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Botulinum toxin in wound treatment – literature review

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Abstract

Introduction and purpose

Botulinum toxin has been used in medicine since the 1970s, although many years earlier it was known only as a biological weapon. Originally, it was used in medicine to treat strabismus in children. Over time, it has been used in the treatment of neurological disorders, excessive sweating of the armpits, wrinkles, and bladder disorders. In recent years, botulinum toxin has also begun to be used in wound healing. The paralyzing effect of botulinum toxin on the muscles within the wound eliminates micro-injuries caused by tension in these muscles. The aim of the article is to summarize information on the effects of botulinum toxin on the wound, its impact on inflammation, and the type of scar formation.

Materials and methods

The literature included in the PubMed databases is searched through the words such as botulinum toxin, wounds, and scars.

Description of the state of knowledge:

The literature review shows the breadth of the issue. Scientific studies showing the effect of using botulinum toxin on wounds emphasize its beneficial effects. However, it should be remembered that wound healing has a complex, dynamic course supported by numerous cellular processes that must be closely coordinated to effectively repair damaged tissues. Determinants influencing the course of wound healing include both local and systemic factors. Important factors influencing the process of proper wound healing are specialized wound preparation, pharmacotherapy, and other aspects of therapeutic intervention.

Conclusions

The range of applications of botulinum toxin is constantly expanding. In addition to reducing muscle tension within the wound, it also inhibits the production of inflammatory transmitters, limiting its development, and also reduces the development of microcirculation by preventing the development of vessels in hypertrophic scars.

Keywords: botulinum toxin, wounds, scars, hypertrophic scars, skin ulcers

1. Introduction

Botulinum toxin (BoNT) – one of the most potent biological toxins in the world, in the past, it was known as a food safety hazard and a kind of biological weapon, now it is a highly effective drug in many diseases.[1,2] BoNT has been shown to be effective in neurological movement disorders, neuropathic pain, urology, dermatology, ophthalmology, otolaryngology, gynecology, plastic and general surgery.[3,4,5] It has been shown that reducing tension within the healing wound by denervating the subcutaneous muscle with botulinum toxin results in faster healing and less scarring and colloid formation.[6,7] Botulinum toxin type A inhibits the release of acetylcholine (ACh) in the neuromuscular synapse and causes reversible muscle paralysis. Its administration in the area of the surgical incision induces temporary muscle relaxation and paralysis, which leads to a reduction in tension. Its administration in the area of the surgical incision induces temporary muscle relaxation and paralysis, which leads to a reduction in tension.[7]

2. Objective of the work

The aim of the study is to describe the effects of botulinum toxin and to summarize information from available medical sources on its effect on wound healing.

3. Description of the state of knowledge

Botulinum toxin

Botulinum toxin (BoNT) is a neurotoxic substance produced by Clostridium botulinum, a Gram-positive anaerobic bacterium.[8] BoNT is typically identified in 7 serotypes with 40 subtypes. The most commonly used therapeutic drugs are botulinum toxin type A (BoNT-A) and B (BoNT-B).[1,8]

Pharmacodynamics of botulinum toxin

The main action of BoNT takes place in the neuromuscular synapse, where the toxin causes inhibition of the release of acetylcholine from the presynaptic part and muscle and autonomic paralysis. The entire process occurs in three phases: binding, internalization, and inhibition of neurotransmitter release.[9,10] The binding process involves the attachment of the heavy chain (via the receptor-binding domain) to substances on the surface of the nerve cell.

Subsequently, the toxin is internalized by binding to another surface receptor, reaching the synaptic interior.[1,3,11] In the third stage, BoNT acts on cholinergic presynaptic nerve terminals by cleaving and deactivating SNARE proteins, which, under physiological conditions, participate in the exocytosis of acetylcholine vesicles located at nerve terminals by attaching acetylcholine vesicles to the cell membrane. BoNT thus inhibits the release of acetylcholine, preventing muscle contractions and resulting in local weakness and paralysis.[12] The toxin has a reversible paralyzing effect. Paralysis is observed 2-5 days after BoNT injection, peaking at 5-6 weeks. The effect lasts for approximately 2-3 months.[11,13]

