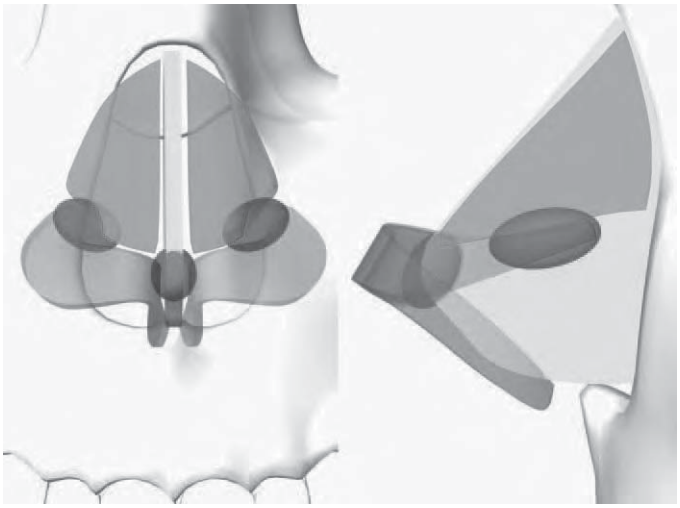

Fig. 1. Illustration of our surgical routine for maintaining nasal tip support in primary open approach rhinoplasty. Paramedian and median supporting ligament reconstruction (*red ellipses*) along with a proper anterior septal angle position is performed in every case.

profile photographs from the alar midpoint to the nasal tip by using methods described previously by Byrd and Hobar.[9] Preoperative right profile and morphed right profile images were identical, which enabled a direct, linear, pixel-based measurement of nasal tip projection. Preoperative and postoperative right profile pictures, however, had subtle differences in framing, distance, and resolution. To minimize the effects of these differences, measured postoperative nasal tip projection was adjusted by using a correction coefficient that was calculated by dividing a fixed distance (superior margin of the tragus to the lateral canthus) on the preoperative photograph by the same fixed distance on the postoperative photographs as described previously in the literature.[10,11] The measured postoperative nasal tip projection multiplied by the correction coefficient gave the adjusted postoperative nasal tip projection that was used in statistical analysis.

Percentage differences between preoperative nasal tip projection and morphed nasal tip projection values were calculated for each patient to better describe and analyze planned changes in nasal tip projection. Percentage differences between preoperative nasal tip projection and adjusted postoperative nasal tip projection values were calculated for each patient to better describe and analyze actual changes in nasal tip projection (Fig. 2). If the planned change in nasal tip projection was an increase greater than 1 percent, those patients were also included in a separate "planned increase in nasal tip projection" cohort.

Analysis of Nasal Tip Rotation and Nasal Profile Proportions

Nasal tip rotation was analyzed by using the nasolabial angle, which was measured at the intercept of the columellar break point to the subnasale line with the superior labial point to the subnasale line. Nasal profile proportions were analyzed by using the Goode ratio. The Goode ratio was calculated by dividing the distance from the alar point to the nasal tip by the distance from the nasion to the nasal tip (Fig. 2). All measurements were performed by the same surgeon (H.U.) to ensure consistency.

Statistical Analysis

Statistical analysis of the data was performed by using SPSS Version 17 (SPSS, Inc., Chicago, Ill.). Nasal tip projections, nasolabial angles, and Goode ratios were compared between preoperative, morphed, and postoperative groups by using the repeated measures analysis of variance test with a Greenhouse-Geisser correction. Post hoc tests using the Bonferroni correction were used to make pairwise comparisons. The linear correlation between planned and actual changes in nasal tip projection was also assessed by calculating the Pearson parametric correlation moment coefficient. The chi-square test was used to examine the differences in distribution tables.

RESULTS

Of the 100 patients in our series, 86 were female and were 14 male. The average patient age was 27.6 years (range, 17 to 55 years), and the

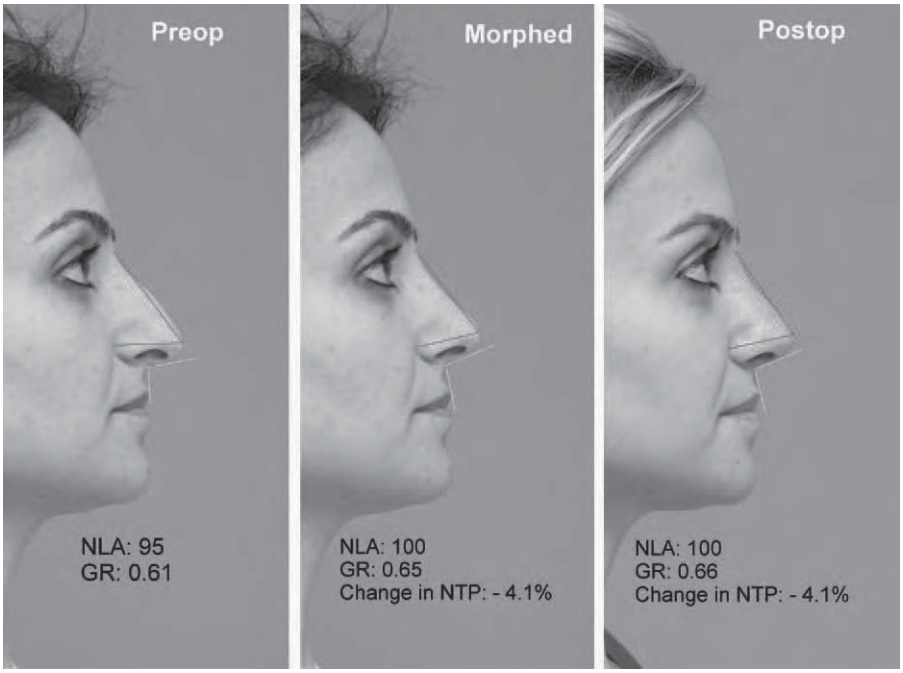

Fig. 2. Analysis of planned and obtained changes in nasal tip projection, nasal tip rotation, and nasal profile proportions. *NLA*, nasolabial angle; *GR*, Goode ratio; *NTP*, nasal tip projection.

average length of postoperative follow-up was 14.8 months (range, 12 to 25 months).

Distribution analysis of planned changes in nasal tip projection demonstrates that, in 32 percent of our cases, a projection increase of more than 1 percent was planned preoperatively. In 19 percent of our cases, a projection increase of more than 3 percent was planned; in only 9 percent of our cases a projection increase of more than 5 percent was planned (Table 2). Distribution analysis of obtained changes in nasal tip projection has shown that, in 42 percent of our cases, a projection increase of more than 1 percent was obtained. In 27 percent of our cases, a projection increase of more than 3 percent was obtained; and in only 15 percent of our cases, a projection increase of more than 5 percent was obtained (Table 3). The differences in distribution between planned and obtained changes were not statistically significant in any interval.

In the overall study population, the preoperative nasal tip projection (211.2 ± 37.7 pixels) was not significantly different from morphed nasal tip projection (210.4 ± 35.7 pixels) and postoperative nasal tip projection (213.1 ± 37.4 pixels) (Table 4). In the overall study population, the Pearson parametric correlation coefficient between planned and obtained changes in nasal tip projection was 0.791, which was indicative of a strong, statistically significant correlation ($p < 0.0001$). Primary rhinoplasty did not demonstrate a universal trend toward either an increase or a decrease in nasal tip projection.

In the "planned increase in nasal tip projection" cohort, both morphed nasal tip projection (212.6 ± 45.3 pixels) and postoperative nasal tip projection (214.5 ± 45.8 pixels) were significantly higher than preoperative nasal tip projection (204.8 ± 45.9 pixels) ($p < 0.0001$). There

Table 2. Distribution of Planned Changes in Nasal Tip Projection (*n* = 100 patients)

Planned Change	No. (%)
>5% decrease	10 (10)
>3% decrease	23 (23)
>1% decrease	32 (32)
Difference <1%	36 (36)
>1% increase	32 (32)
>3% increase	19 (19)
>5% increase	9 (9)

Table 3. Distribution of Actual Changes in Nasal Tip Projection (*n* = 100 patients)

Actual Changes	No. (%)
>%5 decrease	6 (6)
>3% decrease	17 (17)
<1% decrease	31 (31)
Difference <1%	26 (26)
>1% increase	42 (42)
>3% increase	27 (27)
>5% increase	15 (15)

Table 4. Repeated Measures Analysis of Variance, Nasal Tip Projection, Overall Study Population*

(I) Time	(J) Time	Mean Difference (I – J)	*p*†
Preoperative NTP (211.2 ± 37.7), *n* = 100	Morphed NTP	0.790	1.000
	Postoperative NTP	–1.880	0.124
Morphed NTP (210.4 ± 35.7), *n* = 100	Preoperative NTP	–0.790	1.000
	Postoperative NTP	–2.670‡	0.002‡
Postoperative NTP (213.1 ± 37.4), *n* = 100	Preoperative NTP	1.880	0.124
	Morphed NTP	2.670‡	0.002‡

NTP, nasal tip projection.
*Descriptive statistics and pairwise comparisons, based on estimated marginal means.
†Bonferroni adjustment for multiple comparisons.
‡The mean difference is significant at the 0.05 level.

was no statistically significant difference between morphed nasal tip projection and postoperative nasal tip projection (Table 5). In the planned increase in nasal tip projection cohort, the Pearson parametric correlation coefficient between planned and actual changes in nasal tip projection was 0.781, which was indicative of a strong, statistically significant correlation ($p < 0.0001$).

In the overall study population, both morphed Goode ratio (0.66 ± 0.039) and postoperative Goode ratio (0.67 ± 0.039) were significantly higher than preoperative Goode ratio (0.61 ± 0.048) ($p < 0.0001$). Postoperative Goode ratio was also higher than morphed Goode ratio (0.67 versus 0.66) ($p < 0.0001$); this difference was interpreted as a desirable shift toward the ideal nasal projection/length proportion of 2:3 (0.67) (Table 6). In the overall study population, both morphed nasolabial angle (101.5 ± 6.1) and postoperative nasolabial angle (101.5 ± 6.7) were statistically significantly higher than the preoperative nasolabial angle (92.4 ± 10.3) ($p < 0.0001$). As seen with tip projection, there was no statistically significant difference between morphed nasolabial angle and postoperative nasolabial angle (Table 7).

Three patients (3 percent) in our series had minor columellar contour irregularities (Fig. 3). None of these patients belong to the planned increase in nasal tip projection cohort. Two of these patients underwent a columellar revision procedure that was performed under local anesthesia. Columellar irregularities were successfully corrected by revising transcolumellar scars and by using short columellar strut grafts. Two patients in our series had revisions for reasons other than columellar deformities, including one unilateral vestibular V-Y procedure for correction of alar asymmetry and one revision of right lateral osteotomy. The total revision rate was 4 percent, and all revisions were performed after obtaining the late postoperative photographs.

Of 100 patients, 92 responded to the postoperative survey. Patients were asked to rate their current satisfaction with the functional elasticity of their nose during (1) activities of personal hygiene such as cleaning and wiping, and (2) activities of social interaction such as kissing and smiling on a scale from 1 (not satisfied) to 5 (extremely satisfied). The average score of satisfaction during activities of personal hygiene was 4.5, and the average score of satisfaction during activities of social interaction was 4.8

DISCUSSION

In the normal nasal anatomy, an anterior septal angle of sufficient height keeps the feet of the medial crura off the anterior nasal spine; the medial crura do not bear a significant load;

Table 5. Repeated Measures Analysis of Variance, Nasal Tip Projection, and Planned Increase in the Nasal Tip Projection Cohort*

(I) Time	(J) Time	Mean Difference (I – J)	*p*†
Preoperative NTP (204.8 ± 45.9), *n* = 32	Morphed NTP	–7.791‡	0.0001‡
	Postoperative NTP	–9.684‡	0.0001‡
Morphed NTP (212.6 ± 45.3), *n* = 32	Preoperative NTP	7.791‡	0.0001‡
	Postoperative NTP	–1.894	0.074
Postoperative NTP (214.5 ± 45.8), *n* = 32	Preoperative NTP	9.684‡	0.0001‡
	Morphed NTP	1.894	0.074

NTP, nasal tip projection.
*Descriptive statistics and pairwise comparisons, based on estimated marginal means.
†With Bonferroni adjustment for multiple comparisons.
‡The mean difference is significant at the 0.05 level.

Table 6. Repeated Measures Analysis of Variance, Goode Ratio, and Overall Study Population*

(I) Time	(J) Time	Mean Difference (I – J)	*p*†
Preoperative GR (0.61 ± 0.048), *n* = 100	GR morphed	–0.053‡	0.0001‡
	GR postoperatively	–0.060‡	0.0001‡
Morphed GR (0.66 ± 0.039), *n* = 100	GR preoperatively	0.053‡	0.0001‡
	GR postoperatively	–0.007‡	0.0001‡
Postoperative GR (0.67 ± 0.039), *n* = 100	GR preoperatively	0.060‡	0.0001‡
	GR morphed	0.007‡	0.0001‡

GR, Goode ratio.
*Descriptive statistics and pairwise comparisons, based on estimated marginal means.
†With Bonferroni adjustment for multiple comparisons.
‡The mean difference is significant at the 0.05 level.

Table 7. Repeated Measures Analysis of Variance, Nasolabial Angle, Overall Study Population*

(I) Time	(J) Time	Mean Difference (I – J)	*p*†
Preoperative NLA (92.4 ± 10.3), *n* = 100	NLA morphed	–9.020‡	0.0001‡
	NLA postoperatively	–8.980‡	0.0001
Morphed NLA (101.5 ± 6.1), *n* = 100	NLA preoperatively	9.020‡	0.0001‡
	NLA postoperatively	0.040	1.000
Postoperative NLA (101.5 ± 6.7), *n* = 100	NLA preoperatively	8.980‡	0.0001‡
	NLA morphed	–0.040	1.000

NLA, nasolabial angle.
*Descriptive statistics and pairwise comparisons, based on estimated marginal means.
†With Bonferroni adjustment for multiple comparisons.
‡The mean difference is significant at the 0.05 level.

and the columella is soft, mobile, and flexible. Although relying on medial crural strength for nasal tip support/projection can be a surgically successful strategy in selected rhinoplasty cases, it is also associated with clinically significant but infrequently discussed sequelae such as nasal tip rigidity and loss of columellar flexibility.[10,12]

Rohrich et al. have classified columellar strut grafts and proposed a clinical algorithm for their application.[12] They recommended using either type 3 (long and floating) or type 4 (long and fixed) columellar strut grafts for increasing nasal tip projection. Although nasal tip projection can be stabilized or increased with the use of a long columellar strut, this occurs at the expense of a rigid and fixed columella. In our experience, patients who undergo reconstruction with a long, fixed columellar strut may complain of difficulty wiping their nose, or the nose may be felt like a stick on the cheek when kissing.

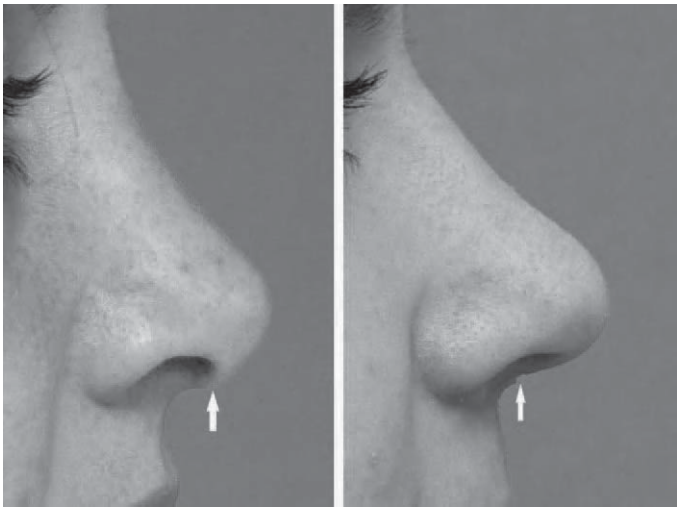

Fig. 3. Examples of postoperative columellar contour irregularities in the absence of columellar strut grafts. Unwanted bowing of intrinsically weak medial crura (*left*) and columellar notching beneath the transcolumellar incision (*right*).

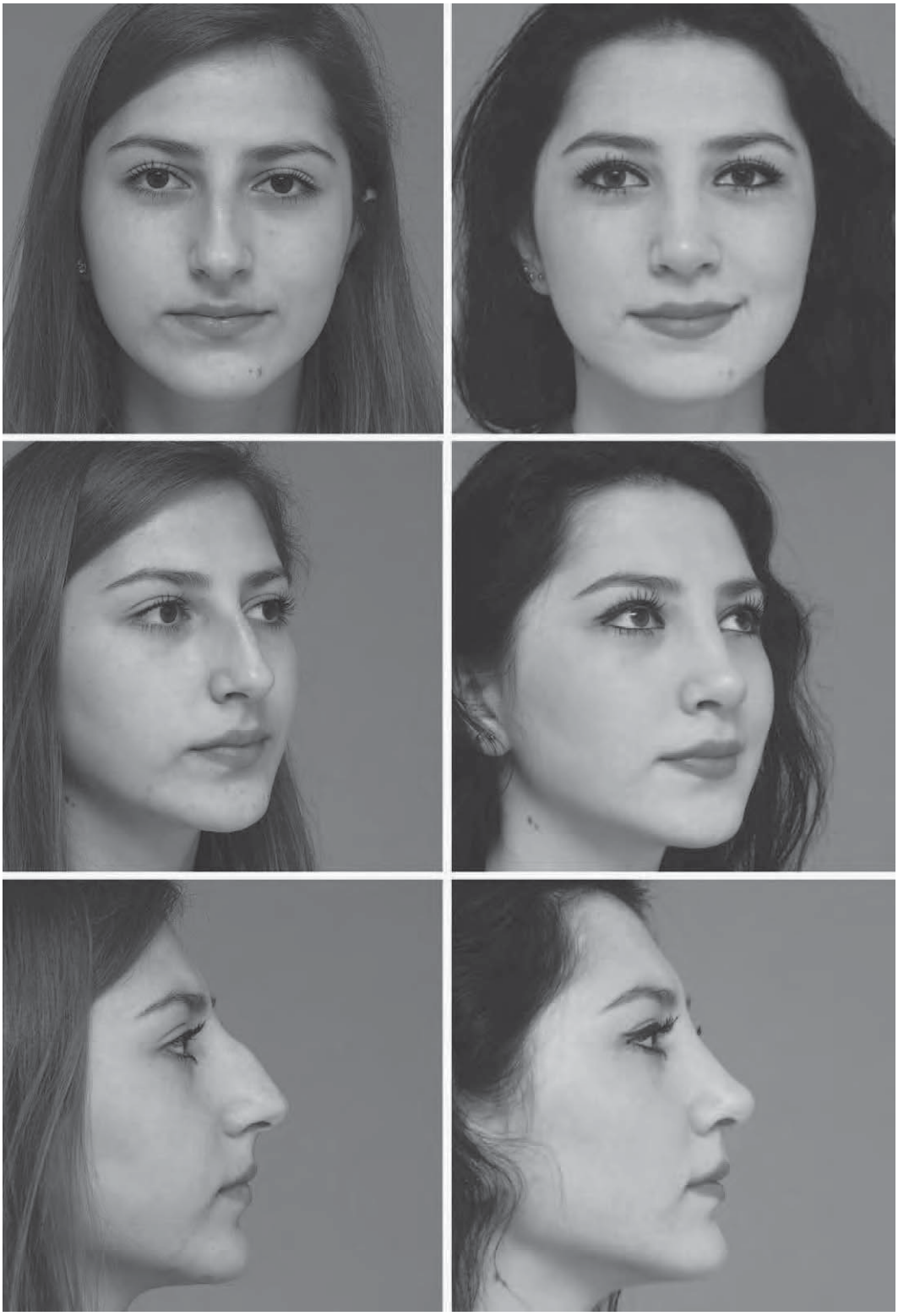

Fig. 4. A 22-year-old female patient with tension nose. Preoperative (*left*) and 12-month postoperative (*right*) photographs after primary open rhinoplasty.

