BioChemMed

Technium BioChemMed Vol. 10, pp.11-22 (2024)
ISSN: 2734-7990
www.techniumscience.com

Electronic Detection and Diagnosis of Complex Regional Pain Syndrome

Ashly Hahn*, Ridgewood High School, United States of America, ashly070712@gmail.com

Noella Park*, United World College of Southeast Asia, Singapore, noellapark08@gmail.com

*Co-first Authors

Abstract

Complex Regional Pain Syndrome (CRPS) is a chronic disease that is random and abrupt. As a result, an accurate diagnosis and treatment have yet to be identified due to numerous symptoms and causes. Currently, the method of diagnosis of CRPS is the Budapest Criteria. However, this criterion relies on the patient and doctor's subjective judgments. Therefore, we propose to develop a diagnostic method for CRPS, integrating reliable and objective analysis of common patterns that occur in patients' genetic and neuronal structures. Utilizing a zinc oxide biosensor, it was shown the patients' blood samples with high concentrations of MMP-9, and neuroinflammation occurs as the immune system is impaired. By analyzing blood samples, we quantified the number of CD4+ T cells that penetrate nerves, with direct human CD4+ T cell counting. We compared the structure of neuron cells and identified damage in CRPS patients that were located primarily on dendrites. The significance of using DNA detection and neuron biochips will dramatically improve the diagnosis and treatment of CRPS.

Keywords: complex regional pain syndrome; zinc oxide biosensor; MMP-9; CD4+ cell; neuron biochip; electronic detection

1. Introduction

Complex Regional Pain Syndrome (CRPS) is a disease that contains neuropathic pain. However, experts need clarification on the major cause of this disease. Since the treatment has yet to be reported clearly, the treatment has a limitation and does not have a direct positive effect on the disease. Three main treatments are used for CRPS patients to reduce that pain: medication, physical therapy, and mental therapy. Lyrica capsule, which Pfiz

Technium BioChemMed Vol. 10, pp.11-22 (2024)
ISSN: 2734-7990
www.techniumscience.com

er produces, is currently the most commonly used medication for relieving n europathic pain.

Moreover, scientists reported that physiotherapy does not affect trea ting the pain or disability of CRPS patients[1]. Even though there are trea tments that patients could try to reduce the pain, CRPS is an incurable, or phan disease meaning that the pharmaceutical industry will not develop reme dies. Since so few people have this disease, many people are still unaware of it. Also, the medicines on the market are known to have common side effe cts such as swelling and depression.

The major external symptoms of CRPS are long-lasting pains and sensit ivity to the temperature or touch. The pain normally starts from the end of the arm or leg. These result from internal inflammation, tissue damage, or nerve damage. CRPS is classified into 2 types based on the different cause s: type I and type II. CRPS Type I arises after injuries or illnesses, whil e Type II is caused by damage to a specific nerve[2]. The severity of CRPS is divided into 3 stages by the Budapest Criteria, created, and published b y the International Association for the Study of Pain (IASP). By the final stage, the affected parts are permanently deformed due to the atrophy of mu scles and tendons and are covered with dry skin that undergoes color chang e.

Therefore, since there is still no accurate diagnosis and treatment f or CRPS, it is important to improve fast and accurate diagnostic methods to prevent severe pain. Recent studies suggest that cells responsible for woun d healing and infection protection, such as MMP-9 and CD4+ T cells, or abno rmal neuronal cells, may play a role in the development of CRPS[3, 4, 5]. C onsequently, in this proposal, we investigated the correlation between the mutations in the blood samples and the potential of CRPS by using biochip-b ased analysis methods.

2. Materials and Methods

2.1 Detection of MMP-9 using ZnO biochip

MMP, which stands for Matrix Metalloproteinase, is a type of DN A that contains zinc and is dependent on calcium. Specifically, MMP-9 is a gene that contributes significantly to pathological processes su ch as wound healing and cell migration[6]. The concentration of MMP-9 in the human body fluctuates significantly during the healing of the human respiratory epithelium. It also indicates that wound healing is impaired when there is a change in the concentration of MMP-9 enzyme in the body. Therefore, we proposed that the MMP-9 enzyme may play a role in the etiology of CRPS, which can start with a small bruise and

nerve injury. We aimed to explore the potential impact of MMP-9 on CRPS by measuring its concentration.