The medical application of botulinum toxin

Injected into muscle tissue, botulinum toxin relaxes muscles through neuromuscular blockade. This property is utilized in the treatment of dystonia, tics, cerebral palsy, spasticity, tremor disorders, and motor dysfunctions of the urinary and gastrointestinal tracts. Botulinum toxin has the ability to reduce sweat, saliva, and tear production when injected into exocrine glands, which is used in the treatment of hyperhidrosis and sialorrhea.[14] Literature also demonstrates that BoNT preparations have been applied in the treatment of cancer pain, post-radiation and postoperative pain, as well as neuropathic pain, diabetic neuralgia, trigeminal neuralgia, and migraines.[2,3,11] Botulinum toxin is widely used in aesthetic medicine, where its muscle-relaxing effect can reduce wrinkles caused by muscle overactivity.[14]

Wound healing

The wound-healing process is a crucial physiological mechanism aimed at maintaining the integrity of the skin after injury. It occurs in 3 phases: hemostasis/inflammation, proliferative phase, and remodeling phase.[15] Despite innate reparative abilities, many cellular repair reactions may be compromised, hindering wound closure. This compromise is most often a result of systemic pathological changes, such as advanced age or uncontrolled diabetes.[16] Imperfect wound healing can also have local sources, including wound location, prolonged

inflammation, wound infection, and delayed epithelialization.[17] Non-healing wounds, scars, and keloids expose patients to significant discomfort, anxiety, and reduce their quality of life. They contribute to decreased self-esteem and a sense of worth.[18,19] Wound healing requires the collaboration of various factors, such as professional dressings and modern wound treatment methods that accelerate the healing process, prevent infection, and reduce wound hypoxia.[19] Tension exerted perpendicular to the wound by muscles at its base causes microtrauma, prolonging the inflammatory phase. It leads to increased accumulation of collagen and glycosaminoglycans, enhanced fibroblast response, poor wound healing, and increased scarring. Various surgical techniques, such as deep sutures or undermining wound edges, have been developed to reduce muscle tension.[6,13] None of these methods completely eliminates muscle tension.[13,17] Review and research studies confirm the beneficial effects of using botulinum toxin type A (BoNT-A) to induce paralysis, known as chemoimmobilization. This procedure appears to have benefits in scar prevention, improving wound healing processes, and alleviating existing scars and keloids.[20].

4. Medical literature review

Kucukkaya D. et al. examining thirty adult female Wistar rats, observed a reduction in the degree of contraction within the wound area in individuals subjected to BoNT-A injections. It was found that BoNT-A reduces wound and graft contraction, as well as reduces the number of sebaceous cells and hair follicles.[21]

Zhou N. et al. investigated the effect of botulinum toxin type A on microcirculation in models of hypertrophic scars on rabbit ears. They demonstrated that BoNT-A injections immediately after modeling inhibit the expression of VEGF, reducing angiogenesis and thereby limiting the formation of hypertrophic scars.[22]

Major challenges in wound treatment include burns, which commonly lead to complications such as infections, inflammatory reactions, excessive protease expression, and scar formation. Oryana A. et al. investigated the impact of botulinum toxin type A (BoNT-A) and aprotinin (AP) separately or in combination on the healing process of burn wounds in rats. Both BoNT-A and AP significantly reduced the expression of interleukin-1β and transforming growth factor β1, while increasing the basic fibroblast growth factor.AP proved to be more effective

than BoNT-Aby reducing protease production, enhancing tissue organization and maturation, and improving the cosmetic appearance of wounds. The best results were achieved using a combination of BoNT-A and AP in the healing of burn wounds. Combined treatment significantly alleviated inflammation, reduced collagen density, and resulted in minimal scar formation. [23]

Facial wounds

Gassner HG. et al. conducted a study on the use of chemodenervation induced by botulinum toxin to improve the quality of scars on the foreheads of primates. Half of the incision was injected with 7 units of botulinum toxin diluted in 0.9% saline solution, while the other half was injected with a 0.9% saline solution. The authors reported a significantly better cosmetic outcome in the toxin-treated group, as well as a significantly lower level of inflammation observed in histopathological examination.[24]

