Design for Biosecurity

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Lecture 58

Cell Based Biosensors - Part 3

Welcome back to this class. In the previous session, I mentioned how we could mimic the brain's electrical activity on a chip and recreate some of the key events that occur within the brain. Today, we will explore this concept through a case study focused on how a neurotoxin affects the substantia nigra, and whether it influences other parts of the brain. One way to investigate this, without having to harm or kill thousands of animals, is through the use of advanced technology on a chip, a direction where modern research is heading.

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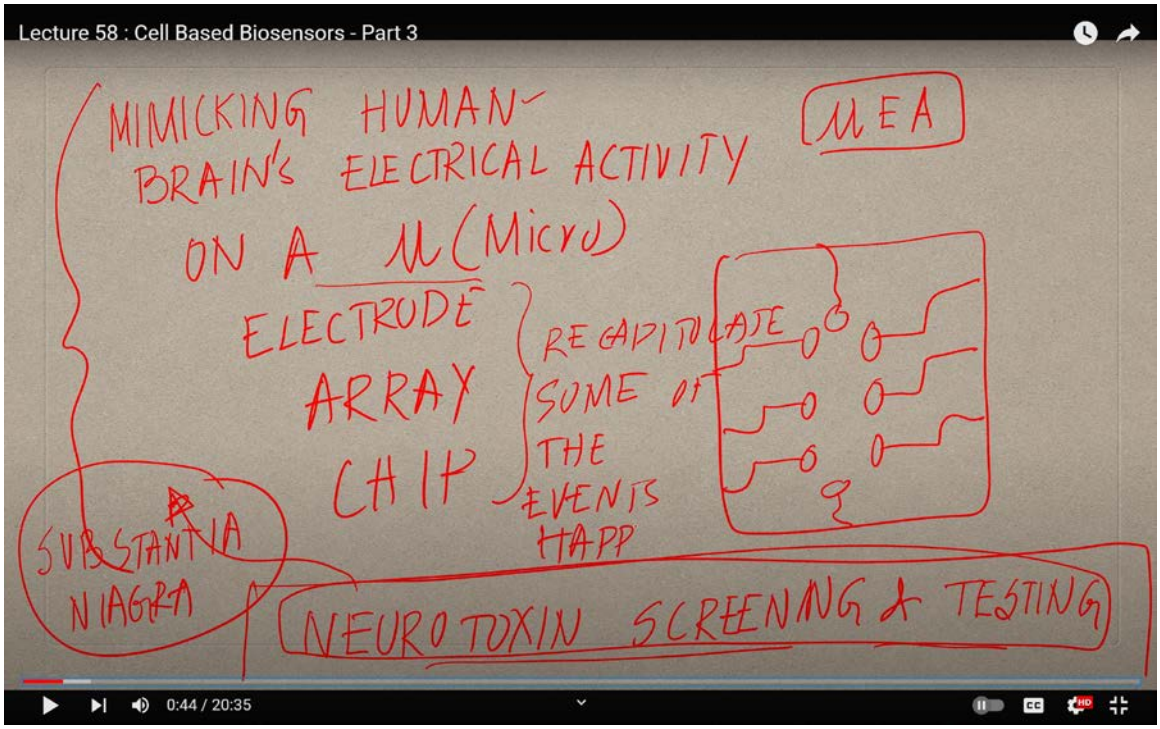

For instance, you could use a rodent model or stem cells to develop substantia nigra neurons and hippocampal neurons. Stem cells are a great source for these types of neurons, or you

could collect the cells from a small number of rodents. Using cutting-edge photolithographic techniques, we can develop and lay down an extracellular matrix in the form of a circuit. The goal here is to establish a connection between the neurons of the substantia nigra and those of the hippocampus.

Imagine you have a custom-designed microelectrode array chip. These dotted lines on the chip represent where neurons will grow. The extracellular matrix is printed on both the electrode surface and the non-electrode surface, and the electrodes are marked E1, E2, E3, and so on. In this simplified example, you'll also see small tunnels, large enough to allow neuronal processes to grow through, but too small for the cell body to cross. Now, you would take cells from the substantia nigra and plate them on one side of the chip. Since most of the chip is designed to be non-cell-adhering, the neurons will naturally grow along the designated lines, clustering on top of the electrodes.

In a similar way, you would plate hippocampal neurons in another area and allow them to grow. These neurons would follow the same pathways, moving through the tunnels and making connections with the substantia nigra neurons. Once they connect, synapses are formed, creating communication between the two regions. This connection will eventually lead to the generation of electrical activity, where signals are exchanged between the two areas. While this is a conceptual example, it demonstrates how technology allows us to address research questions that were once difficult or impossible to explore.