Since MMPs are proteolytic enzymes that contain zinc, we concluded that a Zinc Oxide (ZnO) electronic biosensor would be suitable for measuring MMP-9 concentrations in patients. Electronic biosensors are inexpensive but effective because of their efficiency. Furthermore, it provides accurate diagnostics as well as fast results with very little technology[7, 8]. The ZnO in the biosensor can provide electrostatic forces, which help to immobilize enzymes and antibodies. A gold-coated substrate with a thin layer of ZnO would create a ZnO nanoparticle electrode. MMP-9 antibodies attached to each nanoparticle would connect and incubate the MMP-9[9]. Based on the number of MMP-9 enzymes connected to the antibody, the concentration comes out by the electrochemical impedance spectroscopy method (EIS). EIS offers kinetic and mechanistic data of various electrochemical systems. Electrochemical measurements are made using electrochemical workstation software controlled by EC-Lab software. ZnO nanoparticles were used as electrodes to enable measurements at around 60 frequencies[9].

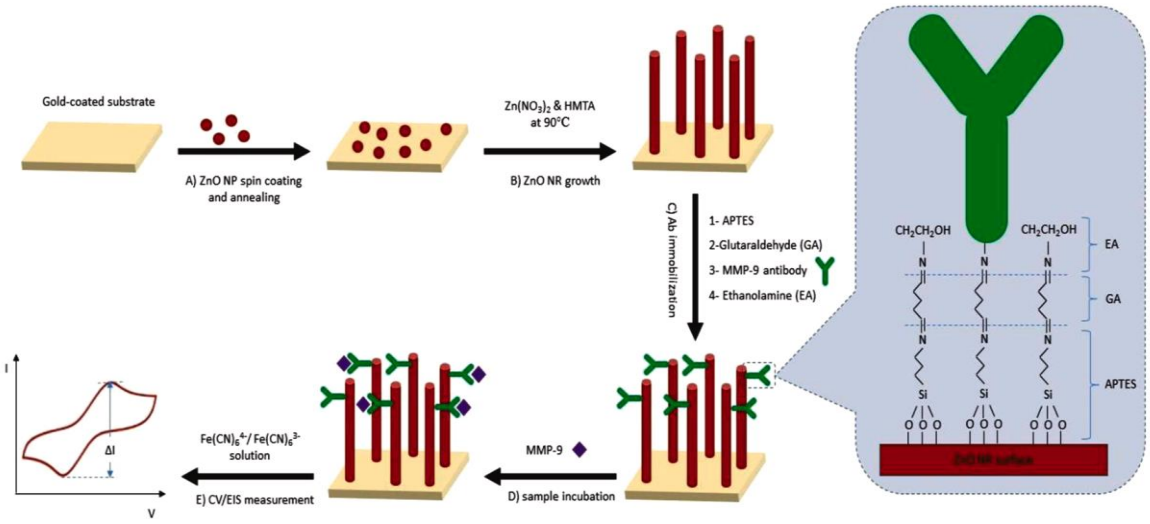

Fig. 1. Illustration of biosensor detecting MMP9. (A) ZnO nanoparticle (B) ZnO nanorod growth; (C) Antibody immobilization; (D) sample incubation; (E) Electrochemical measurement (CV or EIS). The chemical link between the ZnO surface and the antibody is shown on the right side of the illustration.

Technium BioChemMed Vol. 10, pp.11-22 (2024)
ISSN: 2734-7990
www.techniumscience.com