Ziade M. et al. assessed whether early injection of botulinum toxin type A, inducing temporary muscle paralysis, reduces tension vectors at the wound edges and diminishes scarring in facial wounds. Thirty patients with facial wounds were enrolled in the study, randomly assigned to two groups, with or without the injection of BoNT-A within 72 hours post-surgery. BoNT-A was injected into facial muscles directly or indirectly involved in scar formation. During the one-year follow-up, observations were made on 24 patients. No statistically significant differences were found between the two groups in terms of patient scar assessment scores by the Patient Scar Assessment Scale, the Observer Scar Assessment Scale and the Vancouver Scar Scale. However, the median the Visual Analogue Scale rating by the six evaluators was 8.25 for the botulinum toxin-treated group compared to 6.35 for the control group. The result allows confirming the positive impact of BoNT-A on facial scars. [25]

Kima SH. et al. included forty-five patients with a lacerated forehead wound in the study, randomly assigning them to 2 groups, with or without BoNT-A injection. Twenty-four members of the study group received 5 IU/cm BoNT-A injections, while twenty-two patients from the control group received saline solution. Follow-ups were conducted at 1, 3, and 6 months. In all scar assessment scales (Observer Scar Assessment Scale, Stony Brook Scar Evaluation Scales, and Visual Analogue Scale), the results changed favorably in both groups, with greater changes observed in the BoNT-A group compared to the control group.The

researchers observed improvement in the aesthetic, functional, and emotional aspects of scar formation in the groups treated with BoNT-A. [26]

Wounds in plastic surgery

Lower eyelid blepharoplasty is a common cosmetic eyelid surgery. In Asian patients, the risk of hypertrophic and/or widened scars is higher compared to Caucasian patients. Berman B. et al. evaluated whether simultaneous transcutaneous lower eyelid blepharoplasty and BoNT-A injections could improve the quality of eyelid scars. This was a prospective, randomized, double-blind clinical trial involving 40 adults who underwent bilateral transcutaneous lower eyelid blepharoplasty. Patients were randomly assigned in equal proportions to receive injections of BoNT-A or saline into the lateral orbicularis oculi muscle immediately after wound closure. The results on the Vancouver Scar Scale and the Visual Analogue Scale in the experimental and control groups with a carrier were similar, but the width of scars in the experimental group at all measured points was significantly narrower than in the control group. Researchers concluded that scars following transcutaneous lower eyelid blepharoplasty in Asians can be significantly narrowed through simultaneous BoNT-A injection without additional complications. [27]

One of the most popular procedures in plastic surgery is breast augmentation surgery. Disphanurat W. et al. investigated the effectiveness of botulinum toxin type A in improving the appearance of submammary scar after primary breast augmentation. A prospective, double-blind, randomized controlled study involving 27 participants who underwent primary augmentative mammoplasty with subareolar incisions was conducted. Subcutaneous injections of BoNT-A were administered to the experimental group after skin closure. Scars were assessed at 3, 6, 9 months using the Patient and Observer Scar Assessment Scale, along with multispectral imaging analysis. Observations did not reveal significant subjective differences between the experimental and control groups, except that the experimental group showed significantly narrower scars at 6 and 9 months ($p < 0.001$) and better scar surface texture at 9 months ($p = 0.003$) compared to the control group. [28]

Wounds in general surgery

Li YH. et al. treated midline scars after sternotomy in 17 patients with botulinum toxin. Each scar was divided in half, and a randomly selected portion underwent botulinum toxin injection.

After 6 months of observation, the outcome was assessed using the Vancouver Scar Scale. The study showed a significant improvement in scar width and patient satisfaction for the halves of wounds treated with BoNT-A.[29]

Phillips TJ. et al. enrolled 40 patients who underwent total thyroidectomy, hemithyroidectomy, or parathyroidectomy to assess wound healing after botulinum toxin administration. The study demonstrated that administering the medication directly at the surgical site immediately after the operation showed no significant difference in scar extent compared to the control group. However, scars were smaller in individuals with a history of severe scarring.[30]

Chen S. et al. assessed how local injection of botulinum toxin type A prevents the development of postoperative scars. The analysis was conducted on 267 patients enrolled in the study and randomly assigned to the group receiving local injections of BoNT-A (184 patients) and placebo (182 patients). The review provided preliminary evidence confirming the efficacy and safety of BoNT-A in preventing postoperative scars.[31]