The effect of a columellar strut graft on final nasal tip position has been a subject of ongoing debate. Historically, the columellar strut cartilage graft has been assumed to be a technique that increases nasal tip projection. However, in the study by Rohrich et al. of 100

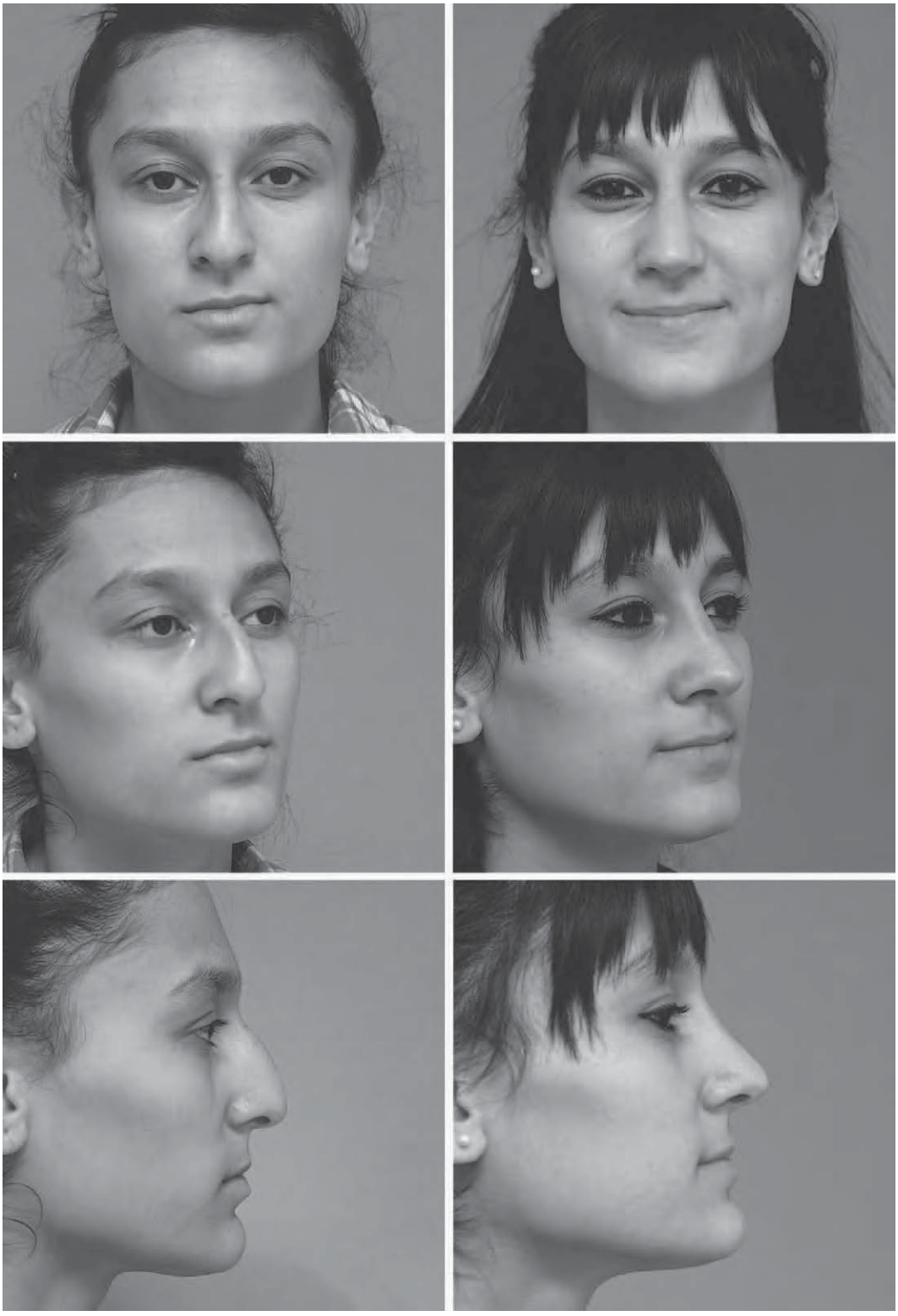

Fig. 5. A 20-year-old female patient with tension nose. Preoperative (*left*) and 9-month postoperative (*right*) photographs after primary open rhinoplasty.

consecutive open rhinoplasties in which a columellar strut was used in each case, tip projection decreased in 65 percent, increased in 27 percent, and was unchanged in 8 percent. Rohrich et al. concluded that the routine use of the columellar strut graft does not necessarily imply an increase

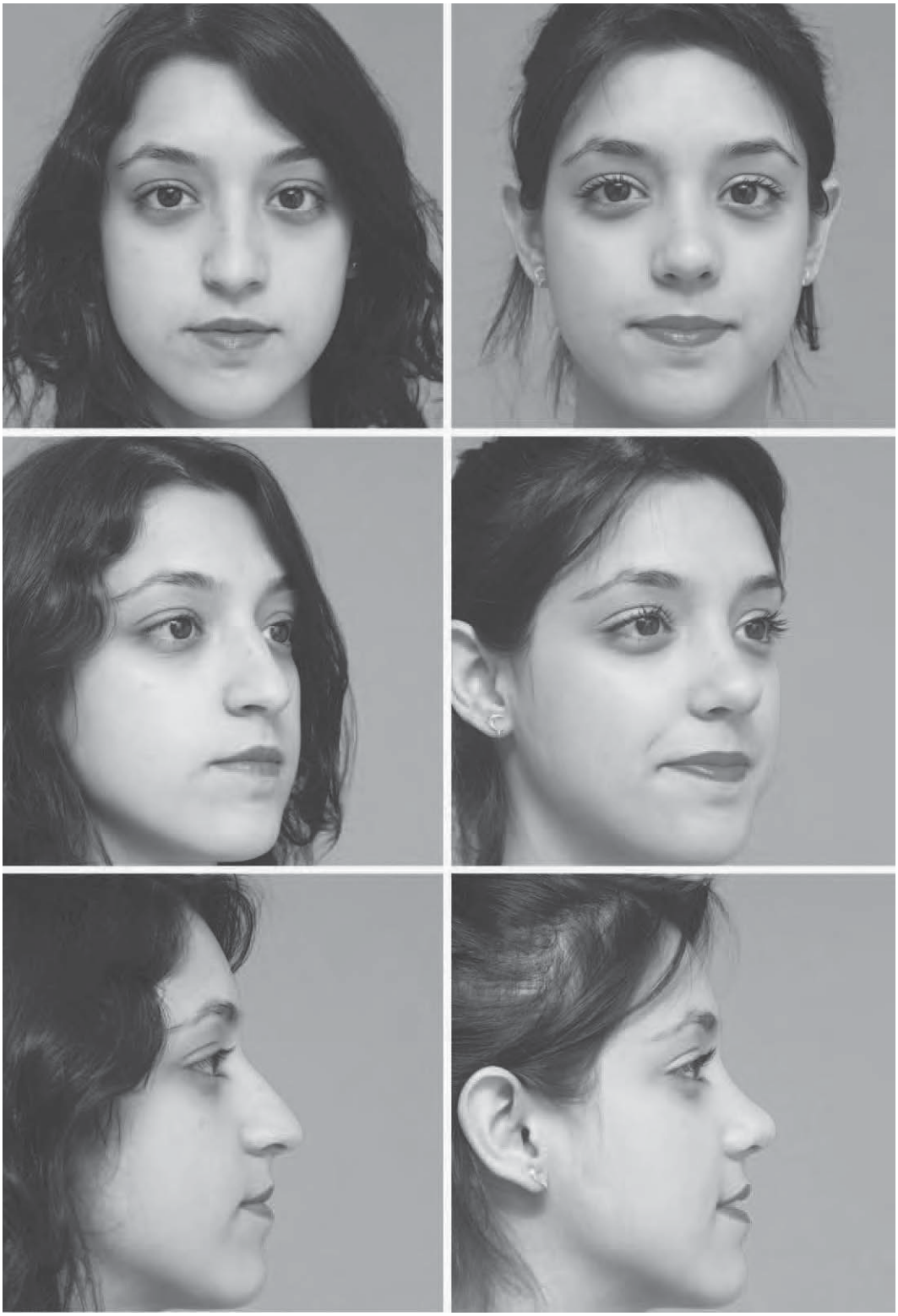

Fig. 6. A 19-year-old female patient with tension nose. Preoperative (*left*) and 10-month postoperative (*right*) photographs after primary open rhinoplasty.

in nasal tip projection but rather serves as a means of unifying the nasal tip and helping to control final tip position.[10]

In our 100 consecutive open rhinoplasties, the preoperatively planned alterations in nasal tip projection, nasal tip rotation, and nasal profile

proportions were achieved with statistically significant accuracy despite not using the columellar strut cartilage graft. Therefore, we conclude that the absence of the columellar strut cartilage graft does not necessarily imply a deterioration in final nasal tip position.

The distribution of nasal tip projection in both our morphed images and our postoperative results was similar to the findings of Rohrich et al. In addition, our results indicate that only a minority of primary rhinoplasty patients in our series needed an increase in nasal tip projection to begin with. In this minority (planned increase in nasal tip projection cohort), the planned amount of increase in nasal tip projection was achieved without using a columellar strut (Figs. 4 through 6).

The need to change nasal tip projection in rhinoplasty patients will differ significantly based on ethnicity, facial form, and the surgeon's aesthetic perspective. Our patient population did not require significant changes in nasal tip projection, so the use of more significant modes of stabilizing the nasal base was not necessary. When a significant increase in nasal tip projection was needed, we preferred using septal extension grafts (4 percent). However, for the surgeon operating on a patient population that tends to be significantly underprojected (such as Asian and black patients) with a weak nasal base, the use of more substantial grafting may be needed to gain adequate nasal tip projection and prevent postoperative loss of nasal tip projection.

In our series of 100 consecutive rhinoplasties, only three patients had minor columellar irregularities. It is not possible to declare whether these irregularities are truly related to an absence of a graft or whether a short strut would be of any preventive value. We did not observe a structural disruption in the columella or nasal tip complex in any of our cases. Unfortunately, it is not possible to make a comparison to other studies because there is no mention regarding the incidence of columellar irregularities in studies in which a columellar strut was used routinely. Our survey results show that the majority of our patients were satisfied with the postoperative flexibility of their columella. Because such information was not provided in previous columellar strut studies, a comparison again is not possible.

Although we favor no strut in the primary open approach rhinoplasty, it should be noted that columellar strengthening techniques work undeniably well in secondary rhinoplasty cases in which the nasal septum is deficient or when a major nasal tip correction or cartilage framework reconstruction is required. In addition, closed rhinoplasty is a different situation, and columellar supporting mechanisms are justified in these primary cases. Finally, a short columellar strut graft to secure the structural integrity of the columella is justified in primary cases, especially when the medial crura are markedly weak or asymmetric. Numerous anatomical studies on lower lateral cartilages have demonstrated that the medial legs of the tripod are often of unequal length and strength. Studies also document the considerable biological variation among different ethnic groups in the quantity of cartilage and its inherent resilience and configuration.[13–16]

We have not attempted to describe a new rhinoplasty technique. The importance of septal support in primary rhinoplasty has already been emphasized in the past,[17,18] as have both central suspension sutures and scroll repair/reinforcement techniques.[19–26] The methods that we have used for evaluating nasal tip projection and rotation have been similarly used in previous studies to justify the efficacy of columellar struts grafts and other nasal tip alteration techniques.[10,11,27,28] We have instead suggested the combined use of previous recognized techniques for nasal tip support that obviate the use of a columellar strut and retain a postoperative flexible columella.

CONCLUSIONS

Before defining the exact role of columellar strut grafting in open rhinoplasty, it is important to understand what would occur if no strut was used. Our study addresses this question by using a methodology that not only evaluates quantitative outcome measures but also compares these with preoperative objectives.

In primary open approach rhinoplasty, preoperative goals regarding nasal tip projection, nasal profile proportions, and columellar integrity can be consistently achieved without using columellar strut grafts if native anatomical support structures of the nasal tip are preserved or reconstructed. The columellar strut graft is a valuable tool when used for clear indications such as a significantly underprojected nose with a weak nasal base, columellar deficiency, deformity, or asymmetry. However, the decision to use a long, fixed columellar strut as a nasal tip positioning tool in primary open approach rhinoplasty should be carefully judged against its potential drawbacks and alternatives.

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PATIENT CONSENT

Patients provided written consent for the use of their images.

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Original Investigation

Aesthetic and Functional Results of Lateral Crural Repositioning

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IMPORTANCE Thin or cephalically malpositioned lateral crura cause nasal obstruction by depressing nasal valves and decrease patient satisfaction with rhinoplasty as a result of nostril asymmetry and alar collapse.

OBJECTIVE To demonstrate the aesthetic and functional efficacy of lateral crural repositioning with lateral strut grafting in patients with cephalic malposition of the lateral crura undergoing primary septorhinoplasty.

DESIGN, SETTING, AND PARTICIPANTS We prospectively selected 80 patients with lateral crural malposition who underwent primary septorhinoplasty performed by the same surgeon from December 1, 2013, through May 30, 2014. The surgeon measured the angle between the lateral crura and midline intraoperatively with a goniometer to confirm malposition (angle, ≤30°). Data analysis was performed from March 13 to 23, 2015.

INTERVENTION All the patients underwent primary rhinoplasty with the open approach. Lateral crural repositioning with lateral crural strut graft was used in all selected patients.

MAIN OUTCOME AND MEASURES Preoperative and 6- and approximately 12–month postoperative scores on the Nasal Obstruction Symptom Evaluation (NOSE) scale (range, 0-20; decreased scores indicate improved functional results) and the Rhinoplasty Outcomes Evaluation (ROE) questionnaire (range, 0-24; increased scores indicate improved aesthetic results).

RESULTS Seventy-five of 80 patients were confirmed to have cephalic malposition intraoperatively. Four patients were excluded owing to selection of different surgical techniques, leaving 71 patients for analysis. The mean (SD) and median postoperative NOSE scores at 6 months (3.18 [3.12] and 2.0) and 12 months (0.39 [1.07] and 0) showed significant improvement compared with the preoperative scores (6.96 [5.10] and 7.0) ($P < .01$ for each comparison). The mean (SD) and median postoperative ROE scores also showed significant improvement at 6 months (21.06 [3.82] and 23.0) and 12 months (23.12 [2.09] and 24.0) compared with preoperative scores (7.03 [3.70] and 6.0) ($P = .001$). However, the changes from preoperative to 12-month postoperative scores (mean [SD] and median) were not significantly different between patients with normal (NOSE scores, 8.41 [4.59] and 90 to 0.28 [0.79] and 0, respectively; ROE scores, 6.97 [3.24] and 6.0 to 23.31 [1.91] and 24.0, respectively) and thin (NOSE score, 6.59 [5.09] and 8.0 to 0.11 [0.33] and 0, respectively; ROE scores, 7.76 [3.82] and 7.0 to 23.29 [1.72] and 24.0, respectively) skin types and those with thick skin types (NOSE scores, 5.52 [5.42] and 4.0 to 0.72 [1.54] and 0, respectively; ROE scores, 6.60 [4.16] and 6.0 to 22.80 [2.53] and 24.0, respectively) ($P > .05$).

CONCLUSIONS AND RELEVANCE Lateral crural repositioning is a useful and versatile technique to achieve successful functional and aesthetic results in a 1-year follow-up. We detected no significant difference by skin type in improvement of nasal function and aesthetic satisfaction.

LEVEL OF EVIDENCE 3.

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Rhinoplasty is one of the most complex of all aesthetic procedures. Despite the numbers of surgical techniques that have achieved satisfactory results, the surgeon's choice of appropriate technique should be based on the anatomic characteristics of the nasal skeleton, presence of nasal obstruction, skin type, and the surgeon's experience. Rhinoplasty is a patient-specific surgery and must be planned according to the patient's skin type, cartilage, and bony tissue characteristics. The shape of the nose and intranasal anatomy should be analyzed, and the anatomic variations that create pathologic conditions should be addressed carefully before every rhinoplasty. Bone and cartilage tissue constituting the nasal skeleton should be evaluated carefully.

Tip refinement is the most important part of rhinoplasty to create an aesthetically attractive nose. The size, shape, and position of the lower lateral cartilages create the appearance of the nasal tip.[1] Furthermore, the positioning and the properties of the lower lateral cartilages affect the air passage of the nose by forming the nasal valve area. The tissue supporting the alar rim is the lateral crus of the greater alar cartilage. Thin or cephalically malpositioned lateral crura cause nasal obstruction by depressing nasal valves and decrease patient satisfaction as a result of nostril asymmetry and alar collapse.

In this study, we evaluated lateral crural position after a repositioning technique with a lateral crural strut graft (LCSG). We investigated the effect of lateral crural repositioning and LCSG on the airway patency and the aesthetic satisfaction of the patients.

Methods

Patient Selection

In this study, we selected 80 patients who presented for primary septorhinoplasty to treat parenthesis tip deformity and malpositioning of the lateral crura from December 1, 2013, through May 30, 2014. The same surgeon (A.E.I.) performed all the procedures and selected the patients for the study according to results of preoperative examinations and photographs. All the patients underwent a detailed preoperative examination of the ear, nose, and throat. We excluded patients with chronic sinusitis, nasal polyposis, asthma, allergic rhinitis, or a previous septoplasty or rhinoplasty. This study was approved by the ethics committee of University of Acibadem, Istanbul, Turkey. Patients gave written and oral informed consent (eFigure 1 in the Supplement).

We measured the angle between the lateral crura and midline intraoperatively with a goniometer to confirm the preoperative selection made by the surgeon (**Figure 1**A and B). We included 75 patients with an angle of 30° or less who were considered to have malpositioned lateral crura. All procedures implemented in the surgery were standardized. Medial oblique and internal osteotomy starting from the aperture piriformis that preserved the Webster triangle and went down and then up to the inner canthus level (high-to-low-to-high) were performed in all the patients. Four patients who required single-sided or asymmetric spreader grafts were excluded from the study, leaving 71 patients who underwent middle vault structuring with bilateral spreader grafts and lateral crural repositioning with LCSG.

We divided the patients into 3 groups according to their skin thickness by intraoperative skin analysis. The patients whose nasal tip definition was restricted owing to expanded skin and subdermal tissue were classified as having a thick skin type. Patients whose tip cartilages were visible and observable despite the soft tissue covering the cartilages were described as having a thin skin type. If the tip cartilages did not affect the tip definition positively or negatively during the surgical procedure, the skin type was accepted as normal. The Nasal Obstruction Symptom Evaluation (NOSE) Scale (range, 0-20; decreased scores indicate improved functional results)[2] and Rhinoplasty Outcomes Evaluation (ROE) questionnaire (range, 0-24; increased scores indicate improved aesthetic results)[3] were administered to all the patients before and at 6 and approximately 12 months (range, 10-15; mean, 12.7 months) after the procedure. We compared the results among the 3 skin type groups.

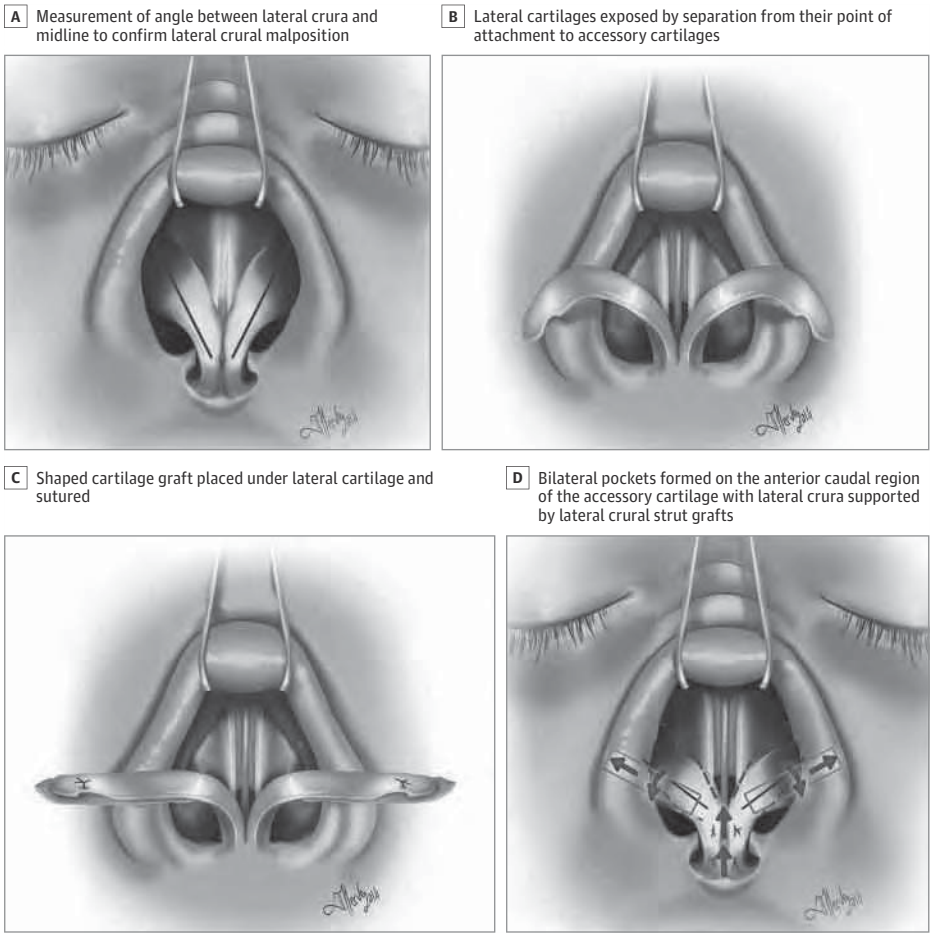
Surgical Technique

An open approach was used for all procedures, and patients underwent radiofrequency ablation for hypertrophic inferior turbinates if necessary. Patients who were assessed as having lateral crural malposition (Figure 1A) by goniometry underwent total release of the lateral crura, repositioning, and LCSG. The cartilage graft was obtained from the septal cartilage through septoplasty, leaving the L-strut, and applied as the LCSG. All the patients underwent medial oblique and high-low-high lateral osteotomies with preservation of the Webster triangle. The middle vault was restructured using bilateral spreader grafts in all patients.

