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BOTOX SPA/FACIAL IS DONE BY BOTULINUM NEUROTOXIN BY BLOCKING OF RELEASE OF NEUROTRANSMITTERS FOR NERVE SIGNALS

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ABSTRACT
Botox, short for botulinum toxin, is a neurotoxic protein produced by the bacterium Clostridium botulinum. It's commonly used in small doses for both cosmetic and medical purposes. Medically, it can treat conditions like migraines, muscle spasms, and excessive sweating. Cosmetically, it's used to temporarily reduce the appearance of wrinkles by paralyzing the underlying muscles. A Botox spa typically refers to a salon or clinic that offers Botox hair treatments, not the cosmetic injections for wrinkle reduction. Botox hair treatments are a type of hair treatment that uses a protein-based solution to smooth, strengthen, and add shine to hair, similar to a hair spa or keratin treatment. These treatments are distinct from the cosmetic Botox injections used to reduce wrinkles on the face. **Botox Hair Treatments (Botox Spa):** To improve hair texture, reduce frizz, add shine, and potentially repair damage. **Process:** Involves applying a protein-based solution to the hair, similar to a hair spa treatment. Not the same as cosmetic Botox: Botox hair treatments do not involve injecting a neurotoxin into the scalp. Cosmetic Botox Injections: To reduce the appearance of wrinkles and fine lines by temporarily paralyzing facial muscles. **Process:** Involves injecting a neurotoxin (Botox) into specific facial muscles. Not related to hair treatments: These injections are not used for hair treatment. Many salons and spas offer hair Botox treatments along with other services like hair cutting, coloring, and other hair treatments.

KEYWORDS: Botox, botulinum toxin, acetylcholine, neurotransmitter, polypeptide.

INTRODUCTION
Botox, short for botulinum toxin, is a neurotoxin used in medicine and cosmetic procedures. It works by temporarily blocking nerve signals to muscles, causing them to relax. This effect is used to treat various conditions, from wrinkles and migraines to muscle spasms and excessive sweating. Botulinum toxin (BoNT) is a large protein, roughly 150 kDa, composed of two polypeptide chains: a light chain (LC) and a heavy chain (HC), linked by a disulphide bond. The heavy chain is further divided into two domains: the N-terminal translocation domain (HN) and the C-terminal receptor-binding domain (HC). These domains are critical for the toxin's mechanism of action, which involves binding to nerve cells, entering them, and ultimately blocking the release of neurotransmitters. Formula: $C_{6760}H_{10447}N_{1743}O_{2010}S_{32,}$ Molar mass: 149323.05 g·mol^{-1}

Light Chain (LC): This ~50 kDa chain acts as a zinc-dependent endopeptidase, specifically targeting and cleaving proteins involved in neurotransmitter release.

Heavy Chain (HC): This ~100 kDa chain is responsible for the toxin's specificity and its ability to enter target cells. It is further divided into two domains:

Translocation Domain (HN): This domain facilitates the movement of the light chain across the cell membrane into the cytoplasm.

Receptor-Binding Domain (HC): This domain binds to specific receptors on the surface of nerve cells, allowing the toxin to target the correct cells and initiate the intoxication process.[1,2]

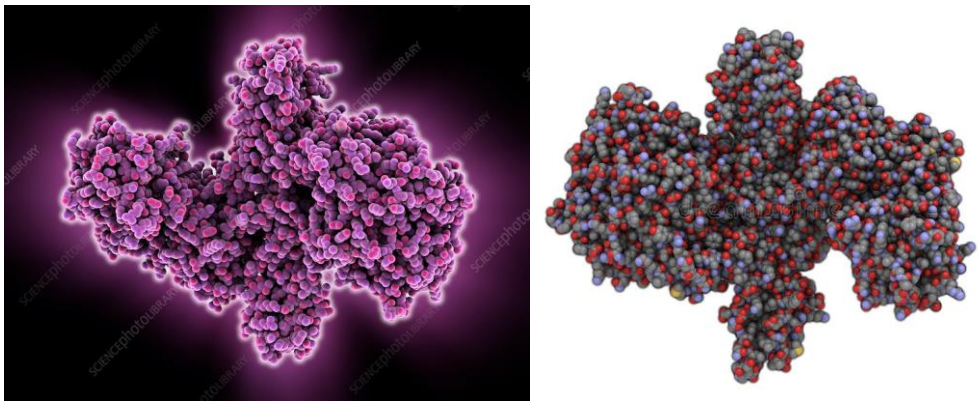

Figure 1: Chain a and Chain B of Botulinum toxin 3D.