Now, to further test this, you introduce a neurotoxin into the environment. The toxin is added close to the substantia nigra side of the chip, and it will naturally affect the cells in that region first. Over time, a small amount of the toxin will diffuse to the hippocampal side, just as it might in a real brain through cerebrospinal fluid. By monitoring this setup, we can observe how the neurotoxin influences the electrical activity in both regions, offering valuable insights into the toxin's effects.

Is this electrical activity being compromised, and how long does it take to show signs of impairment? As I mentioned earlier, with this model, you can conduct extensive chronic experiments to monitor long-term effects. This system can also be adapted for other fascinating studies. For instance, instead of focusing solely on different brain regions, you

could explore how a neuromuscular junction functions. In this setup, you can grow muscle cells on one side and neurons on the other. The neurons will extend processes, forming connections with the muscle, enabling you to study the interaction between them, including the effects of neuromuscular toxins. You can apply toxins directly at the synaptic site, the neuromuscular junction, or on the neuronal side, and observe the resulting changes.

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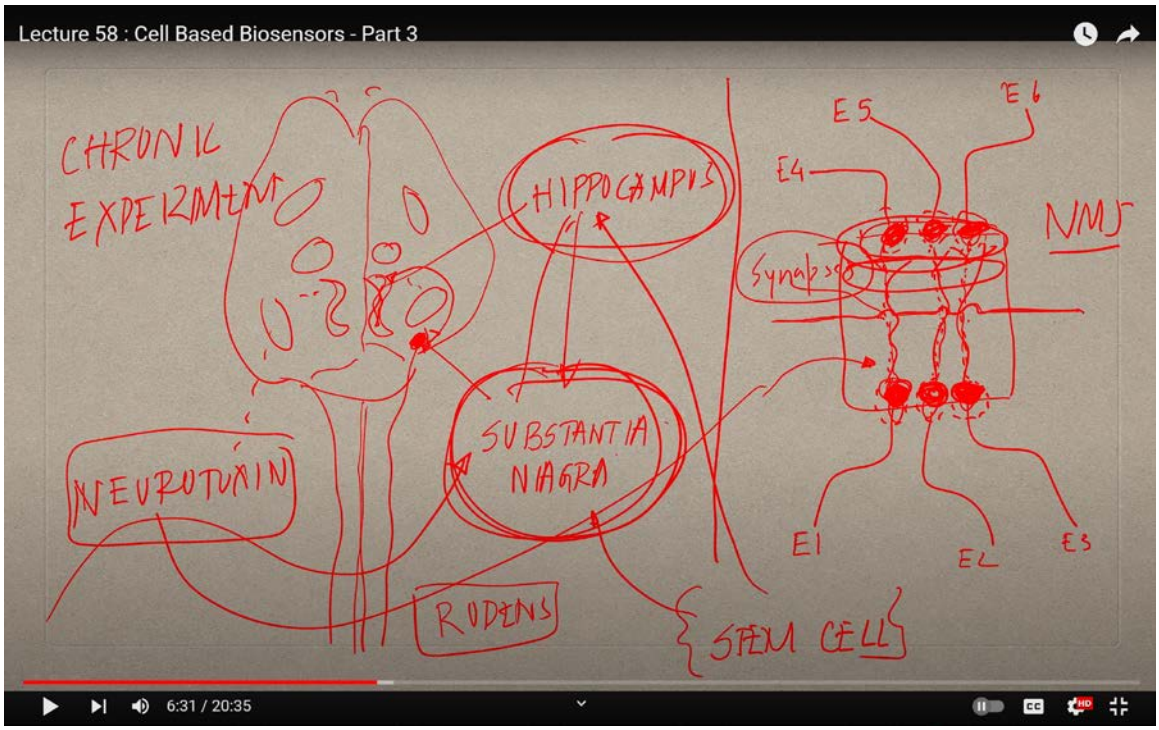

Similarly, in the substantia nigra and hippocampus two-chamber model, you could place toxins in one chamber to see if it affects the other side, enabling you to explore a wide range of toxin interactions. There are countless combinations and possibilities for such experiments. However, to achieve this, you must first understand how to mass-produce planar microelectrode arrays. This requires proficiency with photolithography, cell culture technologies, microsystem fabrication, and electronic interfaces, key tools that are essential for studying systems like these.

