

**Design for Biosecurity**  
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**Indian Institute of Technology, Kanpur**  
**Lecture 29**  
**Botox Therapy**

Welcome back to the fourth lecture of the sixth week. In our previous lecture, we concluded by discussing the basics of botulism and the production of botulinum toxin by Clostridia, which is an anaerobic bacterium. Interestingly, when these spores germinate in anaerobic conditions, they produce the toxin. I emphasized the challenge of detecting botulism because identifying the vegetative cells of Clostridium does not necessarily mean that you are detecting the toxin itself. The toxin is only produced when the spores germinate in an environment lacking oxygen, and these spores are extremely heat-resistant.

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Today, we will delve into Botox Therapy, focusing on what it entails and the neurological

role of botulinum toxin. To understand how botulinum toxin works, it's important to first comprehend the nerve-muscle junction. This is the area where nerve cells communicate with muscle cells, transmitting signals through a neurotransmitter called acetylcholine.

Imagine the structure of the nerve-muscle junction: the nerve ending communicates with the muscle, which is depicted here as a half-circle. This zone, known as the neuromuscular junction (NMJ) or nerve-muscle synapse, is crucial for muscle function. At this junction, neurons secrete acetylcholine, which then binds to muscle receptors and triggers contraction and movement.

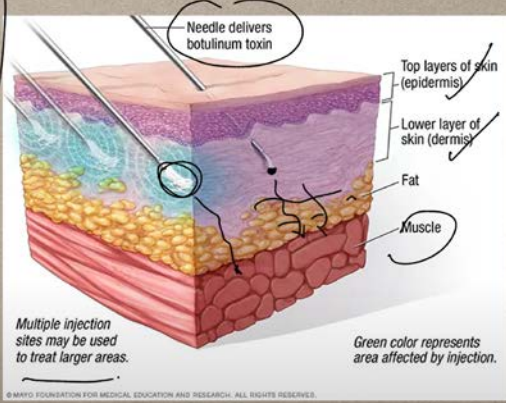
Essentially, muscle contraction occurs at the neuromuscular junction through this communication. The neuron, originating from the spinal cord, forms synapses with the muscle. The signal sent by the nerve leads to muscle contraction, enabling all our muscular movements. This interaction between the nerve and muscle at the NMJ is fundamental to how our bodies move and function.

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Lecture 29

## BOTOX THERAPY

- Botox injections are shots that use a toxin to prevent a muscle from moving for a limited time. These shots are often used to smooth wrinkles on the face. They're also used to treat neck spasms, sweating, overactive bladder, lazy eye and other conditions. Botox shots also may help prevent migraine.



The diagram illustrates a cross-section of human skin and underlying tissue. From top to bottom, the layers are labeled: 'Top layers of skin (epidermis)', 'Lower layer of skin (dermis)', 'Fat', and 'Muscle'. A needle is shown entering the skin from the top left, with a label 'Needle delivers botulinum toxin' pointing to the tip. A green shaded area is shown in the dermis layer, with a label 'Green color represents area affected by injection.' Below the diagram, text reads 'Multiple injection sites may be used to treat larger areas.' At the bottom of the slide, there is a copyright notice: '© MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH. ALL RIGHTS RESERVED.'

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Now, imagine a situation where a molecule interferes with the binding of acetylcholine,

the neurotransmitter responsible for muscle contraction. When acetylcholine cannot bind to its receptors on the muscle, muscle contraction ceases, and the muscles remain relaxed. Over time, many wrinkles that develop with age are due to repetitive muscular movements. If you picture wrinkles on the face, it's easy to see that these are caused by the continuous action of underlying muscles.

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Lecture 29

## WHY IT IS DONE

- Botox shots block certain chemical signals from nerves that cause muscles to contract. The most common use of these injections is to relax the facial muscles that cause frown lines and other facial wrinkles.