Trade names: Botox, Myobloc, Jeuveau, Dyston and others

Other names: BoNT, botox

Biosimilars: abobotulinumtoxinA, daxibotulinumtoxinA, daxibotulinumtoxinA-lanm, evabotulinumtoxinA, incobotulinumtoxinA, letibotulinumtoxinA, letibotulinumtoxinA-wlbg, onabotulinumtoxinA, prabotulinumtoxinA, relabotulinumtoxinA, rimabotulinumtoxinB. CAS Number: Botulinum toxin A: 93384-43-1, Botulinum toxin B: 93384-43-2. **Botulinum toxin-A [Solid, Molecular weight: 69365.94 Da:** [melting point (°C): 85.5-86.5, water solubility >10 mg/ml, isoelectric point: 5.6].

Pharmacodynamics: Botulinum toxin A inhibits the release of acetylcholine, relieving muscle contraction and spasm associated with many conditions, such as incontinence and dystonia. Cosmetically, botulinum toxin A paralyses muscles in the face to temporarily treat wrinkles. The maximum effects of muscle paralysis occur four to seven days after a dose. When injected at therapeutic doses, botulinum toxin A causes partial chemical denervation of muscle tissue, causing local reduction of muscle activity. Muscle atrophy may result, axonal sprouting may begin, and extrajunctional acetylcholine receptors can be formed. Reinnervation of the muscle may occur, reversing muscle denervation caused by botulinum toxin A.

Mechanism of action: Botulinum toxin A is a 150-kDa molecular weight protein consisting of a light chain (50 kDa) and heavy chain (100 kDa) linked by a single disulphide bond. The crystal structure reveals 3 lobes - the light chain, the amino-terminal portion of the heavy chain, and the carboxyl-terminal portion of the heavy chain. Botulinum toxin type A blocks neuromuscular transmission on motor or sympathetic nerve terminals, inhibiting the release of acetylcholine. Botulinum toxins have actions on various regions: the neuromuscular junction, autonomic ganglia, and both postganglionic sympathetic and parasympathetic nerve endings. The heavy chain of the toxin binds selectively at the presynaptic surface of cholinergic neurons in an irreversible fashion. After binding, the toxin-receptor complex is transported into the cell by endocytosis. The disulphide bond between the two chains is cleaved and the botulism toxin enters the cytoplasm. The light chain specifically interacts with SNAP-25 in the nerve terminals to block binding of acetylcholine vesicles with the cell membrane. SNAP-25 is required for successful binding and release of acetylcholine from vesicles in nerve endings.

Botulinum toxin-B [Liquid, Molecular weight: 46871.95 Da] Pharmacodynamics: Botulinum Toxin Type B inhibits acetylcholine release at the neuromuscular junction via a three stage process: 1) Heavy Chain mediated neurospecific binding of the toxin, 2) internalization of the toxin by receptor-mediated endocytosis, and 3) ATP and pH dependent translocation of the Light Chain to the neuronal cytosol where it acts as a zinc-dependent endoprotease cleaving polypeptides essential for neurotransmitter release.

Mechanism of action: Botulinum Toxin Type B binds to and cleaves the synaptic Vesicle Associated Membrane Protein (VAMP, also known as synaptobrevin) which is a component of the protein complex responsible for docking and fusion of the synaptic vesicle to the presynaptic membrane, a necessary step to neurotransmitter release.

BoNTs are synthesized as a single chain and then cleaved into the dichain structure with the disulphide bond. The three-dimensional structure of BoNT/A, the most studied serotype, has been extensively characterized, revealing details about how these domains interact and how they contribute to the toxin's overall function.