The next level of advancement involves translating these models into practical applications. For example, one major challenge we face is the paralysis caused by many of

these toxins. Paralysis results in muscles not functioning or moving. To understand this, recall the chain of events that I've mentioned before. The process begins when a neuron generates an action potential, which travels to the neuromuscular junction, triggering the release of the neurotransmitter acetylcholine. Acetylcholine crosses the synaptic cleft, binding to specific receptors on the muscle, which in turn opens cation channels, allowing sodium ions to flow into the muscle.

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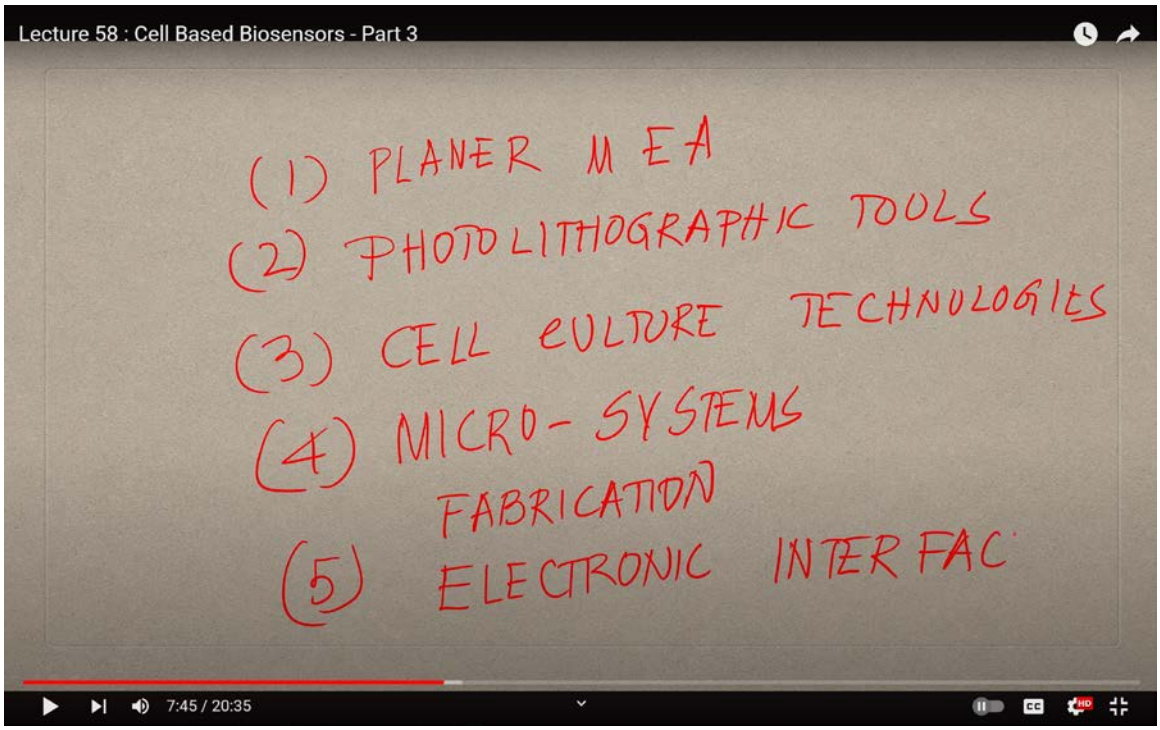

This influx of sodium generates a new action potential in the muscle. While the first action potential occurred in the neuron, there is a chemical transmission followed by an action potential generated within the muscle. This muscle action potential activates the sarcoplasmic reticulum (SR) via ryanodine and dihydropyridine receptors (DHPR), leading to the release of calcium from the SR. This calcium spike, although transient, plays a critical role in the interaction between actin and myosin filaments, ultimately resulting in muscle contraction. This entire sequence, from neuronal excitation to muscle contraction, is known as the excitation-contraction coupling theory, where excitation and contraction are tightly linked.

Now, this whole process can be recapitulated using microelectrode arrays or patch clamp electrodes. But I also want to introduce a different kind of sensor, one that utilizes cantilevers. Since muscles generate varying degrees of force, cantilevers can be used to measure these forces dynamically, providing another innovative approach to studying muscle activity and response.