Vestibular mucosa located under the lower lateral cartilage was dissected from the cephalic to the caudal edges, and the mucosal connection at the cephalic end was separated from the cartilage by leaving the skin connection at the anterior caudal region of the lateral cartilage. Lateral cartilages were exposed by separating them from their point of attachment to the accessory cartilages (Figure 1B). Cartilage obtained from the septum was 3 to 4 mm wide and 15 to 25 mm long. The shaped cartilage graft was placed under the lateral cartilage with its 5-mm tip brimming over the cephalic end of the lateral crura, and it was sutured from the 2 ends with 5/0 polyglactin 901 (Vicryl; Ethicon) (Figure 1C). Bilateral pockets were formed on the anterior caudal region of the accessory cartilage by pointing the tip of the scissors toward the lateral canthus, and the lateral crura supported by the LCSGs were placed in these pockets in contact with the anterior nasal aperture (Figure 1D). The increase in the intercrural angle was confirmed by goniometry.

Lateral crural strut grafts were fixed to the vestibular skin by suturing the skin with 5/0 polyglactin 910 sutures after placement of the newly formed lateral crura with the strut grafts in preformed pockets. The cephalocaudal interrotation of the lateral crura was obtained by applying hemitransdomal suturing after repositioning of the lateral crura for each patient during tip-plasty.[4,5] All patients underwent additional tip suturing (patients with thin and normal skin types) or cap grafts (pa-

Figure 1. Surgical Technique



tients with thick skin type) to make the tip definition standard in every patient according to their skin types and so as not affect overall aesthetic satisfaction. Finally, columellar strut grafts were applied in all the patients to provide the desired rotation, projection, and nasal tip support.

Statistical Analyses

Data analysis was performed from March 13 through 23, 2015. We used commercially available software programs (2007 Number Cruncher Statistical System [NCSS] and 2008 Power Analysis and Sample Size) for statistical analyses. In addition to descriptive statistical methods (mean [SD], median, frequency, proportion, minimum, and maximum), we used a normal distribution, 1-way analysis of variance for quantitative data comparisons among 3 or more groups and the Tukey Honestly Significant Difference test[6,7] to determine the group from which differences arose. For comparisons of 3 or more groups with nonnormal distribution, we used Kruskal-Wallis and Mann-Whitney tests, respectively. We used the Wilcoxon signed rank test to evaluate intragroup changes according to skin type. We compared qualitative data using the Pearson χ^2 and Fisher-Freeman-Halton tests.[8] Level of significance was $P < .05$. Unless otherwise indicated, data are expressed as mean (SD).

Results

Based on goniometry of the angle of the lateral crural axis and midline, lateral crural repositioning and LCSG were applied in 71 cases, included 64 women (90%) and 7 men (10%). Patients ranged in age from 17 to 42 years, with a mean (SD) age of 26.5 (5.9) years. Postoperative follow-up ranged from 10 to 15 months, with a mean duration of 12.7 months (**Table 1**).

No statistically significant differences were detected among the skin type groups by age ($P = .48$), sex distribution ($P = .21$), or duration of follow-up ($P = .61$). We found statistically significant differences in NOSE scores among the skin type groups in preoperative evaluations ($P = .10$) or at 6 ($P = .53$) or 12 ($P = .19$) months after the procedure.

For the entire patient group, mean (SD) NOSE scores were 6.96 (5.10) preoperatively, 3.18 (3.12) at 6 postoperative months, and 0.39 (1.07) at 12 postoperative months. The mean decreases in NOSE scores from the preoperative to 6-month postoperative evaluations (−3.77 [4.76]), from the preoperative to 12-month postoperative evaluations (−6.56 [5.04]), and from the 6- to 12-month postoperative evaluations (−2.78 [3.26]) were all statistically significant ($P < .01$ for each comparison) (**Table 2**).

Table 1. Evaluation of Demographic Characteristics by Skin Type

Characteristic	All Patients (N = 71)	Skin Type Group			P Value
		Thin (n = 17)	Normal (n = 29)	Thick (n = 25)	
Age, mean (SD), y	26.5 (5.9)	28.1 (5.4)	26.0 (6.2)	26.1 (6.0)	.48[a]
Duration of follow-up, mean (SD), mo[b]	12.7 (1.7)	12.4 (1.8)	12.6 (1.8)	12.9 (1.6)	.61
Sex, No. (%)					
Female	64 (90)	14 (82)	28 (97)	22 (88)	.21[c]
Male	7 (10)	3 (18)	1 (3)	3 (12)	

[a] Calculated using 1-way analysis of variance.

[b] Postoperative follow-up ranged from 10 to 15 months.

[c] Calculated using the Fisher-Freeman-Halton test.

Table 2. Evaluation of NOSE Scale and ROE Questionnaire Scores by Skin Type[a]

	All Patients (N = 71)	Skin Type Group			P Value[b]
		Thin (n = 17)	Normal (n = 29)	Thick (n = 25)	
NOSE Score					
Preoperative	6.96 (5.10) [7.0]	6.59 (5.09) [8.0]	8.41 (4.59) [9.0]	5.52 (5.42) [4.0]	.10
Postoperative					
6-mo	3.18 (3.12) [2.0]	2.59 (2.18) [2.0]	3.97 (4.16) [3.0]	2.68 (1.93) [3.0]	.53
12-mo	0.39 (1.07) [0]	0.11 (0.33) [0]	0.28 (0.79) [0]	0.72 (1.54) [0]	.19
P value[c]	.001	.008	.001	.04	NA
Change					
Preoperative to 6-mo postoperative	−3.77 (4.76) [−4.0]	−4.00 (4.88) [−4.0]	−4.45 (6.32) [−5.0]	−2.84 (5.73) [−2.0]	.34
Preoperative to 12-mo postoperative	−6.56 (5.04) [−7.0]	−6.47 (5.07) [−8.0]	−8.13 (4.48) [−18.0]	−4.80 (5.22) [−3.0]	.047
6- to 12-mo postoperative	−2.78 (3.26) [−2.0]	−2.47 (2.21) [−8.0]	−3.68 (4.28) [−20.0]	−1.96 (2.16) [−2.0]	.44
ROE Score					
Preoperative	7.03 (3.70) [6.0]	7.76 (3.82) [7.0]	6.97 (3.24) [6.0]	6.60 (4.16) [6.0]	.60
Postoperative					
6-mo	21.06 (3.82) [23.0]	22.65 (2.32) [24.0]	20.38 (4.69) [23.0]	20.76 (3.29) [22.0]	.07
12-mo	23.12 (2.09) [24.0]	23.29 (1.72) [24.0]	23.31 (1.91) [24.0]	22.80 (2.53) [24.0]	.83
P value[c]	.001	.001	.001	.001	NA
Change					
Preoperative to 6-mo postoperative	14.03 (5.12) [15.0]	14.88 (4.34) [16.0]	13.41 (5.82) [15.0]	14.16 (4.84) [14.0]	.75
Preoperative to 12-mo postoperative	16.09 (3.92) [17.0]	15.53 (4.01) [16.0]	16.34 (3.60) [18.0]	16.20 (4.31) [16.0]	.76
6- to 12-mo postoperative	2.07 (3.50) [1.0]	0.65 (2.84) [0]	2.93 (4.39) [1.0]	2.04 (2.33) [2.0]	.04

Abbreviations: NA, not applicable; NOSE, Nasal Obstruction Symptom Evaluation scale; ROE, Rhinoplasty Outcomes Evaluation questionnaire.

[a] Unless otherwise indicated, data are expressed as mean (SD) [median].

[b] Calculated using the Kruskal-Wallis test, difference among skin type groups.

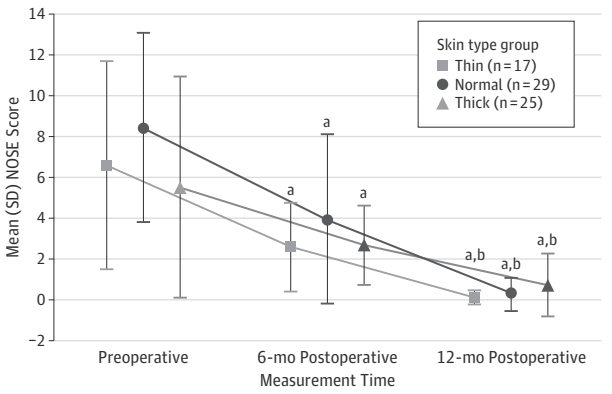
[c] Calculated using the Wilcoxon signed rank test, difference among evaluation periods.

Among patients with a thin skin type, mean (SD) NOSE scores decreased significantly from the preoperative to 6-month postoperative (−4.00 [4.88]; $P = .008$), preoperative to 12-month postoperative (−6.47 [5.07]; $P < .01$), and 6- to 12-month postoperative (−2.47 [2.21]; $P < .01$) evaluations (**Figure 2**). Among patients with a normal skin type, mean NOSE scores decreased significantly from the preoperative to 6-month postoperative (−4.45 [6.32]; $P = .001$), the preoperative to 12-month postoperative (−8.13 [4.48]; $P < .01$), and the 6- to 12-month postoperative (−3.68 [4.28]; $P < .01$) evaluations. Patients with a thick skin type also showed statistically significant decreases in NOSE scores from the preoperative to the 6-month postoperative (−2.84 [5.73]; $P = .04$), preoperative to the 12-month postoperative (−4.80 [5.22]; $P < .01$), and 6- to 12-month postoperative (−1.96 [2.16]; $P < .01$) evaluations. Evaluation of changes in NOSE scores revealed no statistically significant differences between skin types when comparing preoperative and 6-month postoperative scores or 6- and 12-month postoperative scores ($P > .05$) (Table 2).

The patients in this study showed no statistically significant differences according to skin type in ROE scores at the preoperative ($P = .60$) or 6-month postoperative ($P = .07$) evaluations. Postoperative ROE scores among patients with thick skin types were noticeably lower than those of patients with normal skin types, although the differences between skin types in 12-month postoperative ROE scores was not statistically significant ($P = .83$) (Table 2 and **Figure 3**).

Among all patients, mean (SD) ROE scores were 7.03 (3.70) at the preoperative evaluation, 21.06 (3.82) at the 6-month postoperative evaluation, and 23.12 (2.09) at the 12-month postoperative evaluation. The mean increases in scores from the preoperative to 6-month postoperative (14.03 [5.12]), preop-

Figure 2. Nasal Obstruction Symptom Evaluation (NOSE) Scale Score According to Skin Type

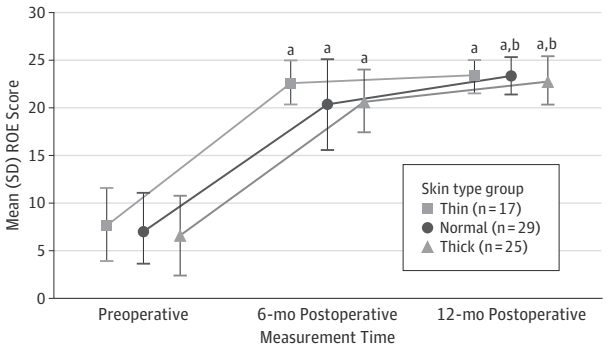


Possible scores range from 0 to 20. Decreased NOSE scores indicate improved functional results.

[a] $P \leq .01$ vs preoperative score.

[b] $P \leq .01$ vs 6-month score.

Figure 3. Rhinoplasty Outcomes Evaluation (ROE) Questionnaire Criteria According to Skin Type



Possible scores range from 0 to 24. Increased ROE scores indicate improved aesthetic results.

[a] $P \leq .01$ vs preoperative score.

[b] $P \leq .01$ vs 6-month score.

erative to 12-month postoperative (16.09 [3.92]), and 6- to 12-month postoperative (2.07 [3.50]) evaluations were all statistically significant ($P < .01$).

In patients with a thin skin type, mean ROE scores increased significantly from the preoperative to 6-month postoperative evaluations (14.88 [4.34]; $P = .001$) (Figure 3). The increase from the preoperative to 12-month postoperative evaluations (15.53 [4.01]; $P < .01$) was also significant, but the change from the 6- to 12-month postoperative evaluations was not (0.65 [2.84]; $P = .36$).

Patients with normal skin thickness showed significant increases in ROE scores from the preoperative to 6-month postoperative evaluations (13.41 [5.82]; $P = .001$) (Figure 3). The increases in ROE scores from the preoperative to 12-month postoperative evaluations (16.34 [3.60]; $P < .01$) and from the 6- to 12-month postoperative evaluations (2.93 [4.39]; $P < .01$) were also significant. In patients with a thick skin type, a significant increase in ROE scores was observed from the preoperative to 6-month postoperative evaluations (14.16 [4.84]; $P = .001$). Increases in ROE scores from the preoperative to 12-month postoperative evaluations (16.20 [4.31]; $P < .01$) and from the 6- to 12-month postoperative evaluations (2.04 [2.33]; $P = .001$) were also statistically significant (Table 2).

Analysis of ROE score differences revealed no statistically significant differences between skin types when comparing the preoperative and 6-month postoperative and the preoperative and 12-month postoperative evaluations ($P > .05$). We found a significant difference between skin types when comparing ROE scores at the postoperative 6- and 12-month evaluations ($P = .04$); patients with thin skin showed a significantly smaller difference in postoperative scores from 6 to 12 months than those with normal or thick skin types ($P = .04$ and $P = .02$, respectively). In patients with normal and thick skin types, no significant differences were detected between ROE scores at the preoperative and 12-month postoperative evaluations ($P = .76$) (Table 2).

Discussion

The terms *cephalic positioning* of the lateral crura and *malposition* were first introduced approximately 30 years ago.[1] Cephalic placement of the lateral crura is described as malposition. The term *malposition* was first introduced by Sheen[1] in 1978. According to this description, the angle of the cephalic-positioned lateral crura and midline is 30° or less.[1] The direction in which the lower lateral cartilage attaches to the accessory cartilages and its direction toward the ipsilateral medial canthus show that the cartilage is malpositioned, which is termed *cephalic malposition*.[9] The direction of the lateral crura toward the ipsilateral lateral canthus and the lateral crural-midline angle being 45° or greater are described as *orthotopic positioning*.[1,9] Sheen[1] and Sheen and Sheen[9] stated that malposition affects nasal tip shape and the constitution of alar rim support. According to the literature, malposition is one of the most common shape deformities of the nasal tip.[10] Malpositioned lateral crura are not parallel to the alar rim, resulting in abnormalities such as a boxy nasal tip, bulbous nasal tip, alar rim retraction, and alar rim collapse.[11] The fact that lateral crura with cephalic malposition causes parenthesis deformity was first introduced in 1992 by Sheen.[12] Many new techniques have been applied to fix noses with parenthesis deformity and cephalic malposition.[13] Our rationale for using intraoperative goniometry in this study was that the measurement provided a more precise patient selection through the correct determination of the angle and enabled us to observe the consistency of preoperative examination findings with intraoperative values. The LCSG was first described by Gunter and Friedman,[11] who claimed that this technique was a multidimensional and rational solution for pathologic situations of the lateral crura such as boxy tip, malposition, alar rim retraction, alar rim collapse, and concave lateral crura. We realized that the distal ends of the goniometer had to be measured by taking the attachment point of the lateral crura to accessory cartilages as a base;

otherwise, inaccurate results would have been obtained. Furthermore, we noticed that the patients with wide lateral crura were considered to have a parenthesis nose in the preoperative evaluations, although their intercrural angle was greater than 45°. Of the 80 patients included in the study, 9 were observed not to have malpositioning by means of intraoperative goniometry and were excluded from the study. Malpositioning of the lateral crura is commonly seen among patients of all skin types undergoing primary rhinoplasty (eFigures 2-5 in the Supplement). If a suitable technique is not used for fixation, patient satisfaction is negatively affected and the rates of revision rhinoplasty increase.[10] Constantian[10] detected alar cartilage malposition in 68% of his patients undergoing primary rhinoplasty and 87% of his patients undergoing secondary rhinoplasty among 200 patients. He concluded that malposition caused boxy and bulbous tip deformities and functional deficits according to the results of the rhinomanometry measurements obtained from the groups undergoing primary and secondary rhinoplasty.[10] According to Sepehr et al,[5] cephalic malpositioning affected tip shape by altering the projection, rotation, and lateral crura length in patients with parenthesis tip deformity and requires the use of different tip-plasty techniques for correction. We deduced that we can achieve the needed rotation and deprojection more easily with the "sliding in" effect of the whole tip complex by lateral crural repositioning and LCSG with the combination of selected tip-plasty maneuvers. The repositioning of the whole lateral crural complex provides a more attractive nasal tip by the change of the tip complex cephalically in the third dimension and a supportive effect to the alar rim region of repositioning the lateral crura laterally. The study by Bared et al[14] found that repositioning of the lower lateral cartilages results in volume loss in the supratip and nasal sidewall junction, and they proved this by 3-dimensional imaging. Lateral crural repositioning with the use of LCSG is a very effective tip-plasty technique in the correction of parenthesis deformity and is a very effective technique for creating an ideal tip complex in patients with different tip abnormalities, such as a drooping, overprojection, underprojection, and very thin or asymmetrical lateral crura, that ineffectively support the alar rims and nasal valve area. By repositioning the lateral crura to the ideal orthotopic position, alar rim support can be achieved, thereby optimizing the appearance of the nostril shape and the tip and positively influencing the ROE score during postoperative follow-up.

The simplest description of lateral crural malposition and its role in nasal valve insufficiency belongs to Sheen and Sheen.[9(pp953-956)] They described collapse of the lateral nasal wall on application of slight pressure as nasal valve insufficiency, which is frequently seen in patients with malpositioning. When we administered the NOSE scale preoperatively to the patients who were included in the study and who were considered to have malposition of the lateral crura, we observed that the scores were elevated, which supported the theory that malpositioning makes a great contribution to nasal obstruction. Constantian[10] confirmed this in his study through rhinomanometry. Alar rim grafts and the application of alar battens are the most frequent procedures implemented in the patients with nasal valve insufficiency in rhinoplasty. Alar rim grafts are used to support the external nasal valve and to correct the asymmetries of the nostrils and slight alar retractions.[15] Alar batten grafts have been found to be effective in long-term follow-up of internal and external nasal valve collapse in previous studies.[16] Toriumi[17] stated that the application of alar rim graft was not required in patients who underwent lateral crural repositioning with LCSG and stated that repositioning with LCSG supports the alar rims.

Toriumi and Asher[18] hypothesized that repositioning with LCSGs may also have a functional benefit compared with other grafts in the valve area, such as alar batten grafts. The statistically significant decrease of the NOSE scale score in our study shows functional improvement with repositioning and LCSG and supports their findings.[18] We also found that repositioning and an LCSG in patients with cephalic malposition support the nasal valve and positively affect the postoperative aesthetic results, as has been reported in previous studies.[5,14,18] Statistically significant decreases in NOSE scale scores and increases in ROE scores postoperatively demonstrated improvement in function and aesthetic satisfaction.

Skin thickness and elasticity are the most important factors that affect the overall satisfaction of patients undergoing rhinoplasty. We also wanted to investigate differences in functional and aesthetic outcomes of repositioning with LCSG in different skin types. We could not find any significant difference in functional or aesthetic outcomes according to the thickness of the skin. Functional and aesthetic outcomes showed significant improvement in all skin types.

Conclusions

Repositioning of cephalically malpositioned lateral crura with an LCSG is functionally and aesthetically effective. The technique can be used with all skin types. Furthermore, this technique can be used to increase overall patient satisfaction in patients with parenthesis deformity, nasal valve insufficiency, nostril asymmetries, or overprojected or underprojected tip and in patients with abnormalities that can be corrected by creating a more stable and symmetrical framework.

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Thermally Confined Micropulsed 1444-nm Nd:YAG Interstitial Fiber Laser in the Aging Face and Neck: An Update

J. David Holcomb, MD

KEYWORDS

• Laser • Lipolysis • Facial • Neck • Contouring • Facelift • Thermal confinement
• Thermal diffusivity

KEY POINTS

- The micropulsed 1444-nm Nd:YAG interstitial fiber laser enables precision contouring of the mid- and lower face and the neck, both as stand-alone procedures (laser-assisted facial contouring [LAFC] and laser-assisted neck contouring [LANC]) and as an adjunct during aging face surgery (laser-assisted facelift [LAFL]).
- Use of the 1444-nm Nd:YAG interstitial fiber laser requires knowledge regarding how to maintain safe clinical thermal confinement during treatment.
- Integrating this technology with facelift surgery facilitates elevation of (extended, if desired) cervicofacial rhytidectomy flaps, enables percutaneous release of major fascial retaining ligaments in the mid- and lower face, may obviate open submentoplasty and platysmaplasty in some patients, and facilitates greater posterior and superior repositioning of flaps for improved outcomes.