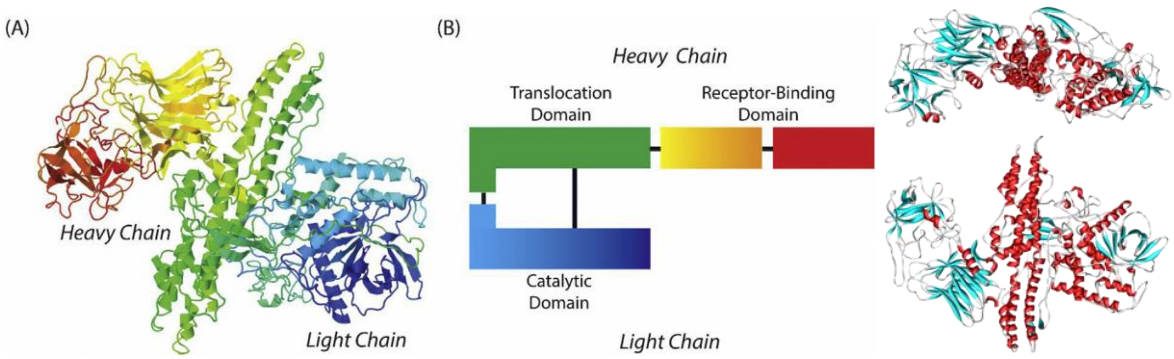

Figure 2: Chain A and Chain B of Botulinum toxin.

Neurological Conditions: Botox is used to treat conditions like cervical dystonia (neck muscle spasms), blepharospasm (uncontrollable blinking), strabismus (misaligned eyes), and muscle spasticity.

Chronic Migraines: It can help prevent headaches in people with chronic migraines.

Overactive Bladder: Botox can be injected into the bladder to treat urinary incontinence and overactive bladder.

Excessive Sweating: It can reduce excessive sweating in the underarms (axillary hyperhidrosis).

Other: It has also been used for other conditions like anal fissures and some digestive disorders.

Cosmetic Uses

Wrinkle Reduction: Botox is most well-known for its cosmetic use in reducing wrinkles and fine lines, particularly in the forehead, around the eyes (crow's feet), and between the eyebrows (glabellar lines). Glabellar lines, also known as frown lines or "11" lines, are the vertical wrinkles that appear between the eyebrows and above the nose. They are often among the first wrinkles to appear on the face, and are primarily caused by the repeated contraction of the corrugator and procerus muscles when frowning or squinting. The procerus muscle is a small, pyramidal-shaped muscle located in the centre of the forehead, between the eyebrows. It plays a role in facial expressions, specifically in actions like frowning and focusing, and can contribute to the appearance of wrinkles on the bridge of the nose

Location: The glabella is the area of skin between the eyebrows and above the nose.

Formation: Glabellar lines are caused by the repeated action of the corrugator and procerus muscles, which contract when we frown or squint. Over time, these dynamic lines can become static, meaning they are visible even when the muscles are relaxed.[3,4]

Figure 3: Wrinkles to be removed by botox.

Appearance: These wrinkles often appear as two vertical lines, resembling the number "11," hence the nickname.

Contributing Factors: In addition to muscle action, other factors like skin aging (loss of elasticity and collagen), fat loss, sun exposure, smoking, and even

emotional expression can contribute to the development and severity of glabellar lines.

Treatment: Various treatments can be used to reduce the appearance of glabellar lines, including:

Botulinum toxin (Botox): Injections temporarily paralyze the muscles, reducing their ability to contract and smooth out the wrinkles.

Dermal fillers: These injections can fill in the lines, making them less noticeable.

Topical treatments: Creams containing ingredients like hyaluronic acid, peptides, retinoids, and antioxidants can help improve skin hydration, elasticity, and overall appearance.

Other treatments: Procedures like chemical peels, microdermabrasion, and laser resurfacing can also be used to address glabellar lines.

Smoothing Dynamic Rhytids: It targets wrinkles caused by muscle movement, like those from frowning or squinting. Rhytids is the medical term for wrinkles, which are creases in the skin. They are a natural part of the aging process and can also be influenced by factors like sun exposure, facial expressions, and genetics.