Let’s take a moment to look at the different types of muscles in the human body. We have three main types: skeletal muscle, cardiac muscle, and smooth muscle. Skeletal muscle is responsible for generating the most force. Within skeletal muscle, there are two types of fibers, intrafusal fibers, which are present in extremely small numbers, and extrafusal fibers, which make up the bulk of the muscle. Then, you have cardiac muscle, which drives most of the force production in the heart (from "cardio" meaning heart, and "myo" meaning muscle). Finally, there are smooth muscles, which are mainly responsible for involuntary actions, such as those in the digestive tract.

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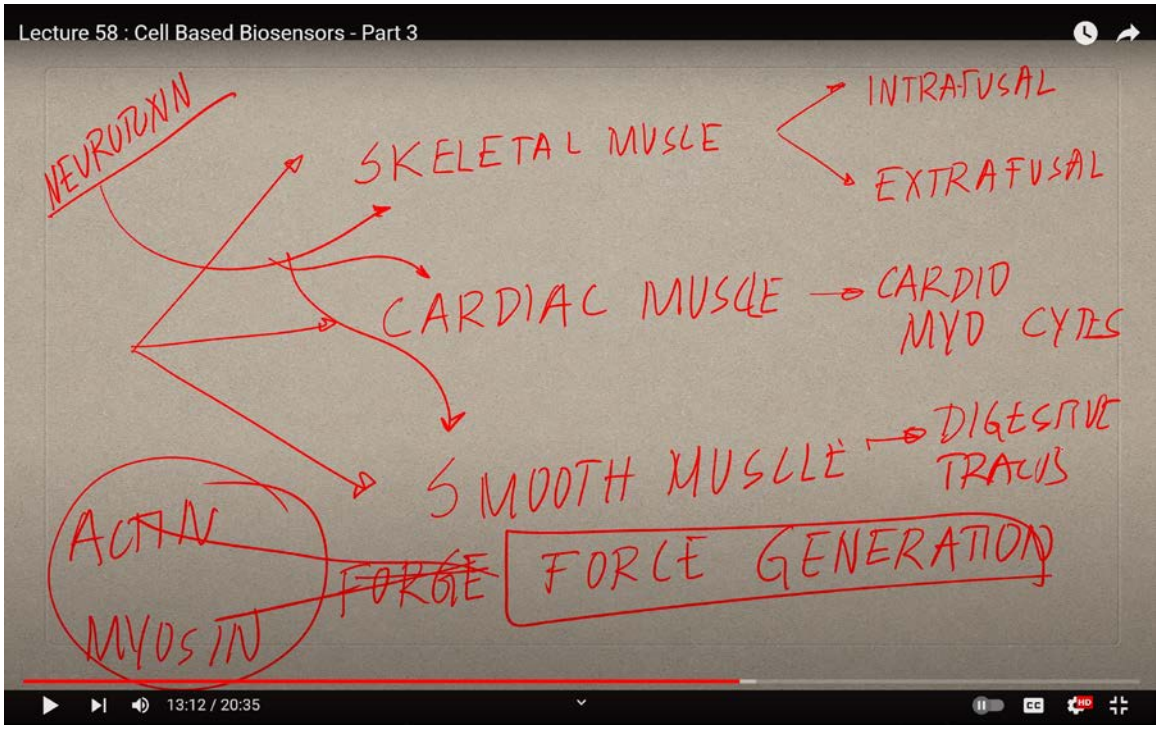

Now, most neurotoxins tend to target either skeletal or cardiac muscles, while smooth muscles are primarily affected in areas where involuntary movements occur. The question we face is: how can we study muscle force dynamics without relying on animals? How can we measure muscle force in a more ethical and advanced way?