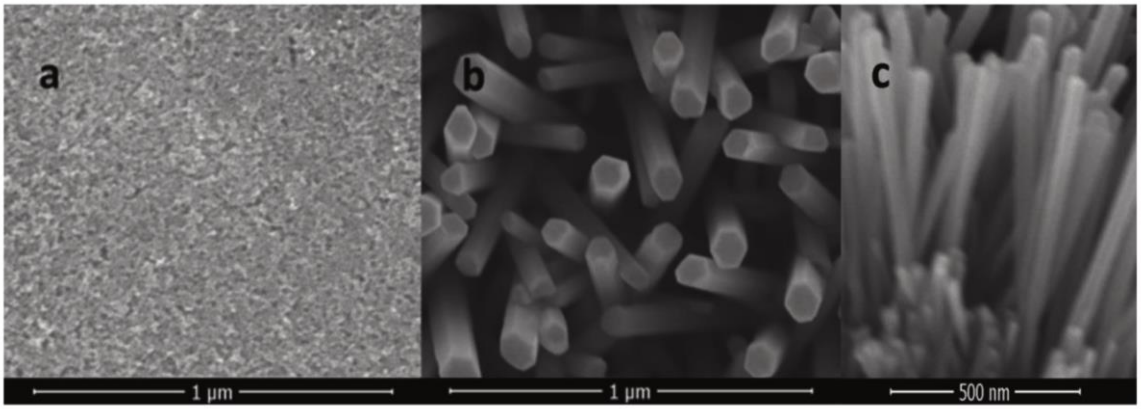

Fig. 2. Images of (a) ZnO nanoparticles, (b) ZnO nanorods c) ZnO nanorods cross-section.

With this technology, it is possible to connect it with the CRPS patients' blood samples. We would use both samples for the biosensor to compare the CRPS patients' and non-CRPS patients' blood samples. Utilizing the ZnO biosensor, we would continuously detect the concentration of MMP-9 in both samples. If MMP-9 levels are significantly lower or higher in CRPS patients compared to the general population, this could indicate that the patient has an injury that the body is unable to passively repair. This, in turn, can affect the suffering of CRPS patients[6].

2.2 Detection of the concentration of CD4+ T cells with the presence of CRPS

CD4+ T cells are a type of lymphocytes and are produced in the thymus. Their major role is producing a long-term immune response to external factors. The concentration of this cell can be measured in one's blood, and it can infer their current health state. If the concentration appears to be lower than the healthy range of 500 to 1200 cells per mm^3, the patient most likely has a diminished immune system. So as for CRPS patients, CD4+ T cell rates are higher than the healthy range, as their immune system is overly active as their body reacts excessively to pain.

CD4+ T cells can be detected through analysis of the patient's blood sample. It is first put under Red Blood Cell (RBC) lysing with an RBC lysis buffer for a sufficient number of times until all the plasma membranes of RBCs break down and RBCs are removed. The solution is put into a centrifugal separation machine, and the white blood cel

Technium BioChemMed Vol. 10, pp.11-22 (2024)
ISSN: 2734-7990
www.techniumscience.com

ls (WBC) are separated from the blood plasma due to the difference in concentration. The WBCs are stored in Phosphate Buffered Saline to pr event the shriveling of cells that occurs from osmosis. The separated WBCs are then dyed with DAPI and AF-488 conjugated anti-CD4 antibodie s, which are fluorescent substances that give an advantage when count ing. A hemocytometer is used complementarity with a microscope in the final stage to manually count the cells. With the collected data, the concentration of CD4+ T cells can be calculated and applied to back u p a more accurate diagnosis for CRPS[10].

2.3 Detection of damaged neuron cells utilizing the neuron biochip

A neuron biochip is used to identify the starting point of nerv e pain, which is the main cause of CRPS Type II[11]. Biochips can pro cess the electrical sign or induce electrical signals by combining or ganic matter and inorganic matter[12]. Biochip is a broad concept of biosensors, which includes biosensors among the types of biochip. Amo ng them, the neuronal biochip used in this proposal has the advantage of culturing neurons using human neuronal cells.