Acetylcholin Binding sites

XX

Bo/Tox

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In this context, botulinum toxin, the substance used in Botox Therapy, plays a key role. This toxin inhibits the release of acetylcholine, thereby preventing muscle contraction. The result is that the muscles remain relaxed, which smooths out wrinkles. Essentially, this is how Botox Therapy can make the skin appear younger and reduce the visible signs of aging.

Botox injections involve administering this toxin to temporarily paralyze specific muscles. These injections are not permanent; they need to be repeated periodically to maintain the desired effect. Botox is commonly used to smooth out facial wrinkles, but it also treats various other conditions such as neck spasms, excessive sweating, overactive bladder, lazy

eyes, and even helps in preventing migraines.

The administration of Botox involves using fine needles to inject the toxin into targeted areas. The toxin is delivered into the dermis and fat layers just beneath the skin's surface, where it affects the underlying muscles. Multiple injection sites may be used to cover a larger area of treatment. The primary mechanism of Botox is to block the chemical signals from nerves that cause muscles to contract, specifically by inhibiting the binding of acetylcholine at the neuromuscular junction.

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The image shows a video player interface for a lecture. The title bar at the top left says 'Lecture 29'. The main content area is a slide with the title 'TYPES OF BOTOX INJECTIONS'. The slide contains two bullet points. The first bullet point states that Botulinum toxin is available in two forms. The second bullet point describes Type A, listing products like Botox, Dysport, Xeomin, Daxxify, and Jeuveau. The third bullet point describes Type B, mentioning Myobloc. Hand-drawn circles and brackets are present on the slide, highlighting 'Type A', 'Type B', and the list of products. The video player controls at the bottom show a play button, a progress bar at 7:51 / 22:29, and other standard icons.

Lecture 29

## TYPES OF BOTOX INJECTIONS

- Botulinum toxin is available in two forms:
- Type A. Type A is mainly used for treating facial wrinkles. Type A products include onabotulinumtoxinA (Botox), abobotulinumtoxinA (Dysport), incobotulinumtoxinA (Xeomin), daxibotulinumtoxinA (Daxxify) and prabotulinumtoxinA (Jeuveau).
- Type B. Type B is often the first choice for treating neck spasms. It's sold as rimabotulinumtoxinB (Myobloc).

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There are different types of botulinum toxins used in Botox Therapy. Type A botulinum toxin, which includes products like Botox, Dysport, and Xeomin, is primarily used to treat facial wrinkles. On the other hand, Type B botulinum toxin, known as Myobloc, is often the preferred choice for treating conditions like neck spasms.

Despite its reputation as a potential bioterrorism agent, botulinum toxin has valuable medical applications, particularly in cosmetic surgery and various therapeutic treatments. Botox injections are effective for managing symptoms of several medical conditions,

though they do not offer a cure. For instance, in cervical dystonia, a condition where neck muscles contract uncontrollably causing discomfort and an abnormal head position, Botox can help alleviate these symptoms and improve quality of life.

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Lecture 29

## BENEFITS OF BOTOX THERAPY

### CERVICAL DYSTONIA

- Botox injections also are used to ease symptoms of some health conditions. It's not a cure. Examples of medical conditions that might be treated with Botox injections include:
- Neck spasms. In this painful condition, the neck muscles contract in an uncontrolled way. This causes the head to twist or turn into an uncomfortable position. The condition also is called cervical dystonia.
- Other muscle spasms. Cerebral palsy and other conditions of the nervous system can cause the limbs to pull in toward the center of the body. Muscle spasms also can cause eye twitching.
- Lazy eye. The most common cause of lazy eye is an imbalance in the muscles used for moving the eye. Lazy eye also is called crossed eyes or misaligned eyes.
- Sweating. Botox might be used for a condition in which people sweat a lot even when they're not hot or working up a sweat. It's called excessive sweating or hyperhidrosis.
- Migraine. Botox injections may help reduce how often you get a migraine. This treatment is used mainly for people who have headaches 15 or more days a month. When you get serious headaches that often, the condition is called chronic migraine. Treatment is needed about every three months to retain the benefit.
- Bladder problems. Botox shots can also help reduce urinary incontinence caused by an overactive bladder.