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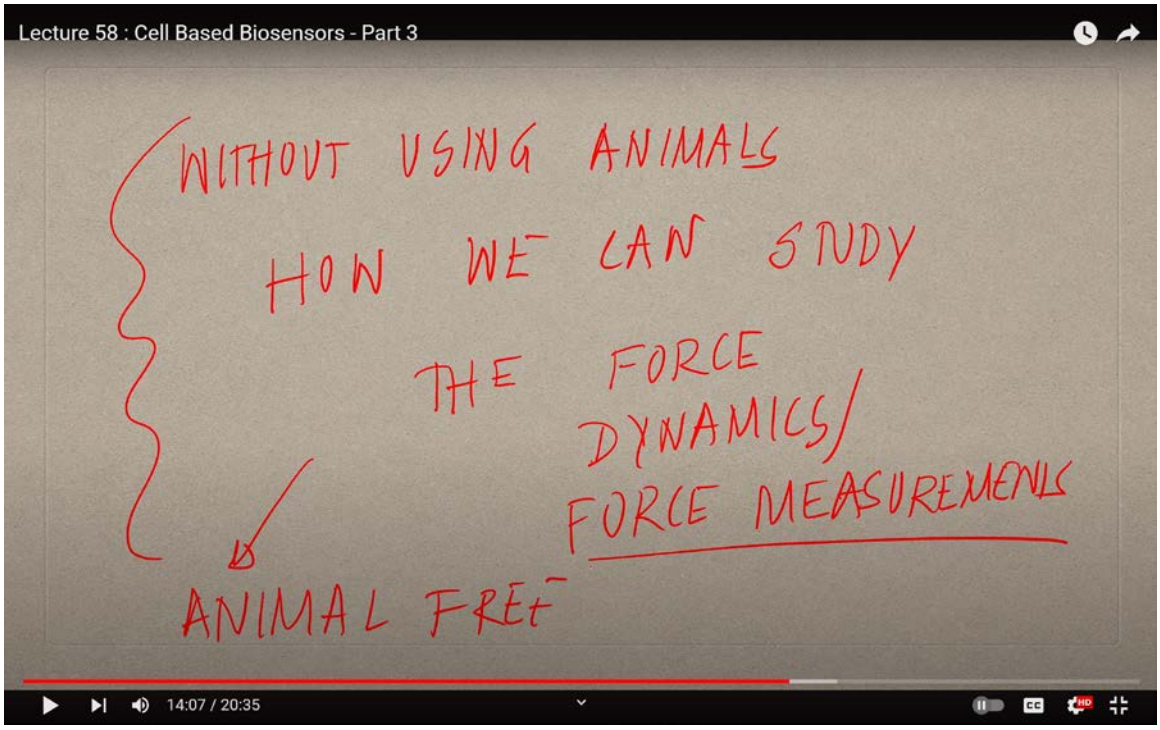

This is where technology, specifically microfabrication and electronic interfaces, comes to the rescue. One such technology is the microcantilever platform. You might wonder, what is a microcantilever? Let me give you a simple analogy. Think about a bridge, or better yet, picture a diving board at a swimming pool. When a diver stands at the edge of the board, the board bends, creating a motion that you can observe. If you shine a light on the tip of the board and use a position-sensitive detector, you can track the motion by measuring how the angle of the light reflection changes. This change in angle (let's call it $\Delta\theta$) tells us about the motion, and from this motion, we can calculate the force that the diver exerts on the board.

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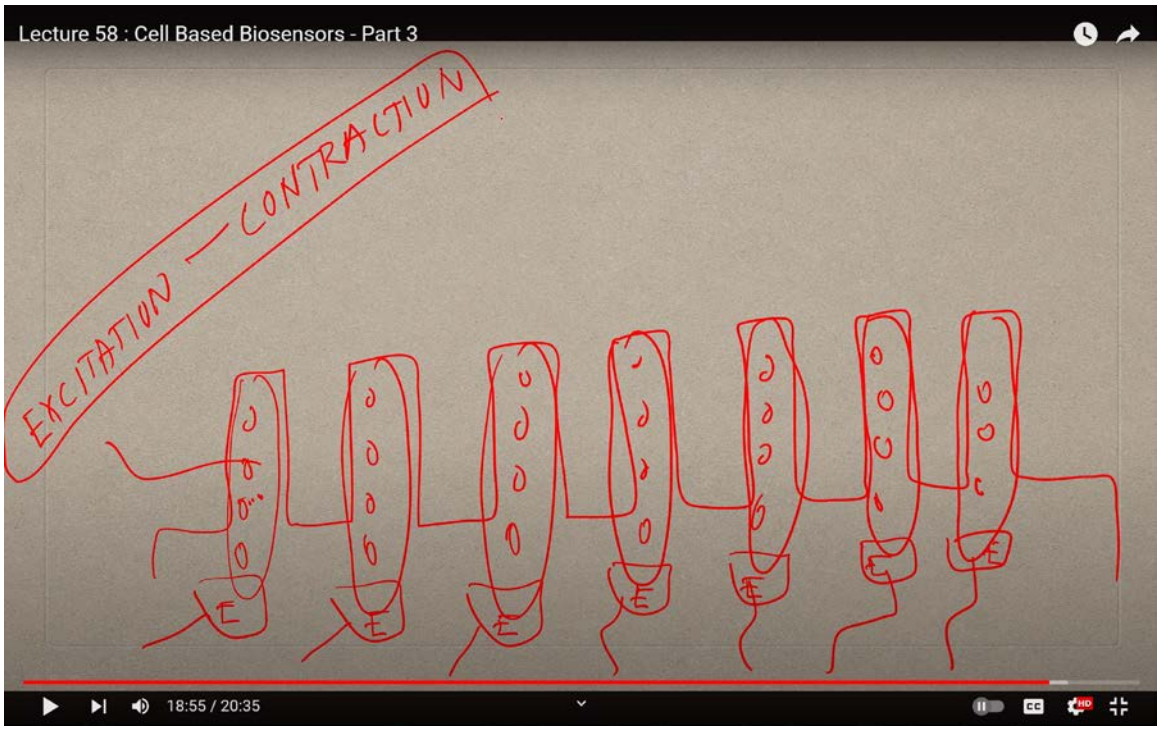