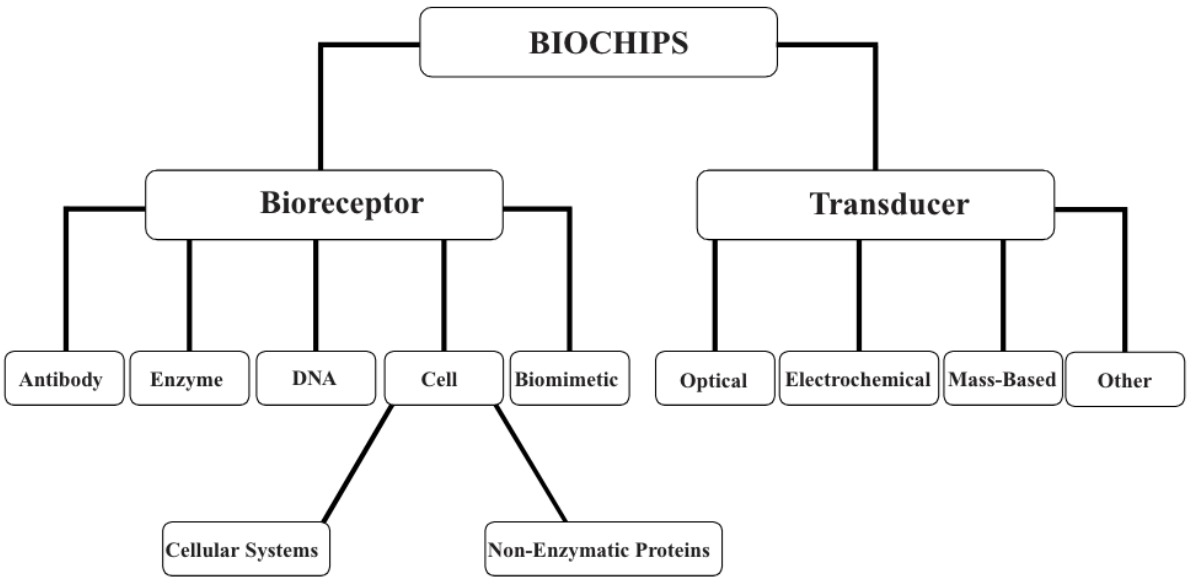

Fig. 3. Classification of biochip

Technium BioChemMed Vol. 10, pp.11-22 (2024)
ISSN: 2734-7990
www.techniumscience.com

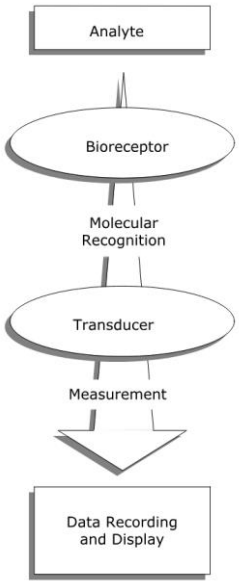

Fig. 4. Principle of biosensor

When polylysine is dotted in a grid on the surface of a neuron biochip, cultured neurons grow along the polylysine[11]. This is because the biocompatible nature of lysine, the amino acid that makes up polylysine's protein, makes it favorable for culturing neurons. The electrical charge of polylysine allows cells to adhere to specific surfaces and be cultured [11]. Using these characteristics of neuron biochips, damaged neuron cells from CRPS patients and neuron cells from normal people would be cultured on different biochips. The damaged neurons of CRPS patients would be detected by using MRI. The amount of axon growth on the biochips when culturing neurons from control and CRPS patients can be compared. By analyzing the culture patterns of the cells from CRPS patients, we can determine that damaged neuronal cells exhibit abnormal cell culture patterns and that these neuronal cells are the starting point for the symptoms of CRPS [11].

3. Expected Results

3.3 Proposal of expected result on MMP-9

When blood samples from CRPS patients were analyzed for MMP-9 concentrations, there was a difference in MMP-9 concentrations from those of the general population. The normal concentration of MMP-9 is 10-100 ng/ml, but CRPS patients had higher levels. Concentrations of MMP-9 in the abnormal range suggest that the body is unable to properly heal wounds or inflammation. These findings led to the conclusion that differences in the concentration of the MMP-9 gene in the patient

s' samples may trigger the onset of CRPS[6]. These differences in MMP-9 gene concentration may also affect the level of pain in CRPS patients.