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Another nervous system condition that can cause involuntary muscle contractions is called vertebroplasty. Additionally, muscle spasms can lead to eye twitching and conditions such as lazy eyes. Lazy eyes, also known as strabismus or misaligned eyes, are often caused by an imbalance in the muscles responsible for eye movement.

In some cases, Botox can be used to manage excessive sweating, known as hyperhidrosis, where individuals perspire excessively even in the absence of heat or physical exertion. Botox injections may also be beneficial for reducing the frequency of migraines, particularly for individuals who experience headaches 15 or more days per month. This treatment can help alleviate symptoms of chronic migraines, and its benefits typically require maintenance treatments every three months.

Botox shots can also address urinary incontinence caused by an overactive bladder,

providing relief from bladder issues. These are some of the primary benefits of Botox therapy.

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Lecture 29

## CRYSTAL STRUCTURE OF THE BOTULINUM TOXIN

- Crystal structure of botulinum neurotoxin A1 (BoNT/A1)17, showing its associated electrical dipole and the organization of individual toxin domains, each of which has a specific function in cell intoxication: the HC domain binds specifically to nerve terminals; the HN domain translocates the L chain into the nerve terminal cytosol; and the L chain is a metalloprotease that cleaves and inactivates specific SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins that are involved in neurotransmitter release, thereby causing nerve paralysis. A peptide belt (shown in dark blue), which surrounds the L domain and the inter-chain disulphide bond (orange), links the L chain to the HN domain.

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Now, let's delve into the crystal structure of botulinum toxin to understand its structural details. The basic structure of botulinum toxin includes various domains with specific functions in cell intoxication. The HC domain of the toxin specifically binds to nerve terminals, while the HN domain facilitates the translocation of the L chain into the nerve terminal cytosol.

To elaborate, the botulinum toxin consists of distinct domains: the HC domain binds to the nerve terminal, and the HN domain assists in transporting the L chain into the cytosol of the nerve terminal. Once inside, the L chain, a metalloprotease, cleaves and inactivates specific SNARE proteins. These SNARE proteins, such as the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNAP-25), are crucial for neurotransmitter release and thus play a key role in nerve function. The peptide belt, shown in dark blue, surrounds the L domain, and the interchain disulfide bonds, depicted in orange, maintain

the toxin's structural integrity.

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## MECHANISM OF ACTION

Botulinum toxin type A (BoNT-A) mechanism of action. The BoNT-A heavy chain is shown in green and the light chain in yellow, linked by a disulfide bond. Acetylcholine (ACh), the neurotransmitter that is blocked by BoNT-A, is shown as red dots within a circular vesicle in the nerve terminal. The effects of chemodenervation via injection of BoNT-A are summarized at macroscopic, microscopic and molecular levels. SNAP 25, soluble N-ethylmaleimide fusion protein/attachment protein; VAMP, vesicle-associated membrane protein. Reused from for non-commercial/educational purposes under a Creative Commons license (Attribution-NonCommercial), Springer Nature.

The diagram illustrates the mechanism of action of Botulinum toxin type A (BoNT-A) at the neuromuscular junction. It shows a motor terminal containing vesicles of acetylcholine (ACh) and SNARE proteins (SNAP-25, VAMP). BoNT-A binds to its receptor and is internalized. The light chain of the toxin cleaves SNAP-25, VAMP, and synaptobrevin, preventing the formation of the synaptic vesicle and blocking ACh release. This leads to muscle paralysis. The diagram also shows the effects of chemodenervation on the muscle cell, including muscle atrophy and denervation atrophy.