The beneficial impact of BoNT on wound healing has also been demonstrated in patients undergoing surgical reconstruction after Mohs micrographic surgery for skin cancer by Flynn TC. Periodic muscle relaxation associated with BoNT facilitated a more effective healing process by immobilizing the target area. BoNT-A and -B have been shown to yield equally effective results.[32]

Cleft lip

The scar formed after surgical treatment of a cleft lip constitutes an aesthetically undesirable complication of the procedure. The functional orbicularis oris muscle beneath the scar maintains a zone of dynamic tension, leading to the stretching of the scar. Sonane'a J. et al. conducted a prospective, randomized controlled trial involving 28 infants with unilateral cleft lip undergoing primary lip correction. The infants were randomly assigned to a group receiving intraoperative injection of BoNT-A or saline (control group). The substances were administered into the adjacent orbicularis oris muscle immediately after completing the cleft lip repair surgery. Experts reassessed the scar after 6 months using a visual analog scale, the Vancouver Scar Scale, and photographic measurements of scar width.Children in the BoNT-A group demonstrated a statistically significantly better outcome on the visual analog scale and

a smaller scar width compared to the control group. However, the difference in the Vancouver Scar Scale score between the two groups was not statistically significant. When comparing patients with cleft lip only to those with cleft lip and palate, no statistically significant differences were found in the scales used and scar width. No complications associated with the use of botulinum toxin A were observed. Researchers concluded that the injection of botulinum toxin type A is a safe and effective adjunct in improving the appearance of scars after cleft lip surgery. The use of BoNT-A improved scar appearance in terms of width, but no improvement in scar pigmentation was observed. [33]

Chang CS. et al. utilized botulinum toxin to improve outcomes in the treatment of cleft lip in 60 patients with unilateral cleft lip undergoing primary cheiloplasty. Six months after injecting BoNT into the adjacent orbicularis oris muscle, scars appeared better and narrower, but no additional benefits were observed regarding scar pigmentation, vascularity, elasticity, or growth.[34]

Skin ulcers

Currently, some sources have mentioned the use of botulinum toxin in the treatment of chronic skin ulcers. It appears to support wound healing by inhibiting the release of norepinephrine and various neurotransmitters that restrain vessel constriction and increase blood flow.[32,35,36] Zhong J. et al. described four different cases of chronic skin ulcer treatment using botulinum toxin. The procedure involved local, multipoint, cyclic, evenly spaced subcutaneous injections of BoNT-A at a depth of 6-8 mm. The ulcerated area was significantly reduced, and the ulceration was healed within 20 to 48 days.[35]

Shenavandeh S. et al. investigated the clinical benefits, changes in capillaroscopy, and cost-effectiveness of local injection of botulinum toxin A and intravenous analogs of prostaglandins (iloprost/alprostadil) in patients with systemic sclerosis and treatment-resistant finger ulcers. In the clinical study, 26 patients with treatment-resistant finger ulcers were assessed. Before treatment and one month after treatment, visual analog scales for pain and Raynaud's phenomenon, skin color, ulcer type, and capillaroscopy were evaluated. Both BoNT-A and prostaglandins aided in the healing and pain control of finger ulcers. Microhemorrhages significantly decreased in both groups in capillaroscopy. However, the

cost of outpatient treatment with botulinum toxin was considerably lower, allowing for greater time and cost savings. [37]

Acne scars

Common acne is a prevalent skin condition, especially among adolescents, affecting 95–100% of boys and 83–85% of girls. Persistent complications of acne include scars, which occur in 95% of affected individuals, resulting from the destruction of collagen fibers and subcutaneous fat. [38] Treatment of atrophic acne scars includes chemical peels, dermabrasion, needling, and lasers. However, each method has its limitations, and ultimately, the improvement in results is not satisfactory when considering the costs, time involved, and potential complications. [39] Ibrahim AM. et al.conducted a study among 30 patients with inflammatory acne to compare the clinical effectiveness and safety of a long-pulsed Nd:YAG laser with a wavelength of 1064 nm to botulinum toxin type A in the treatment of inflammatory acne. Researchers observed that both the long-pulsed Nd:YAG laser (1064 nm) and BoNT-A injections are safe and effective in acne therapy. Nd:YAG laser exhibits longer efficacy and lower recurrence rates compared to the use of BoNT-A. [40]

