EUROPEAN ACADEMIC RESEARCH
Vol. X, Issue 6/ September 2022

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Botulinum toxin A as a treatment for facial rejuvenation

GOVINDAN KESINI CASTHURI, MD, MS
General Surgery Resident
Department of Plastic and Aesthetic Center
The First Affiliated Hospital of Zhejiang University
LI KAI, MD, MS
General Surgery Resident
Department of Plastic and Aesthetic Center
The First Affiliated Hospital of Zhejiang University
LI TING, MD, MS
General Surgery Resident
Department of Plastic and Aesthetic Center
The First Affiliated Hospital of Zhejiang University
WU HUI LING, MD, MS [1]
Professor and Head of the Department
Department of Plastic and Aesthetic Center
The First Affiliated Hospital of Zhejiang University

Abstract

The typical ageing course changes the well-balanced and symmetrical facial structures found in youth, impacting physical beauty and self-esteem, and causing misunderstandings based on facial miscues. Skin ageing is also a health concern, resulting in compromised skin integrity, impaired or delayed wound healing, and a higher risk of infection and skin malignancies. Signs and symptoms of skin ageing might be an indication of the internal ageing course of the human body since they are visible and can be studied noninvasively. Facial rejuvenation is quickly becoming a significant field in aesthetic medicine. It is fundamental for the plastic surgeon to be familiar with the various facial rejuvenation techniques, whether they be surgical or non-surgical. This literature review aims to summarize the ageing process and the use of botulinum toxin A injection in facial rejuvenation.

Keywords: Skin ageing, facial ageing, facial rejuvenation, non-surgical rejuvenative method, Botulinum Toxin A.

INTRODUCTION:

Ageing is an inevitable process. As an individual ages, he or she goes through a complex but expected process of transformation that gradually takes away their

[1] Correspondence: Wu Hui Ling, MD, MS, Professor and Head of the Department, Department of Department of Plastic and Aesthetic Center, The First Affiliated Hospital of Zhejiang University No.79 Qingchun Road, Hangzhou 310003, Zhejiang Province, P.R. China, Email: zywhl@zju.edu.cn

youthfulness.(Akinbiyi et al. 2020) For a great many years, the concept of beauty has been associated with youthfulness. For example, youth is portrayed as smooth, wrinkle-free skin. However, ageing manifests in many other ways, whether it is a recessed hairline, drooping of the upper eyelid, tear trough concavity, worsening nasolabial folds and marionette lines or flabbiness of the cervical skin.(Panda and Chowdhary 2021)Facial aging results from both intrinsic and extrinsic causes. Intrinsic aging is the biological aging process over the years, and it is unstoppable. In addition to intrinsic aging, extrinsic ageing occurs due to sun exposure or environmental harm such as pollution. Together, these processes contribute to a loss of structural stability and physiologic function. As demand for facial rejuvenation increases throughout the years, cosmetic injections are being highlighted more because of their low cost, faster recovery time, and minimal invasiveness.

About 200 years ago, Botulinum toxin (BonT) was discovered and found to be one of the most poisonous substances ever known. They were briefly used as biological warfare, but with further studies were turned into medical treatment. Botulinum toxin injection for cosmetic use was first reported in an article published by R.P Clark in 1989.(Clark and Berris 1989) BonT has several serotypes (A, B, C, D, E, F, and H) and the ones that are used universally are botulinum toxin types A (BonT A) and B.(Dressler 2020)BonT A was introduced not too long ago to counteract the effects of aging. Botulinum toxin type A works by inhibiting the acetylcholine receptor at the neuromuscular junction, causing chemodenervation. This in turn temporarily relaxes the targeted muscle treating dynamic wrinkles and reducing static ones.

Anatomy of the ageing face

From the superficial skin to the deep skeletal layer, the face comprises various tissue layers that all go through different aging processes. Genetic and environmental factors contribute to changes in skin turgor, pigmentation, and strength leading to skin wrinkle formation.(Akinbiyi et al. 2020)

Keratinocytes from the basal layer produce keratins and move upwards toward the epidermis as they mature.(Panda and Chowdhary 2021)By then, they have become prokaryotic and are called corneocytes. This keratinization process takes place in 40-50 days; a period called turnover time.(Panda and Chowdhary 2021) During this epidermal turnover, damaged cells are eliminated and replaced by regenerated, healthy ones. The dermis contains specialized fibroblasts that produce collagen and elastin, two significant proteins that aid in skin strength and turgor, respectively.(Panda and Chowdhary 2021)

Ageing occurs through two distinct processes, intrinsic and extrinsic aging. Intrinsic aging consists of a series of inevitable innate physiological factors that cause in-built degenerative processes in the body. Epidermal cells no longer regenerate at their usual pace, collagen and elastin production declines in the dermis. The effects include visible wrinkles, dried up, thinning and translucent, and loosened skin.(Panda and Chowdhary 2021)

Extrinsic aging occurs due to external factors such as pollution, UV radiation, smoking, sleeping position, chemical exposure, and so on. Along with the internal factors mentioned, these circumstantial factors cause early-onset facial aging. For instance, since the facial skin is the most likely to be exposed to UV radiation, it undergoes photoageing earlier than any other part of the body.(Panda and Chowdhary

2021)Photoageing damages the skin such that it loses its regenerative ability. In addition, collagen formation is impaired with continuous exposure to UV radiation. With elastin production also defective, facial skin becomes loose and wrinkled faster than usual.