INTRODUCTION

Although the use of Nd:YAG fiber lasers in aesthetic surgery has been traditionally referred to as *laser lipolysis*, it is now evident that subcutaneous fat may not or need not be the primary laser target. As such, the use of Nd:YAG fiber lasers has evolved to include ablation and emulsification of subcutaneous fatty tissue, fibrolysis, and shrinkage of fine skin ligaments (ligamentae retinacula cutis) and more dense structural osseocutaneous anchoring ligaments (eg, zygomatic- and mandibular-cutaneous ligaments) as well as postulated direct tissue effects that may contribute to tightening of the skin and of the platysma muscle. Because the use of Nd:YAG fiber lasers goes beyond direct treatment of subcutaneous fat, some laser surgeons now advocate the term, *interstitial laser*, in lieu of laser lipolysis when referencing the use of these devices.

Subcutaneous Nd:YAG fiber laser tissue interaction is influenced by a variety of factors, including laser wavelength, power, pulse duration and total energy applied, target tissue composition, and relative amounts of exogenous water added to the treatment area. Collectively these factors influence opposing characteristics of fiber laser tissue interaction, termed *thermal confinement* and *thermal diffusivity* (discussed later), whereas related clinical implications affect subcutaneous Nd:YAG fiber laser treatment protocols and safety and immediately observed and late tissue effects.

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Evaluation of absorption spectra for Nd:YAG fiber lasers reveals absorption in fat and water is greatest in the mid–1400-nm range, intermediate at 1320 nm, and least at 1064 nm.[1] The relative absorption is on the order of 1 magnitude higher for fat but many orders of magnitude higher for water in the mid–1400-nm range versus 1320 nm and 1064 nm.[1] A minor anhydrous collagen absorption peak present in the mid–1400-nm range may also influence laser energy absorption and laser tissue interaction.[2] Comparison of direct tissue effects reveals that fatty tissue ablation crater depth and fatty tissue ablation efficiency are greatest at 1444 nm, intermediate at 1320 nm, and least at 1064 nm.[2] Differences in tissue absorption and laser tissue interaction among Nd:YAG fiber lasers are summarized in **Table 1**.

Thermal confinement and thermal diffusivity are opposing characteristics of fiber laser tissue interaction that are of critical importance for exerting desired laser tissue effects while avoiding undesired complications. Thermal confinement refers to spatial limitation of tissue heating relatively near the tip of the laser fiber or more broadly within the desired tissue treatment area whereas thermal diffusivity refers to heat distribution away from the source or tip of the laser fiber via conduction.[2] Although the 2 phenomena are simultaneously present, the relative proportions are influenced by laser wavelength, power, and pulse duration as well as target tissue composition, tissue water content, and total laser energy applied to the treatment area—their differential effects on thermal confinement and diffusivity are summarized in **Table 2**.

Thermal imaging studies among the Nd:YAG fiber laser wavelengths demonstrate that thermal confinement is greatest at mid–1400 nm, intermediate at 1064 nm, and least at 1320 nm.[2] Clinically, improved thermal confinement translates to a longer lag period or larger therapeutic window that precedes significant heat accumulation in the larger laser treatment area. The ability of the tissue and exogenous water in the treatment area to maintain thermal confinement is exceeded at the far side of the therapeutic window where thermal diffusivity then prevails with more rapid tissue heating from that point forward. Various tissues have specific tolerances to prolonged heating—irreversible coagulation of the skin may occur with heating to 59°C for as little as 1 second.[3] Excessive thermal diffusion leading to irreversible tissue injury indicates a clinical failure of thermal confinement.

Native target tissue composition affects Nd:YAG fiber laser tissue interaction. Although relative adipocyte may not able to be estimated versus fibrous tissue content prior to laser treatment, this can be inferred based on the tissue response. If the tissues soften during treatment, significant fat emulsification and liquefaction have generally occurred. Significant firming and tightening of the tissues suggest a greater fibrous tissue content with contraction of collagen containing structures; significant fat emulsification and liquefaction may still have occurred despite the firmness but greater mechanical effort may be required for its removal during lipoaspiration.

INTERSTITIAL ND:YAG FIBER LASER–ASSISTED FACIAL CONTOURING

Interstitial Nd:YAG fiber LAFC may be used as a stand-alone percutaneous sculpting procedure for the midface, lower face/jawline, and the female round Asian face.[1] LAFC of the mid- and/or lower face as a stand-alone treatment is generally more successful in female patients. Volumetric sculpting of the mid- and/or lower face (ie, soft tissue reduction) with LAFC complements well-established procedures for soft tissue augmentation and enables synergy through a proportionally greater effect with soft tissue augmentation. Appropriate patient selection should include those with mild to moderate fullness and readily palpable subcutaneous fat but without excessive skin laxity. Patients with skin laxity but no significant subcutaneous fat are not appropriate candidates for the LAFC procedure. Patient age is not a major determining factor with regard to successful outcomes—very good LAFC results have been obtained with patients into their early 70s.

Table 1
Differences in tissue absorption and laser tissue interaction among Nd:YAG fiber lasers

	Mid–1400 nm	1320 nm	1064 nm
Water absorption	Highest[a]	Intermediate	Lowest
Fat absorption	Highest[a]	Intermediate	Lower
Collagen (anhydrous) absorption	Low[a]	—	—
Fatty tissue ablation efficiency	Highest	Intermediate	Least

[a] Absorption peaks for water, fat, and collagen occur in the mid–1400-nm range.

Table 2
Management of thermal confinement and thermal diffusivity

Greater Thermal Confinement	Independent Parameter	Greater Thermal Diffusivity
Mid 1400 nm	**Laser Wavelength**	1320 nm > 1064 nm
Decrease	**Power**	Increase
Shorter	**Pulse Duration**	Longer
Fat > Fibrous	**Target Tissue Composition**	Fibrous > Fat
Increase	**Exogenous Water**	Decrease
Dynamic (moving continuously)	**Laser Fiber Position**	Fixed

Mild to moderate post-treatment inflammatory edema (PIE) is expected. Early on, PIE seems to have blunted or limited the lower facial tissue contouring response; however, lower facial contour improves over time as PIE gradually resolves and the skin contracts. Early on (eg, weeks 2 through 6), weekly lymphatic massage sessions for the LAFC treatment areas may help reduce PIE and improve lower facial contour. Significant PIE may be treated with staged escalating-dose intralesional triamcinolone (eg, 10 mg/mL initially, gradually moving to 40 mg/mL) beginning at post-treatment month 1 or 2 and continuing monthly as needed until final desired contour is achieved or until no further tissue response. Although this approach is successful in a majority of patients with PIE, the origin of persistent lower facial fullness with palpable subcutaneous fullness in partial responders is not known.

Some of the adipocyte lipid content liberated during laser lipolysis may be subject to reuptake by adipocytes that remain at the periphery of the treatment area. A significant increase in body mass index after LAFC could also partially account for a blunted tissue response. It seems more likely, however, that the natural healing response to adipose tissue ischemia and adipocyte necrosis may stimulate a tissue regeneration response, with adipose tissue remodeling involving adjacent adipose-derived stem progenitor cells and formation of neoadipocytes—this phenomenon has been carefully elucidated in animal models for adipose tissue ischemia[4] and nonvascularized fat grafting.[5] Persistent fullness 6 to 12 months after LAFC may be addressed through a touch-up percutaneous LAFC procedure.

LAFC treatment begins with identification and marking of the desired treatment areas. In keeping with anatomic studies of the jowl fat compartment,[6] the desired area of tissue ablation for contouring of the lower face and jawline may include subcutaneous tissue fullness at, below, and well above the caudal border of the mandible (**Fig. 1**A). In many patients, the position of the jowl changes substantially with supine or slight Trendelenburg positioning; therefore, patient marking for LAFC should be done with patients in an upright, seated position to most accurately ensure inclusion of the desired tissue in the outlined treatment areas. The LAFC percutaneous entry point should be at least 1.5-cm posterior to the posterior extent of the intended LAFC treatment zone to ensure that an adequate tissue seal is maintained between the entry point and the treatment zone. If the entry point is placed too close to the LAFC treatment zone, the lipoaspiration step may be more difficult and inefficient because air may easily be drawn into the aspiration syringe.

Ensuring that the desired tissue is treated during LAFC is accomplished via (1) minimizing any positional tissue shift with slight reverse Trendelenburg patient positioning (eg, 20°); (2) limiting exogenous water input with small amounts of local anesthetic used (eg, 3 mL); (3) using hyaluronidase to improve local anesthetic distribution through the tissues; and (4) isolating and stabilizing the target tissue between the user's thumb

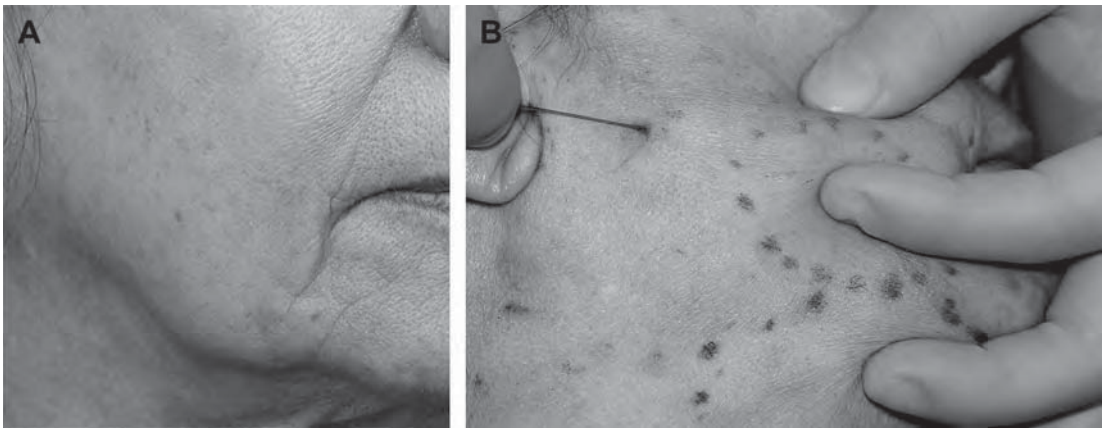

Fig. 1. (*A*) Preoperative view of LAFC treatment area with lower facial fullness and jowling in a 58-year-old woman in upright seated position. (*B*) Intraoperative view of LAFC treatment area in the same patient in 20° reverse Trendelenburg position. LAFC treatment zone is outlined with purple marker. LAFC entry site is located approximately 2 cm posterior to inked posterior extent of LAFC treatment zone and 2 cm above the caudal mandibular margin. The 600-μm bare laser fiber is present at the LAFC entry point and the red aiming beam at the fiber tip is seen faintly between the surgeon's gloved thumb and index finger. Note that the laser fiber is engaged in fatty tissue at the superior extent of the jowl fat compartment.

and forefinger during local anesthesia infiltration, laser energy delivery, and lipoaspiration. Limiting exogenous water infiltration to 3 mL minimizes distortion of the anatomy during treatment and facilitates endpoint identification but also limits thermal confinement. The fatty tissue ablation efficiency of the micropulsed 1444-nm Nd:YAG interstitial fiber laser, however, enables sufficient local tissue effect with preserved thermal confinement within the suggested total energy usage parameters.

The local anesthetic mixture that the author favors includes 0.5% lidocaine; 0.25% Bupivacaine hydrochloride; and 1:200,000 epinephrine and hyaluronidase, 2 to 4 IU per mL (eg, Hylenex recombinant, Halozyme Therapeutics, San Diego, CA, USA). Initially, approximately 1.0 mL of this local anesthetic mixture is used to provide anesthesia to the percutaneous entry site and the intervening tissue toward the LAFC treatment zone as well as a field block that includes the tissue for debulking and contouring. A narrow (eg, 21-gauge) multihole infiltration cannula is then used to deliver 3 mL of local anesthetic to the LAFC treatment area.

With the thermally confined micropulsed 1444-nm Nd:YAG interstitial fiber laser, energy delivery occurs via a 600-μm silica multimode fiber with the fiber used either free (bare) or assembled with a disposable or nondisposable cannula. Prior studies have demonstrated general safety guidelines for energy delivery during LAFC of the lower face when using the micropulsed 1444-nm Nd:YAG interstitial fiber laser and minimal volume local anesthesia (dry technique)—typical parameters include power 5.4 W, pulse energy 180 mJ, pulse duration 100 μs (fixed), pulse rate 30 Hz, and total energy delivered 200 to 300 J.[1,7]

Although the unique thermal signature of the micropulsed 1444-nm Nd:YAG interstitial fiber laser enables safe treatment without the need for internal or external temperature monitoring, it is important to keep the fiber continuously moving through the tissue during active lasing—this facilitates even distribution of laser energy and limits the potential for clinical thermal confinement failure. Certainly some latitude exists with regard to these treatment parameters; however, in a prior study of mid- and lower face LAFC, complications, such as prolonged inflammatory edema and overcorrection, were associated with faster energy delivery (40 Hz) and with a doubling of the total energy delivered (eg, 500 J).[1] Immediately after energy delivery, a similar volume (eg, 3 mL) of room temperature sterile saline is infiltrated into the treatment area as a postcooling or thermal quenching step that attempts to minimize collateral thermal spread to adjacent tissues as well as reduce PIE.

Removal of emulsified tissue and liquefied fat via manual lipoaspiration with a small dual port aspiration cannula (eg, 19 gauge) and a 6-mL syringe (prefilled with 1-mL sterile saline) enables definitive tissue contouring. **Fig. 2** shows the full minimal instrumentation requirement for LAFC. It is not uncommon for a small-diameter aspiration cannula to become blocked with fibrous tissue during lipoaspiration—when this occurs, the cannula is

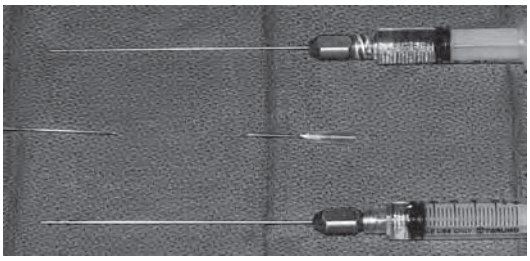

Fig. 2. LAFC instrumentation. (*Top*) 21-Gauge multihole infiltration cannula attached to 6-mL syringe containing 3-mL local anesthetic solution. (*Middle*) 600-μm Bare laser fiber with red diode aiming beam visible (*left*) and 18-gauge needle (*right*). (*Bottom*) 19-Gauge dual port aspiration cannula attached to 6-mL syringe prefilled with 1.0-mL sterile saline.

detached and irrigated until clear and then reattached to the same syringe and the procedure continued. If the syringe becomes filled with air, the seal at the syringe hub may need to be tightened or the percutaneous entry point may be too close to the treatment area. If the latter is the case, the procedure can usually continue with gentle manual occlusive pressure placed over the entry point area. Any air in the syringe can be gently expelled but with care to not also expel any fat already aspirated at this point. At the end of the lipoaspiration, the fat aspirate volume (less 1.0 mL from sterile saline prefilling) from each side is recorded in the treatment record. Mean volumes removed during unilateral lower face LAFC treatment approximate 2.5 mL in 2 studies, with ranges extending from 0.5 mL to more than 5.0 mL.[1,7] **Table 3** outlines major LAFC treatment steps and typical treatment parameters.

Initially, the aspiration cannula should be more superficial (immediately subcutaneous), with ports directed down. Effective debulking in areas of maximal subcutaneous tissue thickness, however, generally requires guiding the cannula into these areas at a deeper level. Although contrary to what has been an accepted tenet for traditional cold liposuction techniques, it is permissible and often helpful to remove some of the immediately subcutaneous fatty tissue adherent to the undersurface of the skin by using the lipoaspiration cannula with the ports directed upward toward the undersurface of the dermis. It is the author's belief that failure to do so may limit the facial contour, related skin contraction, and the ultimate result obtained.

Immediately after treatment, a compression dressing is applied that consists of 1 or 2 layers of 1-inch–thick roll cotton and a compression garment (eg, Universal Facial Band, Design Veronique, Richmond, CA, USA). The wound is evaluated the next day and the cotton is removed but patients are encouraged to wear the compression garment as much as possible for at least 1 week after treatment. Patient expectations must be carefully managed during the recovery and extended post-treatment period (as described previously). **Fig. 3** depicts before and long-term (>2 years) clinical photography following LAFC of the mid- and lower face as well as laser-assisted neck contouring.

INTERSTITIAL ND:YAG FIBER LASER–ASSISTED NECK CONTOURING

Interstitial Nd:YAG fiber LANC may be performed as a stand-alone percutaneous neck contouring procedure. Appropriate patient selection should include those with mild to moderate fullness in the submentum and neck with accumulated subcutaneous fatty tissue in these areas but without excessive skin laxity. Patients with skin laxity but

Table 3
Major LAFC treatment steps and typical treatment parameters

LAFC Treatment Step	Detailed Information
Field block[a]	Include percutaneous access point and target tissue
Infiltrate target tissue[a]	3 mL each LAFC treatment area (21-gauge multihole infiltration cannula)
Apply laser energy	Up to 200 J midface; up to 400 J for jawline Typical laser treatment parameters 5.4 W, 180 mJ, 30 Hz
Postcooling (thermal quenching)	Infiltrate 3-mL room temperature sterile saline (21-gauge multihole infiltration cannula)
Aspiration	Mean 2.5 mL (19-gauge dual port aspiration cannula attached to 6-mL syringe prefilled with 1.0-mL saline)
Compression	Roll cotton and elastic compression garment

[a] Local anesthetic mixture contains 0.5% lidocaine, 0.25% Bupivacaine hydrochloride, 1:200,000 epinephrine, and 2 IU hyaluronidase per mL.

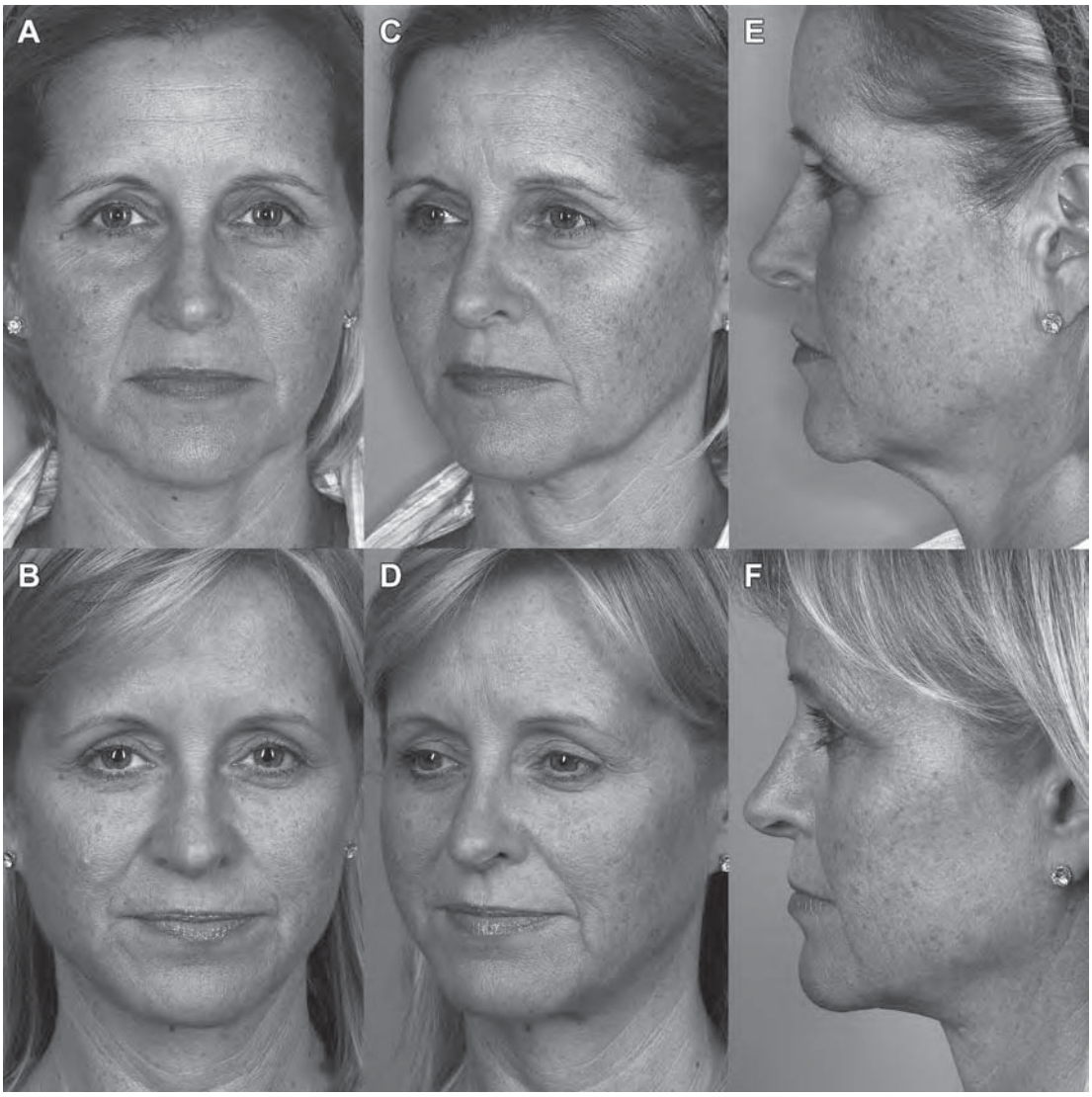

Fig. 3. Before (*A*, *C*, *E*) and 25 months after (*B*, *D*, *F*) photos in a 49-year-old woman after LAFC of the mid- and lower face and LANC (percutaneous). Treatment parameters for the mid- and lower face included 3.0 mL local/tumescent anesthetic, laser power 5.4 W, treatment speed 30 Hz, pulse energy 180 mJ, total energy delivered 400 J and 1.0- to 1.5-mL lipoaspirate. Treatment parameters for the submentum included 12.0-mL local/tumescent anesthetic, laser power 8.0 W, treatment speed 40 Hz, pulse energy 200 mJ, total energy delivered 1000 J and 5.0-mL lipoaspirate.

no significant subcutaneous fat are not appropriate candidates for the LANC procedure. Patient age is not a major determining factor with regard to successful outcomes—very good LANC results have been obtained with patients into their late 60s.