**Macroscopic changes**

- Gross muscle atrophy (ultrasound, MRI)
- Weakness
- Decreased spasticity
- Increased range of motion
- Partial recovery at 6–12 months

**Microscopic changes**

- Denervation atrophy
- Loss/damage to contractile elements
- Change in fiber type: loss of Type I fibres
- Fat infiltration
- Nerve sprouting — NMJ recovery
- Fibrosis
- Reversibility not confirmed in studies to date

**Molecular changes**

- Cleavage of SNAP-25, VAMP, synaptobrevin
- ACh release blocked
- Synaptic vesicle complex does not form
- Light chain cleaves synaptobrevin, SNAP-25, VAMP
- ACh release begins from external sprouts and recovering neuromuscular junction

In terms of mechanism, when botulinum toxin binds to its receptor, it prevents acetylcholine from attaching to its receptor on the muscle. The toxin is internalized into the cell, where it disrupts the machinery responsible for acetylcholine secretion, leading to muscle paralysis. The HN domain facilitates the entry of the L chain into the nerve terminal cytosol, where the L chain then acts as a metalloprotease, cleaving and deactivating the SNARE proteins necessary for neurotransmitter release.

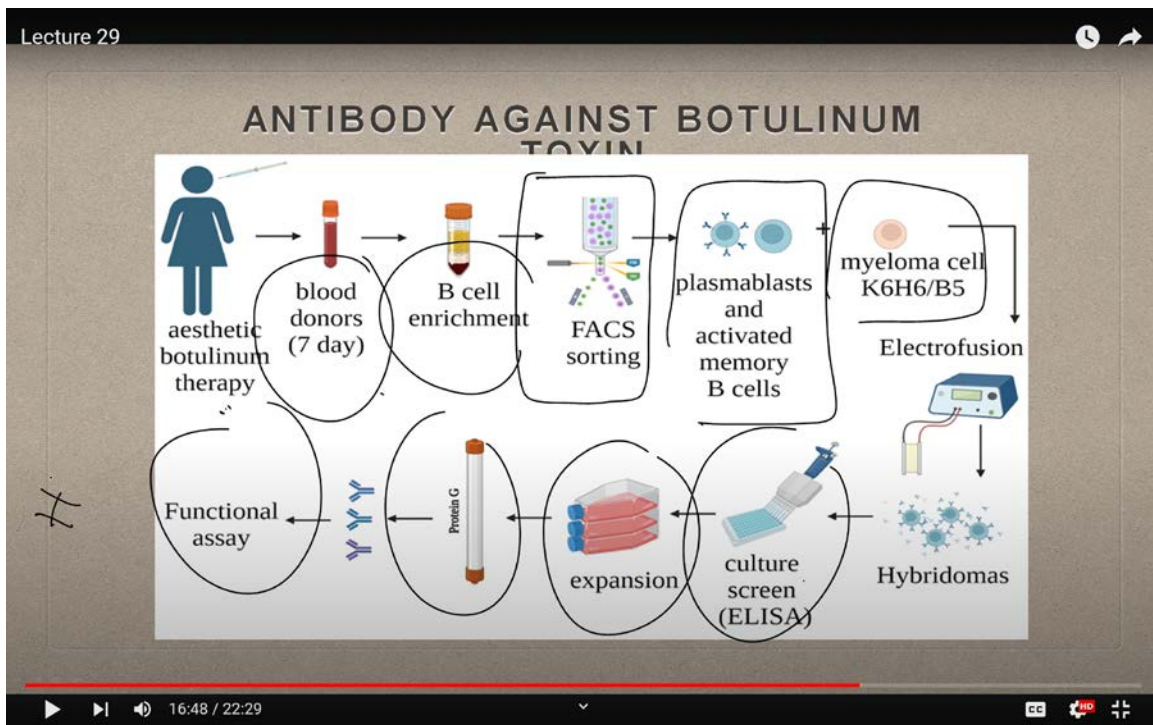
The protein involved in neurotransmitter release, known as SNARE proteins, is cleaved by the botulinum toxin. Once these SNARE proteins are cleaved, the nerve terminal loses its ability to secrete acetylcholine. This results in significant macroscopic changes, such as muscle atrophy, which can be observed using MRI. Additionally, there will be weakness, reduced spasticity, and an increased range of motion. Microscopically, one might see denervation and the loss or damage of contractile elements in the muscle fibers.