The subcutaneous fat is what shapes and adds volume to the face. What defines a youthful face is the complex but harmonious distribution of superficial and deep fat which is greatly compartmentalized. With the combined effect of aging and gravity, fat descends vertically causing loss of volume and creating visible grooves along the face. Fat loss occurs most commonly around the eyes, forehead and mandible as well as around the mouth and chin area.(Panda and Chowdhary 2021) Static wrinkles are caused by the skin stretching out over the bones due to the fat reduction over muscles.(Ilankovan 2014)

The superficial musculoaponeurotic system (SMAS) divides the subcutaneous fat into superficial and deep layers.(Panda and Chowdhary 2021) It connects the facial muscles with the dermis. With aging and the repeated use of our facial muscles, the strength of the SMAS abates and it loses its ability to hold up the muscles, fat, and skin. The facial structures tend to drop because of this loss of strength in combination with gravitational pull.(Panda and Chowdhary 2021)

The facial skeleton acts as a structural cage for soft tissues of the face.(Swift et al. 2021) It has been proven that the facial skeleton keeps remodeling throughout life, growing continually in a vertical direction as well as resorbing in specific areas such as the periorbital region, the midface, around the nose and the mandible.(Ilankovan 2014) The orbital foramen expands with age in both height and width. Resorption occurs more in the superomedial and inferolateral directions causing support loss and volume displacement of the overlying soft tissue.(Ilankovan 2014, Mendelson and Wong 2012, Akinbiyi et al. 2020)

Medially, the maxilla and laterally, the zygoma outline the midface skeleton. The glabellar and maxillary angles continuously decrease with age as the maxilla detrudes causing the malar fat pad to glide down and forwards making the nasolabial fold more obvious.(Ilankovan 2014)

The pyriform aperture expands with age while the borders of the nasal bone retrude.(Mendelson and Wong 2012) Since the anterior nasal spine recedes, it reduces the bony support which contributes to the retraction of the columella leading to the stooping of the nasal tip and giving the illusion of a lengthier nose.(Panda and Chowdhary 2021)

As for the lower third of the face, the mandibular angle knowingly increases while the mandibular height and length reduce with age, giving the appearance of a 'witch's chin'.(Panda and Chowdhary 2021, Mendelson and Wong 2012) Bone resorption below the mental foramen will create a 'prejowl sulcus', a known feature of an aged face.(Ilankovan 2014)

Factors Affecting Ageing

As the organ with the largest contact area between the human body and the external environment, it is no wonder that the skin shows the most visible signs of ageing due to old age, UV exposure and pollution.8 Ageing of the skin is influenced by several factors that can be divided between time-dependent intrinsic factors (such as genetic predisposition, skeletal changes, hormone levels) and extrinsic factors (such as

ultraviolet radiation exposure, smoking, diet, pollution among others). Skin ageing can be divided into chronological aging and photoageing. Chronological ageing occurs throughout the body whereas as the name implies, photoageing occurs on the body's light exposed surfaces. Caused by natural intrinsic factors, chronological ageing is difficult to counter, but delaying photoageing is not impossible because external factors cause it.

Histological changes in skin ageing

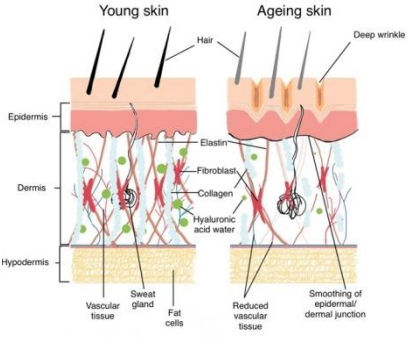

Figure 1. Differences between young skin and ageing skin

Aging is characterized by the cumulative effect of intracellular macromolecular damage, gradual loss of physiological integrity and compromised ability of stem cells to perform tissue regeneration. Skin ageing exhibits through chronological ageing and photoageing, two processes that although complementary, have different clinical mechanisms and features. Chronological ageing naturally happens after a few years and is influenced by various factors such as race, individual and skin site.