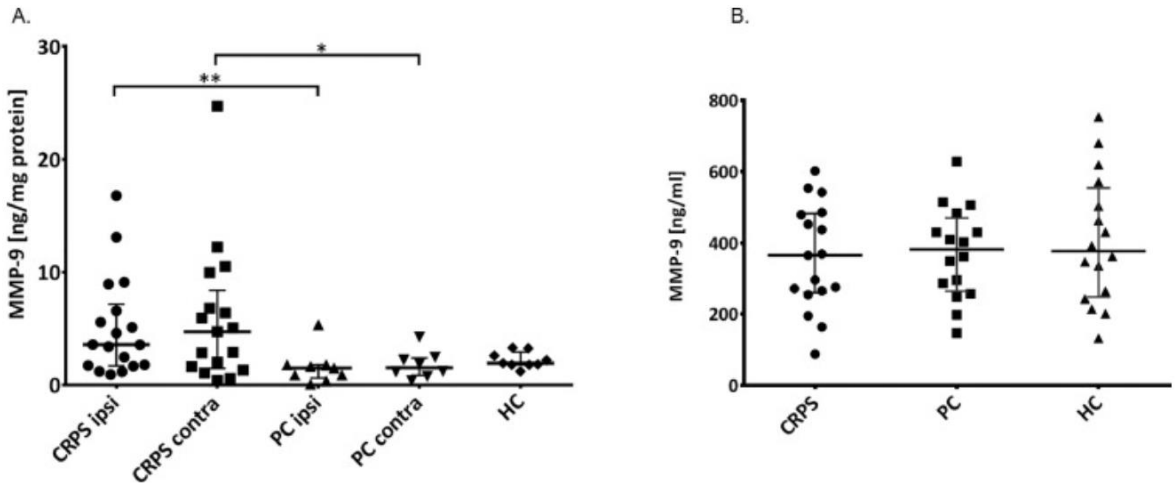

Fig. 5. Higher concentration of MMP9 in CRPS patients

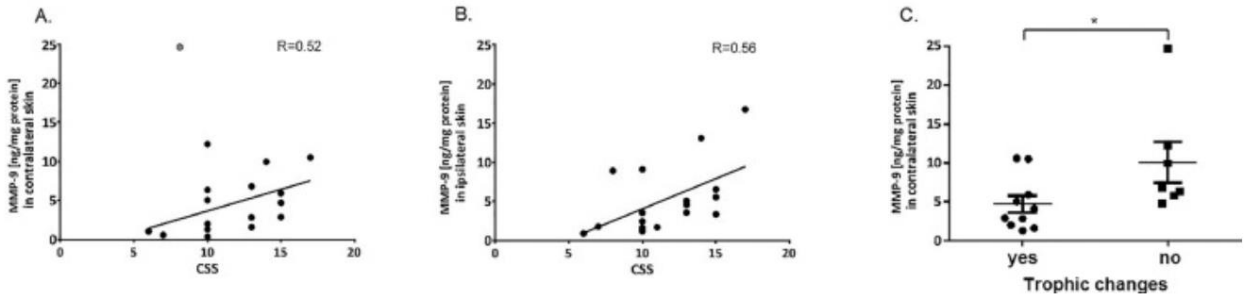

Fig. 6. Correlation between MMP9 and the pain level of CRPS.

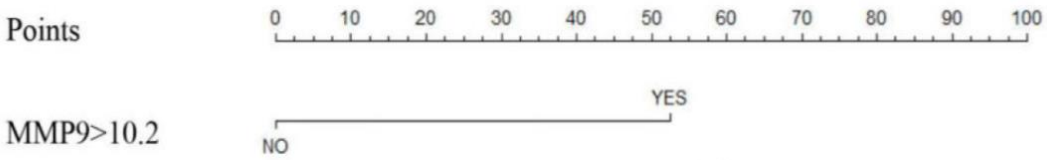

Fig. 7. Vertical projection indicates different variables, horizontal projection indicates the rate of CRPS scale.

3.4 Proposal of expected result on CD4+ T cell

One of the symptoms of CRPS is the weakening of the immune system. Although this can be tested in various ways, we proposed a method that detects CD4+ T cells, as particular mutated forms of these cells can penetrate nerves, mistaking the surface for bacteria or viruses. Although this theory has not been studied in depth yet, the detection of CD4+ T cells is still valid to evaluate the state of the immune sy

Technium BioChemMed Vol. 10, pp.11-22 (2024)
ISSN: 2734-7990
www.techniumscience.com

stem. CRPS patients will have a high concentration of CD4+ T cells in their blood, higher than healthy people with the range of 500 to 1200 cells per mm^3. Thus, this method can be further developed to explain t he correlation between CD4+ T cells and nerve damage and the relation between the immune and nerve systems. However, it should not be used as the sole criteria of diagnosis, as an increase of CD4+ T cells can indicate other internally infectious diseases such as cancer.

3.5 Proposal of expected result on Neuron biochip

When damaged neuronal cells from CRPS patients are grafted onto neuron biochips, the damaged neuronal cells exhibit uneven distributi on and growth[7]. When neurons were cultured on biochips with polylys ine grids, the damaged neurons showed slower growth. In the control g roup, neurons from normal people grew more uniformly and rapidly in t he same environment on neuron biochips. As shown in the figure below, you can see the axon branches of normal human neurons extending unifo rmly at 90 degrees, whereas, in the damaged neurons, you can see the branches extending randomly[11].