On a molecular level, the botulinum toxin disrupts protein SNAREs, blocks acetylcholine

release, and activates inflammatory and fibrotic pathways. The mechanism of action for botulinum toxin type A involves the heavy chain (shown in green) and the light chain (shown in yellow), which are linked by a disulfide bond. This linkage is crucial for the toxin's function.

Botulinum toxin A prevents acetylcholine and neurotransmitter binding, as indicated by the red dots representing the effects of chemical denervation. "Chemical denervation" refers to the chemical-induced loss of nerve function through botulinum toxin injection. This process is summarized at both macroscopic and molecular levels, where SNAP-25, a soluble N-ethylmaleimide-sensitive factor attachment protein, is cleaved, preventing neurotransmitters from binding to the muscle.

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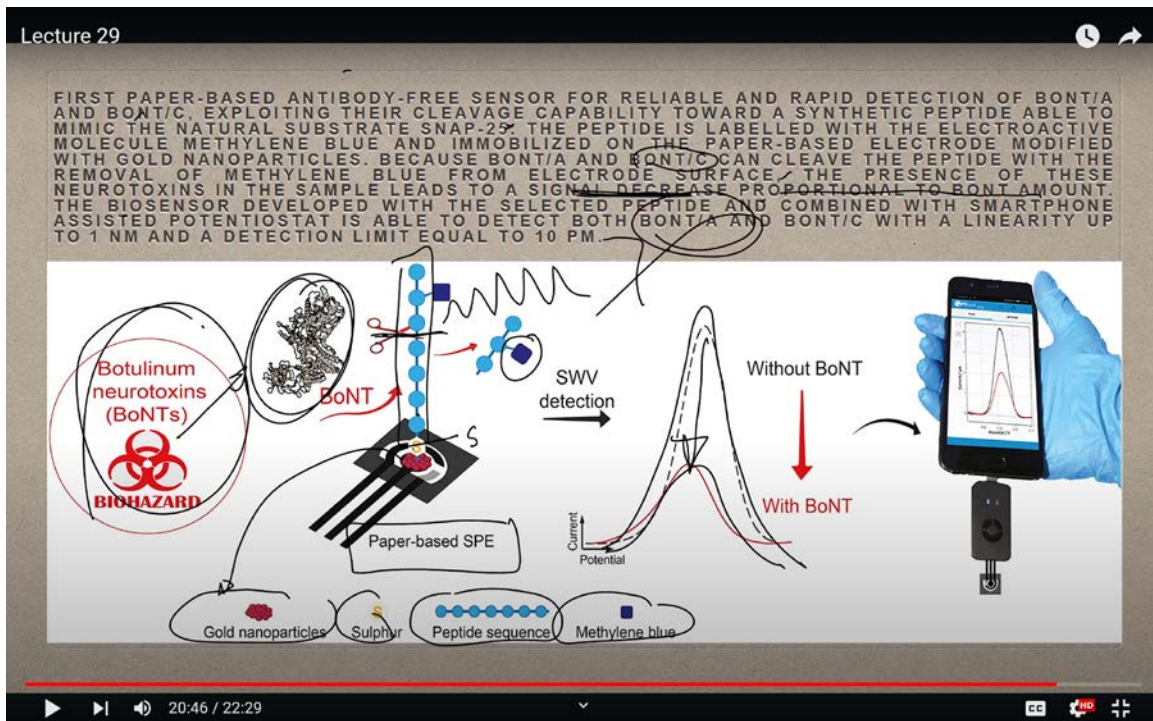
For example, the effect of botulinum toxin can be seen in cosmetic treatments. By preventing muscle contraction, the toxin effectively smooths out wrinkles, resulting in a wrinkle-free appearance. However, this effect is temporary, requiring repeat treatments every two to three months to maintain the desired results.