Skin aged intrinsically manifests by thinning of the epidermis and dermis, appearance of fine lines, reduced number of cells and decreased blood flow as shown in figure 1. On the other hand, photoageing causes skin to be thicker with deeper wrinkles and discoloration, while still reducing dermal thickness. Blood vessels become dilated, cells are infiltrated with inflammatory cytokines and elastic material builds up.(Wilkinson and Hardman 2021) One unfailing structural change in aged skin is the smoothing of the epidermal-dermal junction by more than 33% which occurs due to the dermal papillae loss and an increase in space between layers.(Neerken et al. 2004, Sudel et al. 2005)

This smoothing which usually begins in in the sixth decade, leads to loss of resistance to damaging pressures and an increased susceptibility to trauma. The thinned-out surface between the epidermis and dermis also results in a reduced supply of oxygen and nutrients and an even higher risk of separation between the two strata, a process through which wrinkles may develop.(Farage et al. 2013, Sudel et al. 2005)

Changes on the cellular level include but are not limited to epidermal degeneration, decrease in the number of fibroblasts and collagen fibers found in the dermis, overall decreased molecular activity, thinning out, and disorganized function. Decreased epidermal turnover leads to morphological changes in keratinocytes and corneocytes, keratinocytes become shorter and fatter while corneocytes become bigger. With age, a thinning dermis leads to reduced vascular tissue and cellularity. The

number of mast cells and fibroblasts also decreases which inevitably leads to a decrease in collagen synthesis and therefore a decrease in collagen turnover. A decrease in the number of fibroblasts also means a decline in production of glycosaminoglycans, more specifically hyaluronic acid.

Elastic fibres degrade over time with decreased elastin production and increased calcification. The dermis loses molecular integrity leading to reduced flexibility, torsion extensibility and elasticity.(Wilkinson and Hardman 2021) This occurs faster in women, with an associated susceptibility to tear-type injuries. Although the overall proportion of body fat increases until a person reaches his or her seventies, subcutaneous fat as a whole subsides with age.(Wilkinson and Hardman 2021)

Intrinsic and extrinsic ageing both are the cause of Langerhans cells (LCs) reduction, rete ridges loss at the dermo-epidermal junction, accumulated senescent cells, skin drying out and cross-linking of ECM fibres. Furthermore, augmented production of matrix metalloproteinases (MMPS) result in collagen, elastin and elaunin degradation.

Photoageing

Photoaged skin is illustrated by various histological findings different from those of chronologically aged skin. Under physiologic conditions and especially in developed areas, the human skin is exposed to solar UVA/UVB radiation as well as air pollution from transportation systems. UVA is the primary cause of photoageing since it penetrates deep into the skin, reducing antioxidant mechanisms while UVB is mostly absorbed by the epidermis.(Lee, Hong, and Kim 2021, Sardy 2009, D'Orazio et al. 2013)

UVA fast tracks the breakdown of collagen by increasing matrix metalloproteinases (MMPs) production, resulting in tissue destruction and dermal extracellular matrix (ECM) degeneration. UVA also changes proteoglycan composition of the skin by suppressing the synthesis of hyaluronic acid (HA). Since UVB is mostly absorbed by the epidermis, it can possibly damage the keratinocytes of the epithelial layer, and cause mutations such that they release soluble cytokines which will eventually lead to the appearance of symptoms such as aging, inflammation, apoptosis and carcinogenesis. Keratinocytes, however, are more resistant than fibroblasts when exposed to UVB due to their potent antioxidant properties. They are more resilient to the harmful effects of oxidants and are more susceptible to reactive oxygen species (ROS)-induced apoptosis. Furthermore, senescent skin fibroblasts cause darkening of skin by upregulating melanogenesis which acts as a protective mechanism against photodamage.(Eller, Yaar, and Gilchrest 1994)In a cadaveric study in 1979, it was found that darker skin provides more protection against UV radiation (UVR) than the fairer skin due to increased melanin.(Kaidbey et al. 1979, Lee, Hong, and Kim 2021)

Molecular Mechanism of ageing

Cellular senescence, oxidative stress, gene mutations and chronic inflammation are part of the molecular mechanism of skin ageing.

1. Oxidative stress

According to the free radical theory, ageing occurs as a result of cumulative cellular damage caused by free radical attack. Reactive oxygen species (ROS) help in regulating

cell signaling and defending the skin against harmful bacteria.(Pourzand, Albieri-Borges, and Raczek 2022)When it comes to skin aging and damage, oxidative stress is an essential factor. It achieves senescence by increasing the production of ROS. The combination of UV exposure and the reaction between skin and oxygen produces ROS. ROS accumulation causes DNA damage, stimulates skin's inflammatory response, decreases antioxidant enzymes, activates nuclear factor kappa B (NF-kB) and activator protein 1(AP-1) to hinder collagen production and increases MMPs to unbind collagen and associated proteins in the dermis, eventually leading to skin ageing.(Cao et al. 2020)