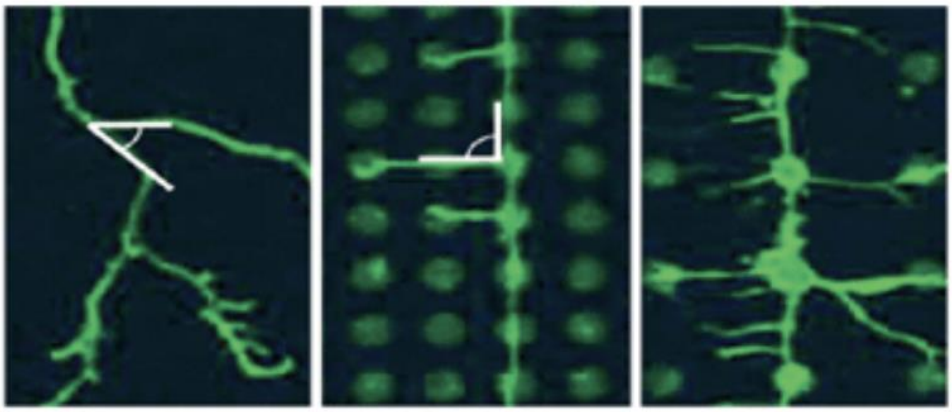

Fig. 8. Neurons grow chaotically on a chip with no pattern (left), wh ile they grow in a dot pattern on a chip with a dot pattern (center a nd right). A single nerve cell connects to more than 10,000 other cel ls via axons, which act as cables, and if the branches that branch ou t from the axons are not properly developed, it can lead to neurologi cal disorders such as autism and neurodegenerative diseases.

Therefore, we conclude that damaged and slow-growing neurons ma y be the main cause of CRPS type II.

3.6 Feasible treatment of deformed neuron cell using Organ-on-a-Chip model

Technium BioChemMed Vol. 10, pp.11-22 (2024)
ISSN: 2734-7990
www.techniumscience.com

Building on the results of the neuron biochip, we devised a method to treat the main cause of CRPS type II using several biotechnological methods. This utilizes the Lab-on-a-Chip (LOC) and Organ-on-a-Chip (OOC) models. LOC technology uses micro or nanofluidics technology. It pursues postenomics research and elucidates biochemical phenomena. It also can contribute to the discovery of the human genetic structure[12]. Using these features of LOC technology, we have come up with a way to rebuild damaged neuronal cells in patients using OOC. OOC technology is based on the lab on a chip technology. We take a tiny sample of an organ, and design it by microfabrication, so we can make a new tissue culture on a biochip.