What are rhytids? Rhytids are simply wrinkles, which are folds or creases in the skin that can appear due to a variety of factors.

Why do they occur? As skin ages, it naturally loses elasticity and collagen, leading to the formation of wrinkles. Sun exposure, repetitive facial movements (like smiling or frowning), and even genetics can also contribute to wrinkle development.

Where do they appear? Rhytids can occur anywhere on the body, but they are most noticeable on the face, neck, and hands. Common areas include the forehead, around the eyes (crow's feet), and around the mouth.

How can they be treated? There are various treatments available to help reduce the appearance of wrinkles, including topical creams (like retinoids), chemical peels, laser resurfacing, and injectable like Botox and fillers.

How it Works: Botox is injected into specific muscles or areas, depending on the intended use. It blocks the release of acetylcholine, a neurotransmitter that signals muscles to contract. This temporary muscle paralysis or relaxation leads to the desired therapeutic or cosmetic effect. The effects of Botox typically last for 3-6 months, and repeat treatments are needed to maintain the results.[5,6]

Important Considerations

Safety: While generally safe when administered by qualified professionals, Botox can cause side effects, including bruising, pain at the injection site, and in rare cases, and more serious reactions.

Qualified Practitioners: It's crucial to have Botox injections administered by a qualified and experienced healthcare professional to ensure proper placement, dosage, and to address any potential complications.

Individual Results Vary: The effectiveness and duration of Botox results can vary from person to person. *Clostridium botulinum* is a gram-positive, rod-shaped, anaerobic, spore-forming, motile bacterium with the ability to produce botulinum toxin, which is a neurotoxin. *C. botulinum* is a diverse group of pathogenic bacteria. Initially, they were grouped together by their ability to produce botulinum toxin and are now known as four distinct groups, C. botulinum groups I–IV. Along with some strains of *Clostridium butyricum* and *Clostridium baratii*, these bacteria all produce the toxin.

Figure 4: *Clostridium botulinum* microbes.

Botulinum toxin can cause botulism, a severe flaccid paralytic disease in humans and other animals, and is the most potent toxin known to science, natural or synthetic, with a lethal dose of 1.3–2.1 ng/kg in humans. *C. botulinum* is commonly associated with bulging canned food; bulging, misshapen cans can be due to an internal increase in pressure caused by gas produced by bacteria. *C. botulinum* is responsible for foodborne botulism (ingestion of preformed toxin), infant botulism (intestinal infection with toxin-forming *C. botulinum*), and wound botulism (infection of a wound with *C. botulinum*). *C. botulinum* produces heat-resistant endospores that are commonly found in soil and are able to survive under adverse conditions.

Microbiology: *C. botulinum* is a Gram-positive, rod-shaped, spore-forming bacterium. It is an obligate anaerobe, requiring an environment that lacks oxygen. However, *C. botulinum* tolerates traces of oxygen due to the enzyme superoxide dismutase, which is an important

antioxidant defence in nearly all cells exposed to oxygen. *C. botulinum* is able to produce the neurotoxin only during sporulation, which can happen only in an anaerobic environment. *C. botulinum* is divided into four distinct phenotypic groups (I-IV) and is also classified into seven serotypes (A–G) based on the antigenicity of the botulinum toxin produced. On the level visible to DNA sequences, the phenotypic grouping matches the results of whole-genome and rRNA analyses, and setotype grouping approximates the result of analyses focused specifically on the toxin sequence. The two phylogenetic trees do not match because of the ability of the toxin gene cluster to be horizontally transferred.[7,8]