Hypertrophic scars

The application of botulinum toxin has been extended to the treatment of hypertrophic scars. [13] In pathological wounds, the inflammatory phase is prolonged, leading to the formation of hypertrophic scars and keloids. These scars are characterized by expanded, itchy, reddish elevations above the skin. [20] The pathophysiology of keloid and hypertrophic scars is not yet fully understood. Their formation is associated with various cytokines, including interleukin 6, 8, and 10, as well as various growth factors, such as transforming growth factor beta and platelet-derived growth factor. Treatment methods for keloid and hypertrophic scars encompass both conventional therapies, such as occlusive dressings, compression therapy, and steroids; surgical and cryosurgical therapies; as well as adjuvant and novel therapies, including radiotherapy, interferon, 5-fluorouracil, tacrolimus, sirolimus, bleomycin, doxorubicin, transforming growth factor beta, epidermal growth factor, verapamil, retinoic acid, tamoxifen, botulinum toxin A, and hydrogel scaffold. [41] Botulinum toxin, by inhibiting neurotransmitters such as glutamate and substance P, reduces the release of inflammatory mediators: bradykinin, prostaglandins, and serotonin. [13]

Xiao et al. injected botulinum toxin type A into hypertrophic scars (an average of 35 units of botulinum toxin type A once a month for three months). They demonstrated improvement in redness, itching sensation, and elasticity assessment in treated patients. Limitations of this study include the absence of a control group and a relatively short observation period.[42]

Elshahed AR. et al conducted a randomized, double-blind, controlled study to assess the safety and efficacy of botulinum toxin type A injections in hypertrophic scars. Thirty patients with old scars (1–15 years) were treated, with sides randomly assigned to receive either BoNT-A or a 0.9% saline solution once a month for three consecutive months. Scars were evaluated using the Vancouver Scar Scale along with standardization of digital photography. The average Vancouver Scar Scale score for scars treated with BoNT-A decreased from 7.29 ± 2.327 before injections to 5.33 ± 2.41 after injections, which was highly significant ($p = 0.01$). In the control group, the decrease was minimal, from 7.29 ± 2.327 before saline injection to 7.10 ± 2.234 after injection ($p = 0.104$). The BoNT-A treated group showed significant clinical and cosmetic improvement. [43]

5. Summary

Thousands of years of evolution have made our skin a multifunctional organ that protects us from the daily onslaught of chemicals, physical, and ultraviolet radiation. In elderly or diabetic patients, the skin's regenerative capacity weakens, resulting in a poorly healing wound. These wounds represent a significant socio-economic burden due to their high incidence and recurrence. Therefore, there is an urgent need to improve the biological and clinical understanding of the mechanisms underlying wound repair and to explore potential methods to improve the wound healing process. Botulinum toxin injections, still a novelty in the medical world for this purpose, represent one such method. The future holds great promise for the development of innovative therapeutic strategies in advanced wound care. Botulinum toxin, through its paralyzing action on the muscles in the wound area, prevents the expansion of the wound and the formation of scars. Additionally, in numerous scientific publications, it has been documented that botulinum toxin exhibits anti-inflammatory, antioxidant properties and inhibits angiogenesis within the wound area.Thanks to its use in the wound healing process, we will be able to obtain a narrower, more aesthetic scar.

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References

1. Anandan Ch, Jankovic J. Botulinum Toxin in Movement Disorders: An Update. Toxins (Basel). 2021 Jan; 13(1): 42. doi: 10.3390/toxins13010042. PMID: 33430071. PMCID: PMC7827923.
2. Dressler D, Johnson EA. Botulinum toxin therapy: past, present and future developments. J Neural Transm (Vienna).2022; 129(5-6): 829–833. doi: 10.1007/s00702-022-02494-5, PMID: 35396965. PMCID: PMC9188496.
3. Park JH, Park HJ. Botulinum Toxin for the Treatment of Neuropathic Pain. Toxins (Basel). 2017 Sep; 9(9): 260. doi: 10.3390/toxins9090260. PMID: 28837075. PMCID: PMC5618193.
4. Solish N, Carruthers J, Kaufman J, Rubio RG, Gross TM, Gallagher CJ. Overview of DaxibotulinumtoxinA for Injection: A Novel Formulation of Botulinum Toxin Type A. Drugs. 2021; 81(18): 2091–2101. doi: 10.1007/s40265-021-01631-w. PMID: 34787840. PMCID: PMC8648634.
5. Freeman MD, Margulies IG, Sanati-Mehrizy P, Burish N, Taub PJ. Nonaesthetic Applications for Botulinum Toxin in Plastic Surgery. Plast Reconstr Surg. 2020 Jul;146(1):157-170. doi: 10.1097/PRS.0000000000006908. PMID: 32590660.
6. Dhawan A, Dhawan S, Vitarella D. The Potential Role of Botulinum Toxin in Improving Superficial Cutaneous Scarring: A Review. J Drugs Dermatol. 2018 Sep 1;17(9):956-958. PMID: 30235381.