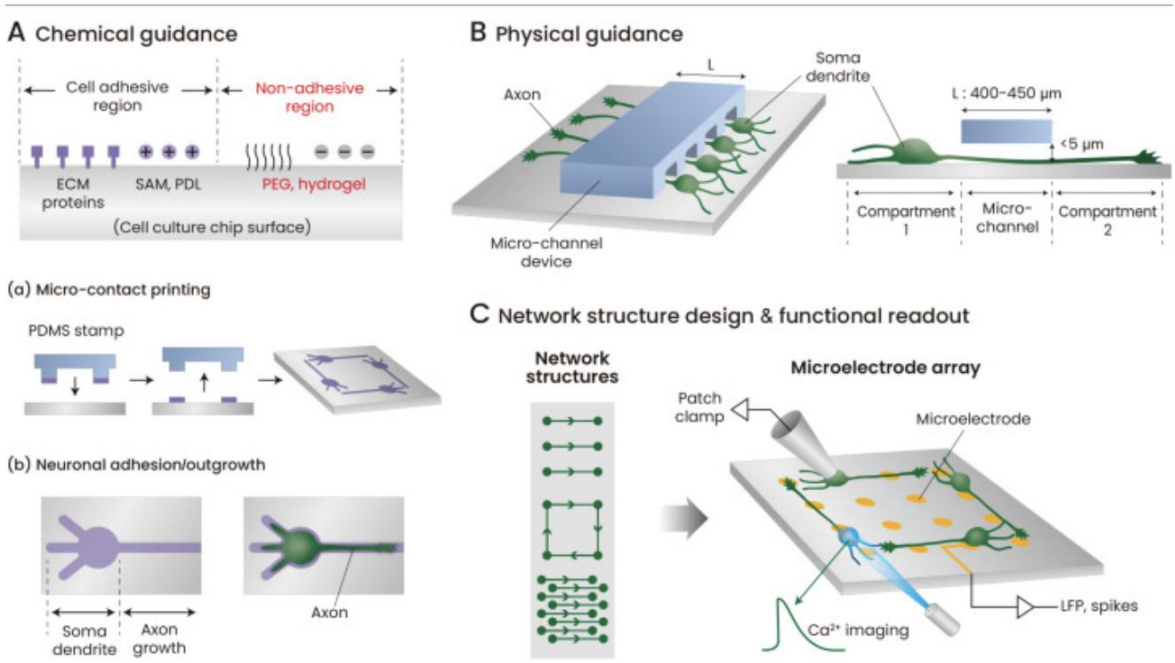

Fig. 9. Neuronal network chip design and analysis.

By using this technology, we can make a neuron cell OOC to mimic the original neuron cell function[13]. This reconstructed neuron will transfer the damaged neuron to the new one, and naturally cure the neuropathic pain. The new Neuron-on-a-Chip technology can be used as a disease model that can be used to observe and study the exact progression of diseases that develop in specific neurons in real time to find precise treatments. It also has the potential to develop into a medical technology that can be customized to each patient based on their characteristics.

Technium BioChemMed Vol. 10, pp.11-22 (2024)
ISSN: 2734-7990
www.techniumscience.com

4. Discussion

With a rare disease like CRPS, it's important to know how to accurately diagnose it and what causes it. However, the exact cause and complete cure of CRPS is still unknown. Even though there are possible treatments, these treatments are not a cure, but rather a way to reduce suffering. Therefore, it is important to know the exact cause and to find new approaches to the disease. Thus, we organized this proposal to help patients suffering from this rare disease and to contribute to medical progress.

Recent research on CRPS has shown that certain cells and enzymes are responsible for the development and symptoms of the disease. In particular, we focused on the relationship between the MMP-9 enzyme, which has wound healing and pathological significance, CD4+ T cells, which are a major contributor to the adaptive immune system, and neuronal cells, which are the starting point of nerve pain. Therefore, we used biochips and biosensors that are capable of analyzing genes and cells to derive results. We found that damaged neuronal cells, along with altered concentrations of the MMP-9 enzyme and CD4+ T cells, can have a significant impact on the pathogenesis of CRPS. Furthermore, by combining neuronal cells with LOC technology, we have devised a therapeutic approach that can prevent CRPS from arising from damaged neurons.

Based on these results, we were able to add new criteria and methods to the diagnosis of CRPS. The steady observation of cell concentrations that directly affect wound healing and the immune system, such as MMP-9 and CD4+ T cells, combined with advanced OOC techniques, is a diagnostic method that could be a step in the right direction against CRPS.

However, this proposal is not a formal medical study and lacks the environment for experimental proof. It also does not directly create or cite a database of patients, which may reduce the accuracy and reliability of the results. Finally, due to the complexity of CRPS, which is still not fully understood, there were clear challenges in aligning both the elaborate structure and biological function to establish this proposal.

5. Conclusion

In summary, we proposed a new diagnostic criterion for CRPS using biosensors and biochips to detect MMP-9 enzyme, CD4+ T cells, and abnormal nerve morphology. However, the application of this diagnostic criterion requires sustained efforts and attention from the experts. Furthermore, f

urther research and direct experiments are essential to increase the relia bility of this criterion and the possibility of applying it to patients in practice.