Serotypes: Botulinum neurotoxin (BoNT) production is the unifying feature of the species. Seven serotypes of toxins have been identified that are allocated a letter (A–G), several of which can cause disease in humans. They are resistant to degradation by enzymes found in the gastrointestinal tract. This allows for ingested toxins to be absorbed from the intestines into the bloodstream. Toxins can be further differentiated into subtypes on the bases of smaller variations. However, all types of botulinum toxin are rapidly destroyed by heating to 100°C for 15 minutes (900 seconds). 80°C for 30 minutes also destroys BoNT. Most strains produce one type of BoNT, but strains producing multiple toxins have been described. *C. botulinum* producing B and F toxin types have been isolated from human botulism cases in New Mexico and California. The toxin type has been designated Bf as the type B toxin was found in excess to the type F. Similarly, strains producing Ab and Af toxins have been reported. Evidence indicates the neurotoxin genes have been the subject of horizontal gene transfer, possibly from a viral (bacteriophage) source. This theory is supported by the presence of integration sites flanking the toxin in some strains of *C. botulinum.* However, these integrations sites are degraded (except for the C and D types), indicating that the *C. botulinum* acquired the toxin genes quite far in the evolutionary past. Nevertheless, further transfers still happen via the plasmids and other mobile elements the genes are located on.

Toxin types in disease: Only botulinum toxin types A, B, E, F and H (FA) cause disease in humans. Types A, B, and E are associated with food-borne illness, while type E is specifically associated with fish products. Type C produces limber-neck in birds and type D causes botulism in other mammals. No disease is associated with type G. The "gold standard" for determining toxin type is a mouse bioassay, but the genes for types A, B, E, and F can now be readily differentiated using quantitative PCR. Type "H" is in fact a recombinant toxin from types A and F. It can be neutralized by type A antitoxin and no longer is considered a distinct type. A few strains from organisms genetically identified as other Clostridium species have caused human botulism: *C. butyricum* has produced type E toxin and *C. baratii* had produced type F toxin. The ability of *C. botulinum* to naturally transfer neurotoxin genes to other clostridia is concerning, especially in the food industry, where preservation systems are designed to destroy or inhibit only C. botulinum but not other Clostridium species.

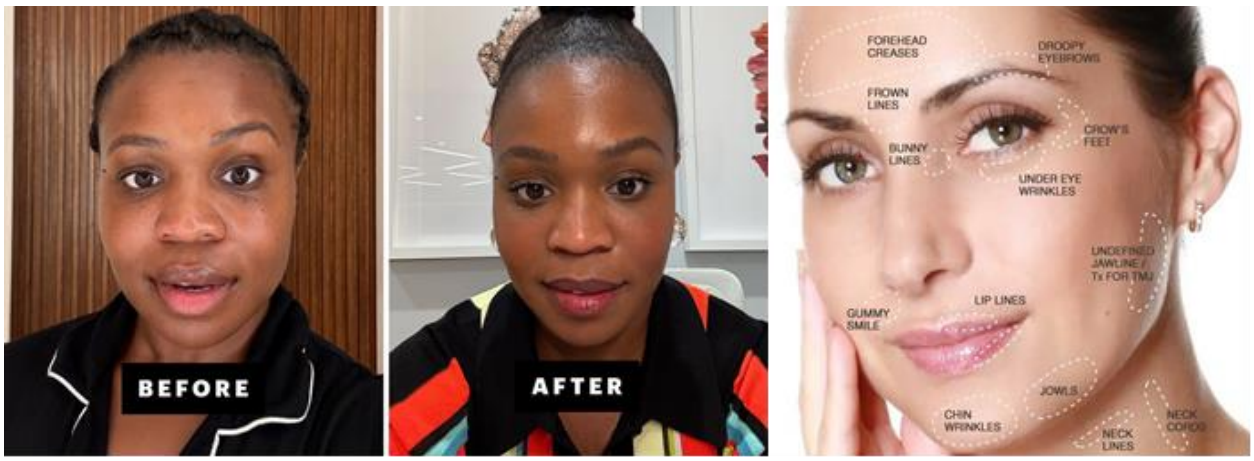

Figure 5: Spa treatment by botox.

Metabolism: Many *C. botulinum* genes play a role in the breakdown of essential carbohydrates and the metabolism of sugars. Chitin is the preferred source of carbon and nitrogen for *C. botulinum*. Hall A strain of *C. botulinum* has an active chitin lytic system to aid in the breakdown of chitin. Type A and B of *C. botulinum* production of BoNT is affected by nitrogen and carbon nutrition. There is evidence that these processes are also under catabolic repression.