Mild to moderate PIE is expected. Early on PIE seems to have blunted or limited the submental/neck tissue contouring response; however, the tissue contour and cervicomental angle improve over time as PIE gradually resolves and the skin contracts. Early on (eg, weeks 2 through 6), weekly lymphatic massage sessions for the LANC treatment area may help reduce PIE and improve submentum/neck contour. Significant PIE may be treated with staged escalating-dose intralesional triamcinolone (eg, 10 mg/mL initially, gradually moving to 40 mg/mL) beginning at post-treatment month 1 or 2 and continuing monthly as needed until final desired contour is achieved or until no further tissue response is seen. Although this approach is successful in a majority

of patients with PIE, the cause of persistent fullness with palpable subcutaneous fullness in partial responders is not known but similar mechanisms may be inferred in both the lower face and neck. Persistent fullness 6 to 12 months after LANC treatment may be addressed through a touch-up percutaneous LANC procedure.

LANC treatment begins with identification and marking of the treatment area. Although patient positioning does not affect the submentum and neck soft tissues as dramatically as in the lower face, marking for LANC is conventionally done with patients in an upright, seated position to most accurately ensure inclusion of the desired tissue in the outlined treatment area. Depending on body habitus, the treatment zone may extend laterally well into the central and toward the posterior aspect of level I in the neck as well as inferiorly to or well beyond the thyroid prominence in the anterior neck. In most patients, the LANC percutaneous entry point is conveniently placed at or just above the submental skin crease.

Ensuring that the desired tissue is treated during LANC is accomplished via the following steps: (1) limiting exogenous water input with small amounts of local anesthetic used (eg, 12–24 mL); (2) using hyaluronidase to improve local anesthetic distribution through the tissues; and (3) isolating and stabilizing the target tissue between the user's thumb and forefinger during local anesthesia infiltration, laser energy delivery, and lipoaspiration. Limiting exogenous water infiltration to approximately 12 mL minimizes distortion of the anatomy during treatment, facilitates endpoint identification, and limits thermal confinement. The fatty tissue ablation efficiency of the micropulsed 1444-nm Nd:YAG interstitial fiber laser, however, enables an adequate local tissue effect with preserved thermal confinement within the total energy usage parameters suggested. The local anesthetic mixture that the author favors for the submentum and neck includes 0.25% lidocaine, 0.125% Bupivacaine hydrochloride, 1:400,000 epinephrine, and hyaluronidase 1 to 2 IU per mL (eg, Hylenex recombinant). Initially, approximately 2.0 mL of this local anesthetic mixture is used to provide anesthesia to the percutaneous entry site as well as a field block that includes the tissue for debulking and contouring. A 1.6-mm multihole infiltration cannula (Tulip Medical Products, San Diego, CA, USA) is then used to deliver the local anesthetic to the LANC treatment area.

As in the lower face, the thermally confined micropulsed 1444-nm Nd:YAG interstitial fiber laser energy delivery occurs via a 600-μm silica multimode fiber with the fiber used either free (bare) or assembled with a disposable or nondisposable cannula. Prior studies have demonstrated general safety guidelines for energy delivery during LANC when using the micropulsed 1444-nm Nd:YAG interstitial fiber laser and minimal volume local anesthesia (dry technique)—typical parameters include power 8.0 to 10.0 W, pulse energy 200 to 250 mJ, pulse duration 100 μs (fixed), pulse rate 40 Hz, and total energy delivered 750 to 2000 J.[7] The mean total energy delivery for LANC in a cohort of approximately 180 neck contouring patients was just over 950 J whereas mean local anesthesia infiltration and lipoaspiration volumes were approximately 12.5 mL each in this same group.[7] **Table 4** outlines major LANC treatment steps and typical treatment parameters.

Because the neck skin is thinner (than in the lower face) and the energy delivery parameters are higher (than in LAFC), it is even more important to keep the fiber continuously moving through the

Table 4
Major LANC treatment steps and typical treatment parameters

LANC Treatment Step	Detailed Information
Field block[a]	Include percutaneous access point and target tissue
Infiltrate target tissue[a]	12–24 (mean 12.5) mL each LAFC treatment area using Tulip 1.6-mm multihole infiltration cannula
Apply laser energy	1000 (+) J Typical laser parameters 8.0–10.0 W, 200–250 mJ, 40 Hz
Postcooling (thermal quenching)	Infiltrate 12-mL room temperature sterile saline (Tulip 1.6-mm multihole infiltration cannula)
Aspiration	Mean 12.5 mL (Tulip 2.1-mm offset triple port aspiration cannula attached to 12 mL syringe prefilled with 1.0-mL saline)
Compression	Roll cotton and elastic compression garment

[a] Local anesthetic mixture contains 0.25% lidocaine, 0.125% Bupivacaine hydrochloride, 1:400,000 epinephrine, and 1 IU hyaluronidase per mL.

tissue while actively lasing during LANC—this facilitates even distribution of laser energy and limits the potential for clinical thermal confinement failure. Some latitude exists with regard to energy delivery and treatment parameters but the author suggests that surgeons proceed with caution with energy delivery totals exceeding 1000 J during LANC with these settings. At higher total energy delivery settings, the neck skin may become slightly to noticeably warm. Immediately after energy delivery, a similar volume (eg, 12 mL) of room temperature sterile saline is infiltrated into the treatment area.

Removal of emulsified tissue and liquefied fat via manual lipoaspiration with a 2.1-mm offset triple port aspiration cannula (Tulip) and a 12-mL syringe (prefilled with 1-mL sterile saline) enables definitive tissue contouring. **Fig. 4** depicts the full minimal instrumentation requirement for LANC. Use of the Tulip Snap Lok facilitates efficient lipoaspiration while allowing a surgeon to focus on tissue contouring. As with performing LAFC, the aspiration cannula may well become blocked during lipoaspiration, so the blockage must be cleared, as discussed previously, and the procedure continued. If the syringe becomes filled with air, then the same remedies can be applied as described previously, taking care not to expel any fat already aspirated at this point. At the end of the lipoaspiration, the fat aspirate volume (less 1.0 mL from sterile saline prefilling) is recorded in the treatment record.

Initially, the aspiration cannula should be more superficial (immediately subcutaneous) with the ports directed down. Effective debulking in areas of maximal subcutaneous tissue thickness, however, generally requires guiding the cannula into these areas at a deeper level. Using a gentle technique, it is helpful to remove some of the immediately subcutaneous fatty tissue adherent to the undersurface of the skin by using the lipoaspiration cannula with the ports directed upward toward the undersurface of the dermis. Generally a yellow or orange emulsion of subcutaneous fatty tissue is obtained. Depending on the volume of lipoaspirate, a second syringe may be needed to complete the procedure. The lipoaspiration portion of the procedure concludes when the desired tissue contour is achieved or when the emulsified fat aspirate return wanes or becomes blood tinged.

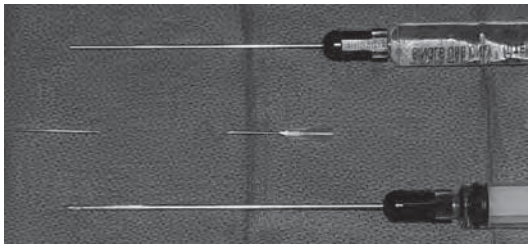

Fig. 4. LANC instrumentation. (*Top*) 1.6-mm Tulip multihole infiltration cannula attached to 12-mL syringe containing local anesthetic solution. (*Middle*) 60-μm Bare laser fiber with red diode aiming beam visible (*left*) and 18-gauge needle (*right*). (*Bottom*) 2.1-mm Tulip triple port (ports offset or nonaxial with only 1 port showing) aspiration cannula attached to 12-mL syringe prefilled with 1.0-mL sterile saline (Tulip Snap Lok not shown).

Persistent dermal to platysma fibrous attachments may represent a potential limiting factor with regard to the ability of the neck skin to adequately contract. After the lipoaspirate is obtained, the cannula is used in a sweeping motion to manually avulse any remaining fibrous attachments that may limit appropriate repositioning of the skin. Occasionally it may be necessary to transition the percutaneous LANC procedure to an open LANC procedure. If significant submental fullness is still evident, then a submental incision may be needed for direct evaluation for platysma muscle laxity and ptosis, bulky fat adherent to undersurface of the skin flap, and excess subplatysmal fatty tissue—if present, these findings are addressed surgically.

Immediately after treatment, a compression dressing is applied in a similar manner as for post-LAFC with a layer or 2 of thick cotton and a compression garment. The wound is evaluated the next day and the cotton is removed, but patients are encouraged to wear the compression garment as much as possible for at least 1 week after treatment. Patient expectations must be carefully managed during the recovery and extended post-treatment period, as described previously. **Fig. 5** shows interim results (3 months) in a patient with substantial submental fullness and skin laxity.

INTERSTITIAL ND:YAG FIBER LASER–ASSISTED FACE AND NECK LIFT—TREATMENT METHOD

Adjunctive use of the thermally confined micropulsed 1444-nm Nd:YAG interstitial fiber laser during face and neck lift surgery involves incorporation of the LAFC and LANC procedures where indicated with additional use of the laser for development of skin flaps and for lysis of osseocutaneous anchoring ligaments in the mid- and lower face. It should be appreciated that the jowl may encompass a significant volume of tissue above and below (with aging) the caudal margin of the mandible and that the jowl position may change significantly with patient positioning for facial

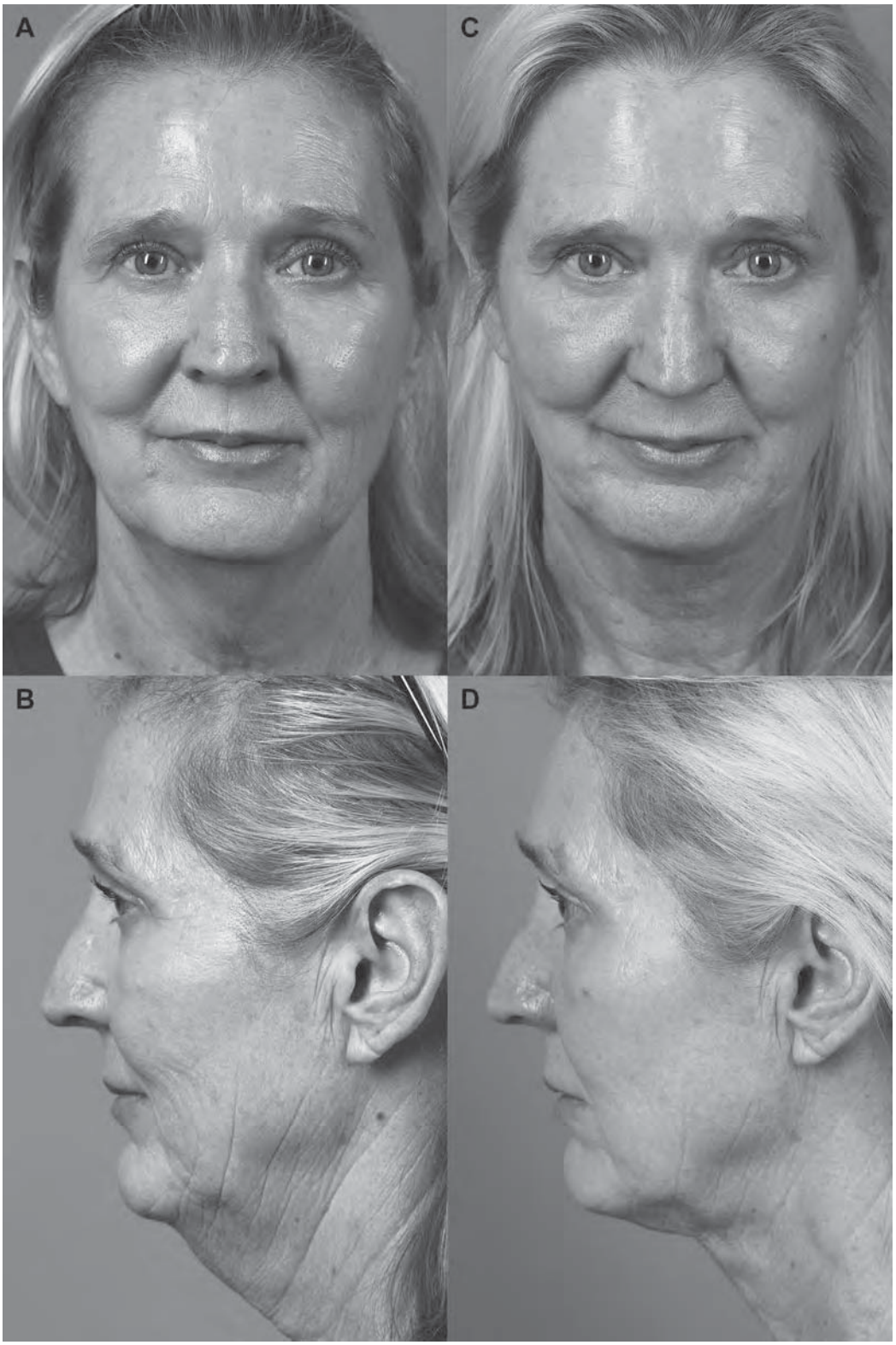

Fig. 5. Before (*A*, *C*) and after (*B*, *D*) photos in a 58-year-old woman 3 months after LANC (percutaneous). Treatment parameters included 18.0-mL local/tumescent anesthetic, laser power 10.0 W, treatment speed 40 Hz, pulse energy 250 mJ, total energy delivered 2000 J, and 20.0-mL lipoaspirate.

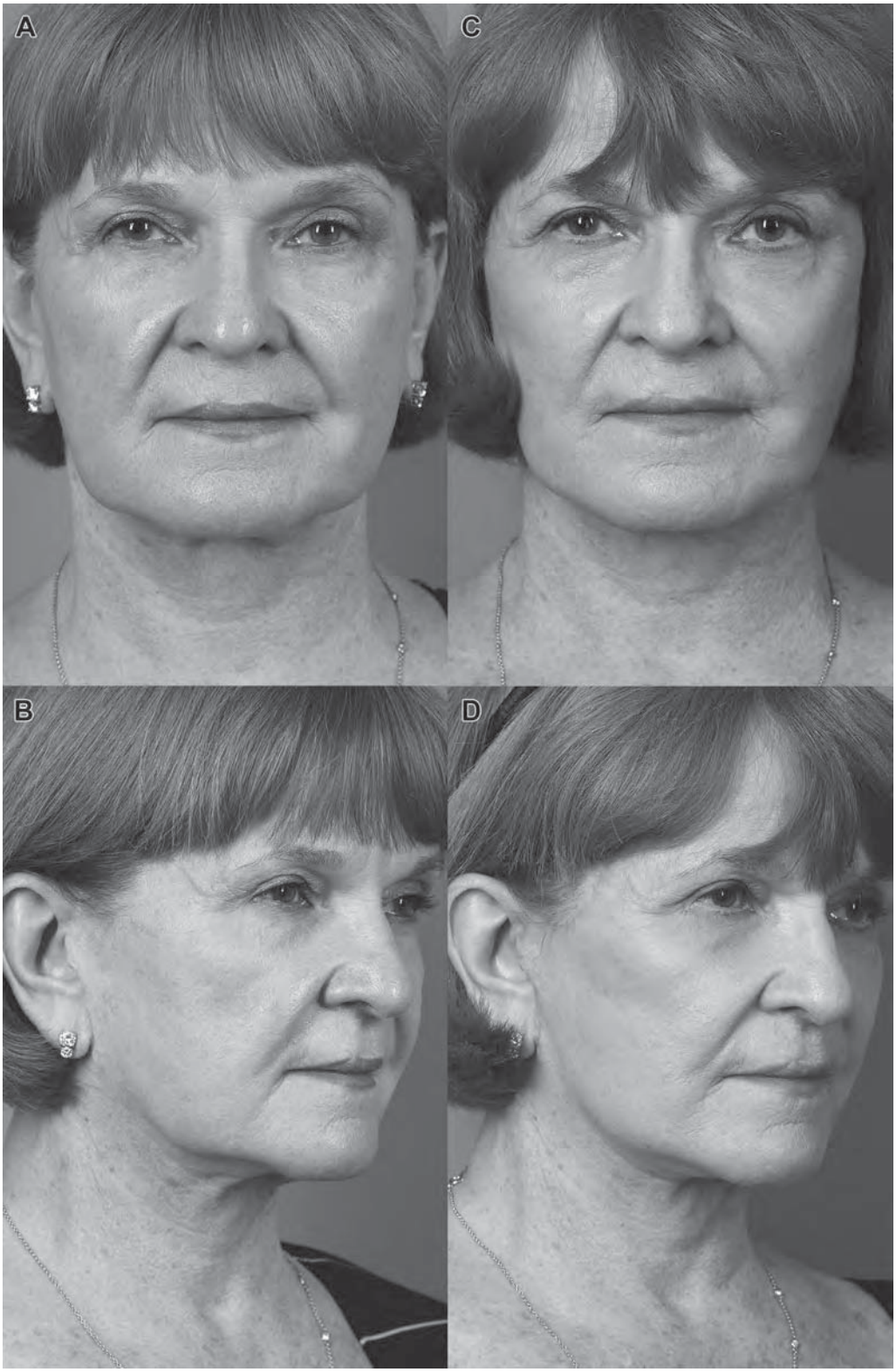

Fig. 6. Before (*A*, *C*) and after (*B*, *D*) photos in 67-year-old woman 1 month after temple and cheek rhytidectomy tuck with LAFC of the mid- and lower face and LANC (percutaneous). Treatment parameters for the mid- and lower face included 2.0- to 3.0-mL local anesthetic, laser power 5.4 W, treatment speed 30 Hz, pulse energy 180 mJ, total energy delivered 200 to 300 J, and 1.0- to 2.0-mL lipoaspirate. LANC treatment parameters included 12.0-mL local/tumescent anesthetic, laser power 5.4 W, treatment speed 30 Hz, pulse energy 180 mJ, total energy delivered 150 J, and 5.0-mL lipoaspirate.