7. Wang YX, Wang Y, Zhang Q, Zhang RD. Current Research of Botulinum Toxin Type A in Prevention and Treatment on Pathological Scars. Dermatol Surg. 2023 May 1;49(5S):S34-S40. doi: 10.1097/DSS.0000000000003770. PMID: 37115998.
8. Park J, Chung ME. Botulinum Toxin for Central Neuropathic Pain. Toxins (Basel). 2018 Jun; 10(6): 224. doi: 10.3390/toxins10060224. PMID: 29857568. PMCID: PMC6024683.
9. Matak I, Lacković Z. Botulinum toxin A, brain and pain. Prog Neurobiol. 2014 Aug-Sep:119-120:39-59. doi: 10.1016/j.pneurobio.2014.06.001. PMID: 24915026.
10. Evans DM, Williams RS, Shone CC, Hambleton P, Melling J, Dolly JO. Botulinum neurotoxin type B. Its purification, radioiodination and interaction with rat-brain synaptosomal membranes. Eur J Biochem. 1986 Jan 15;154(2):409-16. doi: 10.1111/j.1432-1033.1986.tb09413.x. PMID: 3753681.
11. Grenda T, Grenda A, Krawczyk P, Kwiatek K. Botulinum toxin in cancer therapy—current perspectives and limitations. Appl Microbiol Biotechnol. 2022; 106(2): 485–495. doi: 10.1007/s00253-021-11741-w. PMID: 34951660. PMCID: PMC8763801.
12. Castiglione A, Bagnato S, Boccagni C, Romano MC, Galardi G. Efficacy of intra-articular injection of botulinum toxin type A in refractory hemiplegic shoulder pain. Arch Phys Med Rehabil. 2011 Jul;92(7):1034-7. doi: 10.1016/j.apmr.2011.01.015. PMID: 21704782.
13. Al-Qattan MM, Al-Shanawani BN, Alshomer F. Botulinum toxin type A: implications in wound healing, facial cutaneous scarring, and cleft lip repair. Ann Saudi Med. 2013 Sep-Oct; 33(5): 482–488. doi: 10.5144/0256-4947.2013.482. PMID: 24188943. PMCID: PMC6074896.
14. Dressler D. Clinical applications of botulinum toxin. Curr Opin Microbiol. 2012 Jun;15(3):325-36. doi: 10.1016/j.mib.2012.05.012. PMID: 22770659.
15. Wang PH, Huang BS, Horng HC, Yeh CC, Chen YJ. Wound healing.J Chin Med Assoc. 2018 Feb;81(2):94-101. doi: 10.1016/j.jcma.2017.11.002. PMID: 29169897.
16. Wilkinson HN, Hardman MJ. Wound healing: cellular mechanisms and pathological outcomes. Open Biol. 2020 Sep; 10(9): 200223. doi: 10.1098/rsob.200223. PMID: 32993416. PMCID: PMC7536089.
17. Schlessinger J, Gilbert E, Cohen JL, Kaufman J. New Uses of AbobotulinumtoxinA in Aesthetics. Aesthet Surg J. 2017 May; 37(Suppl 1): S45–S58. doi: 10.1093/asj/sjx005. PMID: 28388720. PMCID: PMC5434494.
18. Gouin JP, Kiecolt-Glaser JK. The Impact of Psychological Stress on Wound Healing: Methods and Mechanisms. Immunol Allergy Clin North Am. Immunol Allergy Clin North Am. 2011 Feb; 31(1): 81–93. doi: 10.1016/j.iac.2010.09.010. PMID: 21094925. PMCID: PMC3052954.
19. Han G, Ceilley R. Chronic Wound Healing: A Review of Current Management and Treatments. Adv Ther. 2017; 34(3): 599–610. doi: 10.1007/s12325-017-0478-y. PMID: 28108895. PMCID: PMC5350204.
20. Guo X, Song G, Zhang D, Jin X. Efficacy of Botulinum Toxin Type A in Improving Scar Quality and Wound Healing: A Systematic Review and Meta-Analysis of