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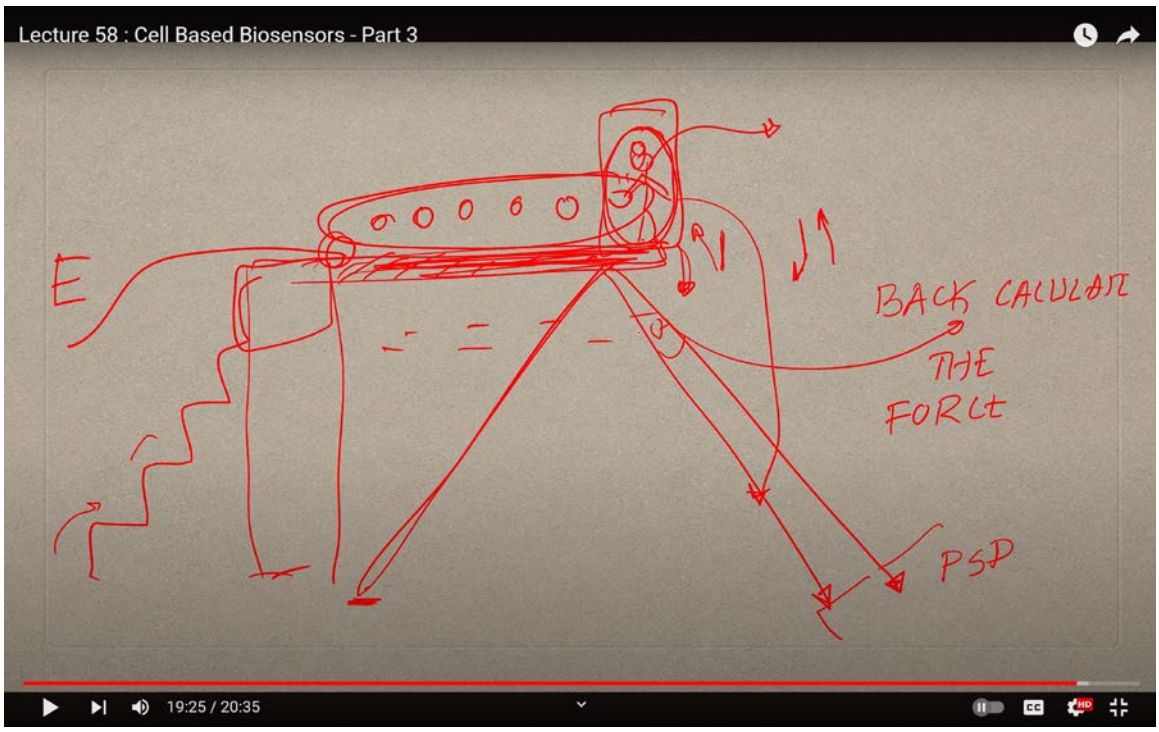

Now, imagine applying this principle on a much smaller scale. In a microcantilever array, each cantilever functions like a tiny diving board. If we could grow individual muscle cells on top of these cantilevers, we could measure the force generated by those muscles.

There are a couple of approaches here: since we aren’t incorporating neurons into the system, we could use electrodes to mimic the neurons' function. By placing electrodes beneath the muscle cells, we could provide electrical stimulation, triggering the muscles to contract.

So, think of this scenario: instead of a diver on a diving board, you have a muscle on the cantilever. Instead of the staircase the diver climbs, you have an electrode. Instead of a light beam, you have a laser, and you use a position detector to measure the movement. By doing this, you can measure the force that the muscle generates.

In the next class, we’ll delve deeper into how this technology can revolutionize our understanding of muscle dynamics and how it could serve as a powerful biosensor for future research. Thank you!