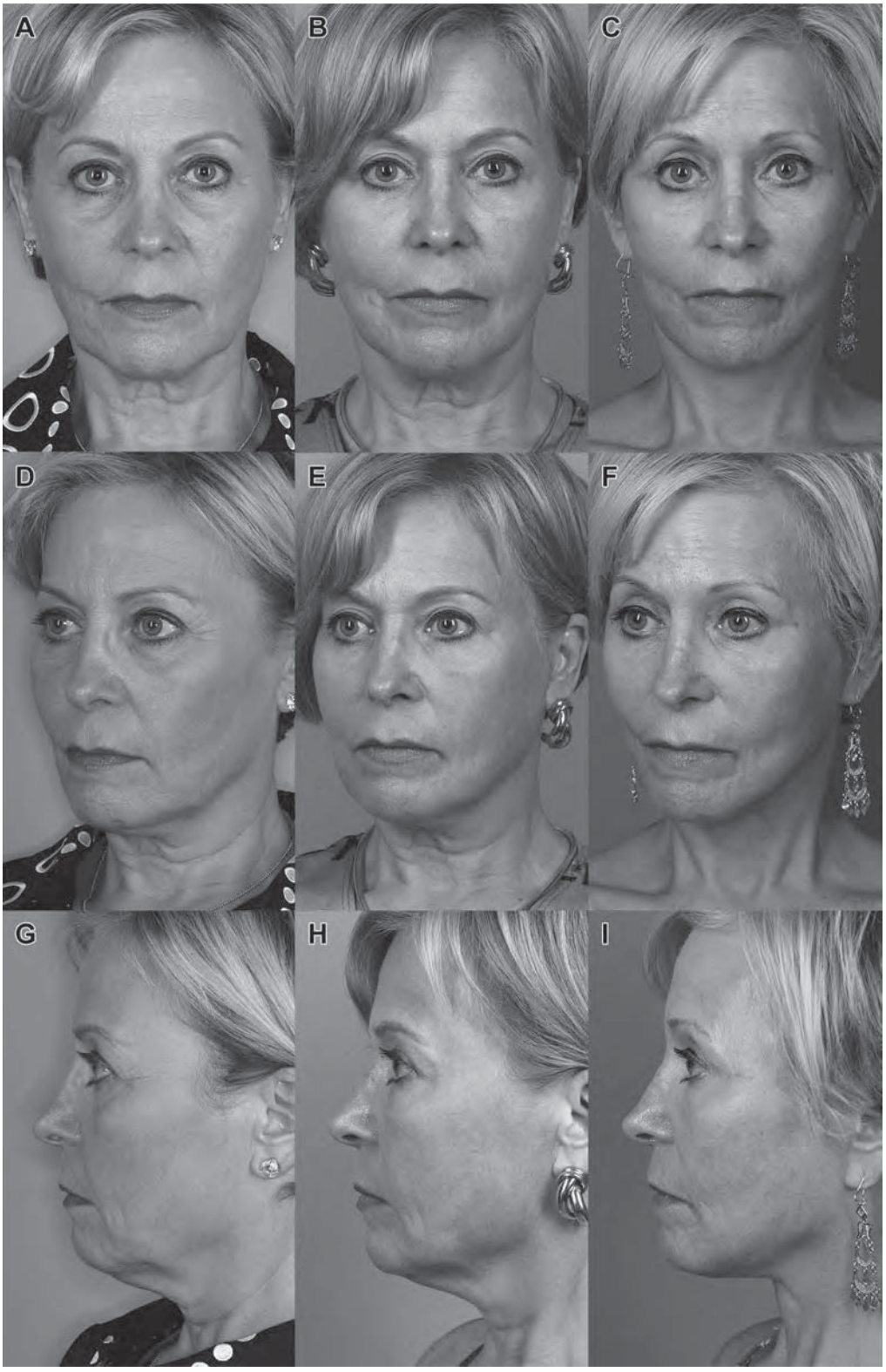

Fig. 7. Before (*A–C*), 6 months after LAFC mid- and lower face with autologous fat grafting and full face CO_2 ablative fractional resurfacing (*D–F*), and then 10 months after LAFL with LANC (percutaneous, converted to open approach but without platysmaplasty) and upper eyelid blepharoplasty (*G–I*) photos in a 59-year-old woman. Treatment parameters for the mid- and lower face included 2.0-mL local anesthetic, laser power 5.4 W, treatment speed 30 Hz, pulse energy 180 mJ, total energy delivered 150 J, and 1.0 to 2.0 mL lipoaspirate. LANC treatment parameters included 10.0-mL local/tumescent anesthetic, laser power 8.0 W, treatment speed 40 Hz, pulse energy 200 mJ, total energy delivered 1000 J, and 11.0-mL lipoaspirate.

surgery. The outline of the jowl may be readily evident with the patient in the upright, seated position for preoperative marking; however, due to tissue laxity and the effect of gravity, the marked tissue slated for contouring and debulking may move both superiorly and posteriorly when a patient is placed in the supine or Trendelenburg position for facial surgery.[7]

Ensuring adequate lower facial contouring may be accomplished by minimizing any positional tissue shift with a slight reverse Trendelenburg patient positioning (eg, 20°) and by isolating and stabilizing the target tissue between the user's thumb and forefinger during local anesthesia infiltration, laser energy delivery, and lipoaspiration. It may be, nonetheless, initially surprising to laser surgeons that the lower face LAFC procedure may involve contouring tissue a significant distance above the caudal margin of the mandible in some patients (see **Fig. 1**B). The central submental and neck tissue is less affected by patient positioning but adequate contouring in this area also requires manual guiding of the laser fiber into the areas of tissue fullness.

Although LANC enables a closed (percutaneous) approach to the neck in some facelift patients, persistent submental fullness and/or significant skin laxity immediately after LANC are indications for converting the initially closed procedure to an open procedure via a submental crease incision. Through this greater access, the effects of the LANC procedure may be assessed and any required surgical intervention (eg, midline imbrication platysmaplasty) may be performed. A recent study suggests that even though a converted open approach may be ideal, in many cases, surgical manipulation of the platysma may be required in only 20% of cases.[7] In cases of excess skin laxity, retrograde dissection at the lateral margins of the LANC treatment area, including subdermal release of the mandibular cutaneous ligament, may be performed via scissor dissection or laser fibrolysis.

The laser may be used to initiate the posterior cervicofacial skin flap dissection via fibrolysis and shrinkage of fine skin ligaments as well as for subdermal release of the zygomatic-cutaneous ligament. Safety of the skin flaps certainly takes precedence over use of the laser for this purpose. With appropriate treatment settings, limits on total energy applied, and proper technique, this application does not require anything other than the normal local anesthetic injection technique. Typical parameters that the author used for laser flap predissection include power 5.4 W, pulse energy 180 mJ, pulse duration 100 μs (fixed), pulse rate 30 Hz, and total energy delivered 200 to 300 J. Even delivery of laser energy at the subdermal level throughout the flap minimizes the risk of a skin flap complication. Laser flap predissection decreases the subsequent physical effort and time required to complete the flap elevation with facelift scissors. With these treatment parameters, bleeding from some vascular perforators, both on the flap and the underlying tissue, requires bipolar cautery for hemostasis.

Laser flap predissection should include subdermal release of the zygomatic-cutaneous ligament as well as connecting the posterior cervicofacial dissection with the LAFC and LANC treatment areas. Fully coalescing the LAFC and LANC treatment areas with the posterior cervicofacial dissection enables greater posterior and vertical repositioning of the skin flaps but also requires more effective management of the skin flaps to harness the potential for improved outcomes.[7] **Figs. 6** and **7** demonstrate how the LAFC and LANC techniques may be integrated into aging face surgery to enhance outcomes.

SUMMARY

Integration of the thermally confined, micropulsed 1444-nm Nd:YAG interstitial fiber laser into minimally invasive and surgical management of the aging face and neck provides numerous benefits and some additional treatment options that are helpful for optimization of the 3-D contours of the mid- and lower face and neck. Currently LAFC may be the best nonsurgical answer to the main limitation faced by soft tissue augmentation (ie, that it does not address adjacent areas of soft tissue fullness). As such, one-sided attempts to enhance the appearance of the face with soft tissue augmentation may result in exaggerated features and excess fullness in attempting to camouflage descended fat in the mid- and lower face. Even subtle soft tissue debulking with LAFC improves the effective proportional enhancement of soft tissue augmentation.

LANC is an effective stand-alone percutaneous procedure for mild to moderate submental and neck soft tissue excess and skin laxity. The LAFL approach expands the use of this Nd:YAG interstitial fiber laser beyond LAFC and LANC to predissection of surgical flaps and release of osseocutaneous anchoring ligaments while also raising the possibility for percutaneous (closed) treatment of the neck and the platysma.

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Laser Skin Treatment in Non-Caucasian Patients

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KEYWORDS

• Laser • Skin • Resurfacing • Ethnic • Fitzpatrick • Hispanic • African American • Asian

KEY POINTS

- Ethnic skin presents a unique challenge for laser skin rejuvenation because of higher density of larger melanosomes, thicker collagen bundles, and increased fibroblast responses.
- Lasers may be safely used in patients with dark skin tones by choosing fractional technologies with longer wavelengths, lower fluences, and longer pulse durations.
- The risks of laser therapy include scarring, postinflammatory hyperpigmentation, and hypopigmentation.
- Developing careful treatment plans based on patient goals and maintaining careful attention to preprocedural and postprocedural management strategies can minimize the risk of complications.
- In the hands of an experienced laser surgeon, laser resurfacing in dark skin types may improve the appearance of fine wrinkles and even skin tone, texture, and pigmentation.

INTRODUCTION

In the last decade, there has been an increase in the use of lasers for facial skin rejuvenation. Owing to improved technologies, patients are able to confront dermatologic concerns in an office-based setting with outpatient procedures. Conditions such as photoaging, acne vulgaris, and dyschromia can be treated with laser therapy, with improved risk profiles and decreased recovery times. Although the demand for facial rejuvenation and cosmetic procedures continues to increase among all ethnic populations and skin types, not all patients and skin types are the same and there is no one-size-fits-all treatment algorithm. In addition, the complications of therapy vary between skin types, and careful attention must be paid to these reaction patterns and specific treatment options.

Skin types and colors are divided into 6 phototypes, Fitzpatrick skin types I through VI, with I being the fairest and VI being the darkest (**Table 1**).[1] Within a single ethnicity, there may be variable phototypes, and it is important to tailor the treatment to the patient. The number of melanocytes is consistent throughout all ethnicities. Melanocytes derive from neural crest cells and transfer melanosomes, which contain melanin, into keratinocytes. The color of skin depends on the density, size, and activity of melanosomes, as darker skin has a higher density of larger melanosomes.[2] In addition, darker skin types, Fitzpatrick types V and VI, have thicker and more compact skin layers with thicker collagen bundles, which increase the epidermal barrier and reduce skin sensitivity (**Fig. 1**).[3,4] This barrier delays skin damage from the environment and ultraviolet

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Table 1
Anatomy of skin types

Fitzpatrick Skin Type	Skin Characteristics	Sun Exposure
I	Pale white skin; blonde or red hair; blue eyes; freckles	Burns easily, never tans
II	White fair skin; blonde or red hair; blue, green, hazel eyes	Burns easily, tans minimally with difficulty
III	Cream white skin; any hair or eye color	Burns moderately, tans moderately and uniformly
IV	Moderate brown skin, Mediterranean	Burns minimally, tans moderately and easily
V	Dark brown skin, Middle Eastern	Rarely burns, tans profusely
VI	Deeply pigmented dark brown to black	Never burns, tans profusely

Adapted from Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol 1988;124:870.

radiation and aging in darker phototypes when compared with lighter skin types. Due to these histologic differences, dark skin is at increased risk for injury due to incidental laser absorption by melanin, problems with postinflammatory hyperpigmentation, and decrease in melanin production leading to hypopigmentation.

Although there are many types of lasers, the fundamental principle is the same: all lasers treat the skin by targeting a specific chromophore. The main chromophores of the skin are hemoglobin, melanin, and water. In general, resurfacing lasers are designed at specific wavelengths that use water as a chromophore to cause targeted thermal damage in the dermis to promote new collagen formation and skin tightening.[5] Other targetable chromophores include melanin, which has a broad, but gradually decreasing, absorption coefficient from 250 to 1200 nm. The selection of a laser with a longer wavelength can allow for targeting of deep melanin or tattoo pigmentation in darker skin types.[4]

Other variables important to lasers include the thermal relaxation time, pulse duration, and energy fluence (**Table 2**). The thermal relaxation time is the time required for a tissue to cool to half the temperature to which it was heated. Heating the tissue for time longer than the thermal relaxation time can cause thermal damage to surrounding tissue. In dark-skinned individuals, it is important to select a pulse duration longer than the thermal relaxation time of the epidermis but shorter than the target chromophore to avoid epidermal blistering, crusting, pigmentation changes, and scarring.[4] The fluence is the joules per square

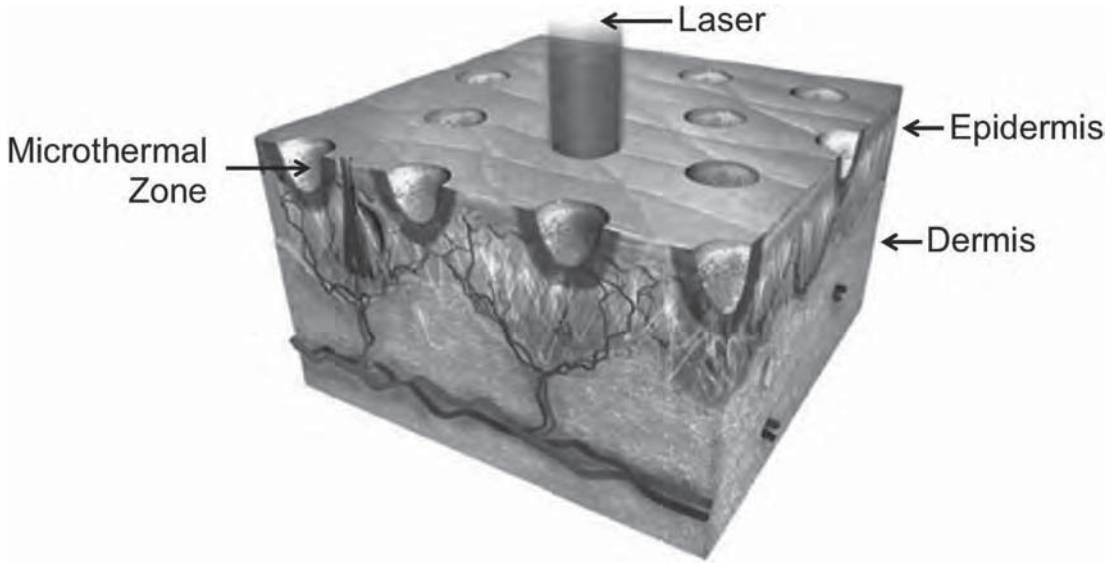

Fig. 1. Layers of the skin. The skin is divided into the epidermis, dermis, and hypodermis. Dark-skinned individuals have increased numbers of larger melanocytes, more compact skin layers, and thicker collagen bundles.

Table 2
Variables of lasers

Variable	Function	Example
Chromophore	Laser target molecule, unique absorption spectrum and peak absorption wavelength	Hemoglobin, melanin, water
Wavelength	Property of light measured in nanometers that influences how chromophores are targeted	Hemoglobin (variable absorption from 300 nm to infrared) Melanin (gradually decreasing absorption from 250 to 1200 nm) Water (1000 to 1 mm)
Thermal relaxation time	Time required for tissue to cool to half the temperature to which it was heated	Melanosome (250 ns) Vessels (2–10 ms) Hair follicles (100 ms)
Pulse duration	Time to heat tissue to target tissue; choose pulse duration less than or equal to thermal relaxation time of target chromophore to avoid damage to surrounding tissue	Pulse duration 10 to 100 ns to target melanosome
Energy fluence	Joules per square centimeter of energy emitted by a pulsed laser device	25 J/cm^2 used by a 1064-nm Nd:YAG for laser hair removal; highest tolerated fluences are 100 J/cm^2 (skin types IV, V) and 50 J/cm^2 (skin type VI)

centimeter of energy emitted from the laser handpiece. The laser fluence may need to be decreased to protect the epidermis to safely treat patients with darker skin types compared with those with lighter skin types. Other helpful strategies in safely treating patient of color include longer wavelengths, longer pulse durations, and skin cooling before, during, and/or after the procedure to avoid overheating the epidermis.[4,6]

CLASSES OF LASERS

The major classes of lasers include ablative and nonablative lasers in both nonfractionated and fractionated varieties (**Table 3**). Ablative lasers target water molecules in the epidermis, causing vaporization of skin cells and retraction of the dermis with collagen formation. Ablative lasers are more aggressive and function similar to a skin peel with prolonged recovery time and higher adverse event profile.[7] Nonablative lasers preserve the epidermis and target the dermal tissues to promote collagen formation. These nonablative treatments are milder and reduce the adverse event profile and recovery time. Fractionated lasers are designed to target microscopic treatment zones, or microthermal zones (MTZs) to create columns of thermal injury with adjacent normal skin.[4] This procedure promotes healing and improves skin texture compared with nonfractionated lasers without the high side-effect profile of ablative lasers. Radiofrequency resurfacing is a nonablative technique that uses a low temperature to penetrate dermal tissues and promote collagen healing.[7] There are several options for laser therapy, and it is important to determine the expectations of your patient while balancing the risks and benefits associated with laser therapy in patient-specific phototypes.

TREATMENT GOALS

Lasers may be considered for a variety of indications, and the goals of the treatment should reflect the patient presentation.

Skin Laxity

There is an increased desire in all patients to achieve more youthful and refreshed facial skin. Over time, facial skin experiences photodamage, which causes wrinkles, texture changes, and abnormal pigmentation. Additional changes over time include soft-tissue volume loss, rhytides, and increased vascularity. The primary environmental factor that affects aging is ultraviolet radiation, but given the protective effects of melanin and a thicker epidermis, individuals with dark skin may experience less skin laxity due to gravity and volume loss compared with others with fair skin.[4]

Dyschromia

The primary concerns of patients may vary depending on ethnicity and skin type (**Fig. 2**). Dyschromia is a common presentation of dark-skinned patients, and it is important to

Table 3
Classes of lasers and clinical outcomes

Laser	Outcomes	Risks
Ablative nonfractionated • 10,600-nm CO_2 laser • 2940-nm Er:YAG laser • Combined CO_2 Er:YAG laser	Dramatic improvement in wrinkle reduction, alleviate acne and atrophic scars[7]	Oozing, bleeding, and crusting (100%)[7]; acne, transient hyperpigmentation and hypopigmentation (IV) (55%–68%)[7,8]; scarring and poor wound healing, permanent skin hypopigmentation[4,7]
Nonablative nonfractionated • 1319-nm pulsed dye laser • 1320-nm Nd:YAG laser • 1540-nm diode laser	Improvement scar severity (29%)[3]; improvement acne scars (10%–50%)[2,9]; atrophic scarring and acne-induced PIH (III–VI) (51%–75%)[5]; limited wrinkle improvement[2]	Minimal, few hours of erythema, no scaling or peeling, no abnormal pigmentation[7]
Nonablative fractionated • 1410-nm laser • 1440-nm Nd:YAG laser • 1540-nm laser • 1550-nm Er laser • 1927-nm thulium fiber laser	Moderate improvement in texture and wrinkles[4]; significant improvement in acne scarring (51%–75%)[3,5] and overall appearance: excellent (30%), significant (59%), moderate (11%)[3,9]; safe in dark skin types because of limited tissue damage and melanocyte stimulation[7]	Moderate downtime; moderate pain[5]; postinflammatory hyperpigmentation (III, IV, V) (3%, 12%, 33%, respectively)[5]; acne (2%)[8,10]; herpetiform eruptions (2%)[8,10]
Ablative fractionated • 10,600-nm fractional CO_2 laser • 2940-nm fractional Er:YAG laser • 1790-nm fractional Er:YSGG laser	Moderate resurfacing power for mild skin laxity and rhytides[2]; moderate improvement in photodamage, scars (37%), and dyspigmentation[2,7]	Moderate downtime, moderate complications[8]; postinflammatory hyperpigmentation (II–V) (44%)[3]; use with caution in skin type VI[2]

Abbreviation: PIH, post-inflammatory hyperpigmentation.
Data from Refs.[2–5,7–10]

distinguish between melasma and postinflammatory hyperpigmentation when considering laser therapy.[4,7,11–13] Options for laser treatment of dyschromia include the Nd:YAG laser and fractional nonablative devices.[7]

Laser Hair Removal

The use of lasers for hair removal relies on melanin absorption within the hair follicle (**Fig. 3**). Laser hair removal may be complicated in patients with dark skin because of unintended epidermal overheating leading to blistering, crusting, and subsequent pigmentary changes.[4] With this in mind, longer-wavelength lasers (1064-nm Nd:YAG) with lower fluences and skin cooling may be used successfully in darker skin types for the treatment of hypertrichosis.[2]

Keloids and Hypertrophic Scarring

Keloids and hypertrophic scars occur more commonly in dark-skinned individuals. Laser treatment of thickened scars may be considered in combination with intralesional steroid injections.[4] The pulsed dye laser has been shown to decrease erythema, improve pain and pruritus, decrease lesion height, and improve hypertrophic scar pliability. These effects may facilitate intralesional steroid injection. However, the pulsed dye laser can target epidermal pigmentation and must be used with caution in patients with dark skin. Keloids may also be treated with the 1064-nm Nd:YAG laser with moderate results of mild keloids. The lesion is injected with intralesional triamcinolone 10 mg/mL up to 3 mL before starting therapy with regular laser treatments (fluence 13–18 J/cm^2, 2000 pulses) for 6 weeks.[2] After 7 weeks, the lesion may be reevaluated and treatment repeated if necessary.

PREPROCEDURAL PLANNING: MEDICAL OPTIMIZATION

Before embarking on laser rejuvenation of facial skin, it is important to emphasize routine skin

Fig. 2. Dyschromia may present as hyperpigmentation or hypopigmentation and is one of the most common treatment goals of laser therapy in ethnic populations.

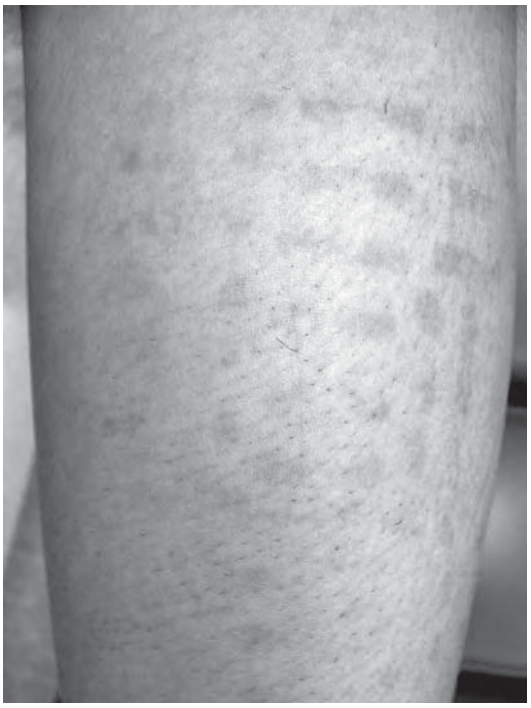

Fig. 3. Laser hair removal. Lasers can be used for treatment of hypertrichosis in all skin types by targeting the melanin chromophore in the hair follicle with a low risk of dyspigmentation.

care to patients to optimize facial skin health before procedures. Sun should be avoided when possible, and mechanical and chemical blockade (broad-spectrum A and B sunscreens) should be used daily. Acne vulgaris can be treated with topical and oral antibiotics, hormonal treatments, and isotretinoin safely in all skin types and should be optimized before starting laser rejuvenation therapy.[8] However, all isotretinoin should be avoided for 6 to 12 months before starting laser therapy due to the possibility of poor healing. In addition, all herpes simplex virus (HSV) outbreaks should be treated with antivirals, and prophylaxis antivirals should be given to patients with HSV before starting laser treatments.