2. DNA damage

Recent studies have shown that UVA exposure can compromise DNA repair in human cells, which is done by causing damage to the DNA repair proteins.(Karran and Brem 2016, Pourzand, Albieri-Borges, and Raczek 2022, Rajapakse et al. 2020) UVA irradiation is capable of damaging DNA both directly and indirectly. In direct DNA damage by UV irradiation, DNA molecules absorb UVB photons resulting in reorganization of the nucleotide sequence leading to DNA strand mutation.(Tyrrell 1996)In indirect DNA damage, DNA absorbs UVA, promoting electron and energy transfer to oxygen molecules and forming oxygen free radicals. Both UVA and UVB have oncogenic effects on the skin, especially when it comes to the fair-skinned population.(Silva, Michniak-Kohn, and Leonardi 2017, Pinnell 2003, Cadet, Douki, and Ravanat 2015, Courdavault et al. 2004, Mouret et al. 2006)

3. Shortening of telomere

Telomeres are short segments of DNA that are thought to be biomarkers of aging. They occur at the very ends of chromosomes and their functions are to maintain chromosomal integrity and control the cell cycle.(Gu et al. 2020) When mitosis occurs, a telomere is shortened. Telomere shortening has been observed in both intrinsic and extrinsic skin ageing.(Gu et al. 2020, Kanaki, Makrantonaki, and Zouboulis 2016)With shortened telomeres, epithelial stem cells now have a weak proliferative capacity. However, this can be remedied by the addition of telomerase, a telomere-elongating enzyme. Telomerase is crucial for telomere preservation and its continuous survival.

4. The role of microRNA

MicroRNAs (miRNA) are small, conserved non-coding type of RNAs that regulate mRNA translation post-transcription and are involved in the skin ageing process. Different strands of miRNAs function differently. MiRNAs in skin ageing regulate molecules in the insulin-like growth factor 1 and mTOR signaling pathways. Chronic UV irradiation changes the expression of miRNA.(Lee, Hong, and Kim 2021, Cao et al. 2020)

5. Advanced glycation end products (AGEs)

Generated from food intake and body synthesis, excess sugar and protein binding produce AGEs by non-enzymatic glycosylation reaction. AGEs tend to accumulate in photoaged skin, affecting protein function especially in the dermis, leading to cross-linking of collagen and elastin to increase stiffness in tissues and reduce skin recoil as well as promote sagging.(Cao et al. 2020, Wilkinson and Hardman 2021)

6. Ageing due to inflammation

Continued exposure to UV radiation causes oxidative stress in epidermal tissue, leading to cell damage, fat disintegration, and eventually cell inflammation. When macrophages cannot control the degree of inflammation anymore, they start secreting pro-inflammatory factors and ROS to expedite dermal inflammation and damage.(Cao et al. 2020)

Other stressors associated with skin ageing

1. Tobacco smoke

In addition to chronic UV exposure, Tobacco smoke also contributes to extrinsic skin ageing. A study reported that smoking is related to the formation of more facial skin lines and decreased volume of the face, demonstrating early skin ageing. Several other studies have proposed different pathological mechanisms by which tobacco smoke can precipitate skin ageing. In one study it was shown that MMP1 mRNA was especially increased in smokers' dermis when compared to non-smokers, resulting in breakdown of collagen and elastic fiber.(Lahmann et al. 2001)Another study showed that tobacco smoke decreases production of procollagen type-I and III but increases MMP1 and MMP3, leading to ECM structure breakdown and irregular deposition of ECM in human cultured skin fibroblasts.(Yin, Morita, and Tsuji 2000)

2. Environmental pollutants

Exposure to any kind of air pollution is associated with a higher risk of extrinsic skin ageing.(Vierkotter et al. 2010)Li et al. reported that Chinese women developed significant facial wrinkles and folds and were at a higher risk of developing fine wrinkles at the back of their hands when they were exposed to indoor air pollution from cooking with solid fuels.(Li et al. 2015)

Changes in the face over time

The facial ageing process starts as early as a person in their twenties. After the age of 20, the body produces 1% less collagen each year. As collagen production decreases, the skin begins to thin out. Thickness decreases about 6.4% every 10 years on average, with a concomitant reduction in epidermal cell quantity.(Farage et al. 2013, Waller and Maibach 2005)

Expression lines start to form from repeated contraction of facial expression muscles, especially in the upper face. Static lines form in the perinasal and perioral region. The skin relaxes more due to loss of elastin giving a sagging skin appearance.

When a person reaches their forties, collagen and elastin levels are still decreasing due to their lower production leading to more wrinkles and sagging. Dynamic wrinkles turn to static wrinkles, meaning expression lines are visible even if there is no muscle contraction. Relaxation and sagging of the skin increase due to changes in the underlying structure also causing volume loss of the middle and lower face and neck. Vertical lines appear and are more pronounced around the mouth.

From age 60 and onwards, dynamic wrinkles persist and become static. Sagging of the whole face and neck becomes more conspicuous. In addition, the skin around the mouth weakens.

Facial muscles

It is imperative to have a sound knowledge of the different facial muscles to allow for precise injection of botulinum toxin. (Fig 2.)