Groups: Physiological differences and genome sequencing at 16S rRNA level support the subdivision of the *C. botulinum* species into groups I-IV. Some authors have briefly used groups V and VI, corresponding to toxin-producing *C. baratii* and *C. butyricum*. What used to be group IV is now *C. argentinense*. Botulinum toxin, or botulinum neurotoxin (commonly called Botox), is a neurotoxic protein produced by the bacterium *Clostridium botulinum* and related species. It prevents the release of the neurotransmitter acetylcholine from

axon endings at the neuromuscular junction, thus causing flaccid paralysis. The toxin causes the disease botulism. The toxin is also used commercially for medical and cosmetic purposes. Botulinum toxin is an acetylcholine release inhibitor and a neuromuscular blocking agent. The seven main types of botulinum toxin are named types A to G (A, B, C1, C2, D, E, F and G). New types are occasionally found. Types A and B are capable of causing disease in humans, and are also used commercially and medically. Types C–G are less common; types E and F can cause disease in humans, while the other types cause disease in other animals. Botulinum toxins are among the most potent toxins known to science. Intoxication can occur naturally as a result of either wound or intestinal infection or by ingesting formed toxin in food. The estimated human median lethal dose of type A toxin is 1.3–2.1 ng/kg intravenously or intramuscularly, 10–13 ng/kg when inhaled, or 1 µg/kg when taken by mouth.[9,10]

Clostridium botulinum is a gram-positive, rod-shaped, anaerobic, spore-forming, motile bacterium with the ability to produce botulinum toxin, which is a neurotoxin. *C. botulinum* is a diverse group of pathogenic bacteria. Initially, they were grouped together by their ability to produce botulinum toxin and are now known as four distinct groups, *C. botulinum* groups I–IV. Along with some strains of *Clostridium butyricum* and *Clostridium baratii*, these bacteria all produce the toxin. Botulinum toxin can cause botulism, a severe flaccid paralytic disease in humans and other animals, and is the most potent toxin known to science, natural or synthetic, with a lethal dose of 1.3–2.1 ng/kg in humans. *C. botulinum* is commonly associated with bulging canned food; bulging, misshapen cans can be due to an internal increase in pressure caused by gas produced by bacteria. *C. botulinum* is responsible for foodborne botulism (ingestion of preformed toxin), infant botulism (intestinal infection with toxin-forming *C. botulinum*), and wound botulism (infection of a wound with *C. botulinum*). *C. botulinum* produces heat-resistant endospores that are commonly found in soil and are able to survive under adverse conditions. Botox results typically last for three to four months. However, this can vary based on individual factors like metabolism, the area treated, and the dosage used. Some individuals may experience results for a shorter period (around 2 months), while others may enjoy them for longer (4-6 months).

Typical Duration: Most people find that Botox effects diminish around the 3-4 month mark, requiring a touch-up treatment.

Individual Variation: Factors like your metabolism, muscle activity, and the specific area treated can influence how long your Botox lasts. For example, Botox for forehead lines might last differently than Botox for crow's feet.

First-Time Experience: Some people find that their first Botox treatment doesn't last as long as subsequent treatments, which may last longer.

Long-Term Effects: While Botox is temporary, regular treatments can lead to muscles becoming less active over time, potentially allowing for longer intervals between injections.

Maintaining Results: To maintain the desired effect, regular injections are necessary. Over time, however, some individuals may find they can space out their treatments. Receiving preventative Botox at 30—and targeting those crow’s feet and brow lines—can significantly delay the onset of deep wrinkles and fine lines, making this the best age to start Botox if you want to feel refreshed and self-assured.[11,12]

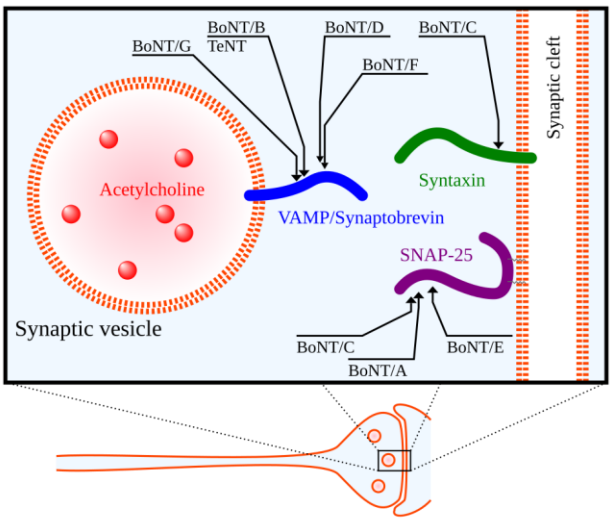

Figure 6: Mode of Action of Botulinum neurotoxin.