Additional topical treatments with melanin suppressors, such as hydroquinones, kojic acid, azeleic acid, or emblica, may be considered for treatment of dyspigmentation and melasma before laser treatments, particularly in dark phototypes where nonablative laser therapies require a series of treatments to achieve satisfactory results.[4]

When considering laser treatment on a patient with dark skin, a test spot adjacent to the intended area of treatment may be performed, as individuals of the same ethnicity and phototype may react differently to the laser depending on variable skin characteristics.[14] Test spots should be started at low-density, low-fluence, and longer-pulse-duration settings. Full response and side effects should be observed at 1 month, at which point scarring and pigment changes will likely be evident.

PROCEDURAL APPROACH: CHOOSING A LASER

For historical reasons, one should note the ablative nonfractionated lasers, including the 10,600-nm carbon dioxide (CO_2) laser, the 2940-nm erbium-doped yttrium aluminum garnet (Er:YAG) laser, and the combined CO_2 Er:YAG laser (see **Table 3**). These lasers target the water molecules in the dermis and vaporize the epidermis. This laser has the most significant outcomes with significant improvement of fine wrinkles and acne scars.[9] However, side effects are a significant issue with this category of devices and include acne, permanent hypopigmentation, temporary hyperpigmentation, skin infections, and scarring. For these reasons, ablative nonfractionated lasers should be used with extreme caution in patients with

Fitzpatrick type IV skin and are contraindicated in phototypes V and VI because of the increased risk of dyspigmentation and scarring (occasionally keloidal).[4]

Due to the side-effect profile of the ablative nonfractionated lasers, a more gentle approach using nonablative technology was developed. The nonablative nonfractionated lasers include the 1319-nm pulsed energy laser, the 1320-nm neodyminum-doped YAG laser (Nd:YAG), and the 1450-nm diode laser. These lasers have had slight improvement with skin resurfacing and good results with acne treatment.[1] There is minimal recovery required with these lasers, little erythema, and minimal peeling. The nonablative nonfractionated lasers often require serial treatment sessions (4–6 treatments) to obtain improvement but can be used safely in patients with dark skin because of decreased risk of scarring and dyspigmentation.[7]

To more effectively treat the skin, nonablative fractionated lasers were developed to combine a more aggressive pulse and the safety of fractionation while still avoiding the epidermal loss incurred with ablative lasers. These include the 1410-nm laser, the 1440-nm Nd:YAG laser, the 1540-nm laser, the 1550-nm erbium laser, and the 1927-nm thulium fiber laser. These nonablative fractionated lasers frequently require several treatments (2–6), with moderate improvements in skin tone and texture with moderate downtime.[7] The targeting of tiny diameter and deep dermal penetration of each MTZ allows for stimulation of collagen formation while avoiding disruption of the epidermal barrier function.[9] These lasers can be used safely in dark phototypes with a small risk of temporary hyperpigmentation.

The ablative fractionated lasers are the most recent addition to the laser family. These lasers were developed in an attempt to increase resurfacing effectiveness while still enjoying quicker healing with fewer complications compared with ablative nonfractionated resurfacing. These include the 10,600-nm fractional CO_2 laser, the 2940-nm fractional Er:YAG laser, and the 2790-nm fractional erbium-doped yttrium scandium gallium garnet (Er:YSGG) laser. These lasers target MTZs with ablation and vaporization of dermal and epidermal tissues. A series of sessions may give resurfacing results nearly comparable to the ablative nonfractionated lasers but with much improved safety profiles.[7] These lasers can improve skin laxity and mild rhytides, but due to the violation of the epidermal layer, there is a risk of infection, scarring, and dyspigmentation and should be used with caution in patients with Fitzpatrick type IV through VI skin.[7]

Radiofrequency technologies achieve mildly improved facial skin tone and texture by denaturing existing dermal collagen and stimulating new collagen through low temperatures and deep tissue penetration. This option decreases the risk of dyspigmentation and scarring, and patient discomfort is minimal.[2,7]

When choosing a laser for hair removal, the 1024-nm Nd:YAG is the safest choice in dark-skinned individuals because the wavelength is poorly absorbed by melanin, which reduces the damage to dark epidermal pigmentation.[2] In addition, the pulse length can be adjusted to deliver the pulse over a longer period to facilitate cooling. Other laser choices for hair removal include the alexandrite and diode lasers at lower fluences and wider pulse widths. As with laser treatment of other skin disorders, multiple treatment sessions may be needed to achieve permanent results. Risks of laser hair removal in Fitzpatrick skin types IV to VI include blistering and temporary dyspigmentation, with a low risk of permanent hyperpigmentation or hypopigmentation.[2,3]

In addition to using lasers with longer pulse duration and longer wavelength to decrease the risk of discoloration or scarring, periprocedural cooling should be considered to decrease thermal damage to surrounding tissues.[3] Contact and noncontact cooling have the added benefit of improving patient comfort during laser therapy while decreasing thermal damage to the epidermis without interfering with laser intensity and direction. Options for contact cooling include skin moistening, application of ice or ice packs, and laser-specific cooling tips.[6]

POSTPROCEDURAL CARE AND FOLLOW-UP

The importance of postprocedural planning and skin care cannot be overstated when managing patients after laser treatment. Because many of these treatments often require several sessions, reducing skin damage between treatments can optimize epidermal healing and dermal collagen regeneration. The skin is more sensitive than usual for a short time after laser treatment, and sun blockade and cooling agents should be used judiciously. Darker phototypes have more reactive and labile fibroblasts compared with skin types I to III, and further dermal injury should be avoided.[2]

After laser treatment and depending on the type of laser used, mild erythema, edema, peeling, and flaking may occur and typically resolve over several days.[10] The period for full recovery depends on the exact type of laser treatment, and postoperative care must be tailored to the treatment administered. The postoperative skin care

routine should include keeping the skin clean and moist to allow for reepithelization and to minimize the potential of scarring. In general, chilled saline-soaked gauze is applied intermittently for the first several days. The treated area should be gently treated with a mild cleanser such as Cetaphil, followed by the application of an oxygen-permeable ointment such as Aquaphor. Patients should be encouraged not to pull or pick at their skin as it starts to flake or peel, as this may increase the likelihood of scarring. Depending on the type of laser or resurfacing technique used, reepithelization typically occurs within a week. Avoidance of sun and the liberal use of sunscreen should be encouraged. Patients should avoid the use of retinoids and other bleaching agents for risk of causing irritation.

Most laser patients feel a sunburnlike sensation for the rest of the day after laser therapy. Topical skin care, oral analgesics, and cooling agents can all be used to improve patient comfort. Topical cooling agents, such as ice packs, are encouraged postprocedurally to improve patient comfort and decrease inflammation. Topical steroids may be considered in patients with persistent erythema.

POTENTIAL COMPLICATIONS AND MANAGEMENT

Careful patient selection combined with conservative and judicious implementation of laser treatments can result in positive outcomes when dealing with patient of color and dark skin types. In this particular subset of patients, the most common postprocedural concerns are related to dyspigmentation and scarring.

Postinflammatory hyperpigmentation is a common occurrence with ablative laser options and is a bothersome side effect in darker phototypes (Fitzpatrick skin types V–VI) (**Fig. 4**).[2,7] There are several options for topical therapies when considering the treatment of hyperpigmentation, such as hydroquinone, azeleic acid, kojic acid, and emblica. Hydroquinone, a common treatment option, is a plant-derived tyrosinase inhibitor and is often used to treat discrete hyperpigmented patches.[4] Deleterious outcomes related to the use of hydroquinone may include hypopigmentation surrounding the treated area because of adjacent bleaching, in a halo effect.[2,10]

Delayed hypopigmentation is a less common complication usually seen after ablative nonfractionated laser resurfacing several months after treatment (**Fig. 5**). This complication is permanent and a major cause for avoiding ablative nonfractionated resurfacing in dark-skinned patients. This condition can be confused with hypopigmentation attributed to the use of retinoids and hydroquinone before laser treatment, which resolves with discontinuation of the medication.[10]

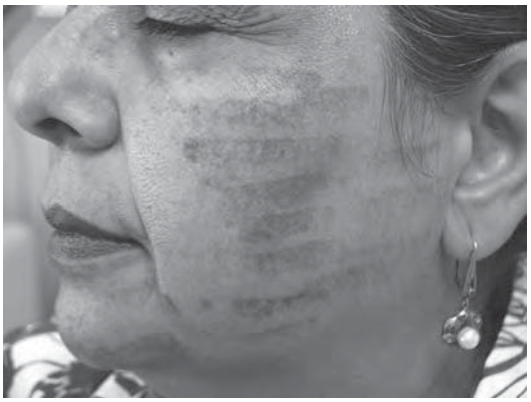

Fig. 4. Posttreatment postinflammatory hyperpigmentation. Postinflammatory hyperpigmentation is common with ablative lasers and may be reduced by using nonablative and fractionated techniques.

In addition to dispigmentation after laser treatment, additional complications such as acneiform eruptions and HSV infections may occur in all skin types (**Fig. 6**).[8] Acne eruptions are more common in patients with acne-prone skin and can be minimized by premedicating with oral antibiotics such as tetracycline. In general, prophylactic antivirals are recommended in patients with a history of orofacial HSV. When treating patients with a history of HSV outbreaks with laser exposure, antivirals should be started before the initiation of laser therapy and continued up to a week after laser application. Laser rejuvenation should not be performed on patients with active HSV infections.

Although bacterial superinfections are uncommon, they should be treated aggressively to minimize scarring and dyspigmentation.[8,10] Bacterial

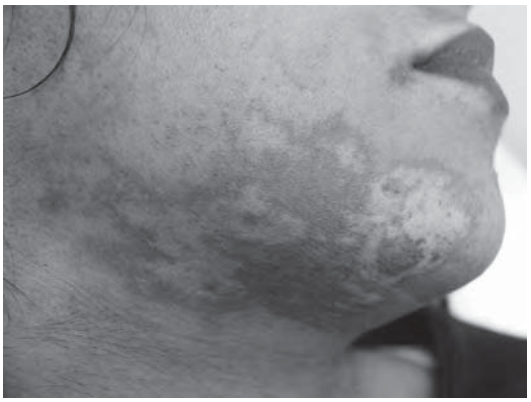

Fig. 5. Posttreatment hypopigmentation. Hypopigmentation after laser therapy is a rare complication that may present several months after treatment.

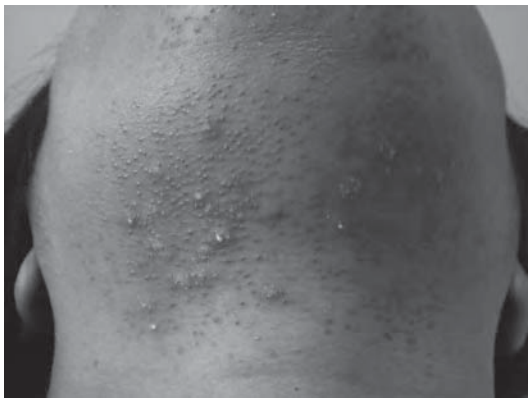

Fig. 6. Posttreatment acneiform eruptions. A common complication after laser therapy is acneiform eruptions and HSV infections. This risk can be minimized with premedication in select patients with skin types predisposed to such infections.

superinfections typically present with pain, increased erythema, exudates, erosions, and crusting. Infections should be cultured and treated with broad-spectrum oral antibiotics to reduce long-term risk of scarring. When treating patients of dark skin types, the development of acne eruptions, HSV infection, or bacterial superinfections can intensify the likelihood of pigment issues and discoloring of the soft-tissue envelope. Every effort should be made to prevent these complications or treat them aggressively should they occur.

SUMMARY

Ethnic skin presents a unique challenge for laser skin rejuvenation because of higher density of larger melanosomes, thicker collagen bundles, and increased fibroblast responses. Special considerations need to be made when considering laser therapy for ethnic patients for the treatment of skin laxity, dyschromia, hypertrichosis, keloids, and hypertrophic scarring. Lasers may be safely used in patients with dark skin tones by choosing fractional technologies with appropriate wavelengths, lower fluences, longer pulse durations, and maintaining careful attention to preprocedural and postprocedural management strategies. When considering the use of lasers, the treatment goals should reflect individual patient complaints and the realistic expectations of laser skin rejuvenation. Patients should be counseled on the risks of laser therapy, including scarring, postinflammatory hyperpigmentation, and hypopigmentation. With this in mind and in the hands of an experienced laser surgeon, laser resurfacing in darker skin types IV through VI may eliminate unwanted hair, improve the appearance of fine wrinkles, and even skin tone, texture, and pigmentation.

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REVIEW ARTICLES

Handling Botulinum Toxins: An Updated Literature Review

Ada R. Trindade De Almeida, MD,* Leticia Cardoso Secco, MD,* and Alastair Carruthers, MD†

BACKGROUND Botulinum toxin (BoNT) has been in use since the late 1970s, and over the last 20 years, its use has been extended to new indications in various areas of medicine. During these years of clinical use, some of the initial ideas have changed, and others have remained stable along with increasing experience with and knowledge about BoNTs.

OBJECTIVE To review the literature and prescribing information on all of the available products and to update the concept of handling toxins (preparations, reconstitution, storage, sterility, and dilution).

METHODS A review (not Cochrane type analysis) of the medical literature based on relevant databases (MEDLINE, PubMed, Cochrane Library, specialist textbooks, and manufacturer information) was performed.

CONCLUSIONS Many of the precautions around BoNT use, often recommended by the manufacturers, are described in the clinical literature as too restrictive. The literature suggests that toxins may be sturdier and more-resistant to degradation than previously understood. *Dr. Ada R. Trindade de Almeida has been a consultant to Allergan, Inc. and participated in clinical trials for Allergan and Galderma. Dr. Alastair Carruthers is a consultant to Allergan, Inc. and Merz GmbH and has been paid to do research for both companies.*

Background

Botulinum toxin (BoNT) has been in use since the late 1970s in ophthalmology,[1] and over the last 20 years, its use has been extended to new indications in various areas of medicine, in particular dermatology. It is an effective treatment for strabismus, hemifacial spasm, blepharospasm, cervical dystonia, spasmodic dysphonia, hyperhidrosis, and facial rejuvenation.[2]

Clostridium botulinum, a Gram-positive anaerobic bacterium, produces seven antigenically different neurotoxins, but only serotypes A and B are commercially available.[3] Serotype A (BoNTA) appears to be the most potent subtype.[4]

BoNTA is naturally produced as a complex of a core neurotoxin protein, along with several hemagglutinin and nontoxin nonhemagglutinin proteins.[3,5] The associated proteins serve to stabilize and protect the neurotoxin molecule from degradation.[6,7]

Under physiologic conditions, the core 150-kDa protein dissociates from the toxin complex, binds to synaptic vesicle protein 2 using the heavy chain component, and enters the neuronal cell by internalization.[8] Once in the cytosolic surface membrane, BoNTA binds to and cleaves the 25-kDa synaptosomal-associated protein (SNAP25), whereas BoNTB binds to and cleaves the vesicle associated membrane protein, a component of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor family[9] involved in exocytic release of the neurotransmitters.[10]

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Initially, it was believed that BoNTA was fragile and susceptible to surface denaturation.[11,12] A variety of conditions have been postulated to affect the potency of BoNTs, and different authors and manufacturers made many recommendations concerning reconstitution and storage of the agent in order not to interfere with its efficacy or duration. During these more than 20 years of clinical use, some of these initial ideas have changed, and others have remained stable with our growing experience with and knowledge about BoNTs.

The objective of this article was to review the medical literature and prescribing information on all of the available products and to update the concept of handling toxins, although this is not a Cochrane type analysis. It will be divided into five sections: preparations, reconstitution, storage, sterility, and dilution.

Preparations

BoNT formulations are not identical or interchangeable.[13] They possess individual potencies, and attention is required to ensure proper use and avoid errors. In April 2009, the FDA established drug names to reinforce these differences and prevent potential serious risks associated with these medications.[14]

Currently, only one form of BoNTB (rimabotulinumtoxinB) is available, under the trade name of Myobloc/Neurobloc (Solstice Neurosciences Inc./Eisai Co., Ltd.) in the United States and Neurobloc in Europe. The U.S. FDA approved it for cervical dystonia in 2001.

There are five sources of BoNTA available worldwide:

1. OnabotulinumtoxinA: Botox/Botox Cosmetic in the United States, Latin America (Allergan, Inc., Irvine, CA), also known as Vistabel in Europe and Vistabex in Italy.

2. AbobotulinumtoxinA: Dysport (Ipsen Ltd., Berkshire, UK) in the United States, Europe, and Latin America and Azzalure in Europe.

3. BoNTA Prosigne (Lanzhou, China) in Asia and Latin America.

4. BoNTA Neuronox (Medy-Tox Inc., South Korea)

5. IncobotulinumtoxinA: Xeomin, (Merz Pharma, Frankfurt) in Canada, Germany, the United States (for therapeutic use), and Latin America and Bocouture in Europe and Latin America.

Another product, BoNTA PureTox (Mentor Corporation, Santa Barbara, CA), similar to Xeomin, is an uncomplexed type A toxin and has completed phase III trials.

BoNTs manufacturer recommendations on supply, dilution, and storage are summarized in Table 1.[15–20]

Reconstitution

Published information about variations in reconstitution methods, including agitation or foam formation and mixtures of toxins with several substances is reviewed in this section.

Unpreserved Saline

OnabotulinumtoxinA is the most studied type of BoNTA, and as a consequence, the majority of the information about handling BoNTs is found with this brand. Initially, the product was thought to be fragile.[11,12] Later, many studies following anecdotal observations confirmed the persistence of activity of BoNTA in different situations.[21–32]

Most manufacturers recommend that BoNTA reconstitution be performed using unpreserved saline.[15–19]

In a recent international consensus on the use of abobotulinumtoxinA,[32] the recommendation was to

TABLE 1. Botulinum Toxin Manufacturer Recommendations on Supply, Dilution, and Storage

	OnabotulinumtoxinA	*AbobotulinumtoxinA*	*BONTA*	*BONTA*	*IncobotulinumtoxinA*	*RimabotulinumtoxinB*
Company	Allergan, Inc.	Ipsen Inc./Medicis Inc.	Lanzhou	Medy-Tox Inc., South Korea	Merz Pharmaceuticals	Solstice Neurosciences Inc./Eisai Co., Ltd.
Commercial names	Botox, Botox cosmetic, Vistabel, Vistabex	Dysport, Reloxin, Azzalure	Prosigne Lantox, Redux	Neuronox, Meditoxin Botulift	Xeomin, Bocoture	Myobloc, Neurobloc
Approvals	Worldwide, including United States and Canada	In more than 65 countries, including United States and Canada	>10 countries including China	Korea, India, Latin South America	Germany, other European countries, Mexico, Argentina, Brazil	United States, some European countries
Type	Type A-Hall strain	Type A (NCTC 2916 strain)	A	A	Type A-Hall strain	B
Active substance	Botulinum toxin type A complex (900 kDa)	Botulinum toxin type A complex (400 kDa)*	Botulinum toxin type A (900 kDa)†	Botulinum toxin type A (940 kDa)	Botulinum toxin type A, free from complexing proteins (150 kDa)	Botulinum toxin type B (700 kDa)
Indications	Blepharospasm, cervical dystonia, glabellar lines, hyperhidrosis, chronic migraine	Blepharospasm, cervical dystonia, glabellar lines	Blepharospasm, cervical dystonia, glabellar lines, hyperhidrosis	Blepharospasm	Blepharospasm, cervical dystonia, cosmetic use in some countries	Cervical dystonia
Mode of action	SNAP 25/SV2	SNAP 25/SV2	SNAP 25	SNAP 25	SNAP 25	Vesicle associated membrane protein
U/vial	100 or 200	300 or 500	100 or 50	100	100 or 50	2,500, 5,000, or 10,000
Pharmaceutical form	Powder dissolved in solution for injection	Powder dissolved in solution for injection	Powder dissolved in solution for injection	Powder dissolved in solution for injection	Powder dissolved in solution for injection	Solution
Excipients	500 μg HSA 0.9 mg NaCl	125 μg HSA 2.5 mg lactose	5 mg gelatin 25 mg dextran 25 mg sucrose	500 μg HSA 0.9 mg NaCl	1,000 μg HSA 4.7 mg sucrose	500 μg/mL HSA NaCl 0.1M Disodium succinate 0.01MHCl (to adjust pH) Water for injection
Transportation and storage before dilution	2–8°C	2–8°C	2–8°C	2–8°C	<25°C	2–8°C

TABLE 1.. Continued

	OnabotulinumtoxinA	*AbobotulinumtoxinA*	*BONTA*	*BONTA*	*IncobotulinumtoxinA*	*RimabotulinumtoxinB*
Dilution, mL	1.0–2.5	1.66–3.32	1.0–2.0	1.0–2.0	1.0–2.0	No reconstitution required
Storage after dilution/temperature	24 hours/2–8°C	4 hours/2–8°C	4 hours/2–8°C	4 hours/2–8°C	24 hours/2–8°C	4 hours (if diluted)/2–8°C

*http://www.accessdata.fda.gov/drugsatfda docs/nda/2009/125274s000ChemR.pdf (accessed October 1, 2010). †Li Biansheng. Injection of Botulinum Toxin A in Correcting Forehead Wrinkles. J Medl For 2005: 26(10):60-6. SNAP-25, synaptosomal membrane-associated protein with the molecular mass of 25 kDa; SV2, synaptic vesicle protein 2; HAS, human serum albumin; NaCl, sodium chloride.

reconstitute the lyophilized powder in unpreserved 0.9% sodium chloride.