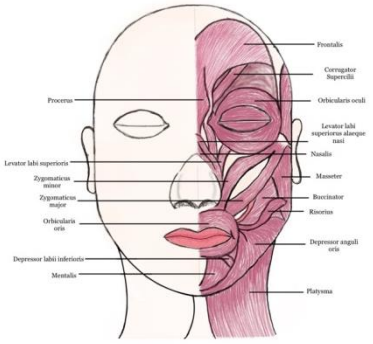

Figure 2. Facial muscles

In the upper face, the frontalis muscle raises the eyebrows and produces transverse forehead wrinkles.35 The corrugator supercilii brings the eyebrows together and produces vertical/ oblique forehead wrinkles (also known as 'number 11s' or 'frown lines'). The procerus, the most superficial muscle of the forehead, produces horizontal or transverse wrinkles by pulling the glabellar skin downwards. The depressor supercilii pulls the eyebrows in an inferior direction in the medial canthal region. In the midface, the orbicularis oculi sphincter muscles, which are as close to the skin surface as possible, control eyelid movements, lower the brows and produce crow's feet wrinkles. Having barely any subcutaneous fat between the fibers of this muscle and the dermis is one of the main reasons why bags under the eyelids are formed. The nasalis causes diagonal or vertical 'bunny lines' on the bridge of the nose when it contracts repeatedly.

The levator labii superiorus alaeque nasi, the levator labii superiorus and the levator anguli oris all contribute to the elevation of the upper lip. The risorius pulls the mouth corners laterally. The depressor anguli oris pulls the corner of the mouth inferiorly and the depressor labii inferioris pulls the lower lip inferiorly. All of these perioral muscles embed into the orbicularis oris, which much like the orbicularis oculi is a sphincter muscle that allows the opening and closing of the mouth.

Mechanism of action

The toxin first binds to specific receptors found on the surface of pre-synaptic cells. This process takes about 30 minutes. Then the plasma membrane of nerve cells 'trap' the toxin-receptor complex, forming a sac enclosing a toxin in a nerve terminal. Following internalization, 50kDa light chain of the toxin is translocated into the cytoplasm of the nerve terminal after cleavage of the disulfide bond. The final step is blocking where the translocated light chain inhibits the release of acetylcholine by slicing the cytoplasmic protein (SNAP-25) essential for binding acetylcholine vesicles to the inner side of the nerve membrane. After the injection, the toxin spreads into the tissue until it attaches selectively and temporarily in the pre-synaptic terminal of the neuromuscular junction

and then binds to the specialized protein-membrane in charge of acetylcholine elimination.(Satriyasa 2019)

Injection techniques

The two main brands of BonT A used in China are Lanbotulinum toxinA or LAN (Hengli®, Lantox®, Prosigne®, Lanzox®, Redux®, Liftox®, HBTX-A and CBTX-A) and Botox (Allergan). Lanbotulinum toxinA was introduced in China in 1997. While Botox was introduced in the early 2000s, it was only in late 2020 that Dysport® (abobotulinumtoxinA) was launched in China. Botox is delivered in 50- or 100-unit vials whereas Dysport is delivered in 300-to 500-unit bottles. Allergan stipulates that Botox should be diluted in 2.5 mL of normal saline while Dysport does not require a specific dilution amount.

Upper face

Forehead lines

The frontalis muscle originates from the epicranial aponeurosis and inserts into the fascia of the facial muscles surrounding the eyes and the skin surrounding the eyes. The frontalis muscle is not a deep muscle and can be located by asking the patient to raise and lower their brows. 2 to 4 intramuscular injections are needed per side with the typical dose ranging from 1 to 2 U per site. Injections are performed 2 cm above the upper orbital rim to avoid causing eyelid and eyebrow ptosis.(Farber et al. 2020, Yu et al. 2020)(Fig 3.). Figures 4-6 show a 30-year-old woman who underwent botulinum A injection for forehead wrinkles followed by an upper blepharoplasty a week later.

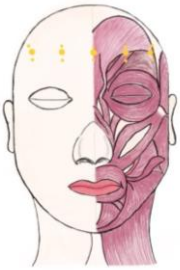

Figure 3. Forehead lines injections

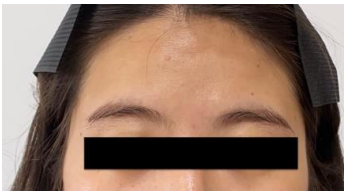

Figure 4. Before Injection, patient at rest

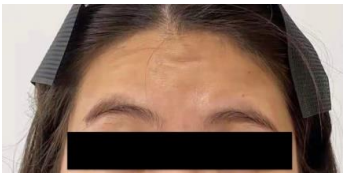

Figure 5. Before injection. Dynamic lines present upon raising eyebrows.