Randomized Controlled Trials. Aesthet Surg J. 2020 Apr 14;40(5):NP273-NP285. doi: 10.1093/asj/sjz165. PMID: 31155638.
21. Kucukkaya D, Irkoren S, Ozkan S, Sivioglu N. The effects of botulinum toxin A on the wound and skin graft contraction. J Craniofac Surg. 2014 Sep;25(5):1908-11. doi: 10.1097/SCS.0000000000000941. PMID: 25102391.
22. Zhou N, Li D, Luo Y, Li J, Wang Y. Effects of Botulinum Toxin Type A on Microvessels in Hypertrophic Scar Models on Rabbit Ears. Biomed Res Int. 2020; 2020: 2170750. doi: 10.1155/2020/2170750. PMID: 32626735. PMCID: PMC7315252.
23. Oryana A, Alemzadeh E. Comparison of botulinum toxin type A and aprotinin monotherapy with combination therapy in healing of burn wounds in an animal model. Mol Biol Rep. 2020 Apr;47(4):2693-2702. doi:10.1007/s11033-020-05367-w. PMID: 32146683.
24. Gassner HG, Sherris DA, Otley CC. Treatment of facial wounds with botulinum toxin A improves cosmetic outcome in primates. Plast Reconstr Surg. 2000 May;105(6):1948-53; discussion 1954-5. doi: 10.1097/00006534-200005000-00005. PMID: 10839391.
25. Ziade M, Domergue S, Batifol D, Jreige R, Sebbane M, Goudot P, Yachouh J. Use of botulinum toxin type A to improve treatment of facial wounds: a prospective randomised study. J Plast Reconstr Aesthet Surg. 2013 Feb;66(2):209-14. doi: 10.1016/j.bjps.2012.09.012. PMID: 23102873.
26. Kim SH, Lee SJ, Lee JW, Jeong HS, Suh IS. Clinical trial to evaluate the efficacy of botulinum toxin type A injection for reducing scars in patients with forehead laceration: A double-blinded, randomized controlled study. Medicine (Baltimore). 2019 Aug;98(34):e16952. doi: 10.1097/MD.0000000000016952. PMID: 31441893. PMCID: PMC6716761.
27. Huang YI, Wallace CG, Hsiao YC, Lee MC, Huang JJ, Chang FCS, Chen ZC, Hu S, Chen JP. Botulinum Toxin to Improve Lower Blepharoplasty Scar: A Double-Blinded, Randomized, Vehicle-Controlled Clinical Trial. Aesthet Surg J. 2021 Aug 13;41(9):1003-1010. doi: 10.1093/asj/sjab024. PMID: 34128526.
28. Disphanurat W, Viarasilpa W, Thienpaitoon P. Efficacy of Botulinum Toxin A for Scar Prevention After Breast Augmentation: A Randomized Double-Blind Intraindividual Controlled Trial. Dermatol Surg. 2021 Dec 1;47(12):1573-1578. doi: 10.1097/DSS.0000000000003198. PMID: 34818273.
29. Li YH, Yang J, Liu JQ, Xie ST, Zhang YJ, Zhang W, Zhang JL, Zheng Z, Hu DH. A Randomized, Placebo-Controlled, Double-Blind, Prospective Clinical Trial of Botulinum Toxin Type A in Prevention of Hypertrophic Scar Development in Median Sternotomy Wound. Aesthetic Plast Surg. 2018 Oct;42(5):1364-1369. doi: 10.1007/s00266-018-1187-x. PMID: 30019241.
30. Phillips TJ, Fung E, Rigby MH, Burke E, Hart RD, Trites JRB, Gassner HG, Taylor SM. The Use of Botulinum Toxin Type A in the Healing of Thyroidectomy Wounds: A Randomized, Prospective, Placebo-Controlled Study. Plast Reconstr Surg. 2019 Feb;143(2):375e-381e. doi: 10.1097/PRS.0000000000005264. PMID: 30688903.