RimabotulinumtoxinB is provided as a ready-to-use sterile liquid; no reconstitution is required.[20]

Preserved Saline

A bilateral, comparative, prospective study in 100 cosmetic patients showed that preserved saline (containing benzyl alcohol) used in reconstitution did not affect the potency of onabotulinumtoxinA and made the injection less painful.[23]

Two other studies confirmed these observations: one comparative, double-blind, side-by-side controlled trial with 20 blepharospasm patients[33] and a single-blind study with 93 individuals injected in the upper face, neck, and axillary regions.[34]

A consensus panel held in 2004 chose preserved saline as the preferred diluent for reconstitution of onabotulinumtoxinA.[22] Another expert panel recognized that benzyl alcohol may act as a local anesthetic but considered that, at low volumes, this effect is usually negligible.[32]

Saline Plus Hyaluronidase

In 2003, Goodman[24] tested the viability of onabotulinumtoxinA when reconstituted with saline admixed with hyaluronidase and whether this mixture could enhance the diffusion of the product. The initial results (after 2 weeks) showed that the mixture maintained the efficacy of onabotulinumtoxinA and that there was greater diffusion of the effect. No long-term results were evaluated.

Lidocaine and Epinephrine

In a double-blind, randomized, controlled study with 10 volunteers for cosmetic indication, Gassner and Sherris[25] showed that, when onabotulinumtoxinA was reconstituted with 1% lidocaine with 1:100,000 epinephrine, all components retained their function. Haubner[35] found similar results in two cases,

reconstituting onabotulinumtoxinA with 2% lidocaine with 1:200,000 epinephrine.

Recently, a brief communication described the injection of a "cocktail" composed of abobotulinumtoxinA, 2% lidocaine with 1:100,000 epinephrine, and hyaluronic acid (Perlane, Medicis, Scottsdale, AZ) for rejuvenation of the upper face. To mix all products, two syringes were connected, and at least 10 back-and-forth mixings were performed. The combined product was injected into five patients in the periocular and glabellar areas in linear threads, small boluses, or serial punctures, and the author concluded that there was no compromise in efficacy or safety.[36]

Another study compared side by side, in 29 patients, onabotulinumtoxinA reconstituted in 2% lidocaine with normal saline for axillary hyperhidrosis.[37] They concluded that it was equally effective over the short and long term. Because injections with lidocaine-reconstituted onabotulinumtoxinA were associated with significantly less pain, it might be preferable for treating axillary hyperhidrosis.

There has been a report of a fatal case of anaphylaxis after the injection of an onabotulinumtoxinA and lidocaine mixture in a woman for chronic neck and back pain.[38] It was not possible to establish which drug was responsible for the reaction, but when using lidocaine to dilute BoNTA, it is advisable to consider that it may increase the possibility of an anaphylactic reaction.

Hantash and Gladstone tested the effect of epinephrine 1:100,000 on onabotulinumtoxinA efficacy.[39] Fourteen subjects were treated and evaluated for up to 6 months. The authors concluded that epinephrine might accelerate the rate of onset of action and enhance the short-term efficacy of onabotulinumtoxinA for the treatment of periorbital rhytides. Other articles reporting authors' personal experience[40,41] have recommended the addition of epinephrine to saline for the reconstitution of abo or onabotulinumtoxinA.

Bupivacaine

Yen and colleagues[42] conducted a randomized, double-blind study with 16 patients treated for glabellar furrows. OnabotulinumtoxinA reconstituted with 0.75% bupivacaine was injected into one corrugator, and the contralateral muscle received the same product reconstituted with unpreserved saline. At 1 week, the side treated with bupivacaine-reconstituted toxin showed greater muscle weakness. At 1 and 3 months, there were no differences between the sides. They concluded that bupivacaine did not affect efficacy or duration of onabotulinumtoxinA but could result in a less-painful procedure. They attributed the faster onset of the paresis to a possible synergistic effect of bupivacaine-induced myotoxicity.

Sterile Water

BoNTA will work if dissolved in sterile water, but this causes intense short-lived pain at the injection site.[43]

Albumin

Bigalke and colleagues[44] demonstrated that human albumin supplementation of abobotulinumtoxinA could allow a dose reduction without decrease of therapeutic efficacy. In another study,[45] 106 patients were treated with abobotulinumtoxinA (diluted in 0.1% albumin solution to a concentration of 25 U/mL) over 5 to 10 years for cervical dystonia, blepharospasm, and hemifacial spasm. The conclusion was that long-term treatment with albumin-supplemented BoNTA was safe, effective, and could help reduce costs.

Foam During Reconstitution

To evaluate the stability of fragments of BoNTs (light chain and the binding domain of the heavy chain) in response to mild agitation, Toth and colleagues[46] performed several in vi tro tests. They used endogenous trypsin-like protease to cleave the 150-kDa toxin into a 50-kDa N-terminal light chain (Lc) and a 100-kDa C-terminal heavy chain. The latter was further proteolyzed into a 50-kDa N-terminal

membrane-spanning domain (Hn) and a 50-KDa C-terminal receptor-binding domain (Hc). After that, they investigated the stability parameters of isolated Lc and the binding domains Hc of BoNT to mild agitation. They found that the recombinant light chains of serotype A (LcA) rapidly lost their secondary structures and that mild stirring denatured Hc domains, but the addition of nonionic detergents completely prevented denaturation. They speculated that BoNT domains underwent surface denaturation due to rapid exposure of hydrophobic residues by mechanical agitation.

A recent experimental study evaluated the effect of onabotulinumtoxinA after vigorous agitation (continuous inversion and straightening of the vial, 30 times per minute) for up to 6 weeks.[47] Eight mice were each injected intraperitoneally with 1 U of the agitated onabotulinumtoxinA on days 1, 3, 5, 7, 14, 21, 28, and 42. The main outcome measure was death of the mice, demonstrating toxin efficacy. At the end of the study, half of each group of mice (4/8 mice) died within 48 hours of the injection (range 16–48 hours), and the conclusion was that the effect of onabotulinumtoxinA is maintained even when it is agitated vigorously for up to 6 weeks.[47]

In a clinical setting, Almeida and colleagues[48] compared muscle paralysis between the sides in a split-face study in which they injected onabotulinumtoxinA gently reconstituted without foam formation on one side of the face (periocular and glabellar areas) and onabotulinumtoxinA rapidly reconstituted with formation of as many bubbles as possible on the other side. They concluded that the presence of foaming during the reconstitution process did not affect the potency or the short- or long-term effects of the product. A consensus panel on onabotulinumtoxinA held in 2004 concluded that this report supports clinical experience, suggesting that the fragility of BoNTA is not as problematic as previously reported.[22]

Kazim and colleagues[49] also studied the duration of onabotulinumtoxinA when reconstituted vigorously. They randomly selected seven patients who had half of their forehead injected with onabotulinumtoxinA reconstituted gently and the other half with onabotulinumtoxinA reconstituted vigorously. (The vial was placed on a vortex Touch mixer at maximum speed for 30 seconds.) They concluded that the effect and duration after 6 months was the same on each forehead side.

The fact that the associated proteins inside the complex of onabotulinumtoxinA might serve to stabilize the neurotoxin molecule and protect it from degradation may explain the different results found between in vitro[46] and in vivo[47–49] studies.

Foreign Body

There have been no reports of injection of nonbiodegradable material concomitantly with the injection of BoNT, but there is a report of the presence of rubber particles in a reconstituted onabotulinumtoxinA vial. The authors and the manufacturer recommend visual inspection of the product before its use.[50]

Storage

RimabotulinumtoxinB must be stored at 2°C to 8°C,[20] and according to Setler,[51] there is no significant loss of activity for up to 30 months under refrigeration, although at room temperature (25°C), it drops to at least 9 months.

Some controversy remains on the storage of reconstituted BoNTA vials. Manufacturers recommend administration within 4 to 24 hours after reconstitution,[15–19] storage at 2°C to 8°C (in the refrigerator), and not freezing after reconstitution, but several publications suggest that these recommendations may be excessively strict.

Shelf Life After Reconstitution

A multicenter, double-blind study demonstrated in 88 patients that reconstitution with nonpreserved saline up to 6 weeks before use did not reduce

efficacy in the treatment of glabellar frown lines for up to 4 months.[52] In the 2004 consensus panel, 73% of panelists stored onabotulinumtoxinA for more than 4 hours.[22]

Lizarralde and colleagues studied 30 patients injected for external canthus dynamic lines with onabotulinumtoxinA reconstituted 1 week before its use. They found clinical results similar to those with freshly reconstituted toxin.[53] Another study[54] demonstrated that onabotulinumtoxinA used after 2 weeks of refrigeration had no changes in time of onset or efficacy in the treatment of lateral orbital rhytides, and a recent experimental trial in mice did not find loss of efficacy in onabotulinumtoxinA vials reconstituted for up to 6 weeks before use.[47]

A study with abobotulinumtoxinA[55] reconstituted 2 weeks before injection in 105 patients found no difference in safety or efficacy. A consensus panel in 2010[32] found that reconstituted abobotulinumtoxinA can be used within 2 to 3 weeks without adverse effects.

Fresh or Frozen

In animal models, Gartlan and Hoffmann[27] and Jabor and colleagues[28] found loss of efficacy if the onabotulinumtoxinA was frozen for longer than 2 weeks. Greene[29] "argued that in vitro experiments may not reflect clinical practice." His opinion that the frozen toxin remained effective for several weeks was similar to clinical study results[30,31] that found no loss of activity or additional side effects for up to 8 weeks.

Parsa and colleagues[4] compared reconstituted onabotulinumtoxinA frozen for up to 6 months with onabotulinumtoxinA not frozen and used within 4 hours after reconstitution in 118 sites in 80 patients and concluded that reconstituted onabotulinumtoxinA may be frozen, thawed, and injected without losing its potency for up to 6 months. Kane found that these conclusions were consistent with anecdotal reports from many injectors.[56]

The ultrastructural alterations of the muscle and nerve after the injection of fresh versus stored onabotulinumtoxinA were evaluated using electron microscopy.[57] Fifteen rabbits were injected with toxin freshly reconstituted or stored for 2 weeks under refrigeration and had muscles and motor nerves harvested at 5 days and 12 weeks. No differences were found at the 5-day evaluation. At 12 weeks, the group that used stored toxin showed less-severe atrophic changes in the muscle, whereas no differences were encountered on nerve evaluation. In the same experimental trial in mice cited above, Shome and colleagues[47] did not find loss of efficacy in onabotulinumtoxinA vials reconstituted and kept refrigerated for up to 6 weeks before use.

Yang and colleagues,[58] in a prospective, double-blind, randomized controlled trial with 40 subjects treated for horizontal forehead rhytides, found no difference in potency or duration of efficacy of abobotulinumtoxinA after 2 weeks of refrigeration or freezing from that of freshly reconstituted abobotulinumtoxinA.

Sterility

Because storing and reusing reconstituted toxin for a period of weeks has become a frequent practice, sterility assessment is an important issue that some authors have addressed.

Alam and colleagues[59] evaluated 127 vials of onabotulinumtoxinA reconstituted using preservative-containing saline, stored, and reused, simulating the routine storage and extraction methods performed in clinical practice. Each vial underwent an average of 4.5 access procedures during a period of up to 7 weeks, and contamination was not evidenced in sterility analysis.

Menon[60] analyzed 11 consecutive bottles of abobotulinumtoxinA left for 4 hours at room

temperature, and no microbial growth was noted. The same results were found in eight vials of this product kept refrigerated for 15 days after reconstitution.[52]

Dilution Issues

There is considerable discussion about the effect of dilution of BoNTs on clinical effect, duration, or diffusion. The package insert for onabotulinumtoxinA and incobotulinumtoxinA recommends dilution of 100 U in 1 to 8 mL of saline (12.5–100 U/mL).[15,19] For abobotulinumtoxinA, 300 U can be diluted in 0.6 to 2.5 mL (120–500 U/mL).[16] RimabotulinumtoxinB can be diluted as much as 6 times using normal saline, but a more-concentrated solution cannot be generated.[43,51,61,62]

Many authors believe that higher dilutions could be associated with greater risk of diffusion to unwanted sites,[12,63–68] unsatisfactory results, or shorter duration, but this is not unanimous in the literature.

To better understand the effect of dilution on diffusion, side effects, and treatment success, studies were reviewed according to indication.

Facial Muscles

Cosmetic Indications

Expert consensus panels for cosmetic indications recommend dilutions between 1 and 3 mL of saline for onabotulinumtoxinA[22] and between 1.5 and 2.5 mL for abobotulinumtoxinA.[32,69]

In 1996, a group reported the use of large volumes os low dose BoNTA[70], with dilutions of 5 and 10 mL of onabotulinumtoxin A. Two years later, Fulton[71] compared injections to one side of the face with dilutions of 10 mL with those of 15 and 20 mL/vial in 10 individuals. He noted that the 10- and 15-mL dilutions were efficacious but had shorter duration. Frequent retreatments were needed to maintain long-term results. He also reported that a great number of individuals were resistant to the toxin, so higher dilutions are no longer recommended.

Hankins and colleagues evaluated 46 patients with glabellar wrinkles injected with different concentrations of onabotulinumtoxinA ranging from 10 and 100 U/mL for up to 21 weeks. They found no differences in effect or duration of treatment, but larger volumes were associated with greater discomfort.[72]

Le Louarn[41] described his dilution technique, in which large volumes of saline mixed with epinephrine were added (2 or 3 times the initial volume) to abobotulinumtoxinA and onabotulinumtoxinA. This diluted toxin was used to treat areas of the face where a higher spread, in his opinion, would be beneficial, such as the frontalis, platysma, and orbicularis oculi muscles.

In a study with 10 volunteers,[73] Hsu and colleagues injected the forehead with 5 U of onabotulinumtoxinA diluted to 20 (5 mL) or 100 U/mL (1 mL). They found that higher-volume injections resulted in greater diffusion and a larger affected area and suggested that muscle contraction influences the pattern of toxin spread.

Carruthers and colleagues studied the effect of different dilutions of onabotulinumtoxinA in the treatment of glabellar rhytides. They followed 80 patients treated with dilutions of 10, 20, 33.3, or 100 U/mL in the glabella (20/group) for 48 weeks. There were no statistical differences in treatment success or adverse effect reports, although six cases of eyebrow ptosis were—all in the higher-dilution group.[74]

Another trial enrolled 20 patients treated for lateral orbital rhytides observed for 90 days. Two different dilutions of onabotulinumtoxinA were used (20 and 100 U/mL); no statistically significant differences were detected in response or adverse effects, even though the lower-dilution group showed a slightly better response.[75]

Prager and colleagues evaluated two different dilutions (25 and 40 U/mL) of incobotulinumtoxinA in the treatment 40 patients with glabellar lines. They also found no significant difference in the initial results or after 4 months of follow up between the two groups.[76]

Blepharospasm

Boyle and colleagues studied the effect of high- and low-concentration onabotulinumtoxinA for the treatment of benign essential blepharospasm. They injected 16 patients with concentrations of 10 and 100 U/mL and followed them for 8 months. No significant differences were reported in efficacy or complications.[77]

Non Facial Muscles

Experimental Studies

Shaari and colleagues treated the tibialis anterior muscle in rats and found that a constant dose of 0.2 U of BoNT resulted in greater paralysis when injected in larger volumes.[78] Ten years later, Kim and colleagues injected 16 rabbits with 10 U of onabotulinumtoxinA at concentrations of 20 and 100 U/mL and drew the same conclusions.[79]

Limb Muscle Dystonias and Spasticity

A group from Korea[80,81] injected a fixed dose of 2.5 U of onabotulinumtoxinA into the human extensor digitorum brevis muscle in two dilutions (5 and 25 U/mL) and measured the compound muscle action potential amplitude. They concluded that a fivefold increase in volume did not enhance the paralyzing effect of onabotulinumtoxinA. Francisco and colleagues treated 13 patients with 60 U diluted to 50 or 100 U/mL for wrist and finger flexor spasticity and found no significant differences either.[82]

Gracies and colleagues[83] conducted a double-blind randomized controlled trial with 21 individuals with spastic elbow flexors using 160 U of onabotulinumtoxinA in two dilutions (20 and 100 U/mL). They found that the higher dilution group (20 U/mL) achieved greater neuromuscular blockade and spasticity reduction than an endplate-targeted (injection to areas known to be dense in endplates) or concentrated solution.

Two studies were conducted in children with cerebral palsy and limb spasticity. One used onabotulinumtoxinA in 38 children at concentrations of 12.5 ($n = 19$) and 50 U/mL ($n = 19$) in the gastrocnemius muscles for reducing ankle plantar flexor spasticity. It was found that, although there were no significant differences in physical evaluation and gait analysis, the large-volume group presented side effects more frequently, making a concentrated solution a better option.[84] In another randomized controlled trial, 22 spastic diplegic or quadriplegic children with cerebral palsy received a fixed dose of abobotulinumtoxinA. Dilutions of 500 U/5 mL and 500 U/1 mL were injected in each gastrocnemius muscle.[85] The more diluted preparation was considered more effective.

Hyperhidrosis

There is no standardized dilution for BoNT treatment of focal hyperhidrosis. Dilutions reported vary from 1 to 10 mL of saline[86–88] for onabotulinumtoxinA, with most clinicians using between 2 and 5 mL. As for abobotulinumtoxinA, reconstitution volumes vary between 1.25 and 10 mL,[86–89] the use of 2.5 to 5 mL being most frequent. In the only study with incobotulinumtoxinA for hyperhidrosis, the dilution used was 10 U/mL.[90]

In an open, comparative study, nine volunteers were injected in the axillary region; 3 U of onabotulinumtoxinA was diluted in 1, 2, 3, 4, and 5 mL of saline solution. Patient assessments using the iodine-starch test showed no difference in anhydrotic halos.[91] Another trial compared the same 5-U dose of abobotulinumtoxinA in three different dilutions (0.2, 0.4 and 0.6 mL) injected in the backs of three patients with compensatory hyperhidrosis. Larger anhydrotic halos were found with the more-diluted

abobotulinumtoxinA group, but after analyzing other interindividual variations, the authors considered differences between the several dilutions to be clinically irrelevant.[92]

Reviewing all published studies where a fixed dose of BoNTA was reconstituted with different volumes, five were favorable to greater diffusion or enhanced effect with higher volumes, whereas nine found no difference in efficacy. Three studies reported more discomfort with higher dilutions, even though they were not statistically significant. Controversy remains. For small muscles such as those located in the face or hand, it seems that there is no difference in results with greater dilutions. For large muscles of the limbs, greater volume might be advantageous. More studies with larger samples are necessary to clarify this.

Conclusion and Summary

There are several different preparations of BoNT licensed worldwide and others to come. Formulations are neither identical nor interchangeable.

Prescribing information on all available products recommend reconstitution in unpreserved saline, but studies in the literature cited suggest that other solutions can be used, such as preserved saline, anesthetics (lidocaine or bupivacaine) with or without epinephrine, and albumin. According to in vivo studies, onabotulinumtoxinA is not as fragile as originally thought, and foam during reconstitution does not inactivate the toxin.

Although manufacturers recommend storage at 2°C to 8°C in the refrigerator, toxin administration within 4 to 24 hours, and not freezing after reconstitution, it appears that some products can be stored for longer periods of time after reconstitution, in the refrigerator or the freezer, without loss of efficacy or contamination.

With regard to the best dilution to be used, when treating limb muscles, perhaps as a result of greater dispersion of the toxin, higher dilutions might be beneficial. For facial muscles, more-concentrated dilutions are preferred, because they produce less discomfort for patients.

Many of the precautions around BoNT use, often recommended by the manufacturers, are described in the clinical literature as too restrictive. The literature suggests that toxins may be sturdier and more resistant to degradation than previously understood.

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