References

1. Smart, K. M., Ferraro, M. C., Wand, B. M., & O'Connell, N. E. (n.d.-b). Physiotherapy for pain and disability in adults with complex regi onal pain syndrome (CRPS) types I and II. *Cochrane Library*, *2022*(8). https://doi.org/10.1002/14651858.cd010853.pub3
2. Harden, R. N., McCabe, C. S., Goebel, A., Massey, M., Suvar, T., Grie ve, S., & Bruehl, S. (n.d.). Complex Regional Pain Syndrome: Practica l Diagnostic and Treatment Guidelines, 5th edition. *Pain Medicine*, *23* (Supplement_1), S1–S53. https://doi.org/10.1093/pm/pnac046
3. Zhu, H., Wen, B., Xu, L., & Huang, Y. (n.d.). Identification of Poten tial Inflammation-Related Genes and Key Pathways Associated with Comp lex Regional Pain Syndrome. *Biomolecules*, *13*(5), 772. https://doi.org /10.3390/biom13050772
4. Janicki, P. K., Alexander, G. M., Eckert, J., Postula, M., & Schwartz man, R. J. (n.d.). Analysis of Common Single Nucleotide Polymorphisms in Complex Regional Pain Syndrome: Genome Wide Association Study Appr oach and Pooled DNA Strategy. *Pain Medicine*, *17*(12), 2344–2352. http s://doi.org/10.1093/pm/pnw133
5. Kang, H., Noh, S., Hwang, J., Lee, J., & Gang, S. (2022). Genetic rol e in the pathogenesis of complex regional pain syndrome: a review. *ww w.jbtr.or.kr*. https://doi.org/10.12729/jbtr.2022.23.4.109
6. Escolano-Lozano, F., Gries, E., Schlereth, T., Dimova, V., Baka, P., Vlckova, E., König, S., & Birklein, F. (n.d.-b). Local and Systemic E xpression Pattern of MMP-2 and MMP-9 in Complex Regional Pain Syndrom e. *the Journal of Pain/Journal of Pain*, *22*(10), 1294–1302. https://d oi.org/10.1016/j.jpain.2021.04.002
7. Campuzano, S., Yáñez-Sedeño, P., & Pingarrón, J. M. (n.d.). Reagentle ss and reusable electrochemical affinity biosensors for near real-tim e and/or continuous operation. Advances and prospects. *Current Opinio n in Electrochemistry*, *16*, 35–41. https://doi.org/10.1016/j.coelec.2 019.03.006
8. Monošík, R., Stred'anský, M., & Šturdík, E. (n.d.). Application of E lectrochemical Biosensors in Clinical Diagnosis. *Journal of Clinical Laboratory Analysis*, *26*(1), 22–34. https://doi.org/10.1002/jcla.2050 0
9. Shabani, E., Abdekhodaie, M. J., Mousavi, S. A., & Taghipour, F. (n. d.-b). ZnO nanoparticle/nanorod-based label-free electrochemical immu

noassay for rapid detection of MMP-9 biomarker. *Biochemical Engineering Journal*, *164*, 107772. https://doi.org/10.1016/j.bej.2020.107772

10. Sher, M., & Asghar, W. (n.d.). Development of a multiplex fully automated assay for rapid quantification of CD4+ T cells from whole blood. *Biosensors & Bioelectronics/Biosensors & Bioelectronics (Online)*, *142*, 111490. https://doi.org/10.1016/j.bios.2019.111490
11. Development of a neuron culture biochip. . . Joint research team of Professor Nam Yoon-ki of KAIST Department of Bio and Brain Engineering and Professor Seon Woong of Korea University College of Medicine | News > Bio News > Trends | BRIC. (n.d.-b). BRIC. https://www.ibric.org/bric/trend/bio-news.do?mode=view&articleNo=8776498&title=%EC%8B%A0%EA%B2%BD%EC%84%B8%ED%8F%AC+%EB%B0%B0%EC%96%91+%EB%B0%94%EC%9D%B4%EC%98%A4%EC%B9%A9+%EA%B0%9C%EB%B0%9C..KAIST+%EB%B0%94%EC%9D%B4%EC%98%A4%EB%B0%8F%EB%87%8C%EA%B3%B5%ED%95%99%EA%B3%BC+%EB%82%A8%EC%9C%A4%EA%B8%B0+%EA%B5%90%EC%88%98%EC%99%80+%EA%B3%A0%EB%A0%A4%EB%8C%80+%EC%9D%98%EB%8C%80+%EC%84%A0%EC%9B%85+%EA%B5%90%EC%88%98+%EA%B3%B5%EB%8F%99%EC%97%B0%EA%B5%AC%EC%A7%84#!/list
12. Azizipour, N., Avazpour, R., Rosenzweig, D. H., Sawan, M., & Ajji, A. (n.d.-b). Evolution of Biochip Technology: A Review from Lab-on-a-Chip to Organ-on-a-Chip. *Micromachines*, *11*(6), 599. https://doi.org/10.3390/mi11060599
13. Hong, N., & Nam, Y. (n.d.). Neurons-on-a-Chip: In Vitro NeuroTools. *Molecules and Cells/Molecules and Cells*, *45*(2), 76–83. https://doi.org/10.14348/molcells.2022.2023