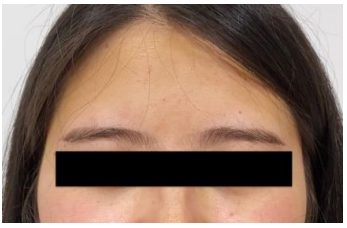

Figure 6. 1 week post injection and blepharoplasty

Glabellar lines

Glabellar lines are caused by three muscle groups: the frontalis, the procerus, and the corrugator supercilii. An X shape formed by two lines joining the inner brows and opposite inner canthus is imagined. The injection is given at the middle point of the 'X'. The next injection should be at the medial part of the eyebrow, on either side, into the Corrugator Supercilii muscle. The total dose typically ranges from 10 to 15 U in this region but can be increased to 40 U depending on wrinkle severity.(Farber et al. 2020, Matsa 2021) (Figs. 7-9)

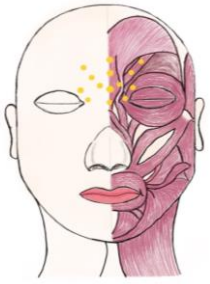

Figure 7. Glabellar lines injections

Figure 8. 55-year-old patient at rest, before injection, static wrinkles present

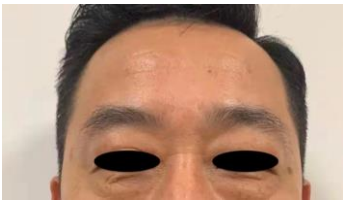

Figure 9. 1 week post injection, wrinkles have faded

Crow's feet wrinkles

For the crow's feet region, about 3 to 4 injections are administered to the eye laterally with a dose of 2 to 5 U per injection. Since the muscle is located superficially, the injection can be performed at the level above the subcutaneous tissue.(Matsa 2021)

Lower face and neck

Orbicularis oris

To treat the vertical wrinkles around the mouth, the orbicularis oculi muscle is injected with botulinum toxin. The needle is advanced superficially above and below the lips at the vermilion border. Botulinum toxin's effect has a wide range and therefore only a small amount should be injected.(Farber et al. 2020)

Platysma

The platysma muscles produce vertical bands upon contraction and contribute to lower lip depression. The platysmal bands can be corrected via injection of the platysma muscle. Injections of 2-2.5 U are carried out throughout each horizontal band with 1-1.5 cm between injection points. The injections must be carried out carefully because deep injections may interfere with swallowing.(Farber et al. 2020, Matsa 2021) (Fig. 10)

Figure 10. Platysmal Band injection (intradermal)

Contraindications

Botulinum toxin is safe in general, but it is contraindicated in patients allergic to eggs or albumin since albumin and lactose are compounded with the botulinum toxin and may cause hypersensitivity reaction. BonT A is also contraindicated in patients who have an infection at the injection site, who are pregnant or breastfeeding, or in a

patient who would be at risk for permanent immobility.(Farber et al. 2020, Guyuron and Huddleston 1994)After 4 to 6 months, the effects are reversed with the formation of new neuromuscular junctions.

Potential side effects

In the forehead region, the main risk of bonT injections is blepharoptosis.(D'Souza and Ng 2020)BonT A injection may cause eyelid ptosis if injected into the corrugator muscle above the eyebrow.(Le Louarn 2001)It is not rare that micro-bleeding or even ecchymosis may happen due to needle injection.(Le Louarn 2001)All these side effects should resolve within days or after the effects of Botulinum toxin have worn out.

CONCLUSION

Botulinum toxin A injection focused on aesthetic improvement is a safe and mature science. The confines of BonT A use are increasing day by day with its safety, efficacy, and reversibility. The side effects are minimal as well as treatable. Whether used as a solitary treatment or as an adjunct therapy to other cosmetic interventions, BonT A injection is an efficient option when it comes to facial rejuvenation.

Declarations:

Ethics approval and consent: This manuscript has been reviewed and approved by the ethics committee of The First Affiliated Hospital of Zhejiang University.

Financial Support: None to declare.

Conflicts of Interest: The authors have no conflicts of interest to declare.

Acknowledgement: We thank the patients for giving their consent for the publication of this report.

REFERENCES:

1. Akinbiyi, T., S. Othman, O. Familusi, C. Calvert, E. B. Card, and I. Percec. 2020. "Better Results in Facial Rejuvenation with Fillers." *Plast Reconstr Surg Glob Open* 8 (10):e2763. doi: 10.1097/GOX.0000000000002763.
2. Cadet, J., T. Douki, and J. L. Ravanat. 2015. "Oxidatively generated damage to cellular DNA by UVB and UVA radiation." *Photochem Photobiol* 91 (1):140-55. doi: 10.1111/php.12368.
3. Cao, C., Z. Xiao, Y. Wu, and C. Ge. 2020. "Diet and Skin Aging-From the Perspective of Food Nutrition." *Nutrients* 12 (3). doi: 10.3390/nu12030870.
4. Clark, R. P., and C. E. Berris. 1989. "Botulinum toxin: a treatment for facial asymmetry caused by facial nerve paralysis." *Plast Reconstr Surg* 84 (2):353-5.
5. Courdavault, S., C. Baudouin, M. Charveron, A. Favier, J. Cadet, and T. Douki. 2004. "Larger yield of cyclobutane dimers than 8-oxo-7,8-dihydroguanine in the DNA of UVA-irradiated human skin cells." *Mutat Res* 556 (1-2):135-42. doi: 10.1016/j.mrfmmm.2004.07.011.
6. D'Orazio, J., S. Jarrett, A. Amaro-Ortiz, and T. Scott. 2013. "UV radiation and the skin." *Int J Mol Sci* 14 (6):12222-48. doi: 10.3390/ijms140612222.
7. D'Souza, Ayman, and Chew Lip Ng. 2020. "Applied Anatomy for Botulinum Toxin Injection in Cosmetic Interventions." *Current Otorhinolaryngology Reports* 8 (4):336-343. doi: 10.1007/s40136-020-00308-4.
8. Dressler, D. 2020. "Therapeutically relevant features of botulinum toxin drugs." *Toxicon* 175:64-68. doi: 10.1016/j.toxicon.2019.12.005.
9. Eller, M. S., M. Yaar, and B. A. Gilchrest. 1994. "DNA damage and melanogenesis." *Nature* 372 (6505):413-4. doi: 10.1038/372413a0.
10. Farage, M. A., K. W. Miller, P. Elsner, and H. I. Maibach. 2013. "Characteristics of the Aging Skin." *Adv Wound Care (New Rochelle)* 2 (1):5-10. doi: 10.1089/wound.2011.0356.
11. Farber, Stephanie E., Mathew T. Epps, Emily Brown, Julie Krochonis, Rena McConville, and Mark A. Codner. 2020. "A review of nonsurgical facial rejuvenation." *Plastic and Aesthetic Research* 7:72. doi: 10.20517/2347-9264.2020.152.