31. Chen S, Yang Y, Zhang D, Liu Y, Li Y. Local Injection of Botulinum Toxin Type A to Prevent Postoperative Scar. J Craniofac Surg. 2020 Mar/Apr;31(2):440-443. doi: 10.1097/SCS.0000000000006144. PMID: 31977704.
32. Flynn TC. Use of intraoperative botulinum toxin in facial reconstruction. Dermatol Surg. 2009 Feb;35(2):182-8. doi: 10.1111/j.1524-4725.2008.34407.x. PMID: 19215253.
33. Sonane J, Sharma RK, John JR, Sharma R. Botulinum Toxin for a Better Scar in Cleft Lip Surgery: A Prospective Randomized Control Trial. J Craniofac Surg. 2022 Jan-Feb;33(1):198-202. doi: 10.1097/SCS.0000000000007836. PMID: 34267122.
34. Chang CS, Wallace CG, Hsiao YC, Chang CJ, Chen KT. Botulinum toxin to improve results in cleft lip repair. Plast Reconstr Surg. 2014 Sep;134(3):511-516. doi: 10.1097/PRS.0000000000000416. PMID: 25158709.
35. Zhong J, Lan Y, Fu S, Zhang J, Lu S, He Y, Zhang JM. Botulinum Toxin A Injection for Treatment of Chronic Skin Ulcer: A Case Series and Literature Review. Int J Low Extrem Wounds. 2019 Mar;18(1):97-103. doi: 10.1177/1534734618816589. PMID: 30696317.
36. Winayanuwattikun W, Vachiramon V. Botulinum Toxin Type A for the Treatment of Skin Ulcers: A Review Article. Toxins (Basel). 2022 Jun; 14(6): 406. doi: 10.3390/toxins14060406. PMID: 35737067. PMCID: PMC9230442
37. Shenavandeh S, Sepaskhah M, Dehghani S, Nazarinia MA. A 4-week comparison of capillaroscopy changes, healing effect, and cost-effectiveness of botulinum toxin-A vs prostaglandin analog infusion in refractory digital ulcers in systemic sclerosis. Clin Rheumatol. 2022 Jan;41(1):95-104. doi: 10.1007/s10067-021-05900-7. PMID: 34471968. PMCID: PMC8409478.
38. Liu L, Xue Y, Chen Y, Chen T, Zhong J, Shao X, Chen J.Prevalence and risk factors of acne scars in patients with acne vulgaris. Skin Res Technol. 2023 Jun;29(6):e13386. doi: 10.1111/srt.13386. PMID: 37357642. PMCID: PMC10240192.
39. Amer A, Elhariry S, Al-Balat W. Combined autologous platelet-rich plasma with microneedling versus microneedling with non-cross-linked hyaluronic acid in the treatment of atrophic acne scars: Split-face study. Dermatol Ther. 2021 Jan;34(1):e14457. doi: 10.1111/dth.14457. PMID: 33107665.
40. Ibrahim AM, Omar GAB, Hamdino M. Long-pulsed Nd: YAG laser (1064 nm) versus intralesional botulinum toxin type (A) in acne vulgaris therapy: a split face study. Int J Dermatol. 2023 Jun;62(6):822-830. doi: 10.1111/ijd.16519. PMID: 36468835.
41. Berman B, Maderal A, Raphael B. Keloids and Hypertrophic Scars: Pathophysiology, Classification, and Treatment. Dermatol Surg. 2017 Jan:43 Suppl 1:S3-S18. doi: 10.1097/DSS.0000000000000819. PMID: 27347634.
42. Xiao Z, Zhang F, Lin W, Zhang M, Liu Y. Effect of botulinum toxin type A on transforming growth factor beta1 in fibroblasts derived from hypertrophic scar: a preliminary report. Aesthetic Plast Su. 2010 Aug;34(4):424-7. doi: 10.1007/s00266-009-9423-z. PMID: 19802513.
43. Elshahed AR, Elmanzalawy KS, Shehata H, ElSaie ML. Effect of botulinum toxin type A for treating hypertrophic scars: A split-scar, double-blind randomized

controlled trial. J Cosmet Dermatol. 2020 Sep;19(9):2252-2258. doi: 10.1111/jocd.13627. PMID: 32668499.