Interestingly, many major cosmetic companies in Europe face ethical restrictions when it comes to animal testing for botulinum toxin due to animal rights regulations. Consequently, these companies may either send samples to countries with less stringent animal ethics laws or utilize synthetic neuromuscular junction models grown in vitro for testing. For the past 25 years, the cosmetic industry has faced challenges in finding suitable testing models for Botox and similar products.

Regarding the development of antibodies against botulinum toxins, the process involves several steps: obtaining blood from botulinum therapy donors, enriching B cells, and performing various techniques such as FAC sorting, plasmoblast and activated B cell memory isolation, myeloma cell electrofusion, ELISA screening, and functional assays. These methods are crucial for developing effective antibodies and ensuring the safety and efficacy of botulinum toxin-based therapies.

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This diagram outlines the overall process for developing antibodies against botulinum toxin, which closely mirrors the techniques discussed in our previous classes on hybridoma

technology and other methods of antibody production. The approach is consistent across different antibody development methods.

Now, let me introduce you to an intriguing biosensor that has been developed: a handheld biosensor designed for detecting botulinum toxin, a potent neurotoxin. This sensor utilizes a paper-based platform that incorporates several key components.

The sensor features a conjugated molecule made of sulfur, which binds to a peptide sequence. This peptide sequence includes methylene blue, an electrical component. The unique aspect of this peptide is its susceptibility to cleavage by botulinum toxin. The sensor also incorporates gold nanoparticles, which enhance the electrical signal.

In this setup, sulfur acts as the conjugating agent, binding gold nanoparticles on one side and the peptide on the other. When botulinum toxin is present, it cleaves the peptide, leading to a significant drop in the electrical signal. Initially, the sensor provides a certain electrical signal, but after the peptide is cleaved, this signal decreases substantially.

This paper-based sensor is noteworthy because it operates without antibodies. It provides a reliable and rapid detection method for toxins by exploiting their ability to cleave a synthetic peptide that mimics the natural substrate of SNAP-25. Essentially, the sensor leverages biological principles by using a peptide designed to replicate the SNAP-25 sequence, which botulinum toxin targets and cleaves.

When the peptide is intact, it maintains a high electrical signature due to the presence of methylene blue. However, when botulinum toxin cleaves the peptide, the methylene blue is released, causing a significant reduction in the electrical signal. This drop in signal indicates that the neurotoxin is actively interacting with the peptide, thus providing a clear and immediate indication of toxin presence.

This is how the biosensor functions: The peptide used as a natural substrate for SNAP-25 is labeled with the electroactive molecule methylene blue and is immobilized on a paper-based electrode that has been modified with gold nanoparticles. When botulinum toxin is present, it cleaves the peptide, removing methylene blue from the electrode surface. This cleavage results in a decrease in the electrical signal detected by the biosensor. The

reduction in signal is directly proportional to the amount of botulinum toxin in the sample.

The biosensor, which incorporates the selected peptide and is paired with a smartphone-assisted potentiostat, is capable of detecting botulinum toxin with linear accuracy down to 1 nanomolar, with a detection limit of 10 picomolar. This integration showcases a streamlined and effective approach to sensor technology. The principle is straightforward: the toxin cleaves the peptide, causing either an increase or decrease in the electrical signal, which can be observed on a handheld device. This allows you to quickly determine whether your sample contains botulinum toxin.

Most of these sensors are paper-based, making them both sustainable and environmentally friendly. They can naturally degrade over time when exposed to various environmental conditions.

In our next class, we will explore additional types of sensors for detecting botulinum toxin before moving on to the next topic. Thank you.