Stopping or pausing Botox treatments: Naturally, lines and wrinkles start to form again during the period of time when treatment has ceased. Once the Botox treatments are resumed, however, smoothness is restored. Patients

who decide to swear off Botox forever will find that their wrinkles steadily return. However, when Botox is administered with care, precision, and a deep understanding of facial anatomy, it's possible to avoid the frozen look with Botox and achieve subtle, natural results. Botox® is commonly used by individuals in their 30s, 40s, and beyond, but there's no strict age range. Botox® is approved for those 18 or older. Some opt for preventative Botox® in their late 20s, while others begin treatment later in life. The decision often depends on personal preferences and specific skin concerns. Yes, Botox is sometimes used in spa facials, though not in the traditional sense of a standard facial. Botox, or botulinum toxin, is primarily known for its wrinkle-reducing effects through muscle relaxation. In a "Botox facial," the toxin is diluted and injected into the superficial layers of the skin, targeting sweat and oil glands. This helps to refine skin texture by reducing oil production, minimizing pores, and potentially reducing breakouts. It also addresses fine lines by relaxing the tiny muscle movements that contribute to their formation.

Traditional Botox: Involves injecting Botox into specific muscles to reduce wrinkles caused by muscle contractions, like frown lines or crow's feet.

Botox Facial: This involves injecting a diluted form of Botox into the top layers of the skin (epidermis and dermis).

Mechanism: The diluted Botox blocks the release of acetylcholine, a neurotransmitter that triggers sweat and oil glands. This reduces oil production and can improve skin texture and minimize pores.

Other Ingredients: Botox facials may also incorporate other ingredients like hyaluronic acid, vitamins, and PRP (Platelet-Rich Plasma), depending on the specific treatment.

Benefits: Beyond oil reduction, Botox facials can also help to reduce fine lines, improve skin brightness, and potentially increase collagen synthesis.[13,14]

Safety: While generally considered safe when administered by qualified professionals, it's crucial to choose a reputable spa or clinic for any Botox treatment. Botox is a popular cosmetic treatment that is used to diminish facial wrinkles and creases. It is commonly used to reduce crow's feet, lip lines, and frown lines. If you are considering Botox, here's what you should know about what it can do for you and what to expect.

CONCLUSION

Botulinum toxin, also called “miracle poison,” is one of the most poisonous biological substances known. It is a neurotoxin produced by the bacterium *Clostridium botulinum*, an anaerobic, gram-positive, spore-forming rod commonly found on plants, in soil, water and the intestinal tracts of animals. First demonstrated the effectiveness of botulinum toxin type A for the management of strabismus in humans. Subsequently, botulinum toxin was approved for the treatment of numerous disorders of spasticiy and a host of other conditions. Currently it is used in almost every sub-specialty of medicine. In 2002, the FDA approved the use of Botox® (Botulinum toxin-A) for the cosmetic purpose of temporarily reducing glabeller forehead frown lines. *C. botulinum* elaborates eight antigenically distinguishable exotoxins (A, B, C1, C2, D, E, F and G). Type A is the most potent toxin, followed by types B and F toxin. Types A, B and E are commonly associated with systemic botulism in humans. All botulinum neurotoxins are produced as relatively inactive, single polypeptide chains with a molecular mass of about 150 kDa with a high degree of amino acid sequence homology among the toxin types. The polypeptide chain consists of a heavy (H) chain and a light (L) chain of roughly 100 and 50 kDa respectively, linked by a disulphide bond. The botulinum toxin neurotoxin complex is also associated with various other nontoxic proteins, which may also have hemagglutinating properties.

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