12. Gu, Y., J. Han, C. Jiang, and Y. Zhang. 2020. "Biomarkers, oxidative stress and autophagy in skin aging." *Ageing Res Rev* 59:101036. doi: 10.1016/j.arr.2020.101036.
13. Guyuron, B., and S. W. Huddleston. 1994. "Aesthetic indications for botulinum toxin injection." *Plast Reconstr Surg* 93 (5):913-8. doi: 10.1097/00006534-199404001-00003.
14. Ilankovan, V. 2014. "Anatomy of ageing face." *Br J Oral Maxillofac Surg* 52 (3):195-202. doi: 10.1016/j.bjoms.2013.11.013.
15. Kaidbey, K. H., P. P. Agin, R. M. Sayre, and A. M. Kligman. 1979. "Photoprotection by melanin--a comparison of black and Caucasian skin." *J Am Acad Dermatol* 1 (3):249-60. doi: 10.1016/s0190-9622(79)70018-1.
16. Kanaki, T., E. Makrantonaki, and C. C. Zouboulis. 2016. "Biomarkers of skin aging." *Rev Endocr Metab Disord* 17 (3):433-442. doi: 10.1007/s11154-016-9392-x.
17. Karran, P., and R. Brem. 2016. "Protein oxidation, UVA and human DNA repair." *DNA Repair (Amst)* 44:178-185. doi: 10.1016/j.dnarep.2016.05.024.
18. Lahmann, C., J. Bergemann, G. Harrison, and A. R. Young. 2001. "Matrix metalloproteinase-1 and skin ageing in smokers." *Lancet* 357 (9260):935-6. doi: 10.1016/S0140-6736(00)04220-3.
19. Le Louarn, C. 2001. "Botulinum toxin A and facial lines: the variable concentration." *Aesthetic Plast Surg* 25 (2):73-84. doi: 10.1007/s002660010100.
20. Lee, H., Y. Hong, and M. Kim. 2021. "Structural and Functional Changes and Possible Molecular Mechanisms in Aged Skin." *Int J Mol Sci* 22 (22). doi: 10.3390/ijms222212489.
21. Li, M., A. Vierkotter, T. Schikowski, A. Huls, A. Ding, M. S. Matsui, B. Deng, C. Ma, A. Ren, J. Zhang, J. Tan, Y. Yang, L. Jin, J. Krutmann, Z. Li, and S. Wang. 2015. "Epidemiological evidence that indoor air pollution from cooking with solid fuels accelerates skin aging in Chinese women." *J Dermatol Sci* 79 (2):148-54. doi: 10.1016/j.jdermsci.2015.04.001.
22. Matsa, Sainath. 2021. "Botulinum Toxin and Fillers for Maxillofacial Esthetics." In *Oral and Maxillofacial Surgery for the Clinician*, edited by Krishnamurthy Bonanthaya, Elavenil Panneerselvam, Suvy Manuel, Vinay V. Kumar and Anshul Rai, 691-705. Singapore: Springer Singapore.
23. Mendelson, B., and C. H. Wong. 2012. "Changes in the facial skeleton with aging: implications and clinical applications in facial rejuvenation." *Aesthetic Plast Surg* 36 (4):753-60. doi: 10.1007/s00266-012-9904-3.
24. Mouret, S., C. Baudouin, M. Charveron, A. Favier, J. Cadet, and T. Douki. 2006. "Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation." *Proc Natl Acad Sci U S A* 103 (37):13765-70. doi: 10.1073/pnas.0604213103.
25. Neerken, S., G. W. Lucassen, M. A. Bisschop, E. Lenderink, and T. A. Nuijs. 2004. "Characterization of age-related effects in human skin: A comparative study that applies confocal laser scanning microscopy and optical coherence tomography." *J Biomed Opt* 9 (2):274-81. doi: 10.1117/1.1645795.
26. Panda, Arun Kumar, and Aarti Chowdhary. 2021. "Non-surgical Modalities of Facial Rejuvenation and Aesthetics." In *Oral and Maxillofacial Surgery for the Clinician*, edited by Krishnamurthy Bonanthaya, Elavenil Panneerselvam, Suvy Manuel, Vinay V. Kumar and Anshul Rai, 661-689. Singapore: Springer Singapore.
27. Pinnell, S. R. 2003. "Cutaneous photodamage, oxidative stress, and topical antioxidant protection." *J Am Acad Dermatol* 48 (1):1-19; quiz 20-2. doi: 10.1067/mjd.2003.16.
28. Pourzand, C., A. Albieri-Borges, and N. N. Raczek. 2022. "Shedding a New Light on Skin Aging, Iron- and Redox-Homeostasis and Emerging Natural Antioxidants." *Antioxidants (Basel)* 11 (3). doi: 10.3390/antiox11030471.
29. Rajapakse, A., A. Suraweera, D. Boucher, A. Naqi, K. O'Byrne, D. J. Richard, and L. V. Croft. 2020. "Redox Regulation in the Base Excision Repair Pathway: Old and New Players as Cancer Therapeutic Targets." *Curr Med Chem* 27 (12):1901-1921. doi: 10.2174/0929867326666190430092732.
30. Sardy, M. 2009. "Role of matrix metalloproteinases in skin ageing." *Connect Tissue Res* 50 (2):132-8. doi: 10.1080/03008200802585622.
31. Satriyasa, B. K. 2019. "Botulinum toxin (Botox) A for reducing the appearance of facial wrinkles: a literature review of clinical use and pharmacological aspect." *Clin Cosmet Investig Dermatol* 12:223-228. doi: 10.2147/CCID.S202919.
32. Silva, Same, B. Michniak-Kohn, and G. R. Leonardi. 2017. "An overview about oxidation in clinical practice of skin aging." *An Bras Dermatol* 92 (3):367-374. doi: 10.1590/abd1806-4841.20175481.
33. Sudel, K. M., K. Venzke, H. Mielke, U. Breitenbach, C. Mundt, S. Jaspers, U. Koop, K. Sauermann, E. Knussman-Hartig, I. Moll, G. Gercken, A. R. Young, F. Stab, H. Wenck, and S. Gallinat. 2005. "Novel aspects of intrinsic and extrinsic aging of human skin: beneficial effects of soy extract." *Photochem Photobiol* 81 (3):581-7. doi: 10.1562/2004-06-16-RA-202.
34. Swift, A., S. Liew, S. Weinkle, J. K. Garcia, and M. B. Silberberg. 2021. "The Facial Aging Process From the "Inside Out"." *Aesthet Surg J* 41 (10):1107-1119. doi: 10.1093/asj/sjaa339.
35. Tyrrell, R. M. 1996. "Activation of mammalian gene expression by the UV component of sunlight--from models to reality." *Bioessays* 18 (2):139-48. doi: 10.1002/bies.950180210.
36. Vierkotter, A., T. Schikowski, U. Ranft, D. Sugiri, M. Matsui, U. Kramer, and J. Krutmann. 2010. "Airborne particle exposure and extrinsic skin aging." *J Invest Dermatol* 130 (12):2719-26. doi: 10.1038/jid.2010.204.
37. Waller, J. M., and H. I. Maibach. 2005. "Age and skin structure and function, a quantitative approach (I): blood flow, pH, thickness, and ultrasound echogenicity." *Skin Res Technol* 11 (4):221-35. doi: 10.1111/j.0909-725X.2005.00151.x.

38. Wilkinson, Holly N., and Matthew J. Hardman. 2021. "A role for estrogen in skin ageing and dermal biomechanics." *Mechanisms of Ageing and Development* 197:111513. doi: https://doi.org/10.1016/j.mad.2021.111513.
39. Yin, L., A. Morita, and T. Tsuji. 2000. "Alterations of extracellular matrix induced by tobacco smoke extract." *Arch Dermatol Res* 292 (4):188-94. doi: 10.1007/s004030050476.
40. Yu, Yijia, Jiaqi Sun, Zeren Shen, Xiaohu Lin, and Jinghong Xu. 2020. "Auxiliary usage of botulinum toxin A in plastic surgery in China." *Journal of cosmetic dermatology* 19 (5):